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Metals in benthic macrofauna and biogeochemical factors affecting their trophic transfer to wild fish around fish farm cages

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HIGHLIGHTS

- Metals in macrobenthos and in tissues of wild fish were studied in 4 fish farms.
- Sediment grain size, metal, organic and chl-*a* contents affect metal bioaccumulation.
- Tolerant species accumulate more metals with higher values than equilibrium ones.
- Benthic ecological and morphological traits affect bioaccumulation of metals.
- Hg is biomagnified and P is bioaccumulated in wild fish through zoobenthos.

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ABSTRACT

Benthic macroinvertebrates and wild fish aggregating in the vicinity of four Mediterranean fish farms were sampled. Concentrations of metals and other elements were measured in macrofaunal taxa and in fish tissues (muscle, liver, gills, bone, gonad, stomach, intestine, and stomach content). Biological and geochemical characteristics play an important role in metal accumulation in benthic invertebrates, and consequently in metal transfer to higher trophic levels. Macroinvertebrates accumulated lower concentrations of most metals and elements than their respective sediment, except As, P, Na, Zn and Cd. Elemental concentrations of benthic organisms increased with increasing sediment metal content, except Cd, and with % silt, refractory organic matter and chlorophyll-*a* of sediment due to the influence of sediment geochemistry on metal bioavailability. Tolerant species were found to accumulate higher concentrations of most metals and elements, except for Cd, than equilibrium species. The ecological and morphological characteristics of the benthic invertebrates can affect the bioaccumulation of metals and elements in macrobenthos. Hg and P were found to increase their concentrations from zoobenthos to wild fish aggregating around fish cages feeding on macrofauna.

1. Introduction

Macroinvertebrates may accumulate metals from their environment through various pathways, including water, diet and/or sediment (Alquezar et al., 2007; Saha et al., 2006; Davydkova et al., 2005; Farag et al., 2007; Callier et al., 2009), through respiratory and digestive surfaces (Won et al., 2008). However, the effectiveness of metal uptake from these sources may vary according to ecological needs and metabolism of animals and also contamination gradients in water, food and sediment as well as other factors such as salinity, temperature and interacting agents (Saha et al., 2006; Eggleton and Thomas, 2004) as well as species-specific physiological attributes (Casado-Martinez et al., 2009). Furthermore, metal accumulation may differ according to the ability of macrofaunal taxa to tolerate environmental disturbance (Banks, 2007; Banks et al., 2013).

When assessing the fate of contaminants in aquatic ecosystems, it is also important to understand the complex interactions between the functional attributes of the benthic organisms and the sedimentary physicochemical processes such as contaminant speciation and sorption kinetics (Hedman et al., 2008). Benthic macrofauna behavior can influence the pathways, rates and relative balance of sedimentary biogeochemical cycles (Aller and Aller, 1998), mainly through their bioturbation activities (Roads and Boyer, 1982; Aller, 1988; Kristensen, 1988; Aller and Aller, 1998; Heilskov et al., 2006).

It is known that sediments beneath and close to fish farms are enriched with metals and other elements (Dean et al., 2007; Kalantzi et al., 2013b) because of either sedimentation of metals contained in

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fish feed and fish feces or due to changes in metal behavior related to modification of environmental conditions. Wild fish are often attracted by fish farms (Dempster et al., 2002, 2005; Tuya et al., 2006; Machias et al., 2004, 2005, 2006; Giannoulaki et al., 2005; Sanchez-Jerez et al., 2008) feeding on unused feed pellets but also on benthic invertebrates. Nevertheless, there is little information on the concentrations of metals in benthic invertebrates thriving in sediments around fish farms and the biogeochemical factors influencing their accumulation, even though macroinvertebrates are an important food source for demersal predators, and play a key role in the bioaccumulation and transfer of metal contaminants to higher trophic levels (Alquezar et al., 2007).

In the present study, the main objective was to assess the concentrations of metals and other elements at the base of the food chain of the demersal fish in the vicinity of fish farms and to assess the influence of biogeochemical factors on the accumulation process. To this end, we collected macrofaunal specimens of various taxa from various distances from different fish farms with varying environmental conditions and analyzed their content of various elements. Our intention was to measure all the metals and elements that could be analyzed by means of the ICP-MS, since up to now, to our knowledge, most of the studies have focused on a limited number of metals (e.g. Pb, Cd, and Hg) leaving a substantial gap regarding the distribution of other elements. The study was designed to test the hypotheses that elemental concentrations in fish farm sites (1) varied among fish prey (macrofauna taxa) and sediments, (2) varied among zoobenthic taxa under different environmental conditions (grain size, redox, sulfide, and organic matter), (3) varied among macrofauna taxa with different levels of sensitivity to disturbance (r–K strategy), (4) varied among taxa with different ecological and morphological traits and (5) were potentially accumulated in demersal fish living around fish cages through their diet on zoobenthos.

2. Materials and methods

2.1. Study areas and samples collection

Metals and other elements in marine macrobenthic communities were investigated at four fish farms in Greece — two located in the Aegean Sea (AEG1 and AEG2) and two in the Ionian Sea (ION1 and ION2). Sampling farms are anonymous in this report because the fish farmers agreed to cooperate in the study if their identities were not revealed. The farms in AEG1 and ION1 are located in shallow exposed straits ca 200–300 m from the shore. The farm in AEG2 lies in a semi-exposed area and the fourth fish farm in ION2 is located in a shallow, closed bay. Sampling was performed during the period of July 2006 to July 2007. Sampling sites are described in detail in Kalantzi et al. (2013a, 2013b).

Macrofauna samples were taken by scuba divers using sampling cores of 9.5 cm internal diameter penetrating down to 15 cm sediment depth. One sample was collected under the cages (0 m) as well as at 5, 10, 25 and 50 m from the edge of the cages downstream in the prevailing current direction. One reference station per site with similar depth and sub-stratum type but not affected by farm wastes 400–1000 m from each farm were also sampled. The sediment cores were sieved through 500 μm mesh and samples were immediately transferred to the laboratory for live sorting. Fauna were counted and sorted to family level or higher level where possible. To obtain adequate material for the analysis, all specimens belonging to the same taxon (family or higher level in some cases) from the same station were merged in one sample; henceforth refer to as "taxon-sample". In total, 87 taxon-samples were analyzed from 23 stations. It has been recommended that purging of gut contents should be avoided when bioaccumulation data are used to determine trophic transfer of contaminants, because under environmental conditions, a predator eats the whole prey and is therefore exposed also to contaminants associated with gut sediments (Van Geest et al., 2010). Therefore, in this study macroinvertebrates were not purged of their gut contents (Chapman, 1985; Miles and Tome, 1997).

Wild fish specimens were also collected around the cages, using nets operated by scuba divers or by local fishermen in order to measure concentration of metals in various tissues (muscle, liver, gills, bone, gonad, intestine, stomach, and stomach content). Immediately after collection, fish were killed in ice and transferred to the laboratory. Fish were dissected using a pre-cleaned, stainless steel knife and tissues of interest were sampled. Stomach content was only available for *Mullus surmuletus*, *Eutrigla gurnardus*, *Micromesistius poutassou*, *Serranus cabrilla*, *Mugil cephalus*, and *Boops boops*. Triplicate sediment samples (4.5 cm inner diameter) were also collected by scuba divers from the sampling stations where macrofauna was sampled in order to measure several environmental variables (percent of silt content, redox potential, sulfide content, refractory organic matter, labile organic matter, chlorophyll-*a*, total organic carbon, and total organic nitrogen) and the concentrations of metals of the surface layer (0–2 cm). Macrofauna specimens, fish tissues and sediment samples were stored in labeled, Ziploc bags at −20 °C until laboratory analysis. All samples were handled very carefully in order to avoid cross-contamination. Detailed determination of physical and geochemical characteristics of sampling sites and of metals and elements in sediment and in tissues of wild fish have been included in Kalantzi et al. (2013a, 2013b).

2.2. Sample analysis

Metal and other elements concentrations were determined using a modification of the method described by the USEPA method 3052 (1996) for microwave-assisted acid digestion of siliceous and organically based matrices. All macrofauna samples were freeze-dried to constant weight and stored under a dry atmosphere until digestion. For dissolution of soft macrofauna tissue, 4–8 ml of conc. HNO_3 and 0.5 ml conc. H_2O_2 were added to 0.001–0.140 g (dry weight) of tissue sample in acid-cleaned Teflon vessels. After pre-digestion for an hour in a sandbath (125 °C), vessels were sealed and placed in a closed, high pressure, microwave system (Multiwave 3000, Anton Paar, Austria). After digestion, samples were diluted with ultrapure water into 10 or 25 ml volumetric flasks depending on initial sample weight and stored in polypropylene sample bottles at 4 °C until further analysis. For the measurement of metal and other element concentrations in the sample digests, an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS,

Table 1
List of ecological and morphological traits and their corresponding codes used in the statistical analysis (Papageorgiou et al., 2009).

Ecological traits	Codes	Morphological traits	Codes
Mobility		Body form	
Mobile	m	Shell structures	s
Semi-mobile	sm	Vermiform (length ⋙ width)	v
Sessile	s	Globulose (with/without extremities) length ≥ width	e
Habitat (living position)		Body size (g)	
Epifauna	ef	Very small: <0.050	1
Surface infauna (<2 cm deep)	i	Small: 0.051–0.0700	2
Sub-surface infauna (>2 cm deep)	si	Medium: 0.0701–0.0800	3
Feeding type		Large: 0.0801–0.1100	4
Suspension feeder	SF	Very large: 0.11<	5
Deposit feeder	DF		
Predator	P		
Scavenger	S		
Omnivore	O		
Bioturbation			
Biodiffuser	bd		
Upward-conveyors	uc		
Downward-conveyors	dc		
Gallery diffusers	gd		
No bioturbation	n		