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ASSESSMENT OF BACTERIAL CONTAMINATION OF IRRIGATED VEGETABLES CULTIVATED ALONG THE KUBANNI IRRIGATION SITE IN ZARIA, NIGERIA

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ABSTRACT
Bacterial contamination of vegetables becomes a significant health challenge. This study identified and categorized bacterial contaminants in vegetables cultivated along the Kubanni irrigation area in order to inform consumers about their safety and quality. Aerobic Colony Count, Gram Stain and Biochemical test was conducted on five vegetables which include cabbage, lettuce, spinach, onion and tomatoes. Samples were obtained from 25 farmlands within the study area. A total of 175 samples of vegetables, soil and irrigation water were analyzed. Result shows that all the vegetables were contaminated with bacteria above the WHO limits of 10^2. Spinach, cabbage, lettuce, onion and tomatoes recorded 5.1 x 10^6, 5.7 x 10^6, 5.2 x 10^6, 4.8 x 10^6, and 4.6 x 10^6 respectively. Cabbage had the highest ACC while tomatoes had the lowest. ACC for the Kubanni River is 4.7 x 10^6 against the WHO limit of <500. Soil recorded highest ACC (6.1 x 10^6) than irrigation water (4.7 x 10^6). Correlation analysis shows that bacteria in all the vegetables and that of soils are positively related except lettuce and tomatoes. It also shows a positive relationship between bacteria in irrigation water and all vegetables except onion and tomatoes. Bacillus is the most prevalence gram positive bacteria with 57 (45%) while Citrobacter and salmonella are the most prevalent gram negative bacteria in vegetables with 38 (30.4%) and 28%. Vegetables should be properly cleaned before consumption.

Keywords: Bacteria, Vegetables, Contamination, Kubanni, Irrigation

INTRODUCTION
Vegetables are organic materials from plant parts such as flowers, fruits, stalks, leaves, roots, and seeds (Amani and Aljahani, 2020). They are regarded as a vital part of both human and animal diets and are significant suppliers of fiber, vitamins, and minerals (Abdulkareem et al., 2012). Produce, particularly vegetables, may occasionally become contaminated as a result of farm activities. Most pollutants that are conveyed to plants come from the environment. Long-term use of human, animal, and municipal waste as manure can surely boost soil fertility but may also change the soil's physical, chemical, and microbiological makeup, increasing the possibility of soil-borne pollutants that might affect crops including vegetables (Vinod et al., 2017). Many pollutants that are present in soil can potentially be transferred to crops during planting operations (Akio et al., 2018). In most regions, a combination of human and natural factors is to blame for the poor soil quality within irrigation fields (Khaled, 2016). The human components include waste production, the use of agricultural chemicals, industrial effluents, sewage, and several other environmental activities (Ameh, and Kawo, 2017). The kind of soil, the region's geology, and various climatic elements are examples of natural factors (Emad, 2015). It is important to state that contaminants will inevitably move to crops and individuals as long as they are present in irrigation water, soil and other components of the environment. According to Ould et al. (2010), irrigation water, soil, fertilizers, manure, insects, and dust are all possible ways for pathogens to enter the vegetables. Also, Nang et al. (2021) stated that organic manure, wastewater and contaminated soil are major sources of vegetable contamination.

Agriculture that uses irrigation needs a reliable supply of water. In many nations, the agricultural industry is severely hampered by the environment's uneven distribution of water resources. In some areas, particularly in peri-urban areas of poor countries, this forces farmers to use wastewater for irrigation as a viable alternative (Ogundele et al., 2017). Over 10% of irrigated farms use wastewater from industrial and municipal waste globally (Hassan et al., 2021).

Fresh vegetables may be contaminated with different pathogenic bacteria from different source within the Kubanni irrigation site. Bacteria may be present in tissue of plants or dispersed in the air, soil, and water. The influx of pathogenic micro-organisms from many sources into the environment may contaminate food (Desta and Diriba, 2016).

The main transportation routes for pathogens that cause food poisoning are soil and wastewater used for irrigation. According to Traore et al. (2022), many residents of African cities pour waste and sewage into waterways (streams and rivers), where it might then spread diseases to people if the water is used to irrigate crops. This may result to changes to vegetables in appearance, texture, taste, and smell as a result of the waste products produced by bacteria as they multiply. Bacterial contaminants make vegetables have strange smell and may be discolored and turn brown, green, or grey. Estimate shows that germs on fresh vegetables cause a large percentage of foodborne illnesses in many parts of the world (Khaled, 2016).

Bacterial contaminants were assessed in the Samaru stream and Shika Dam by Vincent et al. (2012) and Mohammed et al., 2020). However, their study relied solely on irrigation water quality and other elements like soil and vegetables were neglected. There was no attempt to analyze bacteriological quality of vegetables prior to harvest in the Kubanni irrigation site, this study intend to analyze bacterial contaminants in soil, irrigation water and farm produce (vegetables) in the farm. This is in consideration with the amount of liquid waste produced in the catchment area, such as that from the Zango abattoir and other municipal waste that is drained into the river and used for irrigation. Also in this study, isolation and characterization of bacterial contaminants in soil, water and vegetables was carried out. This is because different bacteria have different effects on human health while some can be beneficial to man. None of the previous studies was able to characterize the different bacteria in water, soil and vegetables within the Kubanni irrigation site prior to harvest.

MATERIALS AND METHODOLOGY

The study Area

As seen in Figure 1, the Kubanni Drainage Basin lies between Latitudes 11° 2' 0'' and 11° 12' 0'' North and Longitudes 7° 34' 0'' and 7° 48' 0'' East. Its overall area was around 21 km². Rainfall is 80 mm per hour on average, with an annual total of 1100 mm. Most rainfall occurs as a result of convection (Mangaji et al., 2020). The majority of the vegetation is grassland, with a few isolated trees of both local and alien species, such as shea butter, *Upaca togonensis, Isoberlinia indica, Isoberlinia doka,* and *Isoberlinia tomentosa* (Abdulkadir et al., 2024).

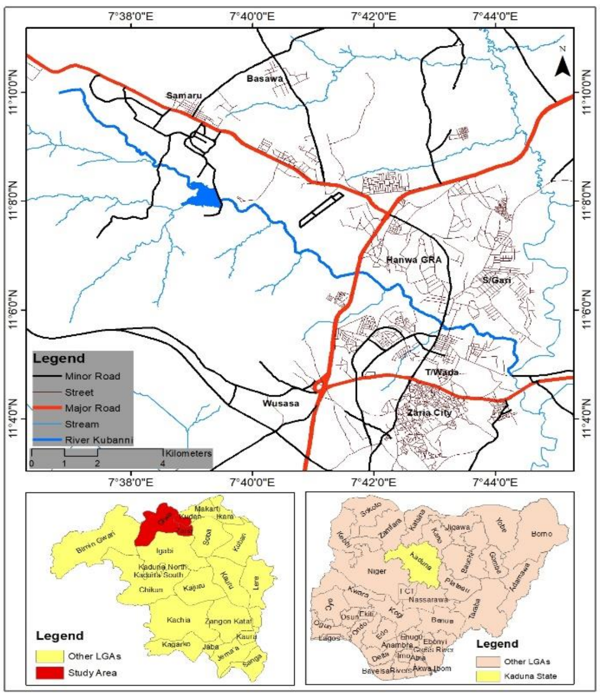

Figure 1: Zaria and its Environ Showing the Study Area
Source: Adapted from the administrative map of Kaduna state.

Sampling Technique

Soil Sampling

Twenty five (25) farmlands were selected as sampling points. A Randomized Complete Block Design (RCBD) approach was employed. In agricultural experiments, it is the norm to group similar experimental units into blocks or replicates. It is employed to account for spatial effects in the field and control variation in an experiment. Slope, color, texture, management, and cropping pattern variations were observed through a visual survey of the field. After the fields were divided into uniform sections, ten samples were taken at random using a soil auger at a depth of 20 cm. This is a result of plants' shallow root systems, which are only 20 cm deep. After carefully mixing all ten samples in a single bucket, they were spread out on a sanitized sheet. The sample was cleared of pebbles, gravel, roots, and surface litter. Four portions of the sample were taken, and one was placed in a plastic bag. The bags were appropriately labeled including date, crop kind, and location (Abdulkareem et al., 2012). The samples were sent right away for processing and examination to Ahmadu Bello University's microbiology lab in Zaria.

Vegetable Sampling

A random sampling technique was employed to gather vegetable samples. A number of units were created out of the farmlands. Ten units were chosen at random to be sampled. Ten samples overall were taken from each farmland, with one sample taken from each unit. A total of 250 vegetable samples were gathered and homogenized to create 25 composite samples using a suitable sampling technique because there are five different vegetable varieties chosen for examination in this study. Five vegetables were chosen since they are the most widely consumed in the research area. They consist of cabbage, tomatoes, onions, spinach, and lettuce.

Water Sampling

Water for irrigation was collected nearer to each of the chosen farms. Twenty-five water samples were purposefully taken for analysis because there are twenty-five farmlands in the research. The water samples were taken 5 cm below the water's surface because it is the depth at which irrigation water is extracted from the river (Yakubu et al. 2017).

Microbial Analysis

To assess the level of bacterial pollutants in irrigation water, soil and vegetables within the study area; Aerobic Colony Count (ACC) method was used for isolation and enumeration of total bacteria from the samples. To characterize the bacteria into gram positive or negative, gram staining was performed. To confirm the type of bacteria identified from each sample, a biochemical test was conducted and seven tests were performed which include Indole, Methyl red (MR), Vogus Proskau (VP), Urease, Catalse, and Triple Sugar Iron (TSI) as done by Danjuma, (2017) and Iyevhobu et al., (2023).

Data Analysis

Descriptive and inferential statistics were both employed in this research. Some of the data were analyzed using Microsoft Excel tables and charts, while SPSS software version 20.0 was used to perform ANOVA and Peason's Correlation coefficient.

RESULTS

Bacteriological Quality of Vegetables, Soil and Water

To assess the bacteriological quality of all the samples, Aerobic Colony Count (ACC) analysis was conducted. The result for vegetable, soil and water are contained in Table 1. It shows that all the vegetable samples analyzed contain some amount of bacteria. Spinach, cabbage, lettuce, onion and tomatoes recorded 5.1 x 10^6, 5.7 x 10^6, 5.2 x 10^6, 4.8 x 10^6, and 4.6 x 10^6 respectively. The ACC results for all the vegetables are above the WHO standard of 10^2. The two main sources of pre-harvest contamination of vegetables considered in this study include irrigation water and soil. The ACC result in Table 1 shows that total viable count for the Kubanni River is 4.7 x 10^6, this is far beyond the WHO standard of <500. Soil had ACC value higher (6.1 x 10^6) than what was observed in irrigation water.

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Table 1: Aerobic Colony Count and the WHO limits

Vegetable Type	No of Samples	Mean total bacteria count (CFU/g)	STD	WHO Limit (CFU/g)
Spinach	25	5.1×10^6	±1.07	10^2
Cabbage	25	5.7×10^6	±1.13	10^2
Lettuce	25	5.2×10^6	±0.94	10^2
Onion	25	4.8×10^6	±0.99	10^2
Tomatoes	25	4.6×10^6	±0.73	10^2
SOURCES OF BACTERIA CONTAMINANTS				
Soil	25	6.1×10^6	±1.08	N/A
Water	25	4.7×10^6	±1.23	<1000

Source: Laboratory Analysis, 2024

Difference between Bacteria contaminants in Vegetables and their Sources

To test the statistical difference between ACC in vegetables and sources of contaminants which include irrigation water and soil, T-test was used. According to the T-test results shown in Table 2, there is a significant difference between the level of bacteria in spinach and soil (0.000248) but not between bacteria in spinach and irrigation water (0.074). There is a significant difference (0.003) between the bacteria in cabbage and irrigation water, but not with soil bacteria (0.091). There is no significant difference between bacteria in lettuce and irrigation water (0.068) but there is with soil (0.002). The level of bacteria in onion does not significantly differ from that of irrigation water (0.37) but does significantly differ from that of soil (0.0001). Furthermore, tomatoes show a significant difference (0.0002) with soil but not with irrigation water (0.401).

Table 2: T-test for total bacteria count

Vegetable Type	Water	Soil
Spinach	0.074	0.000248*
Cabbage	0.002529*	0.09146
Lettuce	0.067921	0.002226*
Onion	0.36767	0.000133*
Tomatoes	0.401294	0.000228*

Source: Author's Analysis 2024 P<0.05, *= there is a significant difference

Relationship between Bacteria Contaminants and their Sources

To assess the relationship between bacteria contaminants in vegetables and their sources (soil and water), Pearson's Correlation Coefficient analysis was performed as shown in Table 3. It shows that bacteria from all vegetables and that of soils are positively related except for lettuce and tomatoes which are negatively correlated. It also shows a positive relationship between bacteria in irrigation water and all the selected vegetables except onion and tomatoes which shows a negative correlation.

Table 3: Correlation Analysis between Vegetables and Sources of contaminants

	spinach	*cabbage*	*Lettuce*	*Onion*	*Tomatoes*	*Soil*	*Water*
Spinach	1						
Cabbage	0.035818*	1					
Lettuce	0.175779	0.048479	1				
Onion	0.111914	0.112603	-0.38926	1			
Tomatoes	-0.42415	-0.09375	-0.25043	0.088256	1		
Soil	0.326225	0.194104	-0.08971	0.096717	-0.30122	1	
Water	0.38767	0.053386*	0.124755	-0.10645	-0.33897	0.055938*	1

Source: Author's Analysis, 2024 *Correlation is significant at 0.05 level (2 tailed)

Characterization of Bacteria in Vegetables, Soil and Water

The two main classes of bacteria which include the gram positive and gram negative bacteria were isolated and characterized using gram staining and biochemical test as shown in Table 4 and 5. The biochemical test conducted include Indole, methyl red (MR), Vogus Proskau (VP), urease, catalse, and Triple Sugar Iron (TSI). This enables us to identify the bacteria type in the samples. Table 4 shows the morphological and biochemical characteristics of gram positive bacteria observed in the study. Gram stain was conducted using Manitol Salt Agar (MSA) for the morphological features, it is one of the medium which allows gram positive bacteria to grow and prevent others from growing.

Table 4: Gram Positive Bacteria

Gram Reaction/Cell Morphology	Indole	MR	VP	Citrate	Catalase	Urease	Possible Organism
+ rod	-	+/-	+/-	+/-	-	-	Bacillus
+ cocci	-	+	+	-	+	+	Staphylococcus
+ cocci	-	+	+	-	-	-	Enterococcus
+ rod	+/-	+	-	-	-	-	Clostridium
+ cocci	-	+	-	-	-	-	Micrococcus

TSI= Triple Sugar Iron, VP= Voges Proskau, MR= Methyl Red

Table 5 shows the morphological and biochemical behaviours observed from bacteria culture under EMB and SSA media after gram staining. Both EMB and SSA culture shows that all the gram negative bacteria had a rod shape appearance and clustered cell arrangement (see Plate III).

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Table 5: Gram Negative Bacteria

Gram Reaction/Cell Morphology	Indole	MR	VP	Citrate	TSI			Urease	Possible Organism
					Slant	Butt	H_2S		
- rod	-	+	-	+/-	R	Y	+	-	Salmonella
- rod	-	+	-	-	Y	Y	+/-	+/-	Citrobacter
- rod	-	+/-	+/-	-	R	Y	-	+/-	Serratia
- rod	-	+/-	+	+	Y	Y	-	+	Klebsiella
- rod	+/-	+	+/-	-	R	Y	-	+	Yersinia
- rod	+	+	-	+/-	R	Y	+	+	Proteaus
- rod	+	+	-	-	Y	Y	-	-	E. coli
- rod	-	-	-	+/-	R	R	-	+/-	Pseudomonas
- rod	+/-	+	-	-	R	Y	-	-	Shigella
- rod	+	+	+/-	+/-	Y	R	-	-	Aeromonas

H_2S= **Hydrogen Sulfide test, R= Red, Y= Yellow**

Prevalence of Gram Positive Bacteria in Vegetables
The prevalence of gram positive bacteria isolated in vegetables within the study area is shown in Figure 2. The gram positive bacteria isolated include bacillus, staphylococcus, enterococcus, clostridium and micrococcus. It also shows that bacillus is the most prevalence among all the vegetable samples which recorded 57 (45%).

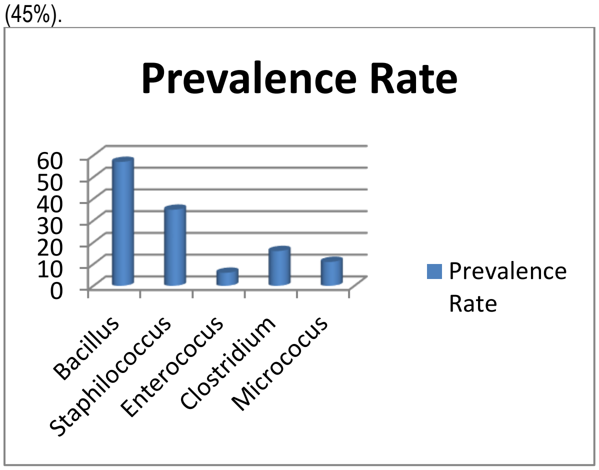

Figure 2: Gram positive Bacteria in Vegetables

Distribution of Gram Positive Bacteria in each of the Selected Vegetables
Figure 3 show that bacillus is the most prevalent bacteria in spinach with 11 (44%). This is followed by staphylococcus with 8 (32%). Enterococcus represents 6 (24%). It is free from other gram positive bacteria. For Cabbage, bacillus is the most prevalence 13 (52%) followed by staphylococcus 6 (24%), clostridium 5 (20%) and micrococcus 1 (4%). Lettuce also had bacillus as the most prevalence 14 (56%) followed by staphylococcus 5 (20%), clostridium 4 (16%) and micrococcus 2 (8%). Bacillus had highest prevalence rate 11 (44%) in onion followed by staphylococcus 9 (36%), clostridium 3 (12%) and micrococcus 2 (8%). In the same way, Tomatoes had bacillus as the most prevalent 8 (32%), followed by staphylococcus 7 (28%), clostridium 4 (16%) and micrococcus 6 (24%).

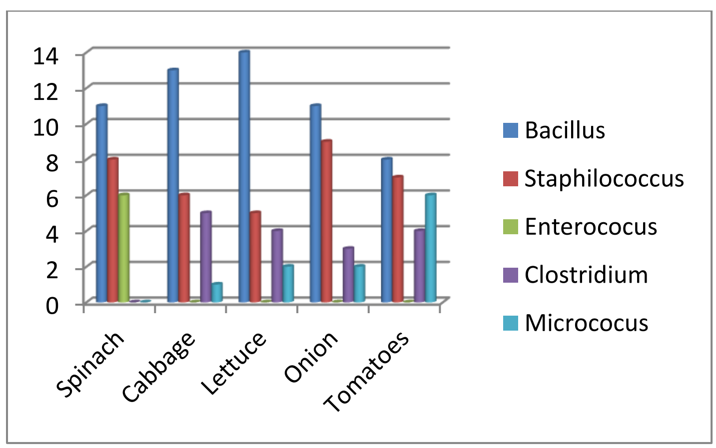

Figure 3: Distribution of Bacteria in Vegetables

Distribution of Gram Positive Bacteria in Soil and Water
Based on the results in Figure 4, irrigation water hosts larger proportion of bacillus and staphylococcus (36%) than soil (32%). Enterococcus was not isolated in both water and soil. Clostridium

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is absent in soil but present in water. Micrococcus is present in both soil and water.

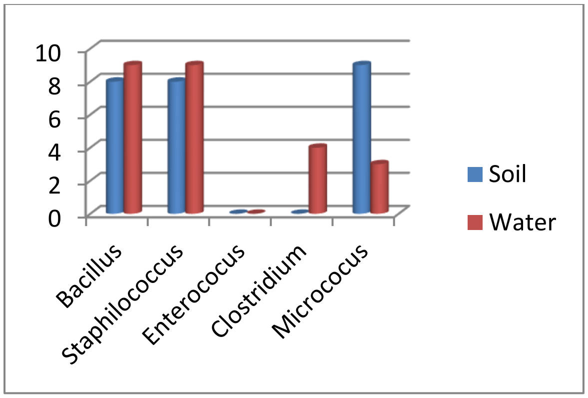

Figure 4: Sources of Gram Positive Bacteria

Prevalence of Gram Negative Bacteria using EMB

Figure 5 contains result for biochemical test using Eusine Methyline Blue (EMB) media. The EMB is a selective culture medium for gram negative bacteria. The gram negative bacteria identified in all the samples include salmonella, citrobacter, serratia, klebsiella, yersinia, proteaus, and *E. coli*. Citrobacter recorded 38 (30.4%) of all bacteria isolated in EMB media from vegetables within the Kubanni irrigation site. Klebsiella species are the second most prevalent gram negative bacteria representing 27.2%. The third most prevalence bacterial contaminant in the selected vegetable is *Serratia species*. It recorded 24 positive cases equivalent to 19.2%. Salmonella represents 12 (9.6%) of all bacteria isolates in vegetables. Proteus was observed only in onion and tomatoes with a prevalence rate of 16%, other vegetables contain no proteaus in them. *E. coli* is only found in tomatoes from 5 samples equivalent to 4% of all the bacteria identified in the selected vegetables. Yersinia had the lowest positive cases in the study area (3.2%).

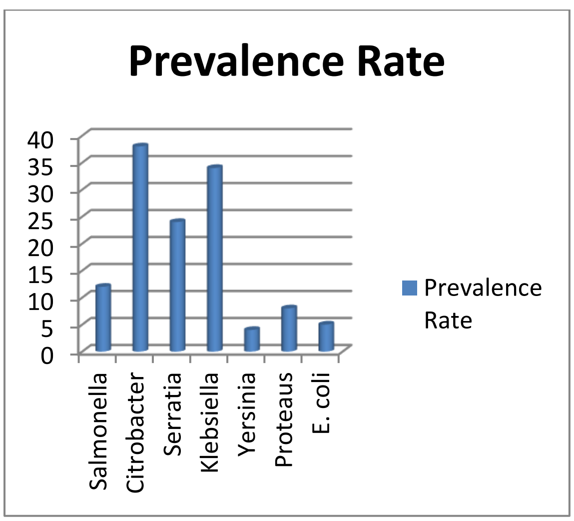

Figure 5: Gram Negative Bacteria from EMB

Distribution of Gram Negative Bacteria in the Selected Vegetables using EMB

As indicated in Figure 6, Citrobacter had the highest population in spinach (10) representing 40% among all the gram negative bacteria isolated in spinach under EMB medium and it is the most prevalent bacteria. This is followed by klebsiella with 9 (36%), serratia is next with 5 (20%) and lastly salmonella with only 1 (4%). Serratia is the most prevalent bacteria in cabbage with 9 (36%), it is followed by citrobacter and klebsiella which recorded 8 (32%) each. In lettuce, citrobacter is the most prevalent bacteria with 9 (36%) followed by serratia with 7 (28%), salmonella had 5 (20%) and klebsiella recorded 4 (16%). Onion had citrobacter and klebsiella as the most prevalent with 9 (36%) each followed by proteaus 4 (16%), serratia 2 (8%) and the least is Yersinia with only 1 (4%). Tomatoes had salmonella as the most prevalent with 6 (24%) followed by *E. coli* 5 (20%), klebsiella and proteaus had 4 (16%) each, Yersinia 3 (12%), citrobacter 2 (8%) and serratia 1 (4%).

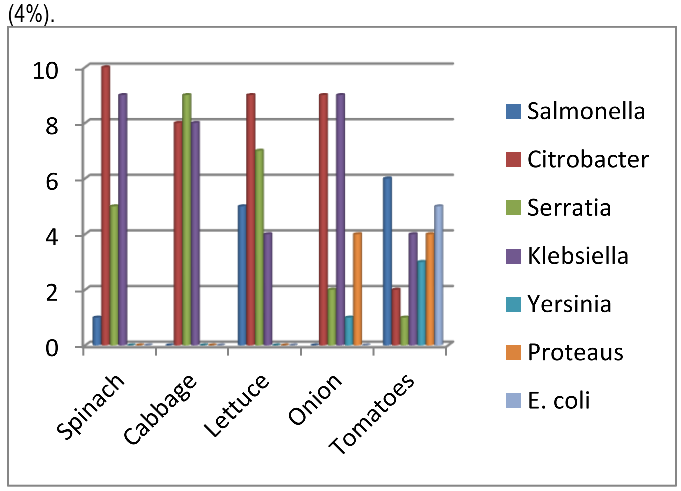

Figure 6: Distribution of gram negative bacteria in Vegetables from EMB

Sources of Gram Negative Bacteria from EMB

As indicated in Figure 7, the two sources of contaminants analysed shows that soil contains no salmonella while irrigation water recorded 8% of all gram negative bacteria cultured using EMB media. Both soils and water contains *Citrobacter species* with 24% and 8% prevalence rate indicating the possible sources of the organism. Serratia was observed only in soil at 16% prevalence rate. Both soil and irrigation water within the study region host *Klebsiella species* with 40% and 16% prevalence rate each. This is an indication that the organism originated from both soil and water.

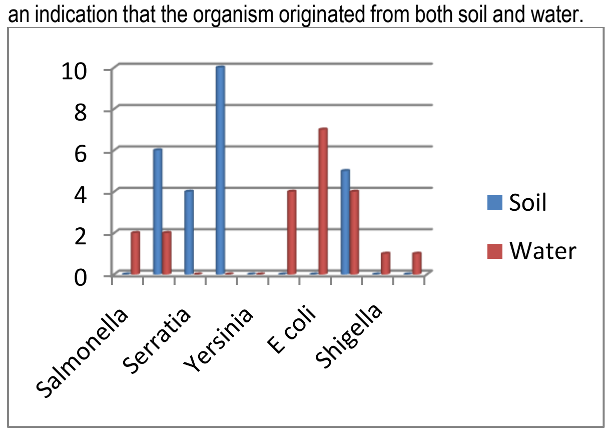

Figure 7: Distribution of Gram Negative Bacteria in soil and Water under EMB

Gram Negative Bacteria in Vegetables using SSA

To further assess the presence of other harmful gram negative bacteria in all vegetable samples in the study area, Salmonella Shigella Agar (SSA) medium was used. The result is shown in Figure 4. The SSA medium is a differential selective media used for the isolation of gram negative bacteria such as salmonella, shigella and others within the gram negative group. This medium is used because some bacteria like salmonella and shigella may fail to grow on EMB medium. As shown in Figure 8, *Salmonella species* is the most prevalence bacteria with 28% in the SSA medium. *Klebsiella species* is the second most prevalence bacteria representing 20% of all the gram negative bacteria identified using SSA medium. Proteaus is the third most prevalent representing 16.4% of all gram negative bacteria under SSA culture. The fourth most prevalent is *Citrobacter species* which represents 15% of all the gram negative bacteria in vegetable samples. Shigella represents 10% of bacteria identified in vegetable samples under SSA medium making it the 5th most prevalent. Population of *Serratia species* under SSA culture stood at 6% among all the gram negative bacteria. It is ranked 6th most prevalent. *Yersinia species* under SSA represents 3% of all the gram negative bacteria and is the 7th most prevalent. *Aeromonas species* is the least contaminant identified in vegetables within the study area. It represents 1.6% prevalence rate among all bacteria contaminants in samples analysed for this study.

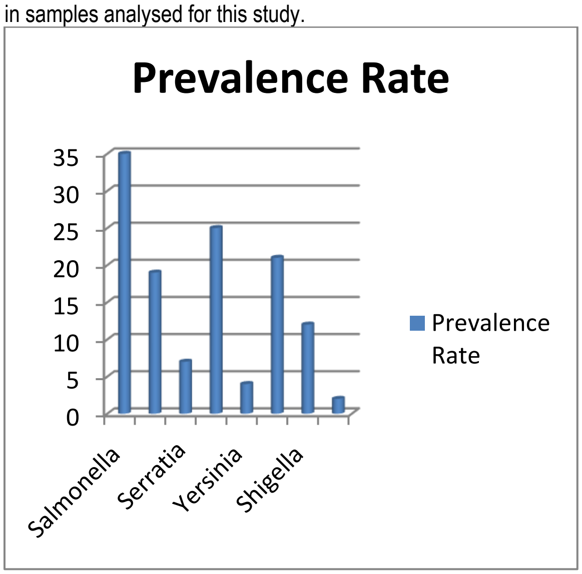

Figure 8: Prevalence of Bacteria from SSA

Distribution of Gram Negative Bacteria in Vegetables from SSA

Figure 9 shows the distribution of bacteria from SSA media in each of the chosen vegetable. Unlike the result for EMB test, the SSA shows that *Salmonella species* are present in all the vegetable samples in different proportions. In spinach, salmonella is the most prevalence with 8 (32%) followed by shigella 7 (28%), klebsiella and Yersinia 3 (12%) each, and the lowest is citrobacter 2 (8%). In cabbage, salmonella and proteus are the two most prevalence with 8 (32%) each followed by citrobacter 5 (20%), and the least is klebsiella 4 (16%). Lettuce had salmonella as the most prevalence with 9 (36%) followed by klebsiella 8 (32%), citrobacter and proteaus each having 4 (16%). The most prevalence bacteria in onion is proteaus with 8 (32%) followed by serratia 5 (20%), citrobacter 4 (16%), salmonella and klebsiella each having 3(12%) and the least is Aeromonas 2(8%). Onion is the only vegetable that contains *Aeromonas species*. Tomatoes had salmonella and klebsiella as the most prevalence with 7 (28%) each followed by shigella 5 (20%), citrobacter 4 (16%), the least are Yersinia and proteaus each having 1(4%). Only spinach and tomatoes are affected with shigella while other vegetables are free from contamination with *Shigella species*.

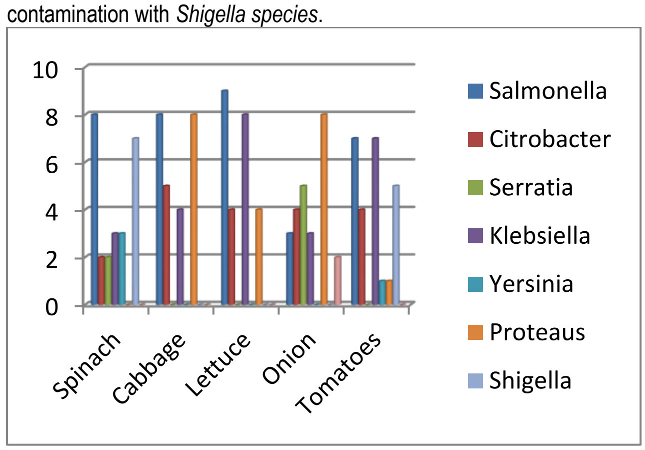

Figure 9: Gram Negative Bacteria in Vegetables from SSA

Gram Negative Bacteria from SSA Culture of Soil and Water Samples

As indicated in Figure 10, Salmonella had soil with 0 (0%), irrigation water 4 (16%); citrobacter: soil 4 (16%), water 5 (20%); Serratia: soil 2 (8%), water 4 (16%); Klebsiella: soil 7 (28%), water 2 (8%); Yersinia: soil 7 (28%), water 0 (0%); Proteus: soil 5 (20%), water 7 (28%); Shigella: soil 0 (0%), water 3 (%) and Aeromonas 0 (0%) for both soil and water.

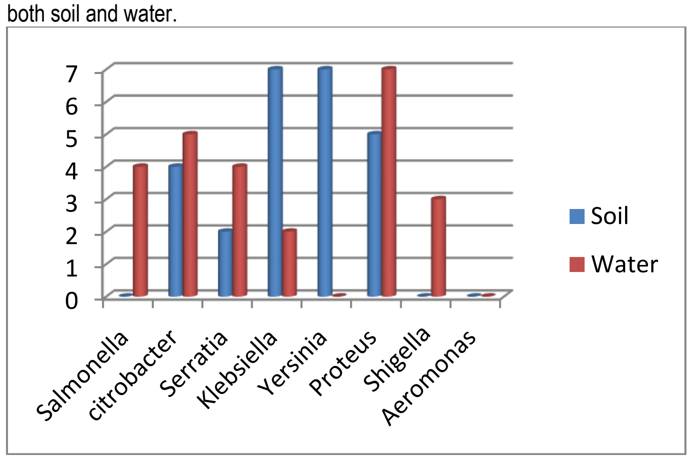

Figure 10: Distribution of Gram Negative Bacteria from SSA

DISCUSSION

In this study, the quality of irrigated vegetables prior to harvest, soil and irrigation water were analyzed. This is followed by characterization of the isolates. The ACC result shows that cabbage recorded highest level of total bacteria count while tomatoes are the least affected by bacteria within the study region. Comparing the result with the findings of Nang et al. (2021), they also observed higher level of bacteria in cabbage than other vegetables cultivated along Vea River in Ghana. This could be due to layering of leaves in cabbage which host larger number of bacteria contaminants than all other vegetables considered in this study. It could also be the case for lettuce; the order of contamination by bacteria based on the result for ACC in Table 1 is cabbage > Lettuce > spinach > onion > Tomatoes. The level of bacteria contaminants observed in this study is far beyond the WHO threshold. This concur with the findings of Vinod et al. (2017) who reported that the level of bacteria contaminants from irrigation water in the Saharanpur province, India is by far beyond the Indian standard for irrigation water quality. Mohammed et al., 2020) also observed higher level of bacteria beyond the WHO/FAO limits in Shika Dam, Zaria, Nigeria. Desta and Diriba (2016) reported that irrigated vegetables were contaminated with bacteria from the Awetu River in Ethiopia. Wastewater used for irrigation from nearby River is suspected to be possible source of bacteria contaminants in vegetables. This could also be the case for vegetables within the Kubanni irrigation field as liquid waste generated within the Kubanni river basin are channeled down the main River which is utilized for irrigation in the study area (see Plate V and VI). The ACC result shows that soil contains highest amount of total viable bacteria than irrigation water within the study area. It may be attributed to intensive use of organic manure by irrigation farmers. Higher level of bacteria observed in vegetables within the Kubanni irrigation site means that consumption of such produce without proper cleaning will pose greater risk.

Based on the result of the correlation analysis, we can conclude that the sources of bacteria observed in spinach and cabbage are from both soil and irrigation water. The source of bacteria in lettuce is irrigation water and other sources but not soil while those observed in onion is from soil and other sources but not irrigation water. However, the sources of bacteria observed in tomatoes are neither from soil nor irrigation water. Other sources of bacteria include air and intruding animals. The result also shows the relationship between bacteria from one vegetable and others that were selected in this study. This is because a mixture of different vegetables in the same farmlands is observed in the study area which increases the chance for cross contamination.

Some of the gram positive bacteria isolated in this study exhibit rod shape morphology while some had cocci (spherical) cell morphology (see Plate I and II). The Gram stain is retained by the thick peptidoglycan coating found in the cell walls of gram-positive bacteria. Gram-positive bacteria identified include Bacillus, Staphylococcus, Enterococcus, Clostridium and micrococcus. Numerous illnesses, such as infections of the skin and soft tissues, lung infections, and gastrointestinal infections, can be caused by gram-positive bacteria. They may also create poisons within them or cause food poisoning (Traore et al., 2022).

.

The EMB and SSA medium were used to culture gram negative bacteria because some salmonella and *Shigella species* may not be able to grow under EMB culture. This will help to ensure a broader characterization of the bacteria in different samples under this study.

Gram-negative bacteria are resistant to the Gram stain because they have an outer membrane made of lipopolysaccharides and a thin layer of peptidoglycan in their cell walls. The gram negative bacteria isolated include salmonella, citrobacter, serratia, klebsiella, Yersinia, proteaus, *Escherichia coli (E. coli)*, pseudomonas, shigella, and aeromonas. Gram-negative bacteria are understood to cause many health challenges such as food poisoning, diarrhea, respiratory infections (like pneumonia or bronchitis), urinary tract infections, bloodstream infections (like sepsis), meningitis, and sexually transmitted infections like gonorrhea and chlamydia (Mohammed et al., 2019).

The biochemical test using MSA medium shows that bacillus is the most prevalence among all the vegetable samples. This is against the findings of Iyevhobu et al. (2023) who reported that staphylococcus is the most prevalent bacteria contaminants in vegetables. This could be due to favourable environmental conditions in the Kubanni irrigation site which enable bacillus to grow and thrive. Some of these requirements include a temperature between 25^{o}C to 37 ^{o}C though some species may require lower or higher temperature but majority of them are within that range. They also require abundant moisture which is available within the Kubanni due to regular supply of irrigation water (Ameh and Kawo, 2017). Continuous use of organic manure from municipal dumpsites including that of human and animal waste (see Plate IV) also provide enough nutrients for bacillus. *Bacillus species* are heterotrophic and hence require abundant organic nutrients to grow. The ability of bacillus to form endospores which makes them highly resistance to hash environmental conditions such as heat, radiation and chemicals could be another reason for higher number of isolates in vegetables within the Kubanni irrigation area.

Some *Bacillus species* are beneficial while some are harmful but overall, they play important role in an ecosystem as they are used in pharmaceutical, agricultural and food processing industries (Sisay, 2017). The beneficial ones are used to produce antibiotics and insecticides. Some pathogenic bacillus includes *Bacillus anthracis* which causes anthrax and *Bacillus cereus* which causes food poisoning (Ulfata et al., 2024). Regular testing, improved agricultural practices, proper cleaning and disinfecting of vegetables are necessary steps to prevent bacillus infection through vegetable consumption within the study area.

Staphylococcus is ranked the second most prevalent bacteria observed in this study which were found in 35 samples representing 28% of all the gram positive bacteria in vegetables. Favourable environmental conditions are the reasons for higher population of staphylococcus observed in the study area. Temperature requirement is between 25-40 ^{o}C. They also require adequate moisture to grow and are abundant through regular supply of irrigation water (Ogundele et al., 2017).

A wide range of infections can be caused by staphylococcus which include respiratory infection like pneumonia, skin infection like boils and abscencess and infection of the borne also known as osteomyelitis (Nang et al., 2021). Effective hand hygiene, cleaning and proper cooking of vegetables can help to reduce infection caused by staphylococcus bacteria within the study area.

Enterococcus had the lowest prevalence rate among all the gram positive bacteria identified in vegetable samples with only 6 (5%). This is because it was only isolated in spinach, the optimum temperature requirement for *Enterococcus species* is between 35-37 ^{o}C (Mohammed et al., 2020). Because the study area is a flood plain, temperature may be lower since the time of data collection for this study was during the Hammatan cold between December to February. Enterococcus can cause many health challenges such as urinary tract infections, meningitis, skin and soft tissue infections and gastrointestinal problems. Proper farming practices like irrigation management, vegetable testing and washing can help to reduce enterococcus contamination.

Clostridium had (16) 13% prevalence rate among all the gram positive bacteria. Population of clostridium is low compared to bacillus because they require high moisture (80%) to grow and are anaerobic (Khaled et al., 2016). The selected vegetables are not cultivated under waterlogged condition and hence, fewer clostridia were observed. Clostridium also forms endospores which makes them highly resistance to environmental conditions like heat, chemicals and radiation.

Some clostridium bacteria are beneficial like *Clostridium butyricum* which produces butyric acid. It is a compound which enhances gut health. Pathogenic clostridium includes clostridium tetani which causes tetanus and *Clostridium perfringens* which causes food poisoning (Daniel et al., 2020). Washing of equipment and improved sanitation can reduce the spread of *Clostridium species* in vegetables.

Micrococcus species represents (11) 9% of all gram positive bacteria identified in vegetables. The population of Micrococcus is low compared to bacillus and staphylococcus. This is because farmers may not be able to supply excess moisture required by micrococcus (60-80%) due to competition for irrigation water within the Kubanni irrigation site. Most farmers irrigate their crops once in every week. They are typically not harmful but may infect people with compromised immune systems (Honorine et al., 2022). They play vital role in an ecosystem in terms of organic matter decomposition and enzyme production. They are used in industries such as pharmaceutical for antibiotic production, cosmetic, food production and biotechnology (Joep, 2017).

The two sources of contamination analysed in this study are soil and irrigation water. Knowledge about the sources of bacterial contamination is crucial for effective management of risk and infections caused by the organism. The presence of bacillus in irrigation water and soil is an indication of possible transmission to vegetable samples within the study area. The presence of staphylococcus in irrigation water and soil also suggests that the bacteria in vegetables originated from there. Both soil and water samples were found to contain zero *Enterococcus species* which means the bacteria observed in vegetables is from another source other than soil and water. Other sources of contaminants include air and excreta from intruding animals (Karshima, 2020).

Clostridium was identified only in irrigation water but absence in soil samples. This means that water is suspected to be the possible source of most of the clostridium species observed in vegetables within the study area. The presence of micrococcus in both soils and water suggest that the organism was transferred to vegetables in the Kubanni irrigation site.

Since both soil and irrigation water was contaminated with different bacteria, it can be reduced through effective waste management, regular environmental monitoring and decontamination of the sources using chemicals.

Higher level of Citrobacter in Vegetables isolated from the EMB culture could be due to favourable environmental conditions within the study area. The optimum temperature required by *Citrobacter species* is between 30-35 ^{o}C. They can survive both anaerobic and aerobic conditions. They need a moderate moisture level usually between 60-80%. Some species of Citrobacter can cause infections in humans, particularly in people with compromised immune systems. Few examples include *Citrobacter freundii* which causes urinary tract infections (UTIs). The symptoms include burning during urination, frequent urinary and abdominal pain. Another example is *Citrobacter braakii* which causes UTIs, pneumonia, cerebral disorder, arthritis, and abdominal pain (Bagudo et al., 2014). Improved hygiene and proper cleaning and cooking of vegetables are some of the easy ways to reduce citrobacter infection within the study area.

Klebsiella have almost a similar environmental requirement with *Citrobacter species* which enables them thrive better in the Kubanni irrigation area. They can cause infections in humans, particularly in people with compromised immune systems. Some few species of Klebsiella include *Klebsiella pneumonia, Klebsiella oxytoca* and *Klebsiella variicola*. Their health effects include Pneumonia UTIs, Bloodstream infection, Meningitis, Wound infections and infections along surgical sites (Herman et al., 2015).

Serratia species were observed in vegetables within the study area. Examples include *Serratia marcescens, Serratia liquefaciens, Serratia rubidaea,* and *Serratia fonticola*. Their major health effects include UTIs, bloodstream infections, pneumonia, meningitis, wound infections and eye infections (Getaneh et al., 2018). Proper hand hygiene, cleaning and cooking of vegetables are important steps to reduce infections caused by *Serratia species*.

Salmonella species isolated in vegetable samples under the EMB culture represents 12 (9.6%) of all bacteria isolates in vegetables. It is the fourth most prevalence bacteria isolated in vegetables within the study area. The result is above the level observed by Raufu et al. (2014) who reported a prevalence rate of 6.3% in vegetables cultivated in Maiduguri, Nigeria. Bagudo et al. (2014) also reported a higher prevalence rate of *Salmonella species* (19.5%) in vegetables cultivated around abattoir area in Sokoto. Infection with salmonella can be severe and life threatening in some people like elderly, young children, pregnant women and people with a weak immune system. Many species of salmonella exist. For example *Salmonella typhi* which causes typhoid fever, *Salmonella paratyphi* causes paratyphoid fever and so on. Some symptoms of salmonella infections include fever, headache, diarrhoea, vomiting and abdominal cramps. Proper handling and adequate cooking of vegetables, hygiene and sanitation, water purification are some of the few ways to reduce infection caused by salmonella bacteria (Kayla et al., 2017).

Proteus is the fifth most prevalence bacterial contaminant of vegetable within the study area. Some common examples of *Proteus species* include *Proteus mirabilis, Proteus vulgaris* and *Proteus hauseri*. Infections caused by Proteus include Urinary tract infections (UTIs), bloodstream infections, meningitis, pneumonia, wound infections and surgical site infections (Gadafi et al., 2020). Improved hygiene, appropriate cleaning and cooking of vegetables can reduce infections caused by Proteus bacteria in the study area.

Presence of *E. coli* in vegetable as shown in Figure 5 is an indication of faecal contamination within the study area. *E. coli* is a common cause of foodborne illness, and outbreaks have been associated with contaminated food and water. They can also produce toxins, such as Shiga toxin, which can cause severe illness (Kayla et al., 2017).

Low population of *Yersinia species* (3.2%) could be due to high temperature in the study area. The optimum temperature requirement for Yersinia is between 25-28 ^{o}C and temperature can sometimes go as high as 38 ^{o}C in the study area. *Yersinia species* in vegetable was only observed in tomatoes and onion (Desta et al., 2016). Other vegetables are free from Yersinia contamination. Some species of Yersinia can cause infections in humans. Examples include *Yersinia pestis, Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. They can cause a wide range of infections such as Bubonic plague, pneumonic plague, Gastroenteritis and bloodstream infections (Herman et al., 2015). Infections caused by Yersinia can be prevented through proper

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hand hygiene, sanitation, cleaning and cooking of vegetables within the study area.

Salmonella was detected only in irrigation water samples under the EMB culture. However, this will be confirmed with further analysis using Salmonella Shigella Agar (SSA) medium. The source of salmonella contaminants in irrigation water could be from liquid wastes especially sewage that are drained into the River from nearby communities.

The source of citrobacter could be from both soil and water contains as indicated in Figure 7. Serratia is absent in water and therefore the source of contamination with *serratia species* could be soil and other environmental components but not from irrigation water. Both soil and irrigation water within the study region host *klebsiella species* with 40% and 16% prevalence rate each. This is an indication that the organism originated from both soil and water. *Yersinia species* were not isolated in both soil and irrigation water using the EMB media. Yersinia is a genus of gram-negative, rod-shaped bacteria that are commonly found in the environment and in the gastrointestinal tracts of animals (Danjuma, 2017) . This means the organism is from a different environmental component other than soil and water used for irrigation. The source of proteaus could be irrigation water but not soil as it recorded zero per cent rate. The source of *E coli* could be irrigation water used for irrigation but not soil due to total absence of the organism in all the soil samples cultured using EMB media. Pseudomonas is present in both soil and irrigation water whereas shigella and aeromonas were only present in water but absent in soils.

The level of *Salmonella species* isolated from the SSA culture in Figure 8 suggests that the bacteria are a serious health threat to consumers of fresh vegetables cultivated within the study area. Common sickness associated with salmonella is typhoid fever caused by *Salmonella typhi*. Consumers of vegetables within the study area have higher risk of typhoid fever and other related illness associated with salmonella infection. The level of salmonella contamination in this study is higher than what was reported by Raufu et al. (2014) who observed a prevalence rate of 6.3% in Maiduguri, Nigeria.

The spread of salmonella species cut across all the selected vegetables in this study as shown in the result in Figure 9. This is because the SSA medium allows effective growth of *salmonella species* than the EMB. However, it shows that lettuce had the highest number of *Salmonella species* than all the selected vegetables. This is similar with the work of Mamdouh et al. (2019) who also observed higher level of salmonella in lettuce than other vegetables in their study of bacterial contamination of vegetables in Qalubia, Giza and Sharkia, along River Nile, Egypt. This is an indication that lettuce harbours more salmonella than other vegetables possibly due to open layers of the leaves unlike cabbage where the leaves are well compacted with little space in between them.

Result in Table 10 shows that salmonella is only present in irrigation water but absent in soil. This suggests that the source of salmonella in vegetables is mainly from irrigation water but not soil. According to Bagudo et al. (2014) abattoir effluents are major sources of salmonella in wastewater used for irrigation. Discharge from the Zango abattoir alongside with municipal liquid waste that is released into the Kubanni River could be the source of salmonella which are transferred to vegetables cultivated in the study area (see Plate V). The source of *Citrobacter species* is from both soil and irrigation water as shown in Table 10. Both soil and irrigation water could be the source of *Serratia species*. It also shows that both soil and irrigation water are sources of contamination caused by Klebsiella but soil accommodates higher population (28%) than water (8%). The source of contamination by Yersinia species is mainly soil (28%) as no amount of the organism was isolated in water. The source of preteaus is from both soil and water as shown in Table 10. Shigella is only found in irrigation water but absence in soils within the study area which implies that water is the main source of the organism. Shigella is a genus of Gram-negative, rod-shaped bacteria that causes shigellosis, a type of bacterial causing dysentery. Other species of Shigella include *Shigella flexneri, Shigella boydii and Shigella sonnei*. They are highly infectious and can cause severe diarrhea, fever, and abdominal pain. They can be found in contaminated food, water, and feces. The source of Aeromonas could be from irrigation water as it was identified in water sample from the EMB culture though absent from the SSA media. According to Raufu et al. (2014), organic manure from human and animal waste as well as solid dumpsites from nearby communities that are used as fertilizer are the main sources of bacterial contaminants in irrigated vegetables. Within the Kubanni irrigation area, many heaps of organic manure are taken to irrigation farmlands (see plate IV).

Conclusion

Vegetables within the Kubanni irrigation area are not safe without adequate washing or cleaning because all the vegetables were contaminated with bacteria above the WHO limits. The main causes of bacterial contamination of vegetables in the research area include wastewater used for irrigation, organic manure from municipal landfills, human and animal waste.

Recommendations

1. The amount of liquid waste that is channeled into the Kubanni River particularly from abattoir and residential areas should be minimized in order to ensure production of bacteria free vegetables within the study area.
2. Alternative fertilizers should be used since it is believed that the main ways that microbes contaminate vegetables in the study area are through organic manure from municipal dumps, human waste, and animal waste.
3. Raising farmers' knowledge of safe agricultural methods can help reduce the threat of bacterial vegetable contamination in the research region.
4. Consumers should avoid eating fresh vegetables that has not been washed or cooked.

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Appendix 1

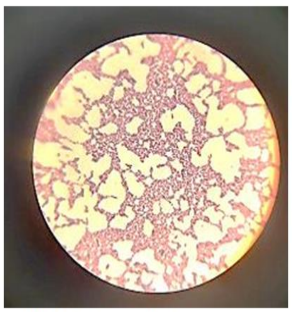
Plate I: Gram Positive cocci

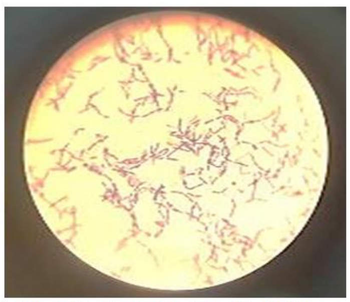
Plate II: Gram Positive Rod shape

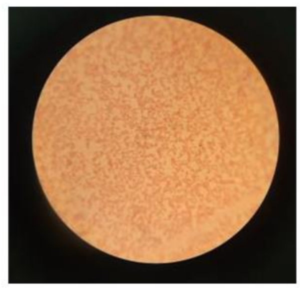
Plate III: Gram Negative


Plate IV: Heaps of Municipal Solid Waste


Plate V: Liquid waste from Residential Areas


Plate VI: Wastewater used for Irrigation