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Assessing lead and cadmium pollution at the mouth of the river Segura (SE Spain) using the invasive blue crab (*Callinectes sapidus* Rathbun, 1896, Crustacea, Decapoda, Portunidae) as a bioindicator organism

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A B S T R A C T

The aim of this study was to evaluate Cd and Pb concentrations in the hepatopancreas, gills, muscle tissue and carapace of the crab *Callinectes sapidus* and in sediments from the mouth of the river Segura (SE Spain), an area that has undergone great anthropogenic change in recent decades. Lead concentrations were higher than Cd concentrations in the hepatopancreas, gills and muscles; no statistical differences were found between the sexes. Cadmium and Pb concentrations in sediments did not exceed the probable effect level in guidelines for the protection of aquatic life. The Biological Sediments Accumulation Factor (BSAF) was higher for Cd than Pb in the hepatopancreas, gills and carapace. For Cd, the hepatopancreas had the highest BSAF of all tissues, followed by gills. As well, the hepatopancreas had the highest Individual Mean Bioaccumulation Index (IMBI). The hepatopancreas and gills of the blue crab could thus be useful tissues for practical field monitoring of metal contamination in this ecosystem.

1. Introduction

The blue crab (*Callinectes sapidus*) has been known to exist in the wild in Europe since 1901 (Mancinelli et al., 2017). In southern Europe its numbers have increased in recent years and it is now regarded as one of the 100 worst invasive alien marine species in the Mediterranean (Streftaris and Zenetos, 2006). The sedentary nature of *C. sapidus* in estuaries and marine bays, together with its habitat fidelity, opportunism and nutritional habits (Hines et al., 1990; Adams and Engel, 2014; González-Wangüemert and Pujol, 2016; Genç and Yilmaz, 2017; Taylor and Calabrese, 2018), mean that this crab is a very suitable species for use in biomonitoring. Indeed, it has been well studied in its native habitats (the west coast of the Atlantic) and is useful in pollutant monitoring (Williams, 1974; Castriota et al., 2012; Adams and Engel, 2014; Taylor and Calabrese, 2018) since, due to its biological and behavioural characteristics, it acts as a sentinel species for heavy metal contamination (Adams and Engel, 2014). According to several authors (e.g. Cossa, 1989; Rainbow and Phillips, 1993), an 'ideal' bioindicator organism should fulfil various requirements: it should be abundant, big enough to provide plentiful samples, and resistant to stress, all characteristics of the blue crab. Other species such as mussels are also regarded as good bioindicators due to their ability to accumulate concentrations of pollutants in their tissues, although they are not present in all ecosystems. In the context of the river Segura, there is a pressing need to identify an efficient biomonitoring species that is sensitive to the local presence of pollutants.

By the 1990s the river Segura was one of the most polluted rivers in Europe due to a combination of a very dense human population (average density 103 inhabitants/km^2) and the local food processing industry (Ródenas and Albacete, 2014). Furthermore, it was almost impossible to dilute this contamination due to the extremely low water flow rates triggered by water extraction for intensive agriculture and the scarce natural flow. This river basin is a good example of industrial agri-food production based on a concentration of intensive agriculture and food-processing companies supplied by local agricultural production (Pascual et al., 2018). According to the Segura Hydrographic Confederation (CHS, 2015), 268,070 ha of land are irrigated in the Segura Hydrographic District, and food, drink and tobacco production generates the

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region's greatest gross value added (€1,416,341,000). In light of this, it is essential that biomonitoring programmes be implemented along the river as a means of validating the performance of water treatment systems and detecting non-point sources of pollutants.

Lead and Cd concentrations have previously been reported from agricultural soils in the Segura valley (Micó et al., 2006) and are thought to have been provoked by local industrial activity, the ever-increasing population and agrochemicals. According to these authors, the concentrations of these pollutants in the Segura valley do not reach toxic levels, although further studies are still required to identify possible increases in sources of contamination. These heavy metals serve no function in the metabolism of crustaceans and their content in tissues is biologically not regulated (Çoğun et al., 2017). They are known to affect the environment and their presence has potentially hazardous long-term implications for crustaceans (Lavolpe et al., 2004; Jerome et al., 2017; Xu and Yang, 2018).

In this context, the aim of this work was to evaluate Cd and Pb concentrations in the tissues of *C. sapidus* collected from the mouth of the river Segura, and then examine their relationship with locally sampled sediments, as a means of determining the usefulness of this species in biomonitoring. For this study, we chose four tissues for metal analysis: (1) the hepatopancreas, as it is the detoxification and main storage organ in crustaceans (Amiard et al., 1987; Chou et al., 2002) and acts as a pointer for both food and water pollution; (2) muscles, as they can be compared with reference values to evaluate toxicological hazards; (3) gills, as they are the filter organs *par excellence* selected due to their close contact with water and ability to expose heavy metal levels in water (Çoğun et al., 2017); and (4) the carapace, a tissue that may signal heavy metal migration.

2. Material and methods

2.1. Sample collection

Samples were collected from the mouth of the river Segura at Guardamar del Segura (Alicante coast, UTM 705558X-4220269Y, Fig. 1). Forty-seven blue crabs (33 males and 14 females) were captured in November and December 2017 by placing a fyke net on the riverbed at night and then recovering it early the next morning. Crabs were euthanized by hypothermia (30–40 min at −20 °C), and their sex and the biometric parameters (weight, length between abdominal segment and rostrum, and width between lateral spines) were recorded. Tissues from each specimen (hepatopancreas, muscle, gill and carapace) were carefully removed from the crab with a surgical knife, transferred to 1.5-ml microtubes and stored at −20° until processed. Finally, sediments (n = 11) were taken directly from the first 10 cm of the riverbed (at the same place as the crabs were collected) and stored in polyethylene containers. In the laboratory, dispersion, agitation, extraction, sedimentation and sieving of sediments for granulometric analysis were carried out. Prior to analysis, sediments were dehydrated at 40 °C until constant weight and then pulverized with a mortar.

2.2. Metal analysis

Samples (tissues and sediments, 0.5 g) were treated with 4 mL of trace mineral grade HNO_3 (69% Suprapure, Merck) and 1 mL of H_2O_2(33% Suprapure, Merck) in special Teflon reaction tubes and heated at 220 °C in a microwave digestion system (UltraClave-Microwave Milestone®) for 20 min, and finally diluted with double deionized water (MilliQ) to 10 ml. Metal concentrations of Cd and Pb were determined using inductively coupled plasma optical emission spectrometry (ICP-OES, ICAP 6500 Duo, Thermo Scientific, with One Fast System). The detection limit (DL) for both Cd and Pb was 0.001 $\mu g\ g^{-1}$. For every sample, two readings were made, the mean of which was used as the concentration value. To check for possible contaminants, one blank sample for every 11 samples was also analysed.

Table 1
Descriptive biometric data (whole population, minimum, maximum and percentiles) in blue crabs from the mouth of the river Segura.

Biometric data	Minimum	Maximum	P-25	P-50	P-75
Weight (g)	6.3	99.7	19.7	36.7	59.4
Carapace width (mm)	22.5	65.0	34.3	45.0	50.0
Carapace length (mm)	45.0	125.0	70.0	85.0	100.0

Multi-element calibration standards (SCP Science, in 4% HNO_3) were prepared with specific concentrations of both metals taking as a reference UNE-EN ISO 11885 for the determination of elements by ICP-OES. As well, intermediate patterns of all elements were prepared. The calibration device was set for each batch, with a minimum of three points for every lot. Each run started out with the calibration standards, continued with samples and intermediate patterns, and finished with the intermediate patterns (10% variation coefficient). The uncertainty and recovery percentages were 4.56 and 95.32 (Cd), and 6.14 and 96.44 (Pb), respectively. Tissue concentrations were expressed in micrograms per gram wet weight ($\mu g\ g^{-1}$, ww); sediment samples, on the other hand, were described in terms of dry weight (dw).

2.3. Data analysis

Statistical analyses were performed using SPSS v.19.0. Several samples had a value below the detection limit (DL). As per international guidelines (GEMs/Food, 1995), the middle bound approach was assumed (the values below the DL were equal to half this limit) for calculating mean concentrations. Given that there were several variables without a normal distribution (Shapiro–Wilk test), all reported statistics are given as median, and minimum and maximum concentrations. The Mann–Whitney U test was used to identify significant differences for each element between males and females, between both metals in each tissue, and between tissues for each metal. Spearman's rank correlation coefficient test for non-parametric variables was applied to establish correlation coefficients between (1) metal concentrations for each tissue and biometric data, (2) metals in each tissue, (3) tissues for each metal, and (4) both metals in sediment samples. In all cases, p values of less than 0.05 were taken to be statistically significant.

To facilitate the understanding of the sediment and biological accumulation correlation, the Biological Sediments Accumulation Factor (BSAF) and Individual Mean Bioaccumulation Index (IMBI) were calculated (Thomann et al., 1995; Maes et al., 2005) to assess, respectively, (a) the efficiency of metal accumulation from the environment by an organism and (b) the degree of this accumulation.

$$BSAF = \frac{[metal\ in\ organism]}{[metal\ in\ sediments]} \qquad IMBI = \frac{\sum_{i=1}^{n} Ci/Cimax}{n}$$

where Ci = the individual metal concentration of the heavy metal, $Cimax$ = the maximum observed concentration of the heavy metal, and n = number of analysed metals. For BSAF, tissue concentrations were expressed in $\mu g\ g^{-1}$ dw.

3. Results and discussion

Descriptive data of biometric measures (weight, carapace length and carapace width) for all the samples are given in

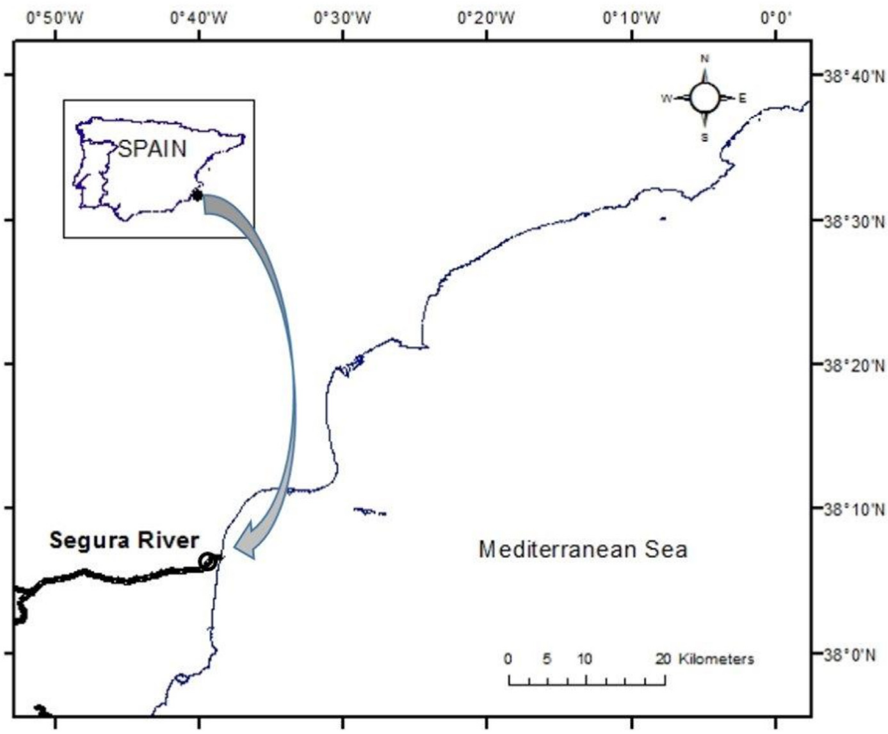

Fig. 1. Location of sampling area (mouth of Segura River, Spain).

Table 2
Concentration of Cd and Pb (median, minimum–maximum, mg kg^{-1}, wet weight) in blue crab tissue from the mouth of the river Segura.

Heavy metal concentration		Hepatopancreas	Gills	Muscle	Carapace
Whole population (n = 47)	Cd	0.045 (nd-0.233)	0.007 (nd-0.073)	<0.001 (nd-0.061)	<0.001 (nd-0.117)
	Pb	0.083 (0.002–0.473)	0.108 (0.029–0.618)	0.027 (nd-0.481)	<0.001 (nd-1.604)
Male (n = 33)	Cd	0.051 (nd-0.206)	0.008 (nd-0.073)	<0.001 (nd-0.014)	<0.001 (nd)
	Pb	0.084 (0.002–0.473)	0.130 (0.031–0.618)	0.027 (nd-0.481)	<0.001 (nd-1.604)
Female (n = 14)	Cd	0.037 (nd-0.233)	0.006 (nd-0.042)	<0.001 (nd-0.061)	<0.001 (nd-0.117)
	Pb	0.073 (0.023–0.192)	0.101 (0.029–0.174)	0.041 (nd-0.162)	<0.001 (nd-0.917)

nd = not detected.

Table 3
Spearman correlations in Cd/Pb concentrations between tissues and biometric parameters (whole population); the carapace (Cd and Pb) and muscle (Cd) were not tested (nt).

	Heavy metal concentrations (Cd/Pb)			
	Hepatopancreas	Carapace	Gills	Muscle
Crab weight	0.094/−0.622**	nt/nt	−0.199/−0.615**	nt/−0.468**
Crab carapace length	0.050/−0.511**	nt/nt	−0.257/−0.593**	nt/−0.458**
Crab carapace width	0.165/−0.416**	nt/nt	−0.199/−0.593**	nt/−0.460**
Hepatopancreas Cd/Pb concentrations	–	nt/nt	0.524**/0.334*	nt/0.136
Carapace Cd/Pb concentrations	–	–	nt/nt	nt/nt
Gills Cd/Pb concentrations	–	–	–	nt/0.450**

**Significant correlation at 0.01.

*Significant correlation at 0.05.

Table 1. There was a close correlation ($p < 0.01$) between carapace length and carapace width ($r = 0.945$), carapace width and weight ($r = 0.884$), and carapace length and weight ($r = 0.939$).

Metal concentrations in tissues from the whole sample and by gender are given in Table 2. For Cd, the percentage of samples with values above the detection limit were 85.1% (hepatopancreas and gills), 17.0% (muscles) and 2.1% (carapaces). For Pb, these percentages were 100% (hepatopancreas and gills), 80.9% (muscles) and 40.4% (carapaces).

No statistical differences by sex were found for either heavy metals or tissues. In all tissues, Pb concentrations were higher than Cd concentrations ($p < 0.05$). The highest Pb concentrations in the samples were detected in the gills; concentrations decreased in the following order: gills>hepatopancreas>muscles>

carapace, with statistical differences between all tissues except between hepatopancreas and gills, and between muscles and carapace. Cadmium concentrations were ordered as follows: hepatopancreas>gills>muscles = carapace, with statistical differences between all tissues.

Table 3 shows the relationship between concentrations of each metal and biometric measurements, and between metal concentrations in tissues. For Cd, significant and positive correlations between hepatopancreas and gill metal concentrations were found. For Pb, there were significant and negative correlations between all biometric data and hepatopancreas, gills and muscles, and significant positive correlations between gills and muscles, and gills and hepatopancreas. No correlations between these two elements in any tissue were found.

Several authors have underlined the ecotoxicological importance of evaluating pollutant accumulation in tissues from sentinel species when selecting which tissues are to be used in biomonitoring (Wallace and Luoma, 2003; Wallace et al., 2003; Richir, 2012). In almost all studies in which Cd and Pb have been analysed in the same tissue, Pb concentrations in hepatopancreas, gills and muscles were higher than Cd concentrations (Table 4). In our study, the highest Pb concentration was detected in gills, although there were no significant differences with Pb concentrations in the hepatopancreas. There was a positive correlation between both tissues and between gills and muscles for Pb (Table 3). Thus, the hepatopancreas and gills could be considered as the most appropriate tissue to be used in biomonitoring. Nevertheless, Pb concentrations in these tissues (also muscles) are correlated negatively with all biometric data. This contradicts the findings reported by Genç and Yilmaz (2017), who found positive relationships between length and Pb concentrations in the same tissues ($p < 0.01$) in *C. sapidus* from Turkey. Differences in allometric parameters (greatest in crabs from Turkey), differences in Pb concentrations in sediments (6.805 $\mu g\ g^{-1}$ in Genç and Yilmaz study *vs.* 14.930 $\mu g\ g^{-1}$ in our study), a dilution effect due to growth in the first stage of crab development, or unequal or continuous periods of exposure to Pb at sampling sites could explain these differences. The highest Cd concentrations were detected in the hepatopancreas, and there were statistical differences with Cd concentrations in the gills and a positive relationship between both these tissues (Table 3). For this metal, Genç and Yilmaz (2017) reported a positive relationship between Cd levels in *C. sapidus* and a biometric measurement (carapace length), which do not agree with our results. The Cd concentrations in sediments were lower in our study (1.016 $\mu g\ g^{-1}$ in Genç and Yilmaz study *vs* 0.341 $\mu g\ g^{-1}$ in our study), which could explain this difference.

The hepatopancreas and gills are the two organs that are usually recommended as good environmental indicators of pollution in aquatic ecosystems; muscles (muscle meat from appendages and abdomen), on the other hand, are an important tissue for detecting risk in food (Commission Regulation EU 2015/1005). Several authors (Table 4) have used *C. sapidus* as a sentinel of metal pollution, and Cd and Pb concentrations have been studied in its hepatopancreas, gills and muscle tissue. If we consider the moisture percentage reported by several authors (Küçükgülmez et al., 2006; Kuley et al., 2008; Zotti et al., 2016) and the moisture percentage obtained from samples (79.8 ± 2.3, muscle; 78.4 ± 7.5, hepatopancreas; 84.5 ± 2.0, gills; and 18.3 ± 0.9, carapace), our results were lower than those of other authors (Table 4), except for results for Pb in muscle tissue reported by Adams and Engel (2014) from Florida (USA). Thus, we believe that, due to the low concentrations in sediments, crabs from the river Segura are less exposed to Pb and Cd.

Sediments were mostly sand (53.23 ± 10.29%). The median concentrations of Cd were 0.341 (0.138–0.469 $\mu g\ g^{-1}$, dw) and of Pb 14.930 (6.841–20.063 $\mu g\ g^{-1}$, dw). The relationship between both heavy metals was significant ($r = 0.891$, $p < 0.01$). Metal concentrations in sediments between sources of contamination and the riverbed are poorly known. Recently, García-Alonso et al. (2015) reported concentrations of Cd and Pb in sediments at different altitude points in the Segura basin that reveal a clear increase from higher (upstream) to lower (downstream) areas. The Cd concentrations reported by these authors near the mouth of the river (Orihuela, 0.2±0.0 $\mu g\ g^{-1}$ dw) were slightly lower than those found in our study (0.341 $\mu g\ g^{-1}$ dw), while Pb concentrations (24.4±1.1 $\mu g\ g^{-1}$ dw) were higher (14.930 $\mu g\ g^{-1}$ dw in our study). Veses et al. (2014) reported a wider range of heavy metal concentrations in sediment in the river Segura (0.09–0.97 and 6.0–41.5 $\mu g\ g^{-1}$ dw for Cd and Pb, respectively). Finally, other authors have reported lower Cd and Pb concentrations in studies performed in coastal areas (Paches et al., 2019). Cadmium and Pb concentrations in this matrix were lower than the reference values proposed for soils by several authors (Table 5) (Pérez et al., 2000; Micó et al., 2007), or similar in the case of Cd (Pérez et al., 2002). Concentrations obtained in our study were lower (Pb) or similar (Cd) (Table 6) to those reported in Alicante by other authors (López and Grau, 2004; Micó et al., 2006, 2007), and lower than in adjacent regions to our sampling stations (Boluda et al., 1988; Andreu, 1991; Errecalde et al., 1991; Andreu and Gimeno-García, 1996) and in other rivers described as polluted (river Ebro Ramos et al., 1999). Finally, our concentrations were also (Tables 5 and 6): (a) lower than or similar to other regions of Spain (Cala et al., 1985; Aller and Deban, 1989; Moreno et al., 1992; Campos, 1997; Marín et al., 2000), (b) lower than the recommended levels according to European legislation (Council Directive 86/278/EEC), (c) lower than other polluted fluvial systems in the Mediterranean (e.g. Turkey, Genç and Yilmaz, 2017 for Cd), and (d) lower than other climatic regions (Iran Rahmanpour et al., 2016). When we compared our results with the limits established by the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (Canadian Council of Ministers of the Environment, CCME, 2019), we found that the Cd and Pb concentrations in sediments in the Segura river basin were lower than those established by the Interim Sediment Quality Guidelines (ISQG) for both metals in freshwater (0.6 and 35 $\mu g\ g^{-1}$ dw, Cd and Pb respectively). Thus, no adverse biological effects are to be expected in crabs as a result of exposure to these sediments in the study area. Finally, Pb was predominant in sediment samples and the high positive correlations between this element and Cd points to a possible common source area for these two metals and a similar distribution in the study area.

To understand the relationships between sediments, tissue metal concentrations and the individual mean (multi-metal) bioaccumulation (BSAF and IMBI) were calculated. The BSAF medians for Cd and Pb, respectively, were 0.354 and 0.015 (hepatopancreas), 0.121 and 0.041 (gills), 0.006 and 0.007 (muscles), and < 0.001 (Pb carapace; no data for Cd). The IMBI medians were 0.217 (hepatopancreas), 0.148 (gills), 0.042 (muscles) and 0.002 (carapace). According Dallinger (1993), our BSAF results enable us to catalogue *C. sapidus* as a 'deconcentrator'. However, this contradicts Genç and Yilmaz (2017), who concluded that blue crabs reflect pollution levels better than two other species (*Anguilla anguilla* and *Mugil cephalus*) due to the higher BSAF found in the Köyceğiz Lagoon System (Turkey), an ecosystem with greater Cd concentrations in sediments (1.016 $\mu g\ g^{-1}$) but lower Pb concentrations (6.805 $\mu g\ g^{-1}$).

It is worth noting that in our study males had a higher IMBI in hepatopancreas and gills, and females in muscle tissue, albeit with no significant differences. Adams and Engel (2014) have reported significant differences between sexes, with females having higher Cd concentrations in muscles. However, no statistical differences between males and females were found (either

Table 4
Cd and Pb concentrations ($\mu g\ g^{-1}$) in tissues of *C. sapidus* from different countries and locations.

Tissue	Location (country)	n	Weight (g)	Length (cm)	Cd	Pb	Reference
Hepatopancreas	Köyceğiz Lagoon System (Turkey)	60	225.55 ± 76.79	17.85 ± 1.65	0.893 ± 0.12[a]	1.386 ± 0.13[a]	Genç and Yilmaz (2017)
	Mersin Bay (Turkey)	5[f]	25.9 ± 4.60	12.4 ± 1.20	11.2 to 48.2[a,e]	17.2 to 85.2[a,e]	Çoğun et al. (2017)
	Pensacola Bay (USA)	7 to 15	*ns*	10.2[c]	0.01 to 4.60[e,b]	0.15 to 0.39[e,b]	Karouna-Renier et al. (2007)
	Mouth of river Segura (Spain)	47	40.6 ± 23.4	8.5 ± 2.0[c]	0.059 ± 0.055[b] 0.159 ± 0.148[a] 0.045[d,b]/0.121[d,a] (*nd*-0.233)[b] (*nd*-0.629)[a]	0.113 ± 0.106[b] 0.305 ± 0.286[a] 0.083[d,b]/0.224[d,a] (0.002–0.473)[b] (0.005–1.276)[a]	This work
Gills	Köyceğiz Lagoon System (Turkey)	60	225.55 ± 76.79	17.85 ± 1.65	0.189 ± 0.01[a]	2.230 ± 0.29[a]	Genç and Yilmaz (2017)
	Mediterranean coastal (Turkey)	10 to 15	91.6 to 213.1[e]	6.35 to 8.71[e]	0.04 to 0.10[e,b]	*na*	Mutlu et al. (2011)
	Mersin Bay (Turkey)	5[f]	25.9 ± 4.60	12.4 ± 1.20	6.9–22.3[a,e]	102.1–272.1[a,e]	Çoğun et al. (2017)
	Mouth of river Segura (Spain)	47	40.6 ± 23.4	8.5 ± 2.0[c]	0.010 ± 0.013[b] 0.057 ± 0.075[a] 0.007[d,b] /0.040[d,a] (*nd*-0.073)[b] (*nd*-0.419)[a]	0.136 ± 0.118[b] 0.780 ± 0.677[a] 0.108[d,b]/0.619[d,a] (0.029–0.618)[b] (0.166–3.544)[a]	This work
Muscle	Iskenderun Bay (Turkey)	15[f]	123.1 ± 19.7	6.2 ± 0.4	1.055 to 2.509[a,e]	2.667 to 4.304[a,e]	Türkmen et al. (2006)
	Mediterranean coastal (Turkey)	10 to 15	91.6 to 213.1[e]	6.35 to 8.71[e]	0.03 to 0.08[e,b]	*na*	Mutlu et al. (2011)
	Atlantic coast of Florida (USA)	51	*ns*	12.35 ± 2.94[c] (122[d])	0.029 ± 0.027[b] (0.026[d,b])	0.02 ± 0.025[b] (0.011[d,b])	Adams and Engel (2014)
	Pensacola Bay (USA)	7 to 15	*ns*	10.2[c]	0.05 to 0.09[e,b]	0.23 to 0.41[e,b]	Karouna-Renier et al. (2007)
	Mersin Bay (Turkey)	5[f]	25.9 ± 4.60	12.4 ± 1.20	0.1–2.5[a,e]	1.1–5.1[a,e]	Çoğun et al. (2017)
	Mersin Bay (Turkey)	30[f]	115.27–386.10	12.50–18.50[c]	0.44 to 1.07[a,e]	0.24 to 0.56[a,e]	Ayas and Ozogul (2011)
	Acquatina Lagoon (Italy)	6	173.00 ± 26.78	13.60 ± 0.76[c]	0.1[b]	0.1[b]	Zotti et al. (2016)
	Mouth of river Segura (Spain)	47	40.6 ± 23.4	8.5 ± 2.0[c]	0.003 ± 0.009[b] 0.011 ± 0.034[a] <0.001[d,b]/ <0.004[d,a] (*nd*-0.061)[b] (*nd*-0.229)[a]	0.054 ± 0.082[b] 0.203 ± 0.308[a] 0.027[d,b]/0.102[d,a] (nd-0.481)[b] (nd-1.808)[a]	This work

[a]Dry weight.
[b]Wet weight.
[c]Specifically carapace length.
Data shown are mean, except:
[d]Median.
[e]Range of means.
[f]By group.
na = not analysed.
ns = not shown.
nd = not detected.
This work = whole population.

in the allometric parameters or in Cd and Pb concentrations), even though females were larger and heavier, a finding that could be explained by the low heavy metal concentrations in the sediments and the small sample size (age). A wider-ranging selection of sizes and sexes in biomonitoring models could be of importance in future studies.

Table 5
Pb and Cd reference values proposed by other authors (mg kg^{-1} dry weight).

Reference	Pb	Cd
Pérez et al. (2000)	88	0.8
Pérez et al. (2002)	30	0.3
Micó et al. (2007)	28	0.7
Council Directive 86/278/EEC	50–300	1–3

Table 6
Cd and Pb concentrations (mean values mg kg^{-1}, dry weight) in soils from other locations.

Location	Cd	Pb	Reference
Alicante (S)	0.34	22.8	Micó et al. (2007)
Alicante (S)	0.25	17.8	López and Grau (2004)
Alicante (S)	0.38	19.6	Micó et al. (2006)
Valencia (S)	*na*	42	Boluda et al. (1988)
Valencia (S)	0.4	41	Andreu (1991)
Valencia (S)	0.5	38	Errecalde et al. (1991)
Valencia (S)	0.6	47	Andreu and Gimeno-García (1996)
River Ebro (S)	0.58	17.25	Ramos et al. (1999)
León (S)	0.7	13	Aller and Deban (1989)
Vega de Aranjuez (S)	1.4	36	Cala et al. (1985),
Madrid (S)	0.07	50	Moreno et al. (1992)
Vega de Granada (S)	2.3	64	Campos (1997)
La Rioja (S)	0.3	22	Marín et al. (2000)
Köyceğiz Lagoon System (T)	1.02	6.81	Genç and Yilmaz (2017)
River Arvand (I)	0.15–3.67	20.05–38.12	Rahmanpour et al. (2016)
River Segura (mouth) (S)	0.34	14.93	Our results

S = Spain; T = Turkey; I = Iran; *na*: not analysed.

According to current legislation (Directive 2010/63/EU), the use of animals in all scientific investigation must be both necessary and rational. Thus, the use of an invasive species in biomonitoring is of great interest. Firstly, authorization for capture and use in experiments of the invasive American blue crab on the Mediterranean coast is not required. During its life cycle, adult blue crabs prefer waters with low salinity in estuaries (Sumer et al., 2013), so this species is present in the mouth and riverbeds of the river Segura (SE Spain) where it is relatively easy to capture. The increase in the surface temperature of Mediterranean waters may have a positive effect on the survival rate of this crab's overwintering populations (Mancinelli et al., 2017). In addition, it is a predator and opportunist species, with a wide and varied trophic range including fish, macroalgae, small crustaceans, molluscs, gastropods, detritus and plant matter Gómez-Luna et al. (2009), Seitz et al. (2011). To date, the predators, parasites and pathogens of this crab in the Mediterranean Sea and southern European waters have not yet been identified (Mancinelli et al., 2017). Like other crabs, this alien species could act as a vector transmitting pollutants to higher trophic levels such as invertebrates, fish, reptiles, birds and mammals, like otters that prey on them (Guillory and Elliot, 1999; Mistri et al., 2020). Thus, the biomonitoring of this species will contribute to knowledge of possible sources of pollutants in predators. In terms of control, the eradication of the blue crab is the best method but could hinder biomonitoring in addition that the time and monetary resources required for eradicating aquatic invaders such as the blue crab are huge and currently unfeasible (Lampert et al., 2014; Mancinelli et al., 2017). To summarize, indigenous populations of this species have long been used as pollution indicators (e.g. heavy metals Weinstein et al., 1992; Adams and Engel, 2014) as they live in surface sediments and feed on benthic prey that typically live in polluted areas. *Callinectes sapidus* could establish a stable population at the mouth of the river Segura and, given that its biological characteristics satisfy several of Naser's (2011) criteria for sentinel species, we believe that this crab is a valuable species for monitoring heavy metal pollution in water and sediments.

Although the quality of this river's waters has improved in recent years, the industrial and agricultural contamination it bears still needs to be studied (Ródenas and Albacete, 2014); moreover, the presence of native species such as *A. anguilla* and *Luciobarbus sclateri* (Verdiell-Cubedo and Parrondo-Celdrán, 2018a; Verdiell Cubedo and Parrondo Celdrán, 2018b) means that further studies of the quality of this ecosystem are required. In conclusion, this study upholds the usefulness of *C. sapidus* for practical field monitoring of metal contamination at a local scale and so this invasive species could become an important source of environmental data from this particular ecosystem. Specifically, our results show that, of all the tissues analysed, the hepatopancreas had the highest heavy metal concentrations and the highest individual mean bioaccumulation index (IMBI). The biological sediment accumulation factor (BSAF) was also highest for Cd in the hepatopancreas, although for Pb it was highest in the gills. In future biomonitoring studies larger and heavier specimens should be selected.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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