НАУЧНИ ТРУДОВЕ И УЧЕБНИ МАТЕРИАЛИ

на доц. Мона Станчева, дхн, Катедра Химия, Фармацевтичен Факултет,
Медицински Университет - "проф. д-р Параскев Стоянов" - Варна

За участие в конкурс за академичната длъжност "професор" по научна специалност "Биоорганична химия, химия на природни и физиологично активни вещества",
обявен за нуждите на Катедра Химия в ДВ 36/16.04.2013 г.

В обявения конкурс за "професор" доц. М. Станчева участва със:

- 52 научни публикации
- Автореферат на дисертационен труд "Устойчиви органични замърсители и тежки метали в черноморски риби", за присъждане на научна степен "доктор на химическите науки" по научната специалност: Биоорганична химия, химия на природни и физиологично активни вещества
- 58 участия в научни форуми – конференции, конгреси, симпозиуми
- Монография "Устойчиви органични замърсители в храни"
- Три учебни помагала
- Шест учебни програми на лекционни курсове

Научните изследвания в представените по-горе научни публикации, участия в научни форуми, в автореферата на дисертационния труд и монографията са в областите: Състав на храни, Безопасност на храни и Катализа.

Резюмета на публикации

Състав на храни

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Fatty acid and fat soluble vitamins composition of raw and cooked Black Sea horse mackerel

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Abstract. The fat soluble vitamins, as well as n3 and n6 fatty acids (FA) are essential compounds of fish lipids and exclusively provided by the diet. Fish is sometimes eaten raw, but it is usually thermal processed before consumption. Temperature processing of fish tissue enhances its taste, inactivates pathogenic microorganisms and increases its shelf life. The fat soluble vitamins (vitamins A, D_3 and E) and fatty acids are considered to be susceptible to oxidation during heating (cooking) process. The aim of the present study was to evaluate the effect of steaming (10 min at 90°C) and frying (5 min on the each side with sunflower oil) on fat soluble vitamins and fatty acids composition in Horse mackerel (*Trahurus mediterraneus*) fish fillets. Vitamins A, D_3 and E were analyzed simultaneously using RP-HPLC. The fatty acid composition was analyzed by GC-MS. The amounts of vitamin A (retinol) in cooked fish fillets (for both heat treatments) decreased significantly, compared to their content in the raw samples. In contrast vitamin D_3 (cholecalciferol) content affects only by steaming, while changes on vitamin E (alpha-tocopherol) was observed solely after frying process. The highest content of monounsaturated fatty acids (MUFA) were observed after steaming, whereas fried samples presented higher values of polyunsaturated fatty acids (PUFA) due to significant increase in linoleic acid (C18:2n6). During steaming did not reduce significant n3 and n6 PUFA levels, while frying caused a large reduction of n3 PUFAs. The ratio of n3/n6 was markedly lower in fried samples than in raw and steamed mackerel. In conclusion the Black Sea Horse Mackerel is a good source of vitamin D_3, vitamin E and n3 PUFAs. After steaming and frying process there were minimum losses in the contents of cholecalciferol and alpha-tocopherol, while retinol was reduced nearly a half. The process of frying affects most significantly three fatty acids groups, whereas after steaming was observed little influence on fatty acids profile.

Keywords: *Trahurus mediterraneus*, steaming, frying, vitamins, fatty acids, human health

1. Introduction

The Black Sea appears to be one of the important fish basins influencing greatly the economy of all Black Sea countries. The small pelagic species Horse mackerel (*Trachurus mediteraneus, Aleev 59*), which inhabits the western and north-western parts of the Black Sea [1], is of key importance for Bulgarian fisheries for economic and social reasons (number of fishermen involved) and as livelihood support for population. This carnivore species represents about 50% of the Bulgarian summer pelagic catches, and a considerable percentage of the Black Sea total catches and plays an important role to provide essential nutrients for the population.

Marine fish, especially carnivores, are characterized by low levels of omega-6 (n6) fatty acids (linoleic acid LA, C18:2 n6) and high levels of omega-3 (n3) PUFAs (eicosapentaenoic acid, EPA C20:5n3; docosahexaenoic acid, DHA C22:6n3) in particular, which are essential for the human health [2]. Fat soluble vitamins are essential components of fish lipids and are exclusively provided by the diet. Vitamins A and E act as natural antioxidants in the living organisms. All-trans-retinol is very important for the visual system in humans; alpha-tocopherol (alpha-TP) is significant for the normal reproduction and muscle development and cholecalciferol promotes and enhances the absorption and metabolism of calcium and phosphorus. It is well-

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FAT SOLUBLE VITAMINS AND FATTY ACIDS COMPOSITION OF BLACK SEA CYSTOSEIRA BARBATA

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Brown alga Cystoseira barbata is the most widely distributed seaweed in the Black Sea. There is limited information about fat soluble vitamins content and fatty acids composition of this specie from Bulgarian Black Sea coast. The aim of this study was to determine fat soluble vitamins, pigments, total lipid and fatty acid composition of Cystoseira barbata. Fat soluble vitamins (vitamin E and D), pigments (β-carotene and astaxanthin) and total cholesterol were analyzed simultaneously using HPLC/UV/FL system equipped with RP analytical column. Sample preparation procedure includes alkaline saponification, followed by liquid-liquid extraction. Brown seaweed Cystoseira barbata contained high amounts of α-tocopherol and β-carotene. Lipids were extracted by following the method of Bligh and Dyer. The residual lipid fraction was methylated using base-catalyzed transmethylation with methanolic potassium hydroxide. Fatty acid composition was analyzed by GC/MS. Cystoseira barbata was rich in linoleic (C18:2n6) and eicosopentaenoic acid (C20:5n3) although total lipid content was generally low. High levels of α-tocopherol correlate with high levels of polyunsaturated fatty acids. As an antioxidant α-tocopherol preserves tissue PUFA from oxidation.

UDC Number: 543

Keywords: Cystoseira barbata, vitamins, fatty acids

Introduction

Seaweeds have been used since ancient times as food, fodder, fertilizer and as source of medicine. Nowadays seaweeds represent an inexhaustible source of the raw materials used in pharmaceutical, food industries, medicine and cosmetics. They are nutritionally valuable as fresh or dried vegetables, or as ingredients in a wide variety of prepared foods. In particular, seaweeds contain significant quantities of protein, lipids, minerals and vitamins (Manivannan et al., 2008).

Lipids represent only 1-5% of algal dry matter and exhibit an interesting polyunsaturated fatty acid (PUFA) composition particularly omega 3 and omega 6 acids which play an important role in the prevention of cardio vascular diseases, osteoarthritis and diabetes. Brown algae are rich in fatty acids with 20 carbons: eicosapentaenoic acid (EPA, C 20:5 n3) and arachidonic acid (AA, C 20:4 n6) (Banerjee et al., 2009). Marine algae are rich in PUFAs of the n-3 and n-6 series, which are considered essential fatty acids for humans and animals. Some of these FAs (20:3n-6, 20:4n-6, 20:5n-3) have high biological activity and are converted into eicosanoids. In addition, PUFAs are of interest in cosmetics

FAT SOLUBLE VITAMINS AND FATTY ACIDS COMPOSITION OF BLACK SEA ULVA RIGIDA AND GELIDIUM CRINALE

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Abstract The aim of this study was to determine fat soluble vitamins, pigments, total lipid and fatty acid composition of *Ulva rigida* and *Gelidium crinale*. Fat soluble vitamins (vitamin E and D), pigments (β-carotene and astaxanthin) and total cholesterol were analyzed simultaneously using HPLC/UV/FL system. Generally, red seaweeds *Gelidium crinale* showed higher amounts of α-tocopherol, β-carotene, astaxanthin and total cholesterol, compared to green seaweed. Lipids were extracted by following the method of Bligh and Dyer. Fatty acid composition was analyzed by GC/MS. *Gelidium crinale* was rich in arachidonic (C20:4n6) and eicosopentaenoic acid (C20:5n3), whereas *Ulva rigida* – in linoleic acid (C18:2n6). Palmitic acid was the most abundant in both algae species. Red algae *Gelidium crinale* had higher PUFA concentrations, particularly from the n-3 series, thereby being a better source of these compounds. Moreover, red algae presented healthier Σn-6/Σn-3 and PUFA/SFA ratios than green algae.

Keywords: macroalgae, fatty acids, GC-MS, HPLC

1. Introduction

Seaweeds belong to a group of plants known as algae. They are classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their pigments and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances which can benefit human health. Algae have been used since ancient times as food, fodder, fertilizer and as source of medicine. Nowadays seaweeds represent an inexhaustible source of the raw materials used in pharmaceutical, food industries, medicine and cosmetics. They are nutritionally valuable as fresh or dried vegetables, or as ingredients in a wide variety of prepared foods. In particular, seaweeds contain significant quantities of protein, lipids, minerals and vitamins [1].

Lipids represent only 1-5% of algal dry matter and exhibit an interesting polyunsaturated fatty acid (PUFA) composition particularly omega 3 and omega 6 acids which play an important role in the prevention of cardio vascular diseases, osteoarthritis and diabetes. Some of these FAs (20:3n-6, 20:4n-6, 20:5n-3) have high biological activity and are converted into eicosanoids. In addition, PUFAs are of interest in cosmetics as components of sun lotions and as regenerating and anti-wrinkle products. Because of the huge and renewable biomass and the fact that many of them could easily be cultivated in the sea on a large scale, seaweeds are a potential source of fatty acids for biotechnology and a dietary source of essential fatty [2].

Seaweeds are a good source of some water- (B1, B2, B12, C) and fat-soluble (β-carotene with vitamin A activity, vitamin E) vitamins. Seaweed vitamins are important not only due to their biochemical functions and antioxidant activity but also due to other health benefits such as decreasing blood pressure (vitamin C), prevention of cardiovascular diseases (β-carotene), or reducing the risk of cancer (vitamins E and C, carotenoids) [3].

Bulgarian Black Sea coast is rich in algae, regarding biomass and algal biodiversity. Taxonomically, the species *Ulva rigida* belong to the division Chlorophyta, class Ulvophyceae order Ulvales, family Ulvaceae, genus Ulva. *Celidium crinale* belong to the division Phodophyta, class Florideophyceae order Gelidiales, family Gelidiaceae, genus Gelidium [4].Extracts of Black Sea marine algae were reported to exhibit antioxidative and antibacterial activities [5,6,7]. However, information available about the lipid composition of these macroalgae is scarce. Seaweeds are still under-utilized in Bulgaria because

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Retinol, alpha-tocopherol and fatty acid content in Bulgarian Black Sea fish species

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RESUMEN

Retinol, alfa-tocoferol y contenido en ácidos grasos de especies de peces búlgaros del Mar Negro.

El objeto de la investigación presentada es definir y comparar los lípidos totales, el perfil de ácidos grasos y el contenido de retinol y alfa-tocoferol en el tejido comestible de cuatro especies de peces con importancia comercial del Mar Negro búlgaro —espadín (*Sprattus Sprattus*), gobio de boca negra (*Neogobius Melanostomus*), chicharro (*Trachurus Trachurus*) y sábalo del Mar Negro (*Caspialosa Pontica*). Dos vitaminas liposolubles son analizadas simultáneamente mediante cromatografía líquida de alta eficacia (HPLC). El contenido mayor de retinol se encuentra en el espadín (142.3 ± 4.4 µg/100g), y de alfa-tocoferol en el chicharro (1112.7 ± 39.2 µg/100g). El contenido de ácidos grasos ha sido analizado mediante cromatografía gaseosa/espectrometría de masas (GC/MS). El contenido de ácidos grasos (AG) omega-3 (n3) es considerablemente más alto que el contenido de ácidos grasos (AG) omega-6 en todas las especies analizadas. La proporción n6/n3 está en el intervalo recomendado (0.2-1.5) para el espadín, el gobio de boca negra y el sábalo del Mar Negro. Los niveles relativamente altos de retinol, alfa-tocoferol, relaciones de ácidos grasos, n6/n3 AG y PUFA/SFA muestran que todas estas especies de peces poseen buenas propiedades nutricionales.

PALABRAS CLAVE: Alosa pontica – Mar Negro – Neogobiusrattan – Sprattus sprattus – Trahurus medditeraneus ponticus.

SUMMARY

Retinol, alpha-tocopherol and fatty acid content in Bulgarian Black Sea fish species.

The aim of the present study was to measure and evaluate the total lipids, fatty acid profile, retinol content and alpha-tocopherol content in the edible tissue of four commercially important fish species from the Bulgarian Black sea: Sprat (*Sprattus sprattus)*, Round Goby (*Neogobius rattan)*, Black Sea Horse Mackerel (*Trahurus medditeraneus ponticus)* and Shad (*Alosa pontica*). Fat soluble vitamins were analyzed simultaneously using an HPLC system. The highest content of retinol was established in the Sprat (142.3 ± 4.4 µg/100g) and the highest content of alpha-tocopherol was found in the Black Sea Horse Mackerel (1112.7 ± 39.2 µg/100g). The fatty acid (FA) composition was analyzed by GC/MS. The content of omega 3 (n3) FAs was significantly higher ($p < 0.001$) than the content of omega 6 (n6) FAs in each of the analyzed fish samples. The n6/n3 FA ratio was within the recommended range (0.20–1.50) for Sprat, Round Goby and Shad. Relatively high levels of retinol and alpha-tocopherol, FA composition, n3/n6 FA and PUFA/SFA ratios indicate that these fish species have good nutritional quality.

KEY-WORDS: Alosa pontica – Black sea – Neogobius rattan – Sprattus sprattus – Trahurus medditeraneus ponticus.

1. INTRODUCTION

Fish is an important component of a healthy diet, providing a number of substantial nutrients that are essential for achieving a balanced nutrition for children, adults and the elderly. Fish tissue is a good source of fats, proteins, vitamins and minerals. Lipids of marine fish species are rich sources of fat soluble vitamins and both saturated and unsaturated fatty acids (Tocher, 2003). The fat soluble vitamins are essential nutrients related to a diversity of biologically important processes in the human body. Retinol takes place in photo reception, regulates gene expression and cell proliferation, bone growth and reproduction. The biologically active isomer of vitamin E - alpha-tocopherol acts as an antioxidant protecting membrane structures and lipo proteins from oxidation (Anderson J. and Young L., 2008). Polyunsaturated fatty acids (PUFA) derived from fish lipids are key constituents of membrane phospholipids, precursors for the biosynthesis of biologically important hormone-like substances such as eicosanoids. Seawater fish fatty acid (FA) composition is unique and is characterized by low levels of n6 FA (linoleic acid LA, C18:2 n6) and high levels of n3 PUFA (eicosapentaenoic acid, EPA C20:5n3; docosahexaenoic acid, DHA C22:6n3) (Tanakol *et al.*, 1999; Tocher, 2003; Abas *et al.*, 2009).

On the recommendations of the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) it is advisable to consume annually at least 15-20 kg of fish per capita (FAO/WHO, 1994). In Bulgaria, the Black Sea is the main resource for fishing. It is an unique semi-closed basin with slow water circulation, relatively low salinity (e.g. compared with the Mediterranean) and high eutrophication, inhabited by about 140 fish species of which only 15 are commercially important. The catches in the Black Sea account for approximately 89.7% of the total fish production in

Fatty acids composition of macroalgae from Bulgarian Black Sea coast

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Abstract Lipids and fatty acids (FA) composition of three Black Sea macroalgae *Cladophora vagabunda, Ceramium rubrum* and *Cystoseira barbata* were studied. Fatty acids composition was analyzed by GC/MS. Total lipids content varied widely among the species and ranged between 0.66 and 0.98 g per 100 g fresh weight. Generally, saturated fatty acids were major components (62–71%), with 16:0 as the most abundant saturate (41–57%). Total polyunsaturated FAs and monounsaturated FAs ranged from 28% to 38%. The green alga *Cladophora vagabunda* showed higher C18 PUFAs contents than did C20 PUFAs while for red alga *Ceramium rubrum* the trend was opposite. *Cystoseira barbata* belonging to the group of brown algae showed similar amounts of C18 and C20 PUFAs contents. *Cladophora vagabunda* was rich in linoleic acid and *Ceramium rubrum* in arachidonic acid (AA) while *Cystoseira barbata* was rich in both linoleic acid and eicosopentaenoic acid. All of the studied species had a nutritionally beneficial n6/n3 ratio (1.24–2.84:1).

Keywords: Black Sea algae, fatty acids, GC/MS

1. Introduction

Seaweeds have been used since ancient times as food, fodder, fertilizer and as source of medicine. Today seaweeds are the raw material for many industrial productions like agar, algin and carrageenan but they continue to be widely consumed as food in Asian countries. They are nutritionally valuable as fresh or dried vegetables, or as ingredients in a wide variety of prepared foods. In particular, seaweeds contain significant quantities of protein, lipids, minerals and vitamins [1].

Lipids represent only 1-5% of algal dry matter and exhibit an interesting polyunsaturated fatty acid (PUFA) composition particularly omega 3 and omega 6 acids which play an important role in the prevention of cardio vascular diseases, osteoarthritis and diabetes. The red and brown algae are rich in fatty acids with 20 carbons: eicosapentaenoic acid (EPA, C 20:5 n3) and arachidonic acid (AA, C 20:4 n6) [2]. Marine algae are rich in PUFAs of the n-3 and n-6 series, which are considered essential fatty acids for humans and animals. Some of these FAs (20:3n-6, 20:4n-6, 20:5n-3) have high biological activity and are converted into eicosanoids. In addition, PUFAs are of interest in cosmetics as components of sun lotions and as regenerating and anti-wrinkle products. Because of the huge and renewable biomass, seaweeds are a potential source of FAs for biotechnology and a dietary source of essential fatty acids [3].

The n-3 PUFAs cannot be synthesized by humans and are thus obtained through diet. In view of their promising medical and nutritional applications, they have been extensively investigated. However, the studies on efficient exploitation of natural sources for these compounds are limited. At present, marine fishes and fish oils are the main commercial sources of PUFAs but their suitability for human consumption has been questioned from a biosafety perspective, raising the need to search for alternative sources of high quality PUFAs [4]. Consequently, marine macroalgae have been studied as alternative potential sources, as many of them could easily be cultivated in the sea on a large scale. Also, the PUFAs present in the fishes enter the food chain from different trophic levels as a result of consuming primary producers, such as phytoplankton and seaweeds, which synthesize and store them in good quantities [5].

Bulgarian Black Sea coast is rich in algae, regarding biomass and algal biodiversity. Three species of algae – *Cladophora vagabunda*, *Ceramium rubrum* and *Cystoseira barbata* belonging to the three phyla Chlorophyta, Rhodophyta and Phaeophyta, respectively, were chosen for the

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Fatty acid composition of Bulgarian Black Sea fish species

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Abstract The fatty acid compositions of three Black Sea fish species turbot *(Pseta maxima),* red mullet *(Mullus barbatus ponticus)* and garfish *(Belone belone*) were investigated. This species are considered as preferred for consumption in Bulgaria. Lipid extraction was done according to the Bligh and Dyer method. The fatty acid composition was determined by GC/MS. The saturated fatty acids amounts were 38.32 % for turbot, 35.44 % red mullet and 42.90% for garfish. Monounsaturated fatty acids were found in lowest level in comparison with other groups for garfish (23.65%) and turbot (24.85%) while for red mullet they have a highest value – 37.56%. Omega 3 polyunsaturated fatty acids as eicosapentaenoic (C 20:5 omega 3, EPA) and docosahexaenoic (C 22:6 omega 3, DHA) acids were found in highest levels in turbot (22.26%) and garfish (21.80%) and in lowest values of red mullet (9.35%). The results showed that the fish examined are good source of omega 3 polyunsaturated fatty acids, resulting in a very favourable omega 3 / omega 6 ratios, especially in turbot and garfish.

Keywords: Black Sea fish, fatty acids, PUFA, GC/MS

1. Introduction

Many marine fish are well known to be excellent dietary sources of essential fatty acids such as omega 3 polyunsaturated fatty acids (n-3 PUFA). The data obtained in epidemiological and experimental studies supported beneficial activity of these PUFA especially omega 3 (n-3) such as EPA and DHA in the prevention of human cardiovascular diseases and cancers, lowering of incidents of diabetes, they play a vital role in the development and function of nervous system [1, 2]. Considering all these facts the importance of consuming fish rich in omega 3 (n-3) and omega 6 (n-6) PUFA can be taken into consideration. The WHO / FAO recommendations for total daily diet the n-6/n-3 ratio should be from 1:1 to 5:1 [3].

In Bulgaria the consumption of fish is very low (4.5kg annual per capita) compared with the average European levels (23 kg annual per capita) [4].

From the Black Sea fisheries perspective, the most important demersal fish species are turbot (*Psetta maxima*) and red mullets (*Mullus barbatus*). Turbot and red mullet are distributed all over the shelf of Black Sea. Turbot is a very large, broad bodied, left-eyed flatfish. It is a benthic marine species, living on sandy and muddy bottoms, from shallow waters to 110 m. Turbot migrations are relative short and perpendicular on shore. Turbot is a high-value species and its white meat with low lipid levels is appreciated by consumers. In all Black Sea countries turbot is one of the most valuable fish species [5].

In the waters of Bulgaria and Romania red mullet is not important target for fisheries but due to its taste it is preferred for consumption. It prefers waters with the temperature higher 8ºC and salinity more than 17$^{o}/_{oo}$ [5].

Garfish is an epipelagic migratory species that is widely distributed in the North-Eastern Atlantic, Mediterranean as well as the Black Sea and is generally considered of minor commercial importance. Three subspecies have been recognized: first restricted to the North-Eastern Atlantic, second distributed from the south of France in the Mediterranean Sea to the Canary Islands in the Atlantic and *B. belone euxini* - which is found in the Black Sea and the Sea of Azov. Garfish are mainly found in offshore areas except for the spawning period when they migrate into coastal regions where they are also susceptible to commercial exploitation [6].

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DANUBE RIVER'S COMMON CARP (*CYPRINUS CAPRIO*) AND EUROPEAN CATFISH (*SILLURUS GLANIS*) AS A SOURCE OF FAT SOLUBLE VITAMINS AND FATTY ACIDS

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Abstract

The study presents total content of fat soluble vitamins and their percentage of recommended daily intake for humans per 100 g portion, fatty acids composition, atherogenic (AI) and the thrombogenicity (TI) indices in two freshwater fish species - Common carp (*Cyprinus carpio*) and European catfish (*Sillurus glanis*).

Retinol, cholecalciferol and alpha-tocopherol were analyzed simultaneously using RP-HPLC system. Retinol content in fresh edible tissue of Common carp and European catfish was found - 30.8±3.4 micrograms per 100 grams wet weight for Common carp (μg.100g^{-1}ww) and 1.9±0.1 μg.100g^{-1}ww for European catfish, cholecalciferol - 14.8±1.0 μg.100g^{-1}ww and 3.1±0.1 μg.100g^{-1}ww, and alpha-tocopherol 2764.5±44.0 μg.100g^{-1}ww and 2182.5±31.5 μg.100g^{-1}ww, respectively.

Fatty acid (FA) composition was analyzed by GC-MS. The sum of monounsaturated FA (MUFA) accounted for 50.02 % (catfish) and 23.15 % (carp), while polyunsaturated FA (PUFA) showed the opposite trend - higher level in carp (36.75 %) and lower in catfish (21.64%).

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FATTY ACIDS COMPOSITION AND FAT SOLUBLE VITAMINS CONTENT OF BIGHEAD CARP *(ARISTICHTHYS NOBILIS)*

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ABSTRACT

In the present study fatty acid composition and fat soluble vitamins content were analyzed in two season's samples (spring and autumn) freshwater bighead carp *(Aristichthys nobilis)*.

Analysis of fatty acid methyl esters was performed by gas chromatography system with MS detection. Vitamins A, D_3 and E were analyzed simultaneously using RP-HPLC system. The sample preparation procedure includes saponification and liquid-liquid extraction of the unsaponifiable matter.

The fatty acid and vitamins contents of the investigated fish species showed significant seasonal changes. The spring bighead carp characterized with saturated fatty acid (SFA) (37.5%) and mono unsaturated fatty acids (MUFA) (22.1%), and poly unsaturated fatty acids (PUFA) (40.4%), including essential omega 3 fatty acids (23.0%). The autumn samples showed higher SFA (40.5%) and MUFA (34.8%), and lower PUFA (24.6%), due to reduced omega 3 fatty acids (9.7%).

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Fatty acid composition of common carp, rainbow trout and grey mullet fish species

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Abstract. *The aim of the present study was to determinate the fatty acid composition of two commercially important freshwater fish species - rainbow trout (Oncorhynchus mykiss) and carp (Cyprinus carpio L.) and one Black Sea fish – grey mullet (Mugil cephalus). Lipid extraction was done according to the Bligh and Dyer method. Methyl esters were prepared according to method EN ISO 5508:2000. The fatty acid (FA) composition was analyzed by GC-MS. The total lipid content in rainbow trout was 11.50 g/100g raw weight (r.w.), in carp – 12.74g/100 g r.w. while grey mullet showed a value of 3.80g/100g r.w. In comparison with other groups, the polyunsaturated FA (PUFA) showed the highest level in trout – 43.13 % including ω3 such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids followed by grey mullet –29.1%, whereas the carp presented lowest level – 17.55%. The amounts of total ω6 PUFAs were higher than the total ω3 PUFAs in all analyzed fish samples. A ω3 /ω6 and PUFA/SFA ratios were determined in all three fish species.*

Keywords: fatty acid composition, common carp, grey mullet, rainbow trout, GC-MS

Abbreviations: FA – fatty acid, PUFA – polyunsaturated fatty acid, MUFA – monounsaturated fatty acids, TL – total lipids, SFA – saturated fatty acids, EPA – eicosapentaenois, DHE – docosahexaenoie, ALA – Aa-alphalinoleic, ARA – arahidonic

Introduction

The polyunsaturated fatty acids (PUFAs) are considered as essential for the normal human growth, development, and they play an important role in the prevention and treatment of coronary heart disease, hypertension, diabetes, arthritis, inflammatory and autoimmune disorders and cancer. Fishes are the main food source of omega 3 (ω 3) and omega 6 (ω 6) PUFAs in the human diet. The fatty acid composition of fish lipids, (especially PUFA) varies in response to their habitats as water temperature change, salinity and dietary lipids (Tocher, 2003; Henderson et al.,1987). The Black Sea appears to be one of the important fish basins influencing greatly the economy of all countries around the basin and grey mullet (*Mugil cephalus*) is one of the commercially important species. Due to its economic importance, two of the most widely farmed fish species in our country are the rainbowtrout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio L.)*. These freshwater fishes are the preferred fish species for breeding and consumption because of ther rapid growth and rich and diverse composition of the meat (Videv et al., 2009).

The lipid FA profiles data for different marine and freshwater fish species especially originating from Canada, Norway and Japan are available in literature. However, information about the fatty acids composition of Bulgarian Black Sea and freshwaters fish species is lacking. Two reports were encounted in the literature, in witch FA content in carp and trout were mentioned (Ribanova et al., 2003; Hadjinikolova, 2005).

The purpose of the present study was to compare the level of the fatty acid composition of two commercially important freshwater fish species - rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio)* and one Black Sea fish – grey mullet (*Mugil cephalus).*

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Material and methods

Sampling

Grey mullet fish species was purchased from the Varna local fishery market (2010). Common carp was obtained from Pjasachnik Dam Lake (early spring 2010, Plovdiv region) and rainbow trout – from Dospat Dam Lake (spring 2010, Smolyan region). Three specimen of each species were used for lipid and fatty acid analysis. Table 1 presented biometric and biologic characteristics of the observed fish species.

Standards and reagents

For fatty acids analysis Fatty Acid Methyl Esters (F.A.M.E.) mix standard (SUPELCO F.A.M.E. Mix C4-C24), nonadecanoic acid and methyl ester nonadecanoic acid standards were purchased from Sigma – Aldrich ™. All chemicals used were of analytical and GC grade (Sharlau, Spain).

Sample preparation

Lipid extraction. To define total lipids the tissue was finely cut and three samples were taken in parallel, each of which weighed 5g. Total lipids (TL) were extracted with chloroform/methanol (2:1 v/v) according to Bligh and Dyer method (1959). After phase separation, the chloroform extracts were evaporated until dryness and were quantified by weight. The total lipid content was measured in triplicate by gravimetry.

Fatty acids methyl esters (FAME) analysis.

Fatty acid methyl esters (FAME) were prepared by base-catalysed transmethylation with 2M KOH in methanol using EN ISO

Fatty Acid profile and Vitamin A and E content in Horse mackerel (*Trachurus mediterraneus*)

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The aim of this study was to measure and evaluate of the total lipid, fatty acid profil and Vitamin A and Vitamin E content of Black Sea horse mackerel or scad (*Trachurus mediterraneus pontica*) catch from Bulgarian Black Sea and the same fish species from Greek coast of Mediterranean Sea (*Trachurus mediterraneus mediterraneus)*. The results from analysis showed that the sample of Black Sea scad (Spring 08) contain 5.01 g total lipid per 100 g raw weight and 2.40 g total lipid per 100 g raw weight (Autumn 08) while Greek scad present 7.90 g total lipid per 100 g raw weight (Spring 09).The fatty acid composition was analysed by Gas Chromatography with MS detector. The level of total ω3 polyunsaturated fatty acid was higher than the total ω6 polyunsaturated fatty acid in the all analyzed Black Sea fish species. The vitamins content was determinate by HPLC with UV detector. The results from measure show the differences for Vitamin A and E contents between Black Sea horse mackerel and Mediterranean horse mackerel.

1. Introduction

On the recommendations of the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) it is advisable to consume annually at least 15-20 kg fish per capita [1]. In Bulgaria, the Black Sea is the main resource for fishing. It is a unique semi-closed basin with slow water circulation, relatively low salinity (e.g. compared with the Mediterranean), high eutrophication, inhabited by about 140 fish species only 15 of which are commercially important. Mediterranean horse mackerel (*Trachurus mediterraneus*) ranks second in the annual fish catch. According to a research on that catch (biometric and biological features – Georgiev, Kolarov, 62; Cantis, Jonescu, 79) it is concluded that in the Black Sea (the Marmara Sea as well) exists a subspecies named *Trachurus mediterraneus ponticus, Aleev 59* which is smaller than the Mediterranean horse mackerel (*Trachurus mediterraneus mediterraneus*) and inhabits the western and north-western parts of the Black Sea. The two populations differ in lipid

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FATTY ACID COMPOSITION OF FISH SPECIES FROM THE BULGARIAN BLACK SEA

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Summary. The total lipids and fatty acid profile in the edible tissue of two traditionally consumed fish species from Bulgarian Black Sea coast – shad and red mullet in two seasons are determined. The fatty acid composition was analysed by GC/MS. The total content of omega-3 fatty acids was significantly higher than the total content of omega-6 fatty acids in shad whereas red mullet showed opposite trend. The omega-3/omega-6 FA ratio, an useful indicator for evaluation the relative nutritional value of a given fish, was within the recommended range for the studied Black Sea fish species. Obtained results for FA composition, omega-3/omega-6 and polyunsaturated /saturated fatty acids ratios indicate that these Black Sea fish species in both seasons – spring and autumn are good sources of essential fatty acids.

Key words: *omega-3, omega-6, fatty acids, GC/MS, fish species*

INTRODUCTION

Nowadays marine food and especially marine fish are an important part of healthy diets. This is because marine fish provides us with a large number of nutrients especially omega-3 (ω 3) fatty acids such as eicosapentaenoic (EPA, C 20:5) and docosahexaenoic (DHA, C 22:6) fatty acids. A large body of scientific paper shows that fish consumption has beneficial effects on coronary heart disease, growth and development, blood lipids and other health issue [4, 12].

Fish consumption in Bulgaria is lower than recommended by World Health Organization (WHO) and the Food and Agricultural Organization (FAO) (at least 15-20 kg fish per capita) [5]. Their is a lack of information on the fatty acid (FA) composition of local fish species consumed in Bulgaria. The shad (Alosa pontica) and red mullet (Mullus barbatus ponticus) are traditionally consumed fish species

Polyunsaturated Fatty Acids in Fish Species from Bulgaria

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Abstract: The aim of the present study was to determinate the fatty acid composition of two popular Bulgarian Black Sea fish species - Black Sea goby (*Neogobius rattan*) and sprat (*Sprattus sprattus*) and one freshwater - brown turbot (*Salmo trutta fario*). The fatty acid (FA) composition was analyzed by GC-MS. Lipid extraction was done according to the Bligh and Dyer method. Methyl esters were prepared according to method EN ISO 5508:2000. The total lipid content in sprat was 2.50 g /100 g raw weight (r.w.), in goby – 1.60g/100 g r.w. while brown turbot shown a value of 3.80g/100g r.w. In comparison with other groups, the polyunsaturated FA (PUFA) showed the highest level in trout – 44.26 % including n-3 such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, and the lowest level in sprat – 33.94% and in goby – 37.82%. The level of total n-3 PUFAs was higher than the total n-6 polyunsaturated fatty acid in all analyzed Black Sea fish samples, while the freshwater brown trout shown a significant level of n-6 PUFAs and minimum amount of n-3 group. A n-3 /n-6 ratio was determined in all fish species.

Keywords: sprat, goby, brown trout, FAME, omega3 /omega 6 ratio, Bulgarian fish species.

Introduction

Nowadays the omega-3 (ω-3) polyunsaturated fatty acids (PUFAs) are considered as essential for human's normal growth and development and play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, inflammatory and autoimmune disorders and cancer. Fishes are the main food source of ω-3 and omega-6 (ω-6) PUFAs in the human diet.

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VITAMIN CONTENT AND FATTY ACIDS COMPOSITION OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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ABSTRACT

The aim of present study is to evaluate the composition and the content of fatty acids (FA) and fat soluble vitamins (A, E, D_3) in the edible tissue of farmed rainbow trout from the region of Central Bulgaria.

All-trans-retinol (vit. A), cholecalciferol (vit. D_3) and α-tocopherol (vit. E) were analyzed simultaneously using HPLC system with UV (vitamin A and D_3) and fluorescence detection (vitamin E). The sample preparation procedure includes saponification and liquid-liquid extraction of the unsaponifiable matter. Total lipids were extracted according to Bligh and Dyer method. Analysis of fatty acid methyl esters were performed using gas chromatography system with MS detection.

It was found that the lipid fraction contains substantial amounts of palmitic, palmitoleic, stearic, linolenic, arachidonic and docosahexaenoic fatty acids and fat-soluble vitamins. The retinol content in the fresh edible tissue of rainbow trout *(Oncorhynchus mykiss)* was 22.3±2.0 μg/100g; cholecalciferol – 6.0±0.29 μg/100g and α-tocopherol – 809.1± 56.0 μg/100g.

Linoleic acid (15.81%), docosahexaenoic acid (9.40%) and arachidonic acid (4.21%) were the most dominant polyunsaturated fatty acids, about 33% of total FA content. Palmitic acid (12.93%), tetracosanoic acid (3.76%) and oleic acid (3.57%) were found to be the dominant of the saturated and unsaturated FA in rainbow trout fillets.

Keywords: fat-soluble vitamins, PUFA, HPLC, GS/MS, trout

INTRODUCTION

Fish tissue is a good source of fats, proteins, vitamins and minerals and important component of balanced diet. Omega-3 and omega-6 fatty acids (FA), as well as fat-soluble vitamins are essential compounds of fish lipids and exclusively provided by the diet.

Being component of membrane lipids, the essential FAs maintain the integrity, flexibility and permeability of membranes, provide substrate for the biosynthesis of biologically active eicosanoids. It has been shown that omega-3 FAs exert antihypertensive, antiarrhythmic, antidepressive, and immunomodulatory effect. Acting as an-

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Fatty acid composition and fat-soluble vitamins content of sprat (*Sprattus sprattus*) and goby (*Neogobius rattan*) from Bulgarian Black Sea

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Abstract Sprat and goby are commercially important Bulgarian Black Sea fish species. The fatty acid (FA) composition was analyzed by Gas Chromatography with MS detector. Lipid extraction was done according to the Bligh and Dyer method. The monounsaturated FA accounted were 26.93 % for sprat and 30.38 % for goby and palmitoleic (C 16:1) and oleic (C 18:1) acids were dominants in this group. In comparison with other groups, the polyunsaturated FA showed the high level in goby – 37.60% including eicosapentaenoic (C 20:5 n3, EPA), docosahexaenoic (C 22:6 n3, DHA) acids, and lower level on sprat – 34.33%. The level of n 3 polyunsaturated fatty acid was higher than the total n 6 polyunsaturated fatty acid in the all analyzed Black Sea fish species. HPLC method was used for determination of Vitamin A (all-trans-retinol), Vitamin D_3 (cholecalciferol) and Vitamin E (α–Tocopherol) content. The results from fat-soluble vitamins show the differences between sprat and goby. The present studies suggest that both fish species are good sources of n 3 fatty acids and vitamins A, D_3 and E.

Keywords: Black Sea fish, fatty acids, PUFA, Vitamin A, Vitamin D_3, Vitamin E

1. Introduction

Fish is considered as a valuable source of essential fatty acids, vitamins and low levels on saturated fatty acids and cholesterol. The significance of long chain polyunsaturated fatty acids such as n-3 PUFA has gained attention because of their prevention of human cardiovascular diseases. The vitamins are organic compound that are necessary in very small amounts in the diet and fish is one of the main source of vitamins. Vitamins forms are heterogeneous group of substances and are vital nutrients and the absence of vitamins causes serious physiological problems. They regulate metabolic processes, control cellular functions and prevent different diseases.

Black Sea appears to be one of the important fish basins influencing greatly the economy of all countries around the basin. Bulgarian's fishery catch are mainly based on small pelagic fishes namely sprat (*Sprattus sprattus*), horse mackerel (*Trachurs trachurus*) and others. The fatty acids and vitamins data for different marine fish species especially originating from Canada, Norway, Japan are available in literature .However information about the fatty acids and vitamin contents of Bulgarian Black Sea fish species is lacking. One report was encounted in the literature, in witch was mentioned FA and vitamin E content in sprat and mackerel [16].

The objective of our study was to collect information on fatty acid composition fat-soluble vitamin content of two of commercially important Bulgarian fish species. Black sea sprat (*Sprattus sprattus*) and goby (*Neogobius rattan*) were selected. Their total lipids, fatty acid composition and vitamin A, D_3 and E contents were determined.

2. Experimental

2.1. Sampling of fish species

Samples of the commercially important Bulgarian fish species sprat (*Sprattus sprattus*) and goby (*Neogobius rattan*) from Kavarna (North Bulgarian Black seacoast) were purchased from Varna local fishmarket during non-spawning season (november 2008). Twenty-five specimens with

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Article

Bulgarian Marine and Freshwater Fishes as a Source of Fat-Soluble Vitamins for a Healthy Human Diet

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Abstract: The aim of the present study evaluates the fat-soluble vitamins all-*trans* retinol (vitamin A), cholecalciferol (vitamin D_3) and α-tocopherol (vitamin E) content in the fresh edible tissue of Bulgarian fish species: marine—grey mullet (*Mugil cephalus*) and bonito (*Sarda sarda*), and freshwater—rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*). The sample preparation procedure includes alkaline saponification, followed by liquid-liquid extraction with *n*-hexane. All-*trans* retinol, cholecalciferol and α-tocopherol were analyzed simultaneously using RP-HPLC\UV\FL system with analytical column C18 ODS2 Hypersil™. The fat soluble vitamins content (μg per 100 g wet weight) in the fresh edible fish tissue of analyzed fishes are in the ranges: vitamin A from 2.7 ± 0.4 to 37.5 ± 3.4 μg/100 g ww; vitamin D_3 from 1.1 ± 0.1 to 11.4 ± 0.6 μg/100 g ww; vitamin E from 121.4 ± 9.6 to 1274.2 ± 44.1 μg/100 g ww. Three fat-soluble vitamins occur in higher amounts in rainbow trout and grey mullet species. According to recommended daily intake (RDI), they are a good source of cholecalciferol.

Keywords: *Oncorhynchus mykiss*; *Cyprinus carpio*; *Mugil cephalus*; *Sarda sarda*; fat soluble vitamins; HPLC/UV/FL

1. Introduction

Fish is an essential source of both macronutrients—proteins and fats, and micronutrients—vitamins and minerals. Therefore, fish consumption is an important component of a balanced human diet [1].

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Alpha-tocopherol and ergocalciferol contents of some macroalgae from Bulgarian Black Sea coast

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Abstract The aim of the present study was to determine and compare α-tocopherol and ergocalciferol content in four macroalgae from Bulgarian Black sea coast. *Ulva rigida*, *Cladophora vagabunda*, *Cystoseira barbata* and *Cystoseira crinita* were used for evaluation of corresponding fat soluble vitamins content. The sample preparation procedure includes alkaline saponification, followed by liquid-liquid extraction. Ergocalciferol (vitamin D_2) and α-tocopherol (vitamin E) were analyzed simultaneously using HPLC/UV/FL system (Thermo Scientific Spectra SYSTEM) equipped with RP analytical column. The mobile phase was composed of 97:3 = MeOH:H_2O. Ergocalciferol was monitored by UV detection at λmax = 265nm, while α-tocopherol was detected by fluorescence at λex=288nm and λem=332nm. Alpha-tocopherol content in algal tissues ranged from 1.68±0.38mg/100g d.w. in Cladophora vagabunda to 29.13±1.08mg/100g d.w. in *Cystoseira barbata*. Ergocalciferol was detected only in *Ulva rigida* samples.

Keywords: macroalgae, ergocalciferol, α-tocopherol, HPLC

1. Introduction

Seaweeds belong to a group of plants known as algae. They are classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their pigments and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances which can benefit human health. Algae have been used since ancient times as food, fodder, fertilizer and as source of medicine. Nowadays seaweeds represent an inexhaustible source of the raw materials used in pharmaceutical, food industries, medicine and cosmetics. They are nutritionally valuable as fresh or dried vegetables, or as ingredients in a wide variety of prepared foods. In particular, seaweeds contain significant quantities of protein, lipids, minerals and vitamins [1].

Seaweeds are a good source of some water- (B1, B2, B12, C) and fat-soluble (β-carotene with vitamin A activity, vitamin E) vitamins. Vitamin E is the most abundant fat-soluble vitamin of non-saponifiable lipids in many algae. Seaweed vitamins are important not only due to their biochemical functions and antioxidant activity but also due to other health benefits such as decreasing blood pressure (vitamin C), prevention of cardiovascular diseases (β-carotene), or reducing the risk of cancer (vitamins E and C, carotenoids) [2]. Vitamin E is one of the most important fat-soluble vitamins with a strong antioxidant activity. Its special function is lipid protection from peroxidation. It exists in eight forms: α, β, γ, δ-tocopherols and α, β, γ, δ-tocotrienols. The α-forms showed the highest antioxidant effect [3]. Further, the connection of vitamin E and decrease of blood pressure was reported [4].

Algal products have been used in food, cosmetic and pharmaceutical industries. An expanding market for these products is a fact and is facing a new challenge of growing algae on a large scale without harming any further the marine environment. Bulgarian Black Sea coast is rich in algae, regarding biomass and algal biodiversity. Brown seaweeds (*Cystoseira crinita* and *Cystoseira barbata*) and green seaweeds (*Ulva rigida* and *Cladophora vagabunda*) are widespread along the coastal area. Seaweeds are still under-utilized in Bulgaria because the knowledge about their chemical composition is still limited. It was reported that Black Sea Ulva spp. and Cystoseira spp. extracts have antioxidative and antibacterial activities [5,6,7].

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FATTY ACID COMPOSITION OF BLACK SEA *ULVA RIGIDA* AND *CYSTOSEIRA CRINITA*

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Abstract

IVANOVA, V., M. STANCHEVA and D. PETROVA, 2013. Fatty acid composition of Black Sea *Ulva rigida* and *Cystoseira crinita*. *Bulg. J. Agric. Sci.*, Supplement 1: 42–47

Green alga *Ulva rigida* and brown alga *Cystoseira crinita* are widespread in the Black Sea. There is limited information about lipid content and fatty acid composition of these species from Bulgarian Black Sea coast. The aim of this study was to determine and compare total lipid and fatty acid (FA) composition of *Ulva rigida* and *Cystoseira crinita*. Lipids were extracted by following the method of Bligh and Dyer. Fatty acid composition was analyzed by Gas Chromatography with MS detector. Total lipid content ranged between 0.72 and 0.79 g.100g^{-1} fresh weight. Generally, saturated fatty acids were major components (65–70%), with palmitic acid (C16:0) as the most abundant saturate (56–63%). Total polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) ranged from 29% to 35%. The green alga showed higher C18 PUFAs contents than did C20 PUFAs. *Cystoseira crinita* belonging to the group of brown algae showed similar amounts of C18 and C20 PUFAs contents. The green alga was rich in linoleic acid (LA, C18:2n6) while the brown alga was rich in both linoleic acid (LA, C18:2n6) and eicosopentaenoic acid (EPA, C20:5n3). PUFA/SFA ratio in both species was approximately 0.35. All of the studied species had a nutritionally beneficial n6/n3 ratio (1.01–2.43:1).

Key words: Black sea algae, fatty acids, GC/MS
Abbreviations: FA – fatty acids; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; FAME – fatty acid methyl esters; GC – gas chromatography; MS – mass spectrometry

Introduction

Seaweeds have been used since ancient times as food, fodder, fertilizer and as source of medicine. Today seaweeds are the raw material for many industrial productions like agar, algin and carrageenan but they continue to be widely consumed as food in Asian countries. They are nutritionally valuable as fresh or dried vegetables, or as ingredients in a wide variety of prepared foods. In particular, seaweeds contain significant quantities of protein, lipids, minerals and vitamins (Manivannan et al., 2008).

Lipids represent only 1–5% of algal dry matter and exhibit an interesting polyunsaturated fatty acid (PUFA) composition particularly omega 3 and omega 6 acids which play an important role in the prevention of cardio vascular diseases, osteoarthritis and diabetes. The red and brown algae are rich in fatty acids with 20 carbons: eicosapentaenoic acid (EPA, C 20:5 n3) and arachidonic acid (AA, C 20:4 n6) (Banerjee et al., 2009). Marine algae are rich in PUFAs of the n-3 and n-6 series, which are considered essential fatty acids for humans and animals. Some of these FAs (20:3n-6, 20:4n-6, 20:5n-3) have high biological activity and are

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Retinol, cholecalciferol and alpha-tocopherol contents of Bulgarian Black Sea fish species

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Abstract The aim of the present study is to determine and to compare the content of retinol, cholecalciferol and alpha-tocopherol in edible tissue of two Black sea fishes - Garfish (*Belone belone*) and Turbot (*Psetta maxima*). All-trans-retinol (vitamin A), cholecalciferol (vitamin D_3) and alpha-tocopherol (vitamin E) were analyzed simultaneously using HPLC/UV/FL system (Thermo Scientific Spectra SYSTEM) equipped with RP analytical column. The mobile phase was composed of 97:3 = MeOH:H_2O. Retinol and cholecalciferol were monitored by UV detection at λ_{max} = 325 nm and λ_{max} = 265 nm, respectively. Alpha-tocopherol was detected by fluorescence at λ_{ex}=288 nm and λ_{em}=332 nm. The sample preparation procedure includes alkaline saponification, followed by liquid-liquid extraction. Quantities of all-trans-retinol and cholecalciferol were higher in garfish tissues while alpha-tocopherol content in turbot showed seven times higher values.

Keywords: *retinol, cholecalciferol, alpha-tocopherol, turbot, garfish, HPLC*

1. Introduction

Fish is considered as a valuable source of essential nutrients – macronutrients as proteins and fats, and micronutrients as vitamins and minerals, and is an important component of balanced human diet. Fat soluble vitamins are essential components of fish lipids and are exclusively provided by the diet.

Fat soluble vitamins control a variety of biologically important processes in human body. All-trans retinol (vitamin A), takes place in photoreception and regulates gene expression and cell division, bone growth, teeth development, reproduction etc. Cholecalciferol (Vitamin D_3) promotes and enhances the absorption and the metabolism of calcium and phosphorus. Alpha-tocopherol is vitamin E isomer with the highest biological activity. Its main role is as antioxidant, protecting membrane structures, essential fatty acids, and vitamins A and C against oxidation [1].

Fish production in Bulgaria comes mainly from commercial fishing. The catches in the Black Sea account about 77.3 % of total fish production for the country. Aquaculture production (including fresh water fish farming and marine farming of fish and mussels) accounts approximately 13.8 % of the total fish production [2].

As a delicious Black sea fishes Turbot (*Psetta maxima*) and Garfish (*Belone belone*) are consumed in significant amounts in Bulgaria.

Garfish is characterized with long thin body and long needle like mouths. Adult species can reach up to 1 m in length and are mostly silver in color with a blue to green back side and green skeleton. They are typical pelagic fishes. The garfishes are omnivorous – they prefer to feed on zooplankton and small fishes [3].

Some of the Black Sea fish species have migratory character (like garfish), others are non-migratory and they are subject of perennial fishing. One of this species is the Black sea turbot. *Psetta maxima* is one of the flatfish species. They are characterized with white meat and usually low lipid levels. The turbots live near the marine floor – in softer bottoms of mud and sandy mud. They fed with crustaceans, squid, and with a variety of small

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LIQUID CHROMATOGRAPHY METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF FAT SOLUBLE VITAMINS IN FISH TISSUE

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Reviewed by: assoc. prof. L. Macedonski

ABSTRACT

The aim of the present study was to develop simple and accurate small-scale method for simultaneous determination of retinol, α-tocopherol, ergocalciferol, and cholecalciferol in edible fish tissue. High performance liquid chromatography was the method of choice since it provides rapid, sensitive and accurate detection of all four fat-soluble vitamins and requires small amounts of sample. The sample preparation procedure was improved using single reaction tube for both hydrolysis and extraction of the analytes. The overall recovery exceeded 76% for retinol and ergocalciferol, 93% for alpha-tocopherol, and 83% for cholecalciferol. The method precision (relative standard deviation) was below 10% for all analytes. The method was verified on real fish tissue samples and the results for the tested fat-soluble vitamin contents were in a good agreement with the data given by other authors.

Key words: fat soluble vitamins, HPLC, fish tissue

INTRODUCTION

Lipids of marine fish species are rich source of fat soluble vitamins, related to a diversity of biologically important processes in human body. Vitamin A (retinol), takes place in photoreception, regulates gene expression and cell division, bone growth, reproduction. The biologically active isomer of vitamin E - alpha-tocopherol acts as antioxidant protecting membrane structures and lipoproteins from oxidation. Vitamin D_3 (cholecalciferol) and its plant isomer vitamin D_2 (ergocalciferol) are of vital importance for regulation of calcium and phosphate homeostasis.

Fat-soluble vitamins have been determined so far, in many different samples, by a variety of techniques. Among them the most widely applied are the chromatographic techniques mainly HPLC, which provides rapid, sensitive and accurate methods for vitamin determination and has the advantages of solvent economy and easy coupling with other techniques. It also requires small amounts of sample. However, most of the already reported methods measure individual vitamin content in fish tissue homogenates (1,3,9,12,14,15). Mobile phases consisting of mixtures of three or four types of solvents or phosphate buffers have been reported (2,11,12,13). Detection modes involved in the determination of fat-soluble vitamins include UV, diode array (11,15) fluorimetric (14,17), and electrochemical (12), as well as MS detection (3,6). Concerning sample preparation, it is recommended to use short time and gentle extraction methods, sometimes in a darkened place, since these vitamins are unstable during common procedures.

The aim of the present work was to develop a simple, fast and accurate method for simultaneous determination of four fat-soluble vitamins: retinol (A), cholecalciferol (D_3), ergocalciferol (D_2), and alpha-tocopherol (E) in edible fish tissue.

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MATERIAL AND METHODS

Instrumentation and chemicals: A high-performance liquid chromatograph (Thermo Scientific Spectra SYSTEM) equipped with UV2000 and FL3000 detectors were used. All solvents were of HPLC grade. Methanol and water was obtained by Sigma-AldrichTM, USA. Substances of vitamins A, D_3, D_2 and alpha-tocopherol were all of analytical grade and were supplied by Supelco (Sigma-AldrichTM, USA).

Chromatographic conditions: For the separation of the fat-soluble vitamins, RP-column, type ODS2 Hypersil™ C18 (250mm × 4.60 mm, 5μm), coupled with RP C18 security guard pre-column was used. The column temperature was kept at 25^{0}C. The mobile phase of the HPLC system consisted of 97% methanol (solvent A) and 3% MiliQ water (solvent B). The mobile phase flow rate was 1 ml/min and the injection volume each time was 20 μl.

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ОПРЕДЕЛЯНЕ НА ВИТАМИН А И ВИТАМИН Е ЧРЕЗ ВИСОКО-ЕФЕКТИВНА ТЕЧНА ХРОМАТОГРАФИЯ В РИБА КАЯ (Neogobius fluviatilis) ОТ БЪЛГАРСКОТО ЧЕРНОМОРСКО КРАЙБРЕЖИЕ

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ABSTRACT

This study presents simultaneous determination of Vitamin A (all-trans retinol) and Vitamin E (α-tocopherol) in tissue samples from Bulgarian Black Sea Coast fish species by high-performance liquid chromatography method.

The method was applied to samples of Black Sea goby fish (Neogobius fluviatilis) and includes two stages: extraction of tocopherol and retinol from the fish tissue and subsequent quantitative HPLC determination. Quantitative determination of the fat soluble vitamins in the hexane extracts has been done by HPLC with UV-detection on RP-column Nucleosil (25 cm x 0,46 cm). The elution of tocopherol and retinol from the chromatographic column was done by mobile phase composed of 100% methanol at flow rate 0.9 ml/min. Tocopherol was detected at wavelength 295 nm and retinol at 325 nm.

Mean concentration in fresh material were 47.93 μg/100g for Vitamin A and 0.5 mg/100 g for Vitamin E. Our results are in good agreement with the data from the literature for other fish species.

Keywords: retinol, α-tocopherol, blacksee fish species, HPLC

ВЪДЕДЕНИЕ

Рибата е ценен хранителен продукт – източник на белтъци, калций и фосфор. В нейните липиди се съдържат и голямо количество есенциални полиненаситени висши мастни киселини (ω-3, ω-6 ПНВМК). Други ценни компоненти в рибната тъкан са също важните за човешния организъм мастно- и водо-разтворими витамини А, Е, Д, B_1, B_2, B_{12}, ниацин.

Витамин А (all-trans-ретинол) изпълнява важни функции в организма. Той е необходим за поддържане на нормалното зрение, тъй като е важна съставка на

ПЛОВДИВСКИ УНИВЕРСИТЕТ „ПАИСИЙ ХИЛЕНДАРСКИ" – БЪЛГАРИЯ
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ORGANOCHLORINE POLLUTANTS IN BLUEFISH *(POMATOMUS SALTATRIX)* FROM BULGARIAN BLACK SEA COAST

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ABSTRACT

Concentrations of 14 polychlorinated biphenyls (PCBs) and organochlorine pesticide dichlorodiphenyl- trichloroethane (p,p'- DDT) including its metabolites (p,p'- DDE and p,p'- DDD) were measured in muscle tissue samples of bluefish *(Pomatomus saltatrix)*. Samples were collected from Black Sea (region of Varna, Bulgaria) in the period of 2003–2006. DDTs and PCBs were determined by gas chromatograph equipped with electron-capture detector and mass spectrometry allowing better identification of compounds.

Total PCB concentration (sum of 14 congeners) in muscle tissue of bluefish varied in the range of 1.2 to 384.9 ng/g lipid weight. Concentrations in bluefish ranged from 367.1 to 879.5 ng/g lipid weight for total DDTs (sum of p,p'-DDT, p,p'-DDD and p,p'-DDE).

The levels of DDTs and PCBs in bluefish from region of Varna were found comparable or slightly higher than those found in fish from other parts of the Black Sea coast and from neighbor seas Marmara Sea and Aegean Sea.

Keywords: polychlorinated biphenyls; organochlorine pesticides; fish; Black Sea; Bulgaria

INTRODUCTION

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are long-lived organic chemicals that are generally resistant to chemical and biological degradation processes. Organochlorine compounds (OCs) are among the most dangerous pollutants because of their high liposolubility and tendency to bioaccumulate along the food chain. As a consequence, they are widespread in the biotic compartment of the environment [1].

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ORIGINAL PAPER

ORGANOCHLORINE PESTICIDES IN FISH FROM BULGARIAN REGION OF BLACK SEA

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Summary

Background: The Black Sea basin is a subject to important freshwater discharges and could be polluted with persistent chemicals, including organochlorine pesticides.
Methods: The organochlorine pesticides were determined by capillary gas chromatography with electron-capture detector and mass detector for better identification.
Results and discussion: Four fish species with different feeding behaviour (sprat, scad, shad and goby) were sampled on a seasonal basis from the Bulgarian region of the Black Sea and the concentrations of organochlorine pesticides pp-DDE, pp-DDD and pp-DDT (DDTs) residues were determined for period 2003-2005. All samples contained DDT mainly in the form of its metabolites pp-DDE and pp-DDD. The time trends in concentration and patterns of organochlorine pesticides in those species were compared with results found in year 2008.
Conclusions: The highest concentrations of ΣDDT (265 ng/g f.w.) were found in shad while the lowest concentrations of the same pollutants were found in goby. The average residue levels of DDTs detected in this study were below the Maximum Residue Limits for food of animal origin 1 mg/kg lipid weight for DDTs.
Key words: Organochlorine pesticides; DDT; fish; Black Sea; Bulgaria

Résumé

Introduction: Les flots d'eau douce affluant dans la Mer Noi
pourraient être contaminés par des substances chimiques pers
tantes, notamment de pesticides organochlorés.
Méthode: Les concentrations de DDT et de métabolites ont été d
terminées par chromatographie en phase gazeuse avec détecte
à capture d'électrons et d'un détecteur de masse pour identifi
tion meilleure.
Résultats de la discussion: des échantillons de quatre espèces
poissons au régime alimentaire différent ont été prélevés (le spr
le maquereau, l'alose et le gobie) sur une base saisonière le long
littoral bulgare et les concentrations de pesticides organichlo
p,p-DDE, p,p-DDD et p,p-DDT (DDTs) ont été déterminées pc
la période 2003-2005. Dans tous les échantillons a été determi
un contenu de DDT, et surtout sous la forme de ses métaboli
p,p-DDE и p,p-DDD. Des tendences temporelles dans la conc
tration et la distribution des pesticides organochlorés dans ces
pèces ont été comparées avec les résultats obtenus en 2008.
Conclusions: les plus fortes concentrations de ΣDDT (265 ng
de poids frais) ont été trouvées dans le hareng, tandis que les p
faibles concentrations de polluants ont été observées chez les
bies.
Les concentrations moyennes des résidus de DDTs, détéctées
cette étude sont inférieures aux limites maximales pour alime
d'origine animale 1 mg/kg de poids lipide de DDTs.
Mots clés: pesticides organochlorés, DDT, poissons, Mer No
Bulgarie

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ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS IN FRESHWATER FISH

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ABSTRACT

Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) were determined in muscle tissue of three freshwater fish species: common carp *(Cyprinus carpio)*, grass carp *(Ctenopharyngodon idella)* and bighead carp *(Hypophthalmichthys nobilis)*. Fish samples were collected in 2010 from the Pyasachnik Dam Lake. The OCPs and PCBs were analysed in order to evaluate the status and potential sources of pollution in area of the lake. The species were selected because of their importance to local human fish consumption.

The fifteen congeners of PCBs, p,p'-DDT and its two main metabolites p,p'-DDE and p,p'-DDD were determined by capillary gas chromatography system with MS detection. DDTs were the predominant organohalogenated contaminants in all species, with the p,p' – DDE contributing to more than 67% to the total DDTs. All samples of muscle tissue examined contained detectable levels of p,p'-DDE and p.p' – DDD. The residues of p,p' – DDT were not detected in all samples. The sum of the individual PCB congeners

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POLYCHLORINATED BIPHENYLS IN FISHES FROM BULGARIAN BLACK SEA COAST

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Reviewed by: assoc. prof. D. Dimitrov

ABSTRACT

The concentrations of polychlorinated biphenyls (PCBs) were determined in fish species from Bulgarian Black Sea coast. It had selected three of the most popular fish species in our country - european sprat (*Spr. Sprathus Sullinig*), Black Sea goby (*Neogobius Melanostomu*) and Mediterranean horse mackerel - scad (*Trachurus Mediterraneus*). The fish samples were collected from the region of Varna in year 2003 - 2005 and the edible tissues of the fish species were investigated. The fourteen congeners of PCB were analyzed including the set of 7 indicators PCBs (IUPAC № 28, 52, 101, 118, 138, 153, 180) with Gas Chromatography Mass Spectroscopy method. PCBs were found in all investigated samples. European sprat showed the highest total level of PCBs (187.9 - 220,1 ng/g fat) compared to the other species - goby (66.3 - 192.2 ng/g fat) and horse mackerel (14.7 - 208.2 ng/g fat). The highest level of PCBs was found in year 2005. Our study illustrated that the concentration level of PCBs in analyzed samples was lower compared with those recommended by different European institutions.

Key words: polychlorinated biphenyls (PCBs), fish, Bulgarian Black Sea coast

INTRODUCTION

Polychorinated biphenyls (PCBs) are organic chemicals with common characteristics to organochlorine pesticides (OCPs). They were produced comercially by catalytic chlorination of biphenyls, producing a complex mixture of multiple isomers with different degrees of chorination yielding up to 209 products called congeners. PCB congeners with the same number of chorine atoms are known as homologs and the homologs with different chlorine positions are called isomers.

PCBs were produced from 1930s with particularly large amount up to 1970s and they were used in a wide range of industrial applications due to their excellent physical and chemical properties - thermal stability, chemical inertness, non-flammability, high electrical resistivity.

In 1970s the first evidence was published about detrimental effects on organisms and the environment as a whole. Part of them are potential causes of cancer and have a negative effect on the endocrine, reproductive and the neurological systems (5,10,15,17). Due to toxic effects in humans and aquatic organisms, the use and sale of most OCPs and PSBs has been banned or restricted in many European countries since the mid 1970s.

Today it is known that PCB and OCP are ubiquitous and persistent environmental pollutants with a well known potential toxicity. They may bioaccumulate in the aquatic food web and have been of great concern due to their toxic effects on wildlife and human health. A number of studies (2,3,4,8,11,13,18,19) have shown that the major food sources of these organic pollutants are fat-containing animal products including fish and other seafood. Fish is a suitable indicator for the environmental pollution monitoring because they concentrate pollutants in their tissues. Therefore fish consumption may be a risk for human health, especially for resident population.

Black Sea is a unique ecosystem because it is an inner sea with low salinity, half-isolated from the Mediterranean with hydrology and phytobentos different from the other seas in the same biogeographic region. It is bordered by Bulgaria, Romania, Ukraine Russia, Georgia and Turkey and receives fresh water input from some of the largest rives in Europe (the Danube, Dniester and Dnieper). At least 170 million people live in the Black Sea basin. Although, some studies have assessed the environmental quality of Black Sea (1,7,9,16). Data about the current contamination of PSBs and OCPs are very scarce, no systematic measurements in biological samples. Therefore, comprehensive studies are needed to understand the status of contamination and pollution of these chemicals in the Black Sea.

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УСТОЙЧИВИ ОРГАНОХЛОРНИ ЗАМЪРСИТЕЛИ В СЛАДКОВОДНИ РИБИ

Мона Станчева, Станислава Георгиева, Любомир Македонски

PERSISTENT ORGANOCHLORINE POLLUTANTS IN FRESHWATER FISH

Mona Stancheva, Stanislava Georgieva, Lubomir Makedonski

ABSTRACT: *Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) were determinated in three freshwater fish species: carp (Cyprinus carpio), pike-perch (Sander lucioperca) and bighead carp (Hypophthalmichthys nobilis) from the Tsonevo Dam Lake (district Varna). The species were selected for their importance to local human fish consumption. Analysis of OCPs and PCBs were performed using gas chromatography system with MS detection. DDTs were the predominant organohalogenated contaminants in all species, with the p,p'- DDE contributing to more than 80% to the total DDTs. Lipid-based concentrations of DDTs were higher in carp than in the other species and this was related to its higher lipid content. PCBs were found in detectable levels only in muscle tissues of bighead carp.*

KEYWORDS: *Fish; Polychlorinated biphenyls; Organochlorine pesticides; Bulgaria.*

INTRODUCTION

The production and intensive agricultural or industrial use of organochlorine pesticides (such as DDT and its metabolites DDE and DDD) or polychlorinated biphenyls (PCBs) have led to the widespread contamination of the environment. They are persistent organic pollutants (POPs) and characterised by a high bioaccumulation potential in food chains and therefore may pose a serious threat to upper trophic levels of aquatic communities [1,2]. In biological systems, several of these chemicals are potentially carcinogenic and may cause alternations in endocrine, reproductive and nervous systems [3]. For these reasons, most countries have restricted or banned the use of PCBs and OCPs since 1970s. DDT is a chlorinated pesticide widely used in the past to control the spread of insects and other agricultural pests. In the environment DDT metabolised slowly and the metabolite DDE is particularly persistent compound. Polychlorinated biphenyls have been widely used by a large variety of industries over the past 50 years. However, several studies have demonstrated that they are toxic to a variety of marine organisms [4].

Fish is a suitable indicator for the environmental pollution monitoring because they concentrate pollutants in their tissues directly from water, but also through their diet, thus enabling the assessment of transfer of pollutants through the trophic web [1]. Data on the presence and distribution of organohalogenated contaminants in fish and especially edible fish species are therefore important not

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Organochlorine pesticides and PCBs in marine fish

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Abstract Organochlorine pesticides (such as 1,1,1-trichloro - 2,2 - bis (4-chlorophenyl) ethane (DDT) and its metabolites) and polychlorinated biphenyls (PCBs) are classified as Persistent Organic Pollutants (POPs) and are present in the contamination pattern of marine environments world-wide. Concentrations of PCBs and DDTs were measured in two marine species: garfish (*Belone belone*) and red mullet (*Mullus barbatus*). Samples were collected from Black Sea, Bulgaria during 2007 – 2010. The DDTs and PCBs were determined by gas chromatography - mass spectrometry.
Concentrations in muscle tissue of garfish ranged from 80.89 to 118.04 ng/g wet weight for total DDTs. DDTs concentration in red mullet was found 104.59 ng/g ww. PCB concentrations in garfish varied in the range of 40.04 and 65.62 ng/g ww. In muscle tissue of red mullet PCB concentrations were found 34.12 ng/g ww. The levels of DDTs and PCBs in garfish and red mullet from the Black Sea were comparable with those found in other marine ecosystem.

Keywords: fish, DDT, PCB, Black Sea, Bulgaria

1. Introduction

PCBs and selected organochlorine pesticides are a group of chemicals that have attracted considerable attention due to their high toxicity, persistence in the environment, and ability to bioaccumulate. The combination of these properties means that organisms at the upper levels of the food chain can potentially be exposed to concentrations sufficient to cause adverse effects. [1,2] In biological systems, several of these chemicals are potentially carcinogenic and may cause alternations in endocrine, reproductive and nervous systems [3]. For these reasons, most countries have restricted or banned the use of PCBs and DDTs since 1970s. DDT (1,1,1-trichloro - 2,2 - bis (4-chlorophenyl) ethane) is a chlorinated pesticide widely used in the past to control the spread of insects and other agricultural peststicides. In the environment DDT metabolised slowly and the metabolite DDE is particularly persistent compound. Polychlorinated biphenyls have been widely used by a large variety of industries over the past 50 years. However, several studies have demonstrated that they are toxic to a variety of marine organisms [4].
Fish is a suitable indicator for the environmental pollution monitoring because they concentrate pollutants in their tissues directly from water, but also through their diet, thus enabling the assessment of transfer of pollutants through the trophic web [1,5]. Data on the presence and distribution of organohalogenated contaminants in fish and especially edible fish species are therefore important not only from ecological, but also human health perspective [6,7].

Red mullet are non-migratory species and feed mainly with benthic invertebrates (crustaceans, worms, and molluscs). The garfish *(Belone belone)* is a pelagic, oceanodromous needlefish found in marine waters of the Mediterranean Sea, the Baltic Sea, etc. The fish lives close to the surface, has a migratory pattern and feed mainly with small fish.

The purpose of this study was to determine the levels of persistent organochlorine contaminants in garfish and red mullet from the Bulgarian Black Sea coast and to monitor the accumulation of these pollutants during the period 2007 – 2010.

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Organochlorine Pollutants in Fish from the Bulgarian Region of the Black Sea

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ABSTRACT

Introduction Persistent organochlorine pollutants (POPs) like polychlorinated biphenyls (PCBs) and DDT residues can still be a problem for the aquatic environment and the human health.

Objectives The levels of DDTs and PCB congeners were determined in fish from the Bulgarian Black Sea coast.

Method Four fish species with different feeding behavior: goby (*Neogobius cephalargoides),* sprat (*Sprattus sprattus sulinus*), horse mackerel *(Trachurus Mediterraneus ponticus),* shad *(Alosa pontica pontica)* were sampled from the Bulgarian Black Sea coast during 2007 – 2010. The DDTs and PCBs residues were measured in clean fish extracts by gas chromatography with mass detection.

Results The main metabolite p,p'-DDE was the most frequently detected compound in all fish species and was present in much higher concentrations than the other DDTs (ranging from 119.32 to 1324.44 ng/g fat). PCBs were found in all fish species at concentrations ranging between 135.1 ng/g fat in horse mackerel and 990.8 ng/g fat in goby (calculated as the sum of 15 investigated congeners).

Conclusions The levels of DDTs and PCBs in fish from Bulgarian Black Sea coast were comparable to those found in fish species from the Black Sea and from neighboring seas – the Marmara Sea, the Aegean Sea and the Mediterranean Sea.

Assessment of heavy metal distribution in muscle, skin and gills of two fish species from the Black Sea, Bulgaria

Mona Stancheva, Katya Peycheva, Lubomir Makedonski

Abstract

Heavy metal (Cd, Pb, Cu, Mn, and Fe) concentrations in muscle, gill and liver of two fish species (Pomatomus saltatrix and Alosa pontica) from the western Black Sea were measured by atomic absorption spectrophotometry. Levels of metals varied depending on different tissues in each species. Iron showed the highest level in all examined tissues of both fish species. High concentration of Pb (2.70 and 2.29) and Mn (4.68 and 1.84) were measured in gill tissues for both species, too. The liver of Pomatomus saltatrix showed significant Cu concentrations. Metal levels in tissues were compared with national and international permissible limits. Metals concentrations in both edible and other tissues of the sampled species were within the permissible safety levels for human consumption.

1. Introduction

Heavy metals from geological and anthropogenic sources are increasingly being released into natural waters [1,2]. Contamination of aquatic ecosystem with heavy metals has seriously increased worldwide attention, and a lot of studies have been published on the heavy metals in the aquatic environment [3,4]. Heavy metals are present in the aquatic environment where they bio accumulate along the food chain [5]. Accumulation occurs in different tissues of aquatic animals and may become toxic for fish and also for people when it reaches substantially high level.

Heavy metals can be classified as potentially toxic (arsenic, cadmium, lead, mercury, nickel, etc.), probably essential (vanadium, cobalt) and essential (copper, zinc, iron, manganese, selenium) [6]. Fish are good indicators for the long term monitoring of metal accumulation in the marine environment. Therefore, numerous studies have been carried out on metal accumulation in different fish species [7]. For the last two decades there are no sufficient data about heavy metals pollution of the Bulgarian Black Sea coast.

The Black Sea is the world's largest natural anoxic water basin below 180m in depth. It is a closed sea with a very high degree of isolation from the world's oceans, but it receives freshwater inputs from some of the largest rivers in Europe; the Danube, the Dniester, and the Dnieper [8]. For this reason, the Black Sea is considered one of the most polluted seas, and the increasing concentration of nutrients in recent years have led to a higher degree of eutrophication. The fishery yield has declined dramatically, and the tourism industry has also suffers from serious pollution of the Black Sea.

In this study, toxic and essential elements (Cd, Pb, Cu, Mn and Fe) in the muscle, liver and gills of two different economic fish species from the Black Sea in Bulgaria were determined by AAS after acid digestions. These species were bluefish (*Pomatomus saltatrix)* and shad *(Alosa pontica)*.

Bluefish (*Pomatomus saltatrix*) occurs in oceanic and coastal waters. It is a good food fish; marketed mostly fresh, but also dried or salted, and frozen. It is most common along surf beaches and rock headlands in clean, high energy waters. Feed on other fish,

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Heavy metals and PCBs level of bluefish (*Pomatomus saltatrix)* from Bulgarian Black sea waters

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Abstract The concentration of some heavy metals (Cd, Mn, Fe, Cu and Pb) and polychlorinated biphenyls (PCBs) were determined in muscle tissue of bluefish *(Pomatomus saltatrix)* collected from the coast of Bulgarian Black Sea. Quantitative determination of the PCBs compounds was performed by gas chromatography–mass spectrometry detection (GC–MS), while the heavy metals were determined by atomic absorption spectrophotometry. The validation of the heavy metal procedure was performed by analysis of standard reference material (DORM-2 Dogfish Muscle). Pb and Cd were under the detection limits for the samples from year 2004. The levels of iron showed the highest value trough the two year period of investigation (from 6.51μg/g up to 7.06 μg/g).

The fourteen congeners of PCB were analyzed including the set of 7 indicators PCBs (IUPAC No 28, 52, 101, 118, 138, 153, 180). PCBs were found in all samples with maximum level in year 2004 (Σ PCBs = 9.1. mg/kg product). The levels of these organochlorines are considered to be comparable to baseline levels.

From an ecotoxicological point of view, the concentrations of heavy metals and polychlorinated biphenyls compounds reflect a comparatively clean and pollution-free environment. These concentrations may be, thus, considered as useful background levels to which to refer for comparison within the Black Sea.

Keywords: Heavy metals, PCBs, bluefish, AAS, GC–MS, Black Sea, Bulgaria

1. Introduction

The world-wide contamination by heavy metals and polychlorinated biphenyls (PCBs) is considered to be of great concern due to their toxic effects on humans and wildlife. PCBs constitute a class of 209 compounds with differential biological activity and toxicity as a result of differences in the number and position of chlorine atoms [1]. Reports from the literature suggest that polychlorinated biphenyls, particularly dioxin-like PCBs, have a complex spectrum of toxicological properties, including chloracne, thymic atrophy, liver damage, immunotoxicity and cancers [2, 3] and have, also, been associated with low birth weights and learning and behavioural deficiencies in children of women who consume large quantities of contaminated fish or are occupationally exposed [4]. The PCBs are fat–soluble and therefore they are accumulated in the lipids and foods containing fats.

Aquatic environmental quality currently receives a great deal of attention [5]. Contamination with heavy metals in aquatic system has been a serious concern for over decades. Heavy metals are introduced in those systems through variety of human activities - industrial waste, agricultural and urban sewage. Under certain environmental conditions, heavy metals may accumulate to toxic concentrations and cause ecological damage [6]. Metals such as copper, zinc, iron, manganese and selenium are essential since they play important roles in biological systems. The essential metals can also produce toxic effects at high concentrations [7].

Fish is the final chain of aquatic food web and an important food source for human. Therefore, heavy metals in aquatic environments are transferred through food chain into humans. A well known fact is that fish muscle is not an active tissue in accumulation of heavy metals [8]. On the contrary liver is a good monitor of water pollution with metals since their concentrations are proportional to those present in environment.

Recently, agricultural and industrial developments as well as increase in population have substantially increased the contamination of

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DETERMINATION OF HEAVY METALS IN FISH SPECIES FROM THE BULGARIAN BLACK SEA

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SUMMARY

Background: In this study heavy metals (Cd, Cu, Zn, As, Pb) concentration in edible part of eight most consumed Bulgarian fish species - Atlantic bonito (*Sarda sarda*), Black sea gobies (*Neogobius melanostomus, Neogobius ratan*), bluefish (*Pomatomus saltatrix*), gray mullet (*Mugil cephalus*), Mediterranean horse mackerel (*Thrachurus mediterraneus ponticus*), shad (*Alosa pontica*), sprat (*Sprattus spratus*) and turbot (*Psetta maxima meotica*) collected from north-east coast of Bulgarian Black Sea were determined.

Methods: The samples were digested with a microwave digestion system followed up by Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) determination. The validation of the presented procedure is performed by the analysis of standard reference material (NRCC-DORM 2 Dogfish Muscle). Sampling period was done in 2008.

Results: The levels of Cd and Cu were relatively low in the edible part for all fish types while those for Zn concentration show highest value for sprat. The concentration of As and Pb are within acceptable levels for a food source for human consumption.

RÉSUMÉ

But: Le présent article définit le contenu des métaux lourds (Cd, Cu, Zn, As, Pb) dans les poisons de consommation en masse – la bonite (Sarda sarda), le gobie (*Neogobius melanostomus, Neogobius ratan*), le tassergal (*Pomatomus saltatrix*), le mulet (*Mugil cephalus*), le maquereau (*Thrachurus mediterraneus ponticus*), l'alose (Alosa pontica), le sprat (*Sprattus spratus*) et le turbot (*Psetta maxima meotica*) sélectionnées sur la côte nord de la Mer Noire en 2008.

Méthodes: Les échantillons ont été désintegrés par micro-ondes, le contenu des métaux lourds étant determine par ICP-MS.

Résultats: Le contenu de Cd et de Cu est relativement faible dans tous les échantillons examinés, tendis que le contenu de Zn est le plus élevé dans la partie comestible du sprat.

Mots cléfs: métaux lourds, poissons, Mer Noire, Bulgarie, ICP-MS

INTRODUCTION

The heavy metal pollution of the marine environment has long been recognized as a serious environmental concern [1,2].

Heavy metals can be accumulated by marine organisms thought a variety of pathways, including respiration, adsorption and ingestion [3]. Over the last few decades the marine environment has been contaminated by persistent pollutants of agriculture and industrial origin. Heavy metal contamination has been identified as a concern in coastal environment, due to discharges from industrial waste, agricultural and urban sewage. The levels of heavy metals are known to increase drastically in marine environment through mainly anthropogenic activities. Heavy metals can be classified as potentially toxic (arsenic, cadmium, lead, mercury, nickel, etc.), probably essential (vanadium, cobalt) and essential (copper, zinc, iron, manganese, selenium) [4]. Fish are good indicators for the long term monitoring of metal accumulation in the marine environment. Therefore, numerous studies have been carried out on metal accumulation in different fish species [5]. For the last two decades there are no

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ОПРЕДЕЛЯНЕ НА ТЕЖКИ МЕТАЛИ В СЕДИМЕНТИ ОТ ВАРНЕНСКИЯ ЗАЛИВ И ВАРНЕНСКОТО ЕЗЕРО

Веселина Иванова, Тодорка Сократева, Мона Станчева

HEAVY METALS IN SURFACE SEDIMENTS OF VARNA BAY AND VARNA LAKE (BLACK SEA, BULGARIA)

Veselina Ivanova, Todorka Sokrateva, Mona Stancheva

ABSTRACT: *Surface sediments are used as environmental indicators to reflect the quality of marine and lake systems. The bioaccumulation of sediment-bound metals by benthic species is very important to the food webs and their eventual transfer back to man. In this study the concentrations of some heavy metals (Cd, Cr, Cu, Pb, Mn, Ni and Zn) in shallow sediments from the Varna Bay and Varna Lake were determined. Dried samples were sieved and digested in aqua regia and analyzed by ICP-OES, FAAS and ET-AAS. The validation of the heavy metal procedure was performed by analysis of standard reference material (MESS-3 Marine Sediment Reference Material). Higher concentrations of heavy metals were found in sediments from Varna Lake. Manganese showed the highest concentration amongst other elements in both sampling places, followed by Zn, Cr, Cu, Pb, Cd and Ni.*

KEYWORDS: *heavy metals, sediments, Black sea, Varna*

INTRODUCTION

The world-wide contamination by heavy metals is considered to be of great concern due to their toxic effects on humans and wildlife. Aquatic environmental quality currently receives a great deal of attention. Contamination with heavy metals in aquatic system has been a serious concern for over decades. Heavy metals are introduced in those systems through variety of human activities – industrial waste, agricultural and urban sewage. Under certain environmental conditions, heavy metals may accumulate to toxic concentrations and cause ecological damage [1]. Metals such as copper, zinc, iron, manganese and selenium are essential since they play important roles in biological systems. The essential metals can also produce toxic effects at high concentrations [2].

The bottom is the last acceptor of the substance inflow. Thus, sediments composition influences directly benthic species [3]. Heavy metals entering the water body would be absorbed in sediments, and subsequently might migrate as a result of exchanges between water, sediment, and biota, through biological and chemical processes [4]. Sediments have been widely used as environmental indicators and their ability to trace contamination sources and monitor contaminants is largely recognized. They play an important role in the assessment of metal contamination in natural waters. Indeed sediments show a high capacity to accumulate and integrate on time the low concentrations of trace elements in water and, therefore, they allow

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DETERMINATION OF Cd, Cu, Fe, Mn AND Pb IN EUROPEAN CARP (CYPRINUS CARPIO CARPIO)

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Reviewed by: assoc. prof. N. Negrev

ABSTRACT

The concentrations of five elements (Cd, Cu, Fe, Mn, and Pb) were determined in the muscle, liver, gills, bones and skin of cultured carp (Cyprinus carpio carpio) caught at Tzonevo Dam in region Varna, Bulgaria. The samples analyzed for Cd, Cu, Fe, Mn and Pb by Atomic Absorption Spectrometer. The highest levels of Pb, Mn and Cd were found in the skin of the fish specie (5.26 mg/kg w.w., 8.74 mg/kg w.w. and 0.34 mg/kg w.w., respectively) while Cu and Fe had been accumulated predominantly in the gills (2.68 mg/kg w.w. and 55.54 mg/kg w.w. respectively). Among the metals analyzed Fe was the most abundant in the different tissues, while Cd and Cu where the least abundant. The results obtained in this study were compared with those reported in other studies. The concentration of these five elements in the European carp samples found in the literature showed a similar tendency except for Pb. Metal concentration in the edible part of the examined fish (muscle) was in the safety permissible levels for human consumption set by various health organizations.

Key words: Heavy metals, cultured carp, liver, gills, AAS, Bulgaria

INTRODUCTION

Fish is the final chain of aquatic food web and an important food source for humans. Water pollution leads to contamination of fish species with toxic metals, from many sources, e.g. industrial and domestic waste water, natural runoff and contributory rivers (16,14). In the water basin, pollutants are potentially accumulated in organisms and sediments, and subsequently transferred to man through the food chain (17). For this reason, determination of chemical quality of aquatic organisms, particularly the contents of heavy metals in fish is extremely important for human health (5).
A well known fact is that fish muscle is not an active tissue in accumulation of heavy metals (18). On the contrary liver is a good monitor of water pollution with metals since their concentrations are proportional to those present in environment.
Dam lake ecosystems are vulnerable to heavy metal pollution. Tzonevo Dam is situated in the Valley of Luda Kamchia River and ranks third in size in region Varna, Bulgaria. It is biodiversity is of importance for the local residents. Recently, agricultural and industrial developments as well as increase in population have substantially increased the contamination of Tzonevo dam.

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The aim of this study is to investigate the distribution of selected metals (Cd, Cu, Fe, Mn and Pb) in different tissues (muscle, skin, gills, liver and bones) of the cultured carp (Cyprinus carpio carpio) collected from Tzonevo Dam by using atomic absorption spectrophotometer (AAS).

MATERIAL AND METHODS

Biology and ecology of the fish sample

Cultured carp dwells in middle and lower reaches of rivers and lakes and shallow confined waters and can survive cold winter periods. Carp are omnivorous, with a high tendency towards the consumption of benthic organisms. Zooplankton consumption is dominant in fish ponds where the stocking density is high. Additionally, the carp consumes the stalks, leaves and seeds of aquatic and terrestrial plants, decayed aquatic plants, etc.

Sampling collection

Samples of the fishes were acquired from three locations along Tzonevo Dam. All the fish species were sampled in July 2009. Total length and weight of the samples (total number 9) brought to laboratory on ice after collections were measured to the nearest millimeter and gram before dissection. The biometric data of the fish sample are as follows (mean ±SD): weight 1662.0 ±43.0 g; length 45.5 ±8.0 cm.
Special care was taken to prevent metal contamination of the samples by the laboratory equipment. After biometric measurements, the fishes were immediately dissected, washed

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DETERMINATION OF HEAVY METALS IN BLACK SEA *MYTILUSGALLO PROVINCIALIS* AND *RAPANA VENOSA*

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ABSTRACT

Shellfish living in seas can accumulate heavy metals and thus serve as excellent passive biomonitors. Concentrations of arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and manganese (Mn) in two kinds of shellfish, *Rapana venosa* and *Mytilus galloprovincialis* were determined. Samples were collected at three coastal sites along the Bulgarian Black Sea, including one mussel farm. Shellfish tissues were subjected to microwave-assisted acid digestion followed by appropriate atomic absorption spectrometry (AAS) (Flame AAS for Mn, Electrothermal AAS for Cd, Pb and As). Concentration of total mercury was determined by Direct Mercury Analyzer. Levels of metals varied within species. The results clearly indicated that the concentrations of As exceeded the maximum permissible levels (MLPs) of 2,0 mg/kg according to the Bulgarian Food Codex (2004).

Key words: heavy metals, atomic absorption spectrometry, *Rapana venosa*, *Mytilus galloprovincialis*, Bulgarian Black Sea

INTRODUCTION

Black Sea is the world's largest natural anoxic water basin below 180 m in depth. It is a closed sea with very high degree of isolation from the world's oceans, however, it receives freshwater inputs from some of the largest rivers in Europe such as the Danube, the Dniester, and the Dnieper (12,13). Black Sea is considered as one of the most polluted seas, and recently increasing concentration of nutrients has led to a higher degree of eutrophication.

Heavy metal pollution of the marine environment has been long recognized as a serious environmental concern (2,14). Marine organisms, especially shellfish, are capable of accumulating the metals from the environment in which they live. Heavy metals can be accumulated by marine organisms thought a variety of pathways (18). Over the last few decades the marine environment has been contaminated by persistent pollutants of agricultural and industrial origin. Heavy metal contamination has been identified as a concern in coastal environment, due to discharges from industrial waste, agricultural and urban sewage. Heavy metal levels are known to increase dramatically in marine environment mainly through anthropogenic activities. Shellfishes are good indicators of the long term monitoring of metal accumulation in the marine environment.

There are scanty data about heavy metal pollution in shellfish from the Bulgarian Black Sea coast for the last twenty years. The aim of this study was to determine the levels of lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg) and manganese (Mn) in Black Sea mussel (*Mytilus galloprovincialis*) and rapana (*Rapana venosa*).

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MATERIAL AND METHODS

Black mussel (*Mytilus galloprovincialis*) is a natural biofilter that inhabits tidal areas attached to rocks. *Rapana venosa* is a predatory sea snail that has entered the Black Sea in the middle of the last century and feeds mainly on black mussels.

Sampling

Shellfish samples were obtained at three sites along Bulgarian Black Sea coast such as Varna, Kranevo (shellfish farm) and Krapetz in spring 2011. The collected mollusks were depurated in filtered seawater for approximately 24 h before being transported to the laboratory with ice freezing. The soft tissues of mollusks were excised by stainless steel scalpel blades and then thoroughly rinsed with Milli-Q water to remove extraneous impurities. Total soft shellfish tissue was taken for analysis. After sufficient homogenation by a blender, the samples were kept at -18 °C until analysis. Special care was taken to prevent metal contamination of the samples by the laboratory equipment.

Analytical procedure

All the solutions were prepared with analytical reagent grade chemicals and ultra-pure water (18 MΩ cm) was used for all of them. HNO_3 of superb quality was purchased from Fluka. All the plastic and glassware were cleaned by soaking in 2 M HNO_3 for 48 h. They were rinsed five times with distilled water and then five times with deionised water prior to use. Stock standard solutions of As, Hg, Cd, Mn

Съдържание на полихлорирани бифенили и органохлорни пестициди в черноморски риби - паламуд и карагьоз

Мона Станчева, Станислава Георгиева, Любомир Македонски, Томислав Ризов

Определено е съдържанието на полихлорирани бифенили (ПХБ) и органохлорни пестициди (ДДТ) в мускулна тъкан на два вида морски риби: паламуд (Sarda sarda) и карагьоз (Alosa pontica pontica) чрез използване на Газов хроматограф с електронулавящ детектор. Средната стойност на ПХБ в паламуд е 10.7 ng/g мазнина, а в карагьоз е 40.2 ng/g мазнина. Измерените сумарни концентрации на ДДТ в паламуд и карагьоз са 277.2 и 951.9 ng/g мазнина, съответно.

Levels of Polychlorinated Biphenyls and Organochlorine Pesticides in Black sea fish - Atlantic bonito and Shad from Black sea, Bulgaria

Mona Stancheva, Stanislava Georgieva, Lubomir Makedonski, Tomislav Rizov

Concentrations of polychlorinated biphenyls (PCBs) and organochlorine pesticides (DDTs) were measured in muscle tissue of two species of marine fish: Atlantic bonito (Sarda sarda) and shad (Alosa pontica pontica) using a gas chromatograph with electron-capture detector. The average levels of PCBs in the bonito and shad were 10.7 ng/g fat and 40.2 ng/g fat, respectively. Median concentrations of DDTs in bonito and shad were measured as 277.2 and 951.9 ng/g fat, respectively.

1. Увод

Органохлорни пестициди (ОХП) и полихлорирани бифенили (ПХБ) са били произведени и използвани за земеделски и промишлени цели в големи количества в продължение на дълг период от време. Поради своята висока химическа стабилност и липофилност, тези съединения остават в околната среда за много години. Те са склонни към биоконцентрация в хранителните вериги и представляват определен риск за здравето на дивите животни и хората. Въпреки налагането на строги забрани и ограничения за ползване на органохлорни пестицидии и ПХБ в повечето страни, тези съединения продължават да бъдат откривани в измерими количества в екосистемите, включително в морските организми.

Паламудът, Atlantic bonito (*Sarda sarda*) зимува в Средиземно и Мраморно море. Напролет навлиза в Черно море за хранене и размножаване. Малките рибки се хранят със зоопланктон, а възрастните са хищници.

Карагьозът, Shad (*Alosa pontica pontica*) зимува на юг от българското крайбрежие, но при топли зими се държи разпръснато в откритите райони около нос Емине и Маслен нос. За размножаване навлиза във водите на Дунав до най-

РЕПУБЛИКА БЪЛГАРИЯ

АКТУАЛИЗИРАН НАЦИОНАЛЕН ПЛАН
ЗА ДЕЙСТВИЕ ПО УПРАВЛЕНИЕ НА УСТОЙЧИВИТЕ ОРГАНИЧНИ ЗАМЪРСИТЕЛИ (УОЗ) В РЕПУБЛИКА БЪЛГАРИЯ,

2012 г. ÷ 2020 г.

(А - Н П Д У У О З)

Приет с Решение на Министерски съвет на 5 септември 2012 г.,

Извлечение от Протокол № 33 от заседанието на Министерски съвет на 5 септември 2012 г.

Национален изпълнител и Координатор

МИНИСТЕРСТВО НА ОКОЛНАТА СРЕДА И ВОДИТЕ

София, август 2012 г.

РЕЗЮМЕ

Определено е съдържанието на устойчиви органични замърсители – полихлорирани бифенили (ПХБ) и хлорорганични пестициди (ДДТ и метаболити) в черноморски риби във връзка с оценка на тяхната безопасност като храна. След предварително проучване на морския риболов и състоянието на пазара у нас са подбрани десет вида черноморски риби за изследване: кая, трицона, кефал, сафрид, карагъоз, чернокоп, паламуд, зарган, калкан, барбуна. Пробите за анализ са набирани от три района в периода 2007 – 2010г.

Определени са концентрациите на следните конгенери ПХБ с номера по IUPAC: 28, 31, 52, 77, 101, 105, 118, 126, 128, 138, 153, 156, 169, 170, 180, на базата, на които е определено общото съдържание на ПХБ в пробите и хлорорганични пестициди – ДДТ и метаболитите (ДДД и ДДЕ). Анализирани са 85 рибни проби, като всяка проба е определяна трикратно. За статистическата обработка и анализ на резултатите е използвана програмата SPSS 16. Приложени са следните тестове: за определяне вида на разпределението (Колмогоров – Смирнов), за определяне на статистическите параметри и сравняване на сериите резултати (Стюдент-Фишер). Резултатите са представени като средна аритметична и средна геометрична, отнесени за грам мазнина (ng/g fat) и за грам свежо тегло (ng/g ww).

Резултатите показват, че в изследваните проби риби преобладават метаболитите DDE и DDD, което показва, че по-голямата част от използваното в миналото DDT се е метаболизирало и няма употреба на нови количества DDT в черноморски район на България. В заключение, резултатите от проведеното изследване показват, че замърсяването с PCB и DDT в риби от българското крайбрежие на Черно море е по-ниско или съизмеримо в сравнение с резултати за риби от други региони на Черно море и съседни морета – Мраморно и Средиземно море.

МЕДИЦИНСКИ УНИВЕРСИТЕТ "ПРОФ. Д-Р ПАРАСКЕВ СТОЯНОВ" – ВАРНА
ФАКУЛТЕТ ПО ФАРМАЦИЯ

КАТЕДРА ХИМИЯ

Мона Динкова Станчева

УСТОЙЧИВИ ОРГАНИЧНИ ЗАМЪРСИТЕЛИ И ТЕЖКИ МЕТАЛИ В ЧЕРНОМОРСКИ РИБИ

АВТОРЕФЕРАТ

на дисертационен труд
за присъждане на научна степен
"доктор на химическите науки"

по Биоорганична химия, химия на природните и физиологично активни вещества

Варна, 2012

ВЪВЕДЕНИЕ

Развитието на науката и технологиите в началото на миналия век доведе до производството на широка гама индустриални химикали, като пестициди, торове, лекарства, препарати за промишлено и битово потребление и др. Положителният ефект от използването им в световен мащаб е голям, както за индустрията и селското стопанство, така и за здравето на хората.

След тяхната масовата употреба, много учени проведоха широкомащабни епидемиологични и токсикологични изследвания, които категорично доказаха, че много от тези химикали и техни странични продукти, притежават висока токсичност, опасни са за околната среда и човека. Класически пример в това отношение са хлорорганичните пестициди, използвани масово в селското стопанство през 60-те години на миналия век, които се оказаха силно токсични, с голяма устойчивост и способност да се натрупват в живите организми. Установи се, че те променят биологичната стойност на хранителните продукти и предизвикват неблагоприятни последици за човешкото здраве и околната среда. Това доведе до въвеждане на забрана за производството и употребата им.

Аналогична е ситуацията и с полихлорираните бифенили (ПХБ), произведени в големи количества през периода 1950 – 1970г., широко използвани в различни области на индустрията, в последствие също забранени за производство и употреба. Въпреки забраната, те продължават да попадат в околната среда, като източници са отработени масла, стари електрически съоръжения и домакински електроуреди, при горенето на отпадъци, където се образуват още по-токсичните и опасни диоксини и фурани.

Проблемът със замърсяването на околната среда с посочените и подобни на тях органични съединения, известни като устойчиви органични замърсители (УОЗ) е глобален и решения трябва да бъдат взети в световен мащаб. Това доведе до приемане на Стокхолмската конвенция през 2001г., която беше създадена с цел опазване здравето на хората и околната среда от въздействието на УОЗ. България подписва Конвенцията през 2005г., Национален орган по изпълнението на задълженията по нея е Министерството на околната среда и водите.

Според Конвенцията, **устойчивите органични замърсители** притежават токсични свойства, трудно се разграждат, натрупват се в организмите и хранителната верига, пренасят се по въздуха, водата и чрез мигриращите биологични видове през международните граници могат да се отлагат далече от мястото на тяхното изпускане, акумулират се в екосистемите. Първоначално в конвенцията са включени 12 замърсители, това са хлорорганични пестициди, полихлорирани бифенили, диоксини и фурани, а в

последствие още 10. Общото за всички УОЗ е това, че трайно замърсяват околната среда, по хранителната верига могат да попаднат в човешкия организъм, където се акумулират и да предизвикат тежки разстройства на имунната, репродуктивната нервната системи и др. Това е причината и ООН чрез Програмата за околна среда (UNEP) да предприеме интензивни действия за предотвратяване на тази заплаха.

Въпреки, че експозицията на човека с УОЗ и тежки метали може да се осъществи по различен начин, храната е основния източник за попадането им в

организма на човека. Това са предимно храни от животински произход, богати на мазнини, където тези замърсители се натрупват.

Редица учени определят рибите като един от най-подходящите биоиндикатори за оценка на замърсяването на околната среда, не само поради факта, че водните басейни акумулират в значителни количества УОЗ и тежки метали, но и поради бавното им разграждане в тъканите на рибите. Също така, определянето на УОЗ и токсични метали в рибите е много важно за оценката на експозицията на населението и на здравния риск в даден район. Установено е, че основно чрез консумацията на риба, рибни продукти и други морски организми, тези замърсители попадат в човешкия организъм. Затова морската храна и най-вече рибите като храна, са с висок риск, поради високото си липидно съдържание, способността да натрупват тези замърсители и голямата консумация.

Морската храна, особено рибата, е важен източник на хранителни вещества, които са в основата на балансираното хранене. Тя съдържа висококачествени протеини и липиди, които притежават висока биологична стойност, а също така витамини и важни биогенни елементи. Рибните липиди се характеризират с по-ниски нива на наситени мастни киселини и по-високи на ненаситени. Те са основен източник на изключително важните за организма дълговерижни полиненаситени мастни киселини, между които са омега-3 и омега-6 киселините. Тези незаменими за човешкия организъм киселини се доставят с храната, като основен източник са рибите и други морски храни. Те участват в изграждането на клетъчните мембрани, в синтеза на важните ейкозаноиди, които са свързани с регулирането на обмяната на веществата в клетките, кръвното налягане, както и стимулиране на имунния отговор. Доказано е, че омега киселините имат защитен ефект в превенцията на коронарната болест на сърцето и други сърдечносъдови заболявания, подпомагат дейността на мозъка, оказват влияние върху зрението, остеопорозата, различни видове рак, депресии и пр.

И така, възниква въпроса за ползата и риска от използването на рибата като храна. Безспорно в най-голяма степен ползата е свързана с протеините и незаменимите полиненаситени мастни киселини, докато рискът – с различните замърсители, които рибите акумулират от околната среда, като в най-голяма степен това са УОЗ и тежките метали. От

гледна точка на здравето, рискът трябва да има приоритет и да бъде оценен. Тъй като, нашите черноморски риби не са изследвани за съдържание на УОЗ и тежки метали и не е правена оценка на риска, е разработена дисертацията.

ОСНОВНА ЦЕЛ

Определяне съдържанието на устойчиви органични замърсители – полихлорирани бифенили, ДДТ и тежки метали в черноморски риби, във връзка с тяхната безопасност като храна и за оценка на замърсяването на Черно море.

Ще бъдат представени изследвания и резултати в следните три направления:

- **Определяне съдържанието на ПХБ, ДДТ и метаболити в различни видове черноморски риби**

- **Определяне съдържанието на тежки метали в черноморски риби и някои мекотели**

- **Оценка на безопасността и качеството на черноморските риби като храна**

Във всяко едно от посочените направления са разработени конкретни задачи.

Conference Article Template

PERSISTENT ORGANIC POLLUTANTS - PCBs AND DDTs IN FISH FROM DANUBE RIVER AND FROM BLACK SEA, BULGARIA

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Abstract: Persistent organochlorine pollutants (POPs) like polychlorinated biphenyls (PCBs) and DDT residues (DDTs) can still be a problem for the aquatic environment and the human health. PCBs and DDTs were determined in three freshwater fish species: common carp *(Cyprinus carpio),* catfish *(Silurus glanis),* pike-perch *(Sander lucioperca)* and two marine fish: shad *(Alosa pontica pontica)* and grey mullet *(Mugil cephalus).* The freshwater fish samples were collected from the Danube River and from Black Sea, Bulgaria in 2010. The POPs were analyzed in order to investigate the presence of PCBs and DDTs in fish species from Danube River and compared the results to the levels in marine fish species from Black Sea. The fifteen congeners of PCBs, p,p'-DDT and its two main metabolites p,p'-DDE and p,p'-DDD were determined by capillary gas chromatography system with mass spectrometry detection. DDTs were the predominant contaminants in investigated species, with the p,p'- DDE contributing to more than 67% to the total DDTs. In freshwater fish concentrations of DDTs were found from 19.2 to 30.3 ng/g ww and PCBs concentrations - from 6.2 to 12.6 ng/g ww. The highest levels of PCBs and DDTs were determined in shad. The levels of DDTs and PCBs were determined lower than those found in similar fish species from other aquatic ecosystems.

UDC Number: 543

Keywords: PCB, DDT; fish; Danube River, Black Sea; Bulgaria

Introduction

Polychlorinated biphenyls (PCB) and organochlorine pesticides, such as dichlorodiphenyltrichloroethane (DDT) and its metabolites DDE and DDD, are compounds classified as persistent organic pollutants (POPs), capable of remaining in the environment and able to be transported, to accumulate in animal tissues over long periods of time (UNEP 2001). When released into the environment, POPs may be transported by air or water to areas that are often rather distant from the place of origin (Gouina et al., 2005). Fish is a suitable indicator for the environmental pollution monitoring because they concentrate pollutants in their tissues directly from water, but also through their diet, thus enabling the assessment of transfer of pollutants through the trophic web (Fisk et al., 2001). Data on the presence and distribution of organohalogenated contaminants in fish species are therefore important not only from ecological, but also human health perspective (Binelli and Provini, 2004; Smith and Gangoli, 2002). In biological systems, several of these chemicals are potentially carcinogenic and may cause alternations in endocrine, reproductive and nervous systems (Langer et al., 2003). The river Danube, the second longest river in Europe, flows through several countries from where it receives discharges of agricultural, industrial, and urban effluents (Woitke et al., 2003). The Black Sea receives freshwater inputs from some of the largest rivers in Europe: the Danube, Dniester and Dnieper. There is very little information available on the highly persistent PCBs and DDTs in fish from Danube River (Covaci et al., 2006) and from the Black Sea (Tanabe et al., 1997; Stoichev et al., 2007).

Environmental Monitoring and Assessment

Polychlorinated biphenyls and organochlorine pesticides in fish from Black Sea, Bulgaria

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Abstract

Persistent organochlorine pollutants (POPs) like polychlorinated biphenyls (PCBs) and DDT residues (DDTs) were determined in muscle tissue of four marine fish species: grey mullet (*Mugil cephalus*), bluefish *(Pomatomus saltatrix),* horse mackerel *(Trachurus Mediterraneus ponticus)* and bonito *(Sarda sarda).* The POPs were analysed in order to evaluate the status of pollution in Bulgarian Black Sea coastal area. The fifteen congeners of PCBs, p,p'-DDT and its metabolites p,p'-DDE and p,p'-DDD were determined by capillary gas chromatography system with mass spectrometry detection. ∑PCBs were found at concentrations ranging between 15.34 ng/g ww in horse mackerel and 23.88 ng/g ww in bluefish. TEQs of the 6 "dioxin–like" PCBs were found in the range from 0.07 to 0.15 pgTEQ/g ww and did not exceed the European limit of 6.5 pg TEQ/g ww. ∑DDTs were found from 51.61 to 171.36 ng/g ww in horse mackerel and bluefish, respectively. The levels of PCBs and DDTs were determined comparable to those found in fish species from other aquatic ecosystems. The sum of 6 indicator PCBs in all fish species did not exceed the European maximum limit.

Keywords: PCBs; DDTs; TEQs; marine fish; Black Sea; Bulgaria

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Organochlorine pesticides and PCBs in marine fish

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Abstract Organochlorine pesticides (such as 1,1,1-trichloro - 2,2 - bis (4-chlorophenyl) ethane (DDT) and its metabolites) and polychlorinated biphenyls (PCBs) are classified as Persistent Organic Pollutants (POPs) and are present in the contamination pattern of marine environments world-wide. Concentrations of PCBs and DDTs were measured in two marine species: garfish (*Belone belone*) and red mullet (*Mullus barbatus*). Samples were collected from Black Sea, Bulgaria during 2007 – 2010. The DDTs and PCBs were determined by gas chromatography - mass spectrometry.
Concentrations in muscle tissue of garfish ranged from 80.89 to 118.04 ng/g wet weight for total DDTs. DDTs concentration in red mullet was found 104.59 ng/g ww. PCB concentrations in garfish varied in the range of 40.04 and 65.62 ng/g ww. In muscle tissue of red mullet PCB concentrations were found 34.12 ng/g ww. The levels of DDTs and PCBs in garfish and red mullet from the Black Sea were comparable with those found in other marine ecosystem.

Keywords: fish, DDT, PCB, Black Sea, Bulgaria

1. Introduction

PCBs and selected organochlorine pesticides are a group of chemicals that have attracted considerable attention due to their high toxicity, persistence in the environment, and ability to bioaccumulate. The combination of these properties means that organisms at the upper levels of the food chain can potentially be exposed to concentrations sufficient to cause adverse effects. [1,2] In biological systems, several of these chemicals are potentially carcinogenic and may cause alternations in endocrine, reproductive and nervous systems [3]. For these reasons, most countries have restricted or banned the use of PCBs and DDTs since 1970s. DDT (1,1,1-trichloro - 2,2 - bis (4-chlorophenyl) ethane) is a chlorinated pesticide widely used in the past to control the spread of insects and other agricultural peststicides. In the environment DDT metabolised slowly and the metabolite DDE is particularly persistent compound. Polychlorinated biphenyls have been widely used by a large variety of industries over the past 50 years. However, several studies have demonstrated that they are toxic to a variety of marine organisms [4].
Fish is a suitable indicator for the environmental pollution monitoring because they concentrate pollutants in their tissues directly from water, but also through their diet, thus enabling the assessment of transfer of pollutants through the trophic web [1,5]. Data on the presence and distribution of organohalogenated contaminants in fish and especially edible fish species are therefore important not only from ecological, but also human health perspective [6,7].

Red mullet are non-migratory species and feed mainly with benthic invertebrates (crustaceans, worms, and molluscs). The garfish *(Belone belone)* is a pelagic, oceanodromous needlefish found in marine waters of the Mediterranean Sea, the Baltic Sea, etc. The fish lives close to the surface, has a migratory pattern and feed mainly with small fish.

The purpose of this study was to determine the levels of persistent organochlorine contaminants in garfish and red mullet from the Bulgarian Black Sea coast and to monitor the accumulation of these pollutants during the period 2007 – 2010.

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Organochlorine pesticides and PCBs in marine fish

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Abstract Organochlorine pesticides (such as 1,1,1-trichloro - 2,2 - bis (4-chlorophenyl) ethane (DDT) and its metabolites) and polychlorinated biphenyls (PCBs) are classified as Persistent Organic Pollutants (POPs) and are present in the contamination pattern of marine environments world-wide. Concentrations of PCBs and DDTs were measured in two marine species: garfish (*Belone belone*) and red mullet (*Mullus barbatus*). Samples were collected from Black Sea, Bulgaria during 2007 – 2010. The DDTs and PCBs were determined by gas chromatography - mass spectrometry.
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Keywords: fish, DDT, PCB, Black Sea, Bulgaria

1. Introduction

PCBs and selected organochlorine pesticides are a group of chemicals that have attracted considerable attention due to their high toxicity, persistence in the environment, and ability to bioaccumulate. The combination of these properties means that organisms at the upper levels of the food chain can potentially be exposed to concentrations sufficient to cause adverse effects. [1,2] In biological systems, several of these chemicals are potentially carcinogenic and may cause alternations in endocrine, reproductive and nervous systems [3]. For these reasons, most countries have restricted or banned the use of PCBs and DDTs since 1970s. DDT (1,1,1-trichloro - 2,2 - bis (4-chlorophenyl) ethane) is a chlorinated pesticide widely used in the past to control the spread of insects and other agricultural peststicides. In the environment DDT metabolised slowly and the metabolite DDE is particularly persistent compound. Polychlorinated biphenyls have been widely used by a large variety of industries over the past 50 years. However, several studies have demonstrated that they are toxic to a variety of marine organisms [4].
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DDT in fish from the Bulgarian region of the Black Sea

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In spite of a worldwide reduction in the utilization of organochlorine pesticides (OCPs), they are still a problem for the aquatic environment and human health. The Black Sea is still being polluted with persistent chemicals, including OCPs. Aquatic organisms (sprat, scad, bluefish, shad, belted bonito, goby, and black mussel) with different feeding behaviours were sampled on a seasonal basis from the Bulgarian region of the Black Sea, and the concentrations of 13 OCP residues were determined. Although many of the OCPs were not detected in the samples, in all samples 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT) was present mainly in the form of its metabolites 1,1-dichloro-2,2-bis(4-chlorophenyl) ethane (DDD) and 1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene (DDE). Only about 12% of the total DDT was present as the parent compound pp-DDT, which suggests that it was not being used recently in the region. The total DDT concentrations were generally below 150 $\mu g\,kg^{-1}$ fresh weight, but higher levels—up to 354 $\mu g\,kg^{-1}$ fresh weight—were also measured for fish species with a high fat content. Between-species differences were observed, even when the concentrations were presented on a fat-level basis. DDT concentrations did not show any significant changes over the 2-yr sampling period. Fish sampled in the northern areas of the Bulgarian Black Sea coast seemed to contain higher DDT levels than those from the southern areas, suggesting a major (historical) influence of the Danube River. For permanent monitoring purposes, the utility of Black Sea gobies and scad should be considered.

Keywords: Organochlorine pesticides; DDT; Fish; Black Sea

1. Introduction

Organochlorine pesticides (OCPs) have been widely used in the past, but due to their toxicity, stability, and bioaccumulation, especially in the aquatic food web, they can still be a current problem for human health through fish consumption. Many of these compounds, like 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT), have endocrine-disrupting activities and are possible carcinogens for humans [1–3].

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Content determination of PCBs in Black Sea fish

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Abstract Polychlorinated biphenyls (PCBs) are serious environmental pollutants and poisons for the living organisms. They are dissolved and accumulated in the adipose tissue in animals and humans. PCBs are found in dairy products, fish and meat. Sea fish and mammals accumulate PCBs in quantities that exceed many times those in water. Concentrations of PCBs have been determined of fish samples in different part of Bulgarian Black Sea coast in Varna region located in northeast Bulgaria. The PCBs content was determined in clean fish extracts by means of gas chromatography. The experimental results indicate presence of PCBs in all the investigated samples. All samples analyzed presented concentrations below the maximum allowed by the European Community regarding PCBs. The highest levels of PCBs (139.7 – 187.9 ng/g lw) were observed in European sprat. The concentrations of PCBs in all the collected samples were lower than the levels recommended by the European Union – 200 ng/g lw.

Keywords: PCBs, Black Sea, fish, Gas-chromatography

1. Introduction

Polychlorinated biphenyls (PCBs) are man-made organochloride chemicals that are structurally similar to the pesticide DDT. Their unique electrical insulating properties make them useful in the manufacture of transformers and other electrical components. They have also been used in the production of plastic food containers, epoxy resins, caulking compounds, and various types of wall and upholstery coverings and as ingredients in soap, cosmetic creams, paint, glue, waxes, brake linings, and many other products. In the late 1960s, scientists began to realize that PCBs were also serious environmental poisons [1]. PCBs entered the air, water, and soil during their manufacture, use, and disposal; from accidental spills and leaks during their transport; and from leaks or fires in products containing PCBs. They can still be released to the environment from hazardous waste sites. PCBs do not readily break down in the environment and thus may remain there for very long periods of time. PCBs can travel long distances in the air and be deposited in areas far away from where they were released. In water, a small amount of PCBs may remain dissolved, but most stick to organic particles and bottom sediments. PCBs also bind strongly to soil. PCBs accumulate in fish and marine mammals, reaching levels that may be many thousands of times higher than in water [2].

The main source of human exposure to these compounds is through the consumption of fatty foods such as meat, fish, milk and milk products. PCBs enter the human food chain mainly through the intake of animal fats [3]. Laboratory studies have shown that PCBs interfere with reproduction in rodents, fish, and many species of birds and monkeys. PCBs are soluble in the fat of animals and stored in living tissue [1].

They are suspected of being carcinogenic, but conclusive evidence is lacking.

The aim of our study was is determined of the total PCBs content in some of the most popular consumed fish in our country. The study was

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DETERMINATION OF HEAVY METALS (Pb, Cd, As AND Hg) IN BLACK SEA GREY MULLET (*MUGIL CEPHALUS*)

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Abstract

STANCHEVA, M., L. MAKEDONSKI and E. PETROVA, 2013. Determination of heavy metals (Pb, Cd, As and Hg) in Black Sea grey mullet (*Mugil cephalus*). *Bulg. J. Agric. Sci.*, Supplement 1: 30–34

Gray mullet (*Mugil cephalus*) is commercially important marine school pelagic fast moving fish species especially during warm weather. The M. cephalus is an omnivore and usually inshore, entering estuaries and lagoons, like Varna Lake. The aim of the present study was to determine and compare heavy metal contents (Pb, Cd, As and Hg) in edible tissue and gills of grey mullet (*Mugul cephalus*). The fish samples were collected from two different Black sea areas – Varna Lake and Nesebar. The sample preparation was performed by acid microwave digestion with „Multiwave" system in five stages program. Determination of As, Cd, and Pb were carried out on a Perkin Elmer Zeeman 3030 spectrometer with an HGA-600 atomizer, whereas Hg was analyzed by Milestone Direct Mercury Analyzer. Detected levels of As in the studied regions gives exceed those of other analyzed elements. The samples from both regions showed the higher levels of As in edible tissue than gills, especially from Region of Nesebar (1.1 mg/kg w.w.). The results for other heavy metals are several times lower than arsenic and were found in range 0.01–0.12 mg/kg w.w. All studied elements (except As) presented higher amounts from Varna Lake grey mullet compared with Neserbar region samples.

Key words: Heavy metals, Gray mullet, Black Sea

Introduction

Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to industrial, agricultural and mining activities. As a result, aquatic animals are exposed to elevated levels of heavy metals .

The levels of metals in upper members of the food web like fish can reach values many times higher than those found in aquatic environment or in sediments. Thus contamination in the region is an important issue regarding the health of the aquatic animals and in turn, health of the seafood consumers. Gray mullet (*Mugil cephalus*) is commercially important marine school pelagic fast moving fish species especially during warm weather. The *M. cephalus* is an omnivore and usually inshore, entering estuaries and lagoons, like Varna Lake. The habitat of *M. cephalus*, which is an omnivore, is pelagic, usually inshore, entering estuaries and lagoons. While juveniles feed on invertebrates, adults mostly on detritus, bottom algae and small organisms, occasionally on plankton (Yilmaz, 2005).

This study was undertaken to investigate the current heavy metal contamination in edible tissue and gills in gray mullet (*M. cephalus*) from two stations differing in locations – Varna Lake and Nessebar.

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HEAVY METALS AND PROXIMATE COMPOSITION OF BLACK SEA SPRAT (*SPRATTUS SPRATTUS*) AND GOBY (*NEOGOBIUS MELANOSTOMUS*)

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Abstract

STANCHEVA, M., A. MERDZHANOVA, E. PETROVA and D. PETROVA, 2013. Heavy metals and proximate composition of Black Sea sprat (*Sprattus sprattus*) and goby (*Neogobius melanostomus*). *Bulg. J. Agric. Sci.*, Supplement 1: 35–41

The aim of the present study were to determine and compare the heavy metals content (Pb, Cd, Hg and As) and proximate composition in edible part of two commercially important fish species from Bulgarian Black Sea – sprat (*Sprattus sprattus*) and goby (*Neogobius melanostomus*). Determination of As, Cd, and Pb were carried out on a Perkin Elmer Zeeman 3030 spectrometer with an HGA-600 atomizer, whereas Hg was analyzed by Milestone Direct Mercury Analyzer. The levels of Cd and Pb were relatively low in both analyzed species while those for As concentration show higher value for sprat. The amounts of Hg for sprat and goby are also under permitted levels for fishes for human consumption. Proximate composition of the following nutrients was determined using standard procedures of AOAC (1991): moisture, crude protein and total lipids. Crude protein in fish samples was in the range 17.15–18.10%, while fat content was from 1.60 to 4.30 g.100 g^{-1} w.w. Energy values have been calculated using FAO/WHO specific factors and were in interval 373–437 kJ.100 g^{-1} w.w. Results showed that observed heavy metal contents have lower concentration of mean values than the permissible limits set by FAO/WHO in analyzed samples. It can be concluded that both species studied are safe to be consumed and have a good nutrition quality.

Key words: Heavy metals, proximate composition, Black Sea Sprat, Goby

Introduction

Heavy metals are natural trace components of the marine environment, but they constitute one of the most hazardous substances that could be accumulated in biota. According to Munoz-Olivas and Camara (2001) heavy metals are classified as: potentially toxic (e.g. aluminum, arsenic, cadmium, lead, mercury), probably essential (e.g. nickel, vanadium, cobalt) and essential (e.g. cooper, zinc, selenium). Fish populations with commercially important often live in coastal area environments that contain high levels of heavy metals, coming from industrial and agriculture wastes or human activities. The marine organisms accumulateits from water, food, sediment and some suspended particulate materials. Furthermore fish species accumulate heavy metals to concentrations many times higher than presented in water or sediments andtherefore they have been extensively used for marine pollution monitoring (Agusa et al., 2005; Bat et al., 2012).

The nutritional benefits of fish are mainly due to thecontent of high-quality protein (fish provide 17% of the total animal protein and 6% of all protein consumed byhumans), and other essential nutrients. Thequality of fish tissue is function of their body compositions and energy values, which that vary among different species. Determination of proximate composition as protein contents, carbohydrates, lipids, moisture contents

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BULGARIAN CHEMICAL COMMUNICATIONS
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Determination of heavy metal concentrations of most consumed fish species from Bulgarian Black Sea coast

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In this study some heavy metals (Cd, Ni, Cr, As, Hg Cu, Fe, Mn, Pb and Zn) concentration in edible parts of five most consumed Bulgarian fish species - bluefish (Pomatomus saltatrix), gray mullet (Mugil cephalus), Mediterranean horse mackerel (Trachurus mediterraneus ponticus), shad (Alosa pontica) and sprat (Sprattus sprattus sulinus) collected from two stations across Bulgarian Black Sea coast were determined. The samples were digested with nitric acid followed by appropriate spectroscopic determination (Atomic Emission Spectroscopy with Inductively Coupled Plasma (AES-ICP), Flame Atomic Absorption Spectroscopy (FAAS) or Electrotermal Atomic Absorption Spectroscopy (ETAAS). The level of As in the edible part of gray mullet (Mugil cephalus) has shown a value higher than limits set from various health organizations (1.1 ± 0.1 mg/kg). On the contrary this fish species accumulates the other investigated heavy metals such as Hg, Zn, Fe and Pb to lower extend. The concentration of Zn and Fe showed the highest value for all fish species. With some exceptions the concentration of studied heavy metal elements was within the acceptable levels for food source for human consumption.

Keywords: heavy metals; fish; Black Sea; Bulgaria

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Promoted V_2O_5-TiO_2 Catalyst for Selective Oxidation of *ortho*-xylene to Phthalic Anhydride. I Antimony Doping

Lubomir Makedonski[a*], Marina Moskovkina[b] and Mona Stancheva[a]

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[b]*Konstantin Preslavski University, 9712 Shumen, Bulgaria*

Abstract

A V_2O_5-Sb_2O_3-TiO_2 (anatase) based catalyst for ortho-xylene oxidation to phthalic anhydride has been synthesized. The activity and selectivity of the specimen obtained are comparable with those of industrial catalysts.

Key words: V_2O_5-TiO_2 (anatase) supported oxide catalyst, antimony oxide promoted, oxidation, *ortho*-xylene, phthalic anhydride.

1. Introduction

Vanadium and antimony oxides are important components of some industrial catalysts for selective oxidation of substituted aromatic substances to the corresponding anhydrides and of paraffins to the corresponding saturated acids and nitryls [1-4].

Mixed metal oxides have a wide application as industrial catalysts for selective oxidation of many aromatic compounds and alkenes. Yearly Sb oxides as their component are the matter of interest in catalyst industry [5-12] and this interest prolongs in the contemporary investigations [13-17].

The catalysts used for the preparation of phthalic anhydride are mostly titania-supported monolayer of vanadium oxides. Along with the main reaction product (phthalic anhydride), by-products of partial oxidation such as maleic anhydride, phthalide, *ortho*-toluyl aldehyde, benzoic acid etc are also present in the final mixture. The introduction of antimony oxide enhances the selectivity of the catalyst towards the oxidation of phthalic anhydride [16, 18-19]. Other additives such as phosphorus, tin, antimony etc are introduced with a view to controlling the activity lengthwise the catalysts layer on the basis of the temperature in the hot spot. Regulation of the temperature in the hot spot by some of these additives ensures

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Promoted Vanadium-Titanium Catalysts for Selective Oxidation of ortho-xylene to Phthalic Anhydride. II Lithium and Antimony Oxides doping

Lubomir Makedonski [a] *, Marina Moskovkina [b], Mona Stancheva[a]

[a] *Medical University, 55 Marin Drinov Str., 9002 Varna, Bulgaria*
[b] *Konstantin Preslavski University, 9712 Shumen, Bulgaria*

Abstract

A V_2O_5-TiO_2 (anatase) covered catalyst for partial oxidation of ortho-xylene to phthalic anhydride was prepared. The promoting effect of Li_2O and Sb_2O_3 doping upon the catalyst selectivity was investigated. The optimal quantity of the promoting doping agent was estimated. The catalyst quality at various space velocities was checked. The products of catalytic oxidation were analyzed by gas chromatography. The results were compared with those on unpromoted catalysts.

Key words: V_2O_5-TiO_2 (anatase) supported oxide catalyst, lithium oxide and antimony oxide promoted, oxidation, *ortho*-xylene, phthalic anhydride

1. Introduction

Mixed metal oxides are important industrial catalysts for the selective oxidation and ammoxidation of aromatics and alkenes and often contain Sb oxides as a component [1].

Vanadium and antimony oxides are essential parts of some industrial catalysts for the selective oxidation of substituted aromatics to the corresponding anhydrides and the selective oxidation of paraffins to the corresponding unsaturated acids and nitriles [2-5].

The V_2O_5-TiO_2-Sb_2O_3 system is the basis of modern catalysts for selective oxidation of *ortho*-xylene to phthalic anhydride [6-18].

The promoting effect of the oxides of lithium and antimony regulates the acidic-basic characteristics of the catalysts. The alkali metal ions stabilize the vanadium component in its highest oxidation degree as compared to unpromoted catalysts. It is established [19] that alkali metal ions partially neutralize the acid sites on the catalyst

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Preparation of a vanadium-titanium catalyst for the partial oxidation of *o*-xylene to phthalic anhydride from used industrial samples

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Spent vanadium oxide catalysts, which are not yet completely exhausted can be utilized as regenerated catalysts. The present paper shows that the acid-extracted V_2O_5 from partially deactivated vanadium oxide catalysts for *o*-xylene oxidation is suitable for the synthesis of fresh catalysis samples. The catalytic activity and selectivity have been tested in a laboratory flow apparatus and found to be very close to those of the industrial catalyst.

Key words: spent V_2O_5, V_2O_5-TiO_2 (anatase) catalyst, oxidation of *o*-xylene, catalyst preparation.

INTRODUCTION

The regeneration of industrial catalysts has an increasing application because of the high cost of the catalysts and thanks to serious scientific achievements in this respect. In a series of countries the catalyst mass, having lost its activity, is returned to the producer and used for the preparation of new catalysts. Large amounts of deactivated industrial V_2O_5–TiO_2 (anatase) catalysts for oxidation of *o*–xylene to phthalic anhydride are available. It has been established [1] that under the effect of high temperatures and reduction medium, titania is transformed from anatase to rutile, as a result of which the catalyst activity sharply drops down. After having been used for 2 years under industrial conditions, the catalyst should be replaced by a fresh one. The vanadium component of the contact mass can be regenerated and used for a new catalyst [2].

The purpose of the present investigation was to synthesize a vanadium-titanium oxide catalyst, the vanadium component being extracted as vanadium oxalate from used industrial catalysts for o-xylene oxidation to phthalic anhydride. According to literature data [3, 4], the best catalytic activity and selectivity are displayed the catalyst having the composition 7% V_2O_5–93% TiO_2 (anatase). For such reason, we prepared a catalyst mass with this composition. The selectivity of the catalyst obtained was compared with that of an industrial F 04 25 (BASF) sample.

EXPERIMENTAL

Synthesis method of the catalyst

Supported V_2O_5–TiO_2 (anatase) catalyst samples were preparated form vanadyl oxalate extracted from used industrial catalyst (see Table 1). The method of extraction was described elsewhere [5].

Table 1. Chemical composition of extracted V_2O_5.

Components	Content, wt %
V_2O_5	98.20
SO_4^{2-}	1.20
Ti	0.30
K	0.15
Mg	0.10
PO_4^-	0.05

The TiO_2 (anatase; Merck-AG) had a specific surface area of 9 $m^2.g^{-1}$ and its chemical composition is given in Table 2. The anatase and a surfactant (formamide) were added to an aqueous solution of vanadyl oxalate. The obtained suspension was subjected to ultrasonic treatment in order to achieve homogenization and dispersion of the catalyst mass, which was then applied by pulverization at 200–250°C on an inert (also regenerated) support of porcelain spheres. The catalyst samples thus obtained were dried at 100° C for 1 h and calcined for 2 h at 500°C.

Table 2. Chemical composition of titania.

Components	Content, wt. %
Sulphate (SO_4^{2-})	0.0500
Heavy metals (Pb)	0.0030
Fe	0.0050
As	0.0005

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UTILIZATION OF VANADIUM FROM USED INDUSTRIAL CATALYST FOR THE OXIDATION OF SULPHUR DIOXIDE

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Abstract

Used vanadium oxide catalysts are still not completely exhausted and be utilized as regenerated catalysts. The present paper reports data on the extraction of vanadium and other useful components from used vanadium oxide catalysts for the oxidation of sulphur dioxide under industrial conditions of sulphuric acid production.

The effect of various factors affecting the extraction (extragent concentration, temperature, duration, stirring intensity, solid/liquid phase ratio and grain size) has been investigation and the optimum conditions of acid ($H_2C_2O_4$) extraction of V_2O_5 and other components established.

Introduction

The limited number of investigations on the regeneration of these catalysts seems to be due to their low cost, the irreversible changes occurring in the support and relatively low vanadium content. Hence after finding an optimum method for the extraction of vanadium, the used catalyst may be considered as a possible source of vanadium products.

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UTILIZATION OF VANADIUM FROM USED INDUSTRIAL V_2O_5-TIO_2 CATALYST FOR THE PARTIAL OXIDATION OF O-XYLENE IN AN ULTRASONIC FIELD

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Abstract
Used vanadium oxide catalysts are still not completely exhausted and be utilized as regenerated catalysts. The present paper reports data on the extraction of vanadium from used industrial vanadium oxide catalysts for partial oxidation of o-xylene .The effect of various factors affecting the extraction (extragent concentration, temperature, duration, solid/liquid phase ratio, ultrasonic field) has been investigated and the optimum conditions of $H_2C_2O_4$ extraction of V_2O_5.A vanadium extraction degree of 82% has been achieved an extraction with $H_2C_2O_4$ in the presence of ultrasonic treatment.

Introduction
Some waste products from chemical industry such as used vanadium oxide catalysts for oxidation of o-xylene to phthalic anhydride are an important source of vanadium as raw material. The isolation of these products and their storage may be accompanied by vanadium coming in contact with the atmosphere and the hydrosphere, thus giving rise to certain ecological problems due to the toxicity of vanadium. Used industrial catalysts for o-xylene oxidation to phthalic anhydride are still regenerated and utilized.

Finding an optimum method for extraction of the active V_2O_5 component would facilitate the utilization of used catalysts as a potential source of fresh vanadium oxide catalysts and a lot of vanadium products.

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Prog. Catal, Vol. 11, No. 1-2, pp. 39 – 45 (2002)

Extraction of the Vanadium Component from Used Industrial V_2O_5-TiO_2(anatase) Catalysts for Partial Oxidation of *ortho*-Xylene in an Ultrasonic Field

Lubomir Makedonski[1*], Mona Stancheva[1], Nikolay Nikolov[2] and Marina Moskovkina[2]

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Abstract

Spent vanadium oxide catalysts are still not completely exhausted and can be utilized as regenerated catalysts. The present paper reports data on the extraction of vanadium from used industrial vanadium oxide catalysts for partial oxidation of ortho-xylene .The effect of various factors affecting the extraction (extracting agent concentration, temperature, time of extraction, solid/liquid phase ratio, ultrasonic field) and the optimum conditions for V_2O_5 extraction by $H_2C_2O_4$ has been investigated. A vanadium extraction degree of 82% using $H_2C_2O_4$ as extracting agent in the presence of ultrasonic treatment has been achieved.

Key words: industrial V_2O_5-TiO_2 catalyst, extraction of the vanadium component, spent V_2O_5

1.Introduction

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Spent industrial catalysts for *ortho*-xylene oxidation to phthalic anhydride are still regenerated and utilized. In a series of countries the catalyst having lost its activity and selectivity is used for preparing fresh catalysts. Data on the regeneration of spent catalysts for *ortho*-xylene oxidation are scarce. The problem has not been studied

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Учебно-методична публикация

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Review article

Lubomir Makedonski*, Zlatina Peteva, Katya Peycheva, Mona Stancheva

Laboratory manual in teaching medical chemistry in English language course for the student of medicine and dental medicine

ABSTRACT

Chemistry is an experimental science. It is not an inanimate science, but one that help us to understand the behavior of living systems.
This manual was designed especially to follow the lecture portion of the student class.
The laboratory activities include materials that students may be familiar with, such as household products, drinks, and various medicines. In such way, chemical topics are related to the real world and to the student's own science experience. Some of the lab exercises teach basic skills while others encourage students to extend scientific curiosity beyond the lab.
The laboratory class gives an opportunity to go beyond the lectures and words in the textbook and experience the scientific processes from which conclusions and theories concerning chemical behavior are drawn. The concepts of some experiments have health and biological aspects.

Keywords: chemistry, manual, students of medicine, English program, laboratory work

Introduction

Varna Medical University was established in 1961 when the first students began their academic studies in medicine. The structure of Varna Medical University includes Faculty of Medicine, Faculty of Dental Medicine, Faculty of Pharmacy, Faculty of Public Health Protection and Medical College. The academic and research activities carried out at Varna Medical University are provided by modern facilities

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CHALLENGES IN FOOD CHEMISTRY, May 31 – June 1, 2013, Constanta, Romania

P2. ALPHA-TOCOPHEROL, RETINOL AND ERGOCALCIFEROL CONTENTS OF SOME MACROALGAE FROM BULGARIAN BLACK SEA COAST

Veselina Panayotova, Mona Stancheva, Diana Dobreva

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The aim of the present study was to determine and compare the content of alpha-tocopherol, retinol and ergocalciferol in four macroalgae from Bulgarian Black sea coast. *Ulva rigida, Cladophora vagabunda, Cystoseira barbata* and *Cystoseira crinita* were used for evaluation of fat soluble vitamins content.

Alpha-tocopherol (vitamin E), all-trans-retinol (vitamin A) and ergocalciferol (vitamin D_2) were analyzed simultaneously using HPLC/UV/FL system (Thermo Scientific Spectra SYSTEM) equipped with RP analytical column ODS2 Hypersil™ (250 x 4,6 mm, 5um). The mobile phase was composed of 97:3 = MeOH:H_2O. Retinol and ergocalciferol were monitored by UV detection at λ_{max}= 325nm and λ_{max} = 265nm, respectively. Alpha-tocopherol was detected by fluorescence at λ_{ex}=288nm and λ_{em}=332nm. The sample preparation procedure includes alkaline saponification, followed by liquid-liquid extraction.

Alpha-tocopherol content in fresh algae tissues ranged from 188.6±26.2µg/100g in *Cladophora vagabunda* to 6076.9±189.8µg/100g in *Cystoseira barbata*. The high levels of alpha-tocopherol in brown algae may be partially explained with the high PUFA content found in previous studies [1]. As an antioxidant alpha-tocopherol preserves tissue PUFA from oxidation [2]. Retinol content, presented as total Retinol Equivalent (RE) was found at low amounts in analyzed seaweeds while ergocalciferol was detected only in *Ulva rigida* samples.

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FATTY ACID COMPOSITION AND FAT SOLUBLE VITAMINS CONTENT OF BULGARIAN BLACK SEA FISH SPECIES

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Many studies suggest that marine fishes are one of the most important dietary sources of retinol, cholecalciferol and alpha-tocopherol and also essential fatty acids (FA). They are substantial nutrients controlling a diversity of important processes in human body. The contents of these biologically active compounds in fish tissue are notably depending on the species. There is limited information in the scientific literature about fat soluble vitamins and fatty acid composition of Bulgarian Black sea fishes. The aims of the present study were determined and compared the fatty acid profile and retinol, cholecalciferol and alpha-tocopherol contents in some of most commonly eaten marine fish species in Bulgaria – Sprat, Horse mackerel, Goby, Shad, Grey mullet, Bonito and Turbot.

Total lipids were extracted according to Bligh and Dyer method. Analysis of fatty acid methyl esters were performed according to method EN ISO 5509:2000 using GC/MS. Fat soluble vitamins were analyzed simultaneously using HPLC UV/FL. Retinol and cholecalciferol was monitored by UV detection. Alpha-tocopherol was detected by fluorescence detection.

The sum of monounsaturated FA (MUFA) was higher (30.0%) in Shad, while polyunsaturated FA (PUFA) showed the higher level in Turbot (36.8%). Palmitic acid (C16:0) was the most abundant SFA in all species. The dominant MUFA were C18:1 (n-9) and C16:1 (n-7). The Black sea fish contained high amounts of omega 3 PUFA presented by eicosapentaenoic acid (C20:5, EPA) and docosahexaenioc acid (C22:6, DHA). The EPA and DHA levels were higher in Turbot, Bonito and Horse mackerel. The omega-3/omega-6 ratios were similarly for all analyzed fish species - about 1.45. PUFA/SFA ratios were greater than recommended by WHO.

The fat soluble vitamins content in the fresh edible fish tissue of analyzed species are in the ranges: for retinol from 37.5±3.5μg/100g to 4.3±0.1μg/100g; cholecalciferol – 46.5±4.6 μg/100g – 2.5±0.2 μg/100g and alpha-tocopherol – 3750.5±155.6μg/100g - 21.4±0.6 μg/100g.

All presented Black Sea fishes are good dietary sources of omega-3 and omega-6 PUFAs. The most of analyzed fishes are a better source for vitamin D3 – especially Horse mackerel and Shad. One survey of its fillet provides many times higher amounts compared with established Relative Daily Intake in our country. Regarding to the omega-3/omega-6 and PUFA/SFA ratios and high levels of all analyzed fat soluble vitamins we may conclude that these Black Sea fish species have good nutritional quality.

Keywords: Black sea, fat soluble vitamins, fatty acids, fish

DDT AND ITS METABOLITES IN FISH FROM BLACK SEA, BULGARIA

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The purpose of this study was to determine the levels of p,p'-dichloro diphenyl trichloroethane (DDT) and its metabolites p,p'-dichloro diphenydichloroethylene (DDE) and p,p'-dichloro diphenyldichloroethane (DDD) in muscle tissue of seven marine fish species: goby (*Neogobius cephalargoides*), sprat (*Sprattus sprattus sulinus*), horse mackerel (*Trachurus Mediterraneus ponticus*), grey mullet (*Mugil cephalus*), bonito (*Sarda sarda*), turbot (*Psetta maxima*) and garfish (*Belone belone*). The fish species was selected because of their characteristic feeding behavior and importance to human consumption in Bulgaria. Samples were collected from different parts of Bulgarian Black Sea coast during 2007 – 2011.DDT and its metabolites (DDTs) were analyzed in order to evaluate the status of pollution in Bulgarian Black Sea coastal area.

The DDTs were determined by capillary gas chromatography system with mass spectrometry detection. DDTs were found in all fish species at concentrations ranging between 17.3 ng/g ww in turbot and 79.1 ng/g ww in garfish. In all samples DDT was present mainly in the form of its metabolites p,p- DDE and p,p- DDD, suggesting previous contamination. Statistical analyses were prepared as DDTs concentrations were log10-transformed to approximate a normal distribution of the data. All statistical tests were performed using SPSS 16 software.

The statistical analysis indicated that the differences among mean annual concentrations of ∑DDTs were not statistically significant ($p>0.05$). The experimental results for ∑DDTs showed significant differences between fish species. The levels of DDTs in fish from Bulgarian Black Sea coast were comparable to those found in fish species from the Black Sea and from other marine ecosystem.

Keywords: DDTs, fish, Black Sea, Bulgaria

Polychlorinated Biphenyls in Fish from Black Sea, Bulgaria

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Polychlorinated biphenyls (PCBs) can still be a problem for the aquatic environment and human health. Pollution by PCBs has spread all over the world as evidenced by their detection both in human and wildlife. These lipophilic contaminants are very persistent, widely distributed in the environment and can be accumulate in aquatic organisms. In marine organisms, chlorinated compounds uptake occurs directly from sea and through the food chain.

PCBs were determined in muscle tissue of ten marine fish species: goby, sprat, horse mackerel, shad, grey mullet, bluefish, bonito, turbot, red mullet and garfish. Samples were collected from different parts of Bulgarian Black Sea coast during 2007 – 2011. The PCBs were analysed in order to evaluate the status of pollution in Bulgarian Black Sea coastal area.

The PCBs were determined by capillary gas chromatography system with MS detection. The quality control was performed by regular analyses of certified reference material BB350 (PCBs in Fish oil). Determination of PCBs was made by fish species and by year. PCBs were found in all fish species at concentrations ranging between 7.3 ng/g ww in turbot and 32.8 ng/g ww in shad (calculated as the sum of 15 PCB congeners). Our investigation shown that, the sum of seven indicators PCBs was more at 80% of total PCBs content.

TEQs of the 6 "dioxin–like" PCB congeners were calculated from 0.11 pg TEQ/g ww (in goby) to 1.02 pg TEQ/g ww (in shad) and do not exceed the limit of 8.0 pg TEQ/g ww, according to European Commission. The levels of PCBs in fish from Bulgarian Black Sea coast were comparable to those found in fish species from other marine ecosystems.

Keywords: PCB, fish, Black Sea, Bulgaria

Persistent organic pollutants in fish from Black Sea, Bulgaria

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Persistent organochlorine pollutants (POPs) like polychlorinated biphenyls (PCBs) and DDT and its metabolite (DDTs) can still be a problem for the aquatic environment and human health. Pollution by POPs has spread all over the world as evidenced by their detection both in human and wildlife. Polychlorinated biphenyls and organochlorine insecticides are generally found in environment as complex mixtures, their presence often reflects local anthropogenic impacts. These lipophilic contaminants are very persistent, widely distributed in the environment and can be accumulate in aquatic organisms. In marine organisms, chlorinated compounds uptake occurs directly from sea and through the food chain.

PCBs and DDTs were determined in muscle tissue of ten marine fish species: goby (*Neogobius cephalargoides),* sprat (*Sprattus sprattus sulinus*), horse mackerel *(Trachurus Mediterraneus ponticus),* shad *(Alosa pontica pontica),* grey mullet (*Mugil cephalus*), bluefish *(Pomatomus saltatrix),* bonito *(Sarda sarda),* turbot *(Psetta maxima),* red mullet *(Mullus barbatus)* and garfish *(Belone belone).* Samples were collected from different parts of Bulgarian Black Sea coast during 2007 – 2011. The POPs were analysed in order to evaluate the status and potential sources of pollution in Bulgarian Black Sea coastal area.

The fifteen congeners of PCBs, p,p'-DDT and its two main metabolites p,p'-DDE and p,p'-DDDwere determined by capillary gas chromatography system with MS detection. The quality control was performed by regular analyses of certified reference materials: BCR- 598 (DDTs in Cod liver oil) and BB350 (PCBs in Fish oil) – Institute for Reference Materials and Measurements, European commission. Statistical analyses were prepared as PCBs and DDTs concentrations were log10-transformed to approximate a normal distribution of the data. All statistical tests were performed usingSPSS 16 software. Experimental results were shown by fish species, by year and by sampling sites.

PCBs were found in all fish species at concentrations ranging between 7.3 ng/g ww in turbot and 32.8 ng/g ww in shad (calculated as the sum of 15 PCB congeners). Mean concentrations of DDTs were found from 20.0 to 173.0 ng/g ww in goby and shad, respectively. The main metabolite p,p'-DDE was the most frequently detected compound in all fish species and was present in much higher concentrations than the other DDTs.

The statistical analysis on POPs levels indicated that the differences among mean annual concentrations of ∑PCBs and ∑DDTs were not statistically significant ($p>0.05$). The experimental results for ∑PCBs and ∑DDTs in fish species from different sampling sites showed no significant differences between the Northern, Varnaand Southern coast sampling area.

Dioxin - like PCBs are used in order to estimate the toxicity potential (TEQs) of PCB exposure. TEQs of the 6 "dioxin–like" PCB congeners were calculated from 0.11 pg TEQ/g ww to 0.91 pg TEQ/g ww and don't exceed the limit of 8 pg TEQ/g ww, according to European Commission.

The levels of PCBs and DDTs in fish from Bulgarian Black Sea coast were comparable to those found in fish species from theBlack Seaand from neighboring seas – theMarmaraSea, theAegean Seaand theMediterranean Sea.


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FATTY ACID COMPOSITION OF BLACK SEA ULVA RIGIDA AND CYSTOSEIRA CRINITA

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Abstract

Green alga *Ulva rigida* and brown alga *Cystoseira crinita* are widespread in the Black Sea. There are limited information about lipid content and fatty acid composition of this species form Bulgarian Black Sea coast. The aim of this study was to determine and compare total lipid and fatty acid (FA) composition of these species. Lipids were extracted by following the method of Bligh and Dyer. Fatty acid composition was analyzed by Gas Chromatography with MS detector. Total lipid content varied widely among the species and ranged between 0.72 and 0.79 $g.100g^{-1}$ fresh weight. Generally, saturated fatty acids were major components (6570%), with palmitic acid (C16:0) as the most abundant saturate (5663%). Total polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) ranged from 29% to 35%. The green alga showed higher C18 PUFAs contents than did C20 PUFAs. Cystoseira crinita belonging to the group of brown algae showed similar amounts of C18 and C20 PUFAs contents. The green alga was rich in linoleic acid (LA, C18:2n6) while the brown alga was rich in both linoleic acid (LA, C18:2n6) and eicosopentaenoic acid (EPA, C20:5n3). PUFA/SFA ratio in both species was approximately 0.35. All of the studied species had a nutritionally beneficial n6/n3 ratio (1.012.43:1).

Key words: Black sea algae, fatty acids, GC/MS


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Session 2

DETERMINATION OF HEAVY METALS (Pb, Cd, As and Hg) IN BLACK SEA GREY MULLET (*MUGIL CEPHALUS*)

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Abstract

Gray mullet (Mugil cephalus) is commercially important marine school pelagic fast-moving fish species especially during warm weather. The M. cephalus is an omnivore and usually inshore, entering estuaries and lagoons, like Varna Lake. The aim of the present study was to determine and compare heavy metal contents (Pb, Cd, As and Hg) in edible tissue and gills of grey mullet (*Mugul cephalus*). The fish samples were collected from two different Black sea areas Varna Lake and Nesebar. The sample preparation was performed by acid microwave digestion with "Multiwave" system in five stages program. Determinations of As, Cd, and Pb were carried out on a Perkin Elmer Zeeman 3030 spectrometer with an HGA-600 atomizer, whereas Hg was analyzed by Milestone Direct Mercury Analyzer. Detected levels of As in the studied regions gives exceed those of other analyzed elements. The samples from both regions showed the higher levels of As in edible tissue than gills, especially from Region of Nesebar (1.1 mg/kg w.w.). The results for other heavy metals are several times lower than arsenic and were found in range 0.01 0.12 mg/kg w.w. All studied elements (except As) presented higher amounts from Varna Lake grey mullet compared with Neserbar region samples.

Key words: Heavy metals, Gray mullet, Black Sea

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CONTAMINATION BY HEAVY METAL OF SELECTED BLACK SEA FISH SPECIES

Lubomir Evstatiev Makedonski[1], Mona Dinkova Stancheva[1], Irina Ivanova Karadjova[2]
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The concentrations of Cd, As, Hg, Pb, Zn, Cu, Fe, and Ni elements in edible portions and gill of selected most consumed Bulgarian fish species - sprat (*Sprattus spratus*), horse mackerel (*Thrachurus mediterraneus ponticus*), Black sea gobies (*Neogobius melanostomus, Neogobius ratan*), shad (*Alosa pontica*), bonito (*Sarda sarda*), bluefish (*Pomatomus saltatrix*) and gray mullet (*Mugil cephalus*), collected in 2010 year from Bulgarian Black Sea coast were determined. The element analyses were performed with Inductively Coupled Plasma - Emission Spectroscopy (ICP-ES) following microwave digestion techniques.

Metal accumulation levels of tissues and species were remarkably different. Concentrations (μg g^{-1} wet weight) of heavy metals varied in the following ranges: Cd, 0.006 – 0.015; As 0.041 – 1.1; Hg, 0.05 – 0.16 and Pb 0.03 – 0.08. On the contrary this fish species accumulates the other investigated heavy metals such as Hg, Zn, Fe and Pb to lower extend. The concentration of Zn and Fe showed the highest value for all fish species. The highest concentrations of Cu, Fe, and Zn were measured in gill tissue.

The metal levels in muscle of the species were found below the limits proposed by FAO, WHO, and these are safe for human consumption.

Poster session 3G

Abstract no.: 96

Title:
The effect of storage and processing on Fat-soluble vitamin content in Bulgarian Freshwater and Black Sea fishes

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Abstract text:
Fishes are e good source of fat soluble vitamins necessary for healthy diet. The contents of retinol, alpha-tocopherol, ergoferol, and cholecalciferol (vitamins A, E, D2 and D3) in two of most commonly eaten species of fish in Bulgaria were determined and compared. Fat-soluble vitamin contents in Bluefish (Pomatomus saltatrix), a typical Black sea pelagic fish, and in Brown trout (Salmo trutta fario), a typical Balkan freshwater fish, were analyzed in fresh, frozen and boiled edible fish tissue samples. The sample preparation procedure includes saponification and consequent extraction of fat-soluble vitamines with n-hexane. The extract was dried under nitogen flow and redissolved in methanol. HPLC analysis of methanolic samples was performed on ODS2 Hypersil (250x4,6, 5um) column with a mobile phase of methanol:water = 97:3. The quantification of fat-soluble vitamins was by the method of standard addition. The retinol content in the fresh edible tissue of Black sea Bluefish (26.0±2.0 µg/100g) was almost the same as in the Balkan freshwater fish Brown trout (22.3±2.0 µg/100g). Cholecalciferol (vitamin D3) content was higher in Black sea Bluefish (8.6±1.0 µg/100g) than in Brown trout (6.0±0.29 µg/100g) which was higher in alpha-tocopherol (809.1±56.0). In both fishes, the content of vitamin D2 was under the detection limit of the method. Surprisingly after steaming (15 min. at 80-85oC) there were almost no losses in the content of all fat-soluble vitamins per 100g raw tissue. Key words: fat-soluble vitamins, Bulgarian Black see and freshwater fishes.

Poster session 1B

Abstract no.: 48

Title:
Fat-soluble vitamins in fish from Bulgarian Sea Cost

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Abstract text:
Fat-soluble vitamins in fish from Bulgarian Black sea cost were analyzed. Vitamin-A and vitamin E were determined in edible tissues of five most traditionally consumed fish species - sprat (Sprattus sprattus), Black sea goby (Neogobius rattan), shad (Alosa pontica), horse mackerel (Trahurus Mediterraneus pontica), and leaping mullet (Liza saliens) caught in the spring 2009. The sample preparation procedure include saponification and extraction by n-hexane. Non-soaping components were extracted with n-hexane and the common extract was dried under nitrogen. The dry residue was dissolved in methanol. Both vitamins were analyzed simultaneous using HPLC technique with UV detector (Thermo Scientific Spectra SYSTEM, UV-Vis, ODS2 Hypersil 250 * 4,6 mm, 5u). The mobile phase was 100% methanol with a flow of 0.9 ml/min. Qualities determination were carried (wave number 295 nm for vitamin E and 325 nm for vitamin A) based on their retention times and peak areas of the standards. Reproducibility of the method is determined using the method of standard curve for both vitamins analyzed (5 sample without spikes and 5 spikes samples). Reproducibility ranged between 86,5 to 101,5 % for Vitamin A and between 71,1 to 94,9 % for vitamin E, coefficient of variation is between 3,76 to 7,79 % and between 4,07 and 7,06 %, respectively. The experimental results were statistically analyzed using GraphPad Prism 5 program for vitamin analysis.The results are presented as mean values and standard deviation. The yield for vitamin A and vitamin B in 100 g raw tissue ranged from 0,008 to 0,032 mcg and 308,02 to 1112,73 mcg, respectively. An exception is for the amount of vitamin A in the sample of sprat (192.33 mcg). The whole fish were taken for analysis due to consumption habits.

P-17

DETERMINATION OF Cd, Cu, Fe, Mn AND Pb IN EUROPEAN CARP (*CYPRINUS CARPIO CARPIO*)

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The concentrations of five elements (Cd, Cu, Fe, Mn, and Pb) were determined in the muscle, liver, gills, bones, skin and scale of cultured carp (*Cyprinus carpio carpio*) caught at Tzonevo Dam in Bulgaria in August 2009. The samples were treated with 10 ml ultrapure concentrated nitric acid in Teflon beaker, and heated at 200 ºC on a hot plate for 3 h, until the solution evaporate slowly to near dryness. Two milliliters of 1 N HNO_3 was added to the residue and the solution was evaporated again on the hot plate. By repeating the additional digestion twice, all organic materials in each sample were completely digested. After cooling, 1 N HNO_3 was added to digested residue and was transferred to 25 ml volumetric flasks, then diluted to level with deionized water. Before analysis, the samples were filtered through a 0.45 µm nitrocellulose membrane filter. All samples were analyzed three times for Cd, Cu, Fe, Mn and Pb by Atomic Absorption Spectrometer (Varian Model Spectrometer AA-240).

The highest levels of Pb, Mn and Cd were found in the skin and scale of the fish species (5.26 mg/kg w.w, 8.74 mg/kg w.w and 0.34 mg/kg w.w, respectively) while Cu and Fe had been accumulated predominantly in gills (2.68 mg/kg w.w. and 55.54 mg/kg w.w. respectively). Among the metals analyzed Fe was the most abundant in the different tissues, while Cd and Cu where the least abundant. The results obtained in this study were compared with those reported in our earlier studies. The concentration of these five elements in a sample of bighead carp showed a similar tendency. Fe had been accumulated predominantly in all fish tissues followed by Mn and Cu. According to international criteria, heavy metal concentrations in cultured carp from Tzonevo Dam are below limits for human consumption.

P-18

VITAMIN CONTENT AND FATTY ACIDS COMPOSITION OF BROWN TROUT (*SALMO TRUTTA FARIO*)

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Brown trout is a fresh water fish and a dominant species with an economical importance due to its delicious taste. Fish lipids are well known to be rich in long-chain n-3 and n-6 polyunsaturated fatty acids and fat-soluble vitamins (higher level of vitamin E). These nutrients are very important for human's health and might be obtained from the diet.

There is limited information in the scientifically literature about the lipid composition of trout (*Salmo trutta)* inhabiting Bulgarian waters.

The aim of presented work was to determine fat-soluble vitamins content and fatty acids (FA) composition in raw edible tissue from brown trout (*Salmo trutta fario*).

All-trans-retinol, cholecalciferol and α-tocopherol were analyzed simultaneously using HPLC system with UV (vitamin A and D_3) and fluorescence detection (vitamin E). The sample preparation procedure includes saponification and liquid/liquid extraction of the unsaponifiable matter (Sanchez-Machado at all). Total lipids were extracted according to Bligh and Dyer method. Analysis of fatty acid methyl esters were performed using gas chromatography system with MS detection.

It was found that lipid fraction contain substantial amounts of palmitic, palmitoleic, stearic, linolenic, arahidonic and docosahexaenoic fatty acids and lipo-soluble vitamins. The retinol content in the fresh edible tissue of brown trout were 22.3±2.0 μg/100g; cholecalciferol - 6.0±0.29 μg/100g and α-tocopherol - 809.1± 56.0 μg/100g. The amounts of lipo-soluble vitamins, and especially α–tocopherol content were higher than the amounts of the same vitamins in other fish species. Ahmadnia A. at all, (2008) had found that the vitamin content in fresh tissue of Golden grey mullet is 1.4±0.4 μg/100g for retinol; 1.47±0.22 μg/100g for cholecalciferol and 12.5±2.5 μg/100g for α-tocopherol, respectively.

Linoleic acid (24.41%), docosahexaenoic acid (5.89 %) and arahidonic acid (2.66 %) were the most dominant polyunsaturated fatty acids, about 33% in total FA. Palmitic acid, was fount to be dominant in of an saturated fatty acids and oleic acid showed hight value of monounsaturated fatty acids - 17.16% and 6.58%, respectively.

Our results are in agreement with the findings of other authors and confirm that brown trout, being high in vitamin E and polyunsaturated fatty acids, is an important component of the human's healthy diet.

P-19

ORGANOCHLORINE POLLUTANTS IN BLUEFISH *(POMATOMUS SALTATRIX)* FROM BULGARIAN BLACK SEA COAST

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Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are long-lived organic chemicals that are generally resistant to chemical and biological degradation processes. Fish consumption is the main source of human exposure to different environmental contaminants like PCBs and DDTs. The purpose of this study was to determine the levels of persistent organochlorine contaminants in bluefish from the Bulgarian Black Sea coast and to monitor the accumulation of these pollutants during the period 2003 – 2006. The bluefish *(Pomatomus saltatrix)* enters the Black Sea in spring for feeding and spawning and moves back to the Sea of Marmara in winter. It feeds primarily on fish (anchovy, horse mackerel and young mackerel) and partially on crustaceans (shrims).

Concentrations of 14 PCBs (IUPAC №28, 31, 52, 77, 101, 105, 118, 126, 128, 138, 153, 156, 169, 180) and organochlorine pesticide dichlorodiphenyl-trichloroethane (p,p'- DDT) including its metabolites (p,p'- DDE and p,p'- DDD) were measured in muscle tissue samples of bluefish *(Pomatomus saltatrix)*. Samples were collected from Black Sea - region of Varna, Bulgaria in the period of 2003 – 2006. DDTs and PCBs were determined by gas chromatograph equipped with electron-capture detector or mass spectrometry allowing better identification of compounds.

Concentrations in bluefish ranged from 367.1 to 879.5 ng/g lipid weight for total DDTs (sum of p,p'- DDT, p,p'- DDD and p,p'- DDE). In all samples DDT was present mainly in the form of its metabolites pp'- DDE and p,p'- DDD, suggesting previous contamination. The analysis of bluefish during the study period 2003 – 2006 showed a mean total load of DDT pollutants 595.0 ng/g lipid weight. Total PCB concentration (sum of 14 congeners) in bluefish varied in the range of 1.2 to 490.4 ng/g lipid weight. Concentrations of contaminants in bluefish increased during the period of study 2003 – 2006. The levels of DDTs and PCBs in bluefish from region of Varna were comparable or slightly higher than those found in fish from other parts of the Black Sea coast and from neighbor seas Marmara Sea and Aegean Sea. The experimental data present initial investigations from a profound study of PCBs and DDTs in fish and seafood from Black Sea.

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experimental Surgery and the initiator of general rachi-anaesthesia. The aim of this historical review is to underline his contribution to the field of oncologic surgery with the establishment of new original surgical methods for the treatment of the cancer. The majority of his works were on anatomy especially in the region of the abdominal cavity, the peritoneum, the duodenum and the sigmoid loop which he named pelvic colon. His first goal is the introduction of strict asepsis in Romanian Surgery. His scientific contributions are: the general spinal anaesthesia, the surgery of the cervical part of the sympathetic system and the great gynecological surgery. For the first time he completes the total abdominal hysterectomy with the extirpation of the lymph nodes and the cellular tissue of the pelvis of the iliac fossa even of the lumbar region. According to him this is the only way to avoid the reoccurrence of the cancer in distant lymph nodes. The name Jonnesco is related to needle of Jonnesco, Anaesthesia of Jonnesco, Colon ligaments of Jonnesco, operation of Jonnesco and Neprhropexy of Jonnesco.

04. HEAVY METAL CONTENT OF TWO FISH SPECIES FROM BULGARIAN BLACK SEA WATERS

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In this study two migratory Bulgarian fish species (Atlantic bonito (Sarda sarda) and bluefish (Pomatomus saltatrix)) collected from north-east coast of Bulgarian Black Sea were analyzed. The heavy metal (Pb, Cd, Cu, Mn, and Fe) concentration in their edible parts were determined. The two year sampling period covers the years 2004 and 2005. Quantitative determination of the heavy metals was performed by atomic absorption spectrophotometry after a digestion procedure with HNO3 . The concentrations of trace metals in fish samples indicated that S. sarda was more contaminated that P. saltatrix. The highest concentration was determined for Fe for both species (20.22 µg/g and 6.51 µg/g., respectively) followed by Cu (3.28 µg/g and 1.76 µg/g, respectively). The minimum concentration was observed for Mn for both species (0.77 µg/g and 0.18 µg/g). It may be concluded that consumption of these species from this region is not likely to pose a threat for human health due to the fact that the values of the elements are within the limit values for fish muscles.

05. SERUM OSTEOCALCIN IN POSTMENOPAUSAL PATIENTS ON HEMODIALYSIS

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Introduction. Osteocalcin is a vitamin K-dependent protein secreted by osteoblasts. Serum levels of active osteocalcin are recognized as a marker of bone formation. In states of vitamin K deficiency the fraction of the inactive, under-carboxylated osteocalcin (uc-OC) is increased. The elevated level of uc-OC is supposed to reflect reduced bone mineral density (BMD), however, inverse correlation is not always found with osteoporosis. Chronic kidney disease is associated with bone loss and there is evidence for a sub-optimal status of vitamin K in renal patients.
Aim. The objective of the present study was to measure the serum levels of uc-OC in post-menopausal women with and without osteoporosis and to compare it with the level in hemodialysis (HD) postmenopausal patients.
Methods and patients. 80 menopause women were divided into 3 groups: 1) control group of women with normal BMD (n =25); 2) osteoporosis group (n =29); 3) postmenopausal women on HD (n =26). Osteoporosis was verified by densitometry. Serum uc-OC was measured immunochemically by EIA kit (TAKARA Bio Inc.).
Results. There was no statistically significant difference in serum levels of uc-OC between the control (2.72±0.33 ng/ml) and the osteoporosis group (3.63±0.50 ng/ml). The level of uc-OC in the HD women was 16.40±1.62 ng/ml – significantly higher compared with the control group ($p<0.0001$) and the osteoporosis group ($p<0.0001$).
Conclusion. The considerable increase of uc-OC in HD postmenopausal patients indicates a severe vitamin K deficit in chronic kidney disease. Additional investigation is needed to justify supplementation with vitamin K in these patients.

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different pathogenetic mechanisms, whose diagnosis and treatment raise multiple medical problems.
Material and methods: From 726 necropsy we have 26 patients with congenital malformations and 70 patients with acquired cardiac diseases were studied pulmonary biopsies to estimate the pulmonary hypertension by correlating the data obtained by modern multidisciplinary investigations: histological, histoenzymological, ultrastructural, physiological and clinical techniques.
Results: The incidence of PHT was 8 % from total. We have 58 cases of PHT from 756 necropsy cases. The morphological pictures show a range of vascular lesions similar to those found in Heath and Edwards' classification and they are especially thickenings of the intima, hyperthrophies of the media, narrowings of vascular lumen and later some plexiform lesions and hemosiderosis. Progressive fibrosis processes as well immune pulmonary reactions were made evident. The electronmicroscopical examinations provided new data regarding the thickening of the capillary and alveolary basal lamina, the activity pneumocytes and macrophages that were also certified from the histoenzymological point of view by increase of peroxidases and acid phosphatases activity.
Conclusions: Pulmonary hypertension is a disease that have an important contribute to the prognosis of patients with malformations and acquired cardiac diseases like valvular heart diseases.

075. MEDICINES SUBSTITUTION IN BULGARIAN PHARMACIES

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Medicines substitution in the prescription by pharmacist in some countries is legally authorised and supports the generic medicines entrance thus reducing the pharmacotherapy cost, while in other it is prohibited for regulatory purposes.
Aim: This study is analysing the pharmacy substations practice in Bulgarian pharmacies for 5 medicines – antibiotics, antihypertensive, androgenic, and vasodilatators.
Materials and methods: The study is a prospective inquiry research among 100 pharmacies. The inquiry focuses on the frequency of medicines substitution by type of pharmacy, location, and work experience of pharmacists, as well as reasons for substitution. Every product under consideration was compared with its competitors. Descriptive statistic and factorial analysis were applied.
Results: 90% of the pharmacist's works more than 3 years, 73.8% are in the capital, 31.3% and 33.8% of the pharmacies are in the town centre or near to hospital, respectively. The frequency of antibiotics substitution is in 8.8% for every second Rp, 21.3% for every 3rd Rp, 31.3% for every 5th Rp, and 38.8% never. For sildenafil the frequency of substitution is 18.8% in every 2nd Rp, 16,3% in every 3rd Rp; 20% every 5th Rp, and 45% never. The reasons for substitution are availability of products in 30% of cases; efficacy in 33,8%; professionals opinion in 52,5%; price in 58,5%; patients request in 27,5%; other.
Pharmacists from the capital, mail gender, and located near to hospitals tend to substitute more often medicines.
Conclusion: A variety of factors influence the substitution of the medicines but in general it is a fairly spread practice in Bulgaria.

076. ORGANOCHLORINE POLLUTANTS IN EUROPEAN SPRAT FROM BULGARIAN BLACK SEA COAST

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Fish consumption is the main source of human exposure to different environmental contaminants like PCBs and DDTs. The purpose of this study was to determine the levels of persistent organochlorine contaminants in sprat from the Bulgarian Black Sea coast and to monitor the accumulation of these pollutants during the period 2003 – 2007. The European sprat (Sprattus sprattus) feeds primarily on crustaceans (shrims).This fish species was selected because of its importance for human consumption in Bulgaria.
Concentrations of 14 PCBs (IUPAC №28, 31, 52, 77, 101, 105, 118, 126, 128, 138, 153, 156, 169, 180) and organo-

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23-26 September 2009

Fatsa-Ordu/Turkey

PPIII-66 (Ab332)

FATTY ACIDS PROFIL AND FATSOLUBLE VITAMINS A AND E CONTENT OF SCAD (TRACHURUS MEDITERRANEUS)

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Key Words: Fish, Fatty Acid Composition, Vitamin A and Vitamin E, Bulgarian Black Sea coast

The aim of this study was to measure and evaluate of the total lipid, fatty acid profile and Vitamin A (All-trans Retinol) and Vitamin E (α-Tocopherol) content of scad (*Trachurus mediterraneus*) catch from Bulgarian Black sea and the same fish species from Greek coast of Mediterranean Sea. The Black sea scad were harvest in Spring 08 and Autumn 08, while Greek scad was purchased from the Varna local fish marked in Spring 09. Lipid extraction was done according to the Bligh and Dyer method. Methyl esters were prepared by transmethylation using 2M KOH in methanol and n-hexane.

The results from analysis showed that the sample of Black sea scad contain 6.5 g total lipid per 100 g raw weight (Spring 08), 2.4 g total lipid per 100 g raw weight (Autumn 08) while Greek shad present 7.9 g. total lipid per 100 g raw weight (Spring 09). The fatty acid composition was analyzed by Gas Chromatography with MS detector. In all fish samples the most abundant fatty acids were myristic (C 14:0), palmitic (C 16:0), palmitoleic (C 16:1), oleic (C 18:1), eicosapentaenoic (C 20:5) and docosahexaenoic (C 22:6). Palmitic acid was the dominant saturated fatty acid averaging 55 % of total saturated fatty acids. Oleic acid was the most abundant monoenoic fatty acid. The level of total ω-3 polyunsaturated fatty acids was higher than the total ω-6 fatty acid in the all analyzed fish species. The vitamin content of all-trans retinol and α-tocopherol were determinate by HPLC with UV detector. The results from measure show the differences for all-trans retinol and α-tocopherol contents between Bulgarian horse mackerel and Greek horse mackerel.

5th BLACK SEA BASIN CONFERENCE ON ANALYTICAL CHEMISTRY

BOOK OF ABSTRACTS

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PPIII-65 (Ab331)

LEVELS OF PCBs IN FISHES FROM BULGARIAN BLACK SEA COAST

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Key Words: Polychlorinated biphenyls (PCBs), Fish, Bulgarian Black Sea coast

Concentration of individual and total polychlorinated biphenyls (PCBs) was determined in fish samples from Bulgarian Black Sea coast. Fishes were collected from the region of Varna city during the period of 2003-2005. Varna is located in northern part of Bulgarian Black Sea coast. The edible tissues of the following fish species- shad *(Alonsa pontica),* scad (*Trachurus mediterraneus*) and gobies (*Neogobius melanostomus, Neogobius ratan)* were determined. The samples were analyzed with Gas Chromatography Mass Spectroscopy method-modified and validated. The fourteen congeners of PCB were analyzed including the set of 7 indicators PCBs (IUPAC No 28, 52, 101, 118, 138, 153, 180). PCBs were found in all investigated samples. European sprat showed the highest total level of PCBs (187.9 – 901.4 ng/g fat) compared to the other species-gobies (66.3 – 192.2 ng/g fat) and scad (14.7 – 208.2 ng/g fat). The highest level of PCBs was found in 2005. Our study illustrated that the concentration level of PCBs in analyzed samples was lower compared with those recommended by the European Union.

5th BLACK SEA BASIN CONFERENCE ON ANALYTICAL CHEMISTRY

BOOK OF ABSTRACTS

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ORGANOCHLORINE PESTICIDES IN FISHES FROM BULGARIAN BLACK SEA COAST

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Key Words: Organochlorine pesticides, fishes, Bulgarian Black Sea Coast

In spite of the worldwide reduction of the utilization of organochlorine pesticides (OCP), they can still be a problem for the aquatic environment and the human health. The Black Sea is a closed sea, subject to important freshwater discharges and could be polluted with persistent chemicals, including OCP.

Several fish species with different feeding behavior were sampled on a regular basis from the Bulgarian Black Sea coast and the concentrations of thirteen OCP residues were determined by capillary gas chromatography with electron capture detector.

Many of the OCP, like dieldrin, aldrin, endrin were not detected in the samples. However, in all samples DDT was present mainly in the form of its metabolites pp-DDE and pp-DDD. Only about 10% of the total DDT is present like the parent compound pp-DDT, which suggests it is not being used recently in the region. The total DDT concentrations were generally bellow 150 ng g^{-1} wet weight, but higher levels up to 360 ng g^{-1} wet weight were also measured for fish species with high fat content. Between-species differences were observed even when the concentrations are presented on a fat level basis. The seasonal and regional changes in DDT contents were also investigated.

This first systematic study on OCP levels in fishes from the Black sea will provide useful information both for nutritionist and for environmental scientists. This study of DDT levels in different aquatic organisms from the Black Sea provides useful information both for assessing human health and ecological risks of pesticide pollution. For a future evaluation of the Black Sea pollution with DDT the utility of goby and scad for biomonitoring purposes should be considered.

5th BLACK SEA BASIN CONFERENCE ON ANALYTICAL CHEMISTRY

BOOK OF ABSTRACTS

23-26 September 2009

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PPIII-63 (Ab329)

DETERMINATION OF HEAVY METALS IN FISH SPECIES FROM THE NORTHERN BLACK SEA (BULGARIA) BY ICP-MS

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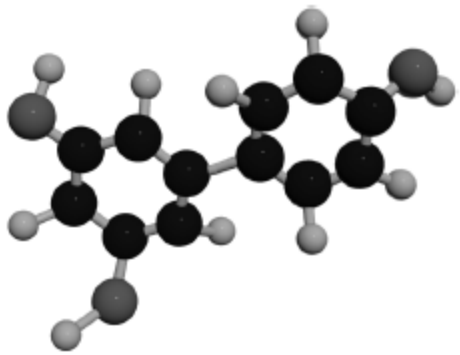
Key Words: Heavy Metals, Fish, Black Sea, ICP-MS analysis

In this study heavy metals (Cd, Cu, Zn, As, Pb) concentration in edible part of five most consumed Bulgarian fish species - scad (*Trachurus mediterraneus*), bluefish (*Pomatomus saltatrix),* Black sea gobies (*Neogobius melanostomus, Neogobius ratan),* atlantic bonito *(Sarda sarda),* shad *(Alonsa pontica)* and sprat *(Sprattus spratus*) collected from north-east coast of Bulgarian Black Sea were determined. The samples were digested with a microwave digestion system followed up by Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) determination. Sampling period was done in 2008 and 2009. The levels of Cd and Cu were relatively low in the edible part for all fish types while those for Zn concentration show highest value for sprat. The concentration of As and Pb are within acceptable levels for a food source for human consumption. Metal concentration differences might be related to diet and feeding habits of benthic and pelagic fish species. Results characterize Black Sea fishes as food product of high nutritive and biological value that should be included in preventive nutrition.

МОНОГРАФИЯ

Мона Станчева

УСТОЙЧИВИ ОРГАНИЧНИ ЗАМЪРСИТЕЛИ В ХРАНИ



Варна, 2013

ВЪВЕДЕНИЕ

През 1962г. в САЩ се публикува книгата „Мълчаливата пролет“ с автор Р. Карсън, която предизвиква голям интерес и дискусии. За някои тя е „най-вредната“ книга, написана в последния век, а за други е точно обратното. В книгата са описани редица вредни ефекти върху околната среда и човека, предизвикани от употребата на пестициди. Авторката аргументирано доказва, че безконтролната употреба на ДДТ и други подобни съединения, използвани в селското стопанство за борба с плевели, насекоми, животни и птици, са вредни за хора. Тя обвинява индустрията, която е произвела в огромни количества тези химикали и държавната власт, която не е упражнила контрол за влиянието им върху хората и околната среда. Отзвукът от тази книга е толкова силен, че президентът Джон Кенеди нарежда разследване по обвиненията, учените извършват редица проучвания, организират се движения в защита на околната среда. Книгата е включена за разглеждане в учебните програми и това допринася в голяма степен за информиране на обществото за ползата и вредата от използването на редица химикали.

Става въпрос за следното: в началото на миналия век бяха произведени широка гама т. н. индустриални химикали, като пестициди, торове, лекарства, препарати за промишлено и битово потребление и др. В световен мащаб положителният ефект от използването им е голям за индустрията, бита, селското стопанство и пр., но много от тези химикали проявиха неочаквани вредни ефекти върху човешкото здраве и околната среда. Класически пример в това отношение са хлорорганичните пестициди, използвани масово в селското стопанство през 60-те години на миналия век, които се оказаха силно токсични, с голяма устойчивост и способност да се натрупват в живите организми. Установи се, че те променят биологичната стойност на хранителните продукти, предизвикват неблагоприятни последици за човешкото здраве и околната среда. Това доведе до въвеждане на забрана за производството и употребата им.

Аналогична е ситуацията и с полихлорираните бифенили (ПХБ), произведени в големи количества през периода 1950–1970г, широко използвани в различни области на индустрията, в последствие също забранени за производство и употреба. Въпреки забраната, те продължават да попадат в околната среда, като източници са отработени масла, стари електрически съоръжения и домакински електроуреди, получават се при горенето на отпадъци, където заедно с тях се образуват още по-токсични и опасни съединения, като диоксини и фурани.

Проблемът със замърсяването на околната среда с посочените по-горе и подобни на тях органични съединения, известни като устойчиви органични замърсители (УОЗ) се оказа глобален и това наложи да бъдат взети мерки в световен мащаб. Съзнавайки, че УОЗ представляват сериозна и нарастваща заплаха за човека и околната среда, през май 1995 г. по програмата на ООН за околна среда UNEP се взема решение за извършване на международна оценка на редица от тези замърсители. В резултат на тази оценка се изработва международен нормативен документ, известен като Стокхолмска конвенция за УОЗ. Конвенцията е приета и открита за подписване през май 2001г. Целта на конвенцията е опазване на околната среда и човешкото здраве от вредното влияние на редица устойчивите органични замърсители.

България подписва Стокхолмската конвенция още през май 2001г., а Народното събрание я ратифицира със закон през март 2005 г. Национален орган по изпълнение на задълженията по нея е Министерството на околната среда и водите. Като страна по конвенцията и в съответствие с нейните изисквания, в България се разработва „Национален план за действие за управление на устойчивите органични замърсители".

Експозицията на човека с УОЗ може да се осъществи по различен начин, но храната е основния източник за попадането им в организма. Това са храни от животински произход, богати на мазнини, където тези замърсители се натрупват.

Редица учени са установили, че моретата и океаните, както и организмите в тях, акумулират в значителни количества УОЗ и там те бавно се разграждат. Така при консумацията на риба и други морски организми, тези замърсители попадат в човешкия организъм. Затова определянето на УОЗ в риби е важно за оценката на експозицията на населението, здравния риск и на замърсяването на водната екосистема.

И така, възниква въпроса за ползата и риска от използването на рибата като храна. Безспорно, в най-голяма степен ползата е свързана с протеините и липидите, съдържащи незаменими полиненаситени мастни киселини, докато рискът – с различните замърсители, които рибите акумулират от околната среда. От гледна точка на здравето, рискът трябва да бъде оценен.

В монографията е направена кратка характеристика на УОЗ, включени в Стокхолмската конвенция, като по-подробно са описани най-използваните в миналото и разпространени в околната среда замърсители – оргонохлорния пестицид ДДТ, неговите метаболити и полихлорираните бифенили (ПХБ). Направен е обзор на изследвания от целия свят за съдържанието на УОЗ в различни видове храни, като специално внимание е обърнато на морските храни. И накрая са представени резултати от изследвания на ПХБ, ДДТ и метаболити в наши черноморски риби във връзка с оценка на безопасността им като храна.

Написването на тази монография е с цел повече информация и знание за УОЗ в храните, защото чрез храните те попадат в организма и могат да предизвикат редица вредни ефекти върху нашето здраве.

Изготвил резюме на научните трудове:

Доц. М. Станчева

МЕДИЦИНСКИ УНИВЕРСИТЕТ – ВАРНА

КАТЕДРА ПО ХИМИЯ

УЧЕБНО ПОМАГАЛО

ЗА УПРАЖНЕНИЯ ПО АНАЛИТИЧНА ХИМИЯ

на Магистър фармацевти

2012 г.

МЕДИЦИНСКИ УНИВЕРСИТЕТ - ВАРНА

КАТЕДРА ПО ХИМИЯ

Т Е Т Р А Д К А

ЗА УПРАЖНЕНИЯ

по Х и м и я

ЗА СТУДЕНТИ ПО МЕДИЦИНА

2012 г.

Lubomir Makedonski
Katya Peycheva
Mona Stancheva

Laboratory Manual

For General and Organic Chemistry
Students of Medicine

PREFACE

To the students

Chemistry is an experimental science. Here you are in a chemistry laboratory with your laboratory book in front of you. Perhaps you have already been assigned laboratory equipment you may never have seen before. Looking around the laboratory, you may see bottles of chemical compounds, balances, burners, and other equipment that you are going to use. This may very well be your first experience with experimental procedures. At this point you may have some questions about what is expected of you. This laboratory manual is written with those considerations in mind.

The activities in this manual were written specially to parallel the chemistry you are learning in the lecture portion of class. Many of the laboratory activities include materials that may be familiar to you, such as household products, drinks, antacids and aspirin. In this way, chemical topics are related to the real world and to your own science experience .Some of the lab teach basic skills; others encourage you to extend your scientific curiosity beyond the lab.

It is important to realize that the value of the laboratory experience depends on the time and effort you invest in it. Only then will you find that the laboratory can be a valuabe experience and an integral part of the chemistry class. The laboratory gives you an opportunity to go beyond the lectures and words in your textbook and experience the scientific processes from which conclucsions and theories concerning chemical behavior are drawn. In some experimets the concepts are correlated with health and biological concepets. Chemistry is not an inaanimated science, but one that help us to understand the behavior of living systems

Using This Laboratory manual

Each experiment begins with learning goals to give you an overview of the topics you will be studying in that experiment. Each experiment is correlated to concepts you are currently learning in your chemistry class. Your instructor will indicate which activities you are to do.

The experimental procedures are written to give you through each laboratory activity. Read and measure carefully, report your data, and follow instructions to complete the necessary calculations. You may also be asked to answer some or all of the follow-up-questions and problems designed to test your understanding of the concepts from the experiment.

While there is no 'best' way, it is important that you carefully prepare for each experiment by *reading the related text material before coming to the laboratory*. In this way you can maximize the laboratory experience.

It is our hope that the laboratory experience will help illuminate the concepts you are learning in the classroom. The experimental process can help make chemistry a real and exciting part of your life and provide you will skills necessary for your future.

УЧЕБНИ ПОГРАМИ

МЕДИЦИНСКИ УНИВЕРСИТЕТ

“Проф. д-р Параскев Стоянов”
Варна

Ф А К У Л Т Е Т по М Е Д И Ц И Н А

Утвърдена с Протокол на ФС № 21 от 11.03.2013 г.

Утвърждавам:

Ректор:

/проф. д-р К. Иванов, дмн/

Декан:

/доц. д-р Р. Радев,дм/

УЧЕБНА ПРОГРАМА

по дисциплината : “ Химия “
включена в учебния план на специалност: “МЕДИЦИНА”
за студентите от I курс,
придобиващи образователно-квалификационна степен “магистър”
с професионална квалификация “ лекар”

Вид на занятията	Семестър	Хорариум-часа седмично	Хорариум-часа **Общо**
Лекции	I II	2 2	60
Семинарни упражнения			
Практически упражнения	I II	2 1	45
Общо часа	I II	4 3	105
Форми на контрол	Текущ контрол Колоквиум		Семестриален изпит
Кредити (ECTS)			

Варна, 2013

УЧЕБНА ПРОГРАМА ПО ХИМИЯ

ЗА СТУДЕНТИ ПО МЕДИЦИНА

I. АНОТАЦИЯ

Обучението по химия се извършва в първи курс – първи и втори семестър.

Учебният материал е съобразен с целите на един курс по Химия, предназначен за студенти по медицина. Усвояването на нови знания и по-задълбоченото разглеждане на редица теоретични въпроси, е направено във връзка с изучаването на други учебни дисциплини, като биохимия, физиология, фармакология и пр.

Разглеждат се основни теоретични въпроси, свързани със строежа и свойствата на веществата, като особено място е отделено на основни групи органични съединения, участващи в метаболизма на живите организми. Специално внимание се обръща на биологичната активност на редица органични съединения, както и значението на някои от тях, като градивни елементи на биополимерите.

Съдържанието на учебния материал, включен в упражненията, е в пряка връзка с лекционния курс. Целта на упражненията е да се осмисли и допълни материала, представен в лекционния курс, както и студентите да придобият определени практически умения. Това се постига чрез семинари върху определени теоретични въпроси, извършване на практически упражнения, които илюстрират някои характерни химични свойства на веществата. Студентите се запознават с основни методи за анализ, използвани в аналитичната, органична химия и клиничната лаборатория.

Проверката на знанията по химия се извършва чрез текущ контрол по време на семестъра и изпит след втория семестър. Текущият контрол включва тестове и контролни задачи.

Изпитът е писмен и устен. Писменият изпит включва определен брой въпроси (25-30) и продължителност 4 часа. Устният изпит е върху писмената работа и допълнителни въпроси. Оценката от изпита се формира от писмения изпит, устния изпит и текущия контрол на студента по време на семестъра.

II. РАЗПРЕДЕЛЕНИЕ НА УЧЕБНИЯ МАТЕРИАЛ ПО СЕМЕСТРИ

Семестър	Седмици	Часове седмично	Часове всичко	Теория	Упражнения
I	**15**	**4**	**60**	**30**	**30**
II	**15**	**3**	**45**	**30**	**15**

III. РАЗПРЕДЕЛЕНИЕ НА УЧЕБНИЯ МАТЕРИАЛ ПО ТЕМИ

П Р О Г Р А М А

на лекциите по Химия

за студенти от специалност Медицина на МУ – Варна

О Б Щ А Х И М И Я

1. Значение на химията в обучението по медицина. Химия, околна среда и здраве.

2. Природа на химичната връзка в кондензирани системи. Междумолекулни взаимодействия – същност, видове и значение. Водородна връзка. Значение на водородната връзка за структурата и свойствата на веществата. Други видове връзки с биологично значение.

3. Разтвори. Колигативни свойства на разтворите. Закони на Раул. Дифузия и осмоза, биологично значение. Слаби и силни електролити. Активност на йоните и йонна сила. Значение на йонната сила.

4. Представи за киселини и основи. Протолитична теория. Сила на протолитите – Ка и Кв. Фактори, от които зависи силата на протолитите.

Автопротолиза. Йонно произведение на водата. Водороден показател /pH/, значение. Методи за определяне на pH.

5. Буфери. Уравнение на Хендерсон – Хаселбах. Буферен капацитет и буферни криви. Значение на буферите за живите организми и за лабораторната практика.

6. Химична кинетика. Фактори, от които зависи скоростта на химичните процеси. Молекулност и порядък на реакциите. Кинетични уравнения на реакции от първи, втори и трети порядък. Графични зависимости.

7. Зависимост на скоростта на химичните процеси от температурата. Активираща енергия. Уравнение на Арениус, приложение. Теория на преходното състояние.

8. Катализа. Механизъм на действие на катализаторите: теория на хомогенната и хетерогенната катализа. Киселинно-основна катализа. Роля на катализаторите за жизнените процеси. Ензимна катализа. Уравнение на Михаелис – Ментен.

9. Химично равновесие. Равновесни константи Кс и Ка. Температурна зависимост на равновесната константа. Равновесие в разтвори на електролити. Произведение на разтворимост.

10. Критерии за определяне посоката на протичане на химичните процеси. Връзка между равновесната константа и свободната енергия. Макроергични връзки. Спрегнати процеси.

11. Адсорбция, механизъм на адсорбцията. Видове адсорбция. Адсорбционни изотерми. Приложение на адсорбционните процеси в медицината.

12. Окислително-редукционни процеси. Характеристика и основни понятия. Уравнение на Нернст. Скорост на окислително-редукционните процеси. Рекдокс-катализатори. Особености на биологичното окисление.

13. Комплексни съединения. Видове комплексни съединения, строеж и изомерия. Химична връзка при комплексните съединения. Приложение и значение на комплексните съединения. Бионеорганична химия.

14. Химични връзки и електронни ефекти в молекулите на органичните съединения. Видове химични реакции и механизми в органичната химия. Структурна и генетична връзка между органичните съединения в биоматерията.

15. Въглеводороди и халогенопроизводни с медико-биологично значение.

16. Алкохоли, феноли и аминоалкохоли с медико-биологично значение. Характерни химични свойства. Представители.

17. Амини. Строеж на мастни и ароматни амини. Химични свойства. Представители.

18. Сяра-съдържащи съединения с медико-биологично значение. Характерни химични свойства. Представители.

19. Алдехиди и кетони. Химични свойства: нуклеофилно присъединяване, окисление, дисмутация, алдолно присъединяване. Биологично важни представители.

20. Карбоксилни киселини – мастни и ароматни. Химични свойства. Нуклеофилно заместване. Механизъм на естерификацията. По-важни представители на монокарбоксилни и дикарбоксилни киселини.

21. Ненаситени мастни киселини. По-важни химични свойства. Представители, трансмастни киселини и омега - киселини.

22. Функционални производни на киселините: киселинни халогениди, амиди, естери, анхидриди, нитрили. Свойства.
23. Мазнини и глицерофосфатиди. Строеж и биологично значение.

24. Въглеродна киселина и производни с медицинско значение. Сулфонамиди. Механизъм на действие.

25. Субституирани карбоксилни киселини. Обща характеристика. Халогенирани киселини. Хидроксикарбоксилни киселини: млечна, ябълчена, винена, лимонена, салицилова. Алдехид- и кетокарбоксилни киселини. По-важни химични свойства. Биологично значение.

26. Аминокиселини. Класификация в зависимост от вида на радикала. Строеж и изомерия. Химични свойства. Киселинно-основни свойства, титрувални криви.

27. Пептиди. Строеж и свойства. Глутатион.

28. Обща характеристика на хетероцикленитe съединения.
Хетероциклени съединения с един хетероатом: група на пирол, природни пиролови багрила, група на индол, група на пиридин.

29. Хетероциклени съединения с два хетероатома. Група на пиразол и имидазол. Група на пиримидин – пиримидинови бази, барбитурова киселина, барбитурати. Група на пурин, пуринови алкалоиди.

30. Алкалоиди – обща характеристика, биологично значение, представители.

31. Въглехидрати – класификация. Строеж на глюкоза, фруктоза, рибоза. Оптична изомерия, диастериоизомери, аномери и епимери. Представители: глюкоза, фруктоза, маноза, галактоза, рибоза и дезоксирибоза. Витамин С.

32. Химични свойства на монозахариди. Реакции на карбонилната група – присъединителни реакции, окисление и редукция. Реакции на хидроксилните групи – естерификация, взаимодействие с основи. Ферментация.

33. Полизахариди. Дизахариди – строеж на захароза, малтоза, лактоза. Химични свойства на дизахариди, редуциращи и нередуциращи дизахариди.

34. Хомополизахариди – нишесте и гликоген. Хетерополизахариди, обща характеристика и представители.

35. Нуклеозиди и нуклеотиди. Строеж на нуклеиновите киселини.

36. Липиди. Класификация на липидите. Фосфолипиди – глицерофосфатиди, свинголипиди, гликолипиди. Строеж, свойства, биологично значение и представители.
37. Липидоподобни - терпени и каротени – строеж и представители. Биологично значение.

38. Стероиди – строеж и биологична активност. Представители: стероли, жлъчни киселини, полови хормони.

39. Витамини. Мастноразтворими витамини. Антиоксидантни свойства.

40. Макро- и микронутриенти, биологично активни вещества в храни. Енергийна стойност на храните.

41. Инструментални методи за анализ. Потенциометрия. Спектрофотометрия. Атомно - абсорбционна спектрофотометрия.

42. Хроматографски методи за анализ. Видове хроматография: колонна, тънкослойна, газова и течна.

УЧЕБНА ЛИТЕРАТУРА

1. Учебник по ХИМИЯ за студенти по медицина
доц. инж. Емил Рачин, Изд. ВМИ – Плевен 2003

2. Учебник по ХИМИЯ за студенти по медицина и стоматология,
под редакцията на проф. Ст. Робев, Изд. АРСО, София, 1996

3. ХИМИЯ, учебник за студенти по медицина и стоматология
Л. Дамянова, Ал. Алексиев, Вл. Лесичков, Хр. Киряков

Изд. Наука и изкуство, София, 1983 и следващите издания.

4. Ръководство за практически упражнения по ХИМИЯ за студенти по медицина и стоматология. Изд. Наука и изкуство, София, 1989

Ал. Алексиев, Л. Дамянова, Б. Чемишев, М. Станчева, Е. Балтова

5. Лекционен курс по химия

П Р О Г Р А М А

за упражненията по Химия на студентите от специалност Медицина

I семестър

№	Тема	Брой учебни часове
1.	Правила за работа в химическа лаборатория. Концентрация на разтворите. Решаване на задачи.	**2**
2.	Концентрация на разтворите. Моларна и нормална концентрация. Решаване на задачи.	**2**
3.	Семинар върху колигативни свойства на разтворите: парно налягане, температура на кипене и замръзване, осмоза и осмотично налягане.	**2**
4.	Водороден показател (pH). Методи за определяне на pH. Буфери. Свойства на буферите.	**2**
5.	Буфери. Уравнение на Хендерсон-Хаселбах. Изчисляване pH на буфери. Буферна крива. Приготвяне на буферни разтвори.	2
6.	Контролно върху колигативни свойства на разтвори, pH и буфери.	**2**
7.	Качествен анализ. Качествен анализ на някои катиони и аниони с биологично значение.	**2**
8.	Количествен анализ.Обемен анализ. Техника на титруване. Киселинно-основен обемен анализ.	**2**

9.	Инструментални методи за анализ. Спектрофотометрия.	**2**
10.	Химична кинетика. Молекулност и порядък на химичните реакции. Опитно определяне порядъка на химична реакция.	**2**
11.	Контролно върху обемен анализ, спектрофотометрия и химична кинетика.	**2**
12.	Опитно представяне на химичните свойства на алкохоли и феноли, алдехиди и кетони.	**2**
13.	Семинар върху карбоксилни киселини и функционални производни на киселините. Мазнини и глицерофосфатиди. Биологично значение.	**2**
14.	Хроматография. Принцип на метода. Видове хроматография – колонна, хартиена и йонообменна.	**2**
15.	Комплексни съединения. Приложения. Бионеорганична химия. Семинар.	**2**

Упражненията са по 2 учебни часа, всяка седмица.

II семестър

№	**Тема**	**Брой учебни часове**
1.	Аминокиселини – строеж, класификация и химични свойства. Построяване на титрувална крива.	**2**
2.	Семинар върху субституирани карбоксилни киселини: хидроксикарбоксилни, алдехид- и кетокарбоксилни киселини. Оптична изомерия.	**2**

3.	Хетероциклени съединения с един хетероатом. Група на пирола, природни пиролови багрила. Групи на индол и пиридин.	**2**
4.	Хетероциклени съединения с два хетероатома. Групи на пиразол, имидазол, пиримидин и пурин. Тавтомерна изомерия. Контролно върху хетероциклени съединения.	**2**
5.	Семинар върху строеж на въглехидрати – монозахариди и полизахариди.	**2**
6.	Химични свойства на монозахариди и полизахариди.	**2**
7.	Биологично активни вещества: алкалоиди, витамини. /практическо упражнение/	**2**

Упражненията са по 2 учебни часа, през седмица.

Текущ контрол през втори семестър:

1.Колоквиум върху карбоксилни киселини, производни и субституирани киселини (на лекция)

2. Колоквиум върху въглехидрати – строеж и химични свойства (на лекция)

Литература:

1. Ръководство за упражнения по Химия
2. Работна тетрадка за упражнения
3. Лекционен курс

Съставил програмата:

доц. М. Станчева

Ръководител Катедра Химия:

доц. М. Станчева

МЕДИЦИНСКИ УНИВЕРСИТЕТ

“Проф. д-р Параскев Стоянов” Варна

Ф А К У Л Т Е Т по Ф А Р М А Ц И Я

Утвърдена с Протокол на ФС № 8 от 21.05.2013 г.

Утвърждавам:

ДЕКАН

/Доц. Д. Иванова, дб/

УЧЕБНА ПРОГРАМА

по задължителната дисциплина “Аналитична химия “
включена в учебния план на специалност “ФАРМАЦИЯ”
за студентите от II-ри курс,
придобиващи образователно-квалификационна степен “магистър”
с професионална квалификация “магистър-фармацевт”

Вид на занятията	*Семестър*	*Хорариум-часа седмично*	*Хорариум-часа Общо*
Лекции	III IV	2 1	45
Практически упражнения	III IV	3 2	75
Общо часа	III IV	5 3	120
Форми на контрол	Текущ контрол Колоквиум		Семестриален изпит
Кредити (ECTS)			

Варна, 2013

II. Анотация

Курсът по аналитична химия има за цел да запознае студентите с класическите и съвременните методи за качествен и количествен анализ.

В теоретичната част се разглеждат равновесните процеси в хомогенна и хетерогенна среда. Задълбочено са представени киселинно-основните равновесия във водна и неводна среда, процесите на разтваряне и утаяване, както и комплексообразувателните и окислително-редукционните процеси.

В лекционният курс са представени видовете количествения анализ,като основно място е отделено на различните видове обемен анализ – киселинно-основен, окислително-редукционен, комплексонометрия и утаечен анализ. Особено внимание е отделено на инструменталните методи за анализ, които имат първостепенно значение при анализ на лекарствените средства, както и прилаганите методи за разтваряне, разделяне и концентриране в аналитичната химия.

Практическите занятия включват: систематика и качествен анализ на йони в разтвор, класически количествени методи за анализ и съвременни инструментални методи, като: потенциометрия, спектрофотометрия, различни видове хроматография и други. Обсъждат се етапите на аналитичното изследване: подготовка на пробата за анализ, избор на аналитичен метод, изпълнение на аналитична задача, представяне и интерпретация на резултатите от анализа. Студентите разработват индивидуални аналитични задачи и представят резултатите от анализа.

През семестрите се провежда текущ контрол на всяко упражнение, както и четири контролни, по две на семестър.Курсът по аналитична химия завършва с изпит, който е писмен и устен, В крайната оценка се отчитат и оценките от текущия контрол по време на семестъра.

III. УЧЕБНО СЪДЪРЖАНИЕ

ЛЕКЦИИ

I семестър

№	*Тема*	*Брой учебни часове*
1	Предмет и задачи на аналитичната химия и на фармацевтичния	2

	анализ. Основни принципи на качествения и количествен анализ. Качествен анализ. Аналитични реакции - основни характеристики. Аналитични групи на катиони и аниони.	
2	Разтвори на електролити и неелектролити. Равновесие в разтвори на електролити. Коефициент на активност, йонна сила, приложение. Равновесие при малко разтворими съединения. Произведение на разтворимост, превръщане на една утайка в друга.Значение.	**3**
3	Представи за киселини и основи. Протолитична теория на Брьонстед-Лаури. Сила на протолитите, протолизни константи. Фактори, от които зависи силата на протолитите. Водороден показател, методи за определяне.	**1**
4	Буферни разтвори. Свойства на буферите. Уравнение на Хендерсон-Хаселбах. Буферни криви. Буферен капацитет. Биологично значение и приложение на буферите.	**2**
5	Количествен анализ. Видове количествения анализ. Обемен анализ - същност и класификация. Основни изисквания към реакциите, използвани в обемния анализ. Изчисления в обемния анализ. Стандартни разтвори, титроустановители, мерителни съдове.	2
6	Киселинно-основен обемен анализ. Титруване на силни и слаби протолити. Титрувални криви. Киселинно-основни индикатори, избор на индикатор.	**2**
7	Комплексни съединения. Строеж на комплексните съединения. Видове комплексни съединения. Стабилност на комплексите съединения, стабилитетни константи. Фактори, от които зависи стабилността на комплексите. Условна стабилитетна константа.	**2**
8	Комплексонометрия. Същност на метода. Комплексони. Метални комплекси с ЕДТА – състав, структура и стабилност. Криви на титруване. Металохромни индикатори. Титруване на метални йони, влияние на pH. Приложение във фармацевтичната практика.	**2**
9	Окислително-редукционни процеси. Уравнение на Нернст. Стандартен и условен редокспотенциал. Фактори, от които зависят	**2**

	редокспотенциалите. Посока на окислително-редукционните процеси.	
10	Окислително-редукционен обемен анализ. Същност и класификация. Перманганометрия. Приложение. Йодометрия – специфични изисквания. Приложение. Окислително-редукционно титруване в неводна среда.	**2**
11	Киселинно основни равновесия в неводни среди. Класификация на разтворителите. Автопротолиза и автопротолизна константа. Определяне на pH в неводни среди. Киселинно - основно титруване в неводна среда. Особености. Приложение в анализа на фармацевтични препарати.	**2**
12	Разтваряне на твърди вещества и утаяване. Фактори, определящи разтворимостта на утайките. Утаечно титруване. Криви на титруване. Определяне на еквивалентния момент, абсорбционни индикатори. Приложение.	**2**
13	Тегловен анализ. Утаечна и тегловна форма. Процеси, водещи до онечистване на утайките. Условия за получаване на чисти утайки и тегловни форми със строго дефиниран състав. Предимства и недостатъци на тегловния анализ.	**2**
14	Адсорбция – същност. Физична и химична адсорбция. Адсорбционни изотерми. Приложение на адсорбционните процеси.	**2**
15	Методи за разделяне и концентриране в химичния анализ. Екстракционни методи – течно-течна и твърдофазна екстракция. Екстракция на органични съединения, многостепенна екстракция. Маскиране в количествения анализ.	**2**

Първи семестър 30 часа

Втори семестър

№	*Тема*	*Брой учебни часове*
1.	Инструментални методи за анализ. Електрохимични методи за анализ. Потенциометрия. Видове електроди – индикаторни и сравнителни, йон-селективни и индеферентни. Стъклен електрод. Потенциометрично определяне на pH. Потенциометрично титруване.	**2**
2.	Спектрални методи за анализ. Приложение на молекулните спектри в аналитичната химия – закон на Буге, Ламбер и Беер. Спектрофотометрия – апаратура. Приложение на закона на Буге, Ламбер и Беер в практиката. Метод на стандартната права.	**2**
3.	Атомно-емисионна и атомно-абсорбционна спектроскопия. Приложения на AAC и ICP. Флуоресцентен анализ. Приложения.	**2**
4.	Хроматографски методи за анализ. Класификация на хроматографските методи. Видове хроматография.	**2**
5.	Газова хроматография. Приложение в аналитичната практика. Масспектроскопия. Принцип на метода. Методи на йонизация. Качествен и количествен масспектрален анализ.	**2**
6.	Течна хроматография. Течна хроматография при високо налягане (HPLC). Приложение в аналитичната практика.	**2**
7.	Статистически методи за обработка на резултатите от анализа – средна стойност, възпроизводимост на резултатите, доверителен интервал. Верифициране и валидиране на аналитичните методи.	**2**
8.	Разработване на аналитична процедура – принципи, етапи.	**1**

Лекции втори семестър 15 ч

Упражнения

I семестър

№	*Тема*	*Брой учебни часове*
1.	Правила за работа в химическа лаборатория. Техники в аналитичната химия – лабораторна стъклария, основни операции. Разтвори – разреждане на разтвори, утаяване и прекристализация. Концентрации на разтвори.	**3**
2.	Качествен анализ. Класификация на катионите в аналитични групи. Аналитични реакции на I-ва група катиони; системен ход.	**3**
3.	Катиони от втора и трета аналитични групи - групови утаители, качествени реакции за катиони от двете аналитични групи.	**3**
4.	Катиони от четвърта и пета аналитични групи – провеждане на качествени реакции за катионите от двете групи.	3
5.	Класификация на анионите по аналитични групи. Качествени реакции на някои аниони с биологично значение. Анализ на разтвор, съдържащ различни аниони.	**3**
6.	Протолитична теория. Сила на протолитите – K_a и K_b. Водороден показател (pH). Методи за определяне. Буферни разтвори, буферни криви, буферен капацитет. Приготвяне на буферни разтвори и определяне на pH стойността им.	**3**
7.	Контролна задача върху качествен анализ, pH и буфери. Количествен анализ. Обемен анализ – същност, видове. Стандартни разтвори и изчисления в обемния анализ.	**3**
8.	Киселинно-основен обемен анализ – стандартни разтвори, киселинно-основни индикатори. Титрувални криви. Титруване на силни протолити.	**3**
9.	Киселинно-основен обемен анализ. Титрувални криви.	**3**

	Титруване на слаби протолити и полипротонни киселини. Определяне на адреналин във фармацевтична субстанция.	
10.	Комплексометричен обемен анализ. Титрувални криви. Стандартни разтвори, металохромни индикатори. Титруване на метални йони с комплексон III. Титруване на магнезиев аспартат (Mg-aspartate) и калциев глюконат (Ca-gluconate).	**3**
11.	Окислително-редукционен обемен анализ – видове. Перман-ганометрия - особености на перманганометричното титруване. Определяне концентрацията на оксалова киселина; определяне процентно съдържание на водороден пероксид в перхидрол.	**3**
12.	Контролна задача върху обемен анализ и концентрации на разтвори.	**3**
13.	Окислително-редукционен обемен анализ. Йодометрия – същност на йодометричните определяния, използвани стандартни разтвори и индикатори. Определяне количеството на витамин С във фармацевтични препарати, количествено определяне на глюкоза.	**3**
14.	Киселинно-основно титруване в неводна среда – принцип, използвани разтвори и приложения. Титруване на бензоена киселина и хлорпромазин хидрохлорид в метанолова среда.	**3**
15.	Тегловен анализ.	**3**

Първи семестър 45 часа

II семестър

№	***Тема***	***Брой учебни часове***
1.	Методи за подготовка на пробите за анализ. Разтваряне, концентриране и разделяне. Екстракционни методи. Течно/течна екстракция. Определяне на токсични елементи в захароза. Отделяне и доказване на водо- и мастно разтворими витамини в мултивитаминна таблетка.	**2**

2.	Електрохимични методи за анализ - видове. Потенциометрия – основен закон, използвани електроди, потенциометрично титруване. Определяне на pKa на слаба едноосновна киселина.	**2**
3.	Потенциометрия – провеждане на титруване на полипротонна (фосфорна) киселина. Построяване на експериментална титрувална крива на фосфорна киселина – области с буферно действие и еквивалентни моменти.	**2**
4.	Спектрофотометрия – същност. Основен закон, абсорбционен абсорбционен спектър, метод на стандартната права. Определяне концентрацията на салицилова киселина.	**2**
5.	Приложение на спектрофотометрията за анализ на фармацевтични субстанции. Спектрофотометрично определяне на витамин B_{12}.	2
6.	Приложение на спектрофотометрията за анализ на разтвори, съдържащи повече компоненти. Определяне на акрифлавин и метиленово синьо в общ разтвор.	**2**
7.	Колоквиум върху: методи за пробоподготовка потенциометрия и и спектрофотометрия.	**2**
8.	Хроматография – същност и видове. Принцип на колонна, хартиена и тънкослойна хроматография. Анализ на хранителни багрила по метода на тънкослойната хроматография. Разделяне на Cu^{2+} и Fe^{3+} йони по метода на кръговата хартиена хроматография. Разделяне на екстракт от зелени растения по метода на колонната хроматография.	**2**
9.	Съвременни хроматографски методи. Газова хроматография –принцип на метода, хроматографска система с масдетекция. Приложения на газовата хроматография.	**2**
10.	Високоефективна течна хроматография – принцип на метода. Устройство на течно-хроматографска система с UV и FL - детектори. Приложения. Определяне на кофеин в различни проби.	**2**
11.	Приложение на високоефективната течна хроматография – анализ на витамини в различни проби.	**2**

12.	Колоквиум върху хроматографски методи за качествен и количествен анализ.	**2**
13.	Атомно-абсорбционна спектрометрия – същност на метода, основни етапи на анализа, устройство на атомно-абсорбционен спектрометър. Приложения на ААС.	**2**
14.	Обработка и представяне на експериментални резултати. Грешки при измерванията. Статистическа обработка на резултатите. Представяне на експерименталните данни – таблично, графично и начини на записване.	**2**
15.	Принцип на добрата лабораторна практика. Контрол на качеството – калибриране на измерването и референтни материали. Валидиране на метод.	**2**

Втори семестър 30 часа

Общо лекции: 45 часа

Общо упражнения : 75 часа

IV. ЛИТЕРАТУРА

1. Основи на химичния анализ, авторски колектив, съставител проф. Р. Борисова, изд. „Водолей“, 2009
2. Омуртаг Будевски, Основи на аналитичната химия, четвърто преработено издание, изд. Ариескомерс, 1995.
3. Бончев П.Р., Увод в аналитичната химия, София, НИ, 1979.
4. Ст.Александров, Аналитична химия, София, Университетско издателство, 2005.
5. Ръководство за практически упражнения по аналитична химия за студенти по фармация, под редакцията на О. Будевски, пето преработено издание, Медицинско издателство “АРСО”-ЕТ, София 1999.
6. Р.Христова, Д. Цалев и др., Ръководство по количествен анализ, София, Университетско издателство, 2003.
7. М. Бонева, Сн. Ганчева, Ръководство по качествен анализ, Шумен, Ун.изд. “Кл.Охридски”, 1993.

Учебната програма е приета на Катедрен съвет с Протокол № 29 от 21 май 2013 год.

Изготвил програмата:

доц. М. Станчева, дх

Ръководител Катедра Химия:

доц. М. Станчева, дх

МЕДИЦИНСКИ УНИВЕРСИТЕТ
"Проф. д-р Параскев Стоянов"
Варна

Медицински колеж

Утвърждавам:

РЕКТОР
/проф. д-р А. Клисарова, дмн/

ДИРЕКТОР:
/доц. Хр. Ганчев, дм/

УЧЕБНА ПРОГРАМА

по дисциплината : " Аналитична химия "

включена в учебния план на специалност: "Помощник – фармацевт"

за студентите от I курс

Вид на занятията	Семестър	Хорариум-часа седмично	Хорариум-часа **Общо**
Лекции	II	1	15
Семинарни упражнения			
Практически упражнения	II	2	30
Общо часа			45
Форми на контрол	Текущ контрол Контролни		Семестриален изпит
Кредити (ECTS)			

Варна, 2008

II. Анотация

Курсът по аналитична химия има за цел да запознае студентите с класическите и съвременните методи за качествен и количествен анализ.

В теоретичната част се разглеждат равновесните процеси в хомогенна и хетерогенна среда. Задълбочено са представени киселинно-основните равновесия, процесите на разтваряне и утаяване, както и комплексообразувателните и окислително-редукционните процеси.

В лекционният курс са представени видовете количествения анализ,като основно място е отделено на различните видове обемен анализ – киселинно-основен, окислително-редукционен, комплексонометрия.

Особено внимание е отделено на физикохимичните методи за анализ, които имат първостепенно значение при анализ на лекарствените средства, както и прилаганите методи за разтваряне, разделяне и концентриране в аналитичната химия.

Практическите занятия включват: систематика и качествен анализ на йони в разтвор, класически количествени методи за анализ и съвременни физико-химични методи. Обсъждат се етапите на аналитичното изследване: подготовка на пробата за анализ, избор на аналитичен метод, изпълнение на аналитична задача, представяне и интерпретация на резултатите от анализа. Предвидено е разработване на индивидуални аналитични задачи и представяне на резултатите от анализа.

П Р О Г Р А М А

за лекции и упражнения по аналитична химия

на специалност "Помощник фармацевти"

летен семестър

ЛЕКЦИИ – 16 часа

№	*Тема*	*Брой учебни часове*
1	Предмет и задачи на аналитичната химия. Основни принципи на качествения и количествен анализ. Качествен анализ. Аналитични реакции - основни характеристики. Предварителен и системен качествен анализ. Качествен анализ на катиони и аниони. Произведение на разтворимост.	2
2	Количествен анализ. Видове количествен анализ. Обемен анализ - същност. Изчисления. Киселинно-основен обемен анализ. Титрувални криви. Киселинно-основни индикатори. Приложения.	**2**
3	Окислително-редукционни процеси в химичния анализ. Окислително-редукционен обемен анализ. Перманганометрия. Йодометрия. Приложения.	**2**
4	Комплексни съединения. Видове лиганди. Стабилност на комплексните съединения. Стабилитетни константи Комплексонометрия. Металохромни индикатори.Титруване на метални йони, влияние на pH.	**2**
5	Физико-химични методи за анализ.	2

	Потенциометрия. Потенциометрично определяне на pH. Потенциометрично титруване.	
6	Спектрофотометрия. Приложение на молекулните спектри в аналитичната химия – закон на Буге, Ламбер и Беер. Атомно-абсорбционен анализ. Приложение.	**2**
7	Хроматографски методи за анализ. Видове хроматография. Газова и течна хроматография. Приложение в аналитичната практика.	**2**
8	Контролно върху хроматография и спектрофотометрия.	**2**
9	Обработка на резултатите от анализа.Систематични и случайни грешки. Валидиране на метода за анализ. Статистическа обработка на резултатите от анализа.	**2**

УПРАЖНЕНИЯ – 30 часа

Сед-мица	***ТЕМА***
I.	Правила за работа в химическа лаборатория. Техники в аналитичната химия.
II.	Качествен анализ на катиони. Аналитични групи. Анализ на катиони с биологично значение.
III.	Системен ход на първа група катиони. Качествен анализ на аниони. Приложение на качествения анализ в медицинската практика.
IV.	Количествен анализ. Обемен анализ. Стандартни разтвори и техника на титруване. Индикатори. Изчисления в обемния анализ.

V.	Киселинно-основен обемен анализ. Титрувални криви. Титруване на силни киселини и силни основи.
VI.	Окислително-редукционен обемен анализ. Перманганометрия. Йодометрия.
VII.	Комплексонометричен обемен анализ. Определяне концентрацията на Mg^{2+} и Ca^{2+} при титруване с ЕДТА.
VIII.	Контролно упражнение върху обемен анализ.
IX.	Инструментални методи за анализ. Спектрофотометрия. Метод на стандартната права. Определяне концентрацията на салицилова киселина.
X.	Спектрофотометрия - приложения. Определяне концентрацията на метални йони и на витамини.
XI.	*Потенциометрия. Потенциометрично определяне на pH.* *Потенциометрично титруване.*
XII.	Атомно - абсорбционна спектрофотометрия. Принцип на метода. Приложение.
XIII.	*Хроматография. Видове хроматография. Принцип на хартиената и на тънкослойната хроматография*
XIV.	*Хроматография – газова и течна.Приложение.*
XV.	Течна хроматография. Приложение.

Упражненията са по 2 учебни часа.

Изготвил програмата:

доц. М. Станчева, дх

МЕДИЦИНСКИ УНИВЕРСИТЕТ

“Проф. д-р Параскев Стоянов”
Варна

Ф А К У Л Т Е Т по Ф А Р М А Ц И Я

Утвърдена с Протокол на ФС №13 от 17.05.2011 г.

Утвърждавам:

Декан:

/доц. Д. Димитров, дм/

УЧЕБНА ПРОГРАМА

по избираемата дисциплина : “ Състав и безопасност на храни “
включена в учебния план на специалност: “ФАРМАЦИЯ”
за студентите от I - IV курс,
придобиващи образователно-квалификационна степен “магистър”
с професионална квалификация “ магистър - фармацевт”

Вид на занятията	Семестър	Хорариум-часа седмично	Хорариум-часа **Общо**
Лекции	*	2	30
Семинарни упражнения			
Практически упражнения			
Общо часа			30
Форми на контрол	Текущ контрол		изпит
Кредити (ECTS) - 2			

Варна, 2012

УЧЕБНА ПРОГРАМА

“СЪТАВ И БЕЗОПАСТНОСТ НА ХРАНИ” – СИД

А. АНОТАЦИЯ

Целта на настоящия курс е да представи съвременна научна информация за състав и безопастност на храни. Храната има не само хранителен и енергиен потенциал, но и силата и разнообразието на биологично-активните вещества съдържащи се в нея.

В курсът се разглеждат основните макро- и микронутриенти, минерални вещества и биологично активни съединения съдържащи се в храните. Биологично активните съединения оказват позитивно или негативно въздействие върху физиологичната и клетъчната активност, отразяваща се върху здравето на човека. Храните съдържат голямо многообразие на биологично активни вещества но в малки концентрации.

Основно място в курса е отделено на витамините – химия, биологично значение, безопастност, съдържание в храни. Студентите ще бъдат запознати със съвременни методи за анализ на мастни киселини, витамини и други биологично активни вещества.

Б. РАЗПРЕДЕЛЕНИЕ НА УЧЕБНИЯ МАТЕРИАЛ ПО СЕМЕСТРИ

Семестър	Седмици	Часове седмично	Часове всичко	Теория	Упражнения
	15	**2**	**30**	**30**	

Лекционен курс

1. Състав на храните. Макронутриенти и микронутриенти. Биологично активни съединения и канцерогени. Енергиина стоиност на храните.

2. Въглехидрати – видове. Съдържание на въглехидрати в различни видове храни. Здравен риск при повишена консумация на въглехидрати.

3. Липиди – видове. Мазнини, глицерофосфатиди и гликолипиди. Холестерол – видове. Здравен риск при повишена консумация на храни богати на мазнини.

4. Висши мастни киселини – наситени и ненаситени. Омега – киселини. Конюгирана линолова киселина.
   Транс-масни киселини – влияние върху човешкия организъм. Нови технологии за намаляване на съдържанието им.

5. Аминокиселини. Белтъчни вещества. Съдържание на белтъчни вещества в различни видове храни. Биологична значение.

6. Минерални вещества – макро- и микроелементи. Съдържание в различни видове храни. Биологична значение. Водно съдържание на харните.

7. Витамини. Въведение във витаминологията. Мастно разтворими витамини. Витамин А – химия, биологично значение, безопастност, съдържание в различни видове храни.

8. Витамин Е и Витамин Д - химия, биологично значение, безопастност, съдържание в храни.

9. Водно - разтворими витамини. Витамин С – химия, биологично значение, безопастност, съдържание в храни. Витамини антиоксиданти.

10. Витамини от групата на В (B_1, B_2, ниацин, B_6, B_{12}, фолиева киселина, биотин) - химия, биологично значение, безопастност, съдържание в храни.
    Методи за определяне на водно разтворими витамини.

11. Биологичноактивни съединения в храни – терминология, класификация, безопастност. Примери за биологичнаактивни храни.

12. Каротеноиди – класификация, биологична роля, съдържание в храни. Методи за определяне.

13. Флавоноиди – класификация, биологична роля, съдържание в храни. Методи за определяне.

14. Хранителни добавки.

15. Законодателство в областта на храните.

Лекциите са по два 2 учебни часа.

Практически упражнения

1. Газ – хроматографски анализ за определяне на мастни киселини в черноморски риби. – 6 часа

2. HPLC анализ на витамини. – 6 часа

3. Атомно-абсорбционен анализ на тежки метали в храни. – 3 часа

Изготвили програмата: 

доц. М. Станчева

Медицински университет

“Проф. д-р Параскев Стоянов”- Варна

Медицински колеж

Варна

Утвърждавам:

Директор:

/Доц. д-р Хр. Ганчев, дмн/

УЧЕБНА ПРОГРАМА

по

ОРГАНИЧНА, НЕОРГАНИЧНА И АНАЛИТИЧНА ХИМИЯ

Специалност: “**Медицински лаборант”**
Образователно-квалификационна степен: “професионален бакалавър”
Хорариум: общо 105 часа лекции, 45 часа, упражнения 60 часа
Семестър в който се провежда преподаването: I$^{-ви}$ и II$^{-ри}$
Семестър в който се провежда изпитът: I$^{-ви}$ и II$^{-ри}$

Преподаватели: доц. Любомир Македонски, д-р хн
доц. Мона Станчева, д-р хн

Варна, 2008

АНОТАЦИЯ:

ОРГАНИЧНА И НЕОРГАНИЧНА ХИМИЯ

Основната цел на обучението на медицински лаборанти по органична и неорганична химия е усвояване на знания за pH, колоидни и буферни разтвори и тяхното приложение в лабораторната диагностика. Студентите трябва да придобият умения за приготвяне на разтвори с определена процентна, моларна и нормална концентрация, които ще са им необходими като специалисти при изследванията в клинична, микробиологична, хистологична и други лаборатории. Студентите трябва да усвояват знания за строежа и свойствата на съединения, които се определят в клиничната лаборатория: триацилглицероли, фосфолипиди, стероли, полови хормони, карбонилни съединения, пурини, белтъци, аминокиселини, въглехидрати, хемоглобин, билирубин и други.

Основните задачи на обучението по обща и органична химия са:

- Овладяване на система от обобщени знания по обща и органична химия,
- Развиване на аналитично мислене и способност за прилагане на придобитите знания в лабораторната диагностика
- Усвояване на практически навици и умения за клиничната практика

Обучението по органична и неорганична химия се провежда през I-ви семестър и включва теоретично обучение – лекции и упражнения.

Контролът и оценката на придобитите знания се осъществява чрез тестове, контролни работи, задачи върху приготвяне на разтвори, определяне на pH-стойности. Обучението по органична и неорганична химия завършва с изпит.

АНАЛИТИЧНА ХИМИЯ

Основната цел на обучението на медицински лаборанти по аналитична химия е запознаване на студентите с методите на качествения и количествения анализ в клиничната лаборатория.

Програмата предвижда изучаване на най-важните аналитични реакции за доказване на катиони и аниони. Теоретичните въпроси за усвояване на материала се изучават в курса по обща химия, който предхожда обучението по аналитична химия.

Форми на обучение: лекции и учебни упражнения. Методи на обучение: лекционно изложение, семинари, онагледяване чрез таблици, извършване на опити, демонстрации, проблемност чрез поставяне на самостоятелни задачи.

Контролът и оценката на знанията се осъществяват текущо чрез предварителен контрол на учебни упражнения, чрез тестове, контролни работи и други.

Обучението по аналитична химия завършва с изпит.

РАЗПРЕДЕЛЕНИЕ НА УЧЕБНИЯ МАТЕРИАЛ ПО СЕМЕСТРИ:

Семестър	Всичко часове	От тях		Часове седмично
		теория	упражнения	
зимен	60	30	30	4
летен	45	15	30	3

П Р О Г Р А М А

за лекции и упражнения по неорганична и органична химия

на специалност "Медицински лаборанти"

<u>зимен семестър</u>

ЛЕКЦИИ – 30 ч.

Седмица	Тема	Брой часове
1	Разтвори. Механизъм на разтварянето. Колигативни свойства на разтворите. Неелектролити и електролити.	2
2	Представи за киселини и основи. Протолитична теория. Автопротолиза.Йонно произведение на водата.	2
3	Водороден показател (pH). Методи за определяне на pH. Буфери. Уравнение на Хендерсон-Хаселбах.	2

4	Колоидни разтвори. Строеж и свойства. Разтвори на високомолекулни съединения.	2
5	Окислително-редукционни процеси. Характеристика и основни понятия. Уравнение на Нернст. Скорост на окислително-редукционните процеси. Редокс-катализатори. Особености на биологичното окисление.	2
6	Комплексни съединения. Строеж. Видове. Приложение и медицинско значение на комплексните съединения.	2
7	Въглеводороди – наситени и ненаситени. Строеж, физични и химични свойства. Представители.	2
8	Арени – бензен и хомолози. Строеж, изомерия, физични и химични свойства. Представители	2
9	Хидроксилни производни на въглеводородите – едновалентни и многовалентни алкохоли и феноли.	2
10	Алдехиди и кетони. Химични свойства: нуклеофилно присъединяване, окисление, дисмутация, алдолно присъединяване. Биологично важни представители.	2
11	Карбоксилни киселини – моно- и дикарбоксилни, наситени и ненаситени, мастни и ароматни. Химични свойства. Хидроксикарбоксилни киселини: млечна, ябълчена, винена, лимонена, салицилова. Биологично значение.	2
12	Въглехидрати. Монозахариди – строеж и химични свойства. Представители: глюкоза, фруктоза, маноза, лактоза, рибоза и дезоксирибоза. Витамин С. Полизахариди. Дизахариди – редуциращи и нередуциращи.	2
13	Аминокиселини. Строеж, изомерия и свойства. Белтъци.	2
14	Хетероциклени съединения с един хетероатом: група на пирола, природни пиролови багрила, група на индола,	2

	група на пиридина	
15	Хетероциклени съединения с два хетероатома: група на пиразола и имидазола, група на пиримидина, група на пурина. Стероли. Полови хормони.	2

УПРАЖНЕНИЯ – 30 ч.

Седмица	**Тема**	**Брой часове**
I	Правила за работа в химическата лаборатория. Разтвори. Концентрация на разтворите. Процентна концентрация. Решаване на задачи.	2
II	Разтвори. Концентрация на разтворите. Моларна концентрация. Решаване на задачи.	2
III	Разтвори. Концентрация на разтворите. Нормална концентрация. Решаване на задачи. Приготвяне на разтвори.	2
IV	Водороден показател. Методи за определяне.	2
V	Буферни разтвори.	2
VI	Въглеводороди. Химични свойства на въглеводородите.	2
VII	Хидроксилни производни на въглеводородите (алкохоли и феноли).	2
VIII	Карбонилни производни на въглеводородите (алдехиди и кетони).	2

IX	Карбоксилни и субституирани киселини. Оптична изомерия.	2
X	Въглехидрати. Монозахариди.	2
XI	Въглехидрати. Полизахариди.	2
XII	Хетероциклени съединения.	2
XIII	Хетероциклени съединения.	2
XIV	Контролно.	2
XV	Аминокиселини и белтъчни вещества.	2

П Р О Г Р А М А

за лекции и упражнения по аналитична химия

на специалност "Медицински лаборанти"

летен семестър

ЛЕКЦИИ – 15 ч.

Седмица	***Тема***	***Брой учебни часове***
1	Предмет и задачи на аналитичната химия. Основни принципи на качествения и количествен анализ. Качествен анализ. Аналитични реакции - основни характеристики. Предварителен и системен качествен анализ - основни принципи.	2

	Качествен анализ на катиони и аниони.	
2	Равновесие в разтвори, равновесни константи, коефициент на активност, йонна сила. Равновесие при малко разтворими съединения. Произведение на разтворимост, превръщане на една утайка в друга.	**2**
3	Количествен анализ. Видове количествен анализ. Обемен анализ- същност. Основни изисквания към реакциите, използвани в обемния анализ.Изчисления в обемния анализ. Киселинно-основен обемен анализ. Титрувални криви. Киселинно-основни индикатори.	**2**
4	Окислително-редукционни процеси в химичния анализ.Окислително-редукционен обемен анализ. Перманганометрия. Йодометрия. Приложения.	**2**
5	Комплексни съединения. Видове лиганди. Стабилност на комплексните съединения. Стабилитетни константи Комплексонометрия. Металохромни индикатори.Титруване на метални йони, влияние на pH.	2
6	Физико-химични методи за анализ. Потенциометрия. Потенциометрично определяне на pH. Потенциометрично титруване. Спектрофотометрия. Закон на Буге - Ламбер - Беер. Приложение в аналитичната химия .	**2**
7	Хроматографски методи за анализ.Видове хроматография. Видове хроматография. Газова и течна хроматография. Приложение в аналитичната практика.	**2**
8	Обработка на резултатите от анализа.Систематични и случайни грешки. Валидиране на метода за анализ. Статистическа обработка на резултатите от анализа – средна стойност, възпроизводимост на резултатите, доверителен интервал..	**1**

УПРАЖНЕНИЯ – 30 ч.

Сед-мица	***ТЕМА***	***Брой учебни часове***
XVI.	Правила за работа в химическа лаборатория. Техники в аналитичната химия.	2
XVII.	Качествен анализ на катиони. Аналитични групи. Анализ на катиони с биологично значение.	2
XVIII	Качествен анализ на аниони. Приложение на качествения анализ в медицинската практика.	2
XIX.	Количествен анализ. Обемен анализ Стандартни разтвори и техника на титруване. Индикатори. Изчисления в обемния анализ.	2
XX.	Киселинно-основен обемен анализ. Титрувални криви. Титруване на силни и слаби киселини и основи.	2
XXI.	Окислително-редукционен обемен анализ. Перманганометрия. Йодометрия.	2
XXII.	Комплексонометричен обемен анализ. Определяне концентрацията на Mg^{2+} и Ca^{2+} при титруване с ЕДТА.	2
XXIII.	Контролно упражнение върху обемен анализ.	2
XXIV	Инструментални методи за анализ. Спектрофотометрия. Метод на стандартната права.Определяне концентрацията на салицилова киселина.	2

XXV.	Спектрофотометрия - приложения. Определяне на витамин B_{12}	2
XXVI	*Хроматография. Видове хроматография. Принцип на колонна, хартиена и тънкослойна хроматография. Приложение.*	*2*
XXVII.	Хроматография – газова и течна. Принципи и приложение.	2
XXVIII	*Контролно върху хроматография и спектрофотометрия.*	*2*
XXIX.	*Атомно абсорбционна спектрофотометрия. Принцип на метода. Приложение.*	*2*
XXX.	Автоматичен анализ.	2

ЛИТЕРАТУРА:

5. ХИМИЯ, учебник за студенти по медицина и стоматология
   Л. Дамянова, Ал. Алексиев, Вл. Лесичков, Хр. Киряков
   Изд. Наука и изкуство, София, 1983 и следващите издания.
6. Ръководство за практически упражнения по ХИМИЯ за студенти по медицина и стоматология. Изд. Наука и изкуство, София, 1989 Ал. Алексиев, Л. Дамянова, Б. Чемишев, М. Станчева, Е. Балтова
3. Лекционен курс по химия

Протокол от:

Съвет на колежа: № /

Изготвил програмата:
доц. Мона Станчева, д-р хн
доц. Любомир Македонски, д-р хн

Ръководител катедра:
доц. Мона Станчева, д-р хн

Изготвил резюме на учебни материали:
Доц. М. Станчева