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Research Article

Metallic Element Contaminant and nutrient assessment in sediments and water at marine fish farms of sea bass and sea bream in the Eastern Mediterranean Sea

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Article information	Abstract
	Concentration of selected Metallic Element Contaminants (MECs) (Cd, Pb, Hg, Cu, Fe, Zn, Mn, Mg, Ca, Na, and K), Total Organic Carbon (TOC), and Total Nitrogen (TN) in the superficial layer of sediments from 4 fish farms in the Eastern Mediterranean Sea were studied in order to evaluate the environmental effects of marine aquaculture and to establish correlations between aquaculture and the accumulation of MECs. The levels of nutrients (Nitrate (NO_3), Nitrite (NO_2), Ammonium (NH_4), Phosphate (PO_4), Silicate (Si), Total Nitrogen (TN), and Total Phosphorus (TP)) in the water column were evaluated. The concentrations of Cd, Pb, and Cu were determined by atomic absorption spectrophotometry. The metallic elements Fe, Zn, Mg, Mn, K, Na, and Ca were measured by flame atomic absorption spectrophotometry. Mercury was analyzed by Direct Analyzer of Mercury. Nutrients analyses were carried out by Autoanalyzer. The levels of MECs obtained varied between 0.04 and 0.25 µg g^{-1} dry weight for Cd; 16.3 and 46 µg g^{-1} dry weight for Pb; and 9 and 46 µg kg-1 dry weight for Hg. The levels of nutrients in µmol/l varied between 0.96 and 2.79 for NO_3; 1.75 and 4.07 for NH_4; and 0.1 and 0.5 for NO_2. The results suggested that MEC enrichment in the sediments may be attributed to fish farm effluents, although metal concentrations were not likely to cause harmful effects in the marine ecosystem. The nutrient levels in the water column could be due to sources arising from various anthropogenic activities within the study area. Moreover, the spatial variability was due to increasing nutrient concentrations at the localities close to nonpoint sources. Continuous monitoring and further studies of the area are recommended to determine long-term effects.

1. Introduction

In the Mediterranean Sea, there has been a substantial increase in fish farming production over the last decades, but there is not enough information about the current and likely impacts of marine aquaculture on the environment. Aquaculture processes introduce metabolic waste products (feces and excreta) and uneaten food into the marine environment. This is in addition to other urban and industrial activities which contribute to the introduction of significant amounts of pollutants

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into the marine environment and directly affect the coastal systems where they are quite often deposited.

In early 1980, one of the first Mediterranean hatcheries of sea bass *Dicentrarchus labrax* and sea bream *Sparus aurata* was built. Recently, expansion of fish farming (sea bass and sea bream) in floating and submerged cages has been observed. Tunisian marine aquaculture production is around 10,000 tons per year (mainly sea bass and sea bream).

Due to their environmental persistence, biogeochemical recycling, and ecological risks, Metallic Element Contaminants (MECs) are of particulate concern worldwide (Liu et al., 2003; Gonzalez-Macias et al., 2006). Some trace elements like copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn) are essential for biota in general to activate vital functions and biological processes. However, when these elements exceed permissible limits, they can cause several health effects (World Health Organization. (1984)). High concentrations of cadmium (Cd), lead (Pb), and mercury (Hg) can affect marine biota and lead to serious risks to human health through the consumption of seafood (Adams et al., 1992; Rowlatt et al., 1994; Mucha et al., 2003; Feng et al., 2004; Zhang et al., 2007).

The rapid development and application of aquaculture in coastal zones during the last 25 years has raised many concerns, either from various organizations or local communities, regarding the potential impacts on the environment (GESAMP, 1990). Aquaculture has been coninually accused of being a potential source of serious environmental impacts on the aquatic environment (Hall et al., 1990).

Furthermore, aquaculture activities could also modify the concentration of MECs in adjacent sediments by introducing high loadings of metals contained in the particular matter present in the effluents, which may be up to several tons per day (Tovar et al., 2000). Metallic Element Contaminants are accumulated in marine sediments, where they are incorporated into several biological and chemical cycles, affecting the water column and biota. On the other hand, chemical reactions can change the concentration of metals in sediments and, as a consequence, in the overlying water (Luoma, 1990).

The most common effects of farming fish in cages which are of greatest concern are mainly based on the issue of local eutrophication. The largest source of waste in aquaculture is organic matter coming from fish feed (Sanz-Lázaro & Marín, 2011).

Generally speaking, approximately 1/4 of the nutrients added via fish feed are incorporated into the fish meat itself, while 3/4 remain in the water (Holmer et al., 2005). This organic matter is relatively rich in organic carbon and nutrients such as nitrogen and phosphorus, released in particulate and dissolved form. The release of dissolved nutrients can provoke an enrichment of surrounding waters, giving rise to an increase in primary production in the affected areas (FAO, 1992).

Phosphorus and nitrogen load from cage fish farming, is expressed in kg per ton of produced fish per season. The feed coefficient used is 1.5, and the content of phosphorus and nitrogen in the feed is considered to be 0.9 and 7.2 % of wet weight, respectively. The desorption from the sediment is considered to be 50 % of the sedimented (particulate) phosphorus and nitrogen (Enell & Ackefors, 1991).

This study seeks to evaluate concentrations of selected MECs (Cd, Pb, Hg, Cu, Fe, Zn, Mn, Mg, Ca, Na, and K), Total Organic Carbon (TOC), and Total Nitrogen (TN) in the superficial layer of sediments from 4 fish farms in the Eastern Mediterranean Sea, with different dates of installation, in order to study the environmental effects of marine aquaculture to establish correlations between aquaculture and the accumulation of MECs.

Additionally, the levels of nutrients (Nitrate (NO_3, Nitrite (NO_2), Ammonium (NH_4), Phosphate (PO_4), Total Phosphorus (TP), Silicate (Si), and Total Nitrogen (TN) in the water column are evaluated.

2. Materials and methods

2.1 Sampling and storage

The sediment sampling campaign was conducted during June 2012. Samplings were carried out at 4 fish farms producing sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). These fish farms are localized in the Hammamet Gulf (Eastern Mediterranean Sea) (**Figure 1**). In Tunisia, marine aquaculture is practiced using modern and innovative techniques using floating cages; a total of 45 sediment samples (5 cm of the uppermost sediment) were collected. For the 4 fish farms, 3 replicates of each sampling station sediments were collected under 3 cages (one cage from the centre and 2 from borders), and 3 replicates of 3 control stations were sampled. The control stations (non-aquafarming zone) were chosen near the fish farms (at distances of 200 m). The controls stations were located downstream of the farms. Sampling depths ranged between 30 m and 40 m. **Table 1** presents the location, fish biomass, age and number of cages of each farm and control site. All the farms used pellets and extruded feeds to produce sea bream (*S. aurata*) and sea bass (*D. labrax*). The same type of composition of the food "Skretting" was administrated to all the farms. The amount of food supplied was 131±15.5 T/cage/year. The quantity distributed depended on several factors: the biomass charge in the cage, size of fish, T° of water, etc. The food had a variable diameter (4±2.8 mm) and was distributed at 2 hour-intervals between 8 a.m. and 8 p.m. For fish farm No 3, food was distributed by an automatic distributor, but in fish farm Nos. 1, 2, and 4, the operation was made manually. For the analysis of sediments collected, scuba divers selected only the uppermost (superficial) layers with a plastic spoon and transferred the samples to polyethylene bags. After returning to the ship, sediments were stored immediately in a freezer (−20 °C) until analysis.

In all, 21 water samples were collected from the farms and the control stations (3 replicas from each station). The sampling was done using a bottle of Nansen. The samples were filtered through a 0.45 μm diameter membrane and then transferred to a polyethylene bottle and placed in a refrigerator at 4 °C prior to analysis.
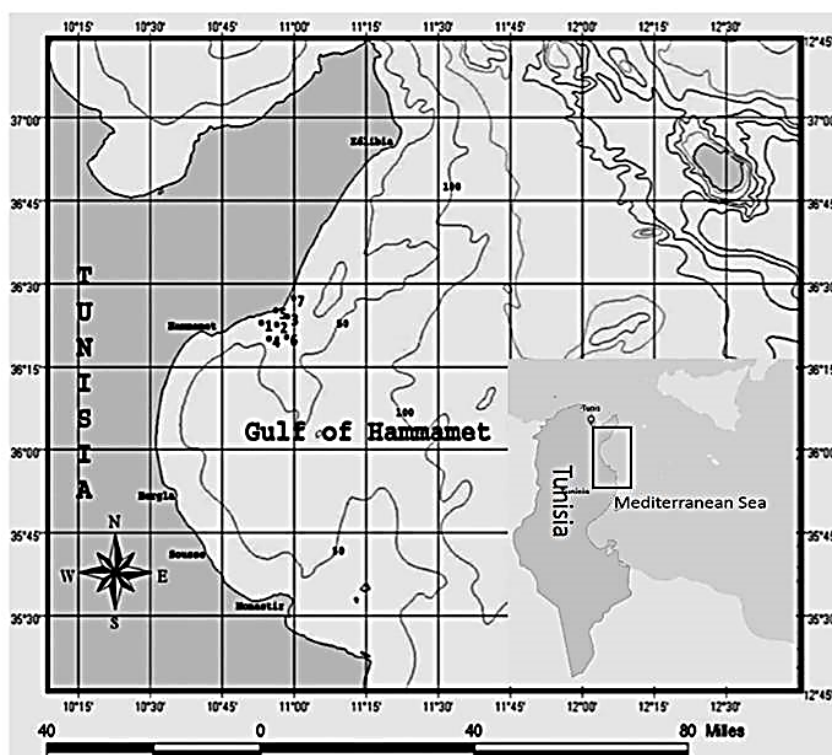

Figure 1 Sampling locations in the Gulf of Hammamet. Surface sediments were taken from fish farms and a non-farming zone. 1: Fish farm1; 2: Fish farm2; 3: Fish farm3; 4: Fish farm4; 5: Control 1; 6: Control 2 and 7: Control 3.

Table 1 Summary of the farm (station) features indicating longitude and latitude of stations, fish biomass, number of cages, and age of farms.

Station	Longitude	Latitude	Fish biomass	Age of farm	Number of cages
1	10°46'24"E	36°25'24"N	400	5	24
2	10°48'49"E	36°22'58"N	700	3	12
3	10°52'26"E	36°27'42"N	600	3	12
4	10°50'49"E	36°20'24"N	400	3	13
5 (Control)	10°49'46"E	36°26'30"N			
6 (Control)	10°53'40"E	36°24'36"N			
7 (Control)	10°47'36"E	36°30'22"N			

2.2 Analytical procedures

Following the freezing step, sediments samples were lyophilized and sieved through vibrating stainless sieves with mesh sizes from 2 mm to 63 μm (Restch, AS2000 model). The fine fraction (< 63 μm) was collected and analyzed. This fraction of sediments was used because it contains higher concentrations of MECs than the sand fraction (Tam & Wong, 2000).

2.3 Determination of Total Organic Carbon (TO C) and Total Nitrogen (TN)

Total Organic Carbon (TOC) and Total Nitrogen (TN) were determined in sediment samples by CHNS elemental analyzer (Froelich, 1980; Hedges & Stern, 1984). The subsamples for TOC were de-carbonated using 1M HCl and dried at 60 °C. The reproducibility was satisfactory, with an average relative standard deviation for replicate analyses of 0.9 % for TOC and 1.3 % for TN.

2.4 Metal analysis of sediment samples

The total element (Cd, Pb, Cu, Zn, Fe, Zn, Mn, Mg, Ca, Na, and K) content in sediment was determined by digesting the samples. Every sample (200 mg) of sediment (fine fraction < 63 μm) was weighed and placed in a Teflon digestion bomb with 5 ml of highly purified concentrated nitric acid (HNO_3) and 6 ml of hydrogen fluoride (HF). The Teflon vessels were closed tightly and placed in a microwave oven for digestion (Milestone, type Ethos) at 200 °C and 362 psi (24 atm) maximum pressure for 20 min. After digestion, samples were diluted using Milli-Q water; 0.8 g of boric acid (H_3BO_3) was added during dilution.

Certified Reference Material (CRM) and blanks were included in the set of samples to check the precision and accuracy of the analysis. A blank was run for each batch of 45 samples to monitor any interference during the sample treatment. The values obtained from the blanks were subtracted from the sample values. The concentrations of Cd, Pb, and Cu were determined by Atomic Absorption Spectrophotometry (AAS) with a graphite furnace and Zeeman correction (Varian 220 Z). Iron, Zn, Mg, Mn, K, Na, and Ca were measured by flame AAS (Varian Spectr-AA10) equipped with an air-acetylene flame (acetylene flow 1.5 l min^{-1}). Five replicates of the reference material (IAEA 405) were digested with the same procedure as the samples. Data reported in this study are calculated as dry weight.

Mercury was analyzed by Direct Analyzer of Mercury (Direct DMA 80 evo). Nutrients in water were analyzed with an autoanalyzer (Technicon) using the colorimetric method, according to Strickland and Parsons (Strickland & Parsons, 1972). We used ammonium-nitrogen type.

2.5 Statistical analysis

Statistical analyses were performed using R 2.15.0 and the STATISTICA (8.0) Software package for Windows. The values for each metal were tested to determine if they were normally distributed using a Kolmogorov-Smirnov test (Roussiez et al., 2005; Veinott et al., 2001). The

multivariate Principal Component Analysis (PCA) was applied in order to explore groups of metals according to their similarities. Also, the PCA procedure allows an easy visualization of the relationships existing amongst the variables in large datasets (Kähkönen et al., 1997; Idris, 2008; Davis et al., 2009; Reid & Spencer, 2009; Passos et al., 2010). A Pearson correlation matrix was used to identify relationships among the metals and to support the results obtained by multivariate analysis. The statistical significance level was set at $p < 0.05$.
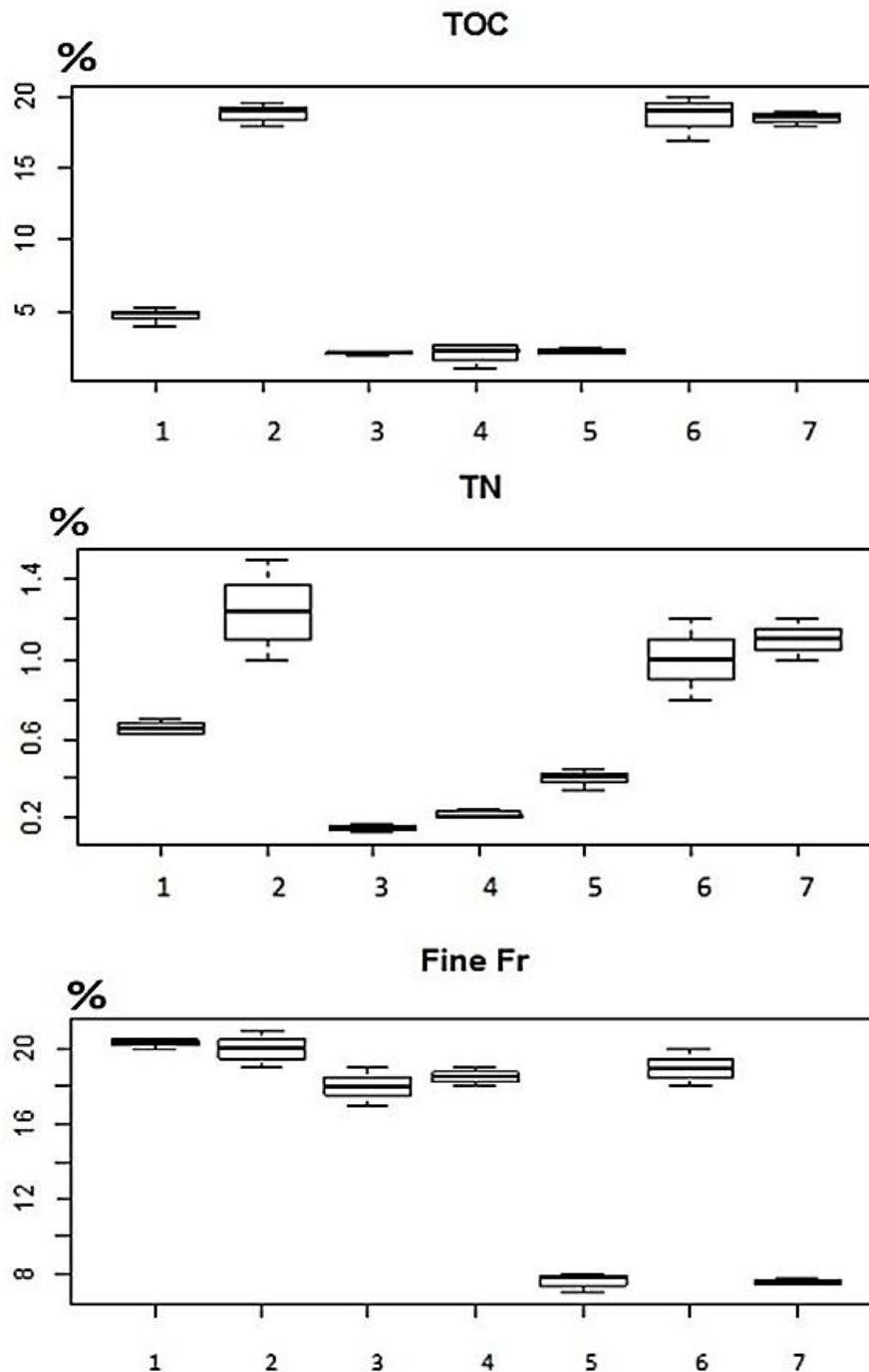

Figure 2 Box plots summarizing the range of TOC, TN, and Fine Fraction contents in the surface sediments from the aqua-farming zone (1, 2, 3, and 4) and the non-aqua-farming zone (Control) (5, 6, and 7).

3. Results and discussion
3.1 TOC, TN, and fine particle content of sediments

The TOC content in sediments ranged between 2 and 17 %, TN in fish farmer sediments varied from 0.15 and 1.26 %, and fine fraction ranged from 8 and 21 % (**Figure 2**). The highest levels were found in control sediments for TOC and TN. The sediments from fish farm 1 were characterized by the highest percentage of fine fraction (Ennouri et al., 2015).

3.2 Concentrations of Metallic Element Contaminants

The concentrations and the ranges of MECs in the superficial layer of sediments from 4 fish farms and control sediments are shown in **Figure 3**. The levels of MECs are summarized in **Table 2**.

The highest concentrations of Cd, Pb, Cu, Zn, and Mn were found in sediments from fish farm 1. The lowest levels of these elements were found in control sediments (Ennouri et al., 2015).

The results obtained in this study showed that the sediments from station 1 (fish farm 1) were characterized by the highest concentrations of Cd, Pb, Hg, Cu, Zn, and Mn and a high percentage of fine fraction. These results could be attributed to the age of this farm (5 years). In fact, this farm was the oldest compared to other farms investigated in this work. The finest fraction of sediments was slightly associated with the area bathymetry and geomorphology.

Table 2 Sediment concentrations for MECs (Cd, Pb, Hg, Cu, Zn, and Mn $\mu g g^{-1}$ dry weight; Fe, Mg, Ca, Na, and K $g k g^{-1}$ dry weight).

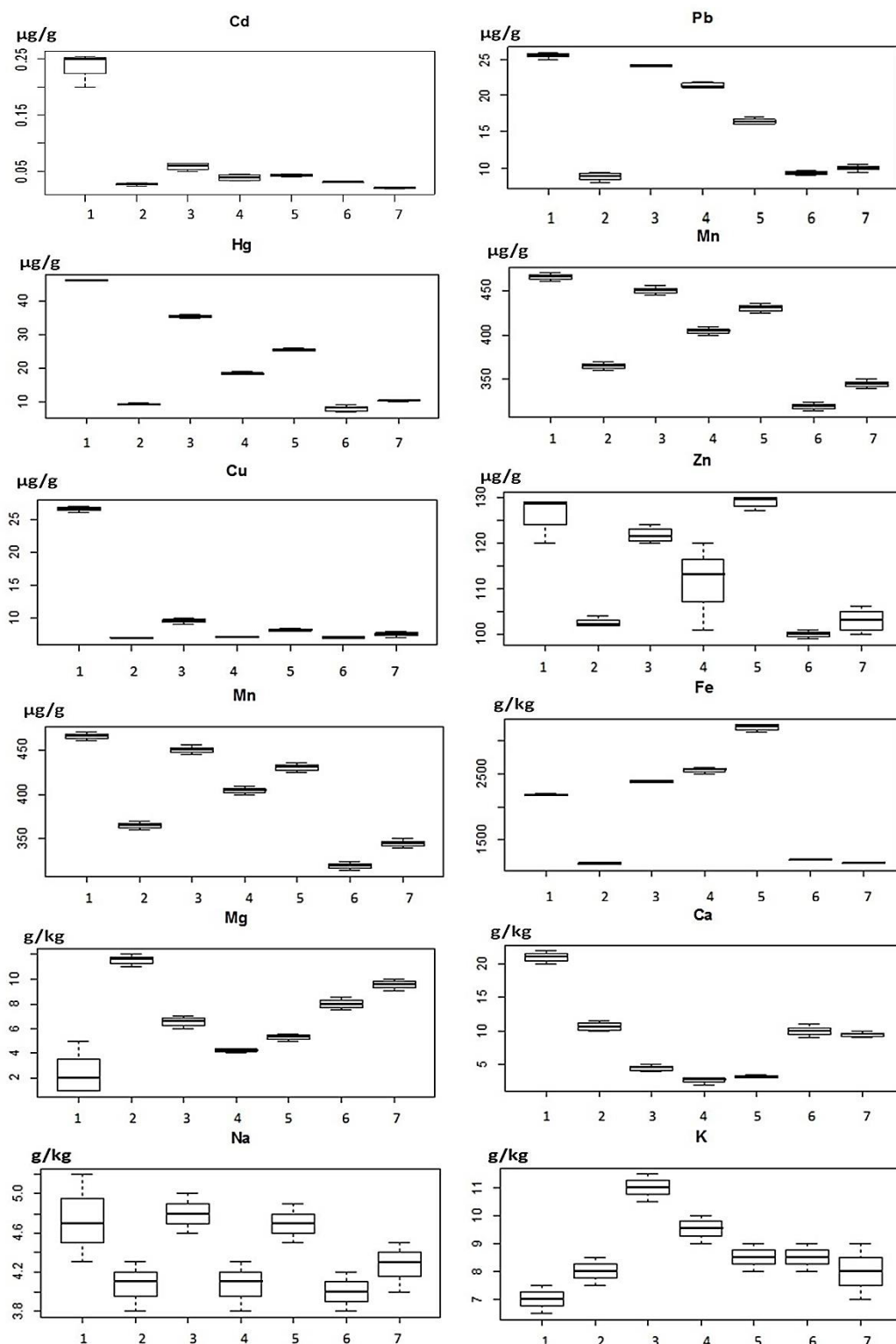| MECs	Concentrations
Cd	0.145±0.148
Pb	31.1±21
Hg	27.5±26.1
Cu	16.4±13.4
Zn	116±19.8
Mn	415±70.7
Fe	2.2±1.5
Mg	7.9±5.2
Ca	12±12.9
Na	4.4±0.5
K	9±2.8

Figure 3 Box plots summarizing the range of MEC (Cd, Pb, Hg, Cu, Zn, and Fe expressed by μgg^{-1}; Mn, Mg, Ca, Na, and K expressed by gkg^{-1}) contents in the surface sediments from the aqua-farming zone (1, 2, 3, and 4) and the non-aqua-farming zone (Control) (5, 6, and 7).

The physical transport of sediment controled this distribution. Several studies have reported accumulations of Zn and Cu in sediments accounted for by aquaculture activities (Mendiguchia et al., 2006; Chou et al., 2002; Sutherland et al., 2007). The sediments from fish farms 2, 3, and 4 were rich in Mn, Zn, Pb, and Na, which presents a positive correlation between them (**Table 3**). They may be discharged simultaneously and have similar behavior (Ennouri et al., 2010). These results can be explained by the common anthropogenic source and the similar environmental properties of these metals (Calace et al., 2005). According to the work of Morillo et al. (2004), metals can be ranked according to the percentages of each one in the acid-soluble fraction (the most mobile and bioavailable) Zn > Mn > Cd > Cu > Pb > Fe.

Table 3 Sediment quality guidelines (SQG) by USEPA for elements (mg/kg).

Marine element	Sediments Non-polluted	Sediments Moderately polluted	Sediments Heavily polluted
Cd	-	-	> 6
Cu	< 25	25 - 50	> 50
Pb	< 40	40 - 60	> 60
Zn	< 90	90 - 200	> 200
Fe	< 17,000	17,000 - 25,000	> 25,000
Mn	< 300	300 - 500	> 500

According to the sediment quality guidelines of the USEPA (Pekey et al., 2004) (**Table 3**), where sediments are classified as non-polluted, moderately polluted, and heavily polluted, the classification of the different farms in this study shows that all sediments from the fish farms investigated were non-polluted by toxic elements. However, sediments from fish farm 1 were moderately polluted by Cu, Zn, and Mn. Samples from fish farms 2, 3, and 4 were moderately polluted by Zn and Mn. Our results show that the aqua-farming zone was more polluted than the non-aqua-farming zone, indicating that the high metal levels in the sediments mainly resulted from the aqua-farming activities. In fact, Chou et al. (2002) reported high levels of Cu and Zn in sediments related to Canadian fish farms. Similar results were observed by Zhang et al. (2012); these researchers revealed that the principal sources of metals were waterway transportation and shipyard discharge.

In addition, high concentrations of Zn, Cu, and Mn in sediments have been reported in areas where intensive aquaculture in cages has been carried out (Schendel et al., 2004; Otero et al., 2005). The metal source in farming zones has been related to the food used (Chou et al., 2002; Uotila, 1991; Belias et al., 2003). Uotila (1991) attributed the accumulation of Zn to fish food, while Cu accumulation was mainly related to the paint used in culture cages. Fish food is the only input of the system during culture; it should contain significant concentrations of Zn, Cu, and Pb (Mendiguchia et al., 2006). Other studies reported that elevated concentrations of elements, such as Cu, Zn, Fe, and Cd, in superficial layers of marine sediments are caused by fish farming and decreased as a result of increasing distance from fish cages. Many other studies indicate that this state is mainly attributed to fish food inputs (Chou et al., 2002; Sutherland et al., 2007; Uotila, 1991; Belias et al., 2003; Kempf et al., 2002; Brooks & Mahnken 2003; Smith et al., 2005; Dean et al., 2007; Jaysankar et al., 2009; Rooney & Podemski, 2010; Russell et al., 2011).

However, high values of Cd, Mn, Pb, Zn, and Fe can be related to other important anthropogenic input, such as atmospheric emission, fly ash, domestic and industrial effluent discharge, and extensive use of antifouling paints by shipping activities (Sarkar et al., 2004). Many others authors demonstrated that the differences found between control stations and fish farm

stations may be attributed to differences in natural background levels as well as, in the case of impacted stations, to differences of element input through fish farm wastes (fish feed and feces) (Brooks & Mahnken, 2003; Dean et al., 2007; Jaysankar et al., 2009). The surface sediment composition under and in the vicinity of the cages is continuously changing, depending on the composition of the solids contained in aquaculture effluents (Mendiguchia et al., 2006). Thus, differences can not only be explained by food but also by other factors such as the environmental conditions under and in the vicinity of fish farms.

3.3 Enrichment by nutrients in the water column

The ranges of nutrients in the water column from the 4 fish farms and the controls are shown in **Figure 4**. The levels of nutrients in $\mu mol/l$ varied between 0.96 and 2.79 for NO_3; 1.75 and 4.07 for NH_4; 0.1 and 0.5 for NO_2; 0.06 and 0.12 for PO_4; 1.74 and 4.79 for Si; 11.99 and 13.93 for TN; and 1.44 and 2.03 for TP.

In fish farm 1, we recorded a higher level of nutrients (nitrate, nitrite, phosphate, and phosphorus) compared to fish farms 2, 3 and 4. This result could be attributed to the age of this farm (5 years). However, they were lower than those found in aquaculture farms in the Mediterranean Sea. High concentrations of nutrients in waters have been reported in the Aegean Sea (Aydin-Onen et al., 2012), where nitrate levels are 7 μmol^{-1}; nitrite levels are 1.5 μmol^{-1}, and phosphate levels are 6.8 μmol^{-1}.

Nitrate is rapidly used by phytoplankton in summer, which explains the negative correlation of nitrate content with temperature (Kormas et al., 2001). Phosphate presents a positive correlation with temperature and salinity. Many authors detected a significant increase in phosphate levels in the cages of aquaculture farms (Aksu & Kocatas, 2007; Rosa et al., 2002).

In fish farms 2, 3, and 4, the highest levels of Ammonium and Total Nitrogen were registered. Some authors have demonstrated that the distribution of ammonium and nitrogen can be related to the variation of physical parameters such as temperature, salinity, and water density (Mantzavrakos et al., 2007). On the other hand, elevated levels may be due to excess feed and fish metabolism in the cages. Also, dynamism in the water column can affect the distribution of nutrients. A similar study attributed the decrease of the concentration of silicate in a water column around aquaculture farms to its ingestion by diatoms (Pitta et al., 2005). The increase of Silicate (Si) concentration can be attributed to remineralization of the biogenic silicate accumulated in marine sediments (Basaran et al., 2010). The enrichment of NH_4 at the vicinity of aquaculture farms was also noted; it is present in the excrement of fish raised on farms (Dosdat, 2001). Ammonium is a toxic element at the highest levels. The presence of this molecule in water usually reflects an incomplete process of degradation of organic matter. This element is the result of the reaction between iron and nitrates, and is an excellent indicator of water pollution induced by organic discharges of agricultural, domestic, or industrial effluents.

Additionally, this study showed a higher level of nutrients in the water around the aquaculture farms than the control sampling. A similar study showed a higher concentration of ammonium, nitrite, phosphate, and silicate near aquaculture farms (Neofitou & Klaoudatos, 2008). The most evident impact of aquaculture farms is the sedimentation of excess food and fecal matter under cages. Similarly, studies conducted in Gulluk Bay (Aegean Sea) showed that nutrients were mainly induced by aquaculture activity in the region (Demirak et al., 2006).

The quality of surface waters is a very sensitive issue, and is affected by various physicochemical factors such as temperature, pH, salinity, conductivity, dissolved oxygen, other dissolved gases, rainfall, turbidity, transparency, and nutrients (Aydin-Onen et al., 2012).

In this work, we confirmed that the levels of nutrient elements increase in the vicinity of fish farm cages proportionally with the increase in the farm's age. This enrichment can be related to the intense aquaculture activity in the area of Benikhiar (Gulf of Hammamet). It is advisable to monitor water columns in the short and long term around aquaculture farms.
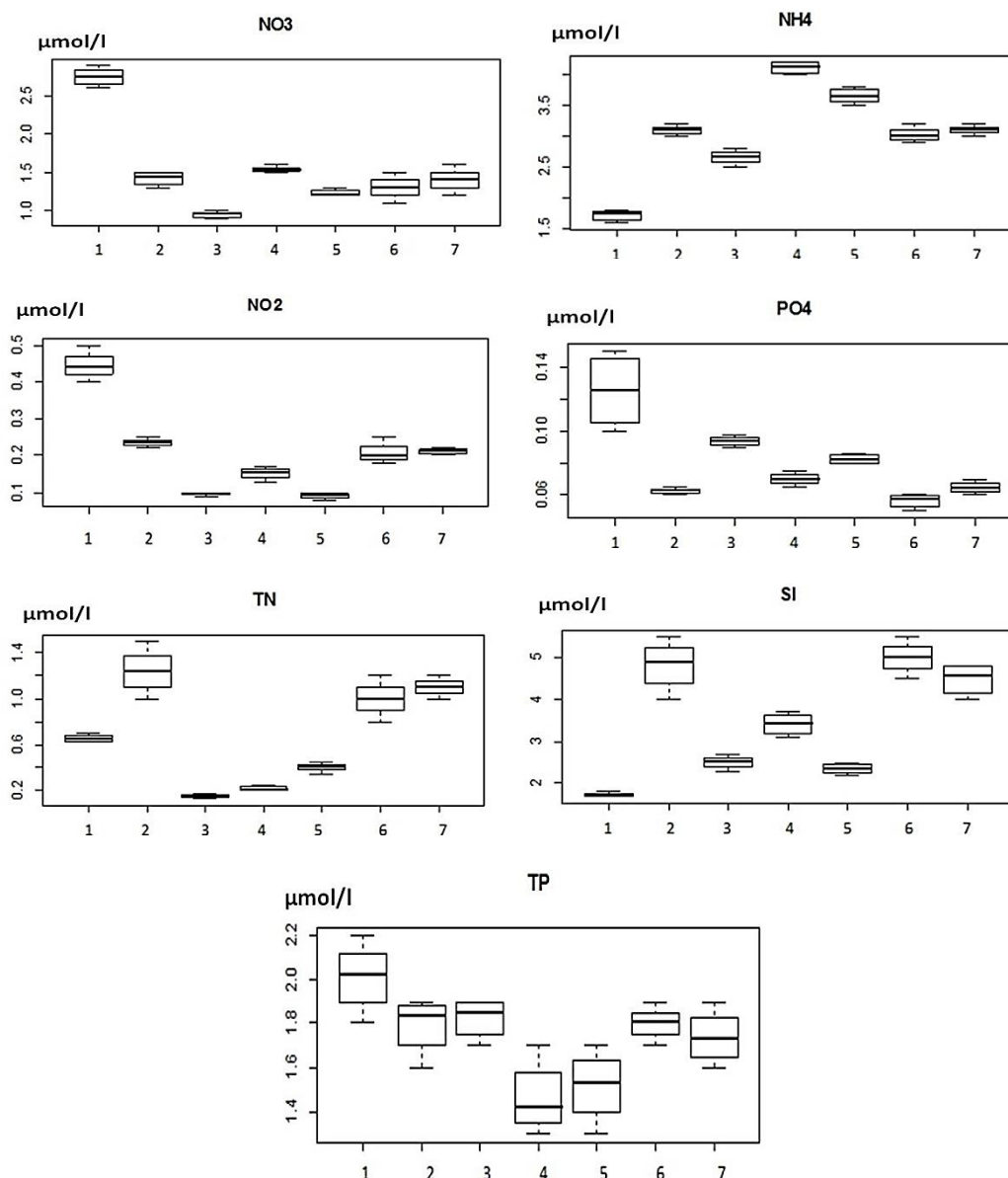
Figure 4 Box plots summarizing the range of nutrient contents in water columns in the aqua-farming zones (1, 2, 3, and 4) and the non-aqua-farming zones (Control) (5, 6, and 7).

3.4 Correlation between Metallic Element Contaminants

Principal Component Analysis (PCA) has been applied to elemental analytical results (14 variables at 45 sediment samples, including all exposed (farm) and control (non-farm) sites together) to interpret the variation of mineral element concentrations in sampling locations. It shows that element concentrations in the fine-grained fraction of samples from the fish farms and non-aquafarming zones (control sediment) could be represented by 2 principal components which accounted for 85.65 % of the total variance. The first component, which accounted for 57.05 % of the variance among metals, had a high correlation with Cd, Pb, Hg, Cu, Mn, Na, and Zn (**Figure 5**). Additionally, the percentage of the variance of the second component represented high positive loads of Ca, fine fraction, and Mg, and a negative correlation with Fe and K. Stations 2, 3, and 4 were clustered in the same group (**Figure 6**). Their sediments were rich in Mn, Fe, and K, but sediments from station 1 were charged by Cu, Cd, Mn, Zn, Pb, Hg, and Na, which presented a positive correlation between them (**Table 4**). Theses correlation suggests that these elements had

common sources, which could have been a natural source. The PCA demonstrated the segregation between station 1 and the other farms. The Pearson correlation analysis (**Table 4**) and PCA (**Figure 5**) showed a significant positive correlation between TOC and TN in all sampling stations.

The results of this study indicate that sediments from the non-farming areas contained the lowest levels of toxic metals.
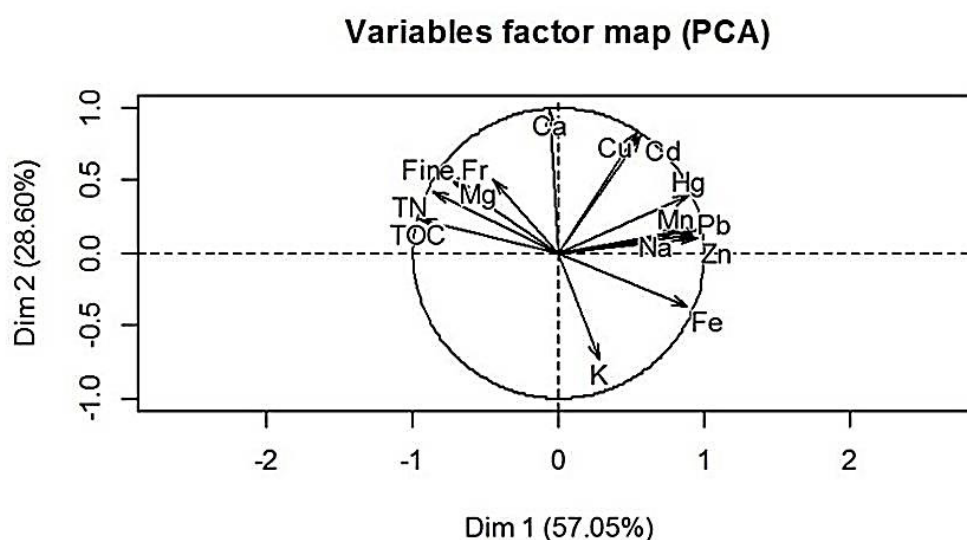

Figure 5 Loading of Metallic Element Contaminants on the principal components.

Table 4 Matrix correlation for METs, TOC, TN and Fine Fraction (Fine Fr) in surface sediments from Tunisian fish farms.

|	Cd	Pb	Hg	Cu	Zn	Fe	Mn	Mg	Ca	Na	K	Fine Fr	TOC	TN
Cd	1.00													
Pb	0.64	1.00												
Hg	**0.82**	**0.90**	1.00											
Cu	**0.99**	0.61	**0.80**	1.00										
Zn	0.61	**0.79**	**0.88**	0.59	1.00									
Fe	0.20	0.68	0.60	0.16	**0.86**	1.00								
Mn	0.64	**0.90**	**0.93**	0.61	**0.93**	**0.76**	1.00							
Mg	-0.06	-0.63	0.39	-0.02	-0.59	**-0.84**	-0.49	1.00						
Ca	**0.78**	0.07	0.33	**0.80**	0.04	-0.41	0.08	0.50	1.00					
Na	0.50	0.67	**0.85**	0.51	**0.86**	0.63	**0.84**	-0.31	0.07	1.00				
K	-0.45	0.32	0.05	-0.48	0.05	0.33	0.19	-0.47	-0.75	0.16	1.00			
Fine Fr	0.17	-0.08	-0.13	0.19	-0.56	**-0.75**	-0.31	0.52	0.51	-0.41	-0.14	1.00		
TOC	-0.35	**-0.88**	-0.75	-0.31	**-0.86**	**-0.93**	**-0.88**	0.83	0.29	-0.67	-0.43	0.48	1.00	
TN	-0.16	**-0.83**	-0.61	-0.12	-0.70	**-0.86**	-0.73	**0.89**	0.47	-0.56	-0.66	0.42	**0.95**	1.00
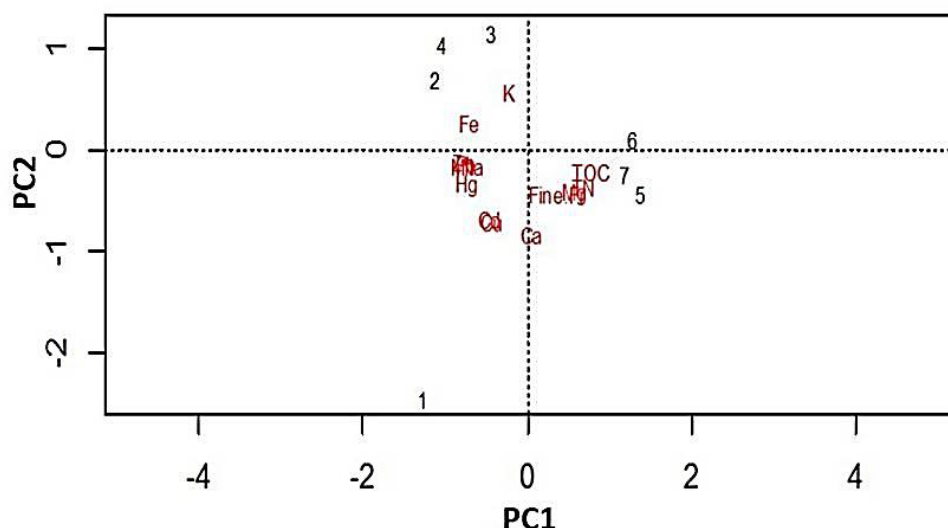
Figure 6 Loading of Metallic Element Contaminants and stations on the principal components.

3.5 Correlation between nutrients

Principal Component Analysis (PCA) was applied to the nutrient analytical results (7 variables at 45 water samples, including all exposed (farm) and control (non-farm) sites together) to interpret the variation of nutrient concentrations in the sampling locations. It showed that nutrient concentrations in water of samples from the fish farms and non-aqua farming zones (control stations) could be represented by 2 principal components which accounted for 81.39 % of the total variance. The first component, which accounted for 53.84 % of the variance among metals, had a high correlation with PO_4, NO_3, NO_2, and TP (**Figure 7**). Additionally, the percentage of the variance of the second component represented high positive loads of TN and a negative correlation SI. Stations 2, 3, and 4 were clustered in the same group (**Figure 8**). Their column waters were rich with TN and NH_4, but waters from station 1 were charged by PO_4, NO_3, NO_2, and TP. The PCA (**Figure 8**) clearly demonstrates the segregation between station 1 and the other farms. Pearson correlation analysis (**Table 5**) and the PCA (**Figure 7**) shows a significant positive correlation between NO_3 and NO_2 in all sampling stations, suggesting that these nutrients had common sources.

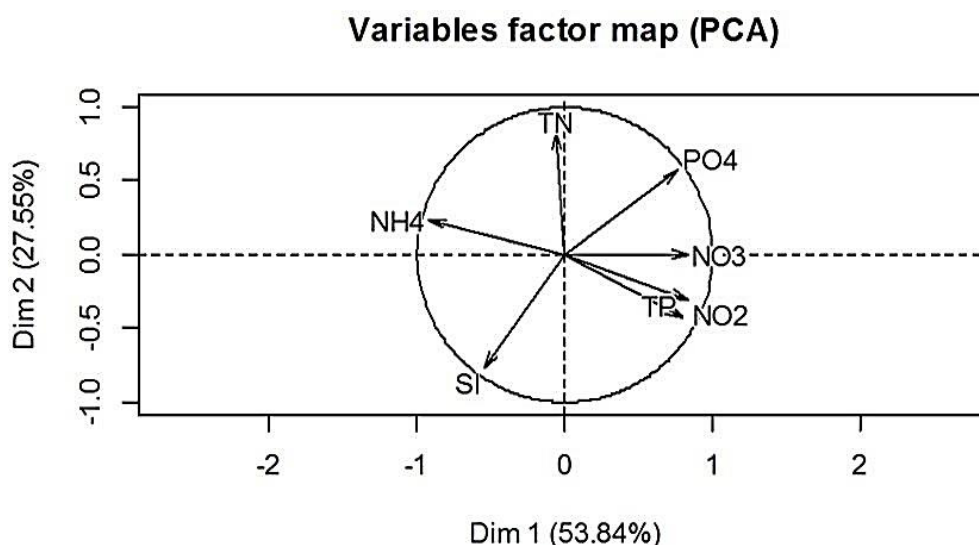## Variables factor map (PCA)

Figure 7 Loading of nutrients on the principal components.
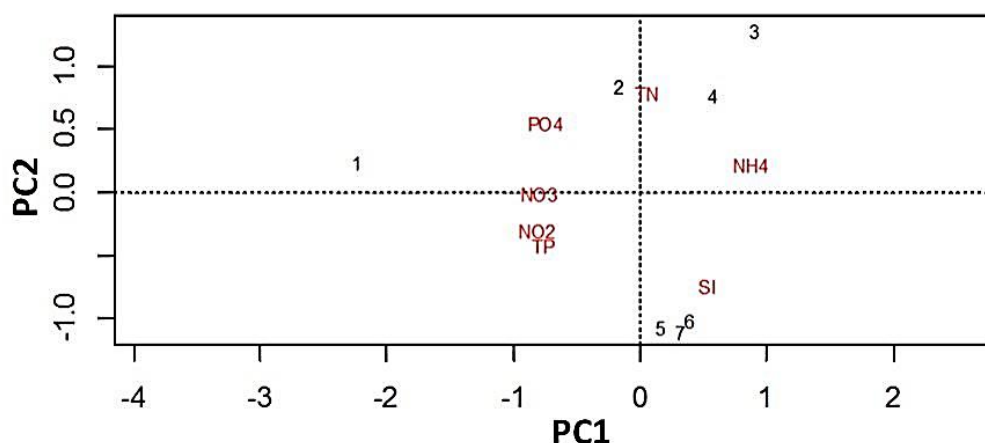
Figure 8 Loading of nutrients and stations on the principal components.

Table 5 Matrix correlation for nutrients in the water column from Tunisian fish farms.

	NO$_3$	NO$_2$	NH$_4$	PO$_4$	SI	TN	TP
NO$_3$	1.00						
NO$_2$	**0.94**	1.00					
NH$_4$	-0.61	-0.73	1.00				
PO$_4$	0.50	0.34	-0.62	1.00			
SI	-0.38	-0.13	0.32	**-0.93**	1.00		
TN	0.03	-0.17	0.24	0.31	-0.41	1.00	
TP	0.44	0.63	**-0.95**	0.44	-0.11	-0.37	1.00

4. Conclusions

The results obtained in this study showed that the sediments from station 1 (fish farm 1) were characterized by a relative highest concentrations of Cd, Pb, Hg, Cu, Zn, and Mn and a relative high percentage of fine fraction. In addition, in fish farm 1, we recorded higher levels of nutrients (nitrate, nitrite, phosphate, and phosphorus) compared to fish farms 2, 3 and 4. These results could be attributed to the age of this farm (5 years). However, they were lower than those found in aquaculture farms in the Mediterranean Sea. The highest levels for TOC and TN were found in the control sediments.

This study clearly showed the low contamination of Tunisian fish farms by MECs and the weak enrichment water with nutrients. It would be useful to monitor, in the long term, both water columns and the sediment environment, to maintain the sustainable development of the aquaculture in the exploited area.

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