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BACTERIAL QUALITY AND CYTOTOXICITY SCREENING OF FRESH VEGETABLES IRRIGATED WITH POLLUTED WATERS

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ABSTRACT

The mutagenic effects of raw vegetables irrigated with polluted water and their bacterial quality were studied. Two vegetables, lettuce (*Lactuca sativa*) and fluted pumpkin (*Telfaria occidentalis)* were planted in sterile soil and irrigated with sewage-polluted stream, rain, tap and well waters, and harvested. The presence of pathogenic bacteria on the vegetable leaf surfaces was determined. The *Allium cepa* assay was then used to evaluate the genetic and acute effects of the vegetable leaf extracts. The heavy metal concentrations of the vegetables were determined using atomic absorption spectrophotometry. Pathogenic bacteria isolated were *Escherichia coli, Staphylococcus aureus, Salmonella paratyphii, Shigella dysenteriae, Klebsiella pneumoniae, Aeromonas hydrophila* and *Enterobacter aerogenes.* In *A. cepa* assay, none of the treatments induced chromosome aberration at the tested concentrations, but retardation of growth and suppression of mitotic activity occurred. The concentrations of heavy metals in the vegetables were lead (0.261-0.531mg/kg), zinc (0.142-1.618mg/kg), cadmium (0.00-0.13mg/kg), copper (0.021-0.057mg/kg), iron (0.711-1.122mg/kg) and chromium (0.00-0.14mg/kg). This study shows that irrigation waters could have effects on the quality of edible vegetables.

Keywords: Mutagenic, Bacterial quality, *Allium cepa*, Chromosome aberration, and Heavy metals.

INTRODUCTION

An important resource of most living organisms is water. It is used in both aquatic and terrestrial environments for various activities, most of which are directed towards balancing the ecological systems of the global environment (Amin, 2002). It is an important natural resource which is abundant in nature. Population explosion, haphazard rapid urbanization, energy utilization and waste generation are some of the factors that rendered many water resources unwholesome and hazardous to man and other living system.

In Nigeria, cases of water pollution by solid wastes are very rampant and this has rendered many surface and ground water unsuitable for drinking and for other purposes (Bakare *et al*., 2003). Since these polluted groundwater, surface water and human waste waters are commonly used for irrigation of

food crops; it is likely that such food crops will pose genetic effect on human system. The food crops irrigated with this water may also be contaminated with pathogenic microorganisms such as *Salmonella* spp., *Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes* and other pathogens that can cause outbreaks of food-borne diseases. It is therefore necessary to investigate the bacterial profile and genotoxic effects of the vegetables on human system through Allium test.

The Allium test has been suggested as a standard short-term test in environmental monitoring (Fiskesjo, 1985). It is an in-vivo plant test that has shown good correlation with other test systems involving general toxicity and genotoxicity. *Allium cepa* is commonly used for this test because it provides an excellent cytological model system due to its relatively large chromosomes that spread easily for observation and cytogenetic studies (El-Shahaby *et al.,* 2003). Allium assay provides an avenue for monitoring and comparing genetic activities in other living systems, such as human genome based on the assumption that any genetic defects observed in the Allium test, consequent upon some treatments, can equally be observed in other genomes that are subjected to such treatments. This test has been used to determine the mutagenic effects of natural water in village communities (Bakare *et al*., 2003); effluent water and waste water from industries and many homes (Amin, 2002; El-Shahaby *et al.,* 2003) and also used to evaluate the mutagenic effects of some leaf extracts. It is therefore envisaged that carrying out Allium test on leaf extracts of raw vegetables irrigated with polluted water will reveal the genotoxic potentials of the vegetables on *Allium cepa* and by inference on the consumers of such vegetables.

The aim of this study is to investigate the mutagenic effect of the vegetable extracts on human system through Allium test, as well as to determine the bacterial quality and the heavy metal content of such vegetables.

MATERIALS AND METHODS

Cultivation and Harvesting

Seeds of lettuce vegetable (*Lactuca sativa*) and fluted pumpkin vegetable (*Telfaria occidentalis*) were planted in plastic pots filled with sterile topsoil (topsoil sterilized in an hot-air oven at 180^{o}C for 2 hours). The plants were raised in the screen house and watered daily with different irrigation waters (sewage-polluted stream, deep well, tap and rain water) using spray irrigation method. The irrigation was done for about 60 days and the vegetables were harvested aseptically.

Isolation of bacteria from harvested vegetables

Bacteriological investigation was carried out on the harvested vegetables. One gram leaf sample of each vegetable was chopped with 10.0ml of sterile peptone water. After then, series of dilutions were made by mixing 1.0ml of the suspension in 9.0ml of the sterile peptone water to obtain 10^{-1} dilution. The dilution was then made to 10^{-2}, 10^{-3} and 10^{-4}. 1.0ml each of the dilutions was inoculated on the Mannitol salt agar, Salmonella-Shigella agar, ampicillin supplemented blood agar, ampicillin supplemented starch agar, MacConkey agar, 5% human blood agar and nutrient agar. The agar plates were incubated aerobically at 37^{o}C for 48 hours. Distinct bacterial colonies were picked and streaked on nutrient agar plates which were then incubated to obtain pure colonies. Bacterial isolates were characterised on the basis of their cultural appearance, microscopic morphology, Gram staining reactions and biochemical properties; and identified using

Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

Cytological studies

This study employed the modified *A. cepa* assay (Fiskesjo, 1985; Bakare *et al*., 2003).

The leaves of the vegetables were carefully detached from their stalks, washed and dried at room temperature for 24 hours. Thereafter, 0.25kg/l and 0.50kg/l concentrations of the leaf extracts were prepared by blending 125g and 250g of leaves samples in 500ml distilled water.

Onion bulbs were purchased from the market. The dried roots at the base of the onion bulbs were carefully prunned, leaving the ring of root primordial intact. The onion bulbs were placed on 250ml beakers filled with the vegetable extracts with the base of the onion bulbs touching the surface of the liquid. Three onion bulbs were set for each treatment of the different samples at room temperature. Three beakers were then filled with tap water and onion bulbs were placed on them. These were used as control. The length and number of roots developed by each bulb were measured at 0, 24, 48 and 72 hours and the means were calculated. The percentage root growth restriction (RGR) in relation to the control, for each sample, was determined. After 72 hours, the developed roots on each bulb were prunned off and the onion bulbs were placed back on their beakers to generate new roots for cytological studies. Roots for cytological studies were harvested when they were about 1.0 to 2.0cm long. The roots were fixed in 1:3 acetic acid/ethanol (v/v) for 24 hours. After fixing, the roots were hydrolysed with 1N HCl for 5 minutes and washed with distilled water.

One root tip was then squashed on each slide, stained with a drop of FLP-porcein for 10 minutes and cover slip was carefully lowered on it to exclude air bubbles. Gentle tapping of the cover slip with the blunt end of a biro pen ensured a good spread of the cells and the excess stain was removed using a filter paper. The slides were then observed under the microscope at ×1000 magnification, for dividing cells and cells carrying chromosomal aberrations. Ten slides were prepared for each concentration.

Data on total cells and total dividing cells were taken from thirty microscope fields on each slide. The data were used to determine the mitotic index of the cells.

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total cells counted}} \times 100$$

(Bakare *et al*., 2000)

Determination of heavy metals in vegetable samples

After harvesting, the vegetable samples were washed with distilled water to eliminate air-borne pollutants. The leaves were detached from their stalks, sliced and dried to eliminate excess moisture. Once dried, each sample was weighed and oven-dried at 60^{o}C for two days. Each oven-dried sample was grounded in a mortar and analysed. Perchloric acid digestion (wet oxidation) method was used, followed by Atomic Absorption Spectrophotometric analysis, as described in the Perkin-Elmer manual for Atomic Absorption Spectrophotometry. One gram of a grounded vegetable sample

was measured into a 125ml Erlenmeyer flask, which has been previously washed with acid and distilled water. The sample was digested with 4.0ml of perchloric acid, 25.0ml of concentrated HNO_3 and 2.0ml of concentrated H_2SO_4 under a fume cupboard. The contents were mixed and heated gently at a low medium heat on a hot plate under perchloric acid fume cupboard. The heating continued until dense white fumes appeared. The solution was then allowed to cool and 50ml of distilled water was added and boiled for about 1minute at medium heat. The solution was cooled and filtered completely into a 100ml Pyrex volumetric flask using Whatman filter paper No.42 and the solution was made to the mark with distilled water. Atomic absorption spectrophotometry (A.A.S.) was used for the determination of the heavy metals (Itanna, 2002).

RESULTS

Bacteriological quality of vegetables

Table 1 shows the bacteria isolated from the vegetable samples. All the vegetable samples contained food-borne pathogens with the exception of vegetables irrigated with rain water, which did not contain food-borne pathogens. Bacteria isolated from the vegetables irrigated with polluted stream water and well water included *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter aerogenes, Salmonella paratyphii, Shigella dysenteriae, Pseudomonas aeruginosa, Streptococcus* spp. and *Aeromonas hydrophilla*. *Salmonella paratyphii, Shigella dysenteriae, Aeromonas hydrophilla* and *Bacillus* spp. were not isolated from the vegetables irrigated with tap water while only *Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus* spp. were isolated from vegetables irrigated with rain water. *Listeria* spp. was not isolated from any sample.

Table 1: Incidence of bacterial isolates on irrigated vegetables

Isolates	Vegetables irrigated with tap water	Vegetables irrigated with rain water	Vegetables irrigated with well water	Vegetables irrigated with stream water
Escherichia coli	+	-	+	+
Staphylococcus aureus	+	+	+	+
Klebsiella pneumoniae	+	-	+	+
Enterobacter aerogenes	+	-	+	+
Salmonella paratyphii	-	-	+	+
Shigella dysenteriae	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+
Streptococcus spp.	-	-	-	+
Aeromonas hydrophilla	-	-	-	+
Bacillus spp.	-	+	+	+

Note:
+: presence
-: absence

Genotoxicity of the vegetables

Mitosis was observed to be normal in the cells of roots obtained from the control (tap water) and treatments. Chromosome counts at mataphase and other stages showed normal cell division without any aberration. Table 2 shows the data on the effect of the vegetable leaf extracts on root growth of *Allium cepa.* As evidenced by the table, there was a gradual inhibition of root growth with increasing leaf extract concentration. The mean root length ranged from 2.12cm in root cells treated with 0.50kg/l of leaf extract of lettuce vegetable irrigated with polluted stream water to 3.20cm in the root cells of the control. Also, the root growth restriction (RGR) values ranged from 66.40% (expressed as growth in % of control) to 88.40%. In all treatments, restricted growth occurred which indicates the toxicity of the treatments. Table 3 shows the data on mitotic activities of the cells at different concentrations and the chromosome aberrations were not observed in the cells of roots treated with the leaf extracts. The mitotic indices in the treated roots were lower than the control. The mitotic index values ranged between 34.61% in the roots treated with 0.50kg/l of leaf extract of *Lactuca* vegetable irrigated with stream water and 45.14% in the roots treated with 0.25kg/l of leaf extract of *Telfairia* vegetable irrigated with rain water. The mitotic index value of the control was 49.53%. There was a gradual decrease in the total number of divided cells. From the results of genotoxicity, the lower mitotic index than the control indicated that the vegetable samples are mitodepressants.

Table 2: Root growth restriction values of *Allium* bulbs treated with different concentrations of vegetable extracts

Samples	Concentration(kg/l)	Mean root length± S.D (cm)	%Root Growth Restriction values
Control (tap water)	-	3.20 ± 0.13^{a}	-
LT	0.25	2.83 ± 0.18^{ac}	88.40
	0.50	2.51 ± 0.07^{c}	78.40
LR	0.25	2.65 ± 0.40^{ac}	82.80
	0.50	2.50 ± 0.20^{c}	78.10
LW	0.25	2.26 ± 0.09^{b}	70.60
	0.50	2.22 ± 0.17^{a}	69.40
LS	0.25	2.17 ± 0.02^{c}	67.80
	0.50	2.12±0.30c	66.40
TT	0.25	2.80 ± 0.70^{ab}	87.50
	0.50	2.74 ± 0.51^{bc}	85.60
TR	0.25	2.62 ± 0.30^{b}	81.90
	0.50	2.53±0.60a	79.10
TW	0.25	2.30 ± 0.40^{b}	71.90
	0.50	2.19 ± 0.14^{b}	68.40
TS	0.25	2.17 ± 0.11^{bc}	67.80
	0.50	2.13 ± 0.20^{bc}	66.60

Means with the same letter(s) in the same column are not significantly different ($P<0.05$)

Note: LS and TS: *Lactuca* vegetable and *Telfaria* vegetable irrigated with stream water respectively.
LW and TW: *Lactuca* vegetable and *Telfaria* vegetable irrigated with well water respectively.
LT and TT: *Lactuca* vegetable and *Telfaria* vegetable irrigated with tap water respectively.
LR and TR: *Lactuca* vegetable and *Telfaria* vegetable irrigated with rain water respectively.

Table 3: Mitotic activities of *Allium cepa* root cells treated with different concentrations of leaf extracts

Samples	Concentrations (kg/l)	Number of divided cells	Number of non-divided cells	Total cells	Mitotic index	Abnormal cells
Control (tap water)	-	212	216	428	49.53	0
LT	0.25	195	239	434	44.93	0
	0.50	197	246	443	44.47	0
LR	0.25	146	187	333	43.84	0
	0.50	158	230	388	40.72	0
LW	0.25	173	280	453	38.19	0
	0.50	123	210	333	36.94	0
LS	0.25	118	215	333	35.44	0
	0.50	154	291	445	34.61	0
TT	0.25	171	238	409	41.81	0
	0.50	152	255	407	37.35	0
TR	0.25	158	192	350	45.14	0
	0.50	154	194	348	44.25	0
TW	0.25	134	164	298	44.97	0
	0.50	130	201	331	39.27	0
TS	0.25	146	226	372	39.25	0
	0.50	149	241	390	38.21	0

Note:
LS and TS: *Lactuca* vegetable and *Telfaria* vegetable irrigated with stream water respectively.
LW and TW: *Lactuca* vegetable and *Telfaria* vegetable irrigated with well water respectively.
LT and TT: *Lactuca* vegetable and *Telfaria* vegetable irrigated with tap water respectively.
LR and TR: *Lactuca* vegetable and *Telfaria* vegetable irrigated with rain water respectively.

Table 4: Phase index of *Allium cepa* root cells treated with different concentrations of leaf extracts

Samples	Concen-trations (kg/l)	prophase	Metaphase	Anaphase	Telophase
Control (tap water)	-	91	30	37	54
LT	0.25	90	30	35	48
	0.50	86	16	39	46
LR	0.25	47	39	44	44
	0.50	62	18	34	44
LW	0.25	70	22	33	48
	0.50	41	11	30	41
LS	0.25	37	12	32	37
	0.50	55	26	35	38
TT	0.25	69	27	31	44
	0.50	65	20	30	37
TR	0.25	46	21	37	54
	0.50	47	16	41	50
TW	0.25	37	20	37	40
	0.50	41	21	33	35
TS	0.25	45	21	31	49
	0.50	61	12	33	43

Note:
LS and TS: *Lactuca* vegetable and *Telfaria* vegetable irrigated with stream water respectively.
LW and TW: *Lactuca* vegetable and *Telfaria* vegetable irrigated with well water respectively.
LT and TT: *Lactuca* vegetable and *Telfaria* vegetable irrigated with tap water respectively.
LR and TR: *Lactuca* vegetable and *Telfaria* vegetable irrigated with rain water respectively.

Levels of Heavy metals in Vegetables

The concentrations of heavy metals found in vegetables irrigated with water from different sources are shown in Table 5. The results showed a low level of zinc and chromium in all irrigated vegetable samples. Cadmium and chromium metals were detected in vegetables irrigated with stream water and well water, and not found in vegetables irrigated with rain water and tap water. The levels of iron detected in the vegetables were between 0.711mg/kg in *Telfaria* vegetable irrigated with rain water to 1.122mg/kg in *Lactuca* vegetable irrigated with stream water.

Table 5: Levels of heavy metals in irrigated vegetables

Heavy metals (mg/kg)	LS	LW	LT	LR	TS	TW	TT	TR	[a] Permissible levels in food (mg/kg)
Pb	0.531	0.451	0.375	0.344	0.371	0.356	0.261	0.327	0.50
Zn	0.566	0.413	0.915	0.645	1.618	1.335	1.079	0.142	5.0
Cd	0.08	0.02	0.00	0.00	0.13	0.04	0.00	0.00	0.05
Cu	0.049	0.047	0.037	0.044	0.057	0.046	0.021	0.035	5.0
Fe	1.122	0.917	1.014	1.011	0.748	0.717	0.741	0.711	15.0
Cr	0.11	0.09	0.00	0.00	0.14	0.04	0.00	0.00	2.30

[a] Source: FAO/WHO Codex alimentarius, 2001

Note:
LS and TS: *Lactuca* vegetable and *Telfaria* vegetable irrigated with stream water respectively.
LW and TW: *Lactuca* vegetable and *Telfaria* vegetable irrigated with well water respectively.
LT and TT: *Lactuca* vegetable and *Telfaria* vegetable irrigated with tap water respectively.
LR and TR: *Lactuca* vegetable and *Telfaria* vegetable irrigated with rain water respectively.

Comparing the two vegetables, it was discovered that *Telfaria* vegetable accumulated more heavy metals than the *Lactuca* vegetable. *Telfaria* vegetable irrigated with stream water had generally the highest concentration of zinc, cadmium, copper and chromium while *Lactuca* vegetable irrigated with stream water had the highest concentration of lead and iron. The amount of lead in *Lactuca* vegetable irrigated with polluted stream water and that of cadmium in the two vegetables irrigated with polluted stream water were above the maximum recommended levels set by Food and Agricultural Organization (FAO) and World Health Organization (WHO) for human consumption. Other metals are within the acceptable levels in the two vegetables. The variation in the metal content observed in the vegetables could depend on the quality of the water used to irrigate them as well as the absorption capacity of each metal by the plant.

DISCUSSION

Microbial food safety is considered as a public health issue and food quality control largely depends on indications of faecal bacteria and other food pathogens. Outbreaks of infectious intestinal diseases linked with vegetables and fruits in many countries have been documented (Li *et al*., 2001). This present study showed that pathogenic bacteria can be transferred to vegetable plants during irrigation. The bacterial pathogens isolated varied with the quality of water used to irrigate them. The bacterial isolates included *Escherichia coli, Salmonella paratyphii, Shigella dysenteriae, Klebsiella pneumoniae, Staphylococcus aureus, Enterobacter aerogenes* and *Aeromonas hydrophila.* The presence of these bacteria in food indicates that such food has been

contaminated with faecal materials and not suitable for human consumption. This indicates that vegetables irrigated with polluted stream water and well water may not be safe for human consumption because they could be contaminated with pathogenic bacteria from the water.

Parameters such as root growth, frequencies of mitosis and abnormal cells can be used to estimate the cytotoxicity, genotoxicity and mutagenicity of substances (Amin, 2002). In the *Allium cepa,* root growth restriction indicates retardation of growth and cytotoxicity, which are followed by suppression of mitotic activity. The present study recorded low mitotic index values for treated onion cells, which showed that cell divisions were depressed in the treated root cells. This agrees with the findings of Bakare *et al*., 1999; Bakare *et al.*, 2000; Bakare and Adekunle, 2001; Amin, 2002 and Bakare *et al., 2003*, who recorded lower mitotic index values in treated onion root cells compared with the controls. A decreased in the mitotic index was also found to be significant with an increase in the vegetable extracts. This indicates the cytotoxic effect of the irrigated vegetable plants especially those irrigated with polluted stream and well waters which had very low mitotic indices. The suppression of mitotic index was probably due to the blocking of G_1, suppressing cells from DNA synthesis or the blocking of G_2, preventing cells from entering mitosis (Amin, 2002). However, the occurrences of low number of metaphase and low metaphase/prophase ratios in most root cells of the treated bulbs indicate that majority of the dividing cells are in the prophase stage. This indicates that after the mitotic suppression, the surviving cells started to divide again, but did not pass to the metaphase stage. From the results, it could be deduced that due to the very low mitotic index values as well as retardation in the growth of the roots of the bulbs treated with the extracts of vegetables irrigated with polluted stream water and well water, these vegetables might be toxic.

The high metallic content of the vegetables irrigated with polluted stream water and well water could have contributed to the observed effects on the root growth and mitotic activities of *A. cepa.* Lead has been reported to reduce root growth and the frequency of mitotic activities in the cells. Cadmium, chromium, copper and zinc have also been reported to inhibit root elongation of different species of plant (Bakare *et al*., 2003).

CONCLUSION

The present study has shown that the use of polluted water in the irrigation of food crops may have effects on the microbiological quality, heavy metals' content as well as cytotoxic effects on such food crops. Therefore, it is suggested that polluted water should be properly treated to remove pathogenic bacteria present in it and heavy metals content should be lowered before being used for irrigation.

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