„Dunărea de Jos” University of Galaţi

Doctoral School of Fundamental Sciences and Engineering

PhD THESIS

SUMMARY

SCIENTIFIC AND NUTRITIONAL ASPECTS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) BIOACCUMULATION IN AQUATIC ORGANISMS ALONG THE BLACK SEA COAST AREA

PhD student,
Nicoleta-Alexandra Bucur (Damir)

Scientific Coordinator,
Prof.dr.eng Elisabeta BOTEZ

Seria I.7: FOOD ENGINEERING Nr.14

GALAŢI

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SCIENTIFIC AND NUTRITIONAL ASPECTS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) BIOACCUMULATION IN AQUATIC ORGANISMS ALONG THE BLACK SEA COAST AREA

PhD student. Nicoleta-Alexandra BUCUR (DAMIR)

INTRODUCTION

Monitoring the marine environment is of particular importance for ensuring food security and safety.This is especially true when it comes to fish and fishery products, as they are undoubtedly an integral part of any healthy diet *(Kissao et al., 2011).* Marine organisms are able to accumulate large amounts of organic pollutants in their tissues, however, this accumulation differs depending on the species and can be transferred to the food chain, sometimes exceeding the accepted thresholds for human consumption *(Chang-Liang Ke et al., 2017).* Industrial and urban activities in coastal areas are the main sources of inorganic and organic pollutants in the marine environment. Among the organic pollutants found in the marine environment, polycyclic aromatic hydrocarbons (PAH) are of particular importance. Polycyclic aromatic hydrocarbons are a group of several hundred organic compounds that consist of two or more aromatic rings condensed in a linear, angular or grouped arrangement and, as indicated on their behalf, contain only carbon and hydrogen *(EO Nwaichia et al., 2016*).

The carcinogenic potential of polycyclic aromatic hydrocarbons is generally associated with the structure and complexity of the molecule, so that a more complex compound has a higher carcinogenic potential. However, due to the fact that polycyclic aromatic hydrocarbons are often found in complex mixtures, the carcinogenicity of individual hydrocarbons is often difficult to specify *(S. Yurchenko et al., 2005).* Polycyclic aromatic hydrocarbons can come from anthropogenic or natural sources. The main anthropogenic sources of polycyclic aromatic hydrocarbons include coal liquefaction plants, carbon black generation, coal tar pitch formation, asphalt manufacturing process, catalytic cracking process and related activities in oil refineries, as well as gas evacuation from vehicles. The release of polycyclic aromatic hydrocarbons into the environment is achieved by incomplete combustion of carbon-containing materials (coal, oil, wood), by direct oil spills or refined petroleum products, by erosion of crude oil-contaminated materials in the aquatic environment *(Hussein I. et al. ., 2016*). In addition to anthropogenic sources, their natural formation is known, and it is known that perylene can be generated by diagenesis under anaerobic conditions and that naphthalene, phenanthrene and perylene can be produced naturally as a result of intense biological activity *(Amos-Tautua and al., 2013).*

Long-term exposure to even low doses of organic contaminants can have endocrine disrupting effects in both animals and humans. Although there are different routes of exposure to this class of organic compounds, it has been established that food accounts for more than 90% of total human exposure *(Husam Alomirah et al, 2011*). According to studies on the exposure of people from different countries to organic pollutants through the consumption of processed foods, it was observed that food intake varies considerably between countries, being influenced mainly by specific eating habits in each country *(Lochan Singh et al., 2016).* Consumption of certain foods of animal origin has been associated with an increased incidence of various cancers, especially colorectal and esophageal cancer. However, there are not many studies to assess the potential carcinogenic risk of meat in relation to the exposure of the population to this risk due to high meat consumption *(Ángel Rodríguez Hernández and Luis D. Boada, 2015*). The cooking process improves the quality of food from microbiological point of view, by inactivating pathogenic microorganisms and improves the digestibility and bioavailability of nutrients in the digestive tract *(A. Malik et al., 2008).*

Fish is prepared in different forms: boiling, frying, baking, marinating, salting, smoking. The most common methods of cooking meat are boiling and frying. Boiling is a very popular method of cooking, which involves immersing food in water, being also a dietary method of cooking. During the cooking process, B vitamins helps the body to use carbohydrates, proteins, fats and minerals, and than are released into the fluid around food. Up to 60% of B vitamins and 50% of vitamin C are lost during cooking. Frying is one of the oldest methods of food preparation. By frying, their sensory quality is improved by forming flavor compounds, attractive colors, crusts and texture. A number of elements are essential for the end point of cooking or cooking time, respectively frying, such as: the degree of browning, crusting, flavour compounds and a certain degree of "inside" cooking, reflected in the color, taste and texture of the products *(Albert CM et al., 2005*).

Due to its nutritional content and taste quality, fish is one of the most valuable foodstuff, being a rich source of iodine needed for the manufacture of thyroid hormons. Fish meat contains important nutrients, so it should be a major intake in the daily diet of humans. Fish is also an important source of fat-soluble vitamins (A, D) and water-soluble B-complex vitamins (B1, B2, B6, B12) *(Barlow S, Chesson et al., 2010).*

The choice for Ph.D thesis "***Scientific and nutritional aspects of polycyclic aromatic hydrocarbons (PAHs) bioaccumulation in aquatic organisms along the Black Sea coast area***" was based on two reasons. The first reason was that these persistent lipophilic compounds classified as potential carcinogenic/mutagenic compounds have a negative impact on the marine environment and implicitly on marine organisms. The second reason was to assess the risks to human health due to excessive consumption of fish and fishery products.

The Ph.D thesis entitled "***Scientific and nutritional aspects of polycyclic aromatic hydrocarbons (PAHs) bioaccumulation in aquatic organisms along the Black Sea coast area*** "aimed the comparative assessment of the content of polycyclic aromatic hydrocarbons (PAHs) in fresh marine organisms from Black Sea coastal area with the accumulation of these compounds as an effect of preparation techniques applicated for the analysis benefits and risks to human health as a result of the consumption of fish and fish products. Therefore, the Ph.D. thesis theme is important and suits perfectly to food industry actual perspectives.

The main scientific objectives of the research conducted during the doctoral studies were:

- Assessment of polyaromatic hydrocarbons and heavy metals content in marine organisms of commercial interest (fish: anchovy, sprat, horse mackerel, gobies; molluscs: rapana and mussels) from the Black Sea coast area;
    - extraction and purification of polycyclic aromatic hydrocarbons in fresh marine organisms;

- analysis the level of polycyclic aromatic hydrocarbons in relation to the maximum limits allowed by the legislation in force;
- determination of heavy metals content in fresh marine organisms;
- identification and quantification of polyaromatic compounds in pelagic and benthic species of commercial interest in the Black Sea coastal area;

- Study of polycyclic aromatic hydrocarbons accumulation (PAHs) as an effect of application some preparation techniques.
  - identification and quantification of interest compounds in preparate marine organisms;
  - determination of polycyclic aromatic hydrocarbons content in preparate marine organisms ;
  - effects assessment of different preparation methods on fish and molluscs quality by analyzing the polycyclic aromatic hydrocarbons content in fresh tissue compared to the processed one;
- Analysis of risks and benefits to human health by monitoring the consumption of marine organisms;
  - risks and benefits researches related to human health as a result of consumption fish;
  - potential toxic and carcinogenic risks assessment associated with high concentrations exposure of polycyclic aromatic hydrocarbons through excessive consumption of fish and fishery products;
  - presentation of the effects related to human exposure to potential toxic and carcinogenic contaminants through fish consumption.

The doctoral thesis includes **3 chapters**. Each chapter contains study of specialized literature and original contributions.

Chapter 1, entitled ***Assessment of polycyclic aromatic hydrocarbons (PAH) content in aquatic organisms along the Black Sea coast area,*** presents data on biology and morphology fish and mollusc species investigated in order to assess polycyclic aromatic hydrocarbons and heavy metals content. In addition, this chapter describes the whole process of analysis (lyophilization, extraction and purification, gas-chromatographic analysis, calculation of polycyclic aromatic hydrocarbon concentrations) of polycyclic aromatic hydrocarbons content in different species of fresh fish and molluscs.

In chapter 2, entitled ***Accumulation of polycyclic aromatic hydrocarbons (PAHs) study as an effect of aquatic organism applicated conservation techniques*****,** is presented the polycyclic aromatic hydrocarbons (PAHs) assessemnt in marine organisms along the Black Sea coast area, following the application of preparation techniques (boiling and frying). Also, is presented the comparative analysis of fresh and thermally prepared products.

In chapter 3, entitled ***Analysis of risks and benefits to human health through the consumption of aquatic organisms,*** the benefits and risks associated fish and molluscs consumption are presented, experimental data on risks and benefits analysis to human health through the consumption of marine organisms. Marine specimens are reliable indicators of polycyclic aromatic hydrocarbons bioaccumulation from environment and have been used to estimate the risks of human exposure to contaminants.

Each chapter of the experimental study comprises the following subchapters: Introduction, Objectives, Materials and Methods, Results and Discussion, Partial conclusions and References.

Chapter 4, ***General Conclusions***, presents the main conclusions of the thesis experiments. The doctoral thesis consists of 150 pages, including 59 figures and 33 tables.

Finally, the original contributions of the Ph.D. thesis and the dissemination of the results are highlighted. Therefore, results have been capitalized through the development of 5 scientific articles, published or in progress of publication of which 3 articles in ISI journals (Journal of Environmental Protection and Ecology, Chemistry Journal, Polycyclic Aromatic Compounds Journal), 1 article published in unlisted journal (Marine Researches) and 1 article published in Conference Proceedings (GLOREP Conference Proceedings). In addition, the research results were presented at 10 international and national conferences.

The experiments within the Ph.D thesis were carried out within the National Institute for Marine Research and Development "Grigore Antipa" (www.rmri.ro), from Constanța.

The thesis was conducted under the scientific coordination of the steering committee with the following structure:

- **Prof. dr. eng. Elisabeta BOTEZ** - Ph.D. supervisor
- **SR gr.I dr Valentina COATU**
- **Prof.dr. chem. Rodica DINICĂ**
- **Prof. dr. eng. Nicoleta STĂNCIUC**

1. ASSESSMENT OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) CONTENT IN MARINE ORGANISMS ALONG THE BLACK SEA COAST AREA

1.1. General aspects

Fish and shellfish, raised in the wild or in aquaculture, have been an important part of the human diet for a long time and are currently 16.7% of the total protein consumed by the world's population (*Rosalee S. Hellberg et al., 2012).* Fish is an essential supplier of high quality nutrients, such as Omega 3 fatty acids, which help reduce the risk of stroke. It also has a role in regulating blood pressure and improving blood integrity and even reduces the risk of certain types of cancer *(Ángel Rodríguez-Hernández et al., 2016).*

1.2. Objectives of chapter 1

The main objective of the study was assessment of polyaromatic hydrocarbons and heavy metals content in marine organisms of commercial interest (fish: anchovy, sprat, horse mackerel, gobies; molluscs: rapana and mussels) from the Black Sea coast area.

The secondary objectives of the study were extraction and purification of polycyclic aromatic hydrocarbons in fresh marine organisms, analysis the level of polycyclic aromatic hydrocarbons in relation to the maximum limits allowed by the legislation in force, determination of heavy metals content in fresh marine organisms and identification and quantification of polyaromatic compounds in pelagic and benthic species of commercial interest in the Black Sea coastal area.

2. ANALYZED MOLLUSCS SPECIES

2.1. ANALYZED MOLLUSCS BIOLOGY

A. Morphological characterization of molluscs

Molluscs are soft-bodied animals, most of them have three regions: head, legs and visceral mass. The head is well developed and distinct from the rest of the body in gastropods and cephalopods. The foot is developed and is located in the ventral part of the body at most of the mollusks. In cephalopods it turned into arms around the head. It is a muscular organ that serves to move. It has different shapes, sole, stake. The visceral mass, where are most of the organs, is bag-shaped, being positioned on the dorsal side of the leg (except for cephalopods) (*Elisabeth Gosling, 2015*).

B. Analyzed mollusks species

Rapana (*Rapana venosa)*

It is part of the *Animalia* kingdom, the *Mollusca* cluster, the *Gastropoda* class. It is a large gastropod, with a globular shell, with an obvious spiral, formed by 3-4 fractures, which grow slowly and regularly, with the last fracture very developed, much dilated (*Luu Thang Pham et al., 2019*). The color is generally reddish-brown, often with brown or more intensely colored stripes.

Mussel *(Mytilus galloprovincialis)*

It is part of the *Animalia* kingdom, the *Mollusca* cluster, the *Bivalva* class. It is the most common bivalve species in the rocky infralittoral areas of the Black Sea, the groups of mussels forming a characteristic association *(Nsikak U.Benson et al., 2017).* The shell is large, relatively thin, but very strong.

2.2. ANALYZED FISH SPECIES BIOLOGY

A. Morphological characterization of fish species

The body of fish, most of the time, is covered with scales and the body shape is very varied (spindle-shaped, tapeworm-shaped, sagittal-shaped, needle-shaped, globular). Some fish have the body flattened ventral, other times the body is flattened laterally, becoming asymmetrical, by migrating both eyes on the same side of the body (*G. Radu et al., 2008).*

B. Analyzed fish species

Anchovy (*Engraulis encrasicolus)*

Anchovy belongs *Clupeiformes* order, *Engraulidae* family. It is a pelagic, gregarious marine species, which makes irregular migrations from the sea to the coast and vice versa, depending on the thermal and food conditions. It feeds very intensely in summer, less in winter, with phytoplankton and zooplankton, overwinter in large flocks, far from the shore, at a depth of 60-70 m, but can come to the surface occasionally (*G. Radu et al., 2008).*

Sprat (*Sprattus sprattus)*

Sprat belongs *Clupeiformes* order*, Clupeidae* family. The body is elongated, compressed laterally, and the operculum is smooth, without stripes. The eyes are large, situated in the center of the head and look sideways. The mouth is small, protractile, oblique upwards, slightly upper, the upper jaw does not touch the posterior edge of the eye. The body is covered by large and thin scales, missing the scales on the head, the ventral hull has 19-22 scales and the lateral line is invisible.

Horse mackerel (*Trachurus mediterraneus ponticus)*

The horse mackerel belongs *Perciformes* order, family *Carangidae*. It has two dorsal fins, the first higher than the second and made up of spiny radially joined by a membrane. In front of the first dorsal, there is a spine hidden under the skin and tilted forward. The anal is preceded by two thick and very sharp spines, joined by a membrane, separated from the rest of the fins. The ventricles are inserted slightly behind the pectorals (*G. Radu et al., 2008).*

Gobius (*Neogobius cephalarges*)

Gobius belongs *Perciformes order*, *Gobiidae family.* The body is elongated, thick and cylindrical in front, compressed laterally in the back. In general, the dorsal profile rises uniformly and fairly smoothly from the tip of the snout to the posterior edge of the operculum. Reproduction takes place in the sea, at the beginning of spring, without all the fish reaching sexual maturity at the same time.

3. INFLUENCING FACTORS ON THE NUTRITIONAL VALUE OF FISH

A number of factors, such as the feeding regime, the rearing system, the way in which the production process and the processing are carried out, influence the composition of the fish meat (*Lie, 2001).* As long as the fish in the aquaculture are fed with adequate food, rich in necessary nutrients and in sufficient quantities, the protein content of each fish species does not seem to be influenced by the food content and the feeding regime *(Morris, 2001).* In wild fish case, the composition of the meat cannot be controlled by the diet, and thus the quality of the fish and fish products will be affected by handling and processing process *(Erikson, 2001).*

3.1. The effects of feeding and rearing of fish and mollusks

Especially in aquaculture, the rearing system and the type of food will be of significant importance, because the fish have to feed on what they receive. This applies to both intensive and freshwater aquaculture. In marine aquaculture, fish oil is traditionally used in feed to provide fish with a sufficient proportion of Omega-3 polyunsaturated fatty acids and to raise fish with a nutrient composition rich in fatty acids (*Steffens și Wirth, 2007).*

3.2. Effects of water temperature and salinity

Along with feeding and rearing system, it was concluded that other factors such as salinity and water temperature influence the composition of fatty acids found in fish.

Following the recent study conducted by *Norambuena et al., 2016* it was concluded that water temperature clearly influences the composition of fatty acids. The research authors have raising salmon sapling in aquariums at temperatures of 10 and 20ºC. After the fish reached maturity, they were analyzed and it was observed that salmon that grew at a temperature of 10ºC had a higher content of Omega-6 fatty acids compared to salmon that grew at a temperature of 20ºC. In terms of water salinity, according to the *Swedish National Food Agency,* the herring from Baltic Sea (*Clupeus harrengus*) is less fatty compared to that found in the Salty North Sea.

4. MATERIALS AND METHODS

4.1. Materials used at polycyclic aromatic hydrocarbons content determination in fresh marine organisms

The analysis of polycyclic aromatic hydrocarbons was performed on two types of mollusks (*Rapana venosa* and *Mytillus galloprovincialis*), three pelagic fish species (*Sprattus* sprattus*, Engraulis encrasicolus, Trachurus mediterraneus ponticus)* and a bental fish species (*Neogobius cephalarges*) in 2016 -2018 period, from different areas along the Romanian Black Sea coast.

Specific sampling locations were selected in the shallow area between Sulina and Vama Veche. After sampling, they were stored in refrigerated boxes and transported to laboratories where they were measured, weighed, washed, eviscerated and reweighed.

4.2. Polycyclic aromatic hydrocarbons determination analytical method

Polycyclic aromatic hydrocarbons determination in marine organisms was done according to internationally agreed methods *(IAEA-MEL: Training manual on the Measurement of Organochlorines and Petroleum Hydrocarbons in Environmental Samples, 1995)*. The main steps of the polycyclic aromatic hydrocarbon analysis method are: preparation extracts for gas chromatographic analysis (extraction and purification) and gas chromatographic analysis.

4.2.1. Polycyclic aromatic hydrocarbons extraction and purification

In molluscs case, the soft tissue was removed from the shell, and the fishes were eviscerated by selecting the dorsal muscle which was used for analysis. After evisceration, marine organisms were preserved by freezing for lyophilization using a LABCONCO FreeZone 2.5.

For priority polycyclic aromatic hydrocarbons analyses about 2 g of the dried tissue were used. Internal standard 9,10-dihydroanthracene was added to the samples for quantifying the overall recovery of the analytical procedures. Samples were Soxhlet extracted for 8 h with 250 ml of methanol. The extracts were then saponified by adding 20 ml of 0.7 M KOH and 30 ml of water and refluxing for 2 h. The resulting mixture was transferred into a separating funnel and extracted three times with hexane – once with 90 ml, twice with 50 ml. The extracts were concentrated by rotary evaporation down to 15 ml using LABOROTA 4001 (*IAEA-MEL: Training manual on the Measurement of Organochlorines and Petroleum Hydrocarbons in Environmental Samples, 1995)*, and then further concentrated to about 5 ml under a gentle flow of clean nitrogen. Finally, the extract was cleaned up and fractionated by passing it through a silica/alumina column.

Elution was performed using 20 ml of hexane to yield the first fraction (containing the aliphatic hydrocarbons), then 30 ml of hexane: methylene chloride (90:10) and followed by 20 ml of hexane: methylene chloride (50:50). These two eluents containing the aromatic hydrocarbons (PAHs) were combined for analysis. The fraction containing PAHs was evaporated under a weak flow of nitrogen to 1 ml and it was subjected to qualitative and quantitative analysis on GC/MS Perkin Elmer Clarus 500.

4.3. Gas chromatographic analysis (GC)

The gas-chromatographic conditions were: capillary column Elite 35 MS, stationary phase: dimethylpolysiloxane (35% diphenyl), length 30 m, inner diameter 0,32 mm, film thickness 0,25 mm; carrier gas - helium, speed - 1 cm^3/min, divided debit 15 cm^3/min, sample volume - 2μl, injector temperature - 300°C, temperature programme – initial temperature 100°C,heating speed - 6°C/1 min, first isotherm - 250°C for 0 min, heating speed- 10°C* 10min, second isotherm - 330°C*10 min, interface temperature-330°C, source temperature - 270°C, analysis method, single ion r. (SIR)(*IAEA-MEL: Training manual on the Measurement of Organochlorines and Petroleum Hydrocarbons in Environmental Samples, 1995)*.

4.3.1. Identification and quantification of interest compounds in fresh marine organisms

Polycyclic aromatic hydrocarbons have been identified based on retention times and characteristic ions.

4.3.2. Polycyclic aromatic hydrocarbons concentrations calculation

For polycyclic aromatic hydrocarbons concentrations calculation will be taken into account the recovery factor – R (1), the sample area, the standard area, the standard concentration, the extract volume and the tissue mass (2) *(IAEA-MEL: Training manual on the*

Measurement of Organochlorines and Petroleum Hydrocarbons in Environmental Samples, 1995).

$$R\% = \frac{(\,Extract\ volume\ x\ \ Standard\ area)}{2} \qquad (1)$$

$$C_{PAH}(\mu g/kg) = \frac{Sample\ area}{Standard\ area} x\ standard\ concentration(ppm)\ x\ \frac{Extract\ volume}{Tissue\ mass} x1000\ (2)$$

4.4. Lipid content determination analytical method

For lipid content analysis about 2 g of dried tissue were used. Lipid extraction from biota samples was performed with 30 ml of solvent (1:1 hexane/acetone) in microwave extraction system for 50 minutes. The extracts were filtered and their volume was measured. From each filtrate obtained was added 250 µL in aluminum trays (which were weighed before) and were placed on a hot plate at 100° C until complete evaporation and then weighed.

For lipid content calculation (3), will be taken into account the dried tissue mass (m), the total volume extract measured (V) and the volume used (v).

$$Lipid\ content = \frac{\mathrm{m(mg)xV(ml)}}{\mathrm{v(ml)}} \qquad (3)$$

4.5. Heavy metals determination analytical method

Sampling methods, conservation, preliminary processing and analysis complied with the reference methods recommended in the marine pollution study *(UNEP, 1993).*

For heavy metals determination, the biological samples were homogenized, weighed and digested with 5 mL nitric acid (65% HNO3, Suprapur, Merck), in sealed teflon vessels (60 mL, Savillex), on an electric plate at 1200° C. Upon completion of mineralization, samples were brought to a volume of 100 ml with deionized water (18.2MΩ.cm, Millipore) and prepared for graphite furnace atomic absorption spectrometric analysis (GF-AAS).

4.5.1. Heavy metals analytical determination by atomic absorption/emission spectrometry

In obtained solutions after biota samples digestion, the metals were analyzed using atomic absorption spectrometer with graphite furnace, type ATI-UNICAM 939Z and SOLAAR M6 Dual Thermo Electron-UNICAM.

4.5.2. Atomic absorption spectrometry with graphite furnace (GF-AAS)

The atomic absorption method is quantitative and relative, it requires a prior calibration. Standard stock solutions at concentration of 1000 ± 2 mg/l element, commercially available (Merck), were used. From these were prepared, by successive dilutions, intermediate stock standard solutions (5000 ng/ml) and standard solutions (highest concentration on the curve, top standard). Depending on the working range of each element (linearity of the calibration curve), they had concentrations of 10 ng/ml (Cd), 25 ng/ml (Pb), 50 ng/ml (Ni, Cu) or 100 ng/ml (Cr). To the standard working solutions is added 5 ml of nitric acid, in order to have the same matrix as the samples after preliminary processing. The solutions thus prepared are stable for a few days at 4ºC + 2ºC *(UNEP, 1993).*

The GF AAS technique has the advantage of being very selective, it allows the quantitative determination of the elements present at trace level (detection limits of 0.010 µg/l Cd; 0.25 µg/l Cr; 0.065 µg/l Cu; 0.03 µg/1 Pb; 0.03 µg/l Mn; 0.065 µg/l Ni). It is important to take into account certain concentration levels must fall within the Lambert-Beer linearity range.

4.5.3. Inductively coupled plasma atomic emission spectrometry (ICP/AES)

Emission spectrometry is a method of physico-chemical analysis based on spectrum study of lines emitted by atoms at high temperatures. The sample is introduced into a source that ensures the thermal or electrical excitation of the atoms. Then the emitted line spectrum is analyzed using high resolution monochromators. The intensity of the spectral lines is measured directly using photomultipliers *(UNEP, 1993).*

5. RESULTS AND DISCUSSION

5.1. Results and discussions on polycyclic aromatic hydrocarbons content in fresh investigated marine organisms

The analysis of polycyclic aromatic hydrocarbons was performed on two types of mollusks (*Rapana venosa* and *Mytillus galloprovincialis*), three pelagic fish species (*Sprattus* sprattus*, Engraulis encrasicolus, Trachurus mediterraneus ponticus)* and a bental fish species (*Neogobius cephalarges*) in 2016 -2018 period, from different areas along the Romanian Black Sea coast.

Polycyclic aromatic hydrocarbons total content in rapana species, during 2016-2018, from the Romanian Black Sea coast varied between 0.0005 and 2776.6374 µg/kg wet tissue. These values are much higher compared to those obtained by *V.Coatu et al, 2016* in *Rapana venosa* taken from Black Sea western sector (65.6-641.6 µg/kg wet tissue). Individual compounds concentrations in *Rapana venosa* ranged from 0.0001 to 2248.495 µg /kg wet weight. The dominant compounds were naphthalene, fluorene and phenanthrene which recorded values of 2248.49, 2776.63 respectively, 180.79 µg/kg wet weight (Figure 5.1.1.).

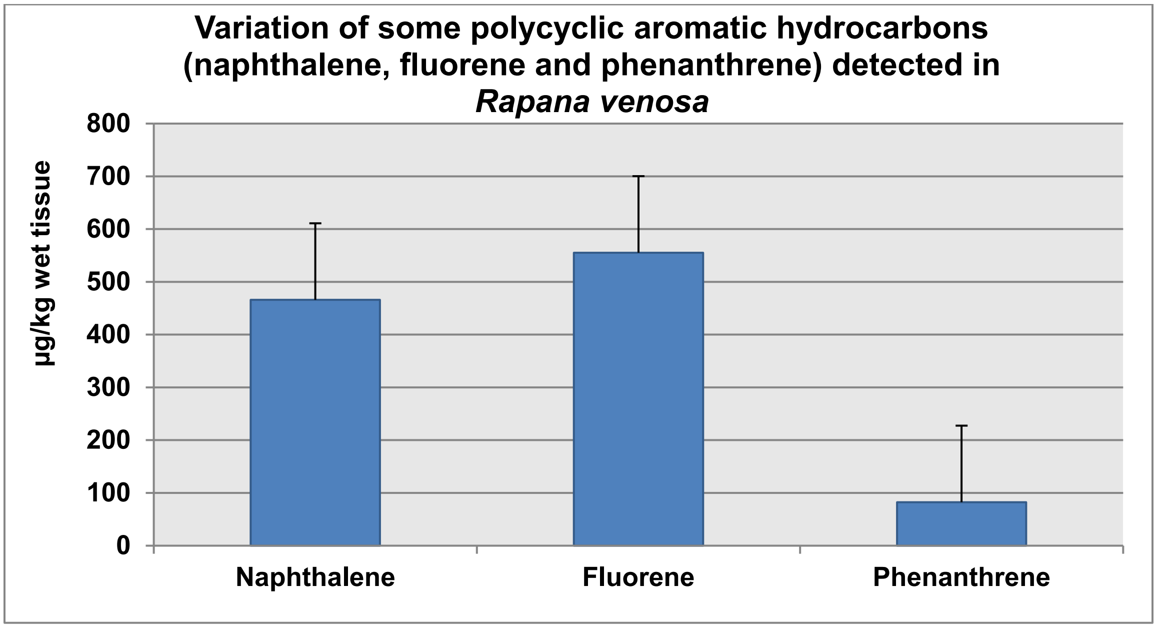

Figure 5.1.1. Variation of some polycyclic aromatic hydrocarbons (naphthalene, fluorene and phenanthrene) detected in *Rapana venosa* from Romanian Black Sea coast area

The total content of polynuclear aromatic hydrocarbons in *Mytilus galloprovincialis* taken from Romanian Black Sea sector varied between 1.2022 and 517.1719 µg/kg wet tissue. These results are comparable to those of *Karacık et al., 2009* in mussels taken from the Istanbul Strait (43.0 - 601.0 µg/kg wet tissue).

Individual compounds concentrations ranged from 0.0001 to 223.8137 µg/kg wet weight. The dominant compounds were naphthalene, fluorene and phenanthrene, recording average values of 34.8503, 24.0559 and 73.8817 respectively, µg/kg wet weight (Figure 5.1.3). The analysis of polycyclic aromatic hydrocarbons total content in *Mytilus galloprovincialis* taken from the Mediterranean Sea revealed values between 26.75 and 107.67 µg/kg wet weight. These concentrations are lower compared to total hydrocarbons content values recorded in mussels taken from the Romanian Black Sea coast area.

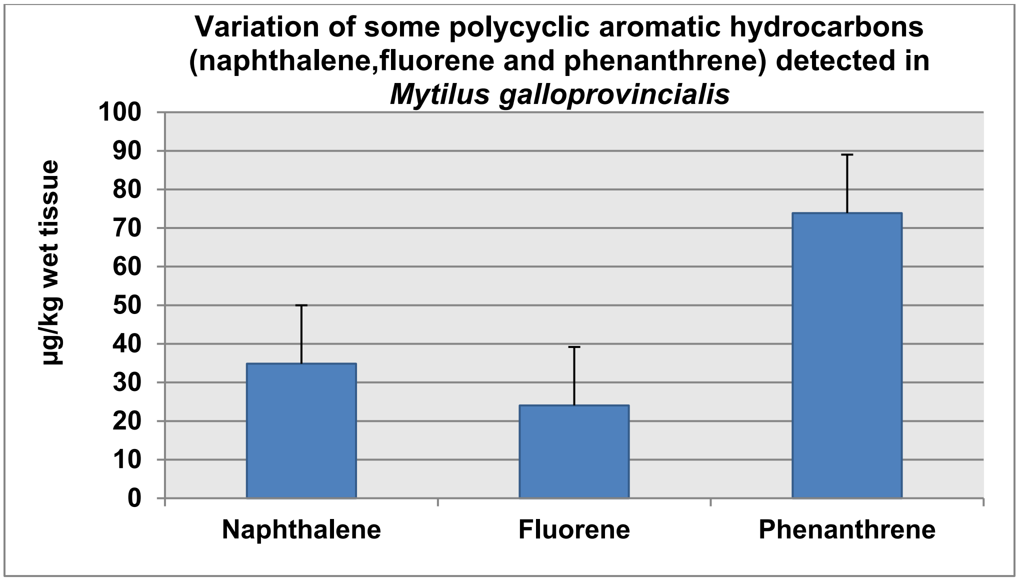

Figure 5.1.3. Variation of some polycyclic aromatic hydrocarbons (naphtalene, fluorene and phenanthrene) detected in *Mytilus galloprovincialis* from Romanian Black Sea coast area

In anchovy (*Engraulis encrasicolus*) from Romanian coast area, individual compounds concentrations varied from 0.0001 at 597.27 µg/kg wet tissue. The dominant compounds were phenanthrene, fluorene and naphthalene recording average values of 597.27, 487.3557, respectively, 20.1421 µg/kg wet tissue (Figure 5.1.5).

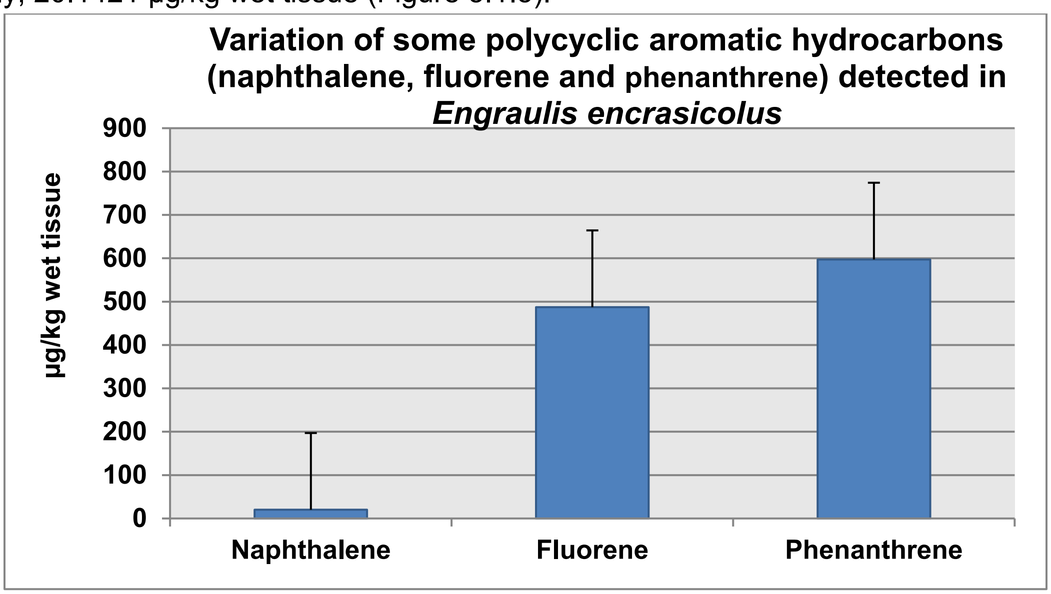

Figure 5.1.5. Variation of some polycyclic aromatic hydrocarbons (naphthalene, fluorene and phenanthrene) detected in *Engraulis encrasicolus* from Romanian Black Sea coast area

In Sprattus sprattus, individual compounds mean values (0.0200 and 2.9226 µg/kg wet tissue) were measured.

The highest concentrations werw recorded for phenanthrene (2.9226 µg/kg wet tissue) and naphthalene (1.4519 µg/kg wet tissue) (Figure 5.1.7.).

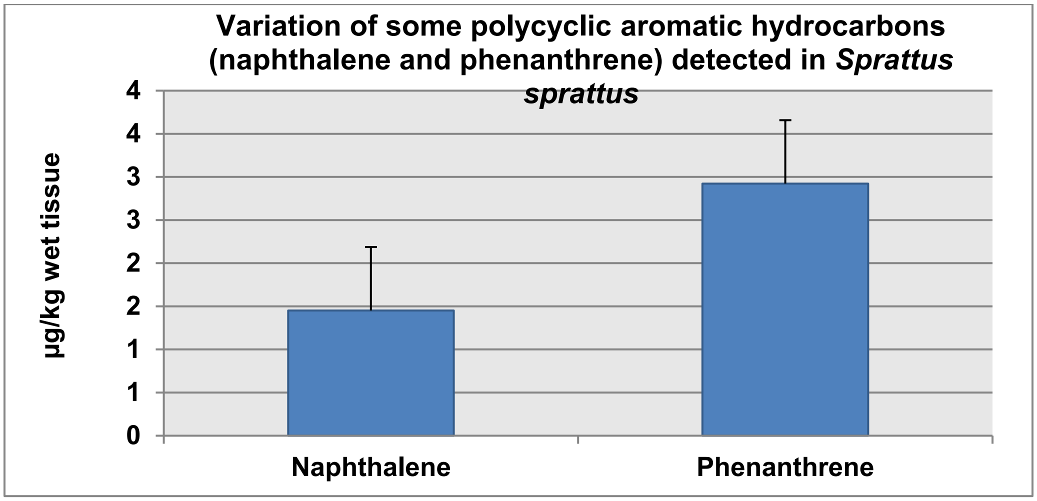

Figure 5.1.7. Variation of some polycyclic aromatic hydrocarbons (naphthalene and phenanthrene) detected in *Sprattus sprattus* from Romanian Black Sea coast area

In horse mackerel samples *(Trachurus mediterraneus ponticus*) from the Romanian Black Sea coastal area, polycyclic aromatic hydrocarbons total content varied from 8.0149 to 926.2241 µg/kg wet tissue. Individual compounds concentrations ranged from 0.0001 to 508.3701 µg/kg wet weight. The average values of the individual compounds varied from 0.679-to 77.1853 µg/kg wet tissue, the highest concentrations being recorded for naphthalene (77.1853 µg/kg wet tissue), phenanthrene (74.8695 µg/kg wet tissue) and acenaphthene (9.5267) (Figure 5.1.9).

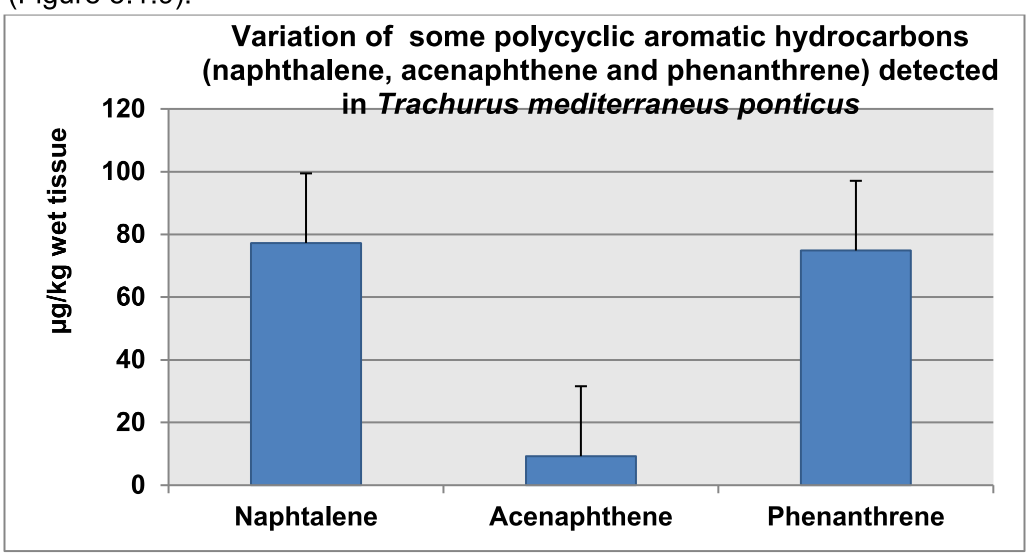

Figure 5.1.9. Variation of some polycyclic aromatic hydrocarbons (naphthalene, phenanthrene and acenapthene) detected in *Trachurus mediterraneus ponticus* from Romanian Black Sea coast area

In gobies (*Neogobius cephalarges)* samples taken during 2016-2018, from the Romanian coastal area, polycyclic aromatic hydrocarbons total content varied from 0.0002 to 171.4768 µg/kg wet tissue. The mean values of the individual polyaromatic compounds varied between detection limit and 85.7384 µg/kg wet tissue, the highest values being recorded for phenanthrene (85.7384 µg/kg wet tissue), acenaphthylene (1.6911 µg/kg wet tissue) and acenaphthene (1.7582 µg/kg wet tissue)(Figure 5.1.11.).

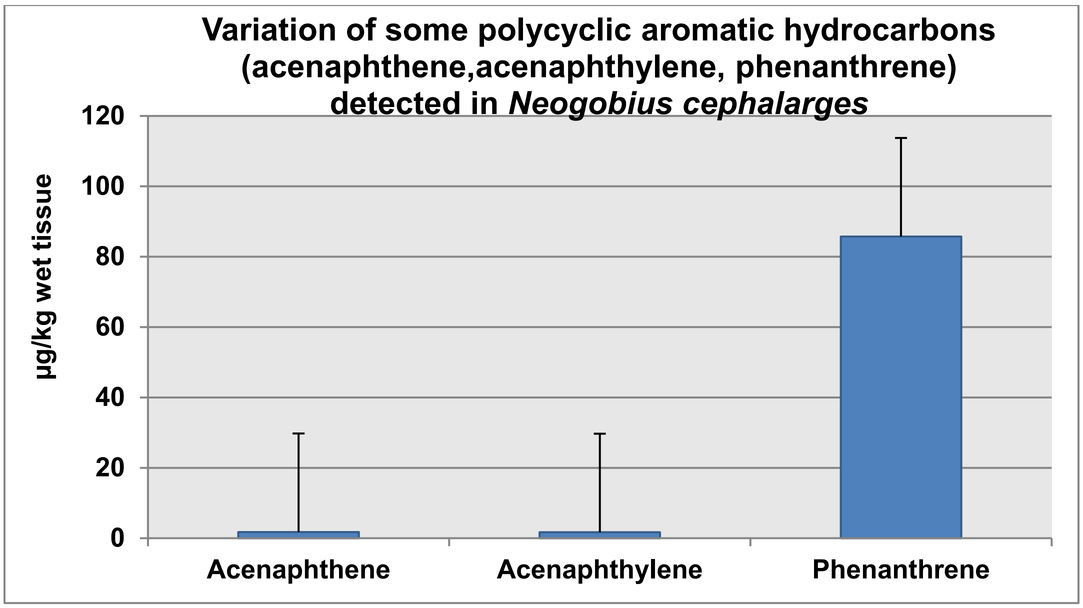

Figura 5.1.11. Variation of some polycyclic aromatic hydrocarbons (phenanthrene, acenapthene and acenaphtyhlene) detected in *Neogobius cephalarges* from Romanian Black Sea coast area

5.2. Results and discussions on lipid content in fresh investigated marine organisms

Among the investigated molluscs, *Rapana venosa* accumulated the largest amount of polyaromatic hydrocarbons. The highest lipid content was measured in *Mytilus galloprovinicialis* (Figure 5.2.1). This is because these mollusks are quite fatty, but this fat is "useful", including polyunsaturated fatty actions with an important role in improving brain function and restoring previous visual acuity.

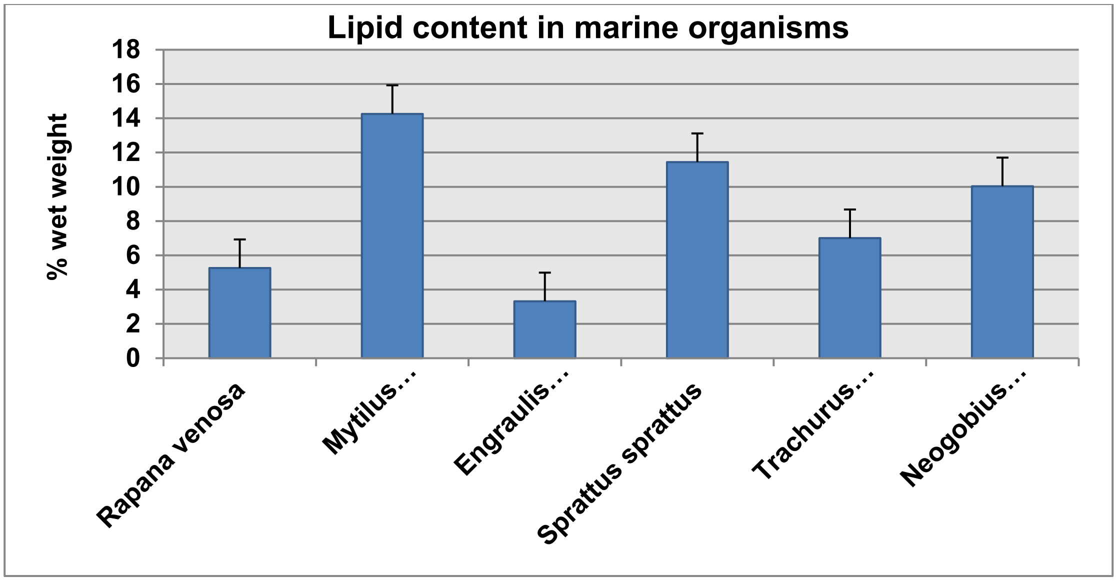

Figure 5.2.1. Lipid content in marine organisms from the Romanian Black Sea coast area, during 2016-2018

The results obtained showed that the lipid content does not correlate with polycyclic aromatic hydrocarbons level accumulated in molluscs and fish species. *Rapana venosa* accumulated the highest amount of hydrocarbons, while *Mytilus galloprovinicialis* had the highest lipid content, and of the fish species analyzed, the highest accumulation of hydrocarbons was found at *Trachurus mediterraneus ponticus* and the highest lipid content was recorded by *Sprattus sprattus.* Despite the low lipid content both species have accumulated a large amount of hydrocarbons, this accumulation can occur through the food chain *(E.O. Nwaichia et al., 2016*).

5.3. Results and discussions on heavy metals content in fresh investigated marine organisms

Compared to the permissible levels *(EC Regulation no. 1881/2006),* for contaminants, in *Rapana venosa* (Figure 5.3.1.) and *Mytilus galloprovincialis* (Figure 5.3.2), lead concentrations were below the threshold in all samples of *Mytilus galloprovincialis* and *Rapana venosa* investigated. Also, mussels had cadmium concentrations below the maximum permissible values for consumption. Slight exceedances of the regulated cadmium level were measured for one Mytilus sample from Sfântul Gheorghe and for one Rapana sample from Portița. The analyzes were performed on whole tissue (sole and viscera). The other investigated elements (copper, nickel, chromium) are in normal variation ranges.

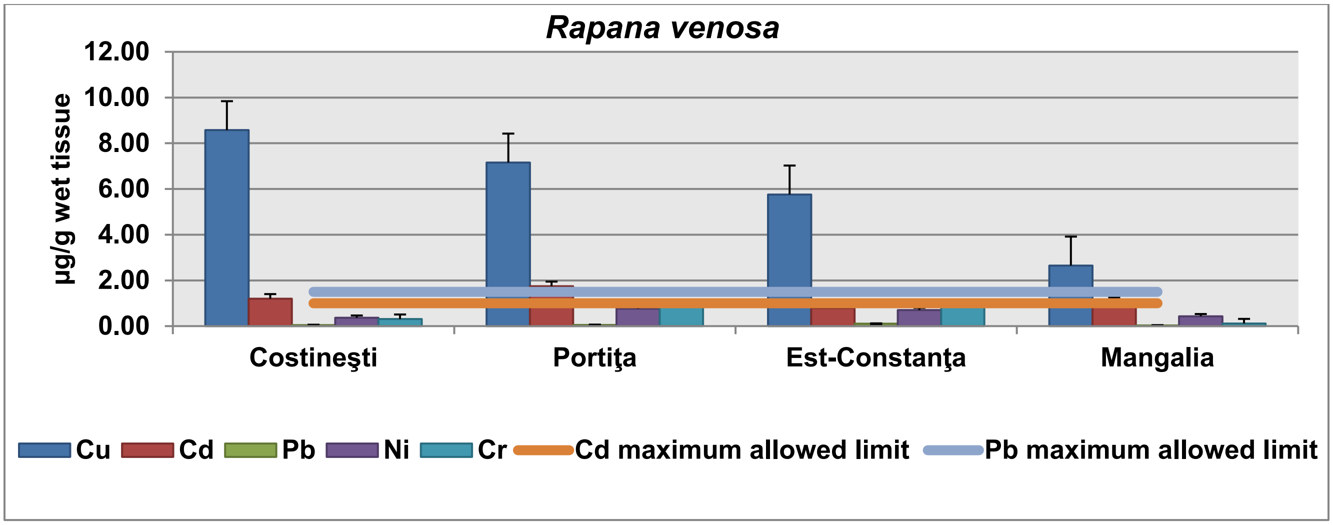

Figure 5.3.1. Heavy metals concentrations in *Rapana* venosa along the Romanian coast area, during 2016-2018

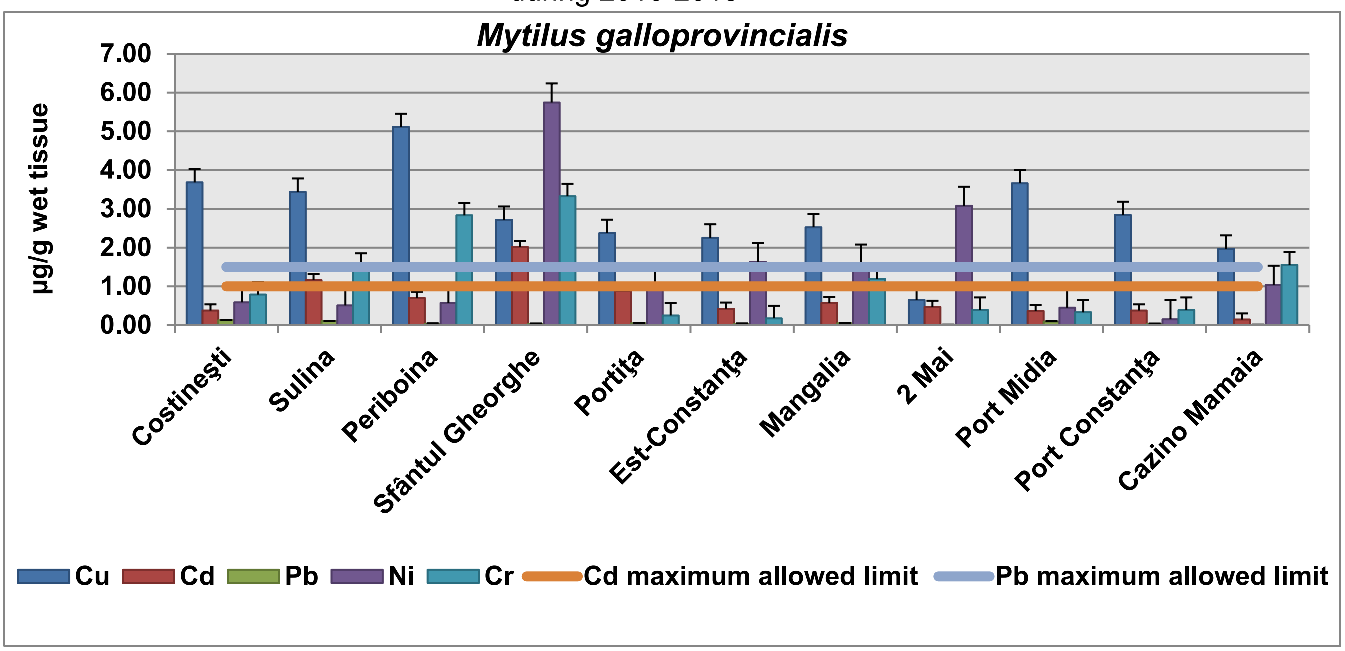

Figure 5.3.2. Heavy metals concentrations in *Mytilus* galloprovincialis along the Romanian coast area, during 2016-2018

Compared to the permissible levels for contaminants, in marine pelagic species *Engraulis encrasicolus* and *Sprattus sprattus* (EC Regulation no. 1881/2006), cadmium and lead concentrations were below the threshold value (Figures 5.3.3, 5.3.4.). Slight exceedances of the regulated lead level were measured in a sample of anchovy (*Engraulis encrasicolus*) from 2 MAI area.Heavy metals concentrations detected in *Trachurus mediterraneus ponticus* and *Neogobius cephalarges,* from the Romanian Black Sea coast area, were below the maximum limit allowed by *EC Regulation no. 1881/2006* (Figures 5.3.5, 5.3.6).

Exceedances of the regulated lead level were measured in a sample of horse mackerel from Mangalia.

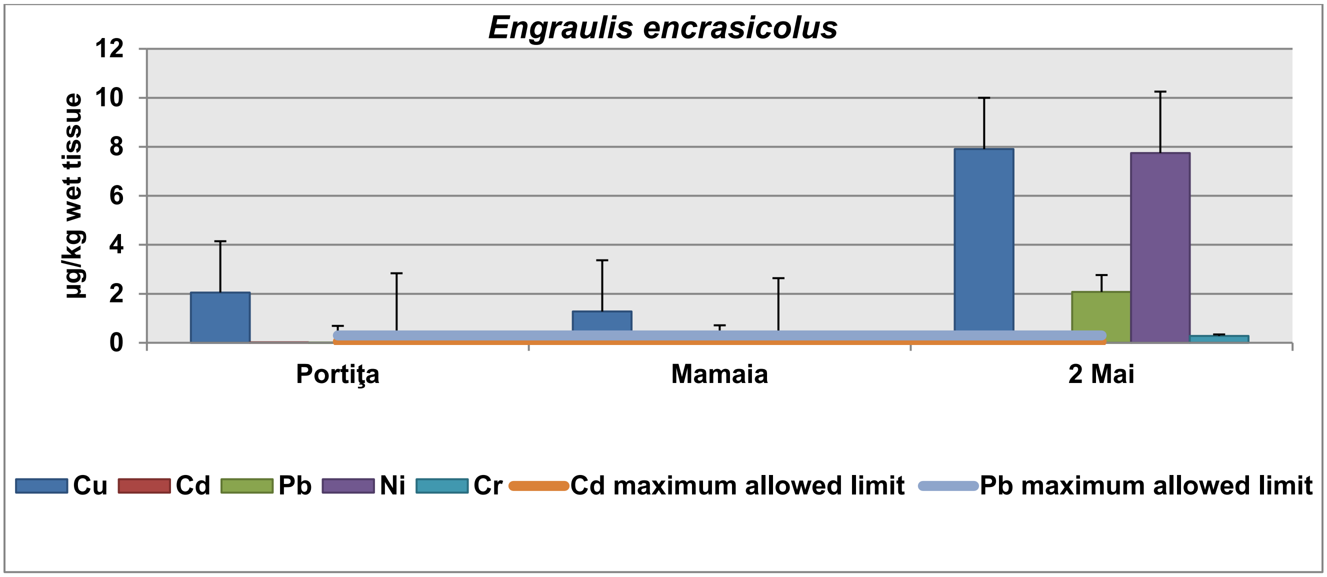

Figure 5.3.3. Heavy metals concentrations in *Engraulis encrasicolus* along the Romanian coast area, during 2016-2018

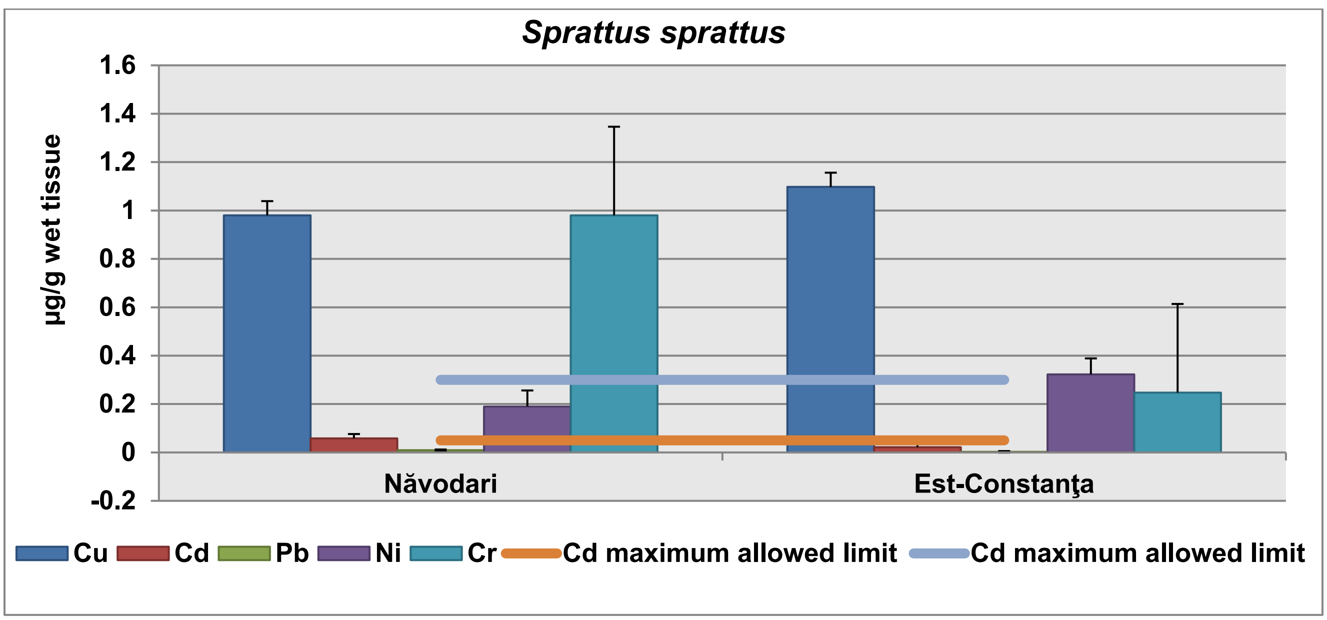

Figure 5.3.4. Heavy metals concentrations in *Sprattus sprattus* along the Romanian coast area, during 2016-2018

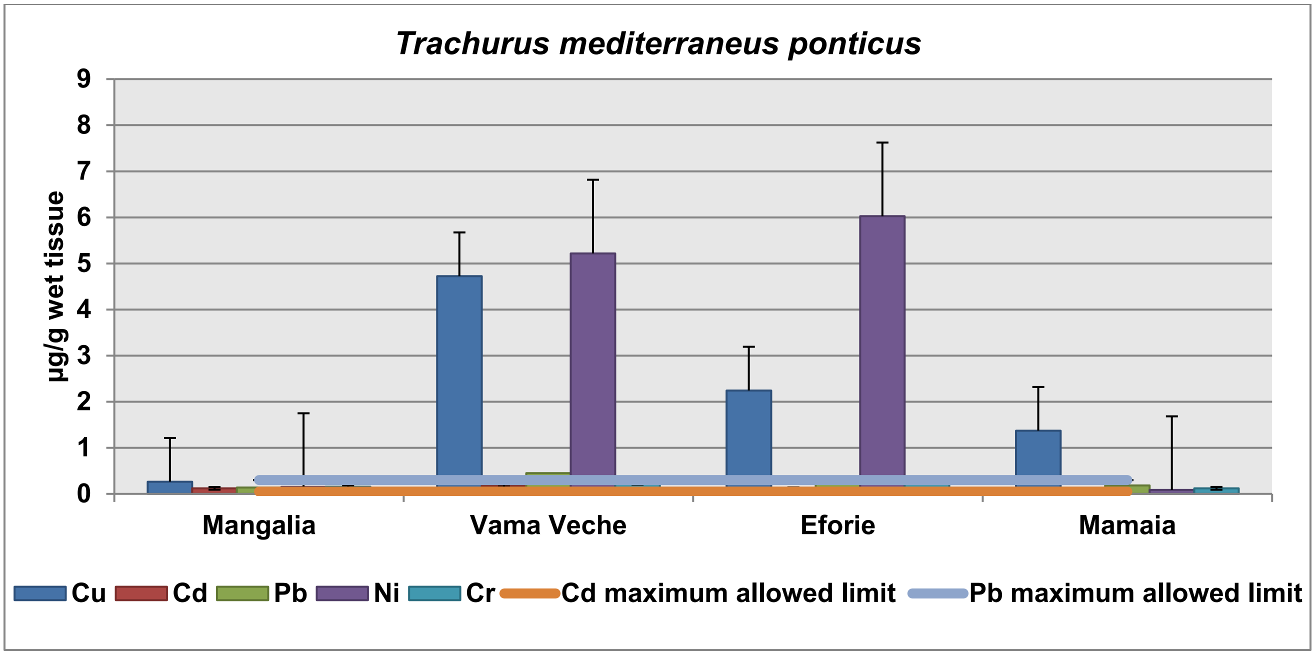

Figura 5.3.5. Heavy metals concentrations in *Trachurus mediterraneus ponticus* along the Romanian coast area, during 2016-2018

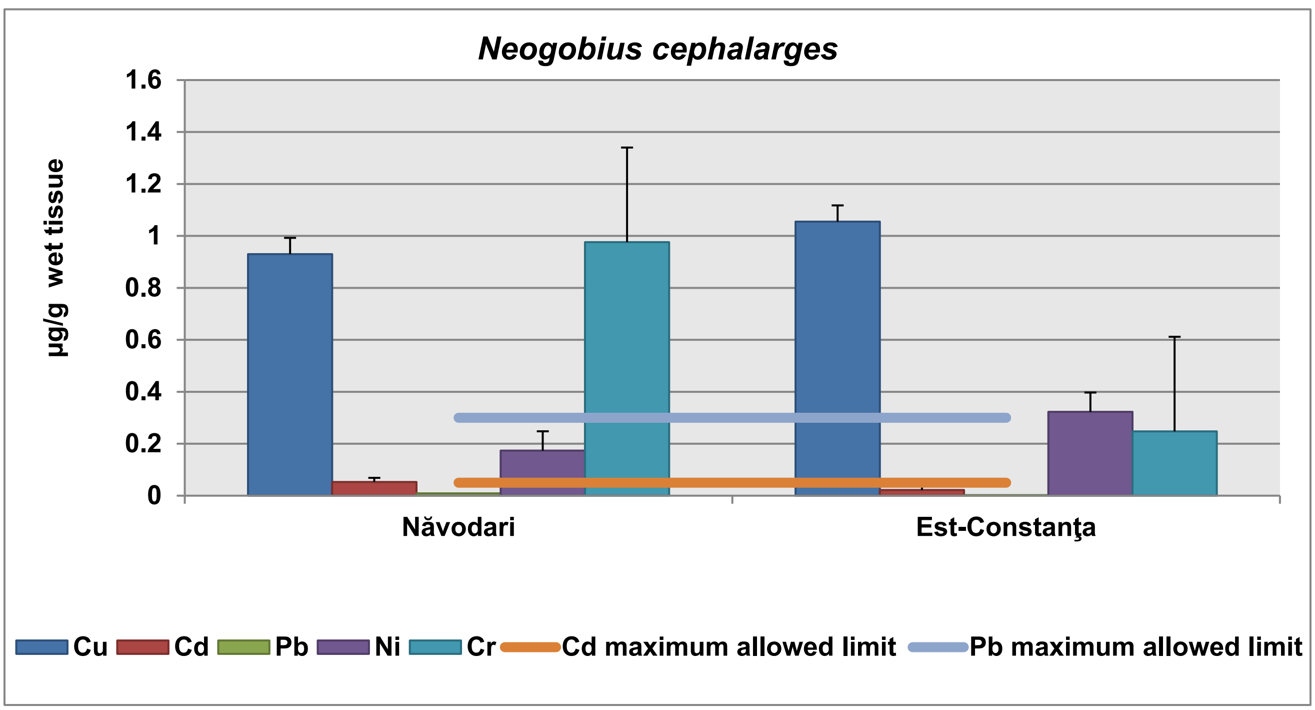

Figure 5.3.6. Heavy metals concentrations in *Neogobius cephalarges* along the Romanian coast area, during 2016-2018

PARTIAL CONCLUSIONS

- This chapter allowed the investigation of three pelagic fish species (anchovy, sprat, horse mackerel), one benthic species (gobius) and two molluscs species (mussels and rapana) taken from different areas along the Romanian Black Sea coast, 2016-2018 period.
- This chapter presents the methodology for sampling, preliminary processing and analysis of fresh marine organisms investigated in order to assess polycyclic aromatic hydrocarbons and heavy metals content.
- Lipid content determination analytical method is also described.
- Study results are a database on the polycyclic aromatic hydrocarbons accumulation in marine organisms, from the Romanian Black Sea coast area.
- Polycyclic aromatic hydrocarbons total content values in molluscs and fish species analyzed from the Romanian Black Sea coast area are higher, compared to other species from different parts of the world.
- According to study results, phenanthrene, fluorene and naphthalene were dominant compounds in molluscs species (*Rapana venosa, Mytilus galloprovincialis*) investigated. The dominant compounds in fish species analyzed, were phenanthrene and naphthalene.
- Heavy metals results, during 2016-2018 period in marine specimens, demonstrate differences in distribution between different sectors of the Black Sea Romanian coast, reflecting the potential impact of natural or anthropogenic pressures, generated by sources and coastal or offshore activities.

6. ASSESSEMENT OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) CONTENT AS AN EFFECT OF THE APPLICATION OF SOME PREPARATION TECHNIQUES ON MARINE ORGANISMS

6.1. General aspects

Marine organisms are a necessary protein source for normal functioning of human body and should be an integral part of the dietary intake of each person with a rational and balanced diet.Although it has an extremely important nutritional potential, both fish and molluscs can accumulate organic pollutants through the biomagnification process. Biomagnification occurs when the chemical enters the food chain at higher trophic levels. Thus, the fatty tissues of animals can accumulate residues of organic compounds. They are transmitted in the food chain and reach higher concentrations, possibly harmful, at higher trophic levels (peak predators), eventually reaching humans. *(Neely, W. B., 1980).*

6.2. Objectives of chapter 2

The main objective of the chapter was to assess polycyclic aromatic hydrocarbons content in marine organisms along the Black Sea coast area, as an effect of some preparation (conservation) techniques applicated.

7. Theoretical considerations regarding the impact of the preparation process on the quality of fish and fish products

7.1. Thermal processing techniques

Heat processing means the process of transforming fresh food into processed food. The preparation of fish and seafood is done by heat processing techniques such as simmering, boiling, frying and gratin.

Structural features of fish meat and seafood are represented by short fine muscle fibers and fine connective tissue. To a lesser extent, it leads to fish meat friability, thus shortening the heat processing time and requiring the adaptation of technical procedures in order to ensure the preparatory quality of the fish (*L.C. Roseiro et al., 2011*).

7.2. The effects of thermal processing techniques on the nutritional quality of fish

Heat processing, in addition to the positive effects, generates a nutritional value reduction. Short-term boiling causes the diffusion of non-protein nitrogen in water, up to 30%, protein substances decrease by 6%, losses of mineral substances reach up to 40-60% *(Y.*

Moradi et al, 2011). Water-soluble vitamins pass into boiling water, and fat-soluble vitamins are partially inactivated.

Heat treatment in the oven and roasting retains almost entirely the protein content (0.1% losses) and minerals, and the vitamin content loss is very small. The fish prepared by frying, can increase the caloric value by soaking in oil, being more difficult to digest.

7.3. Effects of thermal processing techniques on fish amino acid compositions

The quality of fish proteins is influenced by its amino acid content, the essential amino acids ratio and the physiological use of amino acids after digestion, absorption and oxidation. *(Friedman M, 1996).*

According to research, it was concluded that the freezing and unfreezing process has effects on the fish proteins quality. It was observed that, fish freezing followed by fish unfreezing it causes significant decreases in amino acids, and the most affected being L-cysteine.

According to *Oluwaniyi O. et. al., 2017* who studied the effect of thermal preparation techniques such as boiling and frying (over hot coal) using different types of oils, the latter, have an effect on fish amino acid content.

7.4. Impact of the organic contaminants content in fish as a result of thermal processing techniques applicated

Fish is an essential source of nutrients, but by applying the preparation methods, there are changes in the chemical composition and in this way the quality of the final product is affected. Another consequence of preparation processes application is the compounds accumulation with toxic and mutagenic effect such as polybrominated diphenyl ethers (DEPB), hexachlorobenzene (HCB), polycyclic aromatic hydrocarbons (*Gemma Perelló et al., 2016*).

Food processing at high temperatures generates different types of toxic and genotoxic substances, including polycyclic aromatic hydrocarbons. Meat product preaparing by direct exposure to the heat source has proven to be the most harmful method of preparation, along with smoking and frying process.

8. MATERIALS AND METHODS

8.1. Materials used at polycyclic aromatic hydrocarbons content determination in thermal prepared marine organisms

The analysis of polycyclic aromatic hydrocarbons was performed on two types of mollusks (*Rapana venosa* and *Mytilus galloprovincialis*), three pelagic fish species (*Sprattus* sprattus*, Engraulis encrasicolus, Trachurus mediterraneus ponticus)* and a bental fish species (*Neogobius cephalarges*) in 2016 -2018 period, from different areas along the Romanian Black Sea coast.

The main steps of polycyclic aromatic hydrocarbons analyzing method in prepared marine organisms are application of preparation techniques (boiling and frying), preparation of extracts for gas-chromatographic analysis (extraction and purification) and gas-chromatographic analysis.

8.2. Application of preparation techniques on the analyzed marine organisms

Twenty-six samples of fish and twelve samples of molluscs (whole organism, for both fish and molluscs) were sampled in two equal parts, processed by boiling and frying, respectively. A MYRIA MY4007 Multicooker was used to apply both cooking techniques. After applying the preparation techniques, the fish and molluscs were chopped, lyophilized and prepared for gas chromatographic analysis (extraction and purification).

8.3. Polycyclic aromatic hydrocarbons extraction and purification

Polycyclic aromatic hydrocarbons extraction and purification from thermally prepared marine organisms were performed according to the same working protocol applied to fresh marine organisms described in subchapter 4.2.1.

8.4. Gas chromatographic analysis (GC)

The gas-chromatographic conditions were: capillary column Elite 35 MS, stationary phase: dimethylpolysiloxane (35% diphenyl), length 30 m, inner diameter 0,32 mm, film thickness 0,25 mm; carrier gas - helium, speed - 1 cm^3/min, divided debit 15 cm^3/min, sample volume - 2µl, injector temperature - 300°C, temperature programme – initial temperature 100°C,heating speed - 6°C/1 min, first isotherm - 250°C for 0 min, heating speed- 10°C* 10min, second isotherm - 330°C*10 min, interface temperature-330°C, source temperature - 270°C, analysis method, single ion r. (SIR)*(IAEA-MEL: Training manual on the Measurement of Organochlorines and Petroleum Hydrocarbons in Environmental Samples, 1995)*.

8.4.1. Identification and quantification of interest compounds in fresh marine organisms

Polycyclic aromatic hydrocarbons have been identified based on retention times and characteristic ions.

8.4.2. Polycyclic aromatic hydrocarbons concentrations calculation

For polycyclic aromatic hydrocarbons concentrations calculation will be taken into account the recovery factor – R (1), the sample area, the standard area, the standard concentration, the extract volume and the tissue mass (2) *(IAEA-MEL: Training manual on the Measurement of Organochlorines and Petroleum Hydrocarbons in Environmental Samples, 1995).*

$$R\% = \frac{(extract\ volume\ x\ \ standard\ area)}{2}\ (1)$$

$$PAH_C(\mu g/kg) = \frac{Sample\ area}{Standard\ area} x\ \ standard\ concentration\ (ppm) x \frac{Extract\ volume}{Tissue\ mass} x1000\ (2)$$

8.5. Lipid content determination analytical method in thermal prepared marine organisms

Lipid content determination was performed according to the same working protocol applied to fresh marine organisms described in subchapter 4.4.

9. Results and discussions on polycyclic aromatic hydrocarbons content in thermal prepared marine organisms

9.1. Comparative analysis of the total content and individual compounds in fresh and prepared organisms

Mean concentrations of the individual compounds in boiled rapana *(Rapana venosa*) ranged from detection limit (0.0001 µg/kg) to 437.3856 µg/kg wet weight. The dominant compounds were phenanthrene (437.3856 µg/kg wet weight), anthracene (27.3636 µg/kg wet weight), naphthalene (4.5577 µg/kg wet weight). Individual compounds concentrations in fried rapana, varied from 0.0001 to 648.0516 µg/kg wet weight.

The dominant compounds were phenanthrene (648.0516 µg/kg wet tissue), naphthalene (19.4999 µg/kg wet tissue) and anthracene (7.6669 µg/kg wet tissue) (Figure 9.1.1.).

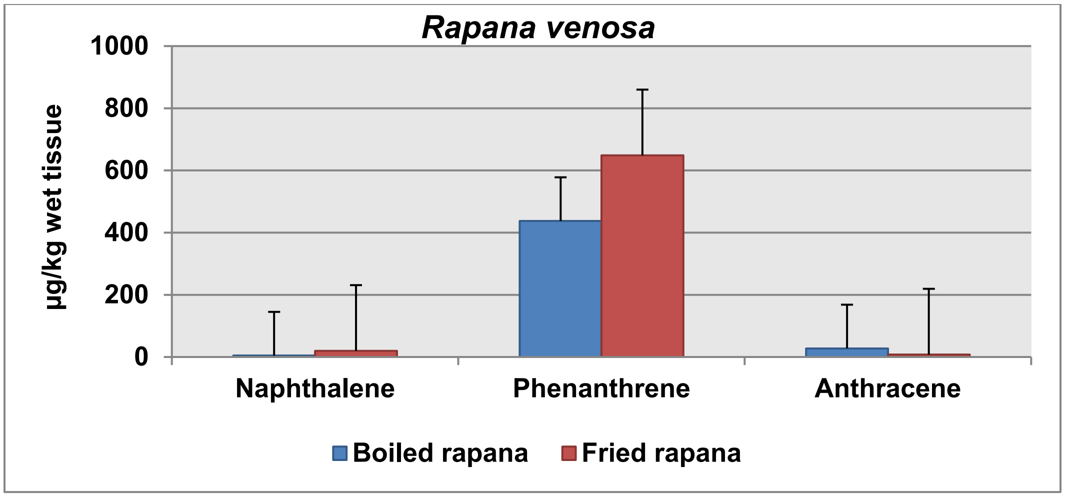

Figure 9.1.1. Variation of some polycyclic aromatic hydrocarbons (naphthalene, phenanthrene and anthracene) detected in boiled and fried rapana

Comparative analysis of polycyclic aromatic hydrocarbons total content in fresh rapana and boiled and fried rapana showed a progressive increase of polycyclic aromatic hydrocarbons content as a result of the preparation techniques application (Figure 9.1.2.). This phenomenon can be explained by rising temperature during the cooking process, by the degree permeation oil and the toxic compounds accumulation during the frying process.

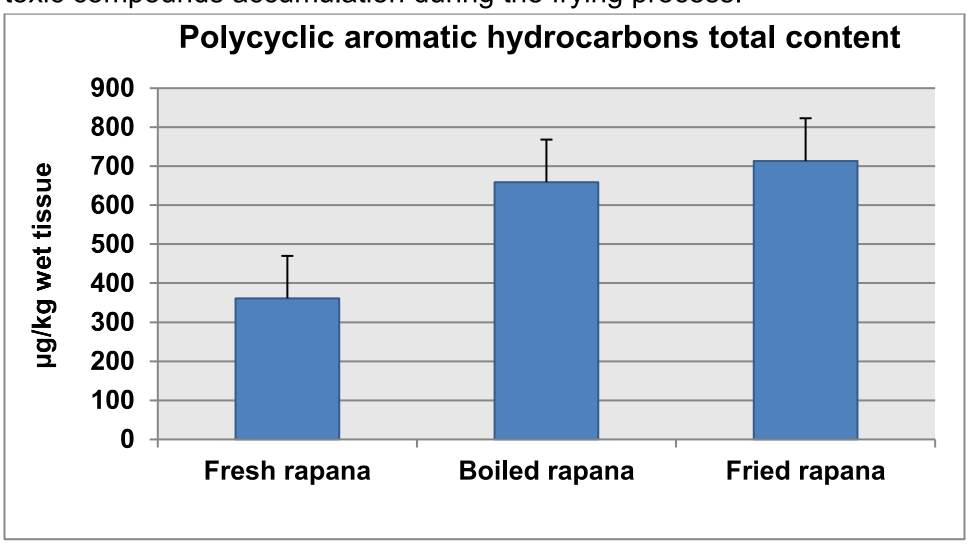

Figure 9.1.2. Polycyclic aromatic hydrocarbons total content in *Rapana venosa* before and after preparation techniques application

Mean values of individual compunds in boiled mussels varied from 0,0001 to 8.1139 µg/kg wet weight. The dominant compounds were anthracene and naphthalene with mean values of 8.1399 and 6.2504 µg/kg wet weight. In fried mussels, mean values of individual compounds varied between detection limit and 137.5013 µg/kg wet weight. The dominant compounds were phenanthrene (137.5013 µg/kg wet weight), anthracene (5.6139 µg/kg wet weight) and naphthalene (6.2504 µg/kg wet weight) (Figure 9.1.3.).

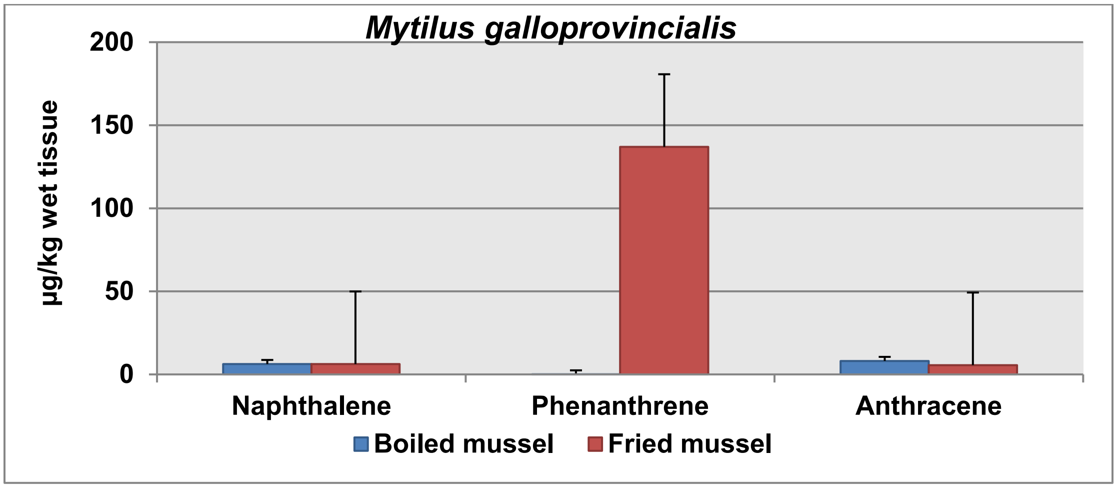

Figura 9.1.3. Variation of some polycyclic aromatic hydrocarbons (naphthalene, phenanthrene and anthracene) detected in boiled and fried mussel

Comparative analysis of polycyclic aromatic hydrocarbons total content in fresh mussel and boiled and fried mussel showed a significant decrease of polycyclic aromatic hydrocarbons content after applying the boiling process, followed by an increase of hydrocarbons level as a result of frying process application (Figure 9.1.4).

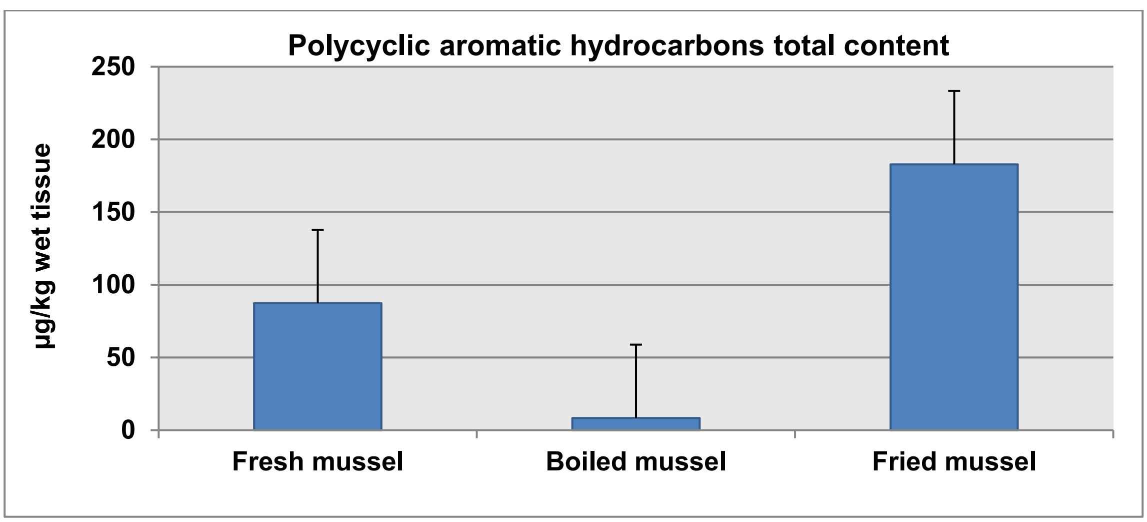

Figure 9.1.4. Polycyclic aromatic hydrocarbons total content in *Mytilus galloprovincialis* before and after preparation techniques application

Mean values of individual compounds in boiled anchovy varied from detection limit (0.0001 µg/kg) to 80.7422 µg/kg ţesut umed, the highest were recorded for phenanthrene

(80.7422 µg/kg wet tissue), anthracene (20.5174 µg/kg wet tissue), naphthalene (7.0130 µg/kg wet tissue). In fried anchovy mean values of individual compounds varied between 0.0001 and 412.6840 µg/kg wet tissue. The dominant compounds were phenanthrene (412.6840 µg/kg wet tissue), anthracene (68.3196 µg/kg wet tissue), naphthalene (37.5999 µg/kg wet tissue) (Figure 9.1.5).

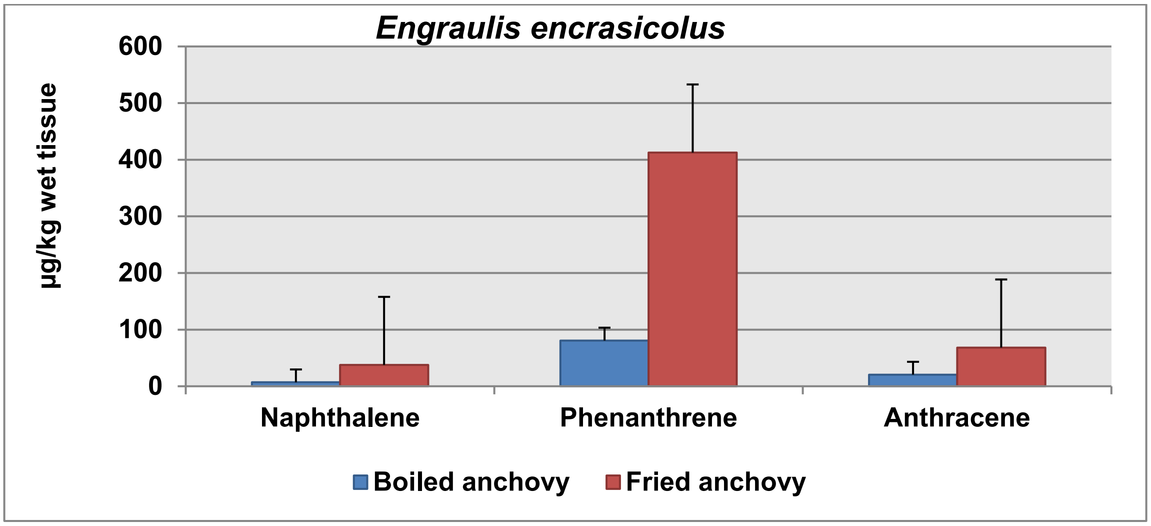

Figure 9.1.5. Variation of some polycyclic aromatic hydrocarbons (naphthalene, phenanthrene and anthracene) detected in boiled and fried anchovy

Comparative analysis of polycyclic aromatic hydrocarbons total content in fresh anchovy and boiled and fried anchovy showed a significant decrease of polycyclic aromatic hydrocarbons content after applying the boiling process, followed by a slight increase of hydrocarbons level as a result of frying process application (Figure 9.1.6.).

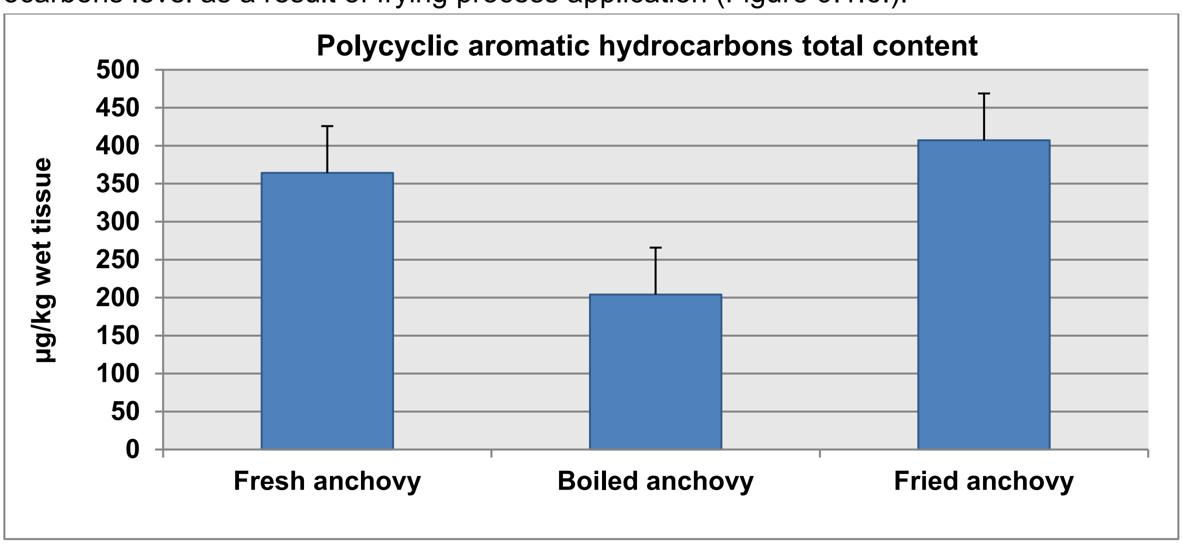

Figure 9.1.6. Polycyclic aromatic hydrocarbons total content in *Engraulis encrasicolus* before and after preparation techniques application

Mean values of individual compounds in boiled sprat varied between detection limit and 75.2622 µg/kg wet tissue, the highest being recorded for phenanthrene (75.2622 µg/kg wet tissue), anthracene (8.8273 µg/kg wet tissue) and naphthalene (8.9969 µg/kg wet tissue).In fried sprat mean values of individual compounds varied between 0.0001 and 125.8516 µg/kg wet tissue. The dominant compounds were naphthalene (3.4762 µg/kg wet tissue), phenanthrene (125,8516 µg/kg wet tissue), anthracene (13.5910 µg/kg wet tissue) and indene(1,2,3-c, d) pyrene (5.9017 µg/kg wet tissue) (Figure 9.1.7.)

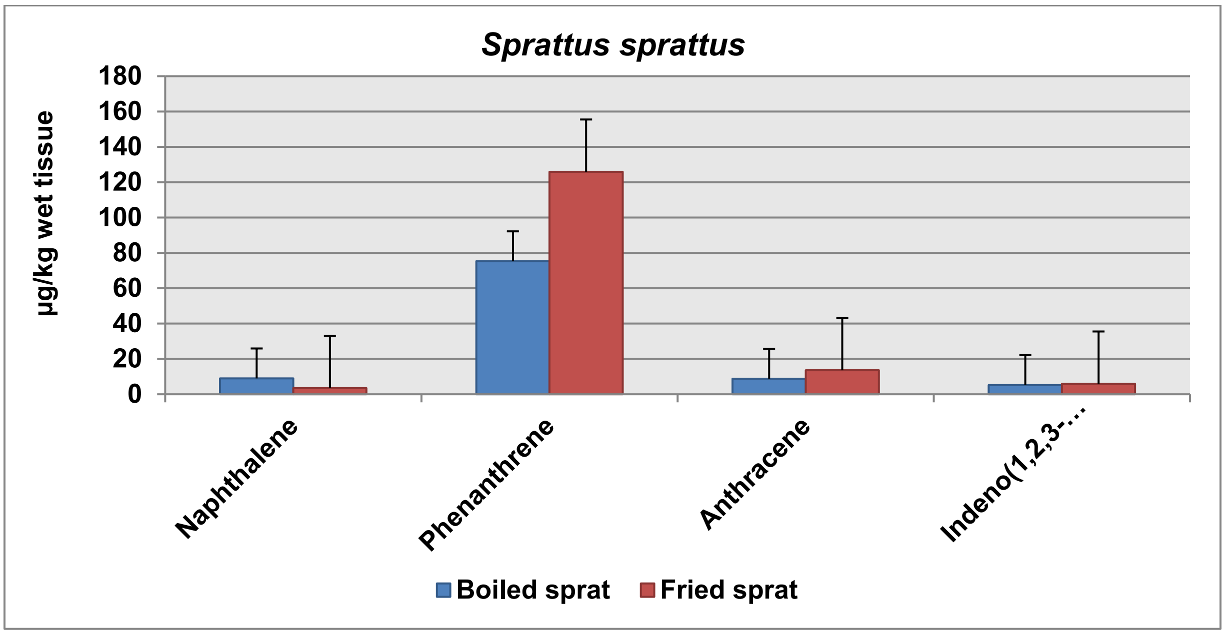

Figure 9.1.7. Variation of some polycyclic aromatic hydrocarbons (naphthalene, phenanthrene anthracene and indene(1,2,3-c,d)pyrene detected in boiled and fried sprat

Comparative analysis of polycyclic aromatic hydrocarbons total content in fresh sprat and boiled and fried sprat showed a progressive increase of polycyclic aromatic hydrocarbons content as a result of both preparation techniques application (Figure 9.1.8).

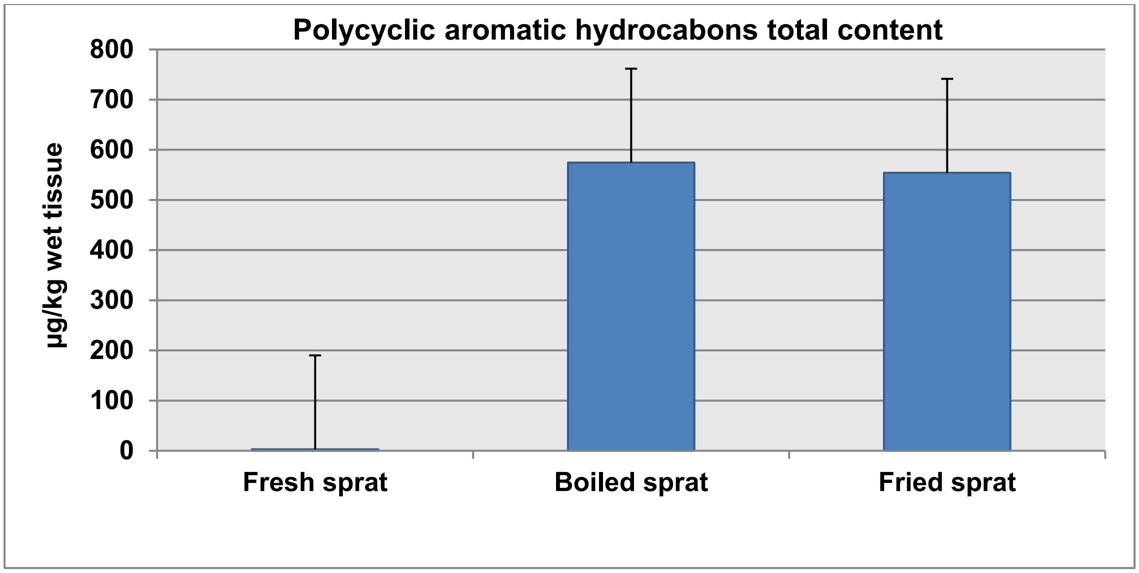

Figure 9.1.8 Polycyclic aromatic hydrocarbons total content in *Sprattus sprattus* before and after preparation techniques application

Mean values of individual compounds in boiled horse mackerel varied from 0.0001 to 74.1810 µg/kg wet weight. The dominant compounds were phenanthrene (74.1810 µg/kg wet tissue), naphthalene (24.0732 µg/kg wet tissue) and anthracene (9.8406 µg/kg wet tissue). In fried horse mackerel mean values of individual compounds varied between detection limit and 101.4083 µg/kg wet tissue. The dominant compounds were phenanthrene (101.4083 µg/kg wet tissue), naphthalene (85.3340 µg/kg wet tissue) and anthracene (45.4437 µg / kg wet tissue) (Figure 9.1.9.).

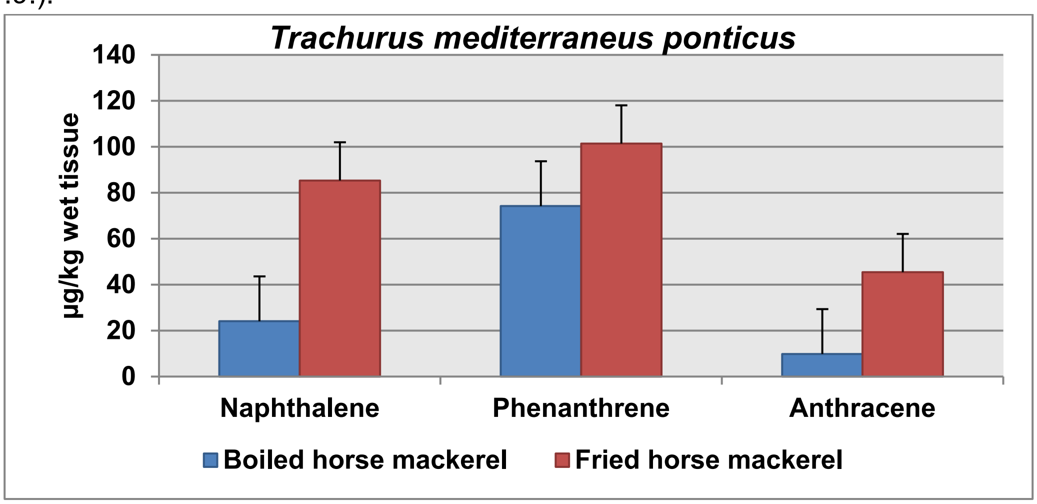

Figure 9.1.9. Variation of some polycyclic aromatic hydrocarbons (naphthalene, phenanthrene and anthracene) detected in boiled and fried horse mackerel

Comparative analysis of polycyclic aromatic hydrocarbons total content in fresh horse mackerel and boiled and fried horse mackerel showed a progressive increase of polycyclic aromatic hydrocarbons content as a result of both preparation techniques application (Figure 9.1.10).

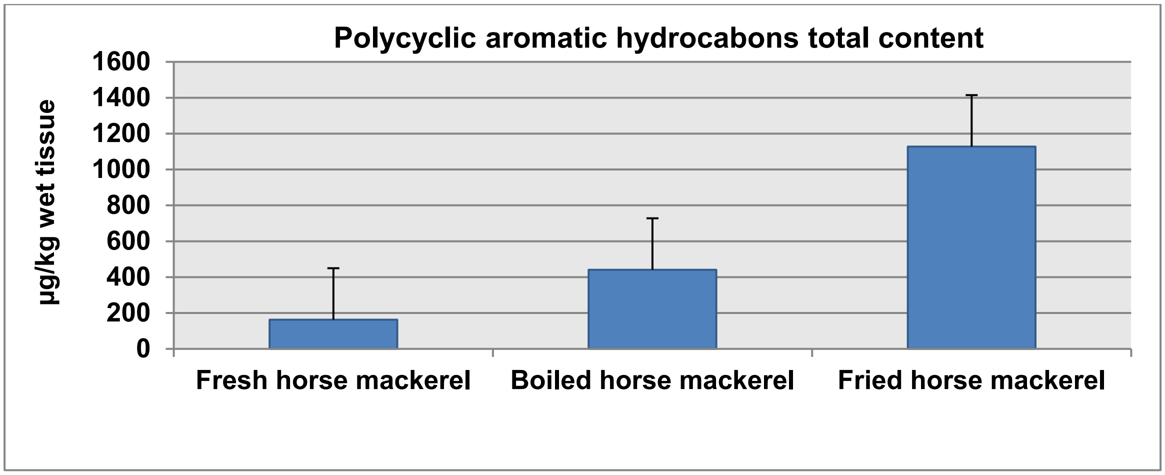

Figure 9.1.10. Polycyclic aromatic hydrocarbons total content in *Trachurus mediterraneus ponticus* before and after preparation techniques application

This increase can be explained by the fish cooking time, by high temperature during cooking, by degree of heating oil during frying. Mean values of individual compounds in boiled and fried gobius varied from 0.0001 to 23.5428 µg/kg wet weight, respectively, from 0.0001 to 35.1607 µg/kg wet tissue. In boiled and fried gobius, the dominant compounds were phenanthrene (23.5428 µg/kg wet tissue),fluorene (22.0233 µg/kg wet tissue), anthracene (18.0151 µg / kg wet tissue) and indene (1,2,3-c, d) pyrene (8.8408 µg/kg wet tissue) (Figure 9.1.11.).

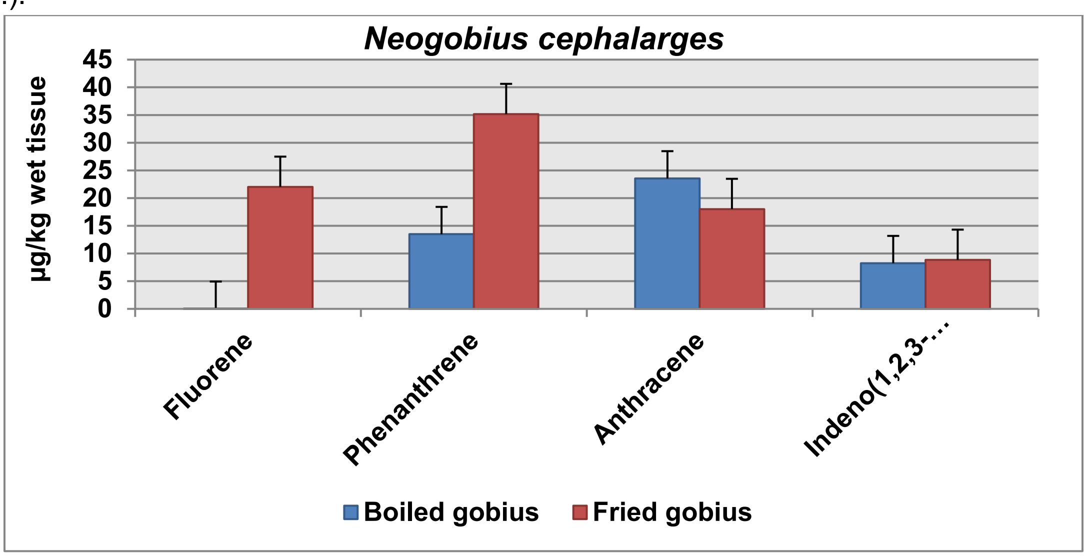

Figure 9.1.11. Variation of some polycyclic aromatic hydrocarbons (fluorene, phenanthrene, anthracene and indene(1,2,3-c,d)pyrene) detected in boiled and fried gobius

Figure 9.1.12 shows polycyclic aromatic hydrocarbons total content values in fresh gobius samples compared to those in boiled and fried gobius samples. The obtained results indicate a polycyclic aromatic hydrocarbons total content modification, by increasing the concentration in boiled and fried gobius.

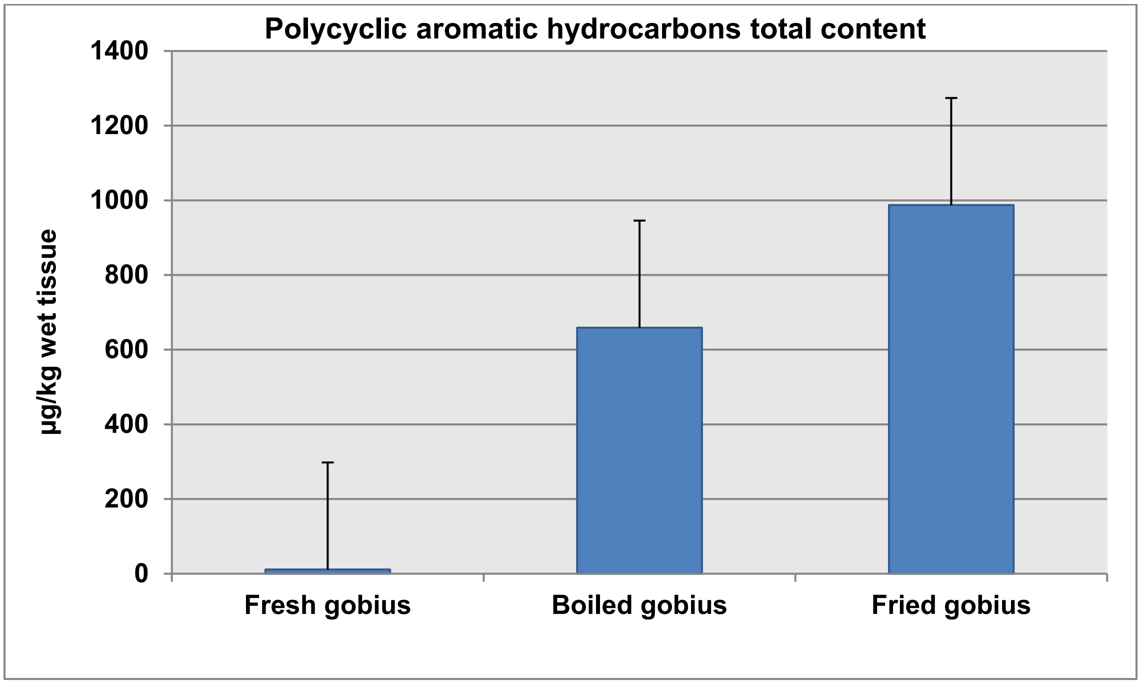

Figure 9.1.12. Polycyclic aromatic hydrocarbons total content in *Neogobius cephalarges* before and after preparation techniques application

Hydrocarbons levels varies depending on the preparation process, noting that, frying is a less healthy cooking method than boiling. During frying, a greater amount of toxic compounds are generated that enter the structure of the fish, thus becoming a direct source of compounds generation with mutagenic effect.

9.2. Results and discussions on lipid content in thermal prepared marine organisms

The results obtained indicated a lipid content visible increase in marine organisms as a result of boiling process application (Figure 9.2.2.). The highest lipid content was determined for *Rapana venosa* and *Sprattus sprattus*, but in *Mytilus galloprovincialis*, lipid level decreased slightly compared to that found in fresh mussels.

Phenomenon explained by the fact that the temperature and cooking time can affect food lipid content. During the boiling process, fatty components can be converted into volatile products such as aldehydes, ketones, alcohols, acids and hydrocarbons and can be evaporated by heat treatment (*Purwaningsih et al.,2015*).

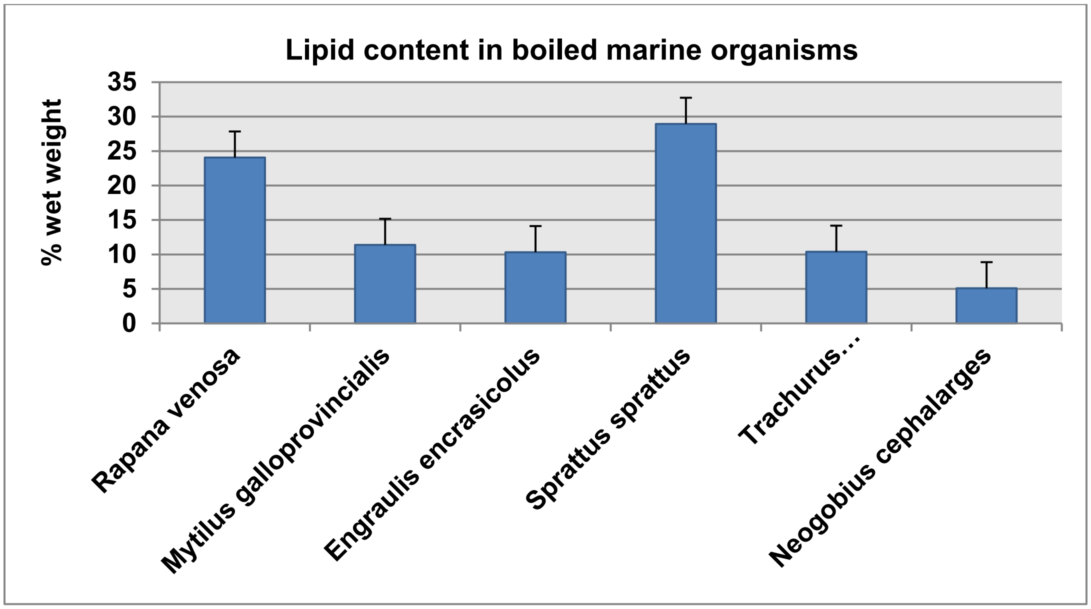

Figure 9.2.2. Lipid content in boiled marine organisms

Lipid level data determined in molluscs and fish species prepared by frying process are visibly higher than those prepared by boiling (Figure 9.2.3.).

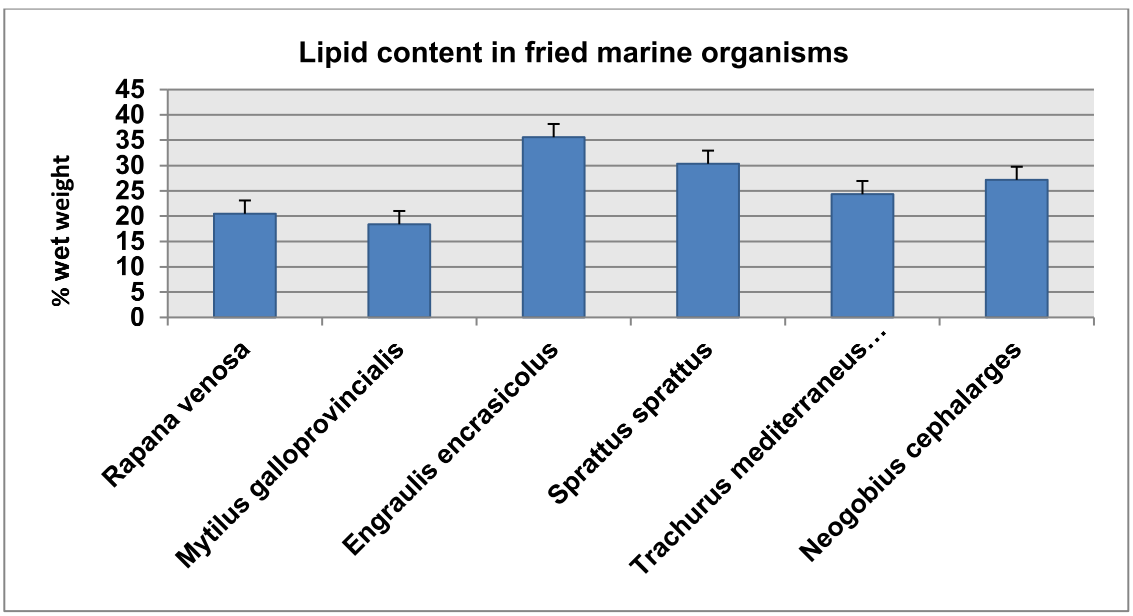

Figure 9.2.3. Lipid content in fried marine organisms

Lipids fish characteristics undergo changes during different thermal preparation processes. But frying produces the greatest changes in fish lipids composition than other thermal preparation methods. For example, during frying process there are higher losses of DHA and EPA (compared to other preparation methods). Moreover, lipid changes that occur during frying are strongly influenced by the frying process time, by fat fish content, by frying oil composition and frying technology type *(Y. Moradi et al.,2011).*

Correlating lipid level with hydrocarbon content found in organisms prepared by frying, it can be seen, that during frying a greater amount of toxic compounds are generated, that enter the fish structure, thus becoming a direct source of mutagenic compounds.

PARTIAL CONCLUSIONS

- In this chapter is presented the polycyclic aromatic hydrocarbons (PAHs) assessemnt in marine organisms along the Black Sea coast area, following the application of preparation techniques (boiling and frying). Also, is presented the comparative analysis of fresh and thermally prepared products.
- Individual compounds, measured higher values (*Rapana venosa*-702.64 µg/kg wet tissue; *Mytillus galloprovincialis*-155.01 µg/kg wet tissue; *Sprattus sprattus*-137.02 µg/ kg wet tissue; *Engraulis encrasicolus* - 503.35 µg/kg wet tissue; *Trachurus mediterraneus ponticus*-183.85 µg/kg wet tissue; *Neogobius chephalarges*-39.19 µg/kg wet tissue) in marine organisms prepared by frying.
- Study results showed that phenanthrene, anthracene and naphthalene were dominant compounds in boiled organisms and in those prepared by frying, the dominant compounds were phenanthrene, naphthalene, fluorene and anthracene and indeno (1,2,3-c, d) pyrene.
- Hydrocarbons level is strongly influenced by the preparation process, frying being a less healthy cooking method than boiling. During frying, a higher amount of polycyclic aromatic hydrocarbons is generated and influences the quality of the final product.
- Investigations results on the lipid content in **boiled** organisms (*Rapana venosa* – 38 % wet tissue; *Mytilus galloprovincialis*- 13.38 % wet tissue; *Sprattus sprattus* – 35.98% wet tissue; *Engraulis encrasicolus* – 18.26 % ţesut umed; *Trachurus mediterraneus ponticus-14.26 %* wet tissue; *Neogobius chephalarges*-4.91% wet tissue) and **fried** organisms (*Rapana venosa* – 35.50% wet tissue; *Mytillus galloprovincialis*- 23.31% wet tissue; *Sprattus sprattus* – 36.04% wet tissue; *Engraulis encrasicolus* – 42.58 % wet tissue; *Trachurus mediterraneus ponticus*-29.45 % wet tissue; *Neogobius chephalarges*-27.12 % wet tissue) showed differences compared to **fresh** marine organisms (*Rapana venosa* – 13.88% wet tissue; *Mytillus galloprovincialis* – 25.59% wet tissue; *Sprattus sprattus – 31.66*% wet tissue; *Engraulis encrasicolus* – 13.62% wet tissue; *Trachurus mediterraneus ponticus-18.74* % wet tissue; *Neogobius chephalarges*-21.45% wet tissue).
- The lipid level is significantly higher after the two thermal preparation techniques are applied. Moreover, lipid changes are strongly influenced by the frying process time, by fat fish content, by frying oil composition and frying technology type.
- As a result of lipid level correlation with hydrocarbon content in prepared marine organisms, it was observed that during both preparation techniques, especially during frying process, a higher amount of toxic compounds is generated, thus becoming a direct source of mutagenic compounds generation.

10. ANALYSIS OF THE RISKS AND BENEFITS OF HUMAN HEALTH THROUGH THE MARINE ORGANISMS CONSUMPTION

10.1. General aspects

Marine specimens are reliable indicators of polycyclic aromatic hydrocarbons bioaccumulation from environment and have been used to estimate the risks of human exposure to contaminants *(Filipa Gomes et al., 2013).*

10.2. Objectives of chapter 3

The main objective of the chapter was to assess the risks and benefits to human health throught marine organisms consumption. To assess human exposure to contaminants (polycyclic aromatic hydrocarbons) by oral ingestion, was used the estimated daily consumption and contaminants absorbtion.

11. THEORETICAL CONSIDERATIONS REGARDING THE NUTRITIONAL VALUE OF FISH

11.1. General information regarding nutritional value of fish and fishery products

Fish and seafood have a high lipid and protein nutritional value and essential micronutrients. In general, aquatic organisms are a rich source of protein and have a lower caloric density, with a high content of long-chain polyunsaturated fatty acids Omega-3 compared to terrestrial organisms. *(Sarvenaz Khalili Tilami et al., 2013).* According to literature, are strong links between fish and seafood consumption and the beneficial effects on human health. Thus, contributing to lowering the coronary cardiovascular risk and inflammatory diseases *(Ismail H.M., 2005).*

11.2. Fish omega-3 polyunsaturated fatty acids influence on human health

Excessive intake of omega-6 fatty acids is associated with adverse effects on human health, such as cardiovascular disease, diabetes, hypertension, depression, neurological dysfunction and immune disorders *(Connor et al, 2000).* Also, during pregnancy and neonatal period, is needed optimal diet that contains an adequate amount of omega-3 fatty acids essential for fetus neuronal development.

The retina and brain of mammals are generally very rich in docosahexaenoic acid, and it is very beneficial for newborns nervous system *(Lauritzen et al., 2001).*

Given the metabolic competition between omega-3 and omega-6 polyunsaturated fatty acids *(Palmquist et al., 2009)* and their opposite properties *(Schmitz and Ecker, 2008)*, it is generally assumed that the intake of omega-6 is too high in current diet. For example, fatty fish contain large amounts of omega-3 fatty acids, and therefore, have an needed ingredient to be included in the human diet.

11.3. Fish protein importance for human health

Aquatic organisms have a higher protein content compared to terrestrial organisms. *(Tacon et al, 2013)*. In addition, aquatic organisms proteins are easy digestible and rich in several essential peptides and amino acids, those are limited in proteins from terrestrial organisms, such as methionine and lysine. However, over the last decade, research has focused on health benefits of eating fish protein (*Rudkowska et al., 2010).* Also, hydrolyzed fish proteins are considered nutritionally superior due to their excellent amino acid composition.

11.4. The importance of vitamin D, selenium, calcium and phosphorus in fish for human health

In addition to its rich composition in lipids and proteins, fish are also a significant source of **D vitamin.** Vitamin D deficiency leads to, among other things, rickets, osteomalacia, low bone mineral density, and therefore, osteoporosis.

According to *Mattila et.al., 1995,* the fish muscle from different species contains between 0.5 and 30 mg of D vitamin/100 g. In addition, it has been shown that farmed salmon has a much lower vitamin D content compared to wild salmon. Also, the cooking method could have an influence on final vitamin D content (*Lu et al., 2007).*

Selenium is toxic in high doses, but is essential as a mineral for both animals and humans. In humans, selenium works as a selenoprotein, as a cofactor to reduce various antioxidant enzymes, such as glutathione peroxidases. Also has cofactor role responsibility for thyroid gland functioning, for three of the four types of thyroid hormones *(Smith et al., 1999).*

Calcium is another mineral with an important role in human nutrition, having an essential role in bone density. Calcium salts provide stiffness to the skeleton, and calcium ions play an important role in many metabolic processes. In addition to dairy products, the most important sources of calcium come from fish and fish bones. The calcium amount found in fish, molluscs and crustaceans varies between 26 and 68 mg/100 g, while the calcium amount in terrestrial organisms is about 14 mg/100 g *(Tacon și Metian, 2013).*

According to research, it is suggested, that the phosphorus fish and seafood content varies between 204 and 230 mg/100g, being a significant phosphorus source, compared to phosphorus amount (176mg/100g) from terrestrial organisms (*Metian et al., 2013).*

12. THEORETICAL CONSIDERATIONS ON THE BENEFITS AND RISKS OF HUMAN HEALTH AS A RESULT OF FISH CONSUMPTION

12.1. Health benefits of molluscs consumption

The benefits of mollusks consumption are due proteins, minerals (iron, zinc, potassium and phosphorus) and vitamins (B6, B12) content, having a positive effect on the body. Due to low content of total and saturated fats, those are omega-3 fatty acids rich, contribute to a healthy heart. According to American Heart Association, omega-3 fatty acids found in mollusks have cardioprotective benefits (*Penny M. Kris-Etherton et al., 2003).* These unsaturated fatty acids decrease triglycerides and fat level in bloodstream, reducing the heart attack risk or sudden death due to heart disease.

12.2. Health benefits of fish consumption

The benefits of eating fish are mainly due to high quality protein content (fish provides 17% of total protein consumed by animals and 6% of protein consumed by humans), vitamins and other essential nutrients. Moreover, unlike fatty meat products, fish doesn't have a high saturated fats content. Fatty fish have a high content of polyunsaturated omega-3 fatty acids: eicosapentaenoic acid (AEP) and docosahexaenoic acid (ADH). The most recognized benefit of eating fish is associated to cardiovascular disease low risk *(W.Becker et al., 2007).*

12.3. Health risks of molluscs an fish consumption

Recent studies showed that, fish and molluscs can be also a potential source of known toxic contamination, such as mercury, polychlorinated dibenzo-p-dioxins, dibenzofurans (DDPC/F), polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers (EDPB), polychlorinated diphenyl ethers (EDPC) and polychlorinated naphthalenes (NPC), and the information on exposure and human health adverse effects is still limited *(Jose L. Domingo et al.,2007).* Very high levels of mercury can damage adults nerve centers and on fetus or young child can disrupt the brain and nervous system development *(Mozaffarian D et al., 2006).*

12.3.1. Fish consumption and heart disease

According to US researchers, the fish consumption frequency is closely correlated with heart disease prevention (*Ana Carolina Fernandes et al, 2012).*In Sweden, studies showed that eating fish three times a week reduces stroke risk in women *(Socialstyrelsen, 1997).*

Therefore, for heart disease prevention, it is recommended eating fish with high level of omega-3 fatty acids , such as trout, salmon, tuna, halibut and mackerel, at least three times a week. Also, the method of cooking may or may not contribute to the benefits of preventing heart disease *(Kris-Etherton PM, 2002).*

12.3.2. Fish consumption, neuropsychological disorders and cataracta

Lu et al., 2012, observed an association between increased fish consumption and low risk of developing cataracts for women over 45, compared to those who consume less frequently. It is clear that, eating fish can prevent cataracts, psychological disorders such as depression, psychotic symptoms.

12.3.2. Fish consumption and cancer

According to a US study, increased fish consumption (> 3/week) is associated with a reduction prostate cancer in men *(Wilson, J.F, 2004).* Therefore, men who are eating fish less than twice a week have a higher risk of developing prostate cancer than those who are eating more often (more than twice a week).

13. MATERIALS AND METHODS

13.1. Assessment of potential toxic and carcinogenic risks to human health through the consumption of fish and fishery products

In order to assess the risks due to fish and fishery products consumption, an survey was done in Constanța, and 200 people, adults (men and women) and children were interviewed.

The information obtained as a result of the survey were about the preferences of fish type consumed, cooking method, the fish freshness purchased, the fish purchase place, the frequency of eating fish and fish amount consumed. Also information on the age and body weight of the respondents were obtained.

According to questions about the fish purchase place, 85% of respondents prefer buying fish from fish market, and only 15% prefer buying fish from supermarket. In general, in fisheries are freshwater and marine species from fishing. However, in supermarket we find oceanic fish species, aquaculture species and fish taste is influenced by the keeping fish conditions. Except mackerel, ocean fishing species, with white meat, are less tasty, compared to those species from Romanian Black Sea coast.

The consumption quality is influenced by the fish freshness and the firmness of its meat.. Fresh fish has its specific smell, sea smell, river smell, lake smell, depending on its origin. The older it gets, the stronger it smells. By nutritional point, frozen fish also its retains proteins content, fats and vitamins are not affected by freezing process *(Afsaneh Farhadian et al, 2010).*

Among the fish species preferred by people of Constanta, the most consumed is horse mackerel (75%), followed by sprat (17%) and gobius (6%) and the least consumed is anchovy (2%).

According to survey results, 59% of Constanța population consumes fish weekly, 19% consumes occasionally, 14% consumes daily and 8% consumes monthly.

Fish consumption **should always be present in our food concerns. It is an excellent protein source and essential fatty acids, vitamins and minerals.** Among the preferred cooking methods by people of Constanta, is frying (48% of respondents). However, this preparation method, is less unhealthy, being even indigestible and harmful to health by accumulating a greater amount of contaminants *(Gemma Perelló et al.,2016).* 52% people of Constanta prefer other fish cooking method such as oven cooking (22%), grilling (16%) and boiling (14%).

Assessment of human exposure to contamination by oral ingestion was estimated using estimated daily consumption and contaminants absorption. Contamination assimilation is defined as fish estimated daily consumption multiplied by the absorption efficiency contaminant in human gastrointestinal tract *(Wang, D.Q. et al., 2013).*

The estimated total daily consumption (EDC) and estimated total daily absorption (EDA) of contaminants in a given fish species were calculated as follows:

$$EDC = \frac{Rfish}{Bw} \sum_{m=1}^{x} C_m \, (m = 1,2, \dots \dots x) \quad (1)$$

$$EDA = \frac{Rfish}{Bw} \sum_{m=1}^{x} (C_m{}^{*}AR_m)(m = 1,2, \dots \dots x) \quad (2)$$

where:

- EDC and EDA are estimated total daily fish consumption and contaminants absorption per body weight through contaminated fish consumption per day (mg/kg per day);
- Rfish represent daily fish consumption rate (kg/day);
- Bw is consumer body weight (kg) and has been calculated to 69.5 kg for adults and 39.5 kg for children;
- Cm means fish concentrations of contaminants (mg/kg), and their values have been measured previously
- ARm is absorption intestinal rate of contaminants in human gastrointestinal tract (%);

13.1.1. Risk coefficient calculation

In order to understand the effects of increased consumption of contaminated fish, the risk assessment index known as the coefficient risk (RC) was applied. This coefficient is defined as the ratio between the daily fish consumption rate (Rfish) and fish maximum allowable rate consumption (CRlma), given the potential carcinogenic and non-carcinogenic effects of the contaminant *(Yu, Y.X., Zhang et al., 2012).*

$$CR = \frac{Rcfish}{RC_{lma}} \quad (3)$$

$CR = Rfish \sum_{m=1}^{x} \frac{1}{RC_{lma}} * (\text{multiple contaminants with different toxic effect endpoints})$ (4)

$CR_{lma} = \frac{NRA*Gc}{\sum_{m=1}^{x} C_m * FC_m}$ (for the carcinogenic effects of multiple contaminants with similar toxic effect endpoints) (5)

$CR_{lma} = \frac{Gc}{\sum_{m=1}^{x} \frac{C_m}{DRF_m}}$ (for the non-carcinogenic effects of multiple contaminants with similar toxic effect endpoints) (6)

where:

- CR_{lma} represent fish maximum allowable rate consumption (g/day);
- NRA is level risk maximum acceptable (10^{-5});
- DRF_m is contaminants reference dose for a non-carcinogenic effect (mg/kg per day);
- FCm indicates cancer factor induced by a carcinogenic contaminant (mg/kg per day) and its value (1) has been taken from the literature *(Ángel Rodríguez-Hernández, 2016).*

13.1.2. Recommended doses for fish consumption

It is very useful for fish and fishery products consumers to know they can eat fish without risking their health. Therefore, the number meals allowed per month was calculated, taking into account potential contaminants with acute carcinogenic and toxic effects *(Liao et Ling, 2003).*

$$NCM = \frac{Rfish*TP}{MS} \quad (7)$$

$$NMR = \frac{NCM}{CR} \quad (8)$$

where:

- NCM is the current number of meals per month for each type of fishery product;
- MS is meal size (225 g fish/meal);
- TP is the time averaging period (month = 30.44 days);
- NMR is the number of maximum recommended meals of each aliment per month.

14. RESULTS AND DISCUSSION

14.1. Polycyclic aromatic hydrocarbons incidence in fish and fishery products

According to results presented in table 14.1.1. it can be seen the differences recorded in contamination level of frequently consumed fish species by the population of Constanta.

Polycyclic aromatic hydrocarbons highest concentration (expressed as $B[a]p_{eq}$) was recorded in horse mackerel (*Trachurus mediterraneus ponticus*), and along with sprat (*Sprattus sprattus*) recorded the highest lipid content (19.78 %, respectively, 31.66% wet weight) of analyzed species. This fact raises some concerns related to potential carcinogenic effects of these compounds, as a result of horse mackerel increased consumption among the population of Constanta.

Tabel 14.1.1. *Concentrations of toxic contaminants associated with carcinogenic and non-carcinogenic effects in fish and seafood most consumed by the population of Constanta*

	$B[a]P_{eq}$ (ng/g wet tissue)		
	Mean±DS	**Median**	**P_{25}-p_{75}**
Anchovy *(Engraulis Encrasicolus)*	0.0001±1.46	0.0001	0.0001
Sprat (*Sprattus sprattus)*	0.06±0.05	0.07	0.03-0,11
Horse mackerel (*Trachurus Mediterraneus Ponticus*)	3.81±10.79	18.9309	9.46-28.39
Gobius *(Neogobius Cephalarges)*	0.0001±0.0	0.0001	0.0001-0.0001

The pollutants accumulation (daily consumption estimation-DCE) in human body through fish consumption was calculated by combining the contamination values and fish consumption mode. The estimates results for both adults and children are presented in table 14.1.2.

Tabel 14.1.2. *Mean values of the daily intake of fish by the population of Constanta*

Middle-bound approach (percentile 50^{th} of consumption)					
Consumption rate (adults)	Anchovy *(Engraulis Encrasicolus)* 8,56 g/zi	Sprat (*Sprattus sprattus)* 28,75 g/day	Horse mackerel (*Trachurus Mediterraneus Ponticus)* 46,95 g/zi	Gobius *(Neogobius Cephalarges)* 18,72 g/zi	Total 102.98 g/day
$B[a]P_{eq}$ (ng/g)	1.23±1.7	5.64±7.1	3.85±4.2	2.69±3.81	13.41±15.6
Consumption rate (children)	5.96 g/day	17.95 g/day	29.76 g/day	12.49 g/day	66.16 g/day
$B[a]P_{eq}$ (ng/g)	0.91±1.8	4.52±5.3	2.75±2.9	1.31±2.1	9.49±11.3
Upper-bound approach (percentile 97.5^{th} of consumption)					
Consumption rate (adults)	Anchovy *(Engraulis Encrasicolus)* 85.92 g/day	Sprat (*Sprattus sprattus)* 89.79 g/day	Horse mackerel (*Trachurus Mediterraneus Ponticus*)	Gobius *(Neogobius Cephalarges)* 83.46 g/day	Total 370.09 g/day

			110.92 g/day		
$B[a]P_{eq}$(ng/g)	4.73±5.6	16.29±8.2	6.72±4.5	5.78±5.2	33.52±43.6
Consumption rate (children)	62.75 g/day	64.57 g/day	78.96 g/day	59.87 g/day	266.15 g/day
$B[a]P_{eq}$(ng/g)	2.61±3.6	8.24±7.21	3.25±4.1	4.56±4.3	18.66±32.4

In order to obtain the risk coefficient with potential toxic of polycyclic aromatic hydrocarbons, it was necessary to calculate the maximum permissible rate (CRlim) of fish and fishery products consumption (g/day) *(Ángel Rodríguez-Hernández, 2016).* If the risk coefficient (CR) is less than 1, there are no obvious health risks due to fish consumption or the contaminant absorption through fish consumption. If fish consumption value is equal or greater than maximum allowable rate value of fish consumption, the population health is exposed to risks *(Yingxin Yuet al., 2014)*.According to results presented in table 14.1.3. the maximum allowed rate values are almost double for adults compared to those obtained for children, the highest value being recorded for *Sprattus sprattus* (1292.0 g/day).

Risk coefficient (CR) values of potentially toxic contaminants varied from 0.000 to 0.0001 for adults, and from 0.0001 to 0.0003 for children. Therefore, doesn't exist obvious risks to human health through fish and fishery products consumption, all values were below the threshold value (RC ≦ 1) of risk coefficient.

Table 14.1.3. *Maximum allowable fish consumption rate (CRlim) expressed in kg/day and risk coefficient (RC) of contaminants with acutely toxic effects in both adults and children.*

$B[a]P_{eq}$		
Adults	**Risk coefficient (RC)**	**Maximum allowable fish or others fishery products consumption rate (CR_{lim}) g/day**
Anchovy *(Engraulis Encrasicolus)*	0.0001	694.9
Sprat (*Sprattus sprattus)*	0,0000	1292.0
Horse mackerel (*Trachurus Mediterraneus Ponticus)*	0.0001	556.5
Guvid *(Neogobius Cephalarges)*	0.0001	694.9
Children		

Anchovy *(Engraulis Encrasicolus)*	0.0001	395.0
Sprat (*Sprattus sprattus)*	0.0001	564.2
Horse mackerel (*Trachurus Mediterraneus Ponticus)*	0.0003	208.6
Gobius *(Neogobius Cephalarges)*	0.0001	395.0

Risk coefficient (CR) values of potentially carcinogenic contaminants varied from 0.0036 to 0.0991 for adults, and from 0.0037 to 0.0560 for children (table 14.1.4).Therefore, doesn't exist obvious risks to human health through fish and fishery products consumption, all values were below the threshold value (RC ≦ 1) of risk coefficient.

Table 14.1.4. *Maximum allowable fish consumption rate (CRlim) expressed in kg/day and risk coefficient (RC) of contaminants with carcinogenic effects in both adults and children.*

	B[a]P$_{eq}$	
Adults	**Risk coefficient (RC)**	**Maximum allowable fish or others fishery products consumption rate (CR$_{lim}$) g/day**
Anchovy *(Engraulis Encrasicolus)*	0.0123	0.6950
Sprat (*Sprattus sprattus)*	0.0991	2.969
Horse mackerel (*Trachurus Mediterraneus Ponticus)*	0.0036	3.0223
Gobius *(Neogobius Cephalarges)*	0.0128	0.6950

Children		
anchovy *(Engraulis Encrasicolus)*	0.0109	0.3950
Sprat (*Sprattus sprattus)*	0.0560	1.1530
Horse mackerel (*Trachurus Mediterraneus Ponticus)*	0,0037	2.0866
Gobius *(Neogobius Cephalarges)*	0.0156	0.3950

In this study, the maximum allowable rate values of fish consumption for carcinogenic effects were lower than those of acute toxicity. Therefore, these values were used to calculate the maximum recommended number of meals from each fish species, because it doesn't present obvious health risks due to fish consumption (an consumption that would allow CR ≦ 1 for all types of fish).

According to results presented in table 14.1.5, it can be seen the fish current consumption exceed the maximum recommended consumption, and therefore, it would be advisable for Constanta population, especially adults to reduce the pelagic fish consumption (anchovy, sprat and horse mackerel).

Tabel 14.1.5. *Recommended maximum number of meals per month of each grouped food item (considering the calculated carcinogenic potential)*

	Adults		Children	
	Current consumption (meals/ month)	Maximum consumption recommended (meals/ month)	Current consumption (meals/ month)	Maximum consumption recommended (meals/ month)
A nchovy*(Engraulis Encrasicolus)*	4.2	3.6	3.8	4
Sprat (*Sprattus sprattus)*	9.1	6.5	7.3	8.7
Horse mackerel (*Trachurus Mediterraneus Ponticus*)	1.2	3.3	1.1	1.5
Gobius *(Neogobius Cephalarges)*	6.3	8.0	5.1	5.6

PARTIAL CONCLUSIONS

- In order to assess the potentially toxic and carcinogenic risks to human health as a result of fish and fishery products consumption, calculating risk coefficient method is highlighted;
- In addition, to estimate the risks due to fish and fishery products consumption, an survey was done in Constanța, and 200 people, adults (men and women) and children were interviewed.
- Regarding the maintenance the health of fish and seafood consumers, is presented the way the marine species recommended doses can be calculated;
- According to fish current consumption values, it can be seen, the Constanta population exceeds the maximum recommended consumption level for pelagic marine species;
- Based on contamination data related to fish and fish products consumption, is indicated that, all maximum rate values were higher than current consumption. Therefore, there are no obvious risks to human health through fish and fishery products consumption;
- Marine species are a rich source of nutrients, vitamins, minerals and other compounds beneficial to human health, mention that are low risks of organic contamination due to increased contaminated fish consumption.

GENERAL CONCLUSIONS

- This research aimed to assess polycyclic aromatic hydrocarbons and heavy metals content from pelagic species (*Engraulis Encrasicolus, Sprattus sprattus, Trachurus mediterraneanus ponticus*), benthic species (*Neogobius cephalarges*) and molluscs (*Rapana venosa and Mytilus galloprovincialis)* from Romanian Black Sea, during 2016-2018 period;
- Thesis experimental data showed that phenanthrene, fluorene and naphthalene were dominant compounds in molluscs species (Rapana venosa, Mytilus galloprovincialis) analyzed. The dominant compounds in fish species analyzed were phenanthrene and naphthalene.
- Heavy metals results, during 2016-2018 period in marine specimens, demonstrate differences in distribution between different sectors of the Black Sea Romanian coast, reflecting the potential impact of natural or anthropogenic pressures, generated by sources and coastal or offshore activities.
- Polycyclic aromatic hydrocarbons accumulation study in fish and molluscs prepared by boiling and frying, allowed the comparative analysis of fresh and thermally prepared organisms.
- Experimental data showed that phenanthrene, anthracene and naphthalene were dominant compounds in boiled organisms and in those prepared by frying, the dominant compounds were phenanthrene, naphthalene, fluorene and anthracene and indeno (1,2,3-c, d) pyrene.
- Individual compounds, measured higher values (*Rapana venosa*-702.64 µg/kg wet tissue; *Mytillus galloprovincialis*-155.01 µg/kg wet tissue; *Sprattus sprattus*-137.02 µg/ kg wet tissue; *Engraulis encrasicolus* - 503.35 µg/kg wet tissue; *Trachurus mediterraneus ponticus*-183.85 µg/kg wet tissue; *Neogobius chephalarges*-39.19 µg/kg wet tissue) in marine organisms prepared by frying.
- Hydrocarbons level is strongly influenced by the preparation process, frying being a less healthy cooking method than boiling. During frying, a higher amount of polycyclic aromatic hydrocarbons is generated and influences the quality of the final product.
- The lipid level is significantly higher after the two thermal preparation techniques are applied. Moreover, lipid changes are strongly influenced by the frying process time, by fat fish content, by frying oil composition and frying technology type.
- Based on contamination data related to fish and fish products consumption, is indicated that, all maximum rate values were higher than current consumption. Therefore, there are no obvious risks to human health through fish and fishery products consumption
- Regarding the maintenance the health of fish and seafood consumers, is presented the way the marine species recommended doses can be calculated
- According to fish current consumption values, it can be seen, the Constanta population exceeds the maximum recommended consumption level for pelagic marine species.

ORIGINAL CONTRIBUTIONS AND PERSPECTIVES FOR CONTINUATION OF RESEARCH

The PhD thesis main objectives were to assess polycyclic aromatic hydrocarbons (PAHs) content in marine organisms of commercial interest (fish): anchovy, sprat, horse mackerel, gobius; molluscs: rapana and mussel) along Romanian Black Sea coast area, accumulation study of those compounds as an effect of some preparation techniques (boiling and frying) applicated, risks and benefits analysis to human health through marine organisms consumption. Therefore, knowing the PAH level accumulation in natural environment, as well as, PAH accumulation level after the certain methods of thermal preparation were applicated, and awareness of benefits and risks of fish controlled consumption are a priority for consumer health.

The original elements of this thesis are represented by the investigated marine species: pelagic species (*Engraulis Encrasicolus, Sprattus sprattus, Trachurus mediterraneanus ponticus*), benthic species (*Neogobius cephalarges*) and molluscs (*Rapana venosa and Mytilus galloprovincialis*).

A novelty element of the thesis is represented by sampling area of marine organisms, the Black Sea. Is a semi-closed sea with unique physicochemical and biological characteristics. The Black Sea biocenosis structure is determined by diversity, spatial distribution, number and species components of biomass, dynamics and relationships between marine species.

Another representative element of PhD thesis is risks and benefits analysis on human health thruough fish and fishery products consumption.

RESEARCH RESULTS DISSEMINATION

A. Articles published in ISI journals

V. Coatu, **N-A. Damir**, A. OROS, L. Boicenco, L.Lazăr, (2018). *Revised Methodology of Black Sea chemical status under water Framework Directive*, Journal of Environmental Protection and Ecology 19, No 2, 601–608;

S. BIRGHILĂ, M.M. BRATU, V. COATU, **N-A Damir,** Levels, *Sources and Risk Characterization of Organochlorine Pesticides in Beer Samples from Romania,* (2020). Revista de chimie, Volum 71, Issue 1, 364-370;

N-A. Damir, V. Coatu, E. Botez, E. Pantea, M. Galaţchi, S. Birghilă, *Assessment of polycyclic aromatic hydrocarbons content in marine organisms of commercial interest from the Romanian Black Sea coast,* Polycyclic Aromatic Compounds (în curs de publicare).

B. Articles published in BDI journals

Valentina Coatu**, Nicoleta Damir,** Andra Oros**,** Hakan Atabay**,** Ertuğrul Arslan Leyla Tolun, Yuriy Denga**,** Yurii Oleinik, (2020). *Comparative Assessment of Organic Pollution in the Rivers Influenced Area of the North-Western, Western, and Southern Part of the Black Sea,* "Cercetări Marine", Issue no. 50, 26 – 46;

C. Articles published in unlisted journals

Coatu Valentina, Oros Andra, **Nicoleta Damir**, Florin Timofte, Luminița Lazăr, (2018); *Bioaccumulation of contaminants in the main links of the pelagic trophic chain on the Romanian Black Sea coast*, Marine Research, Vol.48;

D. Articles published in conference volumes

N-A Damir, V. Coatu, Elisabeta Botez, (2018*). Evaluation of polycyclic aromatic hydrocarbons (PAHs) content in marine organisms in the Romanian Black Sea Coastal Area*, Conference Proceedings "Global and Regional in Environmental Protection" (GLOREP) ;

E. Participation at national conferences

Nicoleta-Alexandra Damir, Valentina Coatu, Elisabeta Botez, (2018). Poster: „*Analysis and identification of the main types of polycyclic aromatic hydrocarbons in the Black Sea Coastal Area*", National conference "Scientific Conference of Doctoral Schools SCDS-UDJG, Perspectives and challenges in doctoral research," Dunărea de Jos" University of Galați, România;

Nicoleta-Alexandra Damir, Valentina Coatu, Elisabeta Botez, (2019). *Assessment of polynuclear aromatic hydrocarbons content in marine organisms of commercial interest at the Black Sea Romanian Coast*", Conferința națională "Scientific Conference of Doctoral Schools SCDS-UDJG, Perspectives and challenges in doctoral research," Dunărea de Jos" University of Galați, România;

Nicoleta-Alexandra Damir, Valentina Coatu, Elisabeta Botez, (2020). Poster: *Assessment of polycyclic aromatic hydrocarbons content from molluscs of commercial interest at the Romanian Black Sea Coast Area in respect with regulated values for human consumption*" Conferința națională "Scientific Conference of Doctoral Schools SCDS-UDJG, Perspectives and challenges in doctoral research," Dunărea de Jos" University of Galați, România.

Nicoleta-Alexandra Damir, Valentina Coatu, Elisabeta Botez, (2021) Poster: *"Polycyclic aromatic hydrocarbons content in benthic fish species (Neogobius Cephalarges) from the Romanian Black Sea Area*" National Conference "Scientific Conference of Doctoral Schools SCDS-UDJG, Perspectives and challenges in doctoral research, "Dunărea de Jos" University of Galați, România;

F. Participation at international conferences

Nicoleta-Alexandra Damir, Valentina Coatu, Elisabeta Botez, (2018). Poster: *''Evaluation polycyclic aromatic hydrocarbons (PAHs) content in marine organisms in the Romanian Black Sea Coastal Area"*, Conferință Internațională "Global and Regional in Environmental Protection" (GLOREP), Timișoara, România.

Nicoleta-Alexandra Damir, Valentina Coatu, Elisabeta Botez, (2019). Poster: "*Assesment of polynuclear aromatic hydrocarbons (PAHs) in fish at the Romanian Black Sea Coast in respect to regulated levels for human consumption*", International Conference Sustainable water ecosystems management (SWEM 2019), Bucureşti;

Nicoleta-Alexandra Damir, V.Coatu, E. Botez (2019); Poster: *Polycyclic aromatic hydrocarbons content in pelagic fish from the Romanian Black Sea area*, International Conference "Euroaliment-Innovative Minds for Future Foods", Galaţi, România;

G. Elements such as books, studies, research published in national and international publishing house

S. Nicolaev, T. Zaharia A.Oros (editori), *The current state of the marine and coastal environment* (2019), editura CD Press Bucureşti, ISBN 978-606-528-447-0, p 214:

Cap. 1.3.2. Contamination indicators/Total petroleum hydrocarbons (V. Coatu, **N. Damir**, p 104-105;

Cap. 1.3.2. Contamination indicators/Polycyclic aromatic hydrocarbons (V. Coatu, **N. Damir**), p 105-108;