Trace element accumulation and trophic relationships in aquatic organisms of the Sundarbans mangrove ecosystem (Bangladesh).

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Highlights

- Trace elements were determined in organisms from the Sundarbans mangrove
- The levels found were similar to those determined in wildlife from other mangroves
- Levels in three edible species were close to threshold limits for human consumption
- Except for Cr, As and Hg, concentration of elements decreased with trophic level
1. Abstract

The Sundarbans forest is the largest and one of the most diverse and productive mangrove ecosystems in the world. Located at the northern shoreline of the Bay of Bengal in the Indian Ocean and straddling India and Bangladesh, the mangrove forest is the result of three primary river systems that originate further north and northwest. During recent decades, the Sundarbans have been subject to increasing pollution by trace elements caused by the progressive industrialization and urbanization of the basins of these three rivers. As a consequence, animals and plants dwelling downstream in the mangroves are exposed to these pollutants in varying degrees, and may potentially affect human health when consumed.

The aim of the present study was to analyse the concentrations of seven trace elements (Zn, Cu, Cr, Hg, Pb, Cd and As) in 14 different animal and plant species collected in the Sundarbans in Bangladesh to study their transfer through the food web and to determine whether their levels in edible species are acceptable for human consumption. $^{\delta^{15}}$N values were used as a proxy of the trophic level.

A decrease in Zn, Cu, Pb and Cd levels was observed with increasing trophic position. Trace element concentrations measured in all organisms were, in general, lower than the concentrations obtained in other field studies conducted in the same region. When examined with respect to accepted international standards, the concentrations observed in fish and crustaceans were generally found to be safe for human consumption. However, the levels of Zn in Scylla serrata and Cr and Cd in Harpadon nehereus exceeded the proposed health advisory levels and may be of concern for human health.

Keywords: Bay of Bengal; heavy metals; fish; crustaceans; plants; stable isotopes
2. Introduction

The Sundarbans mangrove forest, located at the northern shoreline of the Bay of Bengal in the Indian Ocean and straddling India and Bangladesh is one of the most diverse and productive ecosystems in the world (Islam and Wahab, 2005). The mangrove covers the vast delta where the Ganges and Brahmaputra rivers converge and comprises a large expanse of wetlands, ponds and small islands of halophytic mangrove forests with a rich biodiversity. For centuries, the delta has been widely exploited, mainly for agricultural purposes (Getzner and Islam, 2013), and this has produced a severe decimation of fish stocks (Islam and Wahab, 2005).

The vast urbanization and rapid industrialization in the coastal zone of the Indian Sundarbans has resulted in considerable ecological imbalance (Mitra et al., 2012). Untreated domestic and industrial residues have been discharged into the rivers, increasing the levels of a number of chemical contaminants, such as heavy metals (Mitra et al., 2011; 2012), organochlorine pesticides, polychlorinated biphenyls (Binelli et al., 2009; Sarkar et al., 2008 a; b) and polycyclic aromatic hydrocarbons (Guzzella et al., 2005). These changes may have altered the estuary geochemistry and affected the coastal environment (Sarkar et al., 2007).

Chemical contaminants can accumulate in the organs and tissues of aquatic organisms at concentrations higher than those in water. Moreover, some of them can biomagnify in the food web and cause negative biochemical and physiological effects to top predators, including humans (e.g. Aguilar and Borrell, 1994; Al-Reasi et al., 2007; Ikemoto, et al., 2008a; b; Jara-Marini et al., 2009; Kudo et al., 1998). Among these compounds, trace elements occur naturally in aquatic ecosystems due to weathering of rocks and soils (e.g. Garrett, 2013) and, while many of them do not biomagnify through the food web (Aguilar et al., 1999), anthropogenic activities such as industrial development can cause an increase in their environmental concentrations, leading to toxic impacts on ecosystems and organisms (Mitra et al., 2012).

In particular, arsenic (As), a toxic metalloid with geogenic sources (Järup, 2003), is known to be a significant pollutant of soil, water, and biota in the West Bengal delta plain. In this region, most of the As in soils derives from eroded Himalayan sediments which enter solution following reductive release from solid phases under anaerobic conditions (Polizzotto et al, 2008). As a consequence, the degree of groundwater contamination with As and its further spreading into surface soil and food crops has reached a critical level in the Ganges-Brahmaputra delta (Bhattacharya et al., 2013). This area has been described as the most acutely contaminated site by this metalloid worldwide although the degree of As environmental pollution in the area is highly variable (Fattorini et al., 2013; McCarty et al., 2011).

The progressive industrial development in Bangladesh and India and the associated increase of toxic compound levels in the Sundarbans mangrove (Ahmed et al, 2010; 2011, 2015; Kumar et al., 2011; Sarkar et al., 2008a;b; Silva Filho et al, 2010) demands rigorous control over their concentration and biomagnification processes in biota. To determine elemental biomagnification patterns, researchers can infer the trophic level of the species under study through the ratios of the stable isotopes of certain elements. Thus, the relative abundance of \(^{15}\text{N}\) to \(^{14}\text{N}\) (\(\delta^{15}\text{N}\)) in animal tissues are highly indicative of trophic position due to the enrichment of \(^{15}\text{N}\) in consumers relative to their prey. As a consequence, the \(\delta^{15}\text{N}\) of a given animal relative to the \(\delta^{15}\text{N}\) of a primary consumer of known trophic position is accepted as an index of trophic position (Post, 2002) thus producing general and integrated information on the trophic level at which the species feeds; however, it does not provide detailed dietary information, this is, the specific composition of the prey consumed. By contrast, \(\delta^{13}\text{C}\) values (relative abundance of \(^{13}\text{C}\) to \(^{12}\text{C}\)) exhibit considerable variability, with little or no
consistent increase with increasing trophic level (Vander Zanden et al., 2001). Because δ\(^{13}\)C values are conserved as one moves up the food chain but vary at the base, the δ\(^{13}\)C of aquatic consumers can provide information about the sources of energy for higher consumers (Boutton, 1991). δ\(^{13}\)C values differ between plant species that discriminate differently \(^{13}\)C and \(^{12}\)C (Cardona et al., 2007). In addition, nearshore and benthic systems with high nutrient concentrations, and thus high productivity, are typically more \(^{13}\)C enriched than offshore, pelagic systems (Borrell et al., 2011; McMahon et al., 2013). As a consequence of these sources of variation, this index has proven useful in identifying the habitat or segment of the ecosystem where particular organisms feed.

The aim of the current study was twofold: First, to assess the impact of pollution by trace elements in the marine ecosystem of the Sundarbans of Bangladesh and the degree of biomagnification of these elements, if existing, and, second, to evaluate the potential toxicological risk to human health that can result from the consumption of species originating from this region. To do so, we sampled representative species that were situated at various trophic levels in the ecosystem and analysed their tissues for carbon and nitrogen stable isotope ratios as well as for trace elements.

3. Material and methods

3.1 Material

Five individuals from 10 species of fish, 2 of crustaceans, 2 samples of zooplankton (one of ichthyoplankton and one of planktonic crustaceans), and 2 of plants (Table 1) were collected in December 2011 from the Sundarbans area of Bangladesh (Figure 1). The species were selected because of their importance in the mangrove ecosystem and their relevance as human food. Both prey species and predators were selected to create a representation of the food web and thus to enable identification of biomagnification patterns.

Samples of fish and crustaceans were obtained from the local market at Khulna, the main harbour bordering the Sundarbans mangrove (site 1, Figure 1). After determination of body length (Table 1), samples of muscle were taken from the crustacean and fish species, and the rest of their body was discarded. The only exception was Amblyparyngodon mola which, because of its small size, could not be properly dissected and the whole body was taken for analysis. The samples of plants and zooplankton were collected in the field (site 2, Figure 1). Zooplankton was collected with a 1m\(^2\) rectangular, 0.5mm mesh net. Samples were examined immediately after collection and separated into small crustaceans and fish larvae. Five subsamples were taken from each sample group.

All samples were oven-dried (40°C, 72 hours) in situ in a portable food dehydrator (Excalibur Food Dehydrator). Once in the laboratory, a subsample was taken from each and the rest was kept in the biological tissue bank of the University of Barcelona. The sample to be analysed was homogenized and two aliquots separated, one for analysis of the stable isotope composition (δ\(^{15}\)N, δ\(^{13}\)C) and another for analysis of the trace elements. The samples were stored at -20°C until analysis.
Table 1. Number of samples collected by species and species’ mean length.

<table>
<thead>
<tr>
<th>Species</th>
<th>number</th>
<th>Common name</th>
<th>Length (cm)</th>
</tr>
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<td></td>
<td></td>
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<tr>
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<td>individuals</td>
<td>mangrove</td>
</tr>
<tr>
<td><em>Nymphaea pubescens</em></td>
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<td>individuals</td>
<td>water lily</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Planktonic crustaceans</td>
<td>5</td>
<td>samples of various</td>
<td></td>
</tr>
<tr>
<td>Ichthyoplankton</td>
<td>5</td>
<td>samples of various</td>
<td></td>
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<tr>
<td><strong>Crustaceans</strong></td>
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<td></td>
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<td>5</td>
<td>individuals</td>
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<tr>
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<td>individuals</td>
<td>18.0±0.07</td>
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<tr>
<td><strong>Fish</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>18.0±0.76</td>
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<td>26.5±1.56</td>
</tr>
<tr>
<td><em>Pampus argenteus</em></td>
<td>5</td>
<td>individuals</td>
<td>21.7±1.56</td>
</tr>
</tbody>
</table>

3.2. Methods

3.2.1. Stable isotopes

Because lipids cause errors in the analyses by decreasing the δ\(^{13}\)C values, lipids were extracted from the sample previous to the stable isotope analysis. To do so, approximately 1 g of homogenized sample from each specimen was sequentially soaked in a chloroform:methanol (2:1) solution and mixed with a rotator to accelerate the process (DeNiro and Epstein, 1977). The resulting subsample was over-dried and approximately 0.5 mg of it was placed into tin buckets, which were crimped before combustion. Isotope analyses were performed by means of elemental analysis-isotope ratio mass spectrometry using a Thermo Finnigan Flash 1112 (CE Elantech, Lakewood, NJ, USA) elemental analyser, coupled to a Delta C isotope ratio mass spectrometer via a CONFLO III interface (Thermo Finnigan MAT, Bremen, Germany).

Stable isotope abundances were expressed in delta (δ) notation, where the relative variations of stable isotope ratios are calculated in per mil (‰) deviations from predefined international standards according to the equation:

\[
\delta X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000
\]
where X is $^{13}$C or $^{15}$N, and Rsample and Rstandard are the $^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N ratios in the sample and standard, respectively. The standard reference materials were carbon in the V-PDB (Vienna Pee Dee Belemnite) calcium carbonate and nitrogen gas in the atmosphere.

The isotopic ratio mass spectrometry facility at the laboratory of the Centres Científics i Tecnològics of the University of Barcelona (Spain) applies international isotope secondary standards, of known R ratios, supplied by the International Atomic Energy Agency (IAEA, Vienna). Secondary standards for carbon, of known $^{13}$C/$^{12}$C ratio, were: polyethylene (IAEA CH$_2$, $\delta^{13}$C = -31.8‰), graphite (USGS24, $\delta^{13}$C = -16.1‰) and sucrose (IAEA CH$_6$, $\delta^{13}$C = 10.4‰). Secondary standards for nitrogen, of known $^{15}$N/$^{14}$N ratios, were (NH$_4$)$_2$SO$_4$ (IAEA N1, $\delta^{15}$N = +0.4‰ and IAEA N2, $\delta^{15}$N = +20.3‰), and KNO$_3$ (IAEA NO$_3$, $\delta^{15}$N = +4.7‰). All of them were inserted in the analytical runs every 12 samples to calibrate the system and compensate for any drift over time. Replicate assays of standard materials indicated measurement errors of ±0.2 and ±0.3‰ for carbon and nitrogen respectively.

Thresholds for accurate determination of isotopic ratios of Carbon and Nitrogen were 5 ug and 10ug, respectively. Therefore, the amount of tissue processed was well above such thresholds.

3.2.2. Trace elements

Trace elements were analysed in the five individuals of each species of crustaceans and fishes and in one individual of each plant. Samples of zooplankton were too small to conduct both the stable isotope and the trace element analyses and, given that zooplankton is not used for human consumption, priority was given to stable isotope analysis.

Approximately 100 mg of each sample was oven-digested at 90°C overnight in an acid solution (H$_2$O$_2$/HNO$_3$, 1:4 ratio) in Teflon vials. The samples were then diluted (1:8) with ultrapure (Milli-Q) water and transferred to plastic tubes to be analysed via inductively coupled plasma mass spectrometry (ICP-MS) with a Perkin-Elmer Elan 6000 for Cd, Zn, Cu, Hg, Pb, and Cr, and with an Agilent 7500ce with a micro-flow nebulizer (Agilent, Germany) with helium gas in the collision cell (to remove interferences in the ICP-MS measurements) for As. Procedure validation was corroborated using the reference material DORM-3 Fish Protein (National Research Council, Canada). This standard was treated and analysed under the same conditions as the samples. Recoveries of the metals ranged from 95 to 100%. Digestion blanks were also prepared in each sample digestion series (40 samples) to alert for potential contamination during the analytical procedure. For quality control purposes, the standards of the calibration curve were run before and after each sample series.

All the analyses were performed at the Analytical Services of the University of Barcelona (Centres Científics i Tecnològics de la Universitat de Barcelona, CCiT-UB).

3.2.3. Data analyses

Trophic levels (TLs) were estimated from raw $\delta^{15}$N values for each species using the plants as a baseline to estimate shifts in relative trophic levels within the ecosystem, as described by Post (2002). The following equation was used:

$$ TL_{\delta^{15}N} = TL_{baseline} + (\delta^{15}N_{species} - \delta^{15}N_{baseline})/3.4 $$

where $TL_{baseline}$=1, $\delta^{15}N_{baseline}$=3.69‰ (mean $\delta^{15}$N value of Ceriops decandra and...
Concentrations of trace elements in this study are presented as mg kg\(^{-1}\) dry weight. Normality in the distribution of trace element concentrations was tested using the Kolmogorov-Smirnov test. As the test indicated that the data departed from normality in several cases, all series of data were log-transformed for subsequent analyses.

Homogeneity of variances was checked through the Levene’s test. As some variables (i.e. log concentrations of Cu, Zn, Hg and Cr) did not fulfill this condition, comparisons of means among species in these element concentrations were tested by a Welch ANOVA and the Games Howell post-hoc test. For the other elements (i.e. log concentrations of Cd, Pb, As), in which variances were homogeneous, comparisons of means among species were examined with a one-way by analysis of variance (ANOVA) and the post-hoc Tukey test.

Linear regressions were used to assess the relationships between trace element concentrations (logarithm units) and the \(\delta^{15}\)N value, and in this way investigate the potential effect of trophic level on metal concentrations. Plants were not included in these analyses because only one replicate was available from them. A p-value of less than 0.05 was considered to indicate statistical significance. All statistical calculations were performed using the statistical package SPSS15 (SPSS Inc., Chicago, IL, USA).

4. Results

4.1. Isotopic compositions of organisms and trophic level

The isotopic values for all species and the trophic level calculated according to the formula used by Post (2002) are illustrated in Figure 2. \(\delta^{15}\)N values ranged from 3.6‰ in the primary producer Ceriops decandra (trophic level = 1) to 11‰ in Pampus argenteus (trophic level = 3), and \(\delta^{13}\)C values ranged from -30.4‰ in Amblypharyngodon mola to -16.4‰ in P. argenteus.

4.2. Element concentrations in organisms and their relationships with trophic level

Elemental concentrations in plants should be contemplated only as a preliminary result because only one replicate was analysed from each species. The results of Amblypharyngodon mola should also be considered with caution because, despite the fact that 5 replicates were analysed, the whole body and not only the muscle was used for the determination of trace elements. As a consequence, levels of Cd, Cu, Zn and As in this species may have been somewhat overestimated in relation to those of species in which only muscle was analysed (Qiu et al., 2011) but are still considered acceptable for the assessment of interspecific variation.

The concentrations of trace elements in the tissues of different species are presented in Table 2. Zn concentrations ranged from a mean value of 178 mg kg\(^{-1}\) in Scylla serrata to 9.4 mg kg\(^{-1}\) in Tenualosa ilisha. Cu concentrations ranged from a mean value of 61 mg kg\(^{-1}\) in S. serrata to 0.8 mg kg\(^{-1}\) in Panna microdon. As levels ranged from 9 mg kg\(^{-1}\) in S. serrata to 0.081 mg kg\(^{-1}\) in Strongylura leira, and Cr levels ranged from 4 mg kg\(^{-1}\) in Harpodon neherens to 1.3 mg kg\(^{-1}\) in Ceriops decandra.

The remaining metals: Pb, Hg and Cd exhibited concentrations lower than 1 mg kg\(^{-1}\) in all organisms (Table 2). The maximum values of Pb were observed in the plants -
Nymphaea pubescens (0.87 mg kg\(^{-1}\)) and C. decandra (0.30 mg kg\(^{-1}\)), - with the lowest values in high trophic level organisms such as P. microdon and S. leira, with concentrations of 0.03 mg kg\(^{-1}\) in both organisms. Hg levels ranged from 0.6 mg kg\(^{-1}\) in Strongylura leira to 0.02 mg kg\(^{-1}\) in T. ilisha. Cd concentrations were below 0.01 mg kg\(^{-1}\) in both high trophic level (S. leira, Acanthopagrus berda and P. microdon) and low trophic level (Penaeus monodon and Mugil cephalus) organisms. The highest Cd level was found in H. nehereus (0.23 mg kg\(^{-1}\)).

Significant differences were observed among species for all elements (ANOVA (F(11,48) > 17, p < 0.0001; Welch ANOVA (F(11,19) > 10, p < 0.0001). Results from the multicomparisons post hoc tests between species and elements are shown in Table 3.

Table 4 presents the minimum and maximum concentrations of trace elements recorded in the Sundarbans (India or Bangladesh area) by other authors for the same species as in this study. Although the essential metals in Bangladesh Sundarbans’ organisms exhibited similar concentrations to other nearby areas, the non-essential elements exhibited, in general, significantly lower concentrations, and the As exhibited the same range of values.

With respect to the relationship between log-transformed element concentrations and \(\delta^{15}N\) values, which was used to determine the trophic level, we found that Zn, Cu, Pb (p < 0.01) and Cd (p < 0.05) exhibited a significant negative relationship that suggested probable bio-diminution of these metals and Cr, As and Hg did not exhibited any trend (Figure 3).
Table 2. Element concentrations (mg kg$^{-1}$ dry weight) in biological samples, and maximum permitted levels (mg kg$^{-1}$ wet weight) for human intake in fishes and crustaceans. Zn, Cu and As maximum permitted levels are those established in New Zealand; Cr in Hong Kong (Nauen, 1983); and Pb, Cd and Hg in the European Union (European Commission, 2006; 2008). To allow comparison of analytical results in the studied organisms with permitted limits, the latter were converted from wet to dry weights assuming an average 74% water content (Huss, 1995) and depicted between brackets.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Zn (mg kg$^{-1}$)</th>
<th>Cu (mg kg$^{-1}$)</th>
<th>Hg (mg kg$^{-1}$)</th>
<th>Pb (mg kg$^{-1}$)</th>
<th>Cd (mg kg$^{-1}$)</th>
<th>Cr (mg kg$^{-1}$)</th>
<th>As (mg kg$^{-1}$)</th>
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<td>0.0077</td>
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**Maximum permitted levels**

<table>
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<th></th>
<th>fish wet w. (dry w.)</th>
<th>crustaceans wet w. (dry w.)</th>
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<tr>
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<td>40 (154)</td>
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<tr>
<td>Cu</td>
<td>30 (115)</td>
<td>30 (115)</td>
</tr>
<tr>
<td>Hg</td>
<td>0.5 (1.9)</td>
<td>0.5 (1.9)</td>
</tr>
<tr>
<td>Pb</td>
<td>0.3 (1.2)</td>
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</tr>
<tr>
<td>Cd</td>
<td>0.05 (0.19)</td>
<td>0.5 (1.9)</td>
</tr>
<tr>
<td>Cr</td>
<td>1 (3.8)</td>
<td>1 (3.8)</td>
</tr>
<tr>
<td>As</td>
<td>1 (3.8)</td>
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</tr>
</tbody>
</table>
**Table 3:** Results from the multicomparison post hoc tests between pairs of species, split by element. Elements depicted in a given cell are those that showed statistical difference (p<0.05) between the species involved. Results in the top right half of the table correspond to the elements whose variances were homoscedastic (Pb, Cd, As) and which were compared between species with a Tukey test, while results in the low left half of the table correspond to the elements whose variances were not homoscedastic (Zn, Cu, Hg, Cr) and were compared between species with a Games Howell post hoc test.
Table 4. Minimum and maximum concentrations (mg kg\(^{-1}\) dry weight) of trace elements recorded in the Sundarbans (India or Bangladesh area) by other authors for the same species as in this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Species Name</th>
<th>Zn (mg kg(^{-1}))</th>
<th>Cu (mg kg(^{-1}))</th>
<th>Cr (mg kg(^{-1}))</th>
<th>As (mg kg(^{-1}))</th>
<th>Pb (mg kg(^{-1}))</th>
<th>Hg (mg kg(^{-1}))</th>
<th>Cd (mg kg(^{-1}))</th>
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<tr>
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<td>16-80</td>
<td>10-60</td>
<td>5.5-10</td>
<td>0.2-0.5</td>
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<td>0.2-0.3</td>
<td>Mitra et al., 2012</td>
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</tr>
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<td>0.2-0.3</td>
<td></td>
<td>Guhathakurta and Kaviraj, 2000</td>
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<tr>
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<td>P. monodon</td>
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<td>24-35</td>
<td>12-21</td>
<td>1.7-2.9</td>
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<td></td>
</tr>
<tr>
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<td>48-55</td>
<td>18-25</td>
<td>1.6-5.4</td>
<td>0.2-0.6</td>
<td>0.18-0.28</td>
<td>0.003-0.008</td>
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<td>6.28</td>
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<td>Ahmed et al., 2011</td>
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<td>4-18</td>
<td>0.00-0.03-0.009</td>
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<td>0.18-0.28</td>
<td>Current study</td>
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<td>15-63</td>
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<td></td>
<td>Mitra et al., 2012</td>
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</tr>
<tr>
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<td></td>
<td>8-10</td>
<td>1-4</td>
<td>1.2-2.4</td>
<td>3-7</td>
<td>0.02-0.1</td>
<td>0.003-0.009</td>
<td>Current study</td>
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<td>10-23</td>
<td>nd</td>
<td>3.8-21</td>
<td>0.55-1.28</td>
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<td>De et al., 2010</td>
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<tr>
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<td>nt-1.74</td>
<td>0.22-0.60</td>
<td>0-2.1</td>
<td>Kumar et al., 2012</td>
<td></td>
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<tr>
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<td>P. argentius</td>
<td></td>
<td>12-18</td>
<td>0.5-1.2</td>
<td>2.2-4.4</td>
<td>2-6</td>
<td>0.01-0.1</td>
<td>0.003-0.055</td>
<td>Current study</td>
<td></td>
</tr>
</tbody>
</table>
5. Discussion

5.1. Isotopic compositions of organisms and trophic level

The δ¹³C values were highly variable among the studied species. Similarly to results obtained in previous studies in other mangrove ecosystems (Faye et al., 2011; Newell et al., 1995; Schwamborn et al., 2002; Thimdee et al., 2004; Wooller et al., 2003), the primary producers analysed (N. pubescens and C. decandra) exhibited very low δ¹³C values. Conversely, δ¹³C values in the majority of consumers were much higher (Figure 2). Given that δ¹³C values are conserved as one moves up the food chain (Boutton, 1991), we conclude that these plants are not the primary carbon source for most species, consistently with previous studies that indicate that the mangrove vegetation is not the primary energy source for many organisms (Bouillon et al., 2000; Heithaus et al., 2011; Layman, 2007). Rather, these organisms probably rely on phytoplankton, marine algae and/or seagrasses, which normally exhibit higher δ¹³C values than mangrove vegetation (Layman, 2007). The only exception to this appear to be P monodon and A. mola, species that during certain stages of their life inhabit upstream waters, where consumers are known to partially rely on mangrove carbon (Chong et al., 2001; Mamun et al., 2004; Rodelli et al., 1984).

Because δ¹⁵N typically increases with trophic transfer, a larger δ¹⁵N range will probably reflect additional trophic levels within a food web (Layman, 2007; Layman et al., 2007). In the present study, we observed a 7.42% δ¹⁵N range between primary producers and top predators. Assuming a mean δ¹⁵N fractionation of 3.4% with trophic transfer (Post, 2002), this range would span approximately two entire trophic levels (Figure 2). By comparing the results of the analysed fishes with those from previous studies whose trophic levels were calculated using gut contents, as reported in FishBase (http://www.fishbase.org), it can be seen that some species (e.g., L. Calcarifer and H. nehereus) had been reported to exist between one and two trophic levels higher than the trophic levels observed in the present study. Thus, in the present case the food chain length is relatively short, possibly as a result of overfishing (Pauly et al., 1998) or, alternatively, because of the lack of intermediate consumers, which would indicate that the top predators fed on primary consumers of large size, such as S. serrata or P. monodon.

5.2 Arsenic (As)

Approximately 20 years ago, groundwater in Bangladesh was discovered to contain very high levels of As (Smith et al., 2000). The principal cause of this contamination was the proliferation of tub wells that abstract water from subsurface alluvial aquifers to provide bacteriologically safe drinking water. These wells increased the bioavailability of natural As to humans (Nickson et al., 2000) who, after ingesting such contaminated water, suffered several pathologies such as skin diseases and cancer. The World Health Organization (WHO) catalogued this contamination as the largest mass poisoning in history.

To date, As levels in aquatic organisms have not thoroughly been studied in the Sundarbans area, nor has the transfer of As through the food web been well investigated. Scanty studies exist about the chemical forms of As in fish and its bioavailability, both of which are needed to estimate As toxicity (Santra et al., 2013). However, more than 85% of the As found in edible portions of marine fish and shellfish is known to be organic As due to the biomethylation processes encountered by the As in the food chain (Rahman et al., 2012), which result in the conversion of inorganic
forms into less toxic organoarsenic compounds (Mukherjee and Bhupander, 2011; Tu et al., 2011; WHO, 2012).

As levels in organisms in the current study were heterogeneous. The maximum values were observed in S. serrata (8.94 mg kg\(^{-1}\)) and the lowest values were found in plants: C. decandra (0.11 mg kg\(^{-1}\)) and N. pubescens (0.09 mg kg\(^{-1}\)), which typically do not accumulate As (Chowdhury et al., 2015), and S. leira (0.08 mg kg\(^{-1}\)). Sediments may plausibly act as an important exposure source for As into aquatic organisms, particularly benthonic invertebrates such as S. serrata. This higher capacity of crustaceans than fishes to concentrate As has been observed in other As-contaminated areas of the world, such as the Mekong River Delta (southern Vietnam) (Ikemoto et al., 2008b; Tu et al., 2012).

When compared with other products from Bangladesh, the marine products in this study exhibited significantly elevated As concentrations. Al Rmaili et al. (2005) reported mean levels of total As of 0.35 mg kg\(^{-1}\) in freshwater fish foodstuffs imported from Bangladesh, which were nearly one order of magnitude lower than the levels obtained. Moreover, these authors observed that the As content in foodstuffs from the UK was approximately 2- to 3-fold lower than those observed in the products imported from Bangladesh.

When comparing the results of this study with other studies performed in similar organisms from the Sundarbans, the results were variable. Kumar et al. (2012) analysed the As levels in H. nehereus (mean 0.83 mg kg\(^{-1}\)) and P. argenteus (mean 0.57 mg kg\(^{-1}\)) from the Bay of Bengal (Northeast India). These levels were significantly lower (3 to 6 times) than those obtained in the current study (means 2.45 mg kg\(^{-1}\) and 3.61 mg kg\(^{-1}\), respectively). However, another study of speciation of As in the biota of the Hooghly Estuary in the Indian Sundarbans exhibited much higher levels than those of the current survey (e.g., in H. nehereus, 20.62 mg kg\(^{-1}\)) (Fattorini et al., 2013) (Table 4).

One of the species chosen for this study was M. cephalus, which is considered a good indicator of pollution due to its wide distribution. Moreover, this species reflects the benthic feeding strategy and is continually sifting through benthic sediments for food, which increases exposure to contaminated sediments. Waltham et al. (2013) reviewed trace element concentrations in M. cephalus in a large proportion of the geographical range of this species, including many coastal locations around the globe. The As levels observed in M. cephalus in the present study are among the highest levels reported by Waltham et al. and were only surpassed by the levels in M. Cephalus, collected from two coastal locations, the Zheijiang coast (China) and the Anpin Harbour (Taiwan).

Currently, there is no EU-wide regulation for As levels in foodstuff, but some countries such as New Zealand have established a maximum allowable level for inorganic As in fish and crustaceans of 1 mg kg\(^{-1}\) wet weight (~3.8 dry weight) (Nauen, 1983). In this study, only S. serrata and T. ilisha exceeded this limit. However, as previously stated, inorganic As accounts for only 10-15% of the total As in fish and shellfish, so the levels found in this study, although elevated, are considered to pose little risk to the health of consumers.

### 5.3 Heavy metals

There is a complete lack of knowledge of the impact of heavy metals on aquatic organisms in the eastern region of the Sundarbans area.

Heavy metals are natural constituents of the Earth’s crust, but human activities, such as mining and fossil fuel combustion, have altered their geochemical cycles and biochemical balance, thus resulting in their release into the environment. Some metals,
such as zinc (Zn), copper (Cu) and chromium (Cr) are essential elements for living organisms but can result in toxic effects at high concentrations. Others, such as mercury (Hg), lead (Pb), and cadmium (Cd), are non-essential metals and can produce significant toxic effects even at very low levels (e.g. Varotto et al., 2013).

5.3.1. Essential metals (Zn, Cu and Cr)

Cu and Zn levels in organisms from the Bangladesh Sundarbans fell within the same range as those levels found in similar organisms from other localities worldwide (e.g., Celik and Oehlenschläger, 2004; Hossain et al., 2001; Jara-Marini et al., 2009; Tu et al., 2012; Waltham et al., 2013; Zhao et al., 2013). Thus, there does not seem to be significant differences in the concentration of Cu or Zn in aquatic biota among regions despite potential differences in trace metal inputs. Because these elements are essential to organisms, organisms have developed a variety of homeostatic control mechanisms to regulate internal concentrations (Andeson et al., 1978; McGeer et al., 2000; Rejamon et al., 2010). However, the concentrations measured varied according to the species analysed. Thus, crustaceans exhibited higher Cu concentrations than fishes in general. Cu is usually present at the highest concentrations in many molluscs and crustaceans in which the respiratory pigment haemocyanin contains Cu (Barwick and Maher, 2003). Carnivorous fishes exhibited the lowest Cu concentrations, consistent with other studies (e.g., Tu et al., 2012).

If we compare the results of the current study with those of other studies in the same species collected in other parts of the Sundarbans (Bay of Bengal from India or Bangladesh), few differences exist (Table 4). Thus, Zn and Cu concentrations in P. monodon in several different areas of the Indian Sundarbans (Guhathakurta and Kaviraj, 2000; Hossain and Kahn, 2001; Mitra et al., 2012) are within the same range as the present results. Indeed, Zn concentration in P. monodon in Bangladesh (current study) coincides with the mean level of Zn (52 mg kg\(^{-1}\); Mitra 2012) in the zone that covers the western sector of the Sundarbans, an area at the confluence of the Hooghly River and the Bay of Bengal. Conversely, the concentrations found in this study in S. serrata were higher than those found by Ahmed et al. (2011). Moreover, although Zn levels in fish were similar across the entire area, those of Cu were somehow lower in the area analysed in the current study (Table 4).

Cr is essential to normal carbohydrate, lipid and protein metabolism (Pechova and Pavlata, 2007). Cr concentrations found in fish and crustaceans remained relatively constant at approximately 2.4 mg kg\(^{-1}\) and were approximately half again higher than in plants. Few studies have analysed Cr in species of the Sundarbans. A study by Hossain and Kahn (2001) reported mean Cr levels of 2.13 mg kg\(^{-1}\) in P. monodon from the Bay of Bengal, a value similar to that found in the present study (Table 4).

In other geographical areas, similar ecosystems exhibited similar levels. For example, the results obtained by Tu et al. (2012) in southern Vietnam for S. serrata (0.32 mg kg\(^{-1}\)), P. monodon (2.9 mg kg\(^{-1}\)), M. cephalus (3.9 mg kg\(^{-1}\)), and L. calcarifer (2.3 mg kg\(^{-1}\)) are very similar to those observed in this study (2.01, 3.42, 2, and 2.23 mg kg\(^{-1}\), respectively). Similarly, Zhao et al. (2013), in a study performed in a benthic food web in the Yellow Sea in China, reported levels of Cr in the range between 0.68 to 6.70 mg kg\(^{-1}\), which is consistent with the results presented here.

The maximum permitted levels of Zn and Cu in fish and other seafood have not been set at the European level, but several countries have standards specifically recommended for application in their own country (Nauen, 1983). Established Cu legal limits range between 10 and 30 mg kg\(^{-1}\) wet weight (38 and 115 mg kg\(^{-1}\) dry weight approximately), and those of Zn, between 30 and 150 mg kg\(^{-1}\) wet weight (115 and 575
mg kg\(^{-1}\) dry weight approximately). To compare our results with these limits, the concentrations were converted from dry to wet weights, taking an average ratio of 74% water (Huss, 1995). Only the values of Zn in *S. Serrata* exceeded health advisory levels (Table 2). This elevation is a concern because this species is considered one of the tastiest crab species and therefore is in high demanded by many South Asian countries. In addition, in Bangladesh, mud crab is an export fishery that is playing an important role in national and international markets (Ferdoushi et al., 2010).

There are no maximum permitted levels for Cr in food apart from those set in the Hong Kong legislation (Nauen, 1983). In this study, this limit value (1 mg kg\(^{-1}\) wet weight ~3.8 mg kg\(^{-1}\) dry weight) is only surpassed in the Bombay duck, *H. Nehereus* (4.01 mg kg\(^{-1}\)) (Table 2).

### 5.3.2. Non-essential metals (Hg, Pb and Cd)

The levels of the three non-essential heavy metals found in this study are markedly lower than those of essential metals (Table 2).

Overall, the levels of Hg, Cd and Pb observed in this study were similar to those observed in non-polluted areas (Tu et al., 2012; Waltham et al., 2013) and significantly lower than those of known polluted areas (e.g., Tuzen, 2009), which probably reflects the low industrial activity (Mitra et al., 2012).

When compared with other studies performed in similar organisms in the Sundarbans, the concentrations obtained in this study are the lowest (Table 4). Pb in the nearby species of crustaceans and fishes exhibited levels more than two orders of magnitude higher than those in the current study, except for *H. nehereus*, which did not exhibit large differences (Table 4). Hg was only measured in *H. nehereus* and *P. argenteus* of the Indian Sundarbans, where the levels were one order of magnitude higher than the levels in the same organisms in Bangladesh (Table 4). Similarly, Cd exhibited significantly higher levels in the other studies, except for *H. nehereus* in the Bay of Bengal in Bangladesh (Sharif et al., 1991). However, Mitra et al. (2012) only detected Cd in *P. monodon* in one of the four stations studied. This area was Nayachar Island, located facing the Haldia Industrial Complex, which explains the high Cd levels detected (mean 2 mg Kg\(^{-1}\)).

European Union regulations (European Communities, 2006 and 2008) set maximum levels for Hg, Cd and Pb. For crustacean meat, the three metals have maximum permitted levels of 0.5 mg kg\(^{-1}\) wet weight (approximately 1.9 mg kg\(^{-1}\) dry weight). For fishes, the limit depends on the species. Hg limits range between 0.5 and 1 mg kg\(^{-1}\) wet weight (1.9 - 3.8 mg kg\(^{-1}\) dry weight approximately). Cd limits are between 0.05 and 0.3 mg kg\(^{-1}\) wet weight (approximately 0.19 -1.15 mg kg\(^{-1}\) dry weight), and the limit of Pb is 0.3 mg kg\(^{-1}\) wet weight (approximately 1.15 mg kg\(^{-1}\) dry weight).

Almost none of the studied species reached the maximum permitted levels, except for *H. nehereus*, whose Cd levels were slightly above the threshold limits (Table 2).

### 5. 4. Trophic accumulation

The most important factors that affect the trophic transfer of trace elements in aquatic food webs are the assimilation efficiency, the accumulation facility and the excretion rate by aquatic organisms (Soto-Jiménez, 2011). The major source of trace elements
for aquatic organisms is either directly from the water or indirectly through food (Wang, 2002; Zhang and Wang, 2006), with the latter being the most important route for transfer along an aquatic food chain (Wang, 2002). Thus, the importance of metal accumulation from dietary pathways in different marine food webs has gained recognition (Wang, 2002). More concretely, the factors affecting trophic transfer are regulated by the physicochemical properties and/or element speciation and its concentration, the latter being a balance between intake, by whatever means, and regulation (i.e. metabolisation and/or excretion) as well as by the biological variables of organisms, such as geographical region in which they live, habitat, age, sex or state of health (Aguilar et al., 1999; Cardwell et al., 2013; Soto-Jiménez and Páez-Osuna, 2008).

In general, metal concentrations in animals are recognized as being unrelated to the trophic level in the food chain (Wang, 2002). Moreover, Gray (2002) noted that, except for organic mercury (methylmercury), aquatic organisms generally have effective methods for removing metals and difficulties in accumulating and transferring them. Cardwell et al. (2013) reviewed published data in which single and multiple trophic transfers of five metals (Zn, Cu, Ni, Pb, and Cd) were studied in freshwater and marine food chains. The authors concluded that in food chains that consist of primary producers, macroinvertebrate consumers, and fish, Cu, Pb, Cd and Zn, generally do not biomagnify. However, the author noted that in a number of specific food chains, biomagnification of some metals does occur (i.e. Zn).

This is the case of Zn in the study conducted in southern Vietnam (Tu et al., 2012), where slight biomagnification was noted (R = 0.379; p < 0.01), similar to other studies that also observed the same trend (Campbell et al., 2005; Quinn et al., 2003). By contrast, Jara-Marini et al. (2009), in the Gulf of California, and Nfon et al. (2009), in the Baltic sea, observed a slight trend of decreasing levels of Zn with increasing trophic level, a result highly similar to that observed in the present study (see Figure 3).

Furthermore, Cu, Pb and Cd exhibited a significant negative relationship with δ¹⁵N in the present study. The negative correlation of Pb and Cd is consistent with most bibliographic data describing metal trophic transfer in aquatic webs from different areas (i.e., Campbell et al., 2005; Jara Marini et al., 2009; Nfon et al., 2009; Signa et al., 2013), despite some studies reporting biomagnification of Cd (i.e., Croteau et al., 2005; Dietz et al., 2000). Both heavy metals are poorly absorbed from food and exhibit long half-lives in the body by means of inert storage molecules (Pb in bones and Cd joined to metallothioneins) (Nordberg, 1998; Rabinowitz, 1991). These proprieties warrant reduction via trophic transfer (Campbell et al., 2005).

Cu, unlike observed in the present study, and As and Cr, consistent with the results in the present study, generally exhibit no concentration trend within the food web (Barwick and Maher, 2003; Campbell et al., 2005; Hao et al. 2013; Nfon et al., 2009; Tu et al., 2012; Zhao et al. 2013). However, in some areas, such as in the Mediterranean (Signa et al., 2013), As bio-diminution has been observed. This is probably because both organic and inorganic forms of As are easily absorbed from the gastrointestinal tract but are also rapidly depurated, lessening the accumulation and transfer of As through the food web (Campbell et al., 2005). On the other hand, the negative relationship between the Cu concentrations and trophic levels observed in the Bangladesh Sundarbans is probably the result of the accumulation of this metal in crustaceaens situated at lower trophic level than that of fishes.

Hg in marine organisms tends to be transformed to methylmercury, resulting in greater transfer easiness along the food chain than inorganic mercury (Gray, 2002). In the current study, we did not observe a significant trend of biomagnification of Hg throughout the food web (p = 0.603). However, in other studies, Hg typically...
biomagnifies (Atwell et al., 1998; Bowles et al., 2001; Campbell et al., 2005; Ikemoto et al., 2008b; Tu et al., 2012, among others). The biomagnification of Hg is probably lower in tropical marine ecosystems because these systems are characterized by complex food webs and wide biodiversity, which involves many different supplies of different Hg concentrations that may result in similar δ¹⁵N values but highly variable Hg burden for each species (Al-Reasi et al., 2007). Moreover, organisms in tropical areas generally exhibit higher growth rates, which could lead to low biomagnification (Ikemoto et al., 2008b) and to the low number of trophic levels observed in the present food web.

6. Conclusions

Overall, the levels of trace elements found were well below those representing a hazard to the environment and were similar or lower than levels found in other studies in the same region as well as in other mangroves worldwide.

The concentrations in S. serrata (for Zn and As), T. ilisha (for As) and H. nehereus (for Cd and Cr) fell very close or slightly above the accepted threshold limits for human consumption. Therefore, controlling the levels of these elements may be required to protect human health in the future.

None of the trace elements analysed increased in concentration with trophic level. By contrast, Zn, Cu, Pb and Cd concentrations decreased with trophic level, following trends similar to those previously described.

7. Acknowledgments

We thank Víctor Álvarez and Eduard Arrebola for their assistance in the chemical analyses, and the “Centres Científics i Tècnològics” of the University of Barcelona (CCiT-UB) for conducting the stable isotope and trace element analytical runs. Luis Cardona assisted in the classification of the fishes. The Fundació Barcelona Zoo partially funded this study.

8. References


Figure captions

Figure 1. Study area and sampling sites.

Figure 2. Stable isotope bi-plots of the species collected in the Bangladesh Sundarbans. Each point on the graph represents the mean value of 5 individuals of that particular species; bars show the standard deviation around the mean.

Figure 3. Relationship between the concentrations of trace elements (log converted) and those of $\delta^{15}$N (‰).