

HEAVY METAL BIOACCUMULATION AND GENOTOXICITY IN FISH (*MERLUCCIOUS MERLUCCIOUS*, 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,

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 (Araújo 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; Bückner 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 mammals (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/RAMOGÉ, 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/RAMOGÉ (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

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 benthic-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 assess 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 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)

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 micronucleus (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 $\times$ 16 mm (27G $\times$ 5/8 inches) to 0.8 mm $\times$ 40 mm (21G $\times$ 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.

For the micronuclei test, Fish gills dissected by scissor were fixed in acetic acid. The samples were centrifuged at $\times 2000$ 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 $\times 1000$ 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.

Statistical analysis

The obtained results are presented as mean $\pm$ 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 ($0.556 \pm 0.001\%$; $0.520 \pm 0.002\%$, 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 ($0.641 \pm 0.007\%$) and the highest ones in autumn ($0.691 \pm 0.005\%$).

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 ($23.366 \pm 5.098\%$ and $22.233 \pm 4.101\%$, respectively), and winter, when they were the lowest ($7.668 \pm 0.711\%$ 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

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

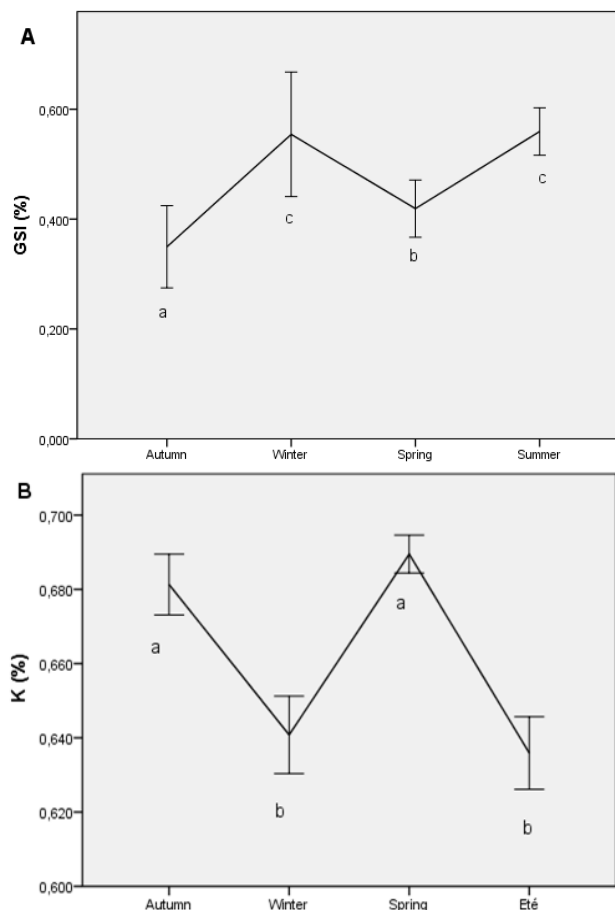


Figure 2. Seasonal variations in the condition factor (mean $\pm$ SD, $n = 120$) of *M. Merluccius* captured at Oran Bay; a, b, c and d indicate that seasonal variation is significant at $p < 0.05$, using the Tukey multiple comparison test among responses in different seasons

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

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.

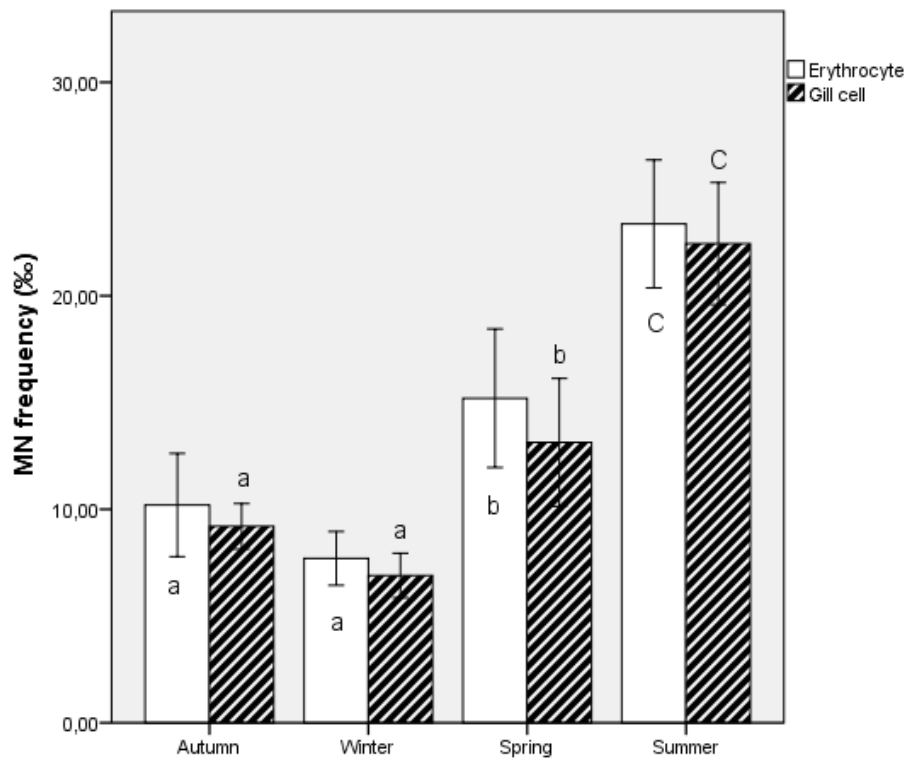


Figure 3. Seasonal variations of micronuclei frequencies in erythrocytes and gill cells (mean $\pm$ SD, $n = 10$) of *M. Merluccius* captured at Oran Bay; a, b, c and d indicate that seasonal variation is significant at p value < 0.05 , using the Tukey multiple comparison test among responses in different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

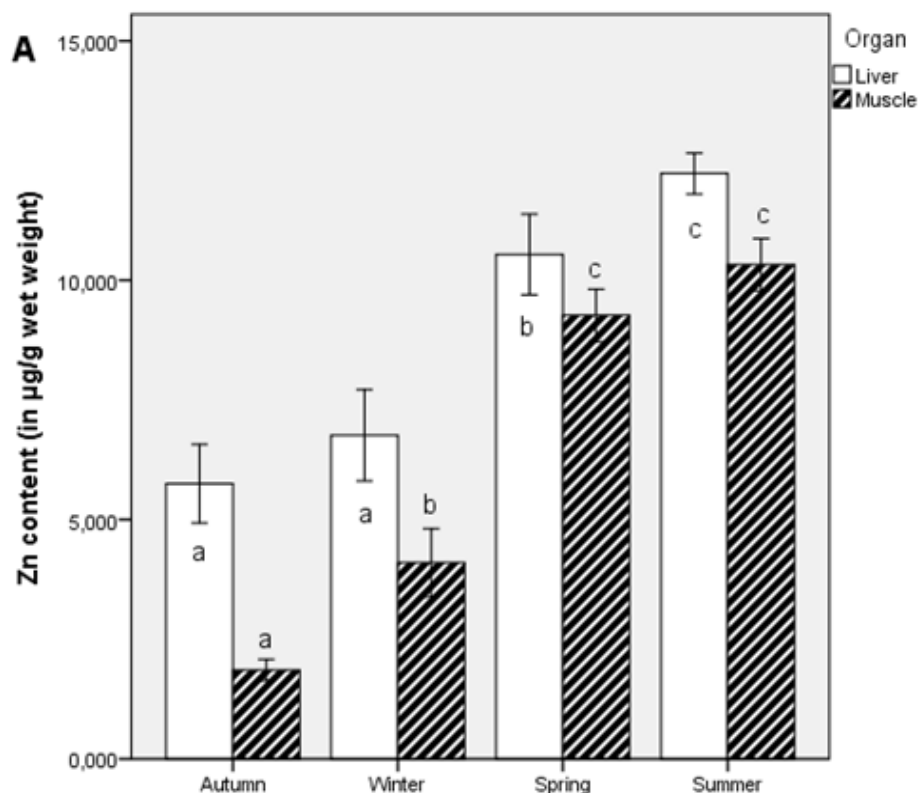
On the other hand, the study of the seasonal evolution of the K condition factor revealed a low value winter and summer and thus a negative correlation with GSI as mentioned in Table 1. The temporal variation in K reflected the effects of both the environmental seasonality and the reproductive cycle 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).

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 than 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).



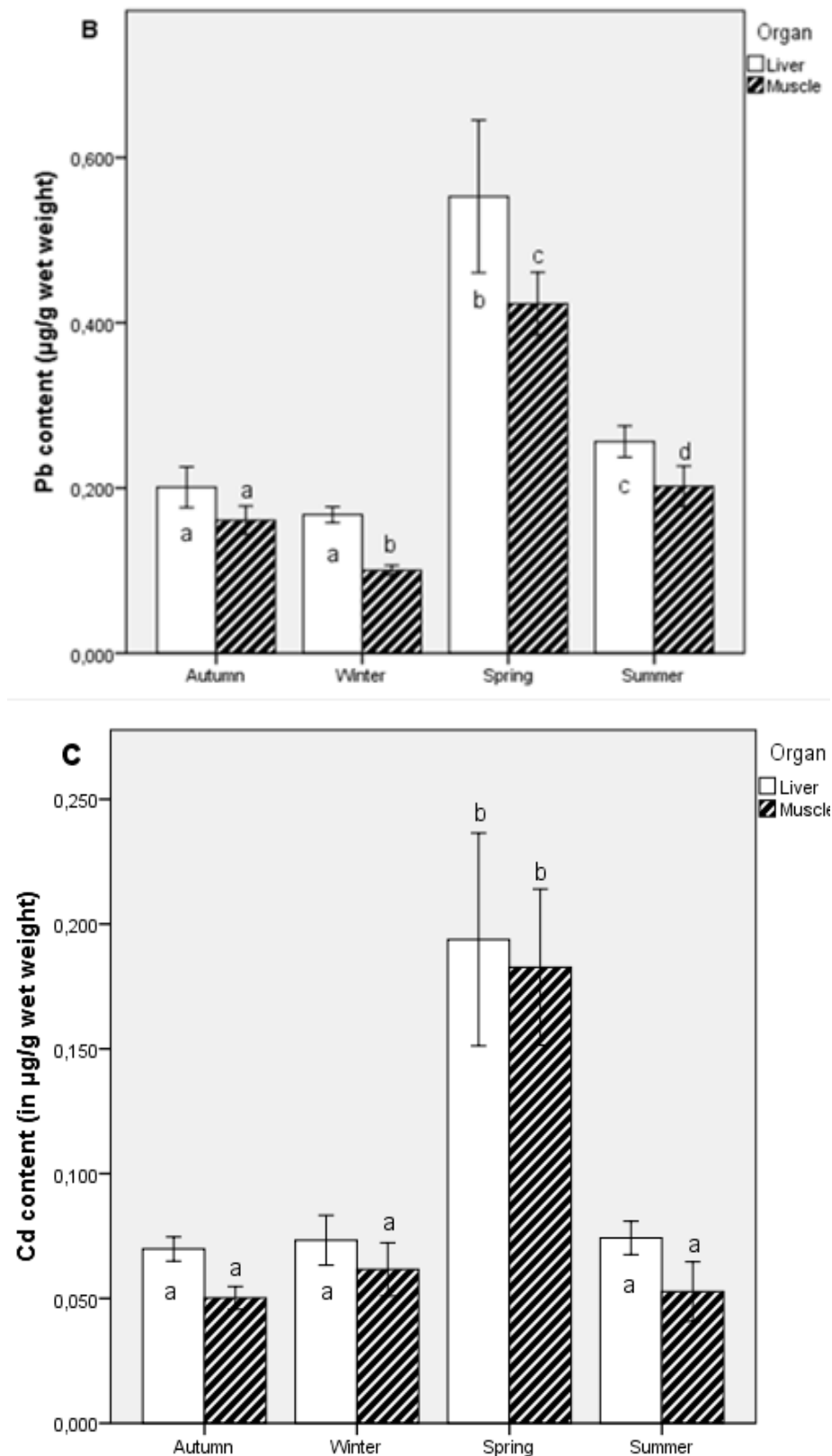


Figure 4. Variation in the average concentrations ($\mu\text{g/g}$ wet weight) of Zn (A), Pb (B) and Cd (C) and 95% of standard deviation (error bars) in the liver and in the muscle of *M. merluccius* sampling on Oran Bay, according to the seasons (The letters a, b, c and d indicate significant seasonal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

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* ($\mu\text{g/g}$ 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.

REFERENCES

- [1] Aldebert, Y., Carriès, C. (1988): Problèmes d'exploitation du merlu du golfe du Lion. – Fuengirola, CGPM, 5ème Consultation technique sur l'évaluation des stocks Baléares et Golfe du Lion, pp. 87-91.
- [2] Al-Sabti, K. (1986): Comparative micronucleated erythrocyte cell induction in three cyprinids by five carcinogenic-mutagenic chemicals. – Cytobios 47(190-191): 147-154.
- [3] Altındag, A., Yigit, S. (2005): Assessment of heavy metal concentrations in the food web of Lake Beysehir, Turkey. – Chemosphere 60(4): 552-556.

- [4] Amiard, J. C., Pinneau, A., Boiteau, H. L., Metayer, C., Amiard-Triquet, C. (1987): Application de la spectrometrie d'absorption atomique zeeman aux dosages de 8 elements traces (Ag, Cd, Cr, Cu, Mn, Ni, Pb, Se) dans des matrices biologiques solides. – *Water Research* 21(6): 693-697.
- [5] Amiard, J. C., Amiard-Triquet, C., Barka, S., Pellerin, J., Rainbow, P. S. (2006): Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. – *Aquatic Toxicology* 76(2): 160-202.
- [6] Araújo, F. G., Moradoa, C. N., Parenteb, T. T. E., Paumgarttenb, F. J. R., Gomesa, I. D. (2018): Biomarkers and bioindicators of the environmental condition using a fish species (*Pimelodus maculatus* Lacepède, 1803) in a tropical reservoir in Southeastern Brazil. – *Brazilian Journal of Biology* 78: 351-359.
- [7] Arkhipchuk, V. V., Garanko, N. N. (2005): Using the nucleolar biomarker and the micronucleus test on in vivo fish fin cells. – *Ecotoxicology and Environmental Safety* 62(1): 42-52.
- [8] Atli, G., Canli, M. (2008): Responses of metallothionein and reduced glutathione in a freshwater fish *Oreochromis niloticus* following metal exposures. – *Environmental Toxicology and Pharmacology* 25(1): 33-38.
- [9] Ayas, D., Köşker, A. R. (2018): The effects of age and individual size on metal accumulation of *Lagocephalus sceleratus* (Gmelin, 1789) from Mersin Bay, Turkey. – *Natural and Engineering Sciences* 3(1): 45-53.
- [10] Barrilli, G. H. C., Rocha, O., Negreiros, N. F., Verani, J. R. (2015): Influence of environmental quality of the tributaries of the Monjolinho River on the relative condition factor (Kn) of the local ichthyofauna. – *Biota Neotropica* 15: 1-9.
- [11] Barros, I. T., Ceccon, J. P., Glinski, A., Liebel, S., Grötzner, S. R., Randi, M. A. F., de Oliveira Ribeiro, C. A. (2017): Environmental risk assessment in five rivers of Parana River basin, Southern Brazil, through biomarkers in *Astyanax spp.* – *Environmental Science and Pollution Research* 24(19): 16228-16240.
- [12] Beiras, R., Bellas, J., Cachot, J., et al. (2018): Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton. – *Journal of Hazardous Materials* 360: 452-460.
- [13] Belhoucine, F., Bouderbala, M., Flower, R., Francour, P., Boutiba, Z. (2012): Hermaphroditism case observed on the hake (*Merluccius merluccius* Linné, 1758) fished in Oran bay (south west Mediterranean sea). – *J. Sci. Halieut. Aquat.* 5: 171-176.
- [14] Belhoucine, F., Alioua, A., Bouhadiba, S., Boutiba, Z. (2014): Impact of some biotics and abiotics factors on the accumulation of heavy metals by a biological model *Merluccius merluccius* in the bay of Oran in Algeria. – *Journal of Biodiversity and Environmental Sciences (JBES)* 5(6): 33-44.
- [15] Benali, I., Boutiba, Z., Merabet, A., Chèvre, N. (2015): Integrated use of biomarkers and condition indices in mussels (*Mytilus galloprovincialis*) for monitoring pollution and development of biomarker index to assess the potential toxic of coastal sites. – *Marine Pollution Bulletin* 95: 385-394.
- [16] Boettcher, M., Grund, S., Keiter, S. (2010): Comparison of in vitro and in situ genotoxicity in the Danube River by means of the comet assay and the micronucleus test. – *Mutation Research* 700:11-17.
- [17] Bonomo, M. M., de Castro Sachi, I. T., Paulino, M. G., Fernandes, J. B., Carlos, R. M., Fernandes, M. N. (2021): Multi-biomarkers approach to access the impact of novel metal-insecticide based on flavonoid hesperidin on fish. – *Environmental Pollution* 268: 115758.
- [18] Bouaziz, A., Bennoui, A., Djabali, F., Maurin, C. (1998): Reproduction du merlu *Merluccius merluccius* (Linnaeus, 1758) dans la région de Bou-Ismaïl. – In: Lleonart, J. (ed.). *Dynamique des populations marines*. CIHEAM, Zaragoza (Cahiers Options Méditerranéennes; n. 35), pp. 109-117.

- [19] Boucetta, S. (2017): Biosurveillance des eaux du littoral Est algérien à travers un mollusque gastropode: *Phorcus* (= *Osilinus*) *turbinatus* (BORN, 1780). – Thèse de Doctorat es sciences. Départements des sciences de la Mer. Université Badji Mokhtar, Annaba.
- [20] Bouhadiba, S., Belhoucine, F., Belhadj, H., Alioua, A., Boutiba, Z. (2017): Quantification of two metallic elements in the Mullet, *Mugil cephalus* Linnaeus, 1758 (Perciformes Mugilidae), fished at the bay of Oran (NW Algeria). – Biodiversity Journal 8(3): 807-818.
- [21] Bücker, A., Carvalho, M. S., Conceição, M. B., Alves-gomes, J. A. (2012): Micronucleus test and comet assay in erythrocytes of the Amazonian electric fish *Apteronotus bonapartii* exposed to benzene. – Journal of the Brazilian Society of Ecotoxicology 7: 65-73.
- [22] Cabral, H., Fonseca, V., Sousa, T., et al. (2019): Synergistic effects of climate change and marine pollution: an overlooked interaction in coastal and estuarine areas. – International Journal of Environmental Research and Public Health 16(15): 2737.
- [23] Canedo, A., de Jesus, L. W. O., Bailão, E. F. L. C., Rocha, T. L. (2021): Micronucleus test and nuclear abnormality assay in zebrafish (*Danio rerio*): Past, present, and future trends. – Environmental Pollution 290: 118019.
- [24] Cant, A., Bonnard, M., Porcher, J-M., Prygiel, J., Catteau, A., Delahaut, L., Palluel, O., Turiès, C., Geffard, A., Bado-Nilles, A. (2022): Integration of Genotoxic Biomarkers in Environmental Biomonitoring Analysis Using a Multi-Biomarker Approach in Three-Spined Stickleback (*Gasterosteus aculeatus* Linnaeus, 1758). – Toxics 10(3): 101.
- [25] Catteau, A., Bado-Nilles, A., Beaudouin, R., Tebby, C., Joachim, S., Palluel, O., Turiès, C., Chrétien, N., Nott, K., Ronkart, S., Geffard, A., Porcher, J-M. (2021): Water quality of the Meuse watershed: assessment using a multi-biomarker approach with caged three-spined stickleback (*Gasterosteus aculeatus* L.). – Ecotoxicology and Environmental Safety 208: 111407.
- [26] Çavas, T., Ergene-Gözükara, S. (2005): Induction of micronuclei and nuclear abnormalities in *Oreochromis niloticus* following exposure to petroleum refinery and chromium processing plant effluents. – Aquatic Toxicology 74: 264-271.
- [27] Cavas, T., Garanko, N. N., Arkhipchuk, V. V. (2005): Induction of micronuclei and binuclei in blood, gill and liver cells of fishes subchronically exposed to cadmium chloride and copper sulphate. – Food and Chemical Toxicology 43: 569-74.
- [28] Copat, C., Arena, G., Fiore, M., Ledda, C., Fallico, R., Sciacca, S., Ferrante, M. (2013): Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: consumption advisories. – Food and Chemical Toxicology 53: 33-37.
- [29] D'Costa, A., Shyama, S. K., Kumar, M. P. (2017): Bioaccumulation of trace metals and total petroleum and genotoxicity responses in an edible fish population as indicators of marine pollution. – Ecotoxicology and Environmental Safety 142: 22-28.
- [30] D'costa, A. H., Shyama, S. K., Praveen Kumar, M. K., Swizzle, F. (2018): The Backwater Clam (*Meretrix casta*) as a bioindicator species for monitoring the pollution of an estuarine environment by genotoxic agents. – Mutation Research/Genetic Toxicology and Environmental Mutagenesis 825: 8-14.
- [31] Deutschmann, B., Kolarević, S., Brack, W., Kaisarević, S., Kostić, J., Kračun-Kolarević, M., Liska, I., Paunović, M., Seiler, T. B., Shaon, Y., Sipos, S., Slo-bodnik, J., Teodorović, I., Vuković-Gačić, B., Hollert, H. (2016): Longitudinal profile of the genotoxic potential of the River Danube on erythrocytes of wild common bleak (*Alburnus alburnus*) assessed using the comet and micronucleus assay. – Science of the Total Environment 573: 1441-1449.
- [32] dos Santos Silva, D., Gonçalves, B., Rodrigues, C. C., Dias, F. C., de Souza Trigueiro, N. S., Moreira, I. S., Rocha, T. L. (2021): A multibiomarker approach in the caged neotropical fish to assess the environment health in a river of central Brazilian Cerrado. – Science of The Total Environment 751: 141632.

- [33] El-Moselhy, K. M., Othman, A. I., Abd El-Azem, H., El-Metwally, M. E. A. (2014): Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. – Egyptian Journal of Basic and Applied Sciences 1(2): 97-105.
- [34] FAO/WHO (2001): Evaluation of certain food additives and contaminants. – Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives.
- [35] FAO/WHO (2011): Evaluation of Certain Food Additives and Contaminants. – Seventy-third [73rd] Report of the Joint FA.
- [36] FAO/WHO (2012): Working document for information and use in discussions related to contaminants and toxins in the GSCTFF. – Joint FAO/WHO Food Standards Programme. CODEX Committee on Contaminants in Foods. Sixth Session, Maastricht, The Netherlands.
- [37] Fenech, M., Chang, W. P., Kirsch-Volders, M., Holland, N., Bonassi, S., Zeiger, E. (2003): HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus as-say using isolated human lymphocyte cultures. – Mutation Research 534: 65-75.
- [38] Galloway, T. S., Goven, A. (2006): Invertebrate Immunotoxicology. – In: Luebke, R. (ed.) Immunotoxicology and Immunopharmacology. CRC Press, Boca Raton, pp. 365-383.
- [39] Gentile, A., Giova, R. (2022): Regularity results for solutions to a class of non-autonomous obstacle problems with sub-quadratic growth conditions. – Nonlinear Analysis Real World Applications 68(4): 103681. DOI: 10.1016/j.nonrwa.2022.103681.
- [40] Gherras, H., Boutiba, Z., Benguedda, W., Shaposhnikov, S. (2016): Active biomonitoring of mussels *Mytilus galloprovincialis* with integrated use of micronucleus assay and physiological indices to assess harbor pollution. – Marine Pollution Bulletin 110: 52-64.
- [41] Ghisi, N. C., Oliveira, E. C., Guiloski, I. C., de Lima, S. B., de Assis, H. C. S., Longhi, S. J., Prioli, A. J. (2017): Multivariate and integrative approach to analyze multiple biomarkers in ecotoxicology: a field study in Neotropical region. – Science of the Total Environment 609: 1208-1218.
- [42] Görür, F. K., Keser, R. E. C. E. P., Akçay, N. İ. L. A. Y., Dizman, S. E. R. D. A. R. (2012): Radioactivity and heavy metal concentrations of some commercial fish species consumed in the Black Sea Region of Turkey. – Chemosphere 87(4): 356-361.
- [43] Grisolia, C. K. (2002): A comparison between mouse and fish micronucleus test using cyclo-phosphamide, mitomycin C and various pesticides. – Mutation Research 518: 145-150.
- [44] Guendouzi, Y., Soualili, D. L., Boulahdid, M., Boudjellal, B. (2017): Biological indices and monitoring of trace metals in the mussel from the southwestern Mediterranean (Algeria): seasonal and geographical variations. – Thalass An Int J Mar Sci. 1(34): 103-112.
- [45] Hallare, A. V., Ocampo, K. A. A., Tayo, P. K. S., Balolong, M. M (2016): Genotoxic stress induced by intensive aquaculture activities in Taal Lake (Philippines) on circulating fish erythrocytes using the comet assay and micronucleus test. – Advances in Environmental Biology 10: 273-283.
- [46] Hartwig, A., Schwerdtle, T. (2002): Interactions by carcinogenic metal compounds with DNA repair processes: toxicological implications. – Toxicology Letters 127(1-3): 47-54.
- [47] Hasan, M. R., Hossain, M. Y., Mawa, Z., Hossain, M. A. (2022): Reproductive biology of *Heteropneustes fossilis* in a wetland ecosystem (Gajner Beel, Bangladesh) in relation to eco-climatic factors: suggesting a sustainable policy for aquaculture, management and conservation. – Saudi Journal of Biological Sciences 29(2): 1160-1174.
- [48] Hayashi, M., Uede, T., Uyeno, K., Wada, K., Kinae, N., Saotome, K., Tanaka, N., Takai, A., Sasaki, Y. F., Sofuni, T., Ojima, Y. (1998): Development of genotoxicity assay systems that use aquatic organisms. – Mutation Research 399: 217-2.

- [49] Hosseini, M., Nabavi, S. M. B., Nabavi, S. N., Pour, N. A. (2015): Heavy metals (Cd, Co, Cu, Ni, Pb, Fe, and Hg) content in four fish commonly consumed in Iran: risk assessment for the consumers. – Environmental Monitoring and Assessment 187(5): 1-7.
- [50] Hussain, R., Ghaffar, A., Ali, H. M. (2018): Analysis of different toxic impacts of fipronil on growth, hemato-biochemistry, protoplasm and reproduction in adult cockerels. – Toxin Reviews 37: 294-303.
- [51] Iftikhar, n., Zafar, Z., Hashmi, I. (2022): Multi-biomarkers approach to determine the toxicological impacts of sulfamethoxazole antibiotic on freshwater fish *Cyprinus carpio*. – Ecotoxicology and Environmental Safety 233: 113331.
- [52] Igbo, J. K., Chukwu, L. O., Oyewo, E. O. (2018): Assessment of polychlorinated biphenyls (PCBs) in water, sediments and biota from e-waste dumpsites in Lagos and Osun States, South-West Nigeria. – Journal of Applied Sciences and Environmental Management 22: 459-464.
- [53] Ismaila, O. S., Samsudeen, O. A., Yusuf, O. A., Gunu, U. C. (2017): Assessment of physicochemical characteristics of selected borehole waters in Oke-Oyi community, Ilorin East Local Government Area, Kwara State. – Journal of Applied Sciences and Environmental Management 21(6): 1127-1129.
- [54] Jabeen, F., Chaudhry, A. S. (2010): Monitoring trace metals in different tissues of *Cyprinus carpio* from the Indus River in Pakistan. – Environmental Monitoring and Assessment 170(1): 645-656.
- [55] JORADP N°25. (2011): Journal Officielle de la République Algérienne N°25. Annexe I: Seuils limites des contaminants chimiques. – Ministère de la Pêche et de Ressources Halieutiques.
- [56] Kaddour, A., Belhoucine, F., Alioua, A. (2021): Integrated use of condition indexes, genotoxic and cytotoxic biomarkers for assessing pollution effects in fish (*Mullus barbatus* L., 1758) on the West coast of Algeria. – South Asian Journal of Experimental Biology 11(3): 287-299.
- [57] Kamel, N. (2014): Effets combinés des facteurs environnementaux et des polluants chimiques chez la moule *Mytilus galloprovincialis*: harmonisation des biomarqueurs d'exposition suivant les recommandations OSPAR et MEDPOL. – Thèse de doctorat en Environnement, Écotoxicologie.
- [58] Kerfouf, A., Alaoui, F. Z., Djoudi, O., Mebarki, Z., Benyahia, M. (2010): Le dessalement de l'eau de mer dans la perspective d'une gestion intégrée et d'un développement durable: Cas de la station de dessalement d'Oran: Algérie Nord Occidentale. – European Journal of Scientific Research 96(2): 245-249.
- [59] Kligerman, D. (1982): Fishes as Biological Detectors of the Effects of Genotoxic Agents. – Heddle, J. (ed.) Mutagenicity: New Horizons in Genetic Toxicology. Academic Press, New York, pp. 435-456.
- [60] Kojadinovic, J., Potier, M., Le Corre, M., Cosson, R. P., Bustamante, P. (2007): Bioaccumulation of trace elements in pelagic fish from the Western Indian Ocean. – Environmental Pollution 146(2): 548-566.
- [61] Kontas, A., Alyuruk, H., Bilgin, M., Uluturhan, E., Ünlüoğlu, A., Darilmaz, E., Altay, O. (2022): Metal bioaccumulation and potential health risk assessment in different tissues of three commercial fish species (*Merluccius merluccius*, *Mullus barbatus*, and *Pagellus erythrinus*) from Edremit Bay (Aegean Sea), Turkey. – Biological Trace Element Research 200(2): 868-880.
- [62] Kumar, A., Nagarajan, R., Prabakaran, K., Bing, C., Rajaram, R. (2018): Human health risk assessment and bioaccumulation of trace metals in fish species collected from the Miri coast, Sarawak, Borneo. – Marine Pollution Bulletin 133.
- [63] Lam, P. K. S., Gray, J. S. (2003): The use of biomarkers in environmental monitoring programmes. – Mar Pollut Bull 46(2): 182-186.

- [64] Larsson, Å., Haux, C., Sjöbeck, M. L. (1985): Fish physiology and metal pollution: results and experiences from laboratory and field studies. – *Ecotoxicology and Environmental Safety* 9(3): 250-281.
- [65] Leclaire, L. (1972): La sédimentation holocene sur le versant meridional du bassin algéro-baleares (précontinent algérien). – Muséum National d'Histoire Naturelle, Paris.
- [66] Lionetto, M. G., Caricato, R., Giordano, M. E. (2019): Pollution biomarkers in environmental and human biomonitoring. – *The Open Biomarkers Journal* 9: 1-9.
- [67] Lloret, J., Planes, S. (2003): Condition, feeding and reproductive potential of white seabream *Diplodus sargus* as indicators of habitat quality and the effect of reserve protection in the northwestern Mediterranean. – *Marine Ecology Progress Series* 248: 197-208.
- [68] Lu, G. H., Wang, C., Zhu, Z. (2009): The dose–response relationships for erod and gst induced by polyaromatic hydrocarbons in *Carassius auratus*. – *Bulletin of Environmental Contamination and Toxicology* 82: 194-199.
- [69] Martin, I. (1991): A preliminary analysis of some biological aspects of hake (*Merluccius merluccius* L. 1758) in the Bay of Biscay. – International Council for the Exploration of the Sea Document, CM/G, Copenhagen.
- [70] Meyer, O. (2003): Testing and assessment strategies, including alternative and new approaches. – *Toxicology Letters* 140-141: 21-30.
- [71] Mimeche, F., Biche, M., Ruiz-Navarro, A., Oliva-Paterna, F. J. (2013): The population structure, age and growth of *Luciobarbus callensis* (Cyprinidae) in a man-made lake in the Maghreb (NE Algeria). – *Limnetica* 32(2): 391-404.
- [72] Minissi, S., Ciccotti, E., Rizzoni, M. (1996): Micronucleus test in erythrocytes of *Barbus plebejus* (Teleostei, pisces) from two natural environments: a bioassay for the in situ detection of mutagens in freshwater. – *Mutation Research* 367: 245-251.
- [73] Mondal, N. K., Ghosh, S., Ray, M. R. (2011): Micronucleus formation and DNA damage in buccal epithelial cells of Indian street boys addicted to gasp ‘Golden glue’. – *Mutation Research* 721: 178-83.
- [74] Okunola, A. A., Bakare, A. A. (2011): Genotoicity and mutagenicty of electronic waste leachate using animal bioassays. – *Toxicological & Environmental Chemistry* 93: 1073-1088.
- [75] Oliver, P., Massuti, E. (1995): Biology and Fisheries of Western Mediterranean Hake (*M. merluccius*). – In: Alheit, J., Pitcher, T. (eds.) *Hake: Biology, Fisheries and Markets*. Chapman & Hall, London, pp. 181-202.
- [76] Omar, W. A., Zaghloul, K. H., Abdel-Khalek, A. A., Abo-Hegab, S. (2012): Genotoxic effects of metal pollution in two fish species, *Oreochromis niloticus* and *Mugil cephalus*, from highly degraded aquatic habitats. – *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 746(1): 7-14.
- [77] OMS-IPCS (1998): Environmental Health Criteria n°200. – International Programme on Chemical Safety, Copper.
- [78] Palazón-Fernández, J. L., Arias, A. M., Sarasquete, M. C. (2001): Aspects of the reproductive biology of the toadfish *Halobatrachus didactylus* (Schneider 1801) (Pisces: Batrachoididae). – *Scientia Marina* 65: 131-138.
- [79] Prabhakaran, P., Goyal, Y., Agarwal, V. (2017): Novel nonlinear droop control techniques to overcome the load sharing and voltage regulation issues in DC microgrid. – *IEEE Transactions on Power Electronics* 33(5): 4477-4487.
- [80] Ramsdorf, W. A., Vicari, T., Almeida, M. I. M., Artoni, R. F., Cestari, M. M. (2012): Handling of *Astyanax sp.* for biomonitoring in Cangiüri Farm within a fountainhead (Iraí River Environment Preservation Area) through the use of genetic biomarkers. – *Environmental Monitoring and Assessment* 184: 5841-5849.
- [81] Rejomon, G., Nair, M., Joseph, T. (2010): Trace metal dynamics in fishes from the southwest coast of India. – *Environmental Monitoring and Assessment* 167(1): 243-255.

- [82] Rodrigue, K. A., Yao, B., Trokourey, A., Kopoin, A. (2016): Assessment of heavy metals contamination in sediments of the Vridi Canal (Cote d'Ivoire). – Journal of Geoscience and Environment Protection 4(10): 65-73.
- [83] Rouabhi, Y. L. (2020): Étude comparative de la biologie et de la bioaccumulation des métaux traces chez deux Mytilidae: *Mytilus galloprovincialis* Lamarck, 1819 et *Perna perna* (Linnaeus, 1758) de la côte ouest algérienne. – Thèse de doctorat. Université de Mons, Belgique.
- [84] Rouane-Hacene, O. (2013): Biosurveillance de la qualité des eaux côtières du littoral occidental algérien, par le suivi des indices biologiques, de la biodisponibilité et la bioaccumulation des métaux lourds (Zn, Cu, Pb et Cd) chez la moule *Mytilus galloprovincialis* et l'oursin *Paracentrotus lividus*. – Thèse de doctorat. Département de Biologie. Université d'Oran, Oran.
- [85] Rouane-Hacene, O., Boutiba, Z., Benaissa, M., Belhouari, B., Francour, P., Guibbolini, S. M., Faverney, C. R. (2018): Seasonal assessment of biological indices, bioaccumulation, and bioavailability of heavy metals in sea urchins *Paracentrotus lividus* from Algerian west coast, applied to environmental monitoring. – Environmental Science and Pollution Research 25(12): 11238-11251.
- [86] Sadekarpawar, S., Parikh, P. (2013): Gonadosomatic and hepatosomatic indices of freshwater fish *Oreochromis mossambicus* in response to a plant nutrient. – World Journal of Zoology 8(1): 110-118.
- [87] Sakamoto, K. Q., Nakai, K., Aoto, T., Yokoyama, A., Ushikoshi, R., Hirose, H., Ishizuka, M., Kazuka, A., Fujita, S. (2003): Cytochrome P450 induction and gonadal status alteration in common carp (*Cyprinus carpio*) associated with the discharge of dioxin contaminated effluent to the Hikiji River, Kanagawa Prefecture, Japan. – Chemosphere 51: 491-500.
- [88] Salam, A., Davies, P. M. C. (1994): Body composition of northern Pike (*Esox lucius* L.) in relation to body size and condition factor. – Journal of Fisheries Research 19: 193-204.
- [89] Setayesh, T., Nersesyan, A., Kundi, M., Mišić, M., Fenech, M., Bolognesi, C., Knasmueller, S. (2021): Impact of infections, preneoplasia and cancer on micronucleus formation in urothelial and cervical cells: a systematic review. – Mutation Research/Reviews in Mutation Research 787: 108361.
- [90] Sommer, S., Buraczewska, I., Kruszewski, M. (2020): Micronucleus assay: the state of art, and future directions. – International Journal of Molecular Sciences 21(4): 1534.
- [91] Sutton, S. G., Bult, T. P., Haedrich, R. L. (2000): Relationships among fat weight, body weight, water weight, and condition factors in wild Atlantic salmon par. – Transactions of the American Fisheries Society 129: 527-538.
- [92] Tabeche, A., Belhoucine, F., Bouhadiba, S., Alioua, A., Belhabib, L., Rouabhi, Y. (2021): Evaluation of the metallic trace element contamination of common sole (*Solea solea*, L. 1758) by the western algerian coast. – Applied Ecology and Environmental Research 19(6): 4641-4664.
- [93] Taleb, Z., Benghali, S., Kadour, A. S., Boutiba, Z. (2007): Monitoring the biological effects of pollution on the Algerian west coast using mussels *Mytilus galloprovincialis*. – Oceanologia 4(49).
- [94] Tollefsen, K. E. (2006): Binding of alkylphenols and alkylated non-phenolics to the rainbow trout (*Oncorhynchus mykiss*) plasma sex steroid-binding protein. – Ecotoxicology and Environmental Safety 56: 215-225.
- [95] Turan, F., Eken, M., Ozyilmaz, G., Karan, S., Uluca, H. (2020): Heavy metal bioaccumulation, oxidative stress and genotoxicity in African catfish *Clarias gariepinus* from Orontes River. – Ecotoxicology 29(9): 1522-1537.
- [96] UNEP/RAMOGÉ (1999): Manual on the Biomarkers Recommended for the MED POL Biomonitoring Program. – UNEP, Athens.

- [97] USEPA (Environmental Protection Agency) (2000): Guidance for Assessing Chemical Contaminant. – In: Données à utiliser dans les avis aux Poissons. Fish sampling and analysis. 3re Ed. Office of Water, Washington DC, [EPA823-R-95-007].
- [98] Viana, L. F., Suárez, Y. R., Cardoso, C. A. L., Crispim, B. D. A., Cavalcante, D. N. D. C., Grisolia, A. B., Lima-Junior, S. E. (2018): The response of neotropical fish species (Brazil) on the water pollution: metal bioaccumulation and genotoxicity. – Archives of Environmental Contamination and Toxicology 75(3): 476-485.
- [99] WHO (World Health Organization). (1989): Heavy Metals-Environmental Aspects. – Environment Health Criteria 85, Geneva, Switzerland.
- [100] Wong, C. K., Wong, P. P. K., Chu, L. M. (2001): Heavy metal concentrations in marine fishes collected from fish culture sites in Hong Kong. – Archives of Environmental Contamination and Toxicology 40(1): 60-69.
- [101] Zhao, S., Feng, C., Quan, W., Chen, X., Niu, J., Shen, Z. (2012): Role of living environments in the accumulation characteristics of heavy metals in fishes and crabs in the Yangtze River Estuary, China. – Marine Pollution Bulletin 64(6): 1163-1171.