LEVELS OF FORMALDEHYDE AND ACETALDEHYDE IN SELECTED BOTTLED DRINKING WATER SOLD IN URBAN AREAS IN TANZANIA

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ABSTRACT

The levels of formaldehyde and acetaldehyde in bottled drinking water and the effect of storage time and storage conditions on their levels were determined. A total of 144 samples of six brands of bottled drinking water were purchased from Dar es Salaam, Iringa, Mwanza and Arusha regions in Tanzania. Analysis was performed using HPLC on the 1st day and after 14 and 42 days of storage in a refrigerator, at room temperature and exposure to sunlight. The levels of formaldehyde and acetaldehyde in the refrigerated samples ranged from 2.46 to 19.25 µg/L and from not detected (ND) to 58.70 µg/L, respectively. In samples stored at room temperature, the levels of formaldehyde and acetaldehyde were up to 23.26 µg/L and 36.10 µg/L, respectively. The highest levels of formaldehyde and acetaldehyde in samples exposed to sunlight were 46.0 µg/L and 187.0 µg/L, respectively. The lowest levels of the aldehydes were found in refrigerated samples and the highest levels were in samples exposed to sunlight. The levels of these aldehydes increased with increased storage time. The levels were below the WHO acceptable limits; however, the findings indicate that the levels could be elevated with increased storage time and exposure to sunlight.

Keywords: Bottled drinking water, formaldehyde, acetaldehyde, storage time, storage conditions

INTRODUCTION

Human bodies obtain water from a variety of sources, such as drinking water, beverages, moisture content of food and water produced by oxidative processes in the body (EFSA 2010). Drinking water may be contaminated by chemicals or microbes. Adverse health effects from chemical contaminants are generally associated with long-term exposures, whereas the effects from microbial contaminants are usually immediate. As a result chemical contamination is often considered a lower priority than microbial contamination (Mona et al. 2008). In recent years, the consumption of bottled drinking water has significantly increased worldwide particularly in urban populations (VWRRC 1996; Mona et al. 2008). In spite of its excessively high price compared to tap water, its consumption in the world has been increasing by an average of 12% each year (Dinelli et al. 2012). In Tanzania, the consumption rates of bottled water range from 0.25 to 2.5 litres per person per day (Kassenga 2007). The increased consumption of bottled water can be attributed to the deterioration on the quality of tap water such that tap water is generally not considered safe for drinking (Kassenga 2007). In addition, taste, convenience and fashion are other reasons for use of bottled drinking water. For many consumers, safety and potential health benefits are the most important considerations because of the belief that bottled water is safer than tap water (Abd El-Salam et al. 2008a; Abd El-Salam et al. 2008b; WHO 2011). Others have the opinion that, because of environmental pollution, bottled water is a safer choice for drinking than their household supply (Momani 2006). Moreover, there are concerns about chlorine by-products and contaminants such as lead, nitrates, and microorganisms in municipal water supplies (Abd El-Salam et al. 2008a). Therefore contamination, awareness and personal perceptions have caused many people to drink bottled water at prices higher than water provided by public systems (Chiarenzelli and Pominville 2008). Few studies on bottled drinking waters have been carried out in Tanzania (Kassenga 2007, Kassenga and Mbuligwe 2009, Mihayo and Mkoma 2012). These studies assessed the microbiological and physico-chemical quality of bottled drinking water. However none of these assessed the aldehydes in bottled water. Studies in various countries have revealed that bottled water can be contaminated by aldehydes (Nawrocki et al. 2002, Tsai et al. 2003, Dabrowska et al. 2003, Mutsuga et al. 2006, Redzepovic et al. 2012). The sources of aldehydes in bottled water include oxidative water treatment processes such as ozonation and chlorination, and migration or formation of aldehydes from the plastic containers which are usually made of polyethylene terephthalate (PET) polymer (Dabrowska et al. 2005, Mutsuga et al. 2006, Ozlem 2008, Bach et al. 2012; Redzepovic et al. 2012). Formaldehyde and acetaldehyde are the dominant carbonyl compounds identified in water (Nawrocki et al. 2002, Dabrowska et al. 2003). Exposure to formaldehyde and acetaldehyde through ingestion may cause cancer, mutation and adverse gastrointestinal effects (Hebert et al. 2010, Richardson et al. 2007). Aldehydes may lead to a change in taste and odour of the bottled drinking water (Dabrowska et al. 2003, Redzepovic et al. 2012). For instance acetaldehyde causes an undesirable, slightly sweet and fruity taste in the bottled drinking water (Mutsuga et al. 2006). Therefore, this study was conducted to determine the levels of formaldehyde and acetaldehyde in bottled drinking water and to evaluate the effect of storage time and storage conditions on levels of these aldehydes.

MATERIALS AND METHODS

Sampling

Six brands of bottled drinking water, designated as A to F, each containing bottles from the same batch were purchased from four regions in the country (Dar es Salaam, Iringa, Mwanza and Arusha) as shown in Table 1. The selection of brands based on their availability and popularity. For each brand, twenty four (24) bottles of drinking water were purchased and the volumes of the bottles ranged from 500 to 600 mL. A total of 144 samples were collected. All brands of bottled water had a validity date of one year. For each brand some bottles were stored at room temperature, some in a refrigerator and some exposed to sunlight.

Table 1: The brands of bottled water purchased for the study

Region of Purchase	Designated Name	Bottle Volume (mL)	Origin of source water	Treatment process
Dar es Salaam	A	500	Underground water	Filtration and sterilization
	B	600	*	Reverse osmosis and ozonation
	C	500	Streams of mount Kilimanjaro	*
Iringa	D	500	Natural spring water	Microfiltration and UV sterilization
Mwanza	E	500	Underground water	Reverse osmosis
Arusha	F	600	Streams of mount Meru	Reverse osmosis, ozonation and UV treatment

*Not described

Preparation of Reagents

Purification of 2,4- dinitrophenylhydrazine (2,4-DNPH) by Recrystallization

A supersaturated solution of 2,4-DNPH was prepared by dissolving 100 mg of 2,4-DNPH in acetonitrile (200 mL). The solution was boiled for one hour. The supernatant was then transferred into a covered beaker on a hot plate and allowed to gradually cool to 40 - 60 °C. The temperature (40 - 60 °C) range was maintained until 95% of solvent evaporated. The solution was decanted and crystals were rinsed twice with acetonitrile (20 mL). The crystals were transferred to another clean beaker. The purification process was repeated. The crystals were placed in an all-glass reagent bottle and acetonitrile (25 mL) was added. The bottle was caped and shaken. Clean pipettes were used to draw 5 mL of saturated 2,4-DNPH stock solution into vials for purity check during analysis.

Preparation of Citrate Buffer, pH = 3

Citric acid solution (1.10 M) was prepared by making up 105.981 g of citric acid to 500 mL with distilled water. On the other hand, 148.405 g of sodium citrate were made up to 500 mL with distilled water to prepare 1 M sodium citrate solution. Citric acid (1.01 M, 80 mL) was added to 20 mL of 1 M sodium citrate and thoroughly mixed to prepare citrate buffer of pH = 3. The pH adjustments were achieved using either NaOH or HCl as needed.

Preparation of Saturated Solution of Sodium Chloride

A saturated solution of sodium chloride (6.54 M) was prepared by making up 76.448 g of sodium chloride to 200 mL with distilled water.

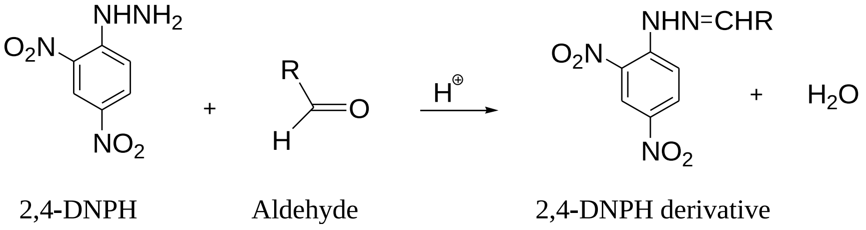
Preparation of HCl and NaOH Solutions

NaOH solution (6.15 M) was prepared by making up 24.589 g of NaOH to 100 mL with distilled water. A 50 mL volume of 37% HCl solution with specific gravity of 1.19 was diluted to 100 mL to prepare 6.04 M HCl.

Sample Preparation and Handling

Sample preparations were carried out on the 1st, 14th and 42nd days of storage in a refrigerator, at room temperature and exposure to sunlight. On each day of sample preparation, a newly opened bottle of drinking water from each storage condition was prepared for analysis. For every water bottle, samples were prepared in triplicate.

A water sample (100 mL) in an Erlenmeyer flask (with a glass stopper) was acidified (pH = 3) and derivatized (Scheme 1) with 2,4-DNPH solution (6 mL). The pH adjustment was performed with HCl and NaOH. The flask was immediately sealed and placed on a heating plate at temperature of 40°C with a magnetic stirrer revolving at a speed of 550 spins per minute, for 1 hour. The resulting dinitrophenylhydrazones were extracted from the solution with liquid-solid extraction on SPE-C18 column. The column was conditioned with 10 mL of citrate buffer (pH = 3) and the sample in which saturated sodium chloride (10 mL) had previously been added, was quantitatively transferred to the column. Elution of derivatives on SPE-C18 column was performed with acetonitrile into a 10 mL volumetric flask in which the sample volume was raised to the notch with acetonitrile, thoroughly mixed, placed in a tightly sealed vial and frozen until analysis.



Scheme 1: Reaction of 2, 4-DNPH with aldehydes.

Analysis of Prepared Samples

All analyses were carried out using a HPLC equipment (SHIMADZU LC-20A model), driven by LC solution software version 1.24. The conditions developed by Redzepovic et al. (2012) were used with modifications to completely separate the desired peaks (formaldehyde-2,4-DNPH and acetaldehyde-2,4-DNPH).

Quality Assurance

The HPLC was calibrated to check the linearity and sensitivity of the detector. A series of aldehyde-DNPH standards were run and their peak areas were plotted against their respective concentration to obtain a calibration curve. Since samples were run in batches, blanks (distilled water in glass bottles) were incorporated in each batch. The reagents and chemicals used were of analytical grade and of high purity. The glassware and equipment were thoroughly washed, rinsed with distilled water and acetonitrile, and then dried. Method detection limits were established based on a 3:1 signal to noise ratio. Recovery studies were performed by spiking brand A with a mixture of formaldehyde and acetaldehyde each with concentrations of 10, 30 and 50 µg/L. The spiked samples were then prepared and analysed in duplicate as other samples. The mean recoveries for formaldehyde and acetaldehyde were 79.5% and 86.5%, respectively.

Data Analysis

Statistical analyses of data were performed using GraphPad InStat software (Motulsky 1998). The concentrations of formaldehyde and acetaldehyde were compared using paired *t*-test.

Concentrations of Formaldehyde in Bottled Drinking Water

The levels of formaldehyde in all tested bottled water samples ranged from not detected (ND) to 46.0 µg/L (Table 2). The highest levels were found in samples exposed to sunlight.

RESULTS AND DISCUSSION

Table 2: Concentrations of formaldehyde in bottled water

Storage condition	Brand name	Concentrations (µg/L, n = 3)					
		1st day		14th day		42nd day	
		min	max	min	max	min	max
Refrigerator	A	10.30	10.39	10.01	10.14	8.57	8.59
	B	2.46	2.56	3.30	3.36	4.59	4.69
	C	9.12	9.21	14.03	14.15	10.07	10.27
	D	10.30	10.50	6.63	6.94	11.10	11.29
	E	3.21	3.34	14.84	15.08	19.06	19.25
	F	3.50	3.61	3.64	3.80	6.62	6.81
Room temperature	A	10.30	10.39	11.05	11.17	ND	ND
	B	2.46	2.56	7.39	7.43	9.90	10.05
	C	9.12	9.21	23.05	23.26	8.42	8.62
	D	10.30	10.50	5.32	5.44	7.15	7.36
	E	3.21	3.34	3.22	3.33	5.37	5.55
	F	3.50	3.61	3.34	3.49	5.54	5.73
Exposure to sunlight	A	10.30	10.39	27.88	27.94	45.48	46.00
	B	2.46	2.56	14.27	14.35	34.18	34.26
	C	9.12	9.21	45.15	45.36	27.35	27.52
	D	10.30	10.50	14.06	14.25	21.67	21.86
	E	3.21	3.34	26.36	26.51	34.25	34.47
	F	3.50	3.61	9.75	9.84	15.34	15.49

ND = Not detected; min = minimum, max = maximum

The mean concentrations of formaldehyde in water samples which were stored in a refrigerator, at room temperature and exposed to sunlight are summarized in Figures 1, 2 and 3 and the standard deviations in each Figure are presented as error bars.

Concentrations of Formaldehyde for Samples Stored in a Refrigerator

The concentrations of formaldehyde in the tested water varied among brands (Figure 1). With increased storage time, the concentrations of formaldehyde increased in B, E and F, decreased in A, increased and then decreased in C, and decreased and then increased in D. Other studies also reported very broad ranges of concentrations of formaldehyde among brands (Dabrowska et al. 2003).

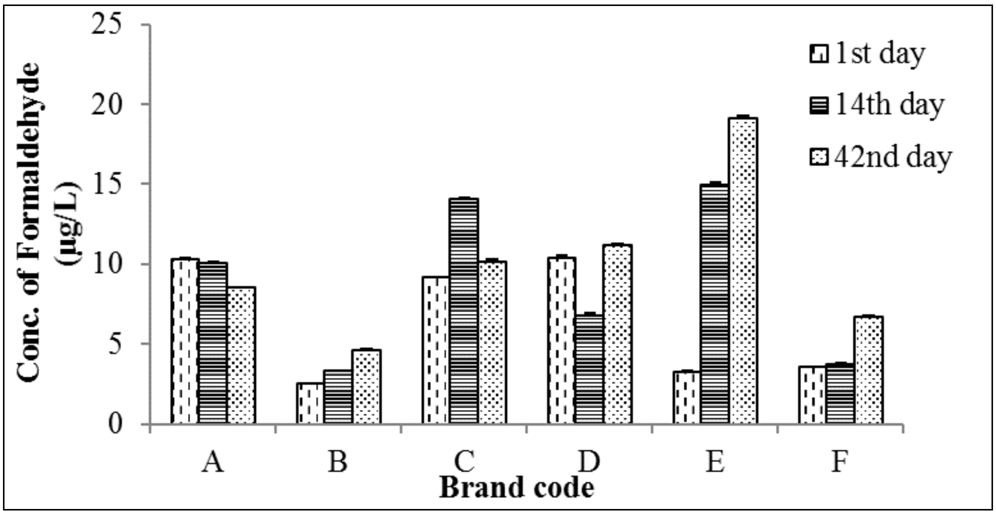


Figure 1: Mean concentrations of formaldehyde in water samples stored in a refrigerator.

On the first day of analysis, brand D had the highest mean level of formaldehyde (10.42 µg/L) while brand B had the lowest mean level (2.52 µg/L). The difference in levels of formaldehyde in the brands may be due to the different origins of the source waters, the varieties of treatment processes employed and different levels of aldehydes in the bottle material. This observation is also in agreement with previous studies (Dabrowska et al. 2003).

For the samples stored in a refrigerator for 14 days, the mean level of formaldehyde was highest in brand E (14.97 µg/L) and lowest in brand B (3.34 µg/L). However, the levels decreased in brands A and D. Although the levels of formaldehyde increased in all other brands, this increase was observed to be high for brands C and E. The increase could probably be due to the migration of formaldehyde from PET bottle as observed by other researchers (Dabrowska et al. 2003, Mutsuga et al. 2006). With increased time of storage to 42 days, it was observed that the formaldehyde levels increased in brands B, D, E and F, but decreased in brands A and C. The conversion of formaldehyde into other species such as methanol might have been the reason for its decrease.

Concentrations of Formaldehyde for Samples Stored at Room Temperature

With increased storage time, the concentrations of formaldehyde increased in B and E, increased and then decreased in A and C, and decreased and then increased in D and F (Figure 2).

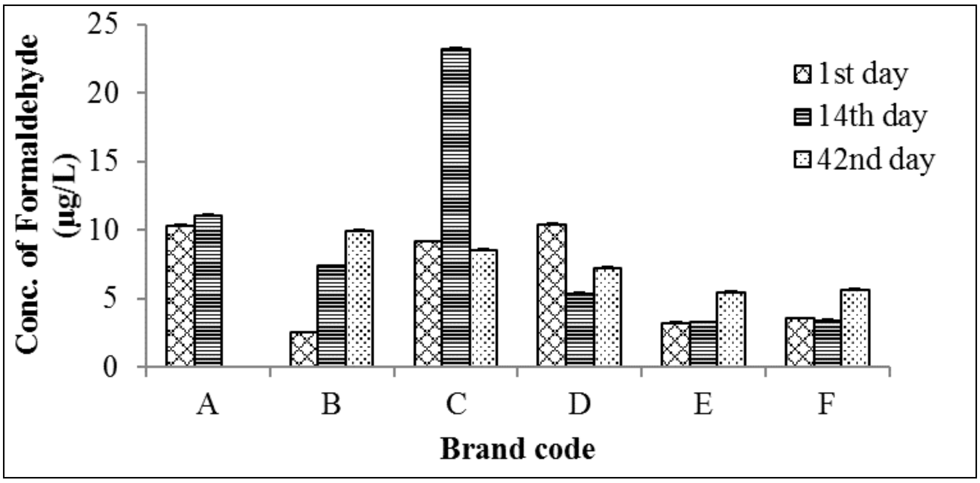


Figure 2: Mean concentrations of formaldehyde in water samples stored at room temperature.

For the samples stored at room temperature for 14 days, the highest mean level of formaldehyde was in brand C (23.18 µg/L) and the lowest in brand E (3.28 µg/L). Moreover, the formaldehyde levels increased in brands A, B, C and E. This could probably be attributed to the migration of formaldehyde from PET bottle as observed by other reserchers (Mutsuga et al. 2005, Mutsuga et al. 2006). In brands D and F, the formaldehyde levels decreased. The decrease in levels of formaldehyde may be due to degradation of formaldehyde by oxygen, traces of metal ions or heterotrophic bacteria present in water (Bach et al. 2012). This is in line with the explanations by Mutsuga et al. (2006). The authors observed that formaldehyde and acetaldehyde disappeared in bottled mineral water stored at 40 °C over a period of time and explained this phenomenon as being due to decomposition of these compounds caused by heterotrophic bacteria present in water. After 42 days, formaldehyde levels increased in brands B, D, E and F but decreased in brands A and C. However, formaldehyde was not detected in brand A after 42 days. This could also be attributed to the decomposition of formaldehyde caused by heterotrophic bacteria present in water

Concentrations of Formaldehyde for Samples Exposed to Sunlight

With increased storage time, the concentrations of formaldehyde generally increased in all brands except in C where it increased and then decreased (Figure 3). For the samples exposed to sunlight for 14 days, the formaldehyde levels increased in all brands. A further increase of storage time to 42 days resulted to a further increase in formaldehyde levels in all brands except in C. Such a variation could be due to the migration of formaldehyde from PET bottles as a result of thermal and photo degradation of the plastic material (Dabrowska et al. 2003, Bach et al. 2012). It can be envisaged that, at high temperatures there could be degradation of organic compounds that could lead to the formation of formaldehyde.

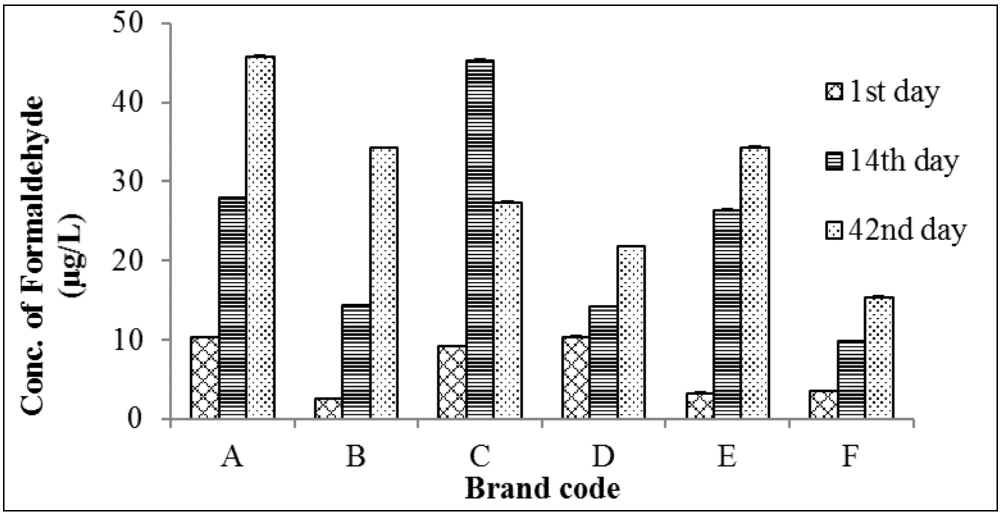


Figure 3: Mean concentrations of formaldehyde in water samples exposed to sunlight.

Influence of Storage Conditions and Time on the Levels of Formaldehyde in Bottled Water

For samples of water stored at various conditions, the levels of formaldehyde at each storage condition (in a refrigerator, 4 ºC at room temperature, 25 - 32 ºC and exposure to sunlight, 32 - 35 ºC) generally increased with an increase in storage time as shown in Figure 4. The levels were lower for the samples stored in a refrigerator than for those exposed to sunlight. For brands A and B that were exposed to sunlight for 14 days, the levels of formaldehyde were three times higher than the levels in the samples stored in a refrigerator for the same period. After 42 days, the level of formaldehyde in brand A exposed to sunlight was five times higher than the levels in the samples stored in a refrigerator while in brand B it was seven times higher. This showed that at high temperatures more formaldehyde migrated from PET bottles into water than at low temperatures.

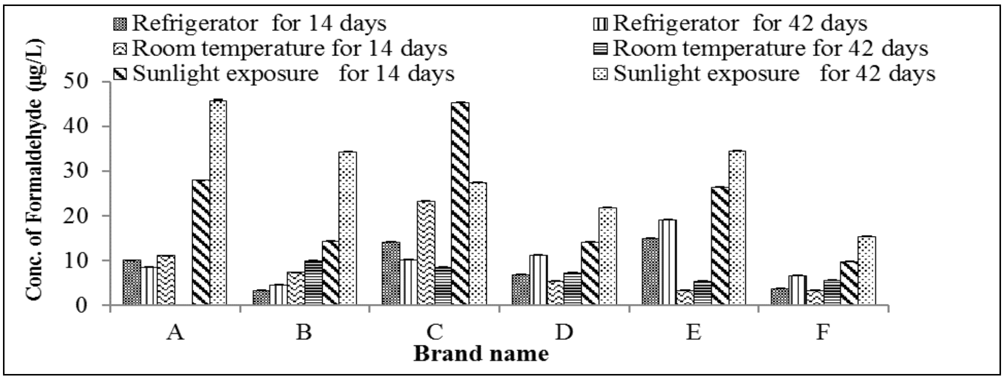


Figure 4: Mean concentrations of formaldehyde in bottled water stored at various conditions.

Figure 4 shows that the levels of formaldehyde in bottled water increased as the storage time increased except for samples presumed to contain heterotrophic bacteria in which the levels of formaldehyde decreased with storage time.

Concentrations of Acetaldehyde in Bottled Drinking Water

The concentrations of acetaldehyde in water samples are summarized in Table 3. The highest level was 187 μg/L. The mean concentrations of acetaldehyde in water samples which were stored in a refrigerator, at room temperature and which were exposed to sunlight are presented in Figures 5, 6 and 7 and the standard deviations in each Figure are presented as error bars.

Table 3: Concentrations of acetaldehyde in bottled water

Storage condition	Brand name	Concentrations (μg/L, n = 3)					
		1st day		14th day		42nd day	
		min	max	min	max	min	max
Refrigerator	A	ND	ND	ND	ND	5.89	6.11
	B	ND	ND	ND	ND	10.51	10.69
	C	ND	ND	7.50	7.69	18.35	18.59
	D	ND	ND	2.61	2.85	6.37	6.65
	E	21.23	21.47	25.84	26.02	58.51	58.70
	F	3.45	3.65	19.85	19.96	33.68	33.94
Room temperature	A	ND	ND	ND	ND	8.63	8.89
	B	ND	ND	ND	ND	5.77	6.06
	C	ND	ND	7.10	7.29	10.11	10.37
	D	ND	ND	ND	ND	3.36	3.59
	E	21.23	21.47	17.73	17.98	35.54	36.10
	F	3.45	3.65	10.93	11.09	24.34	24.57
Exposure to sunlight	A	ND	ND	18.40	18.96	27.96	28.33
	B	ND	ND	7.17	7.35	6.75	7.03
	C	ND	ND	31.77	31.89	46.36	46.75
	D	ND	ND	16.19	16.34	18.75	18.92
	E	21.23	21.47	154.4	154.7	186.7	187.0
	F	3.45	3.65	47.22	47.51	77.87	78.06

ND = Not detected; min = minimum, max = maximum

Concentrations of Acetaldehyde for Samples Stored in a Refrigerator

The concentrations of acetaldehyde generally increased with increased storage time in all the brands tested (Figure 5).

On the first day of analysis, acetaldehyde was not detected in brands A, B, C and D while the highest mean level was in brand E (21.47 μg/L). After storage for 14 days in a refrigerator, the levels of acetaldehyde were different from each brand but increased in all brands except A and B in which acetaldehyde was not detected. A further increase in storage time to 42 days resulted in significant increase in levels of acetaldehyde in all brands. The mean level of acetaldehyde was lowest in brand A (6.00 µg/L) and highest in brand E (58.60 µg/L). The increase in levels of acetaldehyde with storage time may be a result of migration of acetaldehyde from the PET bottles

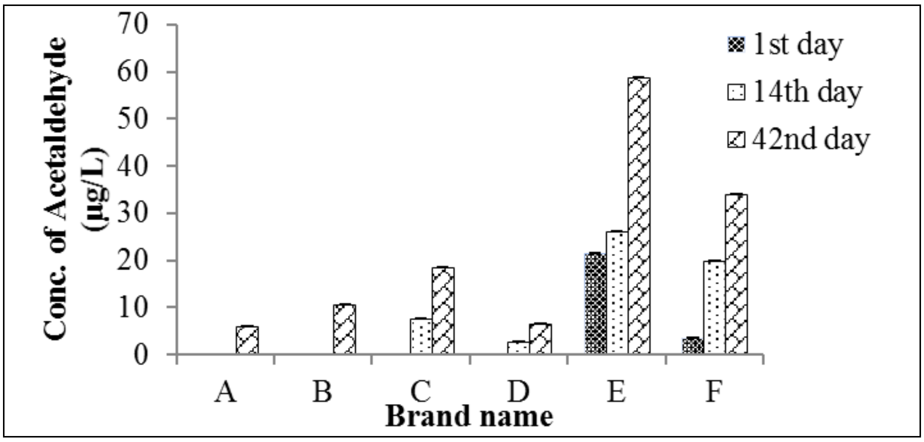


Figure 5: Mean concentrations of acetaldehyde in water samples stored in a refrigerator.

Concentrations of Acetaldehyde for Samples Stored at Room Temperature

The concentrations of acetaldehyde generally increased with increased storage time in all brands as shown in Figure 6. Storage of samples at room temperature for 14 days resulted in an increase in levels of acetaldehyde in brands C and F and a decrease in brand E while it was not detected in brands A, B and D. An increase in storage time to 42 days led to an increase in acetaldehyde levels in each brand and the highest mean level was in brand E (35.88 µg/L) while the lowest in brand D (3.47 µg/L).

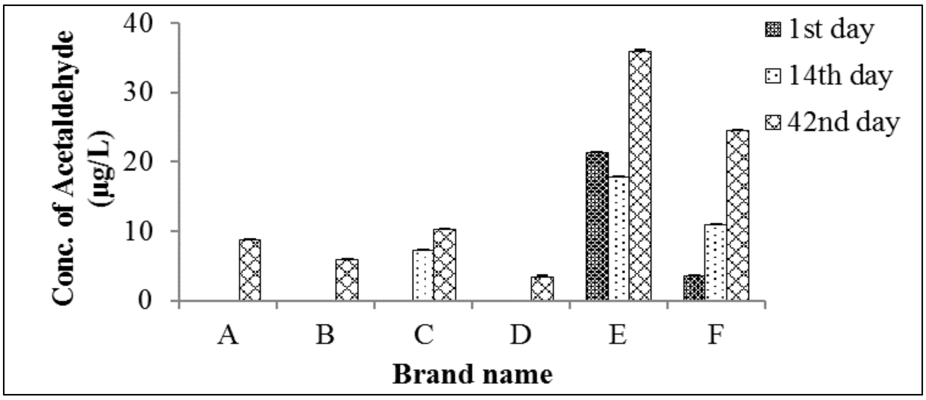
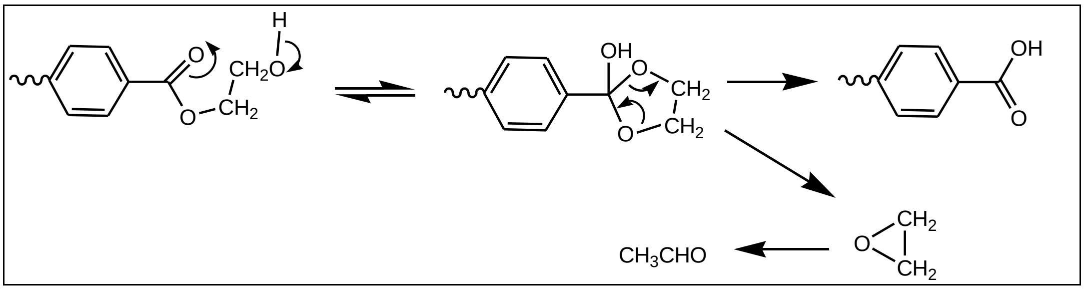


Figure 6: Mean concentrations of acetaldehyde in water samples stored at room temperature.

Concentrations of Acetaldehyde for Samples Exposed to Sunlight

With increased storage time, the concentrations of acetaldehyde generally increased in all brands (Figure 7). After 14 days of exposure to sunlight, brand E had the highest mean level of acetaldehyde (154.53 µg/L) while brand B had the lowest mean level (7.26 µg/L). However, after 42 days of exposure to sunlight, brand E had the highest mean level of acetaldehyde (186.81 µg/L) while brand B had the lowest mean level (6.87 µg/L). High levels of acetaldehyde for samples exposed to sunlight may be due to photo and thermal degradation of PET bottles. The mechanism for the formation of acetaldehyde as a product of photo degradation of hydroxyl end group of PET is shown in Scheme 2 (Ravindranath and Meshelkar 1986; Fechinec et al., 2004). In this process UV light splits the ester bond leaving behind acetaldehyde and PET backbone fragments.



Scheme 2: Acetaldehyde formation (Ravindranath and Meshelkar 1986).

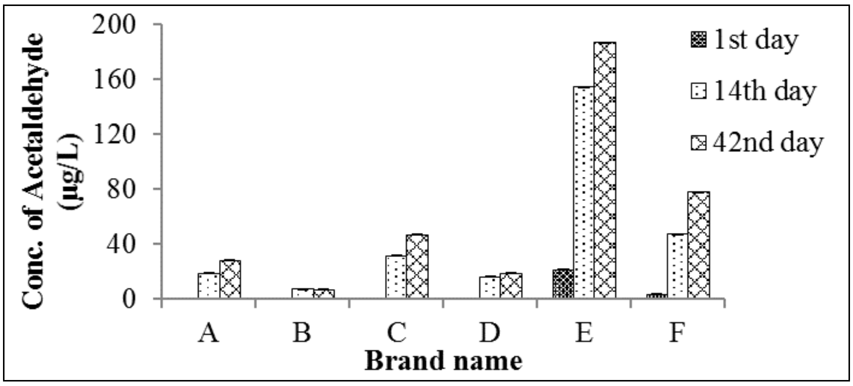


Figure 7: Mean concentrations of acetaldehyde in water samples exposed to sunlight.

Influence of Storage Conditions and Time on the Levels of Acetaldehyde in Bottled Water

The levels of acetaldehyde at each storage condition (in a refrigerator, at room temperature and exposure to sunlight) increased with an increase in the storage time as shown in Figure 8. The levels were lower for the samples stored in a refrigerator than for those exposed to sunlight. Previous studies have established that sunlight and high temperature enhance the migration of carbonyl compounds from PET bottles into their contents (Nawrocki et al. 2002; Ozlem 2008). In addition, it was considered that at high temperatures there might be degradation of organic compounds present that may lead to formation of acetaldehyde (Ravindranath and Meshelkar 1986; Fechinec et al., 2004).

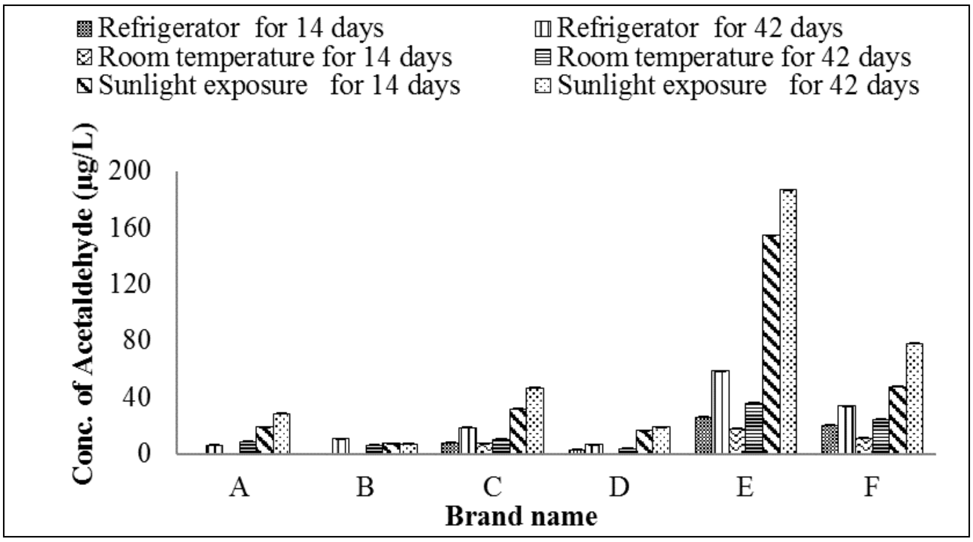


Figure 8: Mean concentrations of acetaldehyde in bottled water stored at various conditions.

Figure 8 shows that the levels of acetaldehyde in bottled water increased as the storage time increased except for samples suspected to contain heterotrophic bacteria in which the level of acetaldehyde decreased with time. Dabrowska et al. (2003) also observed that the levels of acetaldehyde in bottled water increase with increased storage time.

Comparison of Formaldehyde and Acetaldehyde Levels among Brands and with WHO Permissible Limit

The analysis carried out for water samples on the 1st day indicated the presence of formaldehyde in all the brands, but acetaldehyde was detected in brands E and F only. For various brands stored at the same condition, the levels of formaldehyde in some brands were higher than those of acetaldehyde while in other brands the levels of acetaldehyde were higher than those of formaldehyde. The differences in the levels of formaldehyde and acetaldehyde in various brands stored at the same condition can be ascribed to the different origins of source waters, the varieties of treatment processes employed and different levels of aldehydes present in the bottle materials. The differences in levels of formaldehyde and acetaldehyde in the bottle materials may be due to variations in production conditions or techniques such as moulding temperature and de-aeration (Mutsuga et al. 2005). However, the levels of formaldehyde in all brands tested were below the WHO permissible limit (900 µg/L) (WHO 2002). Comparison of concentrations of formaldehyde and acetaldehyde on each analysis day showed that there were no significant differences between the concentrations of formaldehyde and acetaldehyde (*t* = 0.8802–1.843, *p* = 0.083–0.3910, *df* = 17).

CONCLUSIONS

The concentrations of formaldehyde and acetaldehyde in bottled water were up to 46.0 µg/L and 187 µg/L, respectively. The levels of both formaldehyde and acetaldehyde varied among the studied bottled water brands but did not exceed the WHO acceptable limits for drinking water. There were no significant differences between the concentrations of formaldehyde and acetaldehyde in all the samples, but there were variations in their levels for a particular brand of bottled drinking water stored at different conditions. The levels of these aldehydes were found to be lowest in the refrigerated samples and highest in those exposed to sunlight and increased with increased time of storage. Therefore, it is considered that storage conditions and storage time influenced the formation of these aldehydes in the samples.

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