

Levels of phthalate acid esters in carbonated soft drinks bottled in polyethylene terephthalate (PET) bottles kept under various storage conditions in Mwanza City, Tanzania

Abstract

Background: Carbonated soft drinks (CSD), packaged in PET (polyethylene terephthalate) bottles, are among the most widely consumed non-alcoholic beverage globally. People of all ages enjoy CSDs for their flavour, mouthfeel, crispness, freshness, and capacity to both quench thirst and provide mental refreshment. Terephthalic acid, ethylene glycol, and other additives such as phthalates are the materials used to make these PET bottles. Several studies have demonstrated that phthalates may migrate into packaged goods and, when consumed, can cause several ill-health conditions to humans. Type II diabetes, obesity, asthma, male and female reproductive disorders, tumours (including breast cancer), pituitary, hypothalamic, and peripheral hormone disorders, behavioral issues, and neurodevelopmental disorders are among the health issues reported to be associated with phthalates.

Objective: This study aimed to ascertain the presence and concentration of phthalates in PET bottled carbonated soft drinks produced in Mwanza City, and to examine the effects of various storage conditions on the migration of these contaminants into the product.

Methods: Liquid-liquid extraction, clean-up techniques, and chromatographic analysis were used as the methods of determination to identify and quantify the specific phthalates compounds.

Results: The findings demonstrated that after being stored outdoors for four to six months, the migration, especially for dibutyl phthalate (DBP) and di [2-ethylhexyl] phthalate (DEHP), became more apparent. After two, four, and six months of indoor storage, there was no appreciable change in the mean concentrations of DMP (dimethyl phthalate), DEP (diethyl phthalate), DBP (dibutyl phthalate), and DEHP for either brand in this study. Compared to indoor storage, the DBP and DEHP migration increased by up to 24-fold when the two brands were kept outdoors for four and six months. Thus, the safety and quality of carbonated soft drinks were seriously compromised by the phthalate compounds from the PET bottles that these companies were using.

Conclusion: The study's conclusions show that PET packaging, which is influenced by the bottle manufacturing process and raw material formulations for packages of the studied CSDs, are responsible for the migration of phthalates into the products. The storage conditions and time are established to be the primary causes of phthalates migration in bottled carbonated soft drinks. Though the migration figures in this study are lower than those of the other studies from different locations that were compared, it is important to remember that these compounds may interfere with hormones even in small quantities.

Keywords: Polyethylene terephthalate (PET), Carbonated Soft Drinks, phthalate esters, migration, storage time and storage condition

1. INTRODUCTION

Carbonated soft drinks (CSD), the world's second most popular non-alcoholic beverage only after water, are widely consumed due to their crispness, freshness, flavour, mouth feel, thirst-quenching capability, and mental refreshment during times of stress.¹ CSD typically contains carbonated water, caramel colour, phosphoric acid, high fructose corn

syrup or sucrose, and flavours^{1,2,3} as well as a high carbonation level (between 1.5 and 5 volumes of CO₂) and pH level between 2.5 and 4 CSD⁴, along with water, contributes to a healthy and enjoyable diet, as well as the body's quick uptake of salts and water.¹ According to data and analytics firm Strategy Helix, the carbonated soft drinks market in Tanzania is expected to grow by US\$ 393 million between 2021 and 2027, at a compound annual growth rate (CAGR) of 5.1%.⁵

More and more producers are using polyethylene terephthalate (PET) bottles made from terephthalic acid and ethylene glycol to make bottles for drinking water, soft drinks, and other drinking. In 2016, it was estimated that 100 million tons of PET were produced annually.⁶ CSD uses PET bottles for managing CO₂ internal pressures without deforming or booming⁷ and a polymer closure is used to provide a tight seal that can be easily opened and closed. The plastic components used to make these PET bottles and closures are made up of one or more monomers as well as several additives. Phthalate esters (PAEs) are one of the additives (plasticizers) that are frequently used to boost the strength, flexibility, and processability of PET bottles and closures.^{8,9}

According to Salazar *et al.* 2018b¹⁰, PAE consumption in PET ranges from 1 to 5 wt% but this additive is not chemically bonded to the polymer; instead, it binds to the bottle polymer matrix by weak secondary molecular interactions with polymer chains; as a result, they can be released from the bottle, migrate, and pollute soil, water, air, food, and beverage items via interactions between food and packaging.¹¹ This is raising concerns among millions of people who consume bottled water or carbonated soft drinks regularly.¹²

Giuliani *et al.* (2020) state that the main cause of PAE contamination is the migration of plasticizers from plastics into food.¹³ Drinks like bottled carbonated soft drinks may be contaminated during the production and packaging processes. Plastic-lined metal bottle caps and plastic closures made of diverse polymers are two other sources of phthalates suspicions. Numerous studies have connected phthalates and their metabolites to adverse health effects in humans. For instance, Di (2-ethylhexyl) phthalate (DEHP), one of the most significant and frequently used phthalates, have been linked to liver cancer, and many phthalate metabolites, such as monobutyl phthalate, monobenzyl phthalate, and mono (2-ethylhexyl) phthalate, are teratogenic in rodents and other animals.¹⁴ According to other research, these dangerous phthalates have been linked to several health issues in humans, such as obesity, type II diabetes, asthma, neurodevelopmental disorders, cancers (including breast cancer), pituitary, hypothalamic, and peripheral hormone disorders, behavioral issues, and issues relating to both male and female reproduction.^{15,16,17}

Tanzania has seen an increase in the consumption of carbonated soft drinks in plastic bottles over the past few decades, according to HELIX's market growth projections. This rise has also been observed in Mwanza City, where the trade and industries sector has grown steadily over the years, averaging 4% yearly growth and making up just 8% of the GDP.¹⁸ Apart from the consumption volumes, the risk level is raised by the poor storage practices that have been observed, such as transporting and keeping bottled water outdoors in direct sunlight for extended periods and storing it in other non-compliant storage facilities. This study aimed to determine if phthalates were present in Mwanza City-produced PET bottled carbonated soft drinks and to measure the quantity present. It also intended to

ascertain how various storage conditions influenced the quantities of these contaminants in the products.

2 METHODOLOGY

2.1.1 Chemical Reagents and Materials

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). Sodium carbonate 99.5 % and 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.

Other equipment and materials were Agilent Technologies 1260 infinity II HPLC system made in Germany, Fume hood from DAIHAN LABTECH CO. LTD, South Korea, Ultrasonic bath from BRANSONULTRASONIC CORPORATION, USA, Silica gel for chromatography 0.030-0.200mm,60A from Acros Organic B.V.B.A Chemicals Company-German, n-hexane from MRS Scientific Ltd-UK, PTFE Syringe filter with a diameter of 25mm and pore size 0.45µm from ISOLAB Laborgeräte GmbH- Germany, glass HPLC vial with amber color 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.

2.1.2 Sample collection, storage, and preparation

Thirty-two (32) commercially available plastic bottled carbonated soft drinks (n=32) from two randomly selected brands packaged in similar polymer materials and flavors were bought from local markets in Mwanza City using a mixed-levels factorial design. The characteristics of the bottled carbonated soft drinks investigated as shown on their labels are presented in **Table 1**. Each brand's bottled carbonated soft drinks samples were separated into four groups. The first was analyzed soon after purchase, less than two weeks after it was produced. The other three groups of the samples 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. Ambient and room temperature readings were taken using a digital thermometer.

Before sample analysis, each sample of a carbonated soft drink was degassed in an ultrasonic bath at room temperature to remove carbon (IV) oxide, which might hinder the separation of the extraction mixture and thus reduce extraction efficiency.^{19,20}

Table 1: Bottled Carbonated Soft Drinks properties as displayed on labels

Bottle polymer type and Brand	closure polymer type	Volume (mls)	USES	sugar	Carbonated water	Caramel color(E150d)	Caffeine	flavoring
PET-A	LDPE	500	TAKE AWAY	√	√	√	√	√
PET-B	LDPE	500	TAKE AWAY	√	√	√	√	√

2.1.3 Extraction

In this study, the method of determination used was the one developed by Ayofe *et al.*, 2018 using High-performance liquid chromatography (HPLC) for the detection of phthalates compounds (PAEs) in beverage drinks. A 100 mL carbonated soft drink sample was taken from the bottle to a separating glass funnel 250 mL. Phthalates were isolated via a liquid-liquid extraction with dichloromethane (15mLx3). The three serial extracts were combined and further extraction was done twice with 5 mL 0.1M sodium carbonate to get rid of free fatty acids and some aqueous entraps in the organic constituent that had been extracted with DCM. The extract was dehydrated over an anhydrous Na₂SO₄ and the solvent was left to evaporate with time when exposed to a fume cupboard. The residue was re-dissolved in 2 mL DCM before it was cleaned up in a silica gel-packed column.

Internal standard Benzyl benzoate (99%) was spiked into samples before extraction to compensate for loss during extraction, purification, pre-concentration, and detector response. In addition, calibration standards were spiked with internal standards.²¹ The formula used to find the volume of the spiking solution is as follows:²²

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

$$= \frac{0.25 \text{ mg/L} \times 100 \text{ mL}}{100 \text{ mg/L}} = 0.25 \text{ mL} = 250 \text{ } \mu\text{L}$$

The recovery % of internal standard for this study ranged from 50 to 87.8, which is equivalent to 0.125104 μg/mL to 0.219524 μg/mL.

2.1.4 Cleaning technique

A 50 mL glass chromatographic column was filled with 5 g of silica gel. The column was tapped to settle the silica gel for optimum column packing. Following the addition of 1 g of Na₂SO₄ to the top of the silica gel, the column was pre-eluted with 10 mL of n-hexane at a flow rate of roughly 2 mL/min. 3 mL of n-hexane was used to finish the extract transfer down the column after quantitatively transferring the re-dissolved extract into the column. 15 mL of n-hexane was used to elute the column for non-polar components, and the fraction was discarded. The phthalate esters were then eluted with 20 mL of acetone before exposure to the sodium sulphate layer, and the eluate was concentrated to 1 mL while opened in a fume cupboard. Before the HPLC analysis, 2 mL of acetonitrile was added to the concentrate.¹⁹

2.1.5 Separation by Instrumental Analysis

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 x 4.6 mm, 5 μm Agilent C18 analytical column was used to achieve chromatographic separation. The analysis was carried out under gradient conditions, with Water/Acetonitrile as the mobile phase to achieve optimum compound chromatographic separation (refer to **Table 2**), a flow rate of 1 mL/min, an injection volume of 20 μL, and phthalates detected at a wavelength of 228 nm.

Table 2: HPLC mobile phase Gradient run

Acetonitrile (ACN %)	Water (H ₂ O %)	Time(minutes)
50	50	0
90	10	5
100	0	7
100	0	11
90	10	13
50	50	13.1
50	50	15

2.1.6 Calibration

A working standard solution of DMP: DEP: DBP: DEHP (1:1:1:1) phthalates (100 mg/mL each) has been prepared 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 (0.1, 0.2, 0.3, 0.4, 0.5, µg/mL refer to **Table 3**). 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 µg/mL

V_1 = Volume of aliquot to be taken

M_2 = calibration standards concentration

V_2 =volume of aliquot solution=10mls

$$\text{e.g. } 100 \mu\text{g/mL} * V_1 = 0.25 \mu\text{g/mL} * 10\text{mls}$$

$$V_1 = 0.025\text{mls}$$

Table 3: Calibration Standards Calculation for CSD 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)	Volume of aliquot to be taken (in µL)	Final solution volume(in mL)
0.1	0.003	0.01	10	10
0.2	0.006	0.02	20	10
0.3	0.009	0.03	30	10
0.4	0.012	0.04	40	10
0.5	0.015	0.05	50	10

Based on its retention time, phthalate ester was identified, and its quantity was determined using a standard calibration curve (refer to **Table 4**).

Table 4: PAEs HPLC Retention Time and Correlation Coefficient of Bottled Carbonated Soft Drinks samples analyzed

Phthalates compounds	Retention Time(minutes)	Best-fit regression equations	Correlation Coefficient (R ²)
DMP	2.964±0.002	$y=976.43x - 0.6215$	0.9996
DEP	4.193±0.002	$y=960.1x + 0.3287$	0.9965
DBP	6.984±0.002	$y=588.5x + 2.1841$	0.9633
DEHP	10.869±0.002	$y=463.2x + 0.5428$	0.9978
INTERNAL STD	5.786±0.002	$y=1590.3x-0.1836$	0.9992

2.1.7 Phthalates Detection and Quantification

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

For this study, the detection limit was the lowest calibration standard used to create the calibration curve, which is 0.1 µg/mL, similar to 0.003 µg/mL on a sample basis.

2.1.8 Quality Assurance and Quality Control

Several precautions were taken, following 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.^{12,23,24}

The phthalate-free water was pure deionized water kept in a glass bottle. 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 7 or 8 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 equipment 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 results of the study were analysed using Excel, and IBM Statistical Package for the Social Sciences (SPSS) version 26. The determined concentrations are presented with their mean values and standard deviations. To verify that the concentrations in each group were normal, the Shapiro-Wilk test was performed. The non-parametric Mann-Whitney U test was used to compare various storage conditions because of the data's non-normal distribution. The Friedman test was used to compare the concentrations during the exposure periods. A p-value of less than 5% ($p < 0.05$) was used to determine whether the differences were statistically significant.

Following statistical analysis, all phthalate levels found to be below their LOD were given values equal to their individual LOD values divided by the square root of 2. The statistical method for handling data smaller than LOD was developed by Zaki and Shoeib, 2018, Chen *et al.*, 2012, and Serrano *et al.*, 2014.^{12,25,26}

3. RESULTS AND DISCUSSION

The analysis of samples taken before storage, which also served as control samples, showed mean phthalate concentrations of $< 0.003 \mu\text{g/mL}$ for DEP, DBP, and DEHP, with the exception of DMP, which is $0.0059 \mu\text{g/mL}$ above the detection limits of $0.003 \mu\text{g/mL}$. The mean phthalate concentrations in the samples after various storage periods were estimated to be within the range of $< 0.003 \mu\text{g/mL}$ to $0.0326 \mu\text{g/mL}$ for DMP, $0.0410 \mu\text{g/mL}$ for DEP, $0.0442 \mu\text{g/mL}$ for DBP, and $0.0934 \mu\text{g/mL}$ for DEHP. The results of this study (as tabulated in Table 5) suggest that storage time and storage conditions may have an impact on the release of phthalate esters from PET bottles into their contents.

Table 5: Mean Concentrations of DMP, DEP, DBP and DEHP in Carbonated Soft Drinks packed in PET bottles (mean \pm sd)

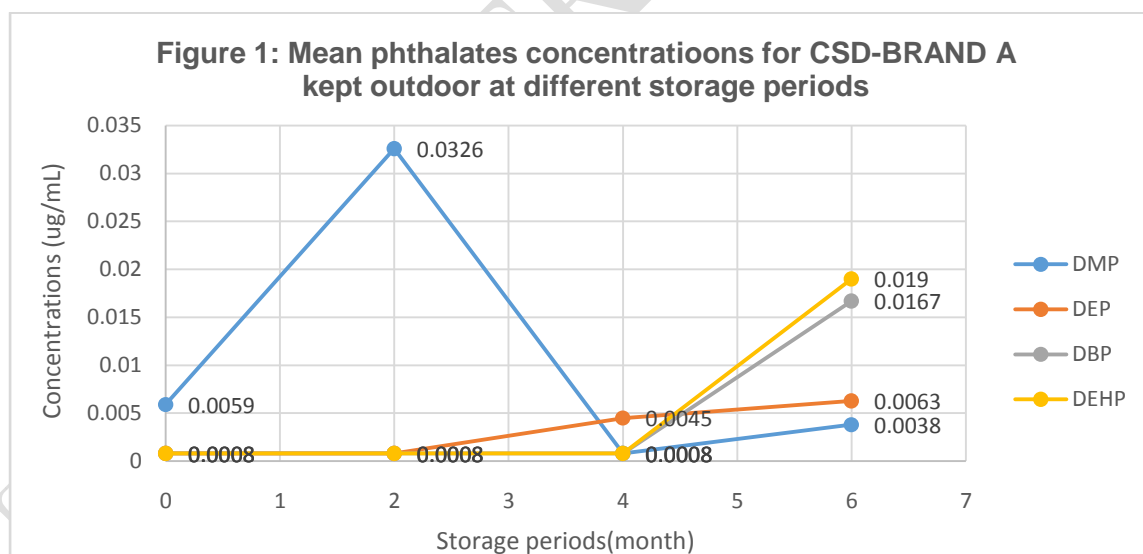
PET bottle	Storage condition	Storage time(Months)	DMP($\mu\text{g/mL}$)	DEP($\mu\text{g/mL}$)	DBP($\mu\text{g/mL}$)	DEHP($\mu\text{g/mL}$)
BRAND A	Outdoor	0	0.0059 \pm 0.0000	0.0008 \pm 0.0000	0.0008 \pm 0.0000	0.0008 \pm 0.0000
		2	0.0326 \pm 0.0016	0.0008 \pm 0.0000	0.0008 \pm 0.0000	0.0008 \pm 0.0000
		4	0.0008 \pm 0.0000	0.0045 \pm 0.0000,	0.0008 \pm 0.0000	0.0008 \pm 0.0000
		6	0.0038 \pm 0.0004	0.0063 \pm 0.0015	0.0167 \pm 0.0024	0.0190 \pm 0.0010
	Indoor/room	0	0.0008 \pm 0.0000	0.0008 \pm 0.0000	0.0008 \pm 0.0000	0.0008 \pm 0.0000
		2	0.0146 \pm 0.0017	0.0410 \pm 0.0000	0.0008 \pm 0.0000	0.0008 \pm 0.0000
		4	0.0121 \pm 0.0000	0.0153 \pm 0.0035	0.0011 \pm 0.0000	0.0008 \pm 0.0000
		6	0.0101 \pm 0.0004	0.0136 \pm 0.0000	0.0008 \pm 0.0000	0.0008 \pm 0.0000
BRAND B	Outdoor	0	0.0008 \pm 0.0000	0.0040 \pm 0.0002	0.0008 \pm 0.0000	0.0138 \pm 0.0005
		2	0.0008 \pm 0.0000	0.0060 \pm 0.0008	0.0008 \pm 0.0000	0.0008 \pm 0.0000
		4	0.0008 \pm 0.0000	0.0070 \pm 0.0004	0.0008 \pm 0.0000	0.0008 \pm 0.0000
		6	0.0008 \pm 0.0000	0.0062 \pm 0.0003	0.0043 \pm 0.0000	0.0108 \pm 0.0000
	Indoor/room	0	0.0008 \pm 0.0000	0.0043 \pm 0.0002	0.0036 \pm 0.0000	0.0839 \pm 0.0016
		2	0.0008 \pm 0.0000	0.0121 \pm 0.0003	0.0089 \pm 0.0010	0.1164 \pm 0.0051
		4	0.0008 \pm 0.0000	0.0062 \pm 0.0004	0.0442 \pm 0.0005	0.0615 \pm 0.0050
		6	0.0008 \pm 0.0000	0.0068 \pm 0.0002	0.0047 \pm 0.0000	0.0934 \pm 0.0024

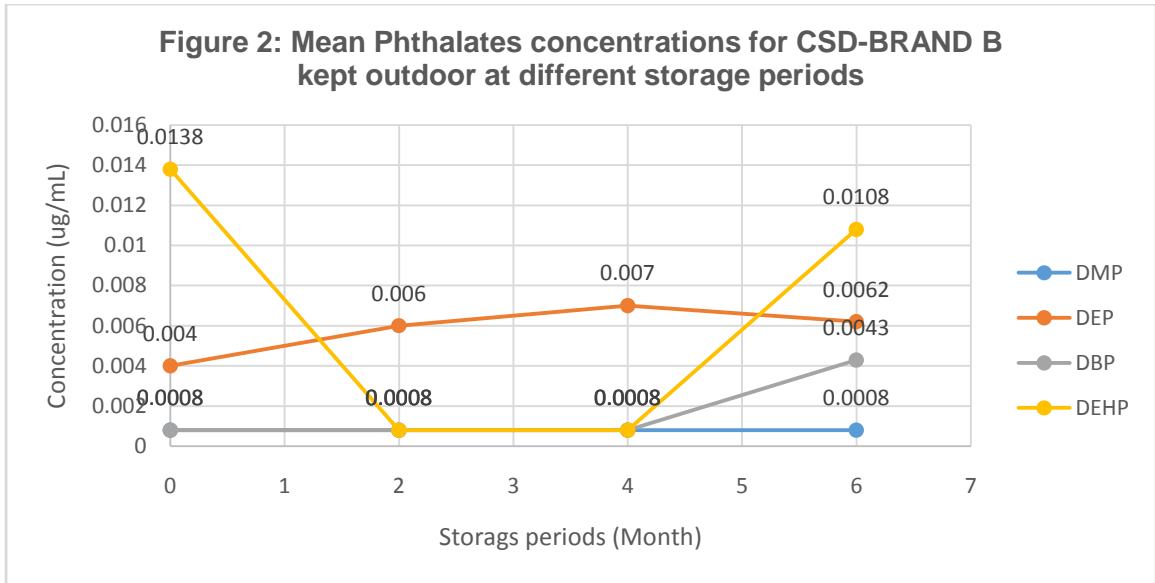
a) The mean phthalate concentrations of CSD bottles kept outdoors for various storage time

The analysis of the samples revealed a trend of change in the mean concentrations of DBP and DEHP, indicating that the migration rate increased with longer storage times, particularly after 4 and 6 months of storage outdoors. For Brand A, this increased from 0.0008 $\mu\text{g/mL}$ to 0.0167 $\mu\text{g/mL}$ for DBP and 0.0008 $\mu\text{g/mL}$ to 0.019 $\mu\text{g/mL}$ for DEHP, or 20.88 and 23.75 times, respectively for CSD bottles kept outdoors (Figure 1). Brand B saw a 5.375-fold increase in DBP from 0.0008 $\mu\text{g/mL}$ to 0.0043 $\mu\text{g/mL}$, and a 0.78-fold decrease in DEHP from 0.0138 $\mu\text{g/mL}$ before storage to 0.0108 $\mu\text{g/mL}$ after 4 and 6 months (Figure 2). This indicates that the phthalate compounds primarily DBP and DEHP migrated within the PET bottles that brand A was using and began to act as the sources of contamination, while Brand B showed the variable trend in the concentrations of DEHP over various storage times, pointing to the possibility for inadequate temperature control of the PET bottle moulds to attain the optimal wall thickness

distribution and bottle perpendicularity during bottle production, which could lead to uneven phthalate distribution in batches used to bottle the same brand. Furthermore, the sample analyzed during one storage time was not the same sample analyzed during the next stage, allowing for the concentration to be influenced by the manufacturing and bottling process, on top of the migration. In addition, this study discovered that phthalates degrade more quickly outside than they do indoors, particularly when exposed to direct sunlight for extended periods. The types of phthalate compounds observed to migrate in this investigation were also reported in related studies carried out in Turkey²⁰ and China²⁷.

Samples left outside for two months showed a notable increase in the DMP mean concentration for Brand A, from 0.0059 $\mu\text{g}/\text{mL}$ to 0.0326 $\mu\text{g}/\text{mL}$ (Figure 1), and after four and six months of storage, the concentration decreased to the same level as the control sample, indicating that the issue was most likely cross-contamination during production rather than migration, similar to the observations for the indoors storage above. The average DEP concentration for both brands does not rise significantly; the maximum concentration was measured at 0.007 $\mu\text{g}/\text{mL}$, which is 7.5 times greater than the control samples after 6 months of storage but far less than the mean concentrations of DBP and DEHP, suggesting very little migration in comparison to DBP and DEHP.

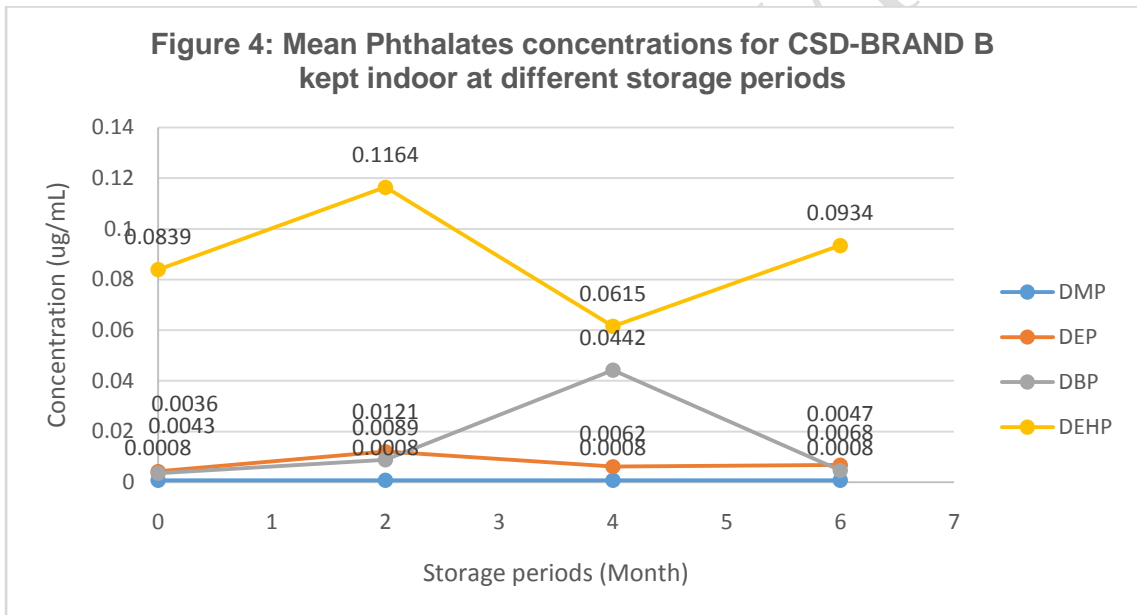
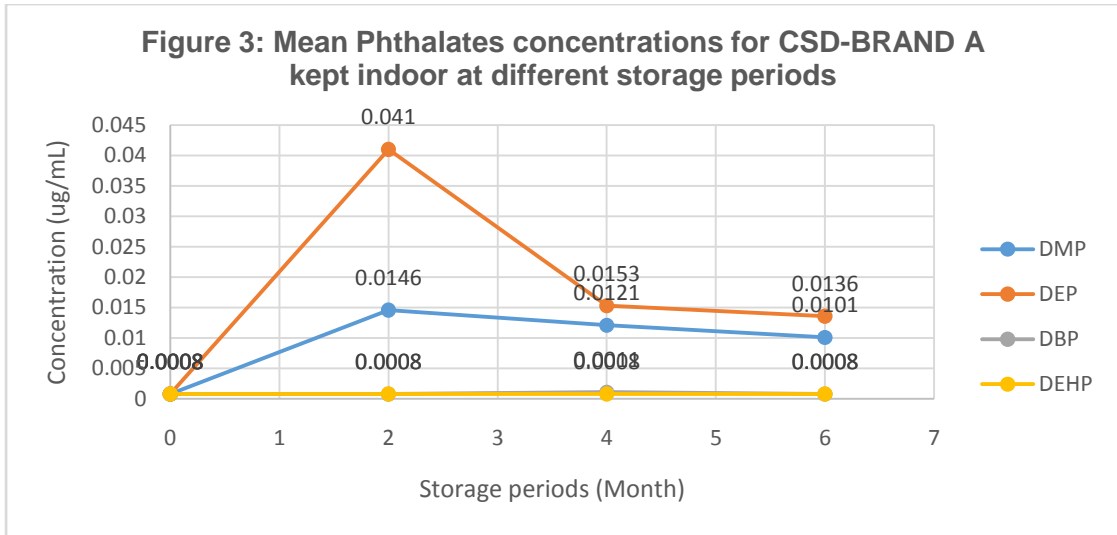




b) The mean phthalate concentrations of CSD bottles kept indoors for various storage time

The sample analysis results indicated that after six months of keeping CSD bottles indoors, there was a significant change in the mean concentrations of DMP and DEP, with increases of 12.6 times and 17 times, respectively, for brand A while for brand B, the mean concentrations of DEHP increased by 1.11 times. The mean DEP concentration for Brand A dropped from 0.0410 $\mu\text{g}/\text{mL}$ to 0.0153 $\mu\text{g}/\text{mL}$ in the second month of storage. This decline is probably the result of the bottle moulding. However, there was no significant change in the mean concentrations of DBP, and DEHP for brand A and DMP, DEP, and DBP for brand B after six months of indoor storage. This indicates that there was a migration related to both DMP and DEP for Brand A and DEHP for Brand B. This data also shows that the manufacturing companies using these PET bottles to package CSD are migrating in terms of DMP and DEP for Brand A and DEHP for Brand B, highlighting the variety of sources for PET bottles.

In addition, following two months of indoor storage, brand B had a mean concentration of 0.116 $\mu\text{g}/\text{mL}$ for DEHP (Figure 4), brand A had a mean concentration of 0.041 $\mu\text{g}/\text{mL}$ for DEP (Figure 3), and brand B had a mean concentration of 0.0442 $\mu\text{g}/\text{mL}$ for DBP (Figure 4) suggesting that contamination may have occurred during production or filling.



The study's findings suggest that keeping carbonated soft drinks outdoors as compared to indoors for extended periods could accelerate the migration of DBP and DEHP, the latter of which exhibits a 24-fold increase in migration. In this study, the temperature outdoors acts as a catalyst for the hydrolysis of phthalates, allowing them to migrate into the carbonated soft drinks that are in bottles.

c) The influence of varying storage times on PAE concentrations

The Friedman test was used to statistically validate the concentrations observed at different storage time points of the same storage condition to concentrations observed before storage time (control sample), without accounting for storage conditions. The results showed p-values of 0.000 (Table 6), indicating that there were significant differences in concentrations at different storage time points of the same storage condition when compared to concentrations observed before storage and after storage.

These results show that longer storage times under the same conditions as this study could lead to a higher migration rate from PET bottles into content, with the mean ranked as DEHP > DBP. Furthermore, these results concur with a prior study by Rastkari *et al.*, 2017 on the effect of temperature, time, and type of packaging on the release of phthalate esters into packed acidic liquids, which showed an increasing trend in phthalate concentrations with longer storage times.²⁸

Table 6: Friedman Test for targeted DBP and DEHP in CSD

	Mean Rank	
Storage Time		2.50
DBP (µg/mL)		1.59
DEHP (µg/mL)		1.91
	Test Statistics ^a	
N		32
Chi-Square		15.782
Df		2
Asymp. Sig.		0.000

a. Friedman Test

d) The influence of storage conditions on the concentrations of PAE

Without taking into account storage time, the concentration changes between various storage conditions (outdoor and indoor temperature) were compared using the Mann-Whitney test, with p-values greater than 0.05 being considered as not significant. The findings revealed that, for DMP, DBP, and DEHP, respectively, the p-values were 0.564, 0.254, and 0.077 (Table 7), suggesting that there were no significant changes in concentrations at different storage condition settings, but the p-value for DEP was 0.043, suggesting that there were significant changes in concentrations at different storage condition settings.

Even though the data for DMP, DBP, and DEHP do not show a statistically significant difference between the two conditions, different storage conditions, such as different temperature setups, a storage room, outdoor storage, and the length of the period for which it was stored, can all increase the phthalate migration rate in PET Carbonated Soft Drinks bottles to varying degrees.^{28,29,30}

Table 7: Mann-Whitney U Test for PET Bottled Carbonated Soft Drinks

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of DMP(µg/mL) is the same across categories of Storage conditions.	Independent-Samples Mann-Whitney U Test	.564 ^a	Retain the null hypothesis.
2	The distribution of DEP(µg/mL) is the same across categories of Storage conditions.	Independent-Samples Mann-Whitney U Test	.043 ^a	Reject the null hypothesis.

3	The distribution of DBP($\mu\text{g/mL}$) is the same across categories of Storage conditions.	Independent-Samples Mann-Whitney U Test	.254 ^a	Retain the null hypothesis.
4	The distribution of DEHP($\mu\text{g/mL}$) is the same across categories of Storage conditions.	Independent-Samples Mann-Whitney U Test	.102 ^a	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .050.

a. Exact significance is displayed for this test.

e) The effect of storage conditions and different storage times on the quantity of phthalate that migrates from packaging materials into carbonated soft drinks

The statistically significant bivariate association of storage conditions and storage time on the leaching effect was assessed using Spearman's rho test due to the non-parametric nature of the data. A p-value of less than 0.05 and a 0.001 were deemed significant, and larger Spearman's rho coefficients indicate a greater degree of association between storage conditions and storage time. The outcomes revealed:

- i. p-values of 0.472 and 0.711, coupled with Correlation Coefficient rho values of 0.132 and 0.068 respectively (Table 8), indicate that there is no statistically significant bivariate correlation between the storage conditions and storage time on the leaching of DMP on bottled carbonated soft drink. Thus, regardless of whether the PET bottles of carbonated soft drinks are stored indoors or outdoors under different storage times for both brands, the Concentration of DMP either slightly increases or does not increase over time.
- ii. p-values of 0.040 and 0.001, coupled with Correlation Coefficient rho values of 0.365* and 0.572** respectively (Table 8), indicate that there is a statistically significant bivariate correlation between the storage conditions and storage time on the leaching of DEP on bottled carbonated soft drink with Positive correlations of 36.5% and 57.2% respectively. For that reason, based on whether the PET carbonated soft drink bottles are stored indoors or outdoors and for a particular period, the concentration of DEP increases over time with positive relationships for both brands.
- iii. p-values of 0.165 and 0.074, coupled with Correlation Coefficient rho values of 0.251 and 0.320 respectively (Table 8), indicate that there is no statistically significant bivariate correlation between the storage conditions and storage time on the leaching of DBP on bottled carbonated soft drink. For both brands, the concentration of DBP thus either increases gradually or does not increase over time, regardless of whether the PET bottles of carbonated soft drinks are stored indoors or outdoors and for different periods of time.
- iv. p-values of 0.061 and 0.697, coupled with Correlation Coefficient rho values of 0.335 and 0.071 respectively (Table 8), indicate that there is no statistically significant bivariate correlation between the storage conditions and storage time on the leaching of DEHP on bottled carbonated soft drink. Hence,

regardless of whether the PET bottles of carbonated soft drinks are stored indoors or outdoors and for varying lengths of time, the concentration of DEHP for both brands either gradually increases or does not increase over time.

This shows that the leaching of DMP, DBP, and DEHP is not statistically significantly correlated with storage conditions or time, while DEP shows a correlation between storage duration and an increase in the concentration of Diethyl phthalate (DEP) in PET bottled carbonated soft drinks. Even though there isn't a statistically significant bivariate correlation, it's relentless to completely rule out the possibility of influencing factors, such as poor storage conditions such as being kept at a cold or high temperature, being exposed to UV light for an extended amount of time, being kept in a storage room or outside, and how long it was kept.^{14,28} Thus, leaching from the polymeric materials under various storage conditions and times was the primary cause of the increase in phthalate concentrations.

Table 8: Spearman's rho test for Plastic Bottled Carbonated Soft Drinks

		Correlations						
Spearman's rho		Storage conditions	Storage Time	DMP($\mu\text{g}/\text{mL}$)	DEP($\mu\text{g}/\text{mL}$)	DBP($\mu\text{g}/\text{mL}$)	DEHP($\mu\text{g}/\text{mL}$)	
	Storage conditions	1.000	0.000	0.132	.365*	0.251	0.335	
	Correlation Coefficient							
	Sig. (2-tailed)		1.000	0.472	0.040	0.165	0.061	
	N	32	32	32	32	32	32	
	Storage Time	0.000	1.000	0.068	.572**	0.320	0.071	
	Correlation Coefficient							
	Sig. (2-tailed)	1.000		0.711	0.001	0.074	0.697	
	N	32	32	32	32	32	32	
	DMP($\mu\text{g}/\text{mL}$)	0.132	0.068	1.000	0.088	-0.222	-.441*	
	Correlation Coefficient							
	Sig. (2-tailed)	0.472	0.711		0.632	0.222	0.012	
	N	32	32	32	32	32	32	
	DEP($\mu\text{g}/\text{mL}$)	.365*	.572**	0.088	1.000	0.269	0.174	
	Correlation Coefficient							
	Sig. (2-tailed)	0.040	0.001	0.632		0.137	0.341	
	N	32	32	32	32	32	32	
	DBP($\mu\text{g}/\text{mL}$)	0.251	0.320	-0.222	0.269	1.000	.691**	
	Correlation Coefficient							
	Sig. (2-tailed)	0.165	0.074	0.222	0.137		0.000	
	N	32	32	32	32	32	32	

DEHP($\mu\text{g}/\text{mL}$)	Correlation Coefficient	0.335	0.071	-0.441 [*]	0.174	.691 ^{**}	1.000
	Sig. (2-tailed)	0.061	0.697	0.012	0.341	0.000	
	N	32	32	32	32	32	32

*. Correlation is significant at the 0.05 level (2-tailed).

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

f) Comparison of Brand A and Brand B's PAE concentration levels

In this study, two brands, denoted as brands A and B, were assessed. The results showed that there were no appreciable differences in the amounts of PAE concentration between the brands, as Tables 9 and 10 demonstrate. The DEHP in Brand B had the highest mean (0.12000 $\mu\text{g}/\text{mL}$) and standard deviation (0.045 $\mu\text{g}/\text{mL}$), suggesting that there was a significant migration of DEHP in Brand B relative to Brand A.

Table 9: Descriptive Statistics of Brand A

PAE	N	Minimum	Maximum	Mean	Std. Deviation
DMP($\mu\text{g}/\text{mL}$)	16	0.000750	0.033715	0.009559	0.010457
DEP($\mu\text{g}/\text{mL}$)	16	0.000750	0.041023	0.007616	0.010677
DBP($\mu\text{g}/\text{mL}$)	16	0.000750	0.018454	0.002773	0.005490
DEHP($\mu\text{g}/\text{mL}$)	16	0.000750	0.019748	0.003032	0.006242
Valid N (listwise)	16				

Table 10: Descriptive Statistics of Brand B

PAE	N	Minimum	Maximum	Mean	Std. Deviation
DMP($\mu\text{g}/\text{mL}$)	16	0.000750	0.000750	0.000750	0.000000
DEP($\mu\text{g}/\text{mL}$)	16	0.003899	0.012342	0.006570	0.002413
DBP($\mu\text{g}/\text{mL}$)	16	0.000750	0.044615	0.007849	0.014481
DEHP($\mu\text{g}/\text{mL}$)	16	0.000750	0.120007	0.047034	0.045716
Valid N (listwise)	16				

g) Comparing the results with other research on PAE concentrations

There is currently no law pertaining to phthalates in carbonated soft drinks in Tanzania or any other agency; however, the US EPA and the World Health Organization (WHO) have recommended maximum allowable concentrations of DEHP only in drinking water of 6 $\mu\text{g}/\text{L}$ and 8 $\mu\text{g}/\text{L}$, respectively. In contrast, the European Union (EU) published Regulation (EU) 2020/1245 to amend and revise Regulation (EU) 10/2011 on plastic materials and articles intended to come into contact with food (the "Food Contact Plastics Regulation"). This regulation states that DEHP can only be present in drinking water at a maximum of 1.5 mg/kg, DBP can only be present in a maximum of 0.3 mg/kg, and BBP can only be present in a

maximum of 30 mg/kg.³¹The study's results are found to be lower than the recommended maximum allowable concentrations when these recommended concentrations are compared to the findings(Table 11).

Furthermore, it is crucial to contrast these results (Table 11) with those of other researchers (Table 12).The results show that the findings are lower than those of the other studies that were contrasted(which did not take into account the brands, flavors, or storage conditions).Though the results of CSD appear to be lower than those of other studies and authority agencies that were compared, it's crucial to keep in mind that these substances have the potential to interfere with hormones.

When comparing this study to one conducted at the same time in 2024 by Evarist *et al.*,³²the mean DMP concentration for 1.5 liters of PET bottled water after six months of outdoor storage was 0.220 µg/mL, while the mean concentration for 0.5 liters of PET bottled CSD was 0.0008 µg/mL. This suggests that the migration of phthalates into the CSD was presumably limited by carbonic acid, which reacts more violently at higher temperatures and increases pressure inside CSD bottles, on top of the carbonation pressure. This observation indicates that there is a far greater phthalates migration risk in PET bottled water, even though the capacity of the bottled water was three times that of the CSD in PET bottles. This presented a significant risk for PET water bottles as compared to PET-bottled CSD.

Table 11: The study's descriptive statistics for Brands A and B

	N	Minimum	Maximum	Mean	Std. Deviation
DMP(µg/mL)	32	0.000750	0.033715	0.005155	0.008540
DEP(µg/mL)	32	0.000750	0.041023	0.007093	0.007633
DBP(µg/mL)	32	0.000750	0.044615	0.005311	0.011077
DEHP(µg/mL)	32	0.000750	0.120007	0.025033	0.039112
Valid N (listwise)	32				

Table 12: The results of other studies

PAE	N	Minimum	Maximum
Shah, et al., 2023³⁰			
DBP(µg/mL)	18	N.D	97.000
DEP(µg/mL)	18	N.D	130.000
Wu et al., 2014³³			
DEP(µg/mL)	12	<LOQ	0.021
DBP(µg/mL)	12	<LOQ	0.065
DEHP(µg/mL)	12	0.015	0.098
Ustun et al., 2015²⁰			
DMP(µg/mL)	10	0.036	0.237
DEP(µg/mL)	10	0.032	0.270
DBP(µg/mL)	10	0.044	1.500

DEHP($\mu\text{g/mL}$)

10

0.387

2.312

Keys:

N.D- Not Detect

<LOQ-Below Limit of quantification

N- Number of samples

4.CONCLUSION

The study's findings indicate that PET packaging is the primary source of phthalates in bottled carbonated soft drinks. These two brands' raw material bottle formulations, manufacturing processes, storage conditions and time, have been found to have an impact on the concentrations of these phthalates compounds in PET bottled carbonated soft drinks. Keeping CSDs outdoors is likely to accelerate the chemical reaction between the carbonic acid in the product and the plastic bottle because the carbonic acid reacts more violently with the packaging material at higher temperatures. Phthalates migrate as a result of this reaction, raising concerns about the safety and quality of carbonated soft drinks.

Ethical Approval

Through letter kumb. Na. AB/307323/01, the Tanzania President's Office-Regional Administration and Local Government (PO-RALG) granted ethical approval for the study.

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