Evaluation of Microbial loads, parasites and Antinutrient factors in *Talinum triangulare* grown on Sewage Dump Site in University of Nigeria, Nsukka Nigeria

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**Abstract:** The use of dumpsites as farm lands is a common practice in urban and sub-urban centers in Nigeria because of the fact that decayed and composted wastes enhance soil fertility and also a wide spectrum of enteric pathogens that may have a negative impact on the environment and human health. This study was conducted to investigate the microbial load, parasite and anti-nutrient factor build-up in vegetables *Talinum triangulare* grown on sewage dump site. Vegetable samples were collected from farms around sewage dump sites (sites A) and a control where there is no dump (sites B). Standard methods of APHA was used to determine microbial load such as Total heterotrophic bacteria, *E. coli*, total coliform, faecal coliform, *Staphylococcus* aureus, *Salmonella* and intestinal parasites. Anti-nutrient factors were determined using standard titrimetric method for phytate and oxalate contents and Pearson method was used for cyanogenic glycoside, tamin, and alkaloid content. Results indicated that all the bacterial counts recorded in this study exceeded the recommended levels by WHO and ICMSF, standards (i.e. 10 to 10\(^5\) cfu/g, 10 femal coliform g-1 and 4.9×10\(^3\) aerobic count g-1) wet weight vegetables. Sewage dump site *Talinum triangulare* samples were mostly contaminated with faecal coliform (12.3 × 10\(^5\) cfu/g). The vegetable samples in dumpsite were significantly (P < 0.05) different from that of the controls. *Ascaris lumbricoides* ova and *Entamoeba histolytica* cyst parasites were mostly found in vegetables in site A than site B. The results also show that the dump site led to the significant (p < 0.05) increase of Antinutrient factors in the vegetables. All the antinutrient factors analysed in sewage dumpsite were not within the threshold in vegetables, except cyanogenic glocoside (1.06) and oxalate (28.11) which were within the Threshold of 0.5 -3.5 mg/kg and 200-500 mg/100g respectively in vegetables. The result indicated a potential health risk of microbial loads, parasites and anti-nutrient in the consumption of vegetables grown on sewage dump site.

**Key words:** Anti-nutrient factor, Microbial load, parasite, Sewage, *Talinum triangulare*

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**I. Introduction**

The use of sewage wastewater for crop production has been increasing worldwide due to the increase in food demand and change in climatic conditions, which makes food production through rainfed agriculture less reliable. The ever increasing worldwide population, especially in urban and peri-urban areas of the developing economies calls for serious thoughts and approaches in meeting the food demand whiles taking care of the environment for sustainable development (Samuel et al., 2013). Vegetables are dietary source of nutrients, micronutrients, vitamins and fibre for humans and are thus vital for health and well-being. A well balanced diets, rich in fruits and vegetables, are especially valuable for their ability to prevent vitamin C and A deficiencies and are also reported to reduce the risk of several diseases.

Vegetables are widely exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing. They therefore harbour a diverse range of microorganisms including plant and human pathogens (Carmo et al., 2004). Use of contaminated sewage water for irrigation of crops is considered to be responsible for transmission of several outbreaks of disease following consumption of such crops. Several cases of typhoid fever outbreak have been associated with eating contaminated vegetables grown in or fertilized with contaminated soil or sewage (Beuchat, 1998).

The sewage treatment plant at the University of Nigeria, Nsukka receives sewage from all the students’ hostels, staff quarters, laboratory, office wastewater, and wastewater from the university clinic. Wastewater contains a lot of nutrients, which increase crop yields without use of fertilizer. However, it contains a variety of...
chemical substances and microbiological loads from domestic and industrial sources (Modasiya et al., 2013; Khaled, 2016). A wide range of microbial pathogens have been found in sewage water and can be transferred to crops during irrigation. Survival of pathogens in the water and surrounding environment is mainly dependent on factors such as nutrient availability, temperature, organic matter content, competition with other microorganisms, pH and radiation. One major cause of vegetable contamination could be the unavailability of hygienic irrigation water. Pathogens can be transmitted to vegetables and cause outbreaks of illnesses when these are consumed.

*Talinum triangulare* is commonly called water leaf, it belongs to the family portulacaeae. It is an herbaceous, annual, coalescent, and glabrous plant widely grown in tropical regions as a leafy vegetable (Catherine et al., 2017). In Nigeria, it is consumed as a leafy vegetable and constituent of sauces (or vegetable soups). Nutritionally, it is a good source of some minerals (e.g., calcium, magnesium, and potassium) and vitamins (e.g., ascorbic acid and pyridoxine). The extract from the leaves and roots is used to cure asthma (Billa et al., 2017). It is known as *Ntie-oka* in igbo, *Gbure* in Yoruba and *Alenyruwa in hausa*.

Anti-nutrients factors are compound found in food that interfere with absorption of beneficial nutrients and minerals, metabolic processes and reduce the bioavailability of nutrients from plants or plant products used as human foods. And causes infertility, Cancer, gastrointestinal and neurological disorder (Awomukwu et al., 2015). Large doses of cyanide prevent cells from using oxygen and eventually these cells die. The heart, respiratory system and central nervous system are most susceptible to cyanide poisoning (Ellenhom and Barcelonx, 1988). Phytate decreases the bioavailability of proteins and essential elements such as Calcium, Magnesium, Zinc, iron, and Phosphorus by forming insoluble complexes, which are not readily absorbed by the gastrointestinal tract with the attendant health problems such as oxalemia (Agbaire and Oyewole, 2012). Oxalate binds to calcium to form insoluble calcium oxalate crystals which may precipitate in the kidney to form kidney stone and oxalemia. Alkaloids are often toxic to man and many have dramatic physiological activities, hence their wide use in medicine for the development of drugs (Harbon, 1973; Okwu, 2005). Alkaloids cause infertility, gastrointestinal and neurological disorder (Olayemi, 2007; Awomukwu et al., 2015). Tannins can bind to proteins and carbohydrates resulting in the reduction in digestibility of these macromolecules and thus inhibition of microbial growth (Dei et al., 2007; Nwogu et al., 2008).

Wastewater contains potentially toxic elements (PTEs) such as zinc, chromium, copper, cadmium, nickel, lead, mercury, and parasitic worms, which can induce severe risks to the human health and the environment (Mark et al., 2017; Shahid, 2017). Soil in and around dumpsite is usually nutrient rich, which improve soil properties such as organic matter, and nutrients, which increases plant productivity, supply of macronutrients (N, P, and K) and can reduce the crop production cost. Thus despite their important and yield benefits, outbreaks of human infections and environmental hazard is associated with the consumption of vegetables grown on sewage dumpsite. Hence, this study was designed to evaluation of microbial loads, parasites and antinutrient factors in *Talinum triangulare* grown on Sewage Dump Site. The results are expected to create awareness among the public on the safety of consuming vegetables grown in such areas.

### II. Material And Methods

#### Study Area

The study was carried out in farm around sewage dumpsite at University of Nigeria, Nsukka local government area in South-East, Enugu State of Nigeria. Nsukka is situated at 6.86° North latitude, 7.39° East longitude, 456 meters elevation above the sea level and has an area of 1810 km². The University of Nigeria, Nsukka lies between longitudes 7°24'E and 7°26'E, and longitudes 6°51'N and 6°53'N (Fig. 1). The sewage plant site is located at the northeast, corner of the university and covers an area approximately 700 m².

#### Study Design

A randomized complete block design with three replications for each test sample was used to assess the microbial contamination, and anti-nutrient constituents on the leafy vegetables of *Talinum triangulare* grown on sewage dumping site. The vegetable samples were collected at random and analyzed for microbial load, presence of a few specific parasites, and antinutrient following standard procedures.
Sampling

Sample collections were carried out according to the methods described by (Chiroma et al., 2014). The vegetable leaves used for the study were harvested fresh from the sites located in UNN and these were done in triplicates for each samples analysis; leaves, and contaminated soil. Control for each were also collected for same analysis. The samples were collected between the months of October and November 2017. Leaves were randomly sampled within the farm to get a representative sample. All samples were collected aseptically in a sterilized universal container and plastic bags. The samples were cooled during transportation using a cooler box to keep the normal conditions of the micro flora of vegetables. Analysis was conducted within 24 hours arrival at the Laboratory.

Microbiological Analysis

All samples were processed following standard methods (APHA, 2012). 10 g of vegetable samples were weighted and added into 90 ml of sterile normal saline in a blender and homogenized for 1-2 minutes. The homogenate 1 ml was mixed with 9 mls of sterile distilled water in a test tube, it was mixed very well, and then 1 ml portion of it was transferred aseptically into another test tube containing 9 mls of sterile distilled water and mixed. The dilution was done in series to the fifth dilution (10⁻⁵). Inocula of 0.1 ml were taken from the third (10⁻³) and inoculums was aseptically placed on the surface of the sterile solid agar to determine the presence of microorganism in each case.

Preparation of Agar medium

The media were prepared by dissolving 26g of Nutrient Agar (NA), 37 g of Eosin-methylene blue (EMB), 63 g of Salmonella-Shigella (SS), 38.5 g of violet red bile agar (VRB) and 111 g Manitol Salt Agar (MSA) in a conical flask properly tight each of the other media prepared was boil to dissolve completely and sterilized by autoclaving at 121°C for15 minutes and about 20 ml volumes of a well-mixed medium was poured aseptically into sterilized petri dishes and allowed to gel before inoculation.

Inoculation and Incubation of Media

Samples were diluted up to 10⁻⁵ dilution following a 10-fold serial dilution technique and were spread on Nutrient Agar (NA) medium, Eosin methylene blue agar, Salmonella shigella agar, violet red bile agar (oxiod) medium, and Manitol Salt Agar (MSA) to determine total heterotrophic bacteria/Aerobic mesophilic bacterial count, Escherichia coli count and total coliform counts, Salmonella shigella count, faecal coliforms, and Staphylococcus aureus. Respectively. All plates showing 30-300 colonies were used for quantitation of bacterial load as cfu/g (Kabir et al., 2014). All media were purchased in dehydrated powder form from jochem Laboratories Ltd., Nigeria. In addition to this parasitological analysis was also undertaken for the vegetable samples using standard procedures.
Parasitological Analyses of Vegetables

In the laboratory, 100g of each fresh vegetable sample was chopped into small pieces and put into a clean beaker containing physiological saline solution (0.85% NaCl), enough to wash the sample vegetable. Fragments of the vegetable sample was removed from the washing saline using sterile forceps and was kept for about 24h for sedimentation to take place. After 24h sedimentation, the top layer of the washing saline was carefully discarded leaving 5 ml of the sediment. This was finally centrifuged at 2000 rotations per minute for 5min and the supernatant discarded. The pellets/residue was mounted on slides, stained with Lugol’s iodine solution and examined under the compound light microscope to examine the samples for intestinal parasites. *Ascaris lumbricoides* eggs, *Entamoeba histolytica* cyst and *Giardia intestinalis* cysts with the following characteristics, oval or spherical in shape and are 45-75µm, oval or spherical in shape 10-20 micro meter in diameter, pear shape with two nuclei, and four pairs of flagella 10-12 µm long respectively. (Abougrain et al., 2009).

Anti-nutrient Analysis

The fresh leaves of the vegetables were sorted to remove damages and defective ones. The sorted fresh samples were washed with running tap water to remove dust and dirt and then with double distilled water. Fresh leaves of the vegetables were also collected in the control site. The cleaned leaves were analyse for phytate, oxalate, cyanogenic glycoside, tannin and Alkaloid content.

Determination of Phytate Content

Titrimetric method of (Akaneme et al., 2014) was used to estimate phytate content in the fresh samples of the vegetables. Exactly 2 g each of the vegetable samples slurry was weighed into a 250 cm³ conical flask, and 100 cm³ of 2% HCl added, and left for 3 hours. The resulting solution was filtered through double layer of hardened whatman No. 1 filter paper. Than 50 ml each of the filtrate was placed in another 250 cm³ conical flask and 100 cm³ distilled water was added in each case to give a proper acidity, while 10 ml of 0.3% ammonium thiocyanate (NH₄SCN) solution was added into each solution as indicator. This was titrated with standard solution of iron (iii) chloride (FeCl₃) which contains 0.00195g/cm³ (1.95 g). The end-point was reached when slightly brownish – yellow colour was observed which persisted for about 5 minute. The concentration of phytic acid in g/100 g sample was calculated using the formula:

\[
\text{Concentration of phytic acid} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100 \times 3.55}{\text{weight of sample}}
\]

Determination of Cyanogenic Glycoside Content

The method of Pearson, (1976) was used to analyze Cyanogenic Glycoside content in the samples. In this method, a reaction of cyanide and alkaline picrate produce a characteristic orange colour. Slurry 0.5g of the sample was weighed into put in a test tube. The sample was macerated in 20 ml of phosphate buffer pH 6 for 10 minutes. The test tube was allowed to stand for an hour with shaking every 10 minutes intervals. It was centrifuged for 5 minutes and 1 ml of the supernatant was transferred into triplicate tubes. Than 4 ml of alkaline picrate was added and boiled for 5 minutes in a water bath. The tube was cooled in cold water and the absorbance was taken using a colourimeter at 470 nm against a reagent blank.

Determination of Oxalate Content

Titrimetric method of Association of official analytical chemist AOAC, (1995), was used to estimate Oxalate content in the fresh vegetables. The samples were ground into slurry and 1.0 g of sample leaves of vegetables were weighed into a crucible dish, and was extracted with 10 cm³ of distilled water, followed by the addition of 1cm³ of concentrated H₂SO₄. This was allowed to stand for an hour. The volume was made up to 50
cm⁻³ with distilled water. The resulting solution was filtered. Also 25 cm⁻³ of the extract was pipetted into a conical flask heat to 90°C and titrated against potassium permanganate KMnO₄ in a burette. A colour change was noted which indicates the end point and the reading of the burette was taken when the red colour remained steady for some seconds. The concentration of oxalate (mg g⁻¹) in each of the sample was got by multiplying the burette reading by 11.5. Oxalate (concentration) = Average volume used × 11.5

**Determination of Tannin Content**

The tannin content in the vegetable samples was determined by the method of Pearson, 1976. The samples were ground into slurry and 0.5 g of the sample was weighed and into a test tube. The sample was macerated in 20ml of methanol for 10 minutes and centrifuged for 5 minutes at 3000 r. p. m. Than 5ml of the supernatant was transferred into triplicate tubes. Also 0.3 ml of 0.1 m ferric chloride in 0.1m hydrogen chloride were added. The solution was mixed and 0.3ml of 0.0008 m potassium ferricyanide was added and was mixed. The absorbance was taken using a colourimeter after 5minutes at 720 nm against a blank.

**Determination of Alkaloid Content**

The Alkaloid content in the vegetable samples was determined by the method of Pearson, (1976). The samples were ground into slurry and 1 g of the sample was weighed, put in a test tube, macerated in 10ml of 20% sulphuric acid and 10ml of ethanol for 10minutes. The tube was allowed to stand for an hour with intermittent shaking, and subsequently centrifuged for 5 minutes. Than 0.5 ml of the supernatant was transferred in triplicate tubes, 2.5 ml of 60% sulphuric acid was added and the two were mixed. Than 2.5 ml of 0.5% formaldehyde was subsequently added and the test tubes were allowed to stand for 3 hours. The absorbance was taken using a colourimeter at 565 nm against a blank.

**Statistical Analysis**

The data obtained were analysed using IBM Statistical Product and Service Solution (SPSS) version 20 and Microsoft excel 2013. The results were expressed as mean ± standard error (SE). One way analysis of variance (ANOVA) was carried out as p<0.05 considered statistically significant.

### III. Results

**Microbiological Analysis on Talinum triangulare Vegetable**

Total bacterial load in the vegetable samples tested from sewage dump site (SDS) and a control site (CS) are shown in (Table 1). The total microbial load in *Talinum triangulare* (TT) on all sewage dump site ranged between $7.1 \times 10^5$ to $12.3 \times 10^6$ cfu/g with faecal coliform more dominate while the control site ranged between $1.2 \times 10^5$ to $2.4 \times 10^5$ cfu/g with *Salmonella* more dominant. There were more microbial loads in the vegetables grown on sewage dump site than the control site which significantly difference (p<0.05) between sites.

<table>
<thead>
<tr>
<th>Microbial load (cfu/g)</th>
<th>Test Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT (A)</td>
</tr>
<tr>
<td>Total Heterotrophic B</td>
<td>$8.3 \times 10^5 \pm 8.8 \times 10^3$</td>
</tr>
<tr>
<td><em>E.coli</em></td>
<td>$9.2 \times 10^4 \pm 3.3 \times 10^3$</td>
</tr>
<tr>
<td>Total Coliform</td>
<td>$1.1 \times 10^6 \pm 5.6 \times 10^3$</td>
</tr>
<tr>
<td>Faecal Coliform</td>
<td>$1.2 \times 10^6 \pm 5.8 \times 10^3$</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>$1.0 \times 10^6 \pm 5.8 \times 10^3$</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>$7.1 \times 10^4 \pm 5.8 \times 10^3$</td>
</tr>
</tbody>
</table>

B = Bacteria; A = Sewage dump site; B = Control site; TT = *Talinum triangulare*; n = 3. Results expressed as Mean ± SE:

**Parasitological Analyses of Vegetables**

The results of this study showed that the parasitic stages recovered from *Talinum triangulare* (TT) presented in Table 2. Out of 12 samples of TT collected from sewage dump, 10 (86.66%) were found to be positive for *Ascaris lumbricoides*. While the control site of TT were 2 (16.66%). 1 (8.33%) of *Entamoeba histolytica* cyst were found in TT. And *Giardia intestinalis* cysts were not found in the vegetable.
Antinutrient factors in vegetable grown on sewage dump site and a control site.

The cyanogenic Glycoside levels in the sewage dump site and control site of *Talinum triangulare* (TT) were 1.06 mg/100g and 0.98 mg/100g respectively. They were all within the threshold in vegetables 0.5 - 3.5 mg/kg. The phytate profile in the sewage dump site (SDS) and Control site (CS) of TT were 7.56 g/100g and 3.30 g/100g respectively. They were all above the threshold in vegetables 0.035%. The oxalate content in SDS and CS of TT were 28.11 mg/g and 9.57 mg/g respectively. They were all below the threshold in vegetables 200 – 500 mg/100g. The Alkaloid content in the SDS samples and CS of TT were 0.13 mg/100g and 0.12 mg/100g respectively. The alkaloid content were all above the threshold in vegetables (0.02%). The Tannin in the SDS and CS samples of TT were: 1.26 mg/100 g and 0.72 mg/100 g. They were all above the threshold in vegetables 0.25 g/l Table 3.

**Table 3: Antinutrient factors on *Talinum triangulare* grown on sewage dump site and a control site**

<table>
<thead>
<tr>
<th>Antinutrient factors</th>
<th>SDS TT</th>
<th>CS TT</th>
<th>TIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanogenic (mg/100g)</td>
<td>1.06 ± 0.00</td>
<td>0.98 ± 0.00</td>
<td>0.5 – 3.5 mg/kg</td>
</tr>
<tr>
<td>Phytate (g/100g)</td>
<td>7.56 ± 0.00</td>
<td>3.30 ± 0.06</td>
<td>0.035 %</td>
</tr>
<tr>
<td>Oxalate (mg/g⁻¹)</td>
<td>28.11 ± 0.13</td>
<td>9.57 ± 0.01</td>
<td>200 – 500 mg/100g</td>
</tr>
<tr>
<td>Alkaloid (mg/100g)</td>
<td>0.13 ± 0.00</td>
<td>0.12 ± 0.00</td>
<td>0.02 %</td>
</tr>
<tr>
<td>Tannin (mg/100g)</td>
<td>1.26 ± 0.00</td>
<td>0.72 ± 0.00</td>
<td>0.25 g/l</td>
</tr>
</tbody>
</table>

SDS = Sewage dump site; CS = Control site; TT = *Talinum triangulare*; TIV = Threshold in vegetables; Results expressed as Mean ± SE: Rows mean value carrying different letter are significantly different (P<0.05). n = 3

IV. Discussion

It was observed that the microbial load on TT from the sewage dump site were higher when compared to TT from non-sewage dumpsite. These may be as a result of the nature of the vegetable, TT grows to 3 feet in height and are directly close to the soil which make them to acquire more microbes from the sewage soil. The sequence of occurrence is Faecal coliform (FC) > Total coliform (TC) > *Staphylococcus aureus* > *Escherichia coli* (E-coli) > Total Heterotrophic bacteria (THB) > *Salmonella in Talinum triangulare* from sewage dump site. The result was in accordance with the findings of Samuel et al., 2013 who recorded highest level of contamination of total coliform, faecal coliform, *E. coli* and helminth eggs on lettuce. Mean levels of total coliforms (TC), faecal coliform (FC), *E. coli* and Helminthes eggson lettuce were 4.1 ± 0.5, 3.7 ± 0.5, 3.3 ± 0.6 log10 CFU·g⁻¹ fresh weight and two helminth eggs respectively. Samuel recorded Total coliform composition of wastewater ranged from 3.19 to 4.82 log CFU/100 ml with a mean of 4.4 log CFU/100 ml. Faecal coliform bacteria ranged from 3.36 to 4.33 log CFU/100 ml with a mean of 4.0 log CFU/100 ml. Mean FC was higher than WHO recommended limit of 3 log CFU/100 ml. Sewage dump site vegetable samples were mostly contaminated with faecal coliform TT (12.3 x 10⁶ cfu/g) due to faeces which is the main content of sewage. The data further showed that all the bacterial counts recorded in this study exceeded the recommended levels by WHO and International Commission on Microbiological Specifications for Food (ICMSF) standards (i.e. 10 to 10⁷ coliforms g⁻¹, 10 fecal coliform g⁻¹ and 4.9x10⁶ aerobic count g⁻¹) wet weight vegetables. Similarly (Drechsel et al., 2006) has
reported that fresh poultry manure used for vegetable production in Kumasi recorded high fecal coliform counts ranging from $3.6 \times 10^3$ to $1.1 \times 10^7$. The result correspond to the findings of (Buck et al., 2003) who reported that the presence of many pathogens in the soil was thought to be from historical application or environmental presence of faeces or untreated sewage. Intestinal parasites are common in fresh vegetables. Hence, consumption of raw vegetables plays an important role in the transmission of human parasitic infection (Tiimub et al., 2012; Farahat et al., 2017). Epidemiological studies have shown that the actual risk of infection for people exposed to wastewater is highest for intestinal nematodes such as roundworm (*Ascaris lumbricoides*).

Large doses of cyanide prevent cells from using oxygen and eventually these cells die. The heart, respiratory system and central nervous system are most susceptible to cyanide poisoning (Ellenhom and Barcelon, 1988). Phytate decreases the bioavailability of proteins and essential elements such as Calcium, Magnesium, Zinc, iron, and Phosphorus by forming insoluble complexes, which are not readily absorbed by the gastrointestinal tract with the attendant health problems such as oxalemia (Akande and Ajayi, 2017). The observation was in accordance with other researchers who also reported higher content in oxalate. Oxalate binds to calcium to form insoluble calcium oxalate crystals which may precipitate in the kidney to form kidney stone and oxalemia. The alkaloid content increases significantly ($p < 0.05$) in all the sewage dump site samples. Alkaloids are often toxic to man and many have dramatic physiological activities, hence their wide use in medicine for the development of drugs (Harborne, 1973; Okwu, 2005). Alkaloids cause infertility, gastrointestinal and neurological disorder (Olayemi, 2007; Awomukwu et al., 2015). Tannins can bind to proteins and carbohydrates resulting in the reduction in digestibility of these macromolecules and thus inhibition of microbial growth (Dei et al., 2007; Nwogu et al., 2008). The results showed that the cyanogenic Glycoside, Phytate, Oxalate, Alkaloid and Tannin content in *Talinum triangulare* samples grown on sewage dump site were generally higher than that of the control samples. The results also indicates that the dump site led to a significant ($p < 0.05$) increased of Antinutrient factors in the vegetable shown on (Table 3).

### V. Conclusion

This research has shown that vegetables grown on sewage dumpsites are contaminated with high antinutrient and microbes. The microbial loads and antinutrient factors on vegetables were above ICSFM and FAO recommended limits for vegetables. People who eat these contaminated vegetables raw or half cooked, stand a high chance of contracting gastrointestinal diseases like typhoid, cholera and dysentery and disruption of numerous biochemical processes. To prevent an eminent outbreak efforts have to be made to discouraged farmers from the use of wastewater for irrigation. The community needs to be informed that consumption of this vegetables can be a mediator for contamination of the pathogen and health problems.

### References


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