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Research Article

A study on the impact of wastewater on the quality water quality from certain wells in the Oued Amlil region of the Taza province, Morocco

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Abstract
Olive mill wastewater, a byproduct of olive oil production, poses a major environmental challenge for Mediterranean countries like Morocco. These residues are rich in polyphenols with notable antimicrobial properties. This study aimed to investigate the impact of anthropogenic factors on the quality of certain wells in the Oued Amlil region of the Taza province, Morocco. It was carried out during two distinct periods by sampling water from six wells: P1, P2, P3, P4, P5 and P6. The first period occurred during the winter season of December 2022, when olive crushing operations for oil production were at their peak. The second period took place in the summer (August 2022), when the activities of the oil mills were stopped. The objective is to assess microbiological quality by determining the microbial load in total germs at 37°C (TG), Fecal Coliforms (FC), Total Coliforms (TC), and Fecal Streptococci (FS), as well as performing a polyphenol assay. The analysis results showed high concentrations of TG (Maximum value = 3100/100ml), FC (Maximum value = 910/100ml), TC (Maximum value = 7120/100ml), and FS (Maximum value = 7000/100ml) in wells P1, P2, and P5, although they are not located on the flow path of groundwater contaminated by the Innaouene River and the local olive oil mills. However, despite their location along this axis, wells P3, P4, and P6 show lower contamination levels. In order to identify similarities between different water sources and/or different points of contamination acquisition, principal component analysis (PCA) was applied to the collected data and showed a significant negative correlation between polyphenols and bacteriological parameters, confirming the hypothesis of polyphenols' bactericidal effect on fecal bacteria. In addition, the degree of contamination varies from one well to another depending on their location with the direction of flow of water in the groundwater and the proximity of sources of pollution

Keywords: *Cynoscion regalis,* Heavy metals, Health risk assessment, Oil, *Pomatomus saltatrix*, Well-water

INTRODUCTION

Waste management remains a global concern as urbanization processes unfold in many developing countries, making waste management a matter of public health and environmental significance in urban areas (Ouyang, 2022). Many rivers in developing countries face persistent pollution from domestic and industrial

discharges (Balali G. I *et al*., 2020). Morocco is one of the developing countries where uncontrolled landfills still exist and where wastewater undergoes little to no adequate treatment before being discharged into rivers, which leads to the release of untreated water into bodies of water (Sghiouer *et al*., 2022). Groundwater is crucial in Morocco's hydraulic heritage (SebouFès Hydraulic Basin Agency (ABHS), 2007). In the Taza province, these aquifers are the main sources of water supply for urban centers like Oued Amlil, Matmata, Tahla, Zerarda, where simple chlorination treatment is often applied, as well as for rural centers like Ghiata El Gharbia, Bouhlou, Ait Amir, which lack any prior treatment. Their exploitation is relatively straightforward, making them essential resources to meet the growing water demand. In recent decades, the Taza region has seen a significant expansion of olive groves and olive and olive oil production in the Taza province. This remarkable increase is shown in Fig. 1, where olive production reached 22.85% of olive oil production between 2018 and 2019 (Fiouz *et al*., 2024).

The evolution of olive oil production has led to the generation of olive mill wastewater (OMW), the wastewater produced by oil mills. This wastewater is temporarily stored in ponds but is often discharged into the natural environment uncontrolled (Zenjari *et al*. 2006), consequently harming the aquatic ecosystem due to its content of soluble phenolic compounds in the aqueous phase. These aromatic compounds harm the environment, particularly through water and soil pollution, causing soil clogging, asphyxiation, and inhibiting living organisms' growth (Elhajjouji *et al*. 2007). This pollution contributes to the economic imbalance of this agro-industrial activity due to the costs associated with its treatment (Yaakoubi, 2021). Margines, liquid residues from olive oil production, pose a major challenge due to their complex organic composition, making them highly polluting. Their presence notably leads to significant greenhouse gas emissions, unpleasant odors, and adverse effects on plant growth and insect development, as highlighted by (Cuomo *et al*., 2016). Furthermore, even a minute quantity of infiltrated phenolic compounds can render groundwater toxic, thus amplifying environmental and health concerns associated with these industrial wastes (Zghari *et al*., 2017). Margines, rich in phenolic compounds, exhibit some toxicity to microflora and aquatic fauna. These compounds impart phyto-toxic and antimicrobial properties to Margines. They act on bacteria by denaturing their cellular proteins, damaging their membranes, and weakening their surface tension, enhancing their antibacterial action (Ranalli, 1991a, b). Notably, even minimal infiltration of these phenolic compounds can render groundwater toxic (Zghari *et al*., 2017). The concentrations of polyphenols present in Margines alone represent a significant risk of groundwater pollution following discharges into rivers, which are conducive environments for the direct recharge of aquifers.

Population growth has led to a serious problem of groundwater quality degradation through the emergence of pollution, which contributes to the overall decrease in water resources and poses a risk to human health, especially when consuming water from unprotected wells. This situation is exacerbated by the insufficient or even absent sanitation infrastructure and the lack of hygiene knowledge in rural areas, as is the case for the free aquifer of Oued Amlil in the Taza province, where the majority of the population directly sources water from the phreatic aquifer which is very vulnerable to pollution. These wells constantly receive liquid effluents, including domestic and industrial wastewater, particularly Margines. These pollutant substances can pose a danger to public health if they continue to be discharged into the environment without prior treatment (El Hajjami *et al*., 2021). The population is forced to use water from these wells as drinking water without concern for its quality. This study aimed to assess the microbiological quality of this water, explore correlations between polyphenols and byproducts of Margines, and detect potential contamination zones negatively affecting the aquifers' water quality.

MATERIALS AND METHODS

Study area

Oued Amlil, a village whose capital is located approximately 30 km west of the city of Taza, along national road No. 6, is an integral part of the Taza province (X=604.475 km ; Y=398.631 km; map of Tahla at 1/50000) (Fig. 2). It lies within a watershed that is part of the Innaouene basin, covering an altitude range from 290 meters downstream to 1169 meters upstream and draining an area of 153.7 km². The rainfall regime in the study area is characterized by two distinct and contrasting seasons: a rainy season spanning approximately 8 months from October to May, with an average precipitation of about 580 mm (mountainous areas receiving more rainfall, up to 1500 mm), and a dry season from June to September, with maximum temperatures reaching around 35°C.

The lithological formations are mainly marly, soft, and impermeable (71.5%). The high drainage density indicates an impermeable lithological nature that promotes surface runoff. The hydrographic network becomes active during heavy, concentrated rainfall (Aiman *et al*., 2021). In the phreatic aquifer of the marly cover of the Fes-Taza corridor, particularly at the present study site, the groundwater level varied from 10 to 20 meters relative to ground level, and the wells tapping into the free aquifer in the Oued Amlil region rarely exceeded a depth of 35 meters. The distance between the sampled wells varied from 1.5 km to 2.5 km.

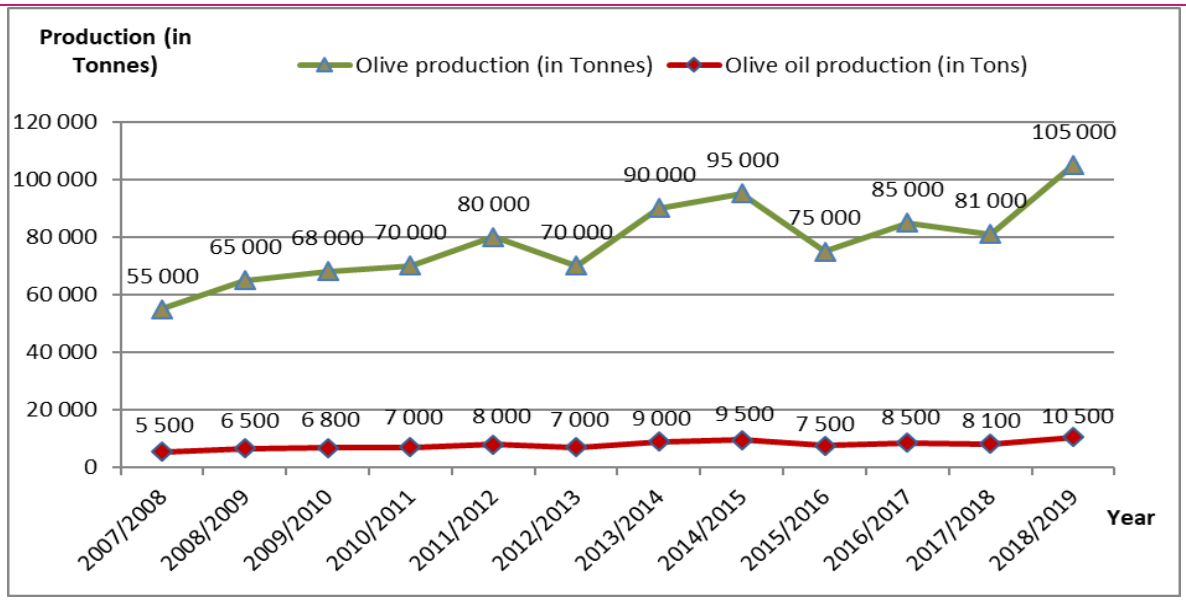

Fig. 1.*Evolution of olive and olive oil production in the province of Taza*

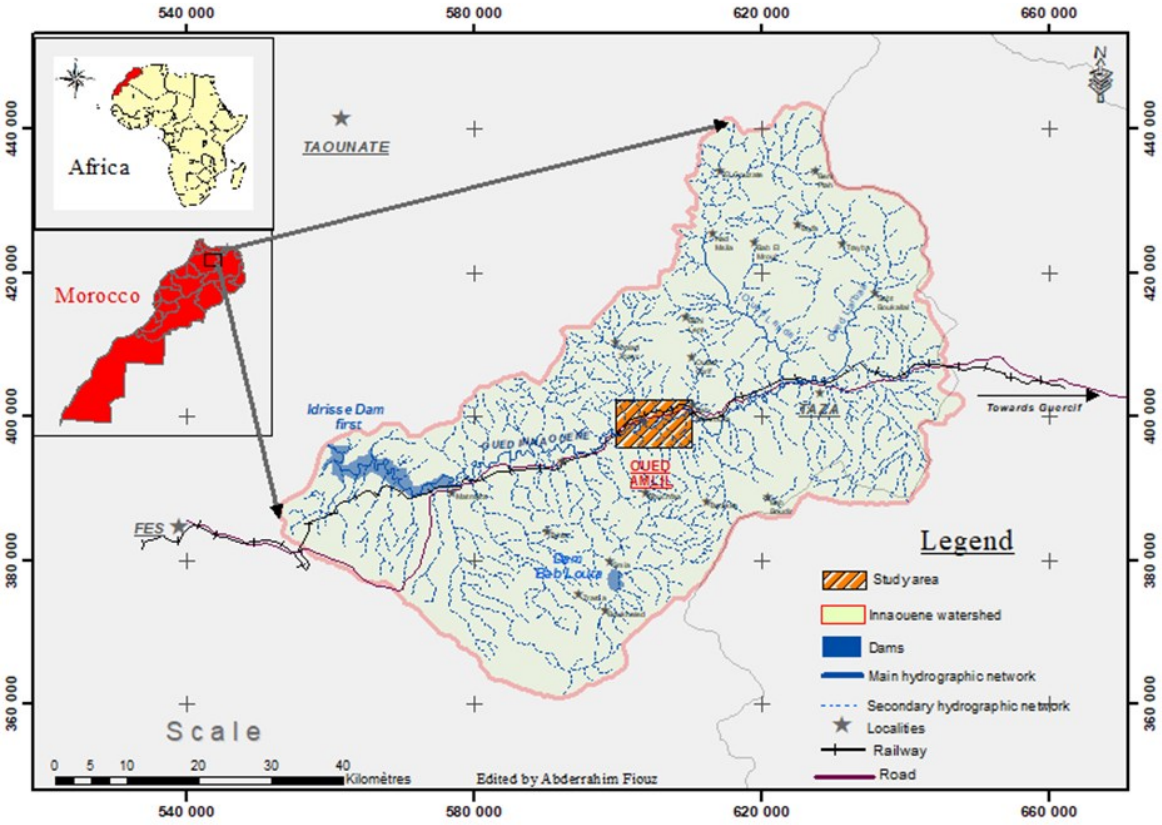

Fig. 2. *Geographical location of the Oued Amlil region, province of Taza*

Experimental protocol

The study used a multiparametric approach based on various criteria to select the wells for sampling. These criteria included accessibility, piezometric level, proximity of wells to potential sources of pollution, protective measures applied to the wells, and the use of water by the local population. Six wells have been identified and included in the analysis, with distances between them ranging from 1.5 km to 2.5 km, as illustrated in Fig. 3 to ensure a complete and meaningful representation of the current study area.

Two sampling campaigns were conducted: the first during the winter period when oil production was at its peak (December 2022) and the second during the summer period when oil mill activities were at a standstill (August 2023). The results are based on two samples taken from each well.

Samples were collected with extreme care using sterile bottles to ensure sample integrity. They were promptly placed in a special cooler and transported to the labor-

atory immediately. There, they were kept at a constant temperature of 4°C until they were ready for bacteriological analysis, which was conducted within 5 hours of sample collection, following the present strict protocols for handling and processing.The study of bacteriological parameters focused on the detection and enumeration of organisms responsible for fecal contamination of the environment by bacteria of fecal origin through discharges resulting from human or agricultural activities, namely total germs at 37°C (TG), fecal coliforms (FC), total coliforms (TC), and fecal streptococci (FS). Enumeration of FC, TC and FS was carried out using the Indirect method of multiple tube fermentation in lactose broth. The number was then statistically deduced using the most probable number (MPN) method (Jean Rodier *et al*., 2009). Filtration was performed on a vacuum filtration manifold. Isolation and enumeration were performed on the solid medium.The method described by De Marco *et al*. (2007; De Marco *et al*., 2007) was used to extract phenolic compounds.

A Principal component analysis (PCA) was performed using the average values of each parameter during the months of December and August. The method described by De Marco, 2007 (De Marco *et al*., 2007) was utilized to extract the phenolic compounds to quantify polyphenols. After storing the contaminated water samples at -20°C, they were thawed at 4°C, acidified to pH 2, and then rinsed three times with hexane to remove lipids.

Data analysis method

PCA was adopted and performed on 6 samples and 5 variables using STATISTICA software (version 10). to investigate the correlations between the microbiological and chemical parameters of the analysed water samples.

RESULTS AND DISCUSSION

Bacteriological and physicochemical analyses of water

The samples collected from wells throughout this study provided valuable results, thus allowing for the tracing of seasonal variations in bacteriological parameters TG, FC, TC, and FS and levels of polyphenols present in the water from the 6 studied wells. The first season corresponds (Month of December) to the intense period of olive crushing for olive oil production, while the second season (Month of August) represents summer when oil mill activities are halted. These data are essential for understanding the impact of different seasons on water quality. The data highlights a significant variation in water contamination levels from wells P1, P2, P3, P4, P5, and P6, both from one season to another and from one well to another (Fig. 4).

The concentrations of bacteriological parameters of fecal origin were higher during the dry season than during the wet season. These results are consistent with those reported in a study on the surface waters of the Upper Sebou in Morocco (Sghiouer *et al*., 2022).

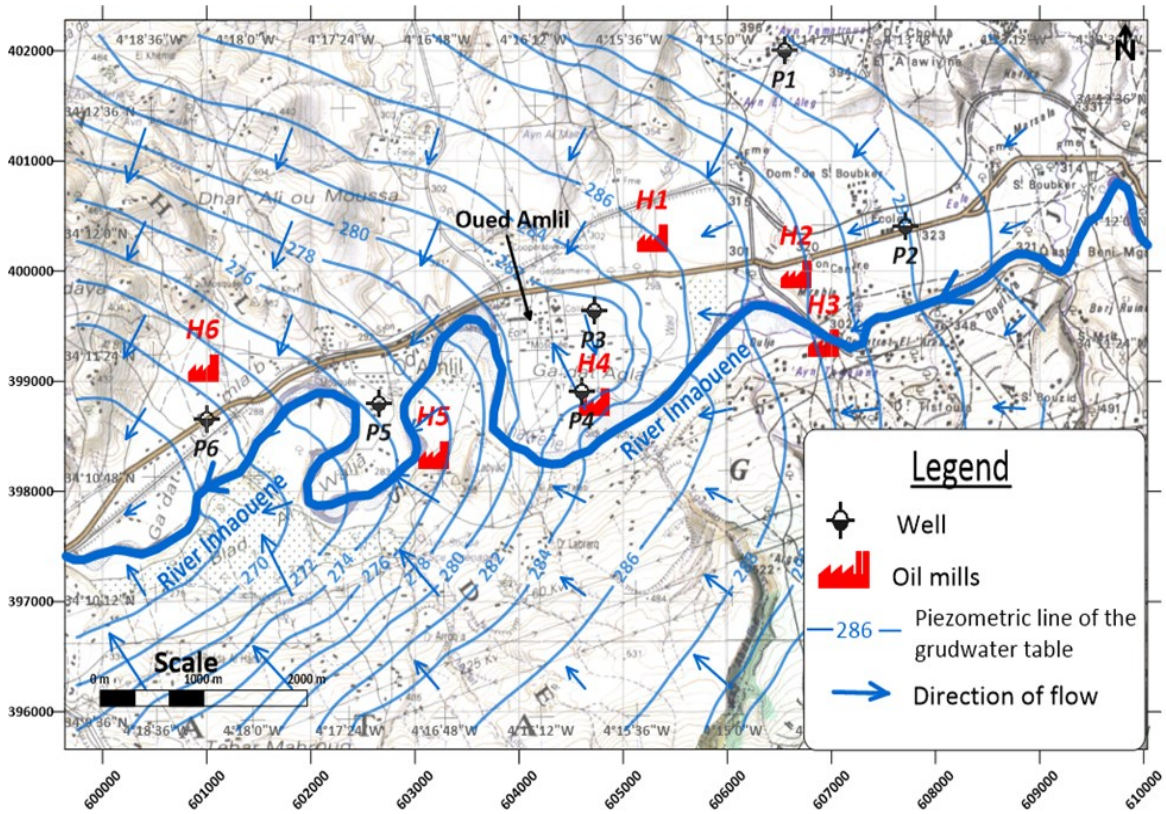

Fig. 3. *Position of the sampled wells and oil mills in the Oued Amlil region, province of Taza*

Table 1. Eigenvalues and percentage of explained variance

Value number	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	4,580143	91,60286	4,580143	91,6029
2	0,332132	6,64263	4,912275	98,2455
3	0,051100	1,02201	4,963375	99,2675
4	0,036425	0,72850	4,999800	99,9960
5	0,000200	0,00400	5,000000	100,0000

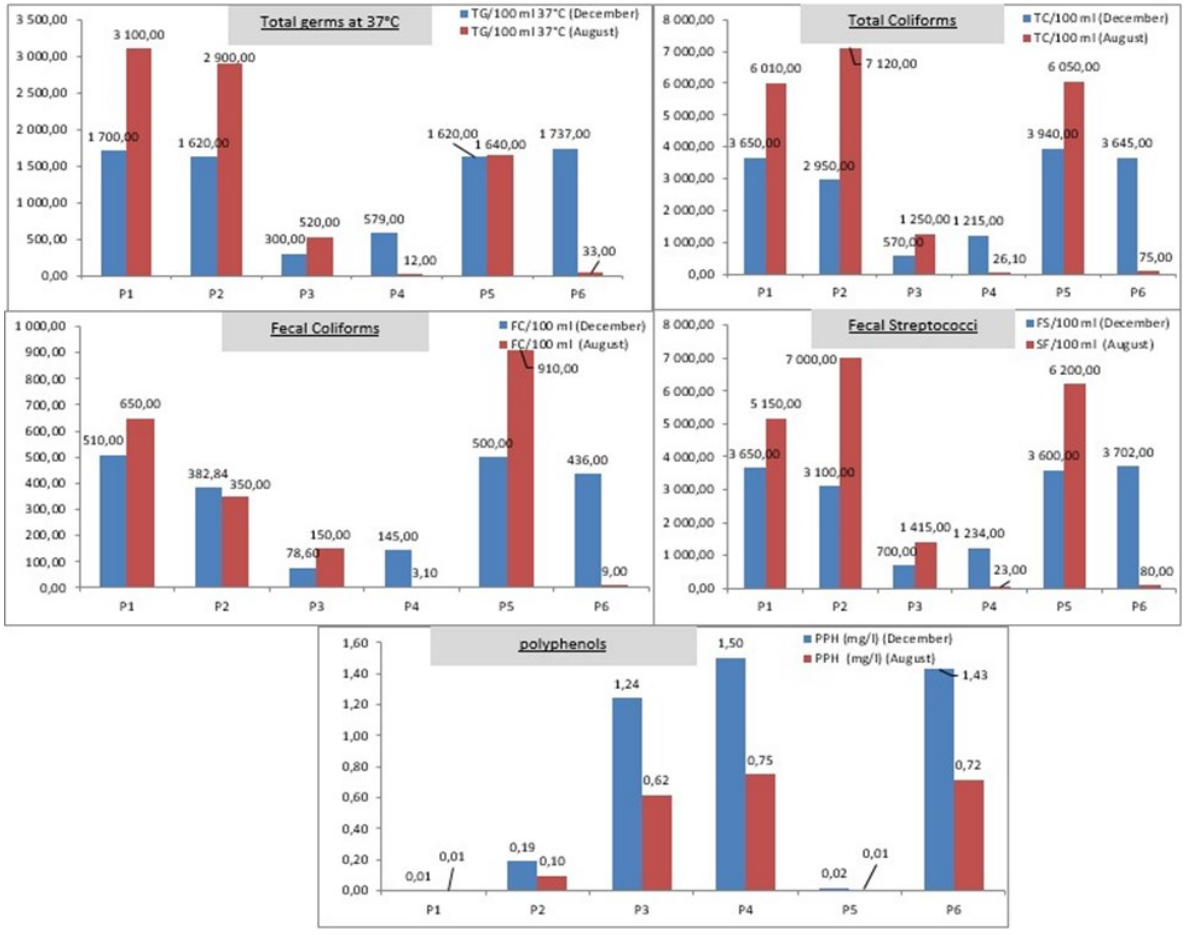

Fig. 4. *Seasonal variations in the number of bacteria and polyphenol levels present in the water of the 6 wells studied.*

Notably, this seasonal variation corresponded to the cessation of industrial activities related to olive oil crushing by the region's modern and traditional oil mills. This correlation suggests a possible implication of liquid effluents, particularly "Margines", from these oil mills in the increase in bacteriological contamination of wells during summer, and their decrease during the olive oil crushing season.

The presence of contamination from human or animal fecal matter, which can originate from conventional septic tanks, untreated wastewater discharges, animal manure runoff, or leaching, remains a common issue in all groundwater aquifers vulnerable to pollution in Morocco in the Moroccan Anti-Atlas (Assouani *et al.,* 2024) and in Souss-Massa (Salah and Driss).

The analysis of the graphs presented in Fig. 4 shows a significant variation in water contamination levels from one well to another and from one season to another.

In the province of Taza, excess volumes of olive mill wastewater (Margines) produced by oil mills during the olive pressing season, which are not retained by evaporation basins, are directly discharged into the environment, leading to significant ecological problems. It is important to note that even minimal infiltration of phenolic compounds can render groundwater toxic (Zghari *et al*., 2017; Allalat *et al*., 2020). The high concentration of polyphenols in the Margines poses a major threat to groundwater pollution, particularly due to discharges into watercourses that directly feed into aquifers. The analysis of dissolved polyphenols in water revealed

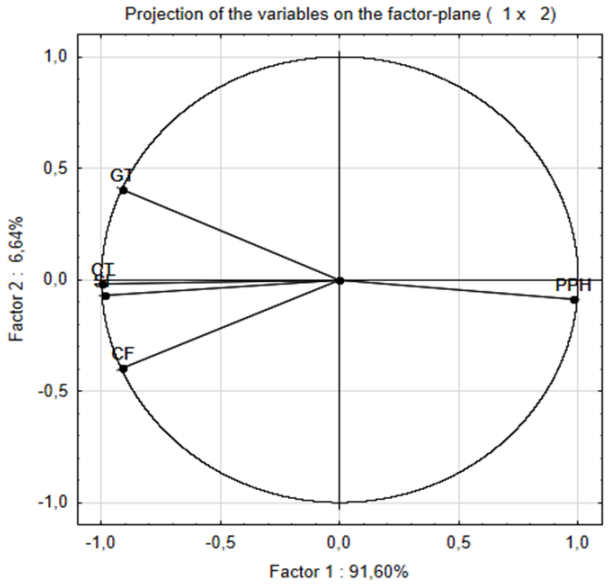

Fig. 5. *Projection of the 6 studied samples on the F1-F2 factorial plane of the PCA of a samples/variables matrix*

contamination in wells P1 to P6, with a maximum concentration of 1.43 mg/l observed in well P6

A remarkable observation, complementary to the one previously mentioned, revealed that wells with high levels of polyphenols are associated with a decrease in bacteriological parameters. This relationship likely suggests a link between the bactericidal activity of polyphenols and the reduction of bacterial growth in the water.

Typology of wells based on bacteriological and physicochemical parameters

The analysis of Principal Component Analysis (PCA) performed on a data matrix consisting of 6 rows representing well samples (P1, P2, P3, P4, P5, and P6) and 5 columns representing bacteriological variables (TG 37°C, TC, FC, FS) and Polyphenols (PPH) analyzed showed that the first component accumulated 91.00% of the captured variability and opposes polyphenols (PPH) with positive coordinates to TG, TC, FC, and FS, which contributed negatively to the expression of axis F1, which accumulated 98.60% of the captured variability (Fig. 5). This means that the bacteriological variables moved in opposite directions compared to polyphenols (PPH).

Table 1 represents the first five factors with their eigenvalues and the different expressed percentages. It can be observed that the 5 factors express 100% of the information, with 91.00% for factor 1 and 6.64% for factor 2. The first two factors total 98.24% of the total expressed variance. The F1-F2 pair alone expressed more than 98.24% of the information. Therefore, the analysis focused only on these two factors since the PCA of a region was valid only if the percentage of cumulative total variance exceeded 70%.

The hierarchical classification of wells, based on their contamination by germs, allowed distinguishing 3 different groups (Fig. 6):

Group G1: This Group brings together the two wells P1 and P2, whose waters showed slight contamination by polyphenols but exhibited significant bacteriological pollution.

Group G2: This includes the wells P3, P4, and P6, whose waters showed moderate contamination by polyphenols and moderate bacteriological pollution.

Group G3: This group is represented by well P5, whose waters contained a very low level of PPH but exhibited high bacteriological pollution.

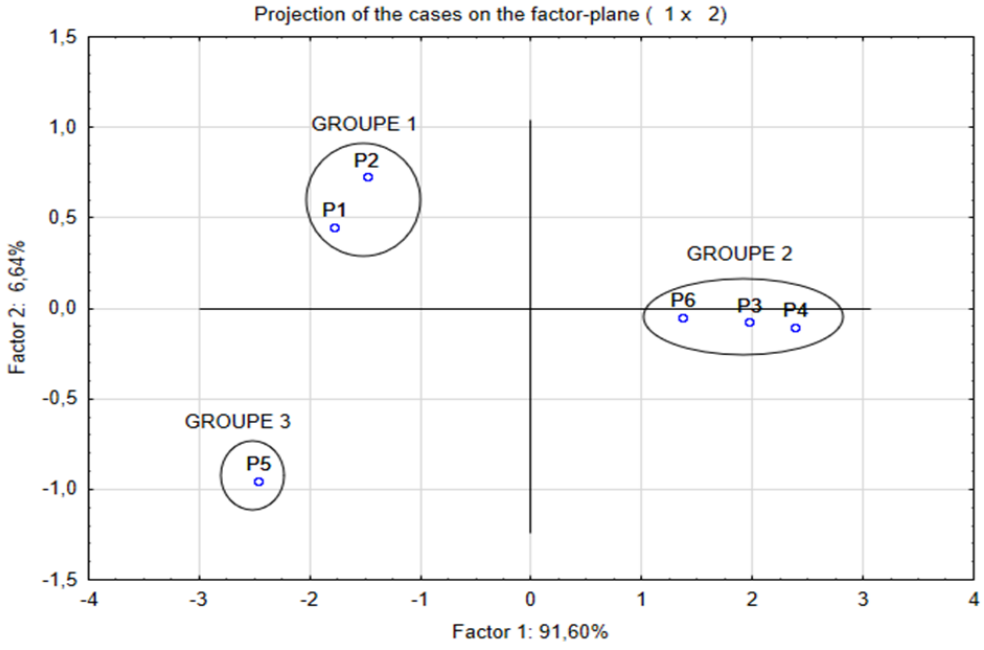

Fig. 6. *Projection of the active observations on the first factorial plane.*

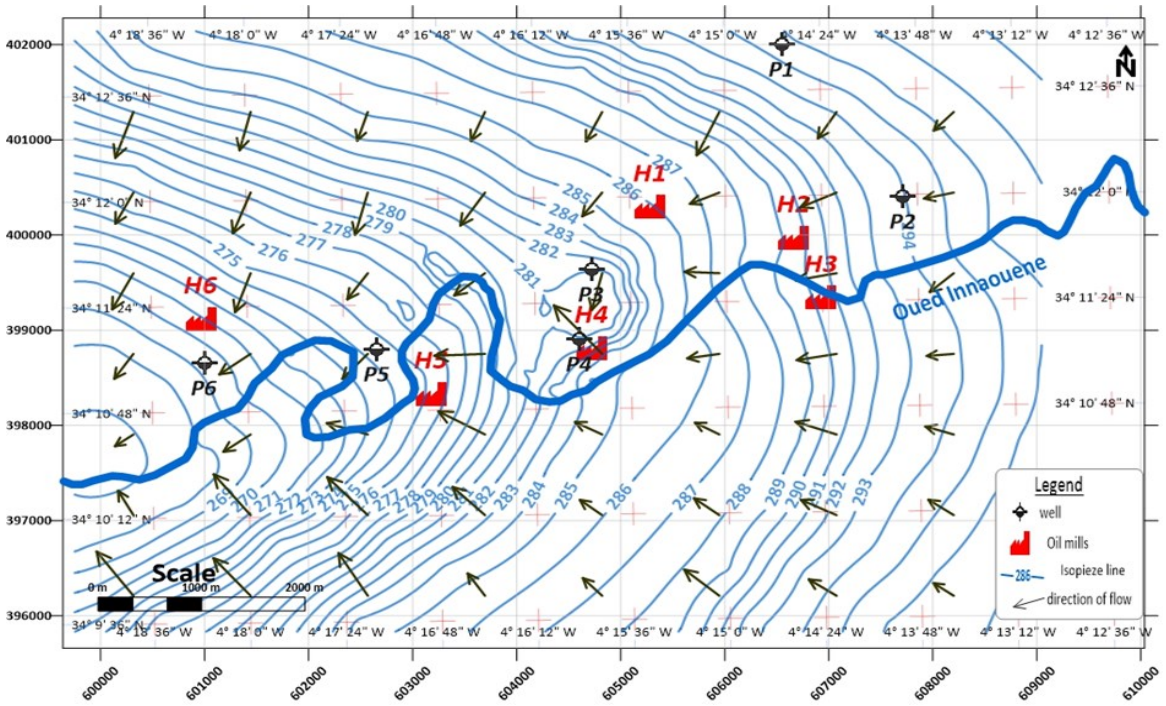

Fig. 7. *Piezometric map of the unconfined aquifer of Oued Amlil, Taza province*

The examination of the bacteriological quality of the water from the analyzed wells revealed contamination of the Oued Amlil aquifer by various environmental pollution sources, including manure leachate, sewage spreading, livestock farming, and the presence of septic tanks. Additionally, the discharge of olive mill wastewater (OMW) without prior treatment during the olive oil milling season appears to contribute significantly to this contamination. Moreover, these wells exhibited high levels of fecal contamination indicators, corroborating the findings of (Fiouz *et al.*, 2024), who mentioned that the water quality of the Oued Innaouene was very poor in the summer season (month of July 2021). These waters infiltrated and actively contributed to the contamination of the sampled wells.

Moreover, PCA revealed a negative relationship between PPH and bacterial contamination indicators (TG, TC, FC, and FS), meaning that an increase in one automatically implied a decrease in the other, as evidenced by their negative contribution to axis F1. Thus, the infiltration of PPHs from olive mill wastewater inhibited the proliferation of polluting bacteria from human activity.The analyses of the direction and flow lines of the piezometric map of the groundwater table (Fig. 7) clearly showed that the transfer of pollutants occurred from the Innaouene River towards the surveyed wells.

Conclusion

This study highlights the potential dangers human activities pose to the environment, particularly groundwater resources. It reveals degradation linked to a high concentration of bacteria, indicating the presence of organic pollution caused by pathogenic germs. This situation poses a risk to public health and threatens residents who rely entirely or predominantly on the water from these aquifers to meet their needs. PCA provided insights into the relationships between various measured parameters. From a bacteriological standpoint, the investigation revealed almost widespread contamination of well water, varying from one well to another depending on their position relative to water flow direction in the aquifer. The data revealed a significant but negative correlation between polyphenols, byproducts of OMW, and bacteriological parameters stemming from human activities, where bacteriological variables moved in opposite directions to polyphenols. This correlation highlights the direct impact of industrial practices on the microbiological quality of groundwater, underscoring the need for more rigorous and sustainable management of these activities to preserve the environment and water resources.

Conflict of interest

The authors declare that they have no conflict of interest.

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