Geochemical and microbiological assessment of groundwater status: a case study

Preziosi E. (preziosi@irsa.cnr.it), Del Bon A., Amalfitano S., Fazi S., Zoppini A., Parrone D.*, Ghergo S.

Istituto di Ricerca Sulle Acque, Consiglio Nazionale delle Ricerche, Via Salaria Km 29.300, Monterotondo, Rome, Italy

*Università degli studi di Roma 3, Dip. Scienze Geologiche, Rome, Italy

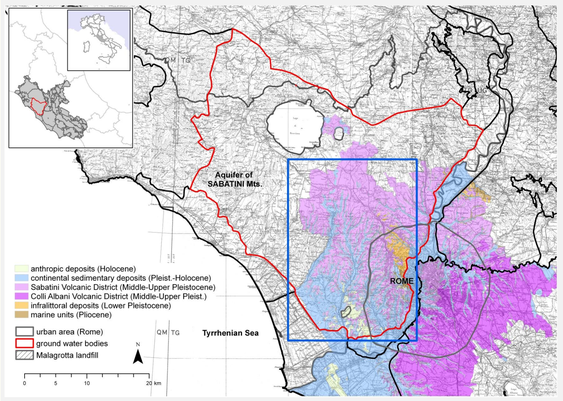
Abstract

The qualitative status of the groundwater resources is drawing increasingly attention in relation to the requirements of the European legislative framework. The monitoring strategies are developed by considering the chemical processes affecting groundwater quality. However, despite the use of biological indicators is a common practice for the qualitative assessment of surface waters, a similar approach is hardly being taken into account by policy makers for subsurface waters. Aquifers are key environments due to the ecosystem capability to ameliorate water quality, e.g. through the natural biodegradation of chemical contaminants.

The objective of this research was to characterize a porous water table aquifer from a geochemical and microbiological point of view, aiming to link the hydro-geochemical properties to distribution patterns of the free-living microbial communities. The broader perspective is to integrate the role of microorganisms in the groundwater evolution processes, with new insights in the knowledge of the different microbial communities inhabiting different aquifer typologies. Moreover, microbiological parameters that could be used as a valuable indicator of groundwater quality are sought.

Sampling site and method

Field data (50 sampling sites)
GPS localization, well depth (m), water table level (m), Eh (mV), T (°C), pH, alkalinity, dissolved oxygen (DO%), conductivity (EC, µS/cm).

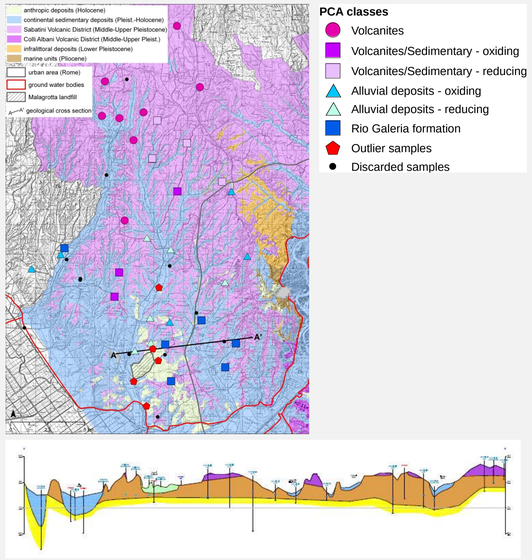


Chemical data
(0.45 µm-filtered samples)
Optical Emission Spectroscopy (ICP-OES) for major cations. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for trace elements. Ionic Cromatography (IC) for major anions. Spectrophotometry for NO_2, PO_4, NH_4, ATP. Shimadzu TOC-5000 analyzer for DOC.

Microbiological data
Flow cytometry for bacterial cell abundance (BAB), live/dead cell ratio (DEAD/BAB%), cells with high and low nucleic acid content (HNA, LNA).
Epifluorescence microscopy associated with Fluorescence *In Situ* Hybridization (CARD-FISH) for the abundance of the major bacterial taxa, cell biovolume. Enzyme Substrate Test for the occurrence of coliforms and *E. coli* cells.

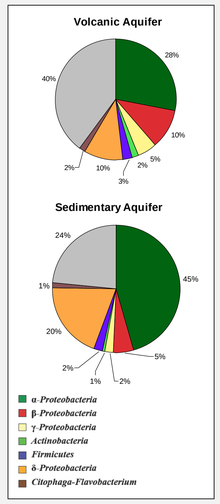
Identified lithotypes

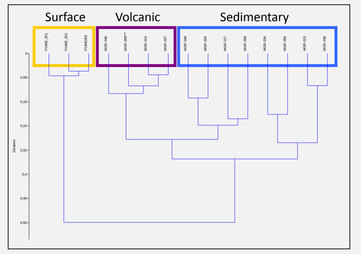
Geological map and localization of samples grouped by the typifying elements and PCA loadings factors.



PCA classes
- Volcanites
- Volcanites/Sedimentary - oxiding
- Volcanites/Sedimentary - reducing
- Alluvial deposits - oxiding
- Alluvial deposits - reducing
- Rio Galeria formation
- Outlier samples
- Discarded samples

		MAINLY VOLCANIC AQUIFER		MAINLY SEDIMENTARY AQUIFER	
	Median concentration per lithotype	Volcanic aquifer	Volcano-sedimentary aquifer	Alluvial deposits	Rio Galeria formation
Physicochemical data	Al (µg/L)	8.05	5.00	2.03	1.75
	As (µg/L)	17.70	8.95	4.95	6.24
	As III/AS	0.02	0.07	0.19	0.15
	B (µg/L)	130.91	92.27	153.91	184.17
	Ba (µg/L)	74.46	74.89	153.21	135.54
	Be (µg/L)	0.12	0.13	0.12	0.12
	Br (mg/L)	0.09	0.09	0.14	0.24
	Ca (mg/L)	39.40	63.80	86.20	136.15
	Cd (µg/L)	0.11	0.11	0.10	0.11
	Cl (mg/L)	28.25	28.05	37.95	68.00
	Cr (µg/L)	1.44	1.84	1.06	1.34
	Cu (µg/L)	1.41	2.21	1.44	2.30
	DO (%)	8.80	7.98	6.25	7.53
	DOC (mg/L)	0.76	0.76	0.68	0.97
	EC (µS/cm)	529.00	553.50	698.00	967.00
	Eh (mV)	220.00	267.00	116.00	190.00
	F (mg/L)	2.07	0.54	0.81	0.69
	Fe (µg/L)	11.33	16.60	48.38	25.47
	HCO3 (mg/L)	206.80	280.60	364.15	477.60
	Hg (µg/L)	0.21	0.22	0.19	0.21
	K (mg/L)	30.10	7.75	8.75	10.85
	Li (µg/L)	21.00	17.00	31.65	20.08
	Mg (mg/L)	13.75	13.90	17.95	30.90
	Mn (µg/L)	2.46	3.77	62.69	4.36
	Na (mg/L)	27.95	34.05	47.70	66.45
	NH4 (mg/L)	0.07	0.14	0.07	nd
	Ni (µg/L)	1.52	2.42	2.73	5.74
	NO2 (mg/L)	nd	nd	nd	0.20
	NO3 (mg/L)	30.81	19.56	4.08	36.78
	Pb (µg/L)	0.31	0.30	0.29	0.30
	pH	7.18	7.46	7.39	7.23
	PO4 (mg/L)	0.44	0.20	0.07	0.05
	Rb (µg/L)	62.15	31.61	22.04	14.27
	Sb (µg/L)	0.71	0.40	0.36	0.39
	Se (µg/L)	0.20	0.45	0.25	0.85
	SiO2 (mg/L)	37.93	30.13	19.00	14.23
	SO4 (mg/L)	16.05	19.15	35.65	97.00
	Sr (µg/L)	411.33	555.67	790.00	1048.00
	T (°C)	16.80	16.65	17.50	17.00
	U (µg/L)	2.28	3.28	2.22	11.22
	V (µg/L)	27.13	15.69	2.31	5.81
	Zn (µg/L)	4.68	20.08	31.26	52.50
Microbiological data	ATP (ng C/ml)	0.06	0.60	0.11	0.13
	BAB (cells/ml)	4.28E+04	3.08E+04	1.20E+05	5.65E+04
	Damaged cells (%)	4.4	3.8	1.9	2.1
	Dead cells (%)	10.9	7.5	4.7	4.5
	Bacteria (%)	42.4	41.3	53.9	38.9
	Archea (%)	12.3	14.9	17.1	22.4
	Alphaproteobact (%)	10.3	11.6	16.9	15.6
	Betaproteobact (%)	4.0	6.9	13.2	2.5
	Deltaproteobact (%)	4.4	6.9	8.4	5.9
	Coliforms (MPN/100ml)	6.3	4.1	0.0	nd
	Ecoli (MPN/100ml)	0.0	0.0	0.0	nd





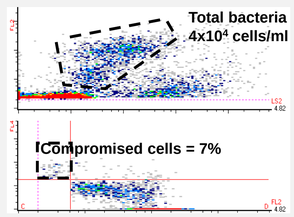
Composition of bacterial community by CARD-FISH with a suite of specific rRNA-target probes: EUB338 I-III specific for the domain of bacteria; ALF1b specific for the *alpha-proteobacteria* subclass; BET42a for *beta-proteobacteria*; GAM42a for *gamma-proteobacteria*; DELTA495a,b,c, for *delta-proteobacteria*; CF319a for *Cytophaga-Flavobacterium*; HGC69a for *Actinobacteria* (Gram-positive bacteria with high GC content of DNA); LGC354a,b,c for *Firmicutes*. Data were expressed as percentage of hybridized cells counts to counts of DAPI-stained cells. Cluster analysis considering the distribution of the most abundant bacterial groups (*Alpha*, *Beta* e *Delta-Proteobacteria*) in surface water (Rio Galeria) and groundwater.

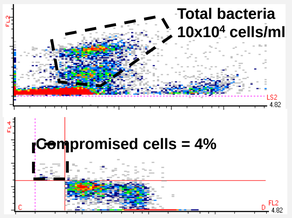
Highlights

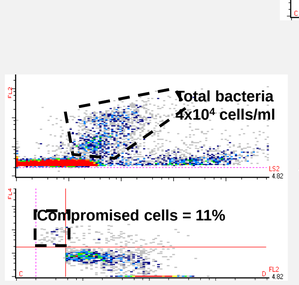
- PCA confirms the existence of 2 main groups of samples (volcanic and sedimentary) subdivided by the redox state (clearly positive, negative /slightly positive)
- Flow cytometry (FCM) properly supported the hydrogeochemical outcomes with multi-parametric micro-biological data for a better understanding of the biogeochemical processes at site level. Volcanic waters show lower contents of total cells and a higher ratio of compromised cells than sedimentary waters: volcanic waters BAB=2-5x10^4; DEAD/BAB%7/15%; sedimentary waters : BAB= 6-10x10^4; DEAD/BAB% < 5%
- FCM results showed that compromised cells percentage was higher in the volcanic samples, enhancing a possible relation with the aquifer geochemistry.
- Differences of the taxa along the flow path were detected with an increasing abundance of Delta-Proteobacteria in the more anoxic sites

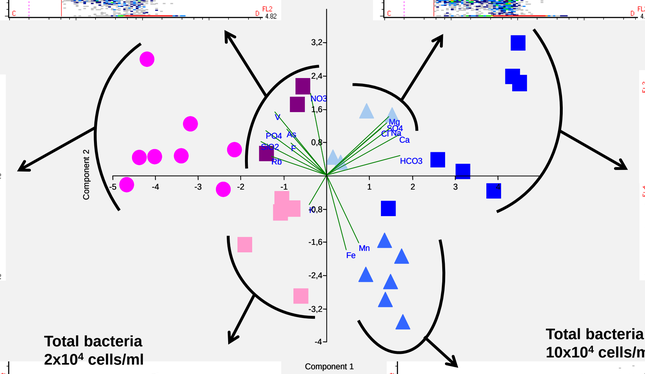
Microbiological characterization

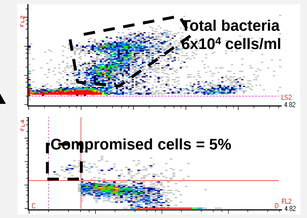
Flow cytometric analyses
Principal component analysis and representative cytograms of the water samples associated with the identified lithotypes.



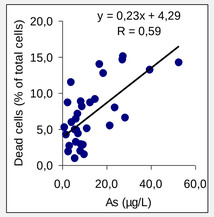


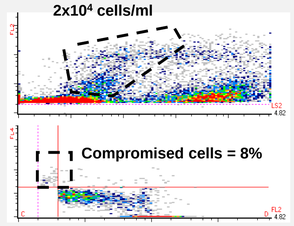


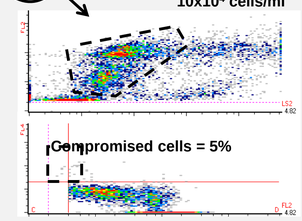




Particle density

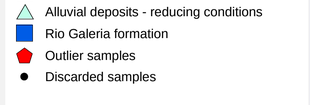




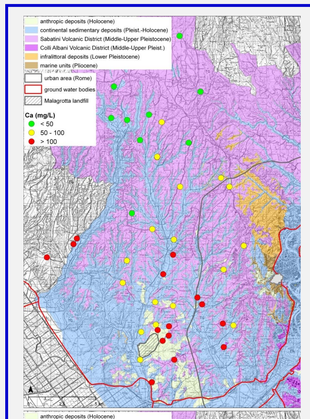


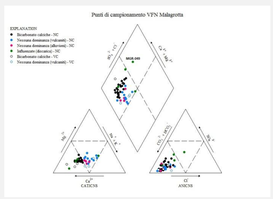
Instrumental settings

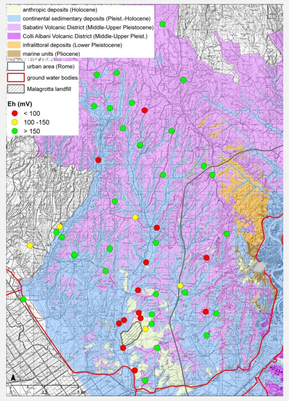
FL2 = Green fluorescence (SYBR Green I; PMT 480V)
FL4 = Red fluorescence (Propidium Iodide; PMT 580V)
FL2 threshold = 20
Subtraction FL4-FL2 = 2%
LS2 = Side light scatter (PMT 400V)
Minimum acquisition time = 90 seconds
Flow cell pressure = 150 mbar
Sample flow rate = 38.9 µl/min

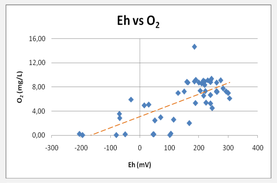


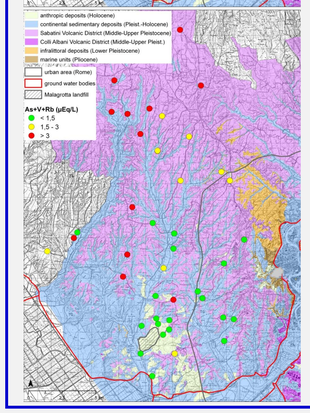
Hydro-geochemical characterization

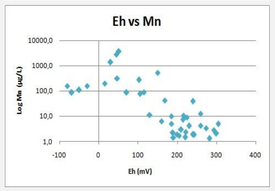


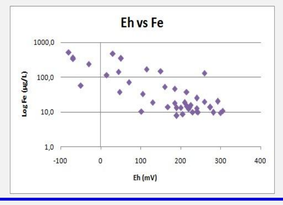












Flowing from north to south ground waters change their composition from nearly alkaline (volcanic aquifer) to clearly earth alkaline bicarbonate waters (sedimentary aquifer).
Eh measurements, although must be considered only qualitative indications of the redox potential of groundwater, show a clear correlation with oxygen and an inverse correlation with Fe and Mn and thus have been considered a good marker for redox state of the aquifers.

Key references

Amalfitano et al., 2009. Flow cytometric analysis of benthic prokaryotes attached to sediment particles. J Microbiol Methods, 79: 246–249.
Falcioni et al., 2008. Evaluating the flow-cytometric nucleic acid double-staining protocol in realistic situations of planktonic bacterial death. Appl Environ Microbiol, 74: 1767–1779.
Manti et al., 2011. Experimental improvements in combining CARD-FISH and Flow Cytometry for bacterial cell quantification. J Microbiol Methods, doi: 10.1016/j.mimet.2011.09.003.
Preziosi et al. 2010. Natural background levels and threshold values derivation for naturally As, V and F rich groundwater bodies: a methodological case study in Central Italy. Environ Earth Sci (2010) 61:885–897, doi: 10.1007/s12665-009-0404-y
Vivona et al., 2007. Occurrence of minor toxic elements in volcanic-sedimentary aquifers: a case study in central Italy. Hydrogeol J, 15: 1183–1196.

European Geosciences Union General Assembly 2012
Vienna, Austria 22 – 27 April 2012