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Evaluation of the Impact of Anthropic Activities on the Physico-Chemical and Microbiological Parameters of the Water of the Wadi Inaouene and Its Tributaries

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ABSTRACT

Water is a basic human requirement that is required in many processes, particularly those performed in households. However, in most circumstances, this critical product does not fulfill commonly recognized safety criteria. Various anthropogenic activities cause various forms of pollution; as a result, their physicochemical and biological components can pose major hazards to the environment and human health. The objective of this research was to evaluate the microbiological parameters along the Inaouene wadi and its tributaries, as well as their correlation with the physicochemical characteristics. In spring 2020, the following physicochemical and microbiological studies were conducted: ICP-AES was used to assess metals. The most polluted stations are located upstream (S1, S3, S4, S5, and S6). The highest concentrations of sulfate, orthophosphate, BOD_5, and nitrogen compounds are found at these stations. Illegal solid and liquid discharges from the tributaries S1 and S5, as well as urban settlements built on the banks of the Inaouen River, are to blame for this pollution, which has a direct and significant impact on water quality throughout the wadi. Furthermore, heavy metals (Zn, Fe, Co, Cr, Ag, and Cu) are abundant upstream of Inaouen and pass downstream. The monitoring of the evolution of microbiological pollution of wastewater has revealed a significant contamination of these waters upstream of the Wadi by the 3 groups of germs indicative of a fecal contamination and the presence of Listeria monocytogenes and salmonella in the upstream stations and especially S1, S3 and S5. The PCA revealed that the indicator bacteria of fecal contamination present negative correlations with dissolved oxygen, thus the lack of dissolved oxygen leads to a proliferation of germs in the water.

Keywords: water quality; pathogenic bacteria; heavy metals, Inaouene.

INTRODUCTION

Water and environmental protection have become two major concerns for all countries in the world. Currently, many countries are experiencing water scarcity problems. In Morocco, surface water constitutes an important part of the country's hydraulic heritage (MEMEE, 2012) and represents a vital resource for the national economy through its use in industry, agriculture and drinking water supply.

Polluted surface water remains a very dangerous factor for human health as it is considered a vector of pathogenic microorganisms wherever it comes in contact with human waste or wastewater from the surrounding locality (UNESCO 2017). Thus, water contaminated with microorganisms becomes extremely dangerous for drinking, bathing and irrigation. Apart from that, sewage in water mainly disturbs the oxygen balance, which is alarming for aquatic flora and fauna (Rezouki et al., 2021b).

According to the WHO, 80% of the diseases that affect the population of the planet are linked in part to the inadequate evacuation of faeces. These diseases are therefore the cause of a high mortality rate in developing countries. The transmission of waterborne diseases depends on three factors: the agent, the environment and the individual (Rodier, 2009; Faouzi et al 2023)

Sebou's second major tributary is the Inaouene wadi. It is situated at the level of the southern Rifian corridor Fez-Taza and drains a catchment area of 3396 km^2 that includes two dams, Driss 1st and Bab Louta. On its right bank, it receives Oued flows from Larbaa, Lahdar, and Amlil. These tributaries collect the runoff from the pre-Ririan hills. The Oued Inaouene receives tributaries on its left bank, which are fed in part by the Tazekka primary massif and the Middle Atlas limestone, which is often karstic in this region: Zerg, Bouhlou, Matmata, and Bouzemlane (Ben Abbou, 2017). This semi-arid region has seen significant agricultural development as well as population growth in recent decades. As a result, water demand has increased significantly, particularly in large agglomerations such as Taza, Oued Amlil, Tahla, and others (El haji et al., 2012). Water from Inaouene is used for irrigation of various organic crops as well as industrial activities in urban agglomerations built along the Oued (Ben Abbou 2014).

This heritage is very much threatened by numerous pathogenic bacteria that can be found in the water through contamination of the latter by animal and human excrement or by sewage (Hbaiz et al., 2022). The metabolism of these unicellular micro-organisms is closely linked to the surrounding environment (nutritive salts, energy, oxygen, temperature...). The presence of undesirable microorganisms in urban and rural areas is caused by changes introduced by man into the environment of the wadis. The current work proposes to establish a diagnosis of the microbiological quality of the water in Inaouene wadi in order to determine the true state of the water quality and the type and source of pollution. The microbiological characterization of waters along the Inaouene wadi, as well as their correlation with physicochemical parameters, is of particular interest to us.

STUDY AREA

Geographic location

The Inaouene wadi is made up of 8.3% of the Sebou basin upstream of the Idriss I dam. It is limited to the East by the catchment area of the middle Mouloya, to the North-West by that of the high Ouergha and to the South by that of the high Sebou. The Oueds Larbaa, Lahdar, and Amlil flow into the Wadi Inaouen on its right bank. These tributaries collect the runoff from the pre-Rifan hills. The Oued Inaouen receives tributaries on its left bank, which are fed in part by the Tazekka primary massif and the Middle Atlas limestones, which are often karstic in this region: Zerg, Bouhlou, Matmata, and Bouzemlane. Taza has 52 wastewater discharges, the vast majority of which are concentrated upstream of Inaouene. The amount of wastewater discharged into the rivers could reach 5,000,000 m^3 / year (Barakat et al., 2016). This flow is increasing from one year to another given the increase in population of the city of Taza.

Geology and hydrogeology

The geology of the watershed is generally formed by tertiary marl formations located in the north and northwest of the watershed. These structures are marked by right bank drifting. This makes the right bank more susceptible to water erosion. A very resistant bedrock composed mainly of carbonate (limestone) formations (Fig. 1) (Nouayti et al., 2019) dominates the eastern and southeastern parts of the basin. A hydrographic network with a hierarchical dendritic (branched) structure and a spatio-temporal heterogeneity of the environment characterizes the watershed. This drainage pattern reflects the combination of geological and topographic effects of the basin. The hydrographic network of Inaouène wadi is composed of a main river (Inaouène) and a series of tributaries distributed on both banks.

MATERIAL AND METHODS

Presentation of the study area

For a more representative sampling of the watercourses, we selected eleven stations along Inaouene wadi, six of which (S1, S2, S5, S7, S9, S10) represent the main tributaries of Inaouene wadi and five stations (S3, S4, S6, S8, S11) on the bed of Inaouene wadi. Station S12 corresponds to the Idriss I dam (Table 1; Figure 1).

Choice of the study stations

Figure 1 represents the location of each station in the watershed of Inaouene wadi, the stations were chosen considering the different activities identified in the study area to be: domestic activity industrial, agricultural and wastewater. These stations were selected to be accessible and to reflect the actual water characteristics of the study area. of the Wadi and its tributaries

Physico-chemical of water

During the year 2019 we collected water samples from Inaouene wadi and its tributaries are made following the protocol of the water quality control laboratory of ONEP (Abouzaid and Duchesne, 1984; Rodier et al., 2016). Eleven sites have been explored along the Wadi (Figure 1 and Table 1) of which 36 samples were taken in polyethylene bottles with a capacity of 1L. According to the Moroccan standard 03.7.059, the water samples were sent directly to the CNRST laboratory in Rabat in eight hours and at 4°C to perform the analyses. The bottles were labeled and accompanied by a card with all the necessary information.

Analyzed parameters

Temperature (T°C), hydrogen potential (pH), electrical conductivity (EC), and dissolved oxygen (O_2 dissolved) were measured directly in situ using a multi-parameter system (Consort C931portable suitable). Rodier's analysis protocol was used to determine sulfates (SO_4^{2-}), orthophosphates (PO_4), and biological oxygen demand (BOD_5) (Rodier, 1997). The total hardness (F°) of the water was determined using volumetric titration with EDTA 0.2N, while the nitrates (NO^{3-}) and ammonium (NH^{4+}) levels were determined using spectrophotometry.

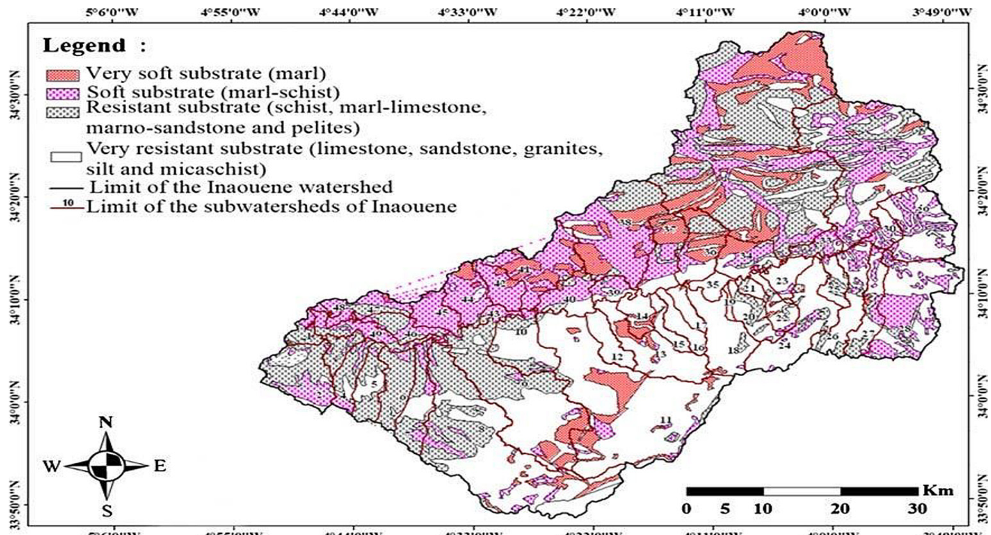

Figure 1. Map of geological formations in the Inaouene watershed (Nouayti et al., 2019)

Table 1. Station names in Wadi Inaouene (Taza, Morocco) (Rezouki et al., 2021a)

Names of the stations		
S1 – Wadi Larba	S5 – Wadi amlil	S9 – Wadi Bouhlou
S2 – Wadi Lahdar	S6 – Wadi Amlil	S10 – Wadi matmata
S3 – Inaouen's Amon	S7 – Wadi Zireg	S11 – Downstream of Inaouen
S4 – Inaouen Amon in Ghiata Al Gharbia	S8 – Middle of Inaouen	S12 – Dam Idriss 1

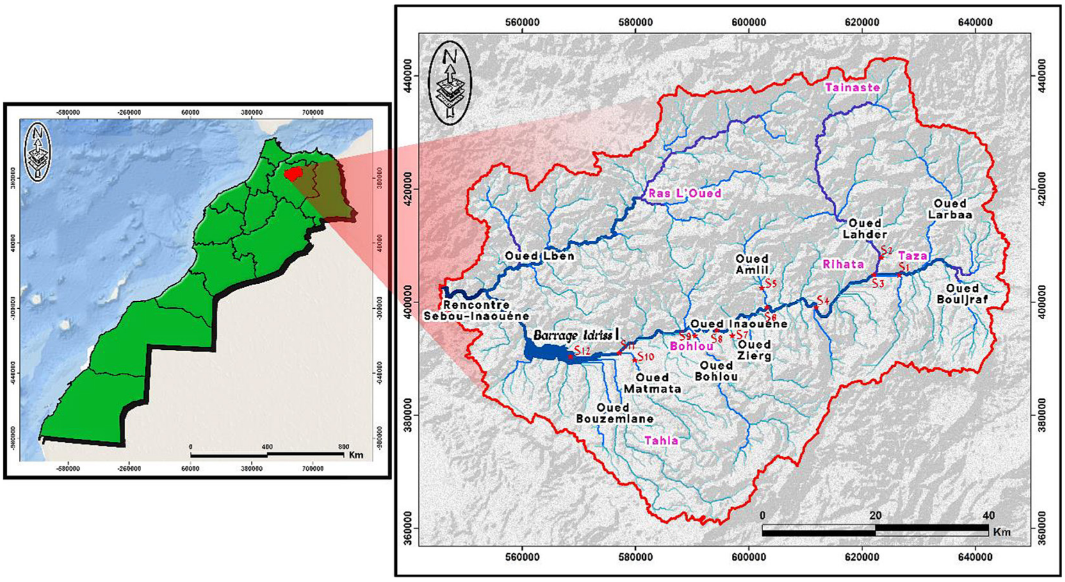

Figure 2. Situation of the stations in the Inaouene wadi watershed

BACTERIOLOGICAL ANALYSIS

Sample collection

For bacteriological analysis, water samples are taken in sterile 500 ml bottles, leaving an air space (2/3) in the bottle to facilitate the resuspension of microorganisms by shaking before inoculation in the appropriate culture media. Samples are taken aseptically with a portable gas flashlight. The samples are transported to the laboratory in a cooler at a temperature of ±4C in order to perform bacteriological analysis, according to Moroccan standards (NM).

Bacteriological analysis

In this work, we were interested in defining the microbiological characterization of water. So, we looked for pathogenic bacteria (Salmonella, Listeria monocytogenes, Escherichia coli) and the main flora (total mesophilic flora, total coliforms, fecal coliforms, Streptococci). The bacteriological analysis was done by two methods: one is the membrane filter and the method of the most probable number (MPN) carried out in the laboratory of CNRST of Rabat. The analyses were carried out according to the standards references mentioned in Table 2.

RESULTS AND DISCUSSION

Physico-chemistry of the water

In Tables 1 and 2, the outcomes of the physico-chemical studies are displayed. Between stations, there are not many significant differences in the average temperature. Station S1 recorded a low of 11.02°C and a high of 19.1°C (station S11). These results demonstrate an upstream and downstream gradient that is gradually growing.

Table 2. Experimental protocols for the enumeration of the main flora

Enumeration of the main germs	Experimental protocol	Reference
Total mesophilic (TMAF)	The culture medium is the nutrient agar PCA (Plate Count Agar). After plating the cultures are incubated (36°C /24 to 48h)	MN 03.7.005 (2007)
Total coliforms (TC)	We used Tryptose Lauryl Sulfate Broth and Brilliant Green Milky Broth (37°C + 1 /48H)	NM 03.7.060 (2012)
Fecal coliforms (FC)	Tryptose Lauryl Sulfate Broth (37°C + 1 /48H) and EC mediul (44°C/24H)	NM 03.7.060 (2012)
Fecal Streptococci (FS)	Azide Glucose Broth (37°C/48H) and Bile Agar with Esculin and Azide (BEA) (44°C/48H)	NM 03.7.252 (2012)
Echerichia coli (E.coli)	Tryptose Lauryl Sulfate Broth (37°C + 1 /48H) and EC mediul (44°C /24H)	NM 03.7.003 (2007)
Salmonella	Selenite dulcitol broth (40°C ± 0.2° / 24 h ± 2 h)	

The local environment, including the geology of the terrain traversed, the hydrology of the ecosystem, and most significantly the climate, has a significant impact on the water temperature (Rodier et al., 1984). The pH values measured are generally alkaline to moderately neutral and range from 7.2 to 8.94. A slight decreasing gradient from the upstream to the downstream was registered. As a factor, pH is affected by environmental conditions, soil rock and substrate type, and water pollution (Rezouki et al., 20121a). Conductivity is one of the physical parameters that can verify the amount of dissolved salts in the water (Rodier et al., 1996; Ougrad et al., 2022) and the presence of water pollution (Ghazali and Zaid, 2013). The water conductivity of Wadi Inaouen and its major tributaries has some important values that differ significantly between the two banks of the wadi Inaouen. On the right bank tributaries, the conductivity is maximum at 1678 s/cm at S5 by cons, while the left bank records minimum conductivities of 110.3 s/cm at S7. From upstream to downstream, the wadi's average oxygen concentration rises from 2.17 mg/l (S1) to 8.01 mg/l (S12). The large pollution loads likely cause the considerable lack of dissolved oxygen upstream of Inaouen from the city of Taza's wastewater. The BOD_5 values upstream of Inaouen are quite high, reaching a maximum value of 491 at S1. These concentrations dramatically decline downstream, with minimum value of 2.12 mg O_2/l being measured at S12. These values are greater above the Moroccan criteria for surface water quality (10–25 mg O_2/l), and as a result, these waters are categorized as low quality. For the downstream stations are considered of good quality. The sulfate (SO_4^{2-}) content shows very important values upstream of Wadi Inaouen, but these values decrease significantly as we go downstream (Table 3). This indicates that the contribution of anthropogenic pollution is not negligible, because of the discharge of domestic water in this element, generally the high content of sulfates is related to the abundance of secondary evaporite formations mainly gypsum ($CaSO_4$, $2H_2O$) and anhydrite ($CaSO_4$) (Rezouki et al., 2021a). Upstream of Inaouen, orthophosphates (PO_4^{3-}) levels are typically high. Annual PO_4^{3-} concentrations in the waters of the six stations ranged from 0.5 mg/l to 8.81 mg/l (Table 3), with station S1 having the highest values. The wastewater discharges from several municipalities near metropolitan areas are the cause of these amounts. The average values of nitrates (NO_3^-) in the water of Inaouen vary between 22.8 mg/l and 0.4 mg/l. The results of the research of 7 heavy metals in the water of wadi Inaouene are presented in table 3, some trace elements are essential for the human body, but if the concentrations exceed the standards it becomes toxic for health and the environment. The concentrations of heavy metals vary spatially significantly from one station to another with high concentrations upstream and gradually decreasing downstream. The heavy metal content,

Table 3. Physico-chemical properties of water in the study sites

Station	T°C	pH	EC	DO	Sulfates	PO_4	NO_3^{2-}	NH_4^+	DBO_5
S1	11.02	7.9	1333	2.17	890	8.81	22.8	0.95	491
S2	11.96	7.88	1534	5.07	272.1	1.3	7.05	0.09	16.74
S3	11.17	7.8	1555	3.39	425.2	7.21	18.09	0.87	373.6
S4	12.7	7.7	1136	3.02	308.8	5.37	17.7	0.65	278.6
S5	14.3	7.35	1678	3.81	633.2	6.6	20.28	0.92	390.45
S6	12	7.2	1145	3.22	315	4.6	17.4	0.62	282.9
S7	15.26	7.94	110.3	6	145	1.22	3.97	0.07	29.1
S8	13.31	7.44	819.4	4.12	389.8	3.82	15.15	0.45	244.9
S9	15.1	7.31	298.6	6.02	141.2	1.45	2.4	0.1	14.02
S10	15.7	7.14	172	7.08	60.95	0.84	1.34	0.07	2.33
S11	19.1	7.24	673	4.40	590	3.35	12.67	0.63	135.4
S12	18.0	7.3	461	8.01	56	0.5	0.4	0.12	2.12
Mean	14.135	7.516	909.608	4.6925	352.270	3.755	11.604	0.461	188.43
Min	11.02	7.2	110.3	2.17	56	0.5	0.4	0.07	2.12
Max	19.1	7.94	1678	8.01	890	8.81	22.8	0.95	491
SD	2.607	0.303	564.430	1.771	252.474	2.795	8.100	0.356	176.926

including Fe, Pb, Cu and Mn, is above the Moroccan and WHO standards (Table 4), which can be explained by the industrial activities in Taza city as well as by the city's domestic liquid discharges. On the other hand, heavy metals, in addition to their possible origins of anthropogenic activities, they may also have a geological origin and have reached the river system by hydro-climatic erosion.

Total mesophilic flora (TAMF)

The total mesophilic floras (TAMF) are the set of microorganisms able to multiply in the air at temperatures of optimal growth between 25 and 40°C. They are used as an indicator of global pollution (EL Haissoufi, 2011). The results shown in Figure 3 show very high values at the upstream of Inaouene compared to the downstream where the values recorded are relatively low. The maximum value of 7.8 106 (Colony forming unit) CFU/100ml was recorded in S1 and the minimum value of 3.7*103 N/100ml was recorded in S12. This pollution upstream could be explained by the domestic discharges that are conveyed from wadi Larbaa and wadi Amlil, which contain discharges from the city of Taza and the neighboring rural areas. For the stations located downstream, the decrease in TAMF could be explained by the phenomenon of self-purification (Figure 3).

During the enumeration of bacteria in the olive growing period there is an absence of them because there is discharge of margines that have an acid character with a pH between 4.5 and 5 (Morillo et al., 2009) and by the presence of phenolic acids and fatty acids that are unfavorable for the development of microorganisms (Achak et al., 2009), but also through the temperature increase and the duration of sunshine in the period of low water that favor their multiplication and enrichment by physical phenomena adsorption, biological activation, dilution, dispersion and sedimentation. The TAMF are less or not pathogenic, revealing a fecal contamination and lead by their abundance to the presumption of a more dangerous contamination (Figarela et al., 2001).

Total coliforms (TC)

Total coliforms (TC), have long been used as indicators of pollution, it groups bacteria naturally present on plants, in the soil, and also the intestines of humans or warm-blooded animals. This group includes various species of the genera Escherichia, Klebsielle, Enterobacter, Citrobacter, and Serratia, since total coliforms are found in both fecal and non-fecal environments, and are not good indicators of fecal contamination and do not represent a direct health risk (Hounsouet al. 2010) with the exception of some strains such as Escherichia coli (E. coli) (Charles, 1990). The spatial distribution of TCs (Figure 3) showed a variation in total coliform counts from one station to another with a maximum value of

Table 4. Heavy metals in different stations

Station	Ag	Fe	Pb	Zn	Cu	Co	Cr
S1	0.051	0.53	0.051	1.95	0.013	<0.01	<0.06
S2	0.046	0.018	0.046	0.005	0.009	<0.01	<0.06
S3	0.052	0.46	0.051	1.81	0.01	<0.01	<0.06
S4	0.048	0.36	0.049	0.405	0.01	<0.01	<0.06
S5	0.046	0.485	0.046	1.87	0.011	<0.01	<0.06
S6	0.036	0.31	0.04	0.7	0.008	<0.01	<0.06
S7	0.037	0.014	0.039	0.0045	0.01	<0.01	<0.06
S8	0.038	0.15	0.038	0.75	0.008	<0.01	<0.06
S9	0.033	0.011	0.031	0.005	0.0077	<0.01	<0.06
S10	0.051	0.023	0.052	0.005	0.011	<0.01	<0.06
S11	0.055	0.12	0.049	0.65	0.01	<0.01	<0.06
S12	0.052	0.023	0.051	0.005	0.011	<0.01	<0.06
Mean	0.045	0.209	0.045	0.679	0.009	0.01	0.06
Min	0.033	0.011	0.031	0.0045	0.0077	0.01	0.06
Max	0.055	0.53	0.052	1.95	0.013	0.01	0.06
SD	0.007	0.206	0.007	0.778	0.001	0	0

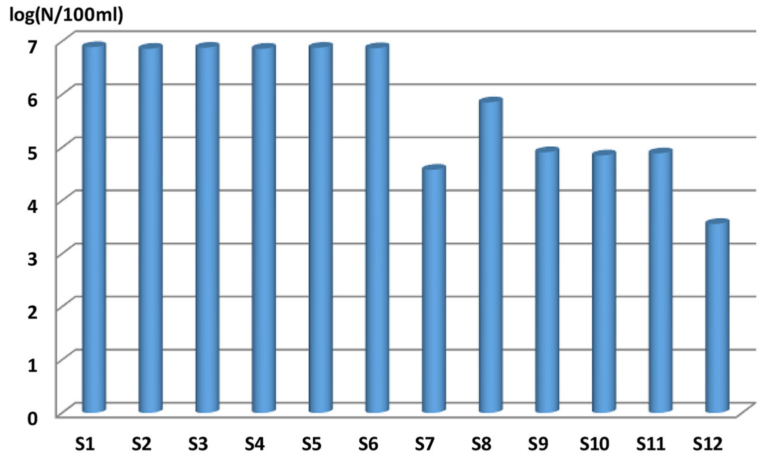

Figure 3. Spatial variation of Total Mesophilic Flora (TMF) in the different stations along the Inaouene wadi

5.7 103 N/100ml recorded at S1 and a minimum value of 3N/100ml recorded at S7 (Figure 4). These high values compared to the standards are mainly due to the contribution of waste pollution from the city of Taza and neighboring rural centers also to the contribution of organic matter and the contribution of soil bacteria or the use of manure on farmland at the level of these peaks (Ben Abou, 2014), the values of CT decreases in some stations because they are far from urban areas.

Fecal coliforms (FC)

Fecal coliforms (FC), also called thermotolerant coliforms, belonging to a subset of total coliforms capable of fermenting lactose at a temperature of 44 – 45°C, the presence of fecal coliforms also indicates the presence of pathogenic microorganisms (El haissoufi et al., 2011). They are good indicators of fecal contamination more than TC (Tallon et al., 2005). The spatial evolution of Fecal Coliforms (FC) in Figure 4 showed the presence of a maximum value of 7.7 106 N/100ml at S1 and a minimum value of 25 N/100ml at S8. The recorded values show a progressive decrease from upstream to downstream of the Wadi. The survival of these coliforms in the environment is generally equivalent to that of pathogenic bacteria. Their presence and density are generally proportional to the pollution degree produced by the faeces, hence the interest of their detection as indicator organisms. (CEAEQ, 2000). The increase in Fecal Coliforms could be a result of the abundance of nutrients, dissolved oxygen and other elements necessary for bacterial growth. However, the absence of Fecal Coliforms in some stations such as S7, S9, S11 and S12 does not mean the total absence of pathogens since the viral infection assessment was not detected.

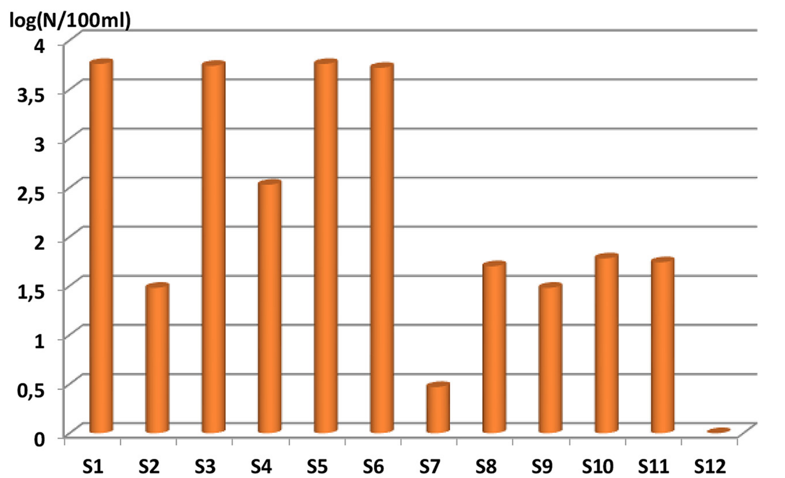

Figure 4. Spatial variation of total coliforms along Inaouene Wadi

Fecal Streptococci

According to WHO, Fecal Streptococci are of human origin (Eddabra, 2011), they are good indicators of fecal pollution due to their high resistance to desiccation and their persistence in various types of water which is higher than other indicator organisms (WHO, 2000). Some bacteria of this group come from animal feces such as Streptococcus Bovis, S. Equinus, S. Gallolyticus and S. Alactolyticus (Havelaar et al., 1986) or even occur on plants (Eddabra, 2011). In Figure 6, the spatial variation of streptococci in the different stations shows a decrease in bacterial abundance going from upstream to downstream, with the recording of a maximum value of $3.2*10^4$ N/100ml at S5 and a minimum value of 10N/100ml in S2 and S11. The number of FS experienced an increase in station S6 which represents, then the number of FS falls towards the downstream of the river under the effect of the power of self-purification along the wadi (Abbou, 2020), this special distribution is due to the discharges of solid waste, domestic and agricultural wastewater in anticipation of the city of Taza and nearby rural centers as well as settlements located on the edge of the wadi tributaries of Inaouene affect its quality. Indeed, the most contaminated points are those located near the housing units of residents

Pathogenic germs

Escherichia coli (E. coli)

E. coli selected as indicators of recent or ongoing fecal bacteriological contamination of streams in this study (Rodier et al., 2009; El Haissoufi et al., 2011). Their interest as indicators lies in the fact that they are always present in the gut of humans and warm-blooded animals and are eliminated in large quantities in feces. Their survival in the environment is generally equivalent to that of pathogenic bacteria such as Salmonella, Campylobacter. Potentially pathogenic microorganisms and indicator bacteria in feces can be excreted directly into waterways by livestock or wildlife that has access to them. Direct discharge can also occur as a result of runoff from manure piles or leaking storage systems, or from untreated domestic sewage discharges. These microorganisms and bacteria can also be transported to watercourses from agricultural land applications, surface runoff or subsurface drains caused by rainfall (Patoine and D'auteuil-Potvin, 2015).

In our study, the contamination by this pathogen E- coli (Table 4) shows the absence of E- coli in all the stations studied except for stations S1, S4, S5 and S6. The potential sources of the selected fecal contamination indicator bacteria (FC and E. coli) are mainly livestock excreta and human wastewater. Other sources such as wildlife and pets may also be present, although of lesser relative importance.

Salmonella

Salmonella are Gram-negative bacteria that belong to the Enterobacteriaceae family, their presence in the water environment is an excellent indicator of fecal pollution of this environment. This germ responds in the natural environment through feces and it seems that domestic or agricultural wastewater represents one of the main

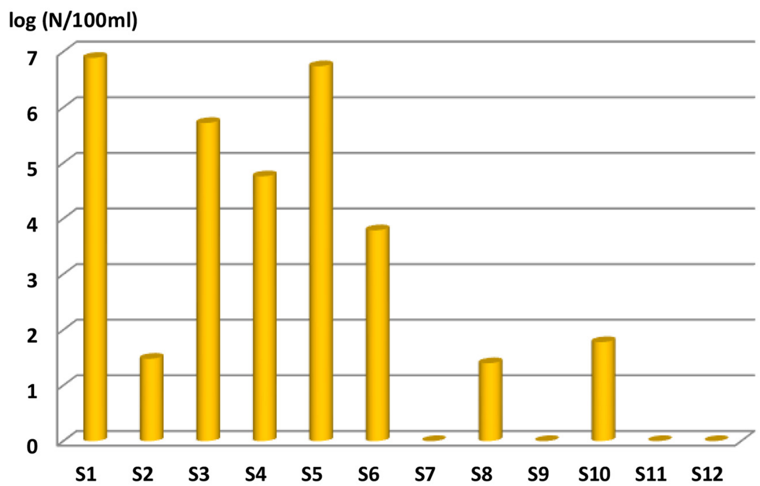

Figure 5. Spatial variation of faecal coliforms in the different stations along the wadi Inaouene

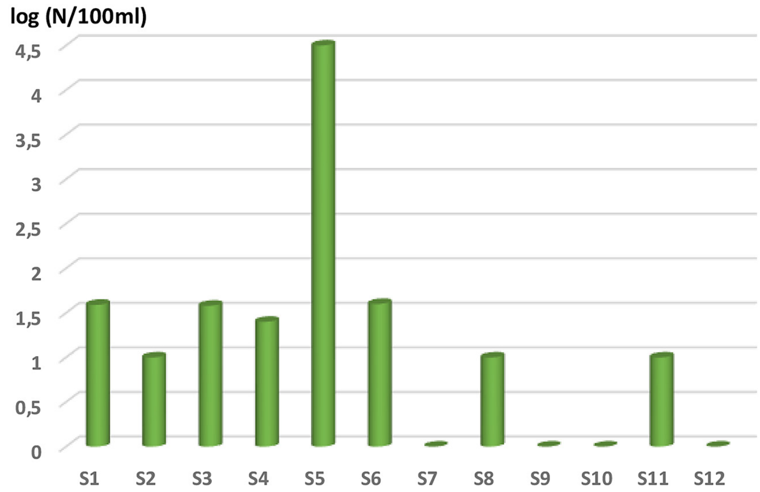

Figure 6. Spatial variation of Fecal Streptococci (FS) in the different stations

sources of potential contamination of surface waters (Haijoubi et al., 2017). Transmission through ingestion of contaminated water or items is the most common in the epidemiology of Salmonella. Contamination of the water environment with these germs is problematic and raises questions about their origin, survivability, and public health significance (Bouchemal, 2017). In our result, Salmonella were only present in station S5 located near the village of wadi Amlil (Table 5).

Listeria monocytogenes

The genus Listeria comprises 8 species including the monocytogenes species, pathogenic for humans and animals and the ivanovii species, pathogenic for animals and rarely for humans. Listeria monocytogenes is responsible for a disease affecting humans and animals (zoonosis) called listeriosis. Bacteria of the genus Listeria are ubiquitous, and their reservoir is mainly environmental: plants, silage products, water, wastewater, animal feces. It is an anthropo-zoonosis that can infect both humans and animals (Hof, 2003). Domestic mammals are most often infected through poor quality silage or hay. Infection with L. monocytogenes in humans occurs primarily through ingestion of contaminated food. Table 3 shows the results obtained after isolation of the pathogens present. The waters studied show contamination by L. monocytogenes in stations S1, S3 and S5 characterized by permanent wastewater discharge.

Origin of the contamination

We calculated the quantitative ratio R to determine the source of the fecal contamination of the water in the study area.

$$R = \frac{\text{FC}}{\text{FS}} \tag{1}$$

They are to relate the densities of Fecal Coliforms (FC), rather than Total Coliforms (TC), with the density of Fecal Streptococci (FS) (Jankovic and Drapeau, 1977), this ratio between Fecal Coliforms and Fecal Streptococci deserves special attention since the human or animal origin of the pollution can be determined by this ratio. When this ratio CF/SF is greater than 4, the pollution is mainly of human origin through the discharge of wastewater (Borrego and Romero, 1982). When the ratio R is lower than 0.7, the source of contamination is animal; domestic livestock, particularly sheep, appear to play a dominant role in

Table 5. Variation in the numbers of pathogens in the different stations of the Wadi Inaouene and its tributaries

Germs	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Listeria monocytogenes	P	A	P	A	P	A	A	A	A	A	A	A
Escherichia coli	p	A	A	p	P	P	A	A	A	A	A	A
Salmonella	A	A	A	A	P	A	A	A	A	A	A	A

Note: P – present, A – absent.

water contamination (Geldreich, 1976). When the CF/SF ratio is between 0.7 and 1, the source of pollution is mixed (human and animal) but predominantly animal, between 1 and 2 uncertain, and between 2 and 4, the source is mixed but predominantly human. (Borrego and Romero, 1982). The results (Figure 7) show a contamination of human origin by wastewater in station S1, while the contamination in stations S7, S9, S10, S11 and S12 is of animal origin, the other stations S2, S5, S8 have a contamination of uncertain origin, and for stations S3 and S4 the origin of the contamination is mixed, with a predominance of humans. This ratio varies according to the temperature and the distance from the source of the contamination. As well as the time of immersion of the contamination in the water, the pH and the toxic substances (Chigbu et al., 2004)

Evaluation of the bacteriological quality of the Inaouene wadi according to Moroccan standards

According to the order of the ME-MPUPHE (2002), defining the quality grid of surface waters which allows classifying the surface waters of the Inaouene watershed according to their quality in chemical elements, using the conventional colors created in the article 8 of this order (Table 6). The bacteriological quality of the water, especially at sites S1 and S5 is classified as very poor due to high concentrations of fecal coliforms under the anthropic impact and wastewater discharges. The Inaouene River flows near the city of Taza and the village of wadi Amlil and is then the main receiver of their stream liquid. This quality gradually improves according to the process of dilution, and

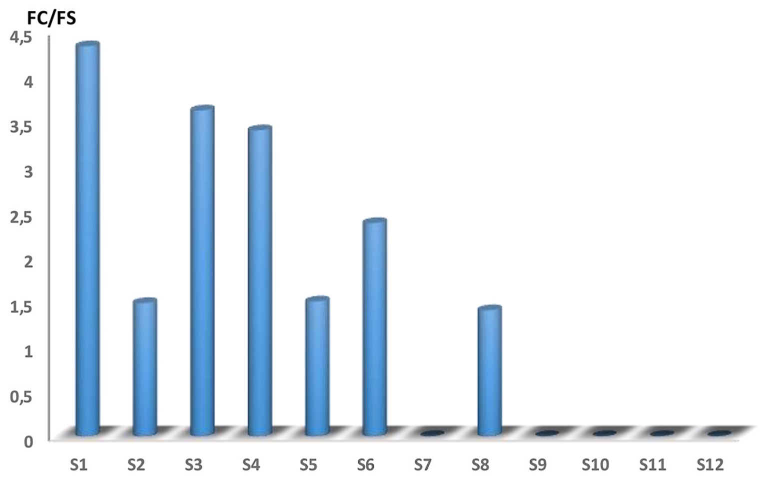

Figure 7. The quantitative ratio R in the different stations

Table 6. Bacteriological water quality in the study area

Stations	Total coliforms (TC)	Fecal coliforms (FC)	Fecal streptococci (FS)
S1	Medium	Very bad	Good
S2	Excellent	Good	Excellent
S3	Medium	Bad	Good
S4	Good	Medium	Good
S5	Medium	Very bad	Bad
S6	Medium	Medium	Good
S7	Excellent	Excellent	Excellent
S8	Good	Good	Excellent
S9	Excellent	Excellent	Excellent
S10	Excellent	Good	Excellent
S11	Good	Excellent	Excellent
S12	Excellent	Excellent	Excellent

Excellent	(blue)	Good	(green)	Medium	(yellow)	Bad	(red)	Very bad	(purple)

then the water becomes average and, lately, seems to be recovered at the level of the dam Idriss 1er.

Explanatory analyses

The physico-chemical and bacteriological analyses showed a significant degree of pollution of the waters, with high values of TC, CF, SF, PO43-, BOD5, Zn, Pb and nitrogenous elements. In order to determine the relationship between the variables and the distribution of the stations according to their physico-chemical and bacteriological characteristics, 21 environmental variables were evaluated by means of a principal component analysis (PCA). The factorial plans F1 and F2 corresponding respectively to 12 stations and the variables are 21 (Figures 8 and 9). This analysis clearly shows the distribution of the stations considering all their characteristics and bacteriological. The F1 and F2 axes give 78.8% of the total information. A positive and highly significant correlation was observed between the indicator bacteria of fecal contamination and the physico-chemical parameters: BOD5, PO43-, NO32-, SO32-, NH4+, Zn, Fe, and Pb. These results are in harmony with those of (Ben abbou, 2017) and can be explained by the natural or anthropogenic presence of suspended

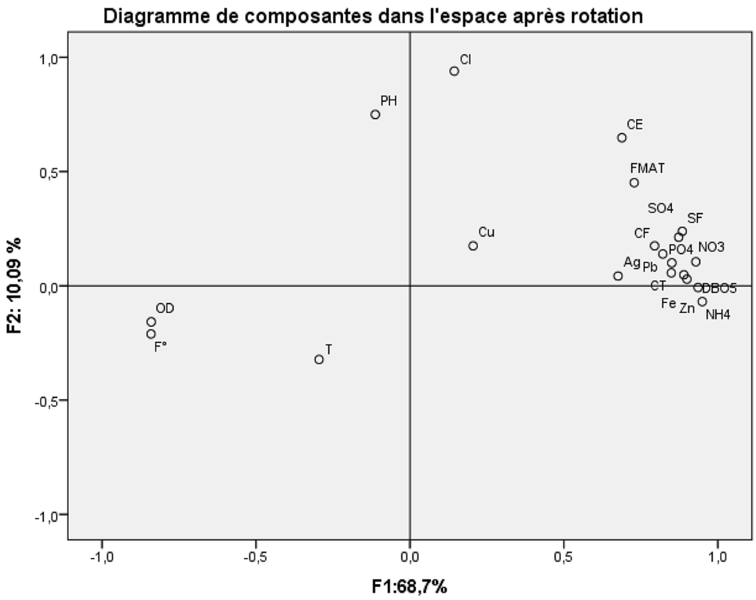

Figure 8. Representations of the variables on the F1*F2 factorial plane

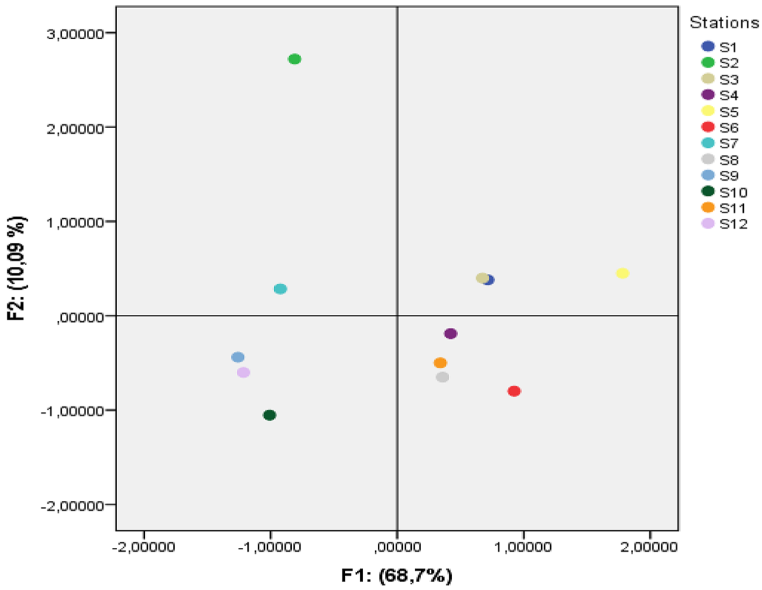

Figure 9. Representation of the study stations on the F1*F2 factorial plane

particles in the water such as clay, silt, organic and inorganic matter, plankton and other micro-organisms that protect bacteria from disinfection (N'diaye et al., 2011).

The PCA revealed that indicator bacteria of fecal contamination correlate negatively with dissolved oxygen, so the lack of dissolved oxygen leads to a proliferation of germs in the water.

CONCLUSIONS

The reuse of wastewater without any prior treatment entails potential health risks as it is rich in pathogenic micro-organisms. The monitoring of the evolution of microbiological pollution of wastewater in twelve wastewater sampling stations has revealed a significant contamination of these waters upstream of the Wadi by the three groups of germs indicative of faecal contamination and the presence of *Listeria monocytogenes* and salmonella in the upstream stations and especially S1, S3 and S5. The bacteriological contamination of the waters of Inaouene wadi is due to discharges of solid waste, domestic and agricultural wastewater from the city of Taza and neighboring rural centers as well as settlements located along the wadi. Indeed, the most contaminated points are those located near the housing units of residents. The waters upstream of Inaouene wadi S1, S3 and S5 polluted by wastewater discharges are not recommended to be used in any activities. On the other hand, the water from the other stations can be reused in the irrigation of vegetable crops on the bank of the Inaouene wadi without any health risk for humans or animals.

REFERENCES

1. Abouzaid, D. 1984. Direction contrôle qualité des eaux (Manuel de prélèvement et d'analyse des eaux). ONEP.
2. Abbou, M.B., Bougarne, L., El Haji, M. 2020. Variation annuelle de la qualité des eaux souterraines de la ville de Taza (Maroc). International Journal of Innovation and Applied Studies, 28(4), 767–781.
3. Achak, M., Ouazzani, N., Mandi, L. 2009. Traitement des margines d'une huilerie moderne par infiltration-percolation sur un filtre à sable. Revue des sciences de l'eau/Journal of Water Science, 22(3), 421–433.
4. Barakat, A., El Baghdadi, M., Rais, J., Aghezzaf, B., Slassi, M. 2016. Assessment of spatial and seasonal water quality variation of Oum Er Rbia River (Morocco) using multivariate statistical techniques. Int. Soil. Water. Conserv. Res., 4, 284–292.
5. Ben Abbou, M., Fadil, F., El Haji, M. 2014. Évaluation de la qualité des cours d'eau de la ville de Taza utilisés dans l'irrigation des cultures maraîchères (Maroc). Journal of Applied Biosciences, 77, 6462– 6473.
6. Ben Abbou, M. 2017. Rejets : impacts sur la qualité des eaux et valorisation par compostage ; bassin versant de l'Ouest Inaouen (Taza, Maroc), Presses Academiques Francophones. ed. Librairie Gallimard, PARIS.
7. Bouchemal, F. 2017. Diagnostic de la qualité des eaux souterraines et superficielles de la région de Biskra (Doctoral dissertation, Université Mohamed Khider-Biskra).
8. Borrego, A.F., Romero, P. 1982. Study of the microbiological pollution of a Malaga littoral area II. Relationschip between fecal coliforms and fecal streptococci. VIè Journée Étude Pollutions, Cannes, France, 561–569.
9. Chigbu, P., Gordon, S., Strange, T. 2004. Influence of inter-annual variations in climatic factors on fecal coliform levels in Mississippi Sound. Water Research, 38.20, 4341–4352.
10. Charles, I.G., Lamb, H.K., Pickard, D., Dougan, G., Hawkins, A.R. 1990. Isolation, characterization and nucleotide sequences of the aroC genes encoding chorismate synthase from Salmonella typhi and Escherichia coli. Microbiology, 136(2), 353–358.
11. CEAEQ. 2000. Recherche et dénombrement des coliformes fécaux; méthode par filtration sur membrane, Centre d'expertise en analyse environnementale. Gouvernement du Québec, CEAEQ, 24.
12. Drapeau, A.J., Jankovic, S., World Health Organization. 1977. Manuel de microbiologie de l'environnement. Organisation mondiale de la santé.
13. Eddabra, R. 2011. Evaluation de la Contamination bacteriologique des eaux usées des stations d'épuration du grand Agadir: isolement, caracterisation moleculaire et antibioresistance des espèces du genre Vibrio (Doctoral dissertation, Strasbourg).
14. Eddabra, R. 2011. Evaluation de la Contamination bacteriologique des eaux usées des stations d'épuration du grand Agadir: isolement, caracterisation moleculaire et antibioresistance des espèces du genre Vibrio (Doctoral dissertation, Strasbourg).
15. El Mahdi, H., Sanae, R., Rachid, F.A., Mustapha, L., Mohamed, F. 2022. Evolution of the Physico-Chemical and Biological Quality of the Waters of Soueir River (Morocco). Ecol. Eng, 4, 202–211.
16. El Haji, M., Boutaleb, S., Laamarti, R., Laarej, L. 2012. Qualité des eaux de surface et souterraine de la région de Taza (Maroc): bilan et situation des eaux. Afrique Science: Revue Internationale des Sciences et Technologie, 8(1), 67–78

17. El Haissoufi, H., Berrada, S., Merzouki, M., Aabouch, M., Bennani, L., Benlemlih, M., Lalami, A.E.O. 2011. Pollution des eaux de puits de certains quartiers de la ville de Fès, Maroc. Revue de microbiologie industrielle sanitaire et environnementale, 5(1), 37–68.
18. El Haissoufi, H., Berrada, S., Merzouki, M., Aabouch, M., Bennani, L., Benlemlih, M., Lalami, A.E.O. 2011. Pollution des eaux de puits de certains quartiers de la ville de Fès, Maroc. Revue de microbiologie industrielle sanitaire et environnementale, 5(1), 37–68.
19. Faouzi, J., Bedoui, I., Rezouki, S., Moubchir, T., Allali, A., Eloutassl, N., Lahkimi, A. 2023 Vertical transfer of bacteriological and parasitological pollutants from irrigation water to soil and crops. Ecological Engineering & Environmental Technology, 24(1), 93–103.
20. Figarella, J., Leyral, G. 2002. Analyse des eaux: Aspects reglementaires et techniques. Ed. Scérén CRDP d'Aquitaine, Paris.
21. Ghazali D., Zaid A. 2013. Study of the physicochemical and bacteriological quality of the waters of the Ain Salama-Jerri spring (Meknes region,Morocco). Larhyss J, 12, 25–36.
22. Geldreich, Edwin, E., Litsky, W. 1976. Fecal coliform and fecal streptococcus density relationships in waste discharges and receiving waters. Critical reviews in environmental science and technology, 6(4), 349–369.
23. Havelaar, A.H., Furuse, K., Hogeboom, W.M. 1986. Bacteriophages and indicator bacteria in human and animal faeces. Journal of applied bacteriology, 60(3), 255–262.
24. Haijoubi, E.H., Benyahya, F., Bendahou, A., Essadqui, F.Z., Behhari, M.E., El Mamoune, A.F., Barakat, A. 2017. Study of the bacteriological quality of water used in the agro-food industry in the North of Morocco. The Pan African Medical Journal, 26, 13–13.
25. Hof, H. 2003. History and epidemiology of listeriosis. FEMS Immunology & Medical Microbiology, 35(3), 199–202.
26. Hounsou, M., Agbossou, E., Ahamide, B., Akponikpe, I. 2010. Qualité bactériologique de l'eau du bassin de l'Ouémé: cas des coliformes totaux et fécaux dans les retenues d'eau de l'Okpara, de Djougou et de Savalou au Bénin. International Journal of Biological and Chemical Sciences, 4(2).
27. Ihsane, O., Zahra, E., Sanae, R., Mohammed, E., Bourhia, M., Ali, G.A., Mohamed, F. 2022. Physicochemical Characterization and Assessment of Magnitude of Pollution to Contribute to Water Sustainability. Sustainability, 14(11), 6689.
28. ME-MPUPHE (Minister of Equipment and the Minister for Planning, Urban Planning, Housing and Environment, (2002). Surface water quality grid. Decree No. 1275–02 of 17 October 2002. Official bulletin, Morocco.
29. Morillo, J.A., Antizar-Ladislao, B. 2009. Monteoliva-Sánchez, M. et al. Bioremediation and biovalorisation of olive-mill wastes. Appl Microbiol Biotechnol, 82, 25–39. https://doi.org/10.1007/s00253–008–1801-y
30. Nouayti, A., Khattach, D., Hilali, M., Nouayti, N., Arabi, M. 2019. Assessment of groundwater quality using statistical techniques in high Basin of Guir (Eastern High Atlas, Morocco). Materials Today: Proceedings, 13, 1084–1091.
31. N'diaye, A.D., Mint, K.M.S., Ould Sid'ahmed Ould Kankou, M. 2013. Contribution A L'etude De La Qualite Physico-Chimique De L'eau De La Rive Droite Du Fleuve Senegal. Larhyss Journal, 12, Janvier, 71–83.
32. Patoine, M., D'Auteuil-Potvin, F. 2015. Contamination bactériologique des petits cours d'eau en milieu agricole : état et tendances, Québec, ministère du Développement durable, de l'Environnement et de la Lutte contre les changements climatiques, Direction du suivi de l'état de l'environnement, 35, 8 annexes.
33. Rezouki, S., Aimad, A., Karim, B., Jamaa, H., Noureddine, E., Mohamed, F. 2021b. The Impact of Physicochemical Parameters and Heavy Metals on the Biodiversity of Benthic Macrofauna in the Inaouene Wadi (Taza, North East Morocco). Journal of Ecological Engineering, 22.
34. Rezouki, S., Allali, A., Touati, N., Eloutassi, N., Mohamed, F. 2021a. Spatio-temporal evolution of the physico-chemical parameters of the Inaouen wadi and its tributaries, 576–587. https://doi.org/10.48317/IMIST.PRSM/morjchem-v9i3.23521
35. Rodier, J., Beuffr, H., Bournaud, M., Broutin, J.P., Geoffray, C., Kovacsik, G., Laport, J., Pattee, E., Plissier, M., Rodi, L., Vial, J. 1984. L'analyse de l'eau, eaux naturelles, eaux résiduaires, eau de mer. 7e édition. Ed. Dunod.
36. Rodier, J., Legube, B., Merlet, N., Alary, C., Belles, A. 2016. L'analyse de l'eau Contrôle et interprétation, 1297.
37. Rodier, J., Bazin, C., Broutin, J.P., Chambon, P., Champsaur, H., Rodier, L. 1996. The Analysis of Water 8th ed. Dunod: Paris.
38. Rodier, J., et al. 2009. L'analyse de l'eau, 9e édition. Dunod (éditeur), Paris, France.
39. Tallon, P., Magajna, B., Lofranco, C., Leung, K.T. 2005. Microbial indicators of faecal contamination in water: a current perspective. Water, air, and soil pollution, 166(1), 139–166.
40. World Health Organization. 2000. The world health report 2000: health systems: improving performance. World Health Organization.