Original Research Article

Levels of phthalate acid esters in drinking water bottled in PET (polyethylene terephthalate) and PC (polycarbonates) bottles - stored under different storage conditions in Mwanza City

Abstract

Bottled drinking water is widely advertised and sold in glass or, to a greater extent, plastic bottles with metallic or plastic caps, and is regularly drank by both children and adults. Mwanza City markets have many brands of bottled drinking water packed in PET and PC plastic bottles whose polymers may contain additives such as phthalates.Certain cancer types, hormone imbalances, and developmental delays have all been connected to these phthalates, which are harmful to human health. This study investigated, the effects of storage conditions, time spent in storage, and type of container polymer on the leaching of phthalates from packaging materials into particular brands of locally produced PC and PET bottled drinking water that are regularly consumed by adults and children and sold in Mwanza City markets.The method of determination was liquid-liquid extraction followed by chromatographic analysis for identification of the specific phthalates compounds.

The results that the lowest mean Di methyl phthalates (DMP) concentration in water packed in PET bottles was 0.085 µg/mL before storage and that the mean DMP concentration increased to 0.235 µg/mL following six months of storage at room temperature and outdoor temperature. Additionally, the 18.9-liter PET bottle migrated higher than the 1.5-liter PET bottle due to its larger surface area for water contact and higher surface-to-volume ratio; however, it was found that production contamination, not migration, was the cause of the higher Di methyl phthalates concentrations in the PC bottles.These findings suggest that storing PET bottles containing drinking water at room and outdoor temperatures for an extended period of time directly impacts phthalate migration. Furthermore, this study shows reusing plastic water bottles raises the risk that the inner surface will deteriorate and release more phthalates, which raises concerns about the safety of drinking water that has been prepared and stored in a plastic bottle.

Keywords: polyethylene terephthalate (PET), polycarbonate (PC), phthalate esters, migration, storage time and storage condition**.**

1. INTRODUCTION

Water is essential for life; it has numerous effects on our lives and is used in a variety of activities, including the manufacture of bottled drinking water, carbonated soft drinks, and other beverages. Global demand for bottled water and other clean, safe drinking water is increasing (21). The increasing use of bottled drinking water in Tanzania is primarily due to the low quality of tap water, and non-tap water sources(17,16,27,18), as well as insufficient progress and frequent failures in public water supply systems (20,15,13).

Increased marketing promotion of bottled water, carbonated soft drinks, and other beverages by manufacturing companies results in increased consumption and sales of these beverages (30,31,32).According to the United Nations University's Institute for

Water, Environment, and Health, the bottled water market grew by 73% from 2010 to 2020, and consumption is expected to rise from 350 billion litres in 2021 to 460 billion litres by 2030 globally (21).

Bottled water is typically marketed and sold in glass or, to a larger extent, plastic bottles with metallic or plastic tops in various kinds of shapes, colours, and materials. Plastic materials used to produce these bottles are composed of one or more monomers and a variety of additives that can eventually migrate into water during the production, filling, or storage of bottled products (8,9). One of the additives is a plasticizer known as phthalates or Phthalate esters (PAEs), which is used to increase the flexibility, strength, and processability of plastic materials (2,7). Polyethylene Terephthalate (PET) bottles, high-density polyethylene (HDPE), and low-density polyethylene (LDPE) bottles are typical examples of such applications (22).

Phthalate esters (PAEs) are not chemically bonded to the polymer, they bind to the bottle polymer matrix by weak secondary molecular interactions; as a result, they can be released from the packaging material, migrate, and pollute soil, water, air, packed food and beverages (1).This is a concern among millions of people who consume bottled water regularly (29). Di (2-ethylhexyl) phthalate (DEHP), one of the most widely used phthalates, has been linked to liver cancer, while Dibutyl phthalate(DBP), Butyl benzyl phthalate (BBP), and many phthalate metabolites, including monobutyl phthalate, monobenzyl phthalate, and mono (2-ethylhexyl) phthalate, are endocrine disruptors and teratogenic in animals such as rodents(14,33,34).Dimethyl phthalates （DMP), lower molecular weight PAEs, and their metabolites are hazardous to development and reduction systems by altering endogenous hormones and their receptors (6).

Furthermore, several organizations, including the World Health Organization (WHO), the European Union (EU), and the Environmental Protection Agency (EPA), have identified phthalates as emerging contaminants (ECs) and also as serious environmental hazardsand highlighted them for monitoring in plastic-packed food products (6).The US EPA and the World Health Organization (WHO) have recommended maximum allowable concentrations of DEHP in drinking water of 8 µg/L and 6 µg/L, respectively. However, Tanzania has no minimum requirements for phthalates in plastic containers or drinking water.

Poor handlingand storage practices observed in most Tanzanian cities, such as transporting and keeping bottled water outdoors in direct sunlight for extended periods of time, storing in non-compliant storage facilities coupled with the growing consumption of bottled water in Mwanza City over the last decades, are a growing public health concern. The purpose of this study was to investigate the presence of and quantify phthalates in bottled water produced locally in Mwanza City, as well as to look into the impact of different storage conditions on the levels of these contaminants.

2.1 EXPERIMENTAL

2.1.1 Reagents and Instrumentation

Dimethyl phthalate (DMP, 99%), diethyl phthalate (DEP, 99.5%), di(2-ethylhexyl) phthalate (DEHP, 99%), dibutyl phthalate (DBP, 99%), and internal standard benzyl benzoate (99%) were all bought from Sigma-Aldrich (Germany). HPLC analytical grade solvents Dichloromethane 99% and acetonitrile 99.9% were obtained from LOBA Chemie Pvt Ltd in

India, while anhydrous sodium sulphate 99% was procured from SPAN LAB in India, and Acetone 99% from SURECHEM PRODUCTS LTD-England.

Rotary evaporator from BUCHI Rotavapor R-300 made in Switzerland, Agilent Technologies 1260 infinity II HPLC system made in German, Fume hood from DAIHAN LABTECH CO. LTD, South Korea,PTFE Syringe filter with a diameter of 25mm and pore size 0.45µm fromISOLAB Laborgeräte GmbH- Germany, glass HPLC vial with amber colouralso from ISOLAB Laborgeräte GmbH- Germany and Filter papers from global life sciences solutions operations UK Ltd from UK.

Also different Laboratory glassware and a 1mL single-use syringe from HENRY SCHEIN INC-USA were used in the study.

2.1.2 Sample collection and storage

Forty-eight (48)samples of commercially available plastic bottled drinking water (N=48) from randomly chosen brands were purchased from local markets in Mwanza City.The characteristics of the bottled waters investigated as shown on their labels are presented in **Table 1**. Each brandof water samples was separated into three groups. The first was analyzed soon after purchase, (less than two weeks after it was produced- used as control samples). The other two groups were kept outside (directly exposed to sunlight with daylight temperature recorded to be 30± 5 °C and at room temperatures (storage) (at about 25± 5 °C) for 2, 4, and 6 months, respectively, between December 2022 and August 2023.Temperature readings were taken using a digital thermometer.

Table 1: Bottled Drinking Water properties as displayed on labels

Bottle polymer type and brand	Closure polymer type	Volume (ltr)	USES	pH	Ca (mg/L)	Mg (mg/L)	Cl (mg/L)
PET-Brand A	LDPE	1.5	TAKE AWAY	7.2	10	6	9
PET-Brand B	LDPE	1.5	TAKE AWAY	7	-	-	9.1
PET-Brand C	LDPE	1.5	TAKE AWAY	6.8	5.6	2.5	4.8
PET-Brand D	LDPE	1.5	TAKE AWAY	7	-	2	6
PET-Brand E	LDPE	18.9	RE-USABLE	7	5	4.1	6.9
PC-Brand F	LDPE	18.9	RE-USABLE	7	7.6	13.9	7.9

2.1.3 Extraction

The samples were extracted using the methodology used by Amiridou and Voutsa, 2011 and Dumitrascu, 2012 (3,5). A 500 mL water sample was taken from the bottle to a separating glass funnelof 1000 mL. Phthalates were isolated via a liquid-liquid extraction with dichloromethane (50mlx3). The three serial extracts were combined and dehydrated over anhydrous Na_2SO_4, then filtered and concentrated ina 10ml solution in a rotary evaporator (BÜCHI Labortechnik AG- Switzerland).The solvent was allowed to evaporate over time in afume hood and 3 mL of Acetonitrile was added to reconstitutethe sample extract prior to HPLC analysis.The rotary evaporator was set to operate at 40°C for the heating bath, 15°C for the chiller, 100 rpm (rotation per minute), and 850mbar for the vacuum.

Internal standard Benzyl benzoate (99%) was spiked into samples before extraction to compensate for loss during extraction, purification, preconcentration, and detector response.In addition, calibration standards were spiked with internal standards (19).The formula used to find the volume of the spiking solution is as follows (24):

$$\textit{The volume of spiking solution} = \frac{\textit{Spike concentrated desired x Volume of sample to which spike is added}}{\textit{Concentration of the spiking solution}}$$

$$= \frac{0.05\ \text{mg/L} * 500\text{mL}}{100\ \text{mg/L}} = 0.25\text{mL} = 250\ \mu\text{L}$$

For this study, the recovery percentage of internal standard ranged from 60 to 160, which is equivalent to 0.03 µg/mL to 0.08 µg/mL.

2.1.4 Chromatographic separation

Chromatographic analysis was performed at the Tanzania Medicines and Medical Devices Authority's (TMDA) Eastern Lake Zone laboratory using an Agilent Technologies 1260 infinity II HPLC system. A quaternary pumping system, an autosampler with an injector, a variable wavelength diode array UV detector, and a computer system with HP chemstation software were all part of the device. The 150 mm×4.6 mm, 5 µmAgilent C18 column was used to achieve chromatographic separation. The column was mounted in a column oven compartment set to 30^0Celsius. The analysis was carried out under gradient conditions, with Water/Acetonitrile as the mobile phase to achieve optimum compound chromatographic separation (**Table 2**), a flow rate of 1 mL/min, an injection volume of 20µL, and phthalates detected at a wavelength of 228 nm.

Table 2: HPLC mobile phase Gradient run

Acetonitrile (ACN %)	Water (H_20 %)	Time(minutes)
50	50	0
90	10	5
100	0	7
100	0	11
90	10	13
50	50	13.1
50	50	15

2.1.5 Calibration

A working standard solution of DMP: DEP: DBP: DEHP (1:1:1:1) phthalates (100 mg/mL each) wasprepared from the certified reference stock solution of DMP, DEP, DBP, and DEHP in Acetonitrile. Benzyl benzoate (1 mg/mL) as internal standard was spiked to aliquots of the working standard solution, then diluted with 90% Acetonitrile to form a 10ml solution. The analytes were identified and quantified in HPLC using a set of six calibration standards (0.25, 0.5,1,2,4,8µ**g/mLTable 3A**). Individual calibration curves were generated from the chromatograms by plotting the phthalate peak area against concentrations of six calibration standards.

$M_1V_1=M_2V_2$

Where M_1=Stock solution concentration in mg/mL=100 µ**g/mL**

V_1= Volume of aliquot to be taken
M_2= calibration standards concentration
V_2=volume of aliquot solution=10mls

e.g. Preparation of **0.25** µ**g/mL** from a stock solution of **100** µg/mL

100 µ**g/mL** * V_1=0.25µ**g/mL** *10mls

V_1 =0.025mls

Table 3A: Calibration Standards Calculation for Drinking Water Samples

Calibration standards on a solution basis, (in µg/mL)	Calibration standards on a sample basis of 500mLs, (in µg/mL)	Volume of aliquot to be taken (in mL)	The volume of aliquot to be taken (in µL)	Final solution volume(in mL)
0.25	0.005	0.025	25	10
0.5	0.01	0.05	50	10
1.0	0.02	0.1	100	10
2.0	0.04	0.2	200	10
4.0	0.08	0.4	400	10
8.0	0.16	0.8	800	10

Based on its retention time, phthalate ester was identified (**Table 3B**), and its quantity was determined using a standard calibration curve.

Table 3B: PAEs HPLC Retention Time and Correlation Coefficient of Bottled Drinking Water samples examined

Phthalates compounds	Retention Time(minutes)	Best-fit regression equations	Correlation Coefficient (R^2)
DMP	2.978±0.2	$y=1420.263x + 1.114$	0.99951
DEP	4.206±0.2	$y=1427.951x + 0.835$	0.99929
DBP	6.988±0.2	$y=930.556x + 0.1398$	0.99994
DEHP	10.825±0.2	$y=704.243x + 3.0575$	0.99884
INTERNAL STD	5.794±0.2	$y=2364.214x + 3.2337$	0.99973

2.1.6 Phthalates Detection and Quantification

The six-point calibration curve was utilized to quantify targeted phthalate esters in sample extracts by comparing the phthalate ester's HPLC retention time and peak area to the standard calibration curve. To keep concentrations within the linear range of the calibration curve, sample extracts were diluted as needed. Those with no discernible peaks or responses were designated as less than the limit of detection (<LOD) of the calibration curve.

The detection limit (LOD) for this study was the lowest calibration standard used to create the calibration curve, which is 0.25 µg/mL, similar to 0.005 µg/mL on a sample basis.

2.1.7 Quality Assurance and Quality Control

Several precautions were taken, in accordance with previously published reports and ISO standards, to minimize phthalates contamination that may occur during all stages of analysis, including sampling, sample preservation and preparation, sample extraction, and sample clean-up (11,23,29).

These included the use of pure deionized water free of phthalates. Every glassware was carefully cleaned and rinsed with acetone. Before and after each set of samples, a full set of calibration phthalate standards were examined and one standard was run as a control after every batch of samples to verify instrument stability. To investigate background contamination, Acetonitrile which was used as the diluent, was tested with each set of samples.The HPLC was calibrated to ensure the detector's linearity and sensitivity. A calibration curve was produced by running a series of phthalate standards and plotting their peak areas against their relative concentrations.

2.2 Statistical analysis

The IBM Statistical Product and Service Solution (SPSS) version 26 software, excel,and descriptive statistics were used to analyse the data for DMP and DEHP. First, all phthalate levels determined to be below their LOD were statistically treated by assigning values equal to their respective LOD values divided by the square root of 2. This statistical approach of data smaller than LOD was established by Serrano *et al*., 2014; Chen *et al*., 2012 and Zaki and Shoeib, 2018 (4,25,29). To determine the normal distribution of data, the Shapiro-Wilk test was utilized while the Levene Statistic test was used to test the Homogeneity of Variance wherebyp-values greater than 0.05 were considered significant.

The Friedman and Wilcoxon Signed Ranks test and the Mann-Whitney test were used as non-parametric test methods, with the Friedman and Wilcoxon Signed Ranks test being used to assess statistically significant changes in concentrations observed at every single storage time point of the same storage condition in comparison with the concentrations noticed without allowing for storage time (less than two weeks after it was a produced-control sample), and the Mann Whitney test wasused to compare changes in the concentrations among different storage conditions (outdoor and room temperature). In the Friedman, a p-value greater than 0.05 was considered not significant whereas a p-value greater than 0.05 was considered significant forMann-Whitney tests.

Finally, Spearman's rho test was used to assess the effect of storage conditions, storage time, and bottle polymer type on the leaching of phthalate compounds into bottled drinking water witha p-value less than 0.05 and 0.001 was considered significant, and larger Spearman's rho coefficients indicate a greater degree of association.

4. Results and Discussion

This study sought to establish the effect of storage conditions, storage time, and container polymer type on the leaching of phthalates DMP, DBP, DEP, and DEHP from packaging materials into selected brands of PET and PC plastic bottled drinking water. DEP and DBP were not detected in all analyzed samples while DMP was detected in all 48 water samples analyzed under varied storage conditions, and DEHP was found in three of the water samples packed in PET and PC bottles kept outside for two months and in control sample (2 weeks from the date of manufacturing) (Table 4 and Table 5).

Table 4:Mean Concentration of DMP and DEHP in water packed in PET bottles(mean ±sd)

The volume of the PET bottle	Storage condition	Storage time(Months)	DMP((µg/mL))	DEHP((µg/mL)
1.5 Ltr	Outdoor	0	0.150 ± 0.056	0.004 ± 0.000

		2	0.200 ± 0.056	0.004 ± 0.000
		4	0.175 ± 0.021	0.004 ± 0.000
		6	0.220 ± 0.000	0.004 ± 0.000
	Indoor/room	0	0.085 ± 0.035	0.004 ± 0.000
		2	0.120 ± 0.000	0.004 ± 0.000
		4	0.220 ± 0.042	0.004 ± 0.000
		6	0.205 ± 0.007	0.004 ± 0.000
18.9 Ltr	Outdoor	0	0.090 ± 0.056	0.010 ± 0.000
		2	0.160 ± 0.000	0.007 ± 0.004
		4	0.225 ± 0.007	0.004 ± 0.000
		6	0.235 ± 0.007	0.004 ± 0.000
	Indoor/room	0	0.100 ± 0.014	0.004 ± 0.000
		2	0.140 ± 0.000	0.004 ± 0.000
		4	0.17 ± 0.028	0.004 ± 0.000
		6	0.235± 0.035	0.004 ± 0.000

Table 5: Mean Concentration of DMP and DEHP in water packed in PC bottles

The volume of the PC bottle	Storage condition	Storage time(Months)	DMP((µg/mL))	DEHP((µg/mL)
18.9 Ltr	Outdoor	0	0.045 ± 0.021	0.004 ± 0.000
		2	0.050 ± 0.028	0.004 ± 0.000
		4	0.050 ± 0.028	0.004 ± 0.000
		6	0.035 ± 0.007	0.004 ± 0.000
	Indoor/room	0	0.045 ± 0.021	0.004 ± 0.000
		2	0.050 ± 0.028	0.004 ± 0.000
		4	0.045 ± 0.021	0.004 ± 0.000
		6	0.060 ± 0.014	0.004 ± 0.000

4.2.1. PAE concentration levels under various storage times and storage conditions.

According to the results, distinct observations were made regarding the concentration levels of PAEs under different storage conditions and times. These observations include:

Themean DMP concentrations in the investigated take-away 1.5ltr PET Bottled drinking water samples were 0.20 µg/mL and 0.22 µg/mL for the plastic bottled samples kept outdoors and 0.12 µg/mL and 0.205 µg/mL for the plastic bottled samples kept under room temperature for 2 and 6 months respectively, while analysis for samples before storage, which also served as control samples, revealed mean DMP concentrations of 0.15 µg/mL and 0.085 µg/mL kept outdoors and at room temperature with increases of 46.667% and 141.176% for outside and room temperature, respectively (Figure 1), indicating higher migration at room temperature compared to outdoor, despite the slightly difference between their mean values. There were no significant variations in DEHP concentration before and after storage for takeaway 1.5ltr PET bottle drinking water, indicating that DEHP was not used as a plasticizer in this polymer container.

In the examined re-usable 18.9-liter PET bottle bottled drinking water samples, the mean DMP concentrations were 0.160 µg/mL and 0.235 µg/mL for plastic bottled samples kept outside, and 0.140 µg/mL and 0.235 µg/mL for plastic bottled samples kept at room temperature for two and six months, respectively. Analysis of samples taken before storage, which were also used as control samples, showed mean DMP concentrations of 0.09 µg/mL and 0.1 µg/mL for samples kept outside and at room temperature, with increases of 161.11% and 135% for outside and room temperature, respectively, showing that outdoor temperature causes higher migration than room temperature (Figure 1).Two samples before storage were identified to containDEHP means concentrations of 0.01 µg/mL and then after two months of storage, the mean concentrations of DEHP was decreased to 0.007 g/mL, and then no changes observed after 4 and 6 months of storage (Table 4) indicating that DEHP was not used as a plasticizer in this 18.9ltr of PET bottles rather than contamination during production or from the source.

The DMP mean concentrations for re-usable 18.9ltr PC bottles show a migration increase of 33.33% while kept at room temperature, but no variation when stored outside (Figure 1).The highest DMP mean concentration observed was 0.06µg/mL and the lowest was 0.035µg/mL (Table 5) with slight decrease and increase percentages showing instability and concentrations observed to be more contaminated during production rather than migration.Also, no significant changes were observed for DEHP mean concentrations demonstrating that both DMP and DEHP were not used as plasticizers in PC bottles used by Mwanza City manufacturers. This is in line with research by Guart*et al*., 2014 and Amiridou and Voutsa, 2011, which demonstrate that Bisphenol A (BPA), as opposed to DMP and DEHP, is a monomer employed in the synthesis of polycarbonate bottles.

The DMP migration from plastic containers to drinking water was 1.5 and 2.4 times greater for 1.5ltr PET bottles and 2.6 and 2.4 times greater for 18.9ltr PET bottles for outdoor and room temperature storage, respectively, from less than two weeks after production to six months of storage, indicating that the 18.9ltr PET bottle has a higher migration rate than the 1.5ltr PET bottle.This is most likely because an empty PET bottle with a 1.5-liter capacity weighed approximately 29–33 grams (in line with the findings of the Islam *et al*., 2018 study) and had a wall thickness of 0.20mm, whereas an empty 18.9-liter bottle weighed approximately 730–750 grams and had a wall thickness of 0.45 mm. Because of this, the 18.9-liter PET bottle has more materials for leaching per unit volume of stored water and a larger surface area for water contact (Moazzen*et al*., 2018). Furthermore, the higher migration rate seen in 18.9-liter reusable PET bottled drinking water indicates that, despite PET plastic water bottles being tougher, stronger, and more transparent than other commodity plastic bottles, reusing them is not recommended as it accelerates the ablation of the inner surface of the bottle, which results in increased migration of phthalates and microplastic particles.

The minimum and maximum mean DMP concentrations identified in 18.9ltr Re usable PC bottles were 0.035 µg/mL and 0.06 µg/mL respectively(Table 5) while the minimum and maximum mean DMP concentration identified in 18.9ltr ReusablePET bottles were 0.085 µg/mL and 0.235µg/mL respectively (Table 4) before and after 6 months storage at room and outdoor temperature, demonstrates that keeping PET bottles with drinking water at room and outdoor temperature for a longer period has a direct influence on phthalate migration.As a result, phthalate migration is a function of both temperature and time.

Unexpectedly, DMP was discovered in PET and PC bottled water manufactured in less than two weeks and employed as a control. This has been linked to contamination during the bottling and water treatment processes, which resulted in using contaminated water containing phthalates and insufficient PET bottle moulds temperature monitoring to attain optimal wall thickness distribution and bottle perpendicularity.

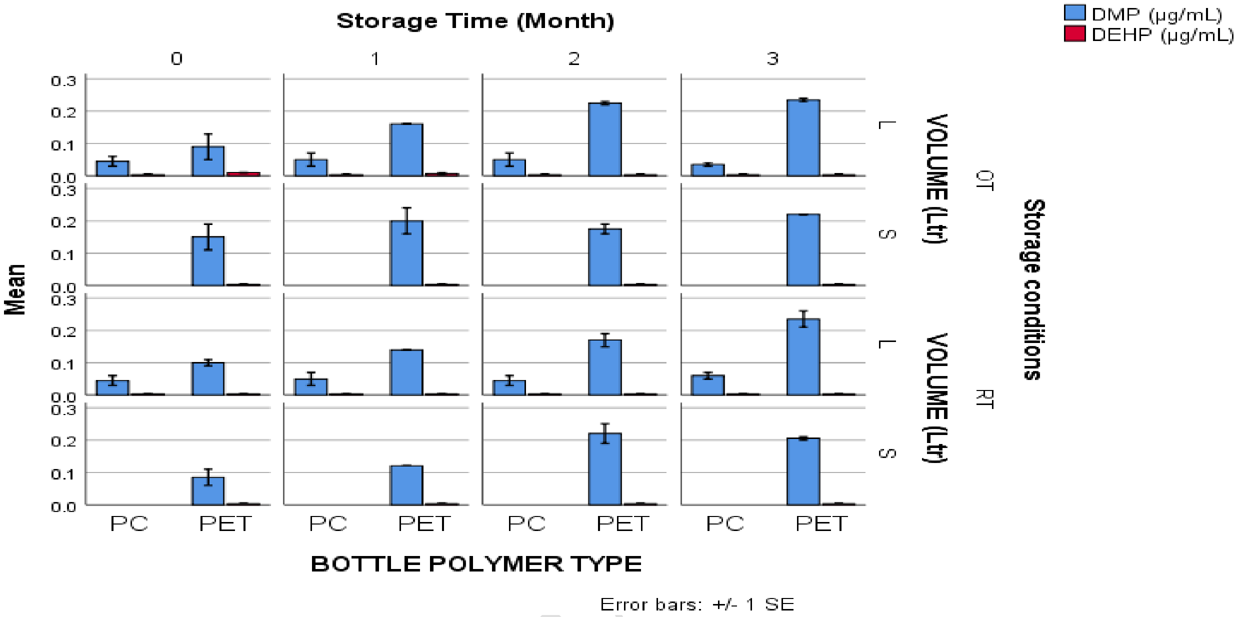


Figure 1: Mean concentrations of DMP and DEHP in µg/mLobserved in PET and PC water samples were analysed at different times and under various storage conditions. Panel RT: storage at room temperature (25 ± 5 °C); and Panel OT: storage outdoors with sun exposure (30 ± 5 °C). The 0 storage time point denotes analysis before storages (2 weeks after production), whereas 1 denotes analysis 2 months, 2 denotes analysis 4 months, and 3 denotes analysis 6 months after storage. PET and PC denotes PET and PC bottles respectively while L and S denotes 18.9 ltr and 1.5ltr volumes of drinking water respectively.

4.2.2. The effect of storage timeon phthalate concentration

The DMP concentrations in PET bottles increased with storage times, at room temperature and in direct sunlight (outdoors) (Figure 1). Also, the results revealed p-values of 0.000 (Table 6), indicating that there were notable variations in concentrations at different storage timesunder the same storage condition.The results show that longer storage times under the same conditions as this study have increased DMP migration from PET bottles, with the lowest mean DMP detected at 0.085 µg/mL before storage rising to the highest mean DMP detected at 0.235 µg/mL after six months (Table 4), a 2.8-fold increase and illustrative of how plastic deterioration over time may change the physical properties of bottled water, particularly in outdoor temperatures. It is therefore usually advised to drink water from plastic bottles within six months to two years of manufacturing (38). This is because plastics should be stored according to their specified shelf life, as exposure to light, heat, and other environmental factors can cause the plastic to deteriorate over time and compromise the water's safety and Quality.

These findings back up previous studies by Zaki and Shoeib, 2018; and Guart*et al*., 2014, which revealed an increase in phthalate concentrationsin PET bottled water as the period of storage increased (8,29).DMP was also found in PET bottled water in studies from Hungary (12), Jordan (28), and Spain (8). Also, this implies that the plastic bottles used to package drinking water produced in Mwanza City factories contain DMP, which isn't frequently employed as a plasticizer.

Table 6: Friedman Test for targeted DMP and DEHP in Bottled drinking water

Mean Rank	
Exposure Time	2.50
DMP (µg/mL)	2.25
DEHP (µg/mL)	1.25
Test Statistics[a]	
N	48
Chi-Square	42.000
Df	2
Asymp. Sig.	0.000

a. Friedman Test

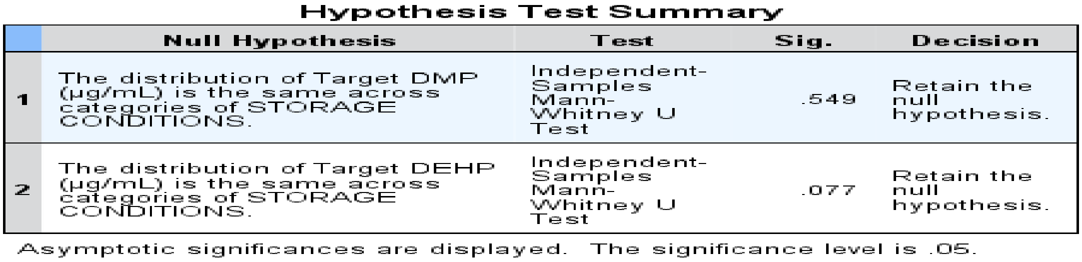
4.2.3. The effects of storage conditions on PAE concentration levels

This study aims to establish a relationship between the level of phthalates concentrations observed and improper handling and storage practices found in most Tanzanian cities, such as leaving PET and PC water bottles in the open during transportation, in direct sunlight for extended periods of time, and non-compliant storage facilities.Results showed that there was no statistically significant difference in DMP and DEHP concentrations at specific times among samples stored indoors and outdoors with p-values of 0.549 and 0.077 respectively.This illustrates how temperature, whether indoors or outdoors, influences a number of the physical properties of drinking water, such as its density, viscosity, decreasing the values of total dissolved substance(TDS), alkalinity, and dissolved chemical content (36).

Despite not being statistically significant, different storage conditionsettings such as refrigerated temperature poured boiled water into a PET bottle, long-term ultraviolet ray exposure, a storage room, outdoor storage, and the length of the period for which it was stored can all increase phthalate migration rate in PET bottlesto varying degrees (26,29).This discovery is congruent with the findings of Zaki and Shoeib (2018), who found a rise in phthalate concentrations after storing plastic bottled water in a refrigerator at 4 °C, a storage room, and outdoor storage for an extended period (29)demonstrating that the migration of phthalates is influenced by temperature and time.

Such results highlighted the importance of paying close attention to the storage conditions of plastic bottles, in particular when transporting, keeping bottled water outside in direct sunlight for extended periods, and storing it in non-compliant storage facilities.Also, it is usually recommended to store water between 41^0 and 78^0 Fahrenheit (5^0 and 26^0 Celsius) since this is the ideal temperature range for the body to absorb water, rehydrate, relaxation, weight loss,and improve digestion (37,39,40).Additionally, PET bottles containing water should be kept out of direct sunlight.

Figure 2:Mann-Whitney test for Bottled drinking water



Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Target DMP (µg/mL) is the same across categories of STORAGE CONDITIONS.	Independent-Samples Mann-Whitney U Test	.549	Retain the null hypothesis.
2	The distribution of Target DEHP (µg/mL) is the same across categories of STORAGE CONDITIONS.	Independent-Samples Mann-Whitney U Test	.077	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

4.2.4. The effects of storage conditions, storage time, and container polymer type on phthalate leaching from packaging materials into bottled drinking water

The following findings were noted after assessing the bivariate relationship between bottle polymer type, storage duration, and conditions on the leaching effect:

The p-values of 0.673 and 0.759, coupled with Correlation Coefficient rho values of 0.115 and 0.083 (Table 7), indicate that there is no statistically significant bivariate correlation between the storage conditions and storage time on the PC polymer leaching DMP on bottled drinking water.

With p-values of 0.190 and 0.000 for DMP, coupled with Correlation Coefficient rho values of -0.238 and 0.799 for storage conditions and storage times, respectively (Table 8), show that there is a statistically significant bivariate correlation between storage time on the PET polymer leaching DMP on bottled drinking water with Positive correlations of 79.9%, but no statistically significant bivariate correlation between storage conditions on the PET polymer leaching DMP on bottled drinking water.

Furthermore, p-values of 0.073 and 0.06 for DEHP, coupled with Correlation Coefficient rho values of -0.322 and -0.336 for storage conditions and storage times, respectively (Table 8), indicate that there is no statistically significant bivariate correlation between storage time and storage conditions on the PET polymer leaching DEHP on bottled drinking water.

This demonstrates an association between storage time and an increase in Dimethyl phthalate (DMP) concentration in PET bottles, indicating that the source is most likely migrating from the PET plastic material regardless of storage conditions.

Table 7: Spearman's rho test for PC Bottled drinking water

Correlations

			PC Bottle	Storage Conditions	Storage Time	DMP (µg/mL)	DEHP (µg/mL)
Spearman's rho	PC Bottle	Correlation Coefficient	.	.	.	.	.
		Sig. (2-tailed)	.	.	.	.	.
		N	16	16	16	16	16
	Storage Conditions	Correlation Coefficient	.	1.000	.000	.115	.
		Sig. (2-tailed)	.	.	1.000	.673	.
		N	16	16	16	16	16
	Storage Time	Correlation Coefficient	.	.000	1.000	.083	.
		Sig. (2-tailed)	.	1.000	.	.759	.
		N	16	16	16	16	16
	DMP (µg/mL)	Correlation Coefficient	.	.115	.083	1.000	.
		Sig. (2-tailed)	.	.673	.759	.	.
		N	16	16	16	16	16
	DEHP (µg/mL)	Correlation Coefficient	.	.	.	.	.
		Sig. (2-tailed)	.	.	.	.	.
		N	16	16	16	16	16

Table 8: Spearman's rho test for PET Bottled drinking water

Correlations

			PET Bottle	Storage Conditions	Storage Time	DMP (µg/mL)	DEHP (µg/mL)
Spearman's rho	PET Bottle	Correlation Coefficient	.	.	.	.	.
		Sig. (2-tailed)	.	.	.	.	.
		N	32	32	32	32	32
	Storage Conditions	Correlation Coefficient	.	1.000	.000	-.238	-.322
		Sig. (2-tailed)	.	.	1.000	.190	.073
		N	32	32	32	32	32
	Storage Time	Correlation Coefficient	.	.000	1.000	.799**	-.336
		Sig. (2-tailed)	.	1.000	.	.000	.060
		N	32	32	32	32	32
	DMP (µg/mL)	Correlation Coefficient	.	-.238	.799**	1.000	-.291
		Sig. (2-tailed)	.	.190	.000	.	.106
		N	32	32	32	32	32
	DEHP (µg/mL)	Correlation Coefficient	.	-.322	-.336	-.291	1.000
		Sig. (2-tailed)	.	.073	.060	.106	.
		N	32	32	32	32	32

**. Correlation is significant at the 0.01 level (2-tailed).

4. Conclusion

DMP was the most predominant phthalate component found in all 48 samples, regardless of storage conditions, when compared to DEHP. The DMP migration from plastic containers to drinking water increased by 2.6 and 2.7 times for outdoor and room temperature storage, respectively, between less than two weeks after production and six months of storage. The minimum mean DMP concentration was found to be 0.035 µg/mL, and the maximum mean DMP concentration was found to be 0.235 µg/mL, indicating cross-contamination of phthalates from packaging to contents and impeding the quality and safety of this significant drink. Meanwhile, the mean DEHP concentration was found to be below the Limit of detection (LOD).

At present, Tanzania lacks legislation concerning phthalates; however, the World Health Organization (WHO) and the US EPA have recommended maximum allowable concentrations of DEHP in drinking water of 6 µg/L and 8 µg/L, respectively. No DMP limits were set by the WHO or Tanzania Bureau of Standards in the course of this study. Regardless of storage conditions or times, none of our samples exceeded the maximum DEHP limit established by the US EPA and the World Health Organization (WHO). Even though the tested bottleddrinking waters had low levels of DEHP, it's vital to keep in mind keep in mind that prolonged use of phthalate-contaminated bottled water may result in adverse effects such as immune system issues, aberrant reproductive function, brain damage, endocrine system disruption, tumour growth, and others (14,35).This begged the question of how safe it is to drink water that has been prepared at home, from a plastic bottle, or straight from the faucet.

Since DMP content increases with storage time, storing plastic bottled water for an extended period of time is likely to impact the migration of phthalate contaminants into bottled drinking water, whether outside or inside. Additionally, this study found a correlation between storage duration and an increase in the concentration of dimethyl phthalate (DMP) in PET bottles as opposed to PC, suggesting that migration from PET plastic materials is most likely the source.

To maintain product safety and quality, it is recommended that drinking water in bottles composed of polymer packaging materials be stored in accordance with manufacturer-specified instructions found on product labels.Reusing plastic water bottles is not a good idea because it causes the inner surface of the bottle to abrade more quickly, producing more microplastic particles and phthalates. Furthermore, since heat effects during storage may accelerate the fragmentation process, first-in, first-out protocols should be followed to guarantee that customers receive the freshest stock.

Ethical considerations

The study received ethical approval from the Tanzania President's Office-Regional Administration and Local Government (PO-RALG) via letter **kumb. Na. AB/307323/01.**

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