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A survey on bioconcentration capacities of some marine parasitic and free-living organisms in the Gulf of Oman

M. Golestaninasab[a], M. Malek[a,*], A. Roohi[a], A.R. Karbassi[b], E. Amoozadeh[a], R. Rashidinejad[a], R. Ghayoumi[b], B. Sures[c]

[a] School of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Iran
[b] Marine Division, Department of Environment, Iran
[c] Department of Aquatic Ecology and Centre for Water and Environmental Research (ZWU), University of Duisburg, Essen, Germany

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ABSTRACT

During the course of the present study 21 rays including *Himantura* cf. *gerrardi* and *Glaucostegus granulatus* infected with the four cestode species *Tetragonocephalum* sp., *Polypocephalus* sp., *Rhinebothrium* sp1., and *Rhinebothrium* sp2. as well as three species of free living animals including the barnacle *Amphibalanus amphitrite*, and the two bivalve species *Saccostrea cucullata* and *Barbatia obliquata* were studied from the Gulf of Oman in respect to their cadmium and lead concentrations. All specimens were analyzed using graphite furnace atomic absorption spectrometry (GFAAS) for metal concentrations. In all cases, Cd and Pb concentrations in cestodes were higher than those in the tissues of the respective fish. The cosmopolitan barnacle species *A. amphitrite* showed the highest bioconcentration for all metals. However, the cestode species and especially *Polypocephalus* sp. could be an additional reliable metal indicator. The combination of "mobile" parasites together with sedentary species (i.e. barnacles) might be the most effective way to indicate local pollution levels and to relate this to an estimation of the average pollution level in larger geographical zones.

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1. Introduction

Marine ecosystems are among the most threatened biological environments. Mainly due to anthropogenic activities marine organisms are continuously subjected to environmental stressors such as chemical pollution with a wide variety of substances. Detection of relevant pollutants and the degree of uptake and bioconcentration in marine biota are the key aspects to exploiting biological indicators for monitoring marine chemical pollution with toxic metals such as lead (Pb) and cadmium (Cd). Biological indicators accumulate the metals in their tissues to notably higher concentrations than the surrounding environment and therefore display a more realistic picture of the health conditions of the ambient environment than solely chemical analyses (Selvin et al., 2008).

One of the major challenges in using aquatic organisms as bioindicators is deciding on the ideal biological marker. Previous studies during the past few decades revealed that different aquatic organisms have different capabilities to accumulate metals in their tissues (see Amoozadeh et al., 2013; Dural et al., 2011). Several criteria such as a high bioconcentration potential, widespread distribution, occurrence in high numbers, ease to collect and identify have been proposed for selecting the best suitable sentinels (see Sures, 2004, and references therein).

Among different organisms, metaozoic parasites were suggested as potential bioindicators, especially as bioconcentration indicators for metals (Sures, 2004). According to Dobson et al. (2008), around 40% of all known species are parasitic and approximately 75% of the links in food webs include a parasite species. This tremendous diversity of parasites along with their well-known capacities to accumulate metals in ecologically significant levels suggests that parasites are suitable bioindicators to evaluate unattended changes in ecosystems. Parasites have many advantages which favors investigations in different areas such as markers of environment health (MacKenzie, 1999; Malek et al., 2007; Palm and Rückert, 2009; Vidal-Martinez et al., 2010), indicators of food resources health (Dural et al., 2011), determinants for boosting biodiversity (Mouritsen and Poulin, 2005), and others. A comprehensive discussion on advantages and disadvantages of parasites as bioindicators of metals and reasons for their preferences on

* Corresponding author at: School of Biology, College of Science, University of Tehran, Enghelab Avenue, Tehran, Iran. Tel.: +98 21 6111 2702; fax: +98 21 6111 2461.
E-mail addresses: mmalek@khayam.ut.ac.ir, masoumeh.malek@gmail.com (M. Malek).

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free-living organisms could be found in MacKenzie (1999) and Sures (2004, 2005).

Recent studies at the University of Tehran on parasites of marine fish revealed that marine parasites are also suitable bioindicators and metal absorption in the parasitic tissue is significantly higher than that in fish (Khaleghzadeh-Ahangar et al., 2011; Malek et al., 2007). Malek et al. (2007) in their study on the shark *Carcharhinus dussumieri* and its parasites, *Anthobothrium* sp. and *Paraorigmatobothrium* sp. (Cestoda) suggested that the latter cestodes may also benefit their hosts by acting as heavy metal filters. This was the first survey on marine cestodes taking into account their potential to act as bioindicators in open waters and was therefore an important step in applying marine cestodes as ideal bioconcentration indicators.

The objectives of the current study were to investigate a range of possible free living and parasitic bioindicators in large and confined zones. In addition to two ray species and their four cestodes species, one species of barnacles and two bivalves species were analyzed for possible metal bioconcentrations. Although it is known that metal bioconcentration occurs in cestodes of the whitecheek shark *Carcharhinus dussumieri* (Malek et al., 2007), this study is the first to evaluate this potential for rays' cestodes. The host fishes and their parasites as well as the free living organisms were selected trying to follow the criteria proposed by Kennedy (1997) and Selvin et al. (2008) to a feasible extent.

The study area, i.e. the Gulf of Oman is among the most important marine systems in the world, because it acts as a main corridor for petroleum transportation, with approximately 35% of the world petroleum passed through this way. Because of this heavy transportation and other anthropogenic activities, the concentrations of the metals cadmium and lead in the sediments were significantly higher than the global baseline levels (Pourrang et al., 2005) which makes this region a suitable area to compare bioconcentration properties of different groups of potential sentinels.

2. Materials and methods

2.1. Sampling

During the course of study 21 rays including, *Himantura* cf. *gerarrdi* ($n = 15$) and *Glaucostegus granulatus* ($n = 6$) infected with four cestodes i.e., *Tetragonocephalum* sp. ($n = 5$), *Polypocephalus* sp. and *Rhinebothrium* sp1., *Rhinebothrium* sp2. and three species of free living animals including the barnacle *Amphibalanus amphitrite*, and the two bivalve species *Saccostrea cucullata* and *Barbatia obliquata* were studied from Jod (N25° 45.8′ 90″ & E59° 51.5′ 57″) and from the Gulf of Oman. All samples were collected during one sampling effort. Rays were collected by local fishermen. After killing and opening the fish their spiral intestines were removed and opened with a mid-ventral incision before transferring them to a plastic bag containing 4% formalin buffered in seawater. Subsequently, they were shaken vigorously for at least 30 s in order to detach parasites from gut. The spiral intestines were then transported to the Zoology laboratory of the University of Tehran where they were investigated for parasites.

Barnacles and bivalves were collected in the 20 cm × 20 cm quadrates by hand. The specimens were preserved in liquid nitrogen and then transferred to the Zoology laboratory of the University of Tehran. For species identification some individuals were fixed in 5% Formalin and subsequently examined in the laboratory. Sediments were collected with a 3 cm × 10 cm tube.

2.2. Sample preparation and metal analyses

Cadmium and lead concentrations in the muscle and intestine of rays and sediments were analyzed by graphite furnace atomic absorption spectrometry (GFAAS) as described in detail in Amoozadeh et al. (2013). Briefly, 1 g sediment (wet weight) was digested with 10 ml of HNO_3 (suprapure grade, Merck) using an open digestion procedure with a subsequent addition of 3 ml of 30% H_2O_2. After cooling, the digestion solution was diluted to 100 ml with distilled water. Eventually remaining particles were removed by filtration. Subsequently, the sample solution was stored at room temperature (about 25 °C) until analysis by GFAAS (Bettinelli et al., 2000; Esen and Balci, 2008). For the analyses of biological samples a portion of 1 g of the respective biota sample was weighed into a digestion vessel and 5 ml of HNO_3 (suprapure grade), and 2 ml of 30% H_2O_2 (suprapure grade) was added to the vessel. In some cases (e.g. barnacles) individuals were pooled to obtain a sample weight of 1 g. For larger specimen such as bivalves, tissues were homogenized before an aliquot of 1 g was used for digestion. Samples were digested according to the procedure of AOAC 999.10 standard program with an Anton Paar multiwave 3000 Microwave system. Cadmium and lead concentrations were analyzed with GFAAS using a Varian Spectra Model AA 400 atomic absorption spectrometer with a Deuterium lamp background correction system. For the analyses phosphoric acid at a concentration of 1 g/l was used as a matrix modifier.

Samples of standard reference materials for Pb and Cd (Fish tissues IAEA-407) were used to check the accuracy of the analysis. Quantitative comparisons of bioconcentration ratios were conducted following calculation of the BCF (bioconcentration factor) as suggested by Sures et al. (1999a) as: $BCF = C_{[parasite]}/C_{[host\ tissue]}$. For free-living organisms as well as fishes, the BCF was calculated following Ali and Fishar (2005) as: $BCF = M_{-tissue}/M_{-sediment}$.

2.3. Statistical analysis

The data were analyzed with SPSS ver. 19 program. Kolmogrov–Smirnov normality test revealed that the data were not normally distributed. Therefore, Mann–Whitney *U* test was applied to investigate significant differences between two groups.

3. Results

15 *Himantura* cf. *gerarrdi* and six *G. granulatus* were collected and examined for infection with cestodes (prevalence is shown in Table 1). Rays were infected with four cestode species i.e. *Polypocephalus* sp. (from *H.* cf. *gerarrdi*), *Tetragonocephalum* sp. (from *H.* cf. *gerarrdi*), and *Rhinobothrium* sp1. (from *H.* cf. *gerarrdi*) and *Rhinobothrium* sp2. (from *G. Granulatus*), and were examined for bioconcentration of Cd and Pb. Metal bioconcentrations in the free-living barnacle *A. amphitrite* and the two bivalve species *S. cucullata* and *B. obliquata* were also examined and compared with rays and their cestodes. Detailed results of examined specimens, bioconcentration factors (BCF), and test values for statistical analyses are summarized in Table 1. Comparison of metal levels in the intestine and muscle between infected and uninfected rays revealed that Cd concentrations in tissues of infected ray species were significantly higher than in the uninfected conspecifics. Regarding Pb, no significant difference was found between mean metal concentrations among the tissues of uninfected and infected hosts. There was no significant difference between Cd concentrations of the cestodes *Polypocephalus* sp. and *Rhinebothrium* sp1. with their respective host tissues. Only the mean Cd concentration in the cestode *Tetragonocephalum* sp. was significantly lower (0.28 times) than that in its respective infected host *H.* cf. *gerarrdi*. Except of *Rhinebothrium* sp1., in all three other cestodes, mean Pb concentrations were significantly higher than those in the muscle and intestine of the respective hosts (11.9, 3.79, and 39.1 times for

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