Groundwater bacteriological quality assessment: impact of urbanization and agricultural activity
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ABSTRACT

Groundwater from boreholes is the major source of bottled water in Algeria. The aim of this study is to determine the bacteriological quality of groundwater that serves bottled water production. A total of 73 groundwater boreholes were sampled and analyzed for the required bacteriological parameters. The analysis was performed in accordance to ISO standards methods. There should be no bacteria growth for each bacteriological parameter to qualify the groundwater of good bacteriological quality. The bacteriological analysis highlighted that 37 of the 73 groundwater samples (51%) were of poor bacteriological quality while 36 of them (49%) were of good bacteriological quality. Total coliforms and E. coli were the major sources of contamination with respectively 35 and 24 contaminated samples, followed in order by Pseudomonas aeruginosa, enterococci and sulfite reducing anaerobic bacteria spores with respectively 8, 7 and 2 contaminated samples. Bacteriological quality was strongly and negatively correlated with urbanization and/or agricultural activity parameter ($r = -0.454$). The performed logistic regression model showed that the presence of urbanization or agricultural activity multiplies significantly ($P < 0.001$) the risk by 7 of being a poor bacteriological quality groundwater. These findings are useful to avoid drill costs and to take the best strategy to protect groundwaters.

Key words | anthropic activity impact, boreholes, coliforms, groundwater quality

HIGHLIGHTS

- Half of groundwaters do not satisfy the bacteriological quality criteria.
- Total coliforms and E. coli are the major sources of contamination in the studied groundwaters.
- The presence of urbanization or agricultural activity has a negative impact on groundwater bacteriological quality.
- Evidence was shown that drilling costs could be avoided by adopting the groundwater quality prediction tools.

INTRODUCTION

Algerian bottled water demand is increasing (Hazzab 2010). The total production comes from groundwater. Its total potential is estimated 2.7 billion m³ in the Atlas northern region and 5 billion m³ in the southern region of the Sahara (Chabour et al. 2018). Groundwater provides 63% of the total water demand in the Northern region and 96% of the water demand in the Sahara region (FAO 2009). Taking into consideration its impact on public health and the economic sector, it is important to assess and monitor the groundwater quality, with focus on the bacteriological...
quality for the prevention against waterborne disease. Groundwater destined for bottling is subject to rigorous bacteriological control before putting it in the market (Ministry of Water Resources 2006). The cost of drilling being important (World Bank 2007), the determination of the factors influencing the bacteriological quality of groundwater is useful in the perspective of avoiding financial waste and providing more rationale to the feasibility studies.

National regulations define the groundwater bacteriological quality parameters and the sample size that should be analyzed for each parameter (Ministry of Water Resources 2006; Ministry of Trade 2016). In this regard, we are generally seeking for fecal contamination indicators. The water is considered to be of good bacteriological quality when there is no growth of bacteria for each evaluated parameter.

Bacteriological pollution of groundwater is generally due to the presence of fecal contamination bacteria indicators, originating mostly from humans or animals (Koffi-Nevry et al. 2012), through intense urbanization, that produces domestic effluents and agricultural activity via animal husbandry or the use of manure and slurry as natural fertilizer (Seiler et al. 2000; Esterhuizen et al. 2012). Several previous studies were carried out to assess the bacteriological and physicochemical quality of groundwater in few regions of Algeria, revealing considerable pollution and concluding on assumptions about the origin of such pollution (Fehdi et al. 2014; Gueroui et al. 2014; Benouara et al. 2016). Sampling size and localization of these studies suffered from several limitations pertaining mainly to small sample sizes and focusing only on one region. Thus, in the current study, an intense effort has been made to collect more samples over many provinces of Algeria, in order to strengthen the results. A study carried out in the city of Bechar did not demonstrate a significant impact of urban domestic effluents on the groundwater bacteriological quality (Kabour et al. 2013). The previous studies highlighted the lack of agricultural activity and urbanization impact on the groundwater bacteriological quality in Algeria and paved the path to this study towards the examination of this question. We collected multiple samples from several boreholes in different provinces of Algeria, carried out a bacteriological analysis. We also constructed a dataset on factors that may influence the bacteriological quality of groundwater, mainly the depth of the borehole, the temperature of water at source, and anthropic activities such as the presence or absence of nearby agricultural or urban activity from the borehole site.

This study aims to assess the bacteriological quality of groundwater in Algeria and to determine the impact of factors that may influence it, in terms of agricultural activity and urbanization.

MATERIALS AND METHODS

Study area and study design

A cross-sectional study was conducted on 73 boreholes located in 25 provinces of Algeria (Figure 1), over a period of one year (from March 2019 to November 2019). Groundwater samples were collected from different water boreholes exclusive for the production of bottled water. The majority of studied boreholes at the Northern provinces were drilled from alluvial aquifers, especially the alluvial aquifer of the Soummam Valley and the alluvial aquifer of the Mitidja. The provinces of the interior and the Saharan Atlas were drilled from karst aquifers mainly the Chott-Chergui karst aquifer and El Outaya-Tolga karst aquifer, except the Hodna aquifer which is an alluvial aquifer. The boreholes of the Saharan and southern provinces like Biskra, Laghouat, ElBayadh and Béchar were drilled from the Terminal Complex aquifer and the Intercalary Continental aquifer which have the greatest depths compared to the previous ones. One sample was collected from each borehole. Depending on the province, there were one to five collected samples per province, except Bejaia province where there were 20 collected samples (Figure 2). These water boreholes cover different areas of the provinces, from desert, agricultural, urban and rural zones. Urban areas are associated with domestic sewage due to human activities, whereas agricultural areas are characterized by an intense presence of farms or cultivated land of fruit trees or vegetables leading to the diffusion of pollution on the ground.

Collection of groundwater samples

Groundwater sampling was carried out by a team comprising biologists and trained health workers in accordance
with the methods described in the ISO standards (ISO 19458:2006), during period of March–April and September–November to avoid the rainy season because of the risk of contaminations by rainwater and the drought season because the water level is very low in the borehole. An information sheet reported temperature of groundwater that was measured at source with a thermometer. Depth of the water borehole (drilling depth) and other information on the vicinity of water borehole (presence of agricultural activity and/or urbanization in the neighborhood) (see Figure 1 | Mapping of the boreholes locations across the Algerian provinces.)
supplementary information, Table S1). The boreholes were purged by a pump through stainless steel pipe. A total of 73 groundwater samples were collected from the study area during early morning hours (07:00–09:00 h). The person collecting the samples should wear sterile gloves. The water should be left to run from the borehole for a few hours by turning on the tap before sampling. The steel tap should be flamed with alcohol and gas torch then samples were collected in sterile polyethylene terephthalate bottles of 500 mL and transported in cooler. They were stored under (5 ± 3)°C and were sent to the laboratory for examination within maximum 12 h from their collection.

**Laboratory analysis of samples**

Groundwater bacteriological analysis was performed using the membrane filtration technique and the standing tube technique to assess the bacteriological status of the groundwater for the required bacteriological parameters, according to the standard methods presented in Table 1.

The membrane filtration technique consisted of passing 250 mL of groundwater samples in sterile funnels using vacuum filtration ramp for each parameter through a cellulose membrane filter with pores of uniform diameter equal to 0.45 μm. After filtration, this membrane filter was inoculated in a Petri dish containing a standard culture medium specific for each bacteriological parameter. We used TTC tergitol-7 agar for total coliforms, Slanetz and Bartley medium for enterococci and cetrimide agar for *Pseudomonas aeruginosa*. At the end of the operation, the Petri dishes were placed in the incubators set at 37°C. The results were collected by counting the colonies 48 h after incubation, except total coliforms that we could collect from 24 to 48 h after incubation. Total coliforms were confirmed by oxidase test which should be negative. *E. coli* was confirmed from total coliforms with indole production at an elevated incubation temperature (44°C) and with a positive β-glucuronidase test. Enterococci were confirmed by esculin hydrolysis in the presence of bile. The membrane containing suspicious colonies was inoculated in a Petri dish of bile-esculin-azide agar and incubated 2 h at 44°C, esculinase positive colonies gave a brown-black halo. Colonies producing blue-green pigment (pyocyanin) in cetrimide were directly counted as *Pseudomonas aeruginosa*. Those

| Parameter | Analysis method | Tolerated limits for a good bacteriological quality |
|-----------|-----------------|--------------------------------------------------|
| Total coliforms | ISO 9308-1:2014 | Absence in 250 mL |
| *Escherichia coli* | ISO 9308-1:2014 | Absence in 250 mL |
| Enterococci | ISO 7899-2:2000 | Absence in 250 mL |
| Sulfite reducing anaerobic bacteria spores | NF T90-4153985 | Absence in 50 mL |
| *Pseudomonas aeruginosa* | ISO 16266:2006 | Absence in 250 mL |

*Parameters and their tolerated limits are set by Algerian regulations (Ministry of Trade 2016).*
which did not produce this pigment were tested for oxidase and fluorescence on King B medium to be confirmed. The standing tube technique was used to search the sulfite reducing anaerobic bacteria spores. After eliminating the vegetative forms by heating 50 mL of groundwater sample distributed in 10 glass test tubes of 20 mL in a water bath at 80 °C for 15 min, 15 mL of a meat-liver agar was incorporated in each glass test tube containing 5 mL of groundwater, the test tubes were sealed hermetically and placed in the incubators set at 37 °C. The results were collected from 16 to 48 h after incubation by counting the black colonies or those surrounded by a black halo. Groundwater is classified of good bacteriological quality when there is no bacteria growth for each one of the evaluated parameters; otherwise it is considered of poor bacteriological quality (see supplementary information, Table S3) (Ministry of Water Resources 2006; Ministry of Trade 2016). The studied parameters, the analysis method, the volume of water to be filtered or incorporated and the tolerated limits are detailed in Table 1.

These applied methods are subject to rigorous and regular control by the quality assurance system adopted by the laboratory. The measurement equipment used such as the thermometer and the pipettes were verified following the protocols recommended by the metrology. Calibrated thermometer was inserted into a tube containing agar inside the incubator to check its good functioning. Displayed temperatures by this thermometer were recorded each 2 h. One bottle of each batch from those used to collect groundwater samples has been tested for sterility. This method consists of introducing 20 mL or 50 mL of molten nutrient agar into the bottle and covering its wall with the agar by rotating it during the cooling. After incubation at (22 ± 2)°C for 5 days, there should be no visible bacteria growth. The membrane filter sterility was checked by inoculating it in tryptic soy broth medium and incubating it at 35 °C for 24 h. Bacteria growth is indicated by the presence of turbidity, specks, or flocculation in the medium; there should be no bacteria growth. To check used mediums’ sterility, one of each medium was incubated without inoculated membrane filter in the incubator at the same incubation circumstances of those with inoculated membrane filter. There should be no visible colonies. Mediums quality control for productivity and selectivity was carried out by testing each one of them with a reference bacteria strain (Table 2).

### Data analysis

In this study, firstly we performed descriptive analysis on all groundwater boreholes data and their bacteriological results. We considered each bacteriological parameter as a dichotomous variable (absence/presence of bacteria colonies) coded to (0/1). We also considered bacteriological quality as a dichotomous variable (good/poor bacteriological quality) coded to (1/0). The presence or the absence of urbanization and/or agricultural activity (Yes/No) is coded to (1/0). Correlation matrix was applied to 09 variables of the groundwater dataset to define the correlations between groundwater temperature at source, borehole depth and all the coded variables mentioned above. Missing values in data of groundwater temperature at source and borehole depth were replaced by their averages values. We also used univariate logistic regression model to evaluate association between urbanization and/or agricultural activity parameter as an explanatory dichotomous variable and the

| Medium                             | Tested bacteria strains                           | Expected results |
|------------------------------------|--------------------------------------------------|------------------|
| TTC Tergitol-7 agar                | *Escherichia coli* ATCC 25922                    | Yellow colonies  |
|                                   | *Enterococcus faecalis* ATCC 19433               | No growth        |
| Slanetz and Bartley agar           | *Enterococcus faecalis* ATCC 29212               | Red colonies     |
|                                   | *Escherichia coli* ATCC 25922                    | No growth        |
| Bile-esculin-azide agar            | *Enterococcus faecalis* ATCC 29212               | Brown colonies with esculin hydrolysis |
|                                   | *Streptococcus pyogenes* ATCC 19615              | No growth        |
| Cetrimide agar                     | *Pseudomonas aeruginosa* ATCC 14207              | Green colonies   |
|                                   | *Escherichia coli* ATCC 8739                     | No growth        |
| Meat-liver agar                    | *Clostridium perfringens* ATCC 13124             | Black colonies   |

*All mediums were incubated at 37 °C for 24 h up to 48 h.*
bacteriological quality as the outcome dichotomous variable of interest. All analyses and graphics were performed using R statistical software version 3.6.3, except bar plots that were performed with XLSTAT version 2014.5.03.

RESULTS AND DISCUSSION

In situ data

The analysis of this data showed an average score for boreholes depth of 145.3 m, a median of 130 m and a range of 27–480 m. A large number of boreholes depth observations are located in the range of 78–220 m except five potential outliers values (Fig. 3) (see supplementary information, Table S2). Borehole depth is a weighting parameter, i.e., great depth protects groundwater from surface contaminations and makes it less vulnerable to anthropogenic influences (Chilton 1996). Usually boreholes are between 90 m and 250 m deep but in some areas it reaches the level of 400 m (Somalia Wash Cluster 2020). In our study, most boreholes were included in this range; great depth gives more protection against anthropogenic influences but implies more drilling costs. Therefore, a tradeoff policy should be adopted. The measurement of the temperature at source of boreholes groundwater revealed an average and median value of 19 °C, a range of 14–26.3 °C, with a concentration in the range of 14–25.5 °C except one outlier value (Fig. 4). Temperature is one of the most important parameters of groundwater quality; temperature influences the growth and survival of microorganisms and affects the rate of its proliferation (Pelczar et al. 2005). Temperatures at source of the groundwater samples were mainly below than the maximum recommended limit by the WHO standards of 25 °C (WHO 2006). This range is suitable for the slowed growth of heterotrophic bacteria when present in the groundwater, both of psychrophilic and thermophilic species. It should be noted that temperatures above 25 °C promote the accelerated growth of microorganisms, thus causing unpleasant tastes and odors (Tardat-Henry 1992). The descriptive analysis indicated that the fraction of boreholes located near an urbanization or an agricultural activity (56.16%) is relatively larger than the rest of the sample (43.84%) (Fig. 5). We cannot avoid entirely the area with no anthropic activities because of the incessant increase of urbanization in Algeria (United Nations 2019) and also the area with the presence of agricultural activity because the crucial economic activity in the sampled rural areas.

Figure 3 | Box plot of boreholes depth.
Bacteriological quality assessment

The results of the bacteriological analysis are presented in Table 3. They showed that overall the half of the sampled groundwater boreholes did not meet the good bacteriological quality standards. Exactly 37 of the 73 groundwater samples (50.68%) were of poor bacteriological quality and 36 of them (49.32%) were of good bacteriological quality (Figure 6). These findings are in accordance with literature (Knobeloch et al. 2013; Ayad & Kahoul 2016). Groundwaters of poor bacteriological quality would not be bottled and the production projects would be cancelled. Thirty-five of the 73 groundwater samples (47.95%) showed the contamination

Table 3 | Summary data of bacteriological parameters

| Statistic            | Total coliforms | E. coli | Enterococci | P. Aeruginosa | SRAS |
|---------------------|-----------------|---------|-------------|---------------|------|
| Samples total size  | 73              | 73      | 73          | 73            | 73   |
| Modality            | Abs             | Prs     | Abs         | Prs           | Abs  |
| Samples size per modality | 38   | 35   | 49   | 24   | 66   | 7   | 65   | 8   | 71   | 2   |
| Frequency per modality (%) | 52   | 48   | 67   | 33   | 90   | 10  | 89   | 11  | 97   | 3   |

Abs: Absence of the parameter, Prs: Presence of the parameter, P. Aeruginosa: Pseudomonas aeruginosa, SRAS: Sulfite reducing bacteria spores.
by total coliforms (Figure 6). This means that total coliforms were the most important source of contamination in our samples and the first one which makes them of poor quality. Total coliforms were also the most common microbiological contaminant in the Pennsylvania study (Swistock et al. 2013). Total coliforms have long been considered to be good indicators of groundwater quality but this group is heterogeneous and contains bacteria of fecal origin and non-fecal origin (nutrient rich water, soils, decaying plants). Therefore, their presence is not always linked to fecal contamination or the presence of pathogen (WHO 1996). Twenty-four of the 73 groundwater samples (32.88%) were contaminated by E. coli (Figure 6). Recent studies of water quality in Algeria and Morocco (Benajiba et al. 2015; Ayad & Kahoul 2016) found the same result. E. coli is the leader of fecal coliforms that are considered as fecal contamination indicator and serve as indicators for measurement of water pollution. Their presence indicates that the borehole is vulnerable to the infiltration of surface water. Therefore they are subject to fecal contamination risk. The presence of E. coli in water indicates recent contamination with feces and may indicates the possible presence of pathogens responsible for diseases (Health Canada 2020). Eight samples (10.96%) were contaminated by Pseudomonas aeruginosa (Figure 6). Although our result differs considerably from those of the study carried out in the north-east of Algeria which revealed a higher contamination rate (Fehdi et al. 2014). It is a bacterium frequently found in water surface and urban wastewater; its origin can be human and possibly fecal. However, fecal carriage is rare. Its presence in these samples can reflect a contamination of groundwater by surface water (ENSP 2004). Seven samples (9.59%) showed the contamination by enterococci (Figure 6). Our results revealed a lower contamination rate than the previous studies (Saibi et al. 2011; Fehdi et al. 2014). Most of enterococci are of fecal origin and can generally be considered in practice as specific indicators of human fecal pollution. They are more resistant than coliforms and E. coli and their presence without E. coli indicates older fecal contamination or predicts pollution of groundwater by runoff (WHO 1996). Only two samples (2.74%) were shown contaminated by sulfite reducing anaerobic bacteria spores (Figure 6). This rarity is in line with previous studies (Bahri & Saibi 2011; Saibi et al. 2011). They are normally present in feces, but in far fewer numbers than E. coli. However, they are not exclusively of fecal origin and
their presence in the environment can have other reasons. These spores can survive in groundwater much longer than coliforms, due to their longevity; they are mainly able to indicate intermittent or remote contamination, and the vulnerability of the aquifer. Therefore, the pollution origin is very far from the borehole in time and space (WHO 1996).

**Correlation matrix**

Correlation matrix was performed on all the studied parameters (Table 4). We focused on strong correlations that may bring some information from our dataset. Bacteriological quality was highly and negatively correlated with total coliforms ($r = -0.947$) and *E. coli* ($r = -0.690$). Thus, total coliforms and *E. coli* were the most influencing groundwater bacteriological quality in our work as it was shown above. Bacteriological quality was also strongly and negatively correlated with urbanization and/or agricultural activity parameter ($r = -0.454$). This supposes that this last parameter may negatively affect groundwater bacteriological quality in the studied area. Therefore, we explored this association by a logistic regression (regression results are presented in a subsequent section). Sulfite reducing anaerobic bacteria spores was highly and positively correlated with enterococci ($r = 0.465$). This is possibly inherent to the fact stipulating that they may come collectively from fecal contamination and being resistant than the other groundwater parameters or their simultaneous absence or presence. It is important to note that the presence of them in our samples was rare, so we need further data collection to demonstrate such fact. Finally, groundwater temperature at source was significantly and positively correlated with borehole depth ($r = 0.352$) because the temperature of earth is rising towards its center; the greatest depth induces high groundwater temperatures, except boreholes less than 10 m that are affected by ambient temperature of the atmosphere (Pedersen et al. 2008). In our study, all our boreholes were more than 10 m deeper. Therefore, the correlation was relatively preserved.

**Logistic regression model**

As it was shown in the correlation matrix (Table 4), correlation coefficients between the bacteriology quality and in situ parameters were not different significantly from 0, except the one between bacteriological quality and urbanization and/or agricultural activity parameter. Therefore, we performed a logistic regression model to evaluate the association between urbanization and/or agricultural activity parameter as an explanatory variable and groundwater bacteriological quality as the outcome variable. The results of the refined model and the related statistical tests are shown in Table 5 and the equation below:

$$B.\text{Quality}^{(1)} = \frac{1}{(1 + e^{-(1.0986 - 1.9810xU/A\text{activity}_2)})}$$

(1): The predicted value of bacteriological quality variable by this model.
(2): The binary value of urbanization and/or agricultural activity variable that could take 0 or 1.

**Table 4 | Correlation matrix coefficients between all the studied parameters**

| Variables       | Depth   | Water T  | U/A.activity | Coliforms | *E. coli* | Enterococci | *P. Aeruginosa* | SRAS | B. Quality |
|-----------------|---------|----------|--------------|-----------|-----------|-------------|-----------------|------|------------|
| Depth           | 1,000   |          |              |           |           |             |                 |      |            |
| Water T         | **0,352** | 1,000   |              |           |           |             |                 |      |            |
| U/A.activity    | -0,214  | 0,073    | 1,000        |           |           |             |                 |      |            |
| Coliforms       | -0,061  | -0,032   | **0,461**    | 1,000     |           |             |                 |      |            |
| *E. coli*       | -0,144  | -0,033   | **0,442**    | **0,729** | 1,000     |             |                 |      |            |
| Enterococci     | -0,220  | -0,248   | 0,194        | 0,339     | **0,465** | 1,000       |                 |      |            |
| *P. Aeruginosa* | -0,076  | 0,004    | 0,222        | 0,190     | 0,315     | 0,333       | 1,000           |      |            |
| SRAS            | -0,136  | -0,267   | 0,148        | 0,175     | 0,240     | **0,515**   | 0,210           | 1,000|            |
| B. Quality      | 0,083   | 0,007    | **-0,454**   | **-0,947**| **-0,690**| -0,321      | -0,346           | -0,166| 1,000     |

The data in bold represent coefficient with significant strong correlation. Depth: Boreholes depth, Water T: Groundwater temperature at source, U/A.activity: Urbanization and/or agricultural activity, Coliforms: Total coliforms, *P. Aeruginosa*: *Pseudomonas aeruginosa*, SRAS: Sulfite reducing anaerobic bacteria spores, B. Quality: Bacteriological quality.
-values showed that the performed logistic regression model evaluating the association between urbanization and/or agricultural activity parameter and the groundwater bacteriological quality were of good fit and adequate. The calculated odds ratio (OR) in this model (Table 5) indicates that the presence of urbanization or agricultural activity around the borehole decreases the risk by 86.2% for being a good bacteriological quality groundwater; the risk is multiplied by seven for being a poor bacteriological quality groundwater. Therefore, the presence of urbanization or agricultural activity has a negative impact on the groundwater bacteriological quality. There was a study in Bechar city that did not reveal clearly the negative impact of anthropic activities (Kabour et al. 2013). Our findings are in line with the study that revealed the negative impact of wastewater through anthropogenic activities (Vijay et al. 2011) and with the study that highlighted the negative impact of cattle grazing through agricultural activity (Wirmvem et al. 2013). The studied association is very complex and there were many confounding factors that should be included in the study to bring a more credible outcome, such as urbanization and agricultural activity accurate distances from boreholes and the hydrogeological underlying structure.

**CONCLUSION**

The results of this study indicate that almost half of groundwater samples did not satisfy the regulatory bacteriological quality criteria, specifically for total coliforms followed in order by *E. coli*, *Pseudomonas aeruginosa*, enterococci and sulfite reducing bacteria spores. It provided an estimation of groundwater bacteriological contamination and the determination of good bacteriological quality groundwaters in the perspective of their bottling to drink or any other safe consumption. Moreover, it defined the most contaminating parameters. Correlation matrix allowed us to better understand the relationships between the studied parameters. Our study highlighted the associated negative impact of urbanization and agricultural activity on groundwater bacteriological quality. Further studies should be performed to bring out separately the partial impact of each one of the two associated factors on groundwater bacteriological quality. From this study, we have developed a good prediction tool of the groundwater bacteriological quality and conjectured assumptions about the origin of the contaminations. We think that our findings could be useful to avoid economic losses in drilling and taking the best strategy to carry out preventive and corrective actions to protect groundwaters, such as a better management of urban development and agricultural activities; by protecting the pipeline of public landfills and building bottled water factories far from agricultural farms and towns. A number of potential limitations and weaknesses need to be considered. The first one is related to the nature of sampling that has not either been carried out equitably nor randomly among all provinces and most of them were in the northern region of the country; Bejaia alone participated with 20 samples while the other provinces participated with one to five samples, the southern region which participated only with six samples. Therefore, the findings could not be generalized to the whole country. The second one pertains to the confounding factors that may have influenced the findings such as urbanization or agricultural activity distances from boreholes and the little knowledge of the underlying hydrogeological structure. Moreover, the sampling was not carried out during the same period. Thus, other additional

**Table 5** | Summary of model parameters

| Model coefficients | Intercept = 1.0986 | khi² (Wald) = 7.2417 | p = 0.0071* |
|--------------------|-------------------|---------------------|-------------|
| ‘U/A.activity’ = − 1.9810 | khi² (Wald) = 13.7947 | p = 0.0002* |
| Odds ratio (OR) | OR(‘U/A.activity’) = 0.1379 | 1-OR = 0.862 | 1/OR = 7.25 |
| Model validation test | Hosmer-Lemeshow test | Khi² = 7.5286 | P = 0.4808** |

*P-value < <0.05 means that coefficients are significant and not different from 0.

**P-value > 0.05 means that the model is of good fit and adequate.
data will be required to perform a powerful analysis to bring better results.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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