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Hydrochemical characteristics and metallic quality in fish in the Loukkos river estuary of Morocco

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Abstract
The region of Larache endures a big problem of pollution of the surface waters. This pollution takes various origins, domestic, agricultural and industrial. The study of the physicochemical characteristics (Water temperature, pH, Salinity, DO, Conductivity, SM, TOC, NO_3, COD and BOD) of the effluents exits of the industrial units of the Loukkos river estuary shows, that these units are responsible of important organic discharges and heavy metals. Indeed, the wastewaters used to drain rice fields are at the origin of important organic discharges which drag strong concentrations in SM and NO_3, also most effluents present a strong salinity (conductivity) and TOC concentration. This high level of pollutants concentration can be a main cause of poisoning for the receiving environment. They especially conjugate themselves by the waters qualities deteriorate of the Loukkos river estuary downstream. The assessment of the metals concentration studied (Fe, Zn, Cu, Cr, Pb and Cd) in the sediments and the superficial waters revealed the presence of a wide metallic contamination, mainly in the river's downstream. Fish species (*Liza ramada, Barbus callensis* and *Anguilla anguilla*) were collected in the Loukkos river estuary, whereas (*Pagellus acarne*, *Sardina pilchardus* and *Diplodus vulgaris*) were collected in the northern Atlantic coast of Morocco in 2006 and 2007. Concentrations of selected metals were measured using an atomic absorption spectrophotometer (AAS). Significant correlations ($P<0.05$) between Cu–Pb were observed in sediments, Fe–Pb, Cr–Pb and Cr–Fe in water and Zn–Fe in fish. The metal levels found in the muscles of fish species were below the legal limits for human consumption, but can induce sever dysfunctioning for the eel.

Key words: Water pollution, water quality, fish, heavy metals, sediment, estuary, Morocco.

1. Introduction
The aquatic ecosystem is threatened more and more by different sources of pollution that decrease its economic potentialities and have ominous repercussions on the human health [1]. More than ever, in this end of millennium, struggle against the water pollution is in the center of discussions and proceedings on a world scale. Hundreds of titles dedicated to this survey have been raised in the press, as well as other symposia, meetings and conventions have taken place to discuss this issue without forgetting the campaigns of sensitization. Among the chemical substances susceptible to constitute a danger to the aquatic life, we signal the heavy metals as the chromium, the lead and the cadmium. These toxic elements are used more and more in the industrial sectors. Some non toxic metals constitute a threat to the aquatic environment because of their non biological deterioration in the sediments.
Morocco is confronted to many environmental problems which can be sometimes a serious menace. They are the result of strong demographic growth, a galloping urbanization and a bad management of natural resources. Water resources in Morocco face problems of quantity and quality. Indeed they are limited because of the semi-arid and arid climate, different discharge of pollutants and wastewater [2]. Some works had led to determinate the metallic levels among fish in the Atlantic coast [3-5] and the Mediterranean one [6,7]. The previous Moroccan studies were incomplete that explains the necessity to lead a new study which determinates efficiently the presence of heavy metals in various compartments of the aquatic environment [8].
The present study comes to complete and to bring new elements to the previous works achieved by [9-11]. The choice has been motivated by the socio-economic importance that this ecosystem represents for the region of

the Larache city and by the strong pollution that knows this region. Indeed, this sector receives lot of polluting discharges because of the numerous agglomerations and industrial units that are installed on its strands and effluents. Otherwise, the unreasoned use of fertilizers and manures in this agricultural region only accentuate the pollution and increase the risk of contamination of the superficial and deep waters of this ecosystem.
In this context, our aims are to study the quality of the superficial waters of the Loukkos river estuary by the spatiotemporal follow-up of a certain number of tracers physical chemical and to value the contribution to the survey of the metallic pollution of the Loukkos river estuary of the Larache Region through the spatiotemporal follow-up of the contamination of water, the sediments and fish by six elements metallic traces as iron (Fe), zinc (Zn), copper (Cu), chromium (Cr), lead (Pb) and cadmium (Cd).

2. Materials and methods

For the realization of this work and taking account of the various activities counted in this zone, five stations, distributed along the Loukkos river estuary, have been chosen (**Figure 1**) and defined as follows: Station 1 (Dhiria): located to the level of the dam at 21 km from the mouth. It receives drainage water from the rice fields that are situated upstream, Station 2 (Ain Chouk): located at 16 km from the mouth, Station 3 (Baggara): located at 9 km from the mouth. It is characterized by the growth of rice plantation, the good extension of agricultural lands and the pruning of vegetation, Station 4 (Grangha): At 3 km from the mouth, it receives urban and industrial discharges of Larache city and Station 5 Port of Larache: located to the level of the mouth of the Loukkos. All stations, however, receive many industrial and domestic effluents from Loukkos River and its surroundings. To value the degree of metallic contamination of the Loukkos River estuary, several collections were made in the different stations so as to analyse the amount of metals in sediments, water and fish. The five chosen stations were visited by only one resumption. During the sampling period were held: 6 campaigns to collect sediments and water samples and 7 campaigns to collect fish samples (3 campaigns for AA and 4 campaigns for (PA, SP, DV, LR and BC; **Table 1**).

Table 1: Campaigns of collections

Month	PCP	Sediment	Water	Fish (n=415) AA (225)	PA (30)	SP (30)	DV (30)	LR (60)	BC (40)
march-06	+	+	+	+	-	-	-	-	-
may-06	+	+	+	+	-	-	-	-	-
juil-06	+	+	+	+	-	-	-	-	-
sept-06	+	-	-	-	-	-	-	-	-
nov-06	+	-	-	-	+	+	+	+	+
janv-07	+	-	-	-	+	+	+	+	+
march-07	+	+	+	-	+	+	+	+	+
may-07	+	+	+	-	+	+	+	+	+
juil-07	-	+	+	-	-	-	-	-	-
campaigns	8	6 (1)	6 (1)	3 (15)	4				
number of samples		n=30	n=30	n=225	n=190				

Remark: PCP: physicochemical parameters / AA: *Anguilla anguilla*, PA: *Pagellus acarne*, SP: *Sardina pilchardus*, DV: *Diplodus vulgaris*, LR: *Liza ramada* and BC: *Barbus callensis*.

Metal analysis has been done on the mineralization after dilution according to the procedure published by several authors [12,13]. The Cu, Cr, Pb and Cd have been determined by atomic absorption spectrophotometry (AAS) in oven on Perkin Elmer 3100 and by flame in the cases of the Fe and Zn. The validity of the analytic methods has been verified by internal control with the help of standard samples (Council National of Research of Canada: BCSS-1) and by external control with the help of an exercise of intercalibration [14]. Statistical analysis of data was carried out with the XL statistical (XL-STAT) program. One-way ANOVA and the Duncan Multiple Comparison Test were used to compare data among stations.

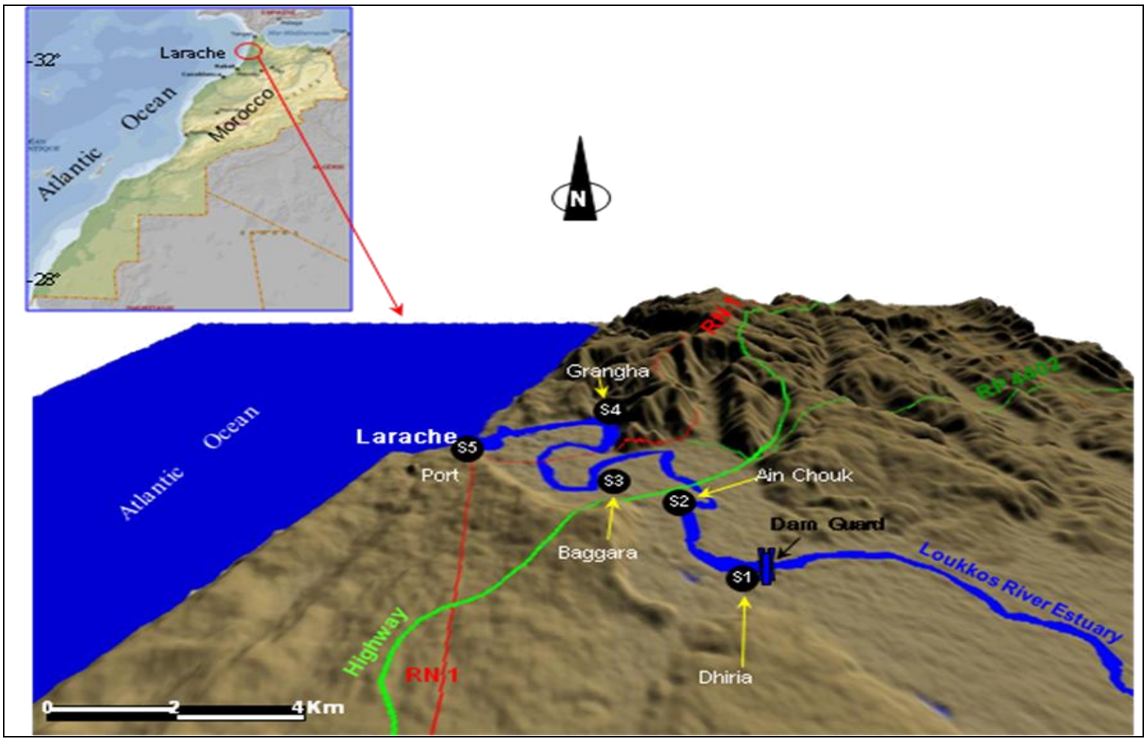

Figure 1: Site of sample collection (Loukkos River estuary in Morocco)

3. Results and discussion

3.1. Spatial evolution of the physicochemical parameters of the water quality

The results of the analysis that were achieved during the years 2006 and 2007 are represented in the **figure 2**.
The mean water **temperature** in upstream was higher than that in downstream in Loukkos river estuary. The same remark has been made by several authors [15-18] that noted an increasing gradient of the downstream to the upstream. This gradient is due to the sunstroke and to the low depth upstream of the estuary. The amplitude variation between the campaigns is low to 30°C. These concentrations are similar to those found in other sites [19,20]. This situation could explain itself by the hydraulic management of the Loukkos basin [21]. Compared to other studies, the water temperature obtained in the Loukkos river estuary was lower than that found in Ganges river where it fluctuates from 15 to 35°C, suggesting that this increase is due to the natural influences and anthropogenic inputs [22].
The **salinity** as well as the **conductivity** that follow opposite the tidal hydrodynamics, defines an increasing gradient of the upstream to the downstream. Indeed, Very high salinity values (34.8 g/l) were found in Loukkos river estuary than those obtained in other studies [22]. Same authors explained this situation by the influence of the natural contributions as well as by anthropogenic inputs. Other authors found that the salinity concentration was similar than that found in our study [10]. This situation also explains itself by the tidal dynamics and the freshwater influences. However, in this study, the concentrations of conductivity (44.58 mS/cm) in Loukkos river estuary were higher than those found at the other sites where they fluctuate between 0.8 and 3.9 mS/cm [22]. But, in the Sebou estuary enters 0 and 29 mS/cm [23].
Slightly lower concentrations of **dissolved oxygen** (DO) were recorded in the Loukkos river estuary than those found in other studies [24]. The mean value of DO in Loukkos river estuary is 8.68 mg/l (**Figure 2**). Similar values were reported by other authors [10]; it varies between 6 and 9 mg/l. Same authors explained this concentration by the elevation abnormal of the temperature that the estuary knew in the beginning of the year 2005 [10]. In another study, these results explained the concentration of the oxygen by the tidal dynamics that generates a brewing continues the mass of water and therefore an enrichment of the phase dissolved in oxygen [25]. Other studies found that the concentration of the DO is low and depends of tidal dynamics [20];[23];[26].
The spatial evolution of the dissolved oxygen content in Loukkos river estuary, showed that the biodegradable organic matter load of rejected and accumulated domestic, industrial and agricultural origin, contribute to a sensitive decrease of the oxygenation of the site in our site and especially in the station 2.

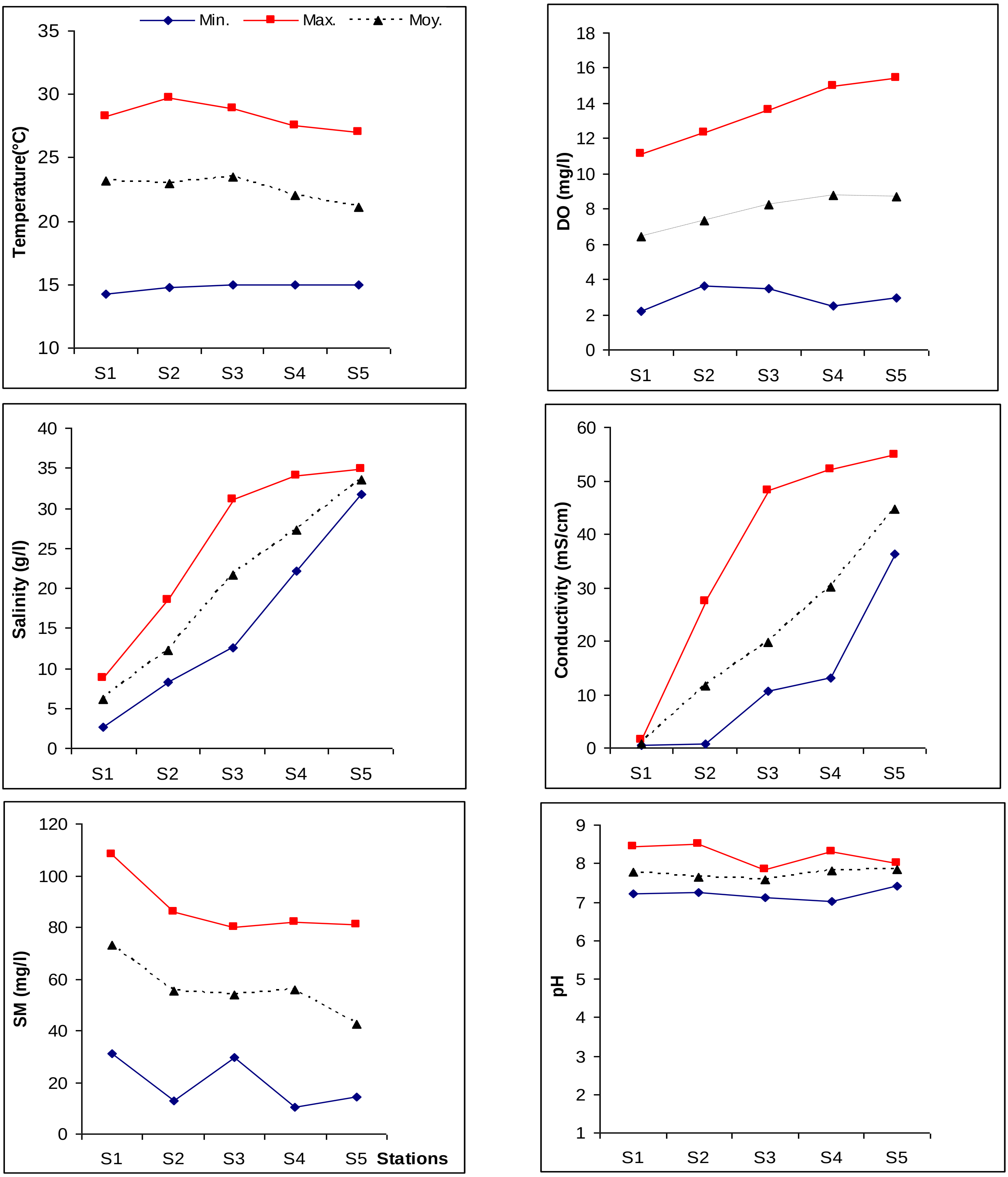

Figure 2 : Spatial evolution of the physicochemical parameters of the water quality (min: minimal, max: maximal and moy: mean)

In spite of these changes in the ionic balance the **potential of hydrogen** (pH) remained close to the neutrality. Indeed pH level varies between 7.02 and 8.52. This can be explained by the fluctuations of the salinity that follow the tidal cycle and the fluctuations of the organic load [11]. This alkalinity is due to the effect tampon of the oceanic waters. Other authors showed that the pH of the water of the estuaries (Sebou and Om-Rbia) is very stamped. The pH is the order of 8 because of the system tampon developed by carbonates and the bicarbonates [19];[24];[27]. The lowest concentrations of the pH that have been recorded to the level of the station 3 can be due to the presence of the organic matter. Several other authors confirmed this result in the same ecosystem by the presence of the biodegradable organic matter [10];[28]. Otherwise, similar pH values were obtained in the Loukkos river estuary than those found by other studies [20];[22].

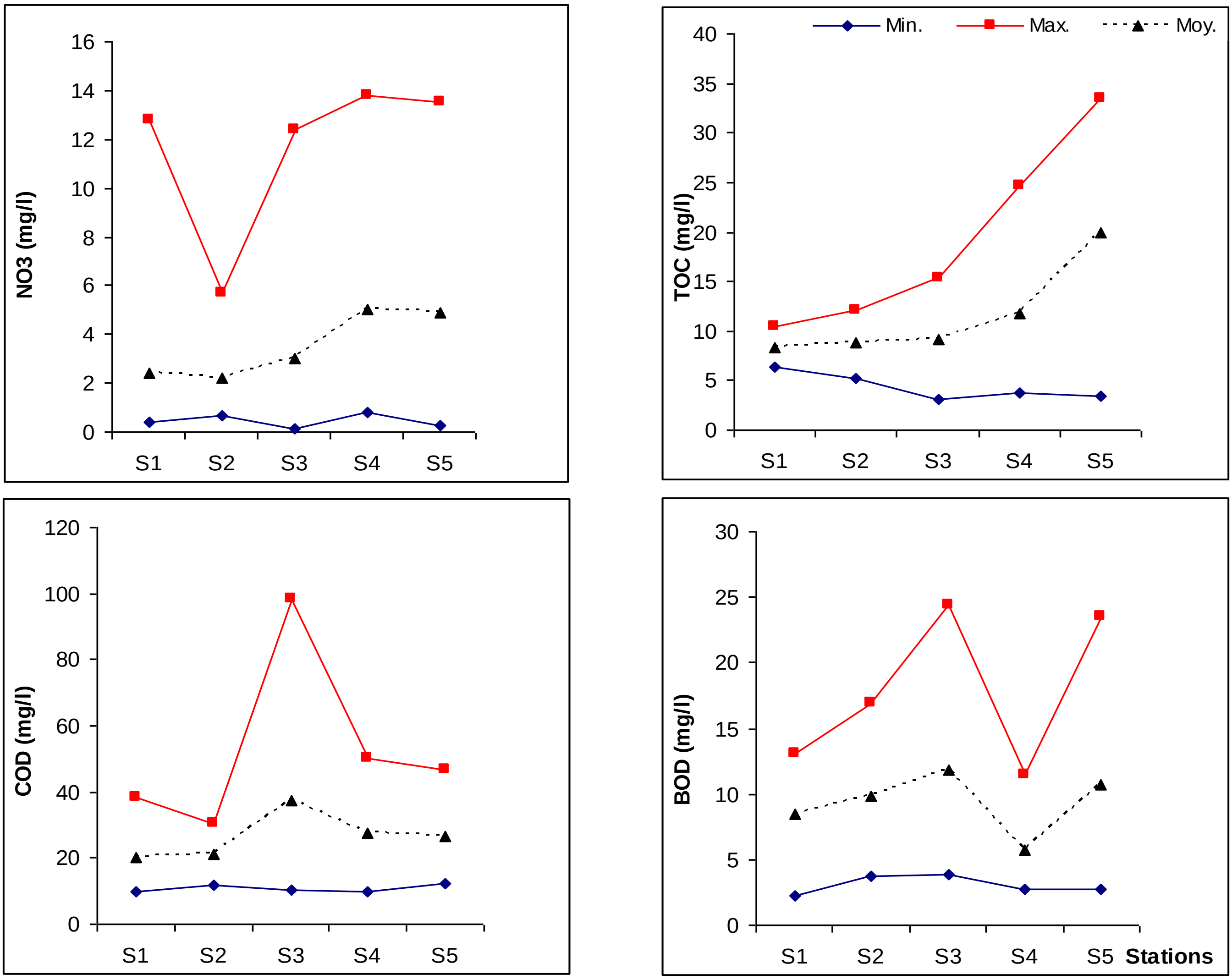

Figure 2 : Spatial evolution of the physicochemical parameters of the water quality (min: minimal, max: maximal and moy: mean ; continuation)

In the Loukkos river estuary, in relation with the relatively low erosive potential of the basin, waters are charged enough by **suspended matter** (SM). Very high SM value (73.27 mg/l) was found in upstream of the Loukkos river estuary. These variations can be affected by the tidal regime and atmospheric fallouts as well as wide variations in hydrological parameters also play important roles. This mean growth of the SM can be attributed to an agricultural activity and to an intense erosion of the Loukkos basin, following brutal stormy rains. Such a rise can also entail a warming up of water, which will be the effect of to reduce the quality of the habitat for the organisms of cold water [29]. Other authors showed that the flood tide agents as the currents bound to the tidal dynamics influences, contribute them also to the increase of the content in SM by the discount in suspension of the fine layer of the sediments [30].

The mean values of the **total organic carbon** (TOC) show the important variations (8.33-19.96 mg/l) denoting a gradient increasing upstream downstream. This situation can probably be attributed to the tidal hydrodynamics and to the release of the dam. This values obtained in Loukkos river estuary were higher than those found in port Jackson estuary. The mean TOC concentration in port Jackson estuary is 4.54 mg/l [20]. This situation can possibly be explained simply by tidal cycle influences.

The mean contents (2.40-4.89 mg/l) of the **nitrate** (NO_3) in the Loukkos river estuary were slightly lower than those previously reported by other authors in the same estuary [5]. These contents have been explained by the presence of the phytoplankton that produces of organic matter. However, other authors found that the NO_3 can reach more elevated values (19.96 mg/l), suggesting the presence of the organic matter.

Chemical oxygen demand (COD) evolves in the same sense that the gradient of mineralization. It has been observed by several authors [31];[19]. The influence of some mineral elements notably the chlorides on the determination of this parameter is translated however by an overestimate of the COD whose assessment remains not significant in the Loukkos river estuary [32]. Several authors explained the lowest concentration of the COD by the presence of the organic matter [22]. The same phenomenon has been described by [33]. Otherwise, in our study higher values have been recorded in the upstream. This confirms the agricultural origin of this contamination.

Slightly higher concentrations of **biochemical oxygen demand** (BOD) were found in Loukkos river estuary (5.75-11.82 mg/l) than those detected by other studies [22]. The mean values ranged from 2.7 to .95 mg/l in the station of Babughat and from 0.75 to 2.8 mg/l in the station of Gangasar.

Higher concentrations of the BOD (5.75-11.82 mg/l) were found in the Loukkos river estuary than those detected in other sites as the river of Ganges where the mean fluctuates between 2.7 and 5.95 mg/l in the Babughat station and between 0.75 and 2.8 mg/l in the Gangasar station [22]. Compared to other estuaries to Morocco, the BOD concentrations of the Loukkos river estuary are lower opposite the usually recorded values [18], suggesting the excess of rejections of the pollutants of organic origin. The quality of the waters of the Loukkos river estuary is characterized by water temperatures without significantly difference between the different stations. The mean neighbouring of 22°C remains bound to the climatic conditions [34] is lower to 30 °C, considered as value limits direct rejections in the receiving environment [35].

The global mineralization of water estimated by the analysis of the conductivity as well as the salinity remains very elevated along the estuary. Indeed, increased water conductivity to 1.5 mS/cm returns the unusable irrigation water for the rice fields [32]. The pH as for remains him close to the neutrality. It is acceptable according to the Moroccan norms (pH=8.5).

The indices of organic pollution, organic matter and dissolved oxygen give an idea on the rejected organic load. Indeed, the spatial profile of the organic matter is inversely proportional than that the dissolved oxygen. The recorded values in the studied sector show a low load in organic matter. Indeed, the BOD/COD ration is the order of 0.36. This result permits to conclude that the waters of the Loukkos river estuary are polluted by a strong enough inorganic pollution. However, the COD/BOD and SM/BOD report are respectively of the order of 2.86 and 6.08, higher values were detected in the Loukkos river estuary, what indicates that oxydable matter (OM = 14.9) in the waters is difficulty degradable. This result could be attributed to the rejection of a textile industry connected to this river. Our results were similar to that presented by several authors [36,37]. But, in another study in the wastewater of the Oujda city, lower results were detected [38].

So, the quality of most of the water sampled in this study can be described as poor, based on guidelines set out for drinking water purposes by the World Health Organisation [39], even though a large number of the population living in Larache area rely on surface water as water supply.

Within sight of the important load produced by the industrial and urban activity very active, several questions oppose notably: the absence of a considerable and substantial impact on the hydrochemical of this Loukkos river estuary since the recorded contents in water cannot translate with accurateness the relative importance of the pollution contributions. This situation could be explained in the intervention of some physicochemical phenomenon as the precipitation, the trapping, the decanting and the storage of the pollutants in the sediments without disregarding the phenomenon of bioaccumulation by fauna.

3.2. Assessment of the spatiotemporal variation of the heavy metals of the sediments

Higher concentrations of Fe and the Zn were detected in the Loukkos river estuary compared to the other heavy metals with a maximal content that can reach until 40.28 mg/g detected in the S5 and 159.90 µg/g recorded in the S1, recorded respectively during the month of July and May of 2006 (**Figure 3**). Higher mean concentrations of Fe (27.20 mg/g) followed from the Zn (115.41 µg/g), of the Pb (69.26 µg/g), the Cr (54.38 µg/g), the Cu (16.85 µg/g) and the Cd (1.11 µg/g) were detected in the Loukkos river estuary. The stations (S4 and S1) showed higher concentration than those detected by other stations in the same estuary, notably in the case of Fe, Cu, Cr and Pb in comparison with those of the station S3 localized more far from the rejection. This enrichment of the heavy metals can be explained by the wastewater contributions of the Larache city that flow in the station S4 and the waters of draining of the rice fields in the station S1. Indeed, the Fe, Zn, Cu and Pb are heavy metals characteristic of a pollution of urban type [40,41].

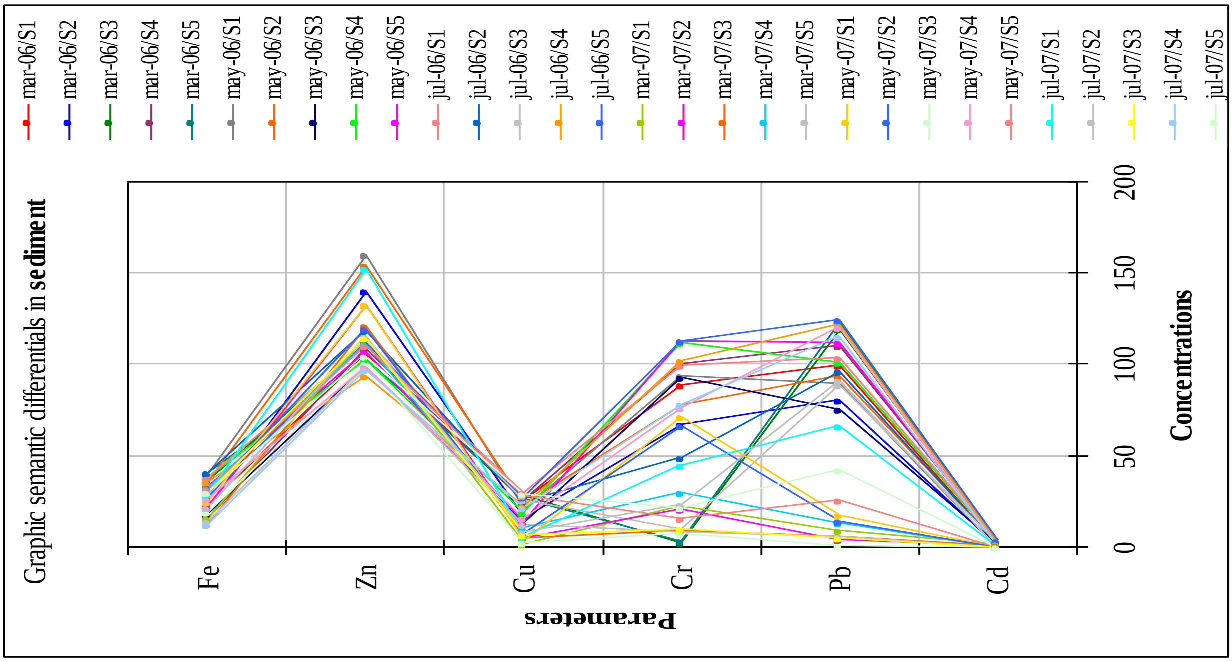

Figure 3 : Graphic semantic differentials of the heavy metals in sediment

Fe (27.20 mg/g) concentrations in the sediments of the Loukkos river estuary were very higher than in unpolluted sediment published by other authors [42]. The mean concentration of Fe was very lower (13.20 mg/g). The excess of Fe could come from the washing of the agricultural soil and the regional geological context as to Sidi Moussa and in Oualidia [30]. Concentrations of Zn (115.41 µg/g) were high when compared to the contents of reference [43]. The mean concentration of Zn was very lower (90 µg/g) underlining a pollution curbed by the Zn. Lower concentrations of Cu (16.85 µg/g) were obtained in the Loukkos river estuary compared with the contents of reference [44]. The mean concentration of Cu was (30 µg/g). The mean values of Pb, Cd and Zn were higher in Loukkos river estuary than in reference which the mean concentrations were 50, 60, and 200 µg/g [45]. The all stations, however, receive many industrial and domestic effluents from Larache areas. So, these stations are significantly contaminated in metals (Pb, Cd and Zn). The level of contamination is considered low for the stations previously with values being located over to the of the values guides of the metallic contents. It is higher to 0.15 µg/g for the Cd, 30 µg/g for the Pb and 90 µg/g for the Zn that represent the concentrations considered as natural in the coast sediments [46].

A significant contamination has been noted for the Cr, Pb and Cd. Indeed, the mean concentrations of Cr (54.38 µg/g) in Loukkos river estuary were consistently higher than in reference detected by several authors in other estuary [47]. However, the spatial evolution of this contamination makes appear a lower of the Cr content in station S3. Metal reduction of Cr can be bound to the different sources such as industrial effluents, municipal sewage and the tidal hydrodynamics of the Loukkos river estuary. Indeed, tidal cycles promote a continuous cycling of mobilisation, release and redistribution of the heavy metals particularly Cr from the sediments. The displacement of this load makes appear an increase of the content in station S4. But, the mean Pb concentrations in the sediments of Loukkos river estuary were slightly higher in stations 4 and 5. This can be explained by the anthropogenic activities as well as atmospheric deposition [48], the corrosion of building materials and traffic-related emissions in urban centres [49]. Higher concentrations of Cd were observed in the sediment of the Loukkos river estuary than in the reference published by several authors [46]. The mean Cd concentrations are slightly lower (0.15 µg/g) and therefore the stations are significantly contaminated by this element (Cd). The sediments of these stations are difficult to interpret. An increase of the Cd in all these stations of the Loukkos river estuary can be explained by the proximity of the harbour zone and the coastal currents. Several authors describe the sediments of the Seine bay as being especially contaminated by the Cd in relation with the rejection of the effluents of an industry of textile connected to this river [50]. However, other authors describe the sediments of the estuary of Bouregreg, due probably to an occasional contribution transported by the Akrach River [51].

For all heavy metals, a higher contamination was detected in our estuary when compared to the other Moroccan estuaries as Bouregreg, Sebou. The mean concentrations of Fe ; Zn ; Cu ; Cr ; Pb and Cd in Bouregreg estuary are slightly lower (1.2; 61; 21; 39; 17 and 0.02 µg/g ; respectively ; [51]. This pollution would be bound, of the least in part, to an agricultural activity based on the massive use of fertilizing and of pesticides, knowing that fungicides are rich in Zn and Fe and that the compounds phosphates contain important quantities of Cd. Moreover, the haulage and the washing of the agricultural lands in rainy period encourage the accumulation of metals in the physical compartments. However, the storage of heavy metals in the sediments is not necessarily definitive. The risks of remobilisation, bio-disponibility and toxicity are to fear and constitute a permanent danger for the whole trophic chain [52].

3.3. Assessment of the spatiotemporal variation of the heavy metals of waters

The aims of this study were, therefore, to investigate the spatial and temporal distributions of dissolved heavy metals, as well as the partitioning between dissolved and particulate phases under sediment and fish in Loukkos river estuary.

The results of this study are the first reliable, systematic measurements of dissolved trace metals in the Loukkos river estuary, and some of the first concentrations of dissolved metals reported in any estuary in Morocco. As expected, concentrations obtained in this study were very lower than the concentrations of Fe, Zn, Cu, Cr, Pb and Cd observed for Morocco coastal. Maximum concentrations of trace metals were, in fact, up to 30 times lower than Morocco coastal, clearly indicating an anthropogenically derived input of trace metals in the estuarine waters. This explanation is similar to that found in other sites published by several authors [53]. However, for all metals, the observed concentrations were below the guideline limits for recreational water quality [39], however, Cr slightly detected an increase values. It has been recognised that availability of metals, and especially of Cr, is of more importance than its total concentration. Speciation of dissolved Cr in natural waters is dominated by interactions with suspended matter, and the formation of relatively stable Cr-SM complexes. These complexes are assumed not to be bioavailable; therefore it is unlikely that Cr poses any ecological risk. The Cr shows the most important contents of all heavy metals of our study with a maximal content that can reach until 9 µg/l recorded in the station S3 during the month of July of 2006 (Fig. 4).

3.4. Levels of heavy metals (Fe, Zn, Cu, Cr, Pb, and Cd) in tissue of fish studies

Fish are widely used as sentinels of contamination in aquatic environment. The levels of metals accumulated in some marine organisms may be many orders of magnitude above the background of certain species as bioindicators of metal pollution. Fish muscle provides low metal content because of its low metabolic activity. However, it is very important to study the metal levels in this tissue in order to estimate metal quantities that enter to human by the consumption of fish.

The bioaccumulation of heavy metals in the fish tissues was the aims of several works of study [54]. The contents of Fe, Zn, Cu, Cr, Pb and Cd are representative in **table 2**. The different metallic contents for an each organ are rarely comparable between them, for the following reasons: The physiological behaviour varies from a species to the other and within the same ecosystem and, within the same species of fish and for a stationary age. The physiological needs vary with the seasons and the variations of the physicochemical parameters of the ecosystem (pH, water temperature, dissolved oxygen, salinity).

Table 2: Contents metallic means expressed in µg/g of the wet weight in the organs (gills, liver and muscles) at the five species of fish studied and in µg/g of the dry weight among the glass eels (AA)

Species	Organs	Fe	Zn	Cu	Cr	Pb	Cd
Pagellus acarne (PA)	Gills	79.95	68.44	43.77	0.05	0.59	0.08
	Liver	76.66	48.04	33.71	0.91	0.42	0.02
	Muscle	33.85	24.10	17.58	0.03	0.04	0.01
Sardina pilchardus (SP)	Gills	85.54	51.67	15.99	0.82	0.63	0.08
	Liver	72.05	41.91	22.51	0.35	0.44	0.07
	Muscle	62.18	37.27	19.17	0.10	0.55	0.08
Diplodus vulgaris (DV)	Gills	89.02	60.57	34.06	0.30	0.25	0.05
	Liver	34.71	72.27	34.13	0.80	1.06	0.01
	Muscle	30.76	34.67	29.49	0.39	0.64	0.01
Liza ramada (LR)	Gills	29.35	39.48	12.26	0.34	0.36	0.05
	Liver	48.34	36.07	24.81	0.68	0.26	0.05
	Muscle	35.44	23.27	10.40	0.14	0.13	0.02
Barbus callensis (BC)	Gills	44.26	88.98	15.92	0.15	0.78	0.46
	Liver	32.47	56.25	33.25	0.45	0.65	0.15
	Muscle	22.43	25.16	22.51	0.09	0.06	0.02
Glass eels (AA)	Tissues	80.35	39.60	0.45	2.66	0.03	0.45

Higher concentrations of Zn and the Fe in fish tissues were detected in the Loukkos river estuary when compared to the other elements (Cu, Cr, Pb and Cd). The mean concentrations of all elements in gills and liver were consistently higher than in muscle (**figure 4**).
The fashion of contamination is also to take. Some divergences can be gotten in the interpretation of the results insofar as the results are valued coins when the assessment rather makes itself on a tissue on a given organ. Furthermore, very high of Fe, Zn and Cu were detected in gills and liver and the lowest concentrations of the Cr, Pb and Cd were recorded in the muscle. The liver confirms its role bioaccumulator in Cu. A big variability of Cr, Pb and Cd was detected between organs, but in any case, the gills show the most elevated values.

3.5. Comparison with the European dietary standards and guidelines
Dietary standards and guidelines applicable in the European for heavy metals in fish were summarised by Ministry of Agriculture, Fisheries and Food. Considering limit value for human consumption of metals, i.e., 200 µg/kg for Cd, 2000 µg/kg for Pb, 20.000 µg/kg for Cu, 50.000 µg/kg for Zn, metal concentrations in muscle appear to be low. Several norms are proposed in this shutter and that are summarized in the table 3.

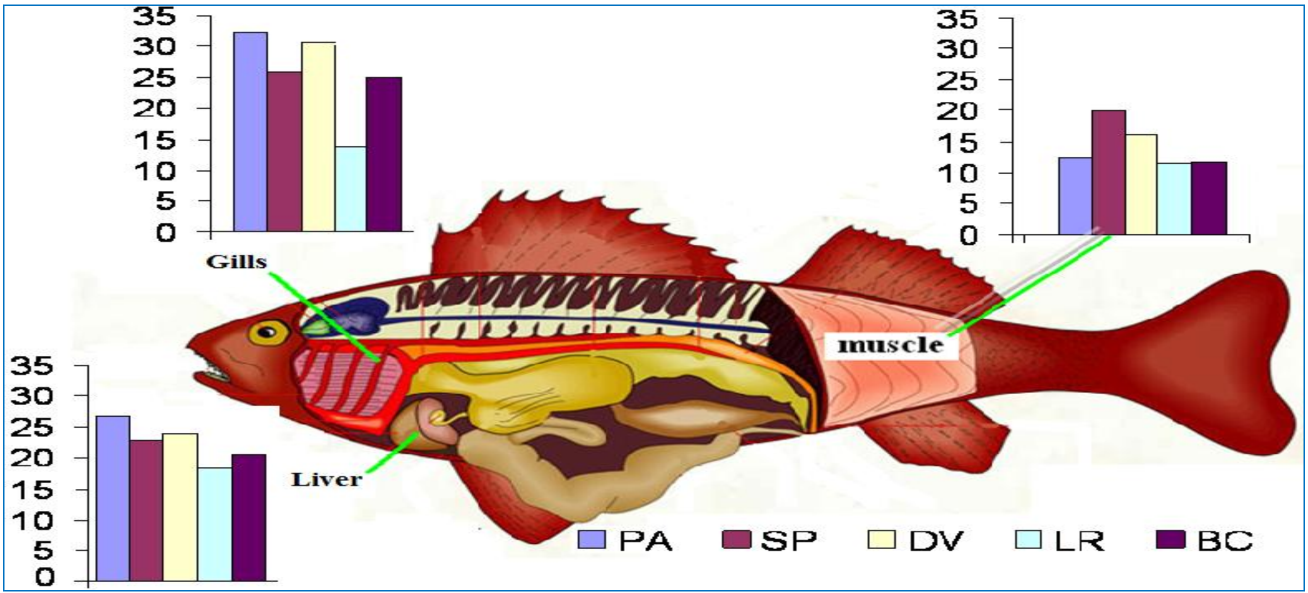

Figure 4 : Concentrations metallic means expressed in µg/g of the wet weight in the organs (gills, liver and muscles) at the five species of fish studied

Table 3: Norms of the contents of some toxic metals expressed in µg/g of the wet weight except AA in dry weight

Norms		Fe	Zn	Cu	Cr	Pb	Cd
Espagne (BOE, 1991)		-	-	20	-	1	-
Canada		-	100	100	-	-	-
Hungary		-	80	60	-	-	-
Australia		-	150	60	-	-	-
OMS/FAO/EPA		-	70	-	-	-	-
CEE/R n° 466/2001		-	-	-	-	0.2	0.05
Present study	PA	63.49	46.86	31.69	0.33	0.35	0.03
	SP	72.05	41.91	22.51	0.35	0.44	0.07
	DV	51.50	55.84	32.56	0.50	0.65	0.02
	BC	33.05	56.80	23.89	0.23	0.50	0.21
	LR	37.71	32.94	15.82	0.39	0.25	0.04
	AA	80.35	39.60	0.45	2.66	0.03	0.45

In fact, the norms change a country to another and those valued in account are especially the norms of the nearest countries. While comparing our results with these norms, we could deduct that the recorded values in the Loukkos river estuary remain below the values critical of contamination. The comparison of the recorded contents in these species of fish studied shows that the contents in essential elements (Fe, Zn and Cu) are higher than those the toxic elements (Cr, Pb and Cd). It is typically bound to their toxicity.

Heavy metals concentrations in the organs of *Diplodus vulgaris* follows the order Zn, Fe, Cu, Pb, Cr and Cd their distribution in the organs follow the pattern liver and muscle. The result of this study reveals the adverse health effect the people in the study area could be exposed to be the consumption of the gills, livers and muscles of *Diplodus vulgaris* which have been shown to accumulate high concentrations of heavy metals as it happens to be the most preferred and heavily consumed fish in human diet in the study area.

3.6. Correlation of the heavy metals between waters, sediments and glass eels

The accumulation of the heavy metals in the sediments can provoke serious problems in the environment. The contamination of the sediment by heavy metals could affect the water quality with an index of contamination (IC=1.03) and the bio-assimilation and bioaccumulation of metals in the aquatic organisms, while resulting from the changes long-term on the human health in particular and the ecosystem in general [55].

The concentrations of metal in the tissues of the glass eels are lower than those in the sediments. The bioaccumulation factors (BAF that is the content of metal in the organism in relation to the one in the sediments; [56];[57] and the bio-concentration factors of cadmium ($BCF_{(Cd)}$ = 0.002) are different when one compares one with and the other in the same tissues for the same metal. The BAF of Fe and Cd showed the more elevated values, for all elements, that are respectively 3.10% and 0.23%. The present results suggest that the content of the toxic metal, as the Cd, in the glass eels can increase with prolonged exhibition, and can put a threat therefore for the human health through the consumption of fish. In general, the metals that are absorbed by the superficial sediments are ingested by the glass eels and can be assimilated with the efficiencies depending on the type of sediment, the animal species and her physiology and metal herself. Metals are not submitted to the assimilation and of where are not bio-available to the benthic organisms [58-60].

The assessment of the heavy metals and their correlation in the Loukkos river estuary reflected the degree of pollution that is considered by a lot of regulating agencies one of the biggest risks for the aquatic environment. In general, the mean contents of Fe, Zn, Cu, Cr, Pb and Cd in the Loukkos river estuary are lower to the norms [39] which the mean concentrations (Fe, Zn, Cu, Cr, Pb and Cd) in the consumption of water are respectively higher (300, 3000, 1000, 50, 10 and 3 µg/l).

While being based on instructions, the direct use of the water of the Loukkos river estuary of without treatment can aggravate the man's health (Savory and Wills, 1991). For example, the rate for Pb in water for domestic use is 0 to 1.70 g/l [61]. To the levels > 100 g/l, the possible neurological damage can appear in the foetuses and the young children [62]. For some metals studied in the superficial sediments, the contents of the heavy metals in our survey were higher than those in the sediments of Port of Montevideo, Uruguay [63], Taylor Creek, south of Nigeria [64], Bouregreg estuary [51] and Om-Rbiâ estuary [19]. The more elevated mean concentrations of the Pb (69.26 mg/kg of the dry weight) and Cd (1.11 mg/kg of the dry weight) have been found in the sediment of the Loukkos river estuary, compared with those recorded in France [44].

For the levels of Cd, Pb and Cr, the correlation between metals seem to be different in the segments of the environment. While, the report between metals exists between Cu–Pb (r = 0.670) for the sediments ($P \leq 0.05$; Fe–Pb (r = 0.564), Cr–Pb (r = 0.560) and Cr–Fe (r = 0.552) for water ($P \leq 0.05$). Among the glass eels ($P \leq 0.05$) showed of the correlation considerable Zn–Fe (r= 0.976) that is higher to the coefficients of the correlation to those returned elsewhere [65-64]. It implies that the understanding for these metals can be a direct mechanism in the glass eels. The aforementioned observations can suggest that the mechanism of sorption of the heavy metals to the sediments of the Loukkos river estuary is ordered mainly by the chemical adsorption, rather than physical or deposition of metals with the sediments. The ions of metal can associate to the ligands by the utilitarian groups as –OH, –NH2, –CO_2H of the organic matter in the sediment and can produce them of the compounds steady organic-metals [67,68].

Conclusion

Whatever the source of metals, the results of the present study showed that concentrations of studied metals were very low all stations when compared to data obtained from uncontaminated waters. Inputs from the catchment area, significant biotic processes, and low concentrations of organic matter, suspended matter, salinity gradients and pH were the essential factors affecting the distribution and biogeochemical behaviour of heavy metals of the Loukkos river estuary. So, what does this all mean? Surface water in Loukkos river estuary based on chemical quality in comparison with World Health Organisation (WHO) standards is unsuitable for drinking because heavy metals of interest exceed permissible limits. The amount of metals in the sediments of the Loukkos river estuary have more to do with the number of water and fish and more to do with what fish studies especially *glass eels* goes on there. The major findings of this study are that heavy metals concentrations in the gills, liver and muscle tissue of

Anguilla anguilla and *Diplodus vulgaris* from Loukkos river estuary were slightly high and in general displayed significant variation from station to station.
Analytical results showed that the heavy metals which were found in glass eels are below the maximum limits set by European legislation for fish. However, the limits for cadmium remain higher in the glass eels and in sediments. This could be a major reason for the disappearance of this organism from aquatic systems. The presence of trace elements at low levels in the aquatic ecosystem could influence the sensibility of glass eels since those elements are often toxic even at a low concentration. Nowadays, glass eel is endangered in most countries of the world especially in European ones.
This situation should spur Moroccan authorities to prohibit their capture and researchers to investigate other possible causes which explain the decline of this organism in aquatic systems such as the negative impact of climatic change, biotope conditions and possibly the organic contamination with chemical substances especially pesticides residues.
Indeed, our research group is studying the effects of chemical contamination to assess completely the situation in Loukkos river estuary.

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