Supplementary material

S1. Description of the sampling sites in the area of the Roman Forum

S1.1. The sources of water inside the Valentini Palace

The sampling points are as follows:

1– Well, in a gap in the marble floor located on the border of the Frigidarium and of the probable apodyterium of the so-called Piccole Terme (VPb), the thermal complex to the north of the block, which was investigated in 2007 and 2009.

2– In the area called Ex Carceri (VPco) to the southwest of the block, archaeological research was carried out in 2011 and 2014.

3– Standpipe in the courtyard of Valentini Palace (VPst) (sampled for comparison with sources).

4– Outcrop in the so-called Ex sala mensa (VPca) area, particularly in one of its annexes to the north, excavated in 2005, 2015, and 2018/2019.

S1.2. The sources of water present inside the Roman Forum and the Mamertine Prison

The sampling points are as follows:

1– Lacus Juturna (J) is currently a square basin with a side of 5.1 m and a height of about 2 m covered in marble, in the center of which remains a rectangular pedestal in the tuffaceous lattice, above which were placed two statues of the Dioscuri.

2– Cesarean Galleries (CG) some square wells from the Caesarian era are present in the Roman Forum; they were the entrances to galleries, where freight elevators were positioned for the gladiatorial games in the Forum.

3– Drilling close to Lapis Niger (D) core excavated in 2010 for archaeological reasons

4– Lapis Niger (LN): groundwater outcrops in the area where a black stone was found, which was attributed to the presence of the tomb of Romulus.

5– Standpipe on the Via Sacra (Fsp), sampled for comparison with sources.

6– Divine Romulus Temple (DR), a square well with a side about half a meter covered by a glass window, is inside

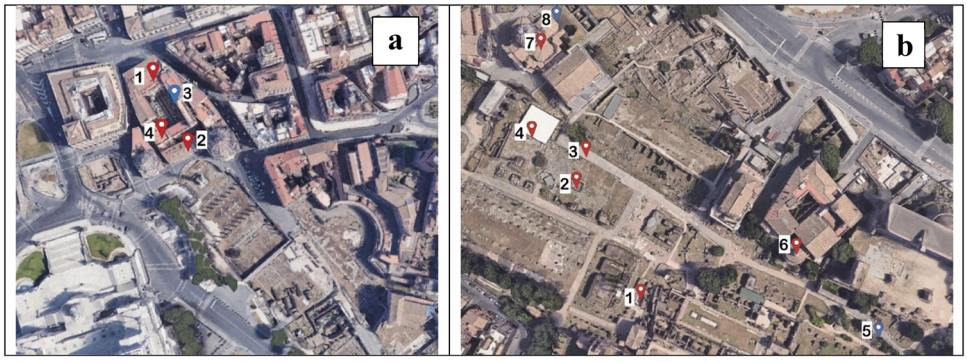

Figure S1: (a) Map of sources inside Valentini Palace: 1 = thermal baths (VPb); 2 = well close to a collapsed column (VPco); 3 = standpipe located in the Valentini Palace courtyard (VPsp); and 4 = outcrop near the former canteen of Valentini Palace (VPca). (b) Map of sources inside the Forum and Tullianum: 1 = Juturnae (J); 2 = Cesarian Galleries (CG); 3 = drilling close to Lapis Niger (D); 4 = Lapis Niger (LN); 5 = standpipe inside Forum (Fsp); 6 = Divus Romulus Temple (DR); 7 = Tullianum (T); and 8 = standpipe outside Tullianum (Tsp).

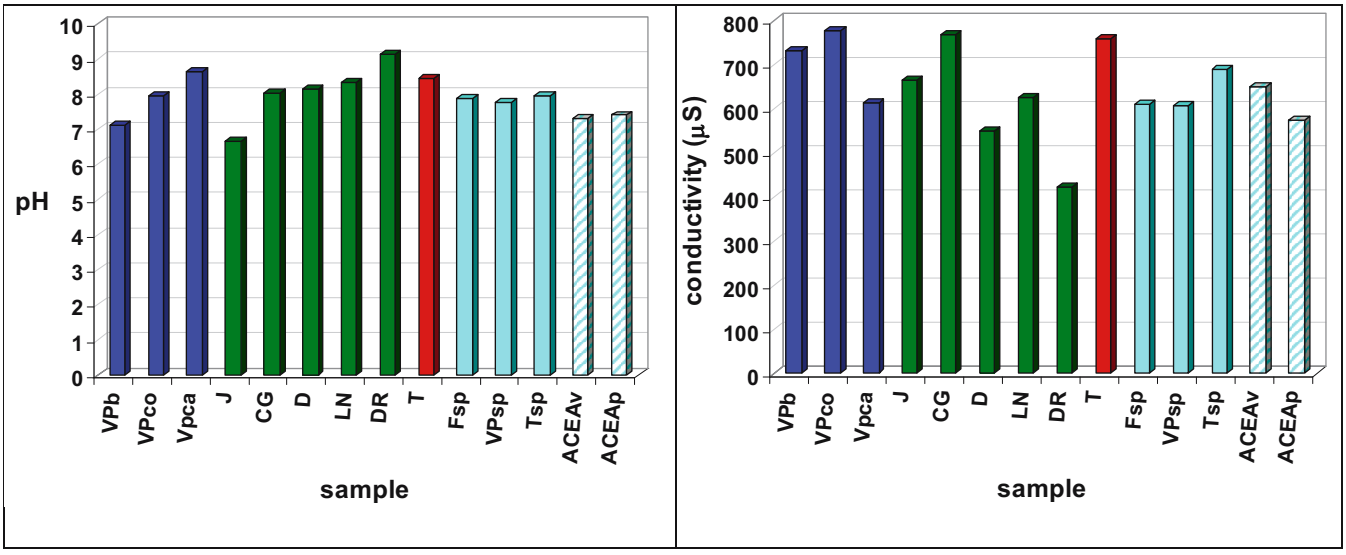

Figure S2: PH (left panel) and conductivity values (right panel) measured in situ in all the considered samples and comparison with data from ACEA aqueducts.

the Temple of the Divine Romulus. Numerous hypotheses have been put forward regarding the origin of the name of the Temple, the best known of which concerns the emperor Maxentius. He allegedly reused the building as a temple dedicated to his son, Valerio Romulus, who died in 309 AD.

7– Tullianum (T) During the analysis campaigns, the pool was almost dry; therefore, the samplings were carried out in an outcrop of water, about 1.50 meters deep, which is located in an area not open to the public where archaeological excavations are underway.

8– Standpipe close to the Mamertino (Tsp), sampled for comparison with sources.

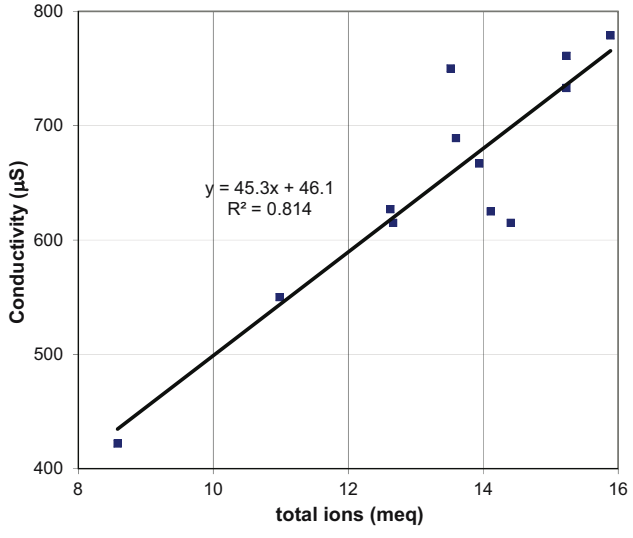

Figure S3: Correlation between conductivity and total ions.

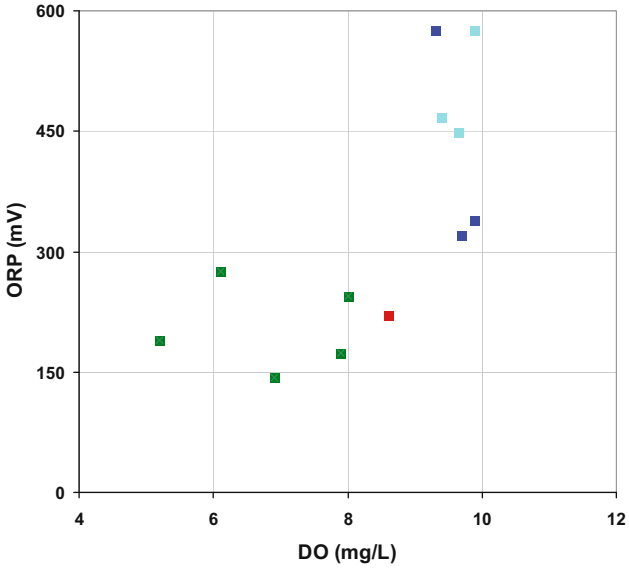

Figure S4: Correlation between Redox Potential (ORP) and Dissolved Oxygen (DO) measured *in situ* in all the considered samples.

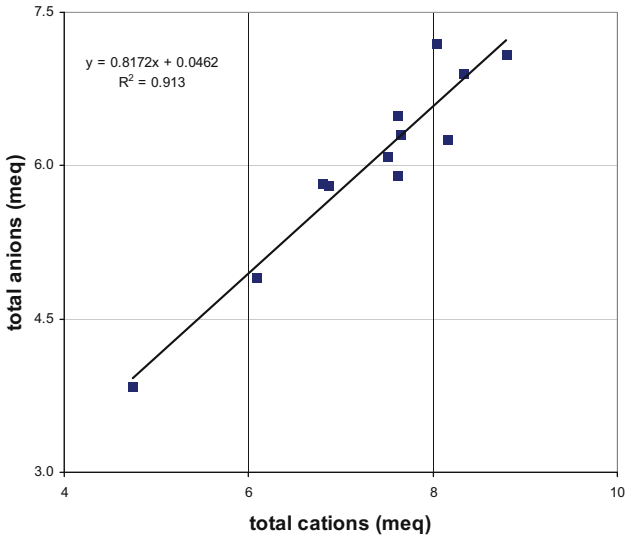

Figure S5: Correlation between total anions and total cations, compliance with electroneutrality.

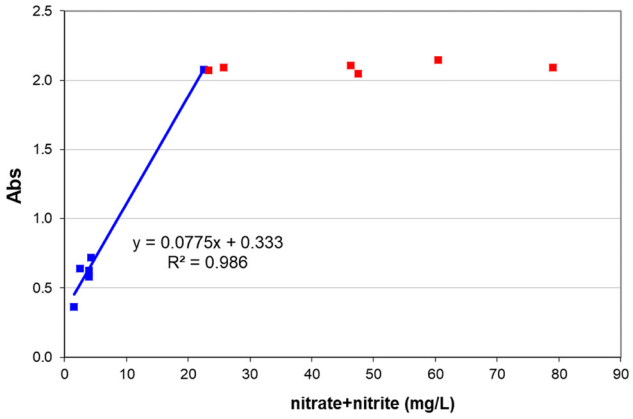

Figure S6: Correlation between absorbance (Abs) at 198 nm, index of nitrate and nitrite presence, and nitrate plus nitrite content by ion chromatography.

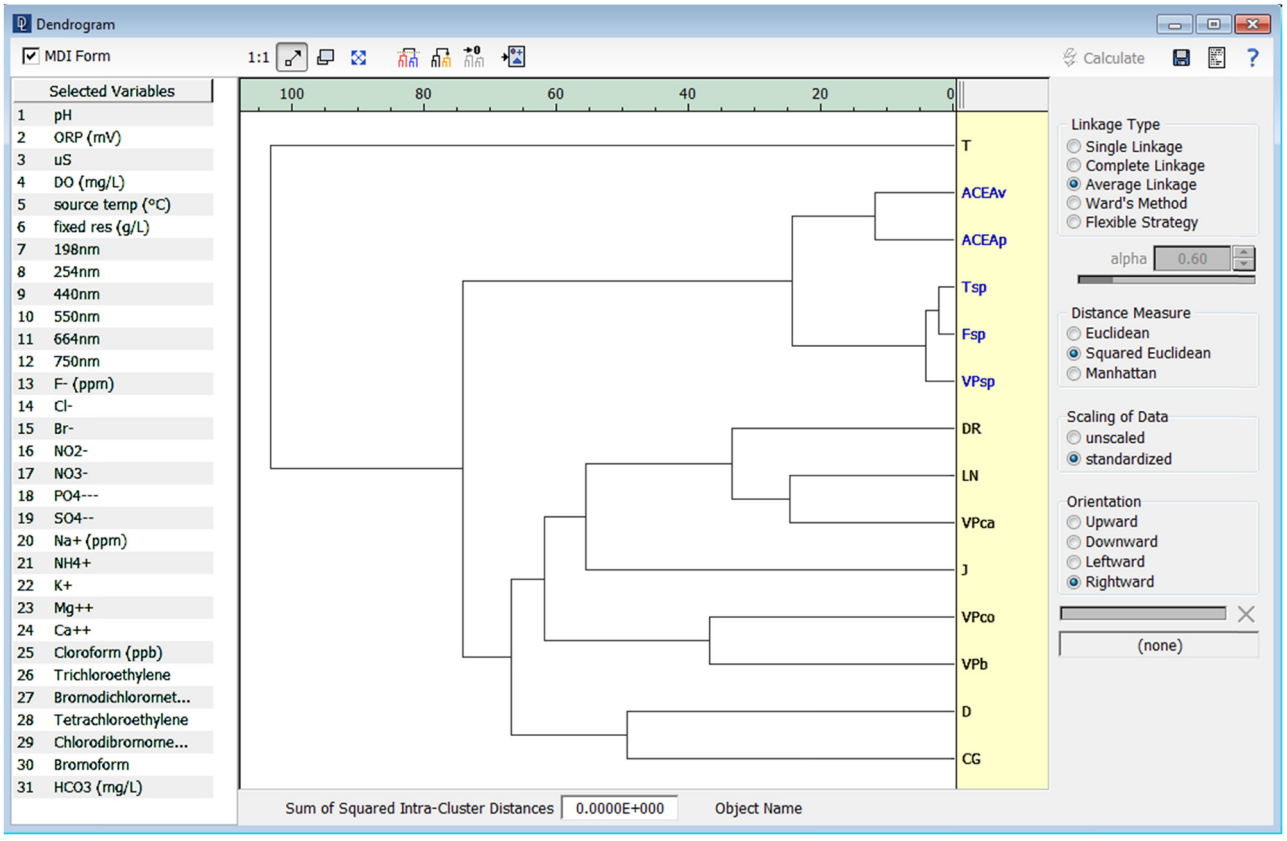

Figure S7: Cluster analysis obtained using all the chemical-physical data.

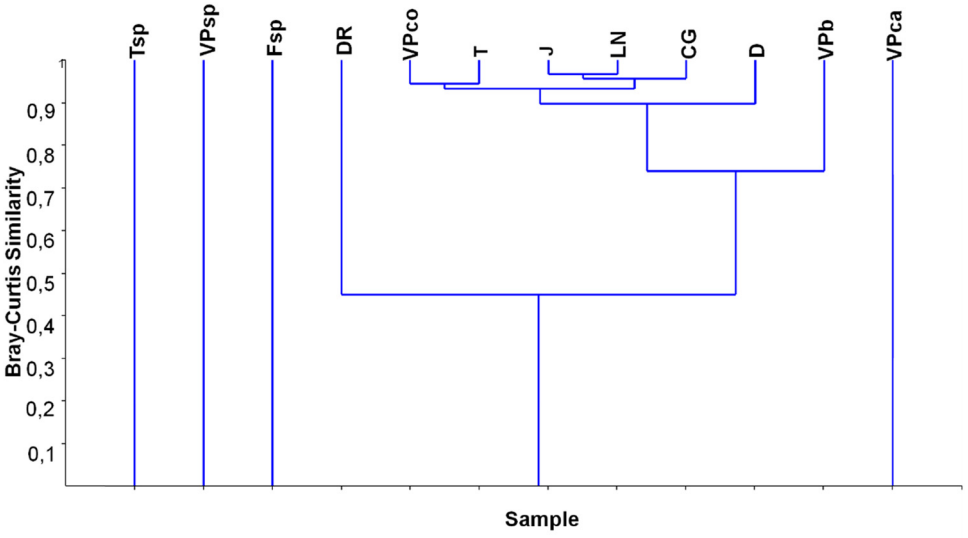

Figure S8: Cluster analysis on microbiological data using Bray-Curtis similarity index.

Table S1: Main analytical parameters obtained for the ion chromatography analysis

	Sensitivity[a]	LOD[b]	LOQ[c]	R^{2d}	RSD%[e]	E%[f]
F^-	24.9	0.03	0.07	0.9946	0.3–5.1	−3.4–5.8
CH_3COO^-	2.0	0.05	0.20	0.9946	3.8–9.5	−5.1–7.0
Cl^-	22.2	0.03	0.10	0.9971	0.5–3.8	−1.8–0.6
Br^-	6.2	0.06	0.10	0.9870	6.6–11	−1.8–0.6
NO_2^-	9.3	0.07	0.10	0.9987	7.5–11	−9.2–13
NO_3^-	10.1	0.05	0.10	0.9993	0.6–1.2	−0.4–0.9
PO_4^{3}	6.0	0.05	0.15	0.9919	3.4–12	−13–11
SO_4^{2}	13.1	0.06	0.10	0.9996	0.1–1.2	−1.3–1.0
Li^+	46.5	0.01	0.05	0.9999	0.7–1.5	−1.0–1.8
Na^+	12.7	0.05	0.10	0.9999	1.1–3.7	−5.0–3.4
NH_4^+	14.1	0.05	0.10	0.9998	0.7–7.9	−5.7–5.6
K^+	6.7	0.06	0.20	0.9998	0.2–5.2	−4.1–6.7
Zn^{2+}	10.5	0.05	0.10	0.9992	1.5–4.6	−3.3–3.5
Mg^{2+}	25.5	0.04	0.10	0.9988	3.5–7.2	−4.0–4.5
Ca^{2+}	13.2	0.05	0.10	0.9990	3.9–5.2	−5.2–7.8
Sr^{2+}	6.49	0.05	0.20	0.9985	3.5–6.8	−3.8–4.8

[a]Sensitivity as slope of the calibration curve (μS·L·s/cm·mg). [b]LOD, limit of detection. [c]LOQ, limit of quantification. [d]R^2, coefficient of correlation. [e]RSD %, percent relative standard deviation. [f]E%, percent accuracy. All data refer to analyzes performed using a 20 μL loop.

Table S2: Anions and cations content found in the analyzed samples (mg/L) and RSD% ranges between 1.0 and 8.2%. Values ranging between LOD and LOQ are reported in Italic

	VPb	VPco	VPca	J	CG	D	LN	DR	T	Fsp	VPsp	Tsp	ACEAv	ACEAp
F^-	0.26	0.34	0.10	0.42	0.16	0.24	0.31	0.56	0.41	0.13	0.11	0.14	0.49	0.12
CH_3COO^-	<LOD	<LOD	<LOD	9.9	<LOD	<LOD	<LOD	<LOD	17	<LOD	<LOD	<LOD	<LOD	<LOD
Cl^-	45.4	44.1	26.7	19.7	20.3	20.1	22.5	18.1	35.5	5.93	6.03	5.96	14.2	6.20
Br^-	<LOD	*0.10*	*0.07*	*0.08*	<LOD	*0.10*	*0.06*	*0.06*	<LOD	*0.07*	<LOD	<LOD	<LOD	<LOD
NO_2	*0.08*	*0.08*	<LOD	*0.06*	*0.07*	*0.09*	*0.08*	<LOD	<LOD	*0.07*	<LOD	*0.10*	<LOD	<LOD
NO_3	46.3	79.0	47.5	23.3	2.46	1.54	25.7	22.5	60.5	3.96	4.28	3.98	14.1	2.93
PO_4^3	0.79	1.07	0.72	*0.14*	2.08	1.48	*0.12*	*0.08*	0.89	*0.10*	<LOD	*0.11*	<LOD	<LOD
SO_4^2	39.7	42.6	30.5	41.4	15.3	15.9	31.6	34.3	45.2	14.9	15.3	14. 8	33.6	16.4
Li^+	*0.04*	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Na^+	39.8	48.7	25.8	29.7	30.2	39.5	40.9	32.7	54.9	3.97	4.35	4.17	19.6	4.10
NH_4^+	0.70	*0.09*	*0.08*	0.61	0.70	0.69	0.69	0.56	0.90	0.50	*0.07*	0.70	<LOD	*0.03*
K^+	38.8	56.1	38.9	37.8	37.5	38.9	38.1	33.1	52.8	1.81	1.70	1.32	19.6	0.98
Zn^{2+}	0.79	<LOD	<LOD	<LOD	<LOD	<LOD	1.03	<LOD	4.13	<LOD	<LOD	<LOD	<LOD	<LOD
Mg^{2+}	16.1	8.72	15.9	14.1	10.3	10.6	11.5	3.56	12.3	19.6	23.0	19.6	17.4	18.4
Ca^{2+}	83.2	90.2	68.6	83.9	89.1	49.2	60.8	43.0	62.3	115	120	113	97.7	105
Sr^{2+}	2.70	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
HCO_3*	244	222	221	263	299	239	249	135	241	363	325	338	401	411

*Obtained by acid-base titration.