Click here to view linked References

Initial phthalates fingerprint and hydrochemical signature as key factors controlling phthalates concentration trends in PET-bottled waters during long storage times

Mercedes Becerra-Herrera[1,2*], Daniel Arismendi[3], Alejandra Molina-Balmaceda[3],
Jorge Uslar[4], Valentina Manzo[3], Pablo Richter[3], Manuel A. Caraballo[5,6,7*]

[1]Department of Chemistry, Faculty of Science, University of Chile, P.O. Box 653, Santiago, Chile.
[2]Larorsur S.L. Polígono Industrial "El Fortiz", Calle A – Nave 16, 21007, Huelva, Spain.
[3]Department of Inorganic and Analytical Chemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, P.O. Box 233, Santiago, Chile.
[4]Dept. of Geology, Univ. de Chile, Plaza Ercilla 803, 8370451, Santiago, Chile.
[5]Department of Mining Engineering, University of Chile, Av. Tupper 2069, 8370451, Santiago, Chile.
[6]AMTC, University of Chile, Av. Tupper 2007, 8370451, Santiago, Chile.
[7]Department of Water, Mining and Environment, Scientific and Technological Center of Huelva, University of Huelva, 21004 Huelva, Spain.

Corresponding authors:
Manuel A. Caraballo, macaraballomonge@gmail.com;
Mercedes Becerra-Herrera, mercedes.becher@gmail.com;
Phones: +562 29787242; +562 29784479

Abstract

Phthalate acid esters (PAEs) concentration in bottled water and different factors (water pH, storage time, sunlight exposure, and temperature) that affect/control them have become hot topics during recent years. Nevertheless, quite contradictory results and disagreements on the effects of these factors have been published. In an attempt to find some consensus on this topic, a comprehensive study considering the combined effect of long storage times (longer than a year) and the water hydrochemical signature (including water pH, elemental composition and the presence/absence of dissolved CO_2) was performed using the four most commonly consumed bottled water brands on the Chilean market. Each water brand was analyzed between 10 or 14 different times, depending on the brand (in total 97 samples were studied).

Following the concept of the hydrochemical signature typically used in hydrogeology to classify types of waters, the notion of a water phthalate fingerprint was proposed. Finally, concerning the effect of long storage times, this study demonstrates that all the trends (increase, decrease or steady) of the Total PAEs concentration are possible; and these trends are controlled by the specific hydrochemical signature and phthalate fingerprint of the bottled water.

Keywords: Phthalates fingerprint, PET-bottled water, hydrochemistry signature, storage time.

1. Introduction

The consumption of bottled water in Chile, as well as worldwide, has steadily increased during the last few decades. According to the International Bottled Water Association, In 2019 the consumption of bottled water just in the USA reached 65,500 million liters (https://bottledwater.org/bottled-water-consumption-shift/). In the last 10 years, consumption of bottled water in Chile has increased by 138%, changing water consumption from 13 liters per person in 2006 to 30 liters per person in 2018 (Daniele et al., 2019).

The most commonly used material to produce water bottles is polyethylene terephthalate (PET) (Koelmans et al., 2019). PET contains some toxic and harmful compounds such as phthalates (PAEs) that may enter the water from the plastic bottles and become a threat to human health (Arfaeinia et al., 2015; Lou et al., 2018; Xu et al., 2020).

Some researchers suggest that PAEs are non-intentionally added substances (NIAS) that can be incorporated into plastic material and potentially migrate into food (Salazar-Beltrán, et al. 2018; Jeddi, et al. 2015; Luo, et al. 2018; Jayaweera, et al. 2020). In this context, PAEs sources have been associated with the origin of the PET container, source of waters, cap-sealing resins, quality of the raw material and chemicals used in the production and contamination in production lines (Jeddi, et al. 2015; Luo, et al. 2018; Giuliani, et al. 2020). Many studies indicate that the initial levels of PAEs are often lower than those found after storage processes or when the bottle is exposed to high temperature (Montuori et al. 2008; Mousa et al. 2013; Zaki et al. 2018; Jeddi et al. 2016; Keresztes et al. 2013; Szendi et al. 2018; Xu, et al. 2020). In this way, direct analysis of the bottle plastics has shown concentrations of DMP, DBP and DEHP between 0.393 - 71 mg/Kg (Salazar-Beltran et al., 2018; Otero et al., 2015). In addition, a positive correlation has been found between the bottles thickness and the levels of DBP in PET bottles and also with the concentration of DBP migrated from bottles to water (Salazar-Beltrán, et al. 2018). Furthermore, recent studies have informed high PAE concentrations in samples packaged in recycled PET bottles which suggest that the bottle material can be contaminated with PVC plastics (Keresztes et al., 2013; Dreolin et al. 2019; Thoden van Velzen et al., 2020). Consequently, these previous results indicate that PAEs (or part of them) can migrate from the polymer matrix to water.

PAEs are very dangerous synthetic compounds, mainly because they can accumulate in the human body and are endocrine disrupting chemicals (EDCs) (Yousefi et al., 2019). The incipient preoccupation caused by EDCs is because they are substances that interfere in the synthesis, secretion, transport, action or excretion of natural hormones in the body, causing anomalies in reproductive development (Casals-Casas et al., 2011), infertility, hormone-dependent cancers, obesity, diabetes and neurodevelopmental disorders, among others (Schug et al., 2011). Although production and use have been regulated in the case of some EDCs, such as phthalates (Błędzka et al., 2014), many of these chemical compounds are still used in a wide range of consumer products. Consequently, six of them (i.e., 2-ethylhexyl phthalate (DEHP), benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), di-*n*-octyl phthalate (DOP), di-isononyl phthalate (DINP), and di-isodecyl phthalate (DIDP)) have been placed on the priority pollutant lists of the United States Environmental Protection Agency (U.S. EPA, 2017) and the European Union (EU, 2011) and on the list of priority pollutants in Chinese waters (CEC, 2007). In addition, the World Health Organization (WHO, 2004) guideline for DEHP in drinking water (WHO/SDE/WSH/03.04/68) has established a maximum allowable concentration in drinking water of 8 μg/L, whereas the U.S. Food and Drug Administration has selected 6 μg/L as the threshold level (U.S. FDA, 2012). The fact that WHO provides guidelines for DEHP in drinking water has a global impact, it is not relevant or needed that each country has its own legislation. However, each country should at least adhere these WHO guidelines. Currently, there is no regulation at all (or specific adherence of the WHO guidelines) for any of these pollutants in South America.

Phthalate concentrations in bottled water and the different factors that affect/control it have become hot research topics. As a result, new review articles summarizing the most recent discoveries appear almost every year (Luo et al., 2018; Abtahi et al., 2019; Akhbarizadeh et al., 2020). As shown by these review articles, plenty of attention has been paid to study the effect that water pH, storage time, sunlight exposure and temperature have on the water PAEs concentration. Additional detailed information on this regard is offered in Table B1 (Supplementary Information). Notwithstanding, the results are quite contradictory and there is still substantial disagreement on the effect that these factors may have on PAE concentrations (Akhbarizadeh et al., 2020). According to two comprehensive reviews (Luo et al., 2018; and Akhbarizadeh et al., 2020), most reported experiments were performed during short periods of time (a few months), whereas the quality warrant for most bottled waters is one year at least. Luo et al. (2018) indicated that long-term monitoring of phthalates in bottled water is necessary, but it is very difficult. As a result, they proposed the use of kinetic models to study the migration of phthalates from plastic bottles to bottled water as a possible alternative. In addition to the factors previously mentioned, some studies have observed that the presence of dissolved CO_2 gas (sparkling water) facilitates the migration of some plastic constituents to water as well as its accumulation (Guart et al., 2014; Bach et al., 2012; Montuori et al., 2008); whereas other studies did not observe any significant differences in the phthalate concentrations between sparkling and still water (Cao et al., 2008). Finally, and to the best of our knowledge, there are no studies considering the effect that the physicochemical parameters and the chemical elemental composition of water (i.e., the hydrochemical signature) may have on the initial concentration of PAEs in bottled waters and their evolution during storage.

As a consequence, despite the prolific (but contradictory) literature on the PAEs concentrations of bottled water and its evolution over time, a comprehensive study considering the combined effect of long storage times (over a year) and the water hydrochemical signature (including water pH, elemental composition and the presence/absence of dissolved CO_2) is missing. Therefore, a combined and comprehensive study considering these factors was performed in the present research study, using the four most commonly consumed bottled water brands on the Chilean market. In addition, this study performs a unique estimation of PAEs in Chilean bottled water and is the first study of this kind in South America.

2. Materials and methods

2.1.Reagents.

Dimethyl phthalate (DMP), diethyl phthalate (DEP), dipropyl phthalate (DPP), DBP, BBP, 2-ethylhexyl adipate (DEHA), DEHP, and DOP were purchased from Sigma-Aldrich (Milwaukee, WI, USA). The individual stock solution of each phthalate was prepared in methanol (MeOH) at a nominal concentration of 100 mg/L. Working multistandard solutions of phthalates at nominal concentrations of 25, 50, 100, 250, 500 and 1000 µg/L were prepared by diluting the individual stock solution with methanol. All solutions were stored at 4°C.

Nitrogen (purity ≥ 99.999%) and helium (99.9999%) were purchased from Linde (Santiago, Chile) and were used in the final extract evaporation and as a carrier in GC-MS, respectively. Dichloromethane (DCM), ethyl acetate (EtOAc) and nitric acid 65% (HNO_3, suprapur® grade) were purchased from Merck S.A. (Santiago, Chile). Water from a

Simplicity® Water Purification System, Millipore (Darmstadt, Germany), was used. The Oasis® HLB sorptive phase from Waters Corporation (Milford, MA, USA) was used in rotating disk sorptive extraction (RDSE).

2.2. *Bottled water sampling campaigns and storage conditions.*

Commercial mineral and purified still and sparkling bottled water samples (*n* = 97) were collected from the four most commonly consumed water brands on the Chilean market (Daniele et al., 2019). Brand 1 y Brand 2 were selected to represent the most commonly consumed Chilean mineral water, while Brand 3 y Brand 4 were selected from the available purified water. Based on the results offered by previous studies (Daniele et al., 2019), the eight types of water selected in the present study (including still and sparkling water) ensure broad ranges of physicochemical parameters and elemental compositions.

The bottled water samples were purchased on the first day of each month from July 2016 to July 2017 from the same supermarket in Santiago, Chile. On the few occasions that the packing dates of the bottled water were the same during 2 consecutive monthly samplings, the repeated sample was not acquired. Avoiding the inclusion of any other variable in the present study (apart from storage time and hydrochemistry), all of the bottled water samples (disregarding the brand) had the same size (500 mL), were packed in PET plastic bottles with high density polyethylene (HDPE) plastic caps with no cap liners and stored in darkness at room temperature (25 ± 4 °C). The aging time of the water within the bottles spanned from 54 to 615 days, and the storage time was calculated by counting the days from the packing date to the analysis date (day when the bottle was opened and physicochemical parameters analysis and phthalate extraction and analysis were performed).

2.3. Chemical analyses and laboratory measurements

The physicochemical parameters (pH, conductivity, and alkalinity), as well as the elemental and phthalate concentrations, were measured for all water samples. For these measurements, several volumes of each bottled water were obtained. First, immediately after opening a bottle, 30 mL of water was transferred to a glass vial to measure the pH and electrical conductivity (EC), while another 40 mL aliquot of water was used to measure the total alkalinity by volumetric titration with HCl 0.01 N and potentiometric determination of the final point at pH 4.5. Second, 50 mL of water was transferred to a plastic bottle and acidified using HNO_3 Suprapur® until reaching a pH value close to 1; the sample was then stored in a refrigerator at 4 °C until analysis. These samples were analyzed for 70 elements by inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma optical emission spectrometry (ICP-OES). Third, a 60 mL aliquot was split into three glass vials (20 mL each) to perform rotating disk sorptive extraction (RDSE) for phthalate extraction and subsequent analysis by gas chromatography mass spectrometry (GC-MS) as described in our previous work (Manzo et al., 2019).

Physicochemical parameters such as pH and EC were measured using a Thermo Orion Star A329 portable meter with the following electrodes: for pH, Orion 8107UWMMD, and for EC, Orion 013010MD. The calibration solutions used were: Orion pH buffers 910104 (pH 4.01), 910107 (pH 7.00), and 910110 (pH 10.01) and EC standards Orion 011007 and Orion 011006. All the electrodes were properly calibrated before each measurement.

Dissolved element analyses were performed by ICP-MS and ICP-OES at an external analytical company (i.e., Bureau Veritas/Acmelabs) using a commercial analytical package (i.e., ICP-MS S0200 analysis for natural waters). The detection limits achieved were lower than the regulatory limits proposed by the World Health Organization (WHO, 2008) and the US Environmental Protection Agency (USEPA, 2017). The specific values of these limits and the results of the elemental analyses are provided in Table A1 (Appendix A). Additionally, to perform quality control on some samples, an ICP-MS iCAP^{TM} RQ was used.

Phthalate analyses are easily affected by cross contamination at different stages from sampling to the final instrumental analyses; therefore, several precautions are typically undertaken to study these compounds (Bach et al., 2012). Specifically, the following precautions were considered in the present study: glass materials (vials, pipettes, bottles, syringes, etc.) were used during all the steps of the analytical process, and the use of any kind of plastic was forbidden; all the solvents and GC-MS vials aluminum septa used in this study were checked independently to ensure they were free of phthalates. The phthalate concentrations were obtained using a Thermo Scientific Trace 1300 gas chromatograph (Milan, Italy) coupled to a Thermo Fisher Scientific ISQ mass-selective detector (Austin, TX, USA) (GC-MS). All the equipment characteristics and chromatography parameters were previously reported by Manzo et al. (2019). That previous study also included the methods for determining accuracy of the method used in this study and for obtaining the detection and quantification limits and relative standard deviation, being them in the ranges 0.01–0.04 µg/L and 1–16%, respectively, for all the analytes (Manzo et al., 2019).

To ensure instrument stability and the feasibility of the results, the following quality control analysis were taken account: a full set of calibration phthalate standards (ranging from 0.10 to 100 µg/L) and the internal standard at 25 µg/L were analyzed before and after each set of samples. In addition, one standard was run as a control after every 9 injections. Blank contributions were taken account making analysis with water storage in glass bottle (Manzo et al., 2019). Phthalates extraction and analyses were performed in triplicate. The mean values and relative standard deviations of the phthalate concentrations in each bottled water sample are shown in Table A1 (Appendix A).

To evaluate the matrix effect and the recovery of working with different kinds of samples, both parameters were studied for each brand (still and sparkling) using the method proposed by Becerra-Herrera et al. (2015). The results were reported in our previous publication, Manzo et al. (2019), in which the methodology was validated.

2.4. Statistical analyses

The generated database with all the elemental and phthalate concentrations (Table A.1, Appendix A) was filtered and adjusted according the following criteria: 1) all the values reported as being below the detection limit (LOD) were changed according to the values resulting from dividing the specific detection limit value by the square root of 2 (Hites, 2019; Serrano et al., 2014); 2) when the compound concentration was between the LOD and quantification limit (LOQ), the value used was the LOD (because the phthalate was detected but unquantified). An additional criterion was used to filter the database before submitting it to any statistical analysis: any set of data with more than 20% of data under the LOQ was not suitable to be included in any statistical analysis (Hites, 2019).

Principal component analysis (PCA) was performed. PCA is a frequently used multivariate statistical technique used in exploratory data analysis to reveal the internal structure of data in a way that best explains their variance. PCA was carried out using the XLSTAT-Pro v.5.1 software and by means of a Spearman's correlation matrix (0.05 significance level, 2 tailed test) for the variables and samples. Bi-plots graphics were used to examine the distribution and tendencies of both the samples and variables of the data matrix.

3. Results

3.1. Bibliographic study of the Total PAE concentrations in bottled water

Most references explain individual phthalate concentrations in bottled waters, but it is important to also pay attention to the Total PAEs (total sum of the individual levels of each phthalate in a sample) concentration obtained in these samples. Thus, before analyzing the results of the individual and Total PAE concentrations found in our research, a bibliographic study of the Total PAEs concentration in bottled water was performed. It is important to mention that DEHP and DBP are usually the major compounds, representing between 80-100% of the Total PAEs, because all the references selected analyzed both of these compounds. As shown by the most recent and comprehensive reviews on the phthalate concentration in bottled water (Luo et al., 2018; Abtahi et al., 2019; Akhbarizadeh et al., 2020), the number of individual phthalates typically measured ranges from 1 to 6, although most studies frequently cover between 3 to 6 different phthalates. A suitable option to homogenize all this valuable information is by generating the Total PAEs. However, this parameter was not calculated in the aforementioned reviews (or in any other, as far as we know). As a result, a bibliographic database including 15 different research works from 14 countries around the world was assembled as part of the present study. The information used to generate the graphic in figure 2 and the corresponding references are included in Table A1 and Appendix A, respectively (Supplementary Information). Table A1 also summarizes other specific information offered in these studies (e.g., country, storage time, pH, conductivity and specific and Total PAEs concentrations). This information supports the idea (already presented in the introduction) that there is still substantial disagreement on the effect that water pH, storage time, sunlight exposure and temperature (among other) may have on PAE concentrations. As shown by the histogram in Figure 2, the data have a non-normal distribution with a high degree of skewness, and most of the data ($75\%_{\text{percentile}}$ = 2.54) are shifted to lower values. As a result, at least seven of the higher values in the database (higher than 5.9) could be considered to be statistical outliers. However, they are maintained in the database because they are validated analytical data representing part of the heterogeneous reality of the Total PAEs concentration of bottled water. The skewness of the data set can also be observed in the significant differences in the mean (2.00) and median (1.10).

Using a bibliographic database (127 samples, 15 different studies, 14 countries), the following classification for Total PAEs in bottled waters was proposed: low = <1 μg/L (50% percentile), intermediate = 1−2.5 μg/L (75% percentile), high = 2.5−6.93 μg/L (95% percentile), and very high >6.93 μg/L (99% percentile). This classification is used in our work to describe the bottled water analyzed the present study according to their Total PAEs concentrations.

3.2. Bottled water hydrochemical signatures

The results obtained for the four bottled water brands in the present study (Table A.2, Appendix A) are in very good agreement with data previously reported by Daniele et al. (2019). As can be inferred from Figure 1, the types of bottled water studied can be classified as follows: 1) Brand 1 is a Ca-Sulphate-Bicarbonate water with high Na and Cl concentrations, 2) Brand 2 is a Ca-Na-Bicarbonate water, 3) Brand 3 is a Na-Chloride-Sulphate water, and 4) Brand 4 is a Ca-Chloride water. As can be deduced from the electrical conductivity values and the elemental concentrations, the two mineral types of water (i.e., Brand 1 and Brand 2) have higher total dissolved concentrations, whereas the two purified water brands (i.e., Brand 3 and Brand 4) have much lower total dissolved concentrations. In accordance with the data presented by Daniele et al., (2019) these types of water show a decrease in their mineralization degree, moving from low mineralization (Brand 1), to very low mineralization (Brand 2 and Brand 4) and oligomineral (Brand 3). As expected, the addition of CO_2 gas to bottled water affected its pH (decreasing it from values ranging 6-8 to a range of 4-5) but not its elemental composition (Figure 1). The possible influence that these very different hydrochemical conditions may have on the concentration of phthalates is discussed in section 4.1.

3.3. Bottled water phthalate fingerprints

Regarding the concentrations of phthalates in the samples in the present study, from the 8 studied compounds, (i.e., **DEP, DBP, DEHP, DEHA, DOP**, DMP, DPP, and BBP), only the first 5 (in bold) were quantified in all the bottled water brands (Figure 3). Generally speaking, DEHP, DBP and DEP are the three major phthalates in all mineral and purified water. On the other hand, DEHA and DOP can be considered to be minor constituents of these waters and were even not detected in a few cases. Notice that the terms major and minor are used here to categorize phthalates within the reality of these water brands, in which all phthalates are already present in trace concentrations if compared with the other dissolved elements (Figure 1).

Seeking to define the phthalate fingerprint of each bottled water brand, the results are discussed in more detail by brand:

- Brand 1: both still and sparkling water characterized by very high concentrations of Total PAEs. Still water, compared with sparkling water, has higher concentrations of all the quantified phthalates, except DEHP. Therefore, following a similar logic as the hydrochemical categorization used in the present study, still water could be categorized as a DEP-DBP-type water with very high Total PAEs, while sparkling water could be labeled as a DEHP-type water with very high Total PAEs.

- Brand 2: both still and sparkling water with an intermediate Total PAEs concentration. All 5 phthalates were detected in both types of water but at low concentrations. Both still and sparkling water could be labeled as DEHP-type water with intermediate Total PAEs.
- Brand 3: still water with and intermediate to high Total PAEs concentration, whereas sparkling water has a low to intermediate Total PAEs concentration. Only three (i.e., DEP, DBP and DEHP) of the 5 typical phthalates were detected in both types of water but at low concentrations. Still water can be categorized as a DEP-type water with intermediate/high Total PAEs, while sparkling water can be labeled a DEHP-type water with low/intermediate Total PAEs.
- Brand 4: still and sparkling waters with quite different phthalates concentrations. The five main phthalates were detected in almost all the samples, although DEHP was the main detected phthalate and predominantly controlled the obtained Total PAEs values. Still water can be considered a DEHP-type water with a high Total PAEs concentration, while sparkling water can be categorized as a DEHP-DBP-type water with a very high Total PAEs concentration.

In addition, despite the high levels of Total PAEs found in the studied Chilean bottled water, if those samples are compared with the DEHP threshold limits proposed by the WHO (WHO, 2008) or the US FDA (U.S. FDA, 2012), only some Brand 4 sparkling water samples are slightly over both limits and most Brand 1 sparkling water samples are over the US FDA limit. All the other water samples are clearly and safely below these international regulatory threshold limits.

4. Discussions

4.1. Hydrochemical control of the bottled water phthalate fingerprint

It is important to observe that almost all the analyzed elements showed a very narrow range of concentration for each bottled water brand (Figure 1 and Table A.2, Appendix A), and no significant variations were observed during the aging experiment (Figures B.1 to B.8, Appendix B). The very small aleatory fluctuations observed in the concentrations of most elements did not exhibit any increasing or decreasing trend, and could be attributed to the natural fluctuations of the water composition at the bottling source. As a result, it can be assumed that the water chemistry (including the main physicochemical parameters as well as the major, minor and trace elements concentrations) of each bottled water brand remained virtually constant during the aging experiment.

By means of PCA, the weight that the main experimental variables had on the phthalate concentration during the aging experiment was studied. To this end, the following variables were considered: pH, electrical conductivity (µS/cm), storage time (days), and Total PAEs (µg/L). Water electrical conductivity is highly correlated with the main dissolved elements (both cations and anions) and can be considered a perfect proxy summarizing the general water chemical composition (McCleskey et al., 2012). This reduction of the database also responds to the need to not have any missing values (not all elements and phthalates were quantified in all samples) in any of the variables, so the PCA study could be properly performed using the 97 water samples available.

As shown in Figure 4a, 94.54% of the original variance in the dataset can be represented by three factors. F1 clearly controls the variability of both electrical conductivity and Total PAEs, whereas F2 and F3 better explain the variability exhibited by storage time and pH value, respectively. Taking also into account the F1, F2 and F3 loading factors for Total PAEs (0.879, -0.221 and 0.016, respectively), it can be inferred that the water elemental composition (represented by the electrical conductivity) strongly controls the initial generation of the water phthalate fingerprint, while storage time has a small effect on Total PAEs variability and water pH does not affect either the Total PAE initial fingerprint or evolution of water over time.

If the database is split into still and sparkling water and each set of samples is submitted to a PCA study, it can be observed how the Total PAEs in still water show the same behavior previously discussed for all the water brands. However, the sparkling water samples show some relevant differences. On the one hand, F1 (61.23%) clearly controls the variability of the electrical conductivity and also the variability of the pH values, while F2 (23.85%) perfectly explains the variability of the storage time. However, the Total PAEs loading factor is 0.707 and 0.477 for F1 and F2, respectively. Therefore, although most of the variability of Total PAEs is explained by F1 (as in the study comprising all the samples), an important amount of the variability is also explained by F2. As a result, the variability of Total PAEs in sparkling water is mainly due to the initial water elemental composition and pH but it is also affected by the storage time.

Once the main principal components have been assigned and discussed, it is very useful to also examine the distribution and tendencies of both the samples and the variables of a data matrix by means of a bi-plot graphic. As shown in Figure 5, single observations (i.e., individual bottled waters) are plotted by groups that reflect the general hydrochemical and phthalate fingerprints of each water brand. For example, because the Brand 1 samples are characterized by high electrical conductivity and a high Total PAEs concentration, they are located on the very right of the F1 axis (i.e., higher F1 factors scores with F1 controlling electrical conductivity and Total PAEs). On the contrary, because the Brand 3 samples are oligomineral, with low electrical conductivity and a low Total PAEs concentration, they are plotted on the very left of the bi-plot graphic (i.e., negative factor scores for F1).

4.2. Influence of the storage time on the bottled water phthalate concentration.

The bi-plot graphic in Figure 5 also shows that the individual observations are distributed following some discernible patterns. On the one hand, Brand 1 still and Brand 4 sparkling waters show a significant tendency (along the aging experiment) to move to higher positive and negative factor scores for F1 and F2, respectively (marked by red dotted arrows) (Figure 5). On the other hand, Brand 3 still and sparkling waters showed a very different tendency of moving to higher negative factor scores for both F1 and F2 (marked by green dotted arrows) (Figure 5). These behaviors could be explained by a tendency to increase (Brand 1 still and Brand 4 sparkling) or decrease (Brand 3 still and sparkling) the Total PAEs concentration as the storage time increased. All the other water samples (i.e., Brand 2 still and sparkling and Brand 4 still) showed a more subtle tendency that could be interpreted as a slight tendency toward increasing Total PAEs as the storage time increased (Figure 5).

Following the previous observations, the studied bottled waters were organized into four different categories depending on the Total PAEs ratio (R_{TPAEs}) at the end of the aging experiment (Total PAEs concentration the last day of the experiment divided by Total PAEs concentration the first day of the experiment).

Using this categorization, the influence that the storage time had on individual phthalate water concentrations was also discussed:

- 1) High phthalate concentrations increase ($R_{TPAEs} \geq 4$ and red dotted arrows in Figure 5): it can be observed how the Total PAEs concentrations of both waters show a fluctuating increase from initial values of 5.8 and 4.6µg/L to final values of 24.8 and 20.6 µg/L after 441 and 501 days of storage (Figure 6). These increments of the Total PAEs concentration are mainly due to the increase over time of the DEP and DBP concentrations and DEHP and DBP concentrations for Brand 1 still and Brand 4 sparkling waters, respectively.
- 2) Moderate phthalate concentrations increase ($1.5 \leq R_{TPAEs} < 4$ and orange dotted arrows in Figure 5): Three waters are included in this category, Brand 1 (sparkling) and Brand 2 (still and sparkling). These three type of waters show very similar R_{TPAEs} values, approximately 2, with the important difference that the increment of Total PAEs in Brand 1 doubles the already high initial concentration from 8.2 to 16.9 µg/L in 324 days; whereas Brand 2 waters (still and sparkling) increase by 1.6 and 2 times from their low initial concentrations of 1.5 and 1.4 µg/L to 2.3 and 2.8 µg/L in 457 and 452 days, respectively (Figure 6).
- 3) No phthalate concentrations increase or decrease ($R_{TPAEs} \approx 1$ and yellow dotted arrow in Figure 5): only Brand 4 still water is included in this category, for which no increasing or decreasing tendency can be observed for any of the detected phthalates.
- 4) Phthalate concentrations decrease ($R_{TPAEs} \approx 0.5$ and green dotted arrows in Figure 5): both still and sparkling Brand 3 waters fell within this category, in which

a decrease in Total PAEs with increasing storage time can be observed. These waters started the aging experiment with already low phthalate concentrations that decreased by one-half, showing final values of 2.2 and 0.6 μg/L after 432 and 448 storage days for still and sparkling waters, respectively.

It is very relevant to observe that increasing or decreasing processes that affect the phthalate concentration in bottled water showed different delay times to modify the initial phthalates concentrations in a substantial way (Figure 6). Specifically, if doubling the initial Total PAEs concentration is considered a clearly discernible increment, some bottled waters achieved this increment just after 259 days (i.e., Brand 1 still) whereas others reached such increase after much longer storage periods like 453 days or even more (i.e., Brand 4 sparkling). On the other hand, it takes approximately 400 days of storage to observe a clear decrease in the phthalate concentration.

5. Conclusions

The following main conclusions can be drawn from the present study:

-The studied Chilean bottled waters showed intermediate to very high concentrations of Total PAEs. However, only some Brand 4 sparkling waters are slightly over the WHO (WHO, 2008) and the US FDA (U.S. FDA, 2012) limits and most Brand 1 sparkling waters are over the US FDA limit.

- The following 5 phthalates fingerprints were defined: 1) DEP-DBP-type waters with very high Total PAEs (Brand 1 still), 2) DEHP-type waters with very high Total PAEs (Brand 1 sparkling), 3) DEHP-type waters ranging from low to high Total PAEs (Brand 2 still and sparkling, Brand 3 sparkling, and Brand 4 still), 4) DEP-type waters with intermediate/high Total PAEs (Brand 3 still), 5) DEHP-DBP-type waters with very high Total PAEs (Brand 4 sparkling).

- It was confirmed, for the first time, that the water elemental composition (not pH) strongly controls the initial generation of the water phthalate fingerprint. As a result, higher water mineralizations induce higher Total PAEs concentrations. The present results also suggest that water pH (more acid for sparkling waters and neutral for still waters) may have some effect on the relative proportion of the detected phthalates but (in most cases) does not affect the Total PAEs concentration of waters of the same brand.
- Long storage times (more than a year) may increase, decrease or not affect the water phthalate concentrations at all. These observations confirm that the discrepancies of the many papers on this topic are because all trends are possible. Specifically, although most waters showed some increments over time on their Total PAEs concentration (with clearly discernible increments taking place between 259 and 453 days of storage, depending on the specific bottled water); some waters with a very low initial mineralization and Total PAEs concentration showed a late decrease in their final Total PAEs concentration (approximately after 400 days of storage). As a result, if the bottled waters are consumed before their expiration date (typically one year), then their Total PAEs concentration will mostly depend on the water initial hydrochemical signatures and phthalate fingerprint.

Acknowledgments

The authors would like to thank FONDECYT (Postdoctoral Projects 3210084 and 3160208, Initiation Project 11190579 and Regular Project 1180742), FONDEQUIP

EQM170141, ANID (Project PAI79170018 and Doctoral fellowship 21200317), and Ramón y Cajal Project RYC2019-026496-I for financial support.

Supporting information description

The following information is included supporting the discussions on the main manuscript: **APPENDIX A. Table A.1.** Information offered in 15 previous manuscripts (e.g., country, storage time, pH, conductivity and specific and Total PAEs concentrations). **Table A2.** Brands (still and sparkling), storage time (days), phthalate concentrations, pH, electrical conductivity, and elemental composition of all samples studies: Brand 1, Brand 2, Brand 3, and Brand 4.

APPENDIX B. Figure B.1. Variations in some elements concentration variations during the aging experiment in Brand 1 still waters. **Figure B.2.** Variations in some elements concentration variations during the aging experiment in Brand 1 sparkling waters. **Figure B.3.** Variations in some elements concentration variations during the aging experiment in Brand 2 still waters. **Figure B.4**. Variations in some elements concentration variations during the aging experiment in Brand 2 sparkling waters. **Figure B.5**. Variations in some elements concentration variations during the aging experiment in Brand 3 still waters. **Figure B.6**. Variations in some elements concentration variations during the aging experiment in Brand 3 sparkling waters. **Figure B.7**. Variations in some elements concentration variations during the aging experiment in Brand 4 still waters. **Figure B.8**. Variations in some elements concentration variations during the aging experiment in Brand 4 sparkling waters. **References used in Figure 2 of the main manuscript and in Table A1.**

Declaration of interest

Declarations of interest: none.

References

Abtahi, M., Dobaradaran, S., Torabbeigi, M., Jorfi, S., Gholamnia, R., Koolivand, A., Darabi, H., Kavousi, A., Saeedi, R., 2019. Health risk of phthalates in water environment: occurrence in water resources, bottled water, and tap water, and burden of disease from exposure through drinking water in Tehran, Iran. Environ. Res. 173, 469–479.

Akhbarizadeh, R., Dobaradaran, S., Schmidt, T.C., Nabipour, I., Spitz, J., 2020. Worldwide bottled water occurrence of emerging contaminants: A review of the recent scientific literature. J. Hazard. Mater. 392, 122271.

Arfaeinia, H., Nabipour, I., Ostovar, A., Asadgol, Z., Abuee, E., Keshtkar, M., Dobaradaran, S., 2016. Assessment of sediment quality based on acid-volatile sulfide and simultaneously extracted metals in heavily industrialized area of Asaluyeh, Persian Gulf: concentrations, spatial distributions, and sediment bioavailability/toxicity. Environ. Sci. Pollut. Res. 23(10), 9871-90. doi: 10.1007/s11356-016-6189-0.

Bach, C., Dauchy, X., Chagnon, M.C., Etienne, S., 2012. Chemical compounds and toxicological assessments of drinking water stored in polyethylene terephthalate (PET) bottles: A source of controversy reviewed. Water Res. 46, 571–583.

Becerra-Herrera, M. Honda, L., Richter, P., 2015. Ultra-high-performance liquid chromatography-Time-of-flight high resolution mass spectrometry to quantify acidic drugs in wastewater. J. Chromatogr. A 1423, 96–103.

Błędzka, D., Gromadzińska, J., Wąsowicz, W., 2014. Parabens. From environmental studies to human health. Environ. Int. 67, 27–42.

Cao, X-L., 2008. Determination of phthalates and adipate in bottle water by headspace solid-phase microextraction and gas chromatography/mass spectrometry. J. Chromatogr. A 1178, 231–238.

Casals-Casas, C., Desvergne, B., 2011. Endocrine disruptors: from endocrine to metabolic disruption. Annu. Rev. Physiol. 73, 135–62.

CEC (Commission of the European Communities): Brussels, 2007. Commission StaffWorking Document on the Implementation of the Community Strategy for Endocrine Disrupters-A Range of Substances Suspected of Interfering with the Hormone Systems of Humans and Wildlife;

Daniele, L., Cannatelli, C., Buscher, J.T., Bonatici G., 2019. Chemical composition of Chilean bottled waters: Anomalous values and possible effects on human health. Sci. Total Environ. 689, 526–533.

Dreolin, N., Aznar, M., Moret, S., Nerin, C. 2019. Development and validation of a lc-ms/ms method for the analysis of bisphenol a in polyethylene terephthalate. Food Chemistry 274, 246–253.

EU (2011) Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come in contact with food. Official Journal of the European Communities.

Giuliani, A., Zuccarini, M., Cichelli, A., Khan, H., Reale, M. 2020. Critical Review on the Presence of Phthalates in Food and Evidence of Their Biological Impact. Int. J. Environ. Res. Public Health, 17, 5655.

Guart, A., Bono-Blay, F., Borrell, A., & Lacorte, S., 2014. Effect of bottling and storage on the migration of plastic constituents in Spanish bottled waters. Food Chem. 156, 73–80.

Hites, R.A., 2019. Correcting for Censored Environmental Measurements. Environ. Sci. Technol. 53, 11059−11060.

Jayaweera, M., Perera, H., Bandara, N., Danushika, G., Gunawardana, B., Somaratne, C., Manatunge, J., Zoysa, K., Thathsara, T. 2020. Migration of phthalates from PET water bottle in events of repeated uses and associated risk assessment. Environ. Sci. Pollut. Res., 27, 39149–39163.

Jeddi, M. Z., Rastkari, N., Ahmadkhaniha, R., Yunesian, M., 2015. Concentrations of phthalates in bottled water under common storage conditions: Do they pose a health risk to children?. Food Research International 69, 256-265.

Jeddi, M. Z., Rastkari, N., Ahmadkhaniha, R., Yunesian, M. 2016. Endocrine disruptor phthalates in bottled water: daily exposure and health risk assessment in pregnant and lactating women. Environmental monitoring and assessment, 188(9), 534.

Keresztes, S., Tatár, E., Czégény, Z., Záray, G., & Mihucz, V. G., 2013. Study on the leaching of phthalates from polyethylene terephthalate bottles into mineral water. Science of the total environment, 458-460, 451-458.

Koelmans, A.A., Mohamed Nor, N.H., Hermsen, E., Kooi, M., Mintenig, S.M., De France, J., 2019. Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. Water Res. 155, 410–422.

Luo, Q., Liu, Z.H., Yin, H., Dang, Z., Wu, P.X., Zhu, N.W., Lin, Z., Liu, Y., 2018. Migration and potential risk of trace phthalates in bottled water: a global situation. Water Res. 147, 362–372.

Manzo, V., Becerra-Herrera, M., Arismendi, D., Molina-Balmaceda, A., Caraballo, M.A., Richter, P., 2019. Rotating-disk sorptive extraction coupled to gas chromatography mass spectrometry for the determination of phthalates in bottled water. Anal. Methods 11, 6111–6118.

McCleskey, R.B., Nordstrom, D.K., Ryan, J.N., Ball, J.W., 2012. A new method of calculating electrical conductivity with applications to natural waters. Geochim. Cosmochim. Acta. 77, 369–382.

Montuori, P., Jover, E., Morgantini, M., Bayona, J. M., Triassi, M., 2008. Assessing human exposure to phthalic acid and phthalate esters from mineral water stored in polyethylene terephthalate and glass bottles. Food Addit. Contam. Part A 25:4, 511-518.

Mousa, A., Basheer, C., Al-Arfaj, A. R. 2013. Determination of phthalate esters in bottled water using dispersive liquid–liquid microextraction coupled with GC-MS. Journal of Separation Sci. 36(12), 2003–2009.

Otero, P., Saha, S. K., Moane, S., Barron, J., Clancy, G., Murray, P. 2015. Improved method for rapid detection of phthalates in bottled water by gas chromatography-mass spectrometry. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences, 997, 229–235.

Salazar-Beltrán, D., Hinojosa-Reyes, L., Ruiz-Ruiz, E., Hernández-Ramírez, A., Luis Guzmán-Mar, J. 2017. Determination of phthalates in bottled water by automated on-line solid phase extraction coupled to liquid chromatography with uv detection. Talanta, 168, 291-297.

Serrano, S.E., Braun, J., Trasande, L., Dills, R., Sathyanarayana, S., 2014. Phthalates and diet: a review of the food monitoring and epidemiology data. Environ. Health 13(1), 43.

Schug, T.T., Janesick, A., Blumberg, B., Heindel, J.J., 2011. Endocrine disrupting chemicals and disease susceptibility. J. Steroid Biochem. Mol. Biol. 127, 204–15.

Szendi, K., Gyöngyi, Z., Kontár, Z., Gerencsér, G., Berényi, K., Hanzel, A. Fekete, J., Kovács, A., Varga, C. 2018. Mutagenicity and Phthalate Level of Bottled Water Under Different Storage Conditions. Expo Health 10, 51–60.

Thoden van Velzen, E. U. Brouwer, M. T., Stärker, C. Welle, F. 2020. Effect of recycled content and rPET quality on the properties of PET bottles, part II: Migration. Packag. Technol. Sci. 33, 359–371.

USEPA. U.S. Environmental Protection Agency, 2017. National Drinking Water Regulations. Retrieved from. Accessible at. https://www.epa.gov/dwstandardsregulations. (Last accessed: 05-18-2020).

U.S FDA, 2012. U.S. Food and Drug Administration. Guidance for Industry Bottled Water Quality Standard: Establishing an Allowable Level for di(2-ethylhexyl) phthalate Small Entity Compliance Guide. Accessible at. https://www.fda.gov/downloads/Food/GuidanceRegulation/UCM302173.pdf .

WHO, World Health Organization (WHO/SDE/WSH/03.04/68), 2004. Di(2-ethylhexyl) adipate in drinking water. Background document for preparation of WHO Guidelines for drinking-water quality. Geneve.

WHO, World Health Organization, 2008. Guidelines for Drinking-water Quality. Fourth Edition. Retrieved from. http://www.who.int/water_sanitation_health/dwq/fulltext.pdf.

Xu, X., Zhou, G., Lei, K., LeBlanc, G.A., An, L., 2020. Phthalate Esters and Their Potential Risk in PET Bottled Water Stored under Common Conditions. Int. J. Environ. Res. Public Health, 17, 141.

Yousefi, Z., Ala, A., Babanezhad, E., Mohammadpour, R.A., 2019. Evaluation of exposure to phthalate esters through the use of various brands of drinking water bottled in polyethylene terephthalate (PET) containers under different storage conditions. Environ. Health Eng. Manag. 6(4), 247–255.

Zaki, G., Shoeib, T., 2018. Concentrations of several phthalates contaminants in Egyptian bottled water: Effects of storage conditions and estimate of human exposure. The Science of the Total Environment 618, 142-150.

Figure caption

Figure 1. Box-plot diagrams showing the concentration distributions of the 5 major (HCO_3^-, SO_4^{2-}, Ca^{2+}, Cl^-, and Na^+) and 2 minor (Mg^{2+}, and Si^{4+}) elements, pH, and electrical conductivity during the experiment. B1 = Brand 1, B2 = Brand 2, B3 = Brand 3, B4 = Brand 4; no superscript = still water, * = sparkling water. Central marks indicate the median; the bottom and top edges of the boxes indicate the 25th and 75th percentiles, respectively; whiskers extend to the 5th and 95th percentiles; and data out of this last range (outliers) are plotted individually using circles.

Figure 2. Total PAEs histogram and box-plot graphic generated from the 127 samples in the Total PAEs bibliographic database. Colors are used to categorize the samples as having low (green), intermediate (yellow), high (orange) and very high (red) Total PAEs contents using the 50%, 75% and 95% percentiles, respectively, as cutoff lines.

Figure 3. Box-plots showing the concentration distributions of the 5 phthalates under study during this experiment. B1 = Brand 1, B2 = Brand 2, B3 = Brand 3, B4 = Brand 4; no superscript = still water, * = sparkling water. The same abbreviations and comments used in Figure 1 apply to this figure. The DEHP threshold values from the World Health Organization (WHO) and the U.S. Food and Drug Administration (FDA) are included. The same scale for Total PAEs previously defined in section 3.2 is applied to the Total PAEs graphic.

Figure 4. Principal component analysis plotting the loadings values obtained for the first and second and first and third factors. Upper graphics (a) were obtained using all the water samples whereas lower graphics (b) were generated using just the still waters.

Figure 5. Bi-plot graphic showing both the samples and main variables of the bottled water database in the present study. Empty symbols correspond to still water, whereas grey

filled symbols are sparkling water. The large black dots mark the position in the graphic of the 4 main variables of the study as a function of F1 and F2.

Figure 6. Phthalate concentration variations during the aging experiment. No superscript = still water, * = sparkling water.

Figure 1

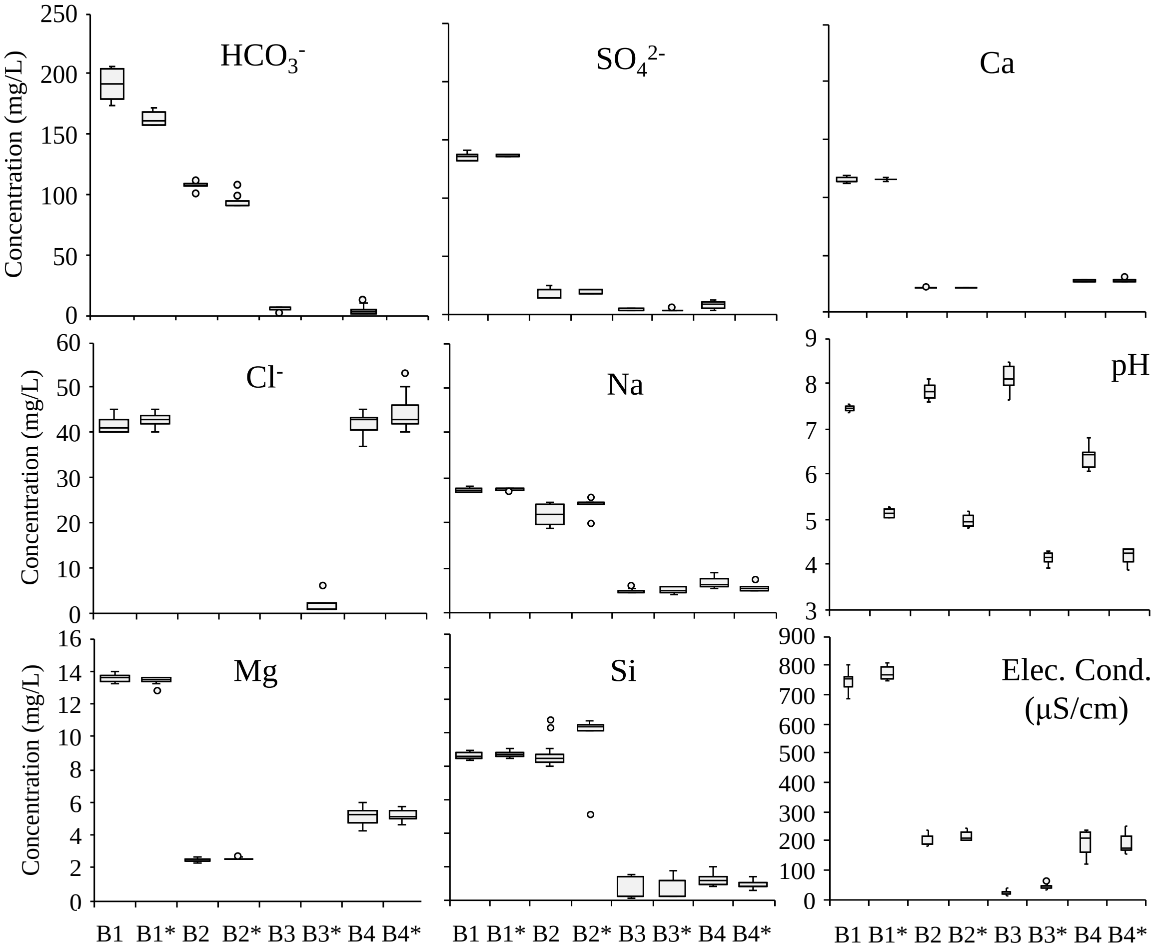

HCO$_3^-$
SO_4^{2-}
Ca
Cl$^-$
Na
pH
Mg
Si
Elec. Cond. (µS/cm)
Concentration (mg/L)
Concentration (mg/L)
Concentration (mg/L)
250
200
150
100
50
0
60
50
40
30
20
10
0
16
14
12
10
8
6
4
2
0
9
8
7
6
5
4
3
900
800
700
600
500
400
300
200
100
0
B1 B1* B2 B2* B3 B3* B4 B4*
B1 B1* B2 B2* B3 B3* B4 B4*
B1 B1* B2 B2* B3 B3* B4 B4*

Figure 2

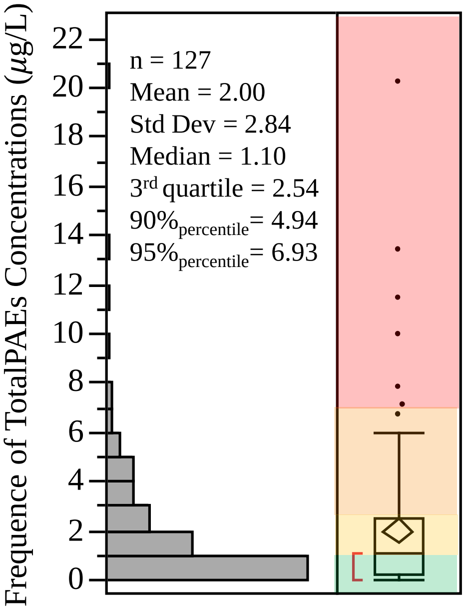

Frequence of TotalPAEs Concentrations (μg/L)
22
20
18
16
14
12
10
8
6
4
2
0
n = 127
Mean = 2.00
Std Dev = 2.84
Median = 1.10
3rd quartile = 2.54
90%percentile= 4.94
95%percentile= 6.93

Figure 3

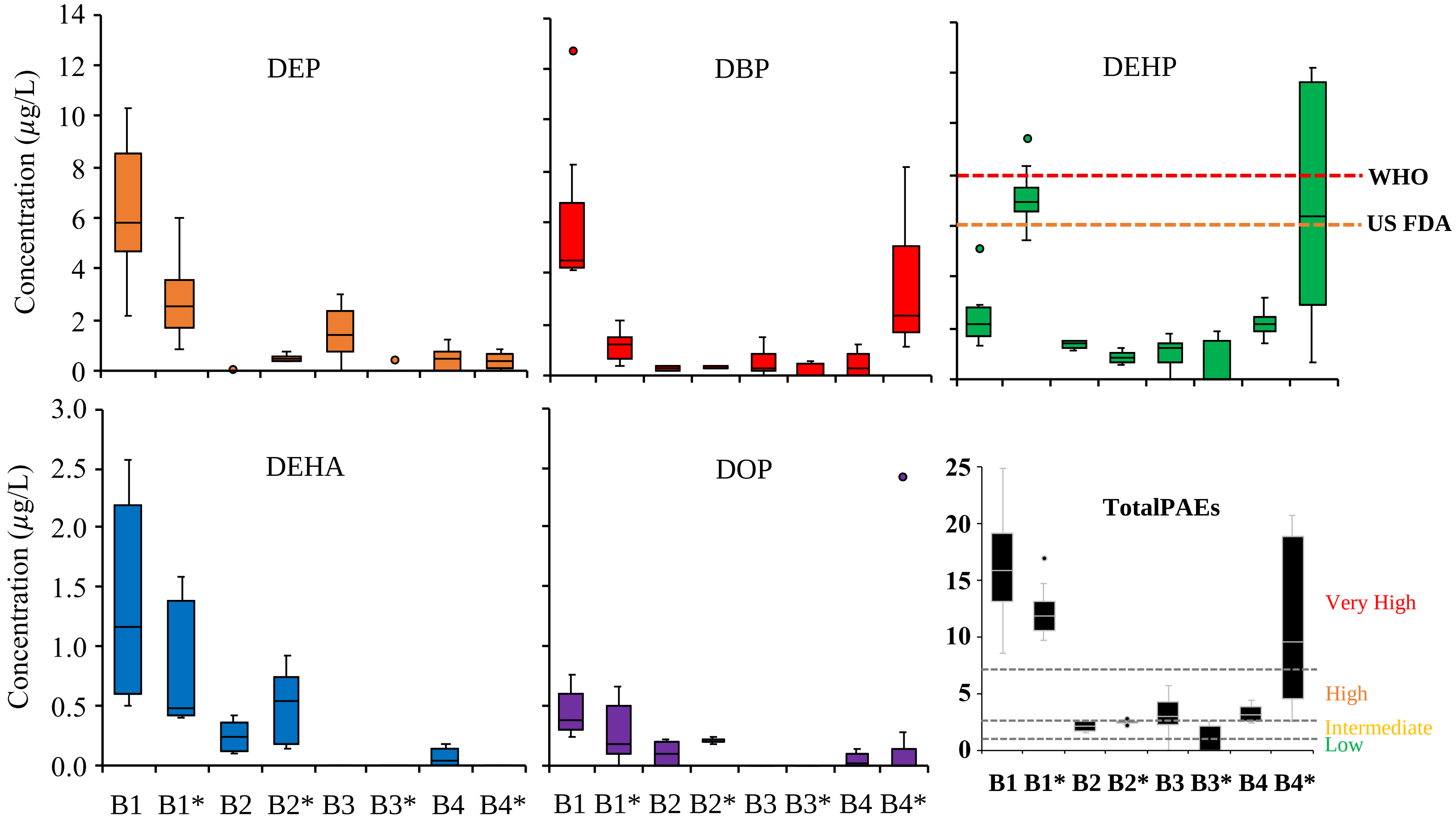

DEP
DBP
DEHP
WHO
US FDA
DEHA
DOP
TotalPAEs
Very High
High
Intermediate
Low
Concentration (μg/L)
Concentration (μg/L)
14
12
10
8
6
4
2
0
3.0
2.5
2.0
1.5
1.0
0.5
0.0
25
20
15
10
5
0
B1 B1* B2 B2* B3 B3* B4 B4*
B1 B1* B2 B2* B3 B3* B4 B4*
B1 B1* B2 B2* B3 B3* B4 B4*

Figure 4

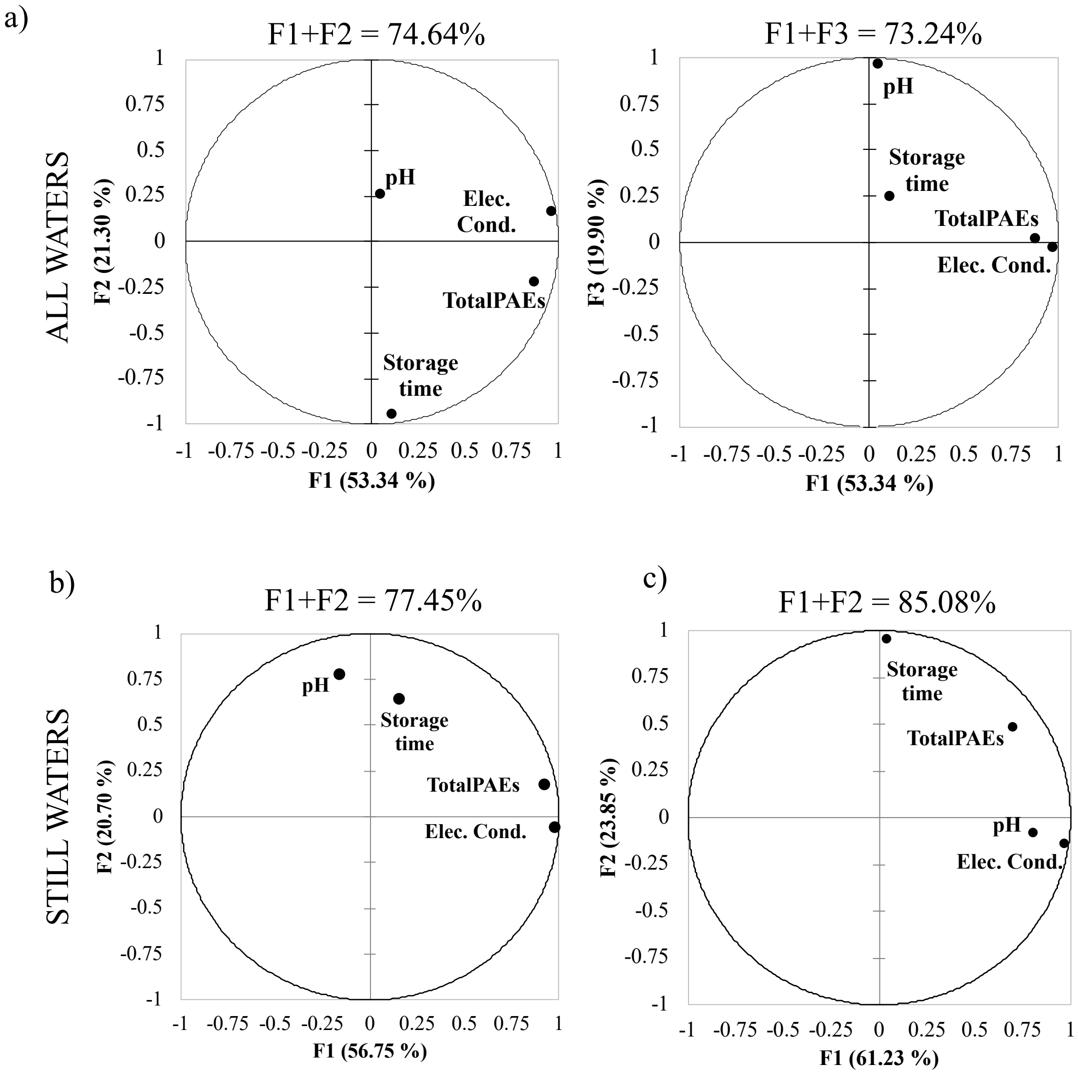


Figure 4. Principal component analysis plotting the loadings values obtained for the first and second and first and third factors. Upper graphics (a) were obtained using all the water samples whereas lower graphics (b) were generated using just the still waters.

Figure 5

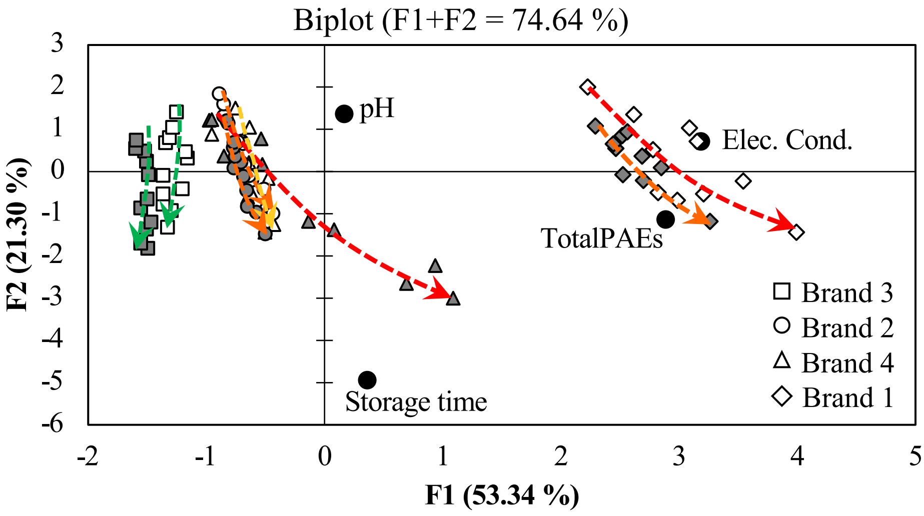
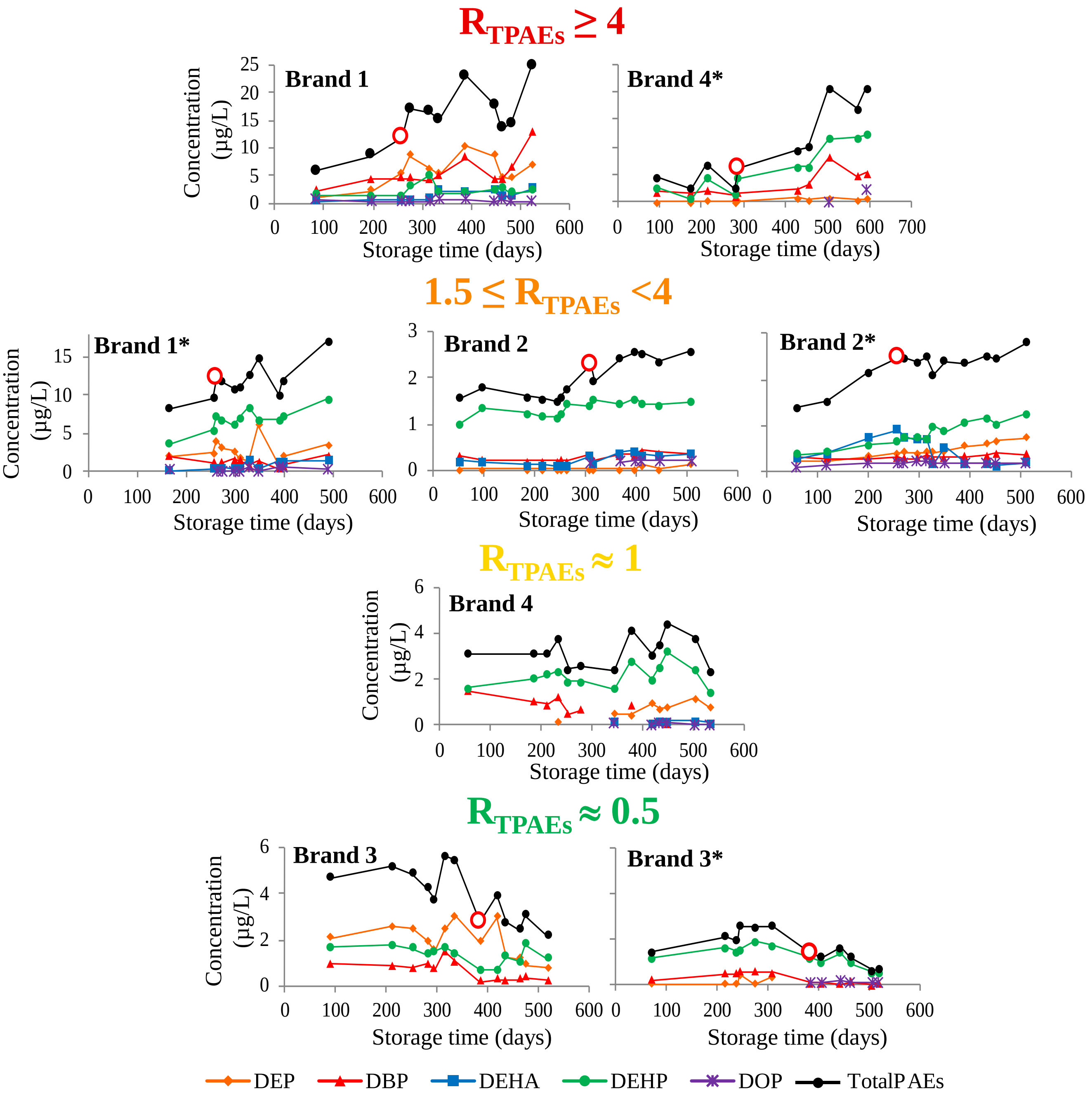

Biplot (F1+F2 = 74.64 %)
F2 (21.30 %)
F1 (53.34 %)
3
2
1
0
-1
-2
-3
-4
-5
-6
-2
-1
0
1
2
3
4
5
pH
Elec. Cond.
TotalPAEs
Storage time
□ Brand 3
○ Brand 2
△ Brand 4
◇ Brand 1

Figure 6


R_TPAEs ≥ 4
Brand 1
Brand 4*
Concentration (µg/L)
25
20
15
10
5
0
0 100 200 300 400 500 600
0 100 200 300 400 500 600 700
Storage time (days)
1.5 ≤ R_TPAEs <4
Brand 1*
Brand 2
Brand 2*
15
10
5
0
3
2
1
0
R_TPAEs ≈ 1
Brand 4
6
4
2
0
R_TPAEs ≈ 0.5
Brand 3
Brand 3*
DEP
DBP
DEHA
DEHP
DOP
TotalPAEs