Bioaccumulation of Heavy Metals in Fish from Dobrudja Aquaculture Farm

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In this study, the influence of pH, temperature and dissolved oxygen on the heavy metals distribution in fish tissue were studied. The bioaccumulation factors of fish tissues were calculated. The heavy metals bioaccumulation was compared in gills, scales, livers, tissue for all the selected fish species: carp (Cyprinus carpio), silver carp (Hypophthalmichthys moilitrix), bighead carp (Aristichthys nobilis) and grass carp (Ctenopharyngodon idella). The samples were taken during 2008, in April and October from aquaculture farm, Sarinasuf. The samples are: water, fish muscle tissue, scales, gill and liver. From the morpho - anatomical fish parts, the heavy metals (Cd, As, Pb, Zn) concentrations in the wet samples were measured. For the water samples, the pH, temperature, dissolved oxygen and the heavy metals (Cd, As, Pb, Zn) concentrations were measured. The lowest bioaccumulation factor was computed for cadmium and the highest, for zinc. The maximum concentration of heavy metals was found in scales.

Key words: heavy metals, bioaccumulation, fish, water

Despite the fact that important progress was achieved in developing countries for environmental management, heavy metals remains a huge bane on human health of flora and fauna (biota) [1-3]. Unlike other classes of pollutants, which can be biodegradable and can be completely destroyed, the metals are not biodegradable [4] and none of them can be destroyed. However these metals can be transformed into more toxic forms or complex forms more stable compounds or more or less toxic combination. In an aquatic ecosystem the metals toxicity can be influenced by a biotic variations of environmental factors such as oxygen, water hardness, *p*H and transparency.

According to Rose KA (1993) a biotic factors are defined as variables that exert a direct effect on each individual of a population. Temperature is an important factor which influence metal toxicity because the most aquatic organisms are poikiloterme [5].

Some studies show that increasing the water temperature leads to the increasing of most substances toxicity, substances that are toxic even in low concentrations and can cause the death of the fish [6]. Other factors that can play an important role are: organic matter, carbon dioxide, metabolic activity, period (time) half-life of metal, solid matter, total organic carbon (TOC), the interaction of pollutants, the stages of organism development and specific variations in metals susceptibility. These factors determine chemical metal speciation and the availability to the aquatic biotic composition [7], variations in toxicity that is directly and simultaneously and/or interactive effects on living organisms. These factors influence the metals toxicity so is vital to determine the physical and chemical factors of water.

Internal organization of the metal can play a role in their transfer from high trophic levels by affecting the assimilation efficiency of consumers. Although these ways of eliminating the metals are more abundant than those of absorption, the metal accumulation installs faster than the removal, because of the presence of some proteins that retain metals in aquatic organism tissues [8].

In Romania, the carp (*Cyprinus carpio*) is widespread in the Danube river and its swamps, hilly areas and lowland rivers of larger lakes and canals, dam lakes, ponds. Carp undertake local migrations throughout the year, from the Danube in plashy, when the river is rising and vice versa when the level of stagnant waters decreases and when their temperature increases. The carp prefers clean water, but he adapted also in suspensions and even eutrophic waters. During the winter, carp is in dormancy both in flowing waters and lakes and in the deepest channels. For breeding, carp seeks areas with low levels and clear water, rich in vegetable substrate. Reproduction is triggered at the water temperature about 14-16^0 C during the April to May period. Regarding of diet, carp is omnivorous. He prefers animal food consisting of molluscs. In the Danube, having in mind that in the last decades clam *Dreissena* has multiplied and intensely spread, carp is localized in areas populated mostly by these shellfish colonies. In the DDBR area, carp was and remains an important species for fishing industry, although in recent decades due to the processes of eutrophication and the intensive fisheries the herds have dwindled [9].

The silver carp (*Hypophthalmichthys militrix*) was brought from China in the form of alevini in 1960 and 1962, in order to grow in ponds for human consumption. Many natural lakes were populated. From here, the species was propagated in the Danube and many other large rivers. The silver carp is the most productive species for aquaculture. He prefers slow flowing freshwaters and stagnant, with glossy and stretched free of vegetation, rich in organic micro algae and suspensions, which feed by filtering. In warm seasons he spends most of the time filtering, when he is slowly moving mass of water in/or near the surface water in groups larger or smaller, usually formed by age. Silver carp retain by the gills filter, for feeding particles from 8 to 10 μm, mostly consisting of plankton algae in all categories, including blue, zooplankton and organic detritus. The silver carp is the most productive and economic species because of his better pace of growth than carp and is no need to be feed, consuming phytoplankton mass [9].

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Bighead carp (*Aristichthys nobilis*) has the same geographical area as the silver carp (*Hypophthalmichthys militrix*). Bighead carp was introduced in Europe in the same time with carp and for the same purpose: increasing productivity in ponds and fish ponds, purification of natural reservoirs. Currently, bighead carp is widespread in temperate areas of all continents, except for Australia. In DDBR is widespread in natural waters having a distribution nearly identical to that of silver carp, but he is more rare. His preferred food consists in zooplankton organisms. He has the ability to chemical detect zooplankton fields. In waters poor in zooplankton, his food consists in organic detritus in proportion up to 70%. The sizes of the retained particles are usually in range between 30-100 μm [9].

Grass carp (*Ctenopharyngodon idella*) was introduced in Europe with silver carp (*Hypophthalmichthys militrix*) and bighead carp (*Aristichthys nobilis*) (having the same geographic area with them), in order to increase productivity in ponds and aquaculture and aquatic vegetation removal of some natural pools. In DDBR grass carp is about as rare as bighead carp. Grass carp prefers fresh water bodies, stagnant or slow flowing expanse waters and rich in aquatic macrophyte vegetation. Food is soft aquatic plants, but he consumes all available plant species, from the reed leaves to the filamentous algae. Also grass carp feeds with the aquatic invertebrates and even organic detritus [9].

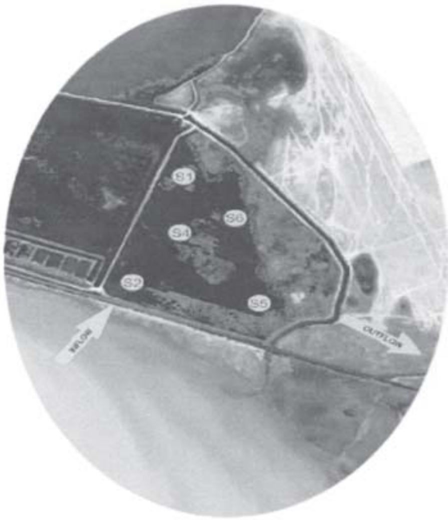

Fig. 1. Sampling points of Sarinasuf area

The Sarinasuf aquaculture farm has water supply natural by Lipoveni channel and also by pumping. The total surface area is 540 ha.

The aims of this study were: to evaluate the influence of physico -chemical factors on the distribution of metals in fish muscle tissue, to calculate and compare the heavy

Table 1
COORDINATES OF SAMPLING POINTS

Sampling points	GPS coordinates
Sarinasuf 1	N 44°99'96''
	E 029°07'98''
Sarinasuf 2	N 44°99'54''
	E 029°08'02''
Sarinasuf 4	N 44°99'96''
	E 029°08'99''
Sarinasuf 5	N 44°99'94''
	E 029°09'10''
Sarinasuf 6	N 44°99'96''
	E 029°08'99''

Table 2
FISH SPECIES AND THEIR CHARACTERISTICS

Sampling point	Period of sampling	Species		Weight (kg)	Length (cm)	Sex
Sarinasuf	april	*Hypophthalmichthys moilitrix*	1	1.10	41.0	male
		Hypophthalmichthys moilitrix	2	1.15	41.5	male
		Ctenopharyngodon idella	1	1.24	42.5	female
		Ctenopharyngodon idella	2	0.84	41.0	male
		Aristichthys nobili	1	0.85	39.0	male
		Aristichthys nobili	2	0.92	40.0	female
		Cyprinus carpio	1	0.54	31.5	male
		Cyprinus carpio	2	0.32	26.5	male
	October	*Ctenopharyngodon idella*	1	3.36	60.0	female
		Ctenopharyngodon idella	2	3.42	58.0	female
		Hypophthalmichthys moilitrix	1	2.42	52.0	male
		Hypophthalmichthys moilitrix	2	2.14	51.0	male
		Cyprinus carpio	1	2.21	46.0	male
		Cyprinus carpio	2	2.22	47.0	female
		Aristichthys nobili	1	2.84	58.0	male

metals bioaccumulation factors in fish muscle tissue and to evaluate the bioaccumulation of heavy metals in some morpho-anatomical fish parts.

Experimental part

Sampling Methods

Our location for water and fish sampling was EC4B basin with an area of 56 hectares. Inside the basin there were established 5 sampling points.

The samples are representative for Sarinasuf farm (fig. 1).

The coordinates were established by GPS and are listed in table 3.

Water Sampling

Samples of the Sarinasuf water lake were collected in accordance with SR ISO 5667/1998. For heavy metals, it was taken 500 mL of water and fixed with 2.5mL concentrated nitric acid. Determination of the total heavy metal concentration is on an unfiltered water sample.

Fish Sampling

For the analysis of heavy metals in the morfo-anatomical fish parts, we collected 2 samples of each species (where was possible). For each fish sample were determined weight, length and sex. The representative sample types were: muscle tissue, liver, gills and scales, which were hacked and homogenized. Selected species and determined characteristics are listed in table 2.

Reagents

All reagents used, have high chemical purity, and are Merck. For the dissolved oxygen, we use: manganese sulphate, alkali-iodide-azide, concentrated sulphuric acid, starch solution, sodium thiosulphate. For the heavy metals extraction we use concentrated nitric acid and hydrogen peroxide, mainly used to increase the oxidizing effect of HNO_3 to destroy matrix residues.

Calibration solutions

For all the heavy metals the flow charts and the calibration curves are made.

The calibration curves were made using the Perkin Elmer Pure Plus Atomic Spectroscopy Standard, certified reference material 10 µg/mL, Multi-element ICP-MS calibration STD.3, matrix 5% HNO_3. The calibration curves are linear and they are made in five points. Using the Excel interface, for each calibration curves, were calculated, the equations and the R^2 coefficients.

The coefficient R^2, for the calibration curves has the values between 0.9994 - 0.9997, that represents a very good correlation between the intensity and the standard concentrations.

Instruments

*p*H and temperature were determined in the field, using the WTW *p*H/cond 340i/SET multi parameter instrument.

Dissolved oxygen in water was determined by titration, using the automatic Metrohm 702SM Titrino.

Microwave digestion, necessary for heavy metals determination was made using the microwave oven Anton Paar, Multiwave 3000.

The heavy metals were analysed using the ICP MS Elan DRC-e. Inductively coupled plasma – mass spectrometry (ICP-MS) is applicable to the determination of small concentrations of a large number of elements. When dissolved constituents are required, samples must be filtrated and acid-preserved before the analysis.

The optimization solution used, was ELAN 6100Setup / Stab./Masscal. Solution. The optimization solution contains the elements: Mg, Cu, Rh, Cd, In, Ba, Ce, Pb, U. The labeled concentrations are 10 ppb for each element.

Sample preparation

The mineralization stage is made differently depending on the type of evidence respecting standards and recommendations suggested by Anton Paar oven manufacturer .

For the water, in vessels of quartz oven Anton Paar we introduced 25 mL of sample and add 6 mL HCl and 2 mL HNO_3. After a short pre-reaction time (10 min), the vessels are hermetically sealed using special device is inserted into the protective sheath and protective cover set and then sit properly in the rotor. It works out after fixing screws that hold the rotor in the position indicated rotor is inserted into the oven. The following meets working memory in the oven. Amounts to 1200 W power in 5.5 min and maintained this power for 4.5 min. The total mineralization is 10 minutes. After completing the program and the cooling time out rotor, open vessels and contents are removed in graduated flask (50 mL) and brought to the mark with bidistilled water.

For the morpho-anatomical fish parts, for the heavy metals determination, in quartz vessels are weighed to the analytical balance 0.25 -0.5 g of fish samples, in the 80 ml quartz digestion vessel, than added 5 mL nitric acids and 2 ml hydrogen peroxide. After 15 min of pre-reaction time, the vessels are hermetic closed with the protective caps, are putting in the protective casings and put into the rotor of microwave oven. After program and the cooling time end, the rotor is draw out, the digestion vessel is open and the content is removed in graduated flask and dilute to 100 mL.

Analysis

Determination of dissolved oxygen in water is based on the reaction of dissolved oxygen in the sample hydroxide manganese (II) fresh precipitate (formed by the additive sodium hydroxide or potassium sulphate with manganese (II)), oxidation and acidification, the compound of high valent manganese in format, with the issuance of an equivalent amount of iodine and the amount of iodine released by titration with sodium thiosulfate.

The ICP MS method measures ions produced by a radio-frequency inductively coupled plasma. Analyte species originating in a liquid are nebulized and the resulting aerosols are transported by argon gas into the plasma torch. The ions produced are entrained in the plasma gas and introduced, by means of an interface into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier.

Results and discussions

The Government Decision nr.202-28 February 2002 contains the technical regulations of surface water that require protection and improvement to support fish life. According with this decision, cyprinid waters are waters that allow or could allow the development of the species of fish cyprinid (*cyprinidae*) or other species.

Reported at this decision, we selected and analyzed *p*H, dissolved oxygen and water temperature in April and October 2008.

The figures 1-3 represent the concentrations variation of indicators selected in the three sampling points.

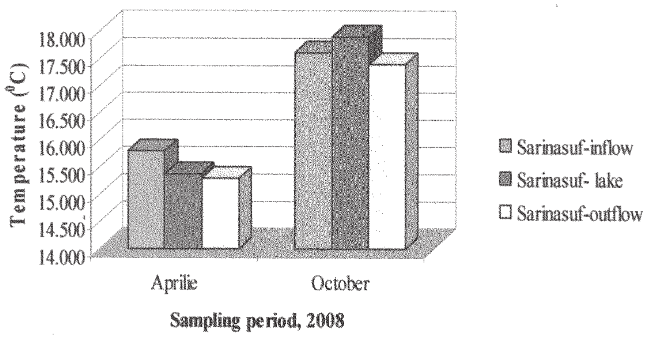

Fig. 2. Sarinasuf, temperature variation values in 2008

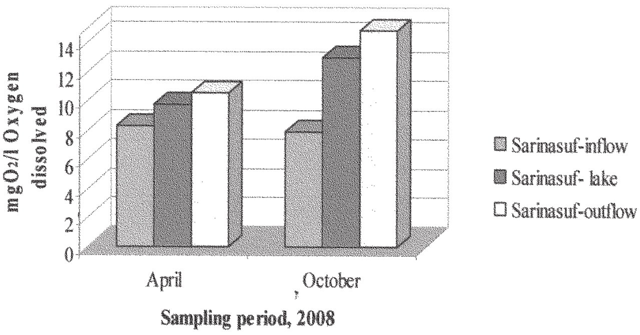

Fig. 4. Sarinasuf, dissolved oxygen concentrations in 2008

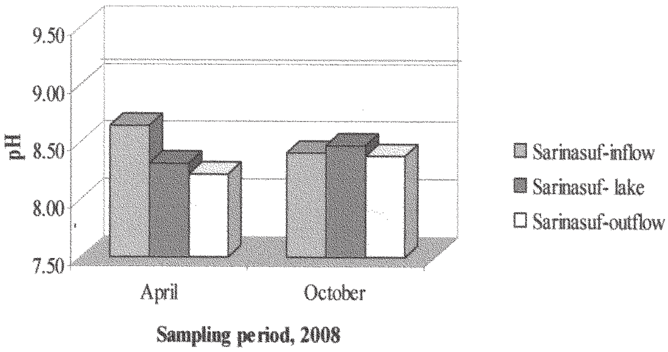

Fig. 3. Sarinasuf, pH variation values in 2008

Values corresponding to the sampling point Sarinasuf lake, represent the average values of the 4 sampling points (north, south, east, west).

Concentrations of dissolved oxygen should not fall below the limit of 4 mg/l. All measured concentrations of dissolved oxygen is above the required minimum of 4 mg/L. Water temperature determined from water samples in spring and autumn, varies in the range of 15.80-17.90 ^{0} C. The optimum *p*H range for cyprinid waters is 6-9 (*p*H units). During the analyzed period, *p*H values of water were 8.23-8.65 field, a field included in the mandatory values at the upper limit.

To study the influence of physical and chemical factors on the distribution of metals in muscle tissue of fish, we took into account dissolved oxygen in water, temperature and *p*H of the water and the metals cadmium, lead, zinc and arsenic in water and fish.

The values are average concentrations (figs.2, 4).

Pearson coefficient values which formed the basis of determining the influence of physical and chemical factors on the distribution of heavy metals in fish muscle tissue, were calculated using the statistical program PRIMER 6 and are listed in the table below (table 3).

Analyzing the influence of *p*H on the distribution of metals in muscle tissue, we obtained negative correlations in all species of selected fish and all metals except for zinc determined in *Hypophthalmichthys moilitrix*.

For the temperature, positive correlations were obtained in almost all species of fish and selected metals except for arsenic determined in *Hypophthalmichthys moilitrix*, zinc determined in *Aristichthys nobilis* and *Cyprinus carpio*, cadmium determined in *Hypophthalmichthys moilitrix*, *Ctenopharyngodon idella* and *Cyprinus carpio*.

Correlations values between dissolved oxygen and metals concentration have negative values, except for lead in Ctenopharyngodon idella and Cyprinus carpio and zinc

Table 3
VALUES OF PEARSON CORRELATION COEFFICIENT

	Temperature	pH	Dissolved oxygen
As- *Hypophthalmichthys moilitrix*	0.496	-0.648	-0.891
As- *Ctenopharyngodon idella*	0.207	-0.309	-0.435
As- *Aristichthys nobilis*	-0.054	-0.791	-0.696
As- *Cyprinus carpio*	0.501	-0.646	-0.924
Pb- *Hypophthalmichthys moilitrix*	0.971	-0.368	-0.425
Pb- *Ctenopharyngodon idella*	0.500	-0.235	0.205
Pb- *Aristichthys nobili*	0.200	-0.822	-0.348
Pb- *Cyprinus carpio*	-0.698	-0.206	0.125
Zn- *Hypophthalmichthys moilitrix*	-0.512	0.003	0.752
Zn- *Ctenopharyngodon idella*	-0.206	-0.150	0.792
Zn- *Aristichthys nobili*	0.318	-0.176	0.337
Zn- *Cyprinus carpio*	0.168	-0.566	-0.473
Cd- *Hypophthalmichthys moilitrix*	0.483	-0.538	-0.941
Cd- *Ctenopharyngodon idella*	0.271	-0.217	-0.482
Cd- *Aristichthys nobili*	-0.064	-0.792	-0.689
Cd- *Cyprinus carpio*	0.537	-0.473	-0.929

Table 4
HEAVY METALS BIOACCUMULATION FACTORS IN FISH MUSCLE TISSUE

	Bioaccumulation factors (BAF)			
	Hypophthalmichthys moilitrix	*Ctenopharyngodon idella*	*Aristichthys nobilis*	*Cyprinus carpio*
Arsenic (mg/kg wet substance)	151	321	141	225
Lead (mg/kg wet substance)	17	24	24	22
Cadmium (mg/kg wet substance)	0.96	2	1	1
Zinc (mg/kg wet substance)	307	170.	130	1207

in Hypophthalmichthys moilitrix, Ctenopharyngodon idella, Aristichthys nobili.

The bioaccumulation factor (BAF) is definited as a ratio of the heavy metal concentration in the living organism and the heavy metal concentration in water (According with the Regulations of The Environmental Protection Agency of USA) [10, 11].

According with the bioaccumulation factor definition, the values for each heavy metal and fish species selected were computed (table 4).

The increasing order of the bioaccumulation factors of heavy metals in fish muscle tissue for all species selected is:

$$BAF_{Cd} < BAF_{Pb} < BAF_{As} < BAF_{Zn}$$

From the morpho-anatomical fish parts there were selected: gills, scales, muscle tissue and liver.

Figure 4 represents the dynamic of the lead average concentrations for the four fish species and selected morpho-anatomical parts. The values determined show that lead is accumulating the most in scales, except for *Cyprinus carpio* which accumulate the most in gills.

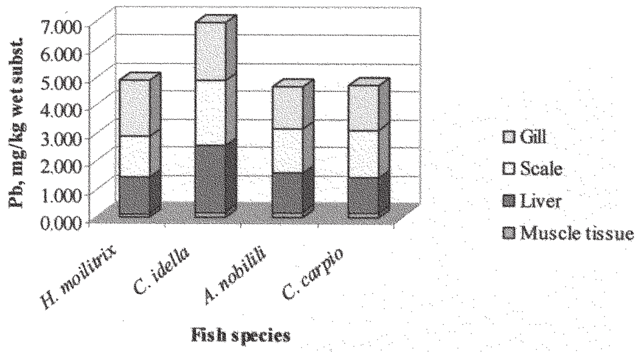

Fig. 5. Accumulation of lead in the morpho-anatomical fish parts

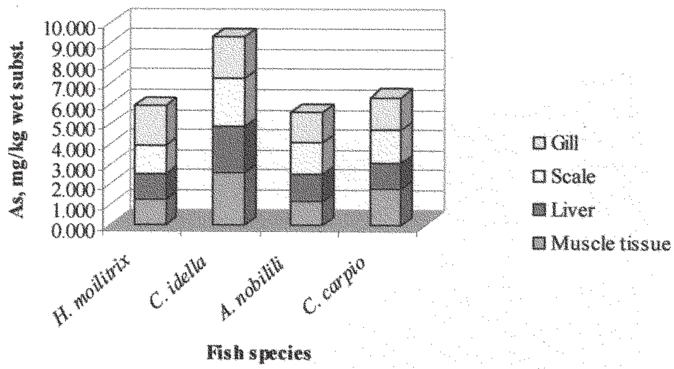

Fig. 6. The accumulation of arsenic in the morpho-anatomical fish parts

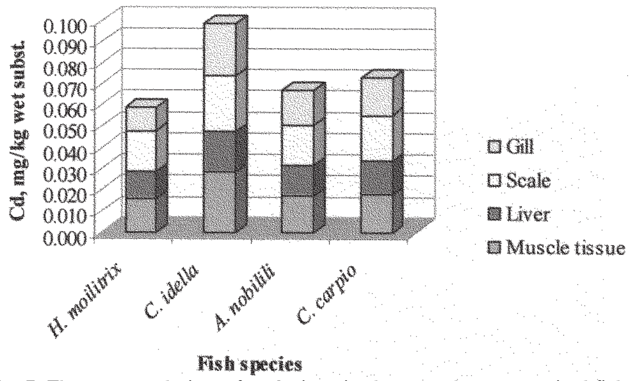

Fig. 7. The accumulation of cadmium in the morpho-anatomical fish parts

Arsenic accumulated in a different way. Thus, the maximum concentrations were obtained in muscle tissue of *Ctenopharyngodon idella* and *Cyprinus carpio* species, the *Hypophthalmichthys moilitrix* in the gills and scales in *Aristichthys nobilis* (fig. 6).

In general, cadmium is accumulating the most in scales. Except for *Ctenopharyngodon idella*, where cadmium is accumulating in most in muscle tissue (fig.7).

Analyzing the average concentrations of zinc, we can see that zinc is accumulating the most in scales, except for *Cyprinus carpio*, that is accumulating more in gills (fig. 8).

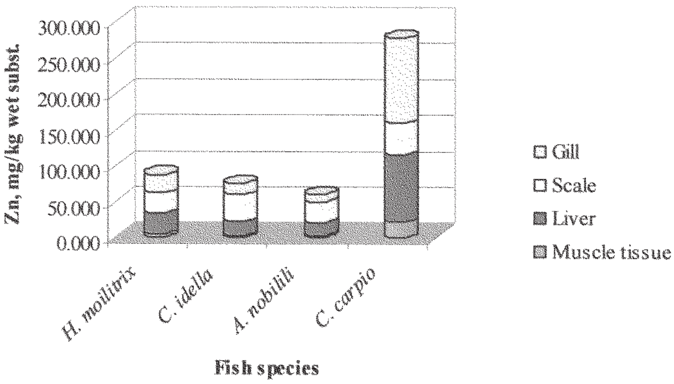

Fig. 8. Accumulation of zinc in the morpho-anatomical fish parts

The fish is often at the top of the food chain and have the tendency to concentrate heavy metals from water [8]. Therefore, bioaccumulation of metals in fish can be considered as an index of metal pollution in the aquatic bodies [12, 13] that could be a useful tool to study the biological role of metals present at higher concentrations in fish [1].

Fish is the major source of heavy metals for birds and mammals.

Accumulation of heavy metals shows harmful for of a number of fish species along the certain stages of life cycle.

Contamination of fish with high lead concentrations decreased the function of the food. Bioaccumulation is the retention of substances (heavy metals) only water through gills or other pathways. Due to their nature, a significant correlation between heavy metal content and weight, length, age and sex of fish - the reported literature - is often very difficult to identify.

To assess the influence of physical and chemical factors on the distribution of heavy metals in muscle tissue of fish, we took into account the following physical and chemical indicators: water temperature, water pH and dissolved oxygen. We selected the heavy metals: cadmium, lead, zinc and arsenic.

The influence of these factors is by direct action on the physiological activity in the changing intensity of metabolic processes, and by acting on microclimate by altering the physico-chemical properties or concentration of pollutants.

At low values of *p*H, the toxicity of heavy metals salts is increasing [14]. The metal concentrations and the values of p*H* should be in an inversely proportional relationship (negative values of the Pearson coefficient), in all species of selected fish and all metals were obtained, except for zinc determined in *Hypophthalmichthys moilitrix*.

The influence of temperature is manifested on the aquatic environment [14].

Thus, it was found that the increasing of temperature has resulted in increasing animal metabolism and increasing oxygen consumption, lower gas solubility in water and decreasing oxygen available in water, increasing gills epithelium permeability and hence, a strong absorption of heavy metals. According with the law of Van Hoff, an increase of 10 degrees of the water temperature within the limits compatible with life, cause a doubling of metabolic intensity and speed of penetration of heavy metals in the body. The metal concentrations must be in direct proportionality relationship with water temperature. In general, we obtained positive correlation.

Water content in dissolved oxygen directly affects heavy metals in water, where they are oxidised. A low concentration of dissolved oxygen in water with high concentrations of metals adversely affect fish, reducing the length of survival through their action on the respiratory organ, gills, reducing gradually their functional capacity, and thus reducing the amount of oxygen absorbed by them. A low concentration of oxygen in water has as effect a low amount of oxygen into the body of fish, so the survival time is limited. Heavy metals are easily oxidising, so a high concentration of dissolved oxygen in water is the consequence of an acceleration of the oxidation of metals, so a decrease in metal concentration in water and therefore an increased fish survival [14].

The fish survival time in a case exposure to a large concentration of heavy metals, decreases with the decrease in dissolved oxygen. In the same time it occurs a decrease in the intensity of oxygen consumption, the increasing of the frequency of respiratory movements. Oxygen saturation, response time is inversely proportional to the consumption of oxygen species. It is expected that with increasing concentration of dissolved oxygen in water, the concentration of metal in the muscle tissue of fish decrease. Negative correlations was obtained at approximately 70% of the metals analyzed.

The bioaccumulation factor is used to describe the chemical compounds accumulation in living organisms, first of all the aquatic organisms, who live in poluted environment. Cadmium has the minimum value and zinc the maximum value. The same trend were observed by Wachs studies in 2000.

The bioaccumulation (at fish) appears only by the heavy metal retention from water by gills or other ways [10].

In fish, lead deposits in active calcification areas such as scales [14, 15]. All our results demonstrate that, except for *Cyprinus carpio,* lead is accumulating in gills.

In general, cadmium and lead concentrations were higher in fish scales than in the other parts of the fish [16]. The cadmium concentrations analysed in morpho-anatomical fish parts, demonstrate that the cadmium is accumulating in scales, except for *Ctenopharyngodon idella*, where cadmium is accumulating more in muscle tissue.

The concentrations of the dangerous inorganic arsenics that are currently present in surface waters enhance the chances of alteration of genetic materials of fish. This is mainly caused by accumulation of arsenic in the bodies of plant-eating freshwater organisms. Birds eat the fish that already contain eminent amounts of arsenic and will die as a result of arsenic poisoning as the fish is decomposed in their bodies. Significant accumulation of arsenic occurred in livers and scales of fish [17].

The selected fish accumulate differently arsenic in liver, scales, gills and muscle tissue.

For *Cyprinus carpio,* Hongwen Sun demonstrates that arsenic is accumulated in intestines, stomach and gills of the fish, and the lowest level of accumulation was found in muscle (Sun and all, 2007). The same trend we observed in the selected morpho-anatomical of *Cyprinus carpio* parts.

The scales appear to be the most important storage muscle tissue of zinc, for *Hypophthalmichthys moilitrix, Ctenopharyngodon idella, Aristichthys nobili.* For *Cyprinus carpio***,** zinc is accumulated more in gills.

The concentrations measured in fish muscle tissue are below limits set by European Union rules for lead (0.2 mg / kg wet substance), cadmium (0.05 mg / kg wet substance), which ensure the conditions of food quality.

Conclusions

Our results suggest that the physical and chemical factors influence the distribution of heavy metals in muscle tissue of fish as follows: in general, the increasing of water pH and oxygen dissolved concentration, determine the decreasing of heavy metals concentrations in fish muscle tissue and the increasing of water temperature determine the increasing of heavy metals concentrations in fish muscle tissue.

For all the selected fish species, the bioaccumulation factor of heavy metals decreases from zinc to arsenic, lead and cadmium.

From the morpho- anatomical fish parts, the scales and the gills accumulated the most of the selected heavy metals: cadmium, arsenic, lead and zinc.

In Sarinasuf aquaculture farm, from the heavy metals point of view, the selected fish species: carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys moilitrix*), bighead carp (*Aristichthys nobilis*) and grass carp (*Ctenopharyngodon idella*) ensure the conditions of food quality.

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