

Impact of sunlight/dark storage on natural spring water bottled in polyethylene terephthalate

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ABSTRACT

The effect of two environmental variables, ultraviolet radiation and external air temperature, on polyethylene terephthalate (PET) bottled water was investigated over 100 days. The high pressure liquid chromatography results showed the migration of carbonyl compounds (acetaldehyde, formaldehyde and acetone) from PET bottles into water over 3 months. Storage of PET bottled water enhanced the process of carbonyl compounds migration especially under sunlight. The combination of these two environmental variables caused an increase of 15% in each carbonyl compound compared with storage in the dark at the laboratory. The maximum ambient Ultraviolet-B, Ultraviolet-A and Photo-synthetically Active Radiation intensity at the solar noon were 1.95, 23.0 W/m² and 100,000 Lux, respectively.

Key words | carbonyl compounds, migration, PET, sunlight, water

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INTRODUCTION

Polyethylene terephthalate (PET) which belongs to the family of polyesters is used in food industry, for products such as packaging and films. It is considered as the most favourable packaging material for drinking water bottles (Conroy *et al.* 1999; Colley *et al.* 1999; Nawrocki *et al.* 2002; Lonnen *et al.* 2005; Leivadara *et al.* 2008; Bach *et al.* 2012, 2013). Previous studies reported changes in the chemical quality of water stored in PET bottles (Nawrocki *et al.* 2002; Schmid *et al.* 2008; Leivadara *et al.* 2008; Rygaard *et al.* 2009; Strube *et al.* 2009; Bach *et al.* 2012). These changes could be caused by contaminants originally existing in the water source or by external contamination from the bottling plant or by the migration of chemicals from the container into the water (especially during storage) (Wegelin *et al.* 2001; Nawrocki *et al.* 2002; Leivadara *et al.* 2008; Ceretti *et al.* 2010; Farhoodi *et al.* 2014). Large numbers of studies have shown that formaldehyde and acetaldehyde are released from PET bottles into water under exposure to sunlight and high temperatures, while a few data have been reported about the migration of acetone (Wegelin *et al.* 2001; Nawrocki *et al.* 2002; Leivadara *et al.* 2008; Rygaard *et al.* 2009; Bach *et al.* 2012, 2013). Bach *et al.* (2012)

found that some carbonyl compounds can migrate from PET bottles by thermo-mechanical and thermo-oxidative degradation of PET during the manufacture of PET bottles and when bottles are exposed to poor conditions of transport and/or storage. There are many factors which could affect the diffusion of carbonyl compounds from PET into the water such as temperature, storage time, sunlight, low pH in carbonated water, and the pressure exerted by CO₂ on the PET wall (Nawrocki *et al.* 2002; Leivadara *et al.* 2008; Bach *et al.* 2012, 2014). Due to the importance of water safety, the World Health Organization (WHO) have suggested a tolerable concentration of 2.6 mg/L for formaldehyde in drinking-water, but have given no indication for acetaldehyde and acetone tolerable concentrations (WHO 2011).

The European Union (EU) stated specific migration limits for some substances (expressed in mg substance per kg food), it was less than 6 mg/kg for acetaldehyde, less than 15 mg/kg for formaldehyde and less than 2 mg/kg for acetone (EU 2011). Different compounds could also migrate from PET like residual oligomers/monomers, inorganic substances such as catalysts (antimony) or some additives

which are used during the fabrication of PET bottles (UV stabilizers) (Monteiro *et al.* 1999; Begley *et al.* 2004; Bradley & Coulier 2007; Cheng *et al.* 2010; Reimann *et al.* 2010; Kim & Lee 2012). Exposure of PET bottles to light and temperature could promote physicochemical changes in the material such as an increase in oxygen permeability which is an important factor controlling the shelf life of a product, and then the quality of the packaged water, or other food and drinks could deteriorate (Berlinet *et al.* 2008).

The aim of this study was to investigate the effect of sunlight and dark storage for up to 3 months on natural spring water bottled in PET – microbiological and chemical aspects – especially the migration of carbonyl compounds (acetaldehyde, formaldehyde and acetone) from the PET into the water in sunny regions.

MATERIAL AND METHODS

Sampling strategy and locations

The natural spring bottled water samples were collected from local Company for Bottling Water-Damascus (ISO-9001-2008). The spring is at a height of 5,000 feet in the Damascus countryside, Syria. The main chemical characteristics of the natural spring water in PET at 25 °C were: pH: 8.10; total dissolved solids (TDS): 175 mg/L; conductivity: 352 $\mu\text{S}/\text{cm}^2$. Forty-five PET bottles from the same batch were concurrently and automatically filled with 1.5 L of water per bottle at the bottling company, water was distributed from the spring source to the bottling apparatus. We divided the bottled water in two groups which contained 24 and 21 PET bottled water, respectively, to be treated by two different processes during the next day.

Solar exposure and dark incubation

The first group of bottled water was placed in the laboratory and it was stored at ambient temperature and in darkness, while the second group was placed on the roof of the laboratory and it was stored under direct sunlight (in the sun). The PET bottled water was stored for 1, 7, 14, 29, 46, 70, 82, and 100 days. The samples were taken in triplicate for each point and for each condition (dark, sun).

Measurement of physical and chemical parameters

The bottles were placed separately on the roof of the laboratory from 10 August until 21 November 2011 with a distance of 40 cm between them to prevent shading. The irradiance was measured at the solar noon across the Ultraviolet-B (UV-B, 280–315 nm), Ultraviolet-A (UV-A, 315–400 nm) and Photo-synthetically Active Radiation (PAR, 400–700 nm) spectral regions using a portable HD2102.2 radiometer (Delta OHM, Italy). Fifty triplicate samples for TDS, conductivity, and pH analysis were analysed using a conductivity meter (Mettler TOLEDO, S30, Switzerland), daily air temperature was measured at the solar noon using a Hygrometer testo 608-H1 (testo, AG, Germany).

Analyses of carbonyl compounds

Reaction solution

A total of 428.7 mg of 2,4-Dinitrophenylhydrazine (DNPH, Merck) 70% (w/w) in water was dissolved in 100 mL absolute ethanol, Citrate Buffer (1M) was prepared by adding 80 mL of citric acid solution (1M) to 20 mL of sodium citrate solution (1M), the reagent standards (acetaldehyde, formaldehyde and acetone) were purchased from Sigma-Aldrich (Eichelberger & Bashe 1992). The reagent solution was purified with 3 mL of 0.2N sulphuric acid solution, blanks were prepared by path distilled water on activated carbon, and then this water was used as a blank, the reagent solution (2,4-DNPH), citric acid and sodium citrate buffer solution were added to the distilled water which was then used as a reagent blank.

Derivatization and extraction

Four millilitres of citrate buffer was added to the aliquot sample and was adjusted to pH 3, then 6 mL of DNPH reagent was added and the sample was sealed and placed in a heated orbital shaker set at 40 °C for 35 min. The DNPH was used to provide a highly sensitive chromophore for ultraviolet or fluorescence detection. The agitation was gently swirl adjusted. Ten millilitres of dilute citrate buffer was pressed through the SPE (solid phase extraction) cartridge (Strata 8B-HCH C18H-E, 500 mg/6 mL from

Phenomenex) and 10 mL of the saturated NaCl solution was added to the reaction vessel after removing from the shaker, then the reaction solution was added to the cartridge train and a vacuum (3 mL/min) was applied to draw the solution through the cartridge. The eluent was diluted to volume with absolute ethanol (Merck), mixed thoroughly and placed in a tightly sealed vial until analysis (Eichelberger & Bashe 1992; Obernosterer *et al.* 1999; Stanisz & Kania 2006).

High pressure liquid chromatography (HPLC) conditions

Triplicate samples of 100 mL were quantitatively transferred to a 250 mL erlenmeyer flask for carbonyl compounds (acetaldehyde, formaldehyde and acetone) analysis using HPLC, HPLC-analytical (Agilent Technologies, 1100 USA), the column was ODS-3 (Octadecylsulphate) column (5 μm , 4.6 \times 150 mm). The elution programme started with 30% methanol with water 10% and TFA(B) (Tetra fluoro acetic acid) (isocratic for 5 min), then raised up to 0% (B) in 18 min (isocratic for 2 min) and then back to 30% (B) in 18 min (isocratic for 6 min). The injection volume was 10 μL with flow rate 0.5 mL/min and the UV/Vis MWD (Ultra Violet/Visible Multi Wave Detector) detection was at 360 nm for all the analysed compounds (Eichelberger & Bashe 1992). Recoveries were calculated from the differences between the spiked and unspiked samples and the mean recovery for each compound was calculated. The limits of detection (LOD) and the limits of quantification (LOQ) for each compound were calculated on the basis of a signal to-noise ratio of 3 and 10, respectively. The LOD and quantification were LOD = 1.89 $\mu\text{g/L}$ and LOQ = 6.32 $\mu\text{g/L}$ for acetone, LOD = 3.68 $\mu\text{g/L}$ and LOQ = 12.29 $\mu\text{g/L}$ for acetaldehyde, and LOD = 1.59 $\mu\text{g/L}$ and LOQ = 5.3 $\mu\text{g/L}$ for formaldehyde.

Microbiological analysis

To control the microbiological quality of the natural spring bottled water during the 100 days of storage, the sampling was carried out at 1, 7, 14, 29, 46, 70, 82, 100 days of storage in both sun and dark incubation. The total bacterial counts as colony forming units (CFU) were determined by a pour plate method (William & Latimer 2005). Each bottle was aseptically opened and a sample of water was poured into a sterile plastic tube. Sample water (0.2 mL) was inoculated

into a 9 mm Petri dish, and then 15 ml of Plate Count Agar 45 °C (Himedia Labs, India) was poured into the inoculated Petri dish, the plates were incubated at 37 °C for 48 h. The total coliforms counts were determined by eosin methylene blue (EMB) (Himedia) agar plates. A volume of 0.2 mL of each water sample was inoculated into a Petri dish and then incubated with 15 ml of EMB at 37 °C for 24 h (William & Latimer 2005). The *Escherichia coli* count was determined by Luria Broth (Himedia Labs, India) at 37 °C for 24 h (William & Latimer 2005).

Statistical analysis

Data were subjected to the statistical analysis of Student's *t*-test, $p > 0.95$ using the SUPERANOVA computer package (Abacus Concepts Inc. Berkeley, CA, USA; 1998). The normality of the distribution of values was tested using a Shapiro-Wilk test.

RESULTS AND DISCUSSION

Physical and chemical analyses during solar exposure and dark incubation

Ambient UV-B at the instantaneous intensity was at a maximum (1.95 W/m^2) at the beginning of the measurement phase in August, the UV-B intensity gradually decreased to reach (1.0 W/m^2) after 1 month, and (0.26 W/m^2) after 3 months (Figure 1). The same trend was observed for

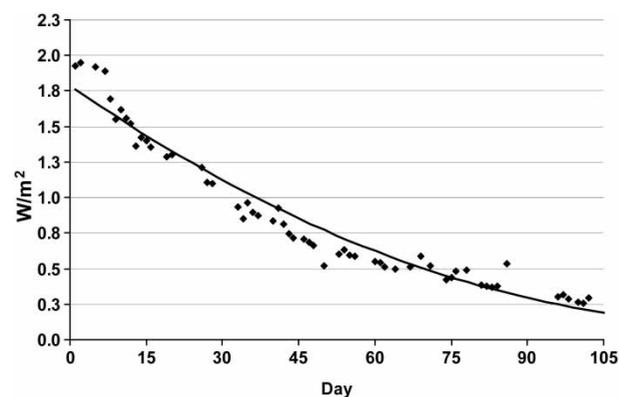


Figure 1 | Measured daily values of UV-B intensity at the solar noon during 100 days (from 10 August to 21 November 2011).

UV-A (Figure 2), with instantaneous maximum intensity (23.0 W/m^2) in August, decreasing to (13 W/m^2) after 1 month, with the minimum value in November (7.0 W/m^2). The ratio UV-B/UV-A was 0.09–0.06. The values of PAR also decreased from 100,000 to 25,000 Lux during the progress of solar exposure incubation. Figure 3 shows the air temperature recorded at the solar noon in the location of the sample incubation. The air temperature was around $42.0 \pm 2.0 \text{ }^\circ\text{C}$ and it starts to decrease after 45 days. The TDS was 175.5 mg/L at the beginning of incubation and it decreased by 6.5% after 100 days of incubation, there was no significant difference between sun and dark incubation. The conductivity also decreased after 100 days of incubation (from 352.0 to $330.0 \text{ }\mu\text{S/cm}^2$) during the sun and dark incubation.

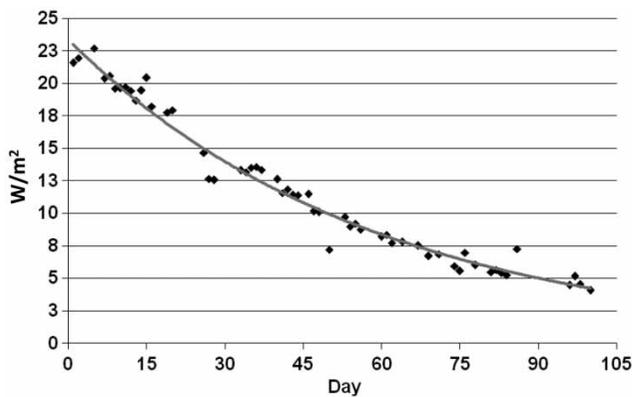


Figure 2 | Measured daily values of UV-A intensity at the solar noon during 100 days (from 10 August to 21 November 2011).

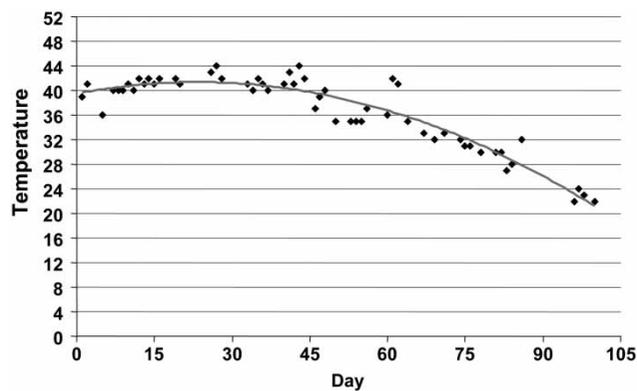


Figure 3 | Daily temperature ($^\circ\text{C}$) recorded at the solar noon during 100 days (from 10 August to 21 November 2011).

Carbonyl compounds analyses

Figure 4 represents a typical chromatogram and demonstrates the evolution in the peak of each carbonyl compound during storage of PET bottled water for 100 days. The concentrations of acetaldehyde in the samples of natural spring bottled water are shown in Figure 5. The concentrations of acetaldehyde increased after 100 days of storage from $64.3 \pm 0.4 \text{ }\mu\text{g/L}$ the first day to 150.0 ± 5.0 and $177.0 \pm 6.0 \text{ }\mu\text{g/L}$ in the dark and sun incubation, respectively. The increase of acetaldehyde concentration was 133 and 175% in the dark and sun incubation, respectively, comparing with the initial concentration. The increase of acetaldehyde concentration after the sun exposure was higher than in the dark incubation. The same trend was shown for the formaldehyde concentration (Figure 6) which increased from $7.1 \pm 0.9 \text{ }\mu\text{g/L}$ on the seventh day of storage (the concentration was lower than the detection limit on the first day) to 19.9 ± 0.4 and $23.7 \pm 1.6 \text{ }\mu\text{g/L}$ in the dark and the sun incubation, respectively, after the same previous period. The increase was 180 and 233% in the dark and sun exposure, respectively, comparing with the initial concentration. The concentration of acetone (Figure 7) also increased during storage from $85.5 \pm 3.5 \text{ }\mu\text{g/L}$ to 196.5 ± 6.4 and $231.5 \pm 7.0 \text{ }\mu\text{g/L}$ in the dark and sun incubation, respectively. The increase of acetone was 130 and 170% in the dark and sun incubation, respectively, comparing with the initial concentration, after 100 days of incubation. Our results about the migration of carbonyl compounds from PET bottles into water confirm the finding that has been reported (Sugaya *et al.* 2001; Wegelin *et al.* 2001; Nawrocki *et al.* 2002; Ceretti *et al.* 2010; Bach *et al.* 2014). The storage conditions (especially time and

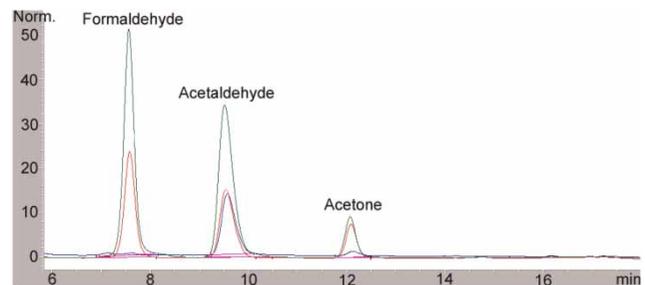


Figure 4 | Representative typical chromatogram demonstrates the evolution in the peak of each carbonyl compounds during storage of PET bottled water for 100 days.

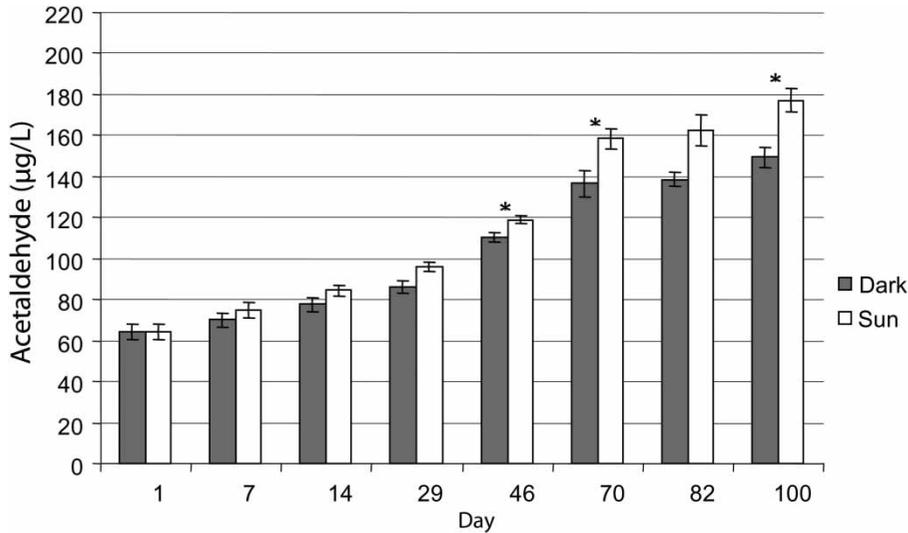


Figure 5 | Average acetaldehyde concentrations of spring bottled water samples during 100 days of storage (from 10 August to 21 November 2011) in the laboratory (Dark) and under sun exposure (Sun). Results are presented as average (with triplicate measurements; error bars indicate standard deviation). *Indicates significant difference between Dark and Sun, according to *t* test, **P* < 0.05.

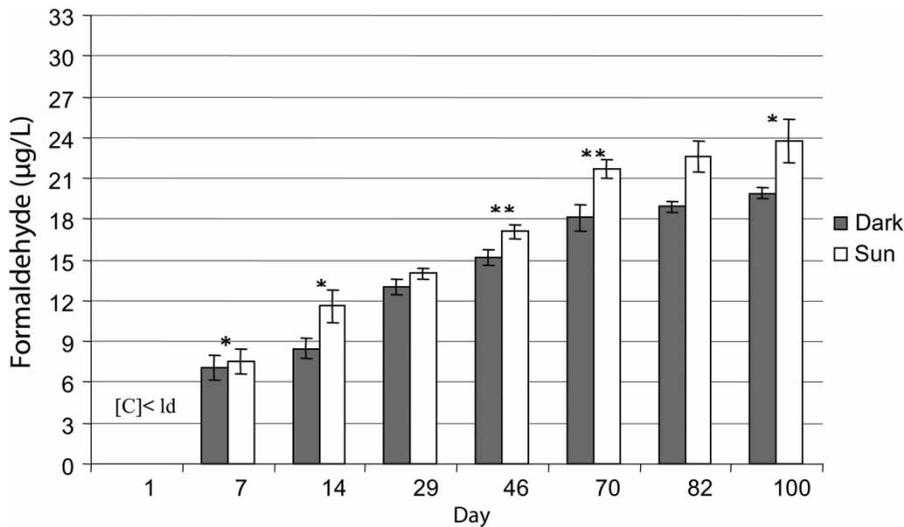


Figure 6 | Average formaldehyde concentrations of spring bottled water samples during 100 days of storage (from 10 August to 21 November 2011) in the laboratory (Dark) and under sun exposure (Sun). [C] < Id : concentration < detection limit. Results are presented as average (with triplicate measurements; error bars indicate standard deviation). *Indicates significant difference between Dark and Sun, according to *t* test, **P* < 0.05; ***P* < 0.01.

temperature) could enhance the process of migration from PET bottles or the formation of aldehyde in PET bottled water (Nawrocki *et al.* 2002; Farhoodi *et al.* 2014). On the contrary, Leivadara *et al.* (2008) did not identify either carbonyl compound or volatile organic compound in bottled water during 3 months of exposure to sunlight and temperatures up to 30 °C without irradiation measurement. Low

concentration of organic compound such as di(2_ethylhexyl) phthalate was detected in PET bottled water by Biscardi *et al.* (2003). The highest concentration of acetaldehyde in mineral water (260 µg/L) was recorded by Sugaya *et al.* (2001), while other studies indicated that the concentration of acetaldehyde did not exceed 2 µg/L after storage under the sunlight for up to 63 days (Wegelin *et al.* 2001; Ceretti

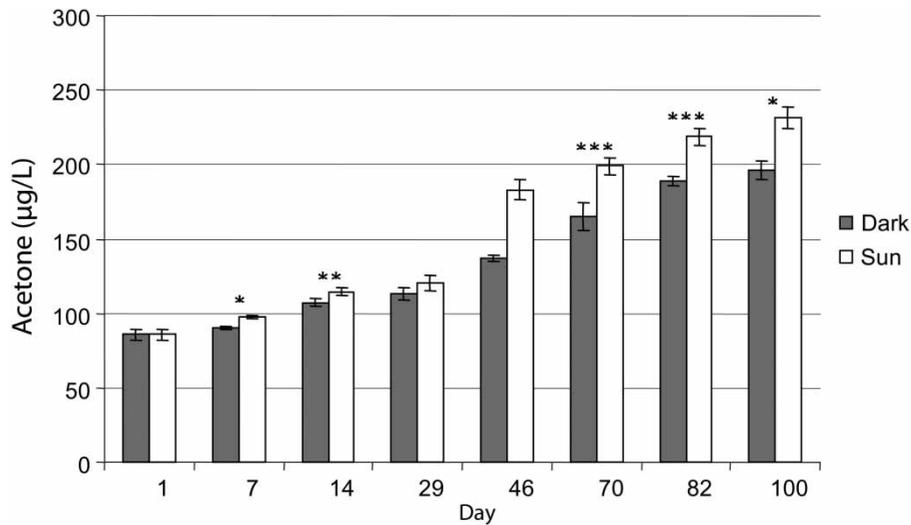


Figure 7 | Average acetone concentrations of spring bottled water samples during 100 days of storage (from 10 August to 21 November 2011) in the laboratory (Dark) and under sun exposure (Sun). Results are presented as average (with triplicate measurements; error bars indicate standard deviation). *Indicates significant difference between Dark and Sun, according to t test, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

et al. 2010). This contrasts with our finding that storage of water in PET bottles for 100 days under the extreme conditions of direct sunlight increased the concentration of acetaldehyde up to 177.0 ± 6.0 µg/L. The concentration of formaldehyde in this study after 100 days of storage ranged within the limit that was indicated by Sugaya *et al.* (2001) and Mutsuga *et al.* (2006). The sunlight has more effect on increasing the formaldehyde concentration than other carbonyl compound concentrations in bottled water after 100 days of storage; formaldehyde concentration was 23.7 ± 1.6 µg/L after 100 days. However, Wegelin *et al.* (2001) found a higher concentration of formaldehyde (44.0 µg/L) than this in water stored for 63 days in a PET bottle in similar conditions. Formaldehyde occurs in industrial effluents and is emitted into the air from plastic materials and resin glues, it could exist in drinking-water by the oxidation of natural organic matter during ozonation and chlorination, and also as a result of release from polyacetal plastic fittings (WHO 2011). Contradictory results for PET bottled water have been reported in the literature review, the differences can be explained by the variety of analytical methods and exposure conditions used (Bach *et al.* 2012).

A few studies have indicated the presence of acetone in PET bottled water. Nawrocki *et al.* (2002) showed that the concentration of acetone was 107.0 and 125.0 µg/L in

mineral and carbonated PET bottled water, respectively, without investigating the effect of storage. The initial value of acetone at the first day of our study was in the same range that was demonstrated by Nawrocki *et al.* (2002), but we have shown that storage could increase the concentration of acetone, especially under direct sunlight. This increase was 286% in the sun exposure, compared to the initial concentration, after 100 days of incubation. Polypropylene caps could be a source of carbonyl compound and particularly acetone in bottled water (Nawrocki *et al.* 2002). Some studies reported that carbonized water seems to be slightly safer for consumption than non-carbonized water (namely natural mineral water, spring water and prepared water) (Leivadara *et al.* 2008).

The decrease of air temperatures, which started after 45 days of storage, may have slowed down the combined effect of storage time and sunlight for the presence of aldehydes in bottled water.

Due to health concerns, there is increasing attention being paid to the quality of bottled drinking water (Liu & Mou 2004). Epidemiological studies on animals have shown different kinds of cancer and damage in the central nervous system, and some diseases due to the chemical quality of bottled drinking water. It is therefore necessary to study the effects on the cells of the concentrations resulting from storage, to determine the safe consumption period.

Microbiological analysis

The results of the microbiological tests of bottled water samples showed that the water was sterile: 0 CFU/100 ml of *E. coli*, 0 CFU/100 ml of total coliforms and less than 10 CFU/100 ml of total count during 100 days of storage. *E. coli* remains an important indicator of faecal contamination for verification of water quality (WHO 2011). According to the recommendation of zero tolerance for coliforms in potable water (Bharath *et al.* 2003), our samples are considered microbiologically safe for human consumption after 100 days of storage under the extreme conditions of the experiments.

CONCLUSION

The storage of natural spring water in PET bottles for 100 days increased the concentrations of carbonyl compounds (acetaldehyde, formaldehyde and acetone) by up to ~150%, compared to the initial concentrations at the first day, when the bottles were stored in the laboratory, while the increase reached up to ~200%, compared to the initial concentration, in PET bottles stored under sun exposure. There is no relationship between the intensities of the high solar radiation and the concentrations of carbonyl compounds; it was a cumulative effect with time. The sun exposure includes two environmental variables, UV radiation and external air temperature, so their combination may mask the individual effects of each. The carbonyl compounds concentration was still considerably lower than the permitted level of migration from PET according to the EU and WHO. It is important to note that none of the literature reviewed reached these referred values. According to the microbiological and carbonyl compounds results under the extreme experiment conditions, it is considered that water stored in PET bottles for up to 100 days is safe to drink.

It is important to expand the research by examining the impact of storage for a longer period such as 1 year (according to the specifications of the manufacturers which are mentioned on the bottle) and by studying the changes in the chemical structure of the PET bottles, since many chemical compounds have been found to migrate from PET into packaged natural spring water in similar climatic conditions.

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