Antibiotic residues and heavy metals in blue crabs (*Callinectes sapidus*) fished in the Mediterranean Sea: a preliminary study

Alessio Manfredi, Patrizio Lorusso, Annamaria Pandiscia, Elisabetta Bonerba, Edmondo Ceci, Giancarlo Bozzo, Domenico Cristofaro, Valentina Terio

Department of Veterinary Medicine, University of Bari Aldo Moro, Valenzano, Italy

Abstract

In recent decades, the Mediterranean Sea has experienced the invasion of the blue crab (*Callinectes sapidus*), which threatens the marine ecosystem and economic activities related to fishing and aquaculture because of its aggressive behavior. Control strategies are being developed to reduce its population. In Italy, a partial solution to the problem is its promotion as a food product. However, to ensure consumer safety, promoting consumption must be accompanied by a careful risk analysis. This study aims to assess heavy metals and antibiotic residues in the appendage muscle of 18 blue crab samples from various Mediterranean sites, selected by sex and size. Heavy metals were quantified using inductively coupled plasma mass spectrometry, and antibiotics were analyzed with the liquid chromatography/triple-quadrupole tandem mass spectrometry multiresidue/multiclass method. In all samples, lead was never detected, while cadmium and mercury concentrations never exceeded the limit of 0.5 mg/kg set by Regulation (EU) 2023/915. Only one sample tested positive for the occurrence of 12 antibiotic residues. The results showed that the blue crab is a species commonly characterized by the accumulation of heavy metals, according to other studies. Therefore, monitoring the concentration of metals in these species is important for food safety and ecosystem management. Data on 12 antibiotic residues detected in a single crab sample require further investigation through extensive sampling in terms of both number and sites, involving a wider area along the Mediterranean Sea coast, to allow for proper risk characterization.

Correspondence: Alessio Manfredi, Department of Veterinary Medicine, University of Bari Aldo Moro, Provincial Road to Casamassima km 3, 70010 Valenzano, Italy.
E-mail: alessio.manfredi@uniba.it

Key words: blue crab, heavy metals, antibiotics, food safety.

Contributions: AM, conceptualization, investigation, writing – original draft; PL, conceptualization, investigation, data analysis; AP, investigation, writing – review and editing; EB, conceptualization, investigation, writing – review and editing, supervision; EC, writing – review and editing, supervision; GB, writing – review and editing, supervision; DC, investigation, data analysis, VT, conceptualization, investigation, writing – review and editing, supervision.

Conflict of interest: the authors declare no potential conflict of interest.

Ethics approval and consent to participate: not applicable.

Availability of data and materials: data and materials are available from the corresponding author upon request.

Conference presentation: this paper was presented at the XXXIII National Conference of the Italian Association of Veterinary Food Hygienists (AIVI), Castellammare di Stabia (NA), September 11-12-13, 2024.

Funding: none.

Received: 23 October 2024.
Accepted: 13 December 2024.
Early access: 8 April 2025.

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0).

©Copyright: the Author(s), 2025
Licensee PAGEPress, Italy
Italian Journal of Food Safety 2025; 14:13287
doi:10.4081/ijfs.2025.13287

Publisher's note: all claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

Introduction

The term "alien species" describes organisms introduced to new environments by human activities, bypassing natural geographic barriers, with the potential to establish, reproduce, and spread, sometimes becoming invasive (Pyšek *et al.*, 2020). According to definitions from the International Union for Conservation of Nature, the Convention on Biological Diversity, and the World Trade Organization, species are deemed invasive if they harm biodiversity, ecosystem services, or human well-being (Pyšek *et al.*, 2020). Currently, over 37,000 alien species have been introduced globally due to human activities, with documented negative impacts on more than 3500 species, including 10% in marine environments (Castriota *et al.*, 2024).

The Atlantic blue crab *Callinectes sapidus* Rathbun 1896 is a species native to the western Atlantic, with a range extending from Nova Scotia, Canada, to northern Argentina, including Bermuda and the Antilles (Castriota *et al.*, 2024). In Europe, it was first reported in the early 20th century on the Atlantic coast of France, where it was introduced through the ballast waters of transoceanic ships (Mancinelli *et al.*, 2017). Subsequently, specimens were detected in the North Sea, Mediterranean Sea, Baltic Sea, Black Sea, and Sea of Azov from 1932 to 1967 (Nehring, 2011). Since 2006, *C. sapidus* has spread rapidly in the western Mediterranean (Adriatic Sea, Ionian Sea, and Spanish coasts), reaching a medium level of population density in 2015 that intensified to high in the period from 2016 to 2023 (Castriota *et al.*, 2024). Nowadays, it has been recognized as one of the 100 most invasive alien species in the Mediterranean Sea and is drawing significant attention due to its rapid spread, substantial population growth, and potential

impacts on ecosystems (Streftaris and Zenetos, 2006; Castriota *et al.*, 2024). In particular, the ability of blue crabs to expand into new areas is primarily attributed to their aggressive behavior, high fecundity, strong swimming ability, and adaptability to different environmental conditions (Castriota *et al.*, 2024). The biological cycle of *C. sapidus* involves distinct developmental stages influenced by temperature and salinity. Males mature in spring, tolerating temperatures of 16-27°C and salinities of 20-36 psu, while females mature in summer, requiring higher salinity (24-38 psu) and tolerating temperatures of 20-28°C. Below 12°C, blue crabs hibernate, reducing metabolic activity (Marchessaux *et al.*, 2023). Therefore, the large metabolic plasticity enables them to thrive in diverse habitats, including estuaries, coastal areas, and lagoons (Kara and Chaoui, 2021; Gil-Fernández *et al.*, 2024). In this regard, it has been demonstrated that, in a climate change scenario, the species' high tolerance to temperature variations may facilitate both population growth in areas where it is already found, particularly in estuaries and lagoons where environmental conditions are highly variable, and its expansion into new, non-native regions (Marchessaux *et al.*, 2022). The feeding habits of *C. sapidus* significantly impact the ecosystems it colonizes. It is an omnivorous generalist, primarily consuming fish, mollusks, and crabs, along with plant material and sediment *via* secondary consumption (Ortega-Jiménez *et al*., 2024). In regions abundant in bivalve mollusks, they can constitute up to 75% of their diet due to their ease of capture (Prado *et al.*, 2022). These habits are evident in the challenges that Italy faces in the shellfish sector, where blue crabs have already caused an estimated €100 million in economic damage and ravaged up to 90% of young clams, threatening the survival of around 3000 family businesses in the Po Delta region (Azzurro *et al.*, 2024). One approach to partially mitigate the economic impact of the *C. sapidus* invasion in the Mediterranean Sea is to promote it as a food product. This initiative must be supported by a comprehensive risk analysis to ensure the safety of this crustacean, thereby protecting consumer health. To date, several studies have examined the microbiological risks linked to the consumption of blue crab meat (Givens *et al.*, 2013; Rodgers *et al.*, 2014; Smalls *et al.*, 2023; Gilstrap *et al.*, 2023); however, only a limited number of investigations have focused on assessing the chemical risks. Furthermore, it is crucial to verify the occurrence of chemical contaminants in the Mediterranean Sea seafood, as this ecosystem is particularly vulnerable to pollution due to its unique hydrogeographical features and the significant impact of anthropic activities that contribute to high levels of contamination (Bonerba *et al.*, 2024). Particularly, this activity contributes to the spread of pharmaceutical contaminants from municipal wastewater effluent into aquatic ecosystems, where they can accumulate in bivalve mollusks through filter feeding (Baralla *et al.*, 2021; Chiesa *et al*., 2018). Since bivalve mollusks are a primary food source for blue crabs, these species may also contain antibiotic residues in their edible tissues, potentially posing health risks to consumers. Therefore, the aim of this study was to evaluate the occurrence of heavy metals and antibiotic residues in the appendage muscle of 18 blue crab samples fished in different areas of the Mediterranean Sea, selected according to sex and size.

Materials and Methods

Sampling

From January to May 2024, 50 specimens of *C. sapidus*, 9 fished in Food and Agriculture Organization (FAO) area 37.2.1 (Adriatic), 9 in FAO area 37.2.2 (Ionian), 32 in FAO areas 37.3.1 (Aegean), 37.1.3 (Sardinia), 37.1.2 (Gulf of Lions), 37.1.1 (Balearic) (8 for each FAO area), were purchased from several large-scale retail distribution in the Apulian and Calabrian regions, and were transported under refrigerated conditions to the Food Safety Unit of the Department of Veterinary Medicine, University of Bari Aldo Moro. Subsequently, 31 male specimens were selected because, reaching larger sizes than females, they are more suitable for commercial purposes. Finally, 18 mature male specimens (with a free and fully expandable abdomen), three from each FAO fishing area, with carapace widths of about 13 cm, were further selected to standardize the data with the bioaccumulation capacity of crabs based on size. Muscle meat from the appendages of these 18 samples was collected and stored at -20°C until the analysis.

Inductively coupled plasma mass spectrometry for the quantitative determination of heavy metals

The quantitative determination of 17 heavy metals [aluminum (Al), iron (Fe), barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), lead (Pb), copper (Cu), tin (Sn), thallium (Tl), vanadium (V), and zinc (Zn)] and 3 metalloids with properties similar to heavy metals [arsenic (As), bismuth (Bi), and selenium (Se)] was carried out using an inductively coupled plasma mass spectrometry (ICP-MS) system.

Therefore, 0.2 g of each sample was weighed into appropriate vessels using an analytical balance and subsequently dissolved into a solution composed of 8.5 mL of nitric acid (HNO_3) 65% and 1.5 mL of hydrogen peroxide (H_2O_2) 30%. The blank was prepared using only nitric acid and hydrogen peroxide to verify their purity. Then, the samples and blank were placed under a fume hood for 15 minutes and transferred to a microwave system for mineralization. Once mineralization was finished, the samples and blank were cooled to room temperature and filtered using Whatman filter paper before ICP-MS analysis was performed according to the method UNI EN 15763:2010 (UNI EN, 2010).

Multiresidues multiclass analytical method for the determination of antibiotics

Determination of the antibiotic residues was carried out using the method described by Joseph *et al*. in the application note entitled "Quantitative Screening of Multiresidue Veterinary Drugs in Seafood Using the Agilent 6470 Triple Quadropole LC/MS" (Joseph *et al.*, 2020). The method was validated according to the Commission Decision 2002/657/EC criteria (Commission of the European Communities, 2002). The molecules investigated were: nalidixic acid; oxolinic acid; AHD (1-aminodantoin), amoxicillin; AMOZ (5-methylmorpholine-3-amino-2-oxazolidone), ampicillin; AOZ (3-amino-2-oxazolidone); carbadox; ceftiofur; ciprofloxacin; chloramphenicol; chlortetracycline; cloxacillin; danofloxacin; dapsone; demeclocycline; dicloxacillin; difloxacin; doxycycline; enoxacin; enrofloxacin; 4-epichlortetracycline; 4-epioxytetracycline; 4-epitetracycline; erythromycin A; florfenicol; florfenicol amine; flumequine; josamycin; lincomycin; marbofloxacin; metacyclin; metronidazole; minocycline; nafcillin; norfloxacin; olaquindox; oxacillin; oxytetracycline; penicillin G; penicillin V; sarafloxacin; SEM (semicarbazide); sum of malachite green and leucomalachite green; spiramycin I; sulfabenzamide; sulfacetamide; sulfaquinoxaline; sulfachloropyridazine; sulfadiazine; sulfadimethoxine; sulfadimidine; sulfadoxin; sulfaguanidine; sulfamerazine; sulfameter (sulfamethoxydiazine); sulfamethyzole;

sulfamethoxazole; sulfamethoxypyridazine; sulfamonomethoxine; sulfamoxol; sulfanilamide; sulfapiridine; sulfatiazole; sulfisoxazole; tetracycline; thiamphenicol; tiamulin; tilmicosin; tylosin A; trimethoprim; tulathromycin A; valnemulin; leucomalachite green; malachite green; virginiamycin M1. For all antibiotics, the detection limit of the method used was 5 µg/kg, while the quantification limit was 10 µg/kg.

Data processing

For descriptive statistics, the concentration of analytes was expressed in µg/kg or mg/kg, considering the dilution factor used during the extraction procedure and the exact sample weight. In addition, mean concentrations, standard deviation, minimum and maximum values (range) of each analyte were calculated for the groups of the three samples belonging to the same FAO Area.

Results

Heavy metals

The concentration of heavy metals in the analyzed samples, categorized by FAO Area, is displayed in Tables 1-3. The Tables show the mean concentration of heavy metals analyzed for the three samples belonging to each FAO Area. Moreover, they include the range of values calculated for each analyte in the same samples.

Antibiotic residues

Of the 18 samples analyzed, no antibiotic residues were detected in 17. However, one sample from FAO Area 37.2.1 contained the presence of residues from 12 different antibiotics, with the corresponding measured concentrations detailed in Table 4.

Discussion

The results of this study revealed that the concentrations of heavy metals have the following decreasing order: zinc > copper > manganese > arsenic > chromium > cadmium > vanadium > mercury > cobalt > selenium > molybdenum. In addition, no traces of beryllium, aluminum, iron, nickel, tin, barium, thallium, lead, or bismuth were detected in all samples analyzed. For cadmium, lead, and mercury, maximum levels of 0.5 mg/kg in muscle meat from appendages are set by Regulation (EU) 2023/915 (European Commission, 2023). In this study, none of the analyzed samples exceeded the regulatory limits for these three metals.

Other studies have quantified the levels of heavy metals in the muscle tissue of *C. sapidus*. Fakhri *et al*. (2021), in a study carried out in the Persian Gulf region, measured levels of chromium, copper, mercury, lead, and zinc. The order of heavy metals in the blue crab's muscle was iron (0.414±0.288 mg/kg) > lead (0.238±0.087 mg/kg) > nickel (0.092±0.039 mg/kg) > cadmium (0.052±0.018 mg/kg) (Fakhri *et al.*, 2021). Genç and Yilmaz (2017), in a study performed in Köyceğiz Lagoon System, quantified in *C. sapidus* muscle tissue the levels of mercury, cadmium, copper, lead, chromium, and zinc. In agreement with our study, the heavy metals with the highest concentration were zinc (43.981±3.44 mg/kg) and copper (18.214±2.60 mg/kg) followed by chromium (1.813±0.41 mg/kg), lead (1.208±0.13 mg/kg), cadmium (0.161±0.24 mg/kg) and mercury (0.090±0.01 mg/kg) (Genç and Yilmaz, 2017). The presence of heavy metals in *C. sapidus* muscle tissue can be linked to several factors, such as the physicochemical conditions of the aquatic environment and the crab's nutritional habits (Fakhri *et al.*, 2021). In particular, aquatic environments receive heavy metals from domestic, industrial, and agricultural sources, concentrating them mainly in sediments (Huang *et al.*, 2020). Blue crabs, as shown in the study conducted by Ortega-Jiménez *et al*. (2024), can feed on sediments that can make up to 32.3 % of their diet. Furthermore, it has been shown how an increase in the ingestion of sediments by this species can occur in heavily contaminated areas where the high presence of contaminants can greatly reduce its predatory activity (Rosas *et al.*, 1994; Belgrad and Griffen, 2016). Therefore, the relative differences in heavy metal concentrations measured in the study conducted by Fakhri *et al*. (2021) and Genç and Yilmaz (2017) could be related to specific pollution sources in the proximity of the sampling areas that caused sediment contamination. In the first case, the authors highlight the significant use of agricultural fertilizers, pesticides, and herbicides near the sampling site, along with the presence of oil platforms and heavy ship traffic (Fakhri *et al.*, 2021). In the second case, the authors report high anthropogenic activity due to tourism in the vicinity of the sample collection area (Genç and Yilmaz, 2017). In our study, however, it is not possible to establish a correlation between the sampled area and heavy metals measured in the blue crabs, as the exact location of specimen collection is unknown. Only the FAO fishing area is known, but it represents a broad region.

Regarding antibiotics, 12 residues were identified in one sample from FAO fishing area 37.2.1, with the following order: chloramphenicol > sarafloxacin > trimethoprim > nalidixic acid > tetracycline. While florfenicol, sulfabenzamide, sulfaquinoxaline, sulfadiazine, sulfadimethoxine, sulfadimidine, and sulfadoxine were found at concentrations below the limit of quantification of the method. The susceptibility of crabs to accumulating antibiotic residues in meat was also demonstrated by the study conducted by Fang *et al*., where in 92 samples of *Eriocheir sinensis*, different antibiotics were detected with the following decreasing concentrations: enroflxacin > ciprofloxacin > sulfaquinoxaline > sulfameter > sulfadoxine > sulfamethoxazole. As in our study, the most represented class of antibiotics was sulfonamides, most likely due to their wide use in aquaculture (Fang *et al.*, 2021).

Currently, Commission Regulation (EU) No 37/2010 sets maximum residual limits (MRLs) for most of them in the muscle of food-producing species (European Commission, 2010). The regulatory framework of Commission Regulation (EU) No 37/2010 addresses the safe use of antibiotics in animal animal-origin food supply chain by establishing species-specific, substance-specific, and tissue-specific (target tissue) MRLs. This legislation was also introduced to mitigate the antibiotic overuse, the consequent spread, and antimicrobial resistance. Specifically, for florfenicol, for antibiotics belonging to the sulfonamide class (sulfabenzamide, sulfaquinoxaline, sulfadiazine, sulfadimethoxine, sulfadimidine, and sulfadoxine), and for tetracycline, the MRL is 100 µg/kg, while, for trimethoprim, it is 50 µg/kg (European Commission, 2010). None of the measured antibiotic concentrations exceeded the limit fixed by the Regulation. However, in the same sample, chloramphenicol was identified at the highest concentration (17 µg/kg). This antibiotic is included in Table 2 of Regulation No (EU) 37/2010, which lists prohibited pharmacologically active substances (European Commission, 2010). Since an MRL is not set for this molecule, the European Commission has established the reference point for action (RPA) (European Commission, 2019). Food of animal origin, containing residues of a pharmacologically active substance in a concentration at or above the RPA, is to be considered not to comply with Union legislation, while food of animal origin containing concentrations below the reference points

Table 1. Mean concentration and range of heavy metals of samples from the Food and Agriculture Organization (FAO) areas 37.2.1 and 37.3.1.

Heavy metals	Samples fished in FAO area 37.2.1 Mean concentration ± SD (mg/kg)	Range (mg/kg)	Samples fished in FAO area 37.3.1 Mean concentration ± SD (mg/kg)	Range (mg/kg)
Be	ND	ND-ND	ND	ND-ND
Al	ND	ND-ND	ND	ND-ND
V	0.07±0.017	0.06-0.09	0.09±0.04	0.05-0.13
Cr	0.16±0.098	0.08-0.27	0.14±0.025	0.12-0.17
Fe	ND	ND-ND	ND	ND-ND
Mn	0.55±0.958	ND-1.66	2.57±0.121	2.44-2.68
Co	0.023±0.032	ND-0.06	0.06±0.078	ND-0.15
Ni	ND	ND-ND	ND	ND-ND
Cu	6.82±2.64	4.82-9.82	9.3±3.318	6.2-12.80
Zn	15.15±3.310	12.57-18.88	10.18±3.604	7.8-14.33
As	1.65±0.222	1.40-1.83	2.08±0.315	1.77-2.40
Se	0.04±0.032	ND-0.06	0.08±0.139	ND-0.24
Mo	0.01±0.017	ND-0.03	0.05±0.035	0.02-0.09
Cd	0.077±0.107	ND-0.20	0.41±0.06	0.35-0.47
Sn	ND	ND-ND	ND	ND-ND
Ba	ND	ND-ND	ND	ND-ND
Hg	0.11±0.056	0.06-0.17	0.01±0.017	ND-0.03
Tl	ND	ND-ND	ND	ND-ND
Pb	ND	ND-ND	ND	ND-ND
Bi	ND	ND-ND	ND	ND-ND

FAO, Food and Agriculture Organization; ND, not detected; SD, standard deviation; Be, beryllium; Al, aluminum; V, vanadium; Cr, chromium; Fe, iron; Mn, manganese; Co, cobalt; Ni, nickel; Cu, copper; Zn, zinc; As, arsenic; Se, selenium; Mo, molybdenum; Cd, cadmium; Sn, tin; Ba, barium; Hg, mercury; Tl, thallium; Pb, lead; Bi, bismuth.

Table 2. Mean concentration and range of heavy metals of samples from the Food and Agriculture Organization (FAO) areas 37.1.3 and 37.1.2.

Heavy metals	Samples fished in FAO area 37.1.3 Mean concentration ± SD (mg/kg)	Range (mg/kg)	Samples fished in FAO area 37.1.2 Mean concentration ± SD (mg/kg)	Range (mg/kg)
Be	ND	ND-ND	ND	ND-ND
Al	ND	ND-ND	ND	ND-ND
V	0.09±0.078	ND-0.14	0.11±0.082	0.04-0.20
Cr	0.39±0.144	0.23-0.51	0.21±0.087	0.15-0.31
Fe	ND	ND-ND	ND	ND-ND
Mn	1.92±0.601	1.33-2.53	8.4±2.82	6.37-11.62
Co	0.08±0.044	0.05-0.13	0.1±0.035	0.08-0.14
Ni	ND	ND-ND	ND	ND-ND
Cu	14.45±2.174	12.57-16.83	9.6±1.089	8.57-10.74
Zn	15.23±1.461	14.10-16.88	11.38±2.113	9.49-13.66
As	2.21±0.790	1.41-2.99	1.77±0.230	1.55-2.01
Se	0.05±0.078	ND-0.14	0.08±0.051	0.04-0.14
Mo	0.06±0.072	ND-0.14	0.04±0.047	ND-0.09
Cd	0.31±0.111	0.21-0.43	0.03±0.026	ND-0.05
Sn	ND	ND-ND	ND	ND-ND
Ba	ND	ND-ND	ND	ND-ND
Hg	0.1±0.078	0.01-0.15	0.09±0.102	0.02-0.21
Tl	ND	ND-ND	ND	ND-ND
Pb	ND	ND-ND	ND	ND-ND
Bi	ND	ND-ND	ND	ND-ND

FAO, Food and Agriculture Organization; ND, not detected; SD, standard deviation; Be, beryllium; Al, aluminum; V, vanadium; Cr, chromium; Fe, iron; Mn, manganese; Co, cobalt; Ni, nickel; Cu, copper; Zn, zinc; As, arsenic; Se, selenium; Mo, molybdenum; Cd, cadmium; Sn, tin; Ba, barium; Hg, mercury; Tl, thallium; Pb, lead; Bi, bismuth.

for action is not to be prohibited from entering the food chain (European Commission, 2019). Therefore, this product could not have been placed on the market due to its RPA being set at 0.15 µg/kg. Anyway, the presence of a high concentration of antibiotic residues in a single specimen is generally linked to a nearby source of pollution at the sampling site. The primary sources of antibiotic contamination in marine environments include hospital effluents, which often merge with municipal wastewater and reach the sea *via* sewage treatment plants, and agricultural fertilizers, derived from contaminated manure, which release antibiotics into soil and water systems. Additionally, aquaculture, through the direct application of antibiotics and industrial antibiotic production, with heavily contaminated discharges, plays a significant role in introducing antibiotics into marine environments (Kraemer *et al.*, 2019). However, in this study, similar to heavy metals, a definitive explanation of a possible source of pollution is challenging to provide, as the only available provenance data for the samples is limited to the FAO fishing area.

Table 3. Mean concentration and range of heavy metals of samples from the Food and Agriculture Organization areas 37.1.1 and 37.2.2.

Heavy metals	Samples fished in FAO area 37.1.1		Samples fished in FAO area 37.2.2	
	Mean concentration ± SD (mg/kg)	Range (mg/kg)	Mean concentration ± SD (mg/kg)	Range (mg/kg)
Be	ND	ND-ND	ND	ND-ND
Al	ND	ND-ND	ND	ND-ND
V	0.08±0.065	0.02-0.15	0.09±0.06	0.03-0.15
Cr	0.37±0.081	0.31-0.46	0.17±0.072	0.11-0.25
Fe	ND	ND-ND	ND	ND-ND
Mn	0.80±0.364	0.41-1.13	ND	ND-ND
Co	0.02±0.026	ND-0.05	0.03±0.015	0.02-0.05
Ni	ND	ND-ND	ND	ND-ND
Cu	15.18±0.507	14.78-15.75	10.70±2.111	8.33-12.37
Zn	18.07±0.56	17.67-18.71	14.89±1.37	13.51-16.25
As	1.17±0.255	0.92-1.43	1.71±0.163	1.57-1.89
Se	ND	ND-ND	ND	ND-ND
Mo	0.01±0.012	ND-0.02	ND	ND-ND
Cd	0.01±0.006	0.01-0.02	0.14±0.066	0.08-0.21
Sn	ND	ND-ND	ND	ND-ND
Ba	ND	ND-ND	ND	ND-ND
Hg	0.02±0.015	0.01-0.04	0.02±0.02	ND-0.04
Tl	ND	ND-ND	ND	ND-ND
Pb	ND	ND-ND	ND	ND-ND
Bi	ND	ND-ND	ND	ND-ND

FAO, Food and Agriculture Organization; ND, not detected; SD, standard deviation; Be, beryllium; Al, aluminum; V, vanadium; Cr, chromium; Fe, iron; Mn, manganese; Co, cobalt; Ni, nickel; Cu, copper; Zn, zinc; As, arsenic; Se, selenium; Mo, molybdenum; Cd, cadmium; Sn, tin; Ba, barium; Hg, mercury; Tl, thallium; Pb, lead; Bi, bismuth.

Table 4. Concentration of antibiotic residues of one sample from the Food and Agriculture Organization (FAO) area 37.2.1.

Antibiotic residues	Concentration (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)
Nalidixic acid	12	10	5
Chloramphenicol	17	10	5
Florfenicol	<LOQ	10	5
Sarafloxacin	13.5	10	5
Sulfabenzamide	<LOQ	10	5
Sulfaquinoxaline	<LOQ	10	5
Sulfadiazine	<LOQ	10	5
Sulfadimethoxine	<LOQ	10	5
Sulfadimidine	<LOQ	10	5
Sulfadoxine	<LOQ	10	5
Tetracycline	11	10	5
Trimethoprim	12.6	10	5

LOQ, limit of quantification; LOD, limit of detection.

OPEN ACCESS

Conclusions

This study provides useful, even if preliminary, data on the presence of antibiotic residues and heavy metals in blue crabs fished in the Mediterranean Sea. The proper characterization of chemical risk, indeed, requires extensive and systematic sampling including different fishing areas, high sample numbers, and comparative assessment of sampled specimen size/age and bioaccumulation. An important limitation of this study is that it focused solely on the muscles of the crab's appendages, in alignment with Commission Regulation (EU) 2023/915. This regulation specifies maximum levels of heavy metals only within the meat from these parts of crabs and crab-like crustaceans (*Brachyura* and *Anomura*) (European Commission, 2023). However, several studies have highlighted that dark crab meat, including digestive organs such as the hepatopancreas, tends to accumulate significant concentrations of heavy metals (Reichmuth *et al.*, 2010; Çoğun *et al.*, 2017). As a result, this study may underestimate the real risk associated with the consumption of blue crabs, especially since other parts are frequently used in home recipes. Therefore, further research is needed to quantify heavy metal levels in crab parts not covered by this study. Finally, to reduce the socioeconomic impact of *C. sapidus*, especially on fishermen who are the most affected group, public initiatives would be necessary to raise awareness about the negative effects of the blue crab on fishing communities and local economies. By providing clear and valuable information, these efforts could guide the development of marine resource management policies aimed at mitigating these impacts. From a business standpoint, it is essential to equip fishers with tools to tackle the economic challenges posed by the blue crab's presence. This could involve developing alternative and more efficient fishing techniques, as well as identifying new revenue opportunities. Fishers could enhance their profitability through various marketing strategies such as direct sales to consumers, selling wholesale to restaurants and retailers, utilizing e-commerce platforms, and promoting innovative blue crab recipes. Moreover, adopting sustainable fishing practices and participating in promotional events could attract environmentally conscious consumers, driving demand for blue crab products. Together, these approaches could contribute to the sustainable management of the issue, alleviating socioeconomic pressures on communities and supporting the well-being of the marine ecosystem (Nardelli *et al.*, 2024).

References

Azzurro E, Bonanomi S, Chiappi M, De Marco R, Luna GM, Cella M, Guicciardi S, Tiralongo F, Bonifazi A, Strafella P, 2024. Uncovering unmet demand and key insights for the invasive blue crab (Callinectes sapidus) market before and after the Italian outbreak: implications for policymakers and industry stakeholders. Mar Policy 167:106295.

Baralla E, Demontis MP, Dessì F, Varoni MV, 2021. An overview of antibiotics as emerging contaminants: occurrence in bivalves as biomonitoring organisms. Animals 11:3239.

Belgrad BA, Griffen BD, 2016. The influence of diet composition on fitness of the blue crab, Callinectes sapidus. PLoS One 11:e0145481.

Bonerba E, Shehu F, Pandiscia A, Lorusso P, Manfredi A, Huter A, Tantillo GM, Panseri S, Nobile M, Terio V, 2024. The EU interreg project "ADRINET": assessment of well-known and emerging pollutants in seafood and their potential effects for food safety. Foods 13:1235.

Castriota L, Falautano M, Perzia P, 2024. When nature requires a resource to be used—the case of callinectes sapidus: distribution, aggregation patterns, and spatial structure in northwest Europe, the Mediterranean Sea, and adjacent waters. Biology 13:279.

Chiesa LM, Nobile M, Malandra R, Panseri S, Arioli F, 2018. Occurrence of antibiotics in mussels and clams from various FAO areas. Food Chem 240:16-23.

Çoğun HY, Firat Ö, Aytekin T, Firidin G, Firat Ö, Varkal H, Temiz Ö, Kargin F, 2017. Heavy metals in the blue crab (Callinectes sapidus) in mersin bay, Turkey. Bull Environ Contam Toxicol 98:824-9.

Commission of the European Communities, 2002. Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (Text with EEA relevance) (notified under document number C(2002) 3044). In: Official Journal, L 221, 17/08/2002.

European Commission, 2010. Regulation of the European Commission of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, 37/2010/EU. In: Official Journal, L 15/1, 20/01/2010.

European Commission, 2019. Regulation of the European Commission of 7 November 2019 on reference points for action for non-allowed pharmacologically active substances present in food of animal origin and repealing Decision 2005/34/EC, 1871/2019/EU. In: Official Journal, L 289/41, 08/11/2019.

European Commission, 2023. Regulation of the European Commission of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006, 915/2023/EU. In: Official Journal, L 119/103, 05/05/2023.

Fakhri Y, Hoseinvandtabar S, Heidarinejad Z, Borzoei M, Bagheri M, Dehbandi R, Thai VN, Mousavi Khaneghah A, 2021. The concentration of potentially hazardous elements (PHEs) in the muscle of blue crabs (Callinectes sapidus) and associated health risk. Chemosphere 279:130431.

Fang L, Huang Z, Fan L, Hu G, Qiu L, Song C, Chen J, 2021. Health risks associated with sulfonamide and quinolone residues in cultured Chinese mitten crab (Eriocheir sinensis) in China. Mar Pollut Bull 165:112184.

Genç TO, Yilmaz F, 2017. Metal accumulations in water, sediment, crab (Callinectes sapidus) and two fish species (Mugil cephalus and Anguilla anguilla) from the Köyceğiz Lagoon system–Turkey: an index analysis approach. Bull Environ Contam Toxicol 99:173-81.

Gil-Fernández A, Rodilla M, Prado P, Falco S, 2024. Early life stages of the invasive Atlantic blue crab Callinectes sapidus in the Western Mediterranean Sea. Estuar Coast Shelf Sci 296:108593.

Gilstrap O, Liu C, Nindo C, Parveen S, 2023. Pilot scale assessment of high-pressure processing (HPP) to enhance microbiological quality and shelf life of fresh ready-to-eat (RTE) blue crab meat. Microorganisms 11:2909.

Givens CE, Burnett KG, Burnett LE, Hollibaugh JT, 2013. Microbial communities of the carapace, gut, and hemolymph of the Atlantic blue crab, Callinectes sapidus. Mar Biol 160:2841-51.

Huang Z, Liu C, Zhao X, Dong J, Zheng B, 2020. Risk assessment of heavy metals in the surface sediment at the drinking water source of the Xiangjiang River in South China. Environ Sci

Eur 32:23.
Joseph S, Zou A, Zhao L, Batoon P, Gan CS, 2020. Quantitative screening of multiresidue veterinary drugs in seafood using the agilent 6470 triple quadrupole LC/MS. Available from: https://www.agilent.com/cs/library/applications/application-veterinary-drug-seafood-dmrm-lcmsms-5994-2832en-agilent.pdf.
Kara MH, Chaoui L, 2021. Strong invasion of Mellah lagoon (south-western Mediterranean) by the American blue crab Callinectes sapidus Rathbun, 1896. Mar Pollut Bull 164:112089.
Kraemer SA, Ramachandran A, Perron GG, 2019. Antibiotic pollution in the environment: from microbial ecology to public policy. Microorganisms 7:180.
Mancinelli G, Chainho P, Cilenti L, Falco S, Kapiris K, Katselis G, Ribeiro F, 2017. On the Atlantic blue crab (Callinectes sapidus Rathbun 1896) in southern European coastal waters: time to turn a threat into a resource? Fish Res 194:1-8.
Marchessaux G, Bosch-Belmar M, Cilenti L, Lago N, Mangano MC, Marsiglia N, Sarà G, 2022. The invasive blue crab Callinectes sapidus thermal response: Predicting metabolic suitability maps under future warming Mediterranean scenarios. Front Mar Sci 9:1055404.
Marchessaux G, Gjoni V, Sarà G, 2023. Environmental drivers of size-based population structure, sexual maturity and fecundity: a study of the invasive blue crab Callinectes sapidus (Rathbun, 1896) in the Mediterranean Sea. PLoS One 18:e0289611.
Nardelli L, Fucilli V, Pinto H, Elston JN, Carignani A, Petrontino A, Bozzo F, Frem M, 2024. Socio-economic impacts of the recent bio-invasion of Callinectus sapidus on small-scale artisanal fishing in southern Italy and Portugal. Front Mar Sci 11:1466132.
Nehring S, 2011. Invasion history and success of the American blue crab Callinectes sapidus in European and adjacent waters. In: In the wrong place - alien marine crustaceans: distribution, biology and impacts. Springer Netherlands, Dordrecht, pp 607-24.
Ortega-Jiménez E, Cuesta JA, Laiz I, González-Ortegón E, 2024. Diet of the invasive atlantic blue crab Callinectes sapidus Rathbun, 1896 (Decapoda, Portunidae) in the Guadalquivir estuary (Spain). Estuar Coast 47:1075-85.
Prado P, Ibáñez C, Chen L, Caiola N, 2022. Feeding habits and short-term mobility patterns of blue crab, Callinectes sapidus, across invaded habitats of the Ebro delta subjected to contrasting salinity. Estuar Coast 45:839-55.
Pyšek P, Hulme PE, Simberloff D, Bacher S, Blackburn TM, Carlton JT, Dawson W, Essl F, Foxcroft LC, Genovesi P, Jeschke JM, Kühn I, Liebhold AM, Mandrak NE, Meyerson LA, Pauchard A, Pergl J, Roy HE, Seebens H, van Kleunen M, Vilà M, Wingfield MJ, Richardson DM, 2020. Scientists' warning on invasive alien species. Biol Rev Camb Philos Soc 95:1511-34.
Reichmuth JM, Weis P, Weis JS, 2010. Bioaccumulation and depuration of metals in blue crabs (Callinectes sapidus Rathbun) from a contaminated and clean estuary. Environ Pollut 158:361-8.
Rodgers C, Parveen S, Chigbu P, Jacobs J, Rhodes M, Harter-Dennis J, 2014. Prevalence of Vibrio parahaemolyticus, and Vibrio vulnificus in blue crabs (Callinectes sapidus), seawater and sediments of the Maryland Coastal Bays. J Appl Microbiol 117:1198-209.
Rosas C, Chavez E-L, Ramirez FB, 1994. Feeding habits and food niche segregation of Callinectes Sapidus, C. Rathbunae, and C. Similis in a subtropical coastal lagoon of the gulf of Mexico. J Crustacean Biol 14:371-82.
Smalls J, Grim C, Parveen S, 2023. Assessments of Vibrio parahaemolyticus and Vibrio vulnificus levels and microbial community compositions in blue crabs (Callinectes sapidus) and seawater harvested from the Maryland Coastal Bays. Front Microbiol 14:1235070.
Streftaris N, Zenetos A, 2006. Alien marine species in the Mediterranean - the 100 'worst invasives' and their impact. Mediterr Mar Sci 7:87.
UNI EN, 2010. Foodstuffs - Determination of trace elements - Determination of arsenic, cadmium, mercury and lead in foodstuffs by inductively coupled plasma mass spectrometry (ICP-MS) after pressure digestion. UNI EN Norm 15763:2010. Italian Standards Body ed., Milan, Italy.