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GROUNDWATER FLOW AND MICROBIAL CONTAMINANT DYNAMICS IN LOW-PERMEABILITY HETEROGENEOUS AQUIFERS: A MULTIDISCIPLINARY STUDY FROM THE NORTHERN APENNINES, ITALY

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EXTENDED ABSTRACT

Gli acquiferi a bassa permeabilità costituiti da marne, arenarie e peliti possono rappresentare sistemi acquiferi strategici, specialmente in aree caratterizzate da una scarsa presenza di corpi geologici ad elevata produttività. La comprensione del funzionamento idraulico di questi sistemi e delle loro dinamiche di contaminazione microbiologica costituisce una sfida scientifica di rilievo per molteplici aree del mondo caratterizzate da assetti litologici, strutturali e antropici analoghi. In questo contesto, l'area di Varano de' Melegari (Appennino Settentrionale, Italia) è stata selezionata come sito di studio rappresentativo per l'analisi delle dinamiche di flusso delle acque sotterranee e della contaminazione fecale. Con un'estensione di circa 15 km², l'area, vocatamente agricola e a ridotta densità abitativa, comprende due formazioni appartenenti alla Successione Epiligure, la Formazione di Monte Piano e la Formazione di Ranzano. All'interno di queste unità sono presenti numerosi pozzi e sorgenti, distribuiti in modo frammentario nel contesto geo-litologico eterogeneo, utilizzati come rete di monitoraggio ai fini dello studio. L'obiettivo principale di questo lavoro, oltre alla comprensione preliminare del comportamento idrogeologico del sistema, è stato quello di identificare i processi che regolano la presenza e la variazione spazio-temporale degli indicatori di contaminazione fecale, sviluppando un quadro conoscitivo applicabile a contesti comparabili. È stato adottato un approccio multidisciplinare che ha integrato: (*i*) rilievi geologici di dettaglio, (*ii*) monitoraggio (un anno idrologico) delle portate e dei livelli piezometrici, e (*iii*) campionamenti trimestrali per analisi batteriologiche (Enterococci fecali) e isotopiche (trizio). Inoltre, lo studio ha previsto un censimento con conseguente mappatura delle possibili fonti di contaminazione fecale ubicate nel territorio analizzato; nello specifico, sono state spazializzate le aree agricole su cui è avvenuta fertilizzazione attraverso concimi a base di letame, i ricoveri di animali (con catalogazione di tipologia e numero di capi), gli ammassi di stoccaggio di letame e la rete fognaria.

L'integrazione dei risultati ottenuti ha consentito di elaborare una prima interpretazione circa il comportamento idraulico del sistema eterogeneo, caratterizzato, nonostante la bassa permeabilità del mezzo, da rapide fluttuazioni sia dei livelli piezometrici sia della portata delle sorgenti, comprese tra 0,0 e 0,3 l/s, in risposta alle precipitazioni. Il gradiente idraulico risulta elevatoe può raggiungere valori nell'ordine del 50% ($i \approx 0,5$), in accordo con quello topografico, come atteso per mezzi a bassa permeabilità. In aggiunta, il basso contenuto di trizio, compreso tra 0,46 e 4,51 UT, ha aperto a possibili scenari interpretativi, circa la coesistenza tra (*i*) componenti di flusso più rapide e più lente e (*ii*) mescolamenti tra acque ascrivibili a circuiti più o meno lunghi e/o profondi all'interno dell'acquifero.

Da un punto di vista microbiologico è stata ulteriormente comprovata la rapida risposta dell'acquifero all'infiltrazione efficacie, tuttavia, il fattore predominante circa la distribuzione spazio-temporale degli indicatori fecali (Enterococci intestinali) è risultato essere associato in via preferenziale alle dinamiche meteo-climatiche (e microclimatiche) locali e, più nello specifico, all'influenza che la temperatura esercita sulla crescita delle colonie batteriche nei suoli locali.

Dallo studio emergono potenziali spunti di affinamento, sia in ambito quantitativo sia in ambito qualitativo, che sono in corso di approfondimento mediante studi condotti a differenti scale di investigazione e supportati da un ampliamento del numero e della tipologia di analisi sul mezzo acquifero e sulla risorsa idrica in esso contenuta.

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ABSTRACT

Low-permeability aquifers hosted in marls, sandstones, and pelites represent strategic but often complex water storage bodies in regions where extensive and highly productive groundwater reservoirs are absent. Understanding the hydrogeological functioning of these systems and their vulnerability to microbial contamination is a key scientific challenge, relevant to many areas worldwide where similar lithological and structural settings occur. In this framework, the area of Varano de' Melegari (Northern Apennines, Italy) was selected as a representative test site to investigate groundwater flow and fecal contamination dynamics within low-permeability aquifers. The study area, covering approximately 15 km², includes two formations of the sedimentary Epiligure Succession, the Monte Piano and Ranzano Formations, and hosts numerous wells and springs used for irrigation, domestic supply, and livestock farming. The main objective of this preliminary work was to identify the processes governing the occurrence and spatial distribution of fecal indicators in such aquifer systems, and to develop a conceptual framework applicable to comparable hydrogeological contexts. A multidisciplinary approach was employed, combining (*i*) detailed geological surveys, (*ii*) long-term monitoring of discharge, hydraulic head, and (*iii*) sampling for bacteriological (fecal indicators), and isotopic (Tritium) analyses. The integrated results provided a preliminary interpretation of the hydraulic behavior of this heterogeneous system. Despite the low permeability of the aquifer, both piezometric levels and spring discharge responded rapidly to rainfall events, with discharge ranging from 0,0 to 0,3 L/s. The hydraulic gradient was consistently high and locally reached values of approximately 50% ($i \approx 0,5$), in agreement with the topographic gradient, as expected for low-permeability media. In addition, the low tritium content (0,46–4,51 TU) suggests the coexistence of both faster and slower flow components, as well as mixing between waters associated with groundwater flow paths of different lengths and/or depths within the aquifer. From a microbiological perspective, the rapid response of the aquifer to effective infiltration was further confirmed. However, the spatial and temporal distribution of fecal indicators (Fecal Enterococci) was found to be primarily controlled by local meteorological and microclimatic conditions, particularly by the influence of temperature on bacterial colony growth in the surrounding soils.

The results also provide new insights for further investigation in both qualitative and quantitative fields for these kinds of aquifers.

Keywords: *low-permeability media, sandstone aquifers, marls aquifers, pelite aquifers, groundwater fecal contamination, groundwater microbial contamination.*

INTRODUCTION

Groundwater represents a strategic resource for low-permeability hydrogeological contexts such as those composed of geological successions made by alternating marls, sandstones, and pelite rock units. Due to their lithological and hydraulic properties, these geological sequences are commonly regarded as aquitards at the regional scales and are therefore rarely used as primary groundwater resources (*e.g.*, Freeze & Cherry, 1979; Kresic, 2023). This is especially true in areas such as Northern Italy, where large alluvial (Po Plain) and carbonate (Alps) aquifers satisfy most groundwater demands (Beretta *et alii*, 2025). Focusing on low-permeability sedimentary aquifers, the test site of Varano de' Melegari (Italian Northern Apennine) has been chosen due to the presence of the Epiligure succession: a group of poorly porous sandstones, marls and pelite units (Pinardi *et alii*, 2023). Hydrogeological behavior of this succession has been investigated in detail in previous studies by Gargini et alii, 2008, Filippini *et alii*, 2024, 2025, and Pugliese *et alii*, 2025, providing a regional framework for the interpretation of groundwater dynamics in similar hydrogeological context. Groundwater quality is of primary importance, especially with respect to microbiological contamination of fecal origin, which may compromise the suitability of the resource for drinking purposes and pose potential risks to human health (Grif *et alii*, 2005). The accommodation of the present work in this specific study area is also linked to an in-depth knowledge about the local dynamics of possible contaminant spills from sewers or agricultural activities. Microbiological contamination in these kinds of media is a complex phenomenon, closely controlled by the geological-hydraulic characteristics of aquifers and recharge processes (Rizzo *et alii*, 2020). An additional source of complexity is represented by the seasonal variability in the availability and transport of fecal microbial contaminants, which is influenced by precipitation-temperature patterns and agro-livestock practices: temporal fluctuations in microbial (fecal) indicator concentrations could be influenced by natural and anthropogenic dynamics change (*e.g.*, seasonal temperatures, precipitations and livestock management) (Bucci *et alii*, 2015). Beyond their role as indicators of groundwater quality, fecal microbial contaminants can also be used as natural tracers of groundwater flow, providing valuable information on recharge pathways, transit times, and surface–groundwater interactions (Boschetti *et alii*, 2014; Bucci *et alii*, 2014; Henrandez-Diaz *et alii*, 2019). The aim of this preliminary work is to improve the understanding of microbiological transport and occurrence mechanisms in these systems, and to provide insights relevant to the protection of groundwater resource. This issue could be crucial for this region and for similar areas worldwide, also in expectation of increasing temperatures (and consequent microbial activity) linked to climate change.

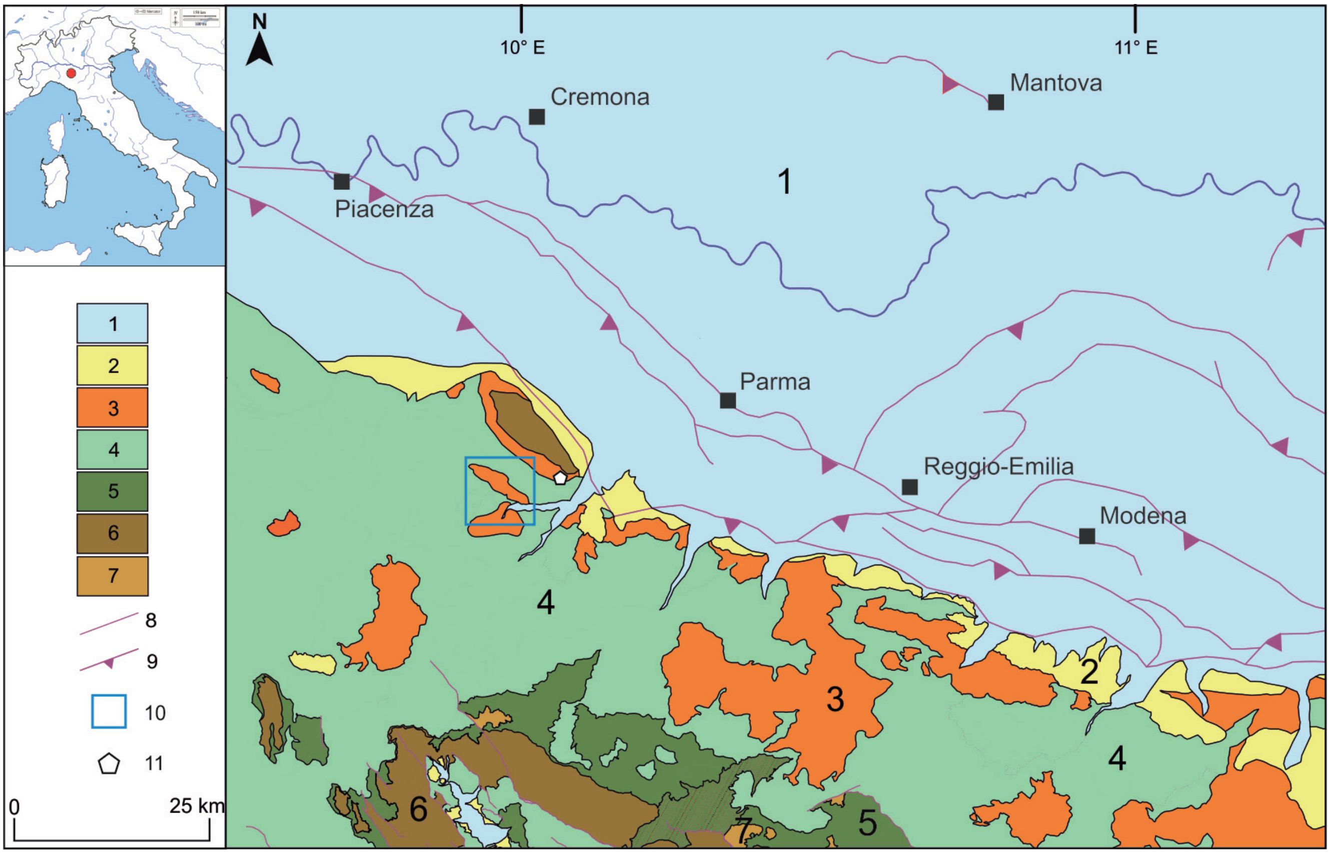

Fig. 1 - Geological setting at regional scale, modified from (Conti et alii, 2020). LEGEND: [1] Quaternary Deposits; [2] Miocene-Pleistocene succession; [3] Epiligurian succession; [4] Ligurian Units; [5] Subligurian Units; [6] Tuscan Nappe; [7] Cervarola/Falterona Unit; [8] Faults; [9] Thrusts; [10] Study area geological context; [11] ARPA official meteorological station

STUDY AREA

The study area is located in the municipality of Varano de' Melegari (Province of Parma), at an altitude ranging from 190 to 430 meters above sea level, within the Northern Apennines (Italy). This portion of the chain is characterized by a complex geological and structural framework (Conti *et alii*, 2020), resulting from the superposition of different tectono-stratigraphic units and the widespread occurrence of fractured sedimentary formations (see Fig. 1).

The geological units cropping out in the area belong to the Epiligure Succession (Ricci Lucchi & Ori, 1985, Piazza *et alii*, 2016, and Tinterri *et alii*, 2017), which was deposited from the Middle Eocene to the Upper Miocene in an unconformable relationship over the older Ligurian units (Cretaceous–Eocene). This regional unconformity records the complex geodynamic evolution of the Apennine orogenic system and strongly controls the stratigraphic architecture of the succession.

Within the Epiligure Succession, the RAN3 unit (Ranzano Formation – Varano de' Melegari Member, *sensu* Di Dio *et alii*, 2005) consists predominantly of fractured sandstone, locally interbedded with subordinate from centimetric to pluri-metric pelite levels. The RAN3 unit is stratigraphically overlayed by the RAN3a unit (Ranzano Formation – Specchio Chaotic, *sensu* Di Dio *et alii*, 2005), a chaotic and heterogeneous assemblage mainly composed of pelitic and marly lithologies. At the bottom, the succession is bordered by the MMP unit (Monte Piano Marls, *sensu* Di Dio *et alii*, 2005), consisting of marly–pelitic sediments.

On the valley floor are observable flat areas characterized by Quaternary and Holocene gravel and sand deposits, linked to recent alluvial dynamics of Ceno Stream and its tributaries (AES8 – Ravenna Subsynthem *sensu* Di Dio *et alii*, 2005).

MATERIAL AND METHODS

A field survey was conducted in the entire study area to characterize and map the overmentioned (hydro)geological units and relatives' groundwater monitoring points. The monitoring network consists of a 18 groundwater points, including 9 wells (with depth varying between 2,20 and 10,50 m below ground level), 3 perennial springs, 4 seasonal springs and 1 artificial drainage system (Table 1).

Groundwater point (ID)	Type	Hydrogeological unit location
VMP1	Well	Sandstone (RAN3)
VMP2	Well	Sandstone (RAN3)
VMP3	Well	Sandstone (RAN3)
VMP4	Well	Sandstone (RAN3)
VMP5	Well	Sandstone (RAN3)
VMP6	Well	Sandstone (RAN3)
VMP7	Well	Sandstone (RAN3)
VMP8	Well	Marls (MMP)
VMP9	Well	Marls (MMP)
VMS1	Seasonal Spring	Sandstone (RAN3)
VMS2	Seasonal Spring	Sandstone (RAN3)
VMS4	Seasonal Spring	Sandstone (RAN3)
VMS5	Seasonal Spring	Sandstone (RAN3)
VMS6	Seasonal Spring	Sandstone (RAN3)
VMS7	Perennial Spring	Sandstone (RAN3)
VMS8	Perennial Spring	Sandstone (RAN3)
VMS9	Perennial Spring	Sandstone (RAN3)
VMD	Artificial Drainage	Pelite (RAN3a)

Tab. 1 - Groundwater sampling points list (location is shown in Figure 2)

Spring flow rate and wells' hydraulic head measurements were carried out to quantify groundwater outflow and to support the interpretation of groundwater flow dynamics over space and time. Surveys were conducted in the field from June 2024 to August 2025 with data registration every month (and in some cases every two weeks). Due to spring intake structures, the flow rate could only be measured in VMS2, VMS8 and VMD.

A groundwater sampling campaign was conducted in June 2024 for the analysis of tritium (3H). Water samples were collected in 16 groundwater monitoring points following standard protocols to avoid contamination and isotopic alteration. Tritium analyses were performed at the laboratory of the Department of Mathematics and Geosciences (DMG), University of Trieste. Using the same method, a composite average rainfall sample for the period from January to June 2024 was collected inside the study area and analyzed (location of the rainwater sampler is shown in Figure 2).

Tritium data were used to investigate groundwater residence times and to identify possible differences in groundwater circulation dynamics within the aquifer.

Meteorological data were collected to support the interpretation of groundwater recharge processes and hydrological variability. Time series of precipitation and air temperature measured at 2 m above ground level from June 2024 to August 2025 were obtained from the official website of ARPA Emilia-Romagna. These data have been collected by the meteorological station located in the near Medesano Municipality (Lat = 44.733399, Long = 10.023631) and were used to characterize the climatic conditions during the monitoring period. This aspect became the basis to evaluate potential relationships between climatic inputs, groundwater discharge and fecal contamination.

Two groundwater sampling campaigns were conducted for microbiological analysis (fecal indicators) in May 2025 (recharge period) and July 2025 (discharge period) in four sampling points representatives of different hydrodynamics features of the test site aquifer (VMS2, VMS7, VMS8 and VMD). Groundwater samples (1 L) were collected in sterile bottles with the protocol used by Ducci *et alii*, 2023. All samples were stored in a refrigerated box and transported to the laboratory. Filtration processes were carried out within 2 h after collection. Indicators of microbial contamination (intestinal *Enterococci*) were determined in groundwater samples using standard methods of water filtration (100 mL) on sterile membrane filters (GN-6 Metricel, pore size 0.45 μm, Pall), with incubation on Slanetz–Bartley agar for 48 h at 37 °C.

In addition, a survey and a mapping was incentered on the definition of (*i*) the farms in the area (quantifying the type of livestock and the number of animals), (*ii*) livestock manure storage piles (estimating the volume), (*iii*) parcels of land fertilized with livestock manure or used for grazing and (*iv*) the layout of the local sewer network.

RESULTS AND DISCUSSION

At first, the spatialization of the measured groundwater hydraulic head in wells, together with the spring elevation, highlighted a hydrogeological context in which groundwater flows in accordance with the topographical slope within the fractured sandstones (RAN unit), both on the SW side and on the northern edge of the aquifer (where it appears unconfined and phreatic). One non-mappable pelite strata has been found interbedded between the RAN3 sandstone, capable of creating a local permeability threshold along the vertical direction, generating the mapped mid-slope springs (Figure 2). In addition, in the NE side of the studied system, the entire sandstone unit could be considered diffusely confined by the pelitic RAN3a unit (Figure 2). RAN3a pelite layer, with its tens of meters thickness, could be considered as a diffuse aquitard at the top of the succession with a low-permeability aquifer behavior on the altered and de-compressed surficial portion. In this sense, a seasonal perched groundwater has been detected at the top of this fine-grained unit thanks to the presence of the artificial drainage (VMD) (Figure 2).

At the bottom of the sandstones, marls (MMP unit) acts as a partial receptor of groundwater flow with a diffuse transfer along the contact surface between these two units. As evidenced by the presence of the wells VMP8 and VMP9, lithogenic and structural discontinuities inside marls unit can favor the permeability of this very low primary porosity medium. At the same way, also the contact surface between fractured sandstones and alluvial high-permeability deposits (AES8) represents a groundwater transfer zone of at the valley floor in the southern edge of the investigated system (Figure 2).

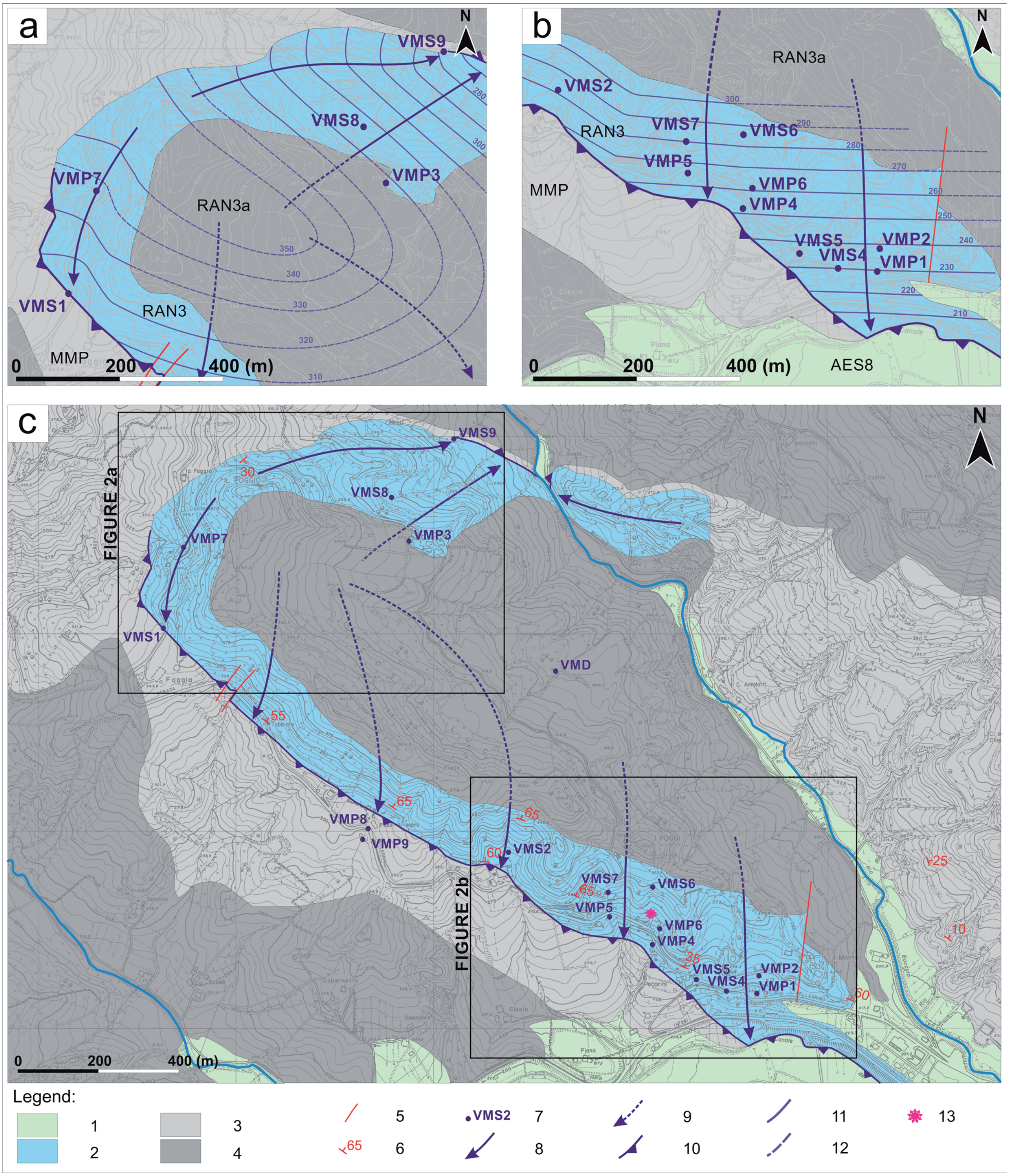

Fig. 2 - Hydrogeological setting resulted (Figure 2a and 2b show two focus areas with groundwater table contour line reconstruction; Figure 2c shows a comprehensive hydrogeological overview of the study area; Figure 2d shows the reconstructed hydrogeological section AA', representative of the studied hydrogeological context. LEGEND: [1] Alluvial Deposits; [2] Sandstone unit; [3] Marls unit; [4] Pelite unit; [5] Fault; [6] Strata bed orientation and inclination angle; [7] Groundwater monitoring point; [8] Groundwater flowlines (unconfined condition); [9] Groundwater flowlines (confined condition); [10] Groundwater transfer contact. [11] Groundwater contour lines and relative elevation in m a.s.l. (unconfined condition); [12] Groundwater contour lines and relative elevation in m a.s.l. (confined condition); [13] Rainwater sampler for Tritium analysis.

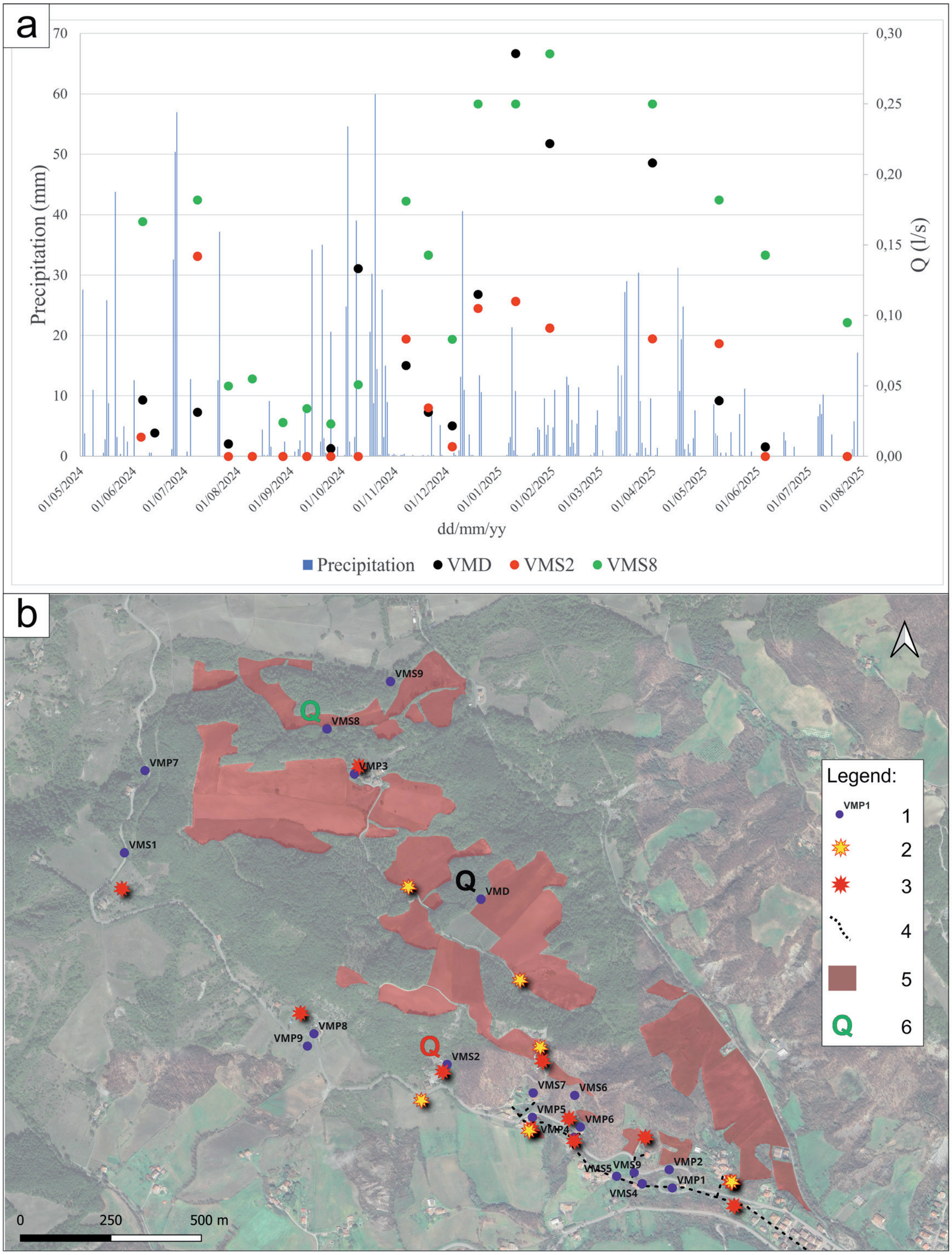

Fig. 3 - Graph showing variability of flow rates (Q) in springs VMD, VMS2 and VMS8 in relation to precipitation, from June 2024 to August 2025. VMS2 is representative of the Sandstone unit in the south-facing slope, VMS8 is representative of the Sandstone unit in the north-facing slope and VMD is representative of the stress-relieved and weathered superficial Pelite unit.Fig. 3b – Map of potential fecal contamination sources within the study area. LEGEND: [1] Groundwater monitoring point; [2] Livestock manure storage pile; [3] Livestock stable; [4] Sewer; [5] parcels of land fertilized with livestock manure; [6] Q symbol indicates the springs for which flow rate monitoring is reported in Figure 3(a)

Flow rate measurement varied for the entire study area from 0,0 to 0,3 L/s during the monitoring period and showed a fast response of the aquifer system to precipitation with peak recharge periods during winter and early spring. Late spring and summer periods, characterized by low rainfall, led to an immediate decrease (VMS8) and/or complete depletion (VMS4, VMD) in flow rate (see, Figure 3a).

Tritium results, on the other hand, showed the complexity of the system in terms of groundwater transit time inside the heterogeneous succession, with the coexistence of different possible flow components, still open to further investigation. These results, compared with the average rainfall value for the six months prior to sampling, would refer to periods of groundwater residence inside the succession in some cases compatible with several decades (see Table 2).

In this hydrogeological context, microbiological analysis revealed a marked variability of fecal indicators (intestinal *Enterococci*) over space and time (see Table 3). In general, an increasing occurrence in perennial spring VMS7 and VMS8 has been observed from spring to summer season (VMS2 and VMD have been deactivated in accordance with their seasonal regime, see Figure 3a). Spring VMS7 resulted the monitoring point with the maximum occurrence of fecal indicators in both the sampling campaigns.

To reconstruct the contamination dynamics, a mapping of potential fecal source permitted to spatialize above the studied territory (*i*) 8 chicken coops with between 15 and 25 head of stock, 2 goat stable with between 10 and 20 head of stock and 2 horse stable with between 2 and 5 head of stock; (*ii*) 6 livestock manure storage piles (with volume ranging from 5-30 m3); (*iii*) 33 hectares (ha) of parcels of land fertilized with livestock manure during autumn; (*iv*) the layout of the local sewer network with a length of 950 meters in the southern edge of the study area (Figure 3b).

The resulting scenario shows a widespread distribution of possible manure releases and permitted to exclude the sewer origin, due to its position downstream to the monitored points. In relation to these aspects, it was not possible to define a logic between the variability of fecal *Enterococci* occurrence in the different aquifer sectors and the availability of potential sources on surface. In fact, the increasing of fecal indicators does not correspond to an increase in manure release (autumnal) and/or an expansion in the number of livestock heads (quite steady).

As evidenced by Bucci *et alii*, 2015, Allocca *et alii*, 2008, Celico *et alii*, 2008, Rizzo *et alii*, 2020, in some cases these kinds of contamination could be unconnected to the spatial and temporal dynamics of fecal source release but better connected with biological cycles of bacteria. Their optimal growth occurs under conditions similar to those of the animal body, at temperatures between 35 and 37 °C (Garcia-Solarche & Rice, 2019). The number of fecal Enterococci colonies in soil (and consequently transported to groundwater by effective infiltration waters) is, for this reason, strongly influenced by surface heat. In the context of this case study, the same behavior has been observed: the significant growth of colonies registered in the sampled groundwater goes hand in hand with the seasonal rise in temperatures (see Figure 4).

Groundwater point (ID)	Tritium Units (TU)
VMP1	3,34
VMP2	3,12
VMP3	2,39
VMP4	3,51
VMP5	3,09
VMP6	2,45
VMP7	4,51
VMS1	4,05
VMS2	3,62
VMS3	3,01
VMS4	2,17
VMS5	3,92
VMS6	3,63
VMS7	0,46
VMS8	2,74
VMD	3,93
Precipitation	8,51

Tab. 2 - Tritium analysis results

Groundwater point (ID)	Fecal Enterococci (CFU/100 ml) May 2025	Fecal Enterococci (CFU/100 ml) July 2025
VMS2	96	inactive
VMS7	128	878
VMS8	4	272
VMD	4	inactive

Tab. 3 - Microbiological analysis results

Observing fecal microbial occurrence diversification in the May campaigns from this temperature-based perspective as well, the microclimate (*sensu* Geiger *et alii*, 1995) component becomes of fundamental importance: fecal indicators in groundwater resulted to be highest in the SW-facing sector of the study area, that is warmer in soil temperature thanks to a better sun exposure. The climatic (or microlimatic) substantial difference between the SW and the NE sides of the studied territory is also confirmed by marked vegetational diversities, with typically Mediterranean species (*Salvia rosmarinus, Olea europaea, Opuntia ficus-indica, Ficus carica*) in the south, that differ from the more continental ones (*Quercus robur, Quercus petrae, Carpinus betulus, Fraxinus excelsior*) on the north-facing side.

This clear interdependence between surface temperature and the increase in fecal indicators in groundwater also indicates at least a very rapid and widespread infiltration and transport within the low-permability aquifer, which must necessarily coexist with the slower circuits evidenced by tritium analyses.

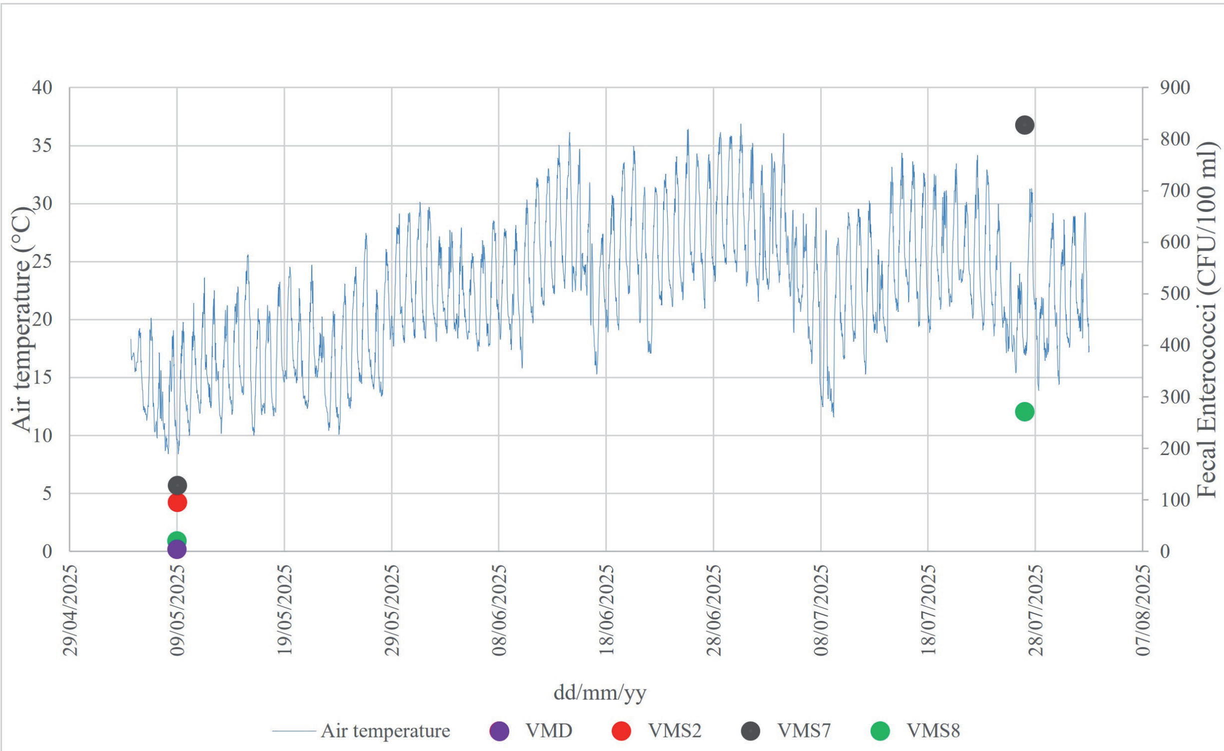

Fig. 4 - Graph showing relation between increasing air temperature and fecal indicators (fecal Enterococci) in groundwater monitored points

CONCLUSION

The work was carried out using a multidisciplinary approach, integrating hydrogeological, isotopic, and microbiological analyses, to investigate the hydraulic behavior of aquifer sequences consisting of low-permeability units and their response to fecal contamination. At first, the study case selected for these aims (Varano de' Melegari, Northern Apennine, Italy) showed that geological bodies made of sandstone, marls and pelite alternance are characterized by scattered outflow points, both perennial and seasonal, due to a widespread hydraulic heterogeneity of the aquifer system.

Despite its low or very low permeability, depending on the single Formation of the sequence, the heterogeneous aquifer is characterized by rapid fluctuations of both the hydraulic head and the springs discharge. Taking into consideration the relatively high hydraulic gradient (coherent with the overall low permeability of the medium), this fast response should be mainly due to the relatively thin unsaturated zone and, then, the short percolation time of the effective infiltration waters from the ground surface towards the groundwater surface. In this scenario, the widespread and significant microbial contamination of fecal origin further supports the existence of short migration pathways, according to the well-known mean survival of fecal bacteria in groundwater (*e.g.*, about 50 for *Escherichia coli*).

Therefore, the low tritium content detected in spring-waters suggest different possible scenarios: (*i*) within the whole heterogeneous medium there is the coexistence of faster and slower pathways and/or (*ii*) there is the mixing between groundwater recharged locally (short pathways) and far from the study site, northeastern of the confined part of the studied aquifer system and/or (*iii*) there is a contribution of rising deep fluids.

To refine knowledge about the possible phenomena synthesized above, the research is still in progress. Based on the results obtained until now, the following activities have been planned: (*i*) expansion of the study area, with emphasis on the northeaster portion of the site, (*ii*) increase in the number of springs and wells to be deeply investigated, (*iii*) hydrochemical investigations, (*iv*) stable isotopes analysis, (*v*) metagenomic investigations.

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