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Heavy metal contamination in European conger (*Conger conger*, Linnaeus 1758) along the coastline of Morocco

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Abstract

Background: In Morocco, fish is an important protein source especially, even not exclusively, for coastal communities and marketed fresh all along the coastline. One of the main targets of coastal artisanal fisheries is the European conger (*Conger conger*, Linnaeus 1758), a widely distributed benthic predatory species of a maximum weight of up to 50 kg. However, information on heavy metal contamination of conger is scarce. Therefore, concentrations of mercury, lead and cadmium were analysed in 108 European conger specimens from nine locations along the Atlantic and Mediterranean coasts of Morocco to describe the spatial distribution of heavy metal contamination.

Results: The average heavy metal concentration in all conger samples under investigation was 246.90 ± 216.83 µg mercury/kg wet mass, 74.14 ± 87.02 µg lead/kg wet mass and 255.12 ± 287.15 µg cadmium/kg wet mass respectively. Mercury and cadmium showed a clear site-specific bioaccumulation in European conger but lead does not. Hence, the effect of fish length bias on contamination was corrected through a generalized linear model (GLM) prior to any spatial comparison.

Conclusions: Different regional hotspots for the three analysed metals are identified and local sources are discussed. Mercury levels in big specimens of European conger exceeded the European threshold level for human consumption at some of the locations under investigation.

Keywords: Pollution, Heavy metals, Bioaccumulation, European conger, Morocco

Background

Fish is generally considered an important source of proteins, minerals, vitamins and polyunsaturated essential fatty acids ([7, 33], and an indispensable food component especially in coastal countries like Morocco. The United Nations Food and Agricultural Organization (FAO) reported an annual per capita fish consumption for Morocco of 19.5 kg in 2019 [18]. In 2018, the total capture fisheries production of Morocco reached 1.4

million tons, rendering it one of the top fisheries producing countries in Africa [46]. The Moroccan coastal zone extends for approximately 3500 km, along the Eastern Atlantic Ocean and the South-western Mediterranean Sea, and represents the main metropolitan and industrial area with more than 60% of the population and 90% of the industrial production.

Despite the proven benefits of fish consumption for human health, potential negative side effects due to environmental pollution are a rising matter of concern: according to WHO ([44]) in fishing regions of countries such as Brazil, Colombia, China and Greenland, where people prefer to eat their local catch, up to 17 out of thousand children suffer mental disabilities related to

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the consumption of Hg contaminated fish. Heavy metals, originating both from natural or anthropogenic sources, are an important component of these worldwide-recognized hazards due to their wide distribution in the aquatic, including the marine environment [10, 13]. As a consequence, metals can be accumulated by marine organisms through exposure to contaminated water and sediments or via the food web. Some of these elements such as mercury (Hg), cadmium (Cd) and lead (Pb) have no known role in biological systems [34]. Though they are natural trace components of the aquatic environment, their levels can locally increase due to, e.g., industrial or mining activities. Concentrations of Hg, Cd and Pb in biota were routinely monitored in the frame of international marine environmental assessments such as described in the last Quality Status Report of the Oslo and Paris Commission performed in the North-East Atlantic [36]. Hg is an element of special concern, because in aquatic environments, its inorganic form is biologically transformed into methylmercury (MeHg), the most toxic organic Hg species, which may cause environmental and health effects in wildlife [13] and biomagnifies in aquatic food webs [26]. People who prefer to eat local seafood in contaminated areas can be at risk of elevated Hg exposure [9]. Increasing Hg concentration in muscle tissue of fish with age is a well-known fact [22, 23], so that older specimens can be expected to exhibit higher Hg contamination than younger ones. Hg accumulation is dominated by MeHg, Lang et al. [25] showed that 94% of the total Hg in fish muscle was MeHg. Hg and MeHg can induce a variety of adverse effects in fish at physiologic, histologic, bio-chemical, enzymatic, and genetic levels [32], partly at environmentally realistic concentrations. In this context, studies have been carried out to determine the level of heavy metal contamination of Morocco's marine ecosystems. Main subject of previous studies on heavy metals was sediments (e.g. [30, 35, 39]) as well as mussels and other molluscs [3, 6, 28, 29]. In addition, different Moroccan fish species were analyzed for various contaminants [1, 4, 8, 43]. As a popular food fish in coastal areas of Morocco, European conger from different origins have been previously a target for heavy metal analyses [8, 14, 42].

The concentrations of Hg, Cd and/or Pb have been revealed in fish species from other regions such as the Aegean Sea [48], the Baltic Sea [22, 37], the North Sea [23, 25], the North Atlantic [24] and the Pacific coast [21].

The European conger (*Conger conger*, Linnaeus 1758) is a marine benthic carnivorous Anguilliform species widely distributed in the North-eastern Atlantic, including the Mediterranean Sea [11], feeding mainly on bottom-living fishes, crustaceans and cephalopods [40]. It can reach 2 m of length and about 50 kg of weight at ages

of up to 20 years [17]. European conger is of high economic importance, especially for small and medium scale coastal fisheries, being caught with hook and line, traps, gill nets but also bottom-trawls [19].

To the authors' best knowledge, no study has provided a spatial overview of contamination with heavy metals in European conger from Morocco. The aim of this study was therefore to bridge this gap and to provide a first overview of the contamination status (Hg, Cd, Pb) of European conger as well as the spatial distribution along the Moroccan coastlines considering bioaccumulation.

Methods

Study area

The Moroccan coastline is an area of increased human activities, mainly along the northern and central Atlantic coast. The northern coastal sector, from Tangier to Casablanca, brings together the largest urban agglomerations and most of the industrial units of the country (textile, tannery, para-chemicals, petrochemicals, agri-food, etc.). The coastal sector from Casablanca to Agadir is characterized by urban agglomerations and industrial infrastructure such as processing units for phosphates, established, e.g., at Safi. The Moroccan coast south of Agadir, is less industrialized. The Mediterranean coastline is also less industrialized compared to the northern part of the Atlantic coast; however, industries, e.g., around the city of Nador, are growing [16, 45]. In the present study samples across the regions described above were taken, covering both the Atlantic and Mediterranean coastline. Each location was selected regarding its environmental and socio-economic characteristics which are summarized in Table 1. Sampling sites are displayed in Fig. 1.

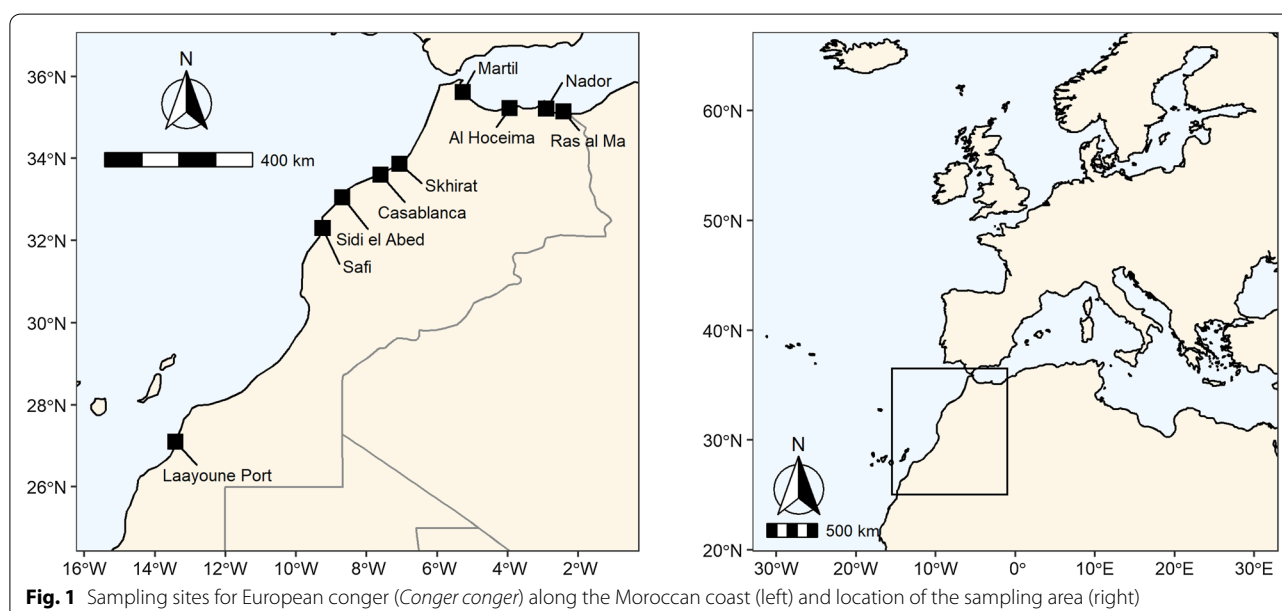
Fish sampling

Sampling was carried out between 2017 and 2019 along the Moroccan coast. Specimens of European conger—in total, 108 individual fish—were collected from artisanal fisheries landing sites and local fish markets, preferably at harbours or directly from fishermen at nine different locations along the Atlantic and Mediterranean coasts of Morocco. The four sampling campaigns took place in 11/2017, 11/2018, 3/2019 and 11/2019 (Table 1; Fig. 1). Fish were kept cool (between 4 and 10 °C) after purchase and were processed the same day. After determining total length and weight, tissue samples were collected as follows: skin was partly removed and a piece of epaxonic white muscle was cut out posterior of the anus using a ceramic knife. In addition, the abdominal cavity was opened and a piece of liver was extracted from each specimen. Muscle and liver samples were weighted and stored in the trifold volume of 99.9% ethanol in plastic

Table 1 Characteristics of the sampling sites and sampling campaigns

Site	Sampling campaign	Fishing area	City size	Socio-economic characteristics
Al Hoceima	11/2018	Coast	small	Fishing; agriculture; quarries, industry: metallurgic
Casablanca	11/2017	Coast	big	Landfills; ship traffic; industry: chemical
Laayoune Port	3/2019	Coast	medium	Fishing; industry: phosphorus
Martil	11/2017 11/2018	Estuary	small	Ship traffic; industry: paper mills, tanneries, chemical
Nador	11/2018	Lagoon	medium	Fishing; ship traffic; agriculture; industry: steel, metallurgic
Ras El Ma	11/2018	Estuary	small	Fishing
Safi	3/2019	Coast	medium	Fishing; agriculture; industry: phosphorus
Sidi El Abed	11/2019	Coast	small	Ship traffic; industry: metallurgic, chemical
Skhirat	11/2017	Coast	small	Fishing

Big city > 1,000,000; medium city > 1,00,000; Small city > 10,000 population; [16, 47]



tubes until being analysed in the lab. The ethanol storage was chosen, because timely sample freezing was not feasible in all regions of Morocco. The possible influence of ethanol storage versus immediately freezing of fish tissue samples was tested before the first sampling has started and was below 10% for Hg (results not shown).

Chemical analytics

Hg determination

The determination of Hg is described in detail in Kammann et al. [22]. Briefly, 2 g of muscle tissue samples were freeze dried using a lyophilizer (LD 1-2, Christ, Osterode, Germany) and subsequently homogenized using an agate mortar or an ultra turrax tube drive dispenser (IKA, Staufen, Germany) to obtain a dry and homogenous sample powder. Total Hg was determined by atomic absorption spectrometry using a Direct Mercury Analyzer

(DMA-80, MLS, Leutkirchen, Germany). 20–30 mg of each sample was weighted into the boat containers of the DMA-80. Direct analysis for total Hg content was performed using a 10-level calibration. The limit of detection (LD) and the limit of quantification (LQ) were calculated from a standard curve according to DIN 32645 [12] with a confidence level of 99%. Considering the sample preparation, an LD of 0.08 µg/kg wet mass (WM) and a LQ of 0.23 µg/kg WM were determined for Hg. No values below these limits were found in any sample under investigation. Precision of the method was 8.2% and recovery was 100.3%.

Cd and Pb determination

Quantification of Cd and Pb was performed in liver samples, defatted according to Smedes [41] which have been subsequently freeze-dried and homogenized as described

above. Dry and fat-free sample powder (1–5 mg) was weighted and placed into the graphite furnace atomic absorption spectrometer (GFAAS) ContrAA 600 (Analytik Jena, Germany) with a continuous source. The GFAAS was calibrated using a 5-level calibration diluted from certified standard solutions in 0.1 M nitric acid. Measurements were performed after adding 5 µg of palladium matrix modifier ($\text{Pd}(\text{NO}_3)_2$ in nitric acid) to each sample. The LD and the limit of quantification (LQ) were calculated from a standard curve according to DIN 32645 [12] with a confidence level of 99%. Considering the sample preparation, a LD of 0.25 (2.61) µg/kg WM and an LQ of 0.74 (7.82) µg/kg WM were determined for Cd (Pb). No values below these limits were found in any sample under investigation. Precision of the method was 15.4 (6.0%) and recovery was 84.1 (96.1)% for Cd (Pb).

Chemicals

Nitric acid, 69% in ultrapure quality and certified standard solutions of Hg were purchased from Carl Roth (Karlsruhe, Germany) in 0.5 M nitric acid. Ultra pure water was obtained from a Purelab Flex 3 device (Elga Veolia; High Wycombe, United Kingdom). Ethanol (99.9%) and palladium-matrix modifier $\text{Pd}(\text{NO}_3)_2$ in nitric acid (15%) were supplied by VWR International GmbH (Darmstadt, Germany).

Analytical quality assurance

The accuracy of the Hg and Cd measurement in fish was determined by analysis of Certified Reference Material (DORM-4) obtained from the National Research Council (NCR) in Canada which was taken through the same analytical procedure as the samples. The reference material NIST-2976 purchased by LGC Standards GmbH, Wesel, Germany was used for Pb. All samples were measured in triplicates. External quality assurance was done by successful participation in laboratory proficiency tests conducted by QUASIMEME (www.wepal.nl) designed for marine environment analytics. Sample preparation steps were carried out under clean benches and clean lab conditions of ISO class 7.

Statistics and calculations

Statistical analyses were carried out using Statistica Version 12.5 (Statsoft Europe, Hamburg Germany): for comparison of contamination levels between the different sites a univariate ANOVA with post hoc test was carried out. We used a Least Significant Difference (LSD) test with $\alpha < 0.05$.

Since contamination levels are likely also related to the different sizes sampled at different sites, further statistical analyses were conducted by fitting separate generalized linear model (GLM) for each contaminant with

gamma distribution and log link, using R statistical software [38] and the lme4 package [5]. The maximum model included site, length and the respective interaction. All nested fixed effects structures were compared based on Akaike's information criterion (AIC, [2]) and significance was tested by likelihood ratio test (lmtest, [49]).

Results

Analytical results are given in Table 2. The average Hg concentration in all conger samples under investigation was 246.90 ± 216.83 µg/kg WM. Highest mean concentrations were determined in fish from Martil (594.65 ± 252.21 µg/kg WM) and Sidi el Abed (542.14 ± 219.87 µg/kg WM). Fish from both sites were significantly higher contaminated than those from Casablanca, Nador or Ras el Ma, respectively, while conger from Martil in addition were significantly higher contaminated with Hg than fish from Laayoune. Fish from Nador exhibited the lowest Hg mean concentration of 69.81 µg/kg WM. The average concentration of Pb in all European conger samples was 74.14 ± 87.02 µg/kg WM. The highest mean concentration of Pb was found in samples from Ras el Ma with 151.31 ± 179.77 µg/kg WM, significantly differing from Pb concentrations in fish from Casablanca. Individuals sampled in Casablanca showed significantly lower Pb concentrations (30.77 ± 23.37 µg/kg WM) compared to fish from Laayoune, Sidi el Abed, Nador and Ras el Ma. The average concentration of Cd in European Conger was 255.12 ± 287.15 µg/kg WM. The highest Cd concentrations were found in samples from Casablanca (537.23 ± 387.90 µg/kg WM) and Laayoune Port (475.70 ± 195.36 µg/kg WM). Both sites differed significantly from lower Cd-contaminated conger in Nador and Ras el Ma in Cd. Individuals from Nador showed significantly lower concentrations (3.56 ± 2.25 µg/kg WM) compared to Casablanca, Laayoune Port, Skhirat and Safi. Mean fish lengths and weights per location are shown in Table 2. The mean length of all fish under investigation was 88.1 cm (86.5 cm for all fish with measurements of Cd). This length corresponds to the age of 5–6 years [31]. European conger from the Mediterranean (compare Table 1) tends to be smaller and lighter than fish caught along the Atlantic coastline in the present sample set. These differences in size have to be considered when bioaccumulation of heavy metals is addressed.

Site specific bioaccumulation of heavy metals

To ensure a meaningful spatial comparison of Hg, Cd and Pb contamination in European conger at different sites, data were checked for bioaccumulation effects. Conditions like food availability, temperature and growth could vary depending on habitat and thus affect the presence and extend of bioaccumulation (i.e., an effect of length on

Table 2 Origin, biometric and heavy metal data of European conger (*Conger conger*) sampled between 2017 and 2019 in Morocco

Site	n	length [cm]	mass [g]	Hg [µg/kg WM]	Pb [µg/kg WM]	Cd [µg/kg WM]
Al Hoceima	5	69.3 (57.0–79.0)	542 (290–760)	164.73 ± 102.96 (80.46–336.01)	52.68 ± 16.80 (34.02–72.77)	97.74 ± 37.00 (41.26–142.60)
Casablanca	10	89.3 (46.0–108.0)	1589 (140–2490)	117.25 ± 71.24 (47.50–261.77)	30.77 ± 23.37 (11.82–87.14)	537.23 ± 387.90 (169.80–1207.70)
Laayoune Port	16 (10)	105.1 (64.0–131.7)	2706 (402–4831)	160.09 ± 91.06 (62.60–384.24)	68.35 ± 36.47 (32.21–156.62)	475.70 ± 195.36 (287.28–920.67)
Martil	12	94.8 (76.5–111.5)	1773 (730–3040)	594.65 ± 252.21 (290.28–1123.08)	53.59 ± 49.33 (17.37–196.11)	139.99 ± 97.58 (14.29–383.80)
Nador	15 (13)	60.5 (42.0–86.5)	357 (101–1010)	69.81 ± 16.07 (44.43–98.83)	73.67 ± 51.13 (33.11–220.98)	3.56 ± 2.25 (0.84–8.47)
Ras el Ma	18 (11)	69.4 (51.0–87.5)	569 (175–1140)	98.60 ± 47.19 (31.09–202.36)	151.31 ± 179.77 (24.08–656.56)	97.66 ± 63.84 (8.19–230.90)
Safi	16	104.3 (91.5–145.5)	2264 (1330–6790)	368.43 ± 153.29 (191.05–862.25)	56.09 ± 26.69 (23.36–112.15)	319.63 ± 90.42 (189.26–467.63)
Sidi el Abed	5 (0)	103.1 (95.0–113.0)	2292 (1690–2910)	542.14 ± 219.87 (164.67–732.62)	104.75 ± 15.97 (83.01–121.87)	n.d
Skhirat	11	101.7 (82.5–144.5)	2209 (960–6160)	322.24 ± 125.13 (112.66–505.89)	40.84 ± 19.63 (17.06–72.91)	414.82 ± 386.39 (59.04–1351.60)
All	108 (78)	88.1 (42.0–145.5)	1578 (101–6790)	246.90 ± 216.83 (31.09–1123.08)	74.14 ± 87.02 (11.82–656.56)	255.12 ± 287.15 (0.84–1351.60)

Total length [cm], mass [g], mercury (Hg), lead (Pb) and cadmium (Cd) [µg/kg wet mass (WM)] as mean values ± standard deviation per site with minima and maxima in brackets. n: number of analyzed individuals for Hg and Pb, in brackets number for Cd if divergent. n.d.: not determined. For locations see Fig. 1

Table 3 Adjusted metal concentration for European conger (*Conger conger*) of 88.1 cm length, according to GLM for mercury (Hg) and cadmium (Cd) given as site means

Site	Hg [µg/kg WM]	Cd [µg/kg WM]
Al Hoceima	297.9	118.3
Casablanca	117.8	467.5
Laayoune Port	98.9	492.0
Martil	457.9	150.2
Nador	93.6	2.3
Ras el Ma	177.4	194.2
Safi	293.7	212.3
Sidi el Abed	303.7	n.d
Skhirat	255.6	267.4

Hg in µg/kg wet mass (WM). Hg was analysed in muscle tissue, Cd in liver. For locations of sites see Fig. 1. n.d.: not determined

contamination, assuming that length is a robust indicator of age). Therefore, a GLM was used to (1) check for and if necessary (2) adjust contaminant concentrations for length (Table 3).

GLM results showed that bioaccumulation effects differed between sites for all contaminants under investigation, i.e., the interaction between length and site was significant for all models ($p < 0.001$ for Hg and Pb, $p < 0.05$ for Cd) and the maximum model was kept. For Hg bioaccumulation was significant in Laayoune Port, Martil, Ras el Ma ($p < 0.01$) as well as Safi and Skhirat ($p < 0.05$); no

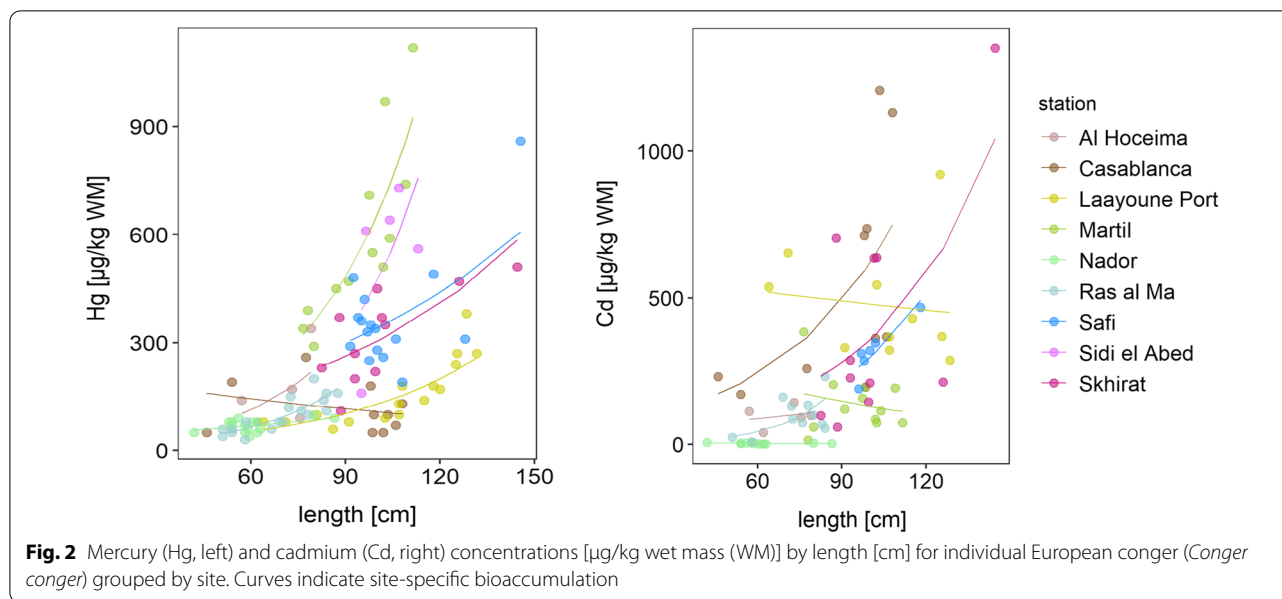
bioaccumulation effect was evident at the other sites. For Cd, bioaccumulation was significant in Casablanca, Ras el Ma ($p < 0.001$) and Skhirat ($p < 0.05$; Fig. 2). Accordingly, Hg and Cd estimates from Table 2 needed to be corrected for the effect of length (per site). GLM predictions of a standard fish of 88.1 cm (mean length of all congers in the data set) were used to perform a spatial comparison without bias from local bioaccumulation processes.

Surprisingly, results showed a significant decrease of Pb contamination with length at several sites but no increase with length (results not shown). It is assumed that bioaccumulation effects are negligible and the mean per site was used for spatial comparison.

After GLM adjusting (Table 3) the ranking of sites by contamination did not change completely compared to the non-adjusted data (Table 2). The two sites with the highest Hg or Cd contaminations remain at the top of the list before and after correction for length. However, the means for Hg and Cd changed, naturally mostly at sites where the mean length of fish differs considerably from the overall mean of 88.1 cm (Tables 2, 3).

Spatial distribution of heavy metals using GLM adjusted results

In Fig. 3, the spatial distribution of heavy metal contamination of European conger in Morocco is shown. Highest values of Hg are reported in Martil, and Sidi el Abed while highest Cd values were reported in Casablanca and Skhirat. Pb concentrations were found to be highest in



Ras el Ma. No general hot spot for heavy metals was evident from the data suggesting different sources for every metal under investigation. Except for a distinct trend towards lower Cd concentrations in the Mediterranean, no general difference in contamination level was found between the Atlantic and Mediterranean coastlines or with city size, as indicated in Table 1. We hypothesize that different industries e.g. in Martil and Sidi el Abed could cause higher environmental contamination. However, it remains unclear how to explain Cd values in Skhirat and Pb values in Ras el Ma, while these are small cities with no known significant industrial activity. Therefore, non-anthropogenic sources for heavy metal contamination have to be considered too.

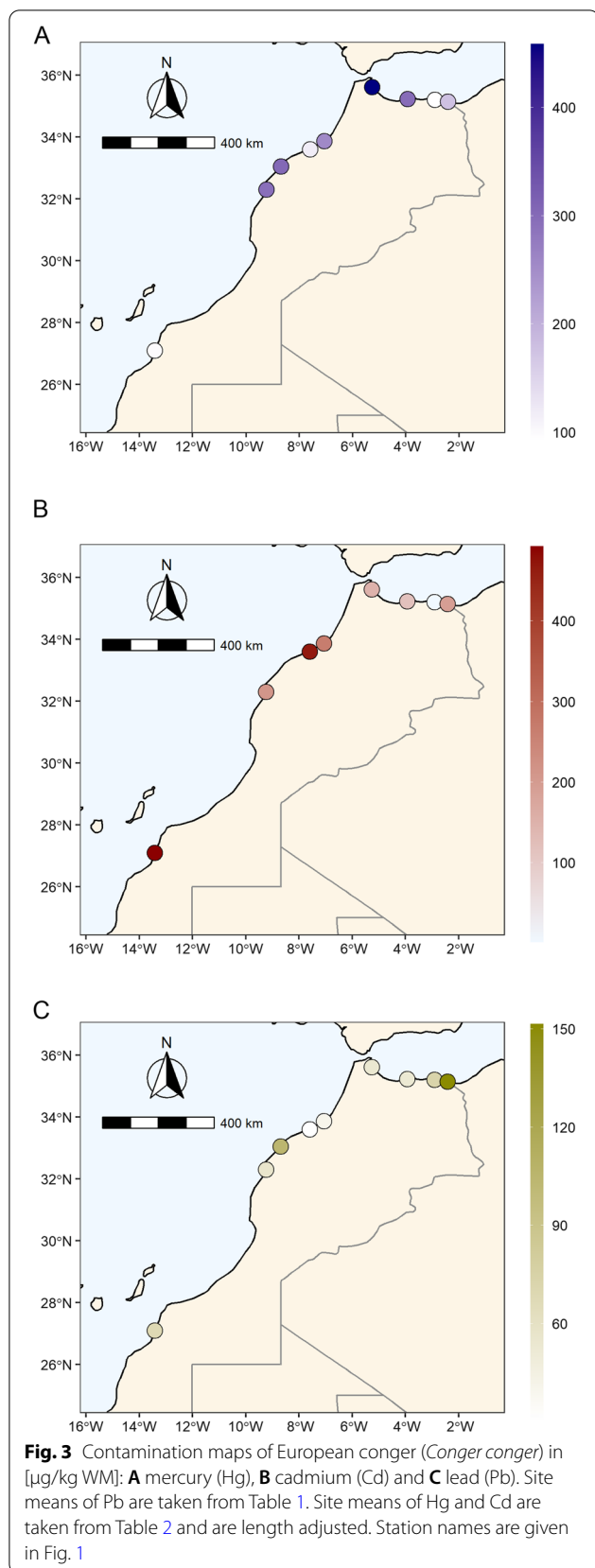
Discussion

The aim of the present study was to investigate the contamination levels as well as spatial distribution of Hg, Cd and Pb in European Conger caught at different regions alongside the Moroccan coasts. The overall contamination levels of the present study are in accordance with Storelli and Barone [42], who reported Hg concentrations in European conger caught in the Adriatic Sea (Mediterranean Sea) from 570 to 3540 $\mu\text{g}/\text{kg}$ WM with a mean value 1140 $\mu\text{g}/\text{kg}$ WM. Dron et al. [14] analyzed European conger from the industrialized Gulf of Fos (France) for heavy metals including Hg and found elevated to moderate contaminant levels of up to 1350 $\mu\text{g}/\text{kg}$ WM, which the authors related to surrounding industries and maritime traffic. The highest value detected in the present study was 1123 $\mu\text{g}/\text{kg}$ WM in a fish from Martil. Lower contaminated specimens from the present study

are in the same range of contamination as reported by Chahid et al. [8] who analysed 14 $\mu\text{g}/\text{kg}$ Cd, 49 Pb and 49 $\mu\text{g}/\text{kg}$ Hg in edible tissue of European conger from southern Morocco. The authors concluded that fish from their study were safe for human consumption. However, in the present study most fish were caught in other regions of Morocco but one location from southern Moroccan Coast (Laayoune Port) was included. In the present study, Hg values of fish from Laayoune Port are considerably higher than reported by Chahid et al. [8].

Compared to environmental threshold values, designed for fish from the North-East Atlantic as used by the International Council for the Exploration of the Sea (ICES) as well as by the Oslo and Paris Commission (OSPAR) for their environmental assessments (e.g. [36]), European Conger from the present study were moderately to significantly contaminated: mean values of heavy metal contamination shown in Table 1 are above background concentration for Hg, Pb and Cd in fish (35, 26 and 26 $\mu\text{g}/\text{kg}$ WM, respectively; [20]) and at the same time below threshold values for ecological effects (500, 1500, and 1000 $\mu\text{g}/\text{kg}$ WM, respectively, [20]). Individual samples of European conger do not exceed ecological effect thresholds for Pb and Cd but some do so for Hg. However, for these thresholds were designed for a different ecosystem this comparison has to be interpreted with care.

The results presented in Fig. 3 indicate different hot spots for every metal under investigation. We assume different sources for contamination to be present along the Moroccan coast; however, Casablanca—the biggest city in the present set of sampling locations—points out the highest Cd values. Our results are partly in accordance



with Benbrahim et al. [6], who analysed Cd, Pb and Hg in mussels along the Moroccan coast and also identified different hotspots for contamination with the three metals. However, the geographical distribution of the three heavy metals described by Benbrahim et al. [6] did not match the results of the present study on European conger.

Bioaccumulation for Hg and Cd at different locations was described in the present study and facilitated adjustment for length. Hg bioaccumulation has been described before in European conger from the Mediterranean Sea [27, 42]. The authors of both studies pointed out potential risks for human consumption regarding larger fish. Our results are in accordance with those findings because some larger specimens (> 100 cm length) exhibited Hg concentration above the maximum levels proposed by the European legislation [15] for most fish species for human consumption of 500 $\mu\text{g}/\text{kg WM}$. Therefore, larger European conger from Sidi El Abed and Martil should be consumed less frequently.

Conclusions

- European conger along the Moroccan coast is moderately to significantly contaminated with Hg, Cd and Pb.
- Bioaccumulation was observed for Hg and for Cd and has to be considered for spatial comparisons of contamination levels.
- The spatial distribution of Cd, Hg and Pb along the Moroccan coastline is different for the three metals.
- Larger European conger specimens above ca 100 cm may exceed the food threshold level for Hg and should be therefore consumed less often.

Abbreviations

Cd: Cadmium; GFAAS: Graphite furnace atomic absorption spectrometer; GLM: Generalized linear model; Hg: Mercury; LD: Limit of detection; LQ: Limit of quantification; LSD: Least Significant Difference; MeHg: Methylmercury; n: Total number of objects; n.d.: Not determined; Pb: Lead; WM: Wet mass.

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Author contributions

UK designed the study, performed data analysis, visualisation and was the main contributor in writing the manuscript. JDP performed statistics and visualisation of data and supported the writing of the manuscript. HM and KR performed fieldwork and chemical analyses. FW and AY provided resources and performed field work. RH designed the study, contributed to writing the manuscript, performed fieldwork and provided biological data. All authors approved the final draft of the manuscript.

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Availability of data and materials

The datasets generated or used during the current study as well as documentation of the statistics are publicly available on gitHub repository, https://github.com/jdpo/R_Conger_Morocco.

Declarations

Ethics approval and consent to participate

All procedures were conducted in accordance with European directive 2010/63/EU on the protection of animals used for scientific purposes.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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