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 analysed 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 μg g^{-1}), Hg (0.01-1.56 μg g^{-1}) and Pb (<d.l.-5.55 μg g^{-1}) resulted in most cases higher than the relative reference threshold, while the levels of Cd (<d.l.-0.64 μg g^{-1}), Cu (0.11-49.0 μg g^{-1}), Ni (<d.l.–11.9 μg g^{-1}) and Zn (1.81-95.6 μg g^{-1}), were below regulation guidelines. Target hazard quotients (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 higher or equal than unit in 39 and 48% of cases. Values of total target hazard quotients (TTHQ) ranged between 0.73 and 9.60 suggesting that the local population could experience adverse health effects due to consumption of local seafood, particularly of demersal and benthic species. The main cancer risk is associated with As exposure, where the calculated TR index resulted in excess in more than 90% of cases. Cancer risk resulted possible also through Cd exposure, since TR_{Cd} values were higher than 10^{-5} in 50% of cases, especially for molluscs. The NMDS model highlighted specie specific bioaccumulation processes and different sensitivity of the species to detect presence of 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 *O. vulgaris, S. mantis* and *S. porcus* species revealed specific 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

Although most of heavy metals play an essential role for organisms, once discharged into the environment by anthropogenic sources they represent significant threat for the ecosystems and directly and/or indirectly for the human health. In particular, heavy metals released from natural and anthropogenic sources can reach the marine environment, move through various biogeochemical cycles, accumulate in sediments and through the food chain yield bioaccumulation and biomagnification (Atwell et al., 1998). Because of their persistence, long biological half-life and high toxicity, heavy metals may pose a serious risk to ecosystems and humans through exposition to periodic food ingestion (Bortey-Sam et al., 2015). Actually, when accumulated in fatty tissues of 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). In fish heavy metals accumulation mainly depends on the state of pollution of the water body. Variable chemical affinities of metals to fish tissues, different uptake, deposition and excretion rates (Jezierska and Witeska, 2006) make the understanding of the distribution of this class of contaminants in marine organisms an important matter of investigation and often a crucial challenge for comprehensive investigation of environmental impact on the marine ecosystem.

To this purpose here, we propose an unprecedented investigation of heavy metals in tissues of several marine organisms collected within 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, the area hosted mercury-cell chlor-alkali plants (Solvay) located in Rosignano (Livorno). It was equipped of treatment facilities in 1976 and only in 2007 the mercury cell was reconverted with the membrane technology (Legambiente, 2007). Several investigations in 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; Barghigiani and De Ranieri, 1992; Barghigiani et al., 2000; 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 et al., 1986). Furtermore, the southern coast suffered of the presence of the Piombino industrial area, which include the harbour, a steel and a metalworking company and the ENEL thermoelectric power plant (ARPAT, 2016).

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. (2002) 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.

Specifically,this study aims to assess levels of heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn) in different species of fish, crustaceans, molluscs and echinoderms collected in four selected sensitive marine-coastal area of Tuscany, (i.e. Lerici, Livorno, Rosignano and Follonica) in order to i) explore the health status of the marine biota, ii) investigate on the diverse sensitivity of the species to detect the presence of bioavailable heavy metals iii) assess cancer and non-cancer risks for human associated with consumption of those marine organisms.

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

In 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 (Table 1). In February 2017 additional specimens of *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; Table 1). Each specimen was measured for total length (TL) and weight 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 analysed 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 8ml of nitric acid (HNO_3) in microwaves oven (CEM Discover SP-D) at T= 160±5 °C, for 4 h. Duplicated samples and reagent blanks (20% of the total number of samples) were prepared and analyzed, to assess reproducibility (better than 12% for all the elements) and detection limits, respectively. Reference Standard Material (TORT-2 *Lobster Hepatopancreas*) was analysed to assess accuracy (% recovery= 89-103%) and precision (routinely better than 7%; RSD%, n= 3).

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, following the formula (USEPA, 1989):

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

where: EF is the exposure frequency (365 days/year); ED represents the exposure duration (70 years), equivalent to the average lifetime; 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, $3x10^{-4}$ for As, $1x10^{-3}$ for Cd, 1.5 for Cr, $4x10^{-2}$ for Cu, $2x10^{-2}$ for Ni and Zn, $4x10^{-3}$ for Pb) (USEPA 2010); WAB is the average body weight of the adult consumer (67 kg); TA is the average exposure time (365 days/year*ED). THQ_{As} was calculated considering the most toxic form of As, Inorganic As (IAs), corresponding to ≈2% of the total As in fish (Storelli and Marcotrigiano, 2000; Martorell et al., 2011). THQ values exceeding unit indicate a potential health risk to the consumers (USEPA, 1989).

Since the exposure to two or more pollutants may result in additive and/or interactive 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:

$$TR = \frac{EF * ED * FIR * C * CSF}{WAB * TA} * 10^{-3} \qquad (3)$$

Since CSF of Cr and Hg have not been published by the USEPA, TR was calculated only for As (CSF= 1.5 kg-day/mg), Cd (CSF= 6.3 kg-day/mg) and Pb (CSF= $8.5*10^{-3}$ kg-day/mg) (USEPA, 2010).

Acceptable risk levels for carcinogens range from 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1000000). In this study we consider 10^{-5} cancer benchmark.

2.4 Statistical analysis

A multivariate statistical analysis (Nonmetric Multi-dimensional Scaling analysis - NMDS) was performed using statistical software R (R 3.3.2). Because of the dataset matrix did not meet the linear conditions, a non-parametric method was applied. NMDS was based on the Euclidean distance matrix, constructed first on the level of metals recorded in all the collected specimens in order to highlight differences among species and subsequently assess dissimilarities among the different sampling areas. The success of the NMDS model was measured by the stress value (Legendre and Legendre, 1998), that correspond to the difference between the distances in the original space and in the estimated ordination space. Final stress values should ideally be smaller than 10% and not larger than 30% to represent data accurately. The results were graphically represented on a two-dimension NMDS ordination plot, where the arrows show the direction of the gradient and the length of the arrow is proportional to the correlation between the vectors (metal concentration) and the ordination. The metals with significant correlation with the axes were circled. The 95% confidence ellipses were shown for each group.

3. Results and discussion

3.1 Heavy metals content in the marine organisms

Metals contents measured in the edible part of the marine organisms are reported in table 1. Mercury levels ranged between 0.03 and 1.52 μg g^{-1} and resulted comparable to data previously reported for the Tuscany coast (0.05-2.48 μg g^{-1}; Gibičar et al., 2009; 0.1-4.2 μg g^{-1}, Barghigiani et al., 2000). The highest values were recorded in fish, where the Hg levels in *Diplodus spp.* from Rosignano (0.59±0.08 μg g^{-1}) and in *S. porcus* from Lerici and Rosignano (0.52±0.08 and 1.52±0.04 μg g^{-1}, respectively) exceeded the threshold fixed by the EU Commission N. 1881/2006 (0.50 μg g^{-1}). These concentrations were comparable to those reported for Augusta Bay (Italy) (range: 0.25-1.26 μg g^{-1} in *Diplodus spp*, 0.80-1.72 μg g^{-1} in *S. porcus*; Bonsignore et al., 2013), an area strongly affected by Hg discharges until the 80s. High Hg levels were also found in *Mullus spp.* from Rosignano (0.39±0.32 μg g^{-1}) which resulted on average lower than data reported for Augusta Bay (0.80±0.19 μg g^{-1}; Bonsignore et al., 2013) but higher than those relative to Abu Khammash (Libya) (0.21±0.17 μg g^{-1}; Bonsignore et al., 2018), where a large chlor-alkali plant is still operating. Arsenic concentrations, with exception of *M. cephalus* (0.42±0.03 μg g^{-1}), resulted higher than the threshold value indicated by the guidelines of the European Community (2 μg g^{-1}; EEC, 2001), ranging between 2.84 and 24.7 μg g^{-1}. These values were comparable with data reported for marine fish collected in areas characterized by intense anthropogenic activities, like Abu Khammash (7.6-45.4 μg g^{-1}; Bonsignore et al., 2018), Portmán (19.8±1.6 μg g^{-1}; Martínez-Gómez et al., 2012) and Valencia (17.7±2.5 μg g^{-1}; Martínez-Gómez et al., 2012). The highest levels were recorded in *O. vulgaris* from Follonica (50.4±16.9 μg g^{-1}) and Rosignano (35.3±17.6 μg g^{-1}). Lead concentrations (range: 0.01-3.56 μg g^{-1}) were within the range reported for marine organisms from Mediterranean Sea (0-01-3.13 μg g^{-1}; Copat et al., 2015). The highest levels were found in species collected in Rosignano and specifically in *Diplodus spp.* (3.21±0.42 μg g^{-1}), *P. marmoratus* (3.56±2.81 μg g^{-1}), *P. lividus* (0.85±0.80 μg g^{-1}) and *Holothuria tubulosa* (0.52±0.32 μg g^{-1}). These concentrations exceeded the limit fixed by the EU Commission N. 1881/2006 (0.3 μg g^{-1}). Zn and Cu concentrations ranged from 2.30 to 58.5 and from 0.17 to 43.7 μg g^{-1}, respectively. All levels resulted below the limit of 30 μg g^{-1}fixed by FAO (1983), with the exception of the highest values to *P. marmoratus* collected in Rosignano. Cadmium levels ranged from 0.001 to 0.01 μg g^{-1} in fish, from 0.06 to 0.35 μg g^{-1} in crustaceans and from 0.003 to 0.02 μg g^{-1} in molluscs. All levels were below the threshold values (Regulation N. 1881/2006: 0.5 μg g^{-1} for crustaceans; 1 μg g^{-1} for cephalopod and molluscs; 0.05 μg g^{-1} for fish). Ni and Cr concentrations ranged from 0.03 to 6.63 μg g^{-1} and from 0.05 to 5.40 μg g^{-1}, respectively. The highest values were relative to *P. marmoratus* collected in Rosignano. The other species showed Ni levels falling within the range reported for fish and seafood from Lazio (0.10-1.23 μg g^{-1}; Papetti and Rossi, 2009), North Eastern Mediterranean Sea (0.11–12.9 μg g^{-1}; Turkmen et al., 2005), Marmara and Aegean Seas (0.02–3.97 μg g^{-1}; Turkmen et al., 2008). There is no a settled threshold for these metals.

3.2 ***Specie and site specific accumulation pattern***

Various species of fish may accumulate different amounts of metals from the same water body (Jezierska and Witeska, 2006). Predatory fish species are known to accumulate more Hg (Voigt, 2004), while the benthivores generally contained more Cd and Zn (Campbell, 1994; Kidwell et al., 1995). Animals feeding on marine algae and crustaceans show higher As concentrations than piscivorous species (Jezierska and Witeska, 2006). High concentrations of Ni are observed mostly in predators, while Pb is accumulated mostly in benthic fish (Ney and Van Hassel, 1983; Campbell, 1994). A statistical model base on Nonmetric Multi-dimensional Scaling analysis (NMDS) was implemented in order to verify specie specific bioaccumulation pattern in the studied species. In particular, species with the highest number of collected specimens (*Mullus spp.*, *S. porcus*, *O.*

vulgaris and *S. mantis*) were taken in account. The model showed that fish (*Mullus spp.* and *S. porcus*) preferentially accumulate Hg and Cr (Fig. 2 a). Both species are characterized by narrow relationship with the bottom sediment and similar ecological features; specifically, *Mullus spp*. usually feeds on small benthic crustaceans, worms and molluscs (Hureau, 1986), while *S. porcus* generally feeds on small fish (gobies, blennies), crustaceans and other invertebrates (Hureau and Litvinenko, 1986). Different behaviour was observed in *O. vulgaris,* which preferentially accumulates As, Pb and Zn (Fig. 2 a). This specie is an active predator that feed primarily on gastropods and bivalves (Altman, 1967) and seems to display seasonal migrations, mainly of vertical orientation. In the early spring, large animals move inshore for spawning; the females tend to disappear during the summer, while the males leave the coastal waters in autumn or early summer. Finally, an evident preference for Cd and Cu was recorded in *S. mantis* (Fig. 2 a), a benthic species, which dig burrows into soft sandy mud or fine sand substrates. It is a nocturnal predator of small crustaceans, molluscs, polychaetes and fish, but studies on stomach content also documented the presence of algae and foraminifera indicating that it is an opportunistic feeder (WoRMS, 2004).

The NMDS model allowed understanding how the constituent species, and their composition, change among the different communities. The element distribution in *S. porcus* specie allowed to highlighted specific differences among sampling sites; specifically, *S. porcus* from Follonica, characterised by the specific occurrence of As and Pb (12.2±3.71 and 0.03±0.03 µg g^{-1} respectively), differed from *S. porcus* collected in Livorno (enriched in Cu and Ni) and Rosignano (mainly enriched in Cr and Hg) (Table 1; Fig. 2b). Since the size of this species was higher in Lerici (22.5±1.00 cm) compared with Follonica (15.1±1.88 cm), Livorno (12.8±2.32 cm) and Rosignano (11.9±2.31 cm) (Table 1), and the Hg/length correlation resulted statistically reliable, samples of *S. porcus* from Lerici were excluded from the NMDS model. Difference between Follonica and Livorno emerged also in the *S. mantis* species; in particular, specimens from Follonica were characterized by high levels of Hg and Cr (0.33±0.10 and 0.34 ±0.12 µg g^{-1} respectively), while the occurrence of Pb resulted discriminant for individuals from Livorno (0.03±0.01 μg g^{-1}). Similarly, significant differences between these two areas were revealed in the species *O. vulgaris;* samples from Follonica were clearly recognized by high As (50.4±16.9 μg g^{-1}), Cd (0.01±0.05 μg g^{-1}) and Pb (0.13±0.06 μg g^{-1}) concentrations, while samples from Livorno resulted enriched in Ni and Cr (0.09±0.06 and 0.18±0.26 μg g^{-1}, respectively) (Table 1; Fig. 2b). Finally, NMDS model based on the levels of metals in *Mullus spp*. species, do not evidence any significant differences among sampling sites, since the confidence ellipses (95%) were overlapping; this could be attributed to an elevated dispersion of the element distributions in this species (Fig. 2b). *Mullus spp*. and *O. vulgaris* species collected in Rosignano showed the widest variability. For this reason, the NMDS model does not evidence any significant differences of this site compared to the other ones. However, it is important to highlight that the mean levels of Hg recorded in *Mullus spp*. and *O. vulgaris* species collected in Rosignano were the highest (Table 1; Fig. 2b). Differences among sampling areas reflected the occurrence of various natural and anthropogenic pressures which rest on the Tuscany coast. Livorno was affected by heavy metals contamination from the ENEL Thermoelectric Power Plant and the AGIP Petroli industrial sewages (Scerbo et al., 1999; Iannelli et al., 2002), while the occurrence of Hg in the central and southern area (Rosignano and Follonica) can be ascribed to the activity of the chlor-alkali plants of Rosignano Solvay, together with the Monte Amiata ore deposits (Dini, 2003) (Fig. 1). Moreover, the incidence of heavy metals in the southern Tuscany resulted also compatible with the occurrence of Cu-Pb-Zn ores and in the Elba Island (Dini, 2003) (Fig. 1). The effect of industrial sewages from Piombino on the levels of metals in organisms from Follonica can not be excluded; indeed, this big industrial pole directly discharges into the Gulf of Follonica (ARAT, 2001).

3.2 Health risk assessment

To define the potential risk for human health, concentration and toxicity information relative to each pollutant need to be integrated with the exposure assessment. For this purpose, THQs (Target

Hazard Quotients) and TRs (lifetime cancer risk) indexes were calculated (eq 1 and 3, respectively) in order to respectively assess non-cancer and cancer risk due to oral exposure. Concerning arsenic, the toxicological profile refers only to inorganic chemical species, since the organic arsenic compounds are relatively non-toxic for human health (ATSDR, 2005). THQ_{As} resulted >1 in Mullus spp. (1.15) and in O. vulgaris (1.41) from Rosignano, in O. vulgaris (2.01) from Follonica and in S. mantis (1.43) collected in Lerici (Table 2). In calculating THQ_{Hg} we assumed that mercury measured in fish was integrally in its methylated form (Mason and Fitzgerald, 1991; Gilmour and Henry, 1991; Horvat et al., 1999). THQ_{Hg} values >1 referred mostly to species collected in Rosignano: *Mullus spp.* (2.35), *S. porcus* (3.12) and *Diplodus spp.* (3.54). Higher concentrations were also found in *Mullus spp.* (1.41), *S. porcus* (2.20) and *S. mantis* (1.99) from Follonica and in *S. porcus* from Lerici (9.14) and Livorno (1.33) (Table 2). THQ_{Cd} and THQ_{Cu}, THQ_{Ni}, THQ_{Pb} were lower than one unit in all cases (Table 2). Although the calculation of the target hazard quotient index does not provide a quantitative estimate on the dangerous health effects of the exposed population, this methodology offers preliminary information on the potential health risk resulting from consumption of the different marine organisms. Specifically, risks resulted associated with exposure to Hg, mainly derived from consumption of fish, and to As come from crustaceans and molluscs (Fig. 3a). Moreover, with the exception of *M. cephalus* from Rosignano and *Mullus spp.* from Livorno, all the TTHQ values resulted in excess, indicating that the resident human population could experience adverse health effects due to consumption of local seafood and fish, especially of demersal and benthic species, since their close relationship with polluted bottom sediment maybe promote the bioaccumulation of hazardous substances.

International Agency for Research on Cancer (2012) has classified arsenic, cadmium and lead are both carcinogenic and non carcinogenic to humans. Excluding *M. cephalus* from Rosignano, the values of TR_{As} resulted higher than $1*10^{-5}$ in all cases (Table 2, Fig. 3b), suggesting that a considerable cancer risk due to uptake of As via fish and seafood consumption could exist. Although the carcinogenic effects of As exposure are not yet clear, it has been proposed that the As mediated intracellular biosynthesis of reactive oxygen species, such as free radicals, may be implied in the carcinogenic process induced via DNA damage (NRC, 2001). Cancer risk resulted possible also through Cd exposure, since TR_{Cd} values were higher than $1*10^{-5}$ in 50% of cases, while TR_{Pb} values never exceeded the benchmark limit (Table 2; Fig. 3b).

4. **Conclusions**

In the study area, the collected organisms variably suffered of excesses of As, Hg and partially Pb, with respect to reference guidelines. Statistical exploration of the multi-species and multi-area dataset provided an important opportunity to verify specie-specific sensitivity to the bioavailable heavy metals in the different environments. Indeed, due to their living and feeding habits, different organisms differently accumulate variable amounts of metals. Specifically, *Mullus spp*. and *S. porcus* showed a specific sensitivity to monitor available contents of Hg, Cr and Ni in the environment; *O. vulgaris* appeared more sensitive to Pb and Zn, while *S. mantis* preferentially accumulates Cd and Cu. Evident differences between the southern (Follonica) and the northern (Livorno) study areas, emerged following the NMDS model and confirmed a highly heterogeneous anthropogenic impact in terms of heavy metals input from the industrial activity.

Calculation of target hazard quotient highlighted potential non-cancer risks associated with Hg exposure derived from consumption of fish (especially those collected in the Rosignano area), while exposure to As might result from consumption of crustaceans and mollusc, mainly from Rosignano and Follonica areas. Cancer risk is associated with exposure to As and Cd, where the calculated TR index resulted in excess in more than 90% and in 50% of cases, respectively.

Figure captions

Figure 1: *Sampling map with the main industries and ore deposits*

Figure 2: *Ordination plots of non-metric multidimensional scaling (NMDS) applied on metals of the collected specimens (a) and of subsets of the different species (b). Ellipses indicate the 95% confidence intervals for each group. Circles indicate parameters significantly correlated to NMDS coordinates.*

Figura 3: *THQ (a) and TR (b) values calculated for the examined metals in order to assess non-cancer and cancer risk due to fish consumption. Lines indicated non-cancer (1) and cancer (10−5) benchmarks.*

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Table 1

Table 1: *Levels of heavy metals measured in fish, crustaceans, cephalopod mollusc and echinoderms collected along the Tuscany coast. The number of specimens per specie and the relative range of length (L) or weight (W) were also reported.*

	Specie	**Site**	**N.**	**L./W.**	**As**	**Cd**	**Cr**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**
				(cm/gr)	$\mu g\ g^{-1}$							
FISH	*Mullus spp.*	Follonica	10	13.0±1.2	17.3±5.10	0.001±0.001	0.14±0.11	0.29±0.09	0.23±0.13	0.06±0.02	0.007±0.04	4.30±1.20
	Mullus spp.	Rosignano	22	13.0±3.1	28.7±10.5	0.01±0.02	0.17±0.12	0.29±0.10	0.39±0.32	0.15±0.10	0.02±0.02	3.70±0.58
	Mullus spp.	Livorno	10	13.5±2.58	10.5±2.24	0.001±0.001	0.10±0.04	0.23±0.04	0.04±0.01	0.09±0.04	0.02±0.03	4.80±1.52
	Mullus spp.	Lerici	10	12.9±0.88	15.0±4.44	0.002±0.001	0.19±0.04	0.56±0.21	0.06±0.04	0.07±0.01	0.01±0.01	5.35±1.81
	S. porcus	Follonica	8	15.1±1.88	12.2±3.71	0.002±0.001	0.07±0.04	0.17±0.03	0.37±0.12	0.03±0.01	0.03±0.03	3.99±0.81
	S. porcus	Rosignano	14	11.9±2.31	4.31±3.15	0.002±0.002	0.30±0.21	0.27±0.19	0.52±0.19	0.15±0.17	0.02±0.01	4.37±0.89
	S. porcus	Livorno	6	12.8±2.32	6.17±0.67	<d.l.	0.31±0.18	0.25±0.09	0.22±0.15	0.21±0.24	0.001±0.004	4.12±1.06
	S. porcus	Lerici	4	22.5±1.00	4.9±1.00	0.002±0.002	0.36±0.25	0.17±0.03	1.52±0.04	0.09±0.003	<d.l.	5.00±0.26
	M. cephalus	Rosignano	5	24.3± 1.2	0.42±0.03	<d.l.	0.34±0.01	0.38±0.01	0.08±0.02	0.26±0.02	0.03±0.014	3.10±0.24
	Diplodus spp.	Rosignano	5	25.5±1.70	1.71±0.79	<d.l.	0.85±0.57	0.63±0.31	0.59±0.08	0.50±0.30	0.04±0.03	3.21±0.42
CRUSTACEANS	*S. mantis*	Follonica	10	13.7±1.34	24.7±8.02	0.24±0.06	0.20±0.10	13.5±5.57	0.33±0.10	0.19±0.05	0.001±0.00	18.2±4.23
	S. mantis	Rosignano	21	13.8±1.67	24.2±7.09	0.26±0.10	0.05±0.05	12.7±2.99	0.14±0.13	0.20±0.09	0.02±0.03	18.0±1.50
	S. mantis	Livorno	10	13.3±1.82	19.13±4.06	0.21±0.03	0.05±0.02	12.1±5.53	0.03±0.007	0.21±0.12	0.03±0.01	20.9±2.06
	S. mantis	Lerici	10	13.8±1.81	35.8±14.1	0.35±0.14	0.34±0.12	20.1±9.50	0.14±0.08	0.30±0.12	0.02±0.02	23.9±3.45
	P. marmoratus	Rosignano	13	0.40±0.02	9.51±5.40	0.06±0.02	5.40±5.95	43.7±7.59	0.04±0.02	6.63±7.45	3.56±2.81	58.5±52.4
MOLLUSC CEPHALOPODS	*O. vulgaris*	Follonica	6	1300±548	50.4±16.9	0.01±0.005	0.06±0.02	1.98±0.57	0.06±0.02	0.06±0.03	0.13±0.06	14.1±1.99
	O. vulgaris	Rosignano	14	806±270	35.3±17.6	0.02±0.05	0.20±0.26	7.04±7.72	0.12±0.06	0.16±0.10	0.08±0.10	15.2±2.22
	O. vulgaris	Livorno	8	322±166	16.0±3.02	0.003±0.001	0.18±0.26	4.13±1.81	0.04±0.01	0.09±0.06	0.04±0.03	17.0±3.46
	O. vulgaris	Lerici	6	450±44.7	23.8±4.19	0.004±0.002	0.08±0.02	4.55±1.52	0.05±0.01	0.075±0.03	0.06±0.05	12.8±1.28
ECHINODERMS	*P.lividus*	Rosignano	25	5.2 ± 0.2	3.74±1.63	0.17±0.11	1.53±0.74	1.58±0.60	0.04±0.013	0.74±0.45	0.85±0.80	20.4±9.25
	H. tubulosa	Rosignano	8		2.84±0.74	0.01±0.000	0.33±0.06	1.07±0.26	0.04±0.04	0.70±0.53	0.52±0.32	2.30±0.39

Table 2

Table 2: *Values of target hazard quotient (THQ), total target hazard quotient (TTHQ) and lifetime cancer risk (TR) calculated for edible organisms. Grey highlighted values exceed recommendations.*

Area	Specie	THQ As	TR As	THQ Cd	TR Cd	THQ Cr	THQ Cu	THQ Hg	THQ Ni	THQ Pb	TR Pb	THQ Zn	TTHQ
Follonica	*Mullus spp.*	0.69	2.80E-04	0.00	3.80E-06	0.06	0.00	1.40	0.00	0.00	2.90E-08	0.13	2.28
	S. porcus	0.49	2.00E-04	0.00	6.40E-06	0.03	0.00	2.20	0.00	0.00	1.50E-07	0.12	2.84
	S. mantis	0.99	4.00E-04	0.14	8.00E-04	0.08	0.20	2.00	0.01	0.00	2.70E-08	0.55	3.97
	O. vulgaris	2.00	8.10E-04	0.00	2.30E-05	0.02	0.03	0.36	0.00	0.02	6.00E-07	0.42	2.85
Lerici	*Mullus spp.*	0.60	2.40E-04	0.00	5.60E-06	0.08	0.01	0.34	0.00	0.00	4.20E-08	0.16	1.19
	S. porcus	0.20	7.90E-05	0.00	7.10E-06	0.15	0.00	9.10	0.00	0.00	4.60E-09	0.15	9.60
	S. mantis	1.40	5.80E-04	0.21	1.20E-03	0.14	0.30	0.82	0.01	0.00	4.00E-08	0.72	3.60
	O. vulgaris	0.95	3.80E-04	0.00	1.20E-05	0.03	0.07	0.32	0.00	0.01	2.90E-07	0.38	1.76
Livorno	*Mullus spp.*	0.42	1.70E-04	0.00	2.90E-06	0.04	0.00	0.26	0.00	0.00	3.40E-08	0.14	0.86
	S. porcus	0.25	9.90E-05	0.00	3.20E-06	0.12	0.00	1.30	0.01	0.00	1.70E-08	0.12	1.80
	S. mantis	0.77	3.10E-04	0.13	7.10E-04	0.02	0.18	0.18	0.01	0.00	1.40E-07	0.63	1.92
	O. vulgaris	0.64	2.60E-04	0.00	6.90E-06	0.07	0.06	0.20	0.00	0.01	1.80E-07	0.51	1.49
Rosignano	*Mullus spp.*	1.10	4.60E-04	0.01	3.30E-05	0.07	0.00	2.40	0.00	0.00	6.30E-08	0.11	3.69
	S. porcus	0.17	6.90E-05	0.00	7.00E-06	0.12	0.00	3.10	0.00	0.00	7.90E-08	0.13	3.52
	M. cephalus	0.02	6.80E-06	0.00	3.20E-06	0.14	0.01	0.46	0.01	0.00	1.50E-07	0.09	0.73
	Diplodus spp.	0.07	2.80E-05	0.00	7.20E-06	0.34	0.01	3.50	0.02	0.01	1.70E-07	0.10	4.05
	S. mantis	0.97	3.90E-04	0.16	8.90E-04	0.02	0.19	0.84	0.01	0.00	6.90E-08	0.53	2.72
	O. vulgaris	1.40	5.70E-04	0.01	7.10E-05	0.08	0.11	0.72	0.00	0.01	3.80E-07	0.45	2.78
	P. lividus	0.15	6.00E-05	0.10	5.90E-04	0.61	0.02	0.23	0.02	0.13	3.90E-06	0.61	1.87

Figure 1

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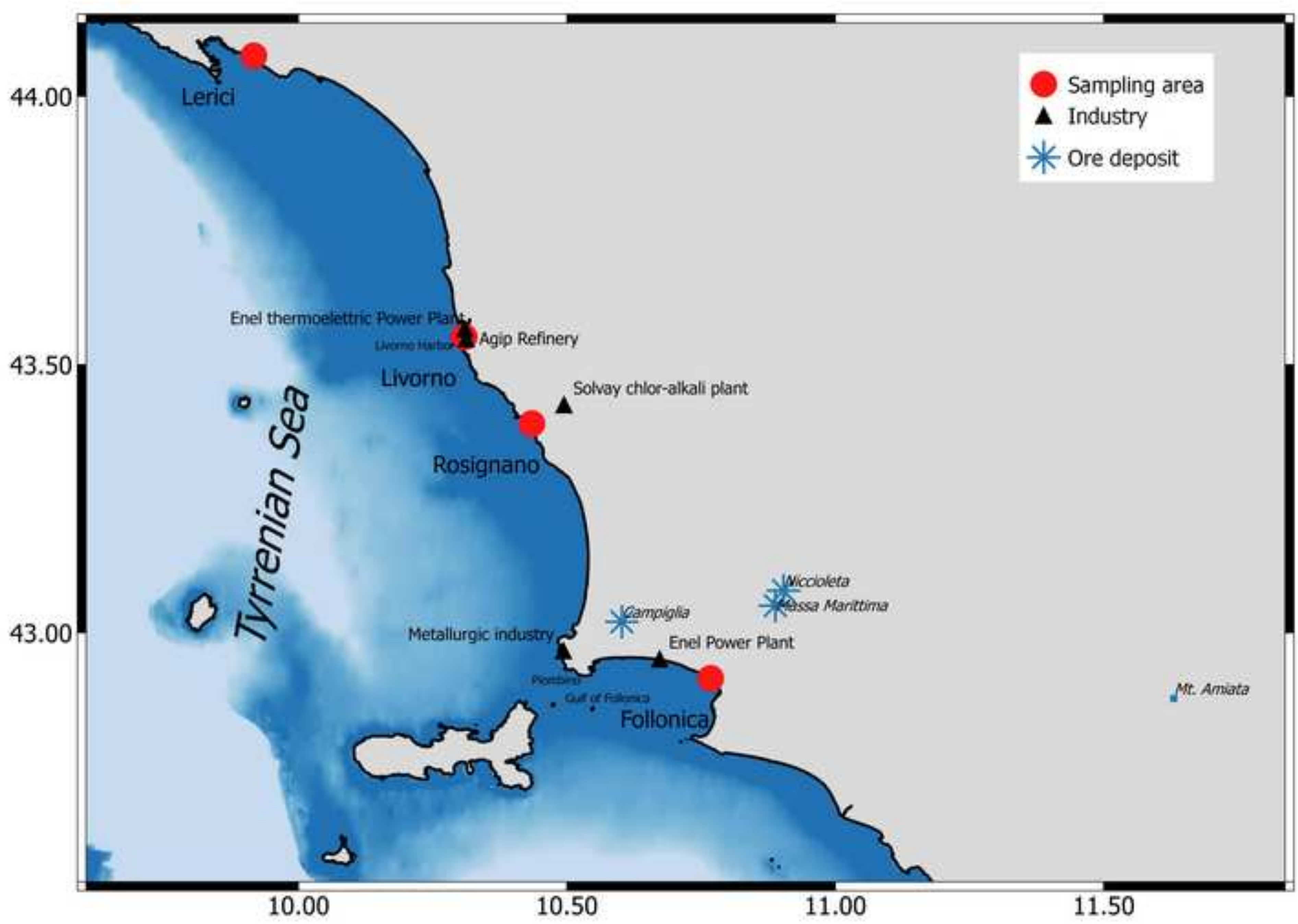

Figure 2

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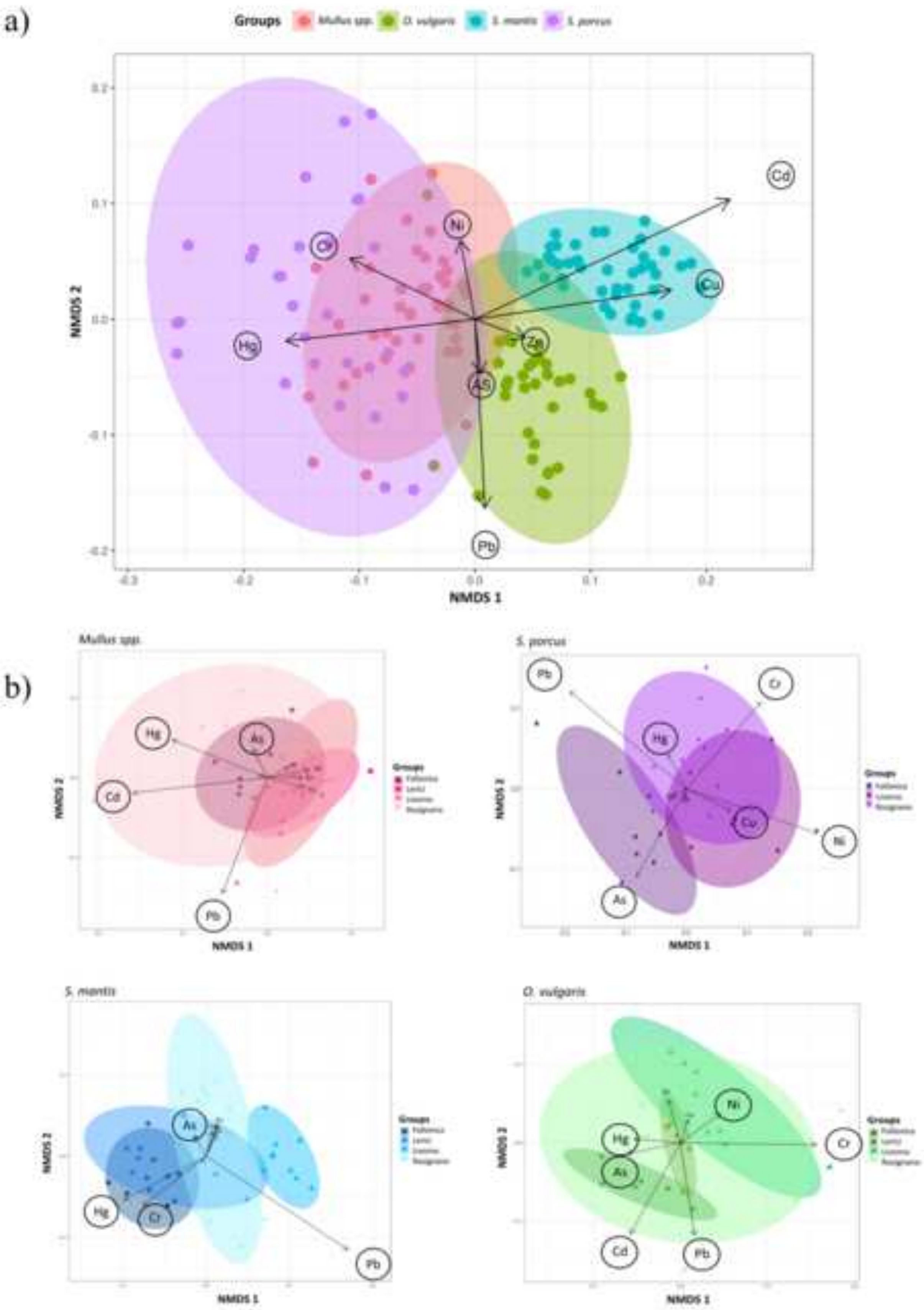
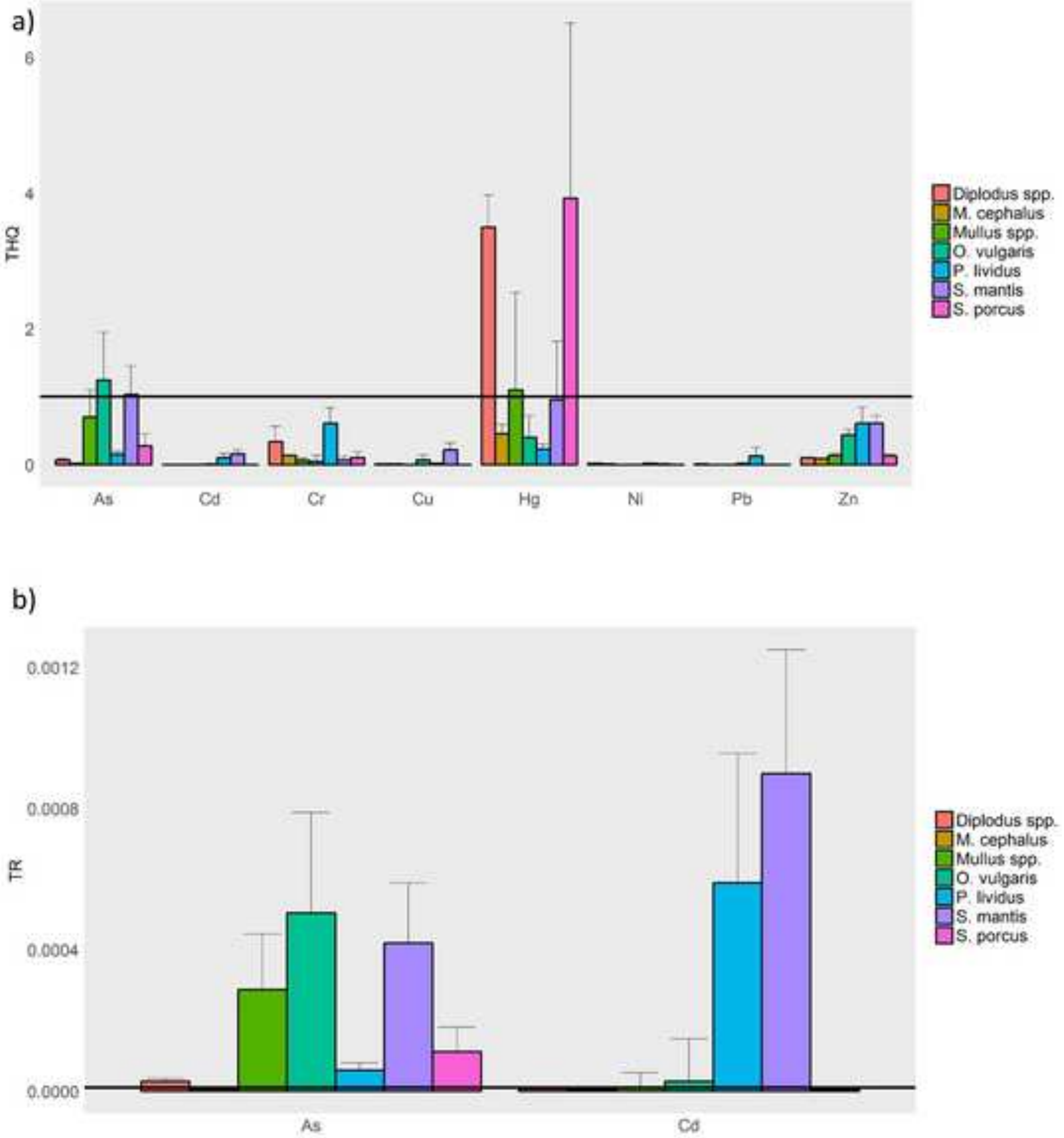


Figure 3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