BACTERIOLOGICAL AND HEAVY METAL CONTAMINANTS OF SOME FRESH VEGETABLES IRRIGATED WITH AWETU RIVER IN JIMMA TOWN, SOUTH WESTERN ETHIOPIA

Conference place: Desalegn Hotel
By: Desta
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- Objectives
- Methodology
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- Conclusion & Recommendation
- Acknowledgement
Introduction

- Fresh vegetables reducing the risk of
  - heart disease, diabetes & cancer
- Risk has been also associated with consumption of fresh vegetables (Beuchat, 2002)
- Outbreaks
  - *S. typhimurium* = tomatoes, USA
  - *E. coli* O157:H7 = lettuce, northern USA
- Wastewater irrigation is a common in 3/4th of the cities in Asia, Africa, & Latin America (Mapanda et al., 2005)
- Microbiological quality of irrigation water is vital to the safety of fresh vegetables (Solomon et al., 2002)
Toxic heavy metals are one of the most serious env’tal concerns (Cui et al., 2004)

Heavy metal-contaminated food
deplete some essential nutrients
gastrointestinal cancer

Awetu River have contaminated via

human & animal faecal materials
wastes disposed from households, hotels & small scale industries (Sofonias & Tsegaye, 2006)
Deneke (2006) were found excessive fecal coliform & trace metals

Comprehensive investigation of the quality & safety of vegetables irrigated with Awetu River encompassing bacteriological & heavy metal parameters is lacking

The present study was

Initiated to investigate the bacteriological & heavy metal quality as well as safety of some fresh vegetables irrigated with Awetu River in Jimma town
OBJECTIVES

General Objective

❖ To investigate the bacteriological & heavy metal contaminants of some fresh vegetables irrigated with Awetu River

Specific Objectives

❖ To determine the microbial quality of water & irrigated vegetables
❖ To isolate & characterize dominant microflora of water & vegetables
To assess microbial safety of water & irrigated vegetables

To evaluate antimicrobial resistance/susceptibility patterns of *S. aureus* & *Salmonella* isolates

To determine some heavy metal levels of water & irrigated vegetables
Methodology

Description of study area
- Conducted at Jimma town

Study design
- Cross sectional study design were used
  - Preliminary survey
  - Laboratory
Heavy metal analysis

Heavy metals were analyzed following standard procedure.

- 30 Samples & corresponding blank samples were digested in the same manner using HNO₃.
- Standard solution for each metal was prepared.
- Samples were measured by FAAS for Cu, Zn, Pb & Fe.
Bacteriological analysis

Sample collection

思います A total of 150 samples were collected.

30 samples of each (water, Lettuce, Carrot, Tomato & Cabbage)

✓ Samples were collected from 3 sites (A, B & C) downstream of Awetu River
Figure 2. Some vegetables irrigated with Awetu River & waste released from Abattoir in to Awetu River from A, B & C sites
Sample preparation

A 25 g each of sample was mixed with 225 ml of 0.1% BPW

Homogenized in a flask for 2 minutes using shaker

One ml of each sample was transferred into 9 ml of PW

The homogenates were serially diluted

A 0.1 ml aliquot was spread-plated and incubated

Colonies were counted between 30 to 300
# Bacterial counts

Table 1. Bacterial counts on various media

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Aliquot</th>
<th>Media</th>
<th>Temperature (°C)</th>
<th>Time (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMB</td>
<td>0.1 ml</td>
<td>PCA</td>
<td>32</td>
<td>24-48</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>0.1 ml</td>
<td>MacConkey agar</td>
<td>32</td>
<td>18-24</td>
</tr>
<tr>
<td>Aerobic spore formers</td>
<td>0.1 ml</td>
<td>PCA</td>
<td>32</td>
<td>24-48</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>0.1 ml</td>
<td>MSA</td>
<td>32</td>
<td>24-48</td>
</tr>
<tr>
<td>Total coliform</td>
<td>10, 1, 0.1 ml</td>
<td>MCB, BGLB</td>
<td>37</td>
<td>24-48</td>
</tr>
<tr>
<td>Fecal coliform</td>
<td>10, 1, 0.1 ml</td>
<td>MCB, BGLB</td>
<td>44.5</td>
<td>24-48</td>
</tr>
</tbody>
</table>

Where; AMB = Aerobic mesophilic bacteria, PCA = Plate count agar, MSA = Mannitol salt agar, MCB = MacConkey broth, BGLB = Brilliant Green Lactose Bile broth
Microbial Analysis

- **Cell Morphology**
  - Cell shape
  - Motility
  - Endospore

- **Biochemical Test**
  - KOH
  - Catalase
  - Oxidase
  - O/F

Done by Using
- Gram staining
- Motility Test
- Endospore Test
Isolation of *Staphylococcus aureus*

Golden yellow colonies from MSA plates were picked

- transferred into 5 ml nutrient broth & incubated at 37°C for 24 hrs
- a loopful of culture was streaked on nutrient Agar supplemented with 0.75% sodium chloride

Finally, Gram stain, catalase & coagulase tests were conducted
Isolation of *Salmonella* and *Shigella* spp.

A 25 g or ml of vegetable or water samples were mixed with 225 ml of BPW and incubated at 37°C for 24 hrs.

One ml pre-enriched broth culture was added to 10 ml of Rappaport Vassiliadis broth (RVB) & incubated at 43°C for 48 hrs.

A loopful of suspension was streaked onto XLD & incubated at 37°C for 24 hrs.

Black colonies surrounded by red color & red or colorless colonies without black center were taken as presumptive *Salmonella* spp. & *Shigella* spp., respectively.
Salmonella & Shigella suspected colonies were further checked by using biochemical tests:

- Triple Sugar Iron Agar
- Lysine Iron Agar
- Urea Agar
- Simmons Citrate Agar
- Sulfide Indole Motility
- Mannitol and Glucose/Sucrose Fermentation
Antimicrobial Susceptibility Testing

Bacterial suspension matched with 0.5 McFarland were prepared and swabbed on MHA and allowed to dry.

Antibiotic discs were placed on the medium using forceps and incubated at 37°C for 18 hrs.

Zones of inhibition were measured and classified as sensitive, intermediate, or resistant.
Data Analysis

- Data were analyzed using SPSS software version 16.0
- Mean values of sample compared using one way ANOVA followed by LSD’s Post Hoc Multiple Comparison Test
- Significance difference was considered at \( p < 0.05 \)
## Result and Discussion

Table 2. Socio-demographic characteristics of vegetable growers with Awetu River

<table>
<thead>
<tr>
<th>Background Characteristics</th>
<th>Grouping</th>
<th>Number of Respondents (n=60)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Female</td>
<td>17</td>
<td>28.3</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>43</td>
<td>71.7</td>
</tr>
<tr>
<td>Age</td>
<td>15-24</td>
<td>10</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>25-34</td>
<td>20</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>35-44</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>45-54</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>55-64</td>
<td>4</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>65-74</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>Marital states</td>
<td>Married</td>
<td>42</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Unmarried</td>
<td>13</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>Divorced</td>
<td>4</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Widowed</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Academic status</td>
<td>illiterate</td>
<td>33</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>elementary school</td>
<td>27</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Secondary school</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3. General information of vegetable growers with Awetu River

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number of Respondents (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
</tr>
<tr>
<td>Awetu River user respondents</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Application of Awetu River</td>
<td></td>
</tr>
<tr>
<td>washing clothes</td>
<td>21</td>
</tr>
<tr>
<td>taking shower</td>
<td>5</td>
</tr>
<tr>
<td>irrigation</td>
<td>59</td>
</tr>
<tr>
<td>Use of irrigated vegetables</td>
<td></td>
</tr>
<tr>
<td>family consumption</td>
<td>53</td>
</tr>
<tr>
<td>source of income</td>
<td>44</td>
</tr>
<tr>
<td>Place of selling irrigated vegetables</td>
<td></td>
</tr>
<tr>
<td>Jimma town</td>
<td>40</td>
</tr>
<tr>
<td>Within the kebele</td>
<td>4</td>
</tr>
<tr>
<td>Type of diseases due to consumption of the vegetables</td>
<td></td>
</tr>
<tr>
<td>Typhiod</td>
<td>10</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>7</td>
</tr>
<tr>
<td>Anemia</td>
<td>2</td>
</tr>
</tbody>
</table>
# Heavy Metal analysis

Table 4. Heavy metal concentration of some water and vegetable samples

<table>
<thead>
<tr>
<th>Sample Types</th>
<th>Heavy Metal Concentration (mg/l) Mean ± S.D</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Cu: &lt;0.001 ± 0.00</td>
<td>Zn: 0.047 ± 0.00</td>
</tr>
<tr>
<td>Safe limits</td>
<td>0.2</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample Types</th>
<th>Heavy Metals Concentration (mg/kg) Mean ± S.D</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lettuce</td>
<td>Cu: 9.506 ± 0.00</td>
<td>Zn: 1067.780 ± 0.00</td>
</tr>
<tr>
<td>Carrot</td>
<td>8.080 ± 0.00</td>
<td>83.447 ± 0.00</td>
</tr>
<tr>
<td>Tomato</td>
<td>11.740 ± 0.00</td>
<td>82.177 ± 0.00</td>
</tr>
<tr>
<td>Cabbage</td>
<td>3.433 ± 0.00</td>
<td>154.640 ± 0.00</td>
</tr>
<tr>
<td>Safe limits</td>
<td>40</td>
<td>60</td>
</tr>
</tbody>
</table>
**Cu** (3.4–11.7 mg kg\(^{-1}\))

- 15.66–34.49 mg kg\(^{-1}\) India (Gupta *et al*., 2008)
- 15.5 & 8.51 mg kg\(^{-1}\) leafy & non-leafy vegetables Bangladesh (Alam *et al*., 2003)

**Zn** (82.2–1067.8 mg kg\(^{-1}\))

- 1,038–1,872 mg kg\(^{-1}\) Zimbabwe (Tandi *et al*., 2004)
- 32.01–69.26 mg kg\(^{-1}\) China (Liu *et al*., 2005)
- 3.00–171.03 mg kg\(^{-1}\) India (Gupta *et al*., 2008)
Pb (16.8–18.9 mg kg\(^{-1}\))

- 0.18–7.75 mg kg\(^{-1}\) China (Liu et al., 2006)
- 3.09–15.74 mg kg\(^{-1}\) India (Sharma et al., 2007)
- 21.59–57.63 mg kg\(^{-1}\) India (Gupta et al., 2008)

Fe (1251.4–5492.2 mg kg\(^{-1}\))

- 111–378 mg kg\(^{-1}\) India (Arora et al., 2008)

Generally heavy metals are important for proper functioning of biological systems. However, their deficiency or excess could lead to a number of disorders (Ward, 1995)
# Bacterial counts

Table 5. Bacterial counts (Mean log CFUg⁻¹ ± S.D) of water and vegetable samples

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>AVERAGE LOG CFUML⁻¹ ± S.D</th>
<th>AVERAGE MPN100ml⁻¹ ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMB</td>
<td>Entero.</td>
</tr>
<tr>
<td>Water</td>
<td>8.58 ± 0.3</td>
<td>7.42 ± 0.2</td>
</tr>
<tr>
<td>Lettuce</td>
<td>6.94 ± 0.5</td>
<td>6.09 ± 0.3</td>
</tr>
<tr>
<td>Carrot</td>
<td>8.06 ± 0.5</td>
<td>7.1 ± 0.4</td>
</tr>
<tr>
<td>Tomato</td>
<td>7.41 ± 0.4</td>
<td>6.24 ± 0.4</td>
</tr>
<tr>
<td>Cabbage</td>
<td>7.66 ± 0.4</td>
<td>6.7 ± 0.5</td>
</tr>
</tbody>
</table>

Where:- AMB = Aerobic mesophilic bacteria, Entero = Enterobacteriaceae ASFs = Aerobic spore formers, Staph= Staphylococci
**AMB** (6.94–8.06 log CFUg\(^{-1}\))
- 2–6 log CFUg\(^{-1}\) (Angelidis *et al.*, 2006)
- 2–10 log CFUml\(^{-1}\) (Blackburn *et al.*, 1996)
- poor & spoiled food (Aycicek *et al.*, 2006)

**Enterobacteriaceae** (6.09–7.10 log CFUg\(^{-1}\))
- 4.84–5.08 log CFUg\(^{-1}\) (Guchi & Ashenafi, 2010)
- sources of foodborne disease (Motarjemi *et al.*, 1993)

**Aerobic spore formers** (5.24–6.54 log CFUg\(^{-1}\))
- 3.47–3.50 log CFUg\(^{-1}\) (Guchi & Ashenafi, 2010)
  - Food poisoning may cause
Staphylococci (2.71–2.97 log CFUg\(^{-1}\))

✓ 4.55–4.97 log CFUg\(^{-1}\) (Guchi & Ashenafi, 2010)

Contamination of foodstuffs during distribution & handling may allow bacterial growth & subsequent production of toxins (Erkan et al., 2008)

Total & fecal coliform (1036, 716 MPN100ml\(^{-1}\))

✓ >1100 MPN100ml\(^{-1}\) (Nipa et al., 2011)

Pathogens may be present due to fecal contamination from humans & animals (Vishwanathan & Kaur, 2001)
Microbial Analysis

Figure 3. Distribution of dominant bacterial isolates from water and vegetable samples
**Bacillus** (Guchi & Ashenafi, 2010)

- High number of *Bacillus* spp. could cause **food poisoning**

**Enterobacteriaceae**

- *Pseudomonas* isolates (Guchi & Ashenafi, 2010)
  - normal flora (Gilbert *et al*., 2000)

**Micrococcus** (Guchi & Ashenafi, 2010)

- Common env’tal bacteria, normal flora of human skin
- Opportunistic pathogens, **meningitis** & prosthetic valve endocarditis (Wharton *et al*., 1986)
Isolation & identification of *S. aureus* and *Salmonella* spp.

Figure 4. *S. aureus* (Left) & *Salmonella* spp. (Right)

- Gram stain
- Catalase
- Coagulase

- Sulfide Indole Motility
- Triple Sugar Iron Agar
- Lysine Iron Agar
- Simmons Citrate Agar
- Urea Agar
Table 6. Frequency distribution of bacterial isolates from water & vegetable samples

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>No. of samples</th>
<th>S. aureus positive</th>
<th>%</th>
<th>Salmonella positive</th>
<th>%</th>
<th>Shigella positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>30</td>
<td>6/30</td>
<td>20.0</td>
<td>7/30</td>
<td>23.3</td>
<td>0/30</td>
</tr>
<tr>
<td>lettuce</td>
<td>30</td>
<td>10/30</td>
<td>33.3</td>
<td>4/30</td>
<td>13.3</td>
<td>0/30</td>
</tr>
<tr>
<td>Carrot</td>
<td>30</td>
<td>5/30</td>
<td>16.7</td>
<td>5/30</td>
<td>16.7</td>
<td>0/30</td>
</tr>
<tr>
<td>Tomato</td>
<td>30</td>
<td>7/30</td>
<td>23.3</td>
<td>6/30</td>
<td>20.0</td>
<td>0/30</td>
</tr>
<tr>
<td>Cabbage</td>
<td>30</td>
<td>8/30</td>
<td>26.7</td>
<td>9/30</td>
<td>30.0</td>
<td>0/30</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>36/150</td>
<td>24.0</td>
<td>31/150</td>
<td>20.7</td>
<td>0/150</td>
</tr>
</tbody>
</table>
**S. aureus** (24.0%, 25.0%)

- 51.5% Lebanon, *(Halablab et al., 2010)*
- bacterial growth & production of toxins which may represent a potential risk to humans *(Erkan et al., 2008)*

**Salmonella spp.** (20.7%, 20.0%)

- 10.0% Ethiopia, *(Guchi & Ashenafi, 2010)*, 11.0% South Africa, *(Ijabadeniyi, 2010)*
- potentially hazardous to consumers *(Cheung et al., 2007)*
### Table 7. Antibiotic susceptibility of *S. aureus* & *Salmonella* spp. isolated from water & vegetable samples

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Disc (µg/ml)</th>
<th>Sensitive</th>
<th>%</th>
<th>Resistance</th>
<th>%</th>
<th>S. AUREUS</th>
<th>Sensitive</th>
<th>%</th>
<th>Resistance</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>10</td>
<td>3</td>
<td>9.7</td>
<td>28</td>
<td>90.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>36</td>
<td>100</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10</td>
<td>31</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>31</td>
<td>86.1</td>
<td>5</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30</td>
<td>31</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>36</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>31</td>
<td>100</td>
<td>7</td>
<td>19.4</td>
<td>29</td>
<td>80.6</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>31</td>
<td>100</td>
<td>29</td>
<td>80.6</td>
<td>7</td>
<td>19.4</td>
<td></td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>25</td>
<td>29</td>
<td>93.5</td>
<td>2</td>
<td>6.5</td>
<td>18</td>
<td>50</td>
<td>18</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Cefuroxime sodium</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>31</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>36</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Penicillin G</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>31</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>36</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>10</td>
<td>31</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>34</td>
<td>94.4</td>
<td>2</td>
<td>5.6</td>
<td></td>
</tr>
</tbody>
</table>
100% resistant to Penicillin G, Ampicillin & Cefuroxime sodium

(Rosina and Estifanos, 2007)

100% resistant to Penicillin G

the production of penicillinase enzyme (Lowy, 2003)

100% Tetracycline, Erythromycin, Penicillin G & Cefuroxime sodium

(Cardoso et al., 2006)

100% resistant of Salmonella Enteritidis for Tetracycline & Erythromycin, Brazil

The co-existence of resistance genes with mobile element

plasmids & transposons facilitates the rapid spread of antibiotic resistance genes among bacteria (Sunde, 2005)
Hygienic quality of the samples were poor b/c their overall mean counts were beyond the standard safe limits.


Bacterial pathogens (*S. aureus* & *Salmonella* spp.)

Chloramphenicol, Gentamycin & Norflaxacin were the drug of choice

- Penicillin G, Cefuroxime sodium & Ampicillin were most resisted antimicrobial agents by *S. aureus* & *Salmonella* spp.

Heavy metals in all vegetables were found above the permissible safe limit of WHO (1984) except Cu

Vegetables assessed in this study shows heavily contaminated with bacterial pathogens & toxic heavy metals

Their consumption could cause health risk
● Health hazards from vegetables could be minimized by using antibacterial chemicals.

● Municipal or industrial waste should not be drained into rivers & farmlands without prior treatment.

● Wastewater should be properly treated with Good Agricultural Practices during production of fresh vegetables.

● Study should be done on the heavy metal levels of the soil.
Acknowledgement

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Derbew Belew (PhD)

Mohd Sayeed ((PhD)

Ketema Bacha (PhD)

Vegetable growers