

Supplementary Material

The unique chemical and microbiological signatures of an array of bottled drinking water

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1 Supplementary Figures and Tables

1.1 Supplementary Figures

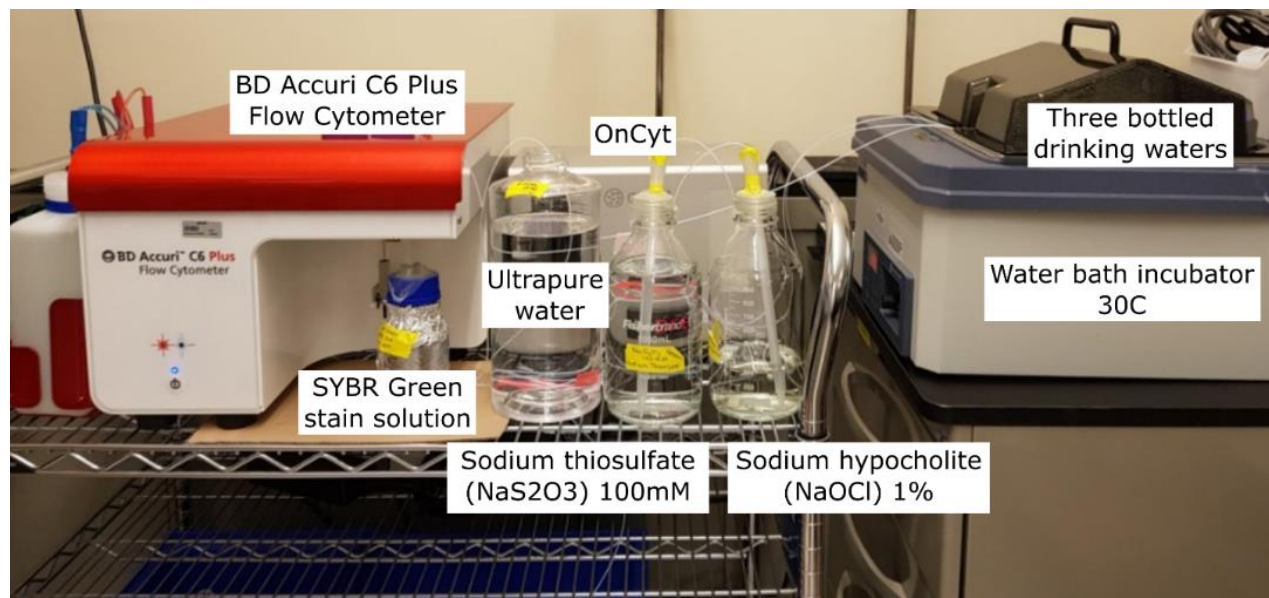


Figure S1: Online flow cytometry system consisting of OnCyt, BD Accuri C6 Plus, and water bath incubator for assessing the microbial growth of bottled drinking waters. Microbial cells in samples are stained with SYBR Green. NaS₂O₃, NaOCl, and ultrapure water were, sequentially, used to clean the system in between samples.

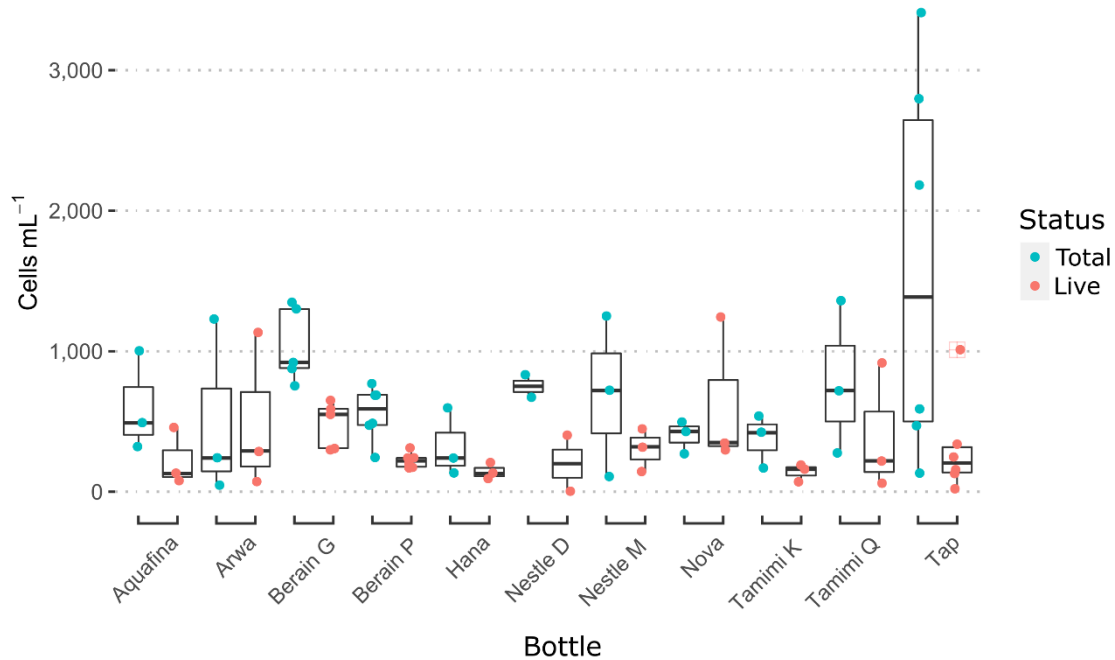


Figure S2: Purified water and tap microbial cell concentrations as measured through flow cytometry with at least three replicates per sample. Each point denotes one replicate.

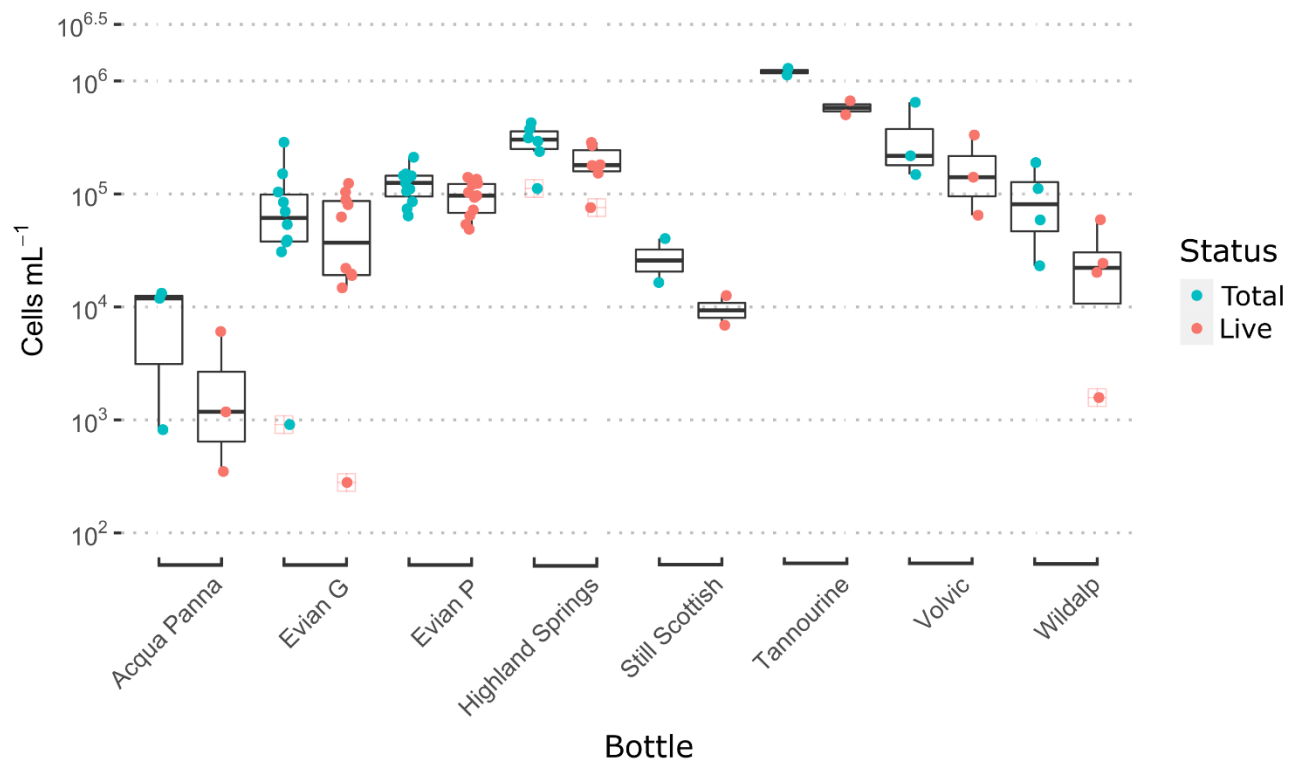


Figure S3: Mineral water microbial cell concentrations, in logarithmic scale, as measured through flow cytometry with at least two replicates per sample. Each point denotes one replicate. Cell values are noted for samples in the low range box plots.

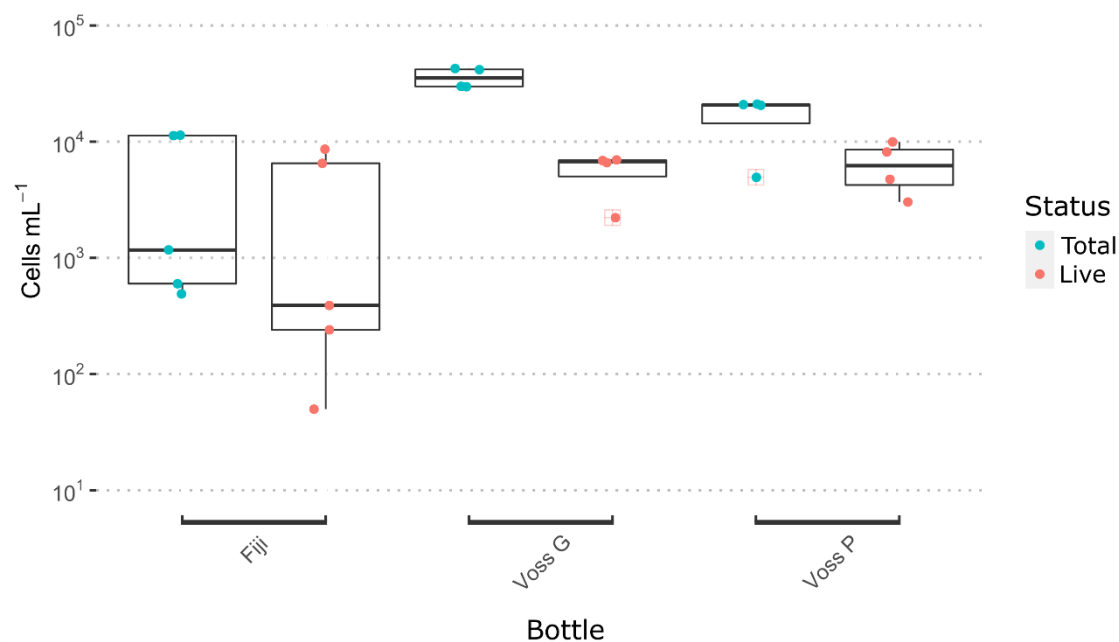


Figure S4: Artesian water microbial cell concentrations, in logarithmic scale, as measured through flow cytometry with at least three replicates per sample. Each point denotes one replicate.

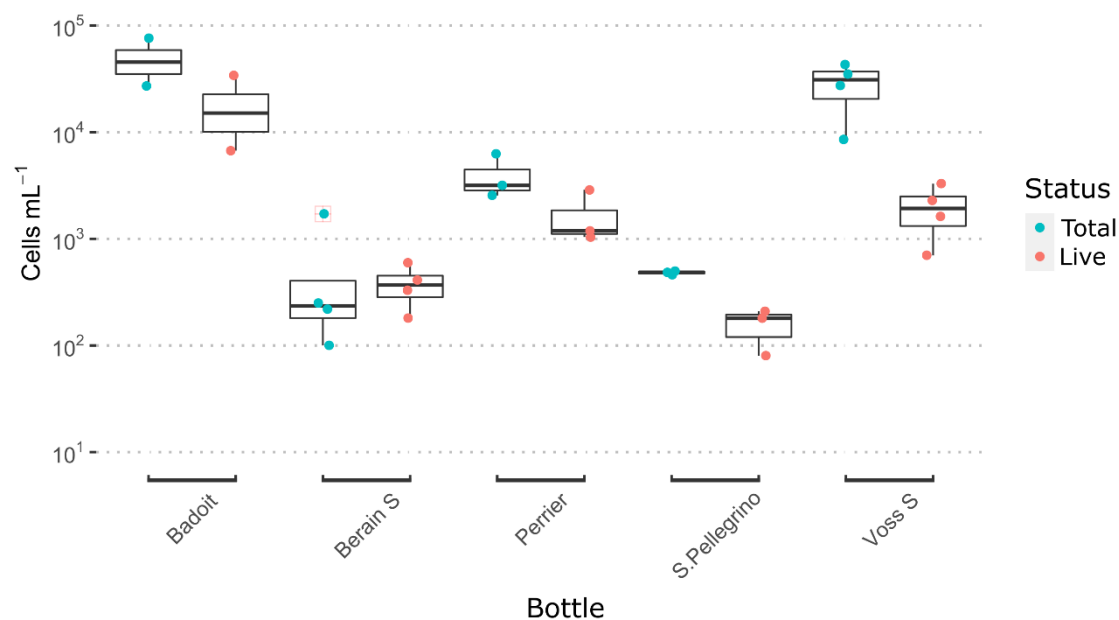


Figure S5: Sparkling water microbial cell concentrations, in logarithmic scale, as measured through flow cytometry with at least two replicates per sample. Each point denotes one replicate.

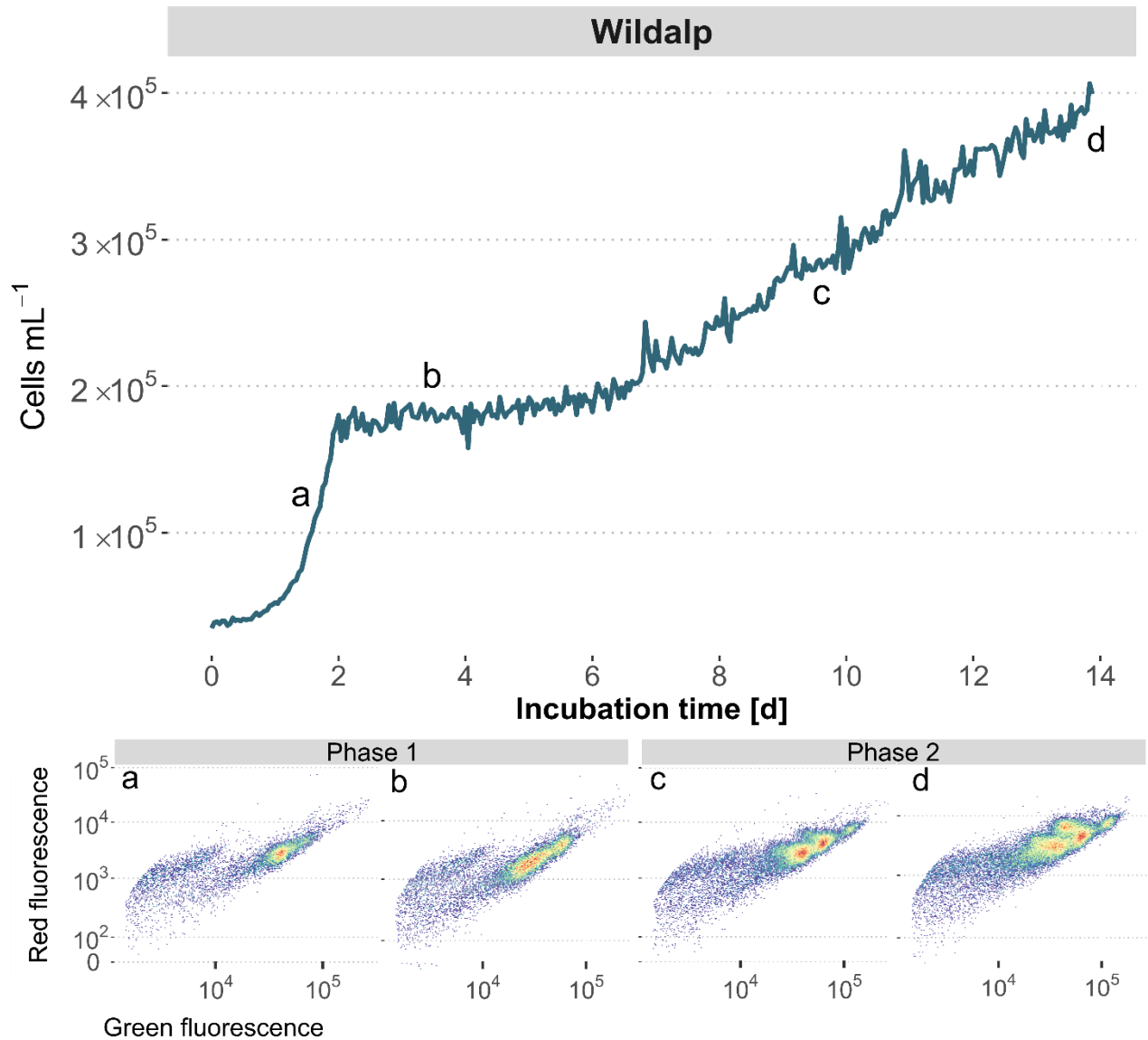


Figure S6: Growth potential test for mineral bottled water sample [Wildalp], with the entire incubation run in two weeks at 30°C , illustrating a diauxic microbial growth phase occurring during the second week. Online flow cytometry plots, or fingerprints, showcase the changing microbial community distribution after a second growth phase. The sample's volume limited the incubation period. The dot plots illustrate only the gated region.

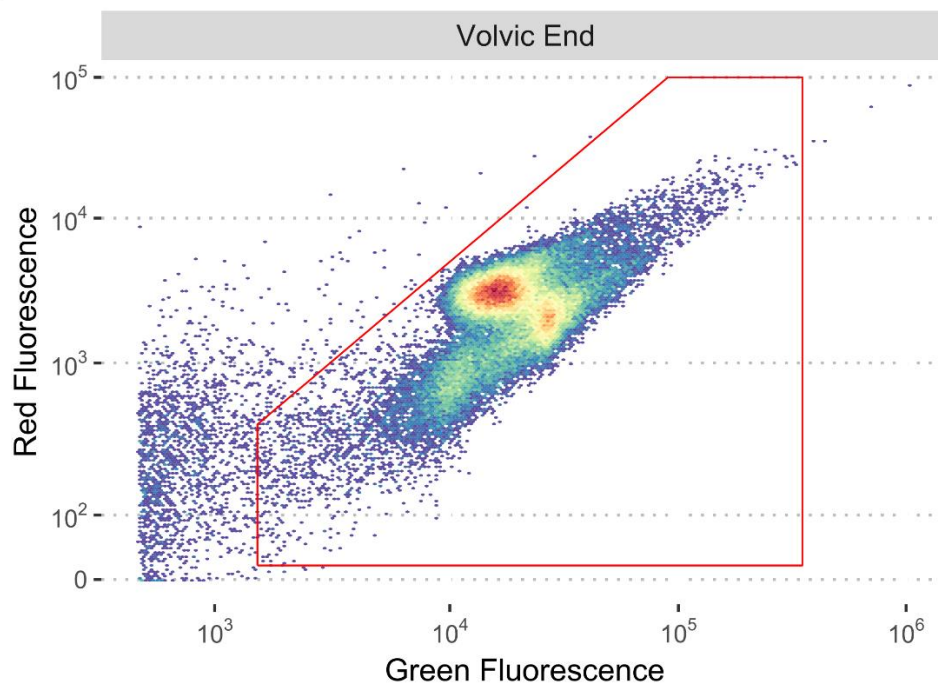


Figure S7: A flow cytometry dot plot indicating the electronic gating used to analyze the data (Hammes et al., 2008). This dot plot presents the mineral water sample Volvic at the end of incubation during the microbial growth experiments, also seen in Figure 6.

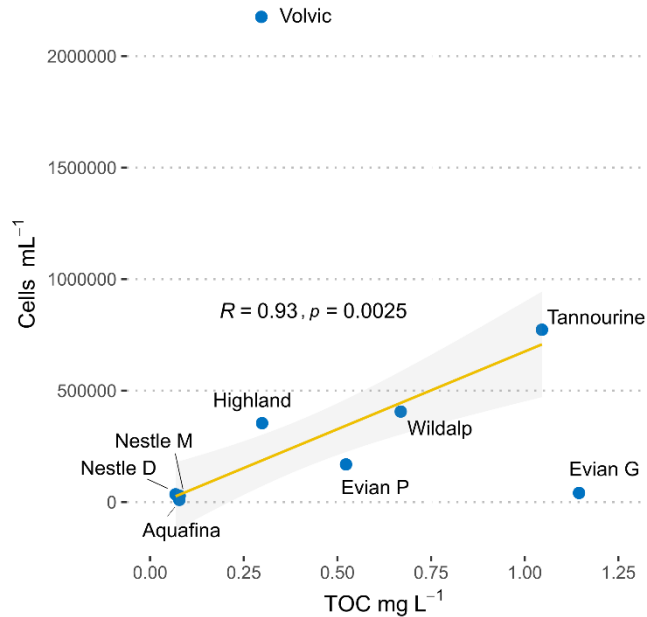


Figure S8: Linear regression showing the correlation between total organic carbon (TOC) and the maximum cell growth in samples analyzed during microbial growth potential tests (Chapters 3.4 – 3.5). Most samples fit the correlation ($R^2 = 0.93$), indicating a relationship between TOC and microbial growth. However, Volvic, with low TOC but high growth, and Evian, with high TOC but low growth, did not conform to the linear trend.

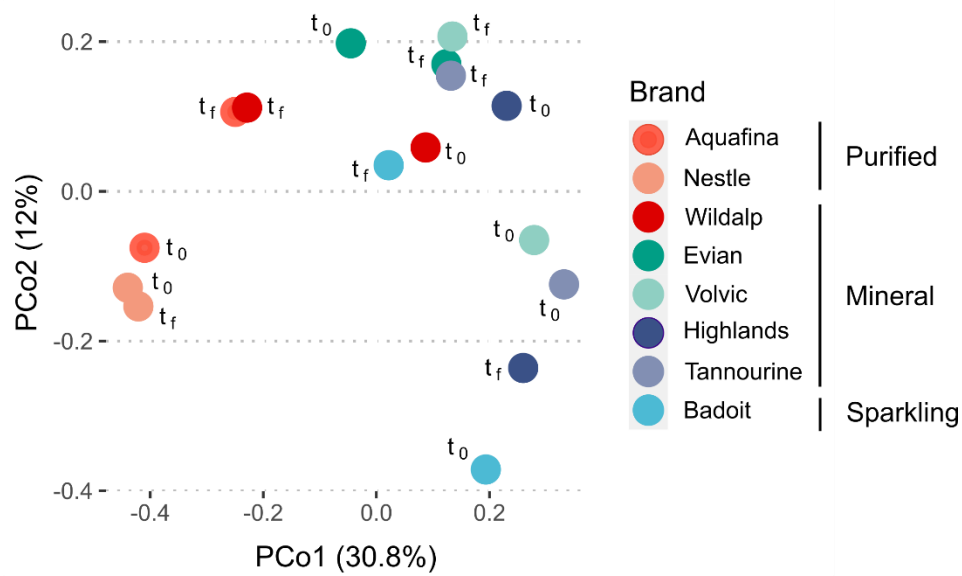


Figure S9: Ordination of phenotypic fingerprints measured by flow cytometry in bottled waters before incubation (t_0) and after incubation for several days when an apparent maximum growth was reached (t_f). Variations are noted between distribution of purified bottled water samples clustering on one side, and mineral bottled water samples on the opposing side.

1.2 Supplementary Tables

Table S1: General information on all bottled water samples collected for analyses including brand name, bottle material, origin location, volume, and production and expiration dates as labeled on the bottles.

No. Brand	No. Bottles Overall	Brand Name	Bottle Material	Water Type	Water Source	Bottling Location, Country	Volume (mL)	Date: Production-Expiration
1	1	Acqua Panna	Glass	Natural mineral water	Toscana, Italy	Sanpellegrino S.p.A. Acqua Panna springs, Florence, Italy *	250	27/03/2019-01/03/2021
2	2	Aquafina	Plastic	Purified water	Groundwater	AlJomaih beverage bottling company. Buraidah, KSA	330	28/07/2020-27/07/2021
3	3	Arwa	Plastic	Purified water	Well water	Saudi Coca cola beverage bottling company. Sudair city, Riyadh, KSA	330	18/03/2020-18/03/2021
4	4	Badoit	Glass	Sparkling natural mineral water	Badoit source	SAEME. Saint Galmier, France	330	20/11/2018-19/11/2020
5	5	Berain	Plastic	Purified water	*	Berain company. Jeddah, KSA	330	22/05/2020-22/05/2021
	6		Glass			Berain company. Riyadh, KSA	300	10/03/2020-10/03/2021
	7		Glass	Sparkling water			750	23/12/2019-23/12/2020
6	8	Evian	Plastic	Natural mineral water	French Alps		330	01/10/2019-01/10/2021

No. Brand	No. Bottles Overall	Brand Name	Bottle Material	Water Type	Water Source	Bottling Location, Country	Volume (mL)	Date: Production-Expiration
	9		Glass			Cachat spring- S.A.E.M.E. Evian, France	330	17/07/2019- 17/07/2021
7	10	Fiji	Plastic	Natural artesian water	Yaqara, Viti Levu, Fiji Islands	Natural waters of viti limited. Yaraqa, Fiji	500	10/12/2019- 10/12/2020
8	11	Hana	Plastic	Purified water	Well water	Hana company for food industries. AlQassim– Buraidah, KSA	330	30/12/2019- 30/12/2020
9	12	Highland Springs	Plastic	Natural mineral water	Drawn from organic land in Scotland	Highland spring Ltd. Perthshire, Scotland, UK *	500	06/02/2020- 06/02/2021
10	13	Nestle Pure Life	Plastic	Purified water	Underground water or desalination water	Springs water factory co. Dammam, KSA	200	17/08/2020- 16/08/2021
	14				Desalination water	Naqiah water factory co. Madinah, KSA	330	16/09/2020- 15/09/2021
11	15	Nova	Plastic	Purified water	Underground well water– Saad City	Health water bottling company. Saad, KSA	200	unknown
	16				Well water– Nufoud Se'ed	HWB co. plant. Seed, KSA	330	22/03/2020- 22/03/2021
12	17	Perrier	Glass	Natural mineral water & CO ₂	Water captured at the Perrier source	N.W.S, SUD. Vergeze, France *	330	27/05/2020- 01/05/2022
13	18	S.Pellegrino	Glass	Sparkling natural mineral water	San Pellegrino, Italy	Sanpellegrino S.p.A. San Pellegrino Terme, localita Ruspino, Italy *	250	09/07/2020- 08/07/2022

No. Brand	No. Bottles Overall	Brand Name	Bottle Material	Water Type	Water Source	Bottling Location, Country	Volume (mL)	Date: Production-Expiration
14	19	Still Scottish Mountain Water by Sainsbury's	Plastic	Still mountain water, underground	Caledonian water-eastern edge of the Campsie Fells	Produced in the UK for Sainsbury's Supermarkets Ltd, London	500	n/a-07/01/2022
15	20	Tamimi Markets Bottled Drinking Water	Plastic	Purified water	Well water– Al Shadida Valley	Tania bottled drinking water factory. AlKharj, KSA	200	03/07/2019-03/07/2020
	21				Well water	Hana company for food industries. AlQassim– Buraidah, KSA	200	22/03/2020-22/03/2021
16	22	Tannourine	Plastic	Natural spring water	Daher & Sarkis Sources	Societe Libanaise des Souces des Eaux de Tannourine s.a.l Lebanon *	330	09/03/2020-09/03/2021
17	23	Volvic	Plastic	Natural mineral water	French Volcanoes	Clairvic spring. Volvic, France *	330	21/10/2019-21/10/2021
18	24	Voss	Plastic	Artesian natural mineral water	Artesian water	Voss production AS. Voss, Vantestrom, Norway *	330	19/12/2019-19/12/2021
	25		Glass				375	11/7/2019-11/7/2021
	26		Glass	Sparkling water			375	28/11/2019-28/11/2021
19	27	Wildalp	Plastic	Natural spring water	Hochschwab Massif spring water-Styrian Alps/Seisensteinquelle	Wildalpen Wasserverwertungs GmbH Sausenbach, Wildalpen, Austria	500	05/03/2019-05/03/2021

* Denotes that water was bottled at the source, as stated on bottle label

Table S2: Average mineral composition for each bottled drinking water samples as stated on bottle labels.

		Average Composition (mg/L)															
Purified	Sample	CO ²⁻	HCO ₃ ⁻	SO ₄ ²⁻	Cl ⁻	F ⁻	NO ₃ ⁻	NO ₂ ⁻	Ca	Mg	Na	K	Fe	BrO ₃ ⁻	SiO ₂	TDS	Total hard- ness
	Aquafina	<1.0	1.3	85	1.4	0.97	<0.50	*	<1.0	20	4	<1.0	0.02	*	*	105	83
	Arwa	<1	7.7	74.5	<1	0.8	<1	*	<1	21.1	3	<1	*	*	*	121	<90
	Berain glass	*	20	9	35	1	0.1	*	22	3	17	5	0.01	<0.005	*	155	65
	Berain plastic	*	50	9	35	1	0.1	*	22	3	17	5	0.01	<0.005	*	155	65
	Hana	0	18	28	32	1	0.2	*	21	9	<5	8	*	<0.005	*	120	84
	Nestle Pure Life Dammam	*	22	10	50	0.8	<1	*	27	2.3	9.5	0.2	<0.02	<0.010	*	120	76 mg/L as CaCO ₃
	Nestle Pure Life Madinah	*	22	10	50	0.8	<1	*	27	2.3	9.5	0.2	<0.02	<0.010	*	120	78 mg/L as CaCO ₃
	Nova	*	26	26	19	1	3	*	11	3.4	17	1.2	*	*	*	120	41
	Tamimi Markets AlKharj	<1	19	40	17	0.9	<1	*	10	10	10	<1	<0.1	<0.010	*	110	65
	Tamimi Markets AlQasim	0	18	28	32	1	0.2	*	21	9	<5	8	*	<0.005	*	120	84

Average Composition (mg/L)																
	Sample	Average Composition (mg/L)														
		CO ²⁻	HCO ₃ ⁻	SO ₄ ²⁻	Cl ⁻	F ⁻	NO ₃ ⁻	NO ₂ ⁻	Ca	Mg	Na	K	Fe	BrO ₃ ⁻	SiO ₂	TDS
Mineral	Acqua Panna	*	106	22	7.8	<0.1	2.9	*	32.2	6.6	6.5	0.8	*	*	6.9	141
	Evian glass	*	360	14	10	0.06	3.8	*	80	26	6.5	1	*	*	15	345
	Evian plastic	*	360	14	*	0.06	3.8	*	80	26	6.5	1	*	*	15	345
	Highland Springs	*	150	5.3	6.1	*	3.1	*	40.5	10.1	5.6	0.7	*	*	*	170
	Still Scottish	*	<240	<28	<11	*	<6	*	<55	<16	<15	<2	*	*	*	<255
	Tannourine	*	160	4	10	<0.1	0.5	*	50	13	4	1	<0.1	*	*	190
	Volvic	*	74	9	15	*	7.3	*	12	8	12	6	*	*	32	130
Artesian	Wildalp	*	170	*	2.6	*	*	0.01	45	13	2	1	*	*	*	*
	Fiji	0	152	1	9	0	1	*	18	15	18	5	*	*	93	222
	Voss glass	*	*	2.1	5.5	0.13	*	*	3.7	0.9	3.8	*	*	*	*	36
	Voss plastic	*	*	2.1	5.5	0.13	*	*	3.7	0.9	3.8	*	*	*	*	36

Average Composition (mg/L)																
Sample	CO ²⁻	HCO ₃ ⁻	SO ₄ ²⁻	Cl ⁻	F ⁻	NO ₃ ⁻	NO ₂ ⁻	Ca	Mg	Na	K	Fe	BrO ₃ ⁻	SiO ₂	TDS	Total hardness
Badoit	*	1250	35	54	1.2	*	*	153	80	180	11	*	*	27	1100	*
Berain sparkling	*	60	9	37	1.1	0.3	*	20	2.3	18	0.9	0.01	<0.005	*	170	60
Perrier	<4	420	25.3	19.5	<0.2	7.3	*	150	3.9	9.6	<1	*	*	*	456	39
S. Pellegrino	*	*	401	49.6	0.5	*	*	166	49.5	30	2.1	*	*	*	853	*
Voss sparkling	*	*	2.1	5.5	0.13	*	*	3.7	0.9	122	*	*	*	*	310	*

TDS signifies total dissolved solids

* Denotes values that were not noted on labels

Table S3: Microbial growth potential experiments performed to analyze the effect of incubation at 30°C on the microbial content for different types of bottled water. Information of samples tested, including type of samples, volume of bottles, and production dates of bottles, is noted.

Incubation Experiment	Bottled Drinking Water Samples	Figure Labels	Volume (mL)	Dates on Bottles
Mineral	1. Highland Springs	-Highland Springs	500	12/04/20
	2. Tannourine	-Tannourine	330	09/03/20
	3. Volvic	-Volvic	330	28/08/19
	4. Wildalp	-Wildalp	500	05/03/19
Sparkling	1. Badoit	-Badoit	330	21/11/19
	2. Perrier	-Perrier	330	24/06/20
	3. S. Pellegrino	-S. Pellegrino	250	09/07/20
Purified	1. Aquafina	-Aquafina	330	26/09/20
	2. Nestle Pure Life-Dammam	-Nestle PL-D	330	10/12/20
	3. Nestle Pure Life-Madinah	-Nestle PL-M	330	22/06/20
Mineral – Evian plastic vs. glass	1. Evian in plastic	-Plastic	330	27/09/19
	2. Evian in glass	-Glass	330	30/08/19

Table S4: Details of bottled water samples filtered for DNA extractions. Microbial community analysis was performed on these select bottled drinking waters based on high cell concentrations measured through flow cytometry in prior experiments. The volumes of bottled waters filtered were determined based on the average DNA content of bacterial cells (Button et al., 2001). The approximate yield of DNA was calculated from the total number of cells determined through FCM. From that, an appropriate volume was estimated for extracting enough DNA (at least 5ng of DNA per mL), assuming 100% retention of cells in the filter used and 100% extraction efficiency.

Sample	Total Volume Filtered (L)	Length of Incubation (days)	Dates Listed on Filtered Bottles
Evian	3.3	0	27/09/2019
Highland Springs	3	0	12/4/2020
Nestle PL (Dammam)	7.6	0	10/11/2020, 01/11/2020
Tannourine	2.6	0	9/3/2020
Volvic	3.3	0	28/08/2019
Wildalp	4	0	05/03/2019, 27/03/2019
Sample Post Incubation	Total Volume Filtered (L)	Length of Incubation (days)	Dates Listed on Filtered Bottles
Evian	3.3	7	27/09/2019, 09/08/2019
Highland Springs	3	9	12/4/2020
Tannourine	2.6	9	10/09/2020
Volvic	3.3	15	28/08/2019
Wildalp	4	9	28/03/2019

Table S5: Concentrations of anions in bottled water samples measured through ion chromatography alongside pH measurements in comparison to the label values and to international and local standards: the World Health Organization (WHO, 2022) and Saudi Arabian Standards Organization (SASO) (Ghrefat, 2013). Percent deviation from label values is noted.

		Concentrations in ppm							pH
Brand name		SO ₄ ²⁻	Cl ⁻	F ⁻	NO ₃ ⁻	NO ₂ ⁻	Br ⁻	BrO ₃ ⁻	
Guidelines	WHO limits	250	250	1.5	50	3	*	0.01	6.5-8.5
	SASO limits	150	150	1.5	50	*	*	0.01	6.5-8.5
Purified	Aquafina	85	1.4	0.97	<0.50	*	*	*	6.5
	Experimental value	79.9	0.3	1.3	0.0	0.3	0.008	ϕ	6
	% Deviation	-6%	-79%	35%	n/a	n/a	n/a	n/a	-8%
	Arwa	74.5	<1	0.8	<1	*	*	*	7
	Experimental value	68.9	0.4	1.3	0.0	0.3	0.0036	ϕ	7
	% Deviation	-8%	0%	65%	n/a	n/a	n/a	n/a	0%
	Berain glass	9	35	1	0.1	*	*	<0.005	8
	Experimental value	9.1	34.0	1.3	0.1	2.7	ϕ	ϕ	9.2
	% Deviation	2%	-3%	31%	0%	n/a	n/a	n/a	15%
	Berain plastic	9	35	1	0.1	*	*	<0.005	8
	Experimental value	10.8	39.6	1.4	0.0	2.3	ϕ	ϕ	7.9
	% Deviation	20%	13%	41%	n/a	n/a	n/a	n/a	-1%
	Hana water	28	32	1	0.2	*	*	<0.005	7.8
	Experimental value	25.8	32.9	1.3	0.0	1.0	0.016	ϕ	7.4
	% Deviation	-8%	3%	35%	n/a	n/a	n/a	0.0	-5%

		Concentrations in ppm							
	Brand name	SO ₄ ²⁻	Cl ⁻	F ⁻	NO ₃ ⁻	NO ₂ ⁻	Br ⁻	BrO ₃ ⁻	pH
Guidelines	WHO limits	250	250	1.5	50	3	*	0.01	6.5-8.5
	SASO limits	150	150	1.5	50	*	*	0.01	6.5-8.5
Purified	Nestle pure life-Dammam	10	50	0.8	<1	*	*	<0.010	7
	Experimental value	11.2	51.7	1.4	0.0	2.1	0.005	φ	7.2
	% Deviation	12%	3%	72%	n/a	n/a	n/a	n/a	3%
	Nestle pure life-Madinah	10	50	0.8	<1	*	*	<0.010	7
	Experimental value	6.2	51.9	1.2	0.0	1.9	φ	φ	7.2
	% Deviation	-38%	4%	52%	n/a	n/a	n/a	n/a	3%
	Nova	26	19	1	3	*	*	*	7.4
	Experimental value	36.8	18.8	1.1	2.3	1.8	2.002	φ	7.2
	% Deviation	41%	-1%	14%	-22%	n/a	n/a	n/a	-3%
	Tamimi markets-AlKharj	40	17	0.9	<1	*	*	<0.010	7.2
	Experimental value	43.5	20.5	1.3	0.0	1.3	0.005	φ	7.4
	% Deviation	9%	20%	48%	n/a	n/a	n/a	n/a	3%
	Tamimi markets-AlQasim	28	32	1	0.2	*	*	<0.005	7.8
	Experimental value	29.2	33.5	1.3	0.1	1.4	0.002	φ	7.4
	% Deviation	4%	5%	30%	n/a	n/a	n/a	n/a	-5%

		Concentrations in ppm							
	Brand name	SO ₄ ²⁻	Cl ⁻	F ⁻	NO ₃ ⁻	NO ₂ ⁻	Br ⁻	BrO ₃ ⁻	pH
Guidelines	WHO limits	250	250	1.5	50	3	*	0.01	6.5-8.5
	SASO limits	150	150	1.5	50	*	*	0.01	6.5-8.5
Mineral	Acqua Panna	22	7.8	<0.1	2.9	*	*	*	8
	Experimental value	19.8	8.4	0.0	2.7	3.6	φ	φ	8.2
	% Deviation	-10%	8%	n/a	-6%	n/a	n/a	n/a	2%
	Evian glass	14	10	0.06	3.8	*	*	*	7.2
	Experimental value	12.7	9.7	0.0	2.9	8.4	φ	φ	7.5
	% Deviation	-9%	-3%	n/a	-23%	n/a	n/a	n/a	4%
	Evian plastic	14	*	0.06	3.8	*	*	*	7.2
	Experimental value	12.3	10.4	0.0	3.2	8.8	0.011	φ	7.8
	% Deviation	-12%	n/a	n/a	-16%	n/a	n/a	n/a	8%
	Highland Spring	5.3	6.1	*	3.1	*	*	*	7.8
	Experimental value	4.8	11.6	0.0	1.9	5.9	0.018	φ	8.2
	% Deviation	-10%	90%	n/a	-38%	n/a	n/a	n/a	5%
	Still Scottish	<28	<11	*	<6	*	*	*	7.4
	Experimental value	8.2	6.0	0.1	2.6	4.1	φ	φ	8
	% Deviation	0%	0%	n/a	0%	n/a	n/a	n/a	8%
	Tannourine	4	10	<0.1	0.5	*	*	*	7.9
	Experimental value	13.5	6.2	0.0	0.6	5.9	0.004	φ	8.2
	% Deviation	237%	-38%	n/a	30%	n/a	n/a	n/a	4%

		Concentrations in ppm							
	Brand name	SO ₄ ²⁻	Cl ⁻	F ⁻	NO ₃ ⁻	NO ₂ ⁻	Br ⁻	BrO ₃ ⁻	pH
Guidelines	WHO limits	250	250	1.5	50	3	*	0.01	6.5-8.5
	SASO limits	150	150	1.5	50	*	*	0.01	6.5-8.5
Mineral	Volvic	9	15	*	7.3	*	*	*	7
	Experimental value	7.7	16.4	0.2	6.2	3.2	0.004	ϕ	7.5
	% Deviation	-14%	9%	n/a	-16%	n/a	n/a	n/a	7%
	Wildalp	*	2.6	*	*	0.01	*	*	7.9
	Experimental value	11.2	3.2	0.0	3.3	5.1	0.009	ϕ	7.9
	% Deviation	n/a	23%	n/a	n/a	50839 %	n/a	n/a	0%
Artesian	Fiji	1	9	0	1	*	*	*	7.7
	Experimental value	1.1	11.3	0.2	0.9	5.1	0.036	ϕ	7.7
	% Deviation	9%	25%	n/a	-15%	n/a	n/a	n/a	0%
	Voss glass	2.1	5.5	0.13	*	*	*	*	6.6
	Experimental value	2.5	5.2	0.1	0.2	1.0	ϕ	ϕ	6.3
	% Deviation	17%	-5%	-47%	n/a	n/a	0.015	0.015	-5%
	Voss plastic	2.1	5.5	0.13	*	*	*	*	6.6
	Experimental value	2.3	4.4	0.1	0.1	0.6	0.026	ϕ	6.3
	% Deviation	7%	-20%	-31%	n/a	n/a	n/a	n/a	-5%

		Concentrations in ppm							
	Brand name	SO ₄ ²⁻	Cl ⁻	F ⁻	NO ₃ ⁻	NO ₂ ⁻	Br ⁻	BrO ₃ ⁻	pH
Guidelines	WHO limits	250	250	1.5	50	3	*	0.01	6.5-8.5
	SASO limits	150	150	1.5	50	*	*	0.01	6.5-8.5
Sparkling	Badoit	35	54	1.2	*	*	*	*	6
	Experimental value	29.5	48.9	1.4	8.2	29.8	φ	φ	5.8
	% Deviation	-16%	-9%	18%	n/a	n/a	n/a	n/a	-3%
	Berain sparkling	9	37	1.1	0.3	*	*	<0.005	5
	Experimental value	11.9	35.4	1.3	0.5	24.2	φ	φ	4.5
	% Deviation	32%	-4%	17%	81%	n/a	n/a	n/a	-10%
	Perrier	25.3	19.5	<0.2	7.3	*	*	*	5.5
	Experimental value	23.4	19.9	0.1	8.2	25.9	0.016	φ	5.3
	% Deviation	-7%	2%	0%	12%	n/a	n/a	n/a	-4%
	S.pellegrino	401	49.6	0.5	*	*	*	*	5.2
	Experimental value	353.9	46.3	0.7	2.2	19.2	0.015	φ	5.3
	% Deviation	-12%	-7%	33%	n/a	n/a	n/a	n/a	2%
	Voss sparkling	2.1	5.5	0.13	*	*	*	*	5.2
	Experimental value	1.5	4.1	0.1	0.1	34.2	φ	φ	5.4
	% Deviation	-27%	-25%	-29%	n/a	n/a	n/a	n/a	4%

		Concentrations in ppm							
	Brand name	SO ₄ ²⁻	Cl ⁻	F ⁻	NO ₃ ⁻	NO ₂ ⁻	Br ⁻	BrO ₃ ⁻	pH
Guidelines	WHO limits	250	250	1.5	50	3	*	0.01	6.5-8.5
	SASO limits	150	150	1.5	50	*	*	0.01	6.5-8.5
Tap	Tap water 1	1.3	50.2	0	0.2	0	0.185	φ	φ
	Tap water 2	1.4	47.7	0	0.2	0	0.239	φ	φ
	Tap water 3	1.3	49.8	0	0.2	0	0.199	φ	φ
	Tap water 4	1.3	49.8	0	0.2	0	0.269	φ	φ
	Tap water 5	1.3	50.1	0	0.2	0	0.334	φ	φ
	Tap water 6	1.3	50.1	0	0.2	0	0.291	φ	φ

* Denotes values that were not provided on the composition labels of bottled waters

φ Denotes values that were not measured

n/a' indicates 'not applicable' in the % deviation rows when the experimental value is below the limit of detection or when there are no label values for comparison

Table S6: Concentrations of cations and trace metals in bottled water samples measured through Inductively coupled plasma-optical emission spectroscopy in comparison to label values and to international and local standards: the World Health Organization (WHO, 2022) and the Saudi Arabian Standards Organization (SASO) (Ghrefat, 2013, Al-Omran et al., 2015). Percent deviation from label values is noted.

		Concentrations in mg/L (ppm)									
Brand name	Ca	Na	Mg	K	Fe	Al	Cu	Ni	Pb	Zn	
WHO limits	*	200	*	*	0.3	0.9	2	0.07	0.01	*	
SASO limits	200	100	150	*	0.3	1	1	*	0.05	5	
Purified	Aquafina	<1.0	4	20	<1.0	0.02	*	*	*	*	*
	Experimental value	0	1.25	24.06	1.16	0.00 u	0.00 u	0.00 u	0	0.00 u	0.00 u
	% Difference	0%	-69%	20%	16%	n/a	n/a	n/a	n/a	n/a	n/a
	Arwa	<1	3	21.1	<1	*	*	*	*	*	*
	Experimental value	0.03	1.53	20.38	4.65	0	0.00 u	0	0	0.00 u	0.00 u
	% Difference	0%	-49%	-3%	365%	n/a	n/a	n/a	n/a	n/a	n/a
	Berain glass	22	17	3	5	0.01	*	*	*	*	*
	Experimental value	20.28	20.06	2.55	4.23	0.00 u	0.09	0	0.00 u	0.01	0.00 u
	% Difference	-8%	18%	-15%	-15%	n/a	n/a	n/a	n/a	n/a	n/a
	Berain plastic	22	17	3	5	0.01	*	*	*	*	*
	Experimental value	22.61	14.19	3.28	6.25	0.00 u	0.00 u	0	0.00 u	0.00 u	0.01
	% Difference	3%	-17%	9%	25%	n/a	n/a	n/a	n/a	n/a	n/a
	Hana water	21	<5	9	8	*	*	*	*	*	*
	Experimental value	18.66	2.81	8.1	7.19	0.00 u	0.00 u	0	0.00 u	0.00 u	0.00 u
	% Difference	-11%	0%	-10%	-10%	n/a	n/a	n/a	n/a	n/a	n/a

Concentrations in mg/L (ppm)										
Brand name	Ca	Na	Mg	K	Fe	Al	Cu	Ni	Pb	Zn
WHO limits	*	200	*	*	0.3	0.9	2	0.07	0.01	*
SASO limits	200	100	150	*	0.3	1	1	*	0.05	5
Purified	Nestle pure life Dammam	27	9.5	2.3	0.2	<0.02	*	*	*	*
	Experimental value	30	8.4	3.29	0.07	0.00 u	0.00 u	0	0.00 u	0.01 u
	% Difference	11%	-12%	43%	-65%	n/a	n/a	n/a	n/a	n/a
	Nestle pure life Madinah	27	9.5	2.3	0.2	<0.02	*	*	*	*
	Experimental value	29.18	8.17	1.86	0.08	0.00 u	0.01	0.00 u	0.00 u	0.00 u
	% Difference	8%	-14%	-19%	-60%	n/a	n/a	n/a	n/a	n/a
	Nova	11	17	3.4	1.2	*	*	*	*	*
	Experimental value	17.23	17.71	5.82	1.91	0.00 u	0.01 u	0.00 u	0.00 u	0.00 u
	% Difference	57%	4%	71%	59%	n/a	n/a	n/a	n/a	n/a
	Tamimi markets AlKharj	10	10	10	<1	<0.1	*	*	*	*
	Experimental value	11.75	9.08	12.98	0.17	0.00 u	0.00 u	0.00 u	0.00 u	0.01 u
	% Difference	18%	-9%	30%	0%	n/a	n/a	n/a	n/a	n/a
	Tamimi markets AlQasim	21	<5	9	8	*	*	*	*	*
	Experimental value	18.92	3.11	8.63	7.12	0.00 u	0.00 u	0.00 u	0.00 u	0.00 u
	% Difference	-10%	0%	-4%	-11%	n/a	n/a	n/a	n/a	n/a

		Concentrations in mg/L (ppm)									
Brand name	Ca	Na	Mg	K	Fe	Al	Cu	Ni	Pb	Zn	
WHO limits	*	200	*	*	0.3	0.9	2	0.07	0.01	*	
SASO limits	200	100	150	*	0.3	1	1	*	0.05	5	
Mineral	Acqua Panna	32.2	6.5	6.6	0.8	*	*	*	*	*	*
	Experimental value	33.79	6.56	7.22	1.23	0.00 u	0.01	0.00 u	0.00 u	0.01	0.01 u
	% Difference	5%	1%	9%	54%	n/a	n/a	n/a	n/a	n/a	n/a
	Evian glass	80	6.5	26	1	*	*	*	*	*	*
	Experimental value	88	7.67	28.09	1.59	0.00 u	0.01	0.00 u	0.00 u	0	0
	% Difference	10%	18%	8%	59%	n/a	n/a	n/a	n/a	n/a	n/a
	Evian plastic	80	6.5	26	1	*	*	*	*	*	*
	Experimental value	77.88	7.23	28.45	1.56	0.00 u	0.00 u	0.00 u	0.00 u	0.00 u	0.00 u
	% Difference	-3%	11%	9%	56%	n/a	n/a	n/a	n/a	n/a	n/a
	Highland Springs	40.5	5.6	10.1	0.7	*	*	*	*	*	*
	Experimental value	38.54	7.87	21.58	1.34	0.00 u	0.00 u	0	0.00 u	0.00 u	0.01
	% Difference	-5%	41%	114%	91%	n/a	n/a	n/a	n/a	n/a	n/a
	Still Scottish	<55	<15	<16	<2	*	*	*	*	*	*
	Experimental value	28.9	10.14	10.28	1.34	0.00 u	-0.01 u	0.00 u	0.00 u	0.00 u	0.01
	% Difference	0%	0%	0%	0%	n/a	n/a	n/a	n/a	n/a	n/a
	Tannourine	50	4	13	1	<0.1	*	*	*	*	*
	Experimental value	57.88	4.18	17.13	0.91	0.00 u	0.00 u	0	0.00 u	0.00 u	0.00 u
	% Difference	16%	4%	32%	-9%	n/a	n/a	n/a	n/a	n/a	n/a
	Volvic	12	12	8	6	*	*	*	*	*	*
	Experimental value	13.33	12.22	9.24	7.1	0.00 u	0.00 u	0	0.00 u	0.00 u	0.00 u
	% Difference	11%	2%	16%	18%	n/a	n/a	n/a	n/a	n/a	n/a
	Wildalp	45	2	13	1	*	*	*	*	*	*
	Experimental value	39.78	1.83	14.79	0.33	0.00 u	-0.01 u	0.00 u	0.00 u	0.00 u	0.01
	% Difference	-12%	-9%	14%	-67%	n/a	n/a	n/a	n/a	n/a	n/a

		Concentrations in mg/L (ppm)								
Brand name	Ca	Na	Mg	K	Fe	Al	Cu	Ni	Pb	Zn
WHO limits	*	200	*	*	0.3	0.9	2	0.07	0.01	*
SASO limits	200	100	150	*	0.3	1	1	*	0.05	5
Artesian	Fiji	18	18	15	5	*	*	*	*	*
	Experimental value	19.44	18.31	15.29	6.25	0.00 u	0.00 u	0	0.00 u	0.00 u
	% Difference	8%	2%	2%	25%	n/a	n/a	n/a	n/a	n/a
	Voss glass	3.7	3.8	0.9	*	*	*	*	*	*
	Experimental value	3.24	3.79	0.69	0.52	0.00 u	0.01	0	0.00 u	0.00 u
	% Difference	-12%	0%	-23%	n/a	n/a	n/a	n/a	n/a	n/a
	Voss plastic	3.7	3.8	0.9	*	*	*	*	*	*
	Experimental value	2.73	2.07	0.6	0.47	0.00 u	0.00 u	0.00 u	0.00 u	0.00 u
	% Difference	-26%	-46%	-33%	n/a	n/a	n/a	n/a	n/a	n/a
	Badoit	153	180	80	11	*	*	*	*	*
	Experimental value	108.68	186.19	91.36	16	0.00 u	0.01	0.00 u	0.00 u	-0.01 u
	% Difference	-29%	3%	14%	45%	n/a	n/a	n/a	n/a	n/a
Sparkling	Berain sparkling	20	18	2.3	0.9	0.01	*	*	*	*
	Experimental value	20.46	14.5	2.12	3.53	0.00 u	0	0	0.00 u	0.01
	% Difference	2%	-19%	-8%	292%	n/a	n/a	n/a	n/a	n/a
	Perrier	150	9.6	3.9	<1	*	*	*	*	*
	Experimental value	170.71	11.39	4.01	0.67	0.00 u	0	0	0.00 u	0.00 u
	% Difference	14%	19%	3%	0%	n/a	n/a	n/a	n/a	n/a
	S.pellegrino	166	30	49.5	2.1	*	*	*	*	*
	Experimental value	176.33	36.45	53.41	3.89	0.00 u	0.01	0	0.00 u	0.00 u
	% Difference	6%	22%	8%	85%	n/a	n/a	n/a	n/a	n/a

		Concentrations in mg/L (ppm)									
Brand name		Ca	Na	Mg	K	Fe	Al	Cu	Ni	Pb	Zn
WHO limits		*	200	*	*	0.3	0.9	2	0.07	0.01	*
SASO limits		200	100	150	*	0.3	1	1	*	0.05	5
Sparkling	Voss sparkling	3.7	122	0.9	*	*	*	*	*	*	*
	Experimental value	3.11	119.61	0.65	0.96	0.00 u	0.01 u	0.00 u	0.00 u	0.00 u	0.00 u
	% Difference	-16%	-2%	-28%	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Tap	Tap water 1	12.02	32.26	0.6	1.89	0.01	0.00 u	0.00 u	0.00 u	0.00 u	0.04
	Tap water 2	15.92	30.98	0.64	1.82	0.01	0.01	0.00 u	0.00 u	0.00 u	0.00 u
	Tap water 3	10.15	32.54	1.51	1.89	0.00 u	0.01	0.00 u	0.00 u	0.00 u	0.01
	Tap water 4	12.19	32.67	0.61	1.91	0.01	0.01	0.00 u	0.00 u	0.00 u	0.1
	Tap water 5	11.66	33.52	0.88	1.94	0.00 u	0.00 u	0.00 u	0.00 u	0.00 u	0.00 u
	Tap water 6	12.11	33.22	0.65	1.93	0.00 u	0.01	0.00 u	0.00 u	0.00 u	0.01

* Denotes values that were not provided on the composition labels of bottled waters

u Denotes values that were under the detection limit

n/a' indicates 'not applicable' in the % deviation rows when the experimental value is below the limit of detection or when there are no label values for comparison

2 Supplementary Methods

Materials and methods for bacterial community analysis targeting 16S V3-4 rRNA

The following information was duplicated as it was provided through supplementary documents supplied by DNASense (Aalborg, Denmark) on 29-03-2021.

1. Library preparation

Bacteria 16S rRNA gene region V3-4 sequencing libraries were prepared by a custom protocol based on an Illumina protocol (Illumina, 2015). Up to 10 ng of extracted DNA was used as template for PCR amplification of the Bacteria 16S rRNA gene region V3-4 amplicons. Each PCR reaction (25 μ L) contained (12.5 μ L) PCR BIO Ultra mix (PCR Biosystems, USA) and 400 nM of each forward and reverse tailed primer mix. PCR was conducted with the following program: Initial denaturation at 95 °C for 2 min, 30 cycles of amplification (95 °C for 15 s, 55 °C for 15 s, 72 °C for 50 s) and a final elongation at 72 °C for 5 min. Duplicate PCR reactions were performed for each sample and the duplicates were pooled after PCR. The forward and reverse tailed primers were designed according to (Illumina, 2015) and contain primers targeting the Bacteria 16S rRNA gene region V3-4: [341F] CCTACGGGNGGCWGCAG and [805R]

GACTACHVGGGTATCTAATCC (Herlemann et al., 2011). The primer tails enable attachment of Illumina Nextera adaptors necessary for sequencing in a subsequent PCR. The resulting amplicon libraries were purified using the standard protocol for Agencourt Ampure XP Beads (Beckman Coulter, USA) with a bead to sample ratio of 4:5. DNA was eluted in 25 μ L of nuclease free water (Qiagen, Germany). DNA concentration was measured using Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, USA). Gel electrophoresis using TapeStation 2200 and D1000/High sensitivity D1000 screentapes (Agilent, USA) was used to validate product size and purity of a subset of sequencing libraries. Sequencing libraries were prepared from the purified amplicon libraries using a second PCR. Each PCR reaction (25 μ L) contained PCR BIO HiFi buffer (1x), PCR BIO HiFi Polymerase (1 U/reaction) (PCR Biosystems, UK), adaptor mix (400 nM of each forward and reverse) and up to 10 ng of amplicon library template. PCR was conducted with the following program: Initial denaturation at 95 °C for 2 min, 8 cycles of amplification (95 °C for 20 s, 55 °C for 30 s, 72 °C for 60 s) and a final elongation at 72 °C for 5 min. The resulting sequencing libraries were purified using the standard protocol for Agencourt Ampure XP Beads (Beckman Coulter, USA) with a bead to sample ratio of 4:5. DNA was eluted in 25 μ L of nuclease free water (Qiagen, Germany). DNA concentration was measured using Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, USA). Gel electrophoresis using TapeStation 2200 and D1000/High sensitivity D1000 screentapes (Agilent, USA) was used to validate product size and purity of a subset of sequencing libraries.

2. DNA sequencing

The purified sequencing libraries were pooled in equimolar concentrations and diluted to 2 nM. The samples were paired-end sequenced (2x300 bp) on a MiSeq (Illumina, USA) using a MiSeq Reagent kit v3 (Illumina, USA) following the standard guidelines for preparing and loading amples on the MiSeq. >10% PhiX control library was spiked in to overcome low complexity issues often observed with amplicon samples.

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