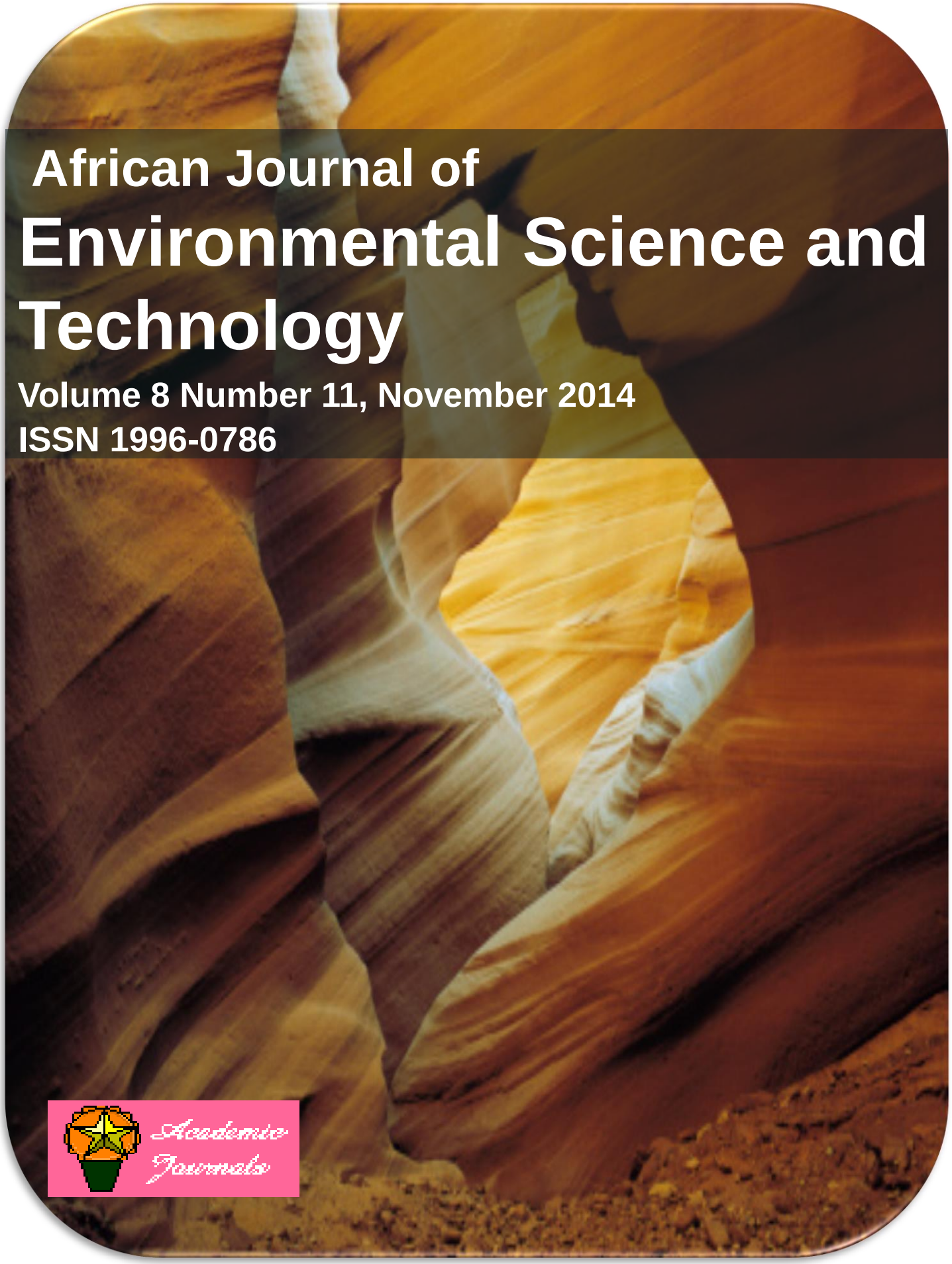

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Full Length Research Paper

Hospital waste generation and management practices in Owerri, Nigeria

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This study is aimed to estimate the rate of waste generations and describe the waste disposal/management practices. The study surveyed the waste generation and management practice of 12 hospital and clinics selected using multistage sampling techniques from the list of hospitals in Owerri Municipal. Wastes generated were weighed and parallel to that, data on patient influx, number of beds, length of stay and waste management practices were collected through a questionnaire. The average waste generated from the survey was 49.8 kg/day and 0.58 kg/bed/day comprising 16% hazardous and 84% non-hazardous. Fifty percent of the wastes generated were disposed openly in municipal bins. Also 41.7% of sharps are disposed mixed with municipal waste and despites high awareness on hazardous nature of hospital waste, only 16.7% of waste are collected in colour coded bags with 33.3% having laid down waste management plans for waste disposal. With the per day waste generation of hospital waste exceeding the per capita per day international generation of waste, that is, 0.25 kg in a country where an average citizen live on less than a dollar per day, there is an urgent need for increasing awareness and education on hospital waste generation and its implication for disposal and management.

Key words: Hospital waste generation, segregation, hazardous, non-hazardous, management, Owerri, Nigeria.

INTRODUCTION

The increasing number of hospitals in Nigeria and the inadequate management of hospital waste have posed a great threat to health and the environment through increasing waste stream. Waste is an issue in the world today, and waste quantities are generally growing (Babatola, 2008). But unfortunately, the lack of available and comparable data for many countries does not always allow reliable comprehensive assessment of waste-related issues. Few data are available on the composition of hospital waste, which is characteristically and extremely heterogeneous in nature (Dehghani et al., 2008).

Any anthropogenic activity is known to generates some wastes and these wastes described with the term

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"hospital waste", refers to waste generated during the diagnosis, treatment, or immunization of human beings or animals or in research activities in these fields or in the production or testing of biological substances (Babatola, 2008). Although a large part of hospital waste usually consists of hazardous/clinical and non-hazardous waste which could be solid or liquids (wastewater), hospital waste in general are by-products of healthcare activities and may include a broad range of materials, from used needles and syringes to soiled dressings, body parts, diagnostic samples, blood, chemicals, pharmaceuticals, medical devices, genotoxic waste, radioactive materials and heavy metals (WHO, 2011).

Johannessen (1997) provided generation rates of health-care waste by regions with Africa excluded. Also, a review of health-care waste generation rates has been provided by the World Health Organization (Pruss et al., 1999) for various country and hospital situations with none for Nigeria. This could be an indication of few available data (from different facilities and states) to estimate the generation of hospital waste in Nigeria.

Depending on the industrial base, waste is likely to vary from country to country (Babatola, 2008) with tendencies for higher generation rates in higher income countries due to industrialization. Although, the quantities of hazardous waste generated have decreased in many countries, an increase has been recorded in some due to changes in definitions such as industrialization and number of hospitals (Pruss et al., 1999).

In middle and low-income countries, health-care waste generation is usually lower than that in high-income countries (Khan et al., 2004). It suffice to state that despite waste management issues suffered by countries like Bangladesh, it produces very small quantity of hospital waste as compared to developed countries like United States of America (4.5 kg/bed/day). The 0.934 kg/bed/day average hospital waste generation rate reported for Sylhet city is in consistence to that of Dhaka city (1.2 kg/bed/day), but much lower than that of developed countries like USA (4.5 kg/bed/day), and France (2.5 kg/bed/day) (Sarkar et al., 2006; Rahman et al., 1999) but yet, poor management of waste has been reported for these developing nations (Fikru, 2004; Yemane and Millogo, 2000).

The actual hospital generation figures can vary greatly on a daily basis and the kilogram per bed per day generation figure itself is difficult to estimate. This variation can be attributed to the different methodologies used to calculate per bed generation figures.

The present low rate of waste collection especially in developing countries does not encourage the hospital to do special efforts to minimise the quantities of waste. In Nigeria, the lack of will by policy makers and implementtation groups to adopt current technology in healthcare waste management is an emerging challenge towards healthcare waste management with correspondent environment and public health implications for incorrect disposal. Hospital wastes are too hazardous to be treated carelessly and poor management of these wastes tends to spread infections and contaminate the entire environment of the hospital and its surrounding.

The basic concept of waste management in a hospital according to Sarkar et al.(2006), do not differ basically from that in hotels, schools and catering establishments since certain areas of the hospital render the same type of basic services; but as earlier stated, some waste generated in the hospital are too hazardous to be treated negligently.

World Health Organization (WHO) (2000) reported that from the total waste generated by healthcare activities, 80% is general waste (non-hazardous) and the remaining is considered as hazardous (infectious waste, pathological waste, sharps, genotoxic waste, chemical waste, wastes with high content of heavy metals, pressurized containers and radioactive waste). This fraction of waste generated at hospitals are known as special or regulated medical waste (Lee et al., 2004), and yet has not attracted the same level of attention as other type of wastes, particularly in developing countries, despite been labelled "hazardous".

Although waste generation depends on numerous factors such as the established waste management methods, type of hospital establishment, hospital specialization, the proportion of reusable items employed in hospital and proportion of patients treated on a day-care basis (Suwanneem, 2002), the level at which these factors influences the quantity of hospital waste generated is not well understood but reflects a loss of materials and energy and imposes economic and environmental costs on hospital management for its collection, treatment and disposal. The rate of waste generation may also differ due to geographical location, season of the year, collection frequency, social status of the patient (income, living standard, awareness about diseases), as well as present hospital management legislation, etc.

Traditionally, hospital wastes have been disposed off with the municipal wastes in landfills. However, since the late 1980's, the spreading trend of immunodeficiency virus (HIV), hepatitis B virus (HBV) and other agents associated with blood bone diseases has raised public awareness and concerns of the disposal of hospital waste. As a result, these wastes are required to be treated in a special way and not to be mixed with municipal waste (Abanobi et al., 2011).

The improper management of hospital waste causes serious problems that impairs human and animal health and ultimately results in economic, environmental and biological losses (Sharholy et al., 2008). Poor management of hospital waste implies a combination of improper handling of waste during generation, collection, storage, transport and treatment and includes; handling without personal protective equipment, poor storage, manual transport for longer distances, use of uncovered

Figure 1. Map of Nigeria showing Imo State (Inserted Right bottom: Map of Imo State Showing Owerri).

containers, exposure times beyond acceptable limits, lack of worker and equipment decontamination procedures, etc.

Proper management of hospital waste is critical to health and wellbeing of urban and rural residents (World Bank, 2003). The management of hospital waste requires its removal and disposal from the healthcare establishments as hygienically and economically as possible, by methods that all stages minimizes the risk to public health and to the environment. The proper collection of hospital waste however, will help reduce the volume of infectious wastes and consequently, the cost of treatment and disposal.

As a general rule, hospital management should coordinate the collection of infectious and other wastes separately, and the local authorities should be responsible for the treatment of infectious waste (Environmental Protection Agency, 1990) under professional supervision.

This study is aimed to estimate the rate of waste generations and describe the waste disposal/management practices. An attempt will also be made to classify these wastes into two general categories, that is, hazardous and non-hazardous.

METHODOLOGY

This study employed a cross-sectional design to assess hospitals for waste generation, disposal and management practice. The study was conducted in Owerri Municipal (See area circled red in the inserted Map) within an interval of two months in 2009 using questionnaire vis-à-vis physical observation to assess waste generation rates, as well as waste handling and disposal practices.

Using a multistage sampling technique, twelve (12) hospitals (healthcare facilities) in Owerri Municipal, either public (government owned) or private (individual owned) were selected using stratified sampling techniques for weighing and estimation of waste generation in kg/bed/day. The waste generated by the selected hospital were collected, weighed and recorded on a special data sheet.

A validated weighing scale was used to measure and generate data on the waste generated in the hospitals. Data were compiled so as to enable the estimation of the generation quantity. The quantities of hospital waste were presented in terms of kg/day and kg/bed/day waste generated.

The physical composition of the waste was also determined. Before segregation, the waste were disinfected with large quantity of bleach known as “JIK” (3.85% m/v sodium hypochlorite) and with the aid of a large forceps, a glove and a nose mask, the wastes were then segregated into hazardous and non-hazardous components. This procedure was a continuous effort to measure and understand the waste generation in hospitals.

The questionnaire (structured) was pretested on five hospitals and the reliability was found to be 0.76 using Cronbach’s alpha. The questionnaire was made up of two sections, to be filled out by the statistic unit (used to collect information on the number of beds, patient influx and average length of stay) and the other by the waste management unit of the surveyed hospitals covering information on waste handling and management practices.

The raw data gathered from the questionnaire was compiled and analyzed using SPSS version 20 and Microsoft excel and the

Table 1. Waste generation.

Hospital	Class of hospital	No. of beds	Bed occupancy (%)	Total weight of waste (kg)/day
Federal Medical Centre	Public	404	98.0	340.0
General Hospital	Public	182	72.5	112.0
St. David Hospital	Private	87	99.8	42.5
Umezuruike Hospital	Private	50	100.0	25.1
Salvation Hospital	Private	19	97.4	14.9
Austin-Grace Hospital	Private	20	50.0	7.1
Ikenegbu Hospital	Private	30	80.0	15.9
Aladinma Hospital	Private	20	97.7	11.2
Ezem Medical Centre	Private	15	93.3	8.0
Lucky Clinic	Private	6	50.0	2.5
Uchendu Clinics	Private	20	45.0	12.7
Area L Health Centre	Public	7	28.6	5.0

Mean waste generation: 49.7475 kg/day and 0.5815 kg/bed/day.

Table 2. Categories/composition of waste.

Hospital	Total weight of waste (kg)/day	Total weight of waste kg/bed/day	Kg/bed/day Hazardous	Kg/bed/day Non-Hazardous
Federal Medical Centre	340.0	0.842	0.152	0.690
General Hospital	112.0	0.615	0.086	0.529
St. David Hospital	42.5	0.489	0.073	0.416
Umezuruike Hospital	25.1	0.502	0.070	0.432
Salvation Hospital	14.9	0.786	0.126	0.660
Austin-Grace Hospital	7.1	0.354	0.046	0.308
Ikenegbu Hospital	15.9	0.530	0.106	0.424
Aladinma Hospital	11.2	0.560	0.095	0.465
Ezem Medical Centre	8.0	0.535	0.064	0.471
Lucky Clinic	2.5	0.408	0.061	0.347
Uchendu Clinics	12.7	0.637	0.089	0.548
Area L Health Centre	5.0	0.720	0.144	0.576

quantitative data presented in tables and figures.

RESULTS

As mentioned in Table 1, according to the categorization of hospital status and class developed in the methodology, about 25% of the hospitals were public/government owned whereas the remaining 75% are all private owned hospitals. The result of the survey on the per day and kg/bed/day waste generation on Table 1, shows that the average waste generation rate of Owerri Municipal is about 46.4 kg/day and 0.58 kg/bed/day.

The waste composition and the waste segregation performed on per day hospital waste generation are shown in Table 2. Hazardous waste generation for the hospitals ranged between 12-20% while non-hazardous range between 80-88% with an average generation of approximately 16 and 84% non-hazardous (Figure 1).

The composition of the waste observed at the temporary waste disposal bin of one of the surveyed hospitals is shown in Figure 2 and photograph of a temporary disposal site not more than 10 m from hospital facilities is shown in Figure 3. Greater percentage (50.0%) of waste generated are shown to be disposed in open dump with about 41.7% of sharps generated disposed openly mixed with other wastes into municipal waste bins (Figure 2).

The means of transportation for hospital was observed in one of the private facilities surveyed is represented pictorially in Figure 4.

Despite report on increased awareness (100%) of hospital staff on hazardous nature of wastes, only 16.7%

Figure 1. Average composition of hospital waste in surveyed facilities.

Figure 2. Composition of hospital waste in a temporary disposal container.

Figure 3. Temporary hospital waste disposal site at General hospital.

Figure 4. Transportation of hospital waste from a surveyed private health facility.

Table 3. Waste handling and management practice.

Variable	Frequency	(%)
Label hospital waste		
Yes	4	33.3
No	8	66.7
Treatment of waste before final disposal		
Yes	2	16.7
No	10	83.3
Use of colour coded waste bags		
Yes	2	16.7
No	10	83.3
Staff awareness of hazardous nature of waste		
Yes	12	100.0
No	0	0.0
Use of personal protective equipment		
Yes	10	83.3
No	2	16.7
Presence of waste management plan		
Yes	4	33.3
No	8	66.7
Inclusion of waste management responsibility for staff		
Yes	6	50.0
No	6	50.0

of the hospitals use colour coding for waste collection. Although there is a greater use (83.3%) of personal protective equipments in waste handling and disposals, only 33.3% of the hospitals have lay down waste management plan with half (50%) including waste management responsibility for their staff and unit heads with only 16.7% applying the colour coding of waste bags or treating waste before final disposal (Table 3).

Waste management from cradle to grave has been given low attention in this surveyed facilities as waste

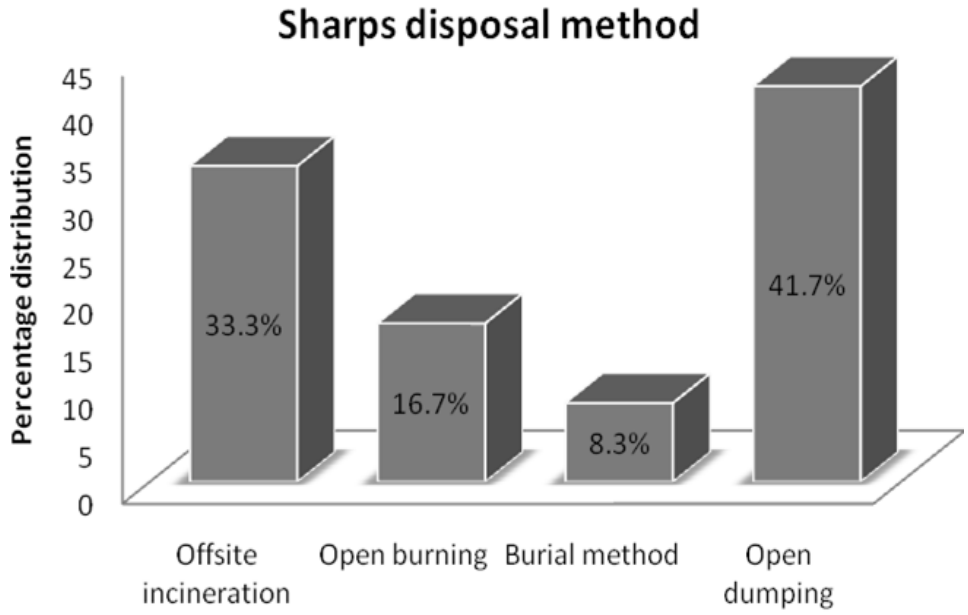

Figure 5. Sharps disposal methods.

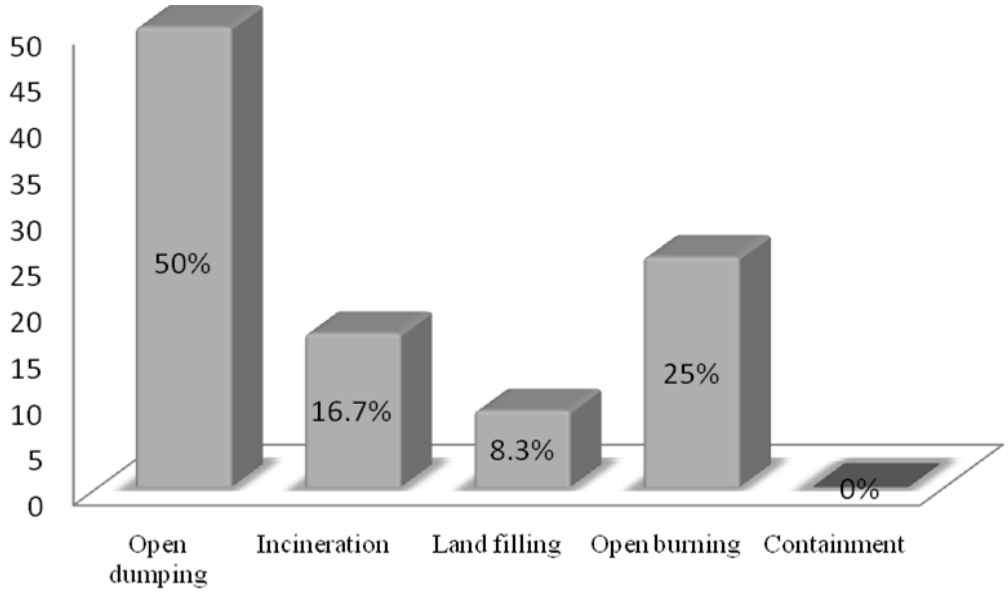

Figure 6. Waste disposal methods.

streams are not properly segregated from collection, carelessly disposed at temporary sites complimented with poor waste transportation and disposal system in some facilities (Figures 4, 5 and 6).

DISCUSSION

Hospital waste is one of the most neglected part of the waste management in Owerri Municipal as well as other cities in Nigeria (Babatola, 2008; Abanobi et al., 2011; Vivan et al., 2012). Neither the government nor the hospital authorities pay proper attention to this issue.

The unhygienic waste disposal by many hospitals and clinics in Nigeria poses serious occupational health hazard and treat to the people living around the vicinity of the hospitals. Most hospitals dispose every kind of wastes (hazardous and non-hazardous) in nearby

municipal dust bins without any pre-treatment whatsoever (Abanobi et al., 2011; Vivan et al., 2012).

In the hospitals surveyed in Owerri Municipal, wastes were collected mostly three times daily in the surveyed hospitals and the average generation rate is 0.58 kg/bed/day. This rate is lower than 0.934 kg/bed/day reported in Sylhet City (Bangladesh), 1.2 kg/bed/day in Dhaka (Bangladesh), 4.5 kg/bed/day in USA, and 2.5 kg/bed/day in France (Rahman et al., 1999). But consistent with a study on medical waste generation of 0.573 kg/bed/day in Lagos (Longe and WIliams, 2006) and 0.81 kg/bed/day in the inpatient wards (Abah and Ohimain, 2011). Several other studies have reported a higher generation rates (Mohanmadi-Baghaee, 2000; Mato and Kaseva, 1999; Askarian and Vikili, 2001; Olubukola, 2009; Mohseni, 2001; Ashrafi, 2005; Bdour, 2007).

Although WHO reported in 1999, the average generation rate of teaching hospital ranges between 4.1 and 8.7 kg/bed/day (WHO, 1999). This is contrary to that reported for University College Hospital, Ibadan (UCH) of 1.3-1.5 kg waste per bed/day and 1.5-2 kg waste per bed/day in Obafemi Awolowo University Teaching Hospital, Ile-Ife (OAUTH) (Toyobo et al., 2012). This can be attributed to the fact that rates may vary from country to country and from hospital to hospital (depending on the level of care and type of services provided).

This study revealed that 16% portion of the hospital waste generated is hazardous. This is much lower than that reported for Denmark (25%) and USA (28%), and consistent with the rate in Dhaka (15.5%) (Rahman et al., 1999), indicating differences in geographical location, living habits and standards, availability of different treatment facilities, and perhaps the ways in which solid wastes are categorized in different countries. This portion of waste labelled “hazardous” requires special attention for their proper disposal. The remaining portion of wastes can be easily disposed off into the municipal dust bins if carefully segregated and treated.

A few changes in material procurement process in hospitals, mandatory staff education in waste segregation, proper hygiene education to the scavengers, treatment of selected hazardous materials, and such other few efforts can get hospitals off the list of major hazardous materials to be disposed off to the municipal dust bins. Once these wastes are properly segregated, the hazardous portion can be treated by different treatment options and properly disposed off.

Medical wastes are sources of contamination and pollution to both humans and the natural environment. Its improper disposal may be hazardous if it enters water supplies or local sources used by nearby communities or wildlife. It also poses risk to scavengers and children if a landfill is insecure (Vivan et al., 2012). Scavengers may be exposed to sharps, pharmaceuticals and chemicals through direct contact with infectious material and recycling of infectious objects from these bins and/or landfills is potentially capable of causing disease and illness in man, through direct contact with their users.

Keeping the healthcare workers safe against occupational health risks arising from hospital waste management, requires a strategic and well implemented and effective waste control measures. This will help protect even patients and the populace who also have chances of contracting infection caused by airborne pathogens or spores harboured in medical waste. Despite the fact that some portion of the hospital waste has been labelled hazardous and increased awareness of health personnel on the risk of health-care waste, waste has been poorly managed in most facilities in Nigeria and other developing countries (Abanobi et al., 2011). And even with the increased level of awareness of hospital staff on the hazardous nature of hospital waste, the hospitals in Owerri has taken little step to ensure the propel disposal of medical waste. Only 16.7% uses colour coding for disposal of waste and 33.3% has any form of laid down waste management plan. Incinerations in most facilities are still done openly at proximal to the facilities and/or openly in dumpsites.

The proper collection and disposal of hospital waste will reduce the volume of infectious wastes and consequently the cost of treatment (Abanobi et al., 2011). As a general rule, waste management especially infectious waste and other hazardous waste should be properly collected by hospital management and treated by local authorities responsible for these wastes (EPA, 1990). And with the pervasive collection, transportation and disposal of hospital waste mixed with municipal waste in Nigeria, there is need to promote compliance with the “National Policy on Injection and Health-care Waste Management” for improved waste management in Nigeria.

Hospital waste management practices in most hospitals are poor (Manyele, 2004) and according to Abanobi et al. (2011) on a study done on medical waste in Owerri, there are neither proper waste management methods employed by hospitals nor proper waste management plans in these hospitals.

Disposal methods are still poor generally with greater percentage (50%) of total wastes and 41.7% sharps disposed into municipal waste stream. A similar improper disposal has been reported by several studies (Babatola, 2008; Abanobi et al., 2011; Vivian et al., 2012).

It is essential that different authorities (government and private) be involved in the monitoring and control of the environment, recognizing the nature of the problem hospital waste posses and develop legislation to regulate hospital sanitation and fortify implementation of existing ones. Some of the problems discovered to be associated with these hospital facilities in term of waste management include: Despite the awareness of hospital personnel on the hazardous nature of hospital waste, there is still negligence on the consequences and impact of improper waste handling and management practices; Practice of colour coding for waste collection before disposal was

poor. The National Policy on Injection and health-care waste management of the Federal Ministry of Health are not properly implemented; Lack of comprehensive waste management plans for the management of hazardous hospital waste with improper managerial interventions on reducing waste generation (inadequate waste minimization interventions); Inadequate disposal of waste (refuse) into municipal waste and liquid waste into municipal poorly channelled and managed sewerage system without treatment; and Reuse of disposables especially plastics after disposal; among several other problems.

Conclusions

Limited concentration has been given to hospital waste and the unhygienic disposal of hospital waste in Owerri Municipal poses a serious health hazard to the city dwellers in general as well as scavengers patrolling municipal bins. The hospitals require hygienic approach in handling, storage; transport, treatment and disposal of their wastes by the methods that at all stages, minimize the risk to public health and to the environment. This approach requires proper knowledge of waste generations and factor influencing them. The average generation rate of 0.58 kg/bed/day including about 16% of hazardous waste is lower than those reported for some developing and developed Nation. Enlightenment of hospital administrators, proper hygiene education to the scavengers, mandatory staff education in waste segregation, and legislation to regulate hospital waste management will help change the traditional habits of different group of people involved in this sector. Waste management system has to be reviewed and improved in conformation with the WHO pollution control standards as reduction in waste generation can only be achieved if waste points are identified, and effective alternatives determined or waste minimization applied.

Recommendation

Based on the findings of this study and in view of the problems discovered during the study, the following recommendations are made to help health care facilities improve and standardize hospital waste management plan. These will serve as baseline for further study and are not limited to:

1. There is need to train and enlightenment all hospital personnel of the potential risk of hospital waste from handling, storage, transport; treatment and disposal. That which has proven effective in hospital waste management in Tanzania (Manyele 2004);
2. Organizing seminars for hospital personnel and waste management personnel by management regulators/agencies on current waste management technologies, enlightening them on the needs to design a (risk free) waste management plans in actualizing good waste management practice;
3. Expert waste management in accordance with the National Policy on Injection Safety and Healthcare Waste Management of the Federal Ministry of Health, 2007 (Federal Republic of Nigeria Gazette 2007) will help properly manage and reduce hospital waste stream;
4. Workable incinerators should be provided by hospitals in accordance with the requirements of the National Policy on Injection Safety and Healthcare Waste Management of the Federal Ministry of Health, 2007 as well as those that conforms to the WHO standards for pollution control, providing cost effective services for smaller hospital establishments. This will enhance waste minimization;
5. Waste segregation from cradle to grave amongst hospitals as means of minimising generation should be encouraged and a well informed and trained staff appointed for separate collection, transport, treatment, and proper disposal of infectious hospital waste;
6. Storing of waste at using the specification of the bins (colour, size, type etc. according to the 2007 National Guideline on Environmental Health Practice in Nigeria, the Biomedical Waste (Management and Handling) Rules 1998/2000) and other applicable laws is necessary;
7. There should be a developed routine for proper waste collection and treatment, transportation and disposals at dumping site with attention to using chemical disinfectants treatment;
8. Provision of protective clothes and safety measures for waste collectors or handlers should be enhanced and sharps management practice that decreases the possibility of injury and spread of infections adopted;
9. It is also necessary to promote a regular monitoring and evaluation of hospital waste management practices and the performance of the systems periodically; and
10. Further studies should exploit the strategies of maximizing waste generation factors, as well as the cost effectiveness of waste management methods and apply it to design appropriate waste management plans for different hospital.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Abah Stephen O, Ohimain Elijah I (2011) Healthcare waste management in Nigeria: A case study. J. Public Health Epidemiol. 3(3):99-110

Abanobi OC, Opara K, Nwakwuo G, Morakinyo OM (2011) Assessment of medical waste management practice in selected hospitals: A case study of Owerri, Nigeria. J. Environ. Health Niger. 8(1): 7-12

Ashrafi D (2005) Evaluation of Medical waste management in Rasht city hospital, Tehran, Iran: MS Thesis of Environmental Health Engineering of Tehran University of Medical Sciences.

Askarian M, Vakili M (2001) Evaluation of Medical waste disposal in Far province. Med. Res. 1:41-53

Babatola JO (2008). A study of hospital waste generation and management practices in Akure, Nigeria. Afr. Res. Rev. 2(3):292-305.

Bdour A (2007). Assessment of medical wastes management practice. A case study of the northern part of Jordan. Waste Manage. 27: 746-759

Dehghani FE, Changani F, Azam K, Dehghani MH (2008). Assessment of medical waste management in educational hospitals of Tehran University Medical Sciences. Iran J. Environ. Health Sci. Eng. 5(2):131-136

Environmental Protection Agency (EPA) (1990). Medical Waste Management in USA, Second Interim Report to Congress. EPA/ 530-SW-90-087A. http://nepis.epa.gov. Accessed 24th July, 2009

Fikru T (2004). Assessment of injection safety at health facilities in four Woredas of Oromia region and SNNPR, report submitted to Injection Safety Project, JSI. Addis Ababa: 27. In Kasahun, Asnake and Fekadu Abebe (2014). Ethiopia: Needs Assessment for Implementation of a Public-Private Partnership in Health Care Waste Management in Addis Ababa City. Arlington, VA: AIDSTAR-One. http://www.aidstar-one.com/sites/default/files/AIDSTAR-One_Report_Ethiopia_Health_Care_Waste_PPP_Assessment.pdf. Accessed 14 August, 2014.

Johannessen LM (1997). Management of health care waste. In: Proceedings in Environment '97 Conference, 16-18 February 1997, Cairo. Dokki-Giza, Egyptian Environmental Affairs Agency.

Khan S, Mohammad S, Masoodi MA, Qureshi W (2004) Current practice of bio-medical waste management in government medical college associated S.M.H.S. Hospital, Srinagar, J & K, India. JK Practitioner 11(3):206-209

Lee BK, Ellenbecker M, Moure-Ersaso R (2004). Alternatives for treatment and disposal cost reduction of regulated medical waste. Waste Manage. 24:143-145

Longe EO, Williams A (2006). A preliminary study of medical waste management in Lagos Metropolis, Nigeria. Iran J. Environ. Health Sci. Eng. 3(2):133-139

Manyele SV (2004). Medical waste management in Tanzania: Current situation and the way forward. Afr. J. Environ. Assess. Manage. 8(1):74-99.

Mato RR, Kaseva ME (1999). Critical review of Industrial and medical waste practices in Dar es Salaam City. Resour. Conserv. Recycl. 25:27-287

Mohanmadi-Baghaee D (2000). Principles of waste management. 1st Edition. Shaharab Company, Tehran. pp. 63-67.

Mohseni A (2001). Evaluation of medical waste collection, transportation and disposal in public and private hospitals in Māzandarān province. J. Māzandarān Univ. Med. Sci. 11:45-52.

Olubukola BO (2009). Comparative Analysis of Health Care Waste Management Practice in Two General Hospitals in Nigeria. http://www.eco-web.com/edi/index.htm. Accessed 28 January, 2011.

Pruss A, Giroult E, Rushbrook D (1999). Safe management of wastes from health-care activities. World Health Organization, Geneva. http://www.who.int/water_sanitation_health/medicalwaste/wastemanag/en/. Accessed 4 July, 2013.

Rahman MH, Ahmed SN, Ullah MS (1999). A study of hospital waste management in Dhaka city. 25th WEDC Conference on Integrated development for water supply and sanitation. Addis Ababa, Ethiopia: 342-345. http://wedc.lboro.ac.uk/resources/conference/25/342.pdf . Accessed 24 July, 2009.

Sarkar SKA, Haque MA, Khan TA (2006). Hospital waste management in Sylhet city. ARPN J. Eng. Appl. Sci. 1(2):32-40.

Sharholy M, Ahmad K, Mahmood G, Trivedi RC (2008). Municipal solid waste in India - A review. Waste Manage. 26(2):459-467.

Suwanneem A (2002). Study on waste from hospital and clinics in Phitsanulok. Online J. Health Allied Sci. 1(3):1-9.

Toyobo AE, Baba AO, Oyeniyi AB (2012). Appraisal of university teaching hospital medical waste management in Nigeria: Case Studies of University College Hospital (UCH) Ibadan and Obafemi Awolowo University Teaching Hospital (OAUTH) Ile-Ife. Univers. J. Educ. Gen. Stud. 1(9):290-297.

Vivan EL, Blamah VN, Ezemokwe I, Okafor CI, Bawa A. (2012) Public health implication of improper hospital waste disposal in Zonkwa district of Zangon-kataf Local Government Area, Kaduna State. J. Res. Environ. Sci. Toxicol. 1(2):23-28.

WHO (1999). Safe management of waste from health-care activities. World Health Organization, Geneva http://who.int/water_sanitation_health/Environmental_sanit/MHCWHanbook.htm. Accessed 27 July, 2009.

WHO (2000). Wastes from Health-care Activities, Fact Sheet No.253 http://who.int/mediacentre/factsheets/fs253/en/. Accessed 5 May, 2013

WHO (2011). Health-care waste management: Health-care waste needs sound management, including alternatives to incineration. Fact sheet N°281. http://www.who.int/mediacentre/factsheets/fs281/en/. Accessed 15 August, 2014.

World Bank (2003). Reducing maternal mortality, learning from Bolivia, China, Egypt, Honduras, Indonesia, Jamaica and Sri Lanka. Human Development Network, Health Nutrition and Population Series. Koblinsky M. Editor. The World Bank, Washington DC

Yemane B, Millogo J (2000). Report of Injection Safety Survey in Ethiopia. Geneva, Switzerland. p. 22.

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Microbiological quality and safety of some selected vegetables sold in Jimma town, Southwestern Ethiopia

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Raw vegetables are major vehicles for the transmission of foodborne infections. In Ethiopia, there is a habit of consuming raw vegetables as salad, particularly tomato (*Solanum lycopersicum* L.), cabbage (*Brassica oleracea* L.), carrots (*Daucus carota* L.), lettuces (*Lactuca sativa* L.) and green peppers (*Capsicum annuum* L.) without adequate treatment. The objectives of this study were to evaluate the microbiological loads, assess the prevalence of some food borne pathogens, and investigate the antimicrobial susceptibility of pathogens. A total of 180 vegetable samples were purchased from different sites and markets of Jimma town and analyzed for their microbial loads following standard microbiological methods. In addition, antibiotic resistance pathogens and prevalence of Salmonella and *Staphylococcus aureus* were also determined. Ninety percent of vegetable samples had aerobic mesophilic counts of ≥ 5 log^{10} CFU g^{-1}. Similarly, 82.2, 92.8 and 97.8% of samples had coliform, Enterobacteriaceae and lactic acid bacteria counts of ≥ 4 log^{10} CFU g^{-1}, respectively. However, most of staphylococci and aerobic spore counts varied between 2 - 3.9 log^{10} CFU g^{-1}, but greater than 74% of yeasts and molds were counted ≤ 2.9 log10 CFU g^{-1}. The aerobic mesophilic flora of the vegetable samples was dominated by *Bacillus* spp. (22.3%) followed by Staphylococcus spp. (17.7%). Salmonella and *S. aureus* were isolated from 23 (12.8%) and 18 (10%) vegetable samples, respectively. All of Salmonella and *S. aureus* isolates showed resistance to ampicillin and penicillin G, respectively. However, they were 100% sensitive to ciprofloxacin and gentamicin. Lettuce had high microbial load and Salmonella were most prevalent in lettuce but *S. aureus* were more prevalent in green pepper. Most of the pathogens were multiple drugs resistant. The use of food grade chemicals to kill pathogens and reduce the microbial load before consumption is recommended.

Key words: Drug resistance, prevalence, pathogens, raw vegetables.

INTRODUCTION

Vegetable is the tender plant part which is not sweet and may be flavored or spiced with condiments before consumption. The consumption of minimally processed food or raw vegetables has been increased tremendously due to their nutritive values in human dietary. Vegetables can be used as salad mixes, side dishes or ingredients in the meals. Fresh products that contain complex and colorful blends incorporating a wide variety of vegetable

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mixes and flavors would especially benefit from sales in all market segments. In general, as consumers continue to lead a healthy lifestyle, there are broad product development opportunities in this category. Currently, supermarkets and the food service outlets are the primary retail outlets for these products (Amoah et al., 2009). Thus despite their nutritional and health benefits, outbreaks of human infections associated with the consumption of fresh or minimally processed vegetables have increased in recent years (Beuchat, 2002).

Since vegetables are produced in a natural environment, they are vulnerable to contamination by human pathogens. The majorities of diseases associated with fresh vegetables are primarily those transmitted by the fecal oral route, and therefore, are a result of contamination at some point in the process (Johnston et al*.,* 2005). Vegetables could be contaminated with bacterial pathogens from human or animal sources including *Salmonella*, *Shigella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus* and *Campylobacter*, and resistance pathogens to different antimicrobials (Al-Binali et al., 2006; Simon et al, 2007; Allende et al., 2008; Elhariry, 2011). As the result, vegetables have been associated with outbreak of foodborne disease in many countries.

The presence of antibiotic resistance both in normal flora and pathogenic microorganisms in fresh vegetables may contribute to horizontal spreading of resistance between different isolates, species and genera. The presence of resistance gene on transferable elements facilitates distribution of resistance and wide spread use of antibiotics allows direct selection or co-selection of resistance (Heuer and Smalla, 2007). Therefore, the presence of antibiotic resistant bacteria in fresh vegetables constitutes an additional concern for consumer safety (Aarestrup et al., 2008; Walsh and Fanings, 2008).

Plate count of aerobic mesophilic microorganisms found in food is one of the microbiological indicators for food quality (Aycicek et al., 2004). These organisms reflect the exposure of the sample to any contamination and in general, the existence of favorable conditions for multiplication of microorganisms. Food borne bacterial pathogens commonly detected in fresh vegetables were coliform bacteria, *S. aureus* and *Salmonella* spp. (Tambekar and Mundhada, 2006).

Coli forms are commonly used bacterial indicator of sanitary quality of foods and water and considered as an indicator of microbial pollution and they are common inhabitant of animal and human guts (Tortora, 1995). The presence of these bacteria poses a serious threat to public health with outbreaks arising from food and water that has been contaminated by human or animal feces or sewage. *S. aureus* is the third most common cause of confirmed food poisoning in the world and the illness is due to the ingestion of preformed enterotoxin produced in foods (Acco et al., 2003).

Ethiopia has highly diversified agroecological zones which are suitable for the production of various types of vegetables. Vegetables are mainly grown by traditional farmers in home gardens. About 27% of the vegetable species recorded from home gardens in Ethiopia were consumed as raw or cooked (Asfaw, 1997). Particularly, in the urban parts of the country eating of raw vegetables becomes more common. Vegetable farmers around Jimma town supply vegetables to the local market but the market place of Jimma town is not well organized. Vegetables are sold in front of shops besides with other goods and on street by street vendors.

In addition, vegetables can be stored in poor quality containers and house before sell for at least one day. This can increase potential contamination of vegetables with animals and human's feces, soil, dusts and other postharvest contaminants (Al-Binali et al., 2006). Contamination of vegetables are of special concern, because it is likely to be consumed raw, without any type of microbiologically lethal processing, thus posing a potential food safety problem.

The present study was under taken to examine the microbiological quality and safety of fresh vegetables particularly tomato (*Solanum lycopersicum* L.), cabbage (*Brassica oleracea* L.), carrot (*Daucus carota* L.), lettuce (*Lactuca sativa* L.), and green pepper (*Capsicum annuum* L.) samples collected from different sites (Kochi, Agip and Merkato) and markets (shops and street venders) of Jimma town, assess the growth potential of standard strains and evaluate the drug resistance ability of *Salmonella* spp and *S. aureus* isolated from these vegetables.

Materials and Methods

Description of the study area

The study was conducted in Jimma town, which is located at 353 km south west of Addis Ababa (Figure 1). The town's geographical coordinates are approximately 7°41' N latitude and 36° 50'E longitude. From a climatic point of view, abundant rainfall makes this region one of the best watered of Ethiopian highland areas, conducive for agricultural production (Alemu et al., 2011).

Study design and study population

The cross sectional study design was used. The sampling sites were Kochi, Agip, and Merkato. The study periods covered from September, 2011 to May, 2012.

Sampling techniques

A simple random sampling technique was used to address representative of the whole population.

Data collection

As the study has survey and experimental parts, data were collected using structured questionnaires.

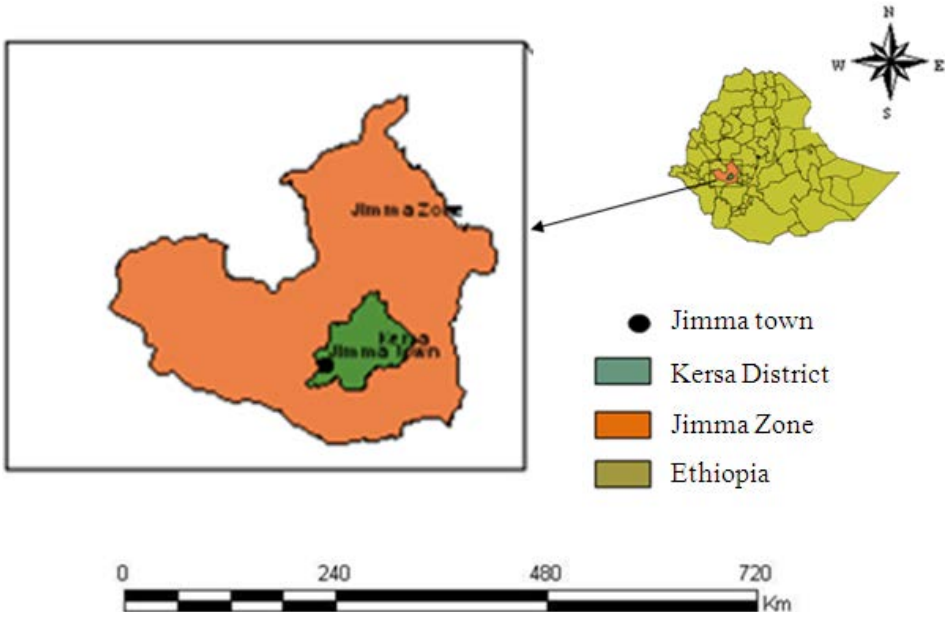

Figure 1. Map of the study area.

Collection of samples

A total of 180 fresh vegetable samples were purchased at different sampling days from local markets of Jimma town, southwest Ethiopia. The samples consisted of 36 each of tomato (*S. lycopersicum* L.*)*, cabbage (*B. oleracea* L.), carrots (*D. carota* L.), lettuces (*L. sativa* L.), and green peppers (*C. annuum* L.). All samples were collected using sterile plastic bags aseptically and immediately brought to the Postgraduate and Research Laboratory of Biology Department, Jimma University, for analysis. Microbiological analysis was conducted within 3 h of sample collection.

Sample preparation

For sample preparation, 25 g samples were aseptically removed from each sample, shredded into approximately 2 - 3 cm pieces using a sterile stainless steel knife and vigorously shaken in 225 ml of sterile 0.1% (w/v) bacteriological (buffered) peptone water (Oxoid) for 3 min separately to homogenize the samples (Shalini, 2010).

Microbiological enumeration

The homogenate from sample preparation in buffered peptone water was used for the following procedures.

Total aerobic mesophilic count

Total viable aerobic mesophilic count of all vegetables samples were determined by plate count using standard plate count agar (PCA) (Oxoid) medium (Shalini, 2010).

Total coliform count

A 0.1 ml of homogenate from 10^{-1} - 10^{-3} dilution was pipetted and spread on violet red bile agar (VRBA) (Oxoid). Red to pink colonies were counted after incubating plates at 32□C for 18 - 24 h (Spencer et al., 2007).

Enterobacteriaceae count

All purple colonies were counted on MacConkey agar (Oxoid) as members of Enterobacteriaceae after incubation for 24 h at 32°C (Spencer et al., 2007).

Staphylococci count

Mannitol salt agar (MSA) (Oxoid) was surface plated with 0.1ml of the homogenate from 10^{-1} - 10^{-2} and incubated at 32°C for 36 h. Then, golden yellow color colonies were aseptically picked and purified (Acco et al., 2003).

Aerobic spore count

Bacterial spores were counted after heating the suspension of vegetable samples for 10 min in water bath at 80°C and incubation at 32°C for 36 to 72 h (Acco et al., 2003).

Lactic acid bacteria (LAB) count

To count LAB, 0.1 ml of 10^{-1} - 10^{-3} dilution of homogenate was spread on de Mann Rogosa Sharpe (MRS) agar (Oxoid) media and incubated at 37°C for 48 h in anaerobic condition using anaerobic jar (Oxoid) (Pal et al., 2005).

Yeasts and molds counts

The yeasts and molds count of all vegetables samples were determined by direct plate count using potato dextrose agar (PDA) supplemented with 0.1 g Chloramphenicol. The plates were incubated

at 25- 28°C for three to five days (Spencer et al., 2007).

Microbial analysis

For microbial analysis, 15 - 20 colonies with different morphology and color were picked randomly from countable plate count plates and were purified by repeated plating and characterized to the family and genus level using the following tests.

Cell morphology

These were carried out by Gram staining techniques and observing under microscope using oil immersion objective. Schefer fulton endospore staining techniques were used to identify the presence or absence of endospore (Krieg, 1981).

KOH-test (test on lipopolysaccharide)

A colony was aseptically picked from the surface of plate count agar plates using an inculcating loop and stirred in the KOH solution for 10 s to 2 min (Gregerson, 1978).

Oxidation Fermentation (O/F) test

This test is used to assess the ability of the isolate to utilize glucose and determine the metabolic way they used as well (that is by fermentation or oxidation) (Hugh and Leifson, 1953).

Catalase test

Catalase taste was carried out after young colonies flooded with a 3% solution of hydrogen peroxide (H_2O_2) (Chelikani et al., 2004).

Cytochrome Oxidase test

This test was conducted following the method outlined by Kovacs (1956).

Detection of pathogens

Salmonella

For detection of *Salmonella*, 25 g vegetable samples were added to 225 ml buffered peptone water, vigorously shaken and the suspension was incubated at 37°C for 24 h for metabolic recovery and proliferation of cells (Deza et al*.,* 2003). From this, 1 ml of culture was transferred into separate tubes each containing 10 ml of Selenite Cystein Broth. The broth was incubated at 37°C for 24 h. After secondary enrichment, culture from enrichment broth was separately streaked on plates of Xylose Lysine Desoxycholate (XLD) (Oxoid) medium. Pink colonies with or without black centers from selective medium was picked, purified and tested biochemically (Cheung et al., 2007).

Staphylococcus aureus

For detection of *S. aureus*, golden yellow colonies from MSA during staphylococci count were picked, purified and preserved. Coagulase test was done by two ways: slide coagulase test and tube coagulase test (Cheesbrough, 2006).

Antimicrobial susceptibility testing of the isolated pathogens

This was investigated on Mueller Hinton Agar (Oxoid) plates following the standardized disk diffusion techniques. The antibiotic discs were placed on the medium by using forceps and incubated at 35°C for 18 h and the zones of inhibition was measured manually with a transparent ruler. The results of the antimicrobial susceptibility were interpreted based on the guidance of National Committee for Clinical Laboratory Standards (CLSI, 2007).

For this tests, Ampicillin (AMP), (10 µg/ml); Chloramphenicol(C), (30 µg/ml); Ciprofloxacin (CIP), (5µg/ml); Gentamicin(CN), (10 µg/ml); Kanamycin (K), (30 µg/ml); Nalidixic acid(NA),(30 µg/ml); Norfloxacin (NOR), (10 µg/ml); Streptomycin (S),(10 µg/ml) and Tetracycline (TE), (30 µg/ml) were used for *Salmonella* and Penicillin G (P), (10 µg/ml); Erythromycin(E), (15 µg/ml); Clindamycin (DA), (2µg/ml); Chloramphenicol(C), (30 µg/ml); Ciprofloxacin (CIP), (5µg/ml); Gentamicin(CN), (10 µg/ml); Kanamycin (K), (30 µg/ml); Streptomycin (S),(10 µg/ml) and Tetracycline (TE), (30 µg/ml) were used for *S. aureus.* The reference strains, *S. aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, sensitive to all the drugs were used in this study.

Statistical analysis

Coefficient of variation (% CV) was calculated and significance of variation in microbial counts within the vegetable samples was analyzed. Mean values of the microbial counts of various vegetable samples from different sites and markets were compared using one way ANOVA and the significance of difference between groups were considered at 95% confidence interval ($p < 0.05$). In addition, the data obtained from the respondents were analyzed by SPSS version 16.

Ethical consideration

Ethical clearance was obtained from Research Review and Ethical committee of College of Natural Science, Jimma University.

RESULTS

Socio-demographic characteristics

A total of 90 farmers and vegetable venders were interviewed. A significant number of the respondents were females (60%) (Table 1). Forty percent of the respondents were within an age group of 30 to 39 years. With respect to the educational status, about 34.4, 32.2, 16.7 and 16.7% of the farmers or sellers attended secondary school, elementary school, capable of reading and writing, and illiterate, respectively (Table 1). Occupationally, the respondents (55.6%) were vegetable sellers and 44.4% were farmers (Table 1).

General vegetable farm and management conditions

The general characteristics of farm and management conditions of vegetables sold in Jimma town are summarized in Table 2. Vegetables sold in Jimma town were 100% cultivated in traditional farming methods by rural farmers. The preferred cultivation seasons of the

Table 1. Socio-demographic characteristics of vegetable farmers and sellers or venders, Jimma town, south western Ethiopia, 2011/12 .

Characteristic		Number of respondents(n=90)	
		Frequency	Percent (%)
Sex	Male	36	40.0
	Female	54	60.0
Age	20-29	24	26.7
	30-39	36	40.0
	40-49	19	21.1
	> 50	11	12.2
Education status	Illiterate	15	16.7
	Read and write	15	16.7
	Elementary school	29	32.2
	Secondary school	31	34.4
Occupation	Farmer	40	44.4
	Vegetable sellers	50	55.6

Table 2. General vegetable farm and management conditions, Jimma town, Southwestern Ethiopia, 2011/12.

Characteristic	Respondents(n=90)	
	Frequency	Percent (%)
Methods of cultivation		
Traditional	90	100
Water source of irrigation		
River	57	63.3
Well	33	36.7
To increase fertility of farm		
Inorganic fertilizers	66	73.3
Animal manure	24	26.7
Harvesting equipments		
Sac	37	41.1
Hand basket	29	32.2
Storage place before selling		
In store room	56	62.2
On the floor in vegetable farm	34	37.8
Transporting containers		
Sac	58	64.4
Plastic bags	32	35.6
How long do you store before sell		
1 day	5	5.6
2 days	29	32.2
3 days	36	40.0
More than 3 days	20	22.2
Consumption habit		
Without heat treatment	49	54.4
With heat treatment	30	33.3
With food grade chemicals	11	12.2

vegetables were found out to be during dry season (41.1%) using irrigation. The water sources for irrigation were river (63.3%) and well (36.7%). A large number of vegetable farmers (73.3%) were used inorganic fertilizers

Table 3. Mean microbiological counts (log CFU g^{-1}) of selected vegetables purchased from shops and vended markets, Jimma town, southwestern Ethiopia, 2011/12.

Microbial group	Vegetables									
	Tomato (T)		Cabbage (Ca)		Carrot (Cr)		Lettuce (L)		Green pepper (G)	
	Mean ± S.D	%C.V	Mean ± S.D	%C.V	Mean ± S.D	%C.V	Mean ± S.D	%C.V	Mean ± S.D	%C.V
AMC	5.3 ± 0.7	13.2	5.7 ± 0.4	7.0	5.5 ± 0.4	7.3	6.0 ± 0.4	6.7	5.4 ± 0.5	9.3
Coliforms	3.4 ± 0.9	26.5	5.2 ± 0.5	9.6	5.0 ± 0.5	10.0	5.2 ± 0.6	11.5	4.7 ± 0.8	17.0
Enterobacteriaceae	4.5 ± 0.9	20.0	5.5 ± 0.5	9.1	5.1 ± 0.7	13.7	5.5 ± 0.6	10.9	5.0 ± 0.6	12.0
Staphylococci	2.8 ± 0.8	28.6	3.4 ± 0.6	17.6	3.5 ± 0.6	17.1	3.7 ± 0.5	13.5	3.8 ± 0.5	13.2
Aerobic Spore	3.6 ± 0.6	16.7	3.5 ± 0.4	11.4	3.7 ± 0.4	10.8	3.7 ± 0.4	10.8	3.4 ± 0.5	14.7
LAB	4.7 ± 0.3	6.4	4.5 ± 0.6	13.3	4.8 ± 0.6	12.5	4.8 ± 0.5	10.4	4.6 ± 0.3	6.5
Yeast	2.5 ± 0.5	20.0	2.5 ± 0.4	16.0	2.6 ± 0.5	19.2	2.9 ± 0.7	24.1	2.5 ± 0.5	20.0
Molds	2.1 ± 0.3	14.3	2.2 ± 0.3	13.6	2.4 ± 0.4	16.7	2.4 ± 0.4	16.7	2.2 ± 0.4	18.2

AMC, Aerobic mesophilic count; LAB, lactic acid bacteria; S.D, standard deviation; C.V, coefficient of variation.

although 26.7% were using animal manure to increase the fertility of the farm land. The vegetable farmers used different materials to harvest the produce including sack (41.1%), hand basket (32.2%) and plastic bags (26.7%). The harvested vegetables were stored at different places before selling. About 62.2% of the vegetable farmers were stored in store room. However, 37.8% of the respondents stored vegetables simply on the floor in the vegetable farms (Table 2).

Vegetables were transported from farm site to market by different means of transportation. Donkey were mostly used (35.6%) followed by horse cart (26.7%), car (23.3%) and humans back (14.4%). Sack and plastic bags were used as transporting containers while 64.4% of the respondents were used sack and 35.6% of vegetable farmers and sellers were used plastic bags. About 63.3% of the respondents were placed vegetables on the bed infront of the shop for sell. On other hand, 35.6% of vegetable sellers vended vegetables on street without using bed or plastic sheet. However, 1.1% of respondents used plastics to vend vegetables on floor. Vegetables were not availed to the consumers as soon as harvested. Therefore, 77.8% of the sellers stored vegetables for up to three days, whereas 22.2% stored for more than three days before sold to consumers. Over 54% of the respondents consumed vegetables without heat treatment. However, 33.3 and 12.2% of the respondents consumed after heat treatment and treating with food grade chemicals, respectively (Table 2).

Microbiological count of raw vegetables

The microbiological load of vegetables sampled in this study was varied with types, sites and markets. The mean microbial counts for selected raw vegetables sold in Jimma town are shown in Table 3. Accordingly, high aerobic mesophilic bacteria counts (6.0 log10 CFU g^{-1}) followed by Enterobacteriaceae (5.5 log10 CFU g^{-1}) and coliforms (5.2 log10 CFU g^{-1}). Lactic acid bacteria (LAB) were the forth dominant bacterial groups, but yeasts and molds were the least dominant (< 3.9 log10 CFU g^{-1}). The maximum aerobic mesophilic bacteria count was recorded in lettuce (7.3 log10 CFU g^{-1}) while the minimum was in carrot (3.3 log10 CFU g^{-1}) samples (Appendix A). Over all, there was significant variation among each microbial counts in tomato samples (C.V > 10%) except LAB. In tomato, lettuce, and green peppers there was significant variation (CV> 10%) within the samples in coliforms counts. The counts of Enterobacteriaceae were significantly different (C.V > 10%) in samples of tomato, carrot, lettuce, and green peppers. *Staphylococcus* spp., aerobic spore formers, and yeast counts significantly varied (C.V > 10%) within samples of all types of vegetable samples. However, LAB was not significantly varied (C.V < 10%); only in tomato and green pepper. On other hand, there was significant variation (C.V > 10%) among yeast and mold counts of all vegetable samples analyzed (Table 3). In general, there was significant variation ($p < 0.05$) between vegetable samples analyzed for various microbial groups. LAB was not significantly different ($p > 0.05$) between vegetables.

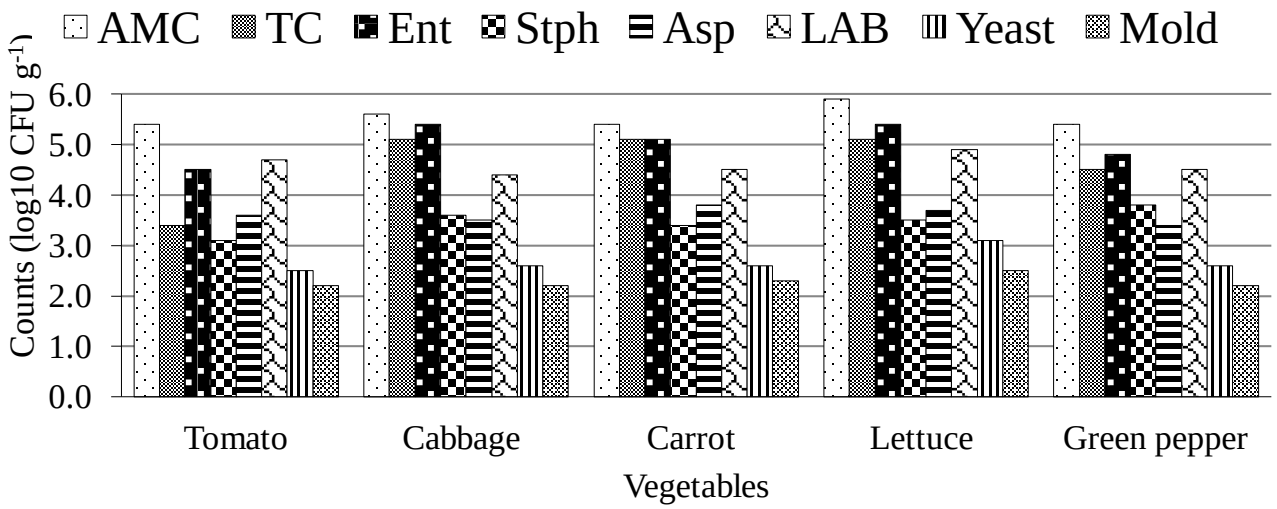

Figure 2. Microbial load of some selected raw vegetables purchased from shops, Jimma town, south western Ethiopia, 2011/12. AMC, Aerobic mesophilic count; TC, total coliforms; Ent, Enterobacteriaceae; Stph, *Staphylococcus* count; ASP, aerobic spores.

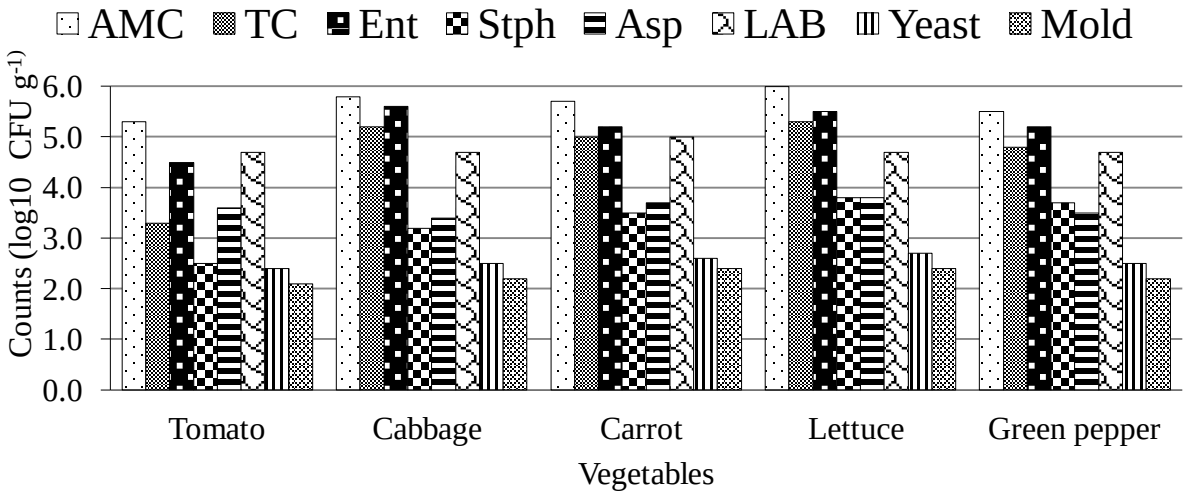

Figure 3. Microbial load of some selected raw vegetables purchased from street venders, Jimma town, south western Ethiopia, 2011/12. AMC, Aerobic mesophilic count; TC, total coliforms; Ent, Enterobacteriaceae; Stph, *Staphylococcus* count; ASP, aerobic spores.

Aerobic mesophilic counts of vegetables analyzed in this study were detected in the range of 3.5 - 6.9, 4.8 - 6.9, 3.3 - 6.1, 5.3 - 7.3, and 3.7 - 6.3 $\log^{10}$ CFU g^{-1} in tomato, cabbage, carrot, lettuce and green pepper, respectively (Appendix A). Most of staphylococci and aerobic spore counts were in the range of 2.0 - 6.5 log10 CFU g^{-1} except in cabbage. Similarly, yeast and mold counts were in the range of 1.9 - 4.2 log10 CFU g^{-1}. However, these ranges were varied based on types of markets and sites from which vegetables were purchased (Appendix A - D).

All vegetable samples purchased from shops contained higher aerobic mesophilic bacterial count than other microbial groups (Figure 2). The counts of coliforms and Enterobacteriaceae were higher in cabbage, carrot and lettuce with counts ≥ 5 log10 CFU g^{-1} (Figure 2). However, counts of the microbial groups of vegetables purchased from shops were ≥ 2 log10 CFU g^{-1}. On other hand, yeast and molds were the least dominant in all vegetables purchased from shops (Figure 2).

Similarly, the aerobic mesophilic bacteria counts of vegetables purchased from street venders were higher than others microbial groups (Figure 3). Likewise, the counts of Enterobacteriaceae and coliforms were ≥ 5 log10 CFU g^{-1} except in tomato. Staphylococci and aerobic spore counts from shops were higher than from

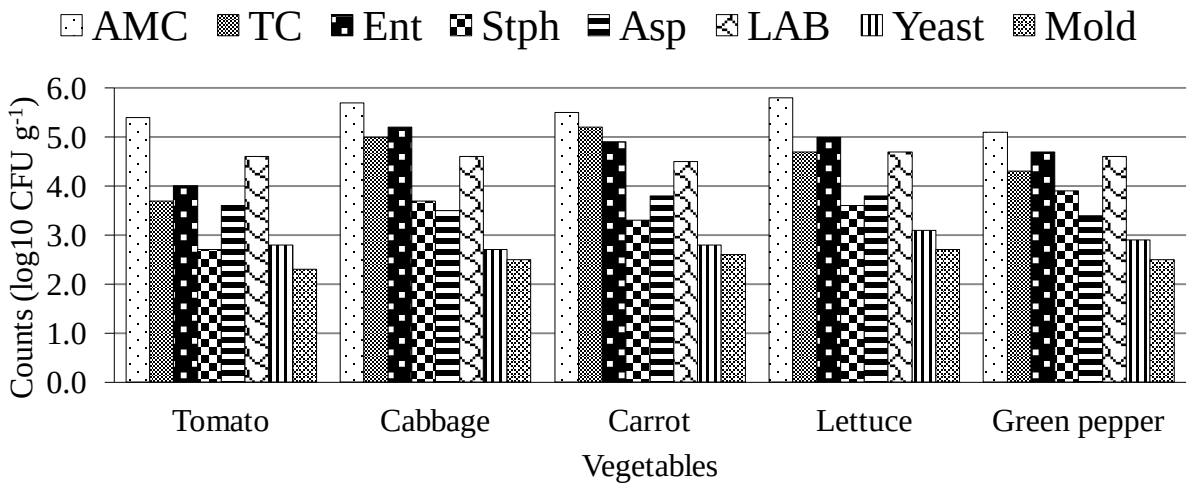

Figure 4. Microbial load of some selected raw vegetables purchased from Kochi site, Jimma town, South western Ethiopia, 2011/12.

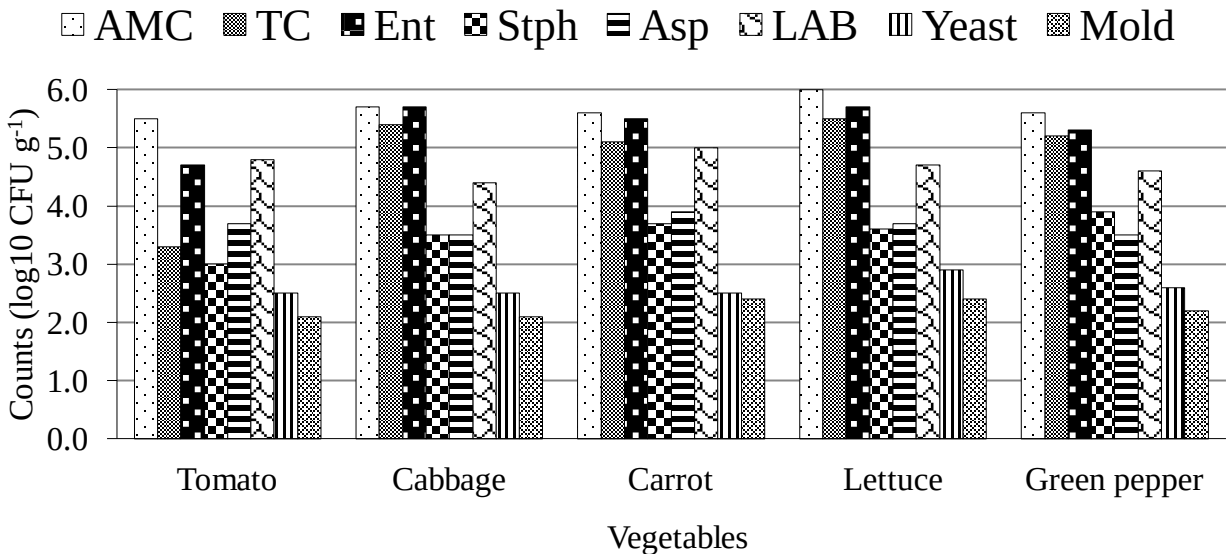

Figure 5. Microbial load of some selected raw vegetables purchased from Agip site, Jimma town, south western Ethiopia, 2011/12. AMC, Aerobic mesophilic count; TC, total coliforms; Ent, Enterobacteriaceae; Stph, *Staphylococcus* count; ASP, Aerobic spores.

street venders with counts ≥ 3 log10 CFU g^{-1} (Figures 2 and 3). But staphylococci counted < 3 log10 CFU g^{-1} in tomato purchased from street venders. Yeasts and molds counts were the lowest in vegetables purchased from street venders and shops (Figure 2 and 3). However, there was no significant variation among counts of aerobic mesophilic bacteria and coliforms among cabbage samples and LAB among tomato and green pepper purchased from different markets. However, counts of the other microbial groups were significantly varied (C.V > 10%) (Appendix A and C). On other hand, the microbial loads of vegetables were analyzed based on the three sampling sites Kochi, Agip and Merkato. Accordingly, the aerobic mesophilic count of both vegetables in Kochi sites were < 6 log^{10} CFU g^{-1} (Figure 4). However, lettuce purchased from both Agip and Merkato contained the maximum aerobic mesophilic bacteria with an average count 6 and 6.1 log^{10} CFU g^{-1}, respectively (Figures 5 and 6). Nevertheless, coliform, Enterobacteriaceae, staphylococci and aerobic spores' counts were ≥ 3 log^{10} CFU g^{-1} in all vegetables purchased from Kochi, Agip and Merkato sites (Figures 4, 5 and 6). However, staphylococci counted < 3 log^{10} CFU g^{-1} in tomato purchased from the three sites (Figures 4, 5 and

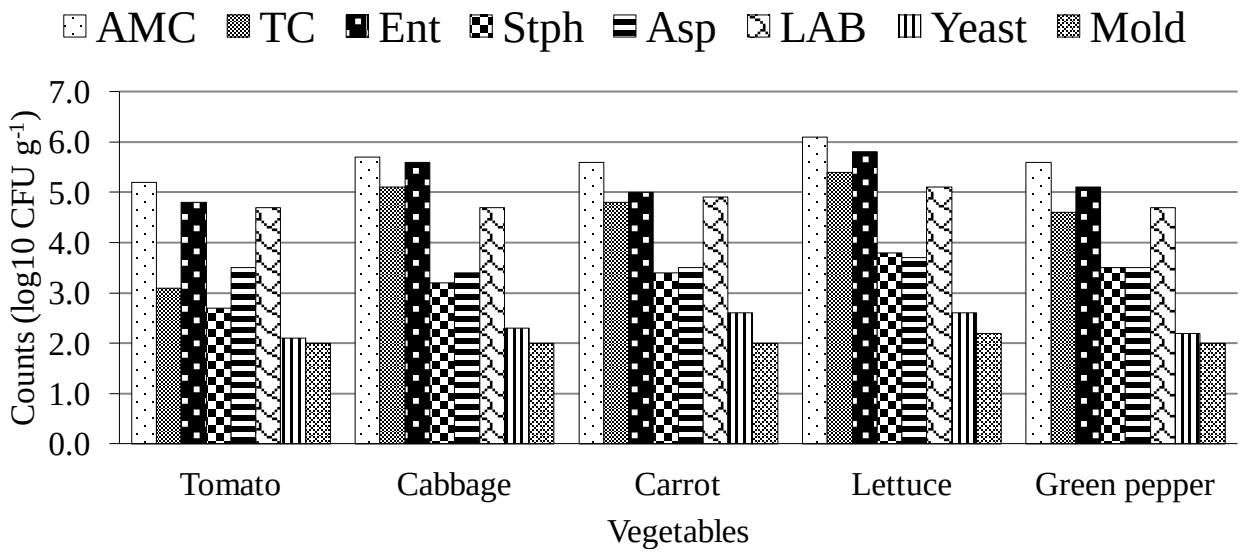

Figure 6. Microbial load of some selected raw vegetables purchased from Merkato site, Jimma town, Southwestern Ethiopia, 2011/12. AMC, Aerobic Mesophilic Count; TC, total coliforms; Ent, Enterobacteriaceae; Stph, *Staphylococcus* count; ASP, Aerobic spores.

6). LAB counts were similar among all vegetables purchased from all sites and counted ≥ 4 log^{10} CFU g^{-1}. But yeast and mold counts were ≤ 3 log^{10} CFUg^{-1} in all samples from Kochi, Agip and Merkato sites. There was no significant variation in counts of aerobic mesophilic bacteria and coliforms among lettuce samples and LAB among tomato purchased from different sites (CV ≤ 10). Counts of other bacterial groups, however, varied significantly (CV>10%) among samples of both vegetable types at both sites (Appendix A - D).

The frequency distribution of different microbiological groups of raw vegetables in Jimma town is as shown in Table 4. Accordingly, 97.2% of tomato and green pepper samples had aerobic mesophilic bacteria counts between 4 - 6.9 log^{10} CFU g^{-1}. However, all of aerobic mesophilic bacteria counts of cabbage and lettuce were higher than 4 log^{10} CFU g^{-1} and 5 log^{10} CFU g^{-1}, respectively. Over 97.7, 92.7 and 82.2% of vegetable samples had LAB, Enterobacteriaceae and coliforms ≥ 4 log^{10} CFU g^{-1}, respectively. The other microbial groups of vegetables were mostly counted between 2 - 3.9 log10 CFU g^{-1}. However, 24.4 and 58.3% of the samples had yeast and mold counts below the detectable level, respectively (Table 4).

Microbial analysis of vegetables

Based on cultural, morphological and biochemical characteristics of the organisms, a total of 1476 bacterial isolates were isolated from 180 vegetable samples. A total of six bacterial genera were identified (Table 5). The number and type of microbial groups isolated from the different vegetable samples were varied (Table 5). *Bacillus* spp (22.3%) was the most frequently isolated group being present in all vegetable types sampled followed by *Staphylococcus* spp. (17.7%), Enterobacteriaceae (15.5%) , *Micrococcus* (14.3%) and *Pseudomonas* (11.6%). *Aeromonas* (9.3%) and other Gram positive (G+) bacteria (9.3%) were the least isolated (Table 5). The most dominant bacterial group isolated from tomato samples were *Bacillus* spp. (29.6%) followed by *Micrococcus* (18.5%) and *Staphylococcus* (13.4%). However, cabbage samples were dominated by Enterobacteriaceae (21.8%) followed by *Bacillus* spp. (20.2%) and *Micrococcus* (15.1%). In carrot, *Bacillus* spp. (26.7%) were the most dominant followed by *Staphylococcus* spp. (19.4%) and Enterobacteriaceae (15.3%). Similarly, *Bacillus* spp. (22.2%) were dominant in lettuce followed by *Staphylococcus* (17.3%) and Enterobacteriaceae (16.4%). On other hand, green peppers were dominated by *Staphylococcus* (21.2%) followed by *Bacillus* spp (16.4%) and *Micrococcus* (15.2%) (Table 5).

Frequency of isolation of Salmonella spp. and S. aureus

Among 180 vegetable samples analyzed 23 (12.8%) samples were positive for *Salmonella* isolates (Table 6). With regard to frequency distribution in each vegetable type, *Salmonella* isolates were highly prevalent in lettuce (16.7%). The frequency distribution of *Salmonella* in both tomato and cabbage were equal (13.9%). On other hand, *Salmonella* were isolated in 11.1% of carrot samples.

Table 4. Frequency distribution of various microbial groups in some selected vegetable samples, Jimma town, south western Ethiopia, 2011/12.

Microbial group	Sample type	Log10 CFU g-1						
		<2 (%)	2-2.9 (%)	3-3.9 (%)	4-4.9 (%)	5-5.9 (%)	6-6.9 (%)	7- 7.9 (%)
Amc	Tomato	0 (0.0)	0 (0.0)	1 (2.8)	8 (22.2)	23 (63.9)	4 (11.1)	0 (0.0)
	Cabbage	0 (0.0)	0 (0.0)	0 (0.0)	2 (5.6)	26 (72.2)	8 (22.2)	0 (0.0)
	Carrot	0 (0.0)	0 (0.0)	1 (2.8)	0 (0.0)	32 (88.9)	3 (8.3)	0 (0.0)
	Lettuce	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	22 (61.1)	13 (36.1)	1 (2.8)
	Green pepper	0 (0.0)	0 (0.0)	1 (2.8)	5 (13.9)	27 (75.0)	3 (8.3)	0 (0.0)
Coliforms	Tomato	6 (16.7)	3 (8.3)	18 (50.0)	8 (22.2)	1 (2.8)	0 (0.0)	0 (0.0)
	Cabbage	0 (0.0)	0 (0.0)	0 (0.0)	13 (36.1)	22 (61.1)	1 (2.8)	0 (0.0)
	Carrot	0 (0.0)	0 (0.0)	0 (0.0)	18 (50.0)	18 (50.0)	0 (0.0)	0 (0.0)
	Lettuce	0 (0.0)	0 (0.0)	0 (0.0)	14 (38.9)	21 (58.3)	1 (2.8)	0 (0.0)
	Green pepper	0 (0.0)	0 (0.0)	5 (13.9)	20 (55.6)	11 (30.6)	0 (0.0)	0 (0.0)
Enterobacteriaceae	Tomato	1 (2.8)	2 (5.6)	7 (19.4)	14 (38.9)	11 (30.6)	1 (2.8)	0 (0.0)
	Cabbage	0 (0.0)	0 (0.0)	0 (0.0)	5 (13.9)	28 (77.8)	3 (8.3)	0 (0.0)
	Carrot	1 (2.8)	0 (0.0)	0 (0.0)	12 (33.3)	23 (63.9)	0 (0.0)	0 (0.0)
	Lettuce	0 (0.0)	0 (0.0)	1 (2.8)	4 (11.1)	27 (75.0)	4 (11.1)	0 (0.0)
	Green pepper	0 (0.0)	0 (0.0)	1 (2.8)	17 (47.2)	18 (50.0)	0 (0.0)	0 (0.0)
Staphylococci	Tomato	14 (38.9)	5 (13.9)	14 (38.9)	3 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)
	Cabbage	0 (0.0)	6 (16.7)	24 (66.7)	5 (13.9)	1 (2.8)	0 (0.0)	0 (0.0)
	Carrot	2 (5.6)	1 (2.8)	26 (72.2)	6 (16.7)	1 (2.8)	0 (0.0)	0 (0.0)
	Lettuce	0 (0.0)	2 (5.6)	26 (72.2)	7 (19.4)	1 (2.8)	0 (0.0)	0 (0.0)
	Green pepper	0 (0.0)	1 (2.8)	26 (72.2)	9 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)
Aerobic spore	Tomato	1 (2.8)	2 (5.6)	23 (63.9)	10 (27.8)	0 (0.0)	0 (0.0)	0 (0.0)
	Cabbage	0 (0.0)	2 (5.6)	30 (83.3)	4 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)
	Carrot	0 (0.0)	1 (2.8)	27 (75.0)	8 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)
	Lettuce	0 (0.0)	1 (2.8)	24 (66.7)	11 (30.6)	0 (0.0)	0 (0.0)	0 (0.0)
	Green pepper	1 (2.8)	2 (5.6)	29 (80.6)	4 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)
Lactic acid bacteria	Tomato	0 (0.0)	0 (0.0)	0 (0.0)	29 (80.6)	7 (19.4)	0 (0.0)	0 (0.0)
	Cabbage	1 (2.8)	0 (0.0)	1 (2.8)	30 (83.3)	4 (11.1)	0 (0.0)	0 (0.0)
	Carrot	1 (2.8)	0 (0.0)	0 (0.0)	22 (61.1)	13 (36.1)	0 (0.0)	0 (0.0)
	Lettuce	0 (0.0)	0 (0.0)	0 (0.0)	23 (63.9)	13 (36.1)	0 (0.0)	0 (0.0)
	Green pepper	0 (0.0)	0 (0.0)	1 (2.8)	31 (86.1)	4 (11.1)	0 (0.0)	0 (0.0)
Yeast	Tomato	14 (38.9)	15 (41.7)	7 (19.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Cabbage	8 (22.2)	19 (52.8)	9 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Carrot	7 (19.4)	21 (58.3)	7 (19.4)	1 (2.8)	0 (0.0)	0 (0.0)	0 (0.0)
	Lettuce	4 (11.1)	21 (58.3)	7 (19.4)	4 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)
	Green pepper	11 (30.6)	14 (38.9)	11 (30.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Molds	Tomato	27 (75.0)	8 (22.2)	1 (2.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Cabbage	24 (66.7)	11 (30.6)	1 (2.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Carrot	17 (47.2)	14 (38.9)	5 (13.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Lettuce	12 (33.3)	18 (50)	6 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Green pepper	25 (69.4)	9 (25)	2 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

AMC, Aerobic mesophilic counts.

However, green pepper contained the least *Salmonella* isolates (8.3%) as compared to lettuce, cabbage, tomato, and carrot samples (Table 6).

Of the total 180 vegetable samples, 18 (10.0%) were positive for *S. aureus* (Table 6). *S. aureus* was prevalent in each vegetable type. In most case, the levels of prevalence were different between vegetables. However, the prevalence of *S. aureus* in both cabbage and lettuce were equal (11.1%). *S. aureus* was most frequently isolated from green pepper (13.9%) followed by cabbage

Table 5. Dominant bacteria in some selected vegetables purchased form shops and vended markets, Jimma town, south western Ethiopia, 2011/12.

Sample type	Number of isolates	Number of different bacterial isolates (%)						
		Enterobacteriaceae	*Pseudomonas*	*Aeromonas*	*Bacillus*	*Micrococcus*	*Staphylococcus*	Other Gram + bacteria
Tomato	216	24 (11.1)	26 (12.0)	20 (9.3)	64 (29.6)	40 (18.5)	29 (13.4)	13 (6.0)
Cabbage	252	55 (21.8)	35 (13.9)	22 (8.7)	51 (20.2)	38 (15.1)	36 (14.3)	15 (6.0)
Carrot	288	44 (15.3)	32 (11.1)	13 (4.5)	77 (26.7)	29 (10.1)	56 (19.4)	37 (12.8)
Lettuce	324	53 (16.4)	44 (13.6)	32 (9.9)	72 (22.2)	44 (13.6)	56 (17.3)	23 (7.1)
Green pepper	396	53 (13.4)	34 (8.6)	50 (12.6)	65 (16.4)	60 (15.2)	84 (21.2)	50 (12.6)
Total	1476	229 (15.5)	171 (11.6)	137 (9.3)	329 (22.3)	211 (14.3)	261 (17.7)	138 (9.3)

Table 6. Prevalence of *Salmonella* and *S. aureus* in raw vegetables, Jimma town, south western Ethiopia, 2011/12.

Sample type	Sample size (180)	Number of *Salmonella* positive samples (%)	Number of *S. aureus* positive samples (%)
Tomato	36	5 (13.9)	2 (5.6)
Cabbage	36	5 (13.9)	4 (11.1)
Carrot	36	4 (11.1)	3 (8.3)
Lettuce	36	6 (16.7)	4 (11.1)
Green pepper	36	3 (8.3)	5 (13.9)
Total	180	23 (12.8)	18 (10.0)

and lettuce. In carrot, the prevalence was 8.3% with least prevalence (5.6%) in tomato samples (Table 6).

Antimicrobial susceptibility patterns of Salmonella isolates and S. aureus

Salmonella isolates were most susceptible to Ciprofloxacin (100%) and Gentamicin (100%) followed by Norfloxacin (95.7%), Chloramphenicol (87%) and Kanamycin (78.3%) (Table 7). On other hand, it exhibited slight resistance to Streptomycin (43.4%), Chloramphenicol (13%) and Kanamycin (21.7%). All *Salmonella* isolates were resistance to Ampicillin (100%) and 82.6% were resistance to Nalidixic acid (Table 7).

Multiple drug resistance patterns of *Salmonella* spp and *Staphylococcus aureus*

Among nine antimicrobial drugs used in this study, both *Salmonella* and *Staphylococcus aureus* showed Multiple Drug Resistance (MDR) to seven of them (Table 8). The highest MDR by *Salmonella* isolates were noted against TE/AMP/NA (26.1%). The maximum MDR registered was resistance to six drugs with the combination of C/K/S/TE/AMP/NA although less frequent (4.3%). Similarly, a total of six MDR patterns were observed among isolates of *S. aureus*. The highest MDR were observed against TE/P/DA (50%) followed by P/DA (22.2%). The maximum MDR registered was resistance to six drugs with the combination of K/S/TE/E/P/DA (5.6%) (Table 9).

DISCUSSION

The current study revealed the possible source of

Table 7. Antibiotic susceptibility patterns of *Salmonella* isolates from raw vegetables sold in Jimma town, Southwest Ethiopia, 2011/12.

Antimicrobial agents	Disk content (µg/ml)	Resistance		Intermediate		Sensitive	
		Number	%	Number	%	Number	%
Ampicillin (AMP)	10	23	100	0	0	0	0
Chloramphenicol (C)	30	0	0	3	13	20	87
Ciprofloxacin (CIP)	5	0	0	0	0	23	100
Gentamicin (CN)	10	0	0	0	0	23	100
Kanamycin (K)	30	2	8.7	3	13	18	78.3
Nalidixic acid (NA)	30	17	73.9	2	8.7	4	17.4
Norfloxacin (NOR)	10	0	0	1	4.3	22	95.7
Streptomycin (S)	10	5	21.7	5	21.7	13	56.5
Tetracycline (TE)	30	15	65.2	0	0	8	34.8

Chloramphenicol, Ciprofloxacin and Gentamycin were the most effective drugs against *Staphylococcus aureus* and shown the same activity level (100%). Kanamycin (94.4%), Erythromycin (88.8%) and Streptomycin (83.3%) were also partly effective against *S. aureus.* On other hand, *Staphylococcus aureus* was 100% resistance to penicillin G followed by Clindamycin (88.9%) and Tetracycline (66.6%) (Table 8).

Table 8. Antibiotic susceptibility patterns of *Staphylococcus aureus* isolated from raw vegetables, Jimma town, south west Ethiopia, 2011/12.

Antimicrobial agent	Disk content (µg/ml)	Resistance		Intermediate		Sensitive	
		Number	%	Number	%	Number	%
Chloramphenicol (C)	30	0	0	0.0	0	18	100
Ciprofloxacin (CIP)	5	0	0	0.0	0	18	100
Clindamycin (DA)	2	14	77.8	2.0	11.1	2	11.1
Erythromycin (E)	15	1	5.6	1.0	5.6	16	88.8
Gentamicin (CN)	10	0	0	0.0	0	18	100
Kanamycin (K)	30	1	5.6	0.0	0	17	94.4
Penicillin G (P)	10	18	100	0.0	0	0	0
Streptomycin (S)	10	2	11.1	1.0	5.6	15	83.3
Tetracycline (TE)	30	8	44.4	4.0	22.2	6	33.3

Table 9. Multiple drug resistance patterns in *Salmonella* and *S. aureus* isolated from raw vegetables, Jimma town, southwest Ethiopia, 2011/12.

Isolate	Number of drug resisted	Drug resisted	Number of resistant isolates	Percent of resistant isolates (%)
Salmonella spp. (23 isolates)	2	AMP/NA	1	4.3
	3	S/AMP/NA	1	4.3
		TE/AMP/NA	6	26.1
	4	K/S/AMP/NA	2	8.7
		S/TE/AMP/NA	2	8.7
		K/TE/AMP/NA	1	4.3
		TE/AMP/NA/NOR	1	4.3
	5	C/S/TE/AMP/NA	2	8.7
		K/S/TE/AMP/NA	1	4.3
		C/S/TE/AMP/NA	1	4.3
	6	C/K/S/TE/AMP/NA	1	4.3
Staphylococcus aureus (18 isolates)	2	P/DA	4	22.2
	3	TE/P/DA	9	50

Table 9. Contd.

4	S/TE/P/DA	1	5.6
5	S/TE/E/P/DA	1	5.6
6	K/S/TE/E/P/DA	1	5.6

AMP, Ampicillin; C, Chloramphenicol; DA, Clindamycin; E, Erythromycin; K, Kanamycin; NA, Nalidixic acid; NOR, Norfloxacin; P, Penicillin; S, Streptomycin; TE, Tetracycline.

pre- and post-harvest contaminants of vegetables. In Jimma, farmers are cultivating vegetables following traditional farming system. Farmers cultivate vegetables during rainy season, dry season and throughout the year. Most of the time, they used water from river and well as source of water for irrigation purpose. Therefore, river could be the main source for contamination of vegetables during pre-harvest in the field since it could contain sludge from different towns and villages (Aycicek et al., 2006). Pathogens from irrigation water may survive in soil and contaminate vegetable which in turn be transported to consumers with the possibility of causing diseases (Halablab et al., 2011). Other possible source of contamination could be animal manure used by farmers to increase the fertility of farm land. In addition, harvesting equipments, storage place, mechanisms of transportation to the market, placement in the market, and length of storage before selling could be the source of post-harvest contamination of vegetables (Natvig et al., 2002).

Extremely high counts of aerobic mesophilic bacteria reflect exposure of the vegetables to contaminants with the existence of favorable conditions for multiplication of microorganisms (Tortora, 1995). This study showed that the counts of aerobic mesophilic bacteria ranged between 3.3 log10 CFU g^{-1} (carrot) to 7.3 log10 CFU g^{-1} (lettuce). In contrary to this, other researchers from different countries reported a varied load of aerobic mesophilic counts in various vegetables. For instance, Chang and Fang (2007) from Taiwan, Vural and Erkan (2008) and Temiz et al. (2011) from Turkey , Eni et al. (2010) from Nigeria and Khiyami et al.(2011) from Saudi Arabia reported that aerobic mesophilic bacteria counts were between 3.3 - 8.6, 6.4 - 7.6, 6.2 - 7.1, 5.9 - 7.5 and 5 - 5.7 log10 CFU g^{-1}, respectively. Moreover, 82% of whole vegetables investigated in Spain revealed aerobic mesophilic bacteria count < 7 log10 CFU g^{-1} (Abadias et al., 2008). In the present study, 97.2% of aerobic mesophilic bacteria counts were < 7 log10 CFU g^{-1}. The difference in the counts between this study and previous reports may probably be due to difference in cultivation areas of vegetables, seasonal and climatic variation and/or difference in the microbial quality of manure and irrigation water used.

Hazard analysis and critical control point total quality management (HACCP- TQM) technical guide lines set the microbial quality standards for raw foods, whereby the food containing < 4, 4.0 - 6.7, 6.7 - 7.7 and > 7.7 log^{10} CFU g^{-1} aerobic plate count are rated as good, average, poor and spoiled food, respectively (Aycicek et al., 2006). Based on these criteria, 2.8% of each tomato, carrot and green peppers were regarded as good whereas, 97.2% were average; but, all of cabbage samples could be regarded as average in its microbial quality. About 97.2 and 2.8% of lettuce samples were rated as average and poor, respectively. Thus, the consumption of street vended vegetables without any treatment could potentially leads to certain health problem. The poor microbial quality of lettuce could be due to the use of animal manure and river water for irrigation. Lettuce is known to serve as a vehicle of foodborne pathogens and toxins of which the principal source of contamination, are the cultivation stages, processing and operation for preparation (Halablab et al., 2011). In agreement with these authors' findings, this study showed that all lettuce samples collected from different sites and markets in Jimma town had higher incidence of aerobic organisms than any other vegetable samples collected from the same location ($p < 0.05$). Accordingly, the total aerobic bacterial count on lettuce ranged from 5.3 - 7.3 log^{10} CFU g^{-1} as compared to tomato, cabbage, carrot and green pepper.

Total coliform and Enterobacteriaceae count can be considered as a hygiene quality indicator especially for fecal contamination. Their presence could indicate the pathogens might be present due to fecal contamination of human and animal origin or irrigation water. In this study, the counts of coliforms in all vegetable samples ranged from 2.0 log^{10} CFU g^{-1} (tomato) to 6.2 ^{log10} CFU g^{-1} (cabbage). In contrary, the coliform counts of salad vegetables in related study ranged from 4.3 - 4.9 log10 CFU g^{-1} (Khiyami et al., 2011). In addition, report from Zambia (Nguz et al., 2005) found coliform counts from vegetable products between 2.2 - 5.9 log^{10} CFU g^{-1} and Temiz et al. (2011) from Turkey reported that average total coliform counts of vegetables were between 3.4 - 4.9 log10 MPN g^{-1}. However, Aycicek et al. (2006) obtained a range of total count of coliforms on vegetable samples from 3.0 to 6.9 log10 CFU g^{-1}. In agreement with what was reported by Aycicek et al. (2006), the coliform counts in the current study were less than 6.9 log10 CFU g^{-1}.

Similarly, the highest counts of Enterobacteriaceae were encountered in cabbage samples collected from Agip venders (6.7 log^{10} CFU g^{-1}) and lowest from cabbage samples purchased from Kochi shops (4.1 log10

CFU g^{-1}). In related study conducted at Addis Ababa,Biniam and Ashenafi (2010) reported counts of Enterobacteriaceae at levels higher than 4 log^{10} CFU g^{-1} in lettuce and green pepper. Similar counts of Enterobacteriaceae were reported from vegetables examined in Morocco (Ibenyassine et al., 2007). Out of 28 vegetable samples collected from Spain, Abadias et al. (2008) found that 78.6% of the samples had Enterobacteriaceae counts < 5 log^{10} CFU g^{-1}. In contrast to this, 95% of Enterobacteriaceae count in current study was < 6 log^{10} CFU g^{-1}. The high coliform and Enterobacteriaceae counts in cabbage samples and other vegetables in this study could be attributed to poor hygiene of vegetable store room, market place, transporting containers, irrigation water and animal manure used by rural farmers to increase fertility of the farm land.

The contamination of vegetables with high level of *Staphylococcus* may cause *Staphylococcus* food poisoning. It has been reported that production of enterotoxin occurs when the counts of *S. aureus* reach 6 log10 CFU g^{-1} (Schelin et al., 2011). In our study, high *Staphylococcus* count was frequently counted between 3.0 - 3.9 log10 CFU g^{-1} in all vegetable samples analyzed. Accordingly, the frequency of isolation of *Staphylococcus* in this study was 38.9 and 66.7% for tomato and cabbage, respectively. In contrast to this, Biniam and Ashenafi (2010) reported over 80% of green pepper and lettuce harbored *Staphylococcus* counts ranging between 4.0 - 6.0 log10 CFU g^{-1}. The relatively low level of *Staphylococcus* count in present study could be due to short period of storage of the vegetables before sell since vegetables were brought to the market from nearby farmers living around Jimma town.

Higher bacterial spore counts from raw vegetables were found in our study than the mean aerobic spore count observed in lettuce and green pepper by Biniam and Ashenafi (2010). In other study, Ijabadeniyi et al. (2011) from South Africa reported aerobic spore count of 1.5 - 2 log10 CFU g^{-1}. In contrary, the aerobic spore count of present study was between 2.0 to 4.5 log10 CFU g^{-1}. Vegetables treated with food grade chemicals do not support the proliferation of spore forming bacteria. The presence of these bacteria at this level could indicate lack of treatment of vegetables with food grade chemicals to enhance the safety level of vegetables. However, the observed counts were not significantly high to pose health risk.

Lactic acid bacteria (LAB) are the biological basis for the production of a great multitude of fermented foods (Lasagno et al., 2002). The most important contribution of these bacteria is to preserve the nutritive qualities of the raw material and inhibit the growth of spoilage and pathogenic bacteria. This inhibition may be due to the production of many metabolites such as organic acids (lactic and acetic acid), hydrogen peroxide, diacetyl and bacteriocins (Ennahar et al., 2000; Lasagno et al., 2002).

In the present study, all vegetable samples had LAB counts < 5 log10 CFU g^{-1}. The high count of LAB are important to lower pH of the vegetables and contributes to accumulate sufficient antimicrobial metabolites to exert inhibitory effect against potential foodborne pathogens that contaminate the raw vegetables. Similarly, Abadias et al. (2008) from Spain reported LAB counts < 5 log10 CFU g^{-1} in all samples examined. Trias et al. (2008) reported the wide distribution of LAB in fresh vegetables of different origins.

Most of vegetable samples (> 74%) in the current study showed yeast and mold counts ≤ 2.9 log10 CFU g^{-1}. Contrary to our observation Meher et al. (2011) reported that the counts of yeasts and molds in carrot and tomato were below detectable level. The presence of molds in vegetables could pose the possible health problems as some may produce mycotoxins and others are known to cause allergies when they are able to produce large numbers of conidia (Seo et al., 2010).

The level of microbial contamination observed in vegetables of our study may be a reflection of poor storage conditions and how long these produce were kept before they were collected. Bacteria on storage materials may transfer to and cross contamination between produce. Different bacteria were identified and number of the bacteria isolated from each of the samples was varied. Some of the bacteria isolated in this study may be part of the natural flora of the vegetables or contaminants from various sources. *Pseudomonas* spp. and *Bacillus* spp. are part of the natural flora and are among the most common vegetable spoilage bacteria (Jay et al., 2005).

The microbial load of different vegetables was varied based on vegetable types, sites of sample collections, and market place. It was observed that level of lactic acid bacteria between different vegetables were similar ($P > 0.05$) although significant difference were observed between vegetables in other microbial counts ($P < 0.05$). Moreover, the high variability of all microbial groups within the samples of each vegetable showed the lack of uniformity in irrigation water, storage container and placement in the market before sell, consistent sanitation practices. Thus, there is an increased potential for vegetables to become contaminated with pathogenic species during production and processing as there was no system for control of microbiological safety of vegetables.

The presence of *S. aureus* and *Salmonella* spp. in vegetables are dangerous to consumers. *Salmonella* spp. was isolated from higher number of lettuce (16.7%) than other vegetable samples. This may be due to having foliar surfaces with many folds and the fragility of leaves (Aycicek et al., 2006).

In other report, too, *Salmonella* spp. was isolated from vegetables particularly lettuce samples (Rajkowski and Fan, 2008). The contamination of vegetables with human pathogen could occur during the growth of the produce using animal manure, contaminated water or cross conta-

mination during the cutting as the cut of vegetable can harbor and support the growth of food borne pathogen due to nutrients leakage from plant cellular material (Eni et al., 2010). The presence of *Salmonella* in 25 g of sample examined is regarded as potentially hazardous to consumers, and is unacceptable for consumption (Cheung et al., 2007). In addition, *S. aureus* was isolated from higher number of green pepper (13.9%). In similar study, Eni et al. (2010) from Nigeria were reported *S. aureus* was the most frequently isolated pathogens from vegetable samples. *S. aureus* is a dangerous pathogen and one of the most causative agents of hospital infectious (nosocomial infections) in human beings. Surface of vegetables may be contaminated by this organism through human handling and other environmental factors and can be able to survive for several weeks. Thus, contamination of vegetables during distribution and handling may allow bacterial growth and subsequently production of toxins which may represent potential risk to humans. Therefore, cleaning and use of the right types and concentrations of food grade chemicals for cleaning should be practiced to make the vegetables fit for consumption. Emergence of drug resistant pathogens is one of the most serious health problems in developing countries. This happens, for instance, when antibiotics are misused or overused (Nuermberger and Bishai, 2004). In our study, all isolates of *Salmonella* spp. and *S. aureus* were resistance to Ampicillin and penicillin G, respectively. The resistance of *Salmonella* to Streptomycin, Nalidixic acid and Tetracycline in this study was lower than reported from Malaysia (Yoke-Kqueen et al., 2008) and Brazil (Geimba et al., 2005). On other hand, all *Salmonella* isolates were sensitive to Ciprofloxacin and Gentamicin (Table 7). Similarly, all *Staphylococcus aureus* were sensitive to Ciprofloxacin and Chloramphenicol (Table 8).

In agreement with our study, Meher et al. (2011) from Bangladesh were reported similar results on susceptibility of *Salmonella* and *S. aureus* to Ciprofloxacin. Most of *Salmonella* isolates (82.6%) and *S. aureus* (88.9%) were multiple drugs resistant. About 30.3% of *Salmonella* isolates were resistant to three antimicrobials, namely TE/AMP/NA and S/AMP/NA. Likewise, 50% of *S. aureus* were resistant to three antimicrobials (TE/P/DA). Such antimicrobial resistance pattern clearly indicates that isolated pathogens were more resistant to easily available and most frequently used antibiotics. Resistance of *Salmonella* and *S. aureus* isolates to specific drugs could possibly be due to dissemination of drug resistance microbes in the environment arising from the misuse of antibiotics among the general population. In other study, Akbarmehr (2012) reported that 28 % of *Salmonella* isolates were resistant to four antibiotics.

Conclusion

There was lack of awareness on feasible sanitation methods to prevent foodborne diseases associated with consumption of fresh vegetables.

The possible source of contamination of vegetables could be irrigation water, animal manure used as fertilizers and water used to wash vegetables as most sellers wash or refresh different vegetables before selling them with the same water again and again.

All samples analyzed in this study were contaminated with high microbial load. The highest microbial load was recorded in lettuce followed by cabbage and carrot which could be attributed to various preharvest and post-harvest sources of contamination. However, there was significant difference in microbial load between vegetable samples.

Out of the total 180 samples of different vegetables, *Salmonella* isolates were found from 23 samples with more prevalence in lettuce than other vegetable samples. Likewise, *Staphylococcus aureus* were encountered from 18 samples with more prevalence in green pepper. This could be an indication of poor hygienic practice and frequent hand contact at the time of harvesting and in the market.

Most of *Salmonella* spp. was resistant to three antibiotics (TE/AMP/NA). Similarly, 50% of *S. aureus* was resistant to three different antibiotics (TE/P/DA).

Recommendations

To limit the introduction of pathogenic bacteria to vegetables through irrigation, the origin of irrigation water should be known. Where wells are used, such wells should be well maintained, and all irrigation sources should be monitored routinely for human pathogens.

Manure used as fertilizer should be treated by composting to eliminate pathogenic microorganisms and farmers should be educated on the need to allow sufficient amount of time between the final manure application and harvest.

Vegetable processors should be educated on the adverse effect of using untreated or polluted water for food processing as these could serve as sources of contamination.

Consumers should treat raw vegetables with food grade chemicals to kill pathogens and significantly reduce the microbial load before consumption.

Different vegetables should be stored separately before consumption to prevent cross contamination and the transfer of drug resistant bacterial pathogens.

In general, to reduce health risk associated to vegetable consumption, intervention mechanisms should be identified and the government should impose strict measures to control or at least minimize the risk of microbial contamination by implementing the Hazard Analysis and Critical Control Point (HACCP).

In the future, the effect of storage time and minimal processing on microbiological quality and safety of vegetables should be analyzed. Vegetables should reach consumers with in short period of time after harvest.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

Aarestrup F, Wegener H, Collingnon P (2008). Resistance in bacteria of the food chain. Epidemiology and control strategies. Expert Rev. Anti Infect. Ther. 6:733-750.

Abadias M, Usall J, Anguera M, Solsona C, Viñas I (2008). Microbiological quality of fresh minimally processed fruit and vegetables, and sprouts from retail establishments. Int. J. Food Microbiol. 123:121-129.

Acco M, Ferreira FS, Henriques JA, Tondo EC (2003). Identification of multiple strains of *Staphyloccocus aureus* colonizing nasal mucosa of food handlers. Food Microbiol. 20:489-493.

Akbarmehr J (2012). Antimicrobial resistance in *Salmonella* isolated from broiler chicken carcasses. Afr. J. Microbiol. Res. 6:1485-1488.

Al-Binali AM, Bello CS, El-Shewy K, Abdulla SE (2006). The prevalence of parasites in commonly used leafy vegetables in South Western Saudi Arabia. Saudi Med. J. 27:613-616.

Alemu A, Tsegaye W, Golassa L, Abebe G (2011). Urban malaria and associated risk factors in Jimma town, south-west Ethiopia. Malar. J. 10:173-200.

Allende A, Selma MV, López-Gálvez F, Villaescusa R, Gil MI (2008). Role of commercial sanitizers and washing systems on epiphy tic microorganisms and sensory quality of fresh-cut escarole and lettuce. Post Harvest Biol. Technol. 49:155-163.

Amoah P, Drechsel P, Abaidoo RC, Abraham EM (2009). Improving food hygiene in Africa where vegetables are irrigated with polluted water. West Africa Regional Sanitation and Hygiene Symposium, Accra, Ghana.

Asfaw Z (1997). Conservation and Use of Traditional Vegetables in Ethiopia. In Traditional African vegetables, Proceedings of the International Plant Genetic Resources Institute (IPGRI), Guarino, L., Ed., International workshop on genetic resources of traditional vegetables in Africa: Conservation and use. Nairobi. pp. 57-65.

Aycicek B, Sarimeh M, Cakiroglu S (2004). Assessment of microbiological quality of meals sampled at the meal serving units of military hospital in Ankara, Turkey. Food Control 15:379-384.

Aycicek H, Oguz U, Karci K (2006). Determination of total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey. Int. J. Hyg. Environ. Health 209:197-201.

Beuchat LR (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes Infect. 4: 413-423.

Biniam G, Ashenafi M (2010). Microbial load, prevalence and antibiograms of *Salmonella* and *Shigella* in lettuce and green peppers. Ethiop. J. Health Sci. 20:41-48.

Chang JM, Fang TJ (2007). Survival of *Escherichia coli O157:H7* and *Salmonella entrica* serovar Typhimurium in iceberg lettuce and the antimicrobial effect of rice vinegar against *E. coli O157:H7*. Food Microbiol. 24:745-751.

Cheesbrough M (2006). District Laboratory Practice in Tropical Countries. Part 2 (2nd ed.). Cambridge: Cambridge University Press.

Chelikani P, Fita I, Loewen P (2004). Diversity of structures and properties among catalases. Cell. Mol. Life Sci. 61:192-208

Cheung PY, Kwok KK, Kam KM (2007). Application of BAX system, Tecra UniqueTM *Salmonella* test, and a conventional culture method for the detection of *Salmonella* in ready-to-eat and raw foods. J. Appl. Microbiol. 103:219-227.

Clinical and Laboratory Standards Institute (CLSI) (2007). Performance standards for Antimicrobial susceptibility Testing: Seventeenth informational supplement. M100-S17.

Deza MA, Araujo M, Garnido MJ (2003). Inactivation of *Escherichia coli* 0157:H7, *Salmonella entritidis* and *Listeria monocytogenes* on the surface of tomatoes by neutral electrolysed water. Lett. Appl. Microbiol. 37:482-487.

Elhariry HM (2011). Biofilm formation by *Aeromonas hydrophila* on green leafy vegetables: cabbage and lettuce. Foodborne Pathog. Dis. 8:80-95.

Eni AO, Oluwawemitan AI, Solomon U (2010). Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria. Afr. J. Food Sci. 4:291-296.

Ennahar S, Sashihara T, Sonomoto K, Ishizaki A (2000). Class IIa bacteriocins: biosynthesis, structure and activity. Microbiol. Rev. 24: 85-106.

Geimba MP, Tondo EC, Brandelli A (2005). Antimicrobial resistance in *Salmonella enteritidis* isolated from foods involved in human foodborne outbreaks that occurred in the south of Brazil. J. Food Saf. 25:173-182.

Gregerson G (1978). Rapid method for distinction of Gram-positive from Gram-negative bacteria. Eur. J. Appl. Microbiol. 5:123-127.

Halablab MA, Sheet IH, Holail HM (2011). Microbiological Quality of Raw Vegetables Grown in Bekaa Valley, Lebanon. Am. J. Food Technol. 6:129 - 139.

Heuer H, Smalla K (2007). Horizontal gene transfer between bacteria. Environ. Biosaf. 6:3-17.

Hugh M, Leifson E (1953). The taxonomic significance of fermentative versus oxidative Gram-negative bacteria. J. Bacteriol. 66:24-26.

Ibenyassine K, Mahand RA, Karakomo Y, Anajjar B, Chouibani M, Enanaji MM (2007). Bacterial pathogens recovered from vegetables irrigated by wastewater in Morocco. J. Environ. Health 9:42-47.

Ijabadeniyi O, Legesse K, Mike V, Elna M (2011). Irrigation water as a potential preharvest source of bacterial contamination of vegetables. J. Food Saf. 31:452-461.

Jay JM, Loessner MJ, Golden DA (2005). Modern food Microbiology (7th edition). Springer science + Business Media, Inc., 233 Springer Street, New York, NY 10013, USA.

Johnston LM, Jaykus LA, Moll D, Martinez MC, Anciso J, Mora B, Moe CL (2005). A field study of the microbiological quality of fresh produce. J. Food Prot. 68:1840-1847.

Khiyami M, Noura A, Basel B, Hassan S (2011). Food borne pathogen contamination in minimally processed vegetable salads in Riyadh, Saudi Arabia. J. Med. Plants Res. 5:444-451.

Kovacs N (1956). Identification of Pseudomonas pyocyanae by the oxidase reaction. Nature 178:703.

Krieg NR (1981). Enrichment and isolation. In: Manual of Methods of Bacteriology, eds. Gerhardt, P., Murray, R.G., Costilow, R.N., Nester, E.W., Wood, W.A., Krieg, N.R. and Briggs Phillips, G., Washington DC: ASM Press. pp. 112-142.

Lasagno M, Beoletto V, Sesma F, Raya R, Font De Valdez G, Eraso A (2002). Selection of bacteriocin producer strains of lactic acid bacteria from a dairy environment. Microbiologia 25:37-44.

Meher NN, Reaz MM, Mahmudul H, Saiful I, Habibur R (2011). Prevalence of multi drug resistant bacteria on raw salad vegetables sold in major markets of Chittagong City, Bangladesh. Middle East J.

Sci. Res. 10:70-77.
Natvig EE, Ingham SC, Ingham BH, Cooperband LR, Roper TR (2002). *Salmonella* enterica serovar Typhimurium and *Escherichia coli* contamination of root and leaf vegetables grown in soils with incorporated bovine manure. Appl. Environ. Microbiol. 68: 2737-2744.
Nguz K, Shindano J, Samapundo S, Huyghebaert A (2005). Microbiological evaluation of fresh-cut organic vegetables produced in Zambia. Food Control 16:623-628.
Nuermberger EL, Bishai WR (2004). Antibiotic resistance in *Streptococcus pneumoniae*: what does the future hold? Clin. Infect. Dis. 38:363-371.
Pal V, Jamuna M, Jeevaratnam K (2005). Isolation and characterization of bacteriocin producing lactic acid bacteria from a south indian special dosa (appam) batter. J. Cult. Collect. 4:53-60.
Rajkowski KT, Fan X (2008). Microbial quality of fresh-cut iceberg lettuce washed in warm or cold water and irradiated in a modified atmosphere package. J. Food Saf. 28:248-260.
Schelin J, Wallin-Carlquist N, Cohn MT, Lindqvist R, Barker GC, Radstrom P (2011). The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. Foodborne Infect. 2:580-592.
Seo Y, Jang J, Moon K (2010). Microbial Evaluation of Minimally Processed Vegetables and Sprouts Produced in Seoul, Korea. Food Sci. Biotechnol. 19:1283-1288
Shalini S (2010). Study on microbiological aspects of fresh fruit and vegetables (including green leafy vegetables) in and around National Capital Region (NCR). Bhaskaracharya College of Applied Sciences. Dwarka, New Delhi, India.
Simon SL, Holzapfel WH, Halasz A (2007). Biogenic amine contents and microbial contamination of leafy vegetables during storage at 5°C. J. Food Biochem. 17: 407-418.
Spencer K, John FT, Spencer AL (2007). Food Microbiology Protocols, Homana Press, Totowa, New Jersey.
Tambekar DH, Mundhada RH (2006). Bacteriological quality of salad vegetables sold in Amravati city (India). J. Biol. Sci. 6: 28 - 30.
Temiz A, Bağcı U, Özmen S (2011). Efficacy of different decontamination treatments on microbial population of leafy vegetables. Gida 36:9-15.
Tortora G (1995). Microbiology. *The Benjamin puplishing co. Ic*., New York, USA. pp. 274-278.
Trias R, Bañeras L, Montesinos E, Badosa E (2008). Lactic acid bacteria from fresh fruit and vegetables as biocontrol agents of phytopathogenic bacteria and fungi. Int. Microbiol. 11:231-236.
Vural A, Erkan ME (2008). Investigation of Microbial quality of some leafy green vegetables. J. Food Technol. 6:285-288.
Walsh C, Fanings S (2008). Antimicrobial resistance in foodborne pathogens. Curr. Drug Targets 9:808-815.
Yoke-Kqueen C, Learn-Han L, Noorzaleha AS, Son AS, Sabrina S, Jiun-Horng S (2008). Characterization of multiple-antimicrobial-resistant *Salmonella enterica* sub sp. Enterica isolated from indigenous vegetables and poultry in Malaysia. Lett. Appl. Microbiol. 46:318-324.

Appendix A. Aerobic mesophilic bacteria and coliforms load of vegetables.

Vegetable sample	Sampling sites and markets	Microbial Counts in log10 CFU g^{-1}										P-value
		Aerobic mesophilic bacteria					coliforms					
		min	max	Mean	SD	%CV	min	max	Mean	SD	%CV	
Tomato	Kochi	3.5	6.6	5.4	0.9	16.7	2.0	5.4	3.7	0.8	21.6	
	Agip	4.5	6.9	5.5	0.5	9.1	2.0	4.6	3.3	0.9	27.3	
	Merkato	4.4	5.8	5.2	0.5	9.6	2.0	4.5	3.1	0.9	29.0	
	Shop	4.4	6.6	5.4	0.6	11.1	2.0	4.6	3.4	0.8	23.5	
	Vender	3.5	6.9	5.3	0.8	15.1	2.0	5.4	3.3	1.0	30.3	
	All sites	3.5	6.9	5.3	0.7	13.2	2.0	5.4	3.4	0.9	26.5	
Cabbage	Kochi	4.8	6.6	5.7	0.5	8.8	4.5	5.7	5.0	0.5	10.0	
	Agip	4.9	6.6	5.7	0.5	8.8	4.5	6.2	5.4	0.5	9.3	
	Merkato	5.3	6.3	5.7	0.3	5.3	4.5	5.8	5.1	0.5	9.8	
	Shop	4.8	6.5	5.6	0.4	7.1	4.5	6.2	5.1	0.5	9.8	
	Vender	5.3	6.6	5.8	0.4	6.9	4.5	5.8	5.2	0.5	9.6	
	All sites	4.8	6.6	5.7	0.4	7.0	4.5	6.2	5.2	0.5	9.6	
Carrot	Kochi	3.3	6.1	5.5	0.7	12.7	4.3	5.8	5.2	0.5	9.6	
	Agip	5.4	5.8	5.6	0.1	1.8	4.4	5.7	5.1	0.4	7.8	
	Merkato	5.3	5.9	5.6	0.2	3.6	4.3	5.4	4.8	0.4	8.3	
	Shop	3.3	6.0	5.4	0.6	11.1	4.5	5.8	5.1	0.5	9.8	
	Vender	5.3	6.1	5.7	0.2	3.5	4.3	5.6	5.0	0.5	10.0	
	All sites	3.3	6.1	5.5	0.4	7.3	4.3	5.8	5.0	0.5	10.0	
Lettuce	Kochi	5.3	6.8	5.8	0.4	6.9	4.2	5.5	4.7	0.4	8.5	
	Agip	5.4	7.3	6.0	0.5	8.3	4.6	5.9	5.5	0.5	9.1	
	Merkato	5.5	6.8	6.1	0.4	6.6	4.6	6.0	5.4	0.5	9.3	
	Shop	5.4	6.8	5.9	0.4	6.8	4.2	5.9	5.1	0.6	11.8	
	Vender	5.3	7.3	6.0	0.5	8.3	4.5	6.0	5.3	0.5	9.4	
	All sites	5.3	7.3	6.0	0.4	6.7	4.2	6.0	5.2	0.6	11.5	
Green pepper	Kochi	3.7	5.8	5.1	0.6	11.8	3.0	5.4	4.3	0.7	16.3	
	Agip	4.5	6.2	5.6	0.4	7.1	4.6	5.9	5.2	0.6	11.5	
	Merkato	4.7	6.3	5.6	0.4	7.1	3.0	5.8	4.6	0.7	15.2	
	Shop	4.5	5.8	5.4	0.4	7.4	3.0	5.8	4.5	0.7	15.6	
	Vender	3.7	6.3	5.5	0.6	10.9	3.3	5.9	4.8	0.8	16.7	
	All sites	3.7	6.3	5.4	0.5	9.3	3.0	5.9	4.7	0.8	17.0	

Min, Minimum; Max, Maximum; SD, Standard Deviation; CV, Coefficient of variation.

Appendix B. Counts of Enterobacteriaceae and Staphylococci load of vegetables.

Vegetable sample	Sampling sites and markets	Microbial Counts in log10 CFU g^{-1}										P-value
		Enterobacteriaceae					Staphylococci					
		Min	Max	Mean	SD	%CV	Min	Max	Mean	SD	%CV	
Tomato	Kochi	2.0	5.4	4.0	1.2	30.0	2.0	4.7	2.7	1.0	37.0	
	Agip	3.5	5.8	4.7	0.8	17.0	2.0	4.2	3.0	0.7	23.3	
	Merkato	3.8	6.4	4.8	0.6	12.5	2.0	3.3	2.7	0.5	18.5	
	Shop	2.0	5.8	4.5	1.0	22.2	2.0	4.7	3.1	0.8	25.8	
	Vender	2.9	6.4	4.5	0.8	17.8	2.0	3.3	2.5	0.5	20.0	
	All sites	2.0	6.4	4.5	0.9	20.0	2.0	4.7	2.8	0.8	28.6	

Appendix B. Contd

Cabbage	Kochi	4.1	5.8	5.2	0.6	11.5	2.8	5.4	3.7	0.8	21.6
	Agip	4.7	6.7	5.7	0.5	8.8	2.8	4.4	3.5	0.5	14.3
	Merkato	5.3	6.0	5.6	0.2	3.6	2.3	3.6	3.2	0.4	12.5
	Shop	4.1	6.1	5.4	0.6	11.1	3.0	4.4	3.6	0.5	13.9
	Vender	4.7	6.7	5.6	0.4	7.1	2.3	5.4	3.2	0.6	18.8
	All sites	4.1	6.7	5.5	0.5	9.1	2.3	5.4	3.4	0.6	17.6
Carrot	Kochi	2.0	5.8	4.9	1.0	20.4	2.0	4.5	3.3	0.7	21.2
	Agip	4.5	5.8	5.5	0.4	7.3	3.0	4.7	3.7	0.5	13.5
	Merkato	4.2	5.7	5.0	0.5	10.0	2.0	5.0	3.4	0.7	20.6
	Shop	2.0	5.8	5.1	0.9	17.6	2.0	4.7	3.4	0.7	20.6
	Vender	4.2	5.8	5.2	0.5	9.6	2.5	5.0	3.5	0.6	17.1
	All sites	2.0	5.8	5.1	0.7	13.7	2.0	5.0	3.5	0.6	17.1
Lettuce	Kochi	3.6	5.7	5.0	0.7	14.0	2.9	4.3	3.6	0.4	11.1
	Agip	5.3	6.3	5.7	0.3	5.3	3.1	4.2	3.6	0.3	8.3
	Merkato	5.3	6.3	5.8	0.3	5.2	2.8	5.7	3.8	0.8	21.1
	Shop	4.2	6.0	5.4	0.5	9.3	2.8	4.3	3.5	0.4	11.4
	Vender	3.6	6.3	5.5	0.7	12.7	2.9	5.7	3.8	0.6	15.8
	All sites	3.6	6.3	5.5	0.6	10.9	2.8	5.7	3.7	0.5	13.5
Green pepper	Kochi	3.4	5.6	4.7	0.6	12.8	3.2	4.8	3.9	0.5	12.8
	Agip	4.5	5.9	5.3	0.5	9.4	3.5	4.9	3.9	0.4	10.3
	Merkato	4.0	5.7	5.1	0.5	9.8	2.8	4.6	3.5	0.5	14.3
	Shop	3.4	5.7	4.8	0.6	12.5	2.8	4.9	3.8	0.5	13.2
	Vender	4.5	5.9	5.2	0.5	9.6	3.0	4.8	3.7	0.5	13.5
	All sites	3.4	5.9	5.0	0.6	12.0	2.8	4.9	3.8	0.5	13.2

Min, Minimum; Max, Maximum; SD, Standard Deviation; CV, Coefficient of variation.

Appendix C. Aerobic spore formers and Lactic Acid Bacteria load of vegetables.

Vegetable sample	Sampling sites	Microbial counts in log10 CFU g^{-1}										P-value
		Aerobic spore					Lactic Acid Bacteria					
		Min	Max	Mean	SD	%CV	Min	Max	Mean	SD	%CV	
Tomato	Kochi	2.0	4.8	3.6	0.9	25.0	4.3	5.4	4.6	0.4	8.6	
	Agip	3.0	4.5	3.7	0.6	16.2	4.5	5.4	4.8	0.3	6.3	
	Merkato	3.0	4.4	3.5	0.4	11.4	4.5	5.4	4.7	0.3	6.4	
	Shop	2.7	4.8	3.6	0.6	16.7	4.4	5.4	4.7	0.3	6.4	
	Vender	2.0	4.5	3.6	0.7	19.4	4.3	5.4	4.7	0.4	8.5	
	All sites	2.0	4.8	3.6	0.6	16.7	4.3	5.4	4.7	0.3	6.4	
Cabbage	Kochi	2.9	4.5	3.5	0.5	14.3	4.5	4.6	4.6	0.0	0.0	
	Agip	2.8	4.2	3.5	0.4	11.4	2.0	5.3	4.4	0.9	20.5	
	Merkato	3.0	3.6	3.4	0.2	5.9	4.1	5.7	4.7	0.4	8.5	
	Shop	2.8	4.5	3.5	0.4	11.4	2.0	5.3	4.4	0.7	15.9	
	Vender	2.9	4.3	3.4	0.4	11.8	4.3	5.7	4.7	0.4	8.5	
	All sites	2.8	4.5	3.5	0.4	11.4	2.0	5.7	4.5	0.6	13.3	
Carrot	Kochi	3.5	4.5	3.8	0.4	10.5	2.0	5.3	4.5	0.8	17.8	
	Agip	3.3	4.4	3.9	0.4	10.3	4.5	5.5	5.0	0.4	8.0	

Appendix C. Contd

	Merkato	2.5	3.7	3.5	0.3	8.6	4.3	5.6	4.9	0.5	10.2
	Shop	2.5	4.5	3.8	0.5	13.2	2.0	5.5	4.5	0.7	15.6
	Vender	3.3	4.4	3.7	0.3	8.1	4.1	5.6	5.0	0.5	10.0
	All sites	2.5	4.5	3.7	0.4	10.8	2.0	5.6	4.8	0.6	12.5
Lettuce	Kochi	2.8	4.8	3.8	0.6	15.8	4.5	5.3	4.7	0.3	6.4
	Agip	3.3	4.2	3.7	0.3	8.1	4.0	5.6	4.7	0.5	10.6
	Merkato	3.3	4.3	3.7	0.3	8.1	4.5	5.8	5.1	0.5	9.8
	Shop	3.2	4.8	3.7	0.4	10.8	4.5	5.6	4.9	0.4	8.2
	Vender	2.8	4.3	3.8	0.4	10.5	4.0	5.8	4.7	0.5	10.6
	All sites	2.8	4.8	3.7	0.4	10.8	4.0	5.8	4.8	0.5	10.4
Green pepper	Kochi	2.0	4.5	3.4	0.6	17.6	4.5	4.6	4.6	0.1	2.2
	Agip	2.5	4.6	3.5	0.5	14.3	4.2	5.2	4.6	0.2	4.3
	Merkato	3.0	4.5	3.5	0.4	11.4	3.5	5.5	4.7	0.6	12.8
	Shop	2.0	4.5	3.4	0.6	17.6	3.5	4.7	4.5	0.3	6.7
	Vender	2.8	4.6	3.5	0.4	11.4	4.1	5.5	4.7	0.4	8.5
	All sites	2.0	4.6	3.4	0.5	14.7	3.5	5.5	4.6	0.5	10.9

Min, Minimum; Max, Maximum; SD, Standard Deviation; CV, Coefficient of variation.

Appendix D. Yeast and Mold load of vegetables.

Vegetable sample	Sampling sites	Microbial counts in log10 CFU g^{-1}										P- value
		Yeast					Molds					
		Min	Max	Mean	SD	%CV	Min	Max	Mean	SD	%CV	
Tomato	Kochi	2.0	3.5	2.8	0.6	21.4	2.0	3.1	2.3	0.4	17.4	
	Agip	2.0	3.5	2.5	0.4	16.0	2.0	2.5	2.1	0.2	9.5	
	Merkato	2.0	2.5	2.1	0.2	9.5	2.0	2.0	2.0	0.0	0.0	
	Shop	2.0	3.5	2.5	0.6	24.0	2.0	3.1	2.2	0.3	13.6	
	Vender	2.0	3.2	2.4	0.4	16.7	2.0	2.7	2.1	0.2	9.5	
	All sites	2.0	3.5	2.5	0.5	20.0	2.0	3.1	2.1	0.3	14.3	
Cabbage	Kochi	2.0	3.2	2.7	0.4	14.8	2.0	3.1	2.5	0.4	16.0	
	Agip	2.0	3.5	2.5	0.5	20.0	2.0	2.8	2.1	0.2	9.5	
	Merkato	2.0	2.8	2.3	0.3	13.0	2.0	2.0	2.0	0.0	0.0	
	Shop	2.0	3.5	2.6	0.6	23.1	2.0	3.1	2.2	0.4	18.2	
	Vender	2.0	3.1	2.5	0.4	16.0	2.0	2.9	2.2	0.3	13.6	
	All sites	2.0	3.5	2.5	0.4	16.0	2.0	3.1	2.2	0.3	13.6	
Carrot	Kochi	2.0	4.2	2.8	0.7	25.0	2.0	3.3	2.6	0.5	19.2	
	Agip	2.0	3.1	2.5	0.3	12.0	2.0	2.8	2.4	0.3	12.5	
	Merkato	2.0	3.0	2.6	0.4	15.4	2.0	2.3	2.0	0.1	5.0	
	Shop	2.0	4.2	2.6	0.6	23.1	2.0	3.0	2.3	0.4	17.4	
	Vender	2.0	3.2	2.6	0.4	15.4	2.0	3.3	2.4	0.4	16.7	
	All sites	2.0	4.2	2.6	0.5	19.2	2.0	3.3	2.4	0.4	16.7	
Lettuce	Kochi	1.9	4.3	3.1	0.7	22.6	2.0	3.3	2.7	0.4	14.8	
	Agip	2.0	4.3	2.9	0.8	27.6	2.0	3.1	2.4	0.4	16.7	
	Merkato	2.0	3.0	2.6	0.3	11.5	2.0	2.5	2.2	0.2	9.1	
	Shop	2.0	4.3	3.1	0.8	25.8	2.0	3.1	2.5	0.4	16.0	

Appendix D. Contd

	Vender	1.9	3.4	2.7	0.4	14.8	2.0	3.3	2.4	0.4	16.7
	All sites	1.9	4.3	2.9	0.7	24.1	2.0	3.3	2.4	0.4	16.7
Green pepper	Kochi	2.0	3.4	2.9	0.4	13.8	2.0	3.2	2.5	0.4	16.0
	Agip	2.0	3.4	2.6	0.5	19.2	2.0	3.3	2.2	0.4	18.2
	Merkato	2.0	2.7	2.2	0.3	13.6	2.0	2.0	2.0	0.0	0.0
	Shop	2.0	3.4	2.6	0.6	23.1	2.0	3.3	2.2	0.4	18.2
	Vender	2.0	3.3	2.5	0.4	16.0	2.0	2.9	2.2	0.3	13.6
	All sites	2.0	3.4	2.5	0.5	20.0	2.0	3.3	2.2	0.4	18.2

Min, Minimum; Max, Maximum; SD, Standard Deviation; CV, Coefficient of variation.

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Full Length Research Paper

Development of a geographic information system (GIS) based road network in Port Harcourt

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The aim of this work was to develop a geographic information system (GIS) based road network map of Port Harcourt city that can be used to analyze traffic congestion within the city and suggest possible solutions. The handheld global positioning system (GPS) was used to acquire geographic coordinates of major locations experiencing traffic jams, bad spots and schools. The transformed GPS coordinates were added to the ArcGIS environment to define the spatial locations. Prior to that, the road map was digitized and geo-rectified. Satellite Imagery from the remote sensing technology was used to acquire data of new roads, for map updating and revision. Geographic information systems (GIS) operations (buffering, overlay and networking techniques) using ArcGIS 9.3 were performed on the road map. The study recommends that: the road network in Borikiri axis of Port Harcourt should be improved by constructing a by-pass to ease the traffic along Harold Wilson road; the width of roads should be increased at T-junctions and cross-junctions; all public facilities especially those located along major roads should have good parking plots before approval for construction. It is also recommended that at proximity of 500 km from a developing area, a boulevard should be constructed at the junction linking such area to the center of the town, for instance, the Wimpey/Iwofe junction. The road network as predicted in this study is expected to contain a minimum of 217,360 cars in 2022 for the identified routes excluding larger vehicles like trucks.

Key words: Road network, Geographic information systems (GIS), traffic congestion, Port Harcourt, global positioning system (GPS).

INTRODUCTION

During the colonial era, the road network of Port Harcourt was planned in such a way that the streets were designed in a grid form. Social and recreational facilities provided were well situated; hence the quality of life of the inhabitants was enhanced. This is obviously due to the fact that transportation networks provide basic

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Table 1. Hourly traffic flow of some routes in Port Harcourt.

Location	2008 traffic flow	2013 traffic flow
Ikwerre Road	1286	1360
East West	771	816
Obi-wale/Rumuigbo	473	500
Rumuepirikom/Ada George	476	504
Rumuokwurushi	983	1040
Eliozu	1116	1181
Aba Road/Woji	3453	3653
Harold Wilson/Churchill	1069	1131
Marine junction/Hospital road	1240	1312
Trans Amadi/Abuloma	1246	1318
LNG	1334	1411
Abonnema wharf	964	1020
Aba Express	1122	1187
East West Tank 1	590	624
East West 2	882	933
Total	17005	17989

Source: Integrated Transport Master Plan, October 2008.

infrastructural framework for rapid economic development. Consequently, Port Harcourt began to experience rapid growth rate.

The rapid increase in population of persons and vehicles without proper planning, design and maintenance of the available roads within the city, as well as the improper location of public facilities resulted in an inadequate transportation network. This is because the volume of traffic outweighs the road capacity, resulting in traffic congestion. According to the UN and its Habitat Organizations, five comprehensive problem fields are relevant for the enhancement of living conditions within a city (UN Habitat, 2003) of which transport is a part. These challenges can be solved basically by employing surveying techniques and GIS. Surveying is the bedrock of any meaningful development. The end-product of its process, the map, is employed in planning. According to Olagbadebo and Dienye (2008), the digital production of maps which aid in improving the legibility, accuracy and updating procedures is achieved using geographic information system (GIS). Hence the development of a GIS based road network map of Port Harcourt for solving problems associated with the road network.

Statement of problem

One of the major problems affecting the road network of Port Harcourt is traffic congestion, and the following factors are responsible for the traffic congestion: Bad spots at close distances along route, absence of alternative routes, flooding as a result of inadequate and poorly maintained and constructed drainage systems, small/sub-standard road width especially on approaching a junction, non provision of parking plots at the location of public facilities such as schools, markets, shopping malls.

The traffic flow data of Port Harcourt reveals that there was an increase in the number of vehicles within the city between the periods 2008-2013. This is obviously as a result of the rural-urban drift (Table 1).

Study area

The study area, Port Harcourt, is named after Lewis Viscount Harcourt in 1913. Port Harcourt lies between longitudes 6°55' and 7°10' East of the Greenwich meridian and latitudes 4°40' and 4°55' north of the equator. The population of the city is estimated at 538,558 people (National Population Census, 2006), while projected population in 2012 was 573,621. The city has one international airport at Omagwa, and a local airport at Air force, two multi-national firms as well as other industrial concerns. Port Harcourt is the chief oil refining city in Nigeria.

Scope of the study

The study is confined to some environments within Port Harcourt. A total of 40 junctions where observed, and six (6) major routes considered. The routes are: i) Harold Wilson Drive; ii) Ada George; iii) Ikwerre Road; iv) Aggrey Road; v) Abuloma Road and vi) Woji Road.

Figure 1. Development in Borikiri.

The spatial location of 221 schools (primary and secondary) where defined within the metropolis; the location of schools being a factor to traffic volume.

Road congestion in Port Harcourt is similar to that of Guwahati, capital city of Assam in Northeastern India. Urbanization peaked without consequent development of the social and physical infrastructure like roads, bridges and settlements (Deka, 2009; Obinna, et al., 2010). Figure 1 is a map showing Borikiri in the southern part of Port Harcourt, developing with only one major road. Traffic snarls take place in most parts of Guwahati city consequent upon the following factors: lack of proportionate attributes of roads, population explosion, and peak number of vehicles, rapid urbanization, and location of social infrastructure, complex land acquisition, and habitation before construction of roads. These challenges could be solved with the application of GIS to surveying and mapping. The prevention of unnecessary traffic, which generates environmental burdens, should be the top priority of municipalities in urban centers (Oluwadare et al., 2009; ITMP, 2008).

According to Matt (2009), Singapore, a southeastern Asian Island since its independence in 1965 realized the need for GIS in 1995 when it formed LTA, having acknowledged the transportation needs of about 4.6 million people. The decision was based on the features and functionality of ESRI's ArcGIS software, aiding Land Transit Authority (LTA) to manage its assets and resources, as well as giving it the freedom to collaborate with other government, private and public agencies having the common interest of a free-flowing transportation system. Singapore LTAuses GIS to integrate transportation data and manage traffic incidents (Transportation GIS Trends, 2009).

Furthermore, Andrew et al. (2011) employed GIS for assessing the road network in Trans-Amadi, Port Harcourt. They concluded that the road network in Trans Amadi was in good condition and the connectivity level was high. They recommended that the study should be carried out on a larger scale considering vehicular movement and impedance at other locations within the city.

METHODOLOGY

Hardware and software selections

Hardware components for data acquisition, manipulation, processing and presentation used for this work included the following: Computer-windows 7 (4.00GB RAM space, 64-bit Operating System, 21″ colour monitor), A0 Scanner (Crystal G600 Wide format), CD-Rom Drive, Hard Drives (flash drive), GPS Map 76, versatile navigator, Plotter (HP Design jet 500 plus 42), and Colour Printer (HP Deskjet 3050A J610series).

The software selected for analyses were ArcGIS 9.3 version and AutoCAD 2007 version. Geographic Calculator (GeoCAL) version 6.3 was used for coordinate system conversions of GPS coordinates in Excel sheet. The coordinates from the Excel sheet where imported into the ArcGIS (Arc Catalog) environment using the 'Add data' tool. Microsoft Word 2007, Microsoft Excel and Power Point 2007 were used for production of the manuscript and presentation.

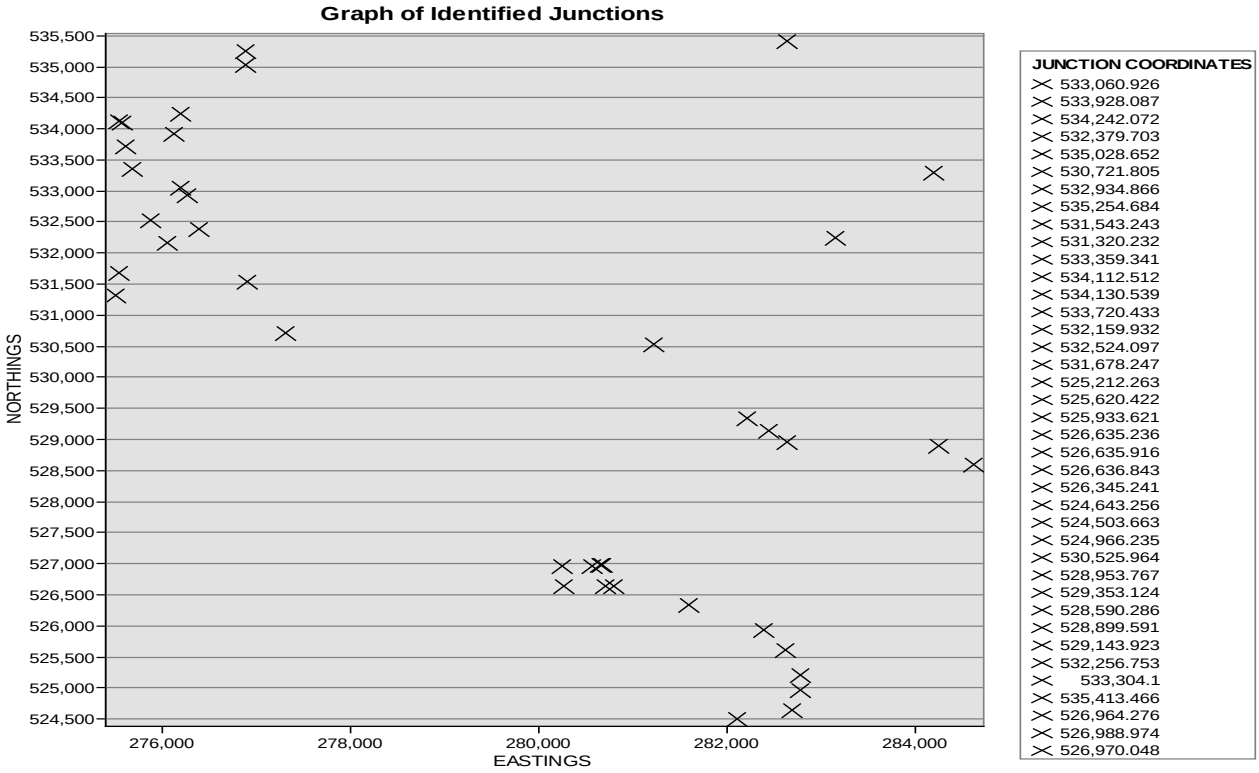

Figure 2. Coordinates of junctions that experience traffic jams.

Data acquisition

The GPS receiver (map 76 versatile navigator) was employed to obtain the coordinate of the junctions (Figure 2), bad spots and schools (Figures 3 an 4). The coordinates (Minna Datum) obtained were converted using Geographic Calculator (GeoCAL) version 6.3 software.

The study also made use of Secondary data derived from the road network map at a scale of 1:20,000 obtained from the Rivers State Geographic Information System (RIVGIS), the population data of Port Harcourt in 2006 obtained from the National Populations Commission (NPC) (2010), and hourly traffic flow rate of vehicles along routes. The satellite imagery of the study area was also obtained. The road map and the imagery were geo-rectified in ArcGIS to geographic coordinates.

Database design

Database design constitutes one of the core tasks in developing any GIS application. It involves the process by which the real world entities and their interrelationships are analyzed and modeled in order to derive the maximum benefits while using the minimum quantity of data (Kufoniyi, 1998, Ghilani and Wolf, 2008). The two stages involved in the database design process are: the design stage, and the implementation stage.

The design stage consists of four elements. These are:

View of reality

For this application, the view of reality includes roads, locations of traffic congestion, built up areas, boundary of the study area.

Conceptual design

In the conceptualization stage, the basic entities were determined, their spatial relationship and the attributes of each entity. This project classified roads as linear features and the boundary of the study area as polygon feature. The road junctions, location of schools and potholes, were taken as point features.

Logical design

The entities or GIS layers and their attributes were translated into a geo-relational data structure. Each layers with the necessary tables and the tables then related or joined together with appropriate cardinalities ranging from one to one (Table 2); one to many (Table 3); and many to many (Table 4).

RESULTS AND DISCUSSION

Spatial analysis

Overlay analysis was used to merge spatial data by combining two or more spatial data sets to produce a new spatial data set where the feature attributes are a union of the input. The road network map was overlaid on the imagery to aid assessment and appreciation of the ratio between road length and total area. The ratio between the total area and the total route length in the network is such that the road density is high.

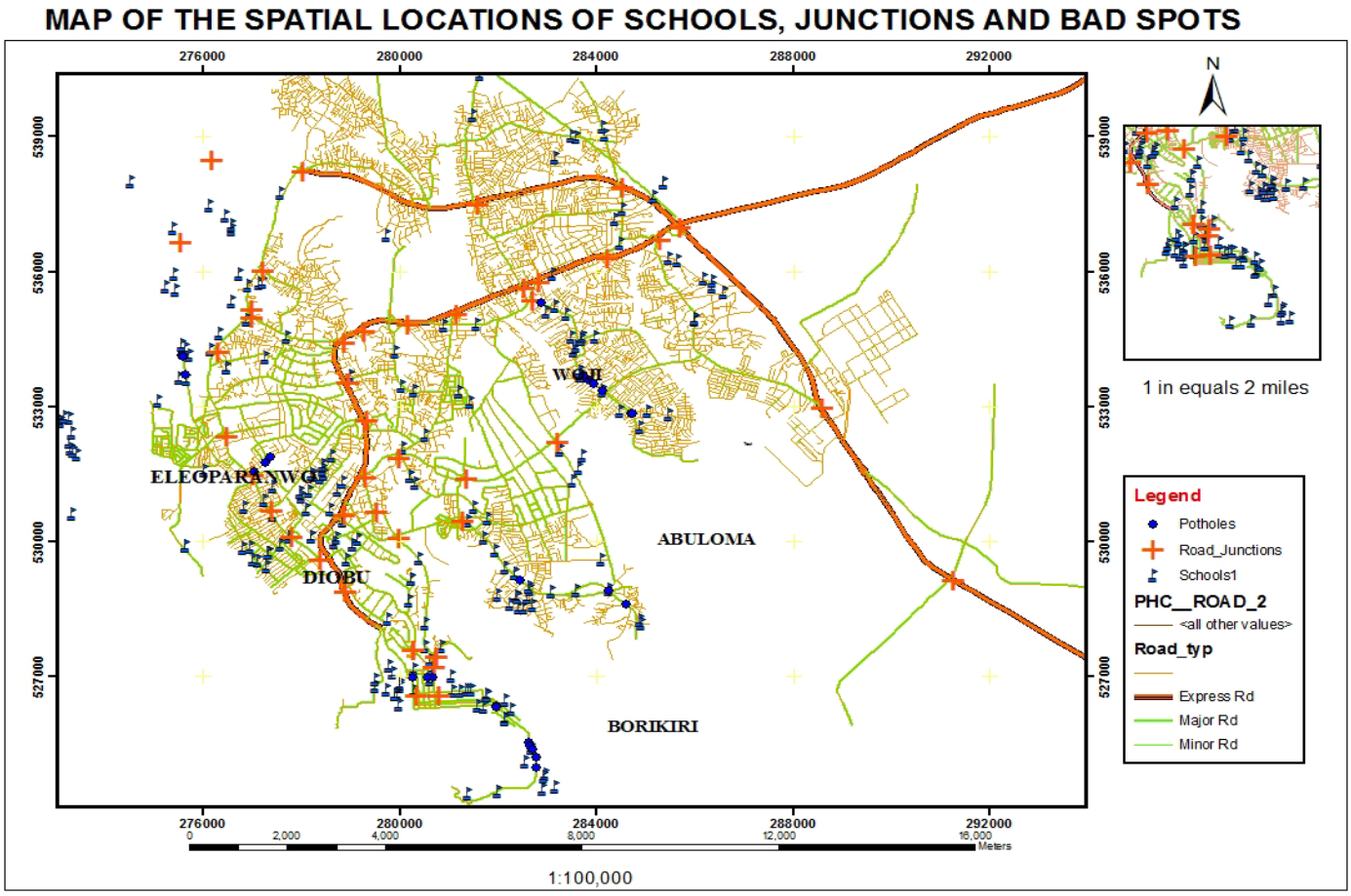

Figure 3. Spatial locations of schools, junctions and bad spots.

Furthermore, overlay operations done in Borikiri shows that, most of the identified bad spots were very close to the identified congested junctions along the Harold Wilson drive. The number of schools along this same route would rather require a smooth flow of traffic which is obviously not certain due to the pot holes at the major junctions and the lack of alternative routes (only one major route) that would ease the congestion of the junctions (Figure 5).

Proximity analysis

Buffering is a means of performing this practical spatial query to determine the proximity of neighbouring features. By point buffering, features (junctions, bad spots) within a prescribed distance from a point, line, or area, are determined. Along the borikiri axis, a buffer of 500m was created at UPE junction (Figure 6). This point in the field is known to be highly congested during peak hours. The buffer captures three (3) junctions, four (4) schools and four major bad spots along the same road. Hence the combination of three factors responsible for traffic congestion is found within the buffered zone. There is no alternative for users to consider in the case of an emergency.

Network analysis

Unlike proximity analysis that searches in all directions from a point, line, or area, network analysis is restricted to searching along a line, such as a route, or throughout a network of linear features, such as the road network. Network analysis can be used to define or identify route corridors and determine travel paths, travel distances, and response times. For example, network analysis may be used to assess the traffic volume impact of a road closure on adjacent roadways.

For this work, the presence of a barrier at the GRA Junction and considering the one way movement of the traffic was adopted. The alternative route is presented thus in Figure 7, having a driving distance of 9361.3 m

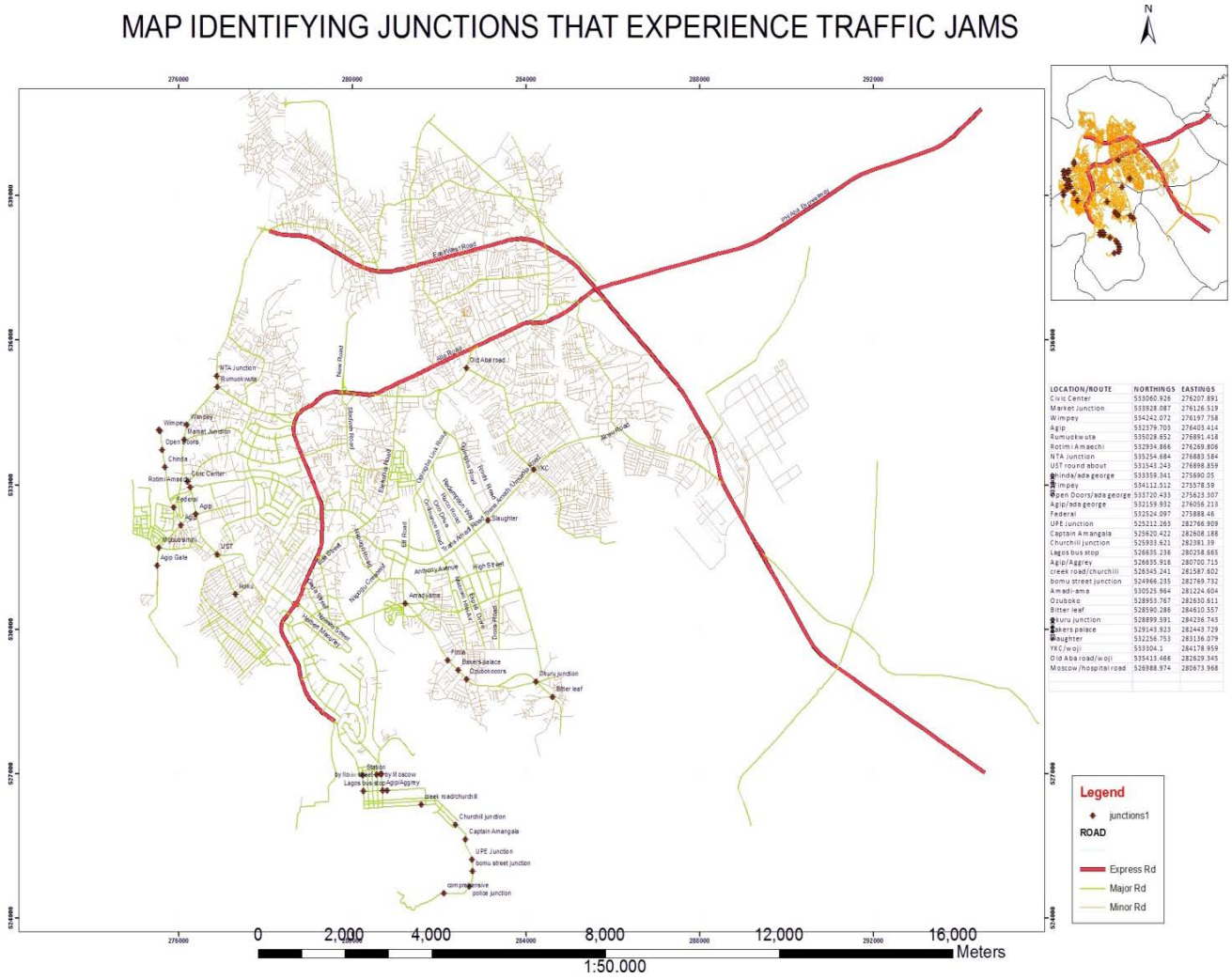

Figure 4. Junctions that Experience Traffic Jams.

Table 2. Road Layer (obtained by digitizing).

RD_ID	RD_NAME	RD_SURFACE	RD_LENGTH
54	Ikwerre Road	Tarred	9775.662721
133	Ada George	Tarred	4412.095238
48	Harold Wilson Drive	Tarred	2198203394
56	Woji	Tarred	3443.434173

Table 3. Traffic Location (junctions).

LO_ID	LO_Name	RD_ID
01	Market Junction	54
02	Wimpey junction	54
11	Wimpey/Iwofe	133
17	UPE	48
05	Ikoku	54
34	YKC	56

Table 4. Location of facility (schools).

LO_ID	ACC_RD	FAC_NAME	BUA_CLASS
01	Ikwerre Road	Community Secondary school, Nkpolu	Dense
11	Ada George	Istan Comprehensive high school	Dense
17	Harold Wilson	State Secondary school UPE	Dense
05	Ikwerre Road	St. Thomas State School.	Dense

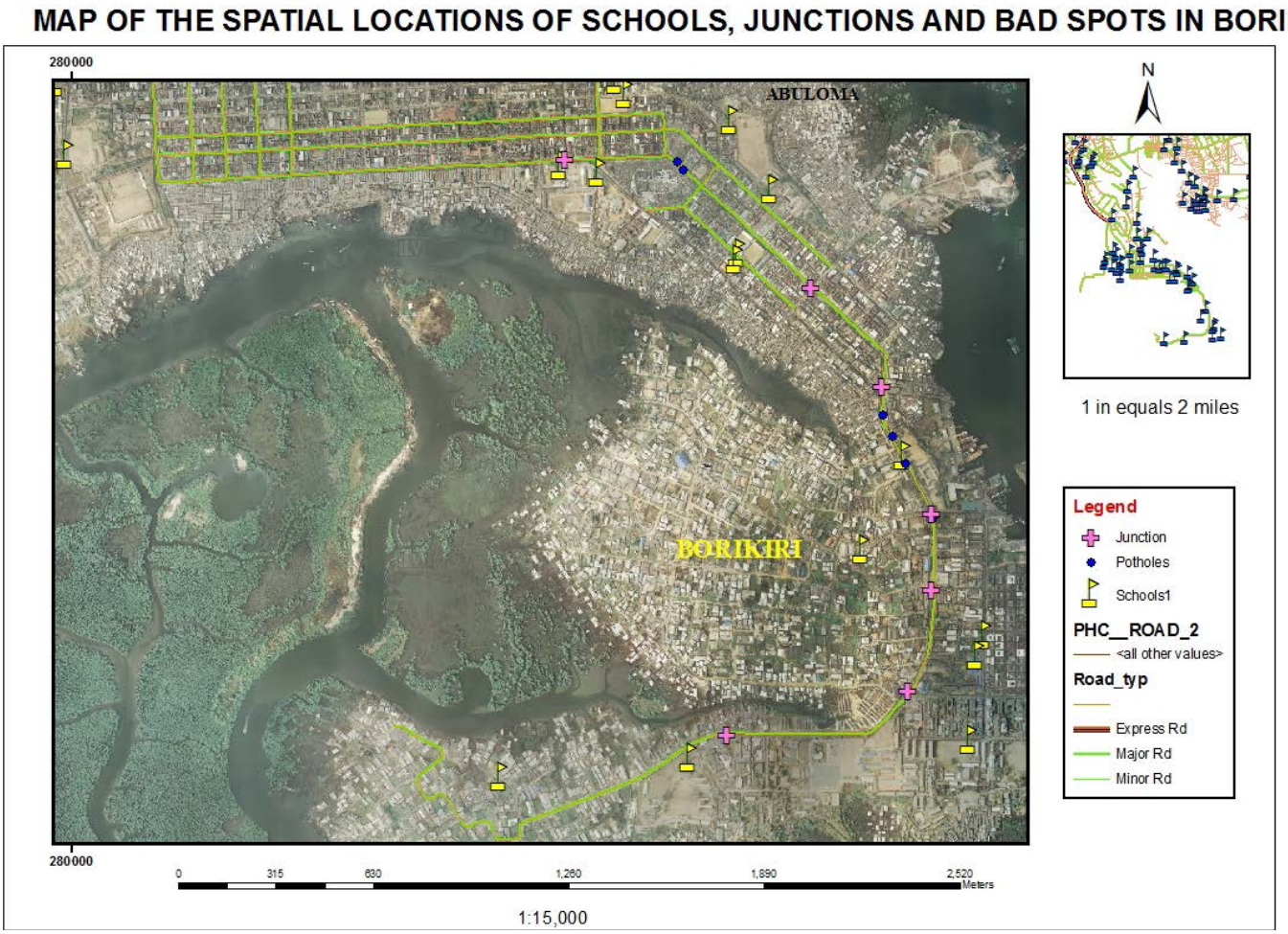

Figure 5. Overlay operation in Borikiri.

from Rumuokwuta to Nwaja at Trans Amadi. One can conveniently determine the travel time based on the distance given by the analysis tool and the travelling speed of the vehicle (Table 5).

Conclusion

The application of Geographic Information System in the development and maintenance of Road Network cannot be overemphasized. Port Harcourt is bound to experience growth in population and a predicted minimum of 7,360 cars in 2022 for the identified routes excluding larger vehicles like trucks; hence there is unavoidable increase in the demand for road usage along these routes.

In relation to estimated projected population figures, a direct proportional increase in the number of vehicles is expected. In 2013 we have an increase of 32.2% in traffic flow. It is expected that there will be an increase of 37.1%

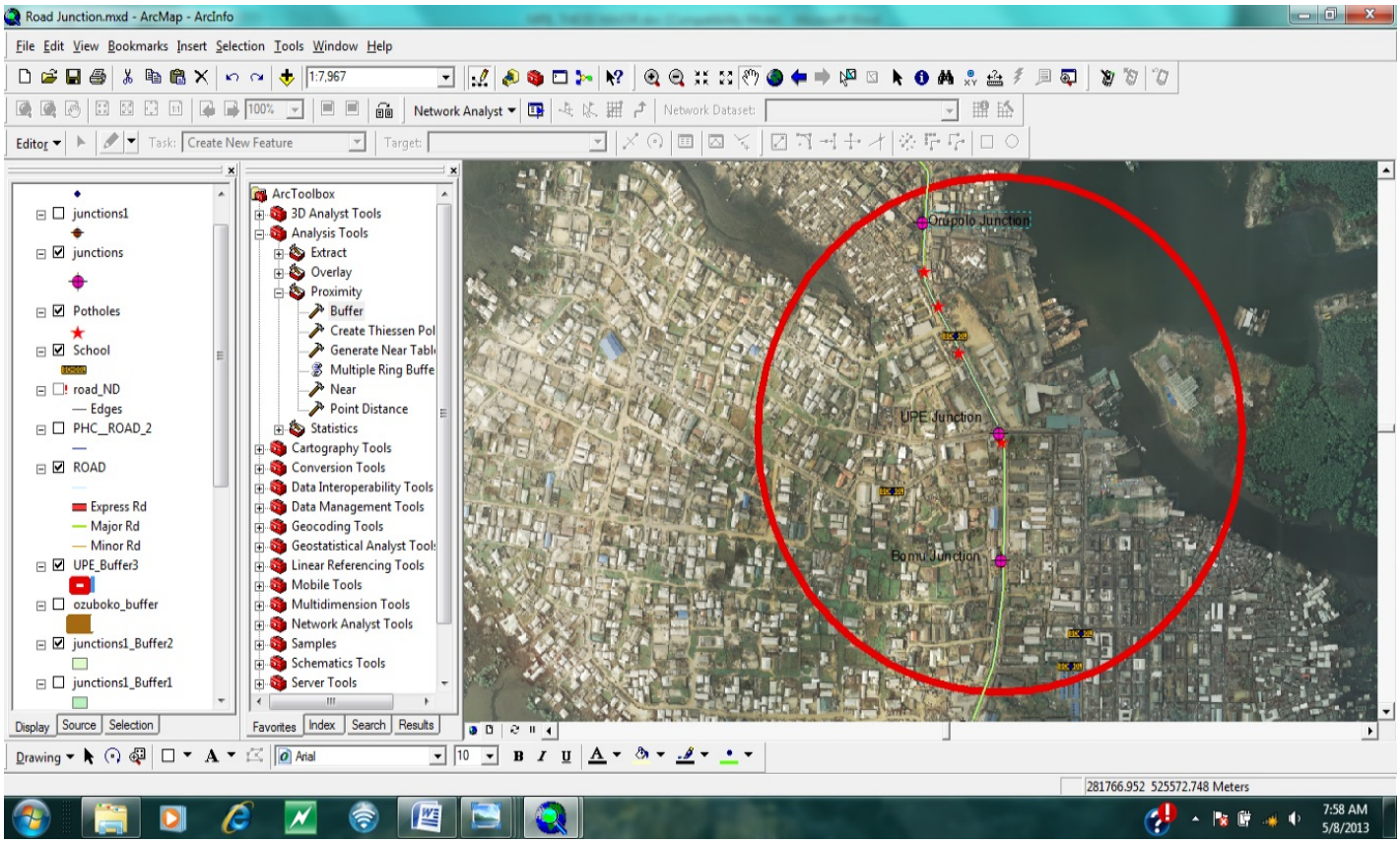

Figure 6. Road Point Buffer (UPE junction Borikiri).

between 2012 and 2022. From calculations made, traffic flow is directly proportional to the estimated population. We had 21.77% increase between 2008 and 2013, 61% increase between 2008 and 2022 and 32.18% increase between 2013 and 2022). From these, it was ascertained that the number of vehicles (private saloon, and bus) expected to ply the identified routes in about 10 years time from 2013 is 217,360. With this, adequate decisions towards the construction and improvement of the road network could be made either by government or other relevant private organizations.

Recommendations

The road network in Borikiri axis of Port Harcourt should be improved by constructing a by-pass to ease the traffic along Harold Wilson road.

All public facilities especially those located along major roads should have good parking plots before approval for construction.

The government should encourage the use of GIS techniques by training and retraining personnel in their various fields of application regarding road usage.

The government should be engaged in projects that would ease traffic flow along the roads through the Ministry of Transport and Ministry of Works. Such projects should include daulization of all major routes, and covering of potholes that develop especially at road junctions.

The width of roads should be extended on approaching major cross junctions with more than 12 conflict points.

It is also recommended that at proximity of 500 km from a developing area, where population is expected to increase, a boulevard should be constructed at the junction linking such area to the center of the town example is the Wimpey/Iwofe junction.

In areas to be developed, the government should ensure a proper road plan is developed prior to construction of buildings. Provisions for taxi parks should be considered.

Conflict of Interests

The author(s) have not declared any conflict of interests.

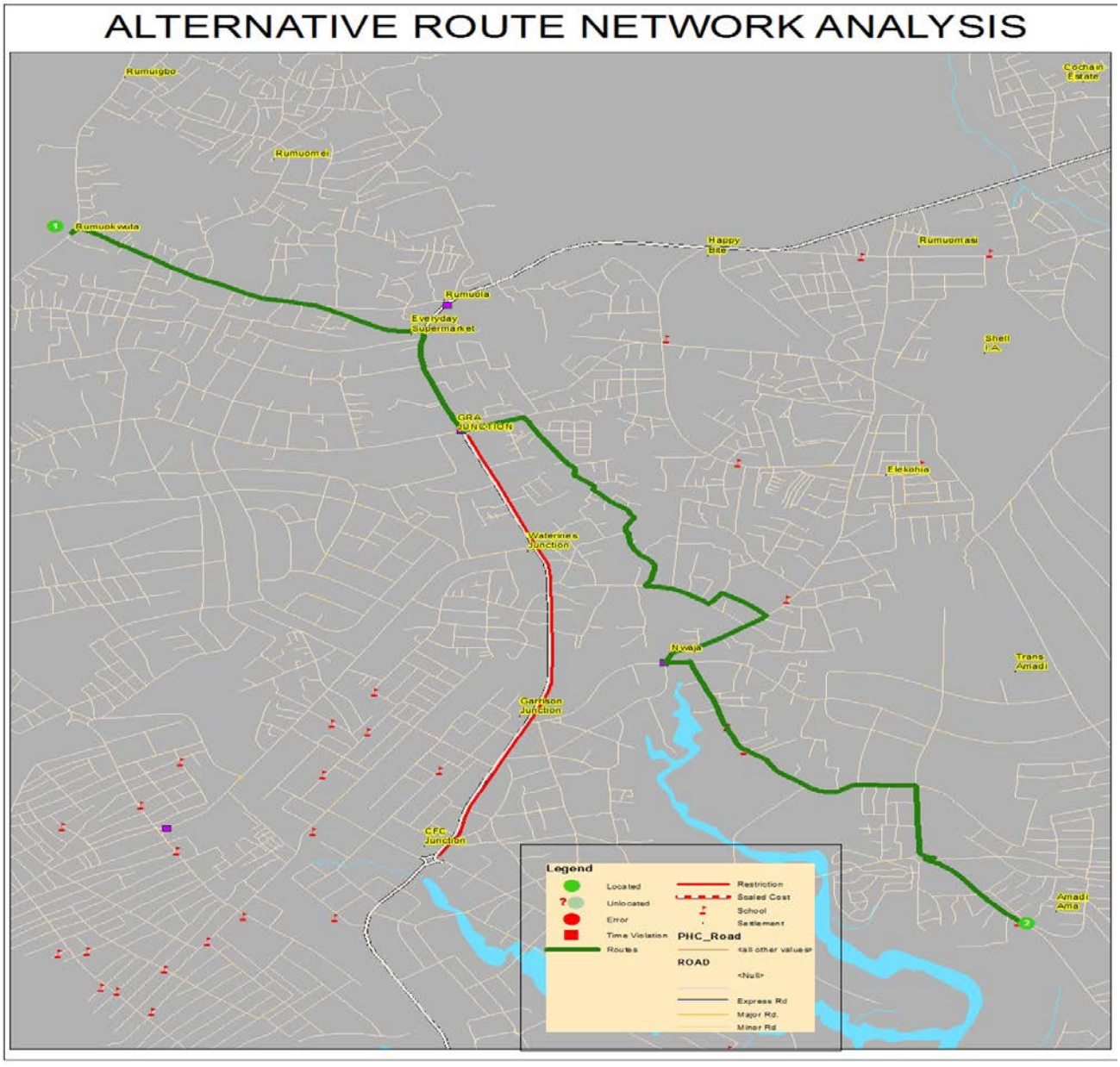

Figure 7. Alternative route analysis map.

Table 5. Direction Details from Rumuokwuta to Nwaja, Trans Amadi.

Driving distance intervals (m)	Driving direction	Cumulative driving distance (m)
8.8	Start (go south west)	0.0
2077.1	Make sharp left turn	8.8
292.1	Turn left at Tumuola Road	2085.9
443.2	Turn right	2378.1
295.7	Turn right	2821.2
118.3	Turn right	3116.9
247	Turn right	3235.2
1301.7	Turn right	3482.2
484.2	Turn right	4783.9

Table 5. Contd.

Driving Distance Intervals (m)	Driving direction	Cumulative Driving Distance (m)
228.7	Turn right	5268.1
139.3	Make sharp right turn	5496.8
289.9	Turn right	5636.1
246.8	Turn right	5925.9
108.5	Turn right	6172.7
314.6	Turn right	6281.2
483.7	Turn right	6595.7
671.9	Turn right	7079.5
852.4	Turn right at National Supply road	7751.3
53.7	Make sharp right turn	8603.7
690.9	Make sharp left turn	8657.4
13	Turn left	9348.3
	Finish at Amadi Ama	9361.3

REFERENCES

Andrew AO, Olatunde SE, Dozie RO (2011). Road Network Assessment in Trans Amadi, Port Harcourt in Nigeria, using GIS. Int. J. Traffic Transp. Eng. 1(4):257-264.

Deka MC (2009). Road Networking System in Guwahati City. [Online] http://www.articlesbase.com/science-articles/road-networking-system-in-guwahati-city-1048408.html [Assesed 3/05/2010]

Ghilani CD, Wolf PR (2008). *Elementary Surveying: An Introduction to Geomatics.* Pearson International Edition. Twelfth Edition pp. 323-326

Integrated Transport Master Plan (ITMP) of Port Harcourt (2008). The Greater Port Harcourt City Development Plan by Arcus Gibbs.

Kufoniyi O (1998). Principles and Applications of Geographic Information systems.

Matt F (2009). Singapore Land Transit Authority Takes the High Road with GIS [PDF][Online]http://esri.com/libaries/newsletters/transportation-gis-trends/trans-gis-fall2009.pdf.p(10-11).(Matt Freeman).

National Population Commission (2010). Federal Republic of Nigeria 2006 Census Population Distribution by State, Local Government and Senatorial Districts, Zone VI-South-south: Priority Table. Abuja: NPC. 3, 61.

Obinna VC, Owei OB, Okwakpam IO (2010). Impacts of Urbanization on the Indigenous enclaves of Port Harcourt and concomitant Policy measure. Soc. Sci. 5 (3):172-186.

Olagbadebo AO, Dienye AS (2008). Application of Digital Map in Road Network in Port Harcourt, Rivers State, Nigeria. Int. J. Environ. Sci. 4 (3):72-78

Oluwadare CO, Alade MA, Afolayan TS (2009). Megacities Development: Issues, Challenges and Sustainability. AGM Technical Session 1:(3-12).

Transportation GIS Trends (2009). GIS for Transportation. Environmental Research Institute (ESRI): Transportation group publication.

UN Habitat (2003). The Challenge of Slums. [Online] Global report on Human Settlements. Nairobi. Available from http://www.earthquakesandmegacities.org [Assessed July 2010].

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Full Length Research Paper

Mortality assessment of *Oreochromis niloticus* fingerlings in varying salinity and influence of salinity changes on acute toxicity of lead

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The setting of safe limits for lead (Pb) into the lagoon and protection of fishes such as *Oreochromis niloticus* has been based on its toxicity ignoring the influence of salinity, an important parameter in the lagoon. The study therefore investigates the salinity tolerance and relative acute toxicity of lead nitrate [$Pb(NO_3)_2$] under fresh water and varying salinity against *O. niloticus* fingerlings. A total of 280 fingerlings were used as test animals and $Pb(NO_3)_2$ as test chemical. Concentrations were 1, 2, 4, 6, 8, 10, 30, 40, 50, 60, 80, 120, 160 and 180mg/l at salinity levels of 2, 12, 18, 22, 32, 35 parts per thousand (ppt) and fresh water. Dose response data were analyzed by Probit. Mortality assessment showed that the fingerlings could not survive at salinities of 22, 32 and 35 ppt within 24 h but survived well in 2 ppt. The 96 h LC_{50} was 130.094 mg/l at 12 ppt but below 12 ppt there was a steady increase in toxicity (3.255 and 6.243 mg/l) in fresh water and 2 ppt respectively. Similarly, the toxicity also increased with an increase in salinity from 12.1 to 18 ppt (113.191 mg/l). The significance of this study shows the need for inclusion of varying salinity in setting of safe limits to confer protection on the delicate biotic components of the rich lagoon biodiversity.

Key words: Lead nitrate, *Oreochromis niloticus*, salinity.

INTRODUCTION

There is paucity of data on the setting of safe limits for discharge of heavy metals such as lead into the lagoon ecosystems, aimed at protecting brackish water organisms at varying salinity conditions. Salinity is one of the major factors (Lawson and Anetekhai, 2011) affecting organisms including fishes such as Tilapia in aquatic medium (Lagoon) and also has the ability to influence the toxicity of pollutants such as heavy metals (Oyewo, 1998; Lawson, 2011). There have been a lot of studies on the influence on the changing salinity on various physicochemical parameters, pollutant toxicity (heavy metals and pesticides) and their effects on fishes (Oyewo, 1998; Chukwu and Okpe, 2006; Breves et al., 2010; Lawson, 2011; Samy et al., 2011). The contamination, persistence, bioaccumulation and biomagnification of heavy metals (manganese, nickel, lead and zinc) in natural brackish are of global concern (Bhupander et al., 2011; Iulia et al., 2012; Marleen et al.,

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2013). Researchers have established high levels of heavy metals in the Lagos lagoon (Oyewo, 1998; Aderinola et al., 2009). Lead (Pb) is one of the most common heavy metals detected in the Lagos lagoon (Oyewo, 1998) which is not useful in living systems and extremely toxic at low concentration thus an indicator of pollution. In Nigeria, the recent increase in the environmental Pb has been attributed to industrial discharges and mining activities. The toxicity of Pb to aquatic organisms and its accumulation in the aquatic biota are well documented. Leblond and Hontela (1999) reported that the 96 h median tolerance limit value for fathead minnows and brook trout were 40 to 70 mg/L in soft water and 440 to 480 mg/L in hard water. The 96-h LC_{50} for Lead acetate in bluegill was found to be approximately 400 mg/L (McKim, 1985).

The principal toxic effects of chronic Pb exposure to fish cause hematological (Małgorzata et al., 2010; Usama et al., 2013), neurological (Talia et al., 2009) and renal (Patel et al., 2006) disorders. In water bodies contaminated with Pb, it has been observed in fishes that it causes the disruption of Na^+, Cl^- and Ca^{2+} regulation during acute exposure, development of black tails and spinal curvature during chronic exposure as well as disruption in hemoglobin synthesis (Sippel et al., 1983; Rogers et al., 2003; Rogers et al., 2005). Concentration of Pb in pond water ranged from 0.03 - 0.16 g mL^{-1} (Himadri and Anilava, 2000). In polluted areas dissolved Pb has been observed in concentration of 50400 $nmolL^{-1}$ (Bowles et al., 2006) while Pb toxicity has been reported for some freshwater species at concentrations as low as 50 nmol L^{-1} (Grosell et al., 2006).

A typical species that inhabit the brackish water is *Oreochromis niloticus.* It is among some of the edible commercial fish species that inhabit the Lagos lagoon and is also widely bred in commercial fish farms. *O. niloticus* can be cultured in fresh water coupled with slight challenges due to fluctuations in salinity and this makes it not so easy to be cultured in the laboratory. Since *O. niloticus is* sensitive to heavy metals (Taweel et al., 2013) it is therefore of interest to study the influence salinity changes will have on the effect of heavy metals such as lead which could be a biological constraint and crucial in setting of safe limits for this fish.

MATERIALS AND METHODS

Test animals

O. niloticus (Nile tilapia, Gnathastomato, Cichlidae) fingerlings of similar sizes (total length; 11 ± 6 cm, mean weight; 20 ± 4 g and age (4 - 6 weeks old) were purchased from Agboola farms and transported to the laboratory in 25 L container into holding tanks (50 X 30 X 35 cm), which contained fresh water from fish pond (opened at the top for aeration) in the laboratory. The fingerlings were kept in the holding plastic tanks, half filled (15 L) with dechlorinated tap water, to acclimatize to laboratory conditions (28 ± 2°C, R.H 70 ± 2%) for a period of seven days before they were used in the bioassays. The fingerlings were fed with fish food (Coppens) at 3% of their body weight and water was changed once every 48 h, aerated continuously with circular plastic bowls (volume = 0 5 L, bottom diameter = 18 cm) were used as bioassay container.

Test chemicals

The heavy metal, lead as [$Pb(NO_3)_2$] investigated in this work was obtained as metallic salts of Fisons laboratory reagents, Analar grades of molecular weight 331.21 g) with 98% purity manufactured by British Drug House (BDH). The choice of Pb as the heavy metal for this study was based on the available and common metal from the results of a chemical survey of industrial effluents that empty into the Lagos lagoon (Oyewo, 1998).

Preparation of treatments at varying salinities

Seawater was obtained in 25 L container, Lagos State and taken to the laboratory, where the salinity (35 ppt) was measured with a salinometer. On the basis of salinity level, computed volumes of the sea water were measured out and mixed with dechlorinated tap water to obtain sea water solutions at pre-determined salinity levels. The salinity of the prepared media was verified with a salinometer for more accurate determination of the salinity was recorded and used in appropriate experiments.

Preparation of treatments for acute toxicity test

A pre-determined amount of Pb compound was weighed (using an oertling 30TD top loading balance) and diluted with given volume of dechlorinated tapwater to obtain a stock solution of known strength. The resultant stock solution was serially diluted to obtain solutions of required concentrations. Actual concentration of Pb ions in each solution of known strength was computed based on molecular weight of test compound. Test media were always made up to 2 L because preliminary studies showed that 10 fingerlings survived well in 2 L of media for a period of 7 days without aeration. In range finding experiments, the fishes were exposed to a wide range of concentrations (600, 300, 100, 50, 25, 5 and 1 mg/l) of the test compound to obtain an effective range of activity, initially relying on trial and error techniques.

Assessment quantal response (mortality)

Fingerlings were taken to be dead if no body movements including the operculum were observed, even when prodded with a blunt glass rod and submerged in water.

Salinity tolerance of *O. niloticus*

Five active fingerlings of similar age and size (as described above) were taken from plastic holding tanks with a sieve (200 µm) and randomly assigned to bioassay containers already holding treatments at varying salinities as described below. Each treatment was replicated twice, giving a total of 10 fingerlings that were exposed per salinity level (35, 32, 22, 18, 12, 2 ppt) and fresh water. Mortality was assessed as described above, once every 24 h for a period of 7 days.

Acute toxicity of ($Pb(NO_3)_2$ against *O. niloticus* in varying salinity treatments

A similar set up as described above in the case of salinity tolerance

Table 1. Acute toxicity concentration at fresh water, 2, 12 and 18 ppt.

Fresh water (mg/l)	2 ppt	12 ppt	18 ppt
1.0	1.0	30.0	40.0
2.0	2.0	50.0	60.0
4.0	4.0	80.0	80.0
6.0	6.0	120.0	120.0
8.0	8.0	160.0	180.0
10.0	10.0	200.0	240.0

Table 2. Salinity tolerance of *O. niloticus* under varying salinity (mortality assessment).

Salinity (ppt)	Time (h)							% Mortality
	24	48	72	96	120	144	168	
35	10	10	10	10	10	10	10	100
32	10	10	10	10	10	10	10	100
22	9	10	10	10	10	10	10	100
18	5	6	7	7	7	7	7	70
12	2	2	3	3	3	3	3	30
2	0	1	1	1	1	1	1	10
Fresh water	0	0	0	0	0	0	0	0

of *O. niloticus* was carried out in this case but mortality was assessed once every 24 h for a period of four days.

Acute Toxicity concentrations

Acute toxicity concentrations under which relative acute toxicity of test heavy metal against *O. niloticus* was investigated were as shown in Table 1.

Statistics

The dose response (mortality) data for freshwater and varying salinities were analyzed with a computer program using Probit analysis after Finney (1971) as adopted by Don Pedro (1989) and used as indices for measuring toxicity of the test organisms to the toxicant. The Indices of measuring toxicity derived from these analyses were Lethal Concentration that caused 95, 50 and 5% response [mortality] of exposed organisms (LC_{95}, LC_{50}, LC_5, respectively) and their 95% confidence limits.

RESULTS

Salinity tolerance of *O. niloticus*

The results of salinity tolerance of *O. niloticus* based on mortality assessment, showed that the fishes could not survive for any appreciable period in treatments with salinity at 22 ppt and above (Table 2). In treatments with salinity of 18 ppt, between 50 - 60 % mortality was recorded in 48 h but from 72 - 168 h, 70 % mortality was observed. At 2 and 12 ppt, mortality occurred at low levels (10 - 20 %) after 24 - 48 h respectively but stabilized at 30% after 72 h at 12 ppt (Table 1). No mortality was recorded in fingerlings exposed to media with fresh water.

Acute toxicity of $Pb(NO_3)_2$ against *O. niloticus* in varying salinity treatments

The acute toxicity of $Pb(NO_3)_2$, based on 96 h LC_{50} values against *O. niloticus* fingerlings at salinity of 12 ppt, was 130.094 mg/l whereas at 2 ppt and freshwater, it was 6.243 and 3.255 mg/l respectively which was significantly (no overlap in 95 % CL of 96 h LC_{50}) lower than the toxicity values at 12 and 18 ppt (113.191 mg/l) treatments evaluated (Figure 1).

Additionally, the relative toxicity of $Pb(NO_3)_2$ based on 24, 48 and 72 h LC_{50} values followed a similar trend of relative toxicity against the fingerlings as 96 h LC_{50} values in all test media (freshwater, 2,12 and 18 ppt) and there was no overlap in 95% confidence limit for 96 h LC_{50} values for all media. Figures 2, displays the Probit line graphs of the toxicity data for the test freshwater fish species under varying salinity conditions indicating increasing toxicity with exposure time.

DISCUSSION

The established salinity tolerance and salinity influence of Pb against *O. niloticus* at varying salinity range reported

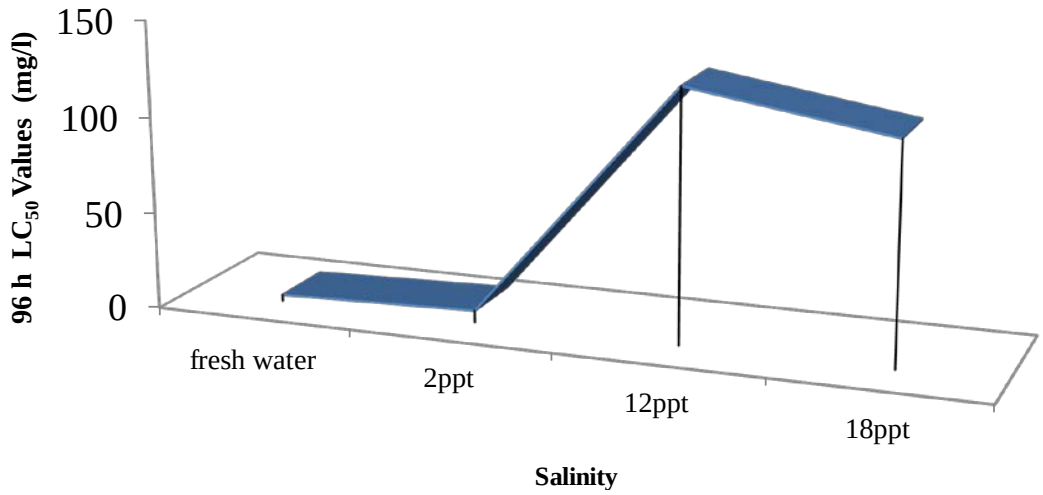

Figure 1. Relationship Between 96 h Lc_{50} values of $Pb(NO_3)_2$ against *O. niloticus* (n = 10) at different salinities.

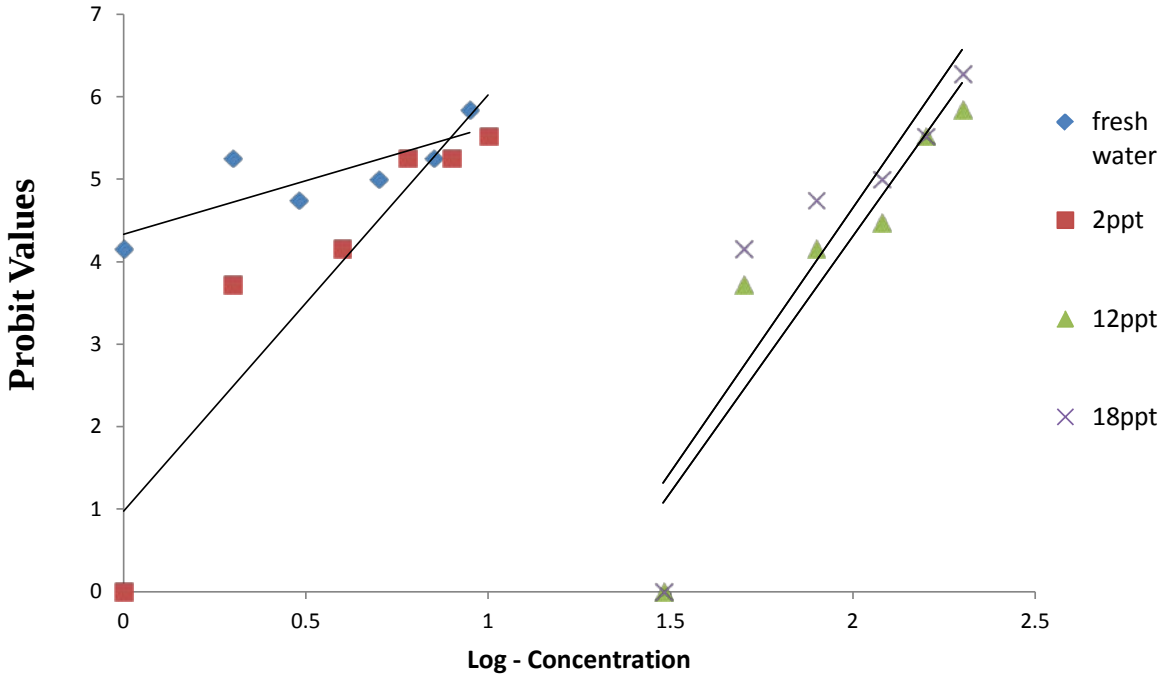

Figure 2. Probit transformed response of *O. niloticus* Log-concentration of $Pb(NO3)_2$ at fresh water, 2, 12 and 18 ppt conditions.

in this study showed that based on mortality assessment, *O. niloticus* fingerlings could not survive for any appreciable period in media with salinity equal to and higher than 22 ppt. This is not surprising because *O. niloticus* is a brackish water species and optimum survival is usually at salinity range between 10 - 15 ppt though can survive in waters with salinity between 0-15 ppt (Kurata, 1959, Thomas and Masser, 1999; Lawson and Anetekhai, 2011). Although the study conducted by Nugon (2003) showed that *O. niloticus* exhibited good survival (81%) in salinity regimes up to 20 ppt, which is in close agreement with the findings of this study and that of Osuala and Bawa-allah (2013).

However in fresh water and at 2 ppt the toxicity of Pb was higher than that observed at 12-18 ppt as depicted by the 96 h LC_{50} values suggesting that the toxicity of Pb against *O. niloticus* fingerlings may have synergized osmotic stress at lower salinities. Moreover, salinity played

a significant role in influencing the acute toxicity of Pb. This probably resulted in the fish being less resistant and unable to regulate the body fluid to restore levels of osmotic pressure to near normal. Additionally it could be due to other toxicity modifying factors such as exposure duration and concentrations of Pb. Chemicals may have multiple effects on populations of organisms such as in mortality, reproductive failure, and productivity (Chukwu and Okpe, 2006). Sensitivity of populations depends upon such factors as age groups and temporal patterns of exposure. Passage of toxins or toxicants into an organism is also highly dependent on the specific physical-chemical characteristics of a given toxicant (Maheswaran et al., 2008; Ololade and Oginni, 2010). $Pb(NO_3)_2$, being the test chemical could be regarded as one of the major reasons for induced mortality due to its lipophilic and surfactant-containing nature (Ezemonye et al., 2007). Most of the test organisms survived initial attack at the early stage of test initiation and at the lower test concentrations of the toxicants. This could be attributed to protective adaptations as well as individual physiological nature of *O. niloticus* (Olalade and Oginni, 2010). However, as exposure progressed to 96 h, inevitable mortality could be due individual physiology and cumulative impact of the chemical toxicity. The results observed in this study are in agreement with other related studies (Omoregie et al., 1990; George and Clark, 2000; Johnson et al., 2005; Scarlett et al., 2005; Ezemonye et al., 2007). It is interesting to note that $Pb(NO_3)_2$ was most toxic to the fish at freshwater conditions, contrary to expectations that since the fish has the ability to survive at extremely low salinity such as in freshwater conditions, they should show more tolerance to the metal in freshwater. This is in agreement with findings of Oyewo (1998) who showed that similar brackish water adapted bony fishes such as *Tilapia guinensis* and *Nerite senegalensis* were known to be most susceptible to heavy metal pollutants including $CuSO_4$ at salinities tending towards the extremities (below 5 ppt and above 25 ppt), but were several folds more tolerant at salinity of up to 15ppt which falls within typical brackish water salinity (10-20 ppt).

The results reported for mean percentage mortality and 96 h LC_{50} values for the fresh water test could probably have been due to the physiology of *O. niloticus* under freshwater conditions, which have a body fluid concentration (about one-third) their surrounding environment, thus constantly taking in water by diffusion through their gills and skin for osmotic balance (Delbeek, 1987). Thus in a situation where there is damage to the skin and other tissues as is the case in exposure to high concentrations of surfactant-containing chemical ($Pb(NO_3)_2$), there is an influx of not only water but also the test chemical leading to a high lethal toxicity of the chemical and death rate of *O. niloticus* under freshwater conditions (Bury et al., 1999; Abel, 2006).

The authors' findings suggested that *O. niloticus* remains a brackish water fish as it retains its innate characteristics which allows it to do well in brackish water as its original natural habitat. Though, it can also strive relatively well in freshwater media because they are euryhaline (being able to adapt to a wide range of salinities due to their ability to osmoregulate especially in habitats where salinity changes regularly).

O. niloticus carry out hyper-hypo osmoregulation switching from hyper osmoregulation to hypo osmoregulation and vice versa during variations in salt content of water in their habitat. However, variations tending towards the extremities may result in stress or death, as was demonstrated with *O. niloticus* in extremely high salinities in this study.

Therefore, the practical significance of this study could be utilized in setting ecologically sound, safe limits for heavy metal (such as Pb) containing discharges into the lagoon ecosystems, aimed at protecting brackish water organisms. It is therefore suggested that data used for setting of safe limits should be based on those obtained from studies carried out over a wide range of salinity conditions.

Additionally this study provides data and information to already existing reviews on the influence of heavy metal pollutant in waters with varying salinity range against *O. niloticus*

Conclusion

This study has shown that salinity affects the acute toxicity of lead on fingerlings of *O. niloticus*. In the freshwater, 2, 12 and 18 ppt experimental media, death caused to *O. niloticus* may be detrimental since this could lead to elimination of potentially reproductive organisms in its natural brackish water habitat. The inclusion of salinity factor in the setting of safe limits would ensure amongst others that the delicate biotic components of the rich Lagoon biodiversity are prudently protected. The findings will assist our relevant agencies on the need to stipulate and implement stiffer regulations to control inflow of nutrient and chemical contaminants into brackish habitats coupled with enforcement of penalties imposed for illegal and unsustainable development that impacts these habitats.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

Abel PD (2006). Toxicity of synthetic detergents to fish and aquatic invertebrates. J. Fish Biol. 6:279-298.

Aderinola OJ, Clarke EO, Olarinmoye OM, Kusemiju V, Anatekhai MA (2009). Heavy metals in surface water, sediments, fish and perwinkles of Lagos lagoon. Am. Eurasian J. Agric. Environ. Sci. 5:609-617.

Bhupander K, Mukherjee DP, Sanjay K, Meenu M, Dev P, Singh SK, Sharma CS (2011). Bioaccumulation of heavy metals in muscle tissue of fishes from selected aquaculture ponds in East Kolkata wetlands. Ann. Biol. Res. 2:125-134.

Bowles KC, Apte SC, Batley GE, Hales LT, Rogers NJ (2006). A rapid Chelex column method for the determination of metal speciation in natural waters. Anal. Chim. Acta. 558:237-245.

Breves JP, Hasegawa S, Yoshioka M, Fox BK, Davis LK, Lerner DT, Takei Y, Hirano T Grau EG (2010). Acute salinity challenges In Mozambique and Nile Tilapia: differential responses of plasma prolactin, growth hormone and brachial expression of ion transporters. Gen. Comp. Endocrinol. 167(1):135-142.

Bury NR, McGeer JC, Wood CM (1999). Effects of Aatering freshwater chemistry on physiological responses of Rainbow Trout to silver exposure. Environ. Toxicol. Chem. 18:49-55.

Chukwu LO, Okpe HA (2006). Differential response of *Tilapia guineensis* fingerlings to inorganic fertilizer under various salinity regimes. J. Environ. Biol. 27(4):687-690.

Delbeek CJ (1987). Osmoregulation: Please give me a drink. J. Fish Biol. 1:3.

Ezemonye LIN, Ogeleka DF, Okieimen FE (2007). Acute Toxicity of industrial detergent (Neatex) and corrosion inhibitor (Norust CR 486) to early stages of Cichlids; *Tilapia guineensis*. Chem. Ecol. 23:1-8.

Finney DJ (1971). Probit Analysis. 3rd Edition University Press, London. 276pp.

George A, Clark JR (2000). Acute toxicity of two Corexit Dispersants. Chemosphere. 40: 897-906.

Grosell M, Gerdes RM, Brix KV (2006). Chronic toxicity of lead to three freshwater invertebrates - *Brachionus calyciflorus, Chironomus tentans, and Lymnaea stagnalis*. Environ. Toxicol. Chem. 25:97-104.

Himadri G, Anilava K (2000). Heavy metal concentration in water, sediment, Shrimp (*Penaeus monodon*) and Mullet (*Liza parsia*) in some brackish water ponds of Sunderban, India Mar. Pollut. Bull. 40 (11): 914-920.

Iulia GD, Marius LM, Alin T, Gabriel C, Laurentiu R, Gabriel LR (2012). Food chain biomagnification of heavy metals in samples from the lower Prut floodplain natural park. Environ. Eng. Manag. J. 11: 69-73.

Johnson TA, Miller LM, Whittle DM, Brown SB, Wieg DM, Kapuscinnsk AR, Leggett WC (2005). Effects of materially transferred Organochlorine contaminants of early life survival in a freshwater fish. Environ. Toxicol. Chem. 24:2594-2602.

Kurata H (1959). Preliminary report on the rearing of herring larvae. Bull. Hokkaido Resour. Fish Res. Lab. 20: 117-138.

Lawson EO (2011). Physico-chemical parameters and heavy metal contents of water from the mangrove swamps of Lagos lagoon, Lagos, Nigeria. Adv. Biol. Res. 5:08-21.

Lawson EO, Anetekhai MA (2011). Salinity tolerance and preference of hatchery reared Nile Tilapia, *Oreochromis niloticus* (Linneaus 1758). Asian J. Agric. Sci. 3(2): 104-110.

Leblond VS, Hontela A (1999). Effects of *in vitro* Exposures to cadmium, mercury, zinc and1-(2-chlorophenyl)-1-(4chlorophenyl)-2, 2-Dichloroethane on Steridogenesis by dispersed interrenal cells of Rainbow Trout (*Oncorhynchus mykiss*). Toxicol. Appl. Phamacol. 157(1):16-22.

Maheswaran R, Devapanl A, Muralidharan S, Velmurugan B, Ignaeimuthu S (2008).Haematological studies of fresh water fish, *Clariasbatradrus* (L) exposed to mercuric chloride. Int. J. Integr. Biol. 2(1): 49-54.

Małgorzata W, Elżbieta K, Monika S, Małgorzata, O (2010). Hematological changes in common Carp (*Cyprinus carpio* L.) after short-term lead (Pb) Exposure. Pol. J. Environ. Stud. 19:825-831.

Marleen E, Isabelle L, Chris MW, Gudrun DB (2013). Physiological effects of waterborne lead exposure in Spiny Dogfish (*Squalus acanthias*). Aquat. Toxicol. 126:373-381.

McKim JM (1985). Early life toxicity tests. In: Fundamentals of aquatic toxicology, hemisphere and New York. pp. 58-59.

Nugon RW (2003). Salinity tolerance of juveniles of four varieties of Tilapia. M.Sc. Thesis, Louisiana state University, USA. p. 76.

Ololade IA, Oginni O (2010). Toxic stress and hematological effects of Nickel on African catfish, *Clarias gariepinus* fingerlings. J. Environ. Chem. Ecotox. 2:14-19.

Omoregie E, Udodike EBC, Keke IR (1990). Tissue chemistry of *O. niloticus* exposed to sublethal concentrations of gammalin-20 and Actellic- 25EC. J. Appl. Sci. 5:33-36.

Osuala FI, Bawa-Allah KA (2013). Influence of salinity changes on acute toxicity of copper sulphate against *Oreochromis niloticus* fingerlings, in laboratory bioassays. Int. J. Environ. Sci. Toxicol. Food Technol. 6:22-27.

Oyewo EO (1998). Industrial Sources and distribution of heavy metal in Lagos lagoon and their biological effects on estuarine animals. Ph.D Thesis, University of Lagos. 247pp.

Patel M, Rogers JT, Pane EF, Wood CM (2006). Renal responses to acute Lead waterborne exposure in the freshwater Rainbow Trout (*Oncorhynchus mykiss*) Aquat. Toxicol. 80:362-378.

Rogers JT, Patel M, Gilmour KM, Wood CM (2005). Mechanisms behind Pb induced disruptions of Na^+ and Cl^- balance in Rainbow Trout (*Oncorhynchus mykiss*). Am. J. Physiol. Regul. Integr. Comp. Physiol. 289(2):R463-R472.

Rogers JT, Richards JG, Wood CM (2003). Ionoregulatory disruption as the acute toxic mechanism for lead in Rainbow Trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 64:215-234.

Samy YE, Mohamed MMA, Mohamed ES, Hamad AE (2011). Production of salinity tolerant Nile Tilapia, *Oreochromis niloticus* through traditional and modern breeding methods: II. application of genetically modified breeding by introducing foreign DNA into fish gonads. Afr. J. Biotechnol. 10(4):684-695.

Scarlett A, Galloway TC, Canty M, Smith EL, Nilsson J (2005). Toxicity of two oil dispersants, Superdispersants - 25 and Corexit 9527, to a range of coastal species. Environ. Toxicol. Chem. 15:1219-1227.

Sippel AJA, Geraci JR, Hodson PV (1983). Histopathological and physiological responses of rainbow Trout (*Salmo Gairdneri Richardson*) to sublethal levels of Lead. Water Res. 17: 1115-1118.

Talia S, Yiming L, Virginia B, Paul BT (2009). Neurotoxic effects and biomarkers of lead exposure. Rev. Environ. Health 24:15-45.

Taweel A, Shuhaimi-Othman M, Ahmad AK (2013). In vivo acute toxicity tests of some heavy metals to Tilapia fish (*Oreochromis niloticus*). J. Biol. Sci. 13(5):365.

Thomas P, Masser M (1999). Tilapia life history and biology. Southern Regional Aquaculture Center. Publication No. 283

Usama MM, Abdel-Basset ME, Salsa MM (2013). Effect of lead on some haematological and biochemical characteristics of *Clarias gariepinus* dietary supplemented with lycopene and vitamin E. Egypt Acad. J. Biol. Sci. 5:67-89.

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Spatial and temporal variations in selected heavy metals in water and sediment from the Mhlathuze Estuary, Richards Bay

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Spatial and temporal variations in water (total, dissolved and particulate), and sediment concentrations of seven heavy metals, Al, Cr, Cu, Mn, Pb and Zn from the Mhlathuze Estuary were analyzed. Effects of environmental factors on the metals in the estuary were also investigated. In water, metals concentrations varied spatially as well as seasonally with highest concentrations of Al Cr, Fe, Mn and Zn recorded in summer. Partitioning of metals was influenced by environmental factors including dissolved oxygen, turbidity and pH with two groups being identified, that is, metals that increased with increasing salinity and metals that increased with increasing turbidity. In sediment, metal concentrations showed little seasonal variation. There were, however, significant spatial differences in metal concentrations, with muddy, high organic areas of the estuary consistently having highest concentrations of metals as compared to other sites. These results suggest a high degree of heavy metal contamination in the Mhlathuze Estuary and also stress the importance of incorporating sediment metal analysis in any assessment of metal pollution in estuarine environments.

Key words: Estuaries, heavy metals, pollution.

INTRODUCTION

Rapid industrial and urban development has previously been linked to high concentrations of heavy metals recorded in the Richards Bay Harbour (Vermeulen and Wepener 1999). The Mhlathuze Estuary is located adjacent to Richards Bay harbour. The estuary and the harbour originally formed a single system called the Richards Bay estuary, but during the development of the deep water port, the original estuary was divided into half by a berm wall (Begg, 1978). The new mouth was created for the Mhlathuze Estuary on the southern side of the harbour. This area was reserved for environmental protection and declared a marine protected area (Begg, 1978).

Industrial activity in the Mhlathuze catchment includes manufacturing of metal products by some industries, while many others use metals in processing their products. The Mhlathuze estuarine system has a potential to be polluted by heavy metals from effluents

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discharged by these industries. Wepener and Vermeulen (1999) reported the estuary as having a low freshwater input in winter and floods occur during summer in the Richards Bay area. These floods initiate in the upper reaches and the freshwater may flush heavy metals into the estuary from the upper reaches.

The adjacent harbour is periodically dredged and the dredger spoil is either discharged on the beach or a few kilometres offshore. In both instances, there is a possibility of the dredger spoil finding its way into the estuary (Cyrus and Wepener, 1998).

The possibility of spoil ingression was predicted by the CSIR (1993) and observed by Begg (1978) who reported the ingression of the fine sediment from the dredger spoil south towards the mouth of the estuary during visual environmental audits. Cyrus and Wepener (1998) also reported the ingression of the fine sediment into the mouth of the estuary. A dramatic increase in the percentage of mud (<63 μm) was observed around the estuary in the area south of the Mhlathuze estuary (CSIR, 1993; Cyrus and Wepener, 1998). The mud fraction is known to be the component of sediment on to which most of the metals will adhere (Newman and Watling, 2007). This ingression brought about physical change and was reported as having impacted on certain components of the biota (CSIR, 1993).

Metals occur in aquatic environments either as dissolved ions or as metals bound to particular matter. The dissolved fraction is usually the bioavailable fraction however it is toxic to biota in estuarine environments (Silva et al., 2006). The particulate fraction is adsorbed to organic matter, organic fluvic and humic acids (Krupadam et al., 2006). A high amount of heavy metals may be locked onto sediments. These are metals that have been scavenged from the water column on to the particulate matter and later onto proximal sediments (Sarkar et al., 2004). In shallow estuaries such as the Mhlathuze Estuary, metals have a potential to be resuspended and thus become bio-available (Wepener and Vermeulen, 2005). They may also be available to benthic and bottom feeding organisms during feeding (Shirneshan et al., 2012). As the normal estuarine function rely on the mixing of both sea water and freshwater from the river, the type of metal species present in the particular system is determined by the chemistry of that freshwater/sea interface.

There is very little information available concerning the environmental and physicochemical processes that regulate the existence of metals in South African estuarine systems. Generally, in estuarine and marine environment, the behavior of heavy metals is governed by a number of physicochemical and environmental factors such as salinity, valence state and association with organic radicals, dissolved oxygen and pH (Cox and Micaela, 2005). These processes can remove metals dissolved in water by adsorption of metals onto particulate matter or cause resuspension of metals from sediment (Sarkar et al., 2004). Any physicochemical change that reduces the hydrophylic complexation of dissolved trace metals enhances the bioavailability of that metal by increasing the concentration of the free metal ions (Krupadam et al., 2006); thereby causing the bioavailable metal becomes toxic to biota. Since physical and chemical factors such as pH and salinity are dynamic in estuaries, metals behave differently across estuarine environments. Mhlathuze estuary is a marine dominated estuary with salinity normally ranging from 20-35 ppt.

In view of possible harbour expansion and the introduction of a metal mining plant in the catchment, the objectives of this study were to determine baseline information on the bioaccumulation of metals in water and sediment of the Mhlathuze Estuary.

MATERIALS AND METHODS

Quarterly water and sediment samples were collected in the Mhlathuze Estuary from April 1996 to December 1997. Samples were collected from the sampling sites (Figure 1).

Water samples

The surface water variables were determined *in situ* at each site using a Surveyor 3 Hydrolab connected to an H_2O water quality multiprobe: pH, water temperature, dissolved oxygen and percentage oxygen saturation, turbidity, and salinity. Water samples collected at each site were analysed by the analytical laboratory of Mhlathuze Water Scientific Services for orthophosphates, sulphates and fluorides. Two surface water samples were collected at each site, one was filtered through a 0.45 μm cellulose acetate filter and the other unfiltered.

The unfiltered sample represents total metal concentrations whereas the filtered sample represents dissolved metal concentrations. The samples were frozen until analysed for metal concentration in the laboratory. The samples were thawed and preconcentration was carried out by acidifying 250 ml water with 10 ml of 55% nitric acid and 5 ml perchloric acid in Erlenmeyer flasks and evaporating to 5 ml on a hotplate (Standard Methods, 1998). Samples were made up to 50 ml with double distilled water.

Metal analyses

Aluminium, Cr, Cu, Fe, Mn and Zn in water and sediment were measured by flame furnace atomic absorption spectrophotometry using a Varian SpectrAA 50B spectrophotometer fitted with a deuterium arc background corrector. Calibration was carried out using matrix matched calibration standards. Analytical accuracy was determined using Standard Reference Material (SRM) of the National Bureau of Standards: standard for trace elements in water (SEM 1643c) and Buffalo River sediment (SRM 2704). Recoveries were within 10% of the certified values.

Statistical analyses

Statistical analyses of the data were performed using Tukey ANOVA multiple comparison test to measure ad hoc significant differences. Significance was regarded at the P-<0.05 significance level. Baseline normalization was performed using Fe as a normalizing metal [metal (μg/g)/Fe (μg/g)]. The principal component analysis (PCA) was used to determine the relationships between

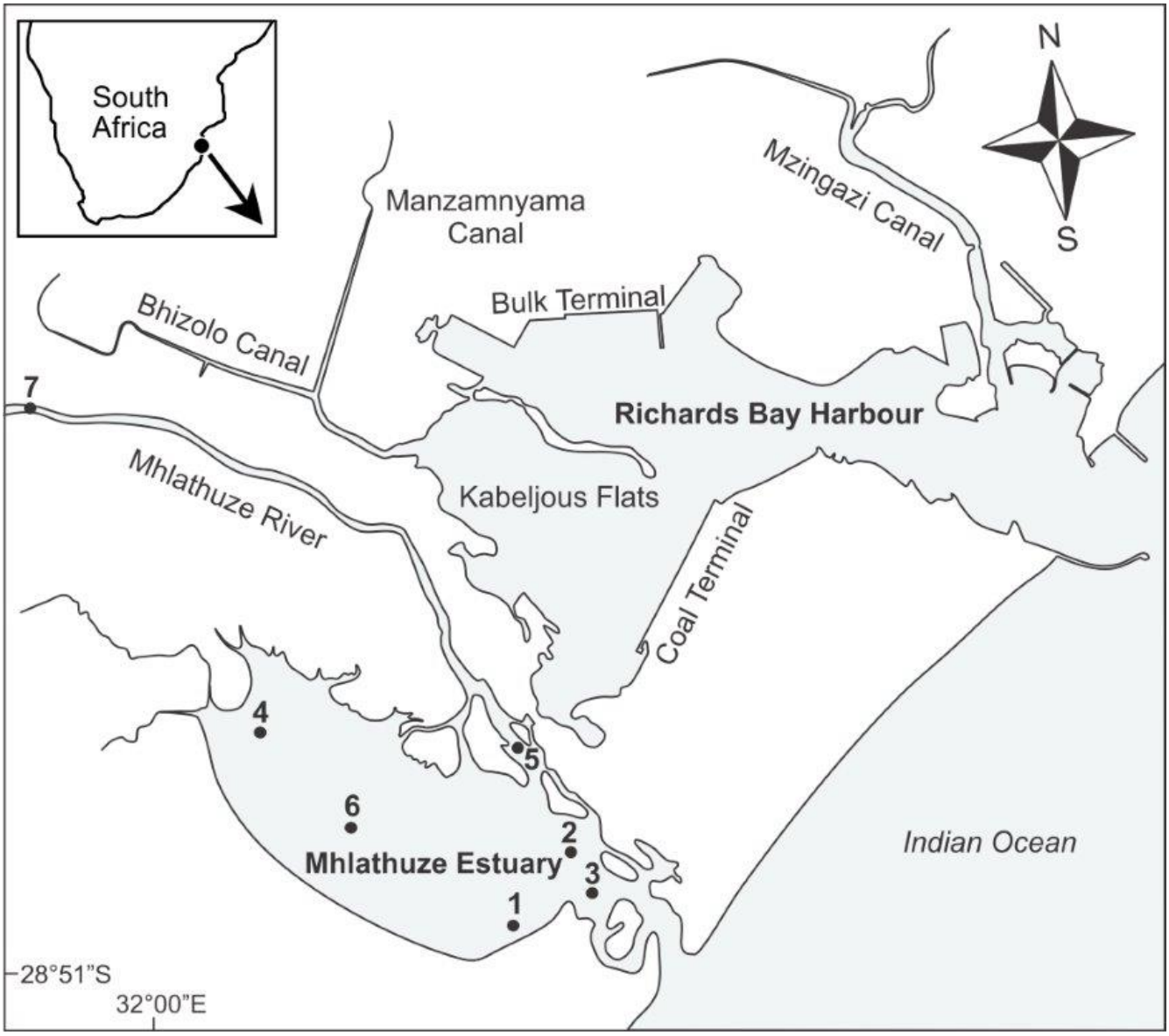

Figure 1. Map of Richards Bay Harbour and Mhlathuze Estuary showing sampling sites.

metals in water, sediment and environmental variables.

RESULTS AND DISCUSSION

Changes in the selected physico-chemical variables of the Mhlathuze Estuary are summarized in Table 1. Temperatures remained fairly constant at all sites during the quarterly surveys reflecting the natural seasonal temperature fluctuations in a subtropical environment. Only the water temperatures at site 4 seemed consistently different from those measured at the other sampling sites during the particular survey. Turbidity varied seasonally with elevated turbidities during Spring and Summer. Turbidities were elevated at site 4 when compared with the historical data. This could be attributed to the deposition of sediments from the catchment into the estuary. The deposition resulted in the buildup of very fine sediments on the bottom of the estuary. Due to its shallow nature, the strong tidal prism and strong prevailing north-easterly or south westerly winds, the soft bottom sediments can be brought into resuspension, with potential ensuing deleterious effectsto the aquatic biota. Although resuspension is a natural process, the spoil from dredging in the harbour has been shown to exacerbate the situation.

Recently, it was shown that ingression of fine sediments into the estuary from the marine environment was causing a build-up of very fine sediments in the

Table 1. Quarterly physico-chemical values recorded at five sites in the Mhlathuze Estuary for the sampling period from April 1996 to December 1997. Historical data in the original estuary before the construction of the harbour are represented by A after the construction of the harbour are presented by B.

Season	Site	Temp (°C)	Turbidity(NTU)	O_2 (mg/l)	O_2 (%)	Salinity (‰)	pH	PO_4 (mg/l)	NO_3 (mg/l)	SO_4 (mg/l)
Historical data A[1]		21.00	6.00	7.58	101.00	28.00	8.10	0.02	0.00	NA
Historical data B[2]		NA	NA	NA	NA	32.8	NA	0.017	0.067	NA
Autumn 1996	1	21.00	3.7	7.07	98.5	36.5	7.87	0.23	ND	2583
Autumn 1996	2	20.09	4.6	6.91	96.9	36.5	7.92	0.09	ND	2667
Autumn 1996	3	22.95	DL	6.84	94.3	36.7	7.79	0.24	ND	2639
Autumn 1996	4	19.91	31.7	6.34	88.2	29.2	7.72	0.16	ND	2222
Autumn 1996	5	19.86	BDL	8.00	89.4	0.8	7.91	0.25	0.80	107
Winter 1997	1	19.09	5.0	8.53	119.5	34.0	4.19	BDL	ND	2600
Winter 1997	2	20.15	19.6	7.42	98.8	30.4	5.40	BDL	ND	3100
Winter 1997	3	17.36	4.4	7.82	108.2	34.5	8.78	BDL	ND	3000
Winter 1997	4	16.31	6.0	7.41	96.8	30.5	7.56	BDL	ND	2600
Winter 1997	5	21.45	6.5	8.31	88.6	8.1	7.49	BDL	ND	410
Spring 1996	1	21.03	6.0	7.63	108.2	34.4	7.82	0.18	ND	3860
Spring 1996	2	20.96	26.0	7.66	109.5	34.4	7.86	0.12	ND	3700
Spring 1996	3	21.88	24.0	7.74	110.3	34.4	7.88	BDL	ND	3140
Spring 1996	4	24.12	5.0	7.48	107.2	34.2	7.78	BDL	ND	3860
Spring 1996	5	25.47	14.0	8.01	108.1	22.2	7.67	BDL	ND	2420
Summer 1996	1	23.62	8.0	7.74	115.3	34.6	8.58	0.10	ND	3300
Summer 1996	2	24.00	5.0	7.88	115.2	34.9	8.44	0.17	ND	2700
Summer 1996	3	25.84	4.0	8.23	12.2	34.9	8.49	0.17	ND	3150
Summer 1996	4	30.41	15.0	7.57	114.8	34.0	8.52	0.20	ND	2850
Summer 1996	5	24.58	28.0	7.94	114.7	14.2	8.44	ND	ND	ND
Autumn 1997	1	22.10	16.0	6.94	101.4	32.3	8.45	BDL	ND	4000
Autumn 1997	2	24.05	12.0	6.80	90.9	32.9	8.40	0.21	ND	3500
Autumn 1997	3	23.80	18.0	7.46	108.1	32.5	8.52	0.09	ND	2900
Autumn 1997	4	23.20	14.0	6.42	88.2	24.2	8.32	0.19	ND	2200
Autumn 1997	5	19.51	26.0	7.50	86.6	0.3	8.25	0.45	0.50	32
Winter 1997	1	19.18	15.0	5.84	105.6	34.2	8.40	0.21	0.07	2881
Winter 1997	2	19.16	22.0	8.01	105.7	35.1	8.40	0.21	0.07	2959
Winter 1997	3	19.04	20.0	8.12	105.1	35.1	8.40	0.20	0.06	3026
Winter 1997	4	14.76	24.0	8.22	94.8	6.5	7.74	0.18	0.06	189
Winter 1997	5	20.22	46.0	9.11	91.2	0.4	7.27	0.11	0.49	24
Spring 1997	1	21.15	14.0	7.62	103.8	35.0	8.82	0.17	BDL	2928
Spring 1997	2	20.03	28.0	7.45	98.4	36.5	8.64	0.13	0.14	1806
Spring 1997	3	18.71	17.5	20.30	102.0	35.3	8.80	0.22	0.12	3137
Spring 1997	4	21.72	10.0	7.03	89.8	29.4	8.36	0.13	0.11	2285
Spring 1997	5	21.42	29.0	21.42	78.4	0.3	8.68	0.06	0.21	32
Summer 1997	1	22.67	20.0	7.16	84.2	4.1	7.70	0.08	0.28	370
Summer 1997	2	22.66	25.0	6.58	76.1	0.2	7.43	0.06	0.46	185
Summer 1997	3	21.76	17.0	7.37	86.5	5.5	7.66	0.10	0.36	477
Summer 1997	4	22.48	45.5	7.36	85.4	0.7	7.78	BDL	0.35	46
Summer 1997	5	22.16	10.00	5.10	59.70	0.20	7.19	0.07	0.28	12

BDL = Below detection limit. ND = No data. [1]Hemens *et al.*.(1971) and [2]Hemens et al. (1976).

mouth of the estuary and embayment (Cyrus and Wepener, 1998). This ingression was attributed to dredging activities in Richards Bay Harbour. Salinity remained constant throughout the system during the

study with the exception of summer 1997 where the salinity at all sites was below 6‰. The reduced salinity during summer 1997 was a result of the flood that occurred during the sampling trip.

Salinity measured at site 5 was consistently lower than other sites during a particular survey. Dissolved oxygen was also constant throughout the estuary. Site 3 which was the site located at the mouth of the estuary had consistently elevated dissolved oxygen concentrations.

This was also the case for sulphate and fluoride. Concentrations at site 5 were consistently different from those measured at any of the other sampling sites during the particular survey.

During summer 1997, sulphate concentrations were lower as compared to those measured in other seasons. Nutrients (nitrates and orthophosphates) showed little variations during the study (Table 1).

During the construction of the harbour, nutrients level increased due to redistribution of anaerobic silt dredge from several meters below the surface.

Hemens et al. (1976) speculated that this was due to the bottom consisting of redeposited silt that had been depleted of its soluble nitrogen, or that the release of nitrogen from dead and decaying plankton had decreased, together with plankton densities, due to the opening of the new mouth.

The present study showed substantially higher nutrient levels than during the 1970s. It is unlikely that runoff from the catchment could contribute to the high nitrate concentrations measured at Sites 5 and 4, since low nutrient levels were recorded in the lower reaches of the river and at Site 1 (Table 1).

According to Wepener and Vermeulen (1999), the source of the nutrients is possibly related to fixation of nitrogen by blue-green algae in the muds of the vast mangrove areas.

Another potential source of nutrients they mentioned was sewage runoff from the surrounding rural settlements was indicated by high faecal coliform counts the authors obtained (Wepener and Vermeulen, 1999).Seasonal and spatial organic content (mean ± standard error) of sediment samples from the Mhlathuze Estuary are presented in Table 2.

Mean organic content were highest during summer 1996 and lowest during autumn 1996. The highest mean organic content was recorded at Site 4 with the lowest levels measured at site 3.

Metals in water

Temporal and spatial variations of metals in water

Metals in the water column generally did not show any significant differences between seasons with the exception of December 1997 where concentrations were significantly different from the rest of the seasons. During Spring 1997, Cu and Pb concentrations were also significantly higher.

The increase in metals during summer 1997 was probably related to resorption of metals bound to suspended particles during river flood conditions that prevailed during the sampling trip. A general trend of a reduction in metals was observed during autumn 1997. This reduction was attributed to dilution of waterborne metals by freshwater due to floods. According to Krupadam (2006) wide variations in water-borne metal concentration within estuaries are normally related to the degree of fresh water contribution or the presence of industrial effluents.

Total metal concentrations in water samples showed little spatial variation (Table 2). Only Pb concentrations significantly differed between Site 3 at the mouth and Site 5 which is located up the Mhlathuze River. This is probably related to the short residence time of water in the estuary.

About 90% of water from the estuary is being drained during each tidal cycle (Begg, 1978). The residence time of water may be too short to allow for major spatial variations of metals in water. The highest Pb concentrations in water recorded at Site 3 suggests the mouth as the source of lead pollution into the estuary. Lead may have been discharged with the fine sediments during dredger spoil on the breach, which were transported into the estuary through the mouth.

The elevated concentrations of Fe, Al, Zn and Cr recorded in the water from the Mhlathuze Estuary are in agreement with the results reported by Vermeulen and Wepener (1999, 2005) for metals in water from the adjacent Richards Bay Harbour (Table 4). When compared with historical concentrations reported from the original Richards Bay Estuary, only Zn concentrations were higher during the present study. Hemens et al. (1976) commented on the high Zn concentrations recorded at that time as “reason for concern”. Their study was, however, conducted before the completion of the harbour.

The high metal concentrations measured in this study could therefore be related to subsequent activities in the catchment and adjacent harbour area. Wave borne metals could also enter the estuary from the harbour via tidal gates. These tidal gates that are no longer in use were built to serve as a connection between the water in the harbour and the estuary. During high tide water can be observed overtopping over the tidal gates (personal observation) and it is therefore highly likely that metals originating in the harbour will find its way into the estuary. The concen-trations of metals from this study were slightly elevated when compared with metal concentrations in the water of other estuaries on the east coast of South Africa (Table4). It must be borne in mind that surveys by Hemens et al. (1970, 1976) were undertaken between 20 and 30 years ago and that these concentrations may have increased in the intervening

Table 2. Comparisons of dissolved metal concentrations in water (A) and metals in sediment (B) in the Mhlathuze Estuary with corresponding metal concentrations in other estuaries on the eastern seaboard of South Africa and in other countries.

Estuary	Al (µg/l)	Cr (µg/l)	Cu Fe (µg/l)	Fe (µg/l)	Mn (µg/l)	Pb (µg/l)	Zn (µg/l)	Reference
Water								
Mhlathuze	990.0	48.0	39.1	907.0	48.2	130.15	66.5	This study
Kynsna	-	0.1	0.2	81	5	0.6	0.3	a
Gamtoos	-	-	0.6	372	41	0.6	1.1	c
Swartkops	-	-	3.9	275	41	1.5	3.6	d
Sundays	-	1.3	3.2	334	18	0.7	2.4	e
Bushmans	-	0.4	1.8	302	10.2	0.2	0.5	f
Kosi Bay	-	-	1.1	-	-	1.0	5.7	i
St Lucia E	-	-	3.3	68.8	-	39.2	2.3	j
St Lucia E	-	-	17	2000	-	-	11.7	i
Richards Bay	-	-	1.7	-	-	1.9	-	k
Richards Bay	-	-	4.0	-	-	4.2	3.8	i
Richards Bay	504.4	23.6	50.8	782.4	80.7	-	85.4	l
Durban	-	-	27	800	-	117	287	i
East London			42.4	183.0	16.3	23.9	27.6	Fatoki
Port Elizabeth			11.3	21.9	4.2	16.8	16.2	Fatoki
Sunderban				175.0		0.20	9.7	GandK
Sediment								
Mhlathuze	18677.4	64.4	12.2	20606.9	13.5	45.6	45.6	This study
Kynsna	-	21	5	-	14	17	17	a
Gamtoos	-	15	5	9180	7	16	16	c
Swartkops	-	5	18	20800	31	55	55	d
Sundays	-	38	16	72	18	57	57	e
Bushmans	-	22	3	7330	5	13	13	f
Kosi Bay	-	150	61	60000	19	72	72	i
St Lucia E	-	7	2	3000	0.8	3.4	3.4	i
St Lucia E	-		9.9	23640	24	98	98	k
Richards Bay	-	74.8	24.04	5814	17.47	87.16	87.16	j
Richards Bay	-	110.3	19.22	31762.7	-	95.54	95.54	l
Richards Bay	31323.4	388	57	40000	117	287	287	i
Durban	-		183.0	18114.0	549.0	332.0	332.0	m
East London			82.3	15182.0	499.0	126	126	m
Port Elizabeth				10068	25.15	3448.4	3448.4	n
Sunderban				16600	29	54	54	o
Victoria		16.0	9.0					

References: a, Watling and Watling, 1982a; c. Watling and Watling, 1982c; d, Watling and Watling, 1982d; e, Watling et al., 1982; f, Watling and Watling, 1983b; g, Watling et al.., 1985, 1985; l, Cloete et al.., 1979; j, Oliff et al.., 1986; k, Connell *et al.*., 1975 l; Vermeulen and Wepener, 1999; m., Fatoki and Mathabatha 2001; n. Guhathakurta and Kariva 2000 , Tanner et al., 2000.

period.

Metals such as Zn and Cr are important constituents of industrial mining and domestic effluents (Khan et al., 2005). High concentrations of these metals may be a direct consequence of anthropogenic contamination from these activities. It is not possible to comment on the high Al levels since there is no historical data (Hemens et al., 1976) to compare with it. However, it is highly likely that the current levels are due to a combination of anthropogenic sources (the nearby aluminium smelter complexes, dredging of sediments in Richards Bay Harbour and natural leaching and weathering processes in the catchment).

Metals in sediment

Metals concentrations in the sediment were in elevated

orders of magnitude above the concentrations recorded in water. According to Soumady and Asokan (2011), sediments represent the most concentrated pool of metals in aquatic environments, metals that can be assimilated by aquatic organisms resident in those sediments. Since those organisms form a primary food source for bottom feeding fish, the accumulation of metals by such fish potentially depends on uptake from food as well as from water (Shulkin et al., 2003; Silva et al., 2006). Various bioaccumulation studies also found concentrations of metals in the estuarine environments which were in orders of magnitude higher in sediments than in water (Allen, 1993; Lim et al., 2012).

No significant temporal differences were recorded for metals in the sediment. This may be due to most metals being present in sediment as precipitates or in an un-dissolved state.

In estuarine systems, these metals are not bio-available except to organisms that are resident in sediment (Williams et al., 1998). Metals would be taken up and absorbed through the skin, or be ingested with food particles in benthic feeders.

Highest metal concentrations in the sediment were recorded for Fe, Al and Zn (Table 4). These metals also displayed the highest accumulation in the particulate matter in the water column. Watling and Watling (1982a-d) also reported high concentrations of Fe and Zn from sediments in Gamtoos, Swartkops, and Sunday River estuaries (Table 4), although concentrations in these estuaries were lower than those in this study.

There were significant spatial variations in metals concentrations (Table 3). With the exception of Al and Pb, most metals differed significantly between Sites 3 and 4. Metals concentrations were highest at Site 4, and with the exception of Cu, all metals had lowest concentrations at Site 3. The reasons could be attributed to the differences in substrate types and the amount of organic material.

Site 4 is in the middle of the embayment and contains mainly fine sediment. It is also the site with the highest organic content when compared with the rest of the sites. Site 3 is at the mouth of the estuary and is dominated by coarse sand. It is also the lowest in terms of organic content (Table 3).

The metals in the Mhlathuze Estuary probably exist as particulate matter or precipitated metals. Their presence in sediment may be predominantly due to a result of anthropogenic activities and ingression with fine dredged spoil from the harbour (Van den Hurk et al., 1997).

The metals are then precipitated onto and into the sediments. According to Sarkar et al., 2004 and Magesh et al. (2011), high loads of heavy metals are normally concentrated in the fine sediments. It is likely that most of the particulate bound metals entered the estuary during the ingression of into the Mhlathuze Estuary which took place when dredger spoil was deposited on the beach north of the estuary (Wepener and Cyrus, 1997; Mackay and Cyrus, 1999). Wave action and the near-shore current would have resulted in contaminated fine silt drifting into the estuary. The very high levels of Al in sediment may either be from geological leaching, as the area is known for its high Al metal content (Kwazulu Natal Business, 2013), or from pollution effects such as dredger spoil (Wepener and Cyrus, 1997).

Concentrations of Cu and Pb in sediment were low, ranging from 20-50 μg/g. These metals exist mostly as dissolved ions in the water column hence their low concentrations in sediment.

Copper is also known to form complexes with organic matter (Sarkar et al., 2004). The highest Cu concentrations in the sediment (at site 4) could be attributed to the complexation of Cu with organic ligands since the highest organic content was also recorded at this site (Table 4). The presence of high organic content in estuaries can decrease toxicity of metals such as copper by binding copper to organic ligands (DePalmer et al., 2011).

Chromium concentrations were elevated in sediments of the Mhlathuze Estuary (Table 4). High concentrations of Cr may be a result of contamination from the harbour due to sediment bound metals entering the estuary as discussed in the previous paragraphs. The results suggest equal amounts of Cr being exchanged between dissolved fractions in pore water and particulate fractions in sediment. This is because the percentage of particulate and dissolved Cr reported in this study were almost equal and also both the fractions were significantly different between seasons. Studies in estuarine areas show that dissolved and particulate Cr are present in almost equal quantities with the flocculation processes increasing Cr^{3+} concentrations in the salinity that are below normal sea water (Zwolsman and van Eck, 1999).

Results of this study show that metals vary spatially and temporally in water at the Mhlathuze estuary. Although there are minor variations of metals in sediment these results, the concentrations are higher than those recorded in other estuaries along in the eastern seaboard (Table 2). Seasonal variation in the Mhlathuze Estuary may be as a result of the metal loads that may be delivered by the river or sea. Spatial differences may be as a result of behaviour of metals in response to gradients in the chemistry of the water body and the nature of the sediment.

The important factor that results in variation of metals between sites closer to the mouth in the Mhlathuze Estuary is the residence time of water in the estuary. The tidal cycle in the estuary affects the sites next to the mouth (Site 1, 2 and 3). Water from these sites is almost completely replaced during a tidal cycle. The quality of water brought in during each tidal cycle would be similar resulting in more or less similar values for sites next to the mouth (sites 1, 2 and 3). Salinity values form these sites are also similar and was close to sea water. Sites 4

Table 3. Spatial heavy metal concentrations (means ± standard error) in water (total dissolved and particulate) (μgL^{-1}) and sediment ($\mu g.g^{-1}$) in the Mhlathuze Estuary. All references to significant differences are made in the text and are not indicated in the table.

Parameter	Turbidity	Salinity	Organic	Dissolved 0_2	pH	Total	Suspended	Dissolved
Aluminium								
Turbidity	1							
Salinity	-0.64*	1						
Organic	0.02	-0.27	1					
Dissolved O_2	0.06	0.11	0.10	1				
pH	-0.18	0.45*	-0.04	0.22*	1			
Total	0.23	-0.24*	-0.19	-0.11	-0.58*	1		
Suspended	-0.28*	0.36*	-0.21*	-0.37*	-0.15	0.117	1	
Dissolved	0.05	0.03	-0.32*	0.13	-0.22*	0.44*	0.35*	1.00
Chromium								
Turbidity	1.00							
Salinity	-0.70*	1.00						
Organic	-0.02	-0.16	1.00					
Dissolved O_2	0.01	0.10	-0.04	1.00				
pH	-0.19*	0.40*	-0.01	0.11	1.00			
Total	-0.02	0.08	-0.28*	-0.17	0.19	1.00		
Suspended	0.24*	-0.29*	0.01	-0.34	0.33*	0.66*	1.00	
Dissolved	0.25	-0.05	-0.29*	-0.02	-0.23*	0.51*	0.26*	1.00
Copper								
Turbidity	1.00							
Salinity	-0.53*	1.00						
Organic	-0.10	-0.26*	1.00					
Dissolved O_2	-0.07	0.12	-0.05	1.00				
pH	-0.10	0.34*	-0.06	0.28*	1.00			
Total	-0.20*	0.44*	0.17	0.14	0.55*	100		
Suspended	-0.07	0.12	-0.46*	-0.16	-0.27*	-0.12	1.00	
Dissolved	0.07	0.30*	-0.06	-0.09	-0.11	0.29*	0.11	1.00
Manganese								
Turbidity	1.00							
Salinity	-0.61*	1.00						
Organic	-0.06	-0.26*	1.00					
Dissolved O_2	0.01	0.17	-0.10	1.00				
pH	-0.19*	0.46*	-0.09	0.19	1.00			
Total	0.11	0.03	-0.41*	-0.06	0.01	1.00		
Suspended	0.48*	-0.51*	0.17	-0.36*	-0.10	0.13	1.00	
Dissolved	0.11	0.03	-0.23*	-0.09	-00.03	.52*	-0.13	1.00
Iron								
Turbidity	1.00							
Salinity	-0.52*	1.00						
Organic	-0.22*	-0.22	1.00					
Dissolved O_2	-0.04	0.22*	-0.20*	1.00				
pH	-0.11	0.43*	-0.04	0.27*	1.00			
Total	0.11	0-.09	-0.30*	0.08	-0.50*	1.00		
Suspended	0.62*	0-.42	-0.23*	-0.13	-0.43*	0.508*	1.00	
Dissolved	-0.07	0.20*	-0.52*	-0.12	0.08	0.36*	0.05	1.00

Table 3. Contd.

Parameter	Turbidity	Salinity	Organic	Dissolved 0_2	pH	Total	Suspended	Dissolved
Lead								
Turbidity	1.00							
Salinity	-0.60*	1.00						
Organic	-0.05	0-.27*	1.00					
Dissolved O_2	0.06	0.18*	-0.13	1.00				
pH	-0.18*	0.44*	-0.02	0.16	1.00			
Total	-0.20*	0.44*	0.05	0.13	0.58*	1.00		
Suspended	-0.11	0.14	0.07	-0.28*	0.27*	-0.00	1.00	
Dissolved	-0.16	0.35*	-0.18*	0.19	-0.06	0.41*	-0.63*	1.00
Zinc								
Turbidity	1.00							
Salinity	-0.62*	1.00						
Organic	0.03	-0.29*	1.00					
Dissolved O_2	0.06	0.13	-0.12	1.00				
pH	-0.20*	0.43*	-0.02	0.24*	1.00			
Total	0.01	0.10	-0.22*	0.32*	-0.11	1.00		
Suspended	0.20*	-0.34*	0.08	-0.39*	-0.04	0.21*	1.00	
Dissolved	0.12	0.01	0.04	-0.23*	0.12	0.16	0.15	1.00

and 5 however were different. Site 4 is in the basin and site 5 up the river. There is limited tidal influence on these sites and consequently they have different levels of metals in comparison to the former sites. The low levels of metals consistently reported in water and sediment up river at site 5, when compared with the other sites lead to the conclusion that the ingression of sediment from the harbour as the source of most of the high metal concentrations found in sediments of the Mhlathuze Estuary. Harbour sediments have been reported by various authors as highly polluted environments, and a possible source of metal contamination in surrounding water bodies e.g. Victoria Harbour, (Tanner et al., 2000) East London and Port Elizabeth (Fatoki and Mathabatha, 2001), Tolo Harbour (Owen and Sandhu, 2000), Richards Bay Harbour (Vermeulen and Wepener, 2005) and Klaipeda Harbour (Galkus et al., 2012).

Temporal variations of metals in sediment could not be observed in the Mhlathuze Estuary. This is because of the relative stability of metals in sediments as well as variation of metals concentrations at different sites. Metals varied spatial because of the different substrates found in the Mhlathuze Estuary and their variability in accumulation of metals. According to Cox and Micaela (2005), heavy metal distribution in estuarine and marine deposits is influenced by sediment texture, clay content, organic carbon, iron hydrous oxides and carbonates. These components differ in the manner in which they adsorb metals (Newman and Watling, 2007). The clay-rich sediment and organic carbon rich sediment tend to contain higher metal content as compared to sand dominated regions. Mud has a very fine texture and it usually accumulates most of the metals. According to Herut and Sandler (2006), clay minerals readily absorb heavy metal and consequently clay-rich sediments tend to contain higher contamination levels than sand-dominated deposits. Fine sediments contribute by having large specific areas with many attachment sites for metals. Organic coating also occurs easily in fine sediments (Mortimer and Rae, 2000). Types of substrate sediments in the Mhlathuze Estuary are described in Cyrus and Wepener (1998). Sites 1, 2 and 4 have high mud and organic content (Table 3). They also had high metals concentration when compared with sandy sites such as sites 3 and 5, which had low concentrations of heavy metals in the sediment (Table 4).

One method used to determine the level of contamination of metals in sediment is the simple ratio between the concentration of a metal and the concentration of the normaliser. The conditions that are considered in selecting a normalizer is that the metal should be abundant, naturally occurring and should not be influenced by anthropogenic sources. Because of these conditions, metals that are considered as suitable for the normalization of metals in sediment are Al and Fe (Newman and Watling, 2007). For this study, Fe was considered more suitable due to the presence of aluminium smelters located around the harbour area with a possibility of estuarine sediment contamination from thesmelters' effluents. An attempt to normalize the sediment metals using Al showed results with very low R2 values, a high number of data points that behaved as

Table 4. Mean seasonal heavy metal concentrations in water (total, dissolved and particulate) (µg.g-1) in the Mhlathuze Estuary. All references to significant differences are made in the text and are not indicated in the table.

Sample	Site 1	Site 2	Site 3	Site 4	Site 5
Organic content	5.12±1.18	4.55±1.05	2.51±0.76	11.17±1.73	4.53±1.73
Aluminium					
Total	1995.8 ±1554.2	42705±3159.8	2060.0 ± 1554.3	5814.2 ±4973.1	2630.0±2029.6
Dissolved	632.8 ±281.9	721.0±403.6	990.3 ± 580.4	1546.5±1090.4	939.3±522.0
Particulate	513.5 ±347.9	564.3±356.6	537.3 ± 234.2	837.0±408.0	332.8±143.5
Sediment	22044.9 ±7119.2	21202.1 ±6081.8	10303.3 ± 3514.6	29118.4±7631.0	17230.9±4012.4
Chromium					
Total	37.5±13.0	61.8±26.7	32.8±13.8	47.0±27.3	26.3±16.2
Dissolved	46.0±10.7	65.3±10.8	46.3±12.1	48.8±17.7	33.5±15.1
Particulate	44.8±14.3	51.0±15.2	50.5±16.8	53.8±20.6	40.0±16.5
Sediment	84.1±15.1	42.4±12.2	41.7±8.9	107.9±16.5	55.6±10.8
Copper					
Total	53.1±10.2	51.3±7.2	48.3±6.6	38.3±9.0	21.8±6.0
Dissolved	42.0±13.4	52.3±16.2	50.8±15.9	38.3±15.9	14.5±4.4
Particulate	2.3±1.2	19.0±11.2	9.3±11.2	9.3±5.9	5.2±3.0
Sediment	12.1±1.8	8.4±2.5	8.8±2.3	22.72.0	11.7±2.7
Iron					
Total	1732.5±1054.9	3900.8±2817.1	1872.5±1241.4	4119.5±3286.1	2585.0±2077.8
Dissolved	655.0±218.8	787.1±292.4	961.8±584.7	1317.5±864.6	730.0±406.7
Particulate	1542.5±869.0	5598.3±4224.3	2067.5±954.3	1142.5±315.4	1797.5±857.7
Sediment	23132.3±2303.6	18715.9±4456.7	12585.8±33473	31280.7±3428.7	21048.5±5641.2
Manganese					
Total	77.8±11.7	110.5±24.7	69.8±12.0	109.0±46.9	82.8±20.2
Dissolved	42.8±8.2	88.0±39.6	45.8±9.1	40.0±6.4	61.3±20.2
Particulate	36.8±8.5	60.8±13.0	43.8±14.9	121.7±58.74	70.5±18.8
Sediment	274.2±44.2	308.2±89.4	165.3±38.7	731.5±141.2	335.5±66.6
Lead					
Total	168.8±22.9	233.5±37.8	240.0±41.5	193.0±55.0	78.8±19.7
Dissolved	167.5±72.2	129.5±35.8	186.0±62.8	130.3±65.8	37.5±12.5
Particulate	59.0±27.76	57.8±23.47	67.0±35.6	62.5±25.97	39.5±19.5
Sediment	18.0±3.8	14.1±3.5	9.1±2.1	18.7±4.2	12.3±2.8
Zinc					
Total	66.8±3.8	76.2±6.4	63.0±4.3	119.5±59.1	43.8±5.2
Dissolved	68.8±8.3	83.5±13.1	74.5±6.5	64.5±18.7	59.8±7.3
Particulate	41.8±10.4	69.0±14.3	47.0±12.5	114.2±59.8	47.0±9.9
Sediment	54.7±4.6	35.0±9.0	33.1±7.1	74.1±4.7	37.7±7.0

outliers and metals Cr, and Zn were not significantly different ($P<0.05$). Normalization plots of metals using Fe are presented in Figure 2. The ratios of metals to Fe were calculated and linear regressions were represented for metals Al, Cr, Mn, Pb and Zn. The R^2 values recorded are displayed in the graph. The regression recorded was significantly different for all metals. The outliers observed in the regression graphs for metals Al, Cr and Cu indicated increased concentrations of metals due to anthropogenic influence. Regression of Al and Fe was the lowest as compared to other metals suggesting an increase of Al from background concentrations. In the comparison of metals and physicochemical parameters using principal component analysis, the two axes retained 60.8% of the variances from the sample data. The multivariate PCA analysis, based on metals in water,

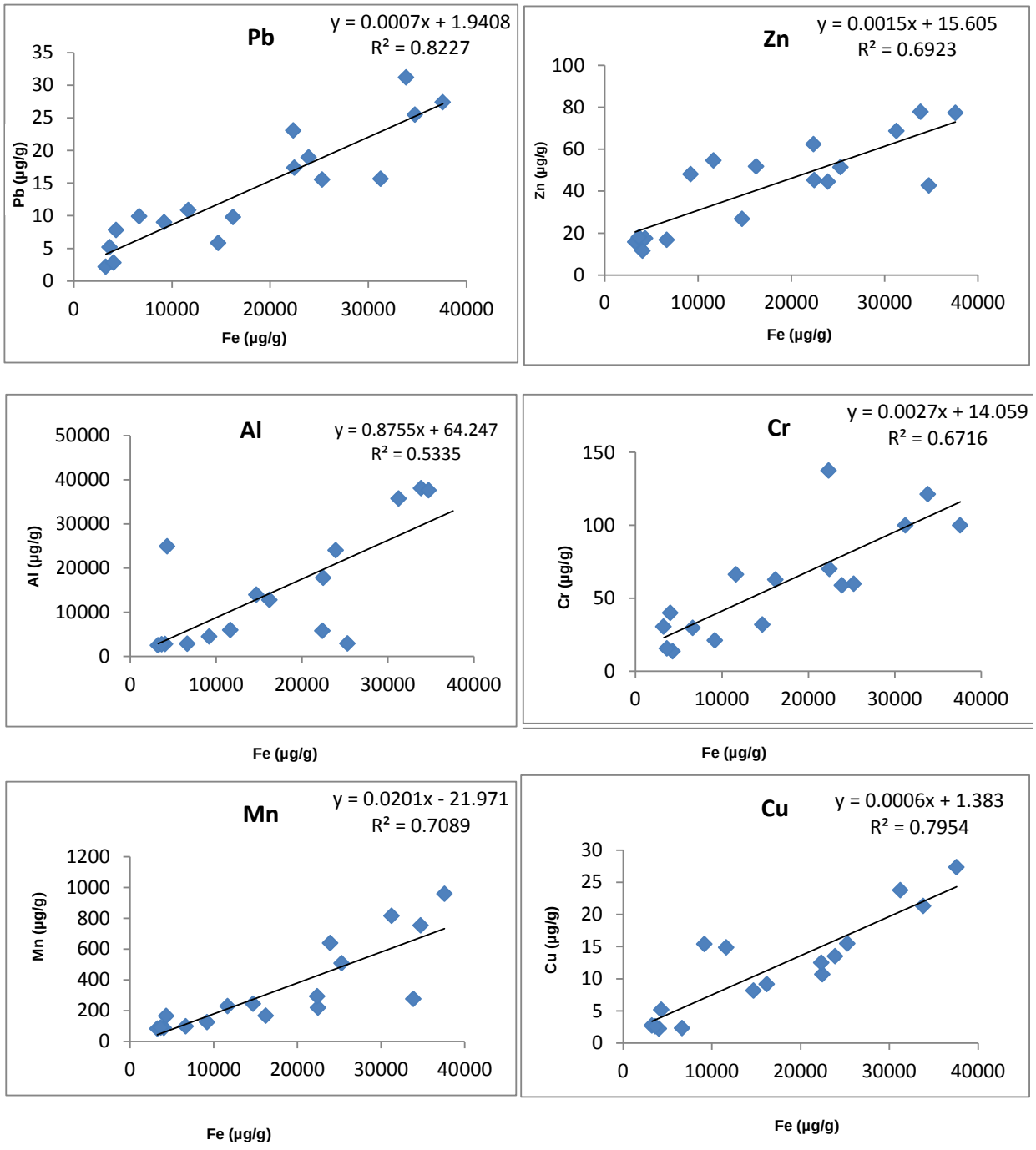

Figure 2. Normalised linear regression plots of Al, Cr, Mn, Pb and Zn concentrations (µg/g) to Fe (µg/g).

sediment and physicochemical data is plotted in Figure 3. The data for metals from the different phases and the physicochemical parameters influencing them formed three distinct groups. A fourth group consisted of nutrients and water quality parameters.

The first group consisted of sediment metals and the organic content. The correlation of metals in sediment suggested the increase of metals in sediment with an increase in the sediment organic content. Sediment metals in the estuary increased and were found in high concentration in the areas such as the basin at Site 4 that had high organic matter. The mouth area, however, had less organic content and low concentrations of metals.

The particulate concentrations of Al, Pb Cu and Zn formed the second group that increased with an increase in dissolved oxygen, pH and temperature. These

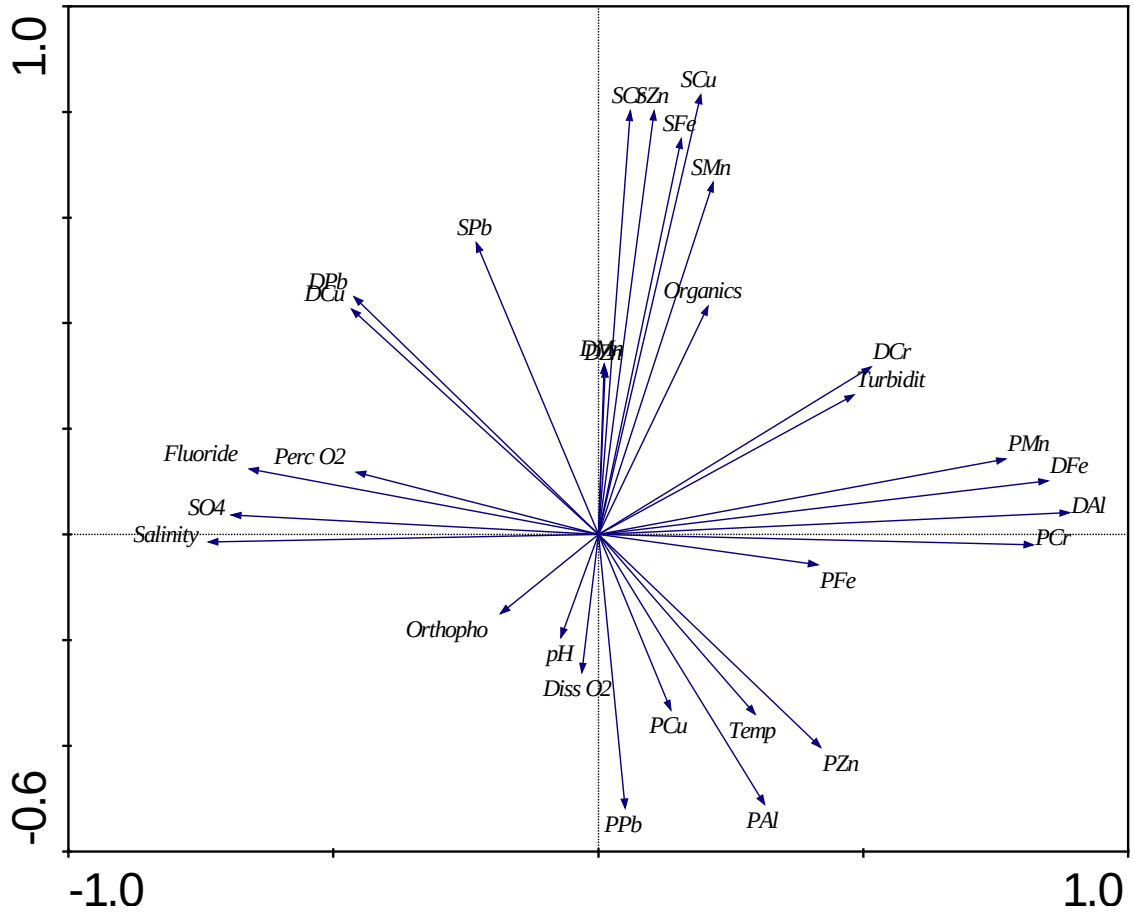

Figure 3. Principal component analysis biplots of particulate (P), dissolved (D) and sediment (S) concentrations (60.8% of variance explained)

conditions were found in the shallow waters towards the mouth of the estuary. This suggests that precipitation is the important process for the increased of these metals in the particulate phase. In the third group, the dissolved concentrations of Al, Cr and Fe and the particulate concentrations of Cr, Mn and Fe were correlated with turbidity, suggesting increasing concentrations of these metals in the basin where turbidity was high due to re-suspension as a result of the shallow nature of the estuary and the very fine bottom sediment. Dissolved Zn and Mn concentrations were both influenced by different factors to the other dissolved metal concentrations. This was also observed in the behaviour of dissolved Mn and Zn in other estuaries on the KZN eastern seaboard (unpublished data) where dissolved concentrations of both Mn and Zn increased in areas of high salinity and high turbidity. The correlations of dissolved Cu and Pb concentrations were probably related to the very low dissolved concentrations of these metals recorded in the Mhlathuze Estuary. The increase in salinity and percentage oxygen at the mouth of the estuary coincided with increases in sulphates and fluoride concentrations as identified by the water quality group. This suggests the sea as the source of the fluorides and sulphates in the estuary as these chemicals are observed in very low concentrations in the upstream sites.

Heavy metals discharged directly from industrial effluents, as well as those diffusing from activities in the harbour and from runoff storm water; result in contamination of water and harbour sediments (Vermeulen and Wepener,1999). Material that was dredged from the harbour was deposited on to the beach north of the estuary mouth. The dredge spoil is then taken up by wind and currents to the estuary mouth thereby transferring metal contamination into the estuary. The harbour authority has been looking at various ways of disposing of the spoil as an environmental exercise to mitigate the potential pollution of the estuary. The method that was recommended by CRUZ (Wepener and Cyrus, 1997; Cyrus and Wepener, 1998) of disposal a few kilometres offshore is environmentally sustainable. This would result in the pollution from the spoil becoming more diffuse and not drifting towards the Mhlathuze Estuary.

The value of an estuary for resource harvest, habitat for fish and other invertebrates and for aesthetic beauty can never be overestimated (Breen and McKenzie, 2001).

The idea of keeping the southern side of the original Richards Bay Estuary for resource protection proved to be a good environmental exercise. It was to ensure perpetuation of biodiversity and resource protection. It is however being affected by activity in surrounding areas. This is shown by the findings of this study in terms of metal pollution in the Mhlathuze Estuary.

Further studies are needed to clearly point to the actual sources of pollutants in the Mhlathuze Estuary. Activities such as mining on the boundary waters (Armah et al., 2010), and periodic dredging of the harbour have been found to have a marked effect on the accumulation of metals in estuarine environments (Van der Hurk et al., 1997; Galkus et al., 2012). A high concentration of these metals has an effect on biota of estuaries. This could be made worse by a number of factors. Plans are underway to expand the Port if Richards Bay. The operation of EXARRO Sands, a heavy metal company that has recently built on the catchment of the Mhlathuze River, has been in operation for about 10 years. Both these and other future developments may impact negatively on the estuary in terms of metal pollution.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

Allen HE (1993). The significance of trace metal speciation for water, sediment and soil quality standards. Sci. Total Environ. 134(Suppl. Part 1):23-45.

Armah FA, Obiri S, Yawson DO, Pappoe AN, Moi BA (2010). Mining and Heavy Metal Pollution: Assessment of Aquatic Environments in Tarkwa (Ghana) using Multivariate Statistical Analysis. J. Environ. Stat. 1(4): 1-13.

Begg G (1978). Estuaries of Natal. Town and regional planning. Report V41.

Breen CN, McKenzie M (2001). Managing Estuaries in South Africa: An Introduction. Institute of Natural Resources, Pietermaritzburg.

Cloete CE (Ed.) (1979) The transfer of pollutants in two southern hemispheric oceanic systems. Proceedings of a workshop held at Plettenberg Bay, South Africa, 23-26 April 1975. South African Scientific Programmes Report. No. 39.

Connell AD, Turner WD, Gardner BD, McClurg TP, Livingstone DJ, Carter JE Geternbach WJN (1975) National marine pollution monitoring programme, East Coast section. Progress Report. No. 2: 118.

Cox, ME, Micaela P (2005). Trace metal distribution within Marine and Estuarine sediments of Western Moreton, Bay Queesland, Australia: Relation to land use and settling. Geogr. Res. 43(2):173-193.

CSIR (1993). Physical impacts of the disposal of dredger spoil at Richards Bay. CSIR Report EMAS-C 93028.

Cyrus DP, Wepener V (1998). The Impact of Dredger Spoil on Selected Components of the Mhlathuze Estuary. Cruz Investigational Report No 52.

DePalmer SGS, Anorld WR, McGeer JC, Dixon DG, Smith DS (2011). Effects of dissolved organic matter and reduced sulphur on copper bioavailability in coastal marine environments Ecotox. Environ. Saf. 74(3): 230-237.

Fatoki OS, Mathabatha S (2001). An assessment of heavy metal pollution in the East London and Port Elizabeth Harbours. Water SA 27:233-240.

Galkus A, Joksas K, Stakeniene R, Lagunaviciene L (2012). Heavy metal contamination of habor bottom sediments. Pol. J. Environ. Stud. 6:1583-1594.

Hemens J, Connell A D, McClurg TP, Simpson DE, Warwick RJ, Muller MS (1976). Richards Bay Sanctuary Area: Report on field surveys in October 1975 and February 1976. Progress Report No 31 Thirty Second Steering Committee Meeting CSIR/NIWR.

Hemens J, Connell AD (1975). Richards Bay: Southern Bay Conservation Area. Progress Report No 29. Thirty Second Steering Committee Meeting CSIR/NIWR.

Hemens J, Simpson DE McClurg TP (1970). Detailed survey of Richards Bay. Progress Report, CSIRINIWR. 20th Steering Committee Meeting: Marine disposal of effluents, 20: 1-28.

Herut B, Sandler A (2006). Normalization method for pollutants in marine sediments: review and recommendations for the Mediterranean IORL Report H18/2006 Submitted to UNEP/MAP 23 pp.

Khan R, Israili SH, Ahman H, Mohan A (2005). Heavy metal pollution assessment in surface water bodies and its suitability for irrigation around the Neyevli Lignitemines and Associated industrial Complex Tamin Nadu India. Mine Water Environ. 24: 155-161.

Krupadam RJ, Smita P, Wate SR (2006). Geochemical fractionation of heavy metals in sediments of the Tapi estuary. Geochem. J. 40: 513-522.

Kwazulu Natal Business(2013). The guide to business and investment in Kwazulu natal.Mining and metals. Downloaded 13 August 2013. http://www.kwazulunatalbusiness.co.za/pls/cms/ti_secout.secout_prov?p_sid=19&p_site_id=130

Lim WY, Aries AZ, Zakaria MP (2012) Spatial variability of metals in surface water and sediment in the Langat river and geochemical factors that influence their water-sediment interactions. Sci. World J. 2012:652150.

Mackay CF, Cyrus DP (1999). A review of the macrobenthic fauna of the Mhlathuze Estuary: Setting the ecological Reserve. S. Afr. J. Aquat. Sci. 24: 111-129.

Magesh NS, Chandrasekar N, Roy VD (2011). Spatial analysis of trace element contamination in sediments from Tamiraparani estuary, South East coast of India. Est. Coast. Shelf Sci. 92: 618-628.

Mortimer RJG, Rae JE (2000). Metal speciation (Cu, Zn, Pb and Cd) and organic matter in oxic and suboxic salt marsh sediments, Servern Estuary. Mar. Pollut. Bull. 40: 377-386.

Newman, BK Watling, RJ (2007). Definition of baseline metal concentrations for assessing metal enrichment of sediment form south-eastern Cape coastline of South Africa. Water SA 33: 675-691.

Owen RB, Sandhu, N (2000). Heavy metal accumulation and anthropogenic impacts on Tolo Habour, Hong Kong. Mar. Pollut. Bull. 40: 174-180.

Sarkar SK, Franciskovic S, Bhattachrrya A, Saha M, Bilinski H (2004). Levels of elements in the surficial estuarine sediments of the Hugli River, northeast India and their environmental implications. Environ. Int. 30: 1089-1098.

Shirneshan G, Bachtiari AR, Kazemi A, Mohamadi M, Kheirabadi N (2012). Oyster *Saccostrea cucullata* as a biomonitor for Hg contamination and the risk for human on the coast of Qeshm Island, Persian Gulf, Iran. Bull. Contam. Toxicol. 88:962-966.

Shulkin VM, Presley BJ, Kavun VI (2003). Metal concentrations in mussel *Crenomytilis grayanus* and oyster *Crassostra gigas* in relation to contamination of ambient sediments. Environ. Int. 29(4): 493-502.

Silva CAR, Smith BD, Rainbow PS (2006). Comparative biomonitors of coastal trace metal contamination in tropical South America (N. Brazil). Mar. Environ. Res. 61: 439-455.

Soumady D, Asokan S (2011). A study on trace metal accumulation in

selected organs of Perna viridis in Nagapattinam coastal waters, Tamil Nadu, India. Int. Pharm. Sci. 1(2):42-47.
Standard Methods (1998). Standard Methods for the Examination of Water Waste Water. (17th ed) American Public Health Association Port City Press, Maryland U.S.A.
Tanner PA, Shing-leong L, Ming pan S (2000). Contamination of heavy metals in marine sediment cores from Victoria Harbour, Hong Kong. Mar. Pollut. Bull. 40:769-779.
van der Hurk P, Eertman RHM, Stronkhorst J (1997). Toxicity of harbour canal sediment before dredging and after offshore disposal. Mar. Pollut. Bull. 34:244-249.
Watling HR, Watling RJ (1982). Metal concentrations in oyster from South African coast. Bull. Contam. Toxicol. 28:460-466.
Watling R,Talbot MMJ-F, Branch EB, Marsland SA (1982e). Metal Survey in South African Estuaries VI. Sundays River. Water SA 8:92-95.
Watling R, Talbot MMJ-F, Branch EB, Marsland SA (1985). Metal Survey in South African Estuaries IX, Buffalo River. Water SA 11:61-66.
Watling RJ, Watling HR (1982a). Metal Surveys in South African Estuaries II. Knysna River. Water SA 8:36-44.
Watling RJ, Watling HR (1982b). Metal Surveys in South African Estuaries Keurbooms and Bietou Rivers (Plattenberg Bay). Water SA 8:14-19.
Watling RJ, Watling HR (1982c). Metal Surveys in South African Estuaries V Kroome and Gamtoos (St Francis Bay). Water SA 8:87-91.
Watling RJ, Watling HR (1982d). Metal Surveys in South African Estuaries I. Swartkop River. Water SA 8:26-35.
Wepener V, Vermeulen L (1999). Comments on the water quality of the Mhlathuze Estuary un relation to determinining the ecological integrity class. S. Afr. J. Aquat. Sci. 24:86-98.
Wepener V, Cyrus DP (1997). The effects of Inter-basin Transfer on the Hydrochemistry, Benthic Invertebrates and Ichthyofauna of the Mhlathuze Estuary and Lake Nsezi: Second Interim Report. CRUZ Investigational Report 43.
Wepener V, Vermeulen LA (2005). A note on the concentrations and bioavailability of the selected metals in sediments of Richards Bay Harbour, South Africa. Water SA 31(4):589-596.
Williams PR, Attrill MJ, Nimmo M (1998). Heavy metal concentrations and bioaccumulation within Fal Estuary A Reappraisal. Mar. Pollut. Bull. 36:463-467.
Zwolsman JJG, van Eck GTM (1999). Geochemistry of major elements and trace metals in suspended matter of the Scheldt Estuary, southwest Netherlands. Mar. Chem. 66:91-111.


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