Isagoge, v. 1, n. 1, p. 131-151, maio, 2021, ISSN 2763-7123

Rio de Janeiro, Brasil

ASSESSMENT OF TRACE ELEMENTS UPTAKE ON THE INVASIVE CRAB *PORTUNUS SEGNIS* HEPATOPANCREAS USING A MULTIVARIATE BIOCHEMICAL APPROACH

AVALIAÇÃO DA CAPTAÇÃO DE OLIGOELEMENTOS NO CARANGUEJO INVASIVO *PORTUNUS SEGNIS* HEPATOPANCREAS USANDO UMA ABORDAGEM BIOQUÍMICA MULTIVARIADA

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Received: 01/05/21 Accepted: 15/05/21 Published: 19/05/21

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Abstract: In the current investigation, we evaluated the biological consequences of trace elements contamination in the two Tunisian gulfs (Gabes gulf and Tunis gulf) on the blue swimming crabs hepatopancreas (*Portunus segnis*). The concentrations of three trace elements (cadmium, copper, and lead) in the hepatopancreas *P.segnis* were evaluated. Additionally, acetylcholinesterase (AChE), metallothioneins (MTs), hydroxide peroxidase (H_2O_2) and advanced oxidation protein products (AOPP) levels, were chosen as measurements to evaluate the environmental effects on the two crabs' populations from different gulfs. Macromolecular (lipids, proteins, and DNA) were also determined in *P.segnis* hepatopancreas. The results of trace elements bioaccumulation in soft *P. segnis* hepatopancreas showed a high pollution in the Gabes gulf as evidence by significant accumulation of cadmium, cooper, and lead. These findings were confirmed by significant increases of metal pollution index (MPI) and metallothioneins (MTs) levels in the hepatopancreas of *P. segnis* from Gabes gulf than these from Tunis gulf. Consequently, the trace elements accumulation in *P.segnis* from Gabes gulf conduct to the generation of lipid peroxidation processes as documented by the high levels of $H_2O_2$and LOOH. A significant decrease of AChE activity was recorded in crabs collected from Gabes gulf as compared to these from Tunis gulf. The present study revealed depletion of proteins and lipids contents, while DNA showed significant degradation on crab hepatopancreas collected from Gabes gulf comparing to Tunis gulf. These evidences must be taken in consideration when using *P. segnis* as an ecological indicator species in the biomonitoring programs.

Keywords: Blue swimming crab. Trace elements. Tunisian gulfs. Macromolecular degradation. Lipid peroxidation. Protein oxidation.

Resumo: Na investigação atual, avaliamos as consequências biológicas da contaminação por oligoelementos nos dois golfos tunisinos (Golfo de Gabes e Golfo de Tunísia) sobre os caranguejos nados azuis hepatopâncreas (Portunus segnis). Foram avaliadas as concentrações de três oligoelementos (cádmio, cobre e chumbo) no hepatopâncreas P.segnis. Além disso, os níveis de acetilcolinesterase (AChE), metalotioninas (MTs), peroxidase hidróxida (H2O2) e produtos de proteína de oxidação avançada (AOPP) foram escolhidos como medidas para avaliar os efeitos ambientais nas populações dos dois caranguejos de diferentes golfos. Macromoleculares (lipídios, proteínas e DNA) também foram determinados em P.segnis hepatopâncreas. Os resultados da bioacumulação de elementos vestigiais em P. segnis hepatopancreas mole mostraram uma alta poluição no Golfo de Gabes como evidência pelo acúmulo significativo de cádmio, cobre e chumbo. Estes resultados foram confirmados por aumentos significativos do índice de poluição de metais (MPI) e dos níveis de metalotioninas (MTs) no hepatopâncreas de P. segnis do Golfo de Gabes do que estes do Golfo de Tunísia. Consequentemente, o acúmulo de elementos vestigiais em P.segnis do Golfo de Gabes conduz à geração de processos de peroxidação lipídica, como documentado pelos altos níveis de H2O2e LOOH. Uma diminuição significativa da atividade de AChE foi registrada em caranguejos coletados do Golfo de Gabes em comparação com estes do Golfo de Tunísia. O presente estudo revelou esgotamento de proteínas e conteúdo de lipídios, enquanto o DNA mostrou degradação significativa no hepatopâncreas de caranguejo coletado do Golfo de Gabes em comparação com o Golfo de Tunísia. Estas evidências devem ser levadas em consideração ao utilizar P. segnis como uma espécie indicadora ecológica nos programas de biomonitoramento.

Palavras-chave: Caranguejo nado azul. Oligoelementos. Golfos da Tunísia. Degradação macromolecular. Peroxidação lipídica. Oxidação de proteínas.

INTRODUCTION

Globally, population growth and the ever-increasing use of coastlines linked to the expansion of human activities exert pressure on the aquatic environments and their inhabiting species (Di Salvator et al., 2013; Barhoumi et al., 2014). The coastal areas were generally considered to be natural reservoirs of land and anthropogenic pollution (Bejaoui et al., 2017). Large amounts of toxic pollutants are released directly and/or indirectly into aquatic ecosystems through natural processes such as winds, runoff, wadis, and rivers (Suaria et al., 2020). Today, more than 41% of maritime areas are destabilized, especially the oceans, which are seriously affected not only by human activities but also by issues related to climate change (Revéret and Dancette, 2010). The Mediterranean, a semi-enclosed sea that contains several marine and coastal ecosystems, presents an important but fragile biological diversity (Poitou 2003). This ecosystem is subjected to a mixture of pressures linked to anthropogenic discharges and climate change, resulting from excessive tourist congregation and an intensely dynamic coastal community (Jebali et al., 2007). The Mediterranean is the most plastic-polluted sea in the world with more than 200,000 tons of waste thrown away each year (Lambert et al., 2020). All these factors disrupt the biodiversity, integrity, and totality of marine systems (Fabres et al., 2012). Tunisia coastal area is a country that opens onto the Mediterranean Sea suffers from intense of industrial activities and heavy power agricultures in the level of coastal areas in contact with the sea (Chalaghmi et al., 2010). Additionally, these coastal are particularly vulnerable to the accumulation of different types of contaminants resulting from progressive technological and economic development, as they are characterized by a low renewal rate (Chalaghmi et al., 2010).

Numerous studies have shown the deleterious effects of climate change on biodiversity with effects on the phenology and distribution of species, on interactions between species, or the functioning of the ecosystem (Ackerly et al., 2010; Asch et al., 2019). Furthermore, it should be noted that invasive species represent an ecological problem because they lead to a modification of the ecological processes of ecosystems, a modification of the structure of communities and the disappearance of native species (Arriaga et al., 2004; Castro et al., 2017). In line with this, the expansion of species outside their range of origin is a phenomenon inherent in the dynamics of terrestrial and aquatic biodiversity (Dogra et al., 2010). During the last three centuries, this phenomenon has gained particular momentum as a result of the propagation of the seas dominated by humans (Kueffer and Kaiser-Bunbury ,2014). Given the adaptability to very diverse climatic conditions and a very large geographical distribution, these invasive species are therefore likely to adapt more easily to climatic changes than native species. Their adaptability is notably due to certain characteristics that allow them to adapt quickly, such as rapid sexual maturity (Padilla and Williams, 2004).

The presence of these species such as the blue crab (Portunus segnis) in large quantities in our ecosystems encourages us to evaluate them, especially since the phenomenon of invasion has significant repercussions on endemic populations, socio-economy, health as well as public preference. The present work, therefore, aimed to develop a multivariate approach for the evaluation of P.segnis redox status collected from two different Tunisian coastline areas (Tunis gulf and Gabes gulf). The current study was conducted on hepatopancreas metabolisms induced by trace elements accumulation. Therefore, this work pointed to examine the stress responses through the analysis of metallothioneins (MTs), hydrogen peroxide (H_2O_2), advanced oxidation protein products (AOPP) levels and acetylcholinesterase (AChE) activity at the level of P. segnis hepatopancreas as a detoxification organ. In an effort to better understand the hepatopancreas damage between the two sampled sites, DNA degradation was also investigated.

METHODOLOGY

In this study, Crabs species were collected from two different gulfs in the Tunisian coast (Tunis gulf and Gabes gulf) in April 2020. Animals were transferred alive on ice directly to the laboratory and those with similar length and weight were selected (14.5 ± 0.31 cm and 140.12 ± 0.21g, respectively). The water parameters were measured in situ three times during the sampling process using a thermometer, salinometer and pH meter (model WTW LF.325). Upon arrival in the laboratory, all crabs were sacrificed and dissected on ice to obtain the hepatopancreas. The samples hepatopancreas (n=6) were weighed and directly stored in liquid nitrogen followed by conservation at -80 °C for trace element analysis. Ten section of crabs hepatopancreas (n=10) were carefully homogenized with Tris-HCL buffer (20mM, pH 7.4) and centrifuged at10000 x g for 20 min at 4 °C. The obtained hepatopancreas supernatants were stored at -80 °C until biochemical determinations. Six others hepatopancreas fractions (n=6) were immediately homogenized in Trizol for DNA damage studies.

To measure the trace element, Freeze-dried hepatopencreas (1 g) were digested with 6 ml of nitric acid (HNO3) and 1 ml of hydrogen peroxide (H2O2) in Teflon reactors operated with a programmable microwave Touch Control Terminal 320 system (Milestone, type Ethos). After digestion, the solutions were cooled at room temperature and transferred and filled to the final volume of 50 mL for analysis. The concentrations of cadmium (Cd), copper (Cu), and lead (Pb), as well as the blanks and internal standards were assessed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Metal concentrations were presented in µg/ mg of dry weight.

The Viarengo et al. (1985) method was used to measure metallothioneins (MTs) levels in hepatopancreas homogenates basing to the spectrophotometric method of using glutathione (GSH) as a standard. The level of MTs was recorded at 412 nm and the data expression was presented as nmol of GSH/mg of protein.

To measure proximate composition, Hepatopancreas protein contents were measured according to the method of Lowry et al. (1951) using bovine serum albumin. Data were expressed as mg/g of muscles tissue. Total lipids were evaluated basing to Frings et al. (1972) method using phosphor-vanillin reagent. The mixture reaction, containing hepatopancreas supernatants and phosphor vanillin, were detected at 540 nm. The standard samples were established using a good olive oil (Sigma, St. Louis, USA). Data were calculated basing to the reference and standard curve, and expressed as mg/g of hepatopancreas tissue.

The Ou and Wolf (1996) method was employed to determine hydrogen peroxide (H2O2) production in the crabs' hepatopancreas using the ferrous oxidation-xylenol orange test. The levels of H2O2were detected at 560 nm and data were calculated as nomoles/mg of protein. Advanced oxidation protein products (AOPP) were analyzed basing to the protocol of Kayali et al. (2006). The hepatopancreas supernatants were mixed with phosphate buffer (0.8 ml; 0.1 M; pH 7.4), potassium iodide (0.1 ml; 1.16 M) and acetic acid (0.2 ml).The AOPP reaction mixture was recorded at 340 nm and data were calculated using the extinction coefficient and expressed as nmoles/mg of protein.

To measure the activity of acetylcholinesterase (AChE) based to Ellman et al. (1961) method, acetylthiocholine iodide was used to the hepatopancreas supernatant as a substrate, in a concentration of 8.25 mM. The activity was measured spectrophotometrically at 412 nm and expressed as nmol of substrate/min/mg protein.

The method of Clarke and Melki (2002) was used for DNA analysis. Crabs hepatopancreas were extracted with cetyltrimethylammonium bromide buffer (CTAB) and centrifuged at 10000× g during15 min, at 4∘C. The purified DNA obtained undergoes migration on an agarose gel (1%) basing to a molecular weight size marker (3 kb DNA ladder). The DNA damage was analyzed through a wavelength at 260 nm according to Sambrook and Russell (2001). Data were expressed as µg/g of hepatopencreas tissue.

To examine the environmental deviation of trace element contents in P.segnis hepatopancreas (Usero et al. 1996), the metal pollution index (MPI) was calculated as follows: MPI = (Cf1 × Cf2...Cfn)1/n, Where Cf1 is the content of the first trace element in the hepatopancreas of P.segnis collected from a particular sampling gulf; Cfn is the content of the nth trace element; and n is the total number of measured trace elements.

All statistical analyses were tested using STATISTICA software. Thehomogeneity of variance and normality wereinitially tested using the Levene's test and Kolmogorov-Smirnov tests, respectively. Significant differences were evaluated through one-way ANOVA, followed by Tukey's post hoc test at $p< 0.05$. Additionally, principal component analysis (PCA) and correlation matrix of Spearman were evaluated by R software version 2.15.2 (R Core Team, 2017).

RESULTS

The results of Trace elements accumulations in *P.segnis* hepatopancreas are reported in Table 1. Our results showed a significant accumulation of Cd (+79 %; $p< 0.001$; one-way ANOVA), Pb (+31 %; $p< 0.01$; one-way ANOVA) and Cu (+25 %; $p< 0.05$; one-way ANOVA) in *P.segnis* hepatopancreas from Gabes gulf as compared to these from Tunis gulf. Similar results were observed for MPI, reveling that *P.segnis* from gabes gulf were characterized by the highest index (64%) when compared to these from Tunis gulf.

Table 1. Trace elements (µg/g of weight) concentrations and metal pollution index (MPI) in *P. segnis* hepatopencreas collected from two Tunisian areas

Sites	Cadmium	Lead	Cooper	MPI
Gulf of Tunis	0.970±0.057	0.284±0.028	0.438±0.057	0.493±0.032
Gulf of Gabes	1.714±0.142***	0.374±0.009**	0.551±0.033*	0.809±0.012***

The data are presented as mean ± SD.

The significant difference is detected at 0.05 :* p<0.05 ;**p<0.01 ;***p<0.001

The levels of MTs in P. segnis hepatopancreas are shown in Figure 1. We observed a significant difference between hepatopancreas crabs as evidence by a statistically enhancement of MTs levels by +16% in P. segnis from Gabes gulf as compared to these from Tunis gulf.

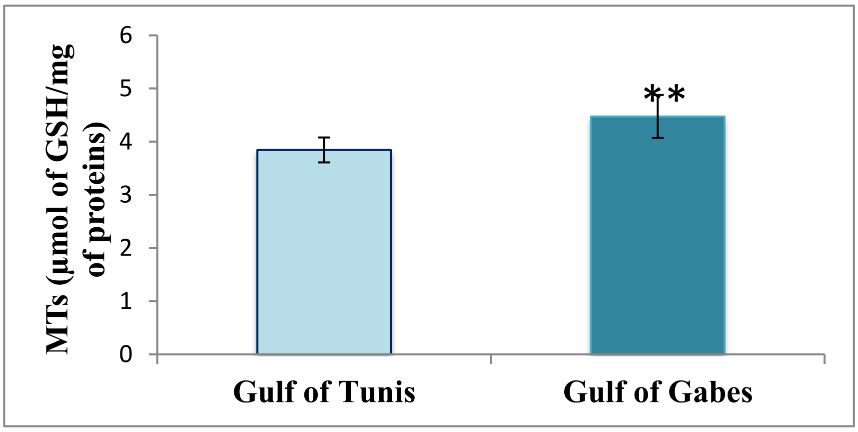

Figure 1. Metallothionein (MT) level in *P. segnis* hepatopancreas collected from Gabes gulf and Tunis gulf. Significant difference is presented at 5% as follows ***p*<0.01.

The hydrogen peroxide levels in P. segnis hepatopancreas collected from the two gulfs are presented in Table 2. The H2O2 levels exhibited a significant change between the two studied populations (p<0.001, one-way ANOVA) with the highest increase by +42% recorded for P.segnis collected from Gabes gulf.

Oxidative damage to protein (AOPP) was higher during the study period in P.segnis hepatopancreas from Gabes Gulf compared to those from Tunis Gulf as evidence by a significant enhancement of +101% (Table 2).

Table 2. Membrane alteration indices changes in *P. segnis* hepatopencreas collected from two Tunisian areas

Sites	H_2O_2 (nmol/mg of protein)	AOPP (nmol/mg of protein)
Gulf of Tunis	3.122±0.645	1.006±0.111
Gulf of Gabes	4.452±0.404***	2.030±0.532***

The data are presented as mean ± SD.

The significant difference is detected at 0.05:*** p<0.001.

H2O2 : hydrogen peroxyde ; AOPP : advanced oxidation proteins products.

Proximate compositions of P.segnis hepatopancreasare reported in Table 3. Our data showed changes in the contents of proteins and lipids between the studied populations (p<0.05, one-way ANOVA). P.segnis collected from Gabes gulf has the lowest contents of lipids (-17 %) and proteins (-10 %) as compared to these collected from Tunis gulf.

Table 3. Proximate composition (mg/g of protein) changes in *P. segnis* hepatopencreas collected from two Tunisian areas

Sites	Proteins	Lipids
Gulf of Tunis	2.793±0.390	38.405±4.559
Gulf of Gabes	2.368±0.399*	40.272±4.126*

The data are presented as mean ± SD.

The significant difference is detected at 0.05:* p<0.05.

As shown in Fig. 2.A, the levels of DNA damage was more accentuated in the hepatopancreas of P.segnis from Gabes gulf as compared to these from Tunis gulf (p<0.01, one-way ANOVA). These data were confirmed by the presence of a smear without tree, indicating accidental DNA degradation of crabs from Gabes gulf (Figure 2.B).

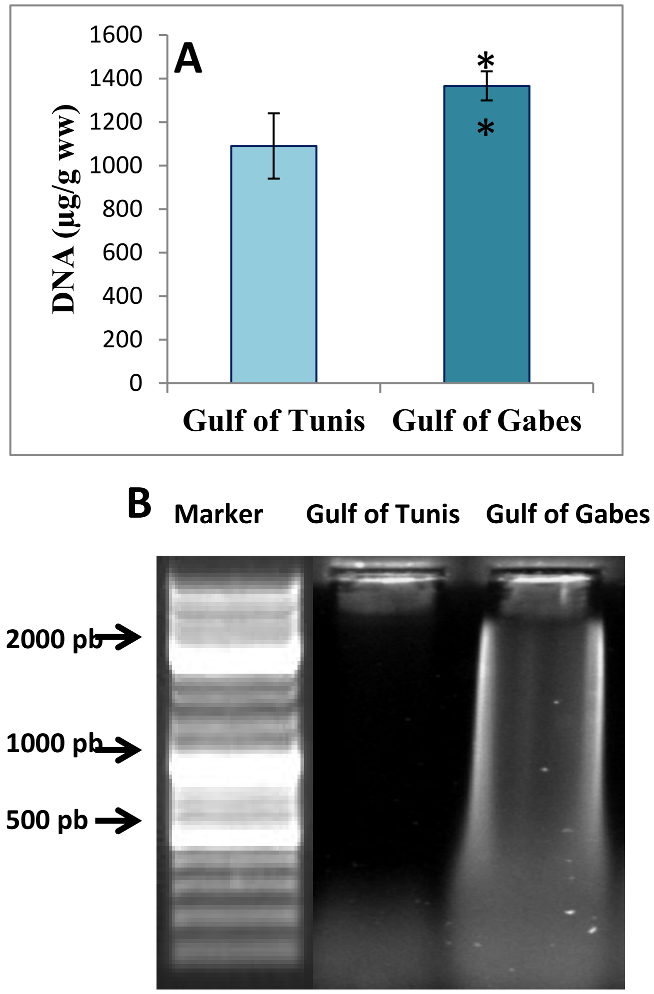

Figure 2. DNA degradation content (A) and structure (B) in *P. segnis* hepatopancreas collected from Gabes gulf and Tunis gulf. Significant difference is presented at 5% as follows ***p*<0.01.

Our results revealed, in P.segnis from the gulf of Tunis, a significant decrease in the hepatopancreas AChE activity by -78% compared to these from the Gabes gulf (Figure 3).

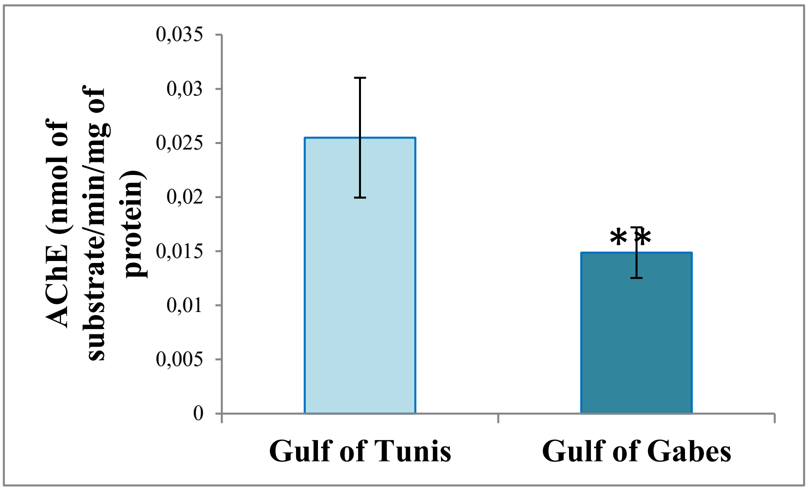

Figure 3. Acetylcholinesterase (AChE) activity in *P. segnis* hepatopancreas collected from Gabes gulf and Tunis gulf. Significant difference is presented at 5% as follows ***p*<0.01.

The results of the studied parameters were combined to perform PCA analysis as represented in Figure 4. This analysis allowed us to retain the two dimensional pattern explaining 84.51% of the total variance. The first axis exhibited the maximum dispersion of the initial cloud point (73.07% of the total dispersion), showing a positive correlation with lipids and negative one with AOPP, H2O2, MTs, Cd, Pb, Cu, MPI and DNA. Nevertheless, only proteins and AChE parameters have contribute positively with the second axe which describes 11.44% of the total dispersion. The PCA clustering depicted a clear separation between the studied populations, confirming the highest perturbation and degradation in P.segnis hepatopancreas from Gabes gulf. This separation was illustrated with an important affinity of trace elements contents, MPI, AOPP, H2O2, MTs, AChE and DNA damage levels in the hepatopancreas of P.segnis from Gabes gulf than these from Tunis gulf.

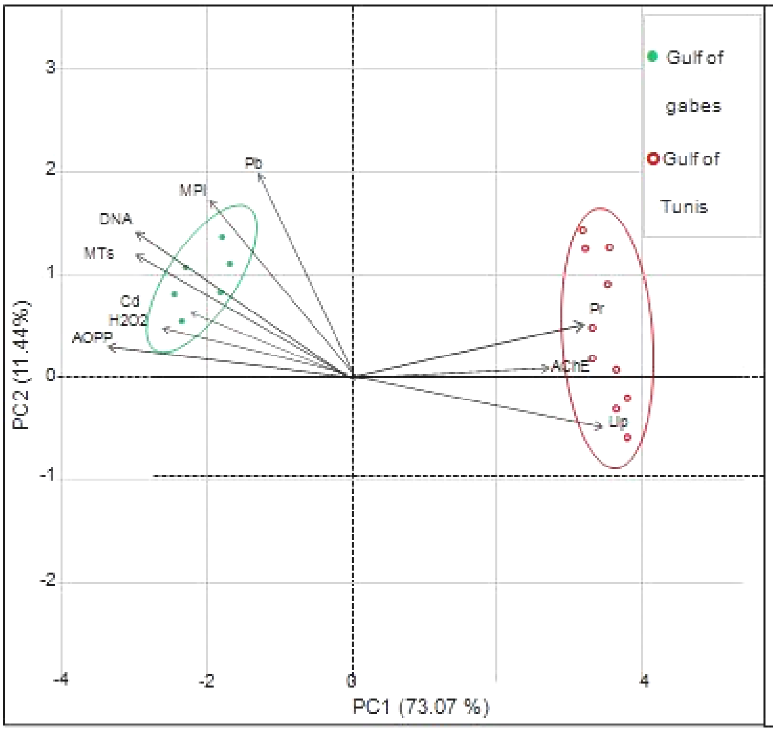

Figure 4. Principal component analysis (PCA) of trace elements, biomarkers and macromolecular on *Portunus segnis* hepatopancreas collected from Gabes gulf and Tunis gulf. Pb: lead; MPI: metal pollution index; MTs: metallothioneins; Cd: cadmium; H2O2: hydrogen peroxidase; AOPP: advanced oxidation protein products; Pr: protein; Lip: lipid; AChE: acetylcholineserase.

DISCUSSION

Aquatic ecosystems are regularly impacted by the continuous amplification of human activities that causing climate change facilitates biological invasions and ecosystem damage. According to the investigation conducted by Shaiek et al. (2018), crabs captured from the Tunisian coastal area were capable to accumulate essential trace elements more powerfully than non-essential ones. With the exception, the crabs from the Gabes gulf showed high levels of Cd, Pb and Cu analyzed in the hepatopancreas, which was proved by the MPI index showing a highly significant correlation with the high pollution of this gulf. In the present study, the choice of hepatopancreas is based on its accumulation power of the metals in comparison to the other organs (such as muscle and exoskeleton) as well as its importance as the most targeted organ of metals uptake (Yilmaz and Yilmaz, 2007; Hosseini et al., 2015). Consistently, the higher levels of metals in crabs collected from the Gabes gulf could disrupt the osmoregulation, gas exchange functions and causing changes at the cell's level. Investigations carried out on the accumulation of TE on Portunus segnis soft tissues in the Mediterranean Sea are limited at the northern part (Italian, and Turkish coastal) (Catalano et al., 2006; Olgunoglu and Olgunoglu, 2016). Only Annabi et al. (2018) have demonstrated that P.segnis muscles, gills, and exoskeleton from Gabes gulf were characterized by the highest amounts of TE.

Trace elements, in particular Cu, are transition metals and powerful inducers of the reactive oxygen species generation (ROS), through Fenton and Haber-Weiss reactions which transform hydrogen peroxide into an extremely toxic free hydroxyl radical (Martinez 1995). ROS are generated as a consequence of several conditions mostly by exposure to xenobiotic such as trace elements (Shanker, 2008). To cope with the adverse effects of ROS, crabs from Gabes gulf have involved defenses mechanism to regulate and eliminate the endogenous pollutants. Among them, metallothionein (MT) play a key role in processes of cellular protection from actions of dangerous agents (Mao et al., 2012). This non-enzymatic antioxidant defense acts in an integrated way to minimize oxidative damage at the molecular level (Hauser-Davis et al., 2020). Since MT induction could enhance its metals tolerance, these metal thiolate cluster proteins (6-7 kDa) have the ability to detoxify the excess accumulation of non-essential and essential trace elements (Yuvaraj et al., 2021). According to our data, we observed that the MT level in the P.segnis hepatopancreas from Gabes gulf were significantly higher than those coming from Tunis gulf. The results of this study provide evidence adaptations of examined crabs, especially those collected from the southern gulf, to deal with trace element accumulation. Similar results of Ghaeni et al. (2015) reported high contents of TE on P.segnis hepatopancreas than muscles. Also, Sarasiab and Hosseini (2014) investigated the contamination status of a wide range of pollutants polychlorinated biphenyl (PCB 101, PCB 153), Mercury (Hg) and methyl mercury (MMHg) through blue swimming crab and found that the concentrations of the analyzed pollutants were highest in hepatopancreas whereas lowest in the muscle and gills.

Cell organelles and cellular components (such as mitochondria, cell membrane, and enzymes) are known to be influenced by trace metals (Jebali et al., 2007; Di Salvatore et al., 2013). Metal ions have been shown to interact with DNA, membrane lipids, and nuclear proteins, thereby causing DNA damage, and cell cycle modulation (Tchounwou et al., 2012). It has also been recognized that metals interchange with membrane lipids and nuclear proteins which cause specific damage (Briffa et al., 2020). This damage can be direct through conformational changes in biomolecules, due to the metal attack. In this issue, our results showed a decrease of protein and lipid contents, observed in P.segnis collected from the Gabes gulf than those from the Tunis gulf. This hypothesis is reinforced by the significant negative correlations found between the concentration of TE and the related compositions. The damages of proximate compositions were also reported in aquatic organisms exposed to the surrounding TE, namely Venerupis decussata (Bejaoui et al., 2019), Catlacatla, Labeorohita and Cirrhinusmrigala (Hussain et al., 2018). On the other hand, the metal can cause indirect damage, which is the result of the ROS overproduction that includes hydroxyl, superoxide radicals, hydrogen peroxide and other oxidants endogenous (Bejaoui et al., 2017). This indirect damage of membrane lipid was elucidated in the present study by a significant increase of H2O2 levels, as the first lipid peroxidation marker, in P.segnis hepatopancreas collected form Gabes gulf. Likewise, the observed excess of AOPP levels in P.segnis collected from the Gabes gulf confirmed the harmful effects on protein function and thus the onset of hepatopancreatic dysfunction. These results are consistent with previous studies of Chetoui et al. (2019) and Hermenean et al. (2017) carried out on the harmful effect of metals on marine organisms. The generation of free radicals could also attack DNA bases; as a result, our data noted a consequent development of DNA smears strongly related to the generation of ROS. Such results were recently reported in several marine organisms under metals exposure (Chetoui et al., 2019).

Acetylcholinesterase (AChE) is an essential enzyme in nerve impulse transmission in aquatic organisms which terminates the diffusion of neural impulses by the rapid hydrolysis of acetylcholine (ACh) into the inactive products of choline and acetic acid (Barnard, 1974). In this regard, AChE inhibition results in a raise of ACh level that generates permanent and excessive stimulation of the neural system. This can lead to several diseases such as tetany, paralysis, and even death (Kirby et al., 2000). The results of the present study showed a depletion of AChE activity in P.segnis hepatopancreas collected from the Gabes gulf than theses from the Tunis gulf that might lead to the accumulation of acetylcholine, which could influence their inflammatory response. Our results argued that the inhibition of AChE activity could be associated with strong anthropogenic pressure from industry and agriculture in this area as previously reported in several investigations (Raaoui et al., 2013; El Zrelli et al., 2018). Additionally, it seems more possible that muscular AChE activity observed in the P.segnis from Gabes gulf could be attributed also to the integrated effect of several classes of contaminants such as carbamates, organophosphates, and organochlorine pesticide. AChE inhibition in Carcinus maenas gills and digestive gland was noted in Bizerte lagoon (Jebali et al. 2011). Also, Ben Khedher et al. (2017) demonstrated that AChE activities in digestive gland, muscle and eyes were significantly lower when crabs were exposed to environmental contaminants.

Taken all these data together, crabs are frequently exposed to many different stressors at the same time, such as adverse environmental conditions and pollutants, and their combination can disrupt the physiological balance of the exposed organisms. Given the scarcity of work, multidisciplinary approaches encompassing the fields of ecotoxicology, ecology and biology of crabs could help to fill the gaps in existing knowledge.

CONCLUSION

The present survey provided basic information on the accumulation of metals and their effects on the hepatopancreas of Portunus segnis in Tunisian waters. For this, an effort was made to present the results in a global context and to elucidate principal component analyzes grouping the parameters were developed. It is evident that the crabs collected from the southern region (Gabes gulf) accumulated more trace elements compared to those from the northern region (Tunis gulf). Our work demonstrated disturbances at the level of macromolecules translated by significant degradations of lipids, proteins and DNA and significant increases of hydrogen peroxyde (H_2O_2) and advanced products of protein oxidation (AOPP). Our study showed that the trace elements accumulated in the hepatopancreas caused inhibition of AChE activity in P.segnis from Gabes gulf. Specifically, given the growing interest in P.segnis as a product of high commercial value, a comprehensive assessment of the soft and edible tissues of crab can support its commercialization and, in turn, donate to its control as an invasive species. Additionally, by placing more importance on P. segnis behavior in polluted environments, further examinations are needed on a larger number of parameters and on a multi-seasonal timescale.

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ACKNOWLEDGMENTS

This work is part of a national project of Dr Safa BEJAOUI with the collaboration of socio-economic partners.

I would like to thank all the co-authors for their contribution to this project and for their invaluable technical support. Special thanks should be addressed to Prof. SOUDAI Nejla and Prof. EL CAFSI Mhamed my research project managers for their professional advice, their invaluable support and for their useful and constructive recommendations on this project.