



# Environmental impact of intensive aquaculture: Investigation on the accumulation of metals and nutrients in marine sediments of Greece

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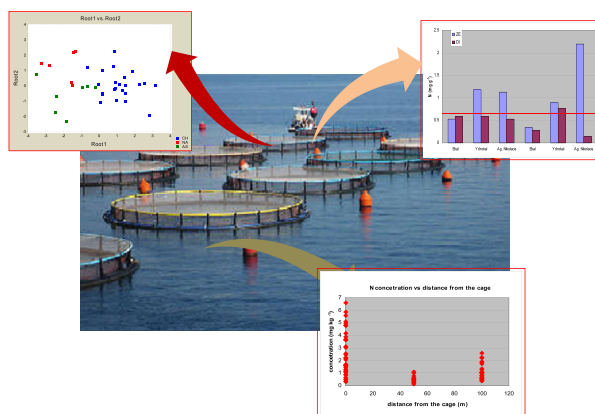
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## HIGHLIGHTS

- Increased levels of C, N, P Cu, Zn, Cd in sediments of aquaculture farms were found.
- Intrinsic differences in C, N, Cd, Mn and Ni between the aquacultures were revealed.
- Non-parametric tests and basic multivariate techniques confirmed farm differentiation.
- Source apportionment revealed natural and anthropogenic origins.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 10 December 2013

Received in revised form 26 March 2014

Accepted 26 March 2014

Available online 17 April 2014

Editor: Eddy Y. Zeng

### Keywords:

Aquaculture

Marine sediments

Non-parametric techniques

PCA

FA

DA

## ABSTRACT

The impact of intensive aquaculture activities on marine sediments along three coastal areas in Greece was studied. The content of nine metals/metalloids (Cu, Cd, Pb, Hg, Ni, Fe, Mn, Zn, As), and three nutrients (P, N and C), that seem to accumulate in marine sediments, was determined under the fish cages (zero distance) and away (50 or 100 m) from them. Elevated concentrations for phosphorus, nitrogen, copper, zinc and cadmium were recorded in the areas where farming establishments are moored. In parallel, the intrinsic differences between the aquaculture facilities and their seasonal variations were investigated. The individual characteristics of each farm (local water currents, facilities' capacity, transferring mechanisms or the geological background) were the determinant factors. On the contrary, significant seasonal differences were not recorded. Statistical techniques, as the non-parametric Mann–Whitney *U* and Kruskal–Wallis tests and principal components analysis (PCA), factor analysis (FA) and discriminant analysis (DA), were used for the evaluation of the results. These chemometric tools succeeded to discriminate the sampling points according to their distance from the cages or the origin of the sample. Variables' significance, correlations and potential accumulation sources were also investigated.

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## 1. Introduction

During the last years, intensive aquaculture has been developed along the Greek islands and continental coastline. This development

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requires further in-deep studies, not as much to the water column, as to the sediments beneath and around the fish cages. Fish farms seem to influence the concentration profiles of organic matter, nutrients (Banas et al., 2008; Mente et al., 2006) and metals (Li et al., 2007; Tovar et al., 2000). Maintenance works, fish food, fecal and metabolic products, contribute to the burden.

The environmental status of marine sediments due to aquaculture activities has been studied scarcely during the last decade. Chou et al. (2002) detected major reduction in waste chemical impact at 50 m distance from fish cages in New Brunswick, Canada. Elevated levels of Cu, Zn and organic carbon were detected. Principal component analysis (PCA) and cluster analysis (CA) were used to explore the chemical data. Mendiguchía et al. (2006) verified the accumulation of selected heavy metals (Cu, Zn, and Pb) and organic matter in marine sediments as a consequence of intensive marine aquaculture. Additionally, total phosphorus was also found to be significantly decreased with increasing distance from fish farms in Sounion (Greece), Alicante (Spain) and Sicily (Italy) (Apostolaki et al., 2007). Dalman et al. (2006) has also determined the elevated concentrations of Zn, Cd and Cu due to nearby aquaculture farms in Gulluk Bay in Turkey. Tovar et al. (2000) evaluated the river water quality in Cadiz Bay (Spain) where extensive aquacultures were carried out. For the characterization of the water quality, the determination of several parameters (pH, temperature, salinity, dissolved oxygen, suspended solids and nutrients) along the river during different seasons was needed. Ammonium and suspended solids were the most significant pollutants. Barasan et al. (2010) confirmed the effects of fish farming on sediments. Significant differences between control and cage stations were found for organic matter and metals, like Fe and Zn. Additionally, the authors used Mann–Whitney *U* and Kruskal–Wallis tests to detect differences among sampling stations, surface–bottom waters and seasons. Neofitou et al. (2010) also studied spatial and temporal effects of fish farming on marine sediment samples and benthic community at two aquacultures in Pagasitikos Gulf (Greece). Analysis of variance (ANOVA) showed only spatial variations, since no significant differences between seasons had been identified in both cases. Recent reviews by Cao et al. (2007), Mente et al. (2006) and Sapkota et al. (2008) verify the environmental impact of aquacultures in the adjacent sediments.

Thus, it is evident that assessing the fate of aquaculture wastes is essential for the sustainability of this industry. In particular, the aim of this study was to investigate by extensive sampling campaigns the enrichment of sediments in organic carbon (C), total nitrogen (N), total phosphorous (P), eight metals (Cu, Cd, Pb, Hg, Fe, Mn, Ni and Zn) and arsenic (As) along three coastal areas in Greece: Chios-Inousses in the northeastern Aegean Sea, and Nafpaktos, and Astakos in central Greece. In parallel, the individuality of each of these facilities along with seasonal variations was investigated. Thus, marine sediments samples were collected below (zero distance) or away (50 and 100 m) from the fish cages in the three aforementioned Greek aquacultures, in order to fulfill the following objectives: (1) to assess concentrations and loads of metal, metalloids and nutrients in marine sediments adjacent to extensive aquacultures in Greece; (2) to compare these data with the respective of distant sampling points; (3) to examine the differences between the geochemical background of the three aquaculture facilities and (4) to examine any seasonal trends.

In order to accomplish the objectives, unsupervised and supervised techniques like PCA/FA (factor analysis) and discriminant analysis (DA), respectively, were used for the assessment of the results. PCA/FA were used for revealing variables' similarities and identifying the factors affecting the enrichment in the study area or discriminating the three facilities. The critical variables were also recorded. DA confirmed the differentiation of the sampling points due to their distance from the aquaculture cages and the facilities' special characteristics or the invariant geological background. It is the first time that the results of many chemometric techniques were used as a strong evidence for demonstrating

the enrichment of metals and nutrients in the sediments under the cages of a fish farm.

## 2. Experimental

### 2.1. Monitoring sites and sampling campaigns

Three facilities were selected: Chios-Inousses (CH), Nafpaktos (NA) and Astakos (AS) (Fig. S1, Table S1 in the Supplementary data), managed for the production of sea breams and sea bass. All the basic farm characteristics are described in Table S1. Factors as years of operations, depth, the winds' direction, the type of sediments and the capacity that may affect the assessment of the results are recorded.

The sampling stations were chosen following patterns proposed by other studies for the environmental impact of aquaculture on sediments (Crawford et al., 2001; Karakassis et al., 2000; Mazzola et al., 1999, 2000; Schendel et al., 2004), where a sample is taken under the farm, a second one is taken at a distance from the farm in order to evaluate the extent of the impact and another sample is taken from a site that is not influenced by the aquaculture activity as "reference" or "control" site.

Ninety (90) sediment samples were totally collected (56 CH, 16 NA and 18 AS) under the fish cages (zero distance) and about 50 and 100 m away from them, along the directions of the main water currents occurred in the wider area due to the predominant winds. Sixteen (16) of them were reference samples taken from sites that were not influenced by the aquaculture activity (Tables S2–S4). In general, the sampling depths varied from 12 to 40 m. All the samples are superficial as they were taken by divers who just filled plastic bottles by scraping the bottom with the bottle. It was not possible to evaluate the vertical profile of the sediment since the sample was mostly a watery sample of the superficial sediment. Sampling was carried out in four campaigns, in December 2005, June and December 2006 and December 2007. Sampling depth was varied (Table S1). All samples were collected by divers in plastic bottles containing 1 kg and kept at  $-20^{\circ}\text{C}$  until analysis.

In order to evaluate the ecological effect of the accumulation of metals and nutrients, benthic samples were collected by divers from the same sampling points in 2011, using corers. Samples were treated and preserved as described in Nicolaidou et al. (1988).

### 2.2. Instruments and methods

Sediment samples were freeze-dried, sieved and the 0.63-mm fraction of each sample was used for the determination of 12 metals–metaloids and nutrients. Specifically, C (TOC) was determined with the chromic acid titration method of Walkey (1947), while N and P were determined with the automated peroxodisulfate oxidation method. Total acid digestion (with HF and aqua regia) was performed for the determination of metals and arsenic (EPA method 3052, 1996). This method succeeds total sample decomposition. Fe and Zn were determined by flame atomic absorption spectrometry (Perkin–Elmer 2380), while Cu, Cd, Pb, Mn, As, and Ni were determined by electrothermal AAS (Perkin Elmer SIMAA 6000, Zeeman THGA). Hg was determined by cold vapor AAS.

The analytical data quality was ensured through blank measurements, analysis of inter-laboratory samples (river sediment/Quality Consult, Italy) and duplicate measurements. Good agreement with the assigned values of the reference material was recorded. All measurements were performed in triplicate and the average was used. A detailed description of all the QA/QC measures is included in the Supplementary material.

Benthic samples of approximately 2 l were collected by divers using core samplers. 2 repetitions were collected from each sampling site, below the farms, at a distance of 50 m and at the control sites. Samples were prepared and preserved according to method described from Nicolaidou et al. (1988).

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