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Toxicity Bio-Indices as Indicators of Water Portability

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ABSTRACT: Genotoxicity testing represents a powerful line of evidence for assessing impacts from chemical contaminants in drinking water. In this study, fifteen brands of sachet water, five of which do not have the National Agency for Food and Drug Administration and Control (NAFDAC) registration numbers, were collected in the Benin metropolitan city square in Edo State of Nigeria and subjected to cytological evaluation using the macroscopic and microscopic parameters in *Allium cepa* L. A series of five small onion bulbs were cultivated in the different sachet water samples and after 48 hours, two root tips from each bulb were harvested and processed for cytological observation using the aceto-orcein squash technique. Results of the root growth inhibition assessment of the onion bulbs after cultivation in the sachet water samples for 96 hours showed that the mean root length ranged between 2.36±0.14 - 5.37±0.30 cm while cytological evaluation revealed that all, except the University-based brand, induced mitotic abnormalities at significant levels. The considerable variations among the examined samples with respect to the cyto-genotoxic parameters indicate that they are a reflection of the amount of chemical constituents present in the sachet water brands. There is therefore need for NAFDAC to enforce drinking water regulatory guidelines and ensure frequent monitoring to safeguard lives of consumers of sachet water in the study area.

Keywords: Sachet water quality, Genotoxicity, Public health, *Allium cepa*

Introduction

Sachet water ("pure water" as it is popularly called) was introduced into the Nigerian market as a readily available and less expensive means of accessing drinking water in packaged forms which has resulted in a big and thriving water industry with several hundreds of million litres of water products consumed every year. There are now several brands of these type of packaged water marketed in Nigeria and other developing nations (Ogan, 1992; Kassenga, 2007). The increased demand for these drinking water products is attributed largely to factors such as inadequate or non-availability of reliable, safe municipal water in the area; creating the impression that sachet water offer a healthy, refreshing and great tasting alternative to ordinary tap water (Ogundipe, 2008).

Prior to the rapid emergence of Privately Owned Water Enterprises (POWE) in recent times, the onus was on the Nigerian Government Owned Public Water Utilities (GPWU) at Federal, State and Local Government to provide portable water to the citizenry, a statutory responsibility that started witnessing a

sharp decline over two decades ago perhaps due to population growth coupled with mismanagement of the economic resources of the nation. The GPWUs provided their supply from conventional water treatments plants that use water from impounded reservoirs, flowing perennial streams, lakes and deep boreholes. As the country population grows and industries increase, the supply of water by the GPWUs became inadequate in quality and quantity. This led to the emergence of some Privately Owned Water Enterprises (POWE) that operated side by side with the GPWUs within the water sector (Onemano and Otun, 2003). One of the most popular POWE in Nigeria is the sachet water sold in polythene sachet otherwise called 'pure water'.

The proliferation of sachet drinking water and its safety for human consumption has become a matter of public interest (CAMON, 2007). The integrity of the hygienic environment, the production conditions and the quality of majority of the sachet water has been questioned. There is a general perception that the POWEs merely undertake minor and in most cases no purification treatment to the water they collect from natural springs, open wells, deep boreholes and supplies from the GPWUs to make the water more potable (Adekunle *et al*., 2004; Dada, 2009). Apart from environmental contaminants, contamination from improper vendor handling also poses threats to the health of unsuspecting consumers, often times without any proper cleaning of the sachets, a major contributing factor to water related diseases which have continued to be one of the major health problems globally (Orisakwe *et al*. 2006, Oladipo *et al*. 2009, Edema *et al*. 2011, Egwari *et al*. 2009).

The National Agency for Food and Drug Administration and Control of Nigeria (NAFDAC) is mandated to enforce compliance with internationally defined drinking water guidelines but the regulation of packaged water industry, aimed at good quality assurance, has remained a challenge to the agency (NAFDAC 2001, 2004). The Benin metropolitan city square is noted for its commercial activities. A large population of traders and other categories of workers depend on sachet water for quenching their thirst on daily basis. Some brands of sachet water sold around this commercial environment do not bear NAFDAC registration mark.

Previous studies undertaken for the assessment of sachet water quality in Nigeria and other parts of Africa have employed physical examination (Dada, 2009), atomic absorption spectrometry (Orisakwe *et al*., 2006), chemical analysis (Edema *et al*., 2001) and microbial studies (Oladipo *et al*., 2009, Egwari *et al*., 2009). Since the complexity of contaminated water makes it almost impossible to carry out a risk assessment based on chemical and microbial analysis alone (WHO, 1976), a comprehensive approach involving the use of plants as standard bioassays alongside physicochemical analysis and other animal tests has been advocated as it has added advantage (Arkhipchuk *et. al*., 2000). Among the seven plant bioassays reviewed by the US Environmental Protection Agency EPA Gene-Tox program in 1980, the *Allium* root tip chromosome aberration assay was one of the protocols adopted and standardised by the International Program on Plant Bioassays (IPPB) for monitoring or testing environmental pollutants, which is currently in operation under the auspices of the United Nations Environment Program UNEP (Ma, 1999).

The *Allium* test is important for conducting research on, among others, drinking water. Its use was recommended as early as in the 1970's by the Royal Swedish Academy of Science and later by the Gene-Tox programme (Barbério, 2013). The advantage of this test in comparison with others is that it does not require preliminary processing of water samples for establishing toxicity and genotoxicity. At the same time, it shows excellent correlation with animal tests *in vivo* which can be extrapolated to humans with reliability (Grant, 1994). In the light of the above, this study was undertaken to assess the nature and extent of toxicity of sachet water packages sold in the Benin metropolitan city square using the *A. cepa* assay.

Materials and Methods

***Sample Collection*:** Fifteen types sachet water used for this study were randomly collected from the Benin Metropolitan City Square, Edo State in March 2012. They were designated BIM, AQC, DCY,

DBR, FMK, HNG, MYR, NRN, NME, RGO, SMN, SJN, SMS, STR, USM and UBN. Five of these water samples did not have NAFDAC registration numbers.

***Physical Assessment of Water Samples and Administration of Questionnaires to Consumers*:** Ten samples were taken from each brand for assessment of the physical properties (turbidity, odour and taste) of the water samples. Two sets of questionnaires were administered on fifty consumers and ten vendors on the organoleptic properties and sales evaluation of sachet water respectively.

The *Allium Test*: The experimental plant used in this study was *Allium cepa* L., the common onion. Approximately equal-sized onion bulbs (2n = 16) were purchased from a local market in Benin City, Edo State and the same batch was used throughout the study. They were sun dried for 6 weeks and dried, mouldy or those that were sprouting green leaves were all discarded. The outer scales were carefully removed, without tampering with the primordial root ring.

For the evaluation of root growth inhibition, seven onion bulbs were utilized for each sachet water sample. The base of each of the bulbs was suspended on the water samples and control inside 100 ml beakers containing about 75 ml of the water sample at 27±1°C in the dark for 96 h. The negative control was tap water obtained from the water factory of *UNIBEN* Enterprises, University of Benin, Ekenhuan Campus. Test samples were changed daily. At the end of the exposure period, the root lengths of 20 roots with the best growth from the best 5 onion bulbs per water sample were removed with a forceps and measured using a meter rule. From the weighted averages for each sample, the percentage root growth inhibition in relation to the negative control was calculated.

For the evaluation of induction of chromosomal aberration, 5 onion bulbs were suspended in the water samples and the control for 48 h., the root tips were cut and fixed in ethanol:glacial acetic acid (3:1, v/v) inside universal bottles and kept at 4°C for 24 h before use. The already fixed root tips were hydrolysed in 1N HCl at 60°C for 5 minutes. The hydrolysed root tips were washed several times with distilled water. Two root tips were squashed on each slide and stained with aceto-orcein for 10 minutes. Excess stains were removed, and the edges of the cover slips were sealed as suggested by Grant (1982). The mitotic index (MI) and the frequency of chromosomal aberration (CA) were calculated for a total of 1000 cells observed with a Nikon Eclipse (E400) light microscope (at 1000x magnification) per slide per water sample for 5 slides. The mitotic index was calculated as the number of dividing cells per total number of observed cells while the frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored and number of dividing cells.

Quantitative data were summarised as means ± standard errors and percentages, which were then subjected to Duncan multiple comparison and Dunetts tests in a one-way ANOVA, using SPSS version 15.0 for Windows 2007. The effects of the water samples and control on root growth, cell division and chromosome aberrations of *A. cepa* were compared. Significant differences were set at $P \leq 0.05$.

Results

Plate 1 shows a consumer purchasing sachet water from a vendor from a section of the market site in the Benin metropolis on a normal work day. Data obtained from the questionnaire randomly administered to 50 consumers of different sachet water brands indicated that 98% depend on sachet water as their main source of drinking water. Ninety-nine per cent neither demand to see NAFDAC registration number nor the expiry date before purchasing the product and only about 2% preferentially buy particular brands of sachet water products. Eleven per cent of the respondents attribute occasional stomach upset to the water they drink from the market while 12% could not precisely link the disorder to the sachet water obtained from the market.

Plate 1: Market site of Benin metropolitan city square showing a lady purchasing sachet water from a water vendor (arrowed)

About 98% of the ten vendors interviewed claimed they sell over ten bags (200 packs) of sachet water daily. The figure increases on hot and sunny days. All the vendors interviewed claimed that they do not demand to see NAFDAC registration number and expiry date before buying from the producers. Only 8% of the vendors go in search of particular brands to satisfy some consumers' requests while 76% testified to consumers' complaints about the quality of the sachet water but these complaints were mainly about chillness of the product and in some cases leakages which they resolve by replacing them. About 7% however agreed that some consumers complain of odour and taste of some of the products. Generally, majority of the respondents drink sachet water without preference for particular brands of sachet water as long as thirst is quenched.

Physical examination of sachet water samples obtained around the Benin metropolitan city square revealed that all the brands were clear and odourless; nevertheless STR, NME, USM, ACC, SMN, DCY and NRN had mild 'chlorinated' taste.

Macroscopic Evaluation of Sachet Water

Plate 2 shows the macroscopic effect of sachet water brands grown on *A. cepa* L. Good root growth were induced by UBN, HNG, MYR, SMS and RGO. The rest were characterized by short roots, particularly in the onion bulbs cultivated in BIM, USM, STR, NRN, AQC, DCY, NME and SMN sachet water.

Plate 2: Macroscopic effects of various sachet water brands obtained in the Benin metropolitan city square grown on *A. cepa* L.

Fig. 1 shows the percentage root growth of control of *A. cepa* grown in the sachet drinking water samples. The percentage root growth of the onion tips grown on UBN sachet water relative to the control (CRL) was the highest with a value of 99.89% and while AQC had the lowest value of 43.95%.

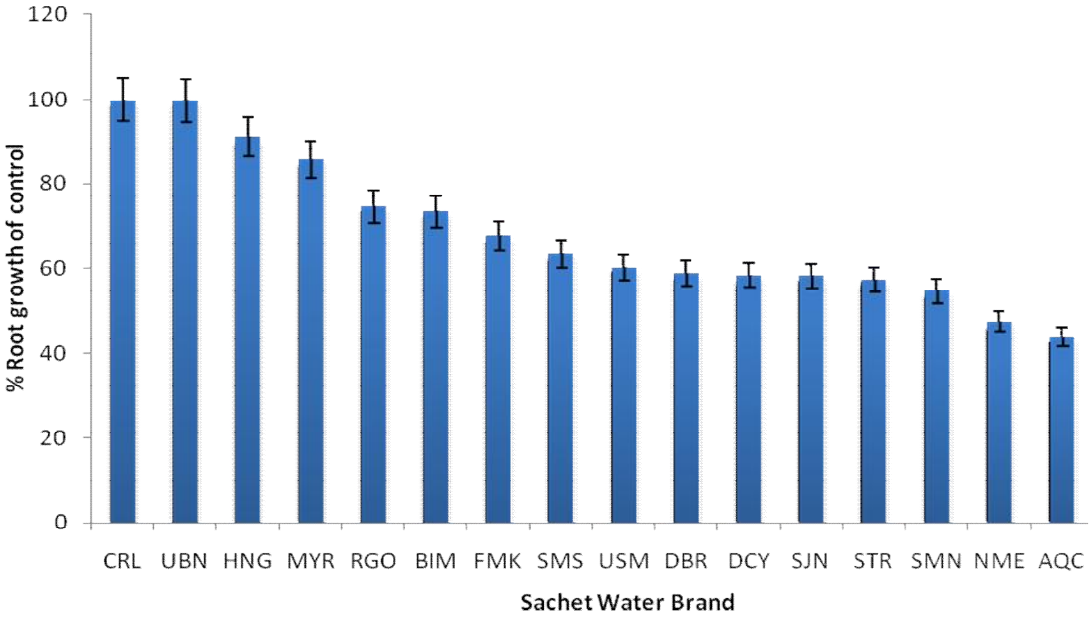

Fig. 1: Effects of different brands of sachet water on root growth of *Allium cepa*

Table 1 shows the mitotic indexes and frequency of mitotic phases in the water sachet samples. AQC brand had the lowest mitotic index of 3.15 while UBN had the highest (10.00) next to the control with a MI of 10.37. It was also observed that there was a relatively uniform percentage prophase, metaphase and anaphase-telophase in the control, UBN and HNG. However, beginning from MYR to AQC, as the mitotic index decreased, there was a corresponding gradual increase in the percentage prophase in the sachet water samples. Similarly, increase in the percentage prophase was accompanied by a decrease in the percentage metaphase and anaphase-telophase stages respectively. For instance, the percentage prophase, metaphase and anaphase-telophase of SMS (MI 7.52±0.36) were 53.19, 28.19 and 18.62 while the values for the same parameters in NRN (MI 3.48±0.86) were 57.47, 25.29 and 17.24 respectively. Conversely, the percentage total aberrant cells increased with decrease in mitotic index. For instance, AQC with an MI of 3.15±0.51 had the highest percentage aberrant cells of 47.47 while UBN (MI 10.00±0.31) had the least frequency of 1% aberrant cells based on the number of dividing cells.

Table 1: Cytological effects of sachet drinking water on cells of *Allium cepa*

Sachet Water Sample	Mitotic Index (% ± SE)	Total mitosis			Chromosome aberrations (CA)						Frequency of aberrant cells (%) based on	
		% Prophase	% Metaphase	% Anaphase-Telophase	Vagrant	Bridge	Fragment	Polar deviation	Stickiness	*Others	Total counted cells	No of dividing cells
Control	10.37 ± 0.92	36.22	34.30	29.48	1	0	0	1	0	2	0.08	0.77
UBN	10.00±0.31	36.60	31.80	31.60	1	0	0	2	0	2	0.10	1.00
HNG	9.12±0.46 [a]	39.04	26.32	34.65	12	3	0	7	0	10	0.64	7.02
MYR	8.66±0.43 [a]	37.64	32.33	24.17	11	6	5	3	0	11	0.71	8.31
RGO	8.43±0.26 [a]	42.65	33.18	24.17	12	6	7	3	0	10	0.76	9.01
BIM	8.09±0.41 [a]	54.32	25.93	19.75	20	8	9	7	1	9	1.08	13.33
FMK	7.86±0.33 [a]	55.98	25.70	15.78	26	12	9	9	1	11	1.36	17.30
SMS	7.52±0.36 [a]	53.19	28.19	18.62	27	17	8	11	0	10	1.46	19.42
USM	7.27±0.32 [a]	54.40	29.12	16.48	27	16	10	10	2	9	1.48	20.33
DCY	7.01±0.26 [a]	55.84	29.92	14.25	24	16	10	10	6	9	1.50	21.37
SJN	5.84±0.22 [a]	51.37	34.25	14.38	24	14	9	10	10	8	1.50	25.69
STR	5.15±0.44 [a]	58.14	26.36	15.50	24	13	8	9	13	8	1.50	29.07
SMN	5.04±0.47 [a]	59.52	28.57	11.91	21	10	8	12	15	9	1.50	29.76
NME	5.01±0.43 [a]	59.76	28.69	12.35	18	12	7	11	20	7	1.50	29.88
NRN	3.48±0.86 [a]	57.47	25.29	17.24	20	10	5	10	26	4	1.50	43.10
AQC	3.15±0.51 [a]	56.96	30.38	12.66	21	9	6	9	26	4	1.50	47.47

[a]Significant difference from negative control ($p < 0.05$).
*Others: Laggards, disoriented chromosomes and spindle disturbance grouped together

The chromosomal abnormalities observed in this study were bridges, stickiness, vagrants and polar deviations (Plate 3). The aberrations were more prevalent in SMS, USM, DCY, SJN, STR, SMN, NME, NRN and AQC sachet water brands.

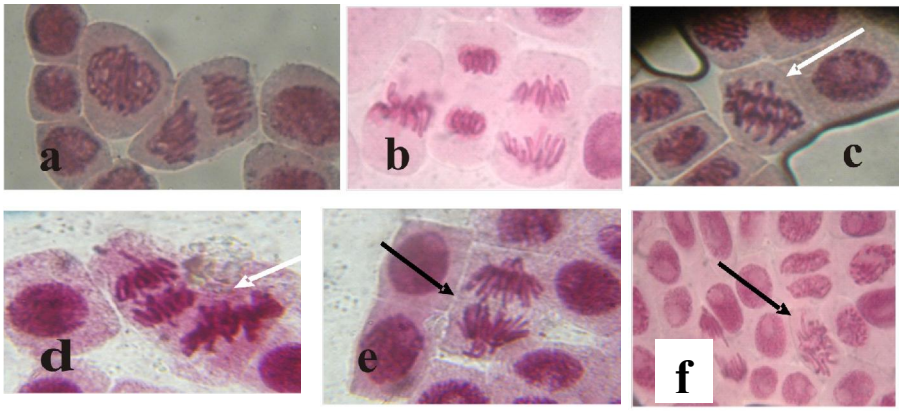

Plate 3: Stages of mitotic division in *Allium cepa* L. root tip cells grown on different brands of sachet water. (a-b) Normal mitotic stages (c) sticky anaphase (d) sticky metaphase (e) chromosome bridge (f) disoriented chromosomes

Discussion

There are about 374,515 inhabitants in Oredo Local Government Area of Edo State (NPC 2006). It is estimated that over 2,000 persons engage in selling, buying and other commercial activities in the Benin Metropolitan City Square on a daily basis (Personal Communication). The objective of this study was to identify sachet water product(s) that meet the set standards by NAFDAC - the regulatory body for drinking water quality. Visits made to some of the production sites of the sachet water brands used in this study revealed that majority of them are produced in questionable hygienic environment and conditions (Personal Communication). The physical properties of the different sachet water samples used for this study show that in general, all the brands were clear and odourless; nevertheless a few had mild 'chlorinated' taste.

In this study the use of *Allium cepa* as a plant bioindicator of cyto-genotoxicity in sachet water was exploited. There was a linear relationship between the macroscopic and microscopic parameters for all the sachet water samples used in the study. In *A. cepa*, whenever there is root growth inhibition, there is always a reduction in the number of dividing cells, a phenomenon attributable to the effects of environmental chemicals acting singly or in their combined states to induce genetic abnormalities in biological systems (Babatunde and Bakare, 2006; Chauhan *et al*., 1998; Godet *et al*., 1993; Kong and Ma, 1999). Our study showed that the sachet water brands with poor root growths were characterised by significantly low number of dividing cells and vice versa. In a recent study, Olorunfemi *et al*. (2013) attributed the presence of Cr, Fe, Mn, Zn and other inorganic compounds in borehole water supplied to hostels in a tertiary institution to the growth inhibition, low mitotic indices and chromosome abnormalities induced in *A. cepa* root meristems. A decrease of over 45% of root growth strongly indicates the presence of toxic substances (Fiskesjö, 1985b) capable of causing sub-lethal effects on plants (Hidalgo *et al*. 1989) and since the inhibition of mitosis is often used for tracing cytotoxic substances (Linnainmaa *et al*., 1978); the gross root growth inhibition induced by most of the test samples and corresponding reduction in mitotic indices observed in *A. cepa* particularly those grown in SMN, NME and AQC brands might be due to the high amounts of chemical impurities in them.

The growth response of *A. cepa* showed that the percentage prophase increased with a corresponding decrease in the percentages of the other stages in most of the sachet water samples. This may be as a result of the blocking of cell division at the end of the prophase stage by the perceived chemical impurities acting as pre-metaphase inhibitors in the test samples.

Chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosomal material. Compared to the control and UBN, the number of chromosome aberrations observed in most of the sachet water samples was significantly higher. The most frequently observed aberrations at anaphase-telophase stages in the root tips of *A. cepa* grown in most of the water samples (none in the control and UBN) were sticky and vagrant chromosomes. Stickiness reflects highly toxic, irreversible effects probably leading to cell death (Fiskesjö, 1988; Liu *et al*., 1992; Türkoglu, 2009). The induction of a large number of vagrant chromosomes indicates that some of these sachet water brands possess chemicals that act as potent spindle inhibitors (Jain and Sarbhoy, 1987). Bridges and fragments are clastogenic effects (Kovalchuk *et al*., 1998, Saxena *et al*., 2005), therefore their presence indicate that the some of the water samples are capable of initiating mutagenic events in the cell (Mishra, 1993).

The study has once again validated the use of Allium test as a reliable test system for monitoring the genotoxicity potential of perceived pollutants. The positive results should be considered as a warning and that consumption of some of the sachet water brands sold at the Benin metropolitan city square may be a risk to the health of the unsuspecting consumers.

In conclusion, NAFDAC should initiate measures to compel sachet water producers in Benin to understudy the University of Benin, producers of the UBN brand.

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