„Dunărea de Jos” University of Galaţi
Doctoral School of Industrial Mechanics and Engineering

PhD THESIS

SYNOPSYS

A research regarding heavy metals pressence in the natural and anthropogenic aquatic ecosystems from South-East Romania

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Eng. Ira-Adeline SIMIONOV

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Series I 4: Industrial Engineering No. 60

GALAŢI
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Table of content

INTRODUCTION

Water is not a commercial product like any other but, rather, a heritage which must be protected, defended and treated as such [1]. Water pollution is an altering process of the physical, chemical or biological quality parameters, and which is produced by human activities. A water body can be polluted not only when it shows visible changes (color changes, iridescent color of petroleum products, unpleasant odors) but also when, although apparently good, it contains, even in a small amount, potentially toxic substances [2]. Omar et al. (2013) characterizes the aquatic environment as the end-point "recipient" of natural or anthropogenic pollutants [3].

Metals have a priority role in terms of water quality monitoring and these elements are included In the lists of potentially toxic substances [2]. Some heavy metals such as mercury, lead or cadmium are compounds that cannot be degraded naturally, having a long remanence time in the environment, context in which their dangerousness is highlighted, a fact sustained by the high accumulation potential at the level of the food chain [2].

The industrial revolution and the technological development are the main events that led to the release of significant quantities of potentially toxic compounds in air, water and soil [4]. Due to the impossibility of degradation of the heavy metals, their negative influence persists over long periods of time and their neutralization can be obtained by dilution, association with organic compounds and mineralization.

Romania’s teritory disposes of all types of water resources and the largest freshwater resource is supplied by Danube River and the inland rivers [2]. Throughout its flowing course, Danube River accumulates discharges from various anthropogenic activities conducted in the 10 countries in which the river crosses. Metal contaminants are released into the Danube basin and some of them reach Danube Delta [5]. Also, in the context of significant increase of water flow there is the real possibility of contamination of downstream areas, after the high mobilization of heavy metals [2].

The domestic and industrial wastewaters exert a significant pressure on the aquatic environment, due to the high loads of the effluents with organic materials, nutrients and dangerous substances [2]. In the romanian rural area, 95.9% of the population is not connected to sewerage systems, so waste water management represents a main problem in the pressent time [6].

The aquatic organisms exposed to these contaminants will accumulate these elements and, sometimes, the concentration of metals related to the body mass of these organisms increases up the food chain. In case of conducted fishing activities in contaminated aquatic areas and, subsequently, by the consumption of fish catches, potentially toxic elements are transferred at the level of the human body, in which these elements can cause a series of diseases and the severity of them depends on the accumulated metal and the accumulated concentration.

Romania’s teritory surface is situated in proportion of 97.4% in the Danube River Basin and the Romanian Danube sector represents the final carrier of wastewater effluents, accumulated from the upstream countries, in the Black Sea [6].

The Black Sea is the main source of marine catches for the riverine countries [7]. In a market study conducted by the Romanian Ministry of Agriculture and Rural Development, it was specified that between the years 2005 and 2013, the average percentage of Romanian fish production was as it follows: 68% aquaculture, 27% fishing in continental waters and 5% marine [8].

Fish are among the most important groups of wild species, from the ecological but also from the economic perspective, representing a valuable food resource for both humans and wildlife [9]. They play a major role in the functioning of aquatic ecosystems [9].

In the same time, fish are recognized bioindicators in the assessment of the aquatic environment pollution with different substances. Therefore, this is possible by monitoring the dynamics of heavy metal concentrations in aquatic organisms.

In order to avoid a polluting disaster, such as the one in Minamata, Japan, manifested by the chronic mercury and cadmium intoxication of human population, following the consumption of contaminated fish, continuous monitoring of environmental pollution is mandatory [10].

The necessity and applicative value of the paper

The present doctoral thesis is meant to bring a significant contribution in regards to the theme related to the presence of heavy metals in the natural and anthropogenic aquatic ecosystems of south-eastern Romania. Thus, in the context of the need for continuous monitoring of the natural and anthropogenic aquatic ecosystems, the present paper contributes for the elaboration of a holistic picture regarding their quality, in order to establish a fast intervention management plan, as well as for the application of an efficient control management, in the cases that require this thing. Therefore, through the elaboration of comparative analyzes between the natural aquatic ecosystems, respectively the anthropogenic ones, clarification of several aspects regarding the bioaccumulation of metals and metaloids at the level of the study area is aimed. The novelty degree and utility of the present doctoral thesis is highlighted by the complexity and the amplitude of the multilevel comparative studies, the geographical areas of sampling, the multitude of fish species analyzed, the use of data processing mechanisms based on the machine learning technique, the actual applicative value of the paper, as well as the obtained elucidating results.

The **general objectives** of this doctoral thesis **“*A research regarding heavy metals pressence in the natural and anthropogenic aquatic ecosystems from South-Est Romania*”** are the following:

- ✓ heavy metals accumulation degree in the organism of different autochthonous fish species, from natural and anthropogenic aquatic ecosystems;
- ✓ water quality assessment in the studied natural and anthropogenic aquatic ecosystems, from the physico-chemical parameters point of view, respectively the concentration of metals with high toxicity potential;
- ✓ classification of the water bodies afferent to the studied aquatic ecosystems in quality classes and the determination of their pollution index;
- ✓ characterization of sediments prelevated from the studied aquatic ecosystems, from the concentration of different metals, respectively of organic carbon and total nitrogen point of view;
- ✓ determination of the estimated daily intake in order to identify the risk to human health, associated with the ingestion of heavy metals, following fish consumption;

correlation and predictive analyzes associated with concentrations of metals in water, sediments and fish biomass.

The thesis is divided into two parts as follows:

I. **DATA ANALYSIS OF SPECIALITY LITERATURE** consists of two chapters and presents recent data on the addressed issue. In these chapters, it has been realised a thorough analysis on the presence of heavy metals in aquatic ecosystems in the macro-system Danube-Danube Delta-Black Sea. The obtained data from the documentation was centralized and processed to generate a clearer picture of how the topic addressed.

II. EXPERIMENTAL ACTIVITY consists of 6 chapters and it includes original investigations carried out during the doctoral stage. Within these chapters, the characterization of the natural and antropogenic ecosystems was realised. Also, the recorded data was used to calculate the pollution index (PI), the estimated daily intake (EDI), various correlations and predictions.

The present PhD thesis contains 239 pages, which includes 40 tables and 397 graphs, the documentation study representing a percentage of 12.5% and a percentage of 87.5% the experimental part.

The research activities of the doctoral thesis were carried out using the infrastructure of the Romanian Center for the Modeling of Recirculating Aquaculture Systems (MoRAS), an integrated part of the multidisciplinary research platform ReForm - UDJG, within the Faculty of Food Science and Engineering, University “Dunărea de Jos” from Galați.

The PhD candidate was involved during the doctoral stage in the project research team of: H2020-MSCA-RISE-2014 with the acronym “ECOFISH”, PN-III-P3-3.6- H2020-2016- 0110 with the title “Researches on the potential transformation of of conventional fish farms in organic farms by establishing a model and a guide of good practices ”, PE no. 124/24.05.2018 with the title "Guide of good practices on the reproduction and post-embryo development of the freshwater fish species from Romania - basic model in the development of the national fisheries sector".

CHAPTER I. Heavy metals pressence in the studied aquatic ecosystems: state of the art**.**

The main objective of this chapter was to present the general properties, particularities and origin of metals in aquatic ecosystems, as well as the legal framework regarding the presence of heavy metals in the aquatic environment.

1.1. General properties of metals

Functional descriptive terms accepted for classifying metals and used in the carried out studies on the environment are: "trace metals", "micro-nutrient" and "heavy metals" [11]. Trace amounts of metal are metals that are present in concentrations of less than 0.1% (<1000 mg kg-1) in the soil. The term "micro-nutrient" is used to describe those elements that are required in small quantities by certain organisms to meet the metabolic functions. The term "heavy metal" is used to describe the elements which have an atomic number greater than 20. Duruibe et al., (2007) and Oves et al. (2012) defined heavy metals as the group metals and metalloids with an atomic density higher than 4g/cm3 or 5 times higher than the density of water [12] [13].

The metals are circulated naturally in the environment and metallic elements are found in all living organisms, where they play a variety of roles. Metals can have structural role, act as stabilizers for biological structures; they are components and control mechanisms of the enzymatic activators of redox systems. Therefore, some metals are essential elements, and their deficiency leads to impaired biological functions. When present in excess, essential metals can also become toxic. Other metals do not possess essential function and can pose toxicity, even in small amounts. Unlike most organic chemicals that can be removed from the tissues by metabolic degradation, metals are indestructible elements and, therefore, have potential of accumulation. Excretion is the main mechanism for removal of metals from tissues. Accumulation in the tissues does not necessarily imply the occurence of a toxic effect, due to the formation of inactive complexes or metal deposits.

1.2. Characteristics of heavy metals found in aquatic ecosystems

The aquatic toxicity and bioavailability of different metals are influenced by abiotic factors such as pH, water hardness, alkalinity and the accumulation of humic substances [14] [15]. The toxicity of metals in water decreases with the increase of alkalinity, pH, salinity, conductivity and temperature [16]. According to Strungaru et al. (2015), the two most important parameters influencing the accumulation metals in the biota are pH and salinity [17]. There is a strong correlation between these parameters and the accumulation of metals, so that an acidic medium results in an increase in the accumulation of metals in biota [17]. For many metals, alkalinity is a co-factor more important than the water hardness [18]. Salts of heavy metals (Mn, Co, Ni, Cr, As, Cd, Pb, Fe, Sn, Sb, Au, Ag, Cu, Hg) are stable and toxic compounds, therefore can represent forms of severe pollution of surface water [19]. Inorganic complexes, organic insoluble or partially soluble forms of metals are less toxic than simple ions [19]. Analyzing their impact on the fish, metals may be classified as essential: iron (Fe), zinc (Zn), copper (Cu), magnesium (Mg), selenium (Se), cobalt (Co), vanadium (Vn) and non-essential elements (potentially toxic trace elements), aluminum (Al), arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), bismuth (Bi) [20]. Burada (2014) stated that some toxic metals have a greater impact on the environment, compared with others, such as Cd, Cr, Cu, Pb, Ni and Zn [18].

Metals in the aquatic environment can cause biodiversity loss by exerting toxic effects on the biota [21] [22] [23]. As pollutants in water bodies, heavy metals are of particular importance because of their inability to decompose, long persistence, bio-accumulation and bio-magnification in the food chain [46, 47].

1.3. The presence of heavy metals in water, sediment and fish body

According to Heath (1995), the distribution of heavy metals in the aquatic environment is as follows: distributed in the water column, precipitated in sediments, accumulated in the benthic substrate, bind to other organic elements or accumulated by organisms, fact confirmed by Burada (2014) [30] [48]. The metallic elements transported in the aquatic systems are subjected to specific processes, manifested under the direct influence of the physico-chemical parameters of the water.

Di Giulio and Hinton (2008) defined the phenomenon of bioavailability as the relative ease of an element to be transferred from the environment to a specific location of an organism of interest and refers to both the water component and the sediment component [45]. The bioavailability of metals in the water column refers to their water-soluble capacity, the soluble form of the elements being one of the most toxic forms for fish.

Purification of the water column is achieved due to important mechanisms such as adsorption and sedimentation processes [49]. Sediments have the ability to immobilize metal ions through specific processes such as adsorption, flocculation and co-precipitation. Therefore, sediments can act as hot spots of high metals concentration [50]. In shallow lakes, metals are more likely to undergo resuspension and may cause secondary contamination to the aquatic environment. Thus, sediments can act as both a decanter and a source of metals in the aquatic environment [51] [52].

A process of particular importance is bioaccumulation in aquatic organisms [56] [57]. The rate of bioaccumulation is influenced by certain factors such as temperature and physiological state of the organism (sex, age, size). Heavy metals, when ingested in excessive amounts, can lead to random connections with cellular biomolecules, such as enzymes or proteins, to form complexes that may compromise their structure and/or function [12]. According to Bat et al. (2015), heavy metals tend to accumulate in organisms at the top of the food chain (such as fish in the aquatic environment), through biomagnification effects [58]. The biomagnification process is a complex process that involves increasing the concentration of toxins upward in the food chain.

1.4. Origin of metals in river-delta-sea macro system

In recent years, rivers, deltas and coastal areas are facing increasing pressures and river pollution is a cross-border issue [67]. The river-sea systems and their transitional aquatic environments (deltas) face multiple pressures arising from the dynamic interaction of physical, chemical and biological processes on natural and anthropogenic triggers. In river environments, heavy metals come from different natural and anthropogenic sources. Over 90% of the content of Cd, Pb, Mn, Ni and Zn present in freshwater and sediments comes from human activities [18].

Natural processes can generate metallic elements in the aquatic environment such as rock erosion and disaggregation (for example, all types of rocks and soils contain low amounts of cadmium and arsenic).

1.5. The legal framework for the presence of heavy metals in aquatic ecosystems

A number of laws have been adopted to protect the environment from contamination with heavy metals and other metalic elements. As a result, the Official Monitor no. 352 from 27/05/2002 published the amendment adopted in the framework of the Basel Convention, regarding the consolidation, revision and adjustment of the lists of hazardous waste, so that on these lists are included the metallic waste or waste containing metallic elements [84]. Regarding the wastewater disposal, Decision 352/2005 from 05/05/2005, published in the Official Gazette of Romania, regulates the specific rules regarding the conditions of discharge in the aquatic environment of the wastewater (Table 4) [85]. Thus, it is decided that the sum of the heavy metal ions should not exceed the concentration of 2 mg/dm3.

CHAPTER II. Heavy metals and their accumulation tendency at the level of aquatic ecosystems

The main purpose of this chapter was to make a thorough documentation of the literature on the addressed issues, centralize and process the obtained data in order to outline a clear picture of the presence of heavy metals in the studied aquatic ecosystems.

2.1. Natural aquatic ecosystems (natural ecosystems from Romania)

2.1.1. Danube River

Pollution of Danube River is determined by the following: point sources (municipal, industrial and agricultural), diffuse sources (farms and congestion), the modifying effects of water abstraction, regulating or morphological changes [71]. A number of scientific papers on the presence of heavy metals in the muscle tissue of fish from the Danube River were reviewed in order to outline an accurate picture of the current situation [44] [92] [93] [95] [96] [97] [98]. The data were summarized by Simionov et al. (2016) [99]. The data related to the heavy metals from the Danube River fish meat (73.5 to 1503 km) have been reported in 5 species of fish, as follows: *Alosa immaculata* (Bennett, 1835), *Cyprinus carpio* (Linnaeus, 1758), *Silurus glanis* (Linnaeus, 1758), *Sander lucioperca* (Linnaeus, 1758), *Acipenser ruthenus* (Linnaeus, 1758).

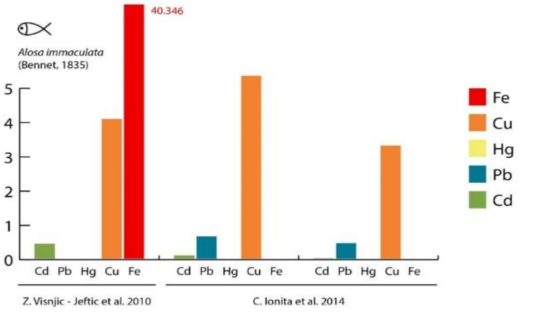


Fig. 2.1. Concentration of heavy metals in *Alosa immaculata* from Danube River [99]

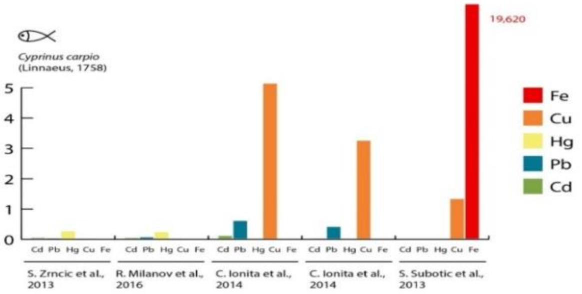


Fig. 2.2. Concentration of heavy metals in *Cyprinus carpio* from Danube River [99]

Following the examined scientific data, the maximum concentration of cadmium is reported in *Alosa immaculata* - 863 km (0.433 mg/g dry weight), followed by *Cyprinus carpio* - from 73.5 km (0.010 mg/g dry weight) and Silurus glanis - km 1173, 1169 and 1170 (0.01 mg/g dry weight) (Figures 2.1., 2.2., 2.3., 2.4., 2.5.) [99].

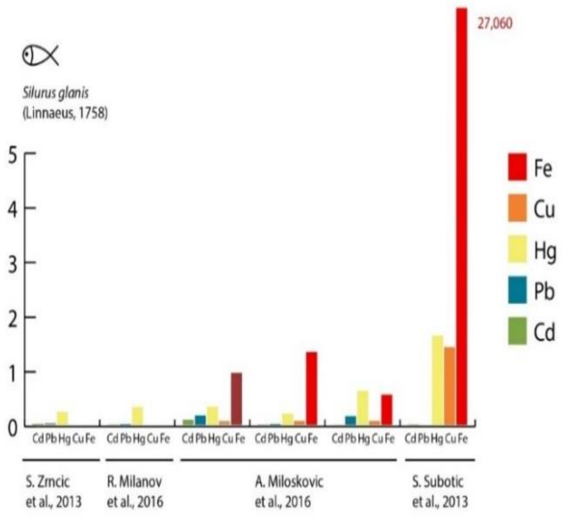


Fig. 2.3. Concentration of heavy metals in *Silurus glanis* from Danube River [99]

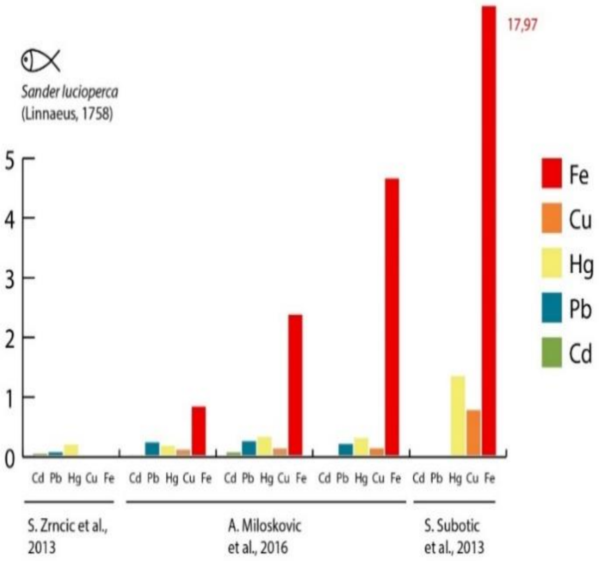


Fig. 2.4. Concentration of heavy metals in *Sander lucioperca* from Danube River [99]

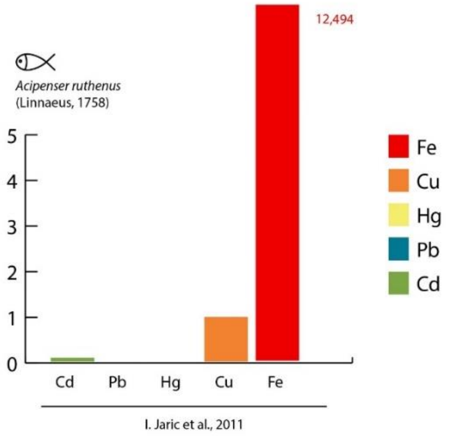


Fig. 2.5. Concentration of heavy metals in *Acipenser ruthenus* from Danube River [99]

2.1.2. Danube Delta

Regarding the accumulation of heavy metals in Danube Delta, a number of authors have conducted studies on this subject [18] [30] [100] [102] [103] [104] [105].

Tudor et al. (2006) sampled the muscle of several fish species from 9 different locations in Danube Delta and recorded mean values as follows: for Cd 0.19±0.06 mg kg^{-1} in the muscle tissue of prusian carp, 0.20±0.08 mg kg^{-1} in the muscle tissue of pike-perch, for Pb 2.4 mg kg^{-1} in the muscle tissue of pike, 2.6 mg kg^{-1} in the muscle tissue of prusian carp, 3.9 mg kg^{-1} in the tissue of perch, 1.3 mg kg^{-1} in the muscle tissue of roach [101].

2.1.3. Black Sea

A number of studies have been conducted [27] [39] [46] [58] [117] [118] [119] [120] [121] [122] [123] [124] [125] for determining the level of heavy metals in the Black Sea fish. The sampling areas from these studies are shown in Figure 2.6.

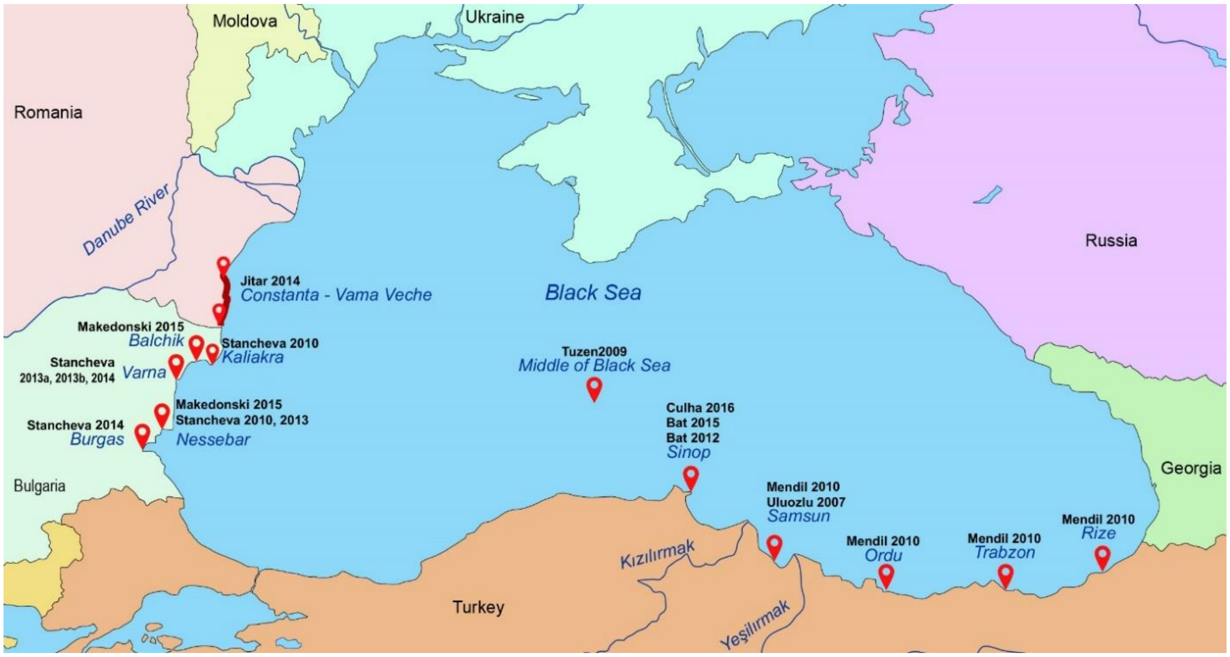


Fig. 2.6. Sampling locations in different studies aimed to assess the concentration of heavy metals in the Black Sea [126].

The highest concentrations of heavy metals in fish tissues from the Black Sea are associated with the flounder (*Mullus barbatus*), followed by the blue fish (*Pomatomus saltatrix*). Also, of all the studied metals, Zn recorded the highest concentrations in all fish species, followed by Fe, Cu and As.

Maximum concentrations of heavy metals in all reviewed studies did not exceed the maximum limits allowed by European legislation. South-Central part of the Black Sea (the Turkish seacoast represented by the sector located between rivers Kizilirmak and Yesilirmak) proved to be more polluted in terms of heavy metals accumulation in fish flesh compared with the South-East (Turkish coastline) and the West (Romanian and Bulgarian coasts).

2.2. Anthropogenic ecosystems

At the level of anthropogenic ecosystems, a number of distinctive features are identified, both in terms of the technical part, which involves their design and functioning, as well as related to the technological part, in conjunction with the ecological one.

2.2.1. Extensive fish ponds

Simionov et al. (2019) reviewed a number of scientific articles regarding the presence of heavy metals in earthen ponds [40] [140] [141] [142] [143] and the data are centralized in Table no. 9.

Tabelul 9. Concentration of metalas in the water and sediment component of earthern fish ponds, according to different authors [140]

Element	Water	Sediment	Reference
Fe	0,32±0,04 mg ml $^{-1}$	-	Onuoha, 2017
	0,41 ± 0,27 mg L^{-1}	38,067±3166 mg kg^{-1} dw	Ben Salem și colab., 2014
Zn	9,90 ± 0,21 mg L^{-1}	371,17 ± 250.24 mg kg^{-1} dw	
	5,39 µg L^{-1}	116,6mg kg^{-1} dw	Ju și colab., 2017
Cu	1,97µg L^{-1}	39,8mg kg^{-1} dw	
	15,10 ± 1,04 mg L^{-1}	177,21±127,42 mg kg^{-1} dw	Ben Salem și colab., 2014
Cr	9.70 ± 0,04 mg L^{-1}	70,02±19,11 mg kg^{-1} dw	
	0,2µg L^{-1}	51,4 mg kg^{-1} dw	Ju și colab., 2017
Mn	0,16 ± 0,11 mg L^{-1}	2877 ± 886 mg kg^{-1} dw	Ben Salem și colab., 2014
	0,08±0,04 mg ml^{-1}	-	Onuoha, 2017
Ni	10,61±1,84 mg L^{-1}	45,61±9,51 mg kg^{-1} dw	Ben Salem și colab., 2014
	1,61 µg L^{-1}	24,1 mg kg^{-1} dw	Ju și colab., 2017
As	< 0,015 mg L^{-1}	1,16 mg kg^{-1} dw	Feldlite și colab., 2008
	0,22µg L^{-1}	5,2 mg kg^{-1} dw	Ju și colab., 2017
Pb	0,14±0,01 mg L^{-1}	37,29±2,65 mg kg^{-1} dw	Ben Salem și colab., 2014
	< 0,01 mg L^{-1}	1,45 mg kg^{-1}	Feldlite și colab., 2008
	0,39 µg L^{-1}	18,3mg kg^{-1} dw	Ju și colab., 2017
Cd	0,0105± 0,0007 mg L^{-1}	1,67±1,49 mg kg^{-1} dw	Ben Salem și colab., 2014
	< 0,003 mg L^{-1}	< 0,003 mg kg^{-1}	Feldlite și colab., 2008

2.2.2. Recirculating aquaculture systems

In recirculating aquaculture systems, the possible sources of heavy metal contamination are erosion of the component parts and the use of fish feed [152] [153]. In muscle and liver tissue of the fish specie *Orechromis niloticus* the concentrations varied, with higher values observed in the liver (Table 10).

Tabelul nr. 10. Concentrations of heavy metals in Orecochromis niloticus, reared in a RAS system [140]

Element (mg kg^{-1} wet weight)	Muscle tissue	Hepatic tissue
Zn	3,23±1,24	3,29±1,30
Fe	ND	3,79±1,79
Cu	ND	28,4±14,7
Cr	0,02±0,02	0,06±0,06
Mn	0,10±0,02	0,17±0,07

Ni	0,01±0,01	0,05±0,07
As	0,85±0,42	0,04±0,02
Cd	0,00±0,00	0,02±0,01
Pb	0,00±0,00	0,01±0,01

CHAPTER III. Materials, methods and investigation techniques

Within this chapter are described the materials, methods and working steps undertaken to determine the different metals in water, sediments and fish tissues, as well as the methods of calculating the pollution index and the estimated daily intake. For the quantification of the elements Ca, Mg, Na, K, Zn and Fe the HR-CS-FAAS (High resolution-Continuum Source-Flame Atomic Absorption Spectrometry) technique was used and for the quantification of the Cu, Mn, Ni, As, Cd, Pb and Cr elements the HR-CS-GF-AAS (High resolution-Continuum Source-Graphite Furnace-Atomic Absorption Spectrometry) technique.

As well, the methodology applied in case of correlation and prediction analysis is described in detail.

The studied natural and anthropogenic aquatic ecosystems are adjacent to the Danube-Danube Delta-Black Sea macro-system and the studied fish material consists of the following fish species: *Abramis brama* (Linaeus, 1758), *Leuciscus aspius* (Linnaeus, 1758), *Alosa immaculata* (Bennett, 1835), *Silurus glanis* (Linnaeus, 1758), *Cyprinus carpio* (Linnaeus, 1758), *Carassius gibelio* (Linnaeus, 1758), *Esox lucius* (Linnaeus, 1758), *Trachurus mediterraneus ponticus* (Steindachner, 1868), *Mugil cephalus* (Linnaeus, 1758), *Platichthys flesus* (Linnaeus, 1758), *Acipenser gueldenstaedtii* (Brandt and Ratzeburg, 1833), *Psetta maxima maeotica* (Linnaeus, 1758).

CHAPTER IV. Evaluation of heavy metals pressence in the studied natural aquatic ecosystems

The main objective of this chapter was to evaluate the studied natural aquatic ecosystems (Danube River, Danube Delta and Black Sea) from the point of view of the water physico-chemical parameters and the presence of different metals in sampled water, sediments and biological material.

4.1. Physico-chemical water parameters and ecological state of natural aquatic ecosystems

Parametrii fizico-chimici ai apei determinați au fost după cum urmează: temperatură (T°C), oxigen dizolvat (O_2), pH, salinitate, nitriți (NO_2^-), amoniac (NH_3), cloruri (Cl^-), bicarbonați (HCO_3^-), electroconductivitate (EC) și totalul de solide dizolvate (TDS). Datorită influenței majore asupra bunăstării biomasei piscicole, în acest rezumat sunt reprezentați grafic doar compușii azotului, respectiv NO_2^- și NH_3.

4.1.1. Inferior sector of Danube River (Lower Danube)

4.1.1.1. Harbour Galați (S3)

The recorded values for the concentrations of NO_2^- and NH_3 in the water sampled from Danube River, Galați Harbor, exceeded the maximum values allowed by the national legislation in force (HG no. 202/2002) (Figures 4.1.4. And 4.1.5.).

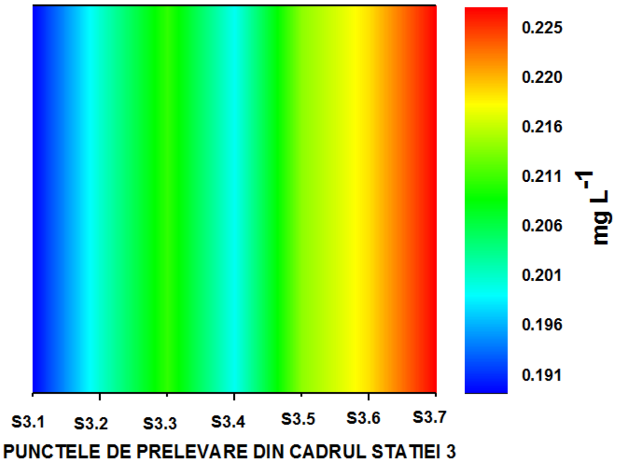


Fig. 4.1.4. Concentration of NO_2^- in the water samples from Station no. 3

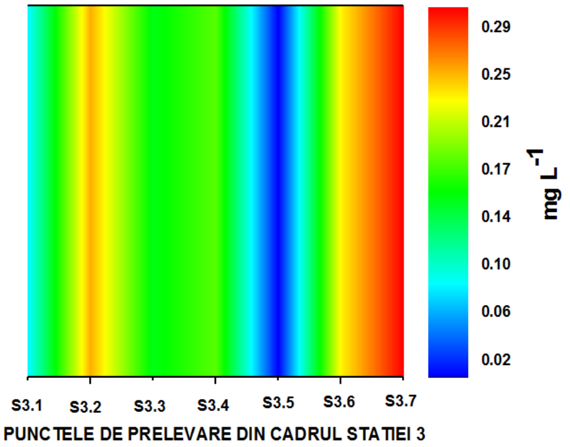


Fig. 4.1.5. Concentration of NH_3 in the water samples from Station no. 3

4.1.1.2. Harbour Tulcea (S2)

The recorded values for the concentrations of NO_2^- and NH_3 in the water sampled from Danube River, Tulcea Harbor, exceeded the maximum values allowed by the national legislation in force (HG no. 202/2002) (Figures 4.1.13. and 4.1.14.).

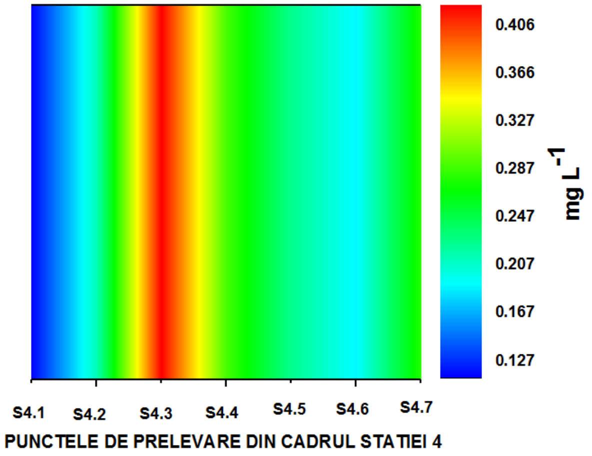


Fig. 4.1.13. Concentration of NO_2^- in the water samples from Station no. 4

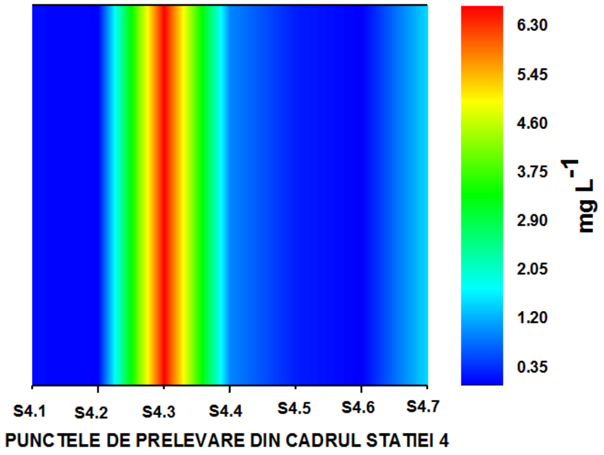


Fig. 4.1.14. Concentration of NH_3 in the water samples from Station no. 4

4.1.2. Danube Delta

4.1.2.1. Barcaz Lake (S6)

The recorded values for the concentrations of NO_2^- and NH_3 in the water sampled from Danube Delta, Barcaz Lake, exceeded the maximum values allowed by the national legislation in force (HG no. 202/2002) (Figures 4.23. and 4.24.).

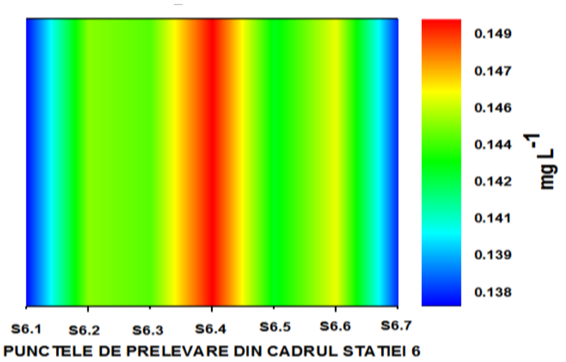


Fig. 4.23. Concentration of NO_2^- in the water samples from Station no. 6

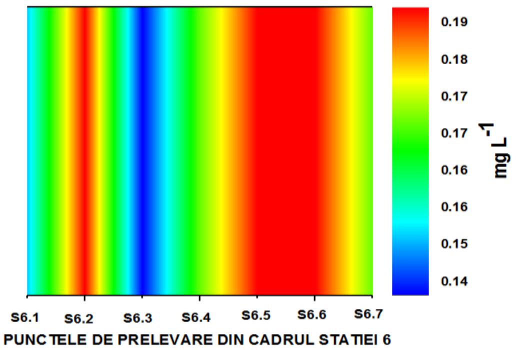


Fig. 4.24. Concentration of NH_3 in the water samples from Station no. 6

4.1.2.2. Soschi Lake (S7)

The recorded values for the concentrations of NO_2^- and NH_3 in the water sampled from Danube Delta, Soschi Lake, exceeded the maximum values allowed by the national legislation in force (HG no. 202/2002) (Figures 4.1.33. and 4.1.34.).

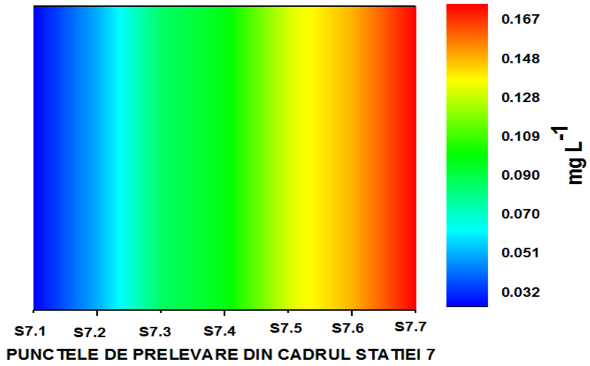


Fig. 4.1.33. Concentration of NO_2^- in the water samples from Station no. 7

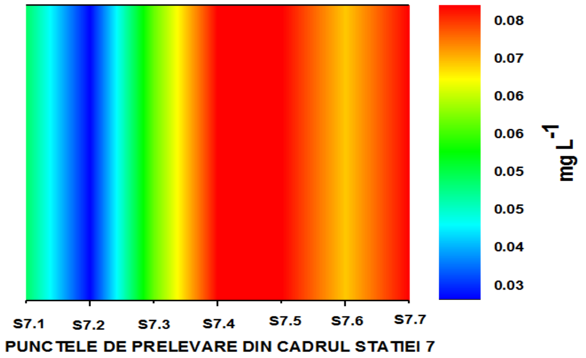


Fig. 4.1.34. Concentration of NH_3 in the water samples from Station no. 7

4.1.3. Black Sea

4.1.3.1. Sf. Gheorghe (S8)

The recorded values for the concentrations of NO_2^- and NH_3 in the water sampled from Black Sea, Sf. Gheorghe, exceeded the maximum values allowed by the national legislation in force (HG no. 202/2002) (Figures 4.1.42. and 4.1.43.).

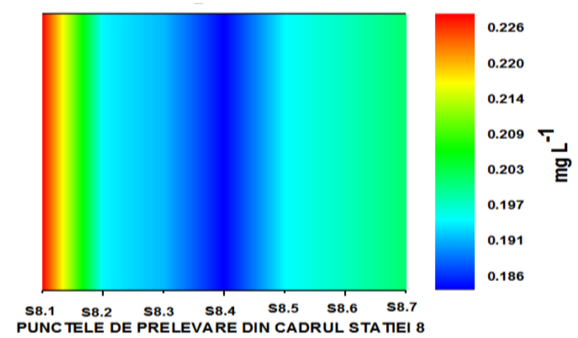


Fig. 4.1.42. Concentration of NO_2^- in the water samples from Station no. 8

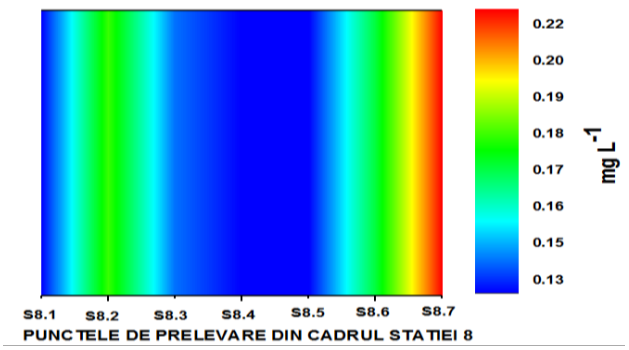


Fig. 4.1.43. Concentration of NH_3 in the water samples from Station no. 8

4.1.3.2. Perișor (S9)

The recorded values for the concentrations of NO_2^- and NH_3 in the water sampled from Black Sea, Perișor, exceeded the maximum values allowed by the national legislation in force (HG no. 202/2002) (Figures 4.1.52. and 4.1.53.).

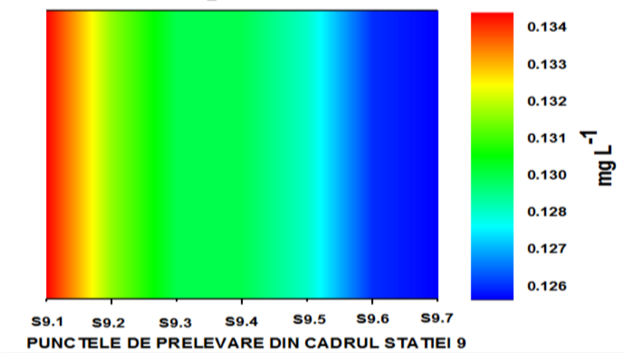


Fig. 4.1.52. Concentration of NO_2^- in the water samples from Station no. 9

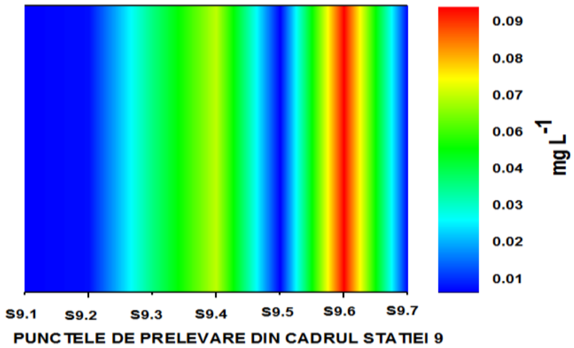


Fig. 4.1.53. Concentration of NH_3 in the water samples from Station no. 9

4.2. Particularities of heavy metal uptake mechanisms in natural aquatic ecosystems

The metals, respectively the metalloids determined from water, sediments and fish material were as it follows: Pb, Cd, As, Ni, Cu, Fe, Zn, Ca, Mg, K and Na. Due to the high toxicity potential, the values of Cd and Pb concentrations are represented graphically in this summary**.**

4.2.1. Inferior sector of Danube River (Lower Danube)

4.2.1.1. Harbour Galați (S3)

The concentration of Pb (Figure 4.2.1.) in the water sampled from the Danube River, Harbour Galați recorded the maximum value (3.57 µg L^{-1}) in point S3.1 and the minimum value (2.74 µg L^{-1}) in point S3.2. Regarding the concentration of Pb in the collected sediments, the maximum value (13.82 µg g^{-1}) was recorded in point S3.1 and the minimum value (7.75 µg g^{-1}) in point S3.3.

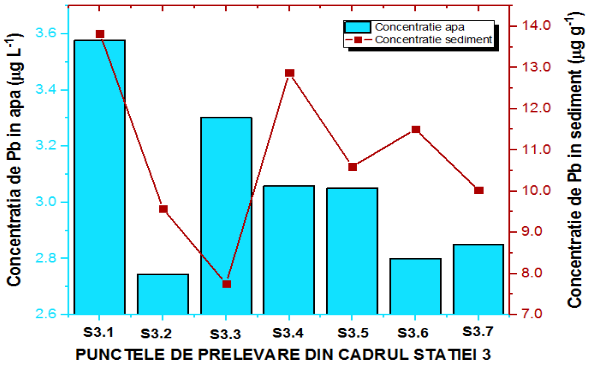


Fig. 4.2.1. Concentration of Pb in water and sediments

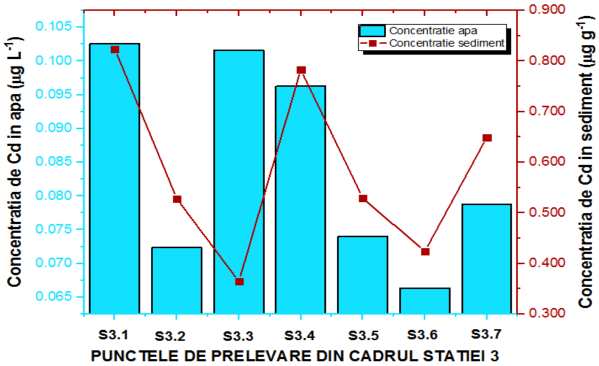
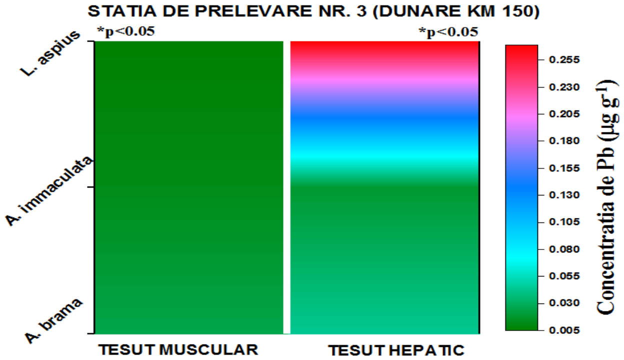


Fig. 4.2.2. Concentration of Cd in water and sediments

The Cd concentration (Figure 4.2.2.) in the water sampled from Danube River, Harbour Galați, registered the maximum value (0.1026 µg L^{-1}) in point S3.1. and the minimum value (0.066 µg L^{-1}) in point S3.6. Regarding the Cd concentration in the analyzed sediments, the maximum value (0.82 µg g^{-1}) was recorded in point S3.1. and the minimum value (0.36 µg g^{-1}) in point S3.3.

The recorded values of the Pb concentration in the muscle tissues sampled in this study did not exceed the maximum level allowed, for human consumption, by the European legislation in force (Figure 4.2.13.) [90]. The values recorded in the liver, for the analyzed fish species (commom bream, shad and asp), increased with 0.73, 0.61, respectively 48.74 more, compared with those recorded in muscles.

The recorded values of Cd concentration in the muscle tissues sampled in this study did not exceed the maximum level allowed, for human consumption, by the European legislation in force (Figure 4.2.12.) [90]. The values recorded in the liver, for the analyzed species (platica, ash and avat) increased by 12.59, 3.96, respectively 10.73 more, compared to those recorded in muscles. The values recorded in the liver, for the analyzed species (commom bream, shad and asp) increased with 0.73, 0.61, respectively 48.74 more, compared with those recorded in muscles.



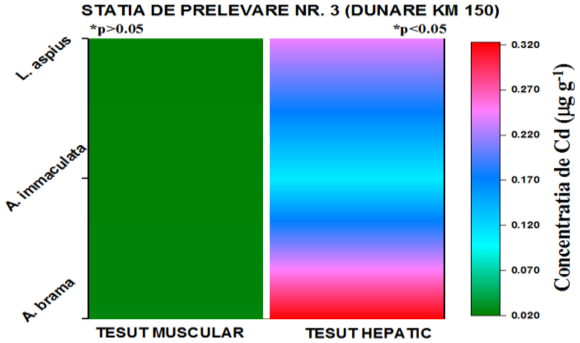


Fig. 4.2.13. Concentration of Pb in different fish species

Fig. 4.2.12. Concentration of Cd in different fish species

4.2.1.2. Harbour Tulcea (S4)

The concentration of Pb (Figure 4.2.22.) in the water sampled from Danube River, Harbour Tulcea, recorded the maximum value (1.503 µg L^{-1}) in point S4.1 and the minimum value (1.276 µg L^{-1}) in point S4.2. Regarding the concentration of Pb in the sampled sediments, the maximum value (5.002 µg g^{-1}) was recorded in point S4.1 and the minimum value (7.75 µg g^{-1}) in point S4.2.

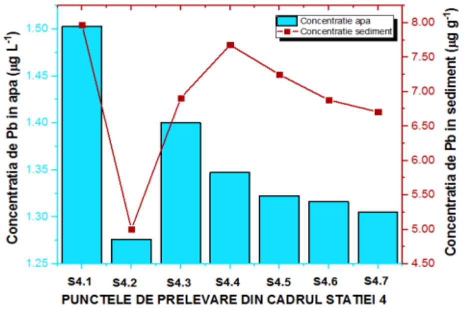


Fig. 4.2.22. Concentration of Pb in water and sediments

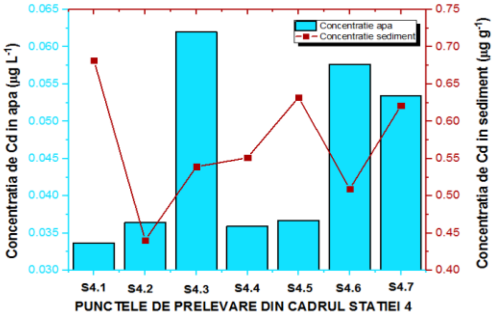


Fig. 4.2.23. Concentration of Cd in water and sediments

The concentration of Cd (Figure 4.2.23.) in the water sampled from Danube River, Harbour Tulcea, registered the maximum value (0.062 µg L^{-1}) in point S4.3. and the minimum value (0.033 µg L^{-1}) in point S4.1. Regarding the Cd concentration in the analyzed sediments, the maximum value (0.681 µg g^{-1}) was recorded in point S4.1. and the minimum value (0.440 µg g$^{-1)}$ in point S4.2.

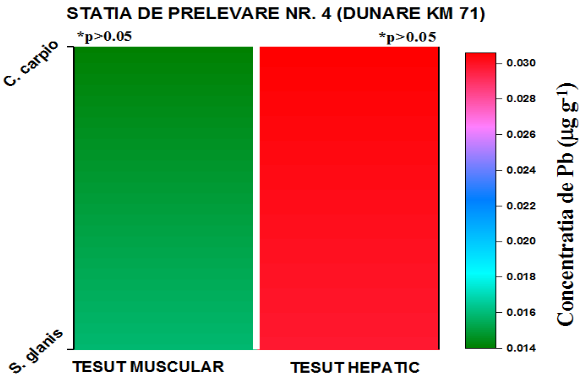


Fig. 4.2.34. Concentration of Pb in different fish species

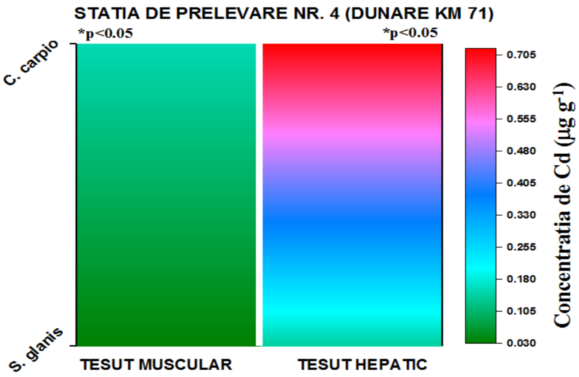


Fig. 4.2.33. Concentration of Cd in different fish species

The recorded values of Pb concentration in the muscle tissues sampled in this study did not exceed the maximum level allowed, for human consumption, by the European legislation in force (Figure 4.2.34.) [90]. The values recorded in the liver, for the analyzed species (catfish and carp) increased with 0.90, respectively 1.21 more, compared to those recorded in muscles.

The recorded values for Cd concentration in the muscle tissues sampled in this study exceed the maximum level allowed, for human consumption, by the European legislation in force (Figure 4.2.33.) [90]. The values recorded in the liver, for the analyzed species (catfish and carp) increased by 3.43, respectively 3.71 more, compared to those recorded in muscles.

4.2.2. Danube Delta

4.2.2.1. Barcaz Lake (S6)

The concentration of Pb (Figure 4.2.42.) in the water sampled from Danube Delta, Lake Barcaz, recorded the maximum value (0.888 µg L^{-1}) in point S6.2 and the minimum value (0.652 µg L^{-1}) in point S6.1. Regarding the concentration of Pb in the collected sediments, the maximum value (9.831 µg g^{-1}) was recorded in point S6.5 and the minimum value (8.472 µg g^{-1}) in point S6.1.

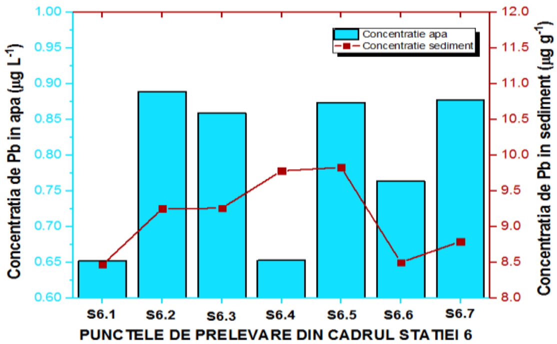


Fig. 4.2.42. Concentration of Pb in water and sediments

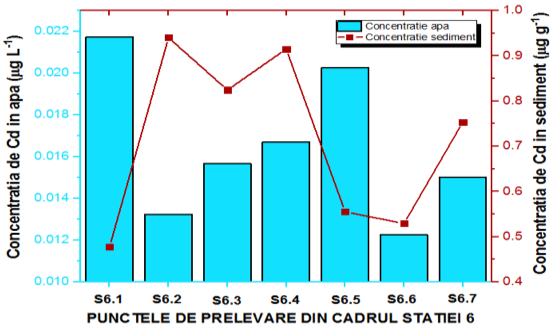


Fig. 4.2.43. Concentration of Cd in water and sediments

The concentration of Cd (Figure 4.2.43.) in the water sampled from Danube Delta Lake Barcaz recorded the maximum value (0.021 µg L^{-1}) in point S6.1. and the minimum value (0.012 µg L^{-1}) in point S6.6. Regarding the Cd concentration in the analyzed sediments, the maximum value (0.940 µg g^{-1}) was recorded in point S6.2. and the minimum value (0.478 µg g^{-1}) in point S6.1.

The recorded values of the Cd concentration in the catfish and carp muscle tissues sampled in this study exceed the maximum level allowed, for human consumption, by the European legislation in force (Figure 4.2.54.) [90]. In case of *S. glanis* and *C. gibelio*, the values recorded in the muscles increased by 33.95, respectively by 0.89 times, compared with those recorded in the liver.

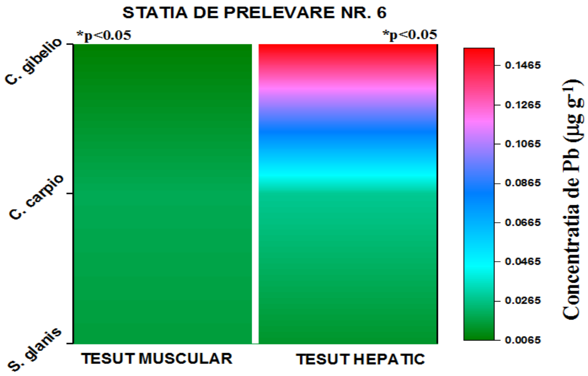


Fig. 4.2.53. Concentration of Pb in different fish species

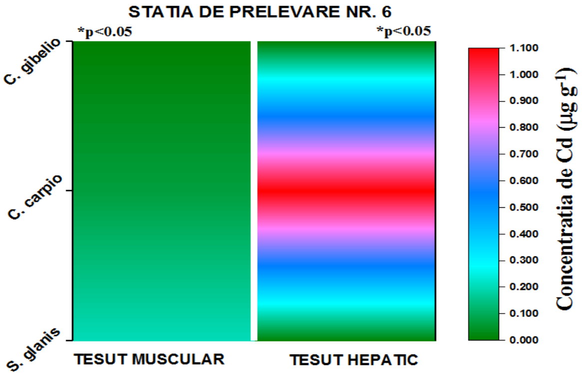


Fig. 4.2.54. Concentration of Cd in different fish species

The recorded values of the Pb concentration in the muscle tissues sampled in this study did not exceed the maximum level allowed, for human consumption, by the European legislation in force (Figure 4.2.53.) [90]. The values recorded in the muscles of *S. glanis* increased 0.19 times compared to those recorded in the liver. In the case of *C. carpio* and *C. gibelio* the values recorded in the liver increased by 0.49, respectively by 22.67 times, compared to those recorded in the muscles.

4.2.2.2. Soschi Lake (S7)

The concentration of Pb (Figure 4.2.62.) in the water sampled from Danube Delta, Lake Soschi, recorded the maximum value (0.6813 µg L^{-1}) in point S7.1 and the minimum value (0.5020 µg L^{-1}) in point S7.6. Regarding the concentration of Pb in the collected sediments, the maximum value (8,592 µg g^{-1}) was recorded in point S7.7 and the minimum value (7,017 µg g^{-1}) in point S7.1.

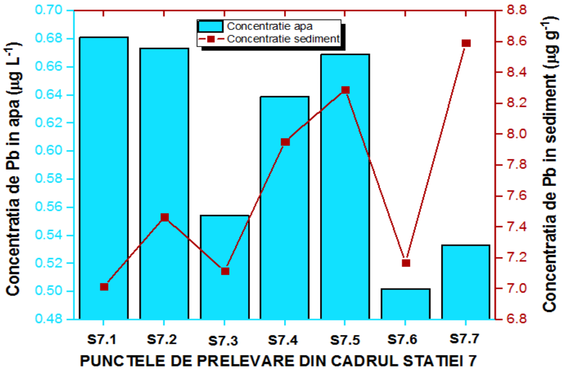


Fig. 4.2.62. Concentration of Pb in water and sediments

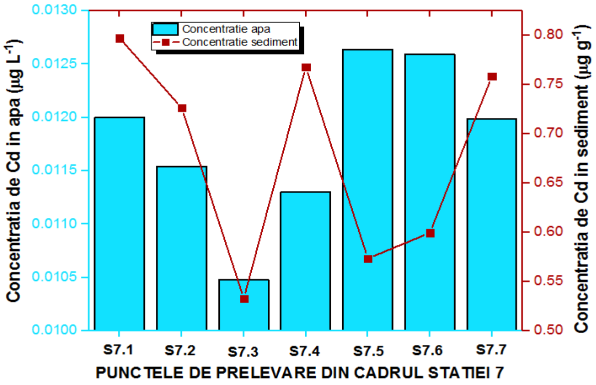


Fig. 4.2.63. Concentration of Cd in water and sediments

The concentration of Cd (Figure 4.2.63.) in the water sampled from Danube Delta, Lake Soschi, registered the maximum value (0.0126 µg L^{-1}) in point S7.5. and the minimum value (0.0104 µg L^{-1}) in point S7.3. Regarding the Cd concentration in the analyzed sediments, the maximum value (0.797 µg g^{-1}) was recorded in point S7.1 and the minimum value (0.532 µg g^{-1}) in point S7.3.

The recorded values of Cd concentration in the muscle tissues of the studied fish did not exceed the maximum level allowed for human consumption, regulated by the European legislation in force (Figure 4.2.74.) [90]. The values recorded in the liver of the analyzed species (catfish, carp, pike, prussian carp) increased by 5.88, 14.38, 0.10, respectively by 3.64 times, compared with those recorded in the muscles.

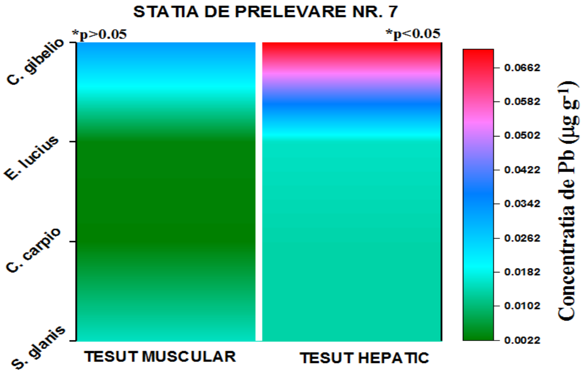


Fig. 4.2.73. Concentration of Pb in different fish species

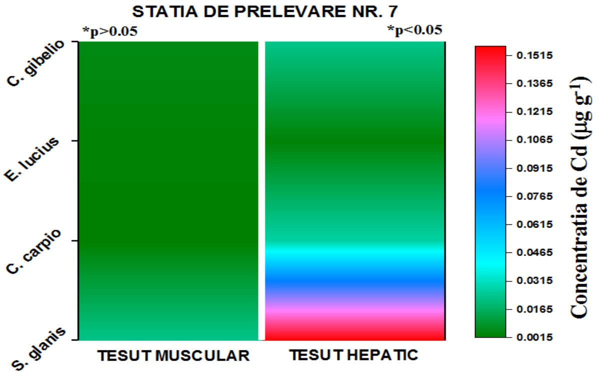


Fig. 4.2.74. Concentration of Cd in different fish species

The recorded values of Pb concentration in the muscle tissues sampled in this study did not exceed the maximum level allowed, for human consumption, by the European legislation in force (Figure 4.2.73.) [90]. The values recorded in the muscle tissue of *S. glanis* increased 0.14 times more than those recorded in the liver. In the case of *C. carpio*, *E. lucius* and *C. gibelio*, the recorded values in the liver increased with 4.80, 4.29, respectively 1.17 times more, compared to those recorded in muscles.

4.2.3. Black Sea

4.2.3.1. Sf. Gheorghe (S8)

The concentration of Pb in the water sampled from Black Sea, Sf. Gheorghe, (Figure 4.2.82.) registered the maximum value (3.105 µg L^{-1}) in point S8.2 and the minimum value (2.975 µg L^{-1}) in point S8.1 . Regarding the concentration of Pb in the collected sediments, the maximum value (3.666 µg g^{-1}) was recorded in point S8.1 and the minimum value (3.090 µg g^{-1}) in point S8.2.

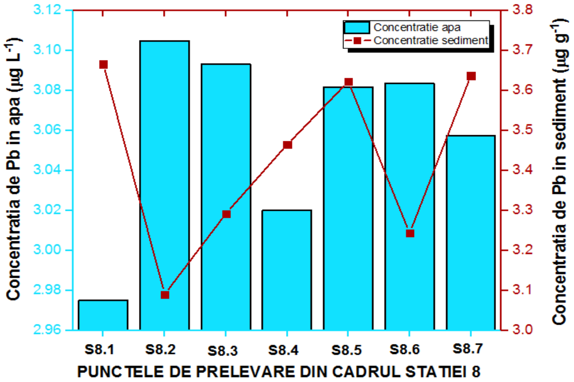


Fig. 4.2.82. Concentration of Pb in water and sediments

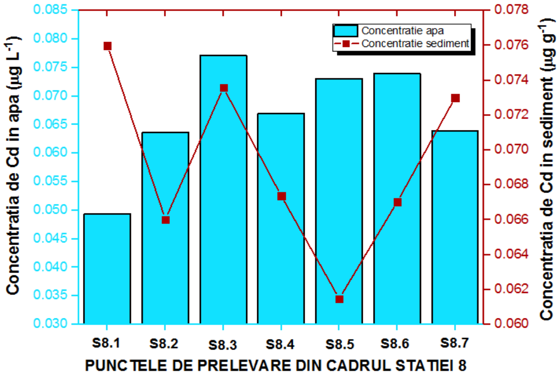


Fig. 4.2.83. Concentration of Cd in water and sediments

The Cd concentration in the water sampled from the Black Sea, Sf. Gheorghe, (Figure 4.2.83.) recorded the maximum value (0.077 µg L^{-1}) in point S8.3 and the minimum value (0.049 µg L^{-1}) in point S8.1. Regarding the Cd concentration in the analyzed sediments, the maximum value (0.075 µg g-^{1}) was recorded in point S8.1 and the minimum value (0.061 µg g^{-1}) in point S8.5.

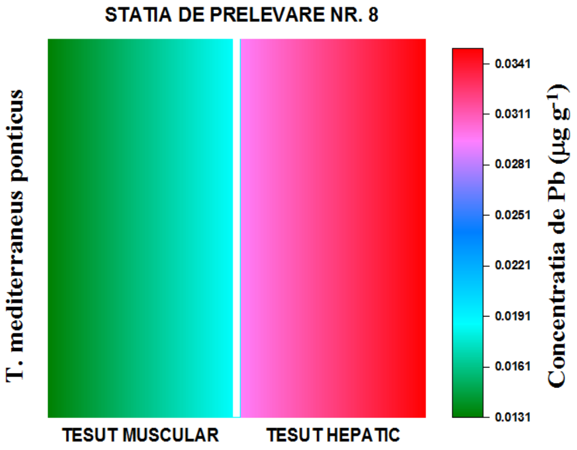


Fig. 4.2.93. Concentration of Pb in mackerel

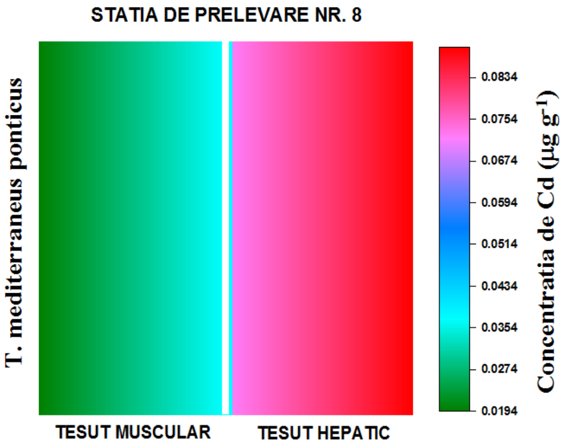


Fig. 4.2.94. Concentration of Cd in mackerel

The values recorded for Cd concentration in the muscle tissue of the analyzed mackerel does not exceed the maximum level allowed for human consumption, regulated by the European legislation in force (Figure 4.2.94.) [90]. The values recorded in the liver of mackerel increased 3.58 times, compared to those recorded in muscle.

The values recorded for Pb concentration in the muscle tissue of the analyzed mackerel does not exceed the maximum allowed level, for human consumption, regulated by the European legislation in force (Figure 4.2.93.) [90]. The values recorded in the liver of mackerel increased 1.66 times more, compared to those recorded in muscle.

4.2.3.2. Perişor (S9)

The concentration of Pb in the water sampled from the Black Sea, Perisor (Figure 4.2.103.) recorded the maximum value (0.957 µg L^{-1}) in point S9.2 and the minimum value (0.503 µg L^{-1}) in point S9.1. Regarding the concentration of Pb in the collected sediments, the maximum value (1.652 µg g^{-1}) was recorded in point S9.2 and the minimum value (1.394 µg g^{-1}) in point S9.3.

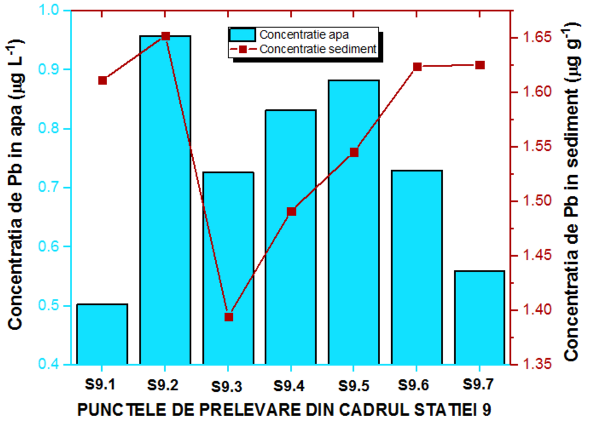


Fig. 4.2.103. Concentration of Pb in water and sediments

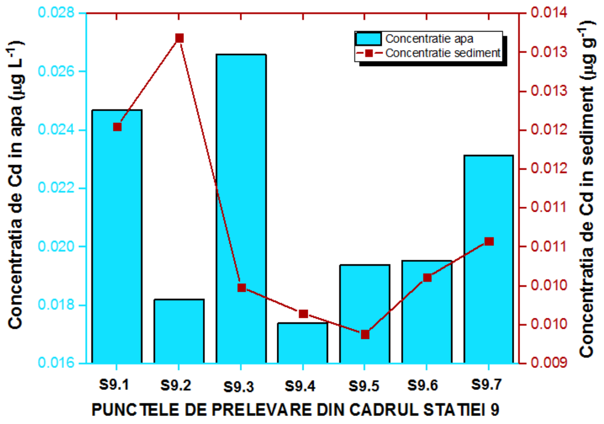


Fig. 4.2.104. Concentration of Cd in water and sediments

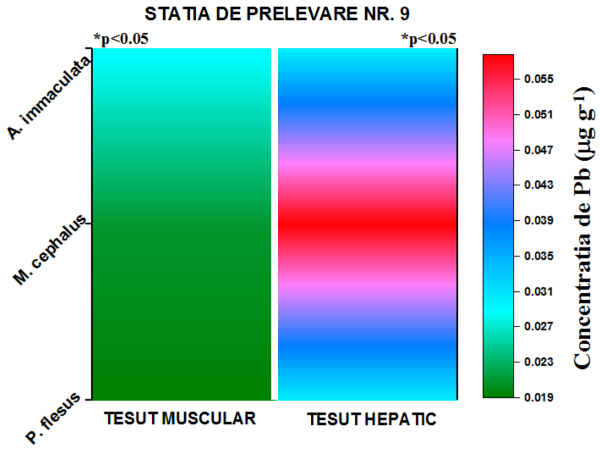


Fig. 4.2.114. Concentration of Pb in different fish species

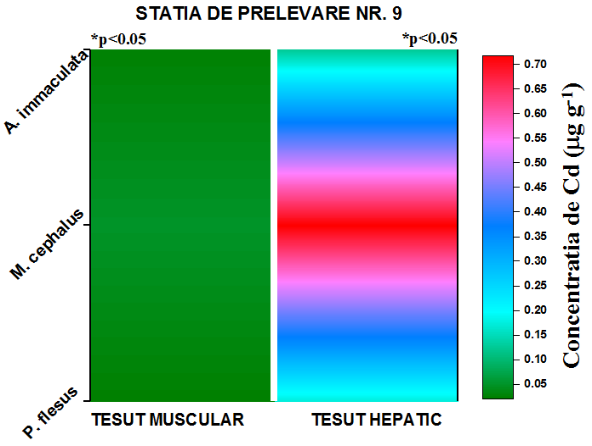


Fig. 4.2.115. Concentration of Cd in different fish species

The Cd concentration in the water sampled from Black Sea, Perisor (Figure 4.2.104.) recorded the maximum value (0.026 µg L^{-1}) in point S9.3 and the minimum value (0.017 µg L^{-1}) in point S9.4. Regarding the Cd concentration in the analyzed sediments, the maximum value (0.013 µg g^{-1}) was recorded in point S9.2 and the minimum value (0.009 µg g^{-1}) in point S9.5.

The recorded values of Cd concentration in the muscle tissues of the analyzed fish species did not exceed the maximum level allowed, for human consumption, by the European legislation in force (Figure 4.2.115.) [90]. The values recorded in the liver of the analyzed species (european flounder, mullet and shad) increased with 7.14, 13.81, respectively 4.31 times, compared with those recorded in the muscles.

The recorded values of Pb concentration in the muscle tissues of the analyzed species did not exceed the maximum level allowed, for human consumption, by the European legislation in force (Figure 4.2.114.) [90]. The values recorded in the liver of the analyzed species (european flounder, mullet and shad) increased with 0.57, 1.75, respectively by 0.02 times, compared with those recorded in the muscle.

4.2.3.3. Constanța

The values recorded in the case of Pb concentration in the turbot body were below the detection limit (ND), in all the analyzed tissues.

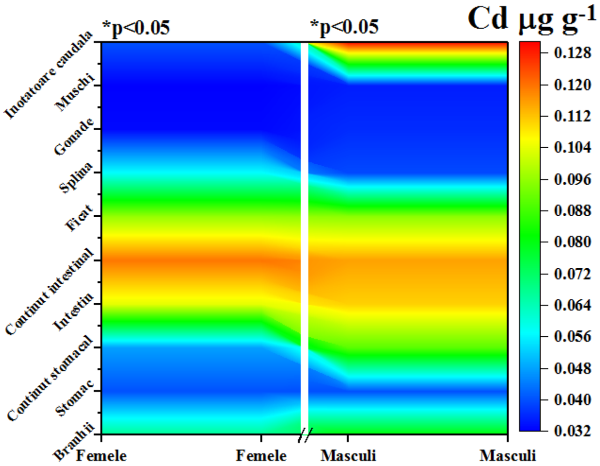


Fig. 4.2.124. Concentration of Cd in different turbot tissues

The Cd concentration values recorded the highest average in the intestinal content, gills and the caudal fin, both in the females and in the male group (Figure 4.2.124).

4.3. Welfare indicators evaluation of the biological material

It is well known that metals induce oxidative stress and the assessment of oxidative damage and antioxidant defense system in fish is a way of assessing the contamination of the aquatic environment with heavy metals [187].

4.3.1. Inferior sector of Danube River (Lower Danube)

4.3.1.1. Harbour Galați

Increased enzymatic activities were registered (CAT, SOD and GPx) in *A. immaculata*, compared to *L. aspius* and *A. brama* species. Regarding MDA and glycogen concentration, *A. brama* and *L. aspius*, recorded higher values.

4.3.1.2. Harbour Tulcea

Increased enzymatic activities of CAT, SOD and GPx were recorded in *S. glanis,* compared to *C. carpio*. Regarding MDA and glycogen concentration, *C. carpio* recorded the highest values.

4.3.2. Danube Delta

4.3.2.1. Barcaz Lake

Increased enzymatic activities of CAT, SOD, GPx and MDA were registered in *S. glanis*, compared to *C. carpio* and *C. gibelio*. The highest values of glycogen concentration were recorded in *C. gibelio*, both in the liver and in the muscles.

4.3.2.2. Soschi Lake

Increased enzymatic activities of CAT, SOD, GPx and MDA were registered in *C. carpio* compared to *S. glanis*, *C. gibelio* and *E. lucius*. The highest values of glycogen concentration were recorded in the *C. gibelio*, both in the liver and in the muscles.

4.3.3. Black Sea

4.3.3.1. Sf. Gheorghe

Enzymatic activity (CAT, SOD, GPx, MDA) was evaluated for *T. m. ponticus* (mackerel).

4.3.3.2. Perișor

Increased enzymatic activities of CAT, SOD and GPx was registered in *A. immaculata* compared to *P. flesus* and *M. cephalus*. Regarding MDA and glycogen concentration, *P. flesus* and *M. cephalus* recorded the highest values.

CHAPTER V. Evaluation of the presence of heavy metals in aquatic anthropogenic ecosystems

The main objective of this chapter is to evaluate the studied aquatic anthropogenic ecosystems (fish rearing extensive and intensive systems) in terms of water physico-chemical parameters and the presence of various metals in water, sediments and biological material.

5.1. Physico-chemical parameters of the water from aquatic anthropogenic ecosystems

The determined water physico-chemical parameters were as it follows: temperature (T°C), disolved oxygen (O_2), pH, salinity, nitrites (NO_2^-), ammonia (NH_3), chlorides (Cl^-), bicarbonates (HCO_3^-), electroconductivity (EC) and the total of disolved solids (TDS). Due to the major influence on fish welfare, in this summary only the nitrogen compounds, respectively NO_2^- and NH_3, are represented graphically.

5.1.1. Extensive production systems in aquaculture

Mălina Pond (S2)

The values of NO_2^- and NH_3 concentration were within the optimal range according to the water quality criteria in aquaculture technology (Fig. 5.1.4. and 5.1.4.) [193].

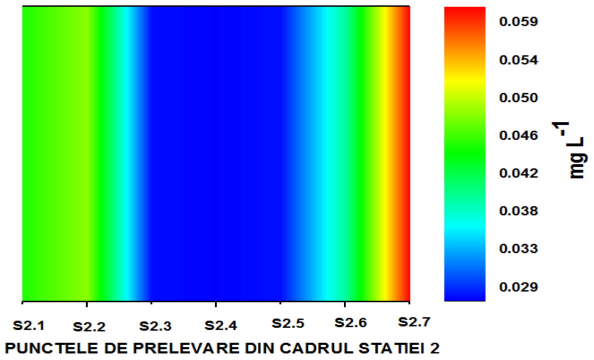


Fig. 5.1.12. NO_2 concentration in water sampled from station no. 2

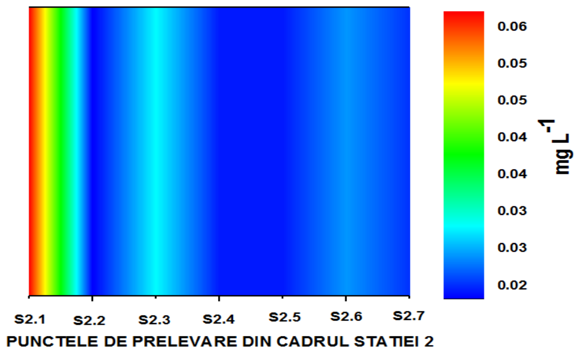


Fig. 5.1.13. NH_3 concentration in water sampled from station no. 2

Pietrei Pond (S5)

The values of NO_2^- and NH_3 concentration were within the optimal range according to the water quality criteria in aquaculture technology (Fig. 5.1.12. and 5.1.13.) [193].

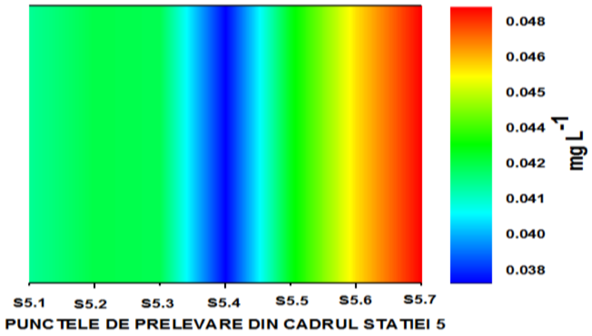


Fig. 5.1.12. NO_2 concentration in water sampled from station no. 5

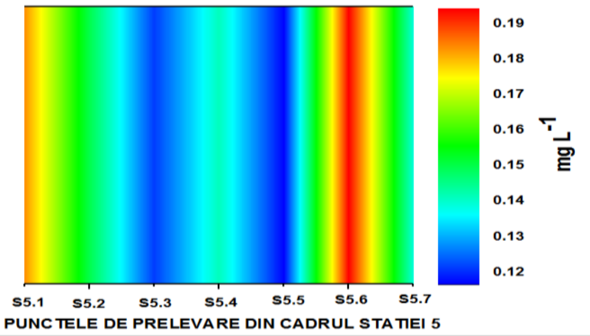


Fig. 5.1.13. NH_3 concentration in water sampled from station no. 5

5.1.2. Intensive production systems in aquaculture

The registered values in the case of NO_2^- și NH_3 concentrations are within the optimal range according to the water quality criteria in aquaculture technology (Fig. 5.1.20. and 5.1.21.) [193].

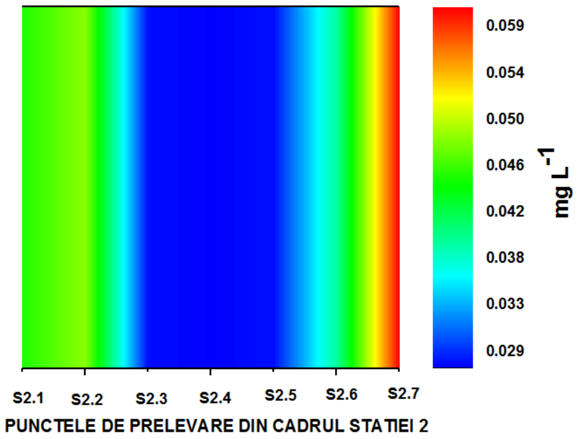


Fig. 5.1.20. NO_2 concentration in water sampled from station no. 1

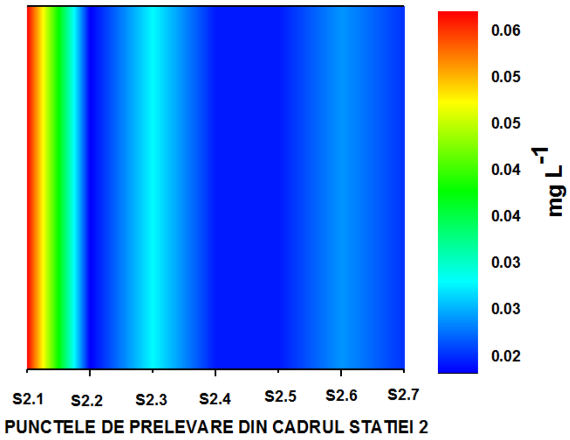


Fig. 5.1.21. NH_3 concentration in water sampled from station no. 1

5.2. Particularities of the heavy metal uptake mechanisms in anthropogenic aquatic ecosystem

The determined metals, respectively metalloids, in water, sediments and fish material were as it follows: Pb, Cd, As, Ni, Cu, Fe, Zn, Ca, Mg, K and Na. Due to the high toxicity potential, in this summary, Cd, respectively Pb, are represented graphically.

5.2.1. Mălina Pond

The concentration of Pb in the sampled water collected from Mălina Pond registered the maxiumum value (0.434 µg L^{-1}) in S2.2 point and the minimum value (0.286 µg L^{-1}) in the S2.4 point (Fig. 5.2.1). In case of Pb concentration in the sampled sediments, the maximum value (10.803 µg g^{-1}) was registered in S2.3 point and the minimum value (9.956 µg g^{-1}) in the S2.1 point.

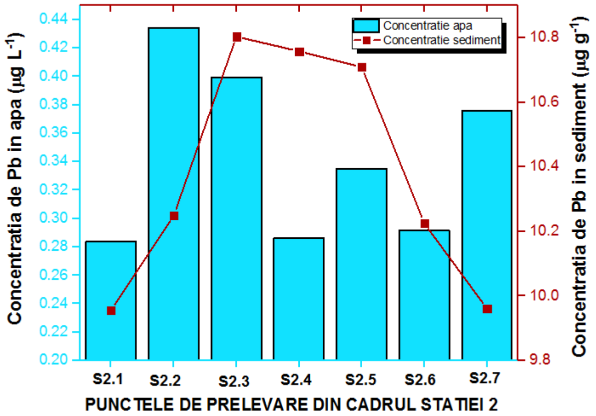


Fig. 5.2.1. Pb concentration in water and sediments

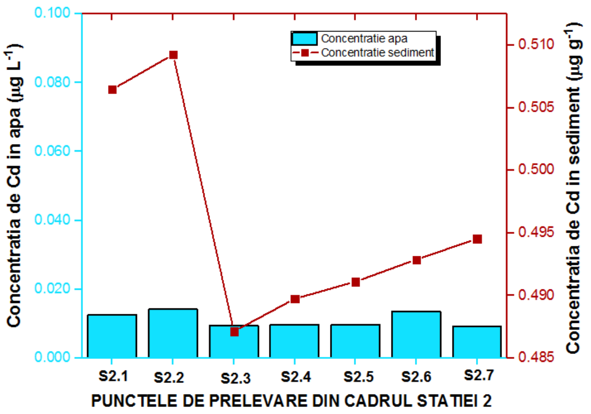


Fig. 5.2.2. Cd concentration in water and sediments

The concentration of Cd in the water sampled from Mălina Pond registered the highest value (0.014 µg L^{-1}) in point S2.2 and minimum value (0.009 µg L^{-1}) in point S2.7. (Figura 5.2.2.). In case of Cd concentration in the sampled sediments, the highest value (0.509 µg g^{-1}) was registered in point S2.2 and lowest value (0.487 µg g^{-1}) in point S2.3.

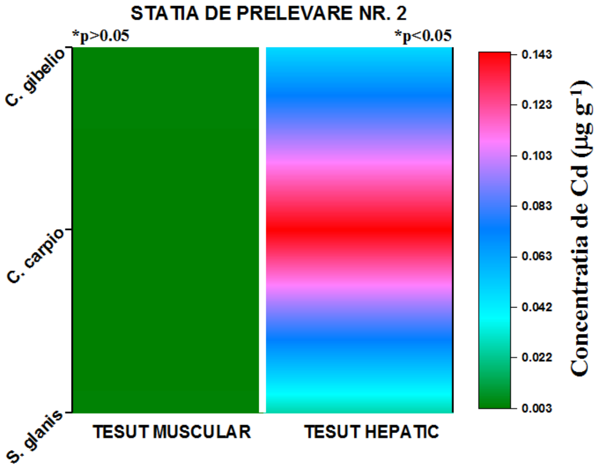


Fig. 5.2.12. Cd concentration in different fish species

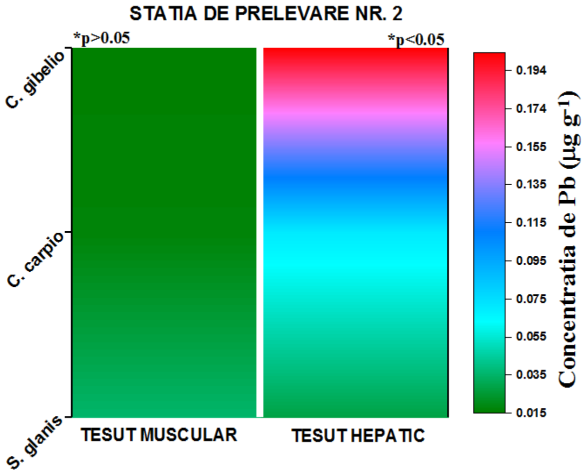


Fig. 5.2.13. Pb concentration in different fish species

The values registered for Cd concentration in the muscle tissue of the analyzed fish species did not exceed the maximum permited level, for human consumption, established by the European legislation in force (Fig. 5.2.12.) [90]. The registered values in the liver of the analyzed fish species (catfish, carp and prusian carp) increased with 7.45, 52.60, respectively 13.33 times more, compared to the ones registered in the muscles.

The values registered for Pb concentration in the muscle tissue of the studied fish species did not exceed the maximum allowed level, for human consumption, established by the European legislation in force (Fig. 5.2.13.) [90]. The values registered for Pb concentration in the muscles of *S. Glanis* have increased 0,27 times more, compared to the ones registered in the liver. In the case of *C. carpio* and *C. gibelio* the values that were registered in the liver have increased 3.30, respectively 12.84 times more, compared to the ones registered in the muscles.

5.2.2. Pietrei Pond

The Pb concentration in the water sampled from Pietrei Pond registered the maximum value (0.759 µg L^{-1}) in S5.2 point and the minimum value (0.655 µg L^{-1}) in S5.1 point (Fig. 5.2.21). In case of Pb concentration in the sampled sediments, the maximum value (13.915 µg g^{-1}) was registered in S5.2 point and the minimum value (9.563 µg g^{-1}) in S5.1 point.

The Cd concentration in the water sampled from Pietrei Pond registered a maximum value (0.0029 µg L^{-1}) in the S5.1 point and the minimum value (0.0010 µg L^{-1}) in the S5.2 point (Fig. 5.2.22). In case of Cd concentration in the sampled sediments, the maximum value (0.779 µg g^{-1}) was registered in the S5.3 point and the minimum value (0.640 µg g^{-1}) in the S5.2 point.

The registered values for Cd concentration in the muscle tissue of the analyzed fish species did not exceed the maximum allowed level, for human consumption, set by the European legislation in force (Fig. 5.2.32) [90]. The registered values in the muscle of *S. glanis* increased with 8.29 times more, compared to the ones registered in the liver. In case of the registered values in the liver of *C. carpio* and *C. gibelio*, they increased 1.73, respectively 11.17 times more, compared to the ones registered in the muscle.

The registered values for Pb concentration in the muscle tissue of the studied fish species did not exceed the maximum allowed level, for human consumption, set by the European legislation in force (Fig. 5.2.33) [90]. The values registered for Pb concentration in the muscle tissue of *C. carpio* increased with 1.89 times more, compared to the ones registered in the liver. In case of *S. glanis* and *C. gibelio*, the values registered in the liver tissue increased with 0.81, respectively 11.43 times more, compared to the ones registered in the muscle.

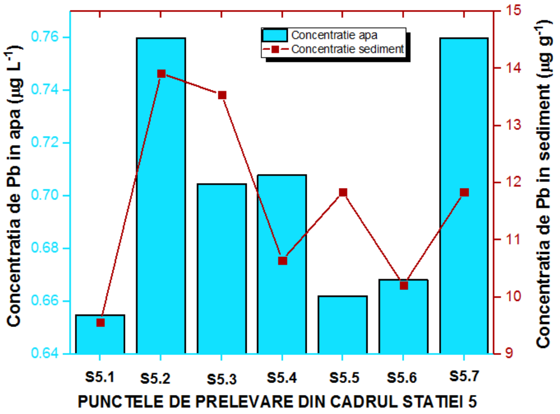


Fig. 5.2.21. Pb concentration in water and sediments

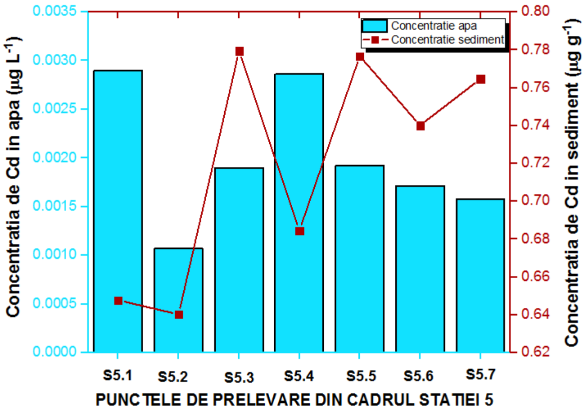


Fig. 5.2.22. Cd concentration in water and sediments

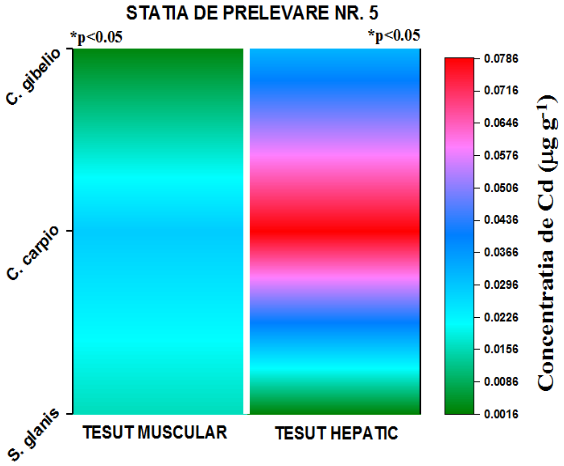


Fig. 5.2.32. Cd concentration in different fish species

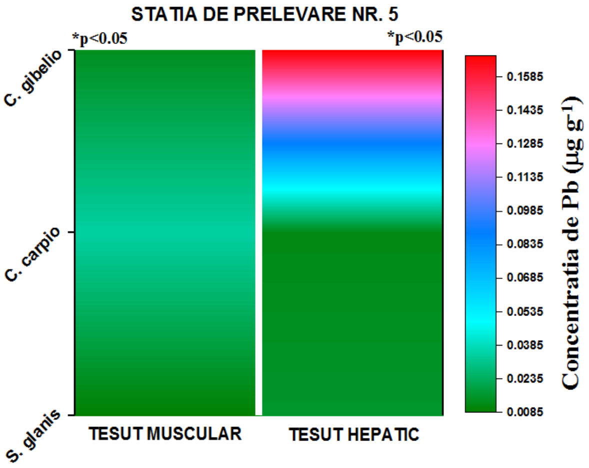


Fig. 5.2.33. Pb concentration in different fish species

5.2.3. Recirculating aquaculture system

The Pb concentration in the water sampled from MoRas system registered the maximum value (0.4713 µg L^{-1}) in the S1.1 rearing unit and the minimum value (0.4448 µg L^{-1}) in the S1.2 rearing unit (Fig. 5.2.41.).

The Cd concentration in the water sampled from MoRas system registered the maximum value (0.0108 µg L^{-1}) in the S1.1 rearing unit and the minimum value (0.0147 µg L^{-1}) in the S1.2 rearing unit (Fig. 5.2.42.).

The registered values for Cd concentration in the muscle tissue of the analyzed russian sturgeon did not exceed the maximum allowed level, for human consumption, set by the European legislation in force (Fig. 5.2.51) [90]. The values registered in the liver of the russian sturgeon have increased 3.29 times more, compared to the ones registered in the muscle.

The registered values Pb concentration in the muscle tissue of the analyzed russian sturgeon did not exceed the allowed maximum level, for human consumption, set by the European legislation in force (Fig. 5.2.52) [90]. The values registered in the liver of the russian sturgeon have increased 12.63 times more, compared to the ones registered in the muscle.

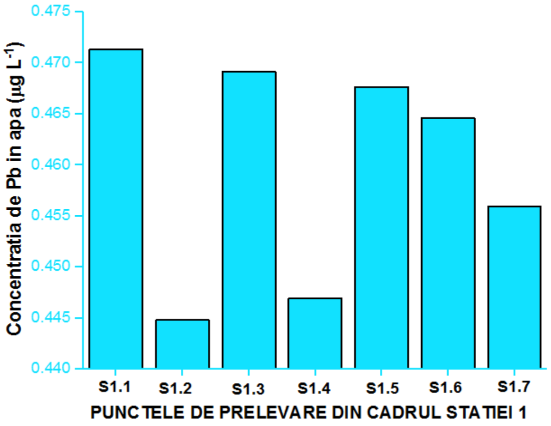


Fig. 5.2.41. Pb concentration in water

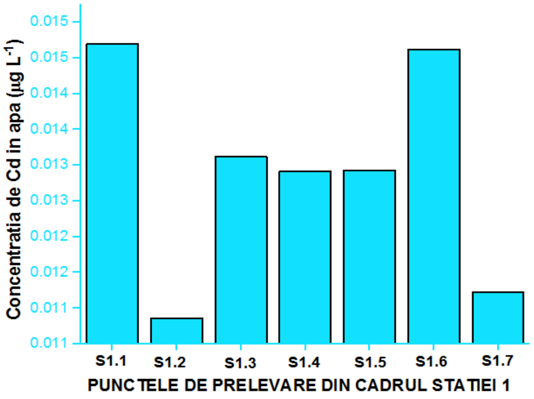


Fig. 5.2.42. Cd contration in water

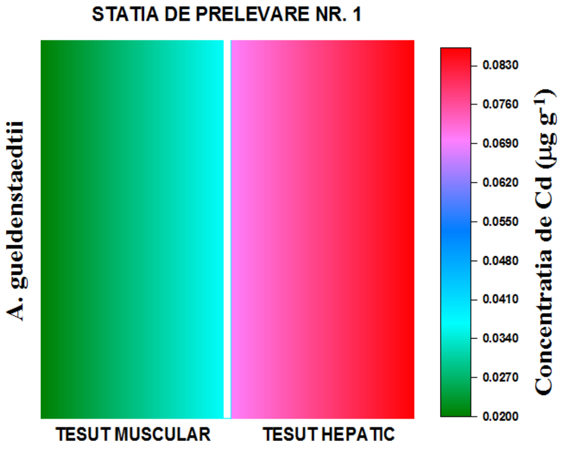


Fig. 5.2.51. Cd concentration in russian sturgeon

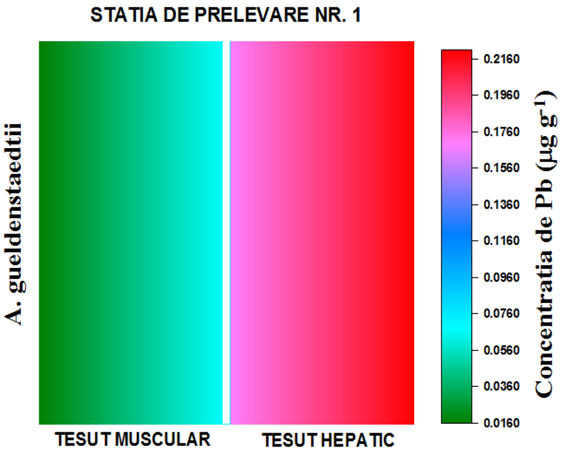


Fig. 5.2.52. Pb concentration in russian sturgeon

5.3. Wellfare indicators evaluation of the biological material

5.3.1. Mălina Pond

It is highlighted a higher enzymatic activity (CAT, SOD, GPx) in the case of the *S. glanis*, compared to *C. carpio* and *C. gibelio*.

5.3.2. Pietrei Pond

It is highlighted a higher enzymatic activity (CAT, SOD, GPx) in the case of the *S. glanis*, compared to *C. carpio* and *C. gibelio*. In case of MDA and glycogen concentration, higher values in *C. gibelio* compared to the rest of the analyzed species, were registered.

5.3.3. Recirculating aquaculture system

In case of the russian sturgeon reared in a recirculating aquaculture system, there have been noted higher enzymatic activities (CAT, SOD, GPx) in the liver tissue, compared to the muscle tissue. In the case MDA and glycogen concentration, higher values were registered in the muscle tissue, compared to the liver tissue.

CHAPTER VI. Comparative study between the analysed aquatic ecosystems

The main purpose of this chapter was to realise a comparative study between the aquatic natural and anthropogenic ecosystems and to reveal the differences between them, in terms of the water physico-chemical parameters, the presence of various analyzed metals, the evaluation of fish wellfare, the pollution index, respectively the estimated daily intake (EDI).

6.1. Comparative study on the water physico-chemical parameters and the ecological state of the analyzed aquatic ecosystems

According to the technical norm issued by the Romanian Government regarding the quality of surface waters which require protection and improvement in order to maintain fish life from

28.02.2002, the registered values for the concentration of dissolved oxygen from the sampled water were within the recommended values for cyprinid technological waters, in all sampling stations [85].

According to HG no. 202/2002 issued by the Romanian Government, the pH of the analyzed water samples were within the recommended values for cyprinid technological waters, in all sampling stations [85].

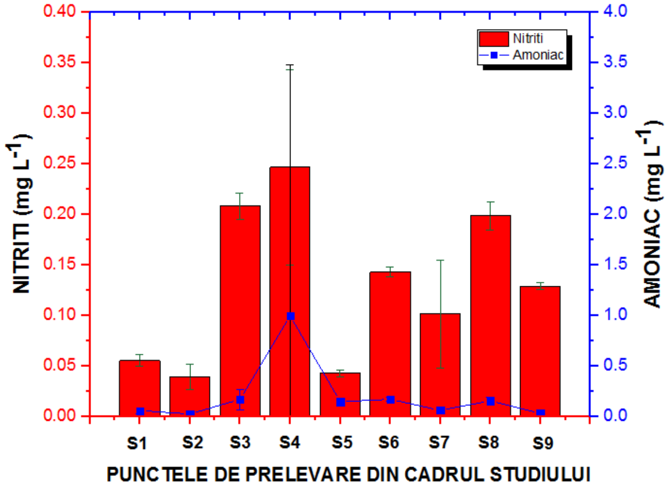


Fig. 6.3. Concentration of NO_2^- and NH_3 from the water, in the sampling stations

According to the HG no. 202/2002 issued by the Romanian Government, the NH_3 concentration exceeds the maximum allowed value (≤ 0.025 mg L^{-1}) from the list of quality indicators for cyprinid waters in all of the stations included in the study (Fig. 6.3) [85]. The same phenomenon was noted in case of NO_2 concentrations. It is highlighted that lower values for NH_3 , respectively NO_2^- concentrations, were registered in the anthropogenic ecosystems (S1, S3 and S5) compared to the natural ecosystems (S3, S4, S8 and S9).

6.2. Comparative study on the particularities of heavy metal uptake mechanisms in the studied aquatic ecosystems

According to the legislative classification of surface waters (Ord. 161/2006), the sampled water from S1, S2, S3, S4, S5, S6, S7, S8, and S9 stations is associated to **Quality Class I** (below 0.5 µg L^{-1}), in case of Cd concentration [66].

As well, the values recorded for Cd concentration in the water samples from the Black Sea (S8 and S9) did not exceed the maximum allowed limits for the marine environment, according to the 1888/2007 Order (20 µg L^{-1}) [89].

According to the legislative classification of surface waters (Ord. 161/2006), the sampled water from the S1, S2, S3, S4, S5, S6, S7, S8, and S9 stations is associated to **Quality Class I** (below 5 µg L^{-1}), in case of Pb concentration [66].

As well, the values registered for Pb concentration in the water sampled from the Black Sea (S8 and S9) did not exceeded the maximum allowed limits for the marine environment, according to the 1888/2007 Order (20 µg L^{-1}) [89].

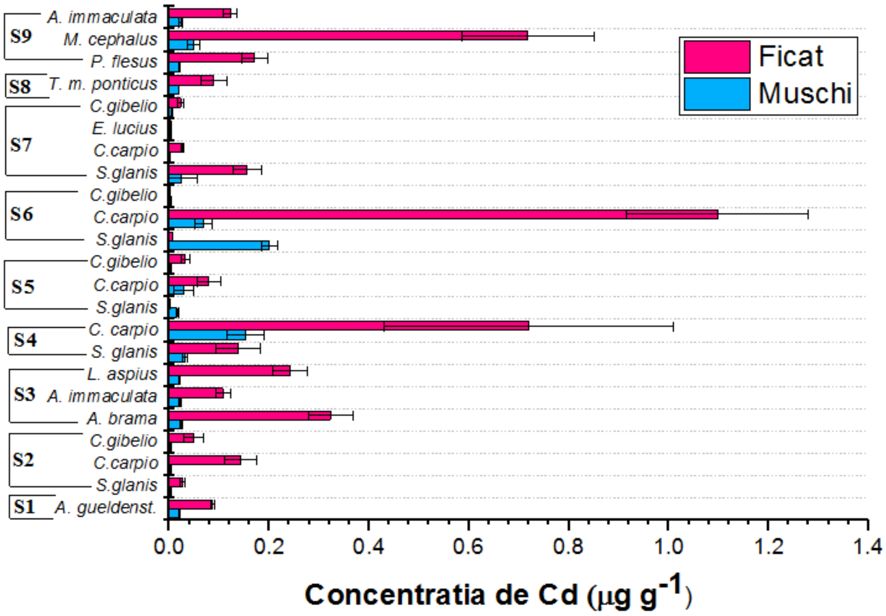


Fig. 6.8. Concentration of Cd in different fish species, in the sampling stations

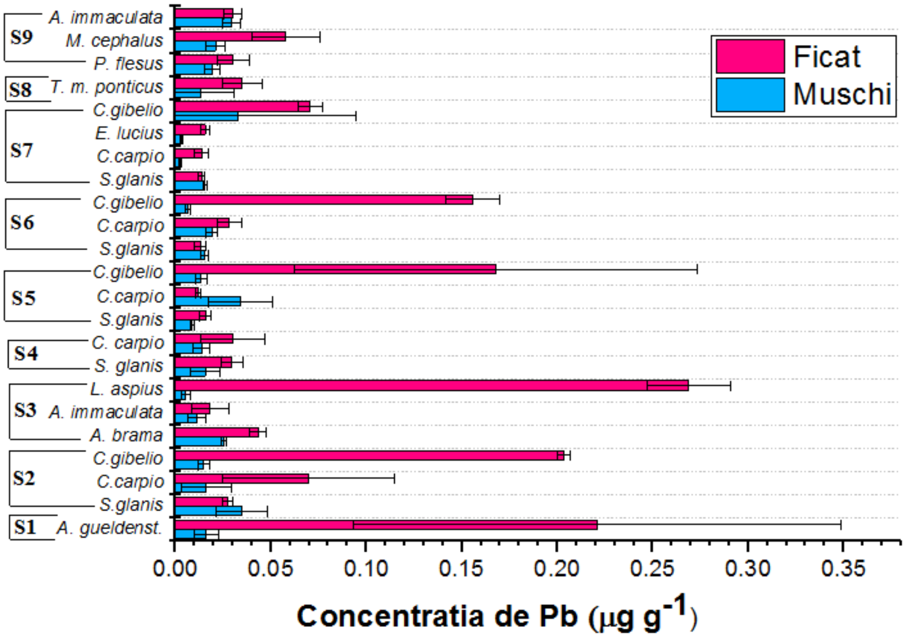


Fig. 6.9. Concentration of Pb in different fish species, in the sampling stations

It was highlighted that fish species from the aquatic anthropogenic ecosystems registered lower values of Cd and Pb concentrations compared to the ones from the natural environment (Fig. 6.8.). This phenomenon is due to the effective water quality control in the aquatic anthropogenic ecosystems and its absence in the natural ecosystems.

The registered values of Pb concentration in the muscle tissue of the analyzed fish species did not exceed the maximum level (0.3 µg g^{-1}) allowed by the European legislation (Fig. 6.9) [90].

6.3. Comparative study on the evaluation of welfare indicators of the biological material sampled from the studied aquatic ecosystems

Of all of the analyzed species, *S. Glanis* from S4 and *A. Immaculata* from S3 registered the highest enzyme activity (in terms of CAT, SOD, GPx) and lipid peroxidation (MDA). As well, in these stations, respectively S4 and S3, there have been recorded high values for concentrations of Fe and Zn, due to strong anthropogenic pressure. As well, *A. Immaculata* from S3 registered high values of As concentration in the liver tissue. It is well known that As is responsible for generating oxidativ stress in fish.

Also, the obvious tendency of fish collected from the natural environment to manifest a higher oxidative stress was observed, compared to the ones produced in aquaculture, a phenomenon which was also reported by Doherty et al. (2010) [154].

6.4. Assessment of ecological status of the studied aquatic ecosystems by calculating the Pollution Index (PI) and determination of estimated daily intake (EDI)

In the context of the presence of Cd, Pb, Ni, Cu and Zn concentrations in the water sampled from the studied aquatic ecosystems, PI registered values < 1 in the majority of the sampling stations, except for Zn in S3, listing an PI value < 5. Therefore, it can be concluded that the presence of Cd, Pb, Ni, Cu and Zn in the sampling water does not have a negative impact over the aquatic ecosystems where they are found.

PI in case of Zn (<5) in the S3 sampling station, Danube River – Harbour Galați, indicates an aquatic environment strongly affected by the presence of this metal (Table nr. 36).

Table no. 36. Interpretation of PI in the sampling stations

Sampling Station	Pollution Index (PI)					
	Cd	Pb	Ni	Cu	Fe	Zn
S1	<1	<1	<1	<1	ND	<1
S2	<1	<1	<1	<1	**>5**	<1
S3	<1	<1	<1	<1	**>5**	**<5**
S4	<1	<1	<1	<1	**>5**	<1
S5	<1	<1	<1	<1	**>5**	ND
S6	<1	<1	<1	<1	**>5**	<1
S7	<1	<1	<1	<1	**>5**	<1
S8	<1	<1	<1	<1	**>5**	<1
S9	<1	<1	<1	<1	**>5**	<1

The PI for Fe concentrations registered in the water column, from all of the sampling stations, has revealed a strongly affected aquatic ecosystem by the presence of this element.

The registered EDI values for various captured fish species were below the maximum intake recommended by the World Health Organization (WHO) and by the Food and Agriculture Organization of the United Nations [247] [248] [249]. In case of EDI values registered for various fish species produced in aquaculture production systems, they were lower compared with the values recommmented by the World Health Organization (WHO) and by the Food and Agriculture Organization of the United Nations [247] [248] [249].

Following the statistical analysis of variance, it has been concluded that EDI values for the fish sampled from the natural environment and the fish produced in aquaculture did not registered significant differences ($p<0.05$). Also, the significantly lower EDI values, compared to the values recommended by WHO and FAO, could be due to the decreased fish consumption recorded in Romania (7 kg per capita), compared to the average European fish consumption (24.33 kg per capita) [175] [254].

Following the analysis of EDI, it can be concluded that the intake of essential elements (Zn and Cu) and elements with toxic potential (Cd, Pb, As) through fish consumption does not represent a risk for the human consumer or pose adverse effects on human health.

CHAPTER VII. Correlations and machine learning

In order to realise the correlation and prediction analyses, the registered data was sorted and selected, taking into consideration the determined factors in the process of metals and metalloids accumulation in water, sediments and animal biomass. Thus, the data was processed in order to be used in the correlation analysis, by eliminating the extreme values.

7.1. Correlation analysis between metals, respectively metalloids from water, sediments and animal biomass

The correlation analyses was carried out by following the next scenarios:

→ Correlation between metals and metalloids from the muscle tissue, liver tissue, as well as muscle tissue vs. liver tissue for fish species with ichthyophagous food regime, respectively non-ichthyophagous, for freshwater species, for marine species, and for species from natural and anthropogenic environment;

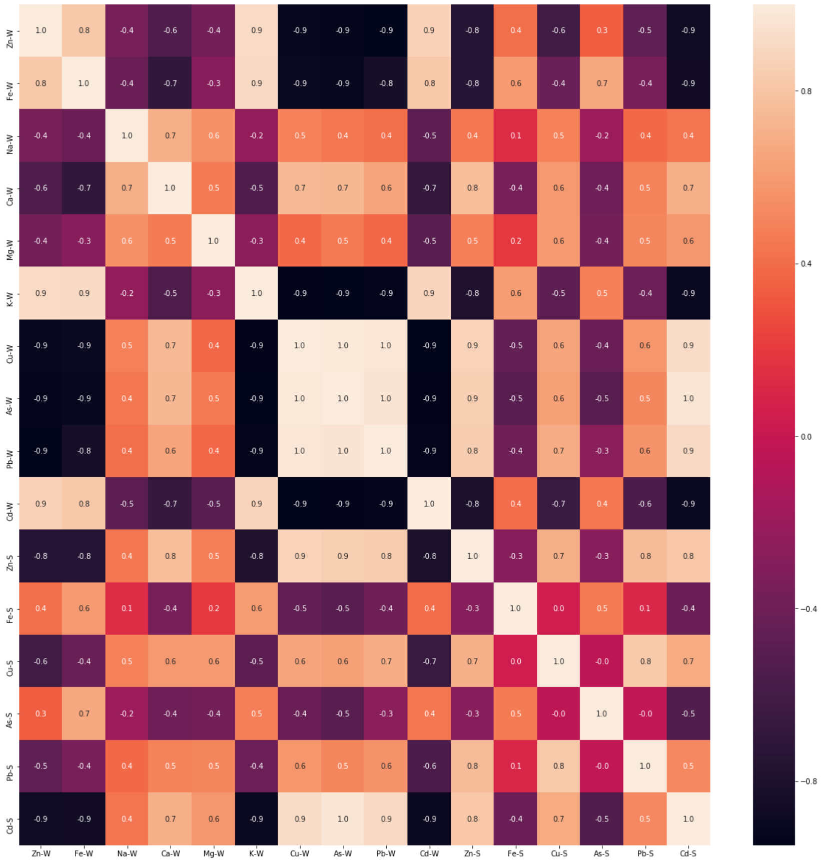
→ Correlation between metals and metalloids in the water sampled from the studied aquatic ecosystems (freshwater, natural and anthropogenic);

→ Correlation between metals and metalloids in the sediments sampled from the studied aquatic ecosystems (freshwater, natural and anthropogenic);

→ Correlation between metals and metalloids between water and sediments from the studied aquatic ecosystems (freshwater, natural and anthropogenic);

→ Correlation between metals and metalloids in water and animal biomass from the studied aquatic ecosystems (freshwater, natural and anthropogenic).

The strongest pozitive correlations between the analyzed metals and metalloids **from water and sediments associated with the anthropogenic environment** were as it follows: Cu_{water} and $Zn_{sediment}$ ($p=0,9$), Ca_{water} and $Zn_{sediment}$ ($p=0,8$), As_{water} and $Zn_{sediment}$ ($p=0,9$), Fe_{water} and $As_{sediment}$ ($p=0,7$), C_{water} and $Cd_{sediment}$ ($p=0,7$) (Fig. 8.5.). In case of negative correlations, the strongest ones occured in the following cases: Cd_{water} and $Cd_{sediment}$ ($p= -0,9$), Zn_{water} and $Zn_{sediment}$ ($p= -0,8$), Zn_{water} and $Cd_{sediment}$ ($p= -0,9$), Fe_{water} and $Cd_{sediment}$ ($p= -0,9$) (Fig. 8.1.5.).



	Zn-W	Fe-W	Na-W	Ca-W	Mg-W	K-W	Cu-W	As-W	Pb-W	Cd-W	Zn-S	Fe-S	Cu-S	As-S	Pb-S	Cd-S
Zn-W	1.0	0.8	-0.4	-0.6	-0.4	0.9	-0.9	-0.9	-0.9	0.9	-0.8	0.4	-0.6	0.3	-0.5	-0.9
Fe-W	0.8	1.0	-0.4	-0.7	-0.3	0.9	-0.9	-0.9	-0.8	0.8	-0.8	0.6	-0.4	0.7	-0.4	-0.9
Na-W	-0.4	-0.4	1.0	0.7	0.6	-0.2	0.5	0.4	0.4	-0.5	0.4	0.1	0.5	-0.2	0.4	0.4
Ca-W	-0.6	-0.7	0.7	1.0	0.5	-0.5	0.7	0.7	0.6	-0.7	0.8	-0.4	0.6	-0.4	0.5	0.7
Mg-W	-0.4	-0.3	0.6	0.5	1.0	-0.3	0.4	0.5	0.4	-0.5	0.5	0.2	0.6	-0.4	0.5	0.6
K-W	0.9	0.9	-0.2	-0.5	-0.3	1.0	-0.9	-0.9	-0.9	0.9	-0.8	0.6	-0.5	0.5	-0.4	-0.9
Cu-W	-0.9	-0.9	0.5	0.7	0.4	-0.9	1.0	1.0	1.0	-0.9	0.9	-0.5	0.6	-0.4	0.6	0.9
As-W	-0.9	-0.9	0.4	0.7	0.5	-0.9	1.0	1.0	1.0	-0.9	0.9	-0.5	0.6	-0.5	0.5	1.0
Pb-W	-0.9	-0.8	0.4	0.6	0.4	-0.9	1.0	1.0	1.0	-0.9	0.8	-0.4	0.7	-0.3	0.6	0.9
Cd-W	0.9	0.8	-0.5	-0.7	-0.5	0.9	-0.9	-0.9	-0.9	1.0	-0.8	0.4	-0.7	0.4	-0.6	-0.9
Zn-S	-0.8	-0.8	0.4	0.8	0.5	-0.8	0.9	0.9	0.8	-0.8	1.0	-0.3	0.7	-0.3	0.8	0.8
Fe-S	0.4	0.6	0.1	-0.4	0.2	0.6	-0.5	-0.5	-0.4	0.4	-0.3	1.0	0.0	0.5	0.1	-0.4
Cu-S	-0.6	-0.4	0.5	0.6	0.6	-0.5	0.6	0.6	0.7	-0.7	0.7	0.0	1.0	-0.0	0.8	0.7
As-S	0.3	0.7	-0.2	-0.4	-0.4	0.5	-0.4	-0.5	-0.3	0.4	-0.3	0.5	-0.0	1.0	-0.0	-0.5
Pb-S	-0.5	-0.4	0.4	0.5	0.5	-0.4	0.6	0.5	0.6	-0.6	0.8	0.1	0.8	-0.0	1.0	0.5
Cd-S	-0.9	-0.9	0.4	0.7	0.6	-0.9	0.9	1.0	0.9	-0.9	0.8	-0.4	0.7	-0.5	0.5	1.0

Fig. 8.1.5. Correlations between metals and metalloids from water and sediments associated with the anthropogenic environment

7.2. Prediction analysis between metals, respectively, metalloids from animal biomass

From the RMSE analysis the best fitting predictions were as it follows:

1. For the database containing the metal values from the muscle tissue of fish collected from the natural environment, the prediction of micro-elements Cd, Zn, Cu, Fe, Pb depending on the macro-elements Na, Mg, Ca, K;
2. For the database containing the metal values from the muscle tissue of fish collected from the freshwater environment, the prediction micro-elements of Cd, Zn, Cu, Fe, Pb depending on the macro-elements Na, Mg, Ca, K;
3. For the database containing metal values from the liver tissue of fish collected from the natural environment, the prediction of micro-elements Cd, Zn, Cu, Fe, Pb depending on the macro-elements Na, Mg, Ca, K;
4. For the database containing the metal values from the liver tissue of fish collected from the freshwater environment, the prediction of micro-elements Cd, Zn, Cu, Fe, Pb depending on the macro-elements Na, Mg, Ca, K;
5. For the database containing the metal values from the liver and muscle tissues of fish collected from the freshwater environment, the prediction of macro-elements Na, Mg, Ca, K from the muscles depending on the macro-elements from the liver;

6. For the database containing the metal values from the liver and muscle tissues of fish collected from the natural environment, the prediction of the macro-elements Na, Mg, Ca, K from the muscles depending the macro-elements from the liver;
7. For the database containing the metal values from the liver and muscle tissues of fish collected from the freshwater environment, the prediction of the micro-elements Cd, Zn, Fe, Cu and Pb from the muscles depending on the micro-elements from the liver;
8. For the database containing the metal values from the liver and muscle tissues of fish collected from the natural environment, the prediction of the micro-elements Cd, Zn, Fe, Cu and Pb from the muscle depending on the micro-elements from the liver (Example Fig. 8.2.36.).

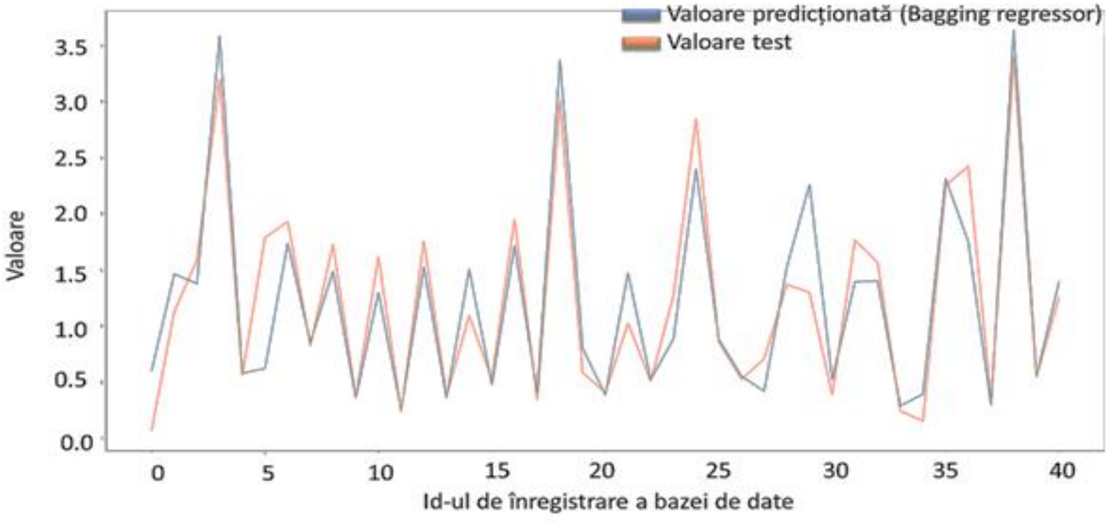


Fig. 8.2.36. Prediction analysis of Cu concentration in the muscle and liver tissue in fish collected from the natural environment

CHAPTER VIII. Final conclusions, personal contributions, future research directions

Final conclusions

The results obtained throughout this study and described within this doctorate thesis lead to a series of relevant conclusions. As a result, the synthesizing and the correlation of the results contribute to a detailed analysis regarding the following aspects:

- The evaluation of water quality within the studied natural aquatic and anthropogenic ecosystems, from the perspective of physico-chemical parameters, respectively the concentration of the heavy metals with high potential of toxicity;
- The classification of the water bodies within the studied aquatic ecosystems into quality classes and the identification of their pollution index;

- The characterization of the sediments from the studied aquatic ecosystems from the point of the concentration level of various metals, as well as organic carbon and total nitrogen;
- The evaluation of the accumulation tendency of heavy metals in the organs of various fish species, from natural and anthropogenic aquatic ecosystems;

The following general conclusions were obtained from the analysis of the experimental results regarding the aforementioned aspects:

- ✓ The **concentration of dissolved oxygen** (5.71-10 mg L^{-1}) and the **pH** (7,6-8,8) from the water collected from all of the sampling stations were within the optimal levels recommended for cyprinid technological waters, in the natural and anthropogenic aquatic ecosystems.
- ✓ The **salinity** of the analyzed water was within the normal limits for freshwater ecosystems (0.2-0.8 PSU), and in the case of the Black Sea (4.5 PSU), the recorded values were lower compared to the values specific for a marine environment, mostly due to the positive freshwater balance specific to the North-West part of the Black Sea, which shows a tendency of decreased salinity with 0,02 PSU $year^{-1}$.
- ✓ From the analysis of the experimental results for the **nitrogen compounds,** the following aspects were highlighted:
  - ○ The NO_2^- and NH_3 concentrations have indicated lower values in the anthropogenic aquatic ecosystems, compared to the natural ecosystems;
  - ○ In relation to the national regulations (HG nr. 202/2002), the concentrations of the nitrogen compounds analyzed from the water column of various aquatic ecosystems within this study had exceeding values over the optimal recommended range for cyprinid waters (≤0.03 mg L^{-1} for NO_2^- and ≤0.025 mg L^{-1} for NH_3) in all of the sampling stations; this phenomenon indicates an inefficient water quality control of the effluents resulted from various anthropogenic activities and discharged in the natural environment;
  - ○ In relation to several rules regarding the water quality in aquaculture which were found in specialty literature (Timmons et al., 2018), the concentrations of the analyzed nitrogen compounds in the water column of various aquatic ecosystems, were within the recommended levels, an aspect which highlights the more restrictiv character of the national norms compared to the international ones;
  - ○ It has been reported a more intense anthropogenic pressure exercised on the S4 station (Danube River, Harbour Tulcea), compared to the others stations in terms of the nitrogen compounds concentration, at the water level.
- ✓ The presence of **metals** and **metalloids** at the water level highlights the following:
  - ○ the analyzed water bodies from the aquatic ecosystems clasify for the **Quality Class I**, in terms of Zn, Cu, Ni, Cd, Pb and As content;
  - ○ the analyzed water bodies from the aquatic ecosystems clasify for **Quality Class V**, in terms of Fe content; Fe represents the only metal with toxic potential, which has been recorded in high concentrations in all of the sampling stations, except S1 (recirculating aquaculture system);
  - ○ in the sampling stations of aquatic anthropogenic ecosystems it has been observed a smaller tendency of accumulation of certain metals, such as: Zn, Fe, Cu, Cd and Ca, compared to the sampling stations of natural aquatic ecosystems. However, exception makes S1 (recirculating aquaculture system), where there has been recorded the highest degree of Zn accumulation; one possible explanation of this phenomenon would be

represented by the administration of fish feed with a significant content of this element. The same phenomenon, of high accumulation of a metalloid in a natural aquatic ecosystem, it is manifested in S5 station (Pietrei Pond): in this case, the phenomenon of high arsenic accumulation can be explained by the fact that agrochemical substances that are administrated on the surface of agricultural soil near the pond contain high amounts of As, which are transported in the water;

- ○ A special conclusion regarding the presence of metals and metalloids in the studied natural aquatic ecosystems relates to station S8 (Black Sea St. Gheorghe) where it has been recorded the highest level of Fe, Cu, Ni and Pb accumulation, compared to the rest of the natural aquatic ecosystems; this phenomenon might be due to the high organic carbon concentrations present in the sediments from the S8 station. It is well known that carbon has the tendency to bind different metals, and after the re-suspension process, they are trained into the water;
- ○ Still, in accordance with the national legislation (HG nr. 1888/2007), the concentrations of certain metals (Cd, Pb, Ni, Zn, Cu), respectively of some metalloids (As) registered in stations S8 (Black Sea St. Gheorghe) and S9 (Black Sea Perișor) are below the maximum permitted limit;
- ○ A particular case regarding the presence of metals and metalloids in the studied natural aquatic ecosystems is represented by the S9 station (Black Sea Perișor), where the lowest concentrations of As and Pb were recorded, compared to the rest of the ecosystems where there is an obvious accumulation tendency of these elements;
- ○ The accumulation trend of the metals has been identified as it follows: Ca>Na>K>Mg>Fe>Zn>Ni>Cu>As>Pb>Cd.

✓ At the sediments level, the interpretation of the results connected to the content of **metal and metalloids**, as well as **organic carbon** and **total nitrogen**, leads to drawing of the following conclusions:

- ○ the sediments collected from the stations associated to the marine environment, respectively S8 (Black Sea St. Gheorghe) and S9 (Black Sea Perișor), have not registered values above the maximum allowed limit by the national legislation (HG nr. 1888/2007), for Cd, Pb, Ni, Zn, Cu and As concentrations; this phenomenon is explained by the reduced anthropogenic pressure exerted on the environment;
- ○ for the same reason, the lowest concentration levels of Zn, Cu, Pb and Cd in the S9 station (Black Sea Perișor) has been recorded;
- ○ the metals accumulation trend has been identified as it follows: Ni>Cu>Fe>Pb>As>Cd>Zn.
- ○ organic carbon and total nitrogen registered the highest values in the S8 station (Black Sea St. Gheorghe), and the lowest in the S9 station (Black Sea Perișor).

✓ The most relevant conclusions regarding the metal and metalloids accumulation in the body of the studied fish species are the following:

- ○ The highest bioaccumulation capacity for metals with toxic potential such as Cd, Pb, Cu and Fe, in the muscle tissue, has manifested in *Silurus glanis* (Linnaeus, 1758), followed by the *Cyprinus carpio* (Linnaeus, 1758);
- ○ The Cd concentration level in the muscle tissue was above the maximum limit allowed by the european legislation (0.05 µg g^{-1}), with values which were 4 times higher in the case of *Silurus glanis* (Linnaeus, 1758) and 3 times higher in the case of *Cyprinus carpio* (Linnaeus, 1758); the aforementioned species were collected from the natural aquatic ecosystems.

- There have been revealed lower values for Cd concentration in the muscle tissue of cultured fish species, compared to the ones from the natural aquatic ecosystems;
- In case of the Pb concentration in the analyzed muscle tissue of fish species, it has been concluded that the values did not exceed the maximum level allowed by the european legislation (0.3 µg g^{-1}); also, it has been concluded the fact that the origin of the Pb in fish bodies is predominantly from the food, due to low concentrations registered in the water;
- The bioaccumulation of As in the body of *Alosa immaculata* (Bennett, 1835), is a process which was manifested, in our opinion, in an atypical way; this element suffers a possible translocation from the muscle to the liver, on the duration of the reproductive migration;
- It has been observed the tendency of raptor species (pike and asp) to accumulate higher concentrations of Ca, compared to peaceful species, due to the involvement of this element in the stimulation of the muscular contraction;
- The marine fish species (European flounder, shad, mullet, turbot, mackerel) had a higher tendency to accumulate As in their muscle tissue, compared to the freshwater ones, which recorded values below the detection limit;
- Regarding the bioaccumulation of various metals in the body of turbot, it is highlighted that the concentration levels in both females and males is almost equal;
- The accumulation trend of the metal concentrations in the muscle tissue of the cultured fish species is as it follows:
- Russian sturgeon: K>Na>Ca>Mg>Fe>Zn>Cu>As>Cd>Pb;
- Catfish: K>Na>Mg>Ca>Fe>Zn>Cu>Pb>Cd>As;
- Carp: K>Na>Ca>Mg>Fe>Zn>Cu>Pb>Cd>As;
- Prussian carp: K>Ca>Na>Mg>Fe>Zn>Cu>Pb>Cd>As;
- The accumulation trend of the metal concentration in the muscle tissue of fish species that were prelevated from natural aquatic ecosystems is:
- turbot: K>Na>Mg>Ca>Fe>Zn>As>Mn>Cu>Ni>Cd;
- catfish: K>Na>Ca>Mg>Fe>Zn>Cu>Cd>Pb>As;
- carp: K>Na>Ca>Mg>Zn>Fe>Cu>Cd>Pb>As;
- prussian carp: K>Na>Ca>Mg>Zn>Fe>Cu>Pb>Cd>As;
- pike: K>Ca>Na>Mg>Zn>Fe>Cu>Pb>Cd>As;
- common bream: K>Na>Ca>Mg>Fe>Zn>Cu>Pb>Cd>As;
- asp: K>Ca>Na>Mg>Zn>Fe>Cu>Cd>Pb>As;
- shad: K>Na>Ca>Mg>Fe>Zn>As>Cu>Pb>Cd;
- mullet: K>Na>Mg>Ca>Fe>Zn>Cu>As>Cd>Pb;
- european flounder and mackerel: K>Na>Mg>Ca>Fe>Zn>As>Cu>Cd>Pb;
- The analysis of the accumulation trend of metals in the muscle tissue of the fish species reveals the fact that the macro-element K is the most abundant, while, at the opposite pole, is situated As, Pb and Cd, which are considered trace elements.
- The accumulation trend of metals in the hepatic tissue of the cultured fish species is at it follows:
- russian sturgeon: K>Na>Fe>Mg>Ca>Zn>Cu>As>Pb>Cd;
- catfish: K>Na>Fe>Mg>Ca>Zn>Cu>Pb>Cd>As;
- carp: K>Na>Fe>Ca>Mg>Zn>Cu>Cd>Pb>As;
- prussian carp: K>Mg>Ca>Fe>Na>Zn>Cu>Pb>Cd>As;
- The accumulation trend of metals in the hepatic tissue in the wild fish was as it follows:
- turbot: K>Na>Mg>Ca>Fe>Zn>As>Cu>Mn>Ni>Cd;

    - catfish: K>Na>Fe>Mg>Ca>Zn>Cu>Cd>Pb>As;
    - carp: K>Na>Fe>Ca>Mg>Zn>Cu>Cd>Pb>As;
    - prussian carp: K>Ca>Fe>Na>Mg>Zn>Cu>Pb>Cd>As;
    - common bream: K>Na>Ca>Mg>Fe>Zn>Cu>Cd>Pb>As;
    - shad: K>Na>Fe>Ca>Mg>Zn>Cu>As>Cd>Pb;
    - asp: K>Mg>Na>Ca>Fe>Zn>Cu>Pb>Cd>As;
    - pike: K>Na>Ca>Mg>Fe>Zn>Cu>Pb>Cd>As;
    - mackerel: K>Na>Ca>Mg>Fe>Zn>Cu>As>Cd>Pb;
    - european flounder: K>Na>Fe>Ca>Mg>Zn>Cu>As>Cd>Pb;
    - mullet: K>Na>Fe>Mg>Ca>Cu>Zn>As>Cd>Pb;
  - The accumulation trend of metals in the liver tissue of the fish species outlines the fact that the macro-element K is the most abundant, while, at the opposite pole there is As and Pb.
  - The bioaccumulation of metals in the muscle and liver tissue of various fish species analyzed in this study have registered significant differences ($p>0.05$); it can be concluded that the bioaccumulation process occurs in a different manner, both interspecific and intraspecific.
- ✓ The main conclusions that were drawn from the ***oxidative stress analysis***, obtained from the evaluation of biochemical indicators registered in the muscle and liver tissue of the analyzed fish species consisted of the following:
  - The majority of the biochemical indicators based on which the oxidative stress (SOD, GPx, MDA and glycogen) was evaluated did not registered significant differences ($p<0.05$) between the muscle and the liver tissue, in both cultured and wild fish species, with only one exception, namely CAT, which registered higher values in the liver. As a result, it can be concluded that the oxidative stress has manifested in the same proportion, in both the muscle and the liver tissue;
  - From the fish species prelevated from the natural environment, the catfish (*Silurus glanis* Linnaeus, 1758) and the shad (*Alosa immaculata* Bennett, 1835) registered the highest values of biochemical indicators which show the level of oxidative stress, respectively of enzyme activity specific to this process;
  - The oxidative stress level, revealed by the mentioned biochemical indicators, it was superior to fish species from natural aquatic ecosystems compared to the cultured species.
- ✓ The calculation of the ***pollution index*** has led to the contouring of the following conclusions:
  - The presence of the Cd, Pb, Ni, Cu, and Zn had no effect on the aquatic ecosystems in which they are present, in all of the studied stations, except for the S3 station (Danube Harbour Galați), which proved to be seriously affected by the presence of Zn.
  - In case of the Fe presence in the studied aquatic ecosystems and following the pollution index calculation, it was revealed that the aquatic ecosystems are seriously affected by the presence of this metal, in all sampling stations included in the study.
- ✓ Following the analysis of the data obtained from the ***estimated daily intake***, it can be concluded that the ingestion of essential elements (Zn, Cu) and elements with toxic potential (Cd, Pb, As) through the consumption of fish does not represent a risk for human consumers from Romania or adverse effects on human health. Also, the differences between the estimated daily intake in the case of wild fish and the ones produced in aquaculture were not significat ($p<0.05$).

Personal contributions and recommendations

The quality of surface waters represents one of the major societal challenges of the XXI century and its degradation represents a threat to human health, a limiting factor of the food production, reducing the functions of the ecosystems and affecting economical growth. The degradation of the water’s quality transposes directly into the environmental, social, and economic problems. The availability of water resources is strongly affected by pollution of the freshwater ecosystems, caused by the discharges of domestic water which is insufficiently treated or non-treated into rivers, lakes and coastal waters. Additionally, newly emerging pollutants from personal care products, pharmaceuticals, pesticides, substances used in households and in the industry, and under the influence of climate changes represent a new challenge in terms of water quality, with an unknown impact, on the long term, on human health and ecosystems.

As such, in the context of the aforementioned aspects, the continuous monitoring of natural aquatic and anthropogenic ecosystems represents an imperative necessity, in order to collect the required data to draw up a holistic picture regarding their quality, as well as the opportunity to intervene quickly and apply an efficient control management in the cases which require it.

At a national level, this work represents an innovation in terms of:

- ✓ *The presence of macro- and micro-elements, some with toxic potential, in the macrosystem Danube-Danube Delta-Black Sea.*
- ✓ *The multilevel analysis regarding the bioaccumulation of metals with toxic and non-toxic potential, in the aquatic ecosystems of Romania.*
- ✓ *The comparative study regarding the quality of natural vs. anthropogenic aquatic ecosystems from Romania.*
- ✓ *The comparative study regarding the quality of the wild vs. cultured fish from Romania.*
- ✓ *The impact of fish consumption on the human consumers from Romania.*
- ✓ *The realisation of mathematical models of predictions type by using the “machine learning” technique.*
- ✓ *Building a database which will be used as a reference in future profile studies, this way, offering the real possibility to obtain the multilevel identification for the accumulation trends of the metals.*

Worldwide, the high degree of novelty and originality of this paper is given by:

- ✓ *the analysis regarding the multilevel traceability of xenobiotics in a macrosystem river-delta-sea and wetlands.*
- ✓ *The analysis of the quality of aquatic ecosystems from the wetlands of international importance and from the existent sites included in the world heritage list of UNESCO.*

Thus, following a detailed study of the available speciality literature it can be asserted that none of the two aforementioned analyses was found to be previously studied.

In conclusion, the paper brings a significant contribution to the elucidation of the addressed phenomena. Also, the recorded results have shown a decrease trend of pollution with metals in the aquatic ecosystems from Romania, a fact that is directly connected to the reduced industrial activity after the year 1989. However, in Romania, an emerging source of diffuse pollution, in the Danube Delta, is represented by the agricultural activity which is practiced in an intensive and super-intensive manner, using agrochemical substances which contain significant amounts of metals. Therefore, I recommend the transition from conventional agricultural practices to the ecological type, particularly in the area adjacent to the wetlands from the Danube Delta, and using agrochemical substances with a low content of Cd, Pb and As. As well, in terms of food safety, it is *recommended the consumption of fish produced in controlled aquaculture systems in order to minimize the potential exposure of human consumers to various concentrations of heavy metals from the consumption of fish and fish products.*

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1. Petrea S. M., **Simionov I.,** Coada M., Cristea V., Zugravu A., Cretu M., Bandi C**.**, **Atmospheric and biological models for aquaponics production systems**; LAP Lambert Academic Publishing (2017); în limba engleză, nr. pag. 152, ISBN-13: 978-620-2-06369-2.
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