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Full Length Research Paper

Comparative evaluation of the physicochemical composition and microbial quality of seven different water sources used in processing root and tuber crops in Umudike, Nigeria

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The physicochemical composition and microbial quality of 7 different water sources (Umudike ukwu bore hole (B5), Umuariaga borehole (B3), Nidoro spring water (S2), Umudike bore hole (B4), Umudike school borehole A (B7), Umudike school bore hole B (B6) and Ahiaeke bore hole (B9) used for root and tuber crop processing in Umudike, Nigeria were investigated using standard techniques. The chloride, sulphate, phosphate, dissolved substances, hardness of water, ammonium nitrate and conductivity in all the water samples, fell within the range recommended by the Nigerian Industrial Standard (NIS) or the World Health Organization (WHO) while the values obtained for pH and nitrites were lower than the range given by WHO and NIS. Most of the samples had higher organic carbon contents than permitted. In terms of dissolved oxygen (D) and total hydrogen (TH), S2 had the highest, B5 and B6 had the least for DO ($P > 0.05$) while B6 had the least in addition for TH. The biochemical oxygen demand (BOD) of B3 was the highest among the water samples analyzed ($P < 0.05$) while that of B5 and B6 were the least ($P > 0.05$). Sensory evaluation of the samples showed that they were clear, tasteless and odorless. Microbial analysis carried out indicated the presence of coliforms in the water samples although this does not give any cause for concern as they fell within the standards for coliform in water. The total plate count of the samples ranged from 8.00 to 19.09cfu/100ml with B9 having the highest while B7 had the least. Some of the microorganisms isolated include: *E.coli, salmonella, shigella, fungi and staphylo*coccus, indicating microbial contamination of these water samples and thus highlights the need for their urgent sterilization. The absence of *Vibrio cholerea* in all the water samples indicate the non-prevalence of cholera in the study area. Finally, most of the water sources used by these communities in processing their root and tuber crops did not meet the recommended standards for nitrites, biochemical oxygen demand, organic carbon and nitrites in water, underscoring the need for regular checks and on these water sources.

Key words: Water, microbial, coliform, physicochemical, root crops, tuber crops.

INTRODUCTION

Water that is of good drinking quality is important for man's continued existence (Lamikanra, 1999; FAO, 1997). Water quality is essentially determined by its physical, chemical as well as microbiological characteristics. However, the quality of water, both for drinking and other uses deteriorates due to inadequacy of treatment plants, direct discharge of untreated sewage into rivers and stream in addition to inefficient management of piped water distribution system (UNEP, 2001). This therefore

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has serious health implications for the users. In addition, in many developing countries, increasing agricultural activities, urbanization and industrialization leads to ever increasing contamination of streams, rivers, lakes and reservoirs which are usually the main sources of drinking water. The ability to control the quality of water is based on routine tests, the results of which are compared with established standards. Chemical and microbial analysis can thus give an idea of the possibility of the water being polluted, the extent of its pollution and the possibility of it containing pathogenic micro-organisms (APHA, 1998).

In the chemical analysis of water, the major parameters commonly analyzed for are Ca^{2+}, Mg^{2+}, CO_3^{2-}, SO_4^{2-}, total hardness as $CaCO_3$, Mg-hardness, as well as minor ions such as: Fe^{2+}, Fe^{3+}, NO_3, NH_3 (Nitrate and Ammonia) and Nitrogen. These ions are important determinable parameters because of their sensitive effects on human health (Oxford, 2002).

National Root Crops Research Institute, Umudike, Nigeria empowered a lot of communities around it by releasing to them, root and tuber crops and which these communities use their own water sources to process. There are indications that some of these water sources used in processing these crops could be polluted or contaminated. It would be worthwhile to investigate the chemical and microbial quality of the sources of water these communities use in processing these root and tuber crops to ascertain if they conform to acceptable standards, in addition to ensuring that they do not convey any health hazard to the user. The occasional reports of food poisoning in some of these areas further underscores the need for this research and this work is aimed at investigating this.

MATERIALS AND METHODS

The water samples that were used for the experiment were collected from 7 different locations in Umudike environment: Umudike ukwu bore hole (B5), Umuariaga borehole (B3), Ndioro spring water (S2), Umudike bore hole (B4), Umudike school borehole A (B7), Umudike school bore hole B (B6) and Ahiaeke bore hole (B9) in Umudike environment. The samples were collected between 9 am and 12 pm day time hours, using 2 liters sterilized plastic containers. The containers were washed with detergents, rinsed with distilled water, 75% alcohol and finally rinsed with de-ionized water. At each sampling point, the containers were rinsed with the water samples to be collected from the various sources. The research work was carried out in line with the ethical standards of Nigerian drinking water as indicated by the Standard Organization of Nigeria reference no: NIS 554: 2007.

Sampling procedures and analysis

The chemical analysis of water samples was carried out at the Chemistry and Biochemistry Laboratories of National Root Crops Research Institute, Umudike, Umuahia, Abia State, Nigeria. Analysis was done approximately 24 h after collection of the samples.

Physicochemical analysis

The conductivity of the water samples was determined using a conductivity meter which was calibrated with a solution of $KMnO_4$, the method of Chikezie et al. (2008) was used in the determination of the total dissolved substances, and results were expressed in ppm, the Winkler method was used in the determination of the biochemical oxygen demand (BOD), and results were expressed in mg/L, the organic carbon contents of the water samples was determined using the rapid titrimetric method, and results were expressed in mg/L, the phosphate contents of the water samples was determined using the Ascorbic acid reduction method, which involves the conversion of the phosphorous formed by colorimetric measurements of the dissolved orthophosphate. The concentration of the phosphates in the water samples was extrapolated from a standard curve and results were expressed in mg/L. The dissolved oxygen was determined using the unmodified winkler method which is the most precise titrimetric method, the total hardness was determined using the titrimetric method, the total hydrogen, nitrate nitrogen and ammonium nitrogen were determined using standard techniques (Chikezie et al., 2008).

Physical/organoleptic analysis

This was determined by physical and sensory evaluation of the water samples for taste, appearance and odour as described by Edema et al. (2001) and Ajayi and Adejumo (2011). The pH of the water samples was determined using the Hana 211 pH meter.

Microbial analysis

Serial dilutions were made by preparing 1 ml of samples in 9 ml of sterile peptone water to obtain diluents. Dilutions were made and appropriate aliquots used on spread plates for total aerobic mesophilic plate counts as described by Ezeama (2001). The total plate count was carried out to provide an estimate of the total number of bacteria in each of the samples that would develop into colonies during the period of incubation on Nutrient agar and MacConkey agar plates. This test detects a broad group of bacteria including the pathogens, non pathogens and opportunistic pathogens. The prepared media was allowed to cool at 45°C before pouring into the Petri dish. It was allowed to solidify before spread plating the dilution factor with a hockey stick. The plates were incubated at 37°C for 24 h in inverted positions to prevent condensation from the lid to the agar, after which the numbers of the colonies formed were counted. The following media were used to isolate some microorganisms: Thiosulphate citrate bile salt agar for *Vibrio cholera*, Kigler iron agar (KIA) for *Salmonella, Salmonella-Shigella* agar for *Salmonella* and *Shigella*, MacConkey agar for *E. coli*, Nutrient agar for *Staphylococcus aureus*, Difico potato dextrose agar for yeast and mould, respectively. These were carried out using standard methods (Prescott et al., 2008).

Coliform count

The most probable number (MPN) method as recommended by APHA (1998), was used in determining the coliform counts of the water samples. The materials and media used for the analysis consisted of the following: Fermentation tubes with aluminum caps, Durham tubes, MacConkey Broth (Single and double strength) inoculating loop, bunsen burner and syringes (10, 5 and 2 ml). The most probable number tube fermentation techniques were performed in three stages: Presumptive test, confirmative test and completed test.

Table 1. Physicochemical composition of water samples from 7 different locations.

Sample	Cl^-	SO_4^{2-}	PO_4^{2-}	TDS	Organic carbon
B5	10.63±0.01^{d}	2.62±2.83^{C}	3.79±0.00^{d}	6.43±3.54^{c}	37.86±0.03^{e}
B3	7.09±0.00^{b}	2.14±0.92b	2.64±0.01^{C}	5.50±0.00^{b}	1.09±0.93^{h}
S2	14.17±0.02^{c}	2.95±0.78f	1.30±0.01^{a}	1.40±0.00^{h}	43.90±0.07^{f}
B4	7.05±0.06^{b}	2.80±0.07^{e}	8.53±0.04^{f}	8.40±0.00^{f}	32.45±0.72^{b}
B7	10.65±0.01^{d}	1.90±0.00^{a}	2.03±0.04^{b}	7.00±0.00^{d}	21.92±0.08^{a}
B6	14.17±0.01^{c}	3.75±0.00^{h}	11.90±0.00^{h}	9.51±0.71^{g}	65.91±0.01^{g}
B9	6.61±0.07^{a}	2.70±0.00^{d}	7.06±0.09^{e}	8.40±0.00^{f}	35.00±1.41^{d}

$^{A-h}$Values with different superscripts in each vertical column are not significantly different from each other ($P > 0.05$); N = 7, Cl = Mg L^{-1}, SO_4^{2-} = Mg L^{-1}, PO_4^{2-} = Mg L^{-1}, N = 3 replicates.

Presumptive tests

Using sterile pipettes, 1 ml of water samples was dispensed into two sets of 5 tubes containing 5 ml of sterile single strength MacConkey broth: 10 ml of each sample was also dispensed into a set of test tubes containing 5 ml of sterile double strength broth. Each fermentation tube contained an inverted Durham tube. Tubes containing single and double strength broth were inoculated with 1 ml of the sterile distilled water to serve as the control. The procedures were carried out aseptically and the tubes were incubated at room temperature for 48 h. After incubation, the results were recorded by looking for the presence of trapped gas bubbles inside the Durham tube as an indication of positive results. The MPN of coliforms in 100 ml of the water sample was estimated by the numbers of positive tubes and the results were checked on the MPN tables.

Confirmative tests

A loop full of the sample from positive tubes were transferred into a plate containing Eosine methylene blue agar (EMB) by streaked method and incubated at 37°C for 24 h. The agar inhibits Gram positive organisms and encourages the growth of Gram negative coliforms.

Completed tests

The organisms that grew on the confirmed test media were inoculated into nutrient agar slants and tubes of MacConkey broth. After incubation at 37°C for 24 h, the broth was checked for production of gas and a Gram stain was made from colonies in the pure cultures while the bacteria isolates were examined for stain reaction. A positive test indicated the presence of coliforms while the reverse was the case for negative test.

Characterization of isolates

The macroscopic examination for physical morphology (colour, texture, odour, etc) and microscopic examination through Gram staining and biochemical tests (coagulase, oxidase, indole, urease, methyl red, citrate utilization and sugar fermentation tests) as reported by previous researchers Baron and Sydney (1990), Benson (1990) and Bitton (1994), were used in identifying all isolates and results were matched with the Bergy's manual of determinative bacteriology (Buchanan and Gibbons, 1974) for confirmation.

Statistical analysis

Results were subjected to statistical analysis using the means ± standard deviations of triplicate experiments. Data was further subjected to one way analysis of variance and results were considered significant at $P < 0.05$ using the Duncan multiple range test.

RESULTS AND DISCUSSION

The physicochemical composition of the water samples as shown in Table 1 indicated that samples S2 and B6 whose chloride contents did not significantly differ from each other ($P > 0.05$), had the highest chloride contents among the other water samples investigated while sample B9 had the least. Chloride is an anion, and anions are essential for osmotic, ionic and water balance in fishes, in addition to contributing to the salinity of water, although excess of it has no known health implication in humans. Values obtained for all the water samples were within the reported standards given by WHO (2007) and NIS (2007) for chlorine content of drinking water.

The values obtained for sulphate in all the water samples investigated as observed in Table 1 were also within the desirable and permissible levels given by WHO (2007) and NIS (2007) for sulphates in drinking water. Phosphate is a generic term for the oxy-anions of phosphorous. Enrichment of water with organic phosphates and nitrates results in an excessive growth of plants and other micro-organisms leading to eutrophication and increased biochemical oxygen demand. Results obtained in Table 1 indicate that B6 had the highest phosphate contents among the water samples analyzed while S2 had the least.

The presence of total dissolved solids (TDS) in the water samples as observed in Table 1 indicates the presence of solid materials or solutes in water. Values obtained for all the water samples investigated showed that they fell within reported standards (NIS, 2007). B6 had the highest TDS while S2 had the least, while that of D4 did not significantly differ from sample D9 ($P > 0.05$). Organic carbon refers to the class of materials that

Table 2. Physical/organoleptic composition of water samples from 7 different locations.

Sample	Total hydrogen	Dissolved oxygen	Biochemical oxygen demand	pH	Conductivity
B5	44.19±0.04^{b}	1.52±0.00^{a}	1.07±0.21^{f}	6.14±0.01^{g}	0.81±0.01^{b}
B3	55.49±0.09^{e}	6.09±0.14^{f}	9.14±0.21^{e}	5.8±0.00^{f}	1.45±0.01^{g}
S2	89.20±0.13^{g}	8.39±0.64^{h}	3.81±0.00^{a}	6.32±0.02^{h}	1.02±0.00^{d}
B4	49.81±0.01^{c}	5.33±0.78^{c}	6.86±0.00^{d}	5.16±0.01^{c}	1.16±0.00^{f}
B7	25.78±0.01^{a}	7.62±0.14^{g}	3.81±0.71^{a}	4.91±0.02^{a}	0.89±0.01^{c}
B6	1.12±0.07^{h}	2.28±0.71^{b}	1.07±0.85^{f}	4.97±0.01^{a}	0.74±0.00^{a}
B9	50.61±0.86^{d}	6.05±6.44^{f}	1.21±0.64^{g}	5.38±0.03^{d}	1.14±0.03^{f}

Values with different superscripts in each vertical column are significantly different from each other ($P < 0.05$). Total hydrogen, dissolved oxygen and biochemical oxygen demand = mg/L; conductivity = uS/cm; N = 3 replicates.

can be extracted from water using organic solvents and it has been implicated in cancer (NIS, 2007). With the exception of sample B3, other samples investigated had higher organic carbon contents than the highest permissive values given by NIS, and this is the major significant finding in this study (Table 1). The high organic carbon contents of some of the water samples investigated underscores the need for regular tests of these water sources used for both industrial and household purposes.

In terms of total hydrogen, S2 had the highest while B6 had the least (Table 2). Dissolved oxygen refers to the oxygen present in the dissolved form in water. It is essential to all forms of aquatic life including those organisms responsible for the self purification process in natural water, as low dissolved oxygen concentrations indicates the eutrophication and biological overloading in aquaculture systems. Results obtained in Table 2 indicate that while B5 and B6 had the least dissolved oxygen, S2 had the highest.

Biochemical oxygen demand (BOD) measures the amount of biodegradable organic material present in a water sample. Polluted and waste water has higher BOD than portable and clean water because more oxygen is required to decompose the waste. B3 had the highest BOD while B5 and B6 had the least. In addition, the BOD of B2 and B7 were observed not to differ significantly from each other ($P > 0.05$). The reason behind the higher BOD content of B3 compared with other water samples remains unclear, especially as it was observed to contain the least organic carbon content among other water samples analyzed.

pH gives an idea of the H^{+} concentration of these water samples. The pHs of all the water samples analyzed were lower than the highest desirable and maximum permissible values given by WHO, 2007; NIS, 2007 and EPA, 2011.

Conductivity measures the rate at which water conducts electricity and increases with ion concentration. Values obtained for all the water samples analyzed fell within the standards of NIS (Table 2).

Hardness of water is due to metallic ions Ca^{2+} and Mg^{2+} which results in lather formation. The total hardness of the water samples as obtained in Table 3 ranged from 25.77 to 111.92. All the other water samples analyzed met the maximum permissible range reported by WHO and NIS. The nitrite level ranged from 2.8 to 8.4 mg/l. Nitrates are ubiquitous in soils and aquatic environment in association with the breakdown of organic matter by bacteria which mineralize and liberate NO^{3-} (Ano and Okwunodulu, 2008). Nitrites can lead to cyanosis and asphyxia (blue baby syndrome) in infants below the age of 3 months. All the water samples analyzed had higher nitrites than reported standards (WHO, 2007; NIS, 2007) and this is another significant finding in this study. The results obtained for the ammonium nitrogen as observed in Table 3 in all the water samples investigated were within the standards given by WHO (2007).

The test for coliform showed that all the samples of water tested positive for the presence of coliform bacteria although at different levels. The standard for good water permits not more than 10 coliform per 100 ml of water (WHO, 2007). The presence of coliform bacteria in the water samples as shown in Table 4 is an indication of fecal contamination of the water or the presence of other pathogenic organisms. However, the water samples fell within the range given by WHO, showing that drinking them may not pose any hazard. The total plate counts of all the water samples analyzed as obtained in Table 4 ranged from 8.00 to 19.09 cfu/100 ml, with sample B9 having the highest while sample B7 had the lease count.

Escherichia coli is the prime indicator of fecal pollution and its presence in water indicates recent contamination and the necessity for purification to render the water safe for consumption. The detection of *E. coli* in sample B5 as obtained in Table 4 indicates fecal contamination of the borehole (Okonko et al., 2008). The isolation of *Salmonella* and *Shigella* spp. in Table 4 is an indication of contact with fecal materials. The greatest danger associated with drinking water is contamination by human excrement. *Salmonella* species have been implicated in several indices of food poisoning such as *Salmonellosis* and typhoid fever caused by *S. typhii*.

The detection of fungi and *Staphylococcus* in some of

Table 3. Total hardness, nitrite nitrogen and ammonium nitrogen of 7 different water sources.

Water sample	Total hardness	Nitrite nitrogen	Ammonium nitrogen
B5	44.20±0.04^{b}	2.80±0.00^{a}	8.40±0.01^{d}
B3	55.49±0.08^{e}	4.20±0.00^{b}	7.03±0.04^{c}
S2	89.20±0.13^{f}	3.51±0.01^{ab}	10.25±0.35^{e}
B4	49.81±0.01^{c}	2.80±0.01^{a}	4.23±0.04^{a}
B7	25.78±0.01^{a}	4.20±0.01^{b}	8.45±0.07^{d}
B6	111.85±0.07^{g}	8.40±0.00^{c}	12.55±0.07^{f}
B9	50.61±0.86^{d}	3.19±0.98^{a}	4.80±0.28^{b}

$^{A-g}$Values with the same superscripts in each vertical column are not significantly different from each other (N = 7). Total hardness = Mg L^{-1}, nitrite nitrogen = Mg L^{-1}, ammonium nitrogen = Mg L^{-1}; N = 3 replicates.

Table 4. Microbial characterization of water samples from 7 different locations.

Sample	Coli	TPC	*Sal.*	*Shig.*	*Staph.*	Fungi	*E.coli*
B5	4.±0.0^{ab}	14.33±1.20^{cd}	4±0.58^{b}	1.67±0.88^{b}	0.33±0.33^{ab}	1.33±0.67^{a}	1.00±0.58^{b}
B3	7.33±0.88^{c}	12.67±0.67^{bc}	1.67±0.88^{ab}	1.00±0.57^{ab}	0.00±0.00^{a}	3.33±0.67^{a}	0.00±0.00^{a}
S2	5.67±0.88^{bc}	10.00±0.58^{ab}	0.33±0.33^{a}	0.00±0.00^{a}	0.67±0.33^{ab}	8.67±0.67^{b}	0.00±0.00^{a}
B4	2.00±0.00^{a}	8.33±0.33^{a}	3.67±0.88	1.00±0.88^{ab}	0.00±0.00^{a}	0.33±0.33^{a}	0.00±0.00^{a}
B7	6.67±1.76^{bc}	12.67±1.45^{bc}	1.67±0.88^{ab}	0.33±0.33^{ab}	1.00±0.58^{b}	1.00±0.58^{a}	0.00±0.00^{a}
B6	6.33±0.67^{bc}	13.67±2.03^{bcd}	2.00±0.58^{ab}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
B9	7.00±1.00^{bc}	17.33±1.76^{d}	1.53±0.88^{ab}	0.67±0.33^{ab}	0.00±0.00^{a}	8.67±1.20^{b}	0.00±0.00^{a}

$^{A-d}$Values with the same superscripts along each column are not significantly different from each other at P > 0.05 (N = 7). S.A = Sample; TPC = total plate count; Sal (*Salmonella*), Shig (*Shigella*), *Staph*. (*Staphylococcus*) and *E. coli* were expressed in percentage. Coliform, TPC and Fungi were expressed in Coliform units/100 ml.

Table 5. Organoleptic properties of water samples from 7 different locations.

Sample	Appearance	Taste	Odour
B5	Clear	Tasteless	Odourless
B3	Clear	Tasteless	Odourless
S2	Clear	Tasteless	Odourless
B4	Clear	Tasteless	Odourless
B7	Clear	Tasteless	Odourless
B6	Clear	Tasteless	Odourless
B9	Clear	Tasteless	Odourless

Results in the table represents the physical properties of water samples from 7 different locations.

the water samples in Table 4 also imply the contamination of some of these water samples. In addition, *Staph* species is known to produce enterotoxin (Okonko et al., 2008). The detection of these microorganisms in some of the water samples indicates pollution or microbial contamination of these water samples and the need for their sterilization. This is of public health significance. All the water samples analyzed were observed not to contain *Vibrio cholerea* which is another significant finding from the study, as it shows that cholorea which can be gotten from water is not endemic in the study area. The sensory evaluation of the water samples as indicated in Table 5 showed that they were all colourless, odourless and tasteless.

Conclusion

This study showed that most of the water sources used by these communities in processing their root and tuber crops did not meet the recommended standards for nitrites, biochemical oxygen demand, organic carbon and nitrites in water, underscoring the need for regular checks and on these water sources. The detection of *E. coli* in

sample B5 and some other micro-organisms in some of the water sources investigated suggest contamination of these water sources, thereby justifying the need for urgent attention to be given to these sources of water used in processing root and tuber crops.

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