

ORIGINAL ARTICLE

# Diatom Diversity and Ecology of the Sebou Watershed (Morocco)

Hafida Jaghror<sup>1</sup>, Karen Serieyssol<sup>2</sup>, Aude Beauger<sup>3</sup>, Khadija Loukili<sup>4</sup>, Mohamed Fadli<sup>5</sup>\*

<sup>1</sup>Laboratoire de Botanique et de Protection des Plantes, Département de Biologie, Faculté des Science, Université Ibn Tofail ; BP. 133, Kenitra, Morocco.

<sup>2</sup>EVS-ISTHME UMR CNRS 5600, Université Jean Monnet, 6 rue Basse des Rives, 42023 St-Etienne cedex 2, France.

<sup>3</sup>Clermont Université, Université Blaise Pascal, GEOLAB, BP 10448, 63000 Clermont-Ferrand, France. <sup>4</sup>CNRS, UMR 6042, GEOLAB, 63057 Clermont-Ferrand, France.

<sup>5</sup>Laboratory of Nutrition, Health and Environment, Faculty of Sciences, University Ibn Tfail. BP. 133, Kenitra, Morocco.

Email: fadli\_fadli@hotmail.fr

## ABSTRACT

This study investigates biodiversity and structure of spring diatoms associations from Sebou Basin, one of Morocco's largest superficial hydrosystems. The physico-chemical results reflect both anthropogenic and natural pollution, mainly from mineral sources. 226 taxa were identified, 92% are pennates. The result of Shannon and equitability indices showed that diatom populations were generally diversified, which depends on degree of specific richness at each station. Thus, the higher number of taxa in site Og (120 species) reflects an ecologically more stable and more diversified ecosystem dominated by *E. minuta*, opposed to S<sub>1</sub> (Sebou) and B<sub>2</sub> (Beht), which have an unbalanced community represented by smaller numbers of taxa (48 in S<sub>1</sub> and 52 in B<sub>2</sub>) strongly dominated by *N. capitellata* and *N. palea*. The Canonical correspondence analysis distinguished three groups: group 1 with S0, B1, Og and In located far from pollution sources, group 2 composed of polluted sites (S1 and B2), while group 3 with of S2, S3, and S6 are located in the lower section of the Sebou Basin. Multi-response permutation procedures confirmed the three site groupings. It was found that the water quality located downstream from the urban rejections is degraded, indicator species analyses showed a succession of changes in diatom species from upstream sites to downstream. Six species were determined as indicator species; Groups 1 and 2 only had one species each, *G. olivaceoides* (86%) and *H. amphioxys* (87%), respectively, while group 3 had four species, *N. recta* (93%), *N. sigma* (95%), *N. tripunctata* (96%) and *D. oblongella* (100%).

**Keywords:** watershed of Sebou (Morocco); diatoms; multi-response permutation procedures (MRPP); indicator species analysis (IS); Canonical Correspondence Analysis (CCA).

# INTRODUCTION

**D**iatoms colonize a wide variety of habitats and are an important part of the biomass in most aquatic ecosystems. They have long been used as the best biological indicators of Europeans waters quality (Prygiel and Coste 1999; Yallop and Kelly 2006; Rimet 2012). Thus, precise knowledge of ecological requirements of certain taxa helps the development of simple indicators systems that characterize and quantify water pollution. in contrast, morocco's diatoms were many studied, but among aquatic phytoplankton and/or in limited areas (Fqih Berrada, Berrada, and Benzekri 1999; El Ouahabi et al 2007; Siddour et al 2007, Loumrhari et al 2009; Gallouli et al 2014). Moreover, morocco's diatoms ecological studies refer to European ecology, while their exact range of ecological conditions of where they occur in Morocco are still unclear. The most important works performed in Morocco are those made by Fekhaoui, Hamada, and Dakki (1988) on Sebou River; on Oued Tensift and its tributaries (Maiffi 1988; Cazaubon and Badri 1994): and on wadis Hassar and Mellah rivers (Fawzi 2002).

#### How to Site This Article:

Hafida Jaghror, Karen Serieyssol, Aude Beauger, Khadija Loukili, Mohamed Fadli (2017). Diatom Diversity and Ecology of the Sebou Watershed (Morocco). *Biolife*. 5(4), pp 564-577. doi: 10.5281/zenodo.7393291 *Received:* 5 October 2017;Accepted; 26 November2017; Available online : 5 December 2017 Figure 1: A. Geographical situation of the Sebou River watershed of (MARA 1970). B. Map showing the location of the study sites



However, no studies have been published for the diatoms found in Sebou Basin that encompasses one of largest superficial waters networks and that suffers sustainable socio-economic development, leading to continue exposure to various pollution (agricultural, industrial and urban) and prejudicing on aquatic life. The objects of this study are:

- Analyze of diatoms and physico-chemical content from this great Moroccan river and its most important tributaries (Innaouéne, Ouergha and Beht).

- Then estimate of ecological preferences of abundant diatoms in order to enrich the bases of Moroccan given.

## **MATERIALS AND METHODS**

#### **Study sites:**

The Sebou Basin (Figure 1A) occup an area of about 40,000 km<sup>2</sup>, it is located between 33° and 35° North, and 4° and 7° West. Sebou River, is a principal water course, originates in the Middle Atlas and flows mainly across Lias carbonated formations (Fedan 1988; Charriérer 1990; Laadila 1996; Dridri and Fedan 2001a, 2001b). Rare Paleozoic buttonholes and Quaternary basalts

outcrops interrupt the area's monotony. The Gharb plain contains thick fluvio-marine beds and sandy-clay alluviums deposited during the Quaternary (Rif, Middle Atlas and Prerif) (Azzaoui 1999).

Otherwise, roughly a fifth of Morocco's total population, 22% of the national rural population and 17% of the urban population live in the Sebou Basin. Similarly, it is important region of the country for its large agricultural surface area, and a diverse industrial complex: oil, sugar and paper mills, tanneries, textiles, oil refining, etc.

In addition, the Sebou Basin contains nearly a third of the nation's surface water ( $\approx$ 33%) and 20% for ground water (ABHS-M 2006).

The studied stations were selected taking into account the:

- Relationship to pollution sources: samples collected upstream and downstream of discharges and then some stations were considered reference stations because of the location far from pollution discharges.

- Ease of access and sampling. A set of 11 stations, located according the longitudinal gradient of the river course (from S0 to S6) (see Figure-1).

- S0: Sebou River, about 20 km upstream from the Fez city. No notable anthropologic activity (troughs or washing).
- **S1:** Sebou River, to 1.5 km downstream of the Oued Sebou confluence with Oued Fes. Downstream from the city of Fez.
- **In:** Innaouéne river (tributary of Wadi Sebou), about 5 km downstream of Idriss 1st water-dam. This is a reference station.
- **S2:** Sebou River, about 8 km upstream from the town of Kariat Ba Mohammed.
- **Og:** Ouergha River (great Oued Sebou tributary), to 10 km upstream of Khnichat and about 20 km downstream of the dam El Wahda. This is a reference station.
- **S3:** Sebou River, to 3 km downstream from the city Mchraa Bel Ksiri.
- **S4:** Sebou river; just downstream of the town of Sidi Allal Tazi.
- **S5:** Sebou River, just downstream retained of the dam custody and influenced by the tides.
- **B1:** Beht River (largest tributary of Wadi Sebu), to 10 km upstream of the releases of the town of Sidi Slimane. No of the anthropologic activities except washing and watering by villagers.
- **B2:** Beht River, located just downstream of discharges of domestic wastewater.
- S6: Sebou River estuary, upstream of the mouth and downstream of the domistic and industrial wastewater of city of Kenitra.

#### Physical and chemical analysis:

Five parameters were measured *in situ*: water temperature, pH and conductivity with a WTW apparatus while salinity and dissolved oxygen were measured with a salininometer and oximeter, reference 320. The 21 water samples were collected in polyethylene bottles and stored in an icebox (4°C) until arrival at the laboratory.

Fourteen physic-chemical parameters were measured and dosed. Chemical oxygen demand (COD) was analyzed immediately (within 24 hours of sampling), while biochemical oxygen. demand (BOD<sub>5</sub>) was analyzed following AFNOR standards. The other parameters (alkalinity, nitrite, nitrate, ammonium, ortho-phosphates, sulfates, chlorides, total hardness, calcium, magnesium, sodium and potassium) were analyzed respecting their shelf life using methods prescribed by AFNOR standards or Rodier et al. (2009).

#### Diatoms sampling, treatment and determination:

Diatom samples were mainly epilithic and were collected from the different sampling stations (Table-1), according to the standard NF T90-354 (AFNOR 2000, 2007). The top of several submerged stones were scraped (approx. 200 cm<sup>2</sup>). The samples were immediately fixed with 10 ml of 10% formalin. In the laboratory, diatom samples were boiled in  $H_2O_2$  and then in HCl, before thoroughly rinsed. Permanent slides are produced by mounting a few drops of the prepared suspension with a drop of Canadian balsam. At least 400 individuals were counted at a magnification of 1000 x using oil immersion. The systematic identification of

diatoms was performed based on the taxa key found in the standard NF T90-354 AFNOR (2003, 2007); Bourrelly (1968); Compére (2000); Lange-Bertalot (2000) and DRIEE-IF (2013).

#### **Diversity and equitability Indices:**

The Shannon-Wiener diversity index (H') expressed in "bit", is amount of information provided by a sample on the population and on the species structures (Daget 1976). It is calculated using the following formula:

#### H'= -∑ (ni/ N) \* Log 2 (ni/ N)

H': species diversity.

N: total populations of species.

ni: Population size of species i.

Equitability also called relative or regularity diversity compares the stand structures. It is defined as the ratio of real diversity to the maximum diversity (H max) and is expressed as follows:

E= H' / Hmax with Hmax= Log2 (S)

Where S is the number of species forming the stand.

Remember that Shannon-Wiener index (H') varies between 0 and H' max (Barbault 1995). A diverse population should have elevated species richness and equal distribution.

#### **Statistical analysis:**

Canonical Correspondence Analysis (CCA), multiresponse permutation procedures (MRPP) and indicator species analysis (IS) were carried out to assess diatom species-environmental relationships (Borcard, Legendre, and Drapeau 1992; Bere and Tundisi 2011b; Birks 2012b; Bertrand, Serieyssol, and Ector 2015) using taxa having  $\geq 1\%$  of the community and occurring in more than one sample. The following physico-chemicals were used in the analysis: dissolved oxygen (DO), conductivity (Cond), sulfate (SO<sub>4</sub>), phosphate (PO<sub>4</sub>), nitrate (NO<sub>3</sub>), ammonium (NH<sub>4</sub>) and pH. However, conductivity data was log 10 transformed prior to analyses. The statistical methods were performed using PCORD 6 (McCune and Mefford 2006) and the following CCA options were selected: the options were columns score standardized by centering and normalizing, scaling of ordination comprise representation of species and samples, scores for graphing samples scores are linear combination of factors, randomization test on, test null hypothesis - no relationship between matrices and number of runs 999. Data from stations  $S_4$  and  $S_5$  were not exploited for their physical and hydraulic characteristics because of poor diatom colonization.

## **RESULTS AND DISCUSSION**

# Physical and chemical variables (Figure 2, 3, 4, 5 and Table 1):

The results of all physico-chemical variables measured at 11 sites are given in Table 2 and Fig 2; 3; 4; and 5. The changes within basin are generally related to nature of substrate but are also related to the urban, industrial and/or agricultural situation. Water temperature varied between 15.5°C at site B1 to 22.7°C at S6 site. The pH varied entre 7.38 at B2 to 8.9 at S4, indicating

Figure-2. Canonical correspondence analysis (CCA) performed on the diatoms collected in nine stations of the Sebou Basin related to major variables. Cond: conductivity; O2: dissolved oxygen; PO: orthophosphates; NO3-: nitrates; NH4+: ammonium; HCO3-: bicarbonate and pH.



alkaline waters. It is due mainly to calcareous marl and limestone bedrock (Fekhaoui and Pattee 1993; El Blidi Fekhaoui 2003), combined insolation and of (temperature), and increase of plant photosynthetic activity in spring (Fawzi 2002; Benabdellouahad 2006). The conductivity varied between 533 µs/cm at Og to 1402 µs/cm at S1, respectively, indicating a moderately accentuated mineralization to elevated (Rodier et al. 2009). The maximum values of the majority of parameters such as COD (113mg/L); BOD (130 mg/L); HCO<sub>3</sub> (415 mg/L); CaCO<sub>3</sub> (360 mg/L); Ca<sup>2+</sup> (132.26 mg/L); Mg<sup>2+</sup> (227.74 mg/L); K<sup>+</sup> (15.60 mg/L);  $PO_4^3$ (164.42  $\mu$ g/L); and NH<sub>4</sub><sup>+</sup> (192.12 mg/L) were observed in site S1 with minimum oxygen-content of 0.29 mg/LO<sub>2</sub>. The sites S0; In; Og; and B1 were the most oxygenated, and less rich in organic and, or mineral parameters. High nitrate values were measured in station S2 (5.67 mg/L of N), with a nitrite peak observed at station S3 (0.86 mg/L of N). However, conductivity and mineral ions analyses (CI; SO<sub>4</sub><sup>2</sup>; Na<sup>+</sup>; CaCO<sub>3</sub>; HCO<sub>3</sub>; Mg<sup>2+</sup>; Ca<sup>2+</sup>) also indicate a lithological origin.

The adjacent agricultural lands and the proximity to urban centers of some stations amplify mainly the sodium and sulfate ions, and also leads to increased chlorides. Makhoukh *et al.* (2011) and Libiad, Khabbach, and Ennabili (2012) made the same remarks in their respective studies of the Moulouya and Innaouéne hydro-systems.

The statistical study showed that the first three axes of the CCA (Fig 2) explained 59.3 % of the data (axis 1 22.8%, axis 2 18.5%, axis 3 18.0%). Three main groups were found; group 1 with S0, B1, Og and In, located far from pollution sources (reference sites). In contrast, Group 2 composed of polluted sites, S1 and B2, are located downstream from Fes and Sidi Slimane cities respectively, while group 3 with of S2, S3, and S6 are located in lower section of Sebou Basin. For group 2, samples are situated in the left half of the graph (Fig 2) indicated by high conductivity and high amounts of carbonate (HCO3), ammonium (NH4) and phosphates (PO). The upper right quadrant had group 1 samples with high dissolved oxygen values while group 3 samples are situated in the lower left quadrant with high amounts of nitrates.

The graph of the first two axis scores (Fig 3) show some of the relative changes that occur from headwater to the ocean. As you move down river starting with group 1 having low conductivity, low concentrations of HCO<sub>3</sub>, PO, NH<sub>4</sub> and NO<sub>3</sub> with high levels of dissolved oxygen to Group 2 has high conductivity and high levels concentrations of HCO<sub>3</sub>, PO and NH<sub>4</sub>, increasing levels of NO<sub>3</sub> and lower levels of O<sub>2</sub> and pH, followed by group 3 with a return to lower conductivity and concentrations of HCO<sub>3</sub>, PO and NH<sub>4</sub>, but with high concentration of NO<sub>3</sub>.

The chemical variation within the three groups (1upstream, 2–polluted sites and 3-downstream) are shown on Figure 4. Groups 1 and 3 have similar profiles with S6 showing increased salinity caused by tidal fluctuation. Within group 2, site S1 showed the greatest amount of pollution.

In Figure 5, the sites are position in the geographic distance from upstream to the sea and variation of the organic pollution and chemical variables are observed along with the different CCA groups the sites belong to; group 1 – upstream, group 2 – most polluted and group 3 – downstream.

Interestingly, when the upstream tributaries (In and Og - group 1) enter the Sebou River, there is a lowering of all of the variables. However, BOD, PO, sulphates (SO4) and chlorides (CI) increase at S1 after Oued Innaouéne (In) waters enter the river but when Oued

Figure-3. Canonical correspondence analysis (CCA) axis scores showing the changes in ecological condition occurring between groups 1, 2 and 3 or from upstream to polluted area and then to downstream zone of the Sebou Basin.



Figure-4: Variation of organic pollution and nitrogenous variables belonging to the different groups (1 – upstream, 2 – pollution and 3 – downstream). Dissolved oxygen (OD mg.L-1), chemical oxygen demand (COD mg.L-1), biological oxygen demand (BOD mg.L-1) HCO3 (mg.L-1) Ca CO3 (mg L-1) Ca (mg.L-1), Mg (mg.L-1), Cond (µs.cm-1), Cl (mg.L-1), Na (mg L-1), K (mg L-1), SO2 (mg L-1), PO (µg.L-1), NO3 (mg.L-1), NO2 (mg.L-1), and NH4 (mg.L-1).



Ourgha water (Og) enter the river, all of the variable seem to decrease from the upstream site (S2) to the downstream site (S3). Interestingly, even the relatively high BOD value had not effect on site S3. There is however an increase in Conductivity, Cl, Na, CaCO<sub>3</sub>, Calcium (Ca),  $HCO_3$  at the estuary site (S6). Chemical trends for the Oued Beht tributary could not be determined considering the large distance between the two upstream sites (B1 and B2) and the estuary site (S6).

Besides that, the maximum values of mineral and organic elements were recorded in Group 2 sites (S1 and B2) reflecting the large amounts of pollution discharged upstream from these sampling stations. This caused excessive oxygen consumption creating critical deoxygenation, accompanied by a pH drop. The reduction of organic matter accompanied by re-oxygenation of waters in group 3, show river self-purification and the dilution effect of well-oxygenate waters entering the river coming from cleaner tributaries (In; Og and B1- group 1). Behavior of nitrogen compounds (NO<sub>3</sub>, NO<sub>2</sub>, and NH<sub>4</sub>) in the basin is related to the reaction of oxidation-reductions. Indeed, the nitrate increase is attributed to nitrification reactions of ammonia nitrogen via nitrite nitrogen (Jaghror 2013). Conversely, a decrease indicates ammonification. Note that the domestic and industrial wastes from Kenitra city are less noticeable in the Sebou Estuary (S6–group 3) in comparison with Fés (S1) or Sidi-Slimane (B2). Indeed, the waters of the station S6 are subjected a continuous stirring by tides, which disperses the pollution and/or is absorbed by soil.

Figure-5. Spatial variation of organic pollution and nitrogenous variables along with the different groups (1 – upstream, 2 – pollution, and 3 – downstream) A. The Sedou River and two tributaries (O. Innaouéne and O. Ourgha), and B. the O. Beht tributary which enter the Sebou River near site S6. The sites are placed in the order as they occur along the river from upstream to downstream. Dissolved oxygen (OD mg.L-1), chemical oxygen demand (COD mg.L-1), biological oxygen demand (BOD mg.L-1), SO2 (mg.L-1), PO (µg.L-1), NO3 (mg.L-1), NO2 (mg.L-1), NH4 (mg.L-1), CI (mg.L-1), Na (mg.L-1), K (mg.L-1), HCO3 (mg.L-1), Ca CO3 (mg.L-1), Ca (mg.L-1), Mg (mg.L-1), and Cond (µs.cm-1).



### **Diatoms analysis**

# Specific richness, index of diversity and evenness (Table 2 and 3):

The spring diatom flora of Sebou Watershed is rich and diverse. 226 species belonging to 57 genera and 5 orders were identified in nine samples. The Pennatophycidae had most represented (209 species classified in 3 orders and 50 genera) while the Centrophycidae had only 17 taxa belonging to 7 genera (only 7% of the harvested diatom). Nitzschia followed by Navicula (47 and 31 species, respectively) had highest species diversity, with 36% of identified species. Gomphonema and Surirella had respectively 17 and 11 species; the other 53 genera had fewer species with 22 having only one. The most common species throughout the Sebou Basin were: Nitzschia palea (Kütz.)W.Sm., Nitzschia frustulum (Kütz.) Grunow var. frustulum and Nitzschia dissipata (Kütz.) Grunow var. dissipata were observed in all sites. The population with highest diatom richness was found at site Og with 120 species (Table3 and 4), that presents 52.4% of the diatom community collected in the Sebou Basin. To the contrary, stations S1 (Oued Sebou) and B2 (Oued Beht) had the lowest number of species (48 and 52 respectively).

Twenty-eight species had a relative abundance  $\geq 5\%$ (table 3) while 92 species had a relative abundance of 1% in at least one sample, indicating that the environment was diverse. The results of Shannon-Wiener index (H') and equitability (E) have same species richness trends for all the stations without showing a high analogy between stations S3 and B1, and do not exhibit significant spatial differences (with the exception of S1 and B2), indicating that the environment is generally diversified. The diversity degree depends on the species richness of each station. The obtained values of diversity ranged between 2.861 for S1 and 5.732 for Og (Table 4), the evenness varied from 0.512 at S1 to 0.840 at S3 and B1. Thus, the highest richness in Og station reflects an ecologically stable and diverse aquatic ecosystem including 73 taxa having lower relative abundance than 1%. At S0 and B1, H' is close to H' max with an diatom community less diverse and less stable than Og. This difference is most likely related to the water depth and high current velocity, as all these stations are located away from human disturbances. Indeed, according to our field observations, the water depth at Og was deeper in comparison with S0, In and B1 sites. The sites S1 and B2 (Group 2) showed the lowest diversity indices values revealing the presence of unbalanced communities and are represented by a small number of taxa. These diversity index values confirm deterioration of water quality at the two stations, which are dominated by Nitzschia capitellata Hust. described in Europe as indicator species of polysaprobic waters, hypereutrophic (Krammer and lange- Bertalot 1988; Van Dam, Mertens, and Sinkeldam 1994; Hofmann, Werum, and Lange-Bertalot 2011), and rich in electrolytes (Taylor, Harding, and Archibald 2007), which is the case for these two sites. Fawzi (2002) also noted in his study a major development of Nitzschia capitellata in moderately alkaline waters, eutrophic, and heavily polluted by ammonium. The increase of both diversity indices and species richness in S2 and S3 (Group 3) indicates that the population resumes a balance following water quality improvement by the self-purifying capacity of the river. The decrease of diversity index and number of taxa colonizing near the estuary (site S6) are due to the reappearance of taxa living in eutrophic conditions prefering brackish waters to salt waters such as



Nitzschia filiformis (W.M. Smith) Van Heurck var. filiformis (Van Dam, Mertens, and Sinkeldam 1994), Cvclotella meneghiniana Kütz (Hürlimann and Niederhauser 2007; Taylor, Hading, and Archibald 2007; Houk, Klee, and Tanaka 2010; Bey and Ector 2013) and Nitzschia clausii Hantzsch (Germain 1981; Van Dam, Mertens, and Sinkeldam 1994; Bey and Ector 2013). The S6 site periodically receives the rising tide from downstream and the upstream waste rejects. However, the real degree of chemical pollution, essentially salinity, compared with organic pollution cannot be full determined as samplings occurred at low tide. The community of group 3 (S2, S3 and S6) indicated freshwater to slightly brackish, even marines conditions, oligosaprobic to mesosaprobic, eutrophic, rich in calcareous and electrolytes as underlined by the authors named above. The main taxa were *Craticula halophila* (Grunow ex Van Heurck) Mann (Krammer and Lange-Bertalot 1986; Taylor, Harding, and Archibald 2007), *Navicula reichardtiana* Lange-Bert. *var. reichardtiana* (Krammer and Lange-Bertalot 1991b; Lange-Bertalot 2001; Taylor, Hading, and Archibald 2007; Lange-Bertalot et al. 2011), *Navicula menisculus* Schumann *var. menisculus* (Lange-Bertalot 2001), *Nitzschia sigma*  (Kütz.) W.M.Smith (Ziemann 1991; Busse, Jahn, and Schulz 1999; Fawzi 2002) and *Navicula gregaria* Donkin (Kelly 1998; Lange-Bertalot 2001; Fawzi 2002).

#### Statistical analysis (Fig 6):

The Canonical Correspondence Analysis showed that taxa preferred water with high dissolved oxygen concentrations were Achnanthidium jackii, Amphora pediculus, Cyclotella ocellata Pantocsek, Gomphosphenia lingulatiformis. Navicula menisculus and Surirella helvetica. Cocconeis pediculus was associated with chloride concentrations and Cyclotella meneghiana, Navicula gregaria and Nitzschia filiformis was observed in areas with nitrates concentrations. Water with high concentrations in phosphorous, ammonium, bicarbonate and conductivity were linked with the following species: Nitzschia capitellata, Nitzschia palea, and Tryblionella constricta (Kütz.) Poulin. Nitzschia Palea is known to be associated with higher concentrations of phosphorus and nitrogen (Chaïb et al, 2011).

Using the three groups determined by the CCA analysis, Multi-Response Permutation Procedures (MRPP) was performed to verify the position of Og in group 1. The greater the T value the greater difference between the groups. The p values indicate that groups are statistically valid (Table 4). MRPP was run three time

with site Og being added to one of three groups. A T value of -4.32 was determined when Og was associated with group 1 (upstream group) while when placed within group 3 (downstream group) a lower T value of -4.09 was observed. When associated with group 2 (polluted group) non-significant result was obtained (Table-4).

Indicator Species analysis (IS) was run using the three groups. It should be noted that those species found in almost all of the samples were excluded from the analysis. Result found that certain species occurred in certain groups in greater than 80%. This marks species movina composition changes that occurred as downstream from group 1 to group 3 (Table-5). Interestingly, five species occurred in all three analyses, relative abundance, relative frequency and indicator species. In the relative abundance (percent of each species in each group defined by topographic position), seven species (Achnanthes coarcta (Breb) Grunow, Fragilaria capucina Desmazieres var. capucina, clavatum Gomphonoma Ehrenb., Gomphonema angustatum (Kütz) Rabenh., Surirella biseriata Breb. in Breb. and Godey, Gomphonoma olivaceoides Hustedt, and Gomphonoma gracile Ehrenb.) were found in > 80%in group 1, three species (Gomphonema angustatum, Surirella biseriata, and Gomphonema olivaceoides) were present in group 2 and one, Gomphonema gracile, in

Figure 6: Canonical correspondence analysis (CCA) axis scores showing the changes of community occurring between groups 1 (dotted circle), 2 (black circle) and 3 (grey circle) or from upstream to polluted area and then to downstream zone of the Sebou Basin (code names are in Table 3).



### Table-1: The physicochemical parameters analyzed in the study sites

Station	O.D (mg/)	COD (mg/l)	BOD (mg/l)	HCO3- (mg/l)	CaCO <sup>-</sup> 3 (mg/l)	Ca <sup>2+</sup> (mg/l)	Mg <sup>2+</sup> (mg/l)	Cond µs/Cm	Cl- (mg/l)	Na⁺ (mg/l)	K⁺ (mg/l)	SO <sub>2</sub> -4 (mg/l)	PO₄ <sup>3-</sup> (µg/l)	NO⁻₃ (mg/l)	NO <sup>-</sup> 2 (mg/l)	NH <sup>4+</sup> (mg/l)	т°С	PH
S0	10.15	40	0	255	280	112,22	167,78	994	355	138	11.70	25.95	19,88	1.98	0,03	0.00	20,3	8,1
S1	0.29	113	130	415	360	132,26	227,74	1402	532,5	138	15.60	237.6 2	164,42	1.51	0,21	192.1 2	20	7,6
In	10.67	17	0	135	240	68,13	171,86	794	149,1	115	11.70	112.6 2	17,23	0.79	0,01	0.00	21	8,48
S2	7.99	9	40	125	280	100,2	179,80	752	560,9	138	3.90	265.4	88,75	5.67	0,22	3.35	17,9	8,13
Og	10.36	70	0	105	200	92,18	107,82	533	142	115	15.60	140.4	14,59	0.62	0,02	0.15	19,1	8,17
S3	9.8	0	25	105	200	7,214	192,79	581	170,4	138	3.90	115.4	64,15	1.84	0,86	0.00	16,8	8,8
S4	9.14	7	15	105	200	76,15	123,85	580	560,9	207	11.70	168.1 7	25,94	1.96	0,30	0.37	16,6	8,9
S5	9.38	24	25	115	240	32,06	207,94	588	355	138	7.80	26.51	30,48	0.84	0,28	0.84	16,5	8,7
B1	14.91	45	0	155	240	79,36	160,64	815	74,55	115	7.80	90.4	16,10	2.56	0,04	0.29	15,5	7,61
B2	8.24	54	50	145	240	65,73	174,27	880	191,7	138	15.60	43.17	58,48	2.52	0,08	3.92	16,7	7,38
S6	4.47	21	22.22	135	280	104,21	175,79	889	376,3	161	3.90	83.73	61,50	5.22	0,12	3.73	22,7	7,6

Table-2. List of abondantes spring diatoms collected with relative abundance (%)	in sites study of the Sebou
Watershed.	

Таха	Abbrevation	S <sub>0</sub>	<b>S</b> 1	In	S2	Og	S <sub>3</sub>	B <sub>1</sub>	B <sub>2</sub>	S <sub>6</sub>
Pennates										
A. jackii Rabenh.	ADJK	1.8	0.3	2.3	0	3.3	2.3	5.5	0.5	0
A. minutissimum Kütz. Czarnecki	ADMI	8.5	0.5	0	0.3	2.3	1.3	4	0.2	0.5
Amphora libyca Ehr.	ALIB	0.5	0.3	0	0.3	0.3	0	5	0.5	0
A. pediculus (Kütz.) Grunow	APED	3	0.5	0.5	0.3	1.8	1	1.5	0.5	0
C. pediculus Ehrenb.	CPED	1.3	1	0	0.5	2.5	1.5	4	1.5	0.5
C. placentula Ehrenb. var. placentula	CPLA	2.5	0	0.5	0.3	1.8	5.8	2.5	0	0.3
C. halophila (Grunow ex Van Heurck) Mann	CHAL	0.3	0	0	0.3	0.3	7.5	0	0	0.8
Cymbella affinis Kütz .var. affinis	CAFF	0.3	0	1.3	0.8	1	2.3	1	0	0.5
C. naviculiformis (Auersw.) Krammer var. naviculiformis	CBNA	0	0	12.5	0	0	0	0	0	0
Encyonema minuta (Hilse in Rabh.) D.G. Mann in Round Crawford and Mann	ENMI	0	0	0	0	15.5	0.8	0	0	0
Encyonopsis microcephala (Grunow) Krammer 1997	ENCM	0.3	0.3	25.8	0.8	3.5	1.5	0	0.2	0
G. intermidium Hustedt	GITM	0.5	0	0	1	0.3	0	7.8	1.2	0
G. pumilum (Grunow) Reichardt and Lange-Bert.	GPUM	0.3	0	13.8	0.5	1	0	3.3	1	0.3
Gomphosphenia lingulatiformis (Lange-Bert. and Reichardt) Lange-Bert.	GPLI	0.5	0	0	1	0.25	0	6.75	1.25	0
N. gregaria Donkin	NGRE	0	0.5	0	1	1.3	12.3	0.5	4.2	11.8
N. menisculus Schumann var. menisculus	NMEN	0.3	0.3	0.5	6.8	1	1	0.3	0	0.3
N. reichardtiana Lange-Bert. var. reichardtiana	NRCH	0	0	0.3	18	0.8	1.3	0	0	0
N. amphibia Grunow f. amphibia	NAMP	0	0	1	2.3	0.3	0	2.3	9.9	0.3
N. capitellata Hust. in A.Schmidt and al.	NCPL	0	56.3	0.3	0	0.3	0	0	38.7	0.3
N. clausii Hantzsch	NCLA	0	0	0	0	1.3	0	0	0	5.8
N. filiformis (W.M.Smith) Van Heurck var. filiformis	NFIL	0	0.3	0	0	0.3	4	0	0	14
N. frustulum(Kutzing) Grunowvar. frustulum	NIFR	2.8	2.5	1	8.5	4.5	5.8	5.3	3.7	1.3
N. palea (Kütz.) W.Sm.	NPAL	1.5	9.8	0.8	1.5	0.8	4.8	3.5	12.9	0.8
N. thermaloides Hustedt	NTHE	0	8.5	0	0	0	0	0	0	0
Surirella angusta Kütz	SANG	1.3	0	0	0.8	0.3	0	6	0	5.5
S. antioquiensis Sala, Ramirez, Plata-Diaz and Vouilloud	SUAN	8.5	0	0	0	0	0.8	0	0.2	0
S. helvetica Brun	SHEL	33.3	0.5	0.3	3.5	2.8	0.5	7.5	0	0
T. constricta (Kütz.) Poulin	TBCO	0	0.3	0	0.8	1.3	1.3	0.3	7.9	0
Centric										
Conticribra weissflogii (Grunow) Stachura-Suchoples and Williams	CTWE	0	0	0	0	0	0	6.3	0	0
C. meneghiniana Kütz	CMEN	0	0.3	0.3	0.3	0.3	1	0	0	6
C. ocellata Pantocsek	COCE	0	0.3	10	3.8	4.8	3	3.3	0 <sub>A.C</sub>	+2.5+

group 3. As for relative frequency (the percent of samples in each group in which a species is present), five species (*Gomphonema olivaceoides*, *Navicula cryptotenella* Lange-Bert., *Gomphomema parvulum* Kütz var. *parvulum* f. *parvulum*, *Amphora pediculus* (Kütz.) Grunow, and *Cymbella affinis Kütz* .var. *affinis*) were found to occur at 100% in group 1 but two of the species (*Gomphonema parvulum* var. *parvulum* f. *parvulum* and *Amphora pediculus*) also occurred at 100% in group 2 along with *Hantzschia amphioxys*, *Nitzschia fonticola* Grunow in Van Heurck, *Nitzschia dubia* W.M.Smith and *Craticula accomoda* (Hustedt) Mann at 100%. Group 3 had nine species (*Cymbella affinis Kütz.var. affinis*,

	S0	S1	In	S2	Og	S3	B1	B2	S6
Total abundance	62	48	70	81	120	82	58	52	67
H'	4.220	2.861	4.195	4.999	5.732	5.338	4.922	3.579	4.914
H'max	5.977	5.585	6.129	6.340	6.907	6.358	5.858	5.700	6.066
E	0.706	0.512	0.684	0.789	0.830	0.840	0.840	0.628	0.810

Table-3: Diatom diversity indices for the sampling sites: Shannon index (H'), index of maximum Shannon (H'max), and the equitability (E)

Table-4. Multi-response Permutation Procedures results. A. Og belonging to group 1 (upstream sites) with a total analysis having a T value = -4.32. B. Og belonging to group 3 (downstream sites) with total analysis having T value = -4.09. C. Og belonging to group2 (polluted sites) had non-significant results.

Α	Group 1	Group 2	Group 3	
Group 1		-2.86	-2.58	e
Group 2	0.0176		-2.23	valı
Group 3	0.0145	0.0001		T
		p value		

В	Group 1	Group 2	Group 3	
Group 1		-2.2	2.32	e
Group 2	0.0001		-2.99	valt
Group 3	0.0221	0.0157		F

Mayamaea atomus (Kütz.) Lange-Bert., Nitzschia recta, Nitzschia sigma, Navicula tripunctata, Actinocyclus normanii (Greg. ex Grev.) Hust. morphotype normanii, Navicula veneta Kütz., Cyclotella meneghiniana Kütz. and Diploneis oblongella (Naegeli) Cleve-Euler). However all but Diploneis oblongella (Naegeli) Cleve-Euler were also found in group 1 and Cymbella affinis Kütz .var. affinis also occurred in group 1 at 100%. Three species not only occurred with relative frequency group 1, but also in group 2 (A. normanii, N. veneta and C. meneghiniana). However, Diploneis oblongella occur only in group 3 at 100%. Finally, only six species were found as indicator species (percent of perfect indication of each species for each group).

Groups 1 only had one species, Gomphonema olivaceum var. olivaceoides (Hustedt) Lange-Bertalot with 86% (Table 5) which preferred oxygenated sites, poor in organic and mineral matter, the same ecology was defined by Bey and Ector (2013) in their study of the rivers from the Rhone-Alpes region. The analysis of diatom assemblages underlined that taxa present in are generally cosmopolitans, colonizing group 1 freshwaters to slightly brackish, oligosaprobic, oligo- to mesotrophic but preferring the calcareous waters with a moderate to high electrolyte contents, according to Van Dam, Mertens, and Sinkeldam 1994; Taylor, Harding, and Archibald (2007); Hofmann, Werum, and Lange-Bertalot (2011); Bey and Ector (2013). The only indicator species of group 2 is Hantzschia amphioxys (Ehr.) Grunow described by Van Dam, Mertens, and Sinkeldam (1994); Hofmann, Werum, and Lange-Bertalot (2011); and Bey and Ector (2013), as an indicator of polluted environments. Furthermore, the main diatoms of group 2 were representatives of the genera of Nitzschia, indicators of heavy metals contamination (Guasch et al.

2009; Tlili et al. 2011), salinity (Hofmann 1997; Rimet 2009), eutrophic (Rimet, Berthon, and Bouchez 2010) and / or organic pollution (Kobayasi and Mayama 1982; Van Dam, Mertens, and Sinkeldam 1994; Martin et al. 2010; Trobajo et al. 2013). while group 3 had four species, Nitzschia recta (93%), Nitzschia sigma (95%), Navicula tripunctata (96%) and Diploneis oblongella (100%). therefore are characteristic of polluted sites by nitrates with high mineralization. Note that among the six indicator species determined, as we consider Diploneis oblongella as the best indicator of group 3 since it was present with a frequency of 100%. Four taxa in this group had a indicator value superior to 80% were Nitzschia recta Hantzsch in Rabenhorst; Nitzschia sigma (Kutzing) W.M.Smith; Navicula tripunctata (O.F.M\_ler) Bory. and Diploneis oblongella (Naegeli) Cleve-Euler. Besides that, in sites S0, Og and B1, oligotrophic and oligosaprobic taxa were identified such as Achnanthidium jackii Rabenh and Surirella helvetica, with more eutrophic and beta-saprobic species like Amphora pediculus (Kütz.) Grunow (Van Dam, Mertens, and Sinkeldam 1994). At site In, meso-eutrophic and oligo-saprobic taxa, Encyonopsis microcephala (Grunow) Krammer and more beta-saprobic and eutrophic taxa like Gomphonema minutum (Ag.) Agardh f. minutum (Van Dam, Mertens, and Sinkeldam 1994) were common. S1 and B2 sites had hypereutrophic and polysaprobic taxa such as Nitzschia palea, and Nitzschia capitellata Hust. (Van Dam, Mertens, and Sinkeldam 1994). Brackish and eutraphentic species were dominant in sites S3 and S6, as Craticula halophila (Grunow ex Van Heurck) Mann and Navicula erifuga Lange-Bert. (Lange-Bertalot 2001). Moreover, Nitzschia filiformis, known to be a species that can support brackish waters and tolerant of strongly polluted conditions, but Cyclotella meneghiniana Kütz.

Table-5: Indicator species analysis presenting only those species having results greater than 80% in a category. A. Relative abundance, which is the percentage of each species in each group defined by topographic position. B. Relative frequency, which is the percentage of samples in each group in which a species is present. C. Indicator values (% of perfect indication) which are a product of the relative abundance and constancy of a species in each group. The species marked in gray are found in A. B. and C.

Α.	Group 1	Group 2	Group 3
Achnanthes coarcta	100	0	0
Fragilaria capucina	100	0	0
Gomphonema clavatum	100	0	0
Gomphonema angustatum	88	13	0
Surirella biseriata	87	13	0
Gomphonema olivaceoides	86	14	0
Gomphonema gracile	80	0	20
Nitzschia umbonata	0	88	12
Hantzschia amphioxys	5	87	7
Nitzschia scalpelliformis	11	0	89
Navicula lanceolata	4	0	96
Nitzschia recta	7	0	93
Nirzschia sigma	5	0	95
Navicula tripunctata	4	0	96
Nitzschia obtusa	0	0	100
Diploneis oblongella	0	0	100
В.			
Gomphonema olivaceoides	100	50	0
Navicula cryptotenella	100	50	0
Gomphonema parvulum	100	100	0
Amphora pediculus	100	100	67
Hantzschia amphioxys	25	100	33
Nitzschia fonticola	75	100	67
Nitzschia dubia	0	100	67
Craticula accomoda	0	100	67
Cymbella affinis	100	0	100
Mayamaea atomus	50	0	100
N. recta	25	0	100
N. sigma	25	0	100
Navicula tripunctata	25	0	100
Actinocyclus normanii	25	50	100
Navicula veneta	25	50	100
Cyclotella meneghiniana	50	50	100
Diploneis oblongella	0	0	100
С.			
G. olivaceoides	86	7	0
Hantzschia amphioxys	1	87	2
Nitzschia recta	2	0	93
Nitzschia sigma	1	0	95
Navicula tripunctata	1	0	96
Diploneis oblongella	0	0	100

and Navicula gregaria which support some pollution but not critical levels of pollution (Taylor, Harding, and Archibald 2007) were also collected.

## Conclusion

The distribution of diatom species along environmental gradients has been the focus of numerous studies, which have been used to determine the structure of communities (Wellborn, Skelly, and Werner 1996; McPeek and Brown 2000). It has underlined that diatom assemblages are driven by local environmental variables rather than regional processes (Soininen 2004). It may be useful, therefore, to describe such patterns and identify environmental drivers of species assemblages within different geographical settings, especially arid regions like North Africa.

The present study attempts to identify the spatial distribution of benthic diatom assemblages and their responses to environmental gradients in a Moroccan hydro-system of the Sebou Basin.

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The species distribution was mainly linked to human impacts and tidal influences in the estuary. The spring diatom communities of Sebou Basin are generally rich and diversified but numerically poor in distribution between taxonomic levels. Nevertheless, the distribution depends on the chemical quality of the water. Indeed, the stations downstream of anthropogenic activities have degraded water quality and low current flow aggravates the situation downstream from the city of Fez, which caused a decrease in taxonomic richness, diversity and stability of diatom community, dominated by pollutionresistant species, instead of pollution-sensitive species well represented in other unpolluted stations. It is hoped that the large treatment plant recently opened downstream of Fez will be beneficial to aquatic life of the Sebou Basin.

## **Conflicts of Interest**

Authors declare that there is no conflict of interests regarding the publication of this paper.

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