Nutrients, trace metals and B-vitamin composition of the
Moulouya River: a major North African river discharging into
the Mediterranean Sea.

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Abstract

We analyzed dissolved nutrient, trace metal and vitamin (B-vitamins and methionine) concentrations in the lower course of the Moulouya River (MR, Morocco) and its estuary. The flow of this African river has changed drastically (a reduction of almost 50 %) in the last 50 years due to the regulation of the river flow through dams and alterations of the course constructed to satisfy population necessities and growing agricultural requirements. Consequently, it has produced a remarkable increase in nitrate concentrations (up to 270 μM) and alteration of N:P ratios within the river, as well as a reduction of overall P and Si efflux to nearby coastal waters. Despite the historical mining activities in the upper MR, concentrations of Pb, Zn and other metals in sediments and waters do not display significant contamination as compared with other Mediterranean rivers, mainly due to the retention by dams of upstream metal contamination. Mean concentrations of dissolved B-vitamins in the river showed lower levels (13 to 55% lower) than those in coastal waters and hence the river does not represent an important B-vitamin source.

1. Introduction

Nutrients and other life-sustaining elements delivered to the coastal zone by large river systems are a major determinant of the functioning of coastal ocean ecosystems. The arrival of terrestrial substances to the marine system depends on both natural and anthropogenic loads from rivers and groundwater, as well as on the biogeochemical transformations that occur preferentially in transitional areas such as estuaries and deltas. These compounds have a key influence on sustaining the productivity of large shelf areas that are affected by the riverine outflows (e.g. Macias et al., 2014).

Because the Mediterranean Sea is a relatively small and semi-enclosed basin where oligotrophic conditions prevail for most of the year (Bosc et al., 2004; Ludwig et al., 2009), influence of freshwater inputs in these regions, associated with major river discharges, affect the water mass balance and the chemical species in coastal waters (Bosc et al., 2004). Most of the knowledge regarding riverine outflows in the Mediterranean Sea is based in studies carried out in large rivers from Southern Europe. For example the Rhone, Ebro and Po Rivers are known to have a major effect on the productivity of the NW Mediterranean (Ludwig et al., 2009; United Nations Environment Programme and Mediterranean Action Plan, 2003). Less well known is the role of North African rivers which are considered as resources for the economic development of the countries in this region. As a result of high, steep and young mountains with erodible rocks, a great amount of sediments are thought to be supplied by Morocco and Algerian rivers (McNeill, 2002). In fact, pre-dam northwest African rivers discharged nearly 20% of the sediments discharged by all African rivers (Probst and Suchet, 1992).

Arguably, the most significant ecological impact on North African rivers is the outstanding reduction in the river flow caused by damming, extraction for irrigation and climate change (Margat and Treyer, 2004). Apart from the obvious hydrological changes, which mainly affect estuarine and coastal areas, the reduction in the transport of particulate material together with changes in land-use, may influence the C, N and P cycles in the river and the delivery of these elements to coastal waters. Strong reduction in river flow may lead to coastal impacts such as shoreline retreat, estuarine water salinization, loss of arable lands and soil erosion, which thereby can alter the estuary topography and coastal stability. Moreover, the continuous inputs of nutrients and metals from urban and agriculture activities may lead to river eutrophication and pollution, which are considered a global threat for ecosystems, water quality, and aquatic chemistry (Cloern, 2001; Rabalais et al., 2009; Smith, 2003). These issues are particularly critical in undeveloped countries due to inadequate treatments of domestic and industrial sewage, and the lack of efficient urban development plans. In Africa these effects could be exacerbated by societal responses to global change pressures (Kitheka et al., 2009).

Three main African rivers discharge into the Western Mediterranean: the Medjerdah (Tunisia), the Chélif (Algeria), and the Moulouya (Morocco). The Moulouya River (MR) is the only African river with active influence in the basin of the Alboran Sea (Ludwig et al., 2009). It drains into a shallow and productive shelf area off of North Eastern Morocco, discharging particulate matter, nutrients and other compounds (Fig. 1). As a result of the damming of the MR, the Alboran Sea has experienced for a period of 20 years (from 1974 to 1994) the strongest reduction in the whole Mediterranean Sea of freshwater

discharged (approximately 57%) (Ludwig et al., 2009; United Nations Environment Programme and Mediterranean Action Plan, 2003; Milliman and Farnsworth, 2011). Set in a region with strong economic development needs, the ecological conservation of the lower course and deltaic area of the MR has been absent from governmental priorities until very recently.

Despite the growing concern on environmental issues in northern Africa and the increasing efforts on environmental monitoring and research, information on both river alteration and its effects on the coastal ocean is fragmented and mostly gathered in unpublished reports. The main objective of this paper is to characterize the chemical composition of waters and sediments of the lower-course and estuary of the MR. While trace metals and nutrient concentrations have been previously reported in the upper course of this river (Bouabdli et al., 2005; Chahboune et al., 2014; Iavazzo et al., 2012b; Makhoukh, 2011; Makhoukh et al., 2013) the concentrations in the lower course, below the last dam, and the contribution to the solutes in the coastal waters of the Mediterranean is uncertain. In addition to inorganic nutrients and trace metals, we have analyzed soluble B-vitamins (thiamin B₁, riboflavin B₂, pyridoxine B₆, biotin B₇ and cobalamin B₁₂) and the amino acid methionine. Vitamins are a major regulator of marine plankton metabolism but many aspects of their sources and fate remain unclear (Sañudo-Wilhelmy et al., 2014 and references therein). Because of their high bacterial activities, freshwater sources (such as rivers and groundwater) are considered important sources of vitamin B₁ and B₆ (Barada et al., 2013; Gobler et al., 2007; Okbami and Sañudo-Wilhelmy, 2005).

Knowledge on the biogeochemical characteristics of the lower MR and its estuary may help the understanding of past and future changes in the biogeochemical budgets of this region and the possible consequences in the Alboran Sea. This basin is a transition zone that plays a key role on the exchanges between the Atlantic Ocean and the Mediterranean Sea through the Strait of Gibraltar (Vargas-Yáñez et al., 2002).

2. Material and Methods

2.1. Site description

With 54,000 km², the Moulouya is the largest Moroccan river basin (Snoussi, 2007) and the second largest estuarine area in the North African coast only behind the Nile Delta. The river flows northeasterly from the Grand Atlas region (2000 m height) to the Mediterranean Sea not far from the Nador Lagoon, after some 650 km (Fig. 1). The lower Moulouya basin corresponds to a vast plain through which the river meanders along ~90 km and connects with the coast through a large estuary, flanked by extensive beaches and dune fields (Fig. 1). The lithology in the middle course is primarily sandstones (Pastor et al. 2015) whereas, the lower course runs through the Guercif basin and the Triffa Plain which is mainly composed of limestone and dolomites (Boudchiche, 1994; Ouahhabi et al., 1986; Naciri T., 1986).

Climate in the basin is typically Mediterranean arid to semi-arid, characterized by large variability in precipitation with yearly accumulated values ranging between 230 and 380 mm/yr (IUCN, 2010; Tekken et al., 2009). Climate in the upper course of the river is continental and rainfall exceeds 600 mm/yr. Available information on annual water discharge by the MR reveals

large variations in mean flux values, most probably indebted to large climate and water use variations during the considered period of flux estimation (Snoussi, 2007).

According to the Agence du Bassin Hydraulique du Moulouya (ABHM), upstream of the Mohamed V dam, the mean annual flow is $34 \text{ m}^3/\text{s}$ (IUCN, 2010). Maximum flow ($80 \text{ m}^3/\text{s}$) at this site is attained during the spring, especially in April, and it coincides with the melting of snow in the High and Middle Atlas. Downstream, the flow is reduced to $5 \text{ m}^3/\text{s}$ and sometimes less (IUCN, 2010). In this lower section, the Moulouya receives on its southern banks the waters of Wadi Sharaa, whose tributary Wadi Zegzel drains the limestone mountains of Beni Snassen. The latter has a mean annual flow of $135 \text{ Mm}^3/\text{s}$, which corresponds in its majority to flashfloods because the base flow barely exceeds $0.1 \text{ m}^3/\text{s}$ (Snoussi, 2007). Also in this area, land drainage from irrigation and from aquifer seeps is estimated to contribute 2 to $3 \text{ m}^3/\text{s}$ to the total flow (IUCN, 2010). Further south, the Wadi Cherraa, that runs through the city of Berkane, is derived for irrigation and is often dry before joining the main course of the Moulouya. The water is accumulated in 5 reservoirs with a total storage capacity of 11000 m^3 (CID, 2012). The city of Berkane, also dumps some $0.024 \text{ m}^3/\text{s}$ of treated waste water to the Moulouya (through a sewage treatment plant 'SWTP' located in the city) as well as some $0.110 \text{ m}^3/\text{s}$ that are discharged untreated (CID, 2012). According to Snoussi et al. (2002) the construction of dams and changes in rainfall have reduced the annual flow and sediment fluxes in the MR up to 47% and 93%, respectively. This supply of sediment is not entirely prevented since high sediment discharges to the sea

may occur when water from the dam is released following most heavy rains (Fig. S1).

The economy of the Moulouya basin depends mainly on agriculture and cattle, but also on industrial activities concentrated in the urban centers, and some incipient tourism activities on the coast (Kitheka et al., 2009). Lead mining in the upper river was an important activity until 1985 when the main mines, already depleted, were abandoned (Iavazzo et al., 2012b). Lead and Zn have been identified among the main metallic contaminants resulting from this activity (Iavazzo et al., 2012a). River damming together with the intensive use of water resources and poor treatment of effluents in the lower MR has produced further environmental consequences affecting, among others, the ecological values and functions of the wetland. Freshwater coming from the upper MR is stored behind the Mohammed V dam (built in 1967), sent to the Mechraâ-Hammadi dam (built in 1956 at the Moulouya gorge, on the western edge of Beni Snassene), from which it is diverted towards cultivated areas through a network of minor superficial channels (Dakki, 2003; Re et al., 2014). According to Snoussi et al. (2008) the MR delta represents one of the most important wetlands of the Mediterranean coast of Morocco, although it presents remarkable recession since the dam was built. The river suffers problems of salinization and contamination by untreated or insufficiently treated domestic and industrial wastewaters, as well as by the intensive use of fertilizers in the increasingly intensive irrigated agricultural areas. These problems have affected regional biodiversity and have decreased the economic values due to cessation of activities such as fishing, aquaculture and shellfish (Khattabi A., 2003).

2.2. Sampling and analysis

Two surveys (24-26 June, 2012 and 22-23 April, 2013) were carried out to perform the chemical characterization of the lower MR. Sediment and water samples were examined at 8 and 9 sites, respectively, between the Mechraâ-Homadi dam and the coastal waters (Stations A - I; Fig. 1b). Stations A and B were located just before and after the dam, respectively, at 90 Km from the river mouth. Water sampling stations were complemented with 26 hydrographic stations (i.e., CTD) located in the estuarine area, in order to obtain detailed characterization of the frontal area between the fresh and seawater. A grid of 21 stations was also sampled on the coast around the mouth of the river (estuarine area; Fig. 1b). This grid included 13 nearshore stations (P1 - P8 and 45 - 49) and 8 outer stations (1 - 8). At each station a YSI CasTaway-CTD was submerged to obtain temperature and salinity profiles. In the stations with shallow water depth, the CTD was left to register stationary at an approximate 0.25 m depth.

Surface water samples were obtained at each station and both surveys to analyze concentrations of nutrients (NO_2^- , NO_3^- , PO_4^{3-} and SiO_2), trace metals (Ag, Cd, Co, Cu, Fe, Mo, Ni, Pb, V, Zn) and chlorophyll-a (Chl-a). Samples for B-vitamins (Thiamine: B₁, Riboflavin: B₂, Pyridoxine: B₆, Biotin: B₇, Cobalamin: B₁₂), the amino acid methionine, and for microphytoplankton identification were taken only in June 2012.

Samples for nutrient analysis were collected in triplicate, filtered through 25 mm Whatman GF/F filters and immediately frozen for subsequent analysis at the laboratory. Concentrations of dissolved nutrients were determined with an autoanalyzer (Alliance Futura) using colorimetric techniques (Hansen and

Koroleff, 1999). The accuracy of the analysis was established using Coastal Seawater Reference Material for Nutrients (MOOS-1, NRC-CNRC) (Table S1). Blanks were prepared using salt water (36 g NaCl/L). Information about limits of detection is included in Table S2.

Samples for trace metal analysis in surface waters were collected using clean protocols (Tovar-Sánchez, 2012). Water samples were filtered through an acid-washed 0.22 µm polypropylene Calyx capsule filter and collected in acid-cleaned 0.5 L LDPE bottles. Trace metal samples were acidified to pH<2 with ultrapure grade HCl (Merck) in a class-100 HEPA laminar flow hood and stored for at least 1 month before extraction. Dissolved metals were pre-concentrated without UV-oxidation by the Ammonium Pyrrolidine Dithiocarbamate (APDC, 99% SigmaAldrich) and diethylammonium diethyldithiocarbamate (DDDC, 97% SigmaAldrich) organic extraction method (Bruland et al., 1979; Tovar-Sánchez, 2012) and analyzed by ICP-MS (PerkinElmer ELAN DRC-e). The accuracy of the analysis was established using Coastal Seawater Reference Material for trace metals (NASS-5, NRC-CNRC), with recoveries ranging from 94% for Co to 109% for Zn (Table S1). Blanks were prepared using ultrapure water and treated like the samples (concentrations and limit of detections are included in Table S2).

Water samples for vitamin and methionine analyses were filtered with acid-washed 0.22 µm polypropylene Calyx capsule filters and collected in acid-cleaned 2 L high-density polyethylene (HDPE) dark bottles and stored frozen until analysis. Dissolved B-vitamins and methionine were extracted and pre-concentrated in solid-phase extraction onto a C₁₈ resin and analyzed using

liquid chromatography/tandem MS following the method described by Sañudo-Wilhelmy et al., (2012).

The concentration of Chl-a in water samples was determined through fluorometric analysis (Parsons et al., 1984). The filters were extracted in 90% acetone overnight and fluorescence was measured on a Turner Designs fluorometer calibrated with pure Chl-a (Sigma Co.).

Microphytoplankton abundance and taxonomy was determined using an inverted light microscope. Water samples (150 ml) were preserved with Lugol's iodine solution. Once in the laboratory, a 25 ml aliquot was settled in a counting chamber for 1 day and subsequently enumerated on the microscope.

Sediment samples were obtained where possible using a Van-Veen grab. All samples were washed, the organic matter was removed using hydrogen peroxide (30%; Jackson, 2005), and then they were dried in the oven for 48 h at 60°C. An approximate amount of 500 g was dry sieved for 15 min. Sediment material for each size fraction was weighed to the nearest 0.01 g and grain size distribution and textural parameters were subsequently calculated. Metal concentrations (Ag, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, V and Zn) were determined by ICP-OES (Perkin Elmer ICP-OES Optima 5300 DV). Before analysis, samples were dried in an oven at 60°C for two days, for grain size correction the sediments were subsequently homogenized in a zirconia ball mill (10 minutes at 170 rpm). Mercury concentrations were determined by a Direct Mercury Analyzer (Milestone DMA-80). Metals were extracted by a microwave acid digestion model Mars 5 (CEM) according to SW-846 EPA Method 3051A (EPA, 2007). Sediment samples (0.2 g) were digested by triplicate in Teflon vessels with 10 ml of nitric acid (65%, Suprapur quality). After digestion

samples were diluted to 50 ml of Milli-Q water and were analyzed. The accuracy of the analysis was checked with marine sediment certified reference materials, MESS-3 and PACS-2 (National Research Council Canada; recoveries are summarized in Table S1). Blanks were prepared using nitric acid (65%, Suprapur quality) and treated like the samples. Detection limits were calculated as three times the standard deviation of the blank values (Table S2).

3. Results and Discussion

3.1. Hydrology and bed sediments

In both surveys (June 2012 and April 2013), river salinities (PSU: Practical Salinity Unit) displayed a similar trend, being < 1 in the upper stations (A to C) and progressively increasing to 2.47 downstream at the agricultural area and the urban areas of Zaïo and Berkane (St. I). These fluctuations of salinity < 3 are not related to the direct influence of seawater, instead they are attributed to the influence of the underlying salinized aquifers (Chettouani and Domou, 1993; Fekkoul, 2013).

Flow measured at SafSaf (see Fig. 1b) by the ABHM (personal communication) indicated that flow was double in April compared to June, when water is intensively used for agriculture (7.18 and 3.50 m³/s respectively). Despite these flow variations, the water mass structure in the estuarine zone and marine area was very similar in both surveys. Fresh water extended to a depth of 2 meters and formed a sharp and intense front near the mouth of the estuary (Fig. 2). The fresh river water flowing out over saltier marine water created a strong halocline that inhibited vertical mixing. Salinity values indicated

little vertical exchange between river water and the underlying marine mass within the estuarine area. Indeed, the estuary maintains a strikingly stable two-layer structure along the last 7 km of the estuary. Poor mixing in the estuarine zone is relevant to the geochemistry of the river because of the influence of salinity on the chemical speciation and in the regulation of particulate matter and its associated elements like P or metals. The settling velocity of cohesive sediment may vary largely in time and space by flocculation effects affecting turbidity and water quality (Winterwerp, 2002). Therefore, processes increasing vertical mixing (i.e., sudden discharges from the dam) could be an important regulator of the elements in this river area. Not only does the weak outflow favor the reduced mixing but, also, other factors such as the reduced tidal mixing and low wave propagation through the estuary mouth prevent water column homogenization. Once in the sea, the low salinity plume extended eastwards in both surveys; however, most of the surveyed area presented lowered surface (0.5 m) salinities, particularly in June when the river plume was less dispersed (Fig. 2).

The particle size composition of riverbed sediments was highly variable throughout the river course. Not surprisingly, sediments at Mechraâ-Hammadi dam (St. A) were almost exclusively composed by cohesive silt and clay-sized particles (100% <63 μm) revealing high fine sediment accumulation at this point (Table S3). A similar material was predominant (99%) in the first station downstream the dam (St. C) but the proportion of this sediment type was rapidly reduced downstream which is attributed to a sediment load reduction and a natural flow modification at the dam. Indeed, beyond this point (St. D to St. I), sediment grain sizes were bimodally composed of a mixture between fine-

grained sediments (possibly transported from the upper course) and coarser-grained materials (fine sands to medium sands), resulting either from relict sediments or from differences in grain-size reduction among lithologies. The sand fraction in these stations accounted for $85 \pm 21\%$ of the total sediment. Sediments in the sea were mainly fine to medium sand (170 to 310 μm) with a percentage of fine fractions $< 0.5\%$ (Table S3). In accordance with this data, only a relatively small percentage of the continental materials from the drainage area should be reaching the sea under the dam-regulated conditions.

Despite the historical mining activities in the upper MR, the average metal concentrations in the sediments of the surveyed area were moderate and below the levels reported for other major Mediterranean rivers (e.g., Ebro, Evros, Nile, Po; Table 1). A previous work reported the elevated concentration of Pb and Zn were restricted to the mining district in the upper course of the river by the different dams located along the river (Makhoukh, 2011). The highest concentrations were measured in those stations with lowest sediment grain size (99-100 % $< 63 \mu\text{m}$; i.e., St. A and St. C; see Table S3 and Fig. S2). Similarly to other aquatic systems, most metals (Al, Cr, Mo, Ni, Pb, V, and Zn) presented good correlation with the fine-grained sediments and, hence, the bulk metal concentrations in sediment decreased with distance from the dam. The crustal enrichment factor (EF_{crust}) reveals sediment contamination by Cu, Mo, Pb and Zn (Fig. 3). Lead is particularly enriched at station A (behind the dam; EF : 6.8), whereas Cu and Zn are enriched after Berkane, suggesting that upstream mining is the source of the former and wastewater from the SWTP contributes to the latter. Molybdenum is highly enriched throughout the study area. The highest EF is calculated for Mo (EF ranging from 11 to 26). High Mo

EF has been also observed in other river and estuarine sediment studies but the sources are unclear (desorption from sediments, anthropogenic inputs, and other internal processes; Tribovillard et al., 2004; Jones, 1974; Adelson et al., 2001).

As expected by the low fine sediment content in the marine environment, the coastal sediments showed the lowest metal concentrations. This indicates that the transport of contaminated sediments to the coast is reduced. These values are of the same order as other non-contaminated coastal and estuarine sediments of the Mediterranean (Barak Herut, 1993).

3.2. Solutes in river and estuarine waters

Mean nutrient, Chl-a, trace metal and B-vitamin (and methionine), concentrations measured along the lower course of the MR and nearby coastal waters are presented in Table 2 (the complete dataset for each station is presented in Table S4). Figure 4 displays the along river trends of NO_x (NO₂⁻ + NO₃⁻), PO₄³⁻ and SiO₂. In June, NO_x concentrations linearly increased from St. B until St. G suggesting important agricultural inputs along the lower Moulouya. High concentrations (50 µM) were also observed along the coast in the shoreline stations but rapidly declined seawards to values ~10 µM. Contrastingly, NO₃⁻ concentrations at Mechraa-Hammadi dam in April were half those in June. In this survey, NO_x increased at the boundary of the agricultural area (St. C) but remained relatively stable downstream (74.7 ± 1.6 µM). This enrichment could be attributed to diffuse seeps from the nutrient enriched aquifer (Fekkoul, 2013; Fetouani et al., 2008).

Phosphate was outstandingly enhanced above the dam in June (13.9 μM) but declined along the course of the river reaching approximately 1.5 μM by the sea. In April, PO_4^{3-} concentrations oscillated between 1 and 3 μM but, as with NO_x , no clear trend was observed. Unlike NO_x and PO_4^{3-} , SiO_2 patterns in the river were consistent in both surveys. However, concentrations were 24% higher in April, when flow was higher, suggesting a natural source of this element associated with river flow variations (Fig. 4). Changes in the nutrient concentrations produced dramatic differences in the N:P ratios in coastal waters with N:P ratios reaching values of 209 ± 178 (median value for coastal stations) when nitrate leaching from agriculture was higher. In contrast, the Redfield ratio was lower (median N:P = 4 ± 20).

Considering flow measurements at SafSaf and the concentrations at this site (stations D and E), the net NO_x , PO_4^{3-} and SiO_2 fluxes at this point were 606 ± 26 , 14.5 ± 3.7 and 426 ± 12 mmol/s in June and 534 ± 5 , 4.0 ± 3.5 and 114 ± 115 mmol/s in April. Nitrate leaching is a highly dynamic process (e.g., Lin et al., 2001) and, besides the seeps from the aquifer, NO_3^- increases in the river could be partially controlled by irrigation practices, which peak in the dry period of the year (June-August) (see Fetouani et al., 2008). However, there is also a more permanent enrichment that could be attributed to domestic discharges and to diffuse seeps from the nutrient-enriched aquifer. Considering the salinity values and NO_3^- concentration in ground waters reported by Fekkoul, (2013), we have estimated a water flow of 1.2 m/s from the aquifer with that could be seeping into the river. This flux is consistent but in the lower range of previous estimates (IUCN, 2010). In the case of PO_4^{3-} , identification of the sources seems more complex. Changes in PO_4^{3-} could be attributed to

variations in the agricultural and/or urban inputs in the upper course of the river and to the sorption of sediment, binding and precipitation with available Fe (Benitez-Nelson, 2000). In any case, the river presents an overall buffering effect on dissolved phosphate concentrations either because it is retained by interactions with particulate matter or by its transformation between inorganic and organic forms by river biota.

Fuelled by the increased nutrient availability, particularly NO_3^- , mean phytoplankton biomass in the river was twofold greater in June with values exceeding 5 mg/m^3 . Biological activity and accumulation are enhanced during summer season, and this could also be favored by the increased temperatures (6.5°C higher in June) and the reduced flow. The uptake in the river has potential effects on the form of nutrient delivery (i.e., variation from inorganic to organic forms) to the coastal sea. However, in the case of the MR, inorganic nutrient concentrations seem sufficient to sustain high coastal standing stocks in both seasons ($> 2 \text{ mg/m}^3$). The freshwater community in this survey was dominated by cyanobacteria (64% of total abundance) whereas diatoms and cryptophytes dominated on April (Fig. 7). Despite the lower biomass in oceanic waters of the Mediterranean Sea in June, mean chlorophyll concentrations on the coast were comparable in both surveys (2.8 ± 0.8 and $2.5 \pm 0.8 \text{ mg/m}^3$; Table 2), showing that the productivity on the coast is sustained even during the oligotrophic season. Phytoplankton biomass was also enhanced in both surveys in the shoreline stations ($>4 \text{ mg/m}^3$) either by shoreline accumulation or by the influence of groundwater seeps through the dune field (Carneiro et al., 2010). The community at the coastal stations in April displayed high dominance of dinoflagellates (82% of total abundance) and diatoms (11%). Conversely,

diatoms were most abundant in the coastal community assemblage (64% of total abundance) (Fig. 7).

Dissolved trace metal concentrations in river and coastal waters varied between surveys and two groups could be differentiated (Table 2, Fig. 5-6). A group of metals (Co, Cu, Ni and Zn), known to be enriched in the upper MR (Bouabdli et al., 2005; Bounakhla et al., 2012), showed higher concentration in April than in June as a consequence of the higher river flow and lower water retention by dams. On the other hand, concentrations of those metals not influenced by the mining activities, such as Ag and Fe, were higher in June than in April, suggesting that the variation of concentrations for these elements is mainly influenced by other sources, like sewage discharge occurring in the lower MR (see below).

In both surveys, concentrations of Ag, Co, Cu, Fe, Ni, V and Zn in the river waters (stations A to I) were higher than those measured in the coastal stations, suggesting that the MR could be a source of these elements to the coastal waters (Tables 2 and S1, Fig. 5). In contrast, concentrations of elements such as Mo and Cd were lower in the river than in coastal waters showing a conservative distribution (Table 2 and Fig. 6). This is consistent with other studies (Tovar-Sanchez and Sañudo-Wilhelmy, 2011) showing that river areas are not a significant source of these elements, but their concentrations in coastal waters are diluted in the areas of mixing among freshwater and seawater. Average Pb concentrations did not show clear differences between fresh (June: 0.2 ± 0.03 nM, April: 0.3 ± 0.1 nM) and coastal waters (June: 0.2 ± 0.01 nM, April: 0.2 ± 0.01 nM) and therefore, despite a previous report of Pb

contamination (Makhoukh, 2011), no enrichments or further sources are identified throughout the lower course of the river.

In MR the concentrations of dissolved Ag, Co, Cu, Fe, Ni, Pb, V and Zn, as a function of salinity showed non-conservative excesses (exponential fits) related to simple mixing of freshwater and seawater (Fig. 5). The increased concentration with distance from the riverine end-member (stations A-B) suggests that levels of these trace elements are influenced by the presence of additional sources. Concentrations of Ag, Fe, V and Zn were enhanced in the middle section of the river (St. E-G), which is under the influence of the effluents from Zaio and Berkane (see Fig. 1). As observed in other rivers, urban effluents are important sources of these metals (Kihampa, 2013). As for sediments, average metal concentrations in the waters of the lower MR were slightly lower than those reported in other Mediterranean rivers (Table S5).

Dissolved ($<0.22\ \mu\text{m}$) B-vitamin concentrations varied widely among river and coastal stations with the maximum concentrations measured in the coastal area (B_1 : 14.2 pM, St 47; B_2 : 8.1 pM St 46; B_6 : 17.4 pM, St H; B_7 : 20.8 pM, St P6; B_{12} : 1.2 pM St 49; methionine: 111.5 pM, St 46). These concentrations are in the range of those reported for oceanic waters (Barada et al., 2013; Sañudo-Wilhelmy et al., 2012, 2014). Freshwater input from the Amazon River was also discarded to be an important source of vitamins of the coast of French Guiana (Barada et al., 2013).

Fig. 7 reveals the along-river concentrations of vitamins and methionine. Different trends were observed in the vitamins analyzed. For example, methionine and vitamin B_2 were enriched in the dam and nearby downstream waters (St. B to D). At these stations cyanobacteria were abundant (up to

114,000 cells/L). Conversely, an increase of vitamin B₁ and B₆ is observed downstream. Other vitamins such as B₁₂ were almost depleted or did not display clear patterns. Opposite responses in the various B-vitamins is not rare since their availability in water is governed by the specificity of the predominant phytoplankton species for those vitamins (Sañudo-Wilhelmy et al., 2012). In our case, different values of various B vitamins (i.e., methionine, B₂ and B₁₂ depletion) were observed in the estuarine and coastal areas where the phytoplankton assemblages changed from dominance of diatoms to dinoflagellates (Fig. 7).

As in previous studies, the origin of the vitamins is uncertain (Sañudo-Wilhelmy et al., 2014). In this study dissolved vitamins B1 and B6 present a negative correlation ($p < 0.05$) with the concentrations of metals in sediments (i.e., Al, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, V and Zn), suggesting a link between sediment composition and vitamin distribution (Table S6). Previous studies have suggested that heterotrophic microbial communities at the water-sediment interface are involved in the generation of vitamins, and are the main source of vitamin to the water column (e.g., B₁₂) (Liu, 2013). Unfortunately, we were not able to characterize the microbial composition of our sediments. However, since bacterial community abundance and composition are conditioned by the particle size of sediments (Jackson and Weeks, 2008), different microbial composition might be influencing the levels and distribution of dissolved vitamins in the MR and its estuary.

3.3. Assessment of the influx in the Alboran Sea

Although highly affected by the mining activities in the upper course, constructions of dams along the MR have conditioned the flow regulation and discharge of trace metals to the Mediterranean Sea (with lower concentrations in waters and sediments than other Mediterranean rivers). The role and contribution of MR to the biogeochemical cycle of trace elements and nutrients of the SW Mediterranean Sea will be conditioned to changes in the water flows regulated by the different dams located along the river. Thus, the growing population in the Moulouya Basin (with 3,371,000 inhabitants in 2004 (IUCN, 2010), along with the expected growth (around 1% during the next decades (Tekken and Kropp, 2012)), the expansion of the irrigated agriculture and the intensified effects of climate change (e.g., decrease of the precipitation in the area of ca. 7% from 1971 to 2005; Tekken and Kropp, 2012), could modify the water regimen of the river. This could imply different inputs of material and elements from the upper river with important consequences in the coastal environment (e.g., loss of canopy, increasing erosion, etc.) (Tekken and Kropp, 2012).

Table 3 shows the estimated annual discharge (mol/year) of studied elements in the MR to the Mediterranean Sea. For this calculation we have used the average dissolved concentrations measured at the last river station (i.e., station I for nutrients and metals and station 49 for vitamins and methionine; Table S4). Using the mean annual flow of 34 m³/s (IUCN, 2010) and the reported concentration of some metals measured in the upper MR after the mining district (Bouabdli et al., 2005) we have estimated the hypothetical discharge to the Mediterranean Sea without the retention effect of the dams (Table 3). We do not have information on pre-dam values of nutrients, but there

is clear evidence that N has increased because of the expansion of irrigated agriculture and fertilization in the lower MR and the flux should be nowadays markedly higher (Bendra et al., 2012; Sardinha et al., 2012; Fekkoul, 2013). Conversely, our estimations reveal that the discharge of metals such as Cd, Cu, Pb and Zn is notably reduced by the dam. Flow to the ocean could be up to 4, 2, 3 and 2 orders of magnitude higher than current levels without this artificial retention.

In conclusion, although having lower concentrations than other river and estuary systems in the Mediterranean, the MR is a source of inorganic nutrients and trace metals (i.e., Ag, Co, Cu, Fe, Ni, Pb, V and Zn) to the Alboran Sea. According to N and P flux estimates shown in Table S7, the Moulouya could be contributing 37% of the inorganic nitrogen and 3% of the inorganic phosphate that are delivered by all the rivers into the Alboran Sea. Fluxes of these elements have notably varied due to extended dam construction in this region and to increasing anthropogenic pressure. Moreover, although sediment-associated metals are retained by the dam and in the river during periods of low discharge, they could be resuspended and transported offshore during flood events. The MR constitutes a significant, but now hazardous, contribution to the sustainment of the productivity of the North African shelf and therefore, to overall Mediterranean budgets.

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Table 1. Comparison of metal concentrations measured in the sediments of the MR (stations A to I) with values reported in the literature from other Mediterranean rivers. Min – Max and (Mean ± SD), Values in µg/g. n.m.: not measured; n.d.: not detected.

River	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Ebro ⁽¹⁾	n.m.	0.2 - 1.2 (0.6 ± 0.3)	n.m.	n.m.	2 - 37 (9 ± 8)	n.m.	0.1 - 1.5 0.3 ± 0.4	n.m.	n.m.	mar-70 (17 ± 20)	21 - 198 (82 ± 63)
Evros ⁽²⁾	n.m.	0.2 - 1.6 (0.4 ± 0.4)	n.m.	n.m.	59 - 710 (170 ± 180)	n.m.	n.m.	n.m.	n.m.	66 - 219 (111 ± 40)	69 - 246 (111 ± 54)
Nile ⁽³⁾	n.m.	1.7 - 3.0 (2.4 ± 0.5)	n.m.	37 - 46 (41 ± 3)	27 - 90 (46 ± 19)	34000 - 81000 (47000 ± 15000)	n.m.	290 - 640 (500 ± 133)	54 - 65 (60.6 ± 3.3)	2 - 685 (90 ± 241)	20 - 262 (97 ± 68)
Po ⁽⁴⁾	6 - 11 (8 ± 2)	0.3 - 1.5 (0.8 ± 0.4)	n.m.	77 - 233 (145 ± 31)	42 - 178 (75 ± 34)	45000 - 53000 (49000 ± 2700)	0.1 - 0.4 0.2 ± 0.1	355 - 1653 (872 ± 284)	99 - 237 (141 ± 33)	26 - 72 (44 ± 14)	91 - 519 (213 ± 106)
Rhone ⁽⁵⁾	0.2 - 3 (1 ± 1)	0.9 - 1.0 (1.0 ± 0.1)	n.m.	66 - 98 (82 ± 15)	36 - 51 (42 ± 8)	14000 - 27000 (22000 ± 5000)	0.1 - 0.3 0.2 ± 0.1	273 - 523 (436 ± 114)	33 - 54 (45 ± 10)	53 - 74 (59 ± 10)	131 - 193 (157 ± 29)
Tafna ⁽⁶⁾	n.m.	1.1 - 1.6 (1.4 ± 0.2)	8.3 - 10.5 (9.4 ± 1.1)	26 - 34 (29 ± 4)	13 - 26 (20 ± 7)	12500 - 13000 (13000 ± 140)	n.m.	279 - 303 (290 ± 11)	n.m.	15 - 30 (23 ± 8)	59 - 120 (85 ± 30)
Moulouya ⁽⁷⁾	6 - 10 (9 ± 1)	0.1 - 1.9 (0.3 ± 0.3)	n.m.	n.m.	12 - 27 (18 ± 4)	n.m.	n.m.	n.m.	n.m.	12 - 157 (63 ± 34)	28 - 192 (78 ± 44)
Moulouya ^(*)	n.d. - 16.9	n.d.	4.8 - 11.1 (8.1 ± 2.1)	sept-63 (32 ± 18)	17 - 58 (38 ± 13)	12000 - 34000 (23000 ± 7200)	0.001 - 0.02 0.01 ± 0.01	292 - 441 (367 ± 58)	13 - 26 (18 ± 4)	6 - 33 (13 ± 9)	23 - 89 (57 ± 24)

1. Ramos et al., (1999); 2. Boubonari et al., (2008); 3. Lasheen and Ammar (2008); 4. Davide et al (2003); 5. Santiago et al., (1994); 6. Amel and Youcef (2014); 7. Makhoukh (2011); *This study

Table 2. Mean concentrations $\pm$ S.E. of dissolved nutrients, Chl-a, trace metals, B-vitamins and methionine in the lower MR (River: stations A to I; n=9) and coastal waters (Sea; n=21) during June 2012 and April 2013. n.m. not measured.

	River						Sea					
	June 2012			April 2013			June 2012			April 2013		
NO₂⁻ (μM)	0.8	$\pm$	0.2	1.0	$\pm$	0.2	0.3	$\pm$	0.1	0.8	$\pm$	0.0
NO₃⁻ (μM)	187	$\pm$	26	67	$\pm$	4.4	105	$\pm$	16	5	$\pm$	1
PO₄³⁻ (μM)	4.9	$\pm$	1.5	0.8	$\pm$	0.1	0.6	$\pm$	0.1	1.0	$\pm$	0.4
SiO₂⁻ (μM)	110	$\pm$	12	140	$\pm$	16.7	38	$\pm$	6	66	$\pm$	9
Chl-a ($\text{mg}\cdot\text{m}^{-3}$)	4.4	$\pm$	0.2	2.0	$\pm$	0.2	2.8	$\pm$	0.2	2.5	$\pm$	0.2
Ag (μM)	4.5	$\pm$	1.1	<0.1	$\pm$	-	3.6	$\pm$	0.3	3.5	$\pm$	-
Cd (nM)	<0.01	$\pm$	-	0.04	$\pm$	0.00	0.1	$\pm$	0.0	0.3	$\pm$	0.0
Co (nM)	1.3	$\pm$	0.2	2	$\pm$	0.2	0.5	$\pm$	0.0	0.7	$\pm$	0.1
Cu (nM)	6.1	$\pm$	0.5	8	$\pm$	0.6	3.5	$\pm$	0.2	5.0	$\pm$	0.2
Fe (nM)	385	$\pm$	59	272	$\pm$	117	30	$\pm$	12	24	$\pm$	3
Mo (nM)	20	$\pm$	2	13	$\pm$	0.37	90	$\pm$	6	135	$\pm$	4
Ni (nM)	8.5	$\pm$	0.8	9.8	$\pm$	0.8	3.7	$\pm$	0.3	5.4	$\pm$	0.3
Pb (nM)	0.2	$\pm$	0.0	0.3	$\pm$	0.1	0.2	$\pm$	0.0	0.2	$\pm$	0.0
V (nM)	12.9	$\pm$	1.4	n.m.	$\pm$	-	7.7	$\pm$	1.2	9.7	$\pm$	-
Zn (nM)	17.2	$\pm$	3.8	54.8	$\pm$	22.9	3.1	$\pm$	0.2	6.0	$\pm$	1.4
B1 (μM)	4.5	$\pm$	1.0	n.m.	$\pm$	-	6.6	$\pm$	1.4	n.m.	$\pm$	-
B2 (μM)	2.0	$\pm$	0.4	n.m.	$\pm$	-	4.1	$\pm$	0.7	n.m.	$\pm$	-
B6 (μM)	5.3	$\pm$	2.2	n.m.	$\pm$	-	8.9	$\pm$	1.4	n.m.	$\pm$	-
B7 (μM)	6.7	$\pm$	1.1	n.m.	$\pm$	-	7.8	$\pm$	1.7	n.m.	$\pm$	-
B12 (μM)	0.3	$\pm$	0.1	n.m.	$\pm$	-	0.6	$\pm$	0.1	n.m.	$\pm$	-
Methionine (μM)	29.5	$\pm$	7.5	n.m.	$\pm$	-	13.8	$\pm$	9.6	n.m.	$\pm$	-

Table 3. Comparison of annual discharge of studied elements in the MR to the Mediterranean Sea calculated with concentrations measured in this study and those reported previous to the dam construction by Bouabdli et al. (2005) in the upper MR (values in mol/yr). For this calculation we have used the average concentrations measured in the last river station (i.e., station I for nutrients and metals, and station 49 for vitamins and methionine).

Element	This study		Pre-dam
NO_2^-	$1.8 \cdot 10^5$	$\pm 3.5 \cdot 10^4$	
NO_3^-	$2.8 \cdot 10^7$	$\pm 2.2 \cdot 10^7$	
PO_4^{3-}	$1.2 \cdot 10^5$	$\pm 2.2 \cdot 10^3$	
NH_4^+	$7.9 \cdot 10^3$		
SiO_2	$2.7 \cdot 10^7$	$\pm 4.5 \cdot 10^6$	
Ag	0.4	± 0.5	
Cd	4	± 5	$1.5 \cdot 10^3 \pm 1.1 \cdot 10^3$
Co	300	± 200	
Cu	1100	± 300	$1.5 \cdot 10^5 \pm 1.3 \cdot 10^5$
Fe	$3.1 \cdot 10^4$	± 40	
Mo	2400	± 500	
Ni	1800	± 900	
Pb	24	± 14	$1.7 \cdot 10^4 \pm 2.6 \cdot 10^4$
V	1100	± 1500	
Zn	1700	± 300	$1.8 \cdot 10^5 \pm 18.5 \cdot 10^4$
B₁-Thiamine	2.2		
B₂-Riboflavin	1.2		
B₆-Pyridoxine	1.4		
B₇-Biotin	0.5		
B₁₂-Cobalamin	0.2		
Methionine	0.3		

Fig. 1. (a) MR basin extending from the Atlas Mountains to the Alboran Sea. Topographical data obtained from ETOPO1 (Amante and Eakins 2009). (b) Schematic map of the lower MR including the sampling points in the river and coastal area. The grid in the river mouth is shown in detail in the inset. c) Cross section of the lower MR indicating the position of the tributaries from Zaio and Berkane and the salinity ranges in each river section. The blue dashed lines indicate sections with different salinity ranges. The red dashed line indicates the estuarine area shown in Fig. 2.

Ref: Amante, C. and B.W. Eakins, 2009. ETOPO1 1 Arc-Minute Global Relief Model: Procedures, Data Sources and Analysis. NOAA Technical Memorandum NESDIS NGDC-24. National Geophysical Data Center, NOAA. doi:10.7289/V5C8276M

Fig. 2. (Top). Vertical salinity distributions along the estuarine area (see Fig. 1) during both surveys showing the fresh river water flowing out over the saltier marine water in the last 7 km of the river. The white dashed line indicates the position of the river mouth. (Bottom) Sea-surface salinity (~ 0.5 m) distribution in the river mouth area.

Fig. 3. Crustal Enrichment factor (EF crustal) for metals in the sediments of the Moulouya River. The line within the box is the median, and the error bars indicate the 5th and 95th percentiles. Horizontal dashed line represents the value 1 of EF crustal.

Fig. 4. Along river concentrations of NO_x , PO_4^{3-} and SiO_2 during June 2012 and April 2013. Dashed lines indicate sections with different salinity ranges.

Fig. 5. Concentrations of dissolved metals as a function of salinity during June 2012 (black dots) and April 2013 (open dots). Each point represents the average $\pm$ SE of a group of stations according to the following salinity ranges (0-1, 1-2, 2-10, 20-30, 30-35 and >35). Solid and dashed lines represent the trend line (exponential least square) that best represent the data distribution in June 2012 and April 2013, respectively.

Fig. 6. Concentrations of dissolved Cd and Mo as a function of salinity during June 2012 (black dots) and April 2013 (open dots). Each point represents the average $\pm$ SE of a group of stations according to the following salinity ranges (0-1, 1-2, 2-10, 20-30, 30-35 and >35). Solid and dashed lines represent the trend line (linear regression) that best represent the data distribution in June 2012 and April 2013, respectively.

Fig. 7. Relative microphytoplankton community composition (Classes) in the MR and coastal stations (mean value). Concentrations of vitamins and methionine in the lower Moulouya. Gray symbols indicate average $\pm$ S.E. concentrations in the coastal sampling points.