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Full Length Research Paper

Evaluation of the microbial attributes of the polluted Alamuyo River on selected surrounding wells

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The physicochemical analysis and microbial load of polluted Alamuyo River in Ibadan, Nigeria and selected wells along its course were evaluated. Toxic effects of water samples obtained from upstream and down stream (F) of the river were also evaluated using *Allium cepa* root assay. The result of the analysis revealed that the water sample contained toxic substances. However, almost all parameters evaluated were within the allowable limit of treated wastewater. High microbial load was observed in river water samples compared to the well water samples throughout the sampling periods with overall highest value observed at Late Rainy Season upstream water sample (LRS-A). MPN of faecal coliform showed that LDS-A, ERS-F and LRS from A – F were significantly different. *Echerichia coli, Bacillus substillis, Staphylococcus aureus, Pseudomonas fragii* were among the microbes observed in the samples. T-test analysis on *A. cepa* root length showed that only 50% of LDS-A, LDS-F and LRS-A were significantly different from control (P < 0.05) though there were reduction in root length in all concentrations tested through out the sampling period except 5% ERSA, 10% ERSF and 10% LRSF. Microscopic evaluation of *A. cepa* cells showed decreasing number of dividing cells and mitotic indices were not dose dependent in most cases. Chromosome fragments, bridge, lag and disturbed spindle were the aberrations observed in this study. Our result showed that water samples from the river contained toxic substances and high microbial load, which have effects on the surrounding wells due to infiltration.

Key words: Toxicity, *Allum cepa*, aberration, allowable limits, evaluation, phytotoxic.

INTRODUCTION

Water is very essential to all living organisms, and man depends on it for different purposes, which include drinking, cooking, gardening, washing, bathing, irrigation, navigation and even dispersal/disposal of waste products. It constitutes about two-third of the earth surface (Gottefried, 1993). Based on salinity, it can be broadly divided into ocean (97%) and fresh water (3%). Most of the latter are not accessible because about 87% of it is locked in the ice caps and glacier, atmosphere, soil and in the deep underground (Paul and Misra, 2004).

WHO (1967) reported that the pollution of surface and underground water is spreading very fast in the world. This may be attributed to population expansion, rapid urbanization, industrial and technological expansion that often leads to generation of enormous wastes from domestic and industrial sources. Indiscriminate discharge of untreated or partially treated wastewaters directly or indirectly into aquatic bodies may render water resources unwholesome and hazardous to man and other living systems (Bakare et al., 2003).

Severe effects of polluted water had been reported in developed countries like USA, Western European nations and Japan (Tibbetts, 2000). Some drinking water was found to contain synthetic organic chemicals, lead, arsenic, faecal wastes and pathogenic organisms. They may have potential of causing cancer, birth defects and varieties of gastrointestinal illness.

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Abbreviations: LDS, Late dry season; ERS, early rainy season; and LRS, late rainy season.

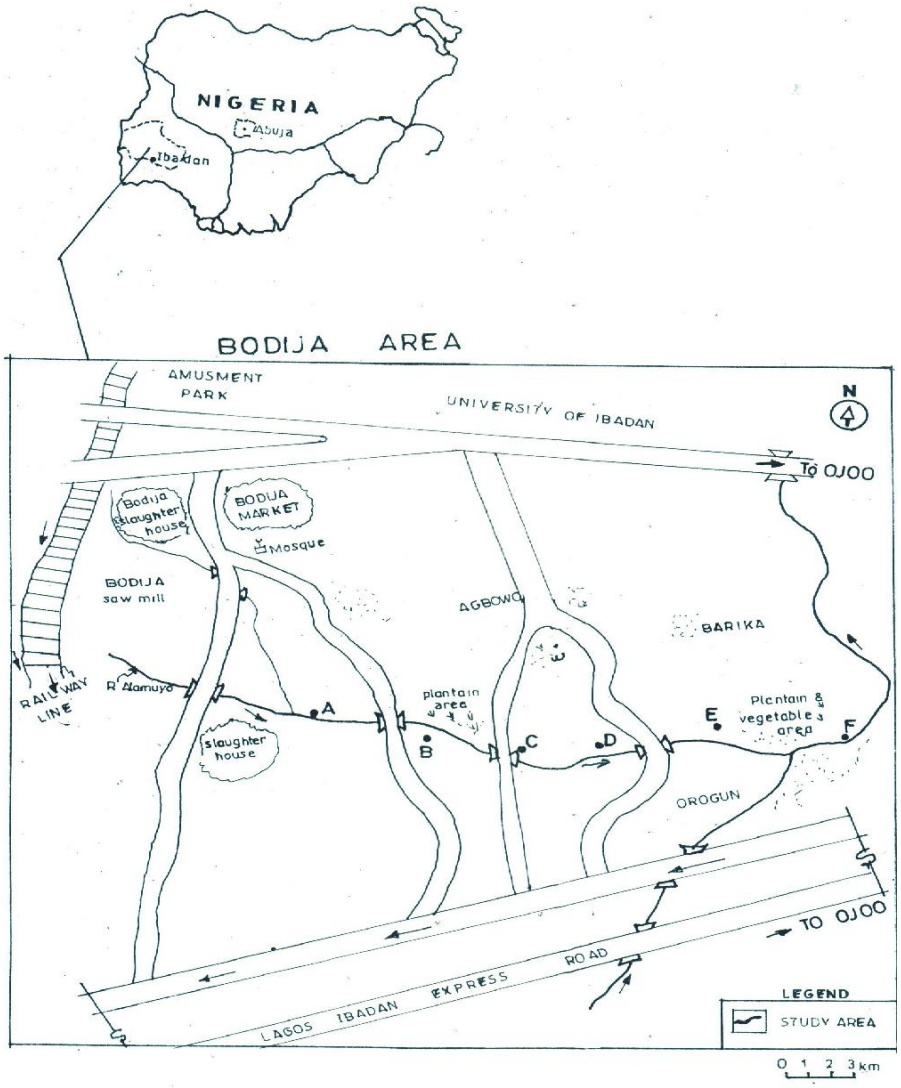

Figure 1. Geographic location of sampling sites (A-F).

In Nigeria, cases of water polluted by oil and solid wastes are very rampant. This was reported to have rendered many surface and underground water unsuitable for drinking and other domestic uses (Odiete, 1999). Other sources of pollutants into aquatic water bodies include industries, domestic wastes and sewage. In the last decades, concern has grown about the adverse effects that the use and disposal of pharmaceuticals might potentially have on human and ecological health (Kummere, 2003). Lateef and Yekeen (2006) showed that disposal of untreated waste waters from pharmaceutical plants is capable of promoting the proliferation and spreading of resistant bacteria and may also be potentially genotoxic. Also wastewater from slaughterhouses had been reported to be very harmful to the environment (Masse and Masse, 2000). Abattoir waste also caused deoxygenation of river (Quinn and McFarlane, 1989) and contamination of ground water (Sangodoyin and Agbawhe, 1992). Report has also shown that indiscriminate disposal of abattoir waste may introduce enteric pathogens into surface and ground water (Meadows, 1995) and that pathogens isolated from the wastewater can survive in the environment and pose danger to humans and animals (Coker et al., 2001).

Although there are reports on the microbial attributes and toxic effects of different industrial wastes as well as leachates (Cameron and Korch, 1980; Cheung et al., 1993; Bakare et al., 2003), there is paucity of knowledge on polluted rivers that receive pollutants from multiple sources. One of such rivers in Ibadan, Nigeria is Alamuyo River (Figure 1). It flows through Bodija, Agbowo, Barika to link a major river called River Orogun. Besides the fact that it receives abattoir effluents from two industries, it also receives domestic wastewater from different tributaries, leachate from saw dust dump site and also serves as means of refuse disposal. At present, the water ob-

tained from the river is not drinkable; however, wells are dug along its course to provide portable water for domestic needs most especially during dry season. Water from the river is also used for irrigation to propagate vegetable, sugarcane, maize and so on. The possible infiltration of water from the river into the wells and its usage for irrigation calls for assessment of the water body.

Therefore, this research sought to evaluate and determine the physicochemical properties of the river and four selected wells along its course, assess their microbial attributes and the toxic potential of water samples from the river using *Allum cepa* assay.

MATERIALS AND METHODS

Sample collection and physicochemical analysis

The samples were collected in the month of February, April, and August 2006, representing late dry season (LDS), early rainy season (ERS) and late rainy season (LRS) respectively. The water samples from the upstream (A) and down stream (F) of the river as well as four selected wells along the course of the river (B, C, D and E) were collected separately into 2 L capacity of pre-cleaned plastic container. Samples for Biological Oxygen Demand (BOD) determination were collected separately into washed BOD bottles with necessary precautions. The temperature, pH and Dissolved Oxygen (DO) were determined on sites while the remaining parameters were analyzed in the laboratory using standard method (APHA, 1995).

Isolation of microorganisms

Total colony count of bacteria was done by the pour plate method using nutrient agar. The water samples (A - F) of each collection time were serially diluted and 0.2 ml of an appropriate dilution was used to inoculate the plate in duplicate. The plates were incubated at 37^{o}C for 24 – 48 h after which the total colony count was determined as described by Nwachukwu (2000). Pure isolates were obtained after several subcultures and the colonies obtained were screened and identified based on the standard taxonomic schemes and description (Buchaman and Gibbons, 1974).

Faecal coliform test

The three tubes procedure using MacConkey broth was used to detect the coliforms and determine the most probable number (MPN) of coliform. 0.1, 1.0 and 10 ml of each of the samples were used to inoculate the MacConkey broth in three replicates, with inverted Durham's tubes inserted for gas collection. Colour change, gas production and turbidity were used as positive indication of faecal coliform (D'Auriac et al., 2000). MPN per 100 ml of water samples were then calculated.

Allum cepa assay

The modified *A. cepa* assay was used to determine the potential genotoxicity of the water samples (Fiskesjo, 1985; Rank and Neilson, 1993; Bakare et al., 2000). Commercially available onion bulbs used for the test were cleaned and sundried for 2 weeks. The outer scale and brownish bottom plate were carefully removed leaving the ring of root primordial intact. Tap water was used to dilute the effluent and also used as the control. Ten and six onion bulbs were placed directly on 50 ml capacity beaker prior filled with 0, 1.0, 5.0, 10.0, 20.0 and 50.0% of each of the sample A and F for macroscopic and microscopic evaluations, respectively. This was repeated for all the sampling periods. The experiment was performed in the dark at 26 ± 1^{o}C with the liquid being changed on daily basis.

Microscopic evaluation

At 48 h, root tips from 5 bulbs from each of the test concentrations and control were fixed in ethanol- glacial acetic acid (3:1, v/v). The root tips were hydrolysed in IN HCl at 65^{o}C for 3 min. Two root tips were squashed per slide and stained with FLP orcein for 15 min for chromosome analysis. One thousand cells (1000) were scored per slide for frequency and different types of chomosomal aberrations in the dividing cells at 1000 x. Five thousand (5000) cells were scored per treatment as well as the control.

Macroscopic evaluation

At 72 h, the length of each root per bulb for each of the test concentrations and the control was measured and recorded in centimetres. The EC_{50} values of the water samples collected from points A and F for all the sampling periods were obtained. T- test was used to compare mean root length obtained for each treatment with that of control.

RESULTS AND DISCUSSION

The results of physicochemical analysis of water samples collected from Alamuyo River (A and F) and wells along its course (B, C, D and E) were as shown in Table 1. Except for temperature, pH, alkalinity and total hardness where there were no specific patterns of variation, values recorded for DO, BOD, SO_4^{2-}, $P\ O_4^{2-}$, NO_3^- Pb^{2+}, Fe^{2+} and Mn^{2+} were found to be higher in water samples obtained from the river compared to those collected from the wells. The reverse was the case for total solid (TS) and total suspended solid (TSS) especially for late dry season water samples. However, these values conformed with the standard for treated waste water (FEPA, 1991) and Standard for Aquaculture (adapted from Chaudhary et al., 2004).

The total bacteria count for all water samples were as shown in Figure 2. The mean total bacteria count ranged between $0.8\ x10^6$ – $32.4\ x\ 10^6$ cfu/ml, 0.9 – $28.0\ x\ 10^6$ cfu/ml and 5.2 – $36.2\ x\ 10^6$ cfu/ml for LDS, ERS and LRS, respectivey. Bacteria load was found to be more in the samples obtained from the river compared to those of the wells throughout the sampling periods. The highest value ($36.2\ x\ 10^6$ cfu/ml) was obtained for LRS river sample (A) while the least bacteria load (0.8) was obtained at well E. Furthermore, well E has the lowest value for each of the sampling periods. This might be due to its not been frequently used and its distance from the river compared to other wells.

All water samples collected from the river as well as

Table 1. Physcochemical analysis of water sample from Alamuyo River and wells along its course.

Parameter	Sampling time	A	B	C	D	E	F	FEPA (1991)	SFA
Temp. (°C)	LDS	24	25	25	25	25	24		
	ERS	24	25	24	24	25	24	_	
	LRS	25.5	25	25	25	25	25		
pH	LDS	6.8	7.1	6.9	7.1	6.9	6.8		
	ERS	6.9	7.1	7.1	7.2	6.9	7.1	6-9	6.7- 8.5
	LRS	6.8	7.1	7.2	7.2	6.9	7.2		
DO (mg/L)	LDS	3.6	2.6	2.0	2.4	3.2	2.8		
	ERS	3.2	2.4	2.0	2.0	3.0	2.2	5-10	
	LRS	2.0	2.2	2.4	2.6	3.4	2.2		
BOD (mg/L)	LDS	3.2	2.4	1.8	1.6	1.8	2.4		
	ERS	2.4	1.8	1.6	1.2	1.4	2.0	50	<10
	LRS	2.2	1.6	1.6	1.4	1.6	1.8		
Total	LDS	416	420	424	460	380	408		
Solid (mg/L)	ERS	432	320	270	300	357	424		
	LRS	484	295	280	285	262	260		
Dissolved	LDS	344	292	286	308	271	328		
Solid	ERS	352	248	212	234	285	346	2000	<500
TDS (mg/L)	LRS	388	237	218	203	196	358		
TSS (mg/L)	LDS	72	128	138	152	109	80		
	ERS	80	72	58	66	60	78	30	
	LRS	96	76	62	82	66	102		
Total	LDS	68	76	64	6.4	76	68		
Hardness	ERS	60	72	68	6.4	72	64		30-180
(mg/L)	LRS	64	58	68	6.4	76	64		
Ca^{2+}	LDS	44	56	40	44	52	48		
Hardness	ERS	40	52	44	40	48	44	-	
(mg/L)	LRS	44	52	40	40	48	44		
Mg^{2+}	LDS	24	20	24	20	24	20		
Hardness	ERS	20	20	24	24	24	20		
(mg/L)	LRS	20	24	29	24	28	20		
Alkalinity	LDS	55	75	70	75	65.0	60		
	ERS	50	65	60	75	60	65		50 - 3000
	LRS	55	62	65	70	60	65		
Cl^- (mg/L)	LDS	0.6	0.6	0.8	0.3	0.4	0.6		
	ERS	0.6	0.4	6.5	0.4	0.3	0.7	2.0	31 - 50
	LRS	7.5	6.5	8.0	4.5	6.5	6.6		
SO_4^{2-} (mg/L)	LDS	12.5	10.0	10.55	9.53	11.0	12.6		
	ERS	10.5	09.0	10.0	15.5	10.5	10.5		
	LRS	11.0	10.0	11.03	11.0	9.5	11.0		
$P0_4^{3-}$ (mg/L)	LDS	1.2	0.7	0.8	0.6	0.9	1.4		
	ERS	1.5	1.0	0.6	0.8	1.1	1.8	20	
	LRS	1.7	1.2	0.6	1.0	1.3	2.1		
$N0_3^-$ (mg/L)	LDS	6.4	2.8	7.2	4.8	3.2	6.8		
	ERS	5.8	3.2	6.8	4.4.	3.6	5.8		
	LRS	6.1	3.6	7.0	4.0	3.8	6.0		
NH_3 (mg/L)	LDS	0.056	0.026	0.060	0.036	0.040	0.068		
	ERS	0.048	0.038	0.048	0.028	0.040	0.056		
	LRS	0.052	0.036	0.044	0.032	0.044	0.060		

Table 1. Contd

Fe^{2+} (mg/L)	LDS	0.9	0.5	0.75	0.5	0.35	1.1		
	ERS	1.1	0.65	0.8	0.6	0.45	1.25		
	LRS	1.3	0.70	0.8	0.75	0.5	1.7		
Pb^{2+} (mg/L)	LDS	0.115	ND	0.010	ND	ND	D 0.110		
	ERS	0.15	0.05	0.15	ND	ND D	0.101	< 1	
	LRS	0.10	0.1	0.15	0.05	ND D	0.15		
Mn^{2+} (mg/L)	LDS	0.55	0.3	0.45	0.35	0.3	0.5		
	ERS	0.65	0.3	0.5	0.5	0.35	0.55	200	
	LRS	0.60	0.35	0.6	0.5	0.45	0.60		

*Standard.
LDS, Later dry season; ERS, early rainy season; LRS, late rainy season; FEPA, Federal Environmental Protection Agency (1991); SFA, Standard for Aquaculture (adapted from Chaudary et al. (2004).
ND = Not detectable.
Co, Cd, Cr, Cu were not detectable.

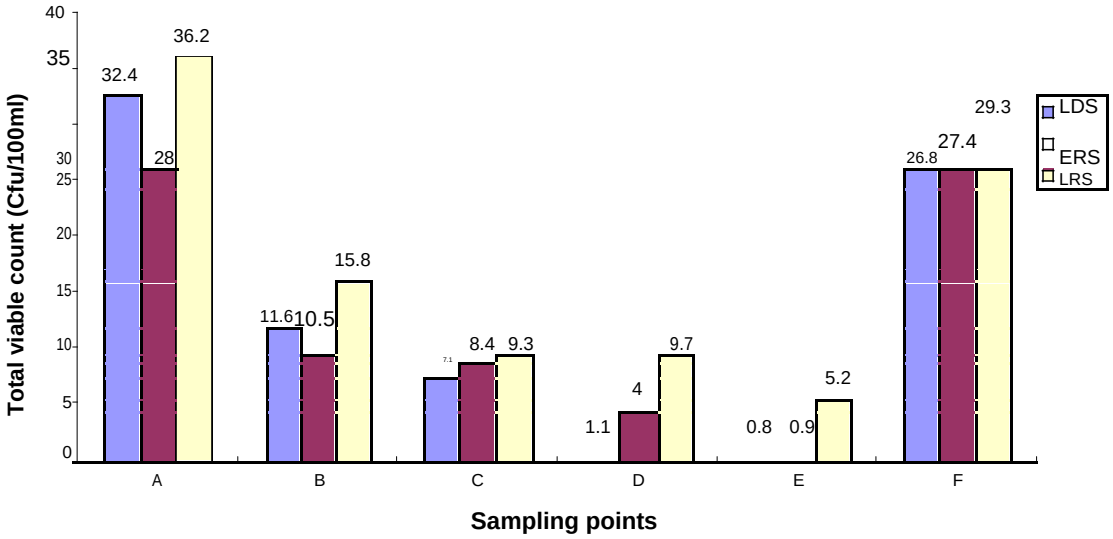

Figure 2. Variations in total bacteria count in water samples from Alamuyo River and the wells along its course.

those of the wells showed presence of faecal contaminants (Table 2). Water samples obtained from the river (A and F) had the highest value of faecal contamination throughout the sampling periods. MPN of coliforms present in the samples varies between (120 – 1100^{+}) for samples obtained from the river and the wells during LRS (Table 2). The prevalent bacteria in the water samples include *Bacillus* sp., *Micrococcus* sp., *Streptococcus* sp., *Staphylococcus* sp., *Serratea* sp and *Escherichia coli* (Table 3).

Results of toxic potential of polluted water samples obtained from the river (A and F) showed the samples to be phytotoxic. Macroscopic evaluation through the *A. cepa* root length showed gradual inhibition with more prominent effects at the highest concentration (50%). T-test analysis showed that only mean root length obtained at 50% LDS-A, LDS-F and LSR-A (Table 4) were significantly different from control ($P < 0.05$). The effective concentration (EC_{50}) obtained for the samples obtained from the river (Figure 3) using *A. cepa* showed that LRS-A < LDS-F < LDS-A while ERS-F, LDS-F and ERS-A have no traceable EC_{50} value.

Table 5 shows the effects of the water samples on *A. cepa* cells (microscropic evaluation). Water collected from the sampling points A and F throughout the period of the sampling periods showed root length inhibition which was prominent at higher concentrations. This indi-cates that the water was mitodepressant. Except at LDS-A, ERS-F and LRS-A, the mitotic index of various concentrations of each sample was dose dependent and their mitotic inhibition increased as concentration increas-ed. The frequency of aberration observed showed no dose

Table 2. MPN of coliforms present in polluted Alamuyo River and selected wells along its course.

Sample	Late dry season	Early rainy season	Late rainy season
A	1100+	1100	1100+
B	290	460	1100+
C	120	53	1100+
D	460	1100	1100+
E	1100	1100	1100+
F	1000	1100+	1100+

Unit per 100 ml of the water sample.
+Significant value of faecal coliforms.

Table 3. Prevalence of Bacterial isolates in water samples collected from Alamuyo River and Wells along its course.

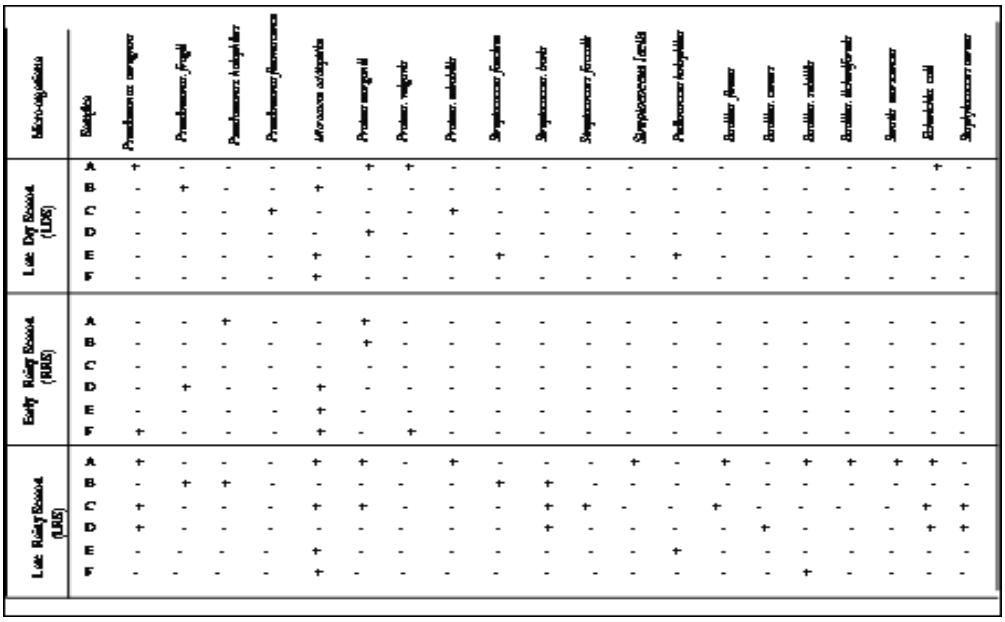

Table 4. Mean root length (cm) of *A. cepa* treated with differnt consentrations of polluted water samples obtained from Alamuyo River.

Conc.	LDSA	LDSF	ERSA	ERSF	LRSA	LRSF
0.0	4.18 (0.12)	3.82 (0.005)	4.46 (0.130)	4.46 (0.130)	3.82 (0.005)	3.82 (0.005)
1.0	3.68 (0.080)	4.27 (0.120)	4.30 (0.140)	4.42 (0.130)	3.73 (0.005)	3.32 (0.004)
5.0	3.67 (0.009)	3.86 (0.080)	4.70 (0.100)	3.79 (0.100)	3.35 (0.003)	3.57 (0.004)
10.0	3.62 (0.090)	2.61 (0.070)	3.56 (0.090)	4.93 (0.120)	3.05 (0.004)	4.05 (0.007)
25.0	3.42 (0.070)	2.71 (0.006)	4.22 (0.080)	3.78 (0.120)	3.06 (0.025)	3.65 (0.003)
50.0	0.55 (0.030)*	1.52 (0.050)*	3.32 (0.190)	3.50 (0.080)	179 (0.004)*	2.96 (0.005)

*Significantly different ($p<0.05$)
Value in parenthesis indicate standard error.

dependency while the most frequently appearing seen aberration was disturbed spindle. However, no aberration was observed in the control. Complete cell arrest was observed only at LRS-F water sample at 20 and 50% concentrations, an indication that the water samples were toxic.

Although the physicochemical parameters of the samples obtained from the river and the wells were within the

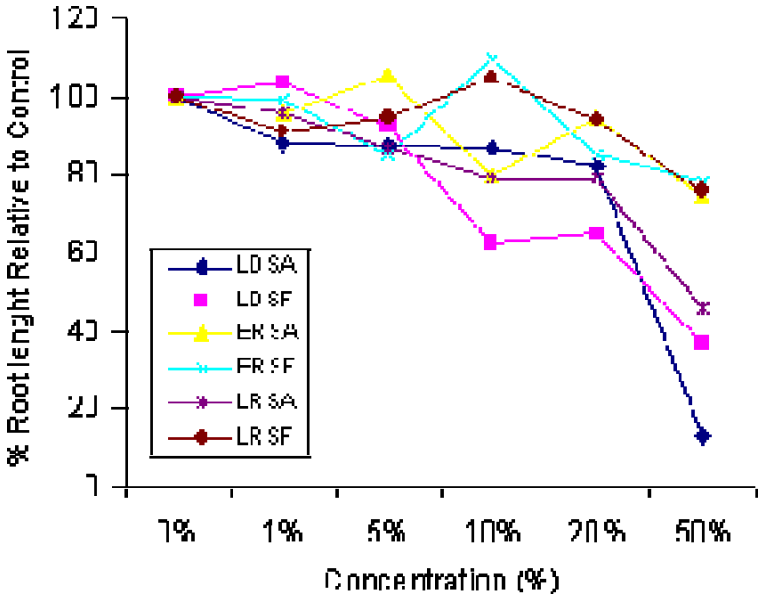

Figure 3. Growth curves and EC50 of polluted water samples tested on *A. cepa* root length.

allowable limit (FEPA, 1991), the samples contained substances that had been established to be cytotoxic. High value of PO_4^{3-}, SO_4^{2-} and NO_3^- in samples from the river compared to the wells might be due to direct discharge of wastes as well as the run off which wash organic substances into the river. This creates enabling environment for the microorganism to thrive. Increased value of BOD relative to DO most especially in the samples obtained from A and F compared to the wells indicates that there were more microbes in the river water sample. This was further corroborated by the high value of bacteria obtained from the river water sample compared to the wells throughout the sampling periods.

Adeyemo et al. (2002) had earlier reported that most slaughterhouses in Nigeria discharged their wastes directly into the river without prior treatment. Bodija abattoir was not left out in this kind of operation. Report has shown that such practices result in the introduction of enteric pathogens into surface water and subsequently leading to contamination of ground water (Meadows, 1995). Pathogens from such environment have ability to survive and pose danger to both animal and human lives (Coker et al., 2001). High faecal contamination observed might also be attributed to direct defaecation into the river, discharge of abattoir wastes and surface water run off. The presence of *E. coli*, *Streptococcocus* and others confirmed human faecal contamination.

Presence of lead (Pb) in both the river and well water samples further confirmed the toxic potential of the samples. Lead can form complexes, which may have individual, synergistic or antagonistic effects in inhibiting cell division as observed in this research. Maitra and Bernstein (1970) reported complete inhibition of growth at 2×10^{-2} M and germination stopped altogether at 10^{-1} M of lead acetate in rice. Pb had been reported to reduce root growth and increased the frequency of mitotic cells in meristematic zones (Lerda, 1992). Although Cd, Cr, Cu and Zn were not detectable in these samples, they are also known to inhibit root elongation (Gorsuch et al., 1995). Besides root growth inhibition triethyl and diethyl lead chloride were found to course disturbances in the spindle fibre mechanism of *A. cepa* at concentration of 10^{-6} - 10^{-7} M (Ahlberg et al., 1972). Similarly, Pb (Lerda, 1992) induced chromosome damage and disturbances of mitotic processes. Complete cell arrest, which had been reported as sign of toxicity by Seetharaman et al. (2004) when *A. cepa* root was treated with various concentrations of nickel (Ni), was also observed in this study.

Our results showed linear correlation between microscopic and microscopic evaluation where the root length inhibition showed that the water sample was phytotoxic. Analysis had shown that well water in the study area are not safe for consumption because of the heavy metal (Pb) and high microbial load which may cause enteric diseases in humans. The use of the Alamuyo River water for irrigation should be discouraged because plants have potentials to bioaccumulate the toxic substance which may be biomaginified along the trophic levels. Adequate provision of water should therefore be ensured in this area in order to prevent the harzads that various toxic components present in the polluted water samples may have on the inhabitant. It is also recommended that the three tiers of government (local, state and federal) in Nigeria should organise enlightenment campaign on the danger that the usage of the water from the River may cause the inhabi-

Table 5. Summary of toxic effects of upstream (A) and downstream (F) water samples of Alamuyo river on *Allium cepa* root cells.

Disturbed Chromosome Conc. (%)	No of dividing cells	Mitotic Index	Mitotic inhibition	Mitotic Spindle	fragment	Chromosome bridge	Chromosome lag	Total Aberrant cells	Freq. of Aberrant Cell %
Late dry season sample A									
0	240	4.80	-	-	-	-	-	-	-
1	150	3.00	37.5	-	06	-	-	06	4.0
5	120	2.40	0.0	-	02	-	-	02	1.7
10	140	2.80	41.7	-	08	02	-	10	7.1
20	160	3.20	33.3	-	-	-	-	-	-
50	10	0.20	95.8	-	-	-	-	-	-
Later dry season sample F									
0	240	4.80	-	-	-	-	-	-	-
1	130	2.60	45.8	02	-	-	-	02	1.5
5	110	2.20	54.2	13	03	-	-	16	14.5
10	90	1.80	62.5	08	02	-	02	12	13.3
20	10	0.20	95.8	02	-	-	-	02	20
50	30	0.60	87.5	-	-	-	-	-	-
Early rainy season sample A									
0	281	5.62	-	-	-	-	-	-	-
1	90	1.80	68.0	6	4	-	-	10	11.1
5	32	0.64	88.6	3	-	-	-	03	9.4
10	10	0.20	96.4	4	-	-	-	04	40.0
20	10	0.20	96.4	-	-	-	-	-	-
50	08	0.16	97.2	2	-	-	-	02	25
Early rainy season sample F.									
0	281	5.62	-	-	-	-	-	-	-
1	146	2.92	48.0	-	-	-	-	-	-
5	70	1.40	78.1	04	-	-	-	04	5.7
10	75	1.50	73.3	09	03	05	-	17	22.7
20	12	0.24	95.7	-	06	-	-	06	50.0
50	06	0.12	97.9	-	-	-	-	-	-
Late rainy season sample A									
0	267	5.34	-	-	-	-	-	-	-
1	140	2.80	47.6	07	-	-	-	8	5.7
5	80	1.60	70.0	03	-	-	-	3	3.8
10	100	2.0	62.5	06	-	-	-	8	8.0
20	78	1.56	70.8	-	-	-	-	-	-

Table 5. Contd.

50	52	1.04	80.5	02	-	-	-	2	3.8
Late rainy seasonsample F.									
0	267	5.34	5.34	-	-	-	-	-	-
1	120	2.40	2.40	03	-	-	-	3	2.5
5	76	1.52	71.5	05	02	-	-	7	9.2
10	43	0.86	83.9	06	-	-	-	6	14.0
20	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-

5,000 *Allium cepa* root cells were scored per concentration.

tants of the area.

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