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Heavy metal bioavailability and effects: II. Histopathology–bioaccumulation relationships caused by mining activities in the Gulf of Cádiz (SW, Spain)

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Abstract

The relationship between bioaccumulation of heavy metals (Zn, Cd, Pb and Cu) and histological lesions in different tissues of organisms is assessed in three different areas located in the southwest of Spain in the Gulf of Cádiz (Ría of Huelva, Guadalquivir estuary and Bay of Cádiz) affected and non-affected by mining activities. Data included in these relationships were obtained along the years 2000 and 2001 to address the impact of the Aznalcóllar mining spill on the Guadalquivir estuary. The bioaccumulation and the histological lesions measured in this seasonal study in the Gudalquivir estuary were linked to derive tissue quality guidelines (TQGs) by means of a multivariate analysis approach (MAA). Sediments collected in the same areas of study were used to expose organisms during the survey carried out in autumn 2001 and to address the relationship between bioaccumulation and histological lesions under laboratory conditions and related to chemicals bound to sediments. Lesions show that the organisms collected in the ría of Huelva and exposed to their sediments were severe, intermediate in the Guadalquivir estuary and absent in the Bay of Cádiz. Results show that the Guadalquivir estuary trends to recover its initial status quo previous to the mining spill. The link between chemical concentration and the lesions measured in the same tissues using MAA permits to derive tissue quality guidelines for two organisms, oysters (*Crassostrea angulata*) and clams (*Scrobicularia plana*) collected in the Guadalquivir estuary and associated with the heavy metals from the mining spill (Zn and Cd). The TQG values expressed as concentrations (mg kg^{-1}—dry weight) not associated with biological effects are for oysters, Zn, 8603, Cd, 3.42; and for clams Zn, 800, Cd, 2.6.

Keywords: Aznalcóllar mining spill; Tissue quality guidelines; Multivariate analysis; Estuaries; Doñana

1. Introduction

The relationship between concentration of contaminants in tissues and toxic effects measured in organisms

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is receiving increased attention during the last decades (Chapman, 1997). These relationships have as a final aim to derive tissue quality guidelines (TQGs) defined as the concentrations of the chemicals measured in the different tissues that are associated or not with the biological effect measured, in a parallelism with the sediment quality guidelines (SQGs) widely used around the world (Riba et al., 2003a). To identify possible toxic agent(s), requires body burden data collected from the same organisms exhibiting toxicity in a weight of evidence evaluation. This toxicity should involve only sublethal measurements (endpoints), otherwise (lethal) the organism is not alive and the bioaccumulation data are not significant for this propose (Chapman, 1997). Coupling tissue residue level with sublethal toxicity responses such as histopathological diseases allows clear identification of possible causative agent(s) and could permit to predict effects of chronic and low-level exposures, especially when surveys are carried out both under field and laboratory conditions and then compared.

The environmental behaviour of heavy metals exhibits direct toxicity, although some of them can be regulated in the organism tissues to greater or lesser degrees. For instance, essential metals such as Cu and/or Zn can produce toxicity both by deficiency or excess of them in the tissues. Most of previous studies establish that metal Bioaccumulation and/or Bioconcentration factors, BAFs—uptake from water and diet—and BCFs—uptake from water only—respectively, are not precise or, even in some case, reliable. It is based on that they are difficult to measure properly and are highly variable (Environment Canada, 1994). However, metals are bioaccumulated through highly specific physiological uptake mechanisms which are generally not conductive to biomagnification and depend on the chemical form of the metal and the properties of the surrounding medium (especially salinity and pH in estuaries—Riba et al., 2003b).

All these complexity pattern that can eclipse the relationship between concentration of metals and toxic effects can be partly neglected if a comparison using the same organism and the same tissues under both field and laboratory conditions is conducted to relate concentration of metals and histological diseases. Using this approach the question Is bioaccumulation–toxicity relationship useful for predicting anthropogenic impacts?, can be addressed.

The main objective of this work is to determine the sublethal effects provoked by an acute event of contamination in the Guadalquivir estuary associated with a mining spill comparing the histopathological lesions to those measured in areas chronically affected by mining activities (ría of Huelva), and in areas with absence of contamination (Bay of Cádiz), both under field and laboratory conditions. Also, an objective of this study is to derive tissue quality guidelines (TQGs) by linking the set of data of metal residues reported by Riba et al. (this issue) and the sublethal effects described in this work. A multivariate analysis approach (MAA) as previously described by DelValls and Chapman (1998) to derive sediment quality guidelines (SQGs) is performed on chemical residues and histopathological lesions measured in the same tissues to derive tissue quality guidelines (TQGs) in the Guadalquivir estuary.

2. Material and methods

2.1. Approach

A detailed description of the surveys, samples and conditions of the bioassays is reported by Riba et al. (see Table 1 and Fig. 1) in this issue. Briefly, two different surveys were used in this study both under field and laboratory conditions. Two different species (estuarine clam *Ruditapes phillipinarum*, 10 days, and juveniles of *Solea senegalensis*, 30 days) were exposed to whole sediments collected in the field. Field surveys were carried out using different species that includes oysters (*Crassostrea angulata*), clams (*Scrobicularia plana*) and fish (*Liza ramada*). Field surveys in the Guadalquivir estuary were conducted during different seasons from years 2000 to 2001. The histological lesions measured in the species collected in the last survey (autumn 2001) were compared to those obtained in the field and in sediment-exposed organisms from different areas of the Gulf of Cádiz (contaminated, ría of Huelva, and uncontaminated, Bay of Cádiz) to establish the potential recovery of the estuary after the spill.

2.2. Histological analysis

Organisms from the field and the toxicity tests were analyzed to determine the histopathological lesions in different tissues. In Table 1 showed in the previous paper describing the bioaccumulation survey (Riba et al., this issue) are described the different tissues and lesions analyzed for each survey either at field and laboratory surveys.

All the organisms (collected in the field and laboratory-exposed) were anaesthetized with 0.05% ethyl-4-aminobenzoate (benzocaine) during 5–10 min; then weight, length and externally examined. All the tissues from all the organisms were obtained by dissection and then fixed in phosphate buffered 10% formaldehyde fixative 24 h and included in paraffin. The histological sections were stained with Haematoxylin–Eosin and Haematoxylin/VOF (Gutierrez, 1967). Sections were reviewed by light microscopy and photographed (Olympus CH20). Damage to the tissues of organisms was semi-quantified by detecting the frequency of the lesions in each detected alteration. The samples of tis-