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Bioaccumulation of heavy metals in fish, crustaceans, molluscs and echinoderms from the Tuscany coast

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ABSTRACT

The concentration of As, Cd, Cr, Cu, Hg, Ni, Pb and Zn were analyzed in the edible part of several species of fish, crustaceans, molluscs and echinoderms collected in sensitive areas of the Tuscany coast (northern Italy). The concentration of As (0.39–78.1 $\mu g\, g^{-1}$) and Hg (0.01–1.56 $\mu g\, g^{-1}$) resulted in most cases higher than reference thresholds. Target hazard quotient (THQ) and lifetime cancer risk (TR) indexes were calculated to assess cancer and non-cancer risk due to oral exposure; the highest THQ values referred to As and Hg, with values ≥ 1 in 39% and 48% of cases, respectively. Total target hazard quotients (TTHQ) values suggested that the local population could experience adverse health effects due to consumption of local seafood, mainly of demersal and benthic species. Cancer risk was mainly associated with As exposure, and with Cd intake, especially through molluscs consumption. The NMDS model highlighted species specific bioaccumulation processes and specific sensitivity of species to different bioavailable heavy metals. Specifically, *Mullus* spp. and *Scorpaena porcus* preferentially accumulate Hg and Cr, *Octopus vulgaris* specimens were discriminated by the presence of Pb and Zn, while an evident preference for Cd and Cu was recorded in *Squilla mantis*. In addition, the distribution of heavy metals in organisms revealed sound differences between Follonica and Livorno sampling sites, demonstrating a highly heterogeneous anthropogenic impact in terms of heavy metals input from the industrial activity resting on land.

1. Introduction

Heavy metals are naturally occurring elements, although their multiple industrial, agricultural, pharmaceutical and technological applications have led to their wide distribution in the environment, raising concerns over their potential effects on ecosystems and human health (Tchounwou et al., 2012). Some heavy metals (Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) are essential elements for the organisms being constituents of several key enzymes and playing important roles in various oxidation-reduction reactions (WHO/FAO/IAEA, 1996). However, an excess amount of these metals may produce cellular and tissues damage (Chang et al., 1996; Tchounwou et al., 2008, 2012). Others metals, such as, Hg, Pb, Cd, As, have no established biological functions and are considered as non-essential and potentially toxic (Chang et al., 1996; Inoue, 2013) at relatively low concentrations.

Heavy metals released from natural and anthropogenic sources can reach the marine environment, move through various biogeochemical cycles and be bioaccumulated and biomagnificated through the food chain (Atwell et al., 1998). Because of their persistence, long biological half-life and potential toxicity, they may pose a serious risk to humans through exposition to periodic food ingestion (Bortey-Sam et al., 2015). Actually, when accumulated in organisms they can affect the digestive, cardiovascular and/or central nervous systems (Crespo-Lopez et al., 2007). In addition to non-cancer effects, some metals (i.e., As, Cd, Pb, Hg) could be accompanied by mutagenic, teratogenic, and carcinogenic outcomes in living organisms (Wong, 1988). Heavy metals accumulation in fish depends on concentration and bioavailability of the contaminants in the seawater. Moreover, physical and chemical factors influence adsorption and precipitation processes, complexation kinetics, chemical speciation, lipid solubility, particulate/water partition coefficients (Hamelink et al., 1994). Also, biological factors such as species, trophic interactions and biochemical/physiological adaptation, play an important role (Verkleji, 1993). Actually, variable chemical affinities of metals to fish tissues, different uptake, deposition and excretion rates make the understanding of the distribution of this class of contaminants in marine organisms an important matter of investigation

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and often a crucial challenge for comprehensive investigation of environmental impact on the marine ecosystem (Jezierska and Witeska, 2006).

To this purpose here, we propose an unprecedented investigation of heavy metals in edible part of several marine organisms collected from different sites of Tuscany (central Italy), which is considered a highly sensitive area because of geological and mineralogical features and anthropogenic activities. Indeed, since 1939, this area hosted an important mercury-cell chlor-alkali plants (Solvay) located in Rosignano (Livorno). This plant was equipped of treatment facilities in 1976 and only in 2007 the mercury cell was reconverted with the membrane technology (Legambiente, 2007). Several investigations carried out in the Rosignano costal-marine area, showed high levels of mercury in sediments, soil, vegetation, air (Baldi and Bargagli, 1984; Bargagli et al., 1987; Barghigiani and Bauleo, 1992; Ferrara et al., 1992), farm products (Barghigiani and Ristori, 1994) and fish (Barghigiani et al., 1991, 2000; Barghigiani and De Ranieri, 1992; Scerbo et al., 2005; Gibičar et al., 2009). High levels of Hg were also found in mussel and limpets from Follonica (Grosseto) and in the southern part of Tuscany coast (Baldi and D'Amato, 1986). Besides, the southern coast suffered of the presence of the Piombino industrial area, which includes the harbour, a steel and a metalworking company and the ENEL thermoelectric power plant (ARPAT, 2016). Furthermore, the northern coast of Tuscany hosts the harbour of Livorno, which collects urban and industrial waste from the ENEL Thermoelectric Power Plant and AGIP Oil (Scerbo et al., 1999). In the harbour of Livorno, Iannelli et al. (2012) reported variable levels of petroleum hydrocarbons and heavy metals. Additionally, Tuscany is the main Italian mining region; almost three millennia of exploitation yielded significant productions of iron, pyrite, base metals, silver, antimony, mercury, gold as well as industrial minerals and super-heated steam (Dini, 2003). Finally, a number of the geothermal power plants represent additional sources of contaminants in the area (Bargagli and Barghigiani, 1991), with high potential for mobilizing natural mercury and transport into the sea.

This study aims to assess levels of heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn) in several economically important species collected in four selected sensitive marine-coastal area of Tuscany (Lerici, Livorno, Rosignano and Follonica) in order to assess cancer and non-cancer risks for human associated with consumption of those marine organisms. Moreover, taking into account that metal accumulation may differ for various species living in the same water body, the available multi-species and multi-area dataset offered an unprecedented chance of exploring the variable sensitivity of the organisms to accumulation of bioavailable heavy metals.

Notably, this work provides key information on the anthropogenic impact on the biotic system according to the European Directive 2008/56/EC (Marine Strategy Framework Directive, MSFD), an integrated policy which aims to protect, preserve and prevent the degradation of the marine environment. In particular, this study explores the descriptor 8 (*Concentration of contaminants at levels not giving rise to pollution effects*) and the descriptor 9 (*Contaminants in fish and other seafood for human consumption do not exceed levels established by Community*) of the MSFD directive.

2. Materials and methods

2.1. Sampling

On December 2015 and October 2016 different species of marine organisms were collected in the Rosignano marine-coastal area (Fig. 1). Fish and crustaceans were collected by specific gill fishing gear, while echinoderms, cephalopods and molluscs were manually collected by scuba diver. On February 2017 additional specimens of some of commercially important species (*Mullus spp*., *Scorpaena porcus*, *Squilla mantis* and *Octopus vulgaris*) were collected from catch landed in the areas of Rosignano, Follonica, Livorno and Lerici (Fig. 1). Each specimen was measured for total length (TL) and weight. All the studied organisms did not present any kind of external sign of abnormal or poor heath status. Specimens were dissected using scissors and stainless steel in order to avoid contamination. Muscle without skin, whole soft tissues and gonads were taken from fish, molluscs and echinoderms, respectively and then stored at − 20 °C until chemical analysis.

2.2. Heavy metals analyses

The total mercury concentration was measured using a direct mercury analyzer (Milestone DMA-80 atomic absorption spectrophotometry). About 100 mg of wet sample were loaded into specific nickel boats and analyzed according to USEPA 7473 Method (2007). The contents of other heavy metals were determined by inductively coupled plasma-atomic emission spectrometry (Thermo iCAP-6000), after acid digestion of wet sample (~ 2 g) with 8 ml of nitric acid (HNO_3) in microwaves oven (CEM Discover SP-D) at T = 160 ± 5 °C, for 4 h. Analytical precision was routinely better than 7% (RSD%; n = 3). Duplicated samples and reagent blanks (20% of the total number of samples) were prepared and analyzed, to assess reproducibility (better than 15% for all the elements) and detection limits, respectively. Reference Standard Material (TORT-2 *Lobster Hepatopancreas*) was analyzed to assess accuracy (% recovery = 89–113%) (Table 1).

2.3. Risk assessment analyses

The target hazard quotients (THQ) index was applied to assess the potential non-cancer risk associated to consumption of the different species of marine organisms sampled. The THQ value was calculated on the base of the metals concentrations recorded in the edible parts of the organisms. THQ values exceeding one unit indicate a potential health risk to the consumers (USEPA, 1989). It was calculated following the USEPA (1989) formula:

$$THQ = \frac{EF*ED*FIR*C}{RfD*WAB*TA}*10^{-3} \quad (1)$$

where: EF and ED represent the exposure frequency (365 days/year) and the average lifetime duration (70 years), respectively; FIR is the fish ingestion rate (36 g day^{-1} for person; FAO, 2005); C is the metal concentration (μg g^{-1}); RfD is the reference oral dose in mg kg_{bw}^{-1} d^{-1} ($1 * 10^{-4}$ for Hg, 3×10^{-4} for As, 1×10^{-3} for Cd, 1.5 for Cr, 4×10^{-2} for Cu, 2×10^{-2} for Ni and Zn, 4×10^{-3} for Pb) (USEPA, 2010); WAB is the average body weight for adult consumer (67 kg); TA is the average exposure time (365 days/year*ED).

THQ_{As} was calculated on the base of inorganic As (IAs), which represent the most toxic form, corresponding to ≈ 2% of the total As (Storelli and Marcotrigiano, 2000; Martorell et al., 2011).

Since the exposure to two or more pollutants may result in additive effects (Hallenbeck, 1993), the total target hazard quotient (TTHQ) was also calculated as the arithmetic sum of each THQ values (Chien et al., 2002):

$$TTHQ = THQ_{(Hg)} + THQ_{(As)} + THQ_{(Cd)} + THQ_{(Cr)} + THQ_{(Cu)} + THQ_{(Ni)} + THQ_{(Pb)} + THQ_{(Zn)} \quad (2)$$

The risk of cancer was estimated as the probability of an individual to develop cancer over lifetime, as result of exposure to potential carcinogens (USEPA, 1989). Among the measured metals, As, Cd Cr, Pb are known to cause risk of cancer and methylmercury (MeHg) is classified as "possibly carcinogenic to humans" (IARC, 2012). The lifetime cancer risk (TR) was calculated by multiplying the daily dose by the cancer slope factor (CSF) derived by response-dose curve for toxicant ingestion, following the formula:

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