ANALYSIS OF HEAVY METALS AND ORGANIC POLLUTANTS FROM SOME DANUBE RIVER FISHES

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Abstract

This paper presents an analysis of pollution with heavy metals (cadmium, cooper, zinc, lead) and pesticides: lindan, dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyldichloroethane (DDD), dichlorodiphenyltrichloroethane (DDT) from four fish species from Danube River: *Alosa pontica, Cyprinus carpio, Abramis brama* and *Esox lucius.* The concentrations of heavy metals were determined with an atomic absorption spectrophotometric method (air / acetylene flame) and the concentrations of pesticides were determined with a gas chromatograph equipped with an electron capture detector (ECD).

Rezumat

Lucrarea prezintă o analiză a poluării cu metale grele (cadmiu, cupru, zinc, plumb) şi pesticide (lindan, DDE, DDD, DDT) din patru specii de peşti din Dunăre: *Alosa pontica, Cyprinus carpio, Abramis brama* şi *Esox lucius.* Concentraţia metalelor grele a fost analizată cu un spectometru de absorbţie atomică cu flacară aer/acetilenă iar pesticidele au fost analizate folosind o metodă gaz cromatografică cu detector cu captură de electroni.

Keywords: heavy metals, organic pollutants, absorption spectrometer.

Introduction

Heavy metals and organic pollutants are persistent and non-biodegradable and they can be bio accumulated through the biologic chains: soil-plant-food and seawater-marine organisms food. Population can be contaminated with organic pollutants and heavy metals by ingestion of contaminated or polluted food and water. The severity of toxic effects depends on the nature, concentration, body resistance and presence of other contaminants [1, 2, 3]. The concentration of these elements in food products varies, depending on their origin, storage conditions and processing technologies.

This paper presents aspects regarding the pollution with heavy metals and pesticides in four fish species from Danube River: *Alosa pontica, Cyprinus carpio, Abramis brama* and *Esox lucius.* The concentrations of heavy metals (cadmium, cooper, zinc, lead) were determined with an atomic

absorption spectrophotometer SHIMADZU AA 6300 (air / acetylene flame) and the concentrations of pesticides (lindan, dichlorodiphenyldichloroethylene, dichlorodiphenyldichloroethane, dichlorodiphenyltrichloroethane) were determined with a FISONS gas chromatograph equipped with an electron capture detector (ECD) [4].

Materials and Methods

The fish and water samples were collected from two zones of Danube River with important industrial activities: Galati and Tulcea, Romania during August 2010. In order to analyse the concentrations of heavy metals, the fresh tissue samples were washed, hashed, dried at 105 °C for 24 hours and mineralized by the wet digestion method (mixture of 66% HNO_3 and 98% H_2SO_4). All samples were homogenised and 2 g of homogenate were weighed, placed in vessels in the ashing unit with 10 mL 65% HNO_3, 5 mL 37% HCl and 2 mL 35% H_2O_2 and gradually heated (Velp DK-6 Heating Digester) (150 ^{0} C for 1 h, 200 ^{0}C for 2 h, 250 ^{0}C for 1 h, 300 ^{0}C for 2 hours). The solutions were allowed to cool at room temperature, transferred into 25 mL volumetric flasks and diluted to the mark with ultra-distilled water [1, 2, 3, 5].

Aqueous samples (500 mL) were filtered using Whatman No. 41 (0.45 mm pore size) filter paper for the estimation of dissolved metal content. Filtrate and as-collected water samples (500 mL each) were preserved with 2 mL nitric acid to prevent the precipitation of metals. Both samples were tenfold concentrated on a water bath and subjected to nitric acid digestion using the microwave-assisted technique, setting pressure at 30 bars and power at 700 Watts [4].

All used reagents were of analytical grade (Merck). The obtained solutions were analysed with an atomic absorption spectrophotometer SHIMADZU AA 6300 (air / acetylene flame) in order to determine the heavy metals concentration: cadmium (λ = 228.8 nm), cooper (λ = 324.7 nm), zinc (λ = 213.9 nm) and lead (λ = 217 nm). A blank digestion solution was used for comparison. A standard solution for each element under investigation was prepared and used for calibration. Triplicate determinations were performed for each solution. Results are expressed as mean ± S.D (standard deviation) of triplicate analysis. Data were statistical evaluated using student t Test [6, 7, 8, 9].

Pesticide residues were extracted from samples with ether and acetone (total lipid extract from fish tissue) and then were purified on fluorisil column with a layer of anhydrous Na_2SO_4. A total of 10 g fluorisil or aluminium oxide was packed in a glass column with ether of oil.

Pesticides were eluted from the column with ethyl ether / ether of oil in the 20 mL fraction. The fraction was concentrated in KUDERNA-DANISH apparatus for concentrating to about 1 mL.

The water samples were collected from the surface. Samples were collected in 1L glass bottles capped with glass caps and then were filtered before analysis. The extraction was performed in 24 hours from the collection of the samples. In this time the samples were kept at 4 ^{0}C.

An aliquot of 500 mL of water was applied through the separation funnel. Afterwards there were added 25 mL of hexane. After mixing and layering, the upper hexane layer was washed again with 25 mL of hexane. The procedure was repeated four times. The extract was transferred to a LABOROTA 4001 rotoevaporator for concentrating to about 20 mL and then was treated with cooper to remove the sulphur compounds with a SONOREX RK 52 ultrasonication bath.

After that the samples concentrated as described above, were transferred onto fluorisil column with a layer of anhydrous Na_2SO_4 for pesticides detection. A total of 10 g fluorisil or aluminium oxide was packed in a glass column with hexane. Pesticides were eluted from the column with dichloromethane/hexane in the 20 mL fraction. The fraction was concentrated in a KUDERNA-DANISH apparatus for concentrating to about 1 mL.

For the analysis of pesticides it was used a FISONS gas chromatograph equipped with an electron capture detection (ECD) and a capillary chromatograph column filled with a mixture of silicone oils (QF-1, OV-11, XE-60) on chromosorb WHP. Conditions: a 1 μL aliquot of extract was injected; column temperature 200 ^{0}C; injector temperature 210 ^{0}C; detector temperature 250 ^{0}C; carrier gas: nitrogen at a flow rate of 4 mL/min.

The maximum levels for certain heavy metals and organic pollutants allowed in marine water according to Ministry of Health Regulations (No. 1888/2007) [10] are: cadmium 20 μg/L, cooper 100 μg/L, zinc 50 μg/L, lead μg/L, lindan 1 μg/L, dichlorodiphenyldichloroethylene (DDE) 1 μg/L, dichlorodiphenyldichloroethane (DDD) 1μg/L, dichlorodiphenyltrichloro-ethane (DDT) 1 μg/L.

Results and Discussion

The concentrations of heavy metals in the analysed water samples are presented in table I and figure 1:

Table I

Heavy metals concentrations of analysed water samples (µg/L)

Water sample	Cadmium	Cooper	Zinc	Lead
Galaţi	18.4 ± 2.6*	112.30 ± 3.8*	47.14 ± 2.0*	21.44± 1.6*
Tulcea	15.7 ± 4.6*	93.50 ± 2.5*	32.58 ± 1.6*	14.31± 2.5*

*p<0.001

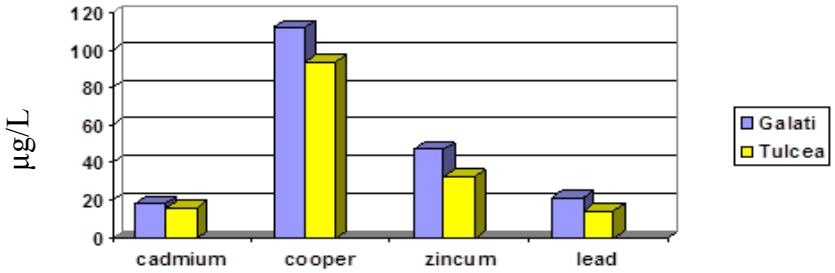

Figure 1.

Heavy metals concentrations of analysed water samples (µg/L)

We remark that the lower concentrations of metals were recorded in water samples collected from Tulcea. The concentrations of Cu (112.30 ± 3.8 µg/L) and Pb (21.44 ± 1.6 µg/L) are over the maximal limits accepted in water samples from Galati.

The concentrations of heavy metals in the analysed fish samples are presented in tables II and III:

Table II

Heavy metals concentrations of analysed fish samples from Galati (µg/g of dry sample)

Fish species	Cadmium	Cooper	Zinc	Lead
Alosa pontica	0.091 ± 1.5*	5.34 ± 3.5*	44.55 ± 2.6*	0.65± 1.4*
Cyprinus carpio	0.084± 2.8*	5.10 ± 4.1*	42.20 ± 1.9*	0.58± 3.1*
Abramis brama	0.053 ± 2.5*	2.77 ± 3.2*	33.27 ± 2.9*	0.27± 1.9*
Esox lucius	0.044 ± 1.7*	2.90 ± 4.2*	23.90 ± 3.7*	0.36± 2.6*

*p<0.05

Table III

Heavy metals concentrations of analysed fish samples from Tulcea (µg/g of dry sample)

Fish species	Cadmium	Cooper	Zinc	Lead
Alosa pontica	0.012 ± 1.8*	3.30 ± 2.3*	41.45 ± 1.6*	0.45± 2.4*
Cyprinus carpio	0.010± 2.5*	3.22 ± 1.1*	39.20 ± 2.9*	0.38± 3.3*
Abramis brama	-	2.15 ± 2.2*	35.77 ± 1.9*	0.29± 1.6*
Esox lucius	-	1.50 ± 2.2*	21.92 ± 2.7*	0.26± 2.5*

*p<0.05

In the fish samples collected from Galati there were found the highest concentrations of heavy metals. In *Alosa pontica* and *Cyprinus carpio* there were recorded the highest concentrations of lead and cooper over the maximal limits accepted in fish samples 0.5 μg/g for lead and 5 μg/g for cadmium.

The concentrations of organic pollutants in the analysed water and fish samples are presented in tables IV, V, VII, and figure 2:

Table IV

Organic pollutants concentrations of analysed water samples (μg/L of dry sample)

Water sample	lindan	DDE	DDD	DDT
Galaţi	1.4 ± 1.6*	0.030 ± 3.2*	-	0.004± 1.2*
Tulcea	0.7 ± 2.5*	0.050 ± 2.5*	-	0.0031± 2.5*

*p<0.001

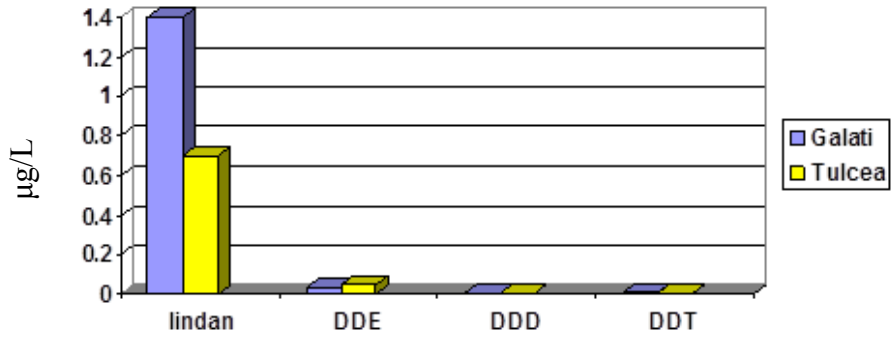

Figure 2.

Organic pollutants concentrations of analysed water samples (μg/L of dry sample)

We also remark that the organic pollutant concentrations are generally higher in the water samples collected from Galati. The lindan (1.4 ± 1.6 μg/L) concentration in the water samples from Galati is over the maximal limit accepted (1 μg/L).

Table V

Organic pollutants concentrations of analysed fish samples (μg/g of wet sample)

Fish species	lindan	DDE	DDD	DDT
Alosa pontica	0.236 ± 1.2*	0.0015 ± 1.3*	-	0.0019± 2.4*
Cyprinus carpio	0.131 ± 2.6*	0.0012 ± 2.1*	-	0.0014± 1.3*
Abramis brama	0.0013 ± 2.1*	0.0007 ± 1.2*	-	-
Esox lucius	0.0017 ± 1.4*	0.0004 ± 2.2*	-	-

*p<0.05

DDE - dichlorodiphenyldichloroethylene, DDD - dichlorodiphenyldichloroethane, DDT – dichlorodiphenyltrichloroethane

Table VI
Organic pollutants concentrations of analysed fish samples (μg/g of wet sample)

Fish species	lindan	DDE	DDD	DDT
Alosa pontica	0.0018± 2.4*	0.0017 ± 2.3*	-	0.0015± 1.4*
Cyprinus carpio	0.0016± 3.3*	0.0005 ± 1.1*	-	0.0008± 2.3*
Abramis brama	0.001± 1.6*	0.0012 ± 2.2*	-	-
Esox lucius	-	-	-	-

*p<0.05

In the fish samples from Galati there were recorded concentrations of organic pollutants higher than the fish samples from Tulcea. Thus, high concentrations of lindan were found in *Alosa pontica* (0.236 ± 1.2 μg/g) and *Cyprinus carpio* (0.131 ± 2.4 μg/g), over the maximal limit accepted (0.002 μg/g).

Conclusions

The results indicate that cooper, lead, lindan and DDT in water and fish samples from Galati are elevated compared to samples from Tulcea. Thus, high concentrations of lindan were found in *Alosa pontica* (0.236 ± 1.2 μg/g) and *Cyprinus carpio* (0.131 ± 2.4 μg/g). In *Alosa pontica* it was found the highest concentration of DDT (0.316 ± 4.8μg/g) and also the highest concentration of lead (0.65 ± 1.4 μg/g) and of cooper (5.34 ± 3.5 μg/g) over the maximal limits accepted. We remark a strong capacity of *Alosa pontica* and *Cyprinus carpio* to concentrate the pollutants from water compared to the other analysed species.

Galaţi is a town with intense industrial activity which can affect the Danube water and the fishes from the river. So, in order to not affect the health of people who consume fish, it is necessary to increase the control over the level of pollutants in the Danube area, near Galati.

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Manuscript received: May 5th 2011