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Original Article

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Abstract

Seasonal changes of heavy metal concentrations in the crustacean *Callinectes sapidus* were determined by the aid of a Buck scientific Accusys 211 Atomic Absorption spectrophotometer in 2020. The heavy metals (copper, cobalt, chromium, iron, manganese, cadmium, lead, nickel, and zinc) were investigated in the tissues of the blue crab *Callinectes sapidus* collected from the Mediterranean Sea coast of Port Said, Egypt. Most estimated heavy metals in hepatopancreas, gill, and muscle tissues are below the permitted level, indicating that blue crab is safe to eat.

1. Introduction

The blue crab Callinectes sapidus is widely distributed along Port Said Mediterranean shore. It is high in proteins, minerals, vitamins and Omega-3 fatty acids. This edible crab is one of the most valuable sources of nutrition for humans (Celik et al., 2006).

The majority of Egypt's Mediterranean shoreline zones are at risk of significant pollution discharges caused by a variety of human activities (Dowidar, 1988).

Run-off and concrete areas, discharges from mining plants and municipal sewer systems, leaching from dumps and formal industrial sites, and atmospheric deposition are the main sources of heavy metal contamination (Singh and Steinnes, 1994; Singh et al., 2007). As a result, evaluating the level of metals in commercial species such as crabs is crucial in assessing the potential risk of blue crab eating to human health.

Crabs are principally susceptible to heavy metal pollution and other toxins because they live in bottom sediments, where contaminants can collect (Cengiz et al., 2011).

Heavy metals are regarded severe contaminants of the aquatic environment and aquatic species because of their toxicity, high persistence, non-biodegradability, and tendency to bio accumulate in organisms.

In recent years, aquatic macro invertebrates such as blue crabs have been used to assess metal bioaccumulation in contaminated areas (Cogun et al., 2017).

The presence of heavy metal residues in food has been related to immunosuppression, hypersensitivity to chemical agents, anemia, chronic renal disease, encephalopathy, cancer, reduced sperm count, and infertility, according to Rubin and Strayer (2008) and Rahman et al. (2014).

The study of heavy metal levels in aquatic species in order to determine whether the concentration is below legal limits and so would not harm consumers (Marti-Cid et al., 2008). The purpose of this study was to investigate the seasonal value of various heavy metals in edible and non-edible blue crab tissues, as well as their safety for human consumption.

2. Materials and Methods

2.1 Research location and specimens collection:

The study area was Port Said, a city in north-east Egypt, stretches over 30 kilometres (19 miles) along the Mediterranean Sea coast, north of the Suez Canal (N, 31° 17'E'31 °31). For the entire study, fifty-six live blue crab samples were taken seasonally from the Mediterranean Sea in Port Said city. The samples were transferred in plastic bags over a layer of ice to the Zoology Department, Factually of Science, Mansoura University.

2.2 Blue crab tissue collection

The crabs were dissected in order to separate and collect the hepatopancreas, muscles, and gills.

2.3 Heavy metal analysis

Hepatopancreas, muscles, and gills samples were digested by taking 0.5g of each sample and adding 4ml of concentrate H2SO4 and leaving it for 24 hours, then putting it on a hot plate with drops of HCLO4 until the digestion was complete and a liquid solution formed.

Before analysis, the digested samples were diluted to 50ml in volumetric flasks with distilled water.

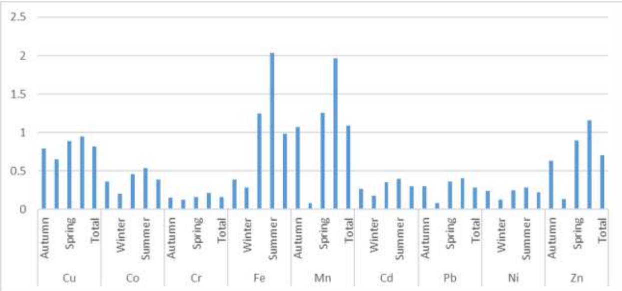

Figure 1 One –Way ANOVA for comparison in terms of seasons.

The heavy metal concentrations was determined using a Buck scientific Accusys 211 Atomic Absorption spectrophotometer from the United States in the Atomic Absorption lab of Mansoura University's Genetic Engineering and Biotechnology Unit.

2.4 Statistical analysis

The data is presented as a mean with standard deviation. One-way analysis of variance (ANOVA) was utilized for statistical analysis, followed by a Post Hoc test (Least Significant Difference, LSD) using SPSS version 20 statistical software. P≤0.05 was considered significant.

Table 3 One- way ANOVA analysis compare heavy metals according to their presence in blue crab tissues.

Metals	H	e	G	il	M	u	F	P value
	M	±	M	±	M	±		
Cu	1.155	.088	.848	.175	.451	.179	42.552	.000
Co	.451	.140	.396	.131	.322	.139	1.781	.193
Cr	.223	.047	.171	.049	.092	.023	20.611	.000
Fe	1.367	1.121	.969	.754	.629	.432	1.628	.220
Mn	1.605	1.441	.945	.592	.725	.417	1.935	.169
Cd	.336	.091	.300	.102	.300	.102	1.386	.272
Pb	.329	.144	.289	.134	.244	.129	.774	.474
Ni	.251	.056	.227	.062	.194	.076	1.561	.233
Zn	.975	.628	.621	.325	.516	.332	2.272	.128

Table 1. Mean levels of copper, cobalt, chromium, iron, and manganese (mean ± SD) in different tissues of *C. sapidus*

Metal / sex		Summer			Autumn			Winter			Spring		
		H	G	M	H	G	M	H	G	M	H	G	M
Cu	♂	1.28± 0.07	1.02± 0.02	0.68± 0.07	1.17± 0.02	0.97± 0.03	0.58± 0.01	1.16± 0.01	0.61± 0.08	0.30± 0.02	1.25± 0.02	0.97± 0.04	0.61± 0.09
Cu	♀	1.19± 0.02	0.99± 0.01	0.57± 0.13	1.04± 0.01	0.70± 0.03	0.29± 0.03	1.04± 0.01	0.62± 0.04	0.17± 0.01	1.11± 0.01	0.91± 0.06	0.45± 0.11
Co	♂	0.63± 0.04	0.54± 0.03	0.51± 0.01	0.45± 0.08	0.40± 0.06	0.32± 0.05	0.26± 0.04	0.21± 0.03	0.19± 0.01	0.54± 0.02	0.50± 0.03	0.38± 0.07
Co	♀	0.56± 0.03	0.51± 0.03	0.48± 0.05	0.43± 0.06	0.34± 0.06	0.24± 0.03	0.24± 0.01	0.21± 0.01	0.11± 0.01	0.52± 0.04	0.45± 0.04	0.37± 0.04
Cr	♂	0.32± 0.02	0.26± 0.01	0.12± 0.01	0.22± 0.01	0.18± 0.01	0.10± 0.01	0.20± 0.01	0.13± 0.02	0.08± 0.01	0.24± 0.03	0.18± 0.02	0.11± 0.01
Cr	♀	0.26± 0.01	0.22± 0.01	0.11± 0.01	0.19± 0.01	0.13± 0.01	0.09± 0.01	0.18± 0.01	0.12± 0.01	0.05± 0.01	0.19± 0.01	0.14± 0.01	0.09± 0.01
Fe	♂	3.19± 0.21	2.16± 0.13	1.19± 0.12	0.49± 0.04	0.44± 0.01	0.31± 0.01	0.44± 0.09	0.32± 0.02	0.20± 0.03	1.78± 0.53	1.14± 0.01	0.96± 0.04
Fe	♀	2.67± 0.51	1.96± 0.51	1.02± 0.01	0.45± 0.07	0.38± 0.03	0.30± 0.01	0.33± 0.09	0.27± 0.03	0.14± 0.01	1.58± 0.51	1.10± 0.01	0.92± 0.01
Mn	♂	4.73± 0.51	1.51± 0.03	1.51± 0.03	1.42± 0.07	1.16± 0.01	0.89± 0.04	0.12± 0.03	0.02± 0.01	0.06± 0.01	1.66± 0.06	1.37± 0.06	0.92± 0.04
Mn	♀	2.09± 0.01	1.28± 0.03	1.03± 0.01	1.26± 0.05	0.93± 0.05	0.76± 0.05	0.15± 0.01	0.03± 0.01	0.09± 0.01	1.41± 0.06	1.27± 0.05	0.91± 0.05

H=Hepatopancreas, G=Gills, M=Muscles.

Table 2. Mean concentrations of cadmium, lead, nickel, and zinc (mean ± SD) in different tissues of *C. sapidus*.

metal / sex		Summer			Autumn			Winter			Spring		
		H	G	M	H	G	M	H	G	M	H	G	M
Cd	♂	**0.44± 0.09**	**0.41± 0.01**	**0.40± 0.07**	**0.28± 0.04**	**0.26± 0.02**	**0.25± 0.01**	**0.27± 0.05**	**0.17± 0.04**	**0.15± 0.03**	**0.42± 0.04**	**0.39± 0.03**	**0.36± 0.05**
Cd	♀	**0.41± 0.04**	**0.40± 0.02**	**0.32± 0.02**	**0.27± 0.04**	**0.26± 0.01**	**0.25± 0.01**	**0.20± 0.01**	**0.15± 0.01**	**0.11± 0.01**	**0.40± 0.02**	**0.36± 0.06**	**0.21± 0.02**
P b	♂	**0.46± 0.06**	**0.40± 0.03**	**0.38± 0.04**	**0.35± 0.05**	**0.32± 0.03**	**0.30± 0.01**	**0.16± 0.01**	**0.07± 0.01**	**0.06± 0.01**	**0.42± 0.04**	**0.38± 0.04**	**0.31± 0.03**
P b	♀	**0.45± 0.08**	**0.39± 0.03**	**0.37± 0.03**	**0.32± 0.03**	**0.31± 0.01**	**0.22± 0.01**	**0.07± 0.01**	**0.06± 0.01**	**0.05± 0.01**	**0.41± 0.07**	**0.36± 0.09**	**0.30± 0.02**
Ni	♂	**0.35± 0.05**	**0.32± 0.02**	**0.26± 0.01**	**0.27± 0.01**	**0.25± 0.02**	**0.23± 0.03**	**0.17± 0.01**	**0.14± 0.01**	**0.08± 0.01**	**0.30± 0.02**	**0.29± 0.03**	**0.27± 0.01**
Ni	♀	**0.30± 0.02**	**0.28± 0.03**	**0.26± 0.01**	**0.26± 0.01**	**0.24± 0.01**	**0.20± 0.01**	**0.16± 0.01**	**0.12± 0.01**	**0.08± 0.01**	**0.27± 0.01**	**0.25± 0.03**	**0.21± 0.01**
Zn	♂	**2.17± 0.21**	**0.95± 0.05**	**0.84± 0.04**	**0.92± 0.03**	**0.60± 0.06**	**0.43± 0.07**	**0.19± 0.01**	**0.15± 0.01**	**0.07± 0.01**	**1.11± 0.01**	**0.84± 0.09**	**0.76± 0.10**
Zn	♀	**1.27± 0.07**	**0.88± 0.05**	**0.84± 0.04**	**0.87± 0.02**	**0.60± 0.06**	**0.39± 0.08**	**0.21± 0.01**	**0.12± 0.01**	**0.04± 0.01**	**1.06± 0.02**	**0.83± 0.05**	**0.76± 0.07**

H=Hepatopancreas, G=Gills, M=Muscle.

3. Results and Discussion

Heavy metal levels in the hepatopancreas, gill and muscle tissues of C. sapidus are provided in Tables 1, 2.

According to obtained results , the total mean concentrations of investigated heavy metal was significantly higher in males than females $p < 0.05$(Cu 0.880± 0. 319 ; Co 0.413 ± 0.146 ; Cr 0.177 ± 0.072; Fe 1.051 ± 0.917; Cd 0.317 ± 0.099; Pb 0.298 ±0.131; Ni 0.227±0.067; Zn 0.752± 0.564) versus in female (Cu 0.756±0.339; Co 0.369±0.143; Cr 0.147±0.06 ; Fe 0.926± 0.793; Cd 0.278± 0.101; Pb 0.276±0.143; Ni 0.221 ±0.068; Zn 0.656 ±0.387), except manganese, which its level is insignificant and higher in males than females $p > 0.05$ during the autumn, spring and summer seasons (total mean 1.251± 1.238 versus in female 0.933±0.609) but during winter females had higher manganese levels (male= 0.068, female= 0.091).

The highest Mn content was found in the hepatopancreas and muscle tissues, with mean values of 1.605 ppm and 0.725 ppm, respectively.

The largest concentration of iron was found in the gill tissues when compared to other tissues, with a concentration of 0.969ppm). The accumulation of the studied heavy metals in hepatopancreas tissue was in the following order: mn> Fe> Cu> Zn> Co> Cd> pb > Ni> Cr.

In gill tissue, the accumulation of the examined heavy metals was Fe> Mn> Cu> z n> Co> Cd> p b>

Ni> Cr, but in muscle tissue, it was M n> Fe> Zn> Cu> Co> Cd> p b> Ni> Cr.

The concentration of heavy metals in blue crab organs varies significantly ($p<0.001$) depending on the season.

The post hoc test (least significant difference) revealed a highly considerable variation($p< 0.001$) in the amounts of heavy metals among hepatopancreas, gill, and muscle tissues, as well as a highly significant difference between seasons, with the exception of chromium, which revealed an insignificant difference($p > 0.05$) between autumn and spring, while there were significant differences between the remaining seasons. In addition, The LSD revealed that there was no substantial difference between autumn and winter, but there were substantial variances between the other seasons. There was no substantial difference in LSD manganese between autumn and spring, but there were substantial differences between the other seasons.

Saber *et al*. (2017) investigated the organochlorine pollutants and heavy metals contamination in Egyptian shellfish

, finding concentrations of cd in crab from Ismailia, Damietta, and Alexandria (0.21, 0.37, 0.36, respectively), with the total mean cd concentration in this study (0.32) being higher than the crab from Ismailia and insignificantly lower than the crabs from Damietta and Alexandria. The Pb concentration in crabs from Ismailia, Damietta, and Alexandria (0.84, 1.49, 1.24, respectively) was significantly lower than the Pb values in crabs from Ismailia, Damietta, and Alexandria .The current study's pb total mean(0.3) was much lesser than Pb values found in crabs from Ismailia, Damietta, and Alexandria.

The total mean of cd (0.32) and p b (0.30) concentrations in this study were greater and lower than those found in crabs taken from fish markets in Kalyobia governorates, Egypt (0.12, 0.40, respectively) (Helmy *et al*., 2018).

Mutlu *et al*., (2017) found that, concentrations of metals in tissues of blue crabs, from Mediterranean wetlands were found to be (Cd ;0.08, Cr ;0.13, Cu;11.7, Fe ;38.2, M n ;2.98, Ni; 0.45, Zn ;20.1) were higher than those in the present study except Cd, Cr which were lower than present study.

Although the non-important metals (cd, pb) have no metabolic role in crustaceans, the elevated concentration of Zn and Cu in the hepatopancreas compared to the gills may represent the elements' vital role as a key component of several coenzymes involved in reproduction.

In crustaceans, the normal permissible values for cd and p b are 3 and 1.5, respectively (USFDA 2003). The heavy metal concentrations in *C. sapidus* in this study were lower than FAO and USFDA standards (2003) and Gutierrez-Pena *et al.* (2018).

The high quantities of metals in *C. sapidus'* hepatopancreas may be due to the creation of low-molecular-weight metal-binding (metallotionine-like) proteins, which have been found in a variety of crab species (Chouvelon *et al*., 2019; Yuzereroglu *et al*., 2010).

The highest concentrations of heavy metals were observed in summer and the lowest concentrations were observed in winter in the present study, which could be related with natural difference of heavy metals in the aquatic environment. These findings are in agreement with those of Turkmen *et al*. (2006) and Cogun *et al*., 2017. Copper, cadmium, zinc, and lead concentrations were found to be highest in the summer and lowest in the winter in a study of seasonal concentrations.

The amounts of heavy metals in *C. sapidus* from the Mediterranean Sea, Egypt, were studied in this study. The goal of this work was to determine the accumulation of Cu, Co, Cr, Fe, Mn, Cd, Pb, Ni, and Zn in *C. sapidus* tissues and their relationship to seasonality. The current study found that blue crab tissues have different abilities to accumulate metals from the environment, that males had higher metal concentrations than females, that higher contamination occurred in the summer and lowest contamination occurred in the winter, and that heavy metal concentrations were higher in the hepatopancreas > gill> muscle. The levels of heavy metals in blue crab muscles in this investigation were lower than the FAO and USFDA (2003) permitted limits, therefore As a result, we may conclude that these metals pose no risk to blue crab muscles when consumed.

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