

Contribution to the evaluation of the physicochemical and metallic quality of the surface waters of the Moulouya River (Lower Moulouya, Eastern Morocco)

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Abstract: The study concerns the analysis of physicochemical and metallic parameters of the waters of the Moulouya River in order to establish a diagnosis of pollution degree of these waters and their possible impact on fish. Water samples were taken from six stations on the lower part of the Moulouya River and a station on the left bank tributary of the Oued Sebra, during the period from July 2017 to May 2018. The results obtained showed high levels of Electrical conductivity, Suspended Matter, Chemical Oxygen Demand, Biochemical Oxygen Demand, Ammonium, Nitrate, Sulfate and Chloride that exceeded the recommended limits for surface waters and fish water in many stations. In addition, the results showed a worrying situation of water quality of the station of Oued Sebra which receives industrial discharges and domestic waste. Furthermore, water analysis for trace metals showed relatively high levels but remains below the Moroccan standard for surface water and fish water. These results indicate that the waters of the Moulouya River can have adverse effects on the health and welfare of fish. Thus, the results of the Principal Component Analysis (PCA) showed an inter-station variability and corroborated the results of the descriptive analysis.

Keywords: Water quality; Physicochemical; Trace metal; Moulouya River; Principal Component Analysis

1. Introduction

The surface water of the Moulouya River and the Mechraâ-Hammadi dam are important sources of water for the eastern region of Morocco. Their water is used for agricultural, industrial, and recreational purposes. They are also a source of drinking water and have a great wealth of aquatic flora and fauna (Mahjoub *et al.*, 2020). However, the continuous inputs of physicochemical and metallic contaminants may lead to pollution of the Moulouya River. These pollutants cause major threats to aquatic ecosystems, alter physicochemical, hydrology and faunal characteristics (Taneez *et al.*, 2021).

The quality concerns about Moulouya River stem from the population pressures in the Moulouya watershed, the local industrial activities of the oil mills and the Sucrafor sugar factory in Zaio, the agricultural activities on the Triffa and Sebra plains, the inadequate treatment capacity and poor

management that result in the discharge of partially treated and untreated sewage from the cities of Taourirt, Zaïo, Aklim, Berkane, and the lack of public awareness of environmental protection. In addition, water quality is affected by natural factors, including geological structure and mineralogy, rainfall and runoff events (Moukrim *et al.*, 2008; Chigor *et al.*, 2013; Malik *et al.*, 2020; Pant *et al.*, 2021). The downstream part of the Moulouya River receives some tributaries of the two banks; the main one is Oued Cherraâ that drains the massive Bni Snassen, followed by Oued Sebra which drains the plain Sebra, and by Oued Lakhmis which drains the plain Triffa.

Fish have specific needs in terms of physicochemical and metallic water quality (Wanja *et al.*, 2020). The deterioration of water quality parameters may have a negative impact on the fish fauna, leading to pathological changes and even mortality under extreme conditions (Person-Le Ruyet *et al.*, 2008; Yildiz *et al.*, 2017), as in the case of July 15th, 2011, where thousands of fish were found dead and floating from the confluence of the Moulouya River and the Oued Sebra to the mouth of the Moulouya (Mahjoub *et al.*, 2020). It is therefore essential to regularly measure these critical water quality parameters to ensure that they remain within the optimal range for surface water and fish water.

The objective of this study is to assess the degree, nature and origin of pollution affecting the surface waters of the Moulouya River, as well as the effects of this pollution on the life and health of the fish fauna of this river. This study also introduces a spatiotemporal evaluation of the physicochemical and metallic pollution of the waters of the Moulouya River.

2. Materials and methods

2.1 Study area and sampling station

The Moulouya River is considered as the longest river of Morocco, with a length of 520 km, it takes its source at 2000 m of altitude near the city of Midelt in the junction zone of the High Atlas and the Middle Atlas and flowing into the Mediterranean (Melhaoui & Boudot, 2009; Makhoukh *et al.*, 2011). The Moulouya watershed has an area of 50000 km², and is composed of three sub-basins: the upper, middle, and lower Moulouya which is the area of our study.

The lower Moulouya is located in the North-East of Morocco and covers an area of 330000 hectares, including the Triffa, Sebra, Bouareg and Garet plains (Fahssi *et al.*, 2016). The formations of the lower Moulouya are composed of granites, basalts, marl, marl-limestone, dolomite limestones, limestones, sandstones, gypsum, and conglomerates (Bouabdli *et al.*, 2005). The climate of this area is semi-arid Mediterranean, characterized by low, extremely variable, and irregular precipitation, with values between 230 and 380 mm/year (IUCN, 2010; Tekken & Kropp, 2012; Fadlaoui *et al.*, 2019). The main tributaries of the lower Moulouya are the Oued Za (permanent tributaries), the Oued Sebra (permanent tributaries), the Oued Lakhmis (temporary tributaries), and the Oued Charaâ (temporary tributaries). Moulouya River is subjected to continuous pumping of water for domestic or agricultural uses, to intense grazing and receives discharges of different nature (Tovar-Sánchez *et al.*, 2016). A very dense vegetation colonizes its banks and shelters a relatively important biodiversity.

Taking into consideration the various activities identified in the study area (agricultural and industrial activities), water samples were taken from seven sampling stations distributed along the Moulouya River, and selected in such a way that they are accessible, representative, and reflect the characteristics of the Moulouya River surface waters in the study area (Figure 1).

The various stations are distributed as follows:

- Station S7 (35°06'44" N 2°20'46" W): It is located 1.5km from the mouth of Moulouya River. It is chosen to evaluate the overall pollution load drained by Moulouya River to the marine area.
- Station S6 (35°03'05"N 2°25'43"W): It is at the level of the ancient bridge of Ras El Ma-Moulouya, which is located downstream from confluence of the Oued Charaâ and Moulouya River. It is chosen to estimate the impact of the contributions of the Oued Charaâ, which drains wastewaters of the city of Berkane on the water of Moulouya River
- Station S5 (34°57'01"N 2°29'49"W): Located at the level of the area of the farms of Aklim region and downstream from confluence of the Oued Lakhmis and Moulouya River. This area is characterized by a good extension of agricultural land. It is chosen to search for possible water contamination caused by agricultural activities, and domestic discharges from the city of Aklim.
- Station S4 (34°56'36"N 2°31'13"W): It is located at the beginning of the farms of the Aklim region, and upstream of the confluence of the Oued Lakhmis and the Moulouya River. It is chosen to investigate the impact of agricultural activities on the waters of Moulouya River.
- Station S3 (34°53'11"N 2°39'38"W): It is located at the confluence of the Moulouya River and the Oued Sebra, which drains the effluent of the Sucrafor sugar factory in Zaio and the domestic water of the city of Zaio. Therefore, it is chosen to estimate the impact of the Oued Sebra on the Moulouya River.
- Station S2 (34°53'14"N 2°39'47"W): Located downstream of Oued Sebra, it is chosen to estimate the impact of discharges from the Sucarfor sugar factory located south of the city of Zaio and wastewater from the city of Zaio on the waters of the Oued Sebra.
- Station S1 (34°44'05"N 2°48'11"W): Is located at the level of Mechraâ-Hammadi Dam.

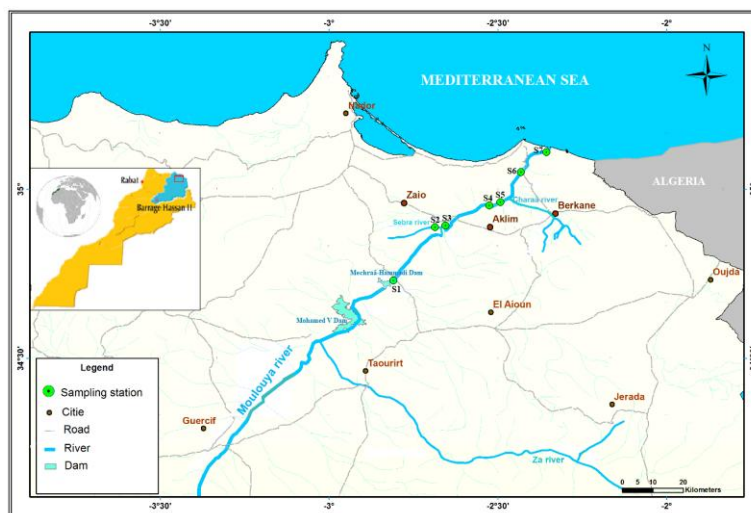


Figure 1. Location of sampling stations in the study area.

2.2. Water sampling and measurement of physicochemical and metallic parameters

In order to characterize and evaluate the physicochemical and metallic quality of the surface water of the Moulouya, we carried out in situ measurements and periodic sampling on an annual cycle once per season (July 2017, October 2017, January 2018 and May 2018). The water samples were collected, using polyethylene bottles pre-cleaned with nitric acid (10%), and rinsed several times with distilled water and three times with water from the site to be sampled. They were then placed in a cooler containing ice flakes of about 4°C, and brought as quickly as possible to the laboratory. At each sampling, water temperature, pH, electrical conductivity, and dissolved oxygen were measured

in situ. The analysis of the physicochemical parameters was carried out in 48h at the laboratory of the agricultural production improvement, biotechnology, and environment and at the Oriental Center of Sciences and Technologies of Water (COSTE). While the water samples intended for the determination of trace metals were filtered through a 0.45 μm membrane filter, acidified with 2 ml of concentrated nitric acid, and stored at room temperature until analysis, where it was carried out at the Regional Laboratory of Analysis and Research of ONSSA in Tangier (LRART-ONSSA). **Table 1** summarizes all physicochemical and metallic parameters measured and the methodology followed.

Table 1. Methods of analysis of the different physicochemical and metallic parameters.

Parameters analyzed	Abbreviation	Unit	Methods of analysis
Water temperature	T	$^{\circ}\text{C}$	Portable multiparameter (WTW 330i)
Potential hydrogen	pH	-	pH-meter (Jenco 6175)
Electrical conductivity	EC	$\mu\text{S}/\text{cm}$	Portable multiparameter (WTW 330i)
Suspended Matter	SM	mg/l	Filtration on filter paper (Whatman) 0.45 μm
Dissolved oxygen	O_2	mg/l	Oximeter (Orion Star A123)
Chemical Oxygen Demand	COD	mg/l	Volumetric methods with ammonium and iron sulfate
Biochemical Oxygen Demand	BOD_5	mg/l	BOD_5 -meter
Ammonium	NH_4^+	mg/l	Determination by spectrophotometric method with sodium phenol nitroprusside reagent
Nitrite	NO_2^-	mg/l	Determination by spectrophotometric method with diazotization reagent
Nitrate	NO_3^-	mg/l	Determination by spectrophotometric method with sodium salicylate reagent
Orthophosphate	PO_4^{3-}	mg/l	Determination by spectrophotometric method with ammonium molybdate reagents
Sulfate	SO_4^{2-}	mg/l	Determination by spectrophotometric method with hydrochloric acid and barium chloride reagents
Chloride	Cl^-	mg/l	Volumetric methods with mercuric nitrate
Calcium	Ca^{2+}	mg/l	EDTA titrimetric method
Magnesium	Mg^{2+}	mg/l	EDTA titrimetric method
Total Hardness	TH	meq/l	Volumetric methods with a complexing reagent EDTA
Lead	Pb	mg/l	Graphite Furnace Atomic Absorption Spectrometry (Varian PERKIN ELMER AS 800)
Cadmium	Cd	mg/l	Graphite Furnace Atomic Absorption Spectrometry (Varian PERKIN ELMER AS 800)
Mercury	Hg	mg/l	Cold Vapor Atomic Absorption Spectrometry (Varian PERKIN ELMER FIMS 100)

2.3 Statistical analysis

The statistical analysis of the data was performed using the IBM SPSS Statistics 21 program. The level of statistical significance was set at 95% ($p < 0.05$). All the water parameters were processed by using Principal Component Analysis (PCA), which corresponds to a widely used method for interpreting hydrochemical data.

3. Results and Discussion

3.1 Physicochemical Parameters

Water Temperature (T) is a limiting factor that has important ecological impacts on the aquatic environment. It affects the existence, survival, growth, metabolic activities, respiration, oxygen consumption, reproduction, and migratory behaviors of fish (Yildiz *et al.*, 2017). In the study area, water temperatures range from 14.2 °C in winter at station S1 to 33.4 °C in summer at station 2 (Figure 2). Referring to the Moroccan surface water grid (SWQG, 2002), the water of the majority of the stations belong to the excellent to class medium of water quality. Therefore, these Cyprinid waters pose no threat to fish life (FWQG, 2003). However, the water of the stations S2, S3, and S7 during the summer season belong to the bad quality class, because they exceed the limit values (30 °C) for both surface water and fish water quality. The variations of water temperature in the Moulouya River have shown that it follows a seasonal pattern, which has been fluctuated according to the atmospheric temperatures. These variations are also related to the depths of the water (water gets colder with depth), and to the presence of riparian vegetation (vegetation cover leads to low sunlight in rivers) (Mayer *et al.*, 2005; Nyanti *et al.*, 2018).

The pH has a great influence on fish welfare, that a sudden drop in water pH can lead to a drop in blood pH (acidosis), which decreases the ability of hemoglobin to carry oxygen (Yildiz *et al.*, 2017). Carter (2008) reported that a pH below 5.5 limited fish growth and reproduction. Moreover, swimming performance of fish is affected by a very low pH of the water (Ye & Randall, 1991; Makori *et al.*, 2017). The analysis of this parameter shows that the water of this part of Moulouya River are slightly neutral to alkaline, with values between 7.44 at station S2 during the summer season, and 8.21 at station S1 during the winter season (Figure 2). This basicity may be mainly related to the marno-dolomitic and limestone lithological nature of the lands crossed by the waters of the Moulouya River and these tributaries, which make them very loaded with carbonates and allow to buffer them (Rezouki *et al.*, 2021). This can also be explained by the massive development of vegetation in the bed of the lower part of the Moulouya River inducing a very significant photosynthetic activity and thus causing an increase in pH. The pH values found in this study, classify the water of Moulouya River as an excellent quality (SWQG, 2002), and indicate that this water does not present a danger for the fish in this area, since the pH is always between 5 and 9 according to the quality grid for fish waters (FWQG, 2003).

The Electrical Conductivity (EC) allows to appreciate the degree of mineralization of a water and thus it is an index of the total ionic content of the water (Joshi *et al.*, 2009). The EC does not directly affect fish welfare, but it is a good indicator of water quality and can affect other water parameters. The values recorded in this study show significant variations; the lowest value is in the order of 1440 µs/cm at station S1 in spring, while the highest value is in the order of 21739 µs/cm at station S2 in summer (Figure 2). This high ionization of the Moulouya River water compared to previous studies (Brahimi *et al.*, 2014; Taybi *et al.*, 2016) could be explained by the decrease in water flow of this river due to the rainfall deficit and because of the depletion of water sources feeding this river (Tovar- Sánchez *et al.*, 2016). In addition, the increase of highly mineralized discharges in this river and the geological nature (limestone formations) of the land crossed by the waters, lead to very high EC values. We also note that this EC increases proportionally in space from upstream to downstream with critical contents of this parameter at station S2, which can be attributed to the urban discharges from the city of Zaio and to the industrial discharges of the refinery of sugar. All of the water in the

stations located downstream of the Mechraâ-Hammadi Dam recorded values higher than the Moroccan standard for surface water (2700 $\mu\text{S}/\text{cm}$) (SWQG, 2002), and for fish waters (Cyprinid waters) (3000 $\mu\text{S}/\text{cm}$) (FWQG, 2003). These EC values classify the waters of the Moulouya River from medium to very bad quality (SWQG, 2002).

Suspended Matters (SM), represent all the mineral particles (sands, clays, silts, etc.) and organic particles (decomposing organic debris, etc.) contained in the water with a diameter higher than 1 μm (Fabris *et al.*, 2021). These SM are responsible for the turbidity of the water, and thus they can disturb the photosynthesis and hinder the respiration of fish (Chapman *et al.*, 2017). They can also accumulate high amounts of toxic matters (trace metals, pesticides, PCB, etc.), and therefore they put the health and welfare of the fish at risk (Bruton, 1985; Weltens *et al.*, 2001; Bilotta *et al.*, 2008; Yildiz *et al.*, 2017). SM values recorded ranged from 3 mg/l (S7) to 747.5 mg/l (S6) (Figure 2). The values obtained in winter (94 to 747.5 mg/l) are higher than those measured in other seasons. These high levels can be attributed to intense erosion of the Moulouya watershed, following strong rainstorms that cause an increase in SM. Despite the high levels of SM during the winter, they remain below 1000 mg/l, which makes it possible to classify the water of the Moulouya River in the excellent to medium quality according to the Moroccan surface water grid (SWQG, 2002). However, these contents are higher than the fish water quality standard (< 50 mg/l) (FWQG, 2003) during the winter season and at stations S2, S4 and S7 during the spring season. This can impede fish respiration and increase their contact with toxic substances such as trace metals and pesticides, which will endanger their lives and the diversity of fish in this area.

Dissolved oxygen (O_2) is a very important parameter in aquatic environments owing to the fact that it governs the majority of chemical and biological processes in aquatic ecosystems and conditions the life of aquatic organisms (Griba *et al.*, 2019; Beddiar *et al.*, 2020). Low dissolved oxygen concentrations (below fish requirements) induces respiratory distress leading to a reduction of metabolic rate, resulting in reduced growth rate, feed efficiency and swimming ability, and ultimately mortality (Jones, 1971; Person-Le Ruyet *et al.*, 2008; Yildiz *et al.*, 2017). In the present study, dissolved oxygen concentrations are marked by the effect of seasonality, since higher levels were recorded in the wet periods (8.15 mg/l at station 6 in winter) than in the dry periods (4.91 mg/l at station 2 in summer) (Figure 2). The variation of dissolved oxygen in the seven stations could be attributed mainly to the water temperature proportionally related to the atmospheric temperature because the low water temperatures in the winter season favor an increase of the dissolution of oxygen, and consequently its concentration in the water increases (Harvey *et al.*, 2011). This was confirmed in the correlation study (Table 2), where O_2 and T are negatively correlated ($r = -0.701$, $p < 0.05$). In addition, the high flow during the wet period induces an increase in the exchange of oxygen with the atmosphere and facilitating the circulation of air in the water and subsequently causes the increase of dissolved oxygen levels (Makhoukh *et al.*, 2011; Fadlaoui *et al.*, 2020). The low dissolved oxygen content recorded at station S2 can be attributed to excessive inputs of organic matter discharged from the Sucarfor sugar factory and the sewers of the city of Zaio. Where excessive amounts of organic matter leads to a significant decrease in dissolved oxygen as a result of its use in the phenomenon of biodegradation by the microorganisms. This explains why we found strongly negative correlations between O_2 and BOD_5 ($r = -0.903$, $p < 0.05$) (Table 2). In general, the O_2 values recorded indicate an excellent to medium oxygenation of the water of the Moulouya River

(SWQG, 2002), and which is favorable to the maintenance of fish species because its values are all higher than the limit value recommended for cyprinid waters (> 3 mg/l) (FWQG, 2003).

As indicated earlier, pollution by biodegradable organic substances is among the factors most responsible for a significant reduction in the oxygen concentration of the water. These substances are oxidized by micro-organisms that consume oxygen from the water for this process (Alaqrbeh *et al.*, 2022). In addition, some chemicals can be decomposed in the absence of micro-organisms. A high input of organic matter and chemicals causes a rapid decomposition of these substances and forms a high dissolved oxygen demand on the water. This will cause to an oxygen deficit in the water and the fish may die from suffocation (Demeke & Tassew, 2016), as in the case of 2011, when thousands of fish were found dead at the level of lower Moulouya. The content of organic substances in water, in terms of their capacity for taking oxygen from the water, can be measured by means of the Biochemical Oxygen Demand within five days (BOD_5 , which allows to characterize the pollution of a water by biodegradable organic matters) and by the Chemical Oxygen Demand (COD, which allows to characterize the overall pollution of a water by organic compounds and also oxidizable chemicals).

BOD_5 values vary from 1.5 mg/l (S4) in the spring season to 314 mg/l (S2) in the summer season. Furthermore, COD values range from 15 mg/l at station S4 in the spring season to 1843 mg/l at station S2 in the summer season (Figure 2). Very high values were detected at station S2 and S3, which confirms the impact of domestic discharges from the city of Zaio and industrial discharges from the Sucrafor sugar factory on the Moulouya River (Mahjoub *et al.*, 2020). The increase in BOD_5 and COD levels in dry periods can be explained by the significant decrease in the flow of the river associated with the warming of the water. Moreover, the levels recorded in the majority of stations exceed the Moroccan standard for fish water (< 6 mg/l for BOD_5 ; < 30 mg/l for COD) (FWQG, 2003), which constitutes an enormous threat to fish life in this river. Referring to the Moroccan surface water standards (SWQG, 2002) the water of this river belong to the excellent to very bad class.

The study of the pollution of aquatic ecosystems by nutrient salts, such as ammonium (NH_4^+), is very interesting, because they promote the excessive development of aquatic plants and lead to the problem of eutrophication, and thus leading to an increase in water turbidity and a decrease in dissolved oxygen levels (Mourão *et al.*, 2020; Che & Wan, 2021). Additionally, NH_4^+ have been suggested to have a depolarizing effect on neurons, by displacement of K^+ , eventually leading to cell death in the central nervous system (Randall & Tsui, 2002). NH_4^+ transforms fairly rapidly to nitrite and nitrate by oxidation and is a good indicator of the pollution of waterways by urban and industrial effluents (Brahimi *et al.*, 2014). NH_4^+ concentrations at the study stations ranged from 0.055 mg/l (S4) in the winter season to 5.28 mg/l (S2) in the summer season (Figure 2). NH_4^+ values found in the dry period are significantly higher than those of the wet period. This may be due to the effect of dilution and the high oxygenation of the water during the winter season, which leads to an increase in the level of oxidation of NH_4^+ , and therefore its values decrease in winter. On the contrary, in the summer season, the low oxygenation of the environment and consequently the incomplete degradation of organic matter maintain high values of NH_4^+ . This was confirmed in the correlation study (Table 2), where NH_4^+ and O_2 are negatively correlated ($r = -0.776$, $p < 0.05$). High levels of NH_4^+ were recorded in station S2 during the summer and autumn seasons (Figure 2), which can be mainly attributed to the discharge of poorly treated wastewater from the Sucrafor sugar factory in

Zaio and from the city of Zaio which is rich in NH_4^+ , from where the water of the Oued Sebra is of good to bad quality (SWQG, 2002). Similarly, the values found for NH_4^+ in the waters of the Moulouya River place these waters in the excellent to medium quality class according to the Moroccan surface water quality grid (SWQG, 2002). On the other hand, most of the stations (S1, S2, S3, S6) have recorded NH_4^+ values that exceed the recommended value for fish ($< 1 \text{ mg/l}$) (FWQG, 2003), which can have adverse effects on the health and welfare of fish.

Nitrite (NO_2^-) is considered to be an intermediate ion between nitrate and ammonium; aerobic bacteria (nitrosomonas genus) oxidize NH_4^+ into NO_2^- (Lewis & Morris, 1986). Nitrite becomes toxic even at low levels to many fish species (Thangam, 2014), it can cause the decrease in oxygen transport capacity of the blood, physiological metabolism disorders, disrupts the ionic homeostasis, immune system damage, induces oxidative stress, histopathological damage, and apoptosis (Cheng *et al.*, 2002; Tseng & Chen, 2004; Kroupova *et al.*, 2005; dos Santos Silva *et al.*, 2018; Valencia- Castañeda *et al.*, 2018; Zhang *et al.*, 2020, 2021). In addition, NO_2^- is a nutrient that causes significant eutrophication (Mourão *et al.*, 2020). The results of this study showed that NO_2^- levels vary from a minimum of 0.014 mg/l in spring to a maximum of 0.771 mg/l in winter, recorded respectively at station 3 and station 2 (Figure 2). These low concentrations may be due to a rapid oxidation of nitrites into nitrates because the waters of the Moulouya River are generally well oxygenated. The levels of NO_2^- recorded are in accordance with the quality grid for Moroccan fish waters (FWQG, 2003), whose content is less than 0.5 mg/l , with the exception of two stations (S2 and S6 in winter) which exceed the limit of the standard. Therefore, nitrite levels in the waters of this river do not have adverse effects on the health and welfare of fish.

Nitrate (NO_3^-) is the final stage of nitrogen oxidation and represents the most stable and abundant nitrogenous form in natural waters (Makhoukh *et al.*, 2011). NO_3^- can cause several health problems for fish, such as growth retardation, histopathological damage, reduced oxygen transport capacity, changes in swimming behavior, and endocrine disorders (Torno *et al.*, 2018; Bjerregaard *et al.*, 2018; Yu *et al.*, 2021). NO_3^- levels in the water of the Moulouya River range from 2.73 mg/l recorded at station 1 to 159.8 mg/l recorded at station 5 (Figure 2). In addition, NO_3^- levels recorded during the winter campaigns for stations S2 (55.3 mg/l), S3 (58.3 mg/l), S4 (57.02 mg/l) and S5 (159.8 mg/l), and during the autumn campaigns for station S6 (72.36 mg/l), are higher than the level suggested by Moroccan and international standards (50 mg/l). These high concentrations of NO_3^- may be due mainly to the excessive use of chemical fertilizers related to intensive agricultural activities developed on the banks of this river (Mahjoub *et al.*, 2020, 2021a), and they could also be due to the oxidation of NH_4^+ into NO_3^- from agricultural diffuse pollution and domestic and industrial discharges (El Ouali Lalami *et al.*, 2010). In addition, the high NO_3^- concentrations in stations S2, S3, S4, and S5 during the winter season may be due to runoff from agricultural lands during rainy periods (mainly in winter). This indicates that the water studied is subjected to a risk of contamination by nitrates and therefore can be classified as excellent to very bad (SWQG, 2002).

Phosphorus is one of the important nutrients, and it is an essential biogenic element for the growth of aquatic plants (Makhoukh *et al.*, 2011). In general, PO_4^{3-} is not toxic to fish, but these high levels in surface water can lead to the problem of eutrophication (Strauch *et al.*, 2019), hence phosphorus concentrations should be limited. The analysis of the results shows that the concentration of PO_4^{3-} in the surface water of the Moulouya River varies between 0.006 mg/l (S6) in the spring season and 0.266 mg/l (S1) in the summer season (Figure 2). This availability of PO_4^{3-} may be due to urban and

industrial discharges from neighboring agglomerations, as well as to agricultural activities associated with the excessive use of phosphate fertilizers in this area (Mahjoub *et al.*, 2021b). These contents remain below the Moroccan standard fixed at 1 mg/l, which makes it possible to classify the water of the Moulouya River in the excellent to good quality class (SWQG, 2002).

Sulfate (SO_4^{2-}) is a chemical element naturally present in surface water but its high concentrations can be toxic to aquatic life in general and to fish in particular in freshwater environments (Karjalainen *et al.*, 2021; Zak *et al.*, 2021). The concentrations recorded in the waters of the Moulouya River vary between a minimum of 326.3 mg/l at station 3 in Summer and a maximum of 1058.4 mg/l at station 2 in Winter (Figure 2). These concentrations remain above the limit values suggested by Moroccan standards for both surface water (< 250 mg/l) and fish waters (< 200 mg/l) (SWQG, 2002; FWQG, 2003). These high values can be explained by the nature of the rocks crossed (abundance of evaporitic sedimentary formations mainly gypsum (CaSO_4)) (Doubi *et al.*, 2013), the anthropic contribution via the discharge of domestic and industrial water loaded with SO_4^{2-} (Brahimi *et al.*, 2014), and also by the fertilizer leaching from agricultural soils in this area (Mahjoub *et al.*, 2020). Referring to the Moroccan standards of surface water, the recorded sulfate levels make it possible to classify the waters of the Moulouya River in a state of bad to very bad quality. In addition, these sulfate values will pose a threat to the life of fish species in this river according to the fish water quality standard (FWQG, 2003).

Chlorides (Cl^-) is one of the main inorganic anions in water, and they are generally in the form of sodium (NaCl), potassium (KCl), calcium (CaCl) and magnesium salts (Makhoukh *et al.*, 2011). High levels of chloride result in an increase in salinity, which can have ecological effects on river water quality and fish assemblages, thus reducing the abundance of freshwater fish (Morgan *et al.*, 2012). In addition, Cl^- can have toxic effects on fish especially when it is combined with other elements (Kasumyan, 2001; Vijayavel *et al.*, 2006; Demska-Zakęś *et al.*, 2021). On the other hand, a number of studies have reported the effect of chloride on the reduction of nitrite toxicity in fish (Mazik *et al.*, 1991; Alcaraz & Espina, 1994; Matsche *et al.*, 2012). The Cl^- contents of the analyzed water samples (Figure 2) ranged from 142 mg/l (S1) to 8455 mg/l (S2). These values indicate the contribution of an anthropogenic input that can be of urban as well as industrial origin (especially for station 2), and they could be due to the nature of the land crossed, and because of the proximity of the sea (for station 7). The strong decrease in the water flow of this river, which it has experienced in recent years, has led to a very significant increase in Cl^- concentrations. Chloride ions are mostly above the Moroccan standards of surface water (750 mg/l), which makes it possible to classify these waters in the excellent to medium grid for station 1, and in the medium to very bad grid for the rest of the stations (SWQG, 2002). Therefore, these high Cl^- concentrations have a potential risk factor for fish welfare.

The total hardness (TH) corresponds to the total concentration, of primarily calcium (Ca^{2+}) and magnesium (Mg^{2+}) presented in the water (Benabbi *et al.*, 2017). Several researchers reported that the toxic effects of elements (particularly heavy metals) on freshwater organisms is linked to water hardness (Pyle *et al.*, 2002; Rathore *et al.*, 2003; Markich *et al.*, 2006; Pourkhabbaz *et al.*, 2011). The increase in water hardness, due to an increase in the concentrations of Ca^{2+} and Mg^{2+} in the water, often tends to reduce the toxicity of heavy metals for fish (Pyle *et al.*, 2002; Pourkhabbaz *et al.*, 2011). In the Moulouya River, total hardness values fluctuate between 11.6 mg/l at station 3 during the summer season, to 89.6 mg/l at station 2 during the spring season (Figure 2). Concerning the

concentration of Ca^{2+} , this ion reaches a maximum value at station S2 during the autumn season which is of the order of 625.3 mg/l, while the minimum content was recorded at stations S1 and S6 at 128.25 mg/l during the summer season (**Figure 2**). Finally for Mg^{2+} , the concentrations of this ion vary between 43.08 mg/l at station 3 in summer and 714.4 mg/l at station 2 in spring (**Figure 2**). The values found of these elements are due to the contact of water with the limestone (rich in Ca^{2+}) and serpentine (rich in Mg^{2+}) rocks of this area (Doubi *et al.*, 2013; Fadlaoui *et al.*, 2020), as well as by runoff and inputs from the Oued Sebra. Strong positive correlations were found between Ca^{2+} and TH ($r = 0.997$, $P < 0.05$), and between Mg^{2+} and TH ($r = 0.999$, $P < 0.05$) (**Table 2**) because the total hardness is almost the sum of Ca^{2+} and Mg^{2+} . Depending on the concentrations of Ca^{2+} and Mg^{2+} found, the water quality of this river varies from excellent to very bad (SWQG, 2002). Furthermore, these relatively high levels of TH can reduce the toxic effect of heavy metals on fish.

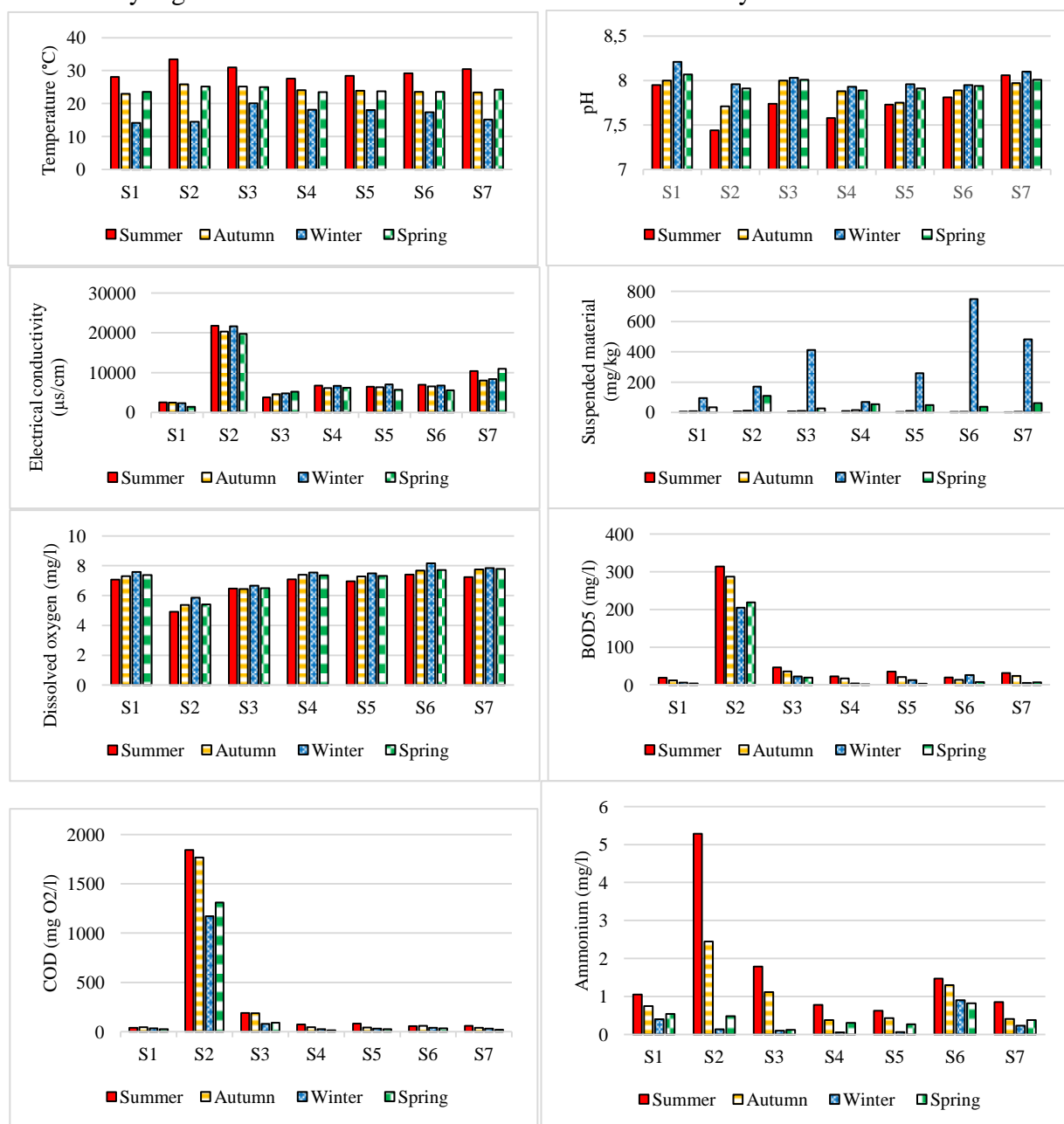




Figure 2. Spatio-temporal variations of physicochemical parameters.

3.2 Trace metals concentrations in water samples

The monitoring of trace metal concentrations is particularly important because of their toxicity, non-biodegradability, persistence, bioaccumulation, and biomagnification in fish (Castro-González & Méndez- Armenta, 2008; Mahjoub *et al.*, 2021b).

Pb is as a potent environmental pollutant. The toxic effects of lead can cause loss of appetite and affect individual growth rates, disturbances of ionic regulation, physiological disorders, spinal and

vertebral deformities, histopathological lesions, neurotoxic action, blood changes, and even death to fish (Köck & Hofer, 1998; Javed, 2012). The results of Pb analyses in surface water of the lower Moulouya during the study period are shown in **Figure 3**. From these results, we can see that the values of Pb are low, whose levels obtained for all the stations vary between 0.077 µg/l at station 5 during the spring and 2.152 µg/l at station 2 during the summer. The increase in Pb concentration at station 2 (downstream of the Oued Sebra) can be explained by diffuse pollution due to the existence of a disused industrial site (Sucrafur sugar factory) and domestic wastewater inputs from the city of Zaio. The Pb contamination in the other stations is probably because of Pb inputs from road and agricultural leaching (Mahjoub *et al.*, 2020) and also to the fact that the waters of the Moulouya drain the Aouli mine tailings (Bouabdli *et al.*, 2005). The levels of Pb are higher in dry periods than in wet periods, this may be owing to the phenomenon of dilution. Moreover, the implementation of a succession of dams on the Moulouya River has limited the inflow of river water loaded with trace metals from the upper and middle Moulouya River to the downstream area. Pb concentrations remain well below the limit values recommended by Moroccan standards for surface water quality (50 µg/l) (SWQG, 2002) and fish water quality (20 µg/l) (FWQG, 2003), which allows this water to be classified as excellent (SWQG, 2002). The presence of this non-biodegradable metal, even at very low values, may have toxicological risks for fish due to the phenomenon of bioaccumulation and biomagnification.

Cd is considered one of the most toxic elements to fish and humans due to its ability to produce a chronic toxic effect even at low concentrations (Rajeshkumar & Li, 2018; Mahjoub *et al.*, 2021b). Cd exposure causes a broad spectrum of toxic effects in fish, including anemia, olfactory nerve damage, impairment of ionic regulation, nasal septum ulceration, enzymatic inhibition, and reproductive failure (Köck & Hofer, 1998; Malik *et al.*, 2020). The spatiotemporal evaluation of Cd levels shows low values in all the stations studied in the Moulouya River. Cd levels showed a minimum value of 0.044 µg/l recorded at station 3 during the spring month, and a maximum value of about 0.248 µg/l recorded at station 2 during the autumn month (**Figure 3**). The contamination by this element can have agricultural origins, because along the Moulouya River is practiced an intense agricultural activity which uses intensively phosphate fertilizers (containing residues of cadmium) and which are responsible, through the phenomenon of runoff and leaching, of an important input of cadmium to the river (Mahjoub *et al.*, 2020, 2021b). Similarly, other discharge points could be the cause, in particular the discharge of urban and industrial wastewater at station 2. Furthermore, a comparison of the Cd levels found with the Moroccan standard set at 5 µg/l places these waters in the excellent class (SWQG, 2002). These revealed levels are all lower than the Moroccan standard for fish waters (FWQG, 2003), and therefore do not present any risk for the fish life of this river. This was confirmed by the study of Mahjoub *et al.* (2020) and Mahjoub *et al.* (2021a) where very low concentrations of Cd were found in the muscles of fish in this river.

Hg is one of the most toxic trace metals in aquatic ecosystems and has a particularly high bioaccumulation capacity (Mahjoub *et al.*, 2021b). Research indicates that Hg exposure can induce a variety of adverse effects in fish such as alterations in the immune system, contributes to cardiomyopathy, inhibitory effect on fish reproduction, neurotoxic effects and a widespread neuronal degradation, reduced the growth and survival, and even death (Berntssen *et al.*, 2003; Mieiro *et al.*, 2010; Penglase *et al.*, 2014; Zheng *et al.*, 2019). **Figure 3** shows that the majority of stations did not record any traces of mercury (S1 in summer and spring; S3 in all months; S4 in summer; S5 and S7

in summer, autumn and spring; S6 in autumn and spring). However, different Hg levels were detected in some stations sampled on the Moulouya River, where Hg concentrations ranged from 0.005 $\mu\text{g/l}$ (S6) in the summer season to 0.184 $\mu\text{g/l}$ (S2) in the autumn season. A high concentration was recorded at station 2, probably due to pollution from industrial sugar and domestic discharges from Zaio City. The Hg values in the waters of the Moulouya River make it possible to place these waters in the excellent class according to the Moroccan surface water quality grid (SWQG, 2002). In addition, these levels remain below the recommended maximum limit for fish waters (FWQG, 2003). However, this metal can be a threat to fish life in this environment because of its high accumulation and amplification capacity in fish, as shown in the study by Mahjoub *et al.* (2021a).

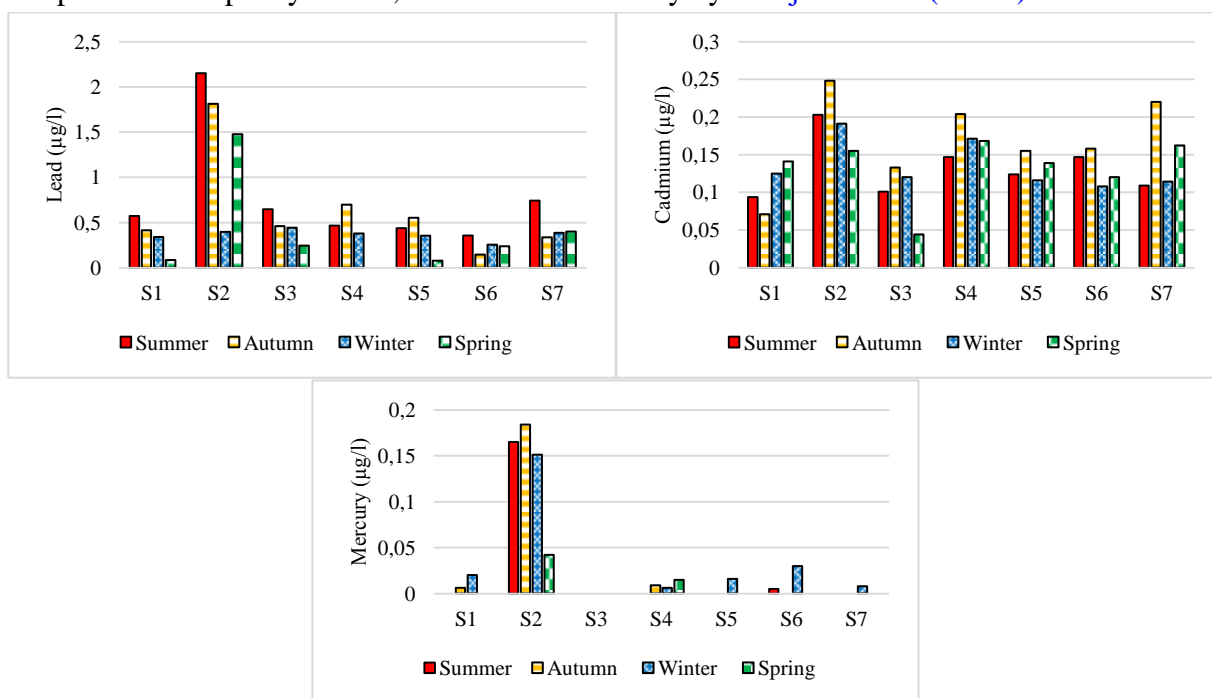


Figure 3. Spatio-temporal variations of metallic parameters.

3.3 Principal Components Analysis

The examination of the matrix of correlations between variables (Table 2) reveals the presence of a significantly high positive correlations between: EC/NO_2^- ; EC/NH_4^+ ; $\text{EC}/\text{SO}_4^{2-}$; EC/Cl^- ; EC/Ca^{2+} ; EC/Mg^{2+} ; EC/TH ; EC/COD ; EC/BOD_5 ; EC/Hg ; EC/Cd ; EC/Pb ; $\text{NO}_2^-/\text{NH}_4^+$; $\text{NO}_2^-/\text{SO}_4^{2-}$; $\text{NO}_2^-/\text{Cl}^-$; $\text{NO}_2^-/\text{Ca}^{2+}$; $\text{NO}_2^-/\text{Mg}^{2+}$; NO_2^-/TH ; NO_2^-/COD ; $\text{NO}_2^-/\text{BOD}_5$; NO_2^-/Hg ; NO_2^-/Pb ; $\text{NH}_4^+/\text{SO}_4^{2-}$; $\text{NH}_4^+/\text{Cl}^-$; $\text{NH}_4^+/\text{Ca}^{2+}$; $\text{NH}_4^+/\text{Mg}^{2+}$; NH_4^+/TH ; NH_4^+/COD ; $\text{NH}_4^+/\text{BOD}_5$; NH_4^+/Hg ; NH_4^+/Pb ; $\text{SO}_4^{2-}/\text{Cl}^-$; $\text{SO}_4^{2-}/\text{Ca}^{2+}$; $\text{SO}_4^{2-}/\text{Mg}^{2+}$; $\text{SO}_4^{2-}/\text{TH}$; $\text{SO}_4^{2-}/\text{COD}$; $\text{SO}_4^{2-}/\text{BOD}_5$; $\text{SO}_4^{2-}/\text{Hg}$; $\text{SO}_4^{2-}/\text{Pb}$; $\text{Cl}^-/\text{Ca}^{2+}$; $\text{Cl}^-/\text{Mg}^{2+}$; Cl^-/TH ; Cl^-/COD ; Cl^-/BOD_5 ; Cl^-/Hg ; Cl^-/Cd ; Cl^-/Pb ; $\text{Ca}^{2+}/\text{Mg}^{2+}$; Ca^{2+}/TH ; $\text{Ca}^{2+}/\text{COD}$; $\text{Ca}^{2+}/\text{BOD}_5$; Ca^{2+}/Hg ; Ca^{2+}/Cd ; Ca^{2+}/Pb ; Mg^{2+}/TH ; $\text{Mg}^{2+}/\text{COD}$; $\text{Mg}^{2+}/\text{BOD}_5$; Mg^{2+}/Hg ; Mg^{2+}/Cd ; Mg^{2+}/Pb ; TH/COD ; TH/BOD_5 ; TH/Hg ; TH/Cd ; TH/Pb ; COD/BOD_5 ; COD/Hg ; COD/Pb ; BOD_5/Hg ; BOD_5/Pb ; Hg/Cd ; Hg/Pb ; Cd/Pb . On the other hand, significantly high negative correlations were found between: $\text{T}^\circ/\text{O}_2$; EC/O_2 ; O_2/NH_4^+ ; $\text{O}_2/\text{SO}_4^{2-}$; O_2/Cl^- ; $\text{O}_2/\text{Ca}^{2+}$; $\text{O}_2/\text{Mg}^{2+}$; O_2/TH ; O_2/COD ; O_2/BOD_5 ; O_2/Hg ; O_2/Pb .

PCA analysis was performed on a data table of seven individuals (stations) and nineteen variables (physicochemical and metallic parameters).

Table 2. Correlation coefficient between the different variables ($P < 0.05$).

	<i>T</i>	<i>pH</i>	<i>EC</i>	<i>O₂</i>	<i>NO₃⁻</i>	<i>NO₂⁻</i>	<i>NH₄⁺</i>	<i>SO₄²⁻</i>	<i>Cl⁻</i>	<i>SM</i>	<i>Ca²⁺</i>	<i>Mg²⁺</i>	<i>TH</i>	<i>PO₄³⁻</i>	<i>COD</i>	<i>BOD₅</i>				<i>Hg</i>	<i>Cd</i>	<i>Pb</i>
<i>T</i>	1.000																					
<i>pH</i>	0.466	1.000																				
<i>EC</i>	0.463	0.614	1.000																			
<i>O₂</i>	0.701	0.548	0.740	1.000																		
<i>NO₃⁻</i>	0.188	0.448	0.206	0.068	1.000																	
<i>NO₂⁻</i>	0.344	0.518	0.882	0.631	0.179	1.000																
<i>NH₄⁺</i>	0.463	0.462	0.793	0.776	0.002	0.943	1.000															
<i>SO₄²⁻</i>	0.611	0.561	0.916	0.934	0.018	0.846	0.895	1.000														
<i>Cl⁻</i>	0.484	0.614	0.984	0.833	0.123	0.886	0.851	0.967	1.000													
<i>SM</i>	0.222	0.137	0.020	0.286	0.440	0.249	0.142	0.098	0.079	1.000												
<i>Ca²⁺</i>	0.453	0.624	0.947	0.873	0.077	0.871	0.870	0.974	0.988	0.175	1.000											
<i>Mg²⁺</i>	0.389	0.601	0.961	0.816	0.090	0.909	0.880	0.954	0.989	0.118	0.993	1.000										
<i>TH</i>	0.406	0.608	0.959	0.832	0.087	0.897	0.875	0.960	0.991	0.138	0.997	0.999	1.000									
<i>PO₄³⁻</i>	0.427	0.695	0.319	0.049	0.423	0.324	0.194	0.156	0.230	0.425	0.160	0.178	0.172	1.000								
<i>COD</i>	0.499	0.594	0.924	0.904	0.028	0.874	0.904	0.988	0.977	0.154	0.994	0.983	0.987	0.141	1.000							
<i>BOD₅</i>															0.999							
	0.510	0.595	0.931	0.903	0.050	0.878	0.904	0.990	0.980	0.134	0.994	0.984	0.987	0.149	6	1.000						
																				1.00		
<i>Hg</i>	0.410	0.608	0.927	0.858	0.033	0.895	0.903	0.965	0.975	0.167	0.994	0.993	0.994	0.152	0.993	0.992			0			
																			0.72	1.00		
<i>Cd</i>	0.144	0.685	0.839	0.434	0.128	0.666	0.490	0.632	0.786	0.169	0.743	0.774	0.769	0.484	0.683	0.683			8	0		
																			0.97	0.70	1.00	
<i>Pb</i>	0.501	0.541	0.933	0.905	0.020	0.819	0.843	0.984	0.979	0.210	0.987	0.972	0.978	0.088	0.987	0.987			4	4	0	

The eigenvalue analysis is used to choose the number of components, and therefore the number of factors/axes to be considered in the analysis.

The obtained results (**Table 3**) showed that the cumulative variance of the first two components (F1 and F2) was 82.674% (70.58% for axis 1 and 12.094% for axis 2), which is sufficient to describe the dependent variable. In addition, the slope of the first two components was greatest, as shown in **Figure 4**. Therefore, this study nominated F1 and F2 as the principal components that reflect 82.674% of the total information. The individuals and the variables are thus projected on the F1-F2 factorial plane. The component diagram of the two principal components is shown in **Figure 5**. The results show that O_2 , pH, T, EC, NO_2^- , NH_4^+ , SO_4^{2-} , Cl^- , Ca^{2+} , Mg^{2+} , TH, COD, BOD_5 , Hg, Cd, and Pb have major roles in the first principal component F1, of which they are positively correlated with the axis defined by the first principal component, except O_2 and pH which are negatively correlated with this axis. O_2 is systematically opposed with COD, BOD_5 , and T at axis F1. This indicates that O_2 decreases when COD, BOD_5 and T increase. This axis then defines a gradient of organic pollution and mineralization from left to right of the axis F1 and a water oxygenation gradient opposing COD, BOD_5 , NO_2^- , NH_4^+ , SO_4^{2-} ... from right to left of this axis. On the other hand, NO_3^- , SM and PO_4^{3-} are major contributors to the second principal component F2, of which NO_3^- and SM are positively correlated with the axis F2, while PO_4^{3-} is negatively correlated with this axis. Therefore, the latter (F2) opposes the waters loaded with NO_3^- and SM to the waters rich with PO_4^{3-} .

Table 3. Eigenvalues, variance, and cumulative inertia of the first two principal components.

	F1	F2
Eigenvalues	13.410	2.298
Variance (%)	70.58	12.094
Cumulative (%)	70.58	82.674

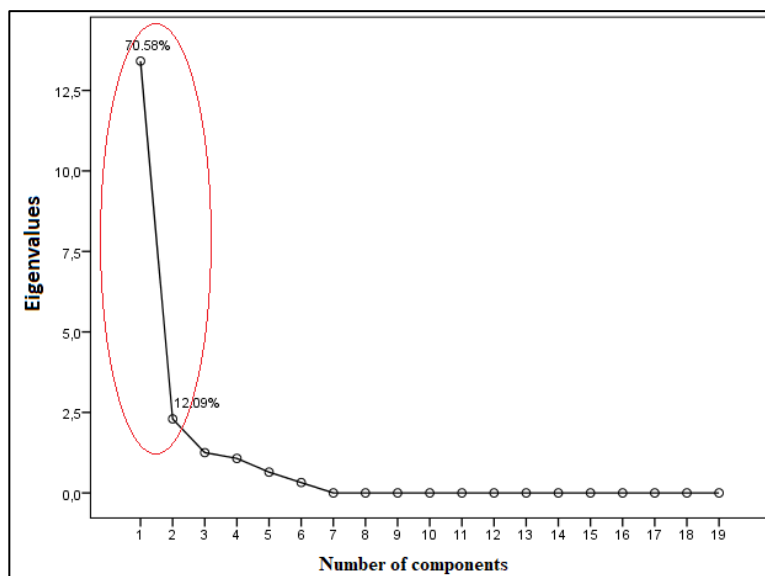


Figure 4. Determination of principal components.

The red circle displays the two components selected as the principal components.

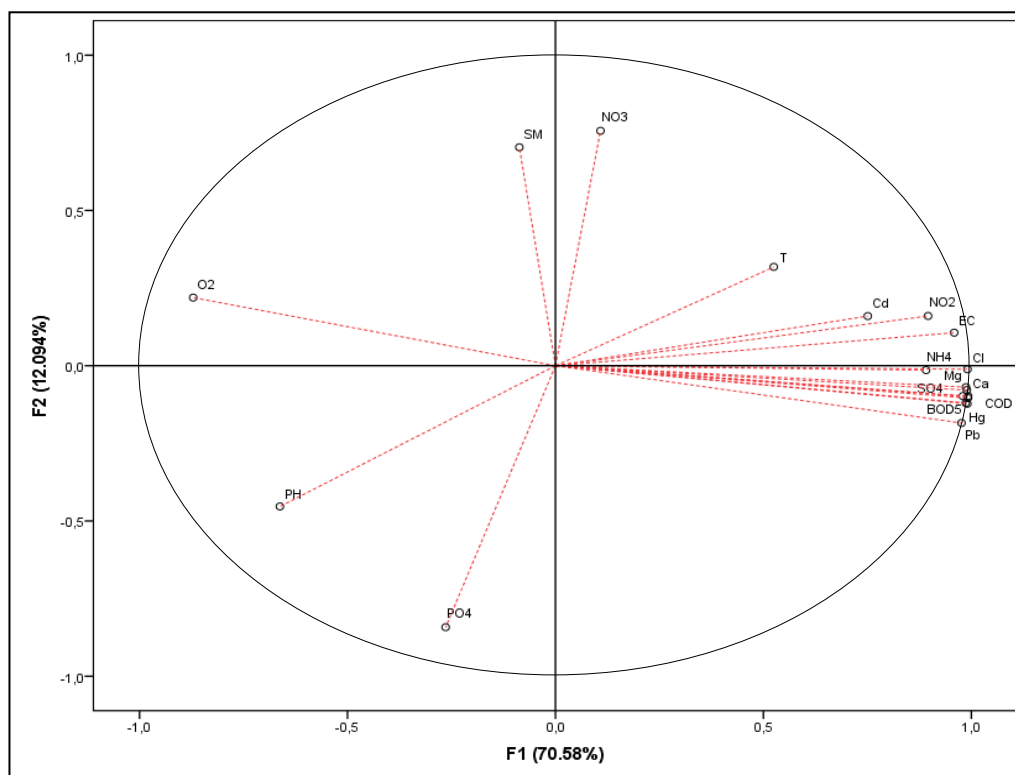


Figure 5. Representation of the variables on the F1 and F2 factorial plan.

The analysis of the projection of individuals on the F1-F2 factorial plane allowed us to define a typology dominated by the individualization of four groups of stations with different water pollution patterns (**Figure 6**):

Group 1: consisting exclusively of station S1 (located upstream of the study area: at the level of Mechraâ-Hammadi Dam), which is negatively correlated with the F2 component. The waters of this group are more loaded with PO_4^{3-} and characterized by a very alkaline pH. The levels of the other parameters remain low. These high PO_4^{3-} values could be attributed to runoff and leaching of fertilized soil of the surroundings areas of Mechraâ-Hammadi Dam (Mahjoub *et al.*, 2021a).

Group 2: it is a group composed of the waters of the stations S3, S4, and S7, which is negatively correlated with the F1 component. The waters of this group are highly oxygenated, slightly mineralized, and less loaded with organic matter and trace metals.

Group 3: this group is made up of S5 and S6 stations positively correlated with the F2 component, and corresponds to the waters of the river that are the most loaded with NO_3^- and SM, and denoting well-oxygenated surface waters. This group of station is the most threatened by pollution related to agricultural activities from Triffa plain.

Group 4: the latter group is represented by a single station S2, which is positively correlated with the F1 component. This group corresponds to the river waters that are the more mineralized and the most loaded with organic matter and trace metals. It determines a very low quality water. S2 station on the Oued Sebra submitted periodically to the industrial discharges of the Sucrafor sugar refinery in Zaio and permanently to the urban discharges from the Zaio city (Mahjoub *et al.*, 2020), the situation is very critical.

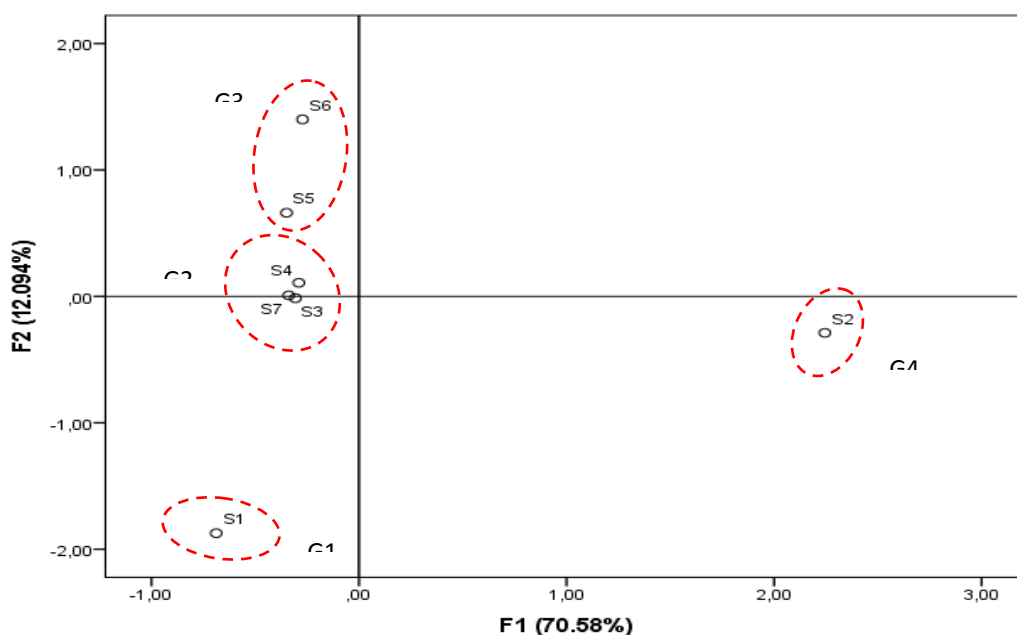


Figure 6. Representation of the sampling stations on the factorial level F1 and F2.

Conclusion

This study focused on the determination of the global quality of the surface water of the Moulouya River and the possible influence of physicochemical and metallic variables on the fish fauna of this river. The results of the present study show that the water of this river is characterized by significant

mineralization, as shown by the high values of electrical conductivity, chlorides, sulfates, calcium and magnesium at all the stations of the Moulouya River. This mineralization is not only a natural phenomenon resulting only from the geological context of the region but it also results from diffuse agricultural pollution (by runoff and leaching) and domestic and industrial discharges. In addition, the results reveal significant organic pollution confirmed by the high values of COD and BOD₅. This pollution could be accentuated by the runoff of salts concentrated on the surface of agricultural soils treated with fertilizers as well as the contributions of industrial and domestic discharges from the units and dwellings that are installed along both banks of the river. In addition, the results of Pb, Cd and Hg, confirm the importance of the dams in the decrease of the capacity of the river to evacuate these trace metals towards the lower Moulouya. The results of this study indicate a worrying situation of the water quality of the Moulouya River and its tributary Sebra, which could have adverse effects on the river fish and also on the health of the surrounding populations.

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Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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