

## Research Article

# Assessment of Bacterial Load in Polyethylene Terephthalate (PET) Bottled Water Marketed in Kathmandu Valley, Nepal

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In recent years, we are having mixed feelings regarding the use of polyethylene terephthalate (PET) bottles for storing water. The aim of this study is to determine any associations between bacterial load and the physical condition of the water bottle. For this study, bottled water was purchased, and parameters like pH, electrical conductivity (EC), total dissolved solids (TDS), heterotrophic plate count (HPC), total coliform count, and *Pseudomonas* spp. count were determined as per the American Public Health Association, 2005. The pH value of water samples tested ranged from 5.2 to 6.8. The majority of samples (96%) were found to contain pH values that were unacceptable as per the Department of Food Technology and Quality Control (DFTQC) guideline. Value of electrical conductivity (EC) ranged from 5 to 199  $\mu\text{S}/\text{cm}$ . HPC revealed that, out of 100 samples, 48 (48%) samples were found to be acceptable as per the DFTQC guideline value (<25 cfu/mL). Among 100 samples, *Pseudomonas* spp. was found to be present in 23% of bottled water. Acidic pH and elevated concentrations of TDS and EC may lead to the survival of extremophiles present in HPC which may lead to degradation of PET. Extremophile bacteria that survive in bottled water for a long time rely on several survival mechanisms including evolutionary development (evo-devo) and solely survive on complex polymers like PET.

## 1. Introduction

The harmful effects of the commercially available and popular commodity plastic of the polyethylene terephthalate (PET) have been known since the 1980s but PET is still being widely used in every corner of the world due to their low cost, ease of availability, processability and recyclability [1]. The PET is a long-chain polymer belonging to the polyester family [2]. The intermediates of PET are terephthalic acid and ethylene glycol which are both acquired using petroleum products/oil feedstock [2]. Pure PET is a shapeless, glass-like material that crystallizes when certain modifying agents are added or when the heat is applied above 72°C [2]. Residual bisphenol A (BPA) is the building block of polycarbonate plastic [3].

Bisphenol A has been found to be leaching from polycarbonate plastic bottles in presence of bacteria, algae etc. when

stored for a long period of time [3] and could lead to effects on the brain, behavior, mammary glands, metabolism of the body, and obesity [4]. A study conducted in Japan detected BPA in bottled waters using high performance liquid chromatography at a concentration of 3–10 ng/l [5]. A typical PET bottle is a major threat to the environment due to the high amount of chemicals used during petroleum production, as well as incorrect usage and disposal [1]. Bottled water also results in a large amount of waste as the bottles of 1 L capacity are used once and then disposed which sooner or later ends up generally in landfill sites. As these polyesters are generally not biodegradable, only ~14% are collected for recycling [6] while the rest are accumulated in the ecosystem as environmental hazards for a long time (possibly up to a millennium) [6–8].

The blooming industry results in profit for the entrepreneurs focusing only on the quantity of the sale rather than the

quality of the bottled water [9–14]. The concept of PET being harmful to the human body is not a recent topic, but its detection in the human body or laboratory animal is still in its infantile stage. The reason may be due to epigenetics [15], metagenomics [15–18], metabolic rate [13, 16], mutations, excretion through unknown routes [4, 5] etc. This is due to lack of ineffective surveillance from governmental and license providing authoritative bodies [9–12, 14, 19–25]. Although in theory, every bottled water industry is aware of food safety and its application in industries but as per the studies across the globe, the practical aspect is almost nonexistent, as its practical implications may be difficult [13].

PET's biological degradation is formidable as it is composed of two simple monomers articulated with ester bonds [6, 8]. As per recent studies [3, 6–8, 26], extremophiles like *Thermobifida fusca*, *Ideonella sakaiensis*, *Fusarium oxysporum*, *F. solani*, and *Pseudomonas* spp. are able to degrade PET by producing enzymes PETase and mono(2-hydroxyethyl) terephthalic acid [MHETase] [6–8, 26]. By producing enzymes PETase [8], *Ideonella sakaiensis* can metabolize a small piece of PET (1.9% crystallinity [6]) which takes 6 weeks as its sole source of carbon. The potential of the microorganisms for degradation of polyesters in general and their full utilization in industries have not been yet utilized. With the aid of genetic engineering [13, 15–18], CRISPR [15] and terephthalic acid can be isolated from PET which would save raw materials (i.e., terephthalic acid and ethylene glycol) for PET and save huge sum of capital [6, 8, 26]. The vertical and horizontal mutation in HPCs may lead to it being resistant to harsh conditions and survival [13, 15–18, 27, 28]. Few studies [29, 30] have also highlighted the decomposition of PET into formaldehyde and acetaldehyde, in the presence of HPCs in the micronutrients in the bottled water. Vertical or horizontal mutations of the PETase can be used to target the natural aliphatic polymer to enhanced selectivity for PET degradation [3, 6, 7, 13, 15–18, 26–28].

Migration of aldehyde compounds from caps to water might not be of high concentration, but upon constant deposition and bioaccumulation in the human body, it may lead to depression, cardiorespiratory failure, and eventually death; after continuous bioaccumulation in the central nervous system [31]. Migration of carbonyl compounds from the polypropylene caps to the bottled water has been reported where concentrations of formaldehyde, acetaldehyde, and acetone changed over the time of extraction and on temperature factors [32]. Acetaldehyde can cause damage at the cellular and genomic levels which is associated with neurological pathological stroke, Wernicke encephalopathy, Alzheimer disease, and alcohol-induced impairment of the brain structure and function, localized cancers, esophageal tumors, brain damage in prenatal infants, alcoholic cardiomyopathy, cancer of the digestive tract, and growth suppression (in chicken embryos) [33, 34]. Formaldehyde—an essential metabolic intermediate in mammalian cells—is produced during the metabolism of amino acids (serine, glycine, methionine, and choline) [35]. Due to the consumption through oral and nasal breathing, formaldehyde depositions and absorption occur in the upper respiratory tract (nasal

passages, oral cavity, trachea, and bronchus), allergies, irritation (eye, nose, and throat), nasopharyngeal cancer, and gastrointestinal tract ulcer; in extremely rare cases, formaldehyde has formed DNA-protein crosslinks, once it reaches the nuclear DNA, and thus due to incomplete repair of DPX chromosome mutations and micronuclei that have been observed in those proliferating cells [35].

As such, human health has been known to be affected across the globe [9–12, 19–25, 30, 31, 36–42]. Many studies [10–12, 19, 23, 24, 38, 40–42] have been carried out in the past concentrating on the microbial flora of bottled water but none of the research has concentrated on the disadvantages from the scratched and/or dented bottled water leading to release of harmful chemical like BPA, acetaldehyde, formaldehyde, and their bioaccumulation in the human body. These studies have all simply concluded that the source of water was contaminated without even once concentrating on the confounding factors of the bottle.

Therefore, this study is aimed at assessing the physiochemical and microbial parameters of the bottled water, along with the physical condition of the bottle. The paper also sheds some light on how extremophiles survive on factors like pH, EC, and TDS. Furthermore, the study also reveals the statistical calculation between HPCs, coliform, and *Pseudomonas* spp.

## 2. Methodology

**2.1. Sample Collection and Investigation.** The study was performed in the Department of Microbiology, St. Xavier's College, Maitighar.

A total of 100 samples (all of the different brands) were selected for the study.

Using the aseptic technique [13, 14], the sample was drawn in four sterilized polypropylene bottles, and headspace was left and was labeled. The collected samples were kept in a mini cooler filled with ice packs and was transported to the microbiology laboratory to be processed.

**2.2. pH.** pH meter (HANNA HI98107) was used to determine the pH of water samples. Buffer solutions of pH 4.0, 7.0, and 9.0 were prepared from tablets of the buffer which were used to calibrate the pH meter [43].

**2.3. Electric Conductivity.** A conductivity meter (HANNA HI 8033) was used to determine the conductivity of water samples in the laboratory. It was calibrated by using conductivity calibration standard (HI 7030) [43].

**2.4. Total Dissolved Solid.** 100 mL of water was filtered through a membrane filter, and then it was subjected to heat in a porcelain basin of known weight at 110°C in a hot air oven, and the basin was transferred to white silica containing desiccator. The change in weight was determined by the weight difference method [43].

**2.5. Heterotrophic Plate Count (HPC).** Using a sterile micropipette, 1 mL of water samples was aseptically dispensed into sterile Petri plates. Then, molted heterotrophic plate count agar ( $\approx 45^\circ\text{C}$ ) was poured and moved in the clockwise and

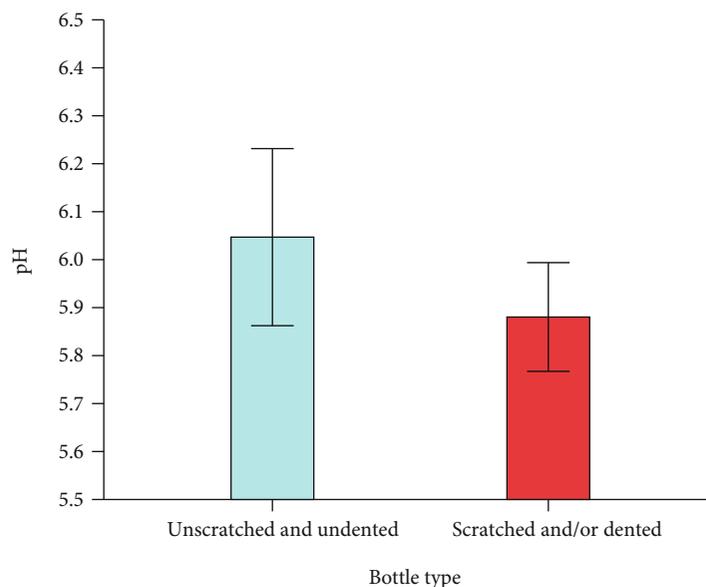


FIGURE 1: Distribution of the pH value with respect to the physical condition of the bottled water vessel.

anticlockwise direction “8.” The plates were incubated for 24 hours at 37°C. The colony count was done after 24 hours. The result was expressed as cfu/mL of water [43].

$$\text{cfu/mL} = \frac{C \times D}{v}, \quad (1)$$

where  $C$  is the total colonies counted,  $D$  is the dilution fold, and  $v$  is the volume of the sample taken.

**2.6. Total Coliform.** Volume 100 mL of water was filtered through a membrane filter apparatus. The membrane filter was inoculated in M-endo agar plates and was incubated for 24 hours at 37°C [27, 28]. The same process was repeated, and the membrane filter was transferred into M-endo agar and was incubated at 44.5°C for 24 hours [17]. The result was expressed as the number of cfu/100 mL of water [43]. The isolates were subjected to biochemical tests. The expression unit is

$$\text{cfu/100 mL} = \frac{C}{100}, \quad (2)$$

where  $C$  is total colonies counted.

**2.7. Pseudomonas Spp.** Measured volume of 100 mL water was filtered through a membrane filter. Membrane filter was inoculated in cetrimide agar for 24 hours at 37°C. The same process was repeated, and the membrane filter was transferred in cetrimide agar and was incubated at 42°C for 24 hours [17]. The result was expressed as the number of cfu/100 mL of water [43]. The isolates were subjected to biochemical tests. The expression unit is

$$\text{cfu/100 mL} = \frac{C}{100}, \quad (3)$$

where  $C$  is the total colonies counted.

**2.8. Quality Control and Data Analysis.** Strict quality control was maintained to obtain reliable physicochemical and microbiological results. Instruments were calibrated before use. The quality of each agar plate was checked by performing the purity test prior to use. Strict aseptic conditions were maintained while executing the methodology. Data analysis was done using Statistical Package for the Social Sciences (SPSS) version 19. The chi square test was used to determine the association between the variables and Cramer’s  $V$  test to determine effect size between the variables.

### 3. Results

A total of 100 samples of bottled water of volume 20 L were collected according to their local availability and popularity in the Kathmandu valley. The samples were examined for physicochemical and microbiological parameters to assess the quality of bottled water.

**3.1. pH.** The pH of the bottled water ranged from 5.2 to 6.8 ( $M = 5.89$ ,  $SD = 0.41$ ). The pH range was found to be 6.0–6.2 ( $M = 6.13$ ,  $SD = 0.12$ ) and 5.2–6.8 ( $M = 5.90$ ,  $SD = 0.41$ ) for unscratched and undented and scratched and/or dented, respectively. The unacceptance criteria as per the DFTQC guideline were 49% from that of unscratched and undented bottled water and 47% from that of scratched and/or dented, respectively. These results are presented in Figure 1 and Table 1.

Levene’s test for equity of variances indicated that the pH and bottle type (unscratched and undented and scratched and/or dented) demonstrated a statistically significant distributed ( $p = 0.089$  at 95% confidence interval).

**3.2. Electrical Conductivity (EC).** Electrical conductivity of the bottled water ranged from 5  $\mu\text{S/cm}$  to 199  $\mu\text{S/cm}$  ( $M = 61.10 \mu\text{S/cm}$ ,  $SD = 62.34$ ). The EC range was found to be 12  $\mu\text{S/cm}$ –78  $\mu\text{S/cm}$  ( $M = 42.33 \mu\text{S/cm}$ ,  $SD = 33.32$ ) unscratched and undented bottled water and 5  $\mu\text{S/cm}$ –

TABLE 1: Association between physical parameters (as per DFTQC guideline), bottle type.

Sn	Physical parameter	Unscratched and undented		Scratched and/or dented		Permissible value as per DFTQC guidelines	Overall ( $M \pm SD$ )	Overall $U$ (%)
		Mean $\pm$ SD	$U$ (%)	Mean $\pm$ SD	$U$ (%)			
1	pH	6.12 $\pm$ 0.12	49	5.90 $\pm$ 0.41	47 (47%)	6.5–8.0	5.89 $\pm$ 0.41	96
2	EC ( $\mu S/cm$ )	42.33 $\pm$ 33.2	—	62.3 $\pm$ 63.78	—	NA	61.10 $\pm$ 62.34	—
3	TDS (mg/L)	7.00 $\pm$ 4.00	0	4.72 $\pm$ 3.93	0 (0%)	250	4.86 $\pm$ 3.93	0

NA=value not available in guideline. SD: standard deviation; U: unacceptable as per DFTQC guidelines; NA: not available.

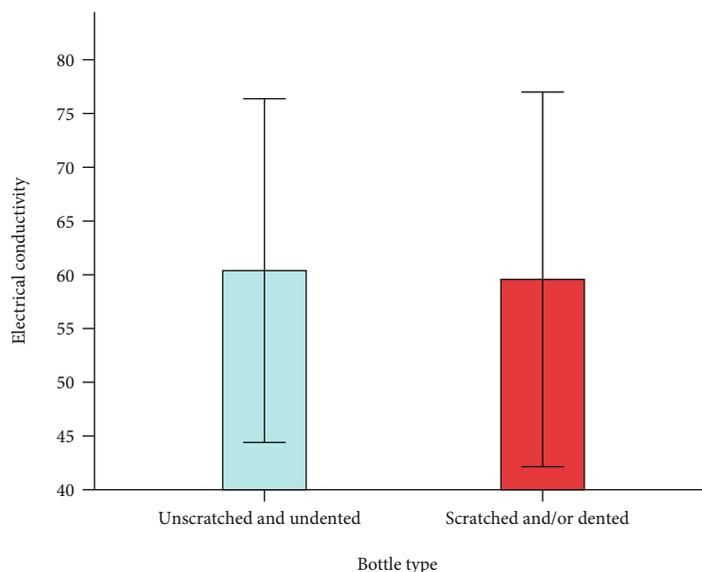


FIGURE 2: Distribution of the EC value with respect to the physical condition of the bottled water vessel.

TABLE 2: Correlation between physical parameters and microbiological parameters.

	Bottle type	pH	EC	TDS	HPC	Coliform count	<i>Pseudomonas</i> count
Bottle type	1	-0.156	-0.007	0.036	0.780 **	0.780 **	-0.441**
pH		1	0.002	0.127	-0.165	-0.165	0.280
EC			1	0.251 *	0.210	0.210	-0.072
TDS				1	0.153	0.153	-0.145
HPC					1	1 **	-0.525**
Coliform count						1	-0.525**
<i>Pseudomonas</i> count							1

\*Correlation is significant at the 0.05 level (2-tail). \*\*Correlation is significant at the 0.01 level (2-tail).

199  $\mu S/cm$  ( $M = 62.30 \mu S/cm$ ,  $SD = 63.78$ ) from that of scratched and/or dented, respectively. These results are presented in Figure 2 and Table 1.

Pearson's correlation implies that there is a significant positive association between EC and TDS (0.251), as seen in Table 2. Levene's test for equity of variances indicated that the EC and bottle type (unscratched and undented and scratched and/or dented) demonstrated a statistically significant distributed ( $p = 0.211$  at 95% confidence interval).

3.3. *Total Dissolved Solid (TDS)*. Total dissolved solid of the bottled water ranged from 1 mg/L to 15 mg/L ( $M = 4.86$  mg/L,  $SD = 3.93$ ). The TDS range was found to be 3 mg/L–11 mg/L ( $M = 7.00$  mg/L,  $SD = 4.00$ ) unscratched and undented bottled water and 1 mg/L–15 mg/L ( $M = 4.72$  mg/L,  $SD = 3.93$ ) from that of scratched and/or dented, respectively. All 100 samples had total dissolved solid value at an acceptable range as per the DFTQC guideline. These results are presented in Figure 3 and Table 1.

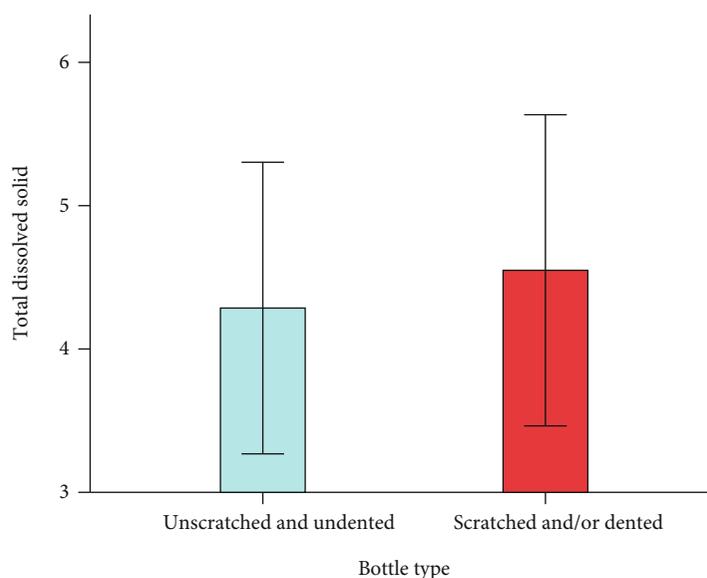


FIGURE 3: Distribution of the TDS value with respect to the physical condition of the bottled water vessel.

Levene's test for equity of variances indicated that the TDS and bottle type (unscratched and undented and scratched and/or dented) demonstrated a statistically significant distributed ( $p = 0.722$  at 95% confidence interval).

**3.4. Heterotrophic Plate Count.** Out of 100 samples, all samples were found to contain heterotrophic bacteria. Out of 100 samples, 48 (48%) samples were found to be within the DFTQC guideline value ( $<25$  cfu/mL) for HPC. The range of HPC was found to be 21 – 1300 cfu/mL. The acceptable HPC resulted in 43% from unscratched and undented bottled water and 5% from that of scratched and/or dented, respectively. The results are presented in Figure 4 and Table 3.

The chi square test shows that there is a significant ( $p < 0.001$ ) association between HPC and bottle type (unscratched and undented and scratched and/or dented). Cramer's  $V$  test revealed that the effect size between HPC and bottle type is strong/large ( $\varphi_C = 0.78$ ). Pearson's correlation implies that there is a significant positive strong association between HPC and coliform count [1], HPC and bottle type ( $r = 0.780$ ), bottle type and coliform count ( $r = 0.780$ ), while there is a significant negative association between HPC and *Pseudomonas* count ( $r = -0.441$ ) as seen in Table 2. Levene's test for equity of variances indicated that the HPC and bottle type (unscratched and undented and scratched and/or dented) demonstrated a statistically significant distributed ( $p = 0.458$  at 95% confidence interval).

**3.5. Total Coliform Count.** Out of 100 samples, 52 (52%) samples were found to be contaminated with coliforms. Only 48 (48%) samples had total coliform within the acceptable range as per the DFTQC guideline. The acceptable coliform count resulted in 43% from unscratched and undented bottled water and 5% from that of scratched and/or dented, respectively. These results are presented in Figure 5 and Table 3.

The chi square test shows that there is a significant association ( $p < 0.001$ ) between total coliform and bottle type,

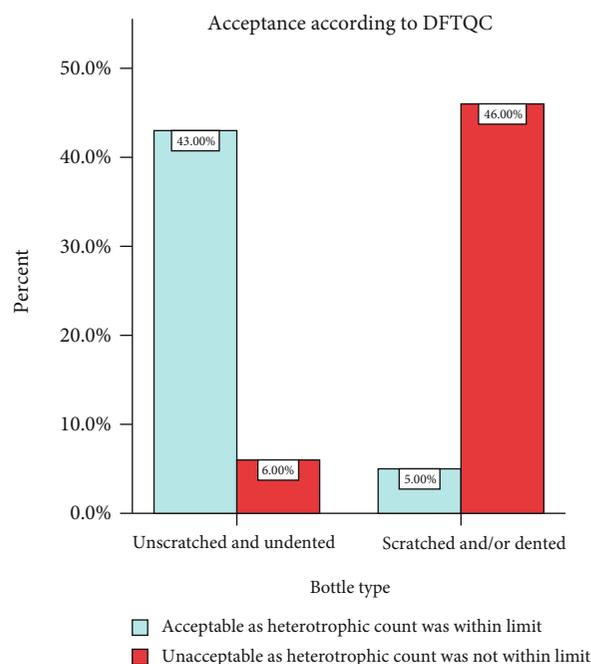


FIGURE 4: Distribution of HPC with respect to the physical condition of the bottled water vessel.

between total coliform and HPC. Cramer's  $V$  test revealed that the effect size between total coliform and bottle type, total coliform, and HPC is strong/large ( $\varphi_C = 0.78$ ) and strong/large ( $\varphi_C = 0.96$ ), respectively. Pearson's correlation implies that there is a significant positive strong association between coliform count and bottle type ( $r = 0.780$ ) and a significant negative association between Coliform count and *Pseudomonas* count ( $r = -0.525$ ), as seen in Table 2. Levene's test for equity of variances indicated that the pH and bottle type (unscratched and undented and scratched and/or dented) demonstrated a statistically significant distributed ( $p = 0.458$  at 95% confidence interval).

TABLE 3: Association between microbiological parameters (as per DFTQC guideline), the volume of bottled water.

Sn	Microbiological parameters	Unscratched and undented		Scratched and/or dented		Permissible value as per DFTQC guidelines
		D	U (%)	D	U	
1	HPC	49	6 (6%)	51	46 (46%)	<25 mg/L
2	Coliform count	49	6 (6%)	51	46(46%)	Nil
3	<i>Pseudomonas</i> spp.	49	2 (2%)	51	21 (21%)	NA

D=No. of water samples in which microorganisms were detected. U: unacceptable as per DFTQC guidelines; NA: not available.

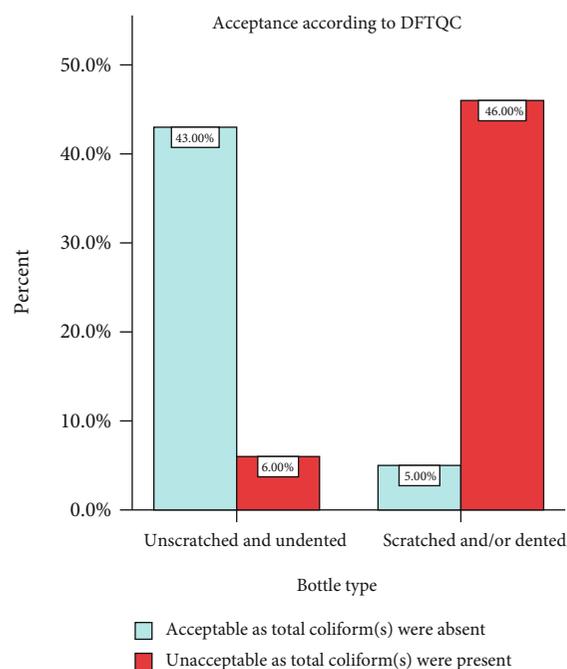


FIGURE 5: Distribution of total coliform with respect to the physical condition of the bottled water vessel.

**3.6. *Pseudomonas* Spp. Count.** Out of 100 samples, 23 samples were found to be contaminated with *Pseudomonas* spp. The acceptable HPC resulted in 47% from unscratched and undented bottled water and 30% from that of scratched and/or dented, respectively. These results are presented in Figure 6 and Table 3.

The chi square test shows that there is a significant association ( $p < 0.001$ ) between *Pseudomonas* spp. and bottle type, *Pseudomonas* spp. and HPC, and *Pseudomonas* spp. and total coliform. Cramer's  $V$  test revealed that the effect size between *Pseudomonas* spp. and bottle type, *Pseudomonas* spp. and HPC, and *Pseudomonas* spp. and total coliform is moderate/medium ( $\phi_C = 0.44$ ), moderate/medium ( $\phi_C = 0.50$ ), and moderate/medium ( $\phi_C = 0.53$ ), respectively. Pearson's correlation implies that there is a significant positive strong association between HPC and bottle type ( $r = 0.780$ ) and coliform count ( $r = 0.780$ ) while there is a significant negative association between bottle type and *Pseudomonas* count ( $r = -0.441$ ) as seen in Table 2. Levene's test for equity of variances indicated that the pH and bottle type (unscratched and undented and scratched and/or dented) demonstrated a statistically significant distributed ( $p = 0.207$  at 95% confidence interval).

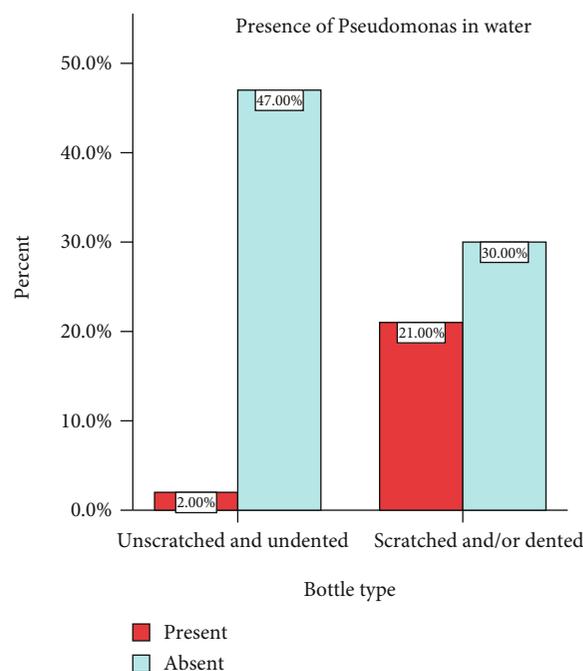


FIGURE 6: Distribution of *Pseudomonas* spp. count with respect to the physical condition of the bottled water vessel.

## 4. Discussion

The pH value of water samples tested ranged from 5.2 to 6.8. The majority of samples 96 out of 100 were found to exhibit pH values which were unacceptable as per the DFTQC guideline. EC levels were found in the range of 5–199  $\mu\text{S}/\text{cm}$ . All the samples were within the acceptable limit as per the DFTQC guideline. In this study, heterotrophic plate count for all the 100 bottled water samples showed 52 (52%) samples were found to be unacceptable according to the guideline of DFTQC. In total, 52% of samples were unacceptable according to the DFTQC guideline for total coliform. Among 100 samples, *Pseudomonas* spp. was found to be present in 23 bottled water.

Low pH values observed in most samples could be associated with treatment procedures and geological location of the water source [36]. pH values of drinking water below 6.5 are known to cause corrosion in pipelines [44] but are drinkable from bottled water. Some studies [45] have found a link between low pH and peptic ulcer [45], gastroesophageal reflux disease [46, 47], low absorption of vitamins [48], and cancer due to absorption of the high amount of

triclosan from toothpaste [27, 28, 49], as such acidic water are to be feared for consumption purposes. Acidic pH has been known to increase the flavor of bottled water which may ultimately lead to the survival of acid tolerant microorganisms [13, 16, 27, 28].

The EC value is in agreement with studies carried out on bottled water by Aris et al. [30], Danso-Boateng and Frimpong [36], Mihayo and Mkoma [50], and Sasikaran et al. [42] where electrical conductivity was found to be in between 2-192  $\mu\text{S}/\text{cm}$ , 5.93-93.2  $\mu\text{S}/\text{cm}$ , 17.4-280  $\mu\text{S}/\text{cm}$ , and 19-253  $\mu\text{S}/\text{cm}$ , respectively. Lower EC values indicate low ionic concentration, and higher EC values indicate higher ionic concentration.

TDS was found to be in the range of 1-15 mg/L. The result of this study concurs with the study of Aris et al. [30] whose mean TDS value was also 17.00 mg/L  $\pm$  41.21. TDS is used to assess mineral content and is dependent on the water source, local geology, treatment scheme, and solubility of minerals [30]. TDS consists of inorganic salts like calcium, potassium, magnesium, sodium, bicarbonates, sulfates, chloride, and also small amounts of organic matter that are dissolved in water [30]. Bottled water containing high TDS that may cause bioaccumulation and elevated mineral content in the urine of the minerals present in the water while bottled water with low TDS may cause severe complications like diuresis and increased elimination of calcium, magnesium, potassium, chloride, sodium ions etc. [30].

HPC result agrees with the result of Venkatesan et al. [51] and da Silva et al. [52] as their studies found 83.3% and 87% samples exceed the guideline value. In this study, the HPC count was in the range of 15-1300 cfu/mL which agreed with the result of Abed and Alwakeel [53], Bashir and Aish [54], and Lalumandier et al. [55] in which the range of HPC was found to be 0-4900 cfu/mL, 0-4800 cfu/mL, and 0-4900 cfu/mL, respectively. HPC is a good indicator of detecting microflora in bottled water/water jug [44]. Although it is a cheap and easy technique to perform, one drawback of HPC is that it detects all viable bacteria (i.e., both pathogenic and nonpathogenic bacteria) [40, 56, 57]. The source of contamination might occur during processing and handling or natural source of water [11, 25]. It has also been pointed out that the higher HPC is an indication of poor manufacturing practices involved in the processing [38] but none have hinted at the fact that bottles (jars) are reused. The chi square test shows that there is a significant ( $p < 0.001$ ) association between HPC and bottle type. Bacteria can grow to the extent of affecting human health due to the storage of bottled water [25]. The decomposition of PET into formaldehyde and acetaldehyde is also brought by the heterotrophic bacteria in the presence of micronutrients in the bottled water [29, 30]. Bottled water with a higher load of HPC may contain pathogens [20, 56, 57], and they might end up causing morbidity to the consumer.

The total coliform result of this study agrees with the study of Halage et al. [58], Timilsina et al. [11], da Silva et al. [52] and Bashir and Aish [54] where 70%, 63.3%, 76.6%, and 75% samples, respectively, were contaminated with coliform. The presence of coliforms in 20L bottled water indicates that the bottled water was not sanitized/disin-

fectured/sterilized before use [40]. Coliforms have also been reported to have the capacity to form biofilms [10]. The chi square test shows that there is a significant association ( $p < 0.001$ ) between total coliform and bottle type, HPC. Improper storage of products provides favorable conditions for the bacteria to grow up to a harmful level [40]. The result of this study agrees with the result of Biadlegne et al. [59] and Meki et al. [60] who established the important fact that water from wholesalers and retailers was more contaminated with total coliform as compared to the water from the industry itself. After being exposed to the environment, these coliforms might have adapted survival mechanisms like integrating plasmid [13, 15, 17] and when consumed it might compete with the normal flora of our intestine leading to morbidity and sometimes even death [13, 15, 17]. Isolation of coliforms in bottled water reflects that morbidity, mortality, endemic, epidemic etc. are just around the corner, and the health of the public should not be taken into consideration [15, 17].

The result of *Pseudomonas* spp. agreed with the result of Falcone-Dias et al. [61] where *Pseudomonas* spp. was detected in 4.3%, 4.5%, and 9.5% of 0.33-0.60 L, 1.5 L, and 20 L bottled water, respectively. *Pseudomonas* spp. isolated from bottled water was also found to be the etiological agent for the outbreak of hospital-acquired infection about 88.9% in ICU patients [37]. *Pseudomonas* spp. survives in water and if the opportunity arises causes pneumonia, among which the mortality rate is 72% [62], but is still not considered a threat by WHO, IBWA, and DFTQC [39, 44, 63]. Biofilm of *Pseudomonas* spp. has a specialized architecture ensuring well-being and survival of cells that can develop on solid surfaces [15, 62]. *Pseudomonas* spp. is fastidious, multidrug resistant, and one of the extremophile bacteria [37, 62]. Thanks to evolutionary development (evo-devo), *Pseudomonas* spp. can survive for several days in water lacking suspended particles as it is able to grow in very low nutrients [15, 17, 62]. PET polymers are not immune to degradation as physical, chemical, and microbial parameters also affect the normal polymer composition [3, 6, 7, 26, 36].

The scratched and/or dented bottle was found to be highly contaminated with the microorganisms, and this could be due to the increased surface area provided by the rough edges (either by scratch or by dent). Self-cleaning bottle is totally demanded in the market and could be even achieved through the knowledge of CRISPR [15], genetic engineering [13, 15-18, 27, 28], and nanotechnology. No one has come up with its solution to date. Due to microbial leaching, even carcinogens could also be present in the sealed water [3, 5, 29, 32, 49]. Bottled water with a high microbial load may cause morbidity to immune compromised persons [56, 57] like those suffering from HIV-AIDS, novel coronavirus, tuberculosis, malnutrition, etc.

## 5. Concluding Remarks

The conclusions of the study are

- (1) Extremophiles present in HPC may lead to degradation of PET in the presence of micronutrients

- (2) Coliforms can survive by utilizing several survival mechanisms like forming biofilms and plasmids, where they might lead to degradation by degrading surface area covered by the biofilms and mutations, respectively
- (3) Extremophile bacteria that survive on bottled water for a long time can incline to several survival mechanisms and solely survive on complex polymers like PET
- (4) PET polymers are not immune to degradation as physical, chemical, and microbial parameters also affect the normal polymer composition
- (5) These extremophile microorganisms can be used for degrading the PET polymers. These extremophiles could also help to make the environment eco-friendlier
- (6) PET polymers can be reused, and raw material costs can be saved if the biological degradation is ceased

### Data Availability

The data can be provided upon request to the corresponding author.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

### Authors' Contributions

BG contributed to the methodology and investigation. BG contributed to the data collection and analysis. DWU and BG contributed to the microbiology and genomic portion. GG and BG contributed to the chemistry and polymer portion.

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