The aquatic ecosystems are characterized by a high biological productivity, a rich biodiversity and a multitude of ecological processes (Higler, 2009). The benthic macroinvertebrates represent an important part of the river biocenosis (Tenkanano, 2017), in the last few years, the knowledge on their occurrence in Moroccan water ecosystems has been a major concern for naturalists, scientists and development officials (Amrani et al., 2006). Benthic macro organisms are widely used today as bioindicators for their ability to reflect different disturbances in aquatic ecosystems. Adandedjan et al. (2013); Lenat & Resh (2001); Bass (2003) and Lencioni & Rossaro (2005) have shown that there are species that are considered excellent tools for the assessment of freshwater pollution and are a good indicator of environmental changes.

Inaouene wadi is one of the main watercourses in Morocco; it plays a very important role in the irrigation of hundreds of agricultural lands, the regulation of the climate, the maintenance of the habitats of benthic macro invertebrates and the local economic development. It is evident that the pollution of these rivers leads to environmental degradation (Smith et al., 2006; Wu et al., 2010), degradation of biodiversity (Dudgeon et al., 2006), as well as a decline in ecosystem services (Ripl and Wolter, 2005).

The composition of faunal communities depends on the interaction of physical and biological factors. Different insect assemblages in a stream are profoundly influenced by noble metals and physical factors such as current velocity, dissolved oxygen, temperature, and water conductivity (Bilton et al., 2001; Zhang et al., 2014; Free et al., 2009; Cai et al., 2017a) and biological factors.
such as plantlets, macrophytes, and periphytons (Cai et al., 2012b; Yan et al., 2014)

The ecosystems and waterways are severely impacted and damaged by economic development and anthropization (Armitage, 1995). All those activities affect the water quality, nutrient levels, and the macroinvertebrate community. The critical factors that influence the spatiotemporal distribution of organisms are an interesting topic in ecological studies (Garrido et al., 1994).

This study aimed to evaluate the level of pollution of aquatic ecosystems and to identify the environmental factors that influence the diversity of macrofauna in the different stations studied along the Inaouene wadi.

**MATERIAL AND METHOD**

**Description of the study area and studied rivers**

The watershed of Inaouène wadi is a sub-basin of the great basin of Sebou, it covers the area of 3320 km². The Inaouene wadi takes its source at Bab Merzoka (Taza) to the downstream where it meets Sebou at the rural town of Al Ouadayne (Molay Yaacoub), it is the second main tributary of the Sebou wadi after Ouargha wadi (Fig. 1).

In order to carry out this study, six stations were explored along the wadi, taking into consideration different criteria such as the source of agricultural and industrial pollution, direction of flow, accessibility etc. (Fig. 1, Table 1). The coordinates were recorded using a 72-hour handheld GPS counter (GARMIN, China).

**Water sampling and analysis**

**Environmental characteristics**

Stream substrates, shown in Table 1, were classified according to their diameter based on the classification of Tacher et al., (2010) fine sand (diameter <2 mm), coarse sand (2–25 mm), stones (25–250 mm) and rocks (> 250 mm), the percentage of substrate cover was assessed visually (Table 1) (Touron-Poncet et al., 2014). The average flow velocity was measured using the formula $V=d/t$ with $V$: average velocity in m/s, $d$: distance between 2 points in meters and $t$: time per second. The current velocities were classified according to the Berg (1948) scale: very slow ($v<10$ cm/s), slow ($10<v<25$ cm/s), moderate ($25<v<50$ cm/s), fast ($v>50$ cm/s) (Table 2).

**Physicochemical characteristics**

The physicochemical analysis of the water was performed on the same day as the insect sampling (AFNOR, 2005): Temperature (T°C), Hydrogen Potential (pH), Electrical Conductivity...
Dissolved Oxygen (DO), measured in situ using a multi-parameter (Consort multi-parameter analyzer). Sulfates (SO$_4^{2-}$), Ortho-phosphates (PO$_4^{3-}$) and Biological Oxygen Demand (BOD$_5$) were determined according to Rodier’s analysis protocol (Rodier 1996). The total hardness (F°) of the water was determined by volumetric titration with EDTA 0.2N, whereas Nitrates (NO$_3^-$) and Ammonium (NH$_4^+$) were determined by spectrophotometry (Table 2). The determination of trace metal elements (Mg, Co, Cu, Fe, Ni, Pb, Cr, and Zn) was performed using Inductively Coupled Plasma Emission Spectrometry (ICP-AES) at the CNRST laboratory in Rabat (Table 3).

Benthos sampling

For the faunistic study, the sampling of the invertebrate macrofauna consists of collecting the greatest faunal diversity representative of the habitats to be studied in order to obtain a most complete assessment of the taxa present in the rivers. Sampling of the benthos is carried out between March and May 2019, the main tool used for sampling the fauna is a “Surber” type net with a mesh opening (500 µm). The samplings carried out thus correspond to the otic facies according to the definition of Tuffery and Vernaux (1967). Each sample is made up of 8 elementary samples following a transect from one bank to the other (except for stations that are too deep).

At each station, sampling was distributed in proportion to the relative extent of the different habitat types, in order to obtain the largest possible number of taxa. Each habitat is characterized by its substrate type and current velocity (Table 1 and 2).

The samples in labeled jars were fixed with a formalin solution of final concentration of 10%. In the laboratory, the samples were rinsed on a 500µm mesh sieve, in order to eliminate as much remaining fine substrate and coarse elements as possible. The identification of the fauna is done under a binocular loupe. The organisms are manipulated with fine tweezers and mounted needles. Determination keys were used to recognize the individuals (Tachet et al., 2006)

### Biological metrics

For each benthic insect family, the abundance-frequency ratio (AF %) was calculated as a percentage of the number of individuals in a considered family to the total number of all individuals.

$$ F = \frac{n}{N} \times 100 $$

(1)

For each benthic insect community, the total taxonomic richness (S) was calculated

$$ D = \frac{\sum n (n-1)}{N(N-1)} $$

(2)

The Shannon-Weaver diversity index $H'$, with $n_i$: the number of taxa i and $N$: the total number:

$$ H' = -\sum (n_i/N) \cdot \ln(n_i/N) $$

(3)

Pielou’s regularity index $J'$, with $H_{max} = \log_2 S$; Magurran 2004):

$$ J' = H'/H_{max} $$

(4)
Family Level Biotic Index (FBI):

\[ FBI = \sum_{i=1}^{F} \frac{n_i t_i}{N} \]  

(5)

FBI and the percentage of tolerant individuals (PTO-ind) were evaluated to describe the diversity and characterize the tolerance status of benthic macroinvertebrate assemblages in different sampling sites as well as the presence of organic pollution.

Statistical analysis

Statistical analyses were performed on the data for both single-variable (correlation tests) and multivariate (similarity tests and principal component analysis) to determine the different correlations between these parameters.

RESULTS

Environmental variables

The variations of the environmental variables measured in the different stations and the different habitat groups are shown in Table 2. The temperature does not show great variations from one station to another with the values varying between 23.1°C and 27.4°C, The pH is relatively basic in all stations varies between 7.8 and 8.5, for The values of water conductivity decreases significantly from upstream to downstream with a maximum value of 2894μs/cm recorded at S2 and a minimum of 214.3 μs/cm recorded at S5, The oxygen content varies from one station to another with a maximum value of 7.08 mg/l recorded at S2 and a minimum value of 2.23 mg/l recorded at S3, BOD₅ also varies from station to station with a maximum value of 520.9 mg O₂/l recorded at S1 and a minimum value of 2.9 mg O₂/l recorded at S6, the sulfate content decreases from upstream to downstream with values varying between 910 and 57, for PO₄³⁻ maximum values of 7.8 mg/l and 6.45 mg/l were obtained in S1 and S3, respectively, and minimum values of 1.02 mg/l, 4.89 mg/l, 1.68 mg/l and 0.6 mg/l were recorded in S2, S4, S5 and S6 respectively. The maximum values of nitrate NO₃⁻ reaching 22.7 mg/l, 21 mg/l and 21.21 mg/l were recorded in S1, S3 and S4 respectively, whereas minimum values of 7.6 mg/l, 7.04 mg/l and 0.55 mg/l recorded in S2, S5 and S6 and for ammonia nitrogen the values vary between a minimum of 0.08 and a maximum of 0.75. As for the speed of the river, it is fast in all the stations.

In this study, the existence of five essential trace elements (Ag, Fe, Pb, Cu, and Zn) was investigated. Minerals are essential elements for aquatic living beings, but if the organism excessively takes these elements from the external environment, they will damage their health. (Mutlu and Kurnaz, 2017), Pb, as an example, is dangerous for different life forms due to its toxicity (Fattori et al., 2002).

The concentrations of heavy metals at the different stations are shown in Table 3. The heavy metal concentrations vary spatially in a significant way from one station to another with high concentrations upstream and gradually decreasing towards the downstream.

Community structure and diversity of insects

With a total of 4140 individuals distributed on 10 orders and 39 families, Ephemeroptera was the most diverse group with 8 families. Diptera

Table 2. Physicochemical characteristics of the water at the study sites

| Station | T (°C) | V (cm/s) | pH | CE (cm/s) | DBOS (mg O₂/l) | Dissolved O₂ (mg/l) | Sulphates (mg/l) | PO₄³⁻ (mg/l) | Nitrates NO₃⁻ (mg/l) | Ammonical nitrogen |
|---------|-------|--------|----|----------|----------------|---------------------|-----------------|-------------|---------------------|------------------|
| S1      | 23.1±0.1* | fast  | 8.2±0.1* | 2000±24* | 520.9±9.95* | 3.02±0.09* | 910±11.37* | 7.8±0.06* | 22.7±0.25* | 0.79±0.1* |
| S2      | 26.9±0.1** | moderate | 8.1±0.1* | 2894±19* | 17.38±4.92* | 5±0.09* | 488±10.45* | 1.02±0.03* | 7.6±0.09* | 0.18±0.07* |
| S3      | 26±0.2* | fast  | 8.05±0.1* | 2099±19* | 516.7±22.2* | 2.23±0.06* | 675±10.01* | 6.45±0.08* | 21±0.43* | 0.65±0.04* |
| S4      | 26.3±0.1** | fast  | 7.9±0.04* | 1859±22* | 447.27±31.2* | 3.02±0.08* | 580.6±14.2* | 4.89±0.06* | 21±0.24* | 0.75±0.1* |
| S5      | 27.4±0.9** | fast  | 8.1±0.03* | 214.3±11.5* | 59.1±1.99* | 5.01±0.09* | 301±11* | 1.68±0.04* | 7.04±0.03* | 0.1±0.04* |
| S6      | 29.5±0.9** | fast  | 7.9±0.01* | 1308±11* | 289±14.64* | 7.40±0.05* | 689±10.75* | 2.5±0.05* | 15.23±0.12* | 0.71±0.1* |
dominate, representing 33.21% of the total abundance followed by Ephemeroptera 11.18%. Trichoptera and Plecoptera represented 2.51% and 2.15% of the total abundance respectively, Coleoptera, Trombidiformes, Hemiptera, Nematelminthes, Megaptra and Odonata were less abundant with 0.27%, 0.024%, 0.15%, 0.19%, 0.17% and 0.15% of the total abundance, respectively. The most abundant families were: Simuliiidae (16.79%), Chaoboridae (10.46%), Baetidae (7.59%) (Table 4). In terms of number of families, station S2 is the least diverse while station S5 is the most diverse.

Biological metrics

Five biological metrics were measured for each station: total taxon richness (D), Shannon-Weaver diversity index (H'), Pielou evenness index (J'), family level biotic index (FBI) and percentage of tolerant individuals PTO-ind. D, J' and H' were generally used to indicate community diversity. FBI and PTO-ind were used to reflect environmental pollution. These parameters varied among habitat types and sampling sites (Fig. 2).

Multivariate analysis of the studied parameters

The Principal Component Analysis (PCA) showed that environmental variables and heavy metals have some influence on the distribution of the 39 families of invertebrate macrofauna. The physico-chemical parameters, such as dissolved oxygen, BOD5, SO42-, PO43-, NH4+ EC, pH and T° as well as the following heavy metals: Pb, Fe, Ag, Zn and Cu were selected as the main parameters affecting the community structure of benthic macroinvertebrates.

The ordination of this PCA in the factorial plane 1–2 (33.5%) allows a clear division of the communities into three major groups (Fig. 3 and 4). Group 1 is composed of nine taxa (Hydropsychidae, Philopotamidae, Helicopsideidae, Polycentropodidae, Brachycentridae, Ameletidae, Ephemerellidae, Leptophelebiidae and Caenidae) that are favored by high dissolved oxygen and temperature levels and mainly identified in the downstream station (S6). Group 2 consists of 9 taxa that develop in the environments characterized by high levels of BOD5, electrical conductivity, pH, NO3–, SO42–, PO43– and NH4+ and the presence of high concentrations of heavy metals (Pb, Ag, Cu, Zn and Fe). These are mainly the taxa: Veliidae, Noteridae, Metretopoidea, Elmidae, Hydrophilidae, Syrphidae, Cera- topogonidae, Baetidae, Chironomidae and Taniopterygidae. These taxa develop especially in the upstream stations (S1, S3 and S4). Group 3 consists of nine taxa (Leuctridae, Odontoceridae, Nemouridae, Capniidae, Dytiscidae, Simuliidae, Hydrachnidae, Nematodes and Pteranarcydae) from station S5 in a rural area, characterized by low levels of BOD5, NO3–, SO42–, PO43– and NH4+ and acceptable values of heavy metals.

DISCUSSION

The degradation of aquatic ecosystems in Morocco presents a major challenge for environmental protection. Development activities, such as urbanization, the development of medium and large-scale industries, and the intensification of agricultural practices near streams and rivers are causing an alteration of water quality and affect the overall health of aquatic ecosystems (Karrouch et al., 2018; Akalu 2011; Beneberu et al., 2014). In the study area, there are several urban, industrial, artisanal and agricultural activities. However, most of these activities do not have any effluent treatment system and discharge their waste directly in the tributaries of the Inaouene wadi (El Haji et al., 2012). Several works conducted in Morocco using physicochemical and

| Station | Ag       | Fe       | Pb       | Zn       | Cu       |
|---------|----------|----------|----------|----------|----------|
| S1      | 0.092±0.007* | 0.72±0.07* | 0.09±0.02* | 1.88±0.038* | 0.012±0.002* |
| S2      | 0.049±0.003* | 0.05±0.001* | 0.049±0.003* | 0.005±0.001* | 0.013±0.025* |
| S3      | 0.06±0.04*  | 0.49±0.015* | 0.045±0.045* | 1.13±0.03*  | 0.01±0.001*  |
| S4      | 0.093±0.015* | 0.42±0.04*  | 0.08±0.004*  | 1.08±0.05*  | 0.011±0.001* |
| S5      | 0.049±0.005b | 0.025±0.004* | 0.05±0.001*  | 0.005±0.001* | 0.011±0.002* |
| S6      | 0.05±0.004a  | 0.24±0.03*  | 0.05±0.002*  | 0.98±0.11*  | 0.012±0.002* |

* denotes significant differences at p < 0.05.
biological methods have shown that there is a serious pollution of rivers and streams, which are the likely causes of diseases in both animals and humans (Zalaghi et al., 2018). In streams in these regions, the dry period leads to the changes in abiotic conditions (parameters) that control the structure of biological communities (e.g., low oxygen, high temperatures, increased deposition of
The diversity of macrofauna observed in this region was significantly less diverse compared with other rivers, including those of the Sebou wadi (Fekhaoui et al., 1993), Boufekrane wadi (Chahlaoui et al., 1996; Aboulkacem et al., 2007; Karrouch, 2010), Boureg Reg wadi (EL Agbani, 1984) and Ouislane (Karrouch, 2010; Aboulkacem, 2007). This could be related to natural and/or anthropic factors influencing this watercourse. Indeed, the discharge of wastewater from the city of Taza through the wadi l’arbaa, as well as domestic discharges from other neighboring villages in the Inaouene wadi could, without doubt, considerably contribute to the distribution and diversity of this watercourse. In the same sense, the low taxonomic richness and abundance also found in different arid and semi-arid bioclimatic regions such as the one considered in this study (Bebba et al., 2015; Sellam et al., 2017) can be explained by unstable environmental conditions, including frequent lack of flow, insufficient running water levels, and high summer temperatures (> 35°C), leading to prolonged dry periods, with a negative impact on biodiversity that tends to be low (Arab et al., 2004). In terms of abundance, insect assemblages were dominated by Diptera, Ephemeroptera and Trichoptera. Diptera were much more...
dominant taxa, as was the case in other arid Mediterranean rivers in North Africa (Sellam et al., 2017). Simuliidae was the most abundant family found in station S3 and S5 with larvae adhering to stable substrates such as stones and sands (Tachet et al., 2010) and their tolerance to moderate levels of pollution (Augusto & Marcos, 2010).

Station S6, which is located in the downstream of Inaouene, is characterized by a high biodiversity compared to the other stations, while

Fig. 3. PCA graphical representation showing the distribution of macro invertebrates according to physicochemical parameters and noble metals. Information: Brach= Brachycentridae, Polyc= Polycentropodidae, Helic= Helicopsychidae, Philo= Philopotamidae, Caeni= Caenidae, Ameie= Ameletidae, Lepto= Leptophlebiidae, Ephem= Ephemereellidae, Hydro= Hydropsychidae, Veli= Veliidae, Noter= Noteridae, Metre= Metretopodidae, Hydrop= Hydrophilidae, Chiro= Chironomidae, Syrph= Syrphydae, Cerat= Ceratopogonidae, Baeti= Baetidae, Taeni= Taenipterygidae, Leuct= Leuctridae, Odont= Odontoceridae, Nemou= Nemouridae, Capni= Capniidae, Dytip= Dytiscidae, Hydra= Hydrachnidae, Nemat= Nematodes, simul= Simuliidae, ptero= Pteronarcydae.

Fig. 4. PCA graphical representation showing the distribution of the study areas.
the fauna of S2 is less diverse. This difference in diversity between sites is probably due to physicochemical parameters, anthropic disturbances of the aquatic ecosystem, some sites having been exposed to domestic pollution. Similarly, Azrina et al. (2006) and Cereghino et al. (2002) noted that taxonomic richness is sensitive to human impact on aquatic ecosystems, particularly aquatic insects, which are good indicators of stream environmental conditions and are therefore very sensitive to pollution.

In the conducted research, six structural metrics were used to describe the tolerance level and diversity of macroinvertebrate assemblages at the study stations. The results showed that the values of these metrics varied from one station to another depending on the variation of physicochemical factors.

The taxonomic richness (D), abandonment (N), and Shannon-Wiener index (H') indicate that the polluted sites tend to have lower diversity and abundance than the unpolluted sites; the family-level biotic index (FBI) and the percentage of tolerant individuals (PTO-ind) indicate that benthic communities at the polluted sites tend to be more tolerant than those at the unpolluted sites. For the different stations, macroinvertebrate diversity and abundance showed significant correlations between the physicochemical and biological parameters. These results show that human activities can have remarkable effects on the characteristics of the benthic community by modifying the abiotic parameters of the habitat. If humans severely disrupt the living conditions of benthic communities, they become more monotonous and more tolerant to pollution. Many studies have highlighted the ecological changes that accompany environmental pollution in large, shallow lakes (Cai et al., 2017; Rodusky et al., 2008).

CONCLUSIONS

The Inaouene wadi is a regime characterized by irregularity of flows and by brutal hydrological events. The annual regime is marked by a weak flow in summer and autumn and by a strong flow in winter and spring. The fauna inventory carried out in the present study constitutes an important first data base. The fauna studied is characterized by a variable taxonomic diversity according to the degree of water pollution.

The fauna recorded in this work is composed of 2029 individuals corresponding to 39 families belonging to 10 orders of faunal groups (Annelids, arthropods). The number of benthic populations showed that Dipteres, Tricopteres, Plecopteres, Ephéméroptreres, are dominant. Odonates, mégaptère, Nemathelmintes, Trombidiformes, Hemipteres and Coleopteres constitute only a small fraction of the collected fauna.

The biological indices (Shannon and Equitability ...,) used for the characterization of the Inaouene wadi waters, better discriminate the study stations according to their pollution level. The results obtained are in good agreement with the results obtained through the study of physicochemical characteristics and heavy metals. The biological diversity of macrofauna in the wadi depends on the physicochemical parameters at each station; it is strongly influenced by anthropogenic activities.

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