



## Heavy metals bioaccumulation in selected tissues of red swamp crayfish: An easy tool for monitoring environmental contamination levels



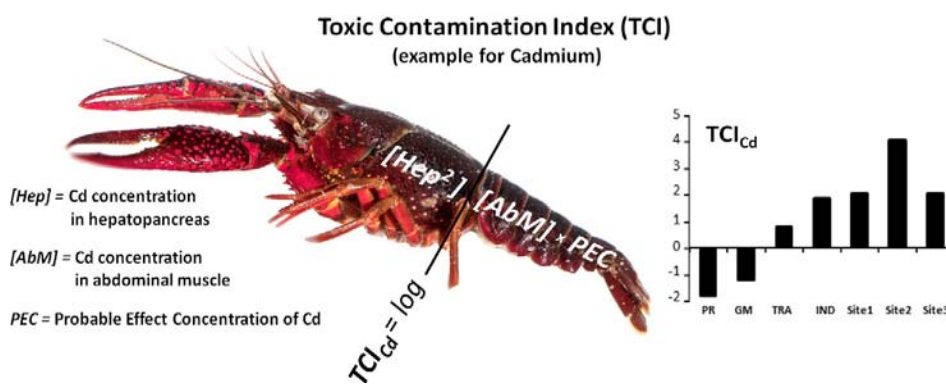
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### HIGHLIGHTS

- The heavy metals are amongst the most frequent pollutants of freshwater sediments.
- The heavy metals bioaccumulation in biota allows to evaluate health risks.
- We studied the metals bioaccumulation in selected tissues of the red swamp crayfish.
- We elaborated an index to assess biological response in contaminated sites.
- The index allows to evaluate the toxicity level by heavy metals in the sediments.

### GRAPHICAL ABSTRACT



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### ABSTRACT

In this paper we explored the heavy metal bioaccumulation (Cd, Cu, Pb and Zn) in *Procambarus clarkii*, a crayfish recently suggested as a potential bioindicator for metals pollution in freshwater systems. The present study is focused on crayfishes populations caught in a heavily polluted industrial and in a reference sites (Central Italy), though the results are generalized with a thorough analysis of literature metadata. In agreement with the literature, the hepatopancreas (Hep, detoxification tissues) of the red swamp crayfish showed a higher concentration of heavy metals in comparison to the abdominal muscle (AbM, not detoxification tissues) in the sites under scrutiny. Hep/AbM concentration ratio was dependent on the specific metal investigated and on its sediment contamination level. Specifically we found that Hep/AbM ratio decreases as follows: Cd (11.7) > Cu (5.5) > Pb (3.6) > Zn (1.0) and Pb (4.34) > Cd (3.66) > Zn (1.69) > Cu (0.87) for the industrial and reference sites, respectively. The analysis of our bioaccumulation data as well as of literature metadata allowed to elaborate a specific contamination index (Toxic Contamination Index, TCI), dependent only on the bioaccumulation data of hepatopancreas and abdominal muscle. In the industrial site, TCI expressed values much higher than the unit for Cd and Cu, confirming that these metals were the main contaminants; in contrast for lower levels of heavy metals, as those observed in the reference site for Cu, Zn and Pb, the index provided values below unit. TCI is proposed as a useful and easy tool to assess the toxicity level of contaminated sites by heavy metals in the environmental management.

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## 1. Introduction

Land-based activities bring increasing pressures on the environment, including the degradation of habitats and a heightened risk for the inhabiting organisms and human population. Industrial production and agriculture combined with population growth and consequent urbanization are the main causes of aquatic ecosystem pollution (EEA, 1999; Gómez-Gutiérrez et al., 2007; Li et al., 2010). Rivers serve as conveyors for pollution, and sediments in particular play an important role in the accumulation and transport of contaminants (Gómez-Gutiérrez et al., 2006; Davutluoglu et al., 2011).

Heavy metals are amongst the most frequent pollutants found in the environment, and the occurrence of high level of these chemicals in the freshwater ecosystem usually indicates the presence of anthropogenic sources i.e. industrial effluents, mining and refining, agricultural drainage, domestic discharges, and atmospheric deposition (Abbas et al., 2008; Klavins et al., 2000; Yu et al., 2001). On the other side, first row transition metals from V to Zn are also necessary elements for terrestrial organisms and ubiquitous in nature. For example Fe is an essential nutrient and even Cd, one of the key monitored pollutants, has some biological role, in the growth of marine microorganisms and in oceanic primary production, as substituent of Zn (Price and Morel, 1990; Abe, 2004). Amongst the other heavier metals, Pb is also widespread in nature. Because of its importance in many anthropic activities, enormous quantities have been produced and released in the atmosphere, soils, sediments and waters, entering in biogeochemical cycles (Patterson, 1965), although not playing any biological role and actually being one of the most toxic elements.

The metal load of a river system is influenced and regulated by a variety of factors such as basin geology, physiography, chemical reactivity, lithology, hydrology, vegetation and biological productivity (Garrels et al., 1975; Warren, 1981; Aurada, 1983; Jain and Sharma, 2006). Stream sediments can act as metal reservoirs, and the sediment-associated metals can be released and accumulated in biological organisms, thus entering the food web (Gibbs, 1977; Jain and Sharma, 2001; Filgueiras et al., 2002; Davutluoglu et al., 2011). Therefore, heavy metals exhibit a rather complex behaviour in river systems (Arnason and Fletcher 2003; Singh et al., 2005; Liu et al., 2009), which pose challenges to the characterization of their mobility, interactions and bioavailability. In particular, the measure of biological availability of a toxicant depends on: (i) the taxon investigated, (ii) the endpoint used and (iii) some characteristics of the sediment/water environment (Admiraal et al., 2000; Mäenpää et al., 2003; Desrosiers et al., 2008; Di Veroli et al., 2014). For that reason, an approach solely based on the measure of physical-chemical parameters may be not fully effective in order to assess the overall effect of the pollution of an ecosystem on its biota (Rosenberg and Resh, 1993). In particular trace pollutants are often elusive due to extremely variable seasonal loads (Archaimbault et al., 2010; Liess and Beketov, 2011). An alternative strategy is based on the study of specific taxa which live mainly in river sediments and can act as bioindicators (Johnson et al., 1992).

The model bioindicator species chosen in present study is *Procambarus clarkii* (Girard, 1852), called the red swamp crayfish. This species has been introduced in Europe in the seventies of last century first in Spain (Hasburgo-Lorena, 1986), afterwards in Italy (Gherardi et al., 1999; Gherardi and Acquistapace, 2007). It is the most diffuse and commercial crustacean species in the world (Hobbs et al., 1989; Huner, 1994) also because is particularly invasive and tolerant to the environmental conditions, due to the flexibility of its biological cycle (Huner and Barr, 1991; Lindqvist and Huner, 1999; Gutiérrez-Yurrita and Montes, 1999; Paglianti and Gherardi, 2004). The red swamp crayfish accumulates heavy metals particularly in the hepatopancreas, antennal gland, gill and exoskeleton tissues (Vogt, 2002). The hepatopancreas is a metabolically very active organ that sequesters contaminants and contributes to the detoxification process. The lowest concentrations of pollutants are observed in the abdominal muscle, the

edible part of the crayfish (Anderson et al., 1997; Alcorlo et al., 2006; Suárez-Serrano et al., 2010; Bellante et al., 2015). For all the above reasons *P. clarkii* is considered a potential good bioindicator for metals pollution in the freshwater system (Pastor et al., 1988; Rincón-León et al., 1988; Madigosky et al., 1991; Naqvi and Howell, 1993; Devi et al., 1996; Bollinger et al., 1997; Devesa et al., 2002; Sánchez-López et al., 2003; Alcorlo et al., 2006; Suárez-Serrano et al., 2010).

The aim of present work is to characterize heavy metals bioaccumulation in selected tissues of crayfishes living in environment at different levels of contamination. Luckily, we had the opportunity to discover a resident spontaneous population of red swamp crayfishes in a heavily polluted industrial site. We exploited the selective bioaccumulation of heavy metals in two different body tissues of the *P. clarkii*: hepatopancreas, that is an important organ of detoxification in decapod crustaceans, and abdominal muscle. Moreover, we analysed the results in comparison with those obtained for a much cleaner ecosystem in the same geographical area and provided a thorough characterization of the environmental chemical conditions of the two sites. The main goal of this paper is to formulate an index, based on our results and on literature metadata, of easy use to assess the toxic contamination level and the restoration effectiveness in the environmental management of contaminated freshwater environment.

The study areas, sampling campaign and analytical protocols are presented in the next section together with the definition of a novel index, based on the selective metal accumulation in the various crayfish tissues. The results of the experimental campaign are illustrated in Section 3 while a discussion of present results in a more general context of literature data is presented in Section 4.

## 2. Materials and methods

### 2.1. Study areas

*P. clarkii* specimens were captured in a heavily polluted industrial and technological area in Central Italy (IND) as well as in the nearby (about 35 km) Lake Trasimeno (TRA). The IND sampling site was schematically described in Fig. 1. Four sampling stations (St. 1–4) were set on two streams (A and B in Fig. 1), which define the IND basin. The hydrological regime of the streams is strongly dependent on precipitation. The four stations were located approximately at 1.5 km from the centre of the industrial area (Fig. 1).

Lake Trasimeno (TRA) is an important freshwater ecosystem in Italy: the largest shallow lake and the fourth largest lake in surface. For its ecological value this biotope is designated as “Natura 2000” Site (Directive No 43 (EEC, 1992): IT5210018 and IT5210070) and as Regional Park. The sediment contamination status of the TRA site was previously described from Di Veroli et al. (2012). Recently the red swamp crayfish population of the Lake Trasimeno has rapidly increased and has become object of professional fishery (Dórr et al., 2006).

### 2.2. Sampling campaign

Three seasonal samplings at stations 1–4 of the IND site from June to November 2012 were conducted (see Appendix 1, Supplementary material). Sediment samples (500 g: 5 sub-samples in different points of the station, about 100 g each) were taken at each station for heavy metals determination with a hand dredge collecting the superficial layer of the bottom sediments (3–5 cm). The sediment samples were preserved in Pyrex glass bottles and kept refrigerated at  $-18\text{ }^{\circ}\text{C}$  (MATT and APAT, 2005).

Crayfish specimens were found and caught at the IND site only in June and July 2012 at St. 2; in this station a total of 10 specimens (4 males and 6 females) were collected. Afterwards, 10 specimens (5 males and 5 females) were collected in September 2012 at the TRA site.

Crayfish specimens were properly cleaned by rinsing with distilled water and kept refrigerated at  $-18\text{ }^{\circ}\text{C}$ . Successively, these specimens

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