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The current situation of inorganic elements in marine turtles: A general review and meta-analysis☆

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Inorganic elements (Pb, Cd, Hg, Al, As, Cr, Cu, Fe, Mn, Ni, Se and Zn) are present globally in aquatic systems and their potential transfer to marine turtles can be a serious threat to their health status. The environmental fate of these contaminants may be traced by the analysis of turtle tissues. Loggerhead turtles (*Caretta caretta*) are the most frequently investigated of all the sea turtle species with regards to inorganic elements, followed by Green turtles (*Chelonia mydas*); all the other species have considerably fewer studies. Literature shows that blood, liver, kidney and muscle are the tissues most frequently used for the quantification of inorganic elements, with Pb, Cd, Cu and Zn being the most studied elements. *Chelonia mydas* showed the highest concentrations of Cr in muscle (4.8 ± 0.12), Cu in liver (37 ± 7) and Mg in kidney (17 μg g^{-1} ww), Cr and Cu from the Gulf of Mexico and Mg from Japanese coasts; *Lepidochelys olivacea* presented the highest concentrations of Pb in blood (4.46 5) and Cd in kidney (150 ± 110 μg g^{-1} ww), both from the Mexican Pacific; *Caretta caretta* from the Mediterranean Egyptian coast had the highest report of Hg in blood (0.66 ± 0.13 μg g^{-1} ww); and *Eretmochelys imbricata* from Japan had the highest concentration of As in muscle (30 ± 13 13 μg g^{-1} ww). The meta-analysis allows us to examine some features that were not visible when data was analyzed alone. For instance, Leatherbacks show a unique pattern of concentration compared to other species. Additionally, contamination of different tissues shows some tendencies independent of the species with liver and kidney on one side and bone on the other being different from other tissues. This review provides a general perspective on the accumulation and distribution of these inorganic elements alongside existing information for the 7 sea turtle species.

1. Introduction

Inorganic pollutants are present in aquatic ecosystems worldwide, deriving from natural sources but also from their extensive anthropic use in agriculture and industry (elements such as Pb, Cd, Hg, Al, As, Cr, Cu, Fe, Mn, Ni, Se and Zn). Coastal and marine contamination is increasing around the world, but the environmental levels of many contaminants which can elicit adverse effects is largely unknown for marine megafauna (cites). Bioaccumulation of these toxic substances has become a concern due to the possibility of their transfer to the food chain and its impact on diverse species of marine wildlife, including marine turtles (Camacho et al., 2014a; Ley-Quiñónez et al., 2011; Storelli and Marcotrigiano, 2003). A better understanding of pollution of marine ecosystems and the consequences for fauna is one of the priorities highlighted by sea turtle specialists from 13 countries in a recent synthesis of threats (Rees et al., 2016).

The accumulation of inorganic elements, particularly non-essential ones (such as Cd, Hg and Pb), may alter normal immune functions and increase the incidence of infectious illnesses in different species such as humans and marine turtles (D'Ilio et al., 2011; Finlayson et al., 2016; Rana, 2014). Several authors mention the importance of investigating the toxicological consequences of

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the marine accumulation of these elements, as well as the effects of bioaccumulation from a spatial and temporal perspective to understand the variations and to provide baseline concentrations for further studies (D'Ilio et al., 2011; da Silva et al., 2016; Day et al., 2007). Furthermore, other essential and trace inorganic elements (such as Al, As, Co, Cr, Cu, Se and Zn) have crucial roles in many essential physiological pathways in most vertebrates, but when their concentrations reach more than these physiological requirements, they can become toxic (Keller et al., 2006).

Due to their longevity, marine turtles can bioaccumulate diverse contaminants (such as metals) through the food chain and direct exposure. With this in mind, sea turtles represent a growing interest species as a potential marine ecosystem pollution bioindicator (Aguirre and Lutz, 2004; Andreani et al., 2008; Godley et al., 1999; Sakai et al., 2000). It has been hypothesized that the accumulation of pollutants varies among the species depending on different factors, including geographic location, life-stage, diet, species, sex, inter-species variance and intrinsic factors (i.e. toxicokinetics and toxicodynamics particular to each species) (Alava et al., 2006; Guirlet et al., 2008; Rainbow, 2002). However, few studies have tested for differences among species or populations (Anan et al., 2001; Andreani et al., 2008; Camacho et al., 2014a; Saeki et al., 2000), which implies a very low power of detection of association between pollutant and life-history traits. This small monophyletic group of animals inhabiting all oceans has been extensively studied, but no meta-analysis to search for any global inter-or intra-species tendency has been undertaken as of yet.

There are 7 extant marine turtle species that are globally distributed (Fig. 1). They utilize occupy several ecological niches in marine estuarine systems and forage across different trophic levels (Wallace et al., 2010, 2011). Olive Ridley turtles (*Lepidochelys olivacea*) are the smallest of the 7 species (35–55 cm of curved carapace length -CCL-in their adult stage), are carnivorous and individuals usually live in oceanic and neritic zones (MTSG, 2007); Leatherback turtles (*Dermochelys coriacea*) are the largest sea turtle (130–180 cm adults CCL), their diet is comprised mainly of jellyfish and they are truly pelagic animals (Stewart and Johnson, 2006). On the other hand, some species change their feeding habits throughout their life. Juvenile Green turtles (*Chelonia mydas*), for instance, switch from an omnivorous to a primarily herbivorous diet (Kubis et al., 2009). Organisms that feed higher on the food chain are generally exposed to higher concentrations of metals (Peakall and Burger, 2003). This varies, however, because some plants can also bioaccumulate high levels of some metals, including certain seaweeds (Peakall and Burger, 2003; Zhou et al., 2008). The indicatory properties of living organisms are used to determine the rate, concentration and range of present and future changes in the natural environment. Bioindicators are organisms that accumulate toxic substances, providing the basis for estimating the concentration of environmental pollution in these substances (Gadzała-Kopciuch et al., 2004). Thus, marine turtles can be useful as sentinel species and bioindicators for diverse pollutants, and they can help us to understand the risk not only to the species themselves but also to the ecosystem at large.

The aim of this work was (i) to review international information on essential and non-essential inorganic elements (Pb, Cd, Hg, Al, As, Cr, Cu, Fe, Mn, Ni, Se and Zn) in different tissues of marine turtles around the world and (ii) to perform a meta-analysis using all the relevant existing information in the seven species to characterize the presence of the inorganic elements worldwide. We have included all the representative existing information about the seven species of marine turtles (super-family Chelonioidea). Six species belong to the family Cheloniidae: Loggerhead (*Caretta caretta*), Green turtle (*Chelonia mydas*), Olive Ridley (*Lepidochelys olivacea*), Kemp's Ridley (*Lepidochelys kempii*), Hawksbill (*Eretmochelys imbricata*), and Flatback marine turtles (*Natator depressus*) and one to the family Dermochelyidae: Leatherback (*Dermochelys coriacea*).

2. Material and methods

2.1. Data collection

To collect the available literature about inorganic elements in sea turtles around the world, we used 4 different search engines: Google Scholar (https://scholar.google.com), PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Bibliovie (http://bibliovie.inist.fr) and Scopus (https://www.scopus.com). We always used different combinations of all the 7 species names (scientific or common name) with "inorganic elements", "metals", "heavy metals", "trace elements", "metalloids" and also the common names for each element one by one. To avoid publication bias, both published and unpublished trials were included, such as conference abstracts and thesis works. For trials with several treatment groups, the eligibility of each individual group was assessed and only those relevant were included. Additionally, when results were represented for age or gender, adults and females were chosen to make the meta-analysis more homogenous. A total of 58 works on the different species and regions were included in this review, the most recent being published in April 2017. One of the works (Sakai et al., 2000), presented very high Hg concentrations reported in $\mu g\ g^{-1}$ ww; we contacted the author and he confirmed that this was a mistake in the table and concentrations of Hg were in $ng\ g^{-1}$ ww.

"Heavy metals" is a term often used as a group name for metals and semimetals (metalloids) that have been associated with contamination and potential toxicity or ecotoxicity. However, many authors debate the appropriate use of this terminology, arguing that it has no geochemical, biological, chemical or toxicological basis (Appenroth, 2010; Duffus, 2002; Hodson, 2004). We will use the term "inorganic elements" to define all the elements included in this review to avoid any ambiguity; "non-essential" for those non-essential elements with a high toxicity risk at low doses (Pb, Cd and Hg) and "essential" for those trace elements which are essential, but potentially toxic at high concentrations (Al, As, Cr, Cu, Fe, Mn, Ni, Se and Zn).

2.2. Analysis and statistics

For the analysis of this dataset, two challenges were met: (i) studied characteristics were a mix of quantitative and qualitative variables, and (ii) not all the same inorganic elements were analyzed in all studies and therefore, many data are missing in the dataset. The first point was resolved with multiple factorial analysis (MFA) using mixed data (qualitative and quantitative). MFA is a principal component method used to explore data with both continuous and categorical variables (Pagès, 2004). In summary, the continuous variables were scaled to unit variance and the categorical variables were transformed into a disjunctive data table and then scaled. This ensures a balance of the influence of both continuous and categorical variables in the analysis. Becue-Bertaut and Pagès (2008) have enhanced MFA using grouping of variables. Missing data have been treated using the procedure described in Josse and Husson (2012) and Audigier et al. (2016) implemented in the R package missMDA (Josse and Husson, 2016). Missing values were imputed with a multiple factor analysis model without the supplementary variables (tissue, species and oceanic basin) to ensure that these factors could be further tested. This procedure ensures that missing data do not influence any conclusions but nor do they reduce power of analysis.

Meta-analysis of the load of inorganic elements (Pb, Cd, Hg, As,