

## Impact of Urban Activities on Water Quality

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### Abstract

Moulouya is the most important river in Boulemane Province. The mining centres abandoned in high Moulouya and the rapid increases in the population with the fast growth of agricultural and industrial activities have caused serious environmental problems, especially on dumping toxic waste materials into the water. 26 samples were collected during April and May 2013 from five sites. The samples were analysed for various physicochemical parameters like: pH, flow velocity, conductivity (EC), temperature, heavy metals (Cu, As, Cd, Pb and Zn), COD (chemical oxygen demand), BOD<sub>5</sub> (biological oxygen demand). Bacteriological analysis was also assessed through: fecal coliform contamination, fecal enterococci, Sulphite reducing bacteria, *Salmonella* spp. and *Vibrio* spp.

The results of this study have shown that the physicochemical and microbiological pollution of the Moulouya River follows a decreasing gradient from Missouri City to Tandite (14.2 mg/l of BOD, 753.2 mg/l of COD, 1856 CFU/100 mL of the total coliform, 1065 CFU/100 mL of the fecal coliform, 2599 CFU/100 mL of the intestinal enterococci). Furthermore, the concentrations of heavy metals in samples met the standards except for copper Cu in Tandite City (0.2mg/l). Statistical approach to principal component analysis (PCA) was made and revealed that the indicator bacterium of fecal contamination presents negative correlations with electrical conductivity.

**KEYWORDS:** Water quality, pollution, Middle Moulouya, physicochemical parameters, bacteriological analysis, Morocco

**Competing Interest:** The authors have declared that no competing interest exists.

### INTRODUCTION

Nowadays, water continues to be at the top of the major preoccupations worldwide. The growing demand for water is estimated to leave over 3.9 billion (roughly 40 %) of the global population living in river basins under severe water stress by 2050 [1]. In Morocco, it is probable that the water

resources per inhabitant can reach around 580m<sup>3</sup>/inhab/year towards 2020 [2]. At this date, about 14 million inhabitants, i.e. almost 35% of the total population of the kingdom, will not dispose of more than 500m<sup>3</sup> /inhab/year [2]. Water resource availability in Morocco is limited due to the annual and the inter-annual variation of precipitations with a huge disparity in their spatial distribution. Among these resources, the watershed's Moulouya is the largest river basin in North Africa [3, 4]. Its basin stretches 32–35°N and 2–6°W, with a hydrological drainage area of ca. 54,000 km<sup>2</sup>[3, 5]. It is the cornerstone of development and a key resource for the global economy of this region [6]. The main human activities in the Moulouya Basin are agriculture (138,000 km<sup>2</sup> of irrigated lands), industry, mining, and grazing [7].

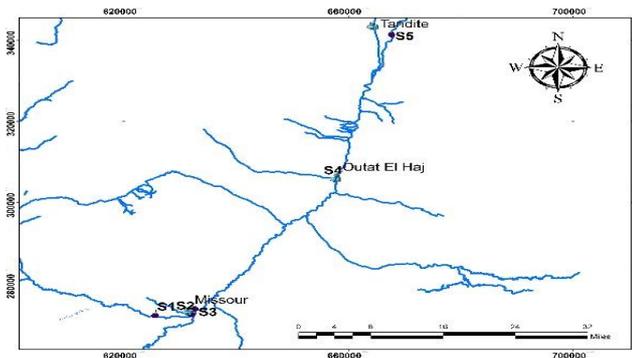
Moulouya basin is subject to the important impacts of wastewater and releases of the mining centers of Aouli, Mibladen and Zaida abandoned without rehabilitation in high Moulouya. This situation has aroused the interest of several researchers. Indeed, studies have been undertaken in recent years and are focused on the high Moulouya as those reported by (El hachimi et al, 2005 and Iavazzo et al, 2013) [8-11]. All of these authors have recorded the contamination of waters in heavy metals such as lead (Pb), Zinc (Zn), copper (Cu), and arsenic (As) primarily in the high part of the Moulouya. Furthermore, the degradation of water resources within the basin of the Moulouya has grown during recent years due to the multiplication of pollution sources (domestic, industrial and agricultural) caused by population growth, intensification of agricultural production and changing lifestyles associated with an increase of consumption. This Pollution is further fueled by recurrent droughts, making sometimes water resources unusable. Thus, the aim of this study was to assess the impact of wastewater on water quality and estimate the possible extension of trace metals from the mining centers abandoned in high Moulouya towards the middle Moulouya.

### MATERIALS AND METHODS

#### Study sites and sample collection

Moulouya is the most important river in Boulemane Province; five sites were chosen based on their location to the

discharges points. We identified site S1 located downstream of Oued Chouf Cherg receiving agricultural sewage sludge (Missour – Sidi Boutayeb), site S2 localized upstream wastewater discharges at approximately 1 km (Missour), site S3 situated downstream from wastewater discharges (Missour - Igly), site S4 located near receiving domestic and industrial wastewater discharges (Outat El Haj) and finally site S5 located in domestic and agriculture wastewater discharges (Tandite).



**Figure 1:** Sampling sites location in Moulouya watercourse, Morocco

The water samples were collected from five different sites at weekly intervals between April and May 2013 using clean sterile glass containers for bacteriological analysis and plastic bottle for chemical analysis (Figure1). The samples were transported to the laboratory carefully using thermo coal box containing ice caps at  $-4^{\circ}\text{C}$ .

## Analytical Methods

### Physico-chemical parameters

Several physicochemical parameters were determined to evaluate the quality of surface water like pH, temperature ( $^{\circ}\text{C}$ ), conductivity (C. E.), nitrite ( $\text{NO}_2^-$ ), chemical oxygen demand, biological oxygen demand and heavy metals. The velocity was determined by measuring the time taken for a floating object to travel a measured distance downstream. Besides, conductivity and temperature were determined in situ using the handheld conductivity meter 330i with parallel temperature display. The quantification of the nitrite ( $\text{NO}_2^-$ ) has been performed by spectrophotometry. Furthermore, chemical oxygen demand was analyzed according to standard method (COD meter, HACH). Also, biological oxygen demand was determined by the manometric respirometry method (OXI TOP IS6) and finally pH was determined in the laboratory using a pH meter (HACH SENSION+ PH31).

Heavy metal concentrations (As, Pb, Zn, Cu and Cd) were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The collection, preservation and analysis of various parameters of water samples from different sampling locations were carried out, by following the standard methods prescribed by Rodier [12].

### Bacteriological parameters

The bacteriological analysis of the various water samples was made according to the Moroccan standard and consisted to enumerate the total coliforms (TC), fecal coliforms (FC), intestinal enterococci (IE) and spore sulfite-reducing anaerobes (SSR): The total coliforms, the fecal coliforms and the anaerobic sulphite-reducing were analyzed according to the official Moroccan method NM03.7.003 and NM 03.7.006 [13, 14]. 100 mL of water was filtered aseptically on membrane nominal porosity ( $0.45\mu\text{m}$ ). The culture media used were Tergitol and Slanetz and Bartley agar added to TTC, and the incubation time was respectively 24 h at  $37^{\circ}\text{C}$  /  $44^{\circ}\text{C}$  for TC, IE and FC. Besides, anaerobic sulphite-reducing was isolated according to official Moroccan method NM 03.7.004 [15]. 50 mL of water was filtered through a sterile membrane and placed on the SPS agar. The plate was incubated at  $37 \pm 2^{\circ}\text{C}$  for 48h.

For *Vibrio cholera*, isolation was made according to NM 03.7.051 [16]. The method consisted of filtering two liters of water through  $0.24\mu\text{m}$  membranes. Membranes were subsequently incubated in 100 mL of alkaline peptone water, pH 8.6, for 6-8 h at  $35^{\circ}\text{C}$ . Two loopfuls of broth were streaked on Thiosulfate Citrate Bile agar (TCBS agar, Difco) and incubated for 18 h at  $37^{\circ}\text{C}$ . Typical colonies (yellow and 1 to 3 mm diameter) were transferred to nutritive soft agar and incubated for 24 h at  $37^{\circ}\text{C}$ . Furthermore, isolation of *Salmonella* was regulated according to NM 03.7.050 [17]. The method consisted of filtering 1l of water through  $0.47\mu\text{m}$  membrane. The membrane was then placed into 50 ml of Rappaport-Vassiliadis broth (RVB) and incubated at  $42^{\circ}\text{C}$  for 24h for selective enrichment of *Salmonella*. Ten microliters of the RVB enrichment were then streaked onto *Salmonella* agar for isolation at  $35^{\circ}\text{C}$  for 24h, and colonies presumptively identified as *Salmonella* was identified by biochemical tests.

### Statistical Analysis

In order to establish the relationship between physicochemical and microbiological parameters and quality of the water in the middle Moulouya and to better assess the impact of human activities on the quality of this river, a statistical Principal Component Analysis (PCA) was applied to all parameters. This statistical method allows transforming the initial quantitative variables, all more or less correlated with each other in new quantitative variables, uncorrelated and called principal components. This method is widely used to interpret the hydro-chemical data [18, 19].

## RESULTS

### Physico-chemical analysis

#### Temperature

The temperature values registered in samples site S1; S2; S3; S4 and S5 were respectively 26.40; 28.98; 27.84; 24.36 and 24.10. The minimum and maximum temperature was recorded in site S4 and S3 with  $19.30^{\circ}\text{C}$  and  $36.60^{\circ}\text{C}$ . The mean and standard deviation of water temperature were 26 and  $\pm 4.57$ .

### Hydric potential

The pH levels found in water samples S1; S2; S3; S4 and S5 were respectively 8.44; 8.36; 8.39; 8.32 and 8.34. The lowest pH value was recorded in S5(7.82), while the highest value was obtained in S1(8.75). The mean and standard deviation of pH in water samples were 8.37 and  $\pm 0.22$ .

### Velocity

The velocity of a water body in stations S1; S2; S3; S4 and S5 were 0.45; 0.33; 0.44; 0.36; and 0.89 respectively. The maximum velocity value was recorded in S5 (2.50m/s) and the minimum value in S3 (0.02m/s). The mean and standard deviation were 0.49 m/s and  $\pm 0.50$ .

### Conductivity

The conductivity values of the investigated sites were 1522.17 $\mu$ s/cm (S1), 1144.6  $\mu$ s/cm (S2), 867.8  $\mu$ s/cm (S3), 1368.8  $\mu$ s/cm (S4), and 1309.4  $\mu$ s/cm (S5). The mean and standard deviation were 1253.31  $\mu$ s/cm and  $\pm 294.13$ . From the analysis, the maximum (1727  $\mu$ s/cm) and minimum values (530  $\mu$ s/cm) were recorded at S1 and S3.

### Biological oxygen demand

The BOD<sub>5</sub> values found in samples in S1, S2, S3, S4 and S5 were 30 mg/l, 34 mg/l, 5 mg/l, 1mg/l, 1 mg/l respectively. The maximum concentration was recorded in site S2 and the lowest concentration was registered in S4 and S5 (Figure 2).

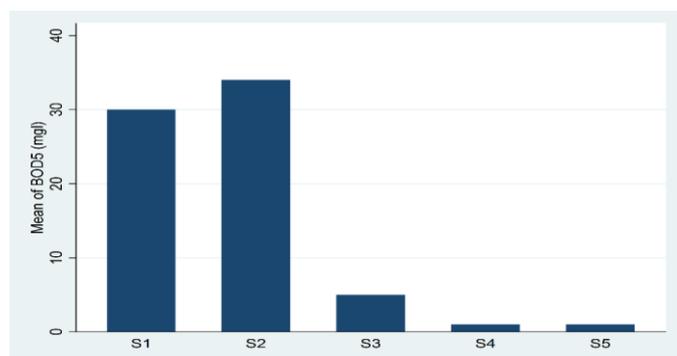


Figure 2. Variation in BOD<sub>5</sub> in different stations

### Chemical oxygen demand

The analyzed COD concentrations in water samples were 1700 mg/l, 1133.33 mg/l, 466.67 mg/l, 300 mg/l and 166 mg/l for stations S1, S2, S3, S4 and S5 respectively (Figure 3). The maximum concentration was recorded in site S1 and the lowest concentration was registered in S5.

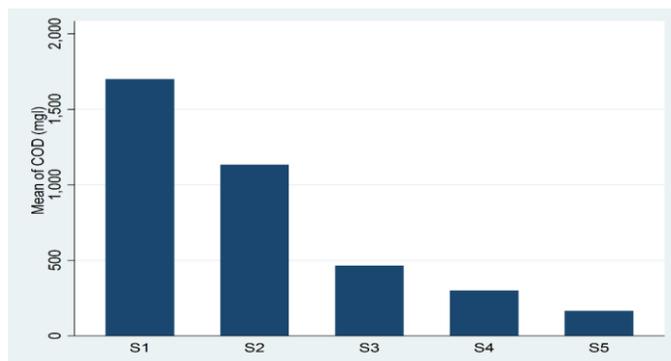


Figure 3. Variation in COD in different stations

### Nitrite

Nitrite concentrations found in water samples were respectively 0.02 mg/l; 0.009 mg/l; 0.011 mg/l; 0.009 mg/l and 0.052 mg/l for stations S1; S2; S3; S4 and S5. The maximum value was recorded at S1 and the minimum at S5.

### Heavy metal

The screening of the heavy metals results showed that all sites were contaminated at least by one heavy metal. The concentration of each heavy metal varied with the variation of the sample site. The result showed that the Cu concentration was respectively 0.167 mg/l; 0.179 mg/l; 0.192mg/l; 0.149mg/l and 0.299mg/l in stations S1; S2; S3; S4 and S5. The maximum Cu value (0.346mg/l) was recorded at S3. Besides, the Pb concentration in the stream was 0.620 mg/l at S1, 0.657 mg/l at S2, 0.598 mg/l at S3, 0.576 mg/l at S4 and 0.539 mg/l at S5.

Thus, the values of Cd in samples S1, S2, S3 and S5 didn't exceed 0.01mg/l. The maximum As value (0.035 mg/l) was recorded at S2. The maximum Pb value (0.733mg/l) was recorded at S4 and the maximum Zn values (0.04mg/l) was recorded at S3 (Figure.4).

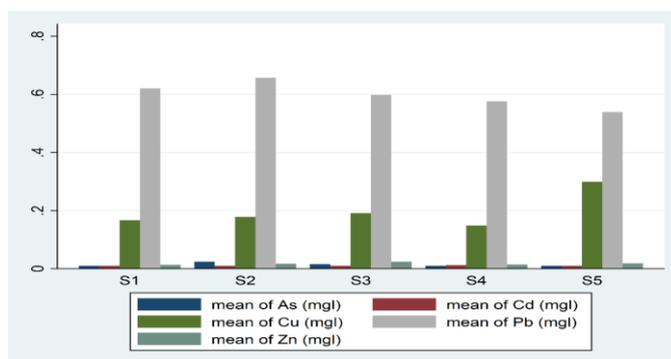
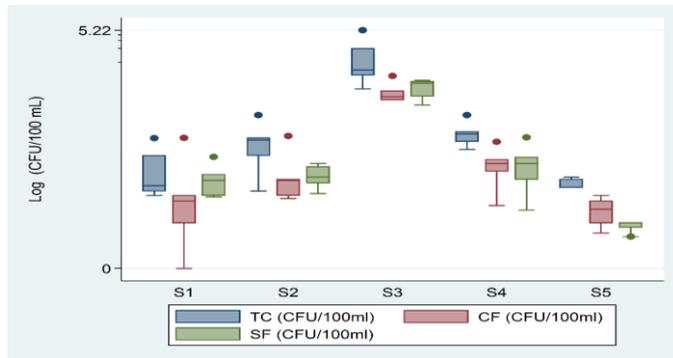


Figure 4. Variation in As, Cd, Cu, Pb and Zn in different stations

### Bacteriological Results

The results of the bacteriological analysis of water in six different locations revealed the presence of the indicator

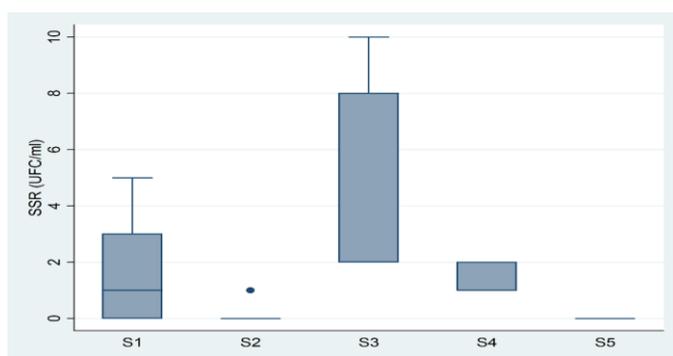
germs of fecal contamination as well as certain pathogenic germs at all sites.



**Figure 5.** Log-mean TC, CF and SF at different sampling points

In terms of bacterial load as can be seen in Figure 5, the total coliform in S3 ( $5.64 \cdot 10^4$  CFU/100 mL) is slightly more concentrated than S4 ( $1.04 \cdot 10^3$  CFU/100 mL), S2 ( $8.07 \cdot 10^2$  CFU/100 mL), S1 ( $2.07 \cdot 10^2$  CFU/100 mL) and S5 (80 CFU/100 mL). Also, the fecal coliform load average (CF) varies between  $1.4 \cdot 10^2$  CFU/100 mL and  $8.06 \cdot 10^3$  CFU/100 mL. The station S3 ( $8.06 \cdot 10^3$  CFU/100 mL) is highly contaminated than S4 ( $2.4 \cdot 10^2$  CFU/100 mL), S2 ( $2.1 \cdot 10^2$  CFU/100 mL), S1 ( $1.4 \cdot 10^2$  CFU/100 mL) and S5 (21.2 CFU/100 mL).

Besides, the intestinal enterococci are highly concentrated in S3 ( $9.49 \cdot 10^3$  CFU/100 mL), S2 ( $1.18 \cdot 10^2$  CFU/100 mL) and S1 ( $1.07 \cdot 10^2$  CFU/100 mL). The lowest values were respectively detected in S5 (8.6 CFU/100 mL), S4 ( $2.67 \cdot 10^2$  CFU/100 mL). The enumeration of the spore of sulphite-reducing anaerobes in the samples is superior at S3 (fig.6).



**Figure 6.** Variation in SSR (UFC/ml) in different stations

Three isolates of suspected salmonella were identified *Salmonella arizona* were detected in Chouf Cherg River (S3). An isolate of suspected vibrio was identified *Vibrio parahemolyticus* was detected in S1, two isolates in S2 and S4, and five isolates in S3. Two isolates of suspected vibrio were identified *V. vulnificus* were detected in S1, five isolates

in S2 and S3, and four isolates in S4 and S5. Five isolates of suspected vibrio were identified *V. alginolyticus* were detected in S1, S2 and S5, three isolates in S3 and four isolates in S4.

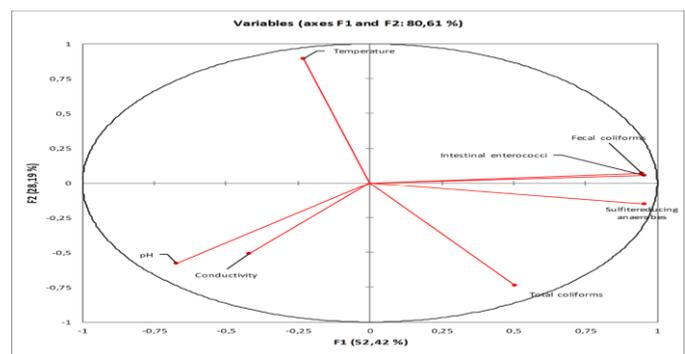
The incidence of *V. parahemolyticus* in S3 was markedly higher than other sites. *V. alginolyticus* was higher in S2 and S5 and a high abundance of *V. vulnificus* was detected in S2 and S3. The ratio of fecal coliform (FC) to fecal streptococci (FS) concentrations has been used to try to determinate fecal contamination source [20]. The results showed that probable fecal contamination source was due to human activities on site S5 (FC/FS= 2.47). Besides, the source contamination was uncertain on site S1 and S2 with respectively values 1.31 and 1.78. Finally, the source contamination was non-human on site S3 and S4 (FC/FS= 85 and 0.90) (Table 1).

**Table 1.** Summary of fecal source related to FC/FS ratios

Stations	FC/FS ratio	Probable fecal Source
S1	1.31	Uncertain
S2	1.78	Uncertain
S3	0.85	Non-human
S4	0.90	Non-human
S5	2.47	Human

### The dynamics of bacterial populations based on the PCA analysis

#### Principal component analysis at the level of Oued Chouf Cherg

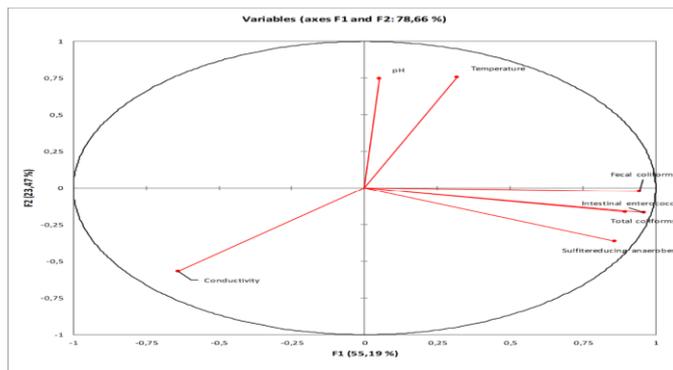


**Figure 7.** The Projection of spatially variable axes F1 and F2 (OuedChoufCherg)

Data obtained from physicochemical and microbiological parameters for PCA, using variables such as temperature, pH, electrical conductivity, the fecal coliforms, intestinal enterococci, bacteria sulphite-reductive and total coliforms were processed. The factorial analysis of F1 and F2 shows that more than 80% are expressed (Figure 7). The axis F1 has a variance equal to 52.42% and it consists of the conductivity, the pH of the medium, the fecal coliforms, intestinal

enterococci, bacteria sulphite-reductive and Total Coliforms. The F2 axis has a variance of 28.19% and seems mainly related to the temperature of the water. The first axis opposed the conductivity to fecal coliforms, intestinal enterococci, bacteria sulphite-reductive and total coliforms. It can be seen that the fecal coliforms, the intestinal enterococci, bacteria sulfite-reductive and total coliforms are strongly positively correlated to this axis.

#### Principal component analysis at the level of OuedMoulouya



**Figure 8.** The Projection of spatially variable axes F1 and F2 (OuedMoulouya)

The factorial analysis of F1 and F2 showed that more than 78.66% are expressed. The axis F1 has a variance equal to 41.84 % and it consists of intestinal enterococci, fecal coliforms, total coliforms the conductivity and spore sulfite-reducing anaerobes (SSR). The F2 axis has a variance of 23.47 % and is expressed by T° and pH of the water (fig.8). The first axis opposes the conductivity to fecal coliforms, to intestinal enterococci, spore sulfite-reducing anaerobes (SSR) and total coliforms. It can be seen that the fecal coliform, intestinal enterococci, spore sulfite-reducing anaerobes (SSR) and total coliforms are strongly correlated positively to this axis. The pH of the environment seems has no major impact on the bacterial load; there are little fluctuations recorded at the level of the pH.

#### DISCUSSION

Aquatic environments such as rivers and streams are considered as ideal reservoirs of bacteria and heavy metals. We showed that all the sites were contaminated by at least one heavy metal. The As, Cd, Pb and Zn values recorded were within the acceptable range of Moroccan irrigation water quality guidelines [21], and they are much smaller compared to the levels observed in Sebou or Fez River [22]. The low level of these elements in S2, S3 and S4 can be explained by the establishment of the dam Hassan II that has decreased the power of evacuation of the pollutants from mining sites of the High Moulouya [9]. But on the other hand, the level of Cu in samples exceeds recommended guidelines in S5 (0.2 mg/l). This result can be justified by the repeated and excessive fertilizer and pesticide applications in surrounding areas. Also, the physico-chemical results showed that the entire sites were

contaminated by several parameters. The majority of the study area samples (92.30%) falls within the desirable limit of 35°C [21]. In fact, water temperature is an ecological factor that has important ecological repercussions [23]. It acts on the density, viscosity, gas solubility in water, the dissociation of dissolved salts, as well as the chemical and biochemical reactions, development and growth of organisms living in water and especially microorganisms [24]. Furthermore, the velocity range of 0.02 - 2.5m/s showed a very large fluctuation range. The velocity of a water body can significantly affect its ability to assimilate and transport pollutants. Water velocity can vary within a day, as well as from day to day and season to season, depending on hydro meteorological influences and the nature of the catchment area [25]. Besides, pH plays an important factor that determines the suitability of water for various purposes, including toxicity to animals and plants. In the present study, pH was found mainly alkaline in all stations. Similar pH values were obtained in some rivers like the Loukkos river estuary [26]. S1 exceeded the permissible limit prescribed by the standards of water quality for irrigation (6.5-8.4)[21].The high alkalinity could be interpreted as being a result of the presence of carbonate rocks (limestone) [27]. The conductivity in all the samples were within the Moroccan standards of quality of surface waters limits (2700µs/cm)[21]. It is a measure of the ability of an aqueous solution to carry an electric current. This ability depends on the presence of ions; on their total concentration, mobility, and valence; and on the temperature of measurement.

The results of the biological oxygen showed that the station S1 and S2 (10mg/l) exceeded the standards of water quality for irrigation [21]. BOD directly affects the amount of dissolved oxygen in rivers and streams. The greater the BOD, the more rapidly oxygen is depleted in the stream. This means less oxygen is available to higher forms of aquatic life. The consequences of high BOD are the same as those for low dissolved oxygen: aquatic organisms become stressed, suffocate, and die [28]. However, the concentrations of chemical oxygen demand (COD) found are higher than those detected for example in Laukkos river estuary [29]. Indeed, the COD in all samples exceeds the standards of water quality for irrigation (40mg/l). In fact, the measure of COD determines the quantities of organic matter found in water. This makes COD useful as an indicator of organic pollution in surface water [30]. The COD test is helpful in indicating toxic conditions and the presence of biologically resistant organic substances [31].

The bacterial load showed that all sites were under the permissible limit of the Moroccan standard except site S3 that is localized downstream the sewage treatment plant considered as the principal contamination source of surface water. The results founded might be due to discharging of domestic wastes containing fecal matters to the river body and open defecation along the sides of the river bank [28]. Besides, pathogenic bacteria research showed that *Salmonella arizona* was detected in Chouf Cherq River (S3). It is the etiologic agent of Salmonellosis in humans causing severe illness in infants, the elderly and immunocompromised patients [32-35]. Salmonellosis symptoms include watery diarrhea, abdominal pain, nausea, fever, headache and

occasional constipation, and can cause Gastroenteritis, peritonitis, pleuritis, Osteomyelitis, meningitis and infection of the blood [36]. *Salmonella typhi* has not been detected in our study. Similar result has been reported in Boufekrane river, that runs through the Boufekrane Town and Meknes City [37-40]. Three species of *Vibrio* were isolated from study sites. *Vibrio vulnificus* has been detected in surface waters at all stations. It is an opportunistic human pathogen that may cause gastroenteritis, severe necrotizing soft-tissue infections and primary septicemia, with a high lethality rate [41]. *Vibrio parahaemolyticus* has been detected in surface waters at all stations except in S5. This species is responsible for sporadic cases and outbreaks of diarrheal illness [42]. Furthermore, *Vibrio alginolyticus* has been detected at all stations except in S5. implicated as a cause of serious wounds especially among vulnerable groups such as older people and those with conditions underlying risk [43]. Moreover, this species has been shown responsible for gastroenteritis and peritonitis in humans [44, 45]. *Vibrio cholera* has not been detected in samples. This finding is similar to those of the previous studies that have examined the presence of *Vibrio* in the Mediterranean coast of the North-East of Morocco [46], Fez and Meknes city [39, 47]. In effect, after the epidemiological data of the Ministry of Health, no cases of cholera have been recorded in Morocco since 1998 [48]. The likely existence of *Salmonella typhi* and *Vibrio cholera* to the viable but non-culturable state would constitute a particular risk of the fact that the surviving bacteria to this state are not detectable by the standard cultivation methods for the enumeration of bacteria. This would call into question the reliability of these methods for the protection of the environment and the public health [37, 49]

## CONCLUSIONS

The results of this study showed that both of Sebou and Moulouya basin were contaminated by heavy metal and pathogenic bacteria. These findings were interesting insofar as they highlight the presence of risk to human and animal lives. Continuous monitoring and treatment process must be regulated. Some steps and awareness programs must need to educate local villagers to safeguard the surface waters.

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