HEAVY METAL BIOACCUMULATION AND GENOTOXICITY IN FISH (*MERLUCCIUS MERLUCCIUS*, LINNAEUS, 1758) FROM THE WESTERN ALGERIAN MEDITERRANEAN COAST

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Abstract. Heavy metals contamination in the coastal ecosystem is a source of major concern due to their toxic nature, persistence, and accumulative behaviours. The present study reports the seasonal variability of the biological responses of European hake (*Merluccius merluccius*, L.1758) collected from the Bay of Oran on the Algerian coast. Besides the condition indexes (gonadosomatic index GSI and Fulton's condition factor K) and the analysis of metallic trace elements: zinc (Zn), lead (Pb) and cadmium (Cd) in tissues, the micronucleus test was performed in erythrocyte and gill cells to assess the genotoxicity status of the fish. The results revealed a significant correlation between the two fish condition indexes, in link with their reproduction cycle. Highlighting the fact that the study site is under intense stress from toxic contaminants, a significant increase (p value < 0.05) in the frequency of micronuclei occurrence, especially, in the summer period is correlated with significantly (p value < 0.05) high levels of Zn, Pb and Cd. Thus, the test of micronucleus and pollution indicators may serve as a valuable tool in biomonitoring studies of the marine environment since they allowed to reveal genotoxic effects of the investigated fish populations and, consequently, the quality of their biotope.
Keywords: *micronucleus test, heavy metals, biomonitoring, Oran Bay, Merluccius merluccius*

Introduction

The Mediterranean Sea is under a great toxicological threat due to its unique hydrographic features and high anthropological activity pressure (Ayas et al., 2018). The agricultural and industrial development of human societies has led to introduced thousands of substances and/or materials in the marine environment that once above certain threshold values might present negative effects on biological components of these ecosystems and, therefore, become pollutants (Beiras, 2018; Cabral et al., 2019). The Algerian coast receives significant inputs of metallic pollutants. Indeed, several studies have highlighted the fact that the Algerian coast is subject to significant anthropogenic pressure (Belhoucine et al., 2014; Boucetta et al., 2016; Bouhadiha et al., 2017; Guendouzi et al., 2017; Rouane-Hacene et al., 2018; Rouabhi et al., 2020).

Aquatic organisms are the most affected by the dissolved toxic metals such as cadmium (Cd), lead (Pb) and zinc (Zn), concentrated in sediments and water (Rodrigue et al., 2016; Kumar et al., 2018). Various studies have shown that xenobiotics cause disturbances in the reproductive system, changes in behavior, disturbances in energy metabolism and the appearance of mutagenic or carcinogenic effects in animals (Meyer,

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2003; D'Costa et al., 2018). Metal-induced genotoxicity is predominantly due to the inhibition of the DNA repair process (Hartwig and Schwerdtle, 2002).

Fortunately, advances in the ecotoxicology field have supplied, for a given organism, a number of biomarkers which assess the exposure and adverse effect of stressors on organisms (Lu et al., 2009; Ramsdorf et al., 2012). A pollution biomarker is defined as an alteration in a biological response occurring at the molecular, cellular or physiological level that can be related to exposure to environmental chemicals and their toxic effects (Lionetto et al., 2019). The biological response of an organism to a stressor following uptake may induce changes at the cellular biochemical levels, resulting in alteration of the cell structure and function, tissues and behavior of the organism (Lam and Gray, 2003).

On the other hand, it is emphasized here that the multi-marker approach may recognize the sensitivity of various biological matrix to a given contaminant as well as the action mechanism behind that change (Iftikhar et al., 2022). Therefore, multi-biomarker approaches are increasingly recommended to reflect the main physiological states of an organism (Araújoa et al., 2018; Catteau et al., 2021).

In order to assess the quality of continental hydrosystem, Teleost fish constitute models of interest (Cavas and Ergene-Gozukara, 2005; Tollefsen et al., 2006; Boettcher et al., 2010; Bücker et al., 2012; Deutschmann et al., 2016; Cant et al., 2022) because of their potential to accumulate metals (Prabakaran et al., 2017). Elements such as Cu, Fe, Co and Zn are essential for fish growth and metabolic activities, however, metals such as Cd, As, Hg and Pb are non-essential and toxic even at low levels of concentration to humans and all biotic life (Altindag and Yigit, 2005; Rejomon et al., 2010; El-Moselhy et al., 2014). If these latter come in contact with fish, different reactions are initiated among chemical and biological systems in the body, that ultimately result into biochemical disturbances. Hence, it is necessary to determine the contaminant action mechanism and potential means to mitigate their impacts (Bonomo et al., 2021).

Since fish respond to toxicants in a similar way to mammalians (Kligerman, 1982), they can be used to screen for chemicals that are potentially dangerous on humans. Moreover, they indicate the risk of human exposure through the aquatic food chain (Hallare et al., 2016; Igbo et al., 2018). Due to their low saturated fat, omega fatty acids, and high protein content, fish consumption has been increased in humans' diet in the last several decades (Copat et al., 2013). However, consumption of contaminated fish and related health risks become crucial all over the world. The consumption of prolonged contaminated fishes by the trace metals results in several adverse effects such as liver damage, food poisoning, cardiovascular diseases and even death (Rejomon et al., 2010; Hosseini et al., 2015).

Indeed, suggested as a sentinel species in many biomonitoring programs (UNEP/RAMOGE, 1999), European hake (*Merluccius merluccius*, L. 1758) represents a good sampling candidate due to its bottom feeding niche, availability in Algerian waters all year round and its consumption by a majority of people living along the coast.

Therefore, the use of multi-marker approach may provide assessing the state of health of *M. merluccius* by merely measuring two biological markers recommended by UNEP/RAMOGE (1999), namely condition indexes (gonadosomatic index GSI and Fulton's condition factor K) and the micronucleus (MN test) identify genotoxic stress.

Condition indexes are the most revealing for a better understanding of the pollution effects on fish. Fulton's condition factors indicate the level of wellness of the fish in their natural environment (Hasan et al., 2022). It is an indirect measure of the nutritional

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status of the fish energy reserves, reflecting recent food availability conditions since it is sensible to stress in natural environment (Sutton et al., 2000; Barrilli et al., 2015). GSI is used to describe the timing and duration of the breeding season (Gentile et al., 2022). Several studies have successfully used GSI values to improve accuracy in determining the maturity stage (Sakamoto et al., 2003; Sadekarpawar and Parikh, 2013).

The MN Test aims to identify cytogenetic damage caused by genotoxic pollutants, such as micronucleus (MN) formation containing acentric chromosome fragments (i.e., without centromere) or whole chromosomes. This test can be conducted by *in vivo* or *in vitro* approaches (Sommer et al., 2020). The MN frequency evaluation provides an assessment index of a serious and near-irreversible genotoxic alteration (Okunola and Bakar, 2011; Mondal et al., 2011; Hussain et al., 2018).

The MN test has been widely used in marine fish due to its simplicity, low cost, high sensitivity, accurate data, and applicability for studies under laboratory and field conditions (Canedo et al., 2021; dos Santos Silva et al., 2021). This original study which rests on bentho-demersal fish species pursues three main objectives: (1) to investigate the seasonal variations of the trace metals concentration's in tissues of the fish and compare their hazardous levels from the maximum permissible dose. (2) to determine the incidence of MN and the integrity of lysosomal membrane in erythrocyte cells of fish samples caught from Oran Bay throughout the seasons. (3) to assessed the relationship between the responses of the two selected biomarkers and pollutants from the tissues of fish during various sampling seasons.

Materials and methods

Study area

The sampling took place in the Bay of Oran (N 35°43', W 00° 38') (*Fig. 1*) located on the the West coast of Algeria and in the South West of the Mediterranean, it belongs to the coastal chain of Tel Septentrional (Djebel Murdjadjo and Djebel Khar) (Leclaire, 1972). The Oran coast is severely affected by the nuisances of the civilized world: industrial activities, intensive tourism and massive urbanization with an ever-increasing level of domestic pollution (Kerfouf et al., 2010).

Figure 1. *Map showing sampling location: Oran Harbour (Oran Bay, Algerian west coast)*

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Recent years, several researches have been performed about impairment of ecosystem in west coast of Algeria for both biological aspects and change in content of the pollutants such as heavy metals and pesticides in the tissues of several marine organisms, especially those of bivalve molluscs (Taleb et al., 2007; Rouane-Hacene et al., 2013; Benali et al., 2015; Gherras et al., 2016; Kaddour et al., 2021; Tabeche et al., 2021).

Fish collection

European hake (*M. Merluccius*), selected as the sentinel organism, is a species widely present and exploited both in the Atlantic and Mediterranean (Aldebert and Carriès, 1988; Martin, 1991; Oliver and Massutí, 1995). Seasonal sampling was carried out along the Oran Bay by professional fishermen. During the study period (October 2019 to July 2020), the collection of the biological material is carried out on 120 individuals of length ranging between 33.5 and 43.7 cm. Blood was immediately drawn from the caudal vein, smeared on clean glass slides and stored in slide boxes. Excess blood was stored in microfuge tubes containing phosphate buffered saline (pH 7.4) at 4 °C. The fish were then stored in clean labelled polythene bags and transported in ice bags to the laboratory for further analyses of the metal content in their tissues.

Condition indexes

At the laboratory, specimens captured ranged on the same length class were analyzed. For each season and each individual, total weight (±0.001 g) and length as well as gonad and liver weights were recorded. Following the work of Lloret and Planes (2003), the two indexes, presented previously for describing the overall physiological condition of the fish, were calculated by means of the following equations:

$$GSI = 100\frac{W_{gonad}}{W_{fish}} \quad \text{(Eq.1)}$$

$$K = 100\frac{W_{fish}}{L^3} \quad \text{(Eq.2)}$$

where *W* (g) and *L* (cm) are the weight and the total length of the fish respectively.

Micronucleus test

The analyses of micronuleus (MN) were performed, each season, with the erythrocytes and gills cells of *M. merluccius* according to the methodology of Al Sabti (1986b).

Sample treatment

Peripheral blood samples were obtained from the caudal vein of the specimens with heparinized 2.5 ml sterile plastic syringes and needles from 0.45 mm × 16 mm (27G × 5/8 inches) to 0.8 mm × 40 mm (21G × 1.5 inches). Thin smears of blood were made on clean slides (3 per fish) by the slide drawn method and air-dried overnight; then their fixation in methanol for 10 min followed. After allowing the slides to air-dry, the smears were stained in 10% Giemsa solution for 25 min and subsequently washed with distilled water and air-dried at room temperature.

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For the micronuclei test, Fish gills dissected by scissor were fixed in acetic acid. The samples were centrifuged at ×2000 rpm for 10 min in order to obtain epithelial cells shed from gill tissue by using acetic acid and pipette. The pellet obtained by this procedure is smeared on the slide and fixed by methanol. Then, it is stained with 5% Giemsa and the slide is covered with entelan after.

Slide analysis

Slides were observed under an optical microscope with camera (Leica DM 2000 Wild M 20 and Olympus BH 2, Germany) using ×1000 resolution, to count the present MN. A total of 1000 erythrocytes were analyzed on each slide, i.e., 3000 for each animal.

Scoring criteria

The MN test in fish is based on analyzing the abnormal small nuclei frequency in cells. MN has similar morphology to the main nucleus, but its diameter ranging from 1/16 to 1/3 of the main nucleus diameter (Setayesh et al., 2021). MN is nuclear entitie independent of the core, present within the cytoplasm, and which cause non-reparable post-mitotic damage (Grisolia, 2002; Kamel, 2014). Non-refractile structures, located on the same focal plane as the main nucleus, resembling nuclei in color and staining intensity and having diameters less than one-third of that of the latter, were thus scored as MN (Fenech et al., 2003). The mean frequencies of MN found in each group were calculated as follows:

$$MN = 1000\frac{N_c}{N_t} \quad \text{(Eq.3)}$$

where N_c and N_t are respectively the number of cells containing a micronucleus and the total number of cells counted.

Trace metal analysis

Preparation of subsamples and analysis were made according to FAO/WHO (2012). For metal analysis, fish was dissected using stainless steel instruments. Muscles, liver and gills were taken out. They are dried in an oven for 24 h at 90 °C, then ground and passed through a sieve. The obtained subsamples are weighed and stored in labeled pillboxes ready for chemical analysis. Composite samples of 2 g were used for subsequent analysis. The samples were digested with ultra pure nitric acid (HNO_3) at 100 °C until the solution become clear. The solution was made up to known volume with deionized distilled water and analyzed for Cd, Pb and Zn using the Atomic Absorption Spectrophotometer (AAS model GPC A932 ver. 1.1) (Amiard et al., 1987). The obtained results were expressed as mg/g wet weight. All reagents were of analytical grade; glassware were soaked in 10% nitric acid and later rinsed with distilled water prior to use in order to avoid metal contamination.

Accuracy and precision were verified by using reference materials (MA-A-2/TM) provided by the International Atomic Agency (IAEA). Analytical results of the quality control samples indicated a satisfactory performance of heavy metal determination within the range of certified values 95-111% recovery for the metals studied.

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Statistical analysis

The obtained results are presented as mean ± standard error. The verification of the normality and that of the homogeneity of covariance matrices were conducted using the Kolmogorov-Smirnov and Levene tests, respectively. The significant variations of the variables (concentrations of Zn, Pb and Cd) and biomarkers recorded at each season were tested by the analysis of variance (ANOVA). The post hoc comparisons were made using an HSD Tukey test to discriminate the differences season at a level of significance (p value < 0.05; p value < 0.001).

The Pearson correlation coefficient was used to check for significant correlation of pollutants from the tissues of fish with the response of biomarkers during various sampling seasons. All statistical analyses were performed using the computer pro-gram SPSS9 (SPSS, Chicago, IL) for PC.

Results

Condition indexes

Gonadosomatic index

The results revealed that the GSI values of *M. merluccius* captured from Oran Bay differs significantly (p value < 0.05) across the seasons; with maximum values in winter and summer (0.556 ± 0.001%; 0.520 ± 0.002%, respectively) (*Fig. 2A*).

Condition factor (K)

The K curves of *M. Merluccius* depicted in *Figure 2B*, showed a decreasing section between autumn and spring. The lowest K values were observed in summer (0.641 ± 0.007%) and the highest ones in autumn (0.691 ± 0.005%).

Micronucleus test

Figure 3 represents the average percentages of MN in cells of *M. merluccius* from Oran Bay. The MN test in erythrocytes and gills cells showed that the corresponding seasonal variations of this biomarker followed a similar pattern. However, the Tukey comparison test revealed significant differences (p value < 0.05) in MN frequencies, especially between summer, when they were the highest (23.366 ± 5.098‰ and 22.233 ± 4.101‰, respectively), and winter, when they were the lowest (7.668 ± 0.711‰ and 6.9 ± 1.113). Fish sampled during spring and autumn had MN frequency values in between the extremes observed for the summer and winter measurements. Moreover, this study revealed a higher sensitivity of erythrocytes as target cells compared to gill cells.

Trace metal analysis

Figure 4 shows the assessment of seasonal variations of metallic trace elements concentration’s in tissues of *M. merluccius*. The highest concentrations of the three metals studied (Zn, Pb and Cd) were recorded in the liver, whatever the season. However, the Tukey comparison test (*Fig. 3*) revealed significant seasonal differences (p value < 0.05). Effectively, the greatest values of concentration of Zn, Pb and Cd were observed in summer (12.236 ± 0.363) (*Fig. 4A*), and spring (0.553 ± 0.005 and

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0.177 ± 0.018) (*Fig. 4B, C*). Also, the mean concentration of elements followed Zn > Pb > Cd in the two organs studied.

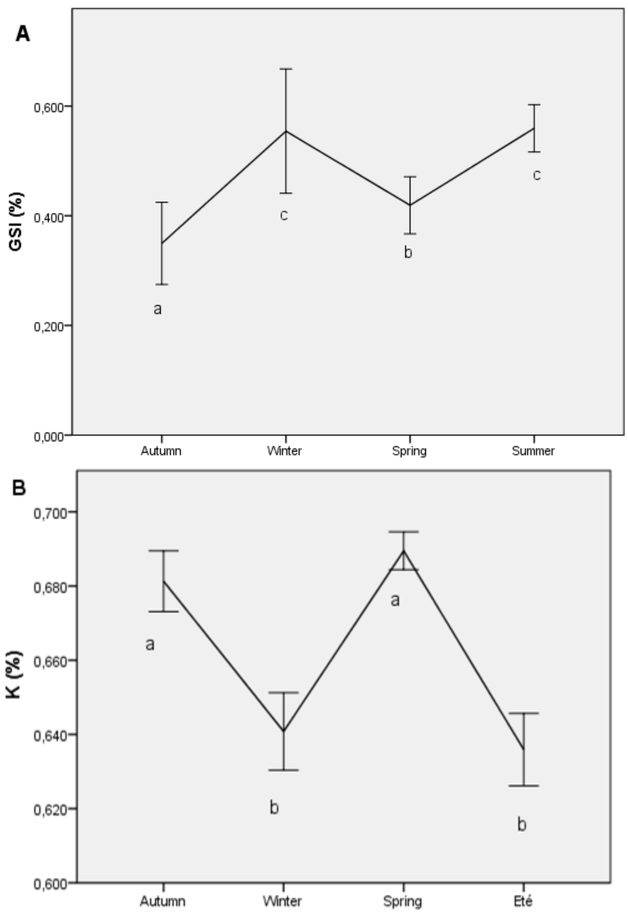

Figure 2. *Seasonal variations in the condition factor (mean ± SD, n = 120) of M. Merluccius captured at Oran Bay; a, b, c and d indicate that seasonal variation is significant at p < 0.05, using the Tukey multiple comparison test among responses in different seasons*

Correlations between the different biologic measures

The correlation coefficients in between condition indexes, MN test and concentrations of heavy metals in liver of *M. merluccius* over the 4 season's period from Oran Bay are given in *Table 1*.

Table 1. *Analysis of the correlation between the different biologic measures in M. merluccius over the 4 seasons period from the Oran Bay*

	GSI	K	MN test	Zn	Pb	Cd
GSI	1	**-0.980****	-0.182	0.187	0.147	0.102
K	**-0.980****	1	-0.196	0.113	0.048	0.119
MN test	-0.182	-0.196	1	**0.673****	**0.555****	**0.601****
Zn	0.187	0.113	**0.673****	1	**0.467****	**0.773****
Pb	0.147	0.048	**0.555****	**0.467****	1	**0.364***
Cd	0.102	0.119	**0.601****	**0.773****	**0.364***	1

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From the data on this matrice, a strong positive correlation was observed among the concentrations of heavy metals and MN test. Also GSI was negatively correlated to K.

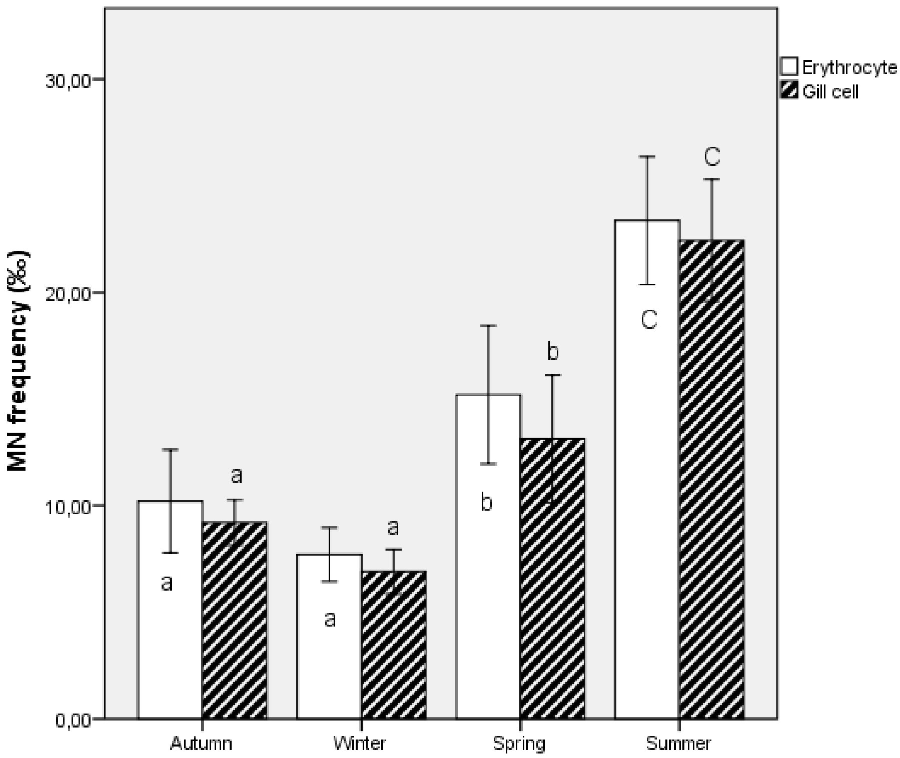

Figure 3. *Seasonal variations of micronuclei frequencies in erythrocytes and gill cells (mean ± SD, n = 10) of M. Merluccius captured at Oran Bay; a, b, c and d indicate that seasonal variation is significant at p value < 0.05, using the Tukey multiple comparison test among responses in different seasons*

Discussion

Condition indexes

The overall goal of this study was to investigate the temporal physiological changes in *M. merluccius* captured from Oran Bay. This was done at first by analysis of the condition indexes. The reproductive period was observed in winter and summer, when tissues showed a marked increase in the GSI. Comparable results were reported by Bouaziz et al. (1998) and Belhoucine et al. (2012) for *M. merluccius* from Bou-Ismail and Oran Bay, respectively on the coast of Algeria. Typically, GSI is at her highest values at the peak of spawning (Galloway and Goven, 2006).

On the other hand, the study of the seasonal evolution of the K condition factor revealed a low value winter and summer and thus a negative correlation with GSI as mentioned in *Table 1*. The temporal variation in K reflected the effects of both the environmental seasonality and the reproductive cycle of the species (Mimeche et al., 2013). K is one of the most important parameters that throws light on the physiological state of the fish in relation to the indication of the sexual maturity onset (Salam and Davies, 1994). This condition is the result of the mobilization of somatic energy reserves needed for the reproductive development and energy in spawned fish, influenced by reduced feeding during this period (Palazón -Fernandez et al., 2001).

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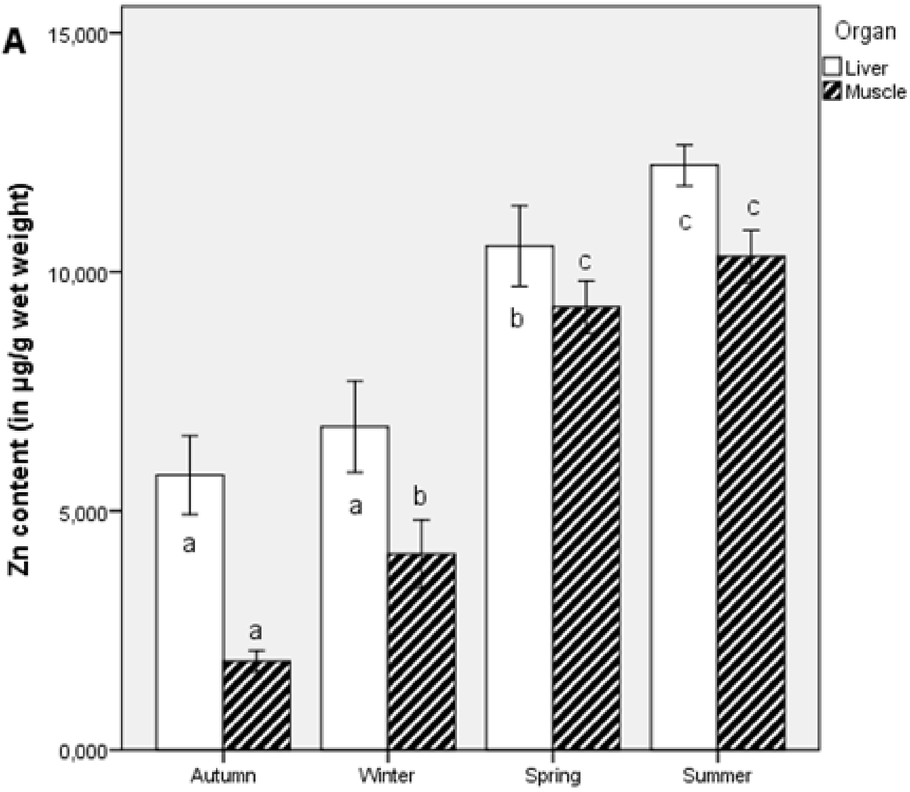
Micronucleus test

Genotoxic effects of the pollutants were examined in MN test carried out with erythrocytes and gill cells of *M. merluccius.* The data obtained in this study revealed seasonal differences in erythrocytes MN frequencies, with high values detected in fish sampled during summer whereas low values were found in those sampled in winter. Seasonal variations in MN frequency in fish were previously demonstrated in several in situ studies. Minissi et al. (1996) reported that it was higher in summer that in autumn in *B. plebejus* erythrocytes. While, Hayashi et al. (1998) observed that MN frequencies in the erythrocytes and gill cells of *C. auratus* and *Z. platypus* were the highest in spring (May). In the same study, this parameter for *L. nuchalis* and *D. temmincki* was found the highest in summer (July). Cavas and Ergene-Gözükara (2005) and Kaddour et al. (2021) found that it was higher in summer than in winter for *M. cephalus* and *M. barbatus*, respectively.

Taken all together, these studies, including the present one, indicate that MN frequencies in fish cells are generally higher in the warmer seasons, especially summer. As suggested by Hayashi et al. (1998) seasonal differences in fish cells MN frequencies might be related to the water temperature. Because fish are poikilothermic animals and their metabolism is highly dependent on the surrounding warmth, the elevated values observed during the hottest seasons might be related to increased metabolism rates and mitotic index caused by the high seawater temperature (Cavas et al., 2005).

Erythrocytes are widely used in the micronuclei experiments. Such studies are recommended because gill cells as well exhibit high sensitivity to the agents promoting formation of micronuclei (Hayashi et al., 1998; Arkhipchuk and Garanko, 2005). Generally, gills are metabolically active tissues because their cells are under the influence of aquatic circulation system (Arkhipchuk and Garanko, 2005).



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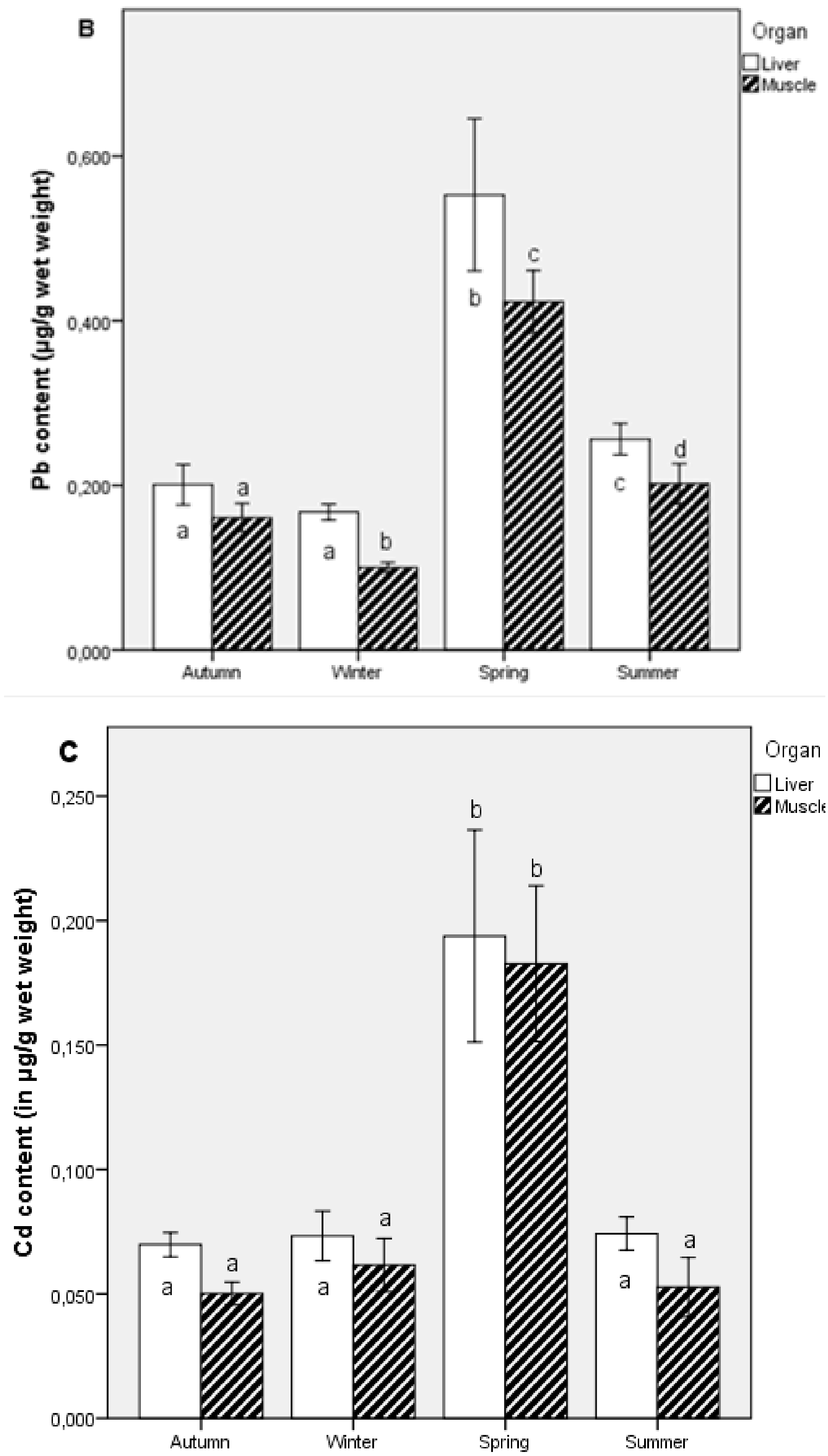

Figure 4. *Variation in the average concentrations (µg/g wet weight) of Zn (A), Pb (B) and Cd (C) and 95% of standard deviation (error bars) in the liver and in the muscle of M. merluccius sampling on Oran Bay, according to the seasons (The letters a, b, c and d indicate significant seasonal differences for the same matrix)*

Also, the Pearson correlation test showed in *Table 1*, the positive correlation between MN test, and the concentrations of trace metals. It is well known that the aquatic organisms exposed to water and sediments contaminated by heavy metals can exhibit genotoxic effects (Turan et al., 2020; Konta et al., 2022). In this regard, D'Costa et al. (2017) and Viana et al. (2018) mentioned that high concentrations of tissue pollutants and higher frequency of micronucleus were positively associated in *A. arius* and *A. lacustris*, respectively, collected from the polluted site. Besides, Ghisi et al. (2017) found a higher frequency of DNA damage in *A. aff. paranae* located in a site downstream of the urban area, which received a mix of pollutants, including industrial and municipal wastewater. Higher levels of DNA damage in *Astya spp*. have also been documented at points disturbed by artificial beaches that received surface runoff, in addition to urban and industrial wastewater (Barros et al., 2017). It should be noted here that, to date, ecological relevance has been demonstrated only through correlation and not mechanistic causality. Thus, The MNT assay performed in cells of *M. merluccius* showed to be an important tool for detecting the current status of the investigated ecosystem concerning genotoxicity.

Trace metal analysis

Trace metals may settle on the surface sediments and can affect benthic feeders, such as catfish. Trace metals, especially Zn, Pb, and Cd which are found to contaminate the water and sediment at Oran Bay, could also be the cause of genotoxicity in *M. merluccius*. This is in agreement with the studies of Omar et al. (2012) and D'Costa (2017) which reported the genotoxic effects of trace metals in *A. arius* and *Mugil cephalus*, respectively.

In *Table 1*, Person correlation test revealed that high levels of genotoxic damage are due to the accumulation of the pollutants in liver and muscle of *M. merluccius* of that present study. Trace metal accumulation in different tissues at high concentrations could occur due to various reasons. Fish of the present study always showed the lowest concentration of metals in muscle. It is well known that accumulation of essential metals in the liver is likely linked to its role in metabolism (Zhao et al., 2012); high levels of Zn hepatic tissues are usually related to a natural binding proteins such as metallothioneins (MT) (Görür et al., 2012) which act as an essential metal store (i.e., Zn) to fulfill enzymatic and other metabolic demands (Amiard et al., 2006; Atli and Canli, 2008).

On the other hand, the liver also showed high levels of non-essential metals such as Cd and Pb; this finding could be explained by the ability of Cd to displace the normally MT-associated essential metals in hepatic tissues (Amiard et al., 2006). Belhoucine et al. (2014); Bouhadiba et al. (2017) and Tabeche et al. (2021) were mentioned a preferential organotropism of trace metals such as Zn, Pb and Cd for the liver in fish have been reported.

In addition, the correlation between different metals within the same fish tissue may have been due to the similar accumulation behavior of trace elements in these fish (Kojadinovic et al., 2007). The significant correlations among most metals reflected a common source of occurrence and its subsequent accumulation in these fish tissues. These inter-metal correlations of this study are indicative of similar biogeochemical pathways for metal bioaccumulation in fish tissues. Jabeen and Chaudhry (2010) also observed the inter-elemental correlations between the essential and non-essential elements in different tissues of *C. carpio* from the Indus River in Pakistan.

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On the other hand, the study of the seasonal evolution of concentrations of metallic trace elements revealed a high value in the summer season. Comparable results were reported by Belhoucine et al. (2014), Bohadiaba et al. (2017) and Tabeche et al. (2021) for *M. merluccius*, *M. cephalus* and *S. solea*, respectively.

According to Wong et al. (2001), physicochemical parameters (pH, salinity, temperature, and dissolved oxygen) might influence the bioaccumulation of metals in marine organisms. physiological parameters of fish are directly correlated with metal uptake. Higher metal bioaccumulation is related to increased metabolism rate with elevated temperature (Larsson et al., 1985; Ismaila et al., 2017). On the other hand, the Algerian coastal population increases considerably during the summer, which can increase direct discharges into water (Belhoucine et al., 2014). Although, the concentrations of Zinc and the purely toxic elements (lead and cadmium) accumulated in the muscle tissue of *M. merluccius* are below the limits recommended by WHO (1989), OMS-IPS (1998), USEPA (2000) and FAO/WHO (2011) (*Table 2*). it may be noted that these fish are consumed throughout the year by the Algerian population and may lead to subsequent bioaccumulation and genotoxicity in humans as well.

Table 2. *Comparison of heavy metal content in the muscle of M. merluccius (µg/g wet weight) from the maximum permissible limit (MPl) international standards*

Heavy metals	Present study	Standards			
		WHO (1989)	OMS-IPS (1998)	USEPA (2000)	FAO/WHO (2011)
Zn	6.83	100	30	120	-
Pb	0.221	2	0.5	4	0.3
Cd	0.103	1	0.1	2	0.1

Conclusion

This study clearly indicated that *M. merluccius* collected from Oran Bay is exposed to genotoxic substances through the significant bioaccumulation of heavy metals in these tissues. Also, the MN test performed in erythrocytes of *M. merluccius* showed to be an important complementary tool for detecting the current status of the investigated ecosystem concerning genotoxicity. Consequently, it might be used as a standard method in a regular monitoring of the coastal pollution. This work should hopefully provide a scientific basis for future laboratory-based studies regarding the genotoxicity of these pollutants either singly or in association with each other and the exact pathways of genotoxic damage can be deduced as future work in the West coast ecosystem of Algeria.

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