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Chapter 5

VOLGA RIVER: POLLUTION, WATER QUALITY, TOXIC CONTAMINATION AND FISH HEALTH

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ABSTRACT

The characteristic of current state of Volga river is given. Concentrations of organic and inorganic toxic substances in water are reported. Basic clinical and postmortem signs of fish intoxication are described; changes in the cellular structure of their organs and tissues, as well as disturbances in hemogenesis, developing under the effect of toxic agents, are characterized. The main disturbances to fish caused by the accumulation of microelements in their organs and tissues are also considered. Based on dose–effect dependencies calculated with respect to the total concentration of toxic substances, standardized to MPC, and fish health criteria, cases that exceed the critical levels of pollutants are demonstrated for the investigated river sections.

Keywords: Volga River, pollution, water quality, metal bioaccumulation, fish pathology.

INTRODUCTION

The *Volga* is the longest river of Europe. It flows through the western part of the Russia. It is Europe's longest river, with a length of 3,690 kilometres (2,293 miles), and forms the core of the largest river system in Europe. Because of the building of dams for hydroelectric power, the Volga is navigable for most of it's 2,293 km (3,692m) length. The Volga river basin which comprises 40% of the population of Russia, 45% of the country's industry and 50% of its agriculture. The biggest environmental problems stem from major industrial complexes, big dams, large cities and maintaining navigability. The problem being faced now is that this system and all of the associated infrastructure is inefficient. Domestic and industrial wastewaters, air-borne pollution of the catchment area, as well as non-sewerage effluents from settlement areas find their way to this water basin. Several studies have proved the contamination of water and accumulation of heavy metals, oil products, polycyclic aromatic hydrocarbons, polychlorirozak biphenyl's, dioxins, and other chemical compounds in bottom sediments (especially in the places of industrial effluents discharge) (Anthropogenic Impact…, 2003; State Report…, 2002, 2003). Water quality problems are most severe in European Russia, especially in the Volga Basin. Of all water withdrawn from natural sources in Russia, 33 percent comes from the Volga. About half of that water returns to the Volga as polluted discharge, accounting for 37 percent of the total volume of such material generated in Russia. The Volga's water does not meet the norms for drinking water and is unsuitable for fish farming or irrigation. The data on water contamination with toxic substances in the Volga River basin are discrepant due to several reasons (different time periods used for the analysis, non-coinciding sampling points, insufficient capacity of measurement instruments, etc.). In the late 1980s and early 1990s, numerous government committees were formed to clean up the Volga. Few of the resulting restorative programs have been implemented, however, and the Volga remains under ecological stress. Lately, due to the overall economic crisis and general industrial decay in the country, the input of pollutants to the Volga River has largely decreased. However, certain studies show that the level of water contamination remains high (Rozenberg, Krasnoshchekov, 1996; State Report…, 2002, 2003). One of the ecological consequences of water contamination with toxic substances in the Volga River basin and unsatisfactory water quality are very frequent cases of fish intoxication. Analysis of scientific papers dealing with the concentration of toxic substances and morbidity in fish in the Volga River basin shows that the situation is alarming. In 1965–1974, 334 cases of mass death of fish have been registered; in 1975–1985, 574 cases; 1986–1988 witnessed mass death of fish caused by 200 emergency and unit discharges of pollutants (Rozenberg, Krasnoshchekov, 1996).The attention of numerous researchers was attracted by very frequent incidences of myopathy (muscle exfoliation) and eggshell weakening in Volga–Caspian sturgeon. In spite of appreciable scientific and practical interest to the problem of the Volga River contamination with toxic elements and diseases in fish caused by such contamination, there are no system studies aimed at assessing the ecotoxicological situation within the investigated river basin.

Main objectives of study:

- To identify the modern levels of contamination of the Volga River water by toxic substances – metals and organic micropollutants;
- To study the accumulation of metals in fish as a consequence of increased concentration of metals in the water environment;
- To reveal the main pathological manifestations of chronic intoxication in fish of the Volga River;
- To assess the ecotoxicological consequences of increased toxic elements in water and the ecosystem health of Volga River on the basis of the pathological investigation of fish;
- To discuss critical levels of water pollution, and compare them with existing levels of pollution.

MATERIALS AND METHODS

In August and September of 2000–2002, comprehensive studies were carried out in 13 sections of the River Volga (Figure 1): the Ivankovskoe reservoir (I, II, III) of the Upper Volga; the Gorkovskoe (IV, V, VI) and Kuibyshevskoe reservoirs (VII, VIII) in the Middle Volga; and the course (IX, X, XII) and delta (XII, XIII) of the Lower Volga. Water was sampled for determination of the concentrations of toxic substances (metals and toxic organic compounds). Fish were examined to study their physiological state in order to reveal different forms of pathology and organ dysfunction. Water samples were always taken at the precise sites where fish were caught for examination. The bream *Abramis brama* (L.), the most widespread fish species in the Volga River basin, was used as a bioindicator. It is a benthic fish that does not make long-distance migrations, which enabled the collection of material for examination from limited sections of the river.

Figure 1. Location of the sections on the Volga River where the investigation was carried out.

Water chemistry. In total, 31 water samples were taken in 13 sections of the Volga River and reservoirs. Water samples were collected into Nalgen® Polyethylene bottles (1l and 60 ml). Bottles were cleaned in the laboratory and rinsed twice with lake water before sampling. After sampling, all samples were kept cool (approximately +4°C) in dark containers and were delivered to the laboratory within 1–3 days.

The analyses carried out on the water samples were as follows. The pH was measured using a Metrohm®pH-meter; conductivity (20°C) by Metrohm®-conductivity; alkalinity using the Gran titration method; and natural organic matter content by the Mn oxidation method. Microelement concentrations were determined using the atomic-absorption in graphite furnace method (GFAAS, "Perkin-Elmer - 5000" model, HGA-400, AAnalyst-800, Corp., Norwalk, USA). Hg was determined using atomic fluorescence (Fl, model Merlin®). Standard solutions with appropriate concentrations for each element were made from 1000 ppm AAS stock standards (Merk, Darmstadt, Germany). In addition, for determination of Hg, Mo, V, Se elemental analysis of the water was carried out by the inductively coupled plasma method using a "Plasma Quad 3" mass-spectrometer manufactured by Fisons Electronic Elemental Analysis (United Kingdom).

"Acidic" and "alkaline" extractions of the water samples (in glass bottles) were obtained with methylene chloride under field conditions. Concentrations of organic micropollutants in these extracts were determined by gas chromatography using a "QP-5000" chromate-mass-spectrometer manufactured by Shimadzu (Japan). The quality of the analytical results was repeatedly tested by intercomparisons during the course of the project (Hovind, 2000; 2002; Makinen, 2002).

An integrated impact dose is determined by summing the excess for each revile concentration of toxic compound to their $MPC_{fishery}$ as follows:

$I_{tox-1} = \sum(C_i / MPC_{fishery.})$

I_{tox} is the integrated toxicity index; C_i is concentration registered in water; $MPC_{fishery}$ is MPC for toxic substances accepted in Russia for fishery and aquatic life.

According to Russian rules of water protection, the water quality may be considered good if I_{tox} is no more than one ($0 < I_{tox-1} \leq 1$).Water quality may be considered good if I_{tox-1} is no more than one.

*Bioaccumulation***.** For determination of the metal content of the bodies of fish, subsamples from a minimum of five individual fish from every site were collected from the gills, liver, kidneys, muscle and skeleton. Samples of fish organs and tissues for metal analyses were dried to their constant weight at 105°C. Dry samples were prepared for analysis by wet digestion in ultrapure nitric acid (10 ml acid for 1 g of tissue). The content of Ni, Co, Cd, Cr, Mn, Pb, Cu, Zn, Al, Sr in fish was determined on an atomic absorption spectrometer, using a graphite furnace HGA-400. Duplicate analyses were used for the purpose of quality control.

In analyzing essential elements (Cu, Zn, Co) additional information about climatic variation along Volga River was also used, that is sums of annual temperature exceeding +10°C taken from climatic map.

Fish pathology. This was aimed at revealing the effects of toxic substances. Fish were studied at 13 river sections; the minimum number of fish observed was 50 of the same age (from 4+ to 6+ years old); all were free of internal parasites in the time period of the investigation (August and early September). Blood samples are taken from live fish tail artery using methods described elsewhere. In the blood samples thus taken, hemoglobin concentration, erythrocyte sedimentation rate (ESR), erythrocyte and leukocyte concentration. Blood smear examination allows the analysis of red blood composition, differential blood count, and the detection of occurrence of pathologic blood corpuscles. (Ivanova, 1976; Krylov, 1980). Macrodiagnostics to determine fish health were carried out under field conditions. The clinical and pathological anatomical signs of intoxication and any abnormalities were documented on the basis of visual examination of the fish during the first hour after fishing.

In the process of visual examination, special attention paid to the following: the intensity of color, the state of pigment (cells–melanophores); the total amount of mucus on the fish body; the state of squama, opercula, oral cavity, anus; the cases of hyperemia, subcutaneous hemorrhages, sores, or hydremia of the body; deformation of skull and skeleton bones; the state of eye crystalline lens and cornea. When the opercula are opened, branchiae are examined, in particular, their color, the presence and the amount of mucus, the state of branchial petals (accretion, adhesion, dilatation, or thinning down). After the abdominal cavity is dissected, the state of fish muscles is studied (color, consistence, hemorrhages, attachment to bones), as well as the presence of exudate in the abdominal cavity, the amount of cavitary fat, its color and density. The topographic location of viscera (liver, kidneys, gonads, spleen, heart, stomach, intestines), their dimensions, color, density, edges, hemorrhages, zones of necrosis, etc. are studied. Mucous membranes of dissected stomach and intestines are examined, in addition to cerebrum, paying special attention to filling of vessels, their color and density. For more precise microdiagnostics, the organs of fish with overt signs of pathology were removed for histological analysis. Histological sections were prepared in the laboratory according to the standard method (Bucke, 1994). For satisfactory histological preparations only freshly killed fish were considered. Gills, kidneys, liver, and gonads were handled rapidly to prevent degenerative changes within the specimen. They were carefully dissected from the body, cut into blocks of $<1\ \text{cm}^3$ and placed in a fixative (Bouin's fluid). Histopathological alterations of organs were evaluated under a light microscope (450×). Diagnosis of disease was confirmed on the basis of histopathological observations. The percentage of sick fish in the stock of each local polluted zone was documented. Fish were detected at various stages of disease ranging from initially insignificant pathological organ changes to serious compromise of the organism. In the process of macrodiagnostics, three stages of disease can be identified (0 denotes healthy individuals):

(1) Low-level disturbance, not threatening the life of the fish;
(2) Medium-level disturbances, causing a critical state in the organism;
(3) Distinct signs of intoxication leading to inevitable death of the organism.

The overall index of morbidity in fish in a given zone of contamination can be presented as:

$$Z = (N_1 + 2N_2 + 3N_3)\,/N_{tot}.$$

Here Z is the morbidity index for fish, $0 \leq Z \leq 3$; N_1, N_2, and N_3 are the numbers of fish in the first, second, and third stages of the disease, respectively; and N_{tot} is the total number of fish examined in the local contamination zone, including healthy individuals. If none of the fish in a given body of water demonstrates any signs of intoxication, then Z = 0. The value of Z will increase with an increase in both the number of sick fish and the severity of their diseases.

Statistics. Statistical data processing was carried out using the regression analysis; the significance of correlation coefficients was determinated by *t*-criteria.

CHARACTERISTIC OF VOLGA BASIN AND ANTHROPOGENOUS LOADS

Geography and Hydrology. The Volga Basin comprises four geographical zones: the dense, marshy forests; the forest steppes; the steppes; and the semi-desert lowlands. It rises in the Valdai Hills of Russia, 225m above sea level north-west of Moscow. It also passes through a chain of small lakes. The Volga and its tributaries form the Volga river system, which drains an area of about 1.35 million square kilometres in Russia**.** The course of the Volga is divided into three parts: the upper; the middle; and the lower Volga. Starting as a small stream, it becomes a bigger river when it is joined by some of its tributaries. The major tributaries are the Oka, the Belaya, the Vyatka, and the Kama, each of which is longer than 1 000 km and has a catchment area exceeding 100 000 km^2.

The variation range of water discharge is great: from13420 m^3/c in flood time, 94 m^3/c in winter low water and 188 m^3/c in summer low water in the Upper Volga to 56500 m^3/c in flood time, 380 m^3/c in winter low water and 600 m^3/c in summer low water in the Lower Volga (Edelstein, 1998).

The Volga's flow is regulated by reservoirs. They accumulate about 70% Volga's flow. The morphometric characteristics of reservoirs are represented in the Table 1. The fierst two of them are included in the Upper Volga, the next tree – in the Middle Volga and the last two – in the Lower Volga. The Oka river falls after the Gorkovskoe reservoir and increases flow in two times, also the Kama river falls into the Kuibyshevskoe reservoir and increases flow in two times.

Table 1. The main characteristics of reservoirs in Volga river (compiled from (Edelstein, 1998))

Reservoir	Year of creation	Volume, km^3	Area, km^2	Maximum depth, km	Length, km	Volume water dropping through dam, km^3/year	Coefficient of water cycle, 1/year
Ivankovskoe	1937	1.1	327	19	120	9.2	7.9
Ribinskoe	1941	25.4	4550	28	112	30.1	1.4
Gorkovskoe	1955	8.8	1590	22	430	46.8	6.0
Cheboksarskoe	1981	4.6	1080	13	340	109.5	24.3
Kuibyshevskoe	1957	57.3	5900	41	510	234.9	4.2
Saratovskoe	1969	12.9	1831	33	336	230.6	19.1
Volgogradskoe	1961	31.4	3117	41	524	236.1	8.0

The Volga's major distributary, the Akhtuba, runs parallel to the main river on its way towards the Caspian Sea. Above Astrakhan, the Buzon River, another main distributary of the Volga, marks the start of the Volga Delta. The mouth of the river is situated on the Caspian Sea at 28m below sea level. As the Volga approaches the Caspian Sea it divides into a delta comprised of about 275 channels covering about 12000 km^2. The Volga Delta has a length of about 160 kilometres. It includes 555 channels and small streams. It is the largest estuary in Europe. It is the only place in Russia where pelicans, flamingoes, and lotuses may be found. The Volga freezes for most of its length for three months each year. Some of the biggest reservoirs in the world can be found along the river.

At the Caspian Sea the Volga is an important source of water for the sea and its famous sturgeon fishery. The Beluga sturgeon is the largest fish found in the Volga. But the water that flows into the Caspian has been used many times upstream by the factories and the farmers.

Anthropogenic impacts. Over half of Russia's industry is located within its drainage. The biggest environmental problems stem from major industrial complexes, big dams, large cities and maintaining navigability. Although the extensive development of the Volga has made a major contribution to the Soviet economy, it also has had adverse ecological consequences. The Volga basin is under pressure from human activities, industrial waste and chemical pollution being the most serious.

The industrial potential of the Volga basin is high and represent all industrial sectors. The most dangerous chemical and petrochemical industries come to the front, in Volga basin main capacities of oil processing (60%) and petrochemistry (70%) of Russia are concentrated. The central region specializes on production of plastic, chemical fibres, lacquers and paints, synthetic dyes, goods of home chemistry, the southern region – mineral fertilizers, caustic soda, polyvinylchloride and caprolactam. 14 enterprises produce pesticides. Should be noted that in 2000 9.3 million tons agricultural production were tested on pesticides and 47 thousand tons (or 0.35%) ones contented pesticides above permitted concentration (Rozenberg, 2009). The machine-building complex comes to the front too, but from 1991 to 2000 emissions of contaminations to the atmosphere have reduced in about 4.5 times and dump of contaminations to Volga – in about 3 times. The fuel and energy complex give maximum emissions to the atmosphere which have reduced in 1.7 times by 2000 (Rozenberg, 2009). The metallurgical complex don't be a ruling one in the Volga basin. The forest, woodworking and pulp and paper industries allocate in the north of Volga basin. Total and comparative dump of polluted water from all industrial source into Volga river are presented in the Table 2. Although the dump of polluted water has been reduced about on one third by 2000, load of wastes per unit of area and per one inhabitant has remained still high as compared with all Russian territory.

Table 2. Total and comparative dump of polluted water into Volga river (compiled from (Rozenberg, 2009))

Region	1991			2000		
	Total from all pollution source	Per unit of area, m^3/km^2	Per one inhabitant, m^3/person	Total from all pollution source	Per unit of area, m^3/km^2	Per one inhabitant, m^3/person
Upper Volga	901	3633	241	496	2000	133

Region	1991			2000		
	Total from all pollution source	Per unit of area, m^3/km^2	Per one inhabitant, m^3/person	Total from all pollution source	Per unit of area, m^3/km^2	Per one inhabitant, m^3/person
Middle Volga without Oka and Kama	4005	7783	218	2533	4923	138
with Oka	9844	13357	238	5997	8137	145
with Oka and Kama	11221	7484	219	7028	4688	137
Lower Volga	1234	4330	158	913	3202	116
All Volga Basin without Oka and Kama	6140	5861	205	3942	3763	132
with Oka and Kama	13356	6572	213	8437	4151	134
Russia	27798	1628	16	20291	1188	12

But most pollution in the Volga River watershed comes from nonpoint sources, or sources that are not easily traced back to a specific "point" like a wastewater treatment or industrial plant. In the Volga River watershed, nonpoint sources include areas used to land-apply manure, feedlots and pastures, and improperly connected or failing septic systems. Rainwater and snowmelt can wash waste from livestock (confined and pastured), pets, and wildlife into the river. To reduce the amount of fecal matter reaching the river, changes in waste and land management will be needed. It will take time to make these changes and to see the effects.

The system of dams and reservoirs has blocked or severely curtailed access for such anadromous species as the beluga sturgeon (famous for the caviar made from its roe) and whitefish (*belorybitsa*), which live in the Caspian Sea but spawn in the Volga and other inflowing rivers, and it has fundamentally altered the habitat of the nearly 70 species of fish native to the river. These changes—along with pollution by industrial and municipal effluents.

As a result of climate change and an increase in the Volga's water temperature, fish like Kilka, a small, Caspian herring, have spread out. This shows that global warming does have a big impact on the river's ecosystem. More than 200 new species now live in the Volga permanently.

WATER CHEMISTRY AND CONCENTRATIONS OF TOXIC SUBSTANCES IN WATER

Water chemistry. Water chemistry not much differs along Volga river. pH values indicate a neutral reaction (the variation range is 6.2-8.0). Mineralization of water is low juding on electroconductivity which slightly increase on averige from 195 μSm/cm in the Upper Volga to 364 μSm/cm in the Lower Volga (Table 3).

Table 3. Concentration of microelements and their MPC values (μg/l) and also pH, conductivity (χ), calcium in water of Volga river (dash denotes the values below the detection limit; here and in tables 2, 3, 4, X is the average value; Min is the minimum value; Max is the maximum value)

Parameter,	Upper Volga		Middle Volga		Lower Volga		MPC
element	X	Min-Max	X	Min-Max	X	Min-Max	
pH	7.7	7.5-7.9	6.8	6.2-7.0	7.4	6.8-8.0	
χ, μSm/sm	195	165-268	255	226-280	364	357-387	
Ca, mg/l	29	25-40	36	32-38	35	32-37	
Hg	<0.05	-	<0.05	-	<0.05	-	0.01
Cd	0.13	0.08-0.20	0.13	<0.02-0.62	0.13	0.02-0.26	5
Pb	0.46	0.02-0.80	<0.02	-	1.72	1.00-3.20	6
Al	272.3	190-400	31.7	8.20-70.9	820.5	440-1480	300
Sr	101.1	85.0-120	190.8	95.7-289	521.3	469-568	400
Ni	1.78	1.20-2.50	1.72	<0.5-5.58	2.22	1.60-3.30	10
Mn	101.6	72.0-150	62.4	12.9-111	27.7	22.7-35.7	10
Zn	3.73	1.30-5.40	1.36	<1-5.11	5.58	2.60-8.70	10
Cu	2.14	1.20-3.80	2.21	0.94-5.68	1.70	1.30-2.30	1
Cr	0.83	0.29-1.70	0.75	0.49-1.18	0.60	0.47-0.75	70
Co	0.31	<0.2-0.60	0.16	0.14-0.19	0.60	0.30-1.30	10
As	2.8	1.8-4.7	1.0	0.7-1.4	1.8	1.2-3.2	50
Mo	0.20	0.16-0.23	0.62	0.43-0.82	0.43	0.38-0.49	1
V	1.36	0.98-1.65	1.21	0.85-1.71	2.35	1.95-2.59	1
Se	0.50	<0.5-0.55	0.84	<0.5-1.16	0.81	<0.5-1.17	2

The calcium and hydrocarbonates dominate in the ion composition, although in the southern region – Lover Volga concentration of more mobile aquatic migrants Na^+, Cl^- and also SO_4^{2-} increase till 15, 26 and 40 mg/l accordingly. The average values of permanganate oxidation and colour index are distributed as follows: in the Upper Volga – 18 mgO/l and 97^{o}Pt-Co, in the Middle Volga – 10 mgO/l and 48^{o}Pt-Co and in the Lower Volga – 15 mgO/l and $<40^{o}$Pt-Co. That means contration of both easy oxidizable organic matter and total and bioavailable nutrients increase toward southern region.

Metals and metalloids. The microelement concentrations in the water were relatively low in the investigated river sections: the concentrations of Mo, Cd, Co, and Cr were less than 1 μg/l, those of Se and Pb varied from less than 1 to 1.7 μg/l, those of Ni, V, and Cu varied from less than 1 to 2.8 μg/l; the concentration of Zn varied from 1 to 6.2 μg/l; and that of As from 1 to 4.2 μg/l (Table 3). Relatively high concentrations of Mn and Sr were observed. The concentration of mercury did not exceed the accuracy of its determination using our technique (< 0.05 μg/l).

Relatively low concentrations of the investigated elements (especially Zn, Ni, Cd and Cu) can be explained by the absence of ferrous and non-ferrous metallurgical plants in the region under consideration, as well as by the overall decrease in the level of Volga River water contamination observed after the recent economic crises.

Comparison of the element concentrations in the Volga River with the respective "background" values for overland flow in European Russia (Petrukhin *et al.*, 1989; Burtseva *et al.*, 1991) showed that the concentration of As was higher than its "background" value in all the investigated areas; the concentrations of Ni and Cd exceeded the background level near the dam of the Kuibyshevskoe Reservoir; whereas the "background' concentrations of Cu and Se were exceeded in the central part of the Gorkovskoe Reservoir.

It is a well-known fact that zones of atmosphere and land contamination can be found within the catchment areas of the Kuibyshevskoe, Saratovskoe and Volgogradskoe reservoirs, as well as in the Lower Volga (Anthropogenic impacts, 2003). This probably explains the exceeding of "background" concentrations by such elements as V, Se, Pb, Ni, and Co. The concentration of Mn in the Ivankovskoe and Gorkovskoe reservoirs, as well as the concentrations of V and Cu in the Kuibyshevsoe Reservoir, the Lower Volga and the Volga River delta, were higher than the respective MPC values (List of Fishery standards, 1999), established for fishery water bodies.Thus, the pattern of element concentration distribution within the investigated areas reflects, primarily, overall diffuse pollution, which is formed against the background of the natural geochemical input of microelements –and is mainly due to pollutant discharge by fuel and energy plants and general economic activity within the catchment area.*Toxic organic compounds.* Dangerous organic substances include oil products, cyclohexane and cyclopentadiene and their derivatives, sebacic acid ether, xylene, phthalates, and dioxanes (Table 4). A high level of water contamination with alkyl derivatives of dioxane was revealed in the Gorkovskoe Reservoir.

Owing to large-scale application of polymer products, phthalates (used as plasticizing agents), and xylene (used for phthalic acid production) were observed in all the investigated sections of the Volga River (especially in the Gorkovskoe Reservoir and the delta downstream of Astrakhan). Dibutyl phthalate, whose concentration in the water varied from 1.3 to 55.7 μg/l (its MPC for fisheries is only 1 μg/l), deserves special attention.

Table 4. Concentration of toxic organic substances and their MPC values (μg/l) in water of Volga river

Toxic organic substances	Upper Volga		Middle Volga		Lower Volga		MPC
	X	Min-Max	X	Min-Max	X	Min-Max	
Hydrocarbons of oil products:							
Alkanes	12.0	1.45-19.3	31.4	6.2-114	10.0	0.9-17.7	
Alkenes	0.1	0-0.4	1.6	0.7-4.0	0.5	0.2-1.8	
Total	12.1	1.45-19.7	33.0	6.2-118	10.5	0.9-19.5	50
Monatomic saturated alcohols	0.8	0-2.0	1.3	0-5.1	1.1	0.1-4.6	500
Ethers of carboxylic acids:							
Dioctyl cebacate	3.9	0-11.6	0	0	0	0	1
Toxic organic	Upper Volga		Middle Volga		Lower Volga		MPC

substances							
	X	Min-Max	X	Min-Max	X	Min-Max	
Carbocyclic compounds:							
Cyclohexane and its derivatives	0	0	0	0	0.1	0-0.8	10
Cyclopentadiene and its derivatives	0	0	0	0	0.4	0-1.0	10
Aromatic compounds:							
Xylene	0.3	0-1.0	1.3	0-2.5	0.5	0.2-1.3	50
Isopropyl benzene	0	0	0	0	0.1	0-02	100
Orthophthalic acids ethers:							
Dibutyl phthalate	2.8	1.3-4.5	25.2	5.7-55.7	32.1	9.6-44.5	1
Dioctyl phthalate	11	4.0-17.7	18.6	0-47.2	1.1	0.5-2.3	10
Heterocyclic compounds:							
Derivatives of 1,3-dioxane	0	0	27.6	2.0-81.7	0	0	10
Sum of chlororganic pesticides (DDT, DDE, α-hexachloran, γ-hexachloran)	0	0	0.01	0-0.04	0	0	0.01

The MPC for oil products was not exceeded in the investigated sections of the Volga River except near the dam of the Gorkovskoe Reservoir, which is probably affected by effluents from petrochemical enterprises located upstream of the Gorkovskoe Reservoir near Yaroslavl city.

Dangerous substances such as chlororganic compounds were not found in the investigated sites, which could be explained by their absence from the water or by their low concentrations (not exceeding the "sensitivity threshold" of the applied method). Analysis of scientific papers and data collected by the Hydrometeorological Service of Russia has shown that, in individual samples of Volga water, the concentrations of certain dangerous substances (such as DDT, DDE, alpha-hexachloran, and gamma-hexachloran) exceed the MPC. In 2002, these substances were also found in the Kuibyshevskoe Reservoir (State Report, 2003).Thus, both organic and inorganic pollutants, for which the Toxicological Harmfulness Value has been established, are found in the Volga River water.

METALS IN FISH AS A REFLECTION OF WATER POLLUTION

The concentrations of metals in fish can reflect levels of pollution more accurately than the indices of contaminant content in water (Moor, Rammamoorthty, 1983; Spry, Wiener, 1991; Moiseenko, Kudryavtseva, 2002). A group of non-essential elements (Hg, Cd, and Pb) is most dangerous for living organisms. The concentrations of these metals in the environment is increasing steadily (Dirilgen, Doğan; 2002; Friedmann *et al.*, 2002; Gochfeld, 2003; Moiseenko *et al.*, 2006*)*.

Mercury. The concentration of Hg in bream organs and tissues varied from less than 0.001 to 0.127 µg/g dry weight (Table 5). This metal accumulates most intensely in the liver, kidneys, and muscles, as confirmed by data presented in the scientific literature (Moore, Rammamoorthty, 1983; Friedmann *et al.*, 2002; Gochfeld, 2003). The highest concentrations of Hg were revealed in bream caught in several sections of the Middle Volga, which is subject to the heaviest anthropogenic load. Comparison of the data obtained by the authors with those from other scientific papers showed that the limits within which the concentration of Hg in bream muscles and liver can vary are comparable with those determined for Lake Balaton (Farkas *et al.*, 2003) and certain water bodies in the Czech Republic (Svobodova *et al.*, 1999). Similar values were cited for certain freshwater and sea fish inhabiting water bodies of the USA (Watras *et al.*, 1998).

Table 5. Concentration of microelements in the organs and tissues of the investigated breams (µg per 1 g of dry weight). Here S_x is the standard error

	Upper Volga		Middle Volga		Lower Volga	
Element	$X \pm S_x$	Min-Max	$X \pm S_x$	Min-Max	$X \pm S_x$	Min-Max
1	2	3	4	5	6	7
gills						
Hg	0,011±0,001	0,005-0,022	0,011±0,002	0,004-0,035	0,005±0,001	0,001-0,015
Cd	0,05±0,01	0,01-0,19	0,01±0,00	0,01-0,04	0,20±0,03	0,05-0,42
Pb	0,33±0,07	0,05-0,46	0,04±0,01	<0,01-0,16	0,07±0,01	<0,01-0,17
Al	52,9±6,7	14,7-110,3	9,38±0,91	4,38-17,3	106,6±15,2	34,6-199
Sr	128±4	96-163	299±24	167-458	790±46	394-1095
Ni	0,23±0,03	0,03-0,48	1,32±0,32	0,30-4,79	1,18±0,14	0,40-2,36
Mn	89,0±5,1	52-134	61,5±3,4	45,0-87,2	28,9±1,4	18,0-39,0
Zn	82,0±1,7	67,4-94,2	81,2±1,8	72,3-90,6	81,4±1,5	75,5-94,4
Cu	2,31±0,07	1,60-2,82	1,95±0,08	1,31-2,63	4,22±0,29	2,91-6,99
Cr	0,28±0,04	0,04-0,66	0,14±0,01	0,06-0,25	0,70±0,14	0,21-2,08
Co	0,10±0,02	<0,01-0,30	0,55±0,05	0,17-0,83	0,32±0,07	<0,01-0,98
muscles						
Hg	0,019±0,003	<0,001-0,041	0,049±0,005	0,023-0,092	0,031±0,004	0,014-0,066
Cd	0,03±0,01	<0,01-0,09	<0,01	<0,01-0,02	0,04±0,02	<0,01-0,22
Pb	0,07±0,02	<0,01-0,18	0,06±0,01	<0,01-0,13	0,02±0,00	<0,01-0,06
Al	4,17±0,54	0,91-7,16	1,54±0,18	0,65-2,79	2,38±0,27	1,06-4,11
Mn	4,10±0,38	1,02-6,89	3,12±0,19	1,98-4,44	1,24±0,15	0,33-2,43
Ni	0,13±0,03	<0,01-0,51	0,22±0,02	0,10-0,43	0,26±0,03	0,12-0,60
Sr	4,63±0,27	2,79-6,21	9,74±1,08	5,15-19,3	16,2±3,28	1,40-42,2
Zn	17,5±0,50	14,1-21,7	20,0±0,9	15,3-25,9	25,0±1,2	17,1-32,5
Cu	0,67±0,03	0,40-0,91	0,75±0,07	0,34-1,24	1,07±0,07	0,67-1,61
Cr	0,10±0,02	<0,01-0,34	0,04±0,01	<0,01-0,10	0,18±0,03	0,05-0,48
Co	0,03±0,01	<0,01-0,10	0,18±0,02	0,01-0,28	0,15±0,02	0,02-0,30
liver						
Hg	0,053±0,005	0,027-0,086	0,048±0,009	0,011-0,127	0,054±0,010	0,001-0,103
Cd	0,25±0,04	<0,01-0,67	0,26±0,04	0,11-0,69	0,35±0,06	0,01-0,59
	Upper Volga		Middle Volga		Lower Volga	

Element	$X \pm S_x$	Min-Max	$X \pm S_x$	Min-Max	$X \pm S_x$	Min-Max
1	2	3	4	5	6	7
liver						
Pb	0,25±0,07	0,01-0,75	0,19±0,04	0,04-0,66	0,06±0,01	0,01-0,15
Al	6,62±1,01	1,59-15,7	6,55±1,02	1,86-14,1	6,06±0,92	3,05-14,3
Sr	0,53±0,08	0,16-1,53	0,82±0,12	0,05-1,66	1,81±0,55	0,46-8,03
Ni	0,20±0,04	<0,01-0,56	0,19±0,03	0,03-0,41	0,33±0,04	0,12-0,63
Mn	8,27±0,34	5,72-11,1	6,93±0,38	4,97-8,94	6,23±0,44	3,56-9,00
Zn	99,7±5,4	51-143	79,6±6,4	51-140	110±10	58-205
Cu	46,7±4,4	7,4-79,8	35,5±7,5	10,3-114,2	89,3±10,4	29,8-155
Cr	0,19±0,05	<0,01-0,73	0,07±0,01	0,02-0,16	0,20±0,04	0,02-0,50
Co	0,09±0,01	<0,01-0,27	0,21±0,03	0,06-0,43	0,16±0,02	<0,01-0,32

Cadmium. The literature cites a high degree of Cd accumulation in living organisms, which is indicative of environmental pollution on local and regional scales (Conto-Cinier *et al.*, 1997). The most intense accumulation of Cd in all the physiological systems of fish was observed for those inhabiting the Lower Volga (Table 5). The accumulation of Cd in fish muscles testifies to long-term pollution of the body of water with this metal (McGeer *et al.*, 2000). The maximum Cd concentration (up to 5.66 µg/g dry weight) was recorded in the kidney. Unfortunately, there are few studies devoted to the analysis of accumulation of this metal in fish kidneys. The concentration of Cd in fish kidneys closely correlates with its concentration in other systems of the fish organism, such as the liver ($r = 0.78$, $p<0.005$), skeleton ($r = 0.71$, $p<0.01$), and gills ($r = 0.56$, $p<0.1$), which demonstrates penetration of Cd into the fish from contaminated water.

Lead. The maximum concentration of Pb in bream was observed in the Upper and Middle Volga (Table 5). Pb is most intensely accumulated by the kidneys, liver, and muscle (the respective concentrations were up to 1.3, up to 0.75, and up to 0.18 µg/g dry weight). It is difficult to explain why, under conditions of a higher concentration of Pb in the Lower Volga water, the maximum accumulation of this element was observed in fish inhabiting the Upper and Middle Volga. This is probably a manifestation of the cumulative effect of river contamination in previous years. In Lake Balaton, the concentration of Pb in bream muscles was higher: 1.6 µg/g dry weight (Farkas *et al.*, 2003). *Aluminum.* According to data from Rosseland *et al.* (1990), a high concentration of Al in the environment (both in dissolved and suspended forms) ensures its intense accumulation in fish (especially in the gills). The maximum concentration of Al in the water and in organs and tissues of bream was observed in the Lower Volga (Tables 3. and 5). Accumulation of Al could be traced in all the body systems of fish, but the highest concentrations of this metal were observed in the gills and skeleton. Bioaccumulation of Al in these organs is demonstrated by the following regression equations:

$$Al_{gills} = 0.072\ Al_{water} + 22.4,\ r = 0.90,\ p<0.001;$$
$$Al_{skeleton} = 0.007\ Al_{water} + 7.19,\ r = 0.74,\ p<0.01.$$

The close dependence of Al in fish gills on Al in water can be explained by the fact that, in the process of breathing, water is filtered through the gills of the fish, and Al settles onto the gill surface. Coagulation of Al on the surface of the gill epithelium, in addition to its inclusion in epithelial cells, has been demonstrated (Rosseland *et al.*, 1990). *Strontium.* This element participates in metabolic processes with Ca. Being more labile and active, Sr gradually disturbs the normal calcification of the skeleton and causes pathological disturbances in bone tissue (Chowdhuury *et al.*, 2000). The highest concentration of Sr in bream inhabiting the Volga basin was found in the Lower Volga sections, where the concentration of Sr in the water was maximal (Table 5), reaching 1500 μg/g dry weight in fish skeleton and 1100 μg/g dry weight in the gills. Sr accumulates not only in fish bones but also in fish muscles, liver, and kidneys. The dependence of the Sr content of bream organs and tissues on its concentration in water can be approximated by the following equations:

$Sr_{gills} = 1.52\ Sr_{water} - 25.0$, r = 0.99, $p<0.001$;
$Sr_{muscles} = 0.026\ Sr_{water} + 2.98$, r = 0.74, $p<0.01$;
$Sr_{liver} = 0.003\ Sr_{water} + 0.191$, r = 0.79, $p<0.005$;
$Sr_{kidneys} = 0.006\ Sr_{water} + 0.672$, r = 0.95, $p<0.001$;
$Sr_{skeleton} = 1.94\ Sr_{water} - 73.6$, r = 0.98, $p<0.001$.

It should be emphasized that the Sr/Ca ratio in water varied along the river course; it was 1/289 in the Upper Volga, 1/186 in the Middle Volga, and 1/66 in the Lower Volga water. In addition to the increase in the absolute value of Sr concentration in the water from the Upper to the Lower Volga, its relative concentration in fish organisms increased even more, which testifies to the replacement of Ca by Sr in bream bones. For example, the Sr/Ca ratio in bream gills was 1/516 in the Upper; 1/266 in the Middle; and 1/83 in the Lower Volga. The respective values for bream skeleton were 1/798, 1/384, and 1/116. Thus, Sr features a high bioaccumulation capacity. *Nickel.* The concentration of this metal in the muscles and liver of bream inhabiting the Volga River did not exceed 0.60 μg/g dry weight; for kidneys, the value was somewhat higher (Table 5). Ni accumulates intensely in fish, mainly in the gills and kidneys (Moiseenko, Kudryatseva, 2002). The ability of this metal to accumulate is confirmed by the regression dependencies between the concentration of Ni in the water and in Volga bream organs and tissues (with the exception of the skeleton):

$Ni_{gills} = 0.343\ Ni_{water} + 0.197$, r = 0.68, $p<0.025$;
$Ni_{muscles} = 0.086\ Ni_{water} + 0.058$, r = 0.82, $p<0.005$;
$Ni_{liver} = 0.051\ Ni_{water} + 0.155$, r = 0.73, $p<0.01$;
$Ni_{kidneys} = 0.206\ Ni_{water} + 0.477$, r = 0.76, $p<0.01$.

Thus, the accumulation of Ni in fish organisms depends on its concentration in the water, but the concentration of this metal in the water and fish of the Volga River is low. *Manganese.* Mn is usually considered to be of low toxicity. According to Musibono and Day (1999), Mn reduces the toxicity of such elements as Cu and Al, i.e. Mn possesses antagonistic properties in multicomponent water contamination. Mn is irregularly distributed in the Volga River water: the concentration of Mn in the Upper Volga was much higher than in the Lower. The concentration of Mn in fish organisms changes similarly: the most intense accumulation of Mn was observed in bream inhabiting the Upper Volga, and the maximum amount of this metal was found in the bream gills and skeleton (Table 5). A significant correlation was revealed between the concentration of Mn in fish organisms and in the respective water (for gills, $r = 0.68$, $p<0.025$; for muscles, $r = 0.67$, $p<0.025$; for liver, $r = 0.61$, $p<0.05$). A close correlation was also revealed between the values of Mn concentration in different tissues and organs of the same fish individual, which testifies to synchronous bioaccumulation of this metal depending on its concentration in the water. *Zinc, chromium, copper, and cobalt.* These are essential elements. No distinct patterns could be traced in the distributions of Zn, Cr, and Co concentrations in the Volga River water. As a rule, Cu, Zn and Co accumulate in fish liver, where active metabolic processes take place. Their maximum concentration was found in the livers of Lower Volga bream (Table 5). It is well established that the rate of metabolic processes in fish is determined by the ambient temperature. If the concentrations of microelements in the water are similar, the rate of their bioaccumulation can depend on the temperature conditions. The availability of essential elements to functionally vital organs of bream inhabiting the Lower Volga is probably affected by the intensification of metabolic processes in warmer water. A correlation was found between the concentrations of Cu, Zn, and Co in fish muscles and the sum of annual temperatures exceeding 10°C (Figure 2), whereas no such correlation could be established between the concentrations of the above elements in the fish organs and in water.

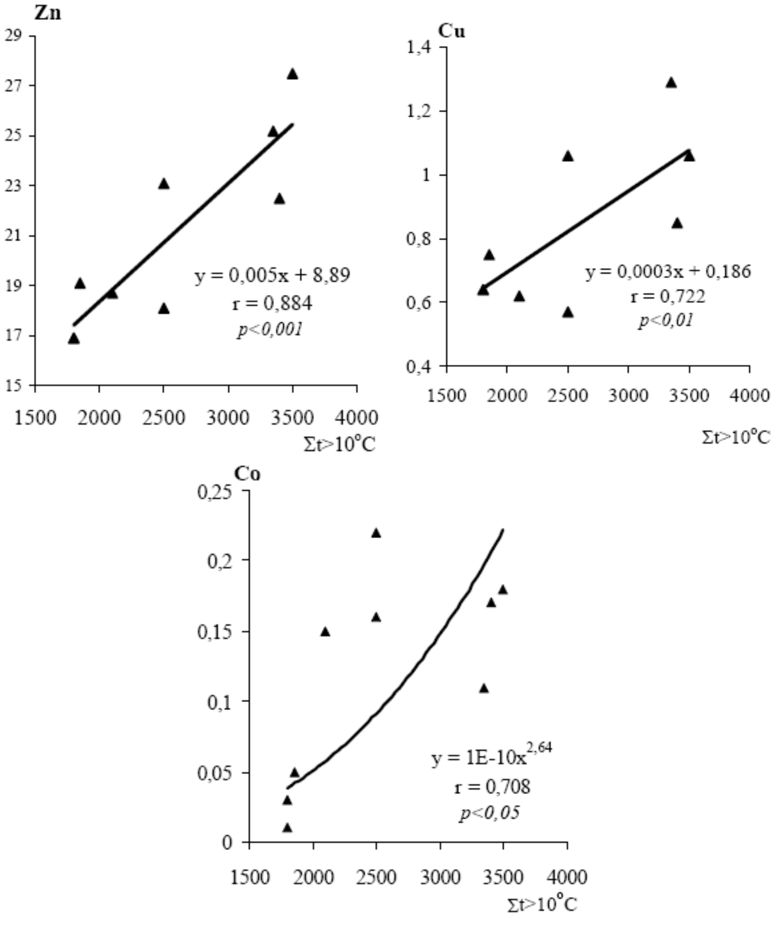

Figure 2. Dependence of essential element concentrations in muscles (□g per 1 g of dry weight) on the sum of temperature values exceeding +10oC (□t>10oC).

Multimetal penetration. The accumulation of microelements in fish organs and tissues causes microelementoses, i.e. changes in the ratio of microelement concentrations in fish organs and tissues. A high correlation was established between the concentrations of certain elements in the bream organs, which testifies to the effect of pollution on the increase in the concentrations of the investigated microelements in fish gills:

$\mathrm{Sr}\rightarrow(\mathrm{r} = 0.87,\ p<0.001)\leftarrow\mathrm{Cd}\rightarrow(\mathrm{r} = 0.96,\ p<0.001)\leftarrow\mathrm{Al}\rightarrow(\mathrm{r} = 0.78,\ p<0.005)\leftarrow\mathrm{Cr}$,

and in fish kidneys:

$\mathrm{Cr}\rightarrow(\mathrm{r} = 0.53,\ p<0.1)\leftarrow\mathrm{Sr}\rightarrow(\mathrm{r} = 0.75,\ p<0.01)\leftarrow\mathrm{Cd}\rightarrow(\mathrm{r} = 0.55,\ p<0.1)\leftarrow\mathrm{Ni}\rightarrow(\mathrm{r} = 0.72,\ ,\ p<0.05)\leftarrow\mathrm{Co}$.

This group of elements accumulates mainly in fish inhabiting the Lower Volga. Accumulation in fish liver of $\mathrm{Hg}\rightarrow(\mathrm{r} = 0.61)\leftarrow\mathrm{Zn}$ is observed in the Middle Volga, whereas accumulation of $\mathrm{Mn}\rightarrow(\mathrm{r} = 0.59)\leftarrow\mathrm{Pb}$ in fish gills and liver is typical of the Upper Volga.

Bone tissues in the investigated fish demonstrated a high degree of correlation between the concentrations of the following elements:

$\mathrm{Pb}\rightarrow(\mathrm{r} = 0.66,\ p<0.025)\leftarrow\mathrm{Hg}\rightarrow(\mathrm{r} = 0.79,\ p<0.005)\leftarrow\mathrm{Ni}$.
$\uparrow$
$\mathrm{Cd}\rightarrow(\mathrm{r} = 0.79,\ p<0.005)\leftarrow\mathrm{Cu}\rightarrow(\mathrm{r} = 0.87,\ p<0.0015)\leftarrow\mathrm{Sr}$.
$\downarrow$
$(\mathrm{r} = 0.76,\ p<0.01)\leftarrow\mathrm{Zn}$.

The correlations established between the concentrations of essential and non-essential microelements prove their joint penetration into the fish organism as a result of multimetal pollution. Based on the analysis of the element distributions in bream and the joint penetration of certain microelements into them, Sr-Cd-Al-Cr-Ni anthropogenic hydrogeoformation can be singled out in the Lower Volga; Hg-Zn hydrogeoformation in the Middle Volga; and Mn-Pb hydrogeoformation in the Upper Volga.

FISH PATHOLOGY

Various deviations from the physiological norm were found in all the fish of investigated river sections.

Gills. In some cases, the gills were pale (their normal color is scarlet) with a clearly distinct anemic ring along the gill arc. The largest number of fish with an anemic ring was caught in the Gorkovskoe Reservoir and in the Lower Volga (downstream of Astrakhan).

Epithelium desquamation in secondary lamellae (Figure 3.c), swelling of the distal parts of filaments, and shortening, curvature, and fusion of secondary lamellae (Figure 3.b) were observed, which resulted in the transformation of the rigidly structured gill into an unstructured mass, with the distal filament alone still functioning. Congestive phenomena (stasis) were found in most of the respiratory lamellae, which is related to the violation of capillary conductivity. Vast hemorrhages were observed between filaments and secondary lamellae (Figure 3.d). In certain filaments, the secondary lamellae were completely destroyed. Extensive lammelar hypertrophy with some proliferation from the bases of the secondary lamellae was recorded.

Liver. Changes in the liver color, dimensions, and texture were observed. All the bream caught in different river sections had increased loose-textured liver with color varying from a mosaic light-brown to pale yellow. In some cases, the liver was liquified; it had clearly distinct parts of necrosis or pronounced signs of atrophy. All the examined fish demonstrated signs of liver disease of differing degrees of severity. Frequent visible disturbances of this organ were typical of fish caught in the Gorkovskoe (up to 92.6% of the fish) and Kuibyshevskoe (up to 54.5% of the fish) reservoirs, as well as in certain sections of the Lower Volga (Table 4). Morphological and functional changes in the liver manifested themselves in the form of lipoid dystrophy (Figure 4.b) and hydropic dystrophy (Figure 4.c), which are symptoms of progressive hepatopathy. In the case of intensified intoxication, lipid and hydropic dystrophy of hepatocytes were often found. Hydropic dystrophy is a variation of protein dystrophy and is related to the disturbance of protein and water exchange. In this case, the permeability of cell membranes increases, vacuoles appear in the cytoplasm due to water ingress, the cellular organelles are destroyed, while the cell itself becomes filled with water and dies. In the case of lipoid dystrophy, fat occlusions, which almost completely fill the cells, appear in the hepatocytes. Diffuse disruptions of bream liver, accompanied by disturbances in the morphological structure of liver lobules and pronounced necrosis of liver tissue, were also diagnosed. Mechanisms of lipoid and protein dystrophy development are similar. Frequently, they develop under the conditions of the organism intoxication or accompany hypoxia. In some microscopic sections, complete necrosis (not that of a "hotbed" character) of the liver tissue was observed (Figure 4.d). Interstitial proliferative inflammation related to hepatocyte necrosis and the appearance of inflammation infiltrates were also diagnosed. In the process of their development, the cells of the infiltrates transform into collagenous fibres of connective tissue. As a result, a thick connective-tissue capsule can appear around the zone of necrosis. Such progressive necrosis and structural reorganization of the tissue can contribute to post-necrotic hepatic cirrhosis, leading, in turn, to hepatic failure. Vast zones of parenchymal hemorrhage, destruction of blood corpuscles and blood vessel walls, as well as proliferation of connective tissue around the blood vessels, were revealed.Signs of chronic congestive hyperemia in liver veins were found. They testify to varicose veins and capillaries, a decrease in blood pressure, and blood flow deceleration. As a result, the supply of the tissues with blood becomes disturbed, and tissue hypoxia occurs. All these processes taking place together can lead to congestive edema. Disturbances revealed in the liver cell structure entail the development of first sclerosis, and then cirrhosis.*Kidneys*. The largest number of fish with pathological disturbances in the kidneys was caught in certain areas of the Gorkovskoe and Kuibyshevskoe reservoirs (Table 4). Pathological disturbances in the kidney tissue manifested themselves in fibrosis, where vast connective-tissue accretions substituted zones of necrosis in the canaliculi and interstitial tissue. In medicine, similar histopathology is typical of interstitial nephritis (fibroelastosis). In the connective tissue between the kidney canaliculi, pronounced interstitial inflammation (a diffuse infiltrate composed of blood cells) was observed (Figure 5.f). Signs of congestive hyperemia in the veins were revealed. Severe degeneration of adipose tissue was also diagnosed (Figure 5.d). In this case, the adipose tissue had a clearly formed structure; lipocytes were organized in groups ("lobules", separated from each other by membranes with blood vessels). The following disturbances were found: destruction of lymphoid tissue (Figure 5.b); proliferative inflammation (Figure 5.c), with zones of necrosis surrounded by thick connective-tissue capsules, separating the disturbed zone from normally functioning tissue and preventing the proliferation of pathology; and the occurrence of interstitial substances in the kidney parenchyma (Figure 5.b), causing compression of healthy tissue, which, in the long run, can lead to the organ atrophy.

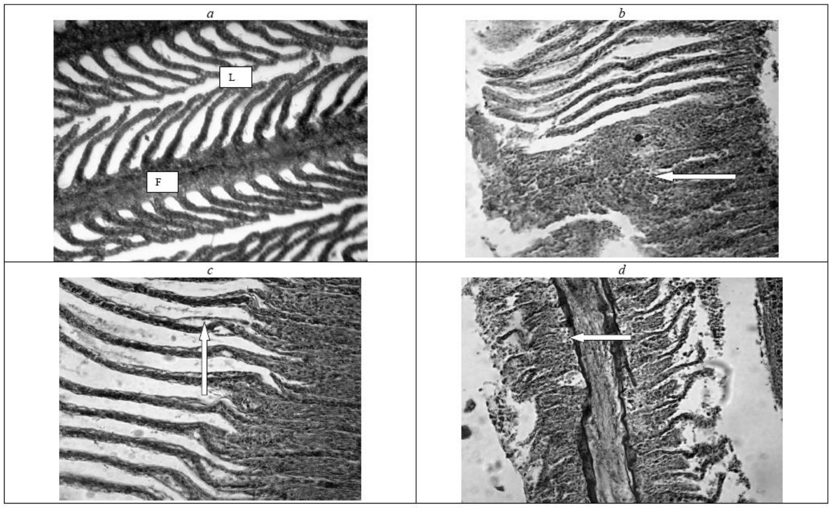

Figure 3. Pathological changes in the gills of bream (arrowed): a – normal structure (F – filament, L - lamellae), x160; b – extensive lamellar hyperplasia with fusion of secondary lamellae, x320; c – separation of epidermis at base of secondary lamellae, x320; d – hemorrhage, x160.

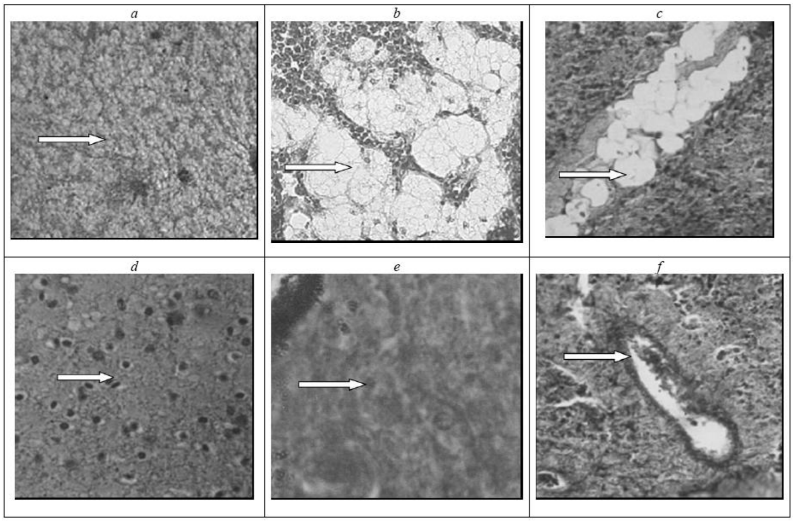

Figure 4. Pathological changes in the liver of bream (arrowed), x320: a – normal structure; b – lipoid dystrophy; c – hydropic dystrophy; d – karyopycnosis and necrosis of hepatocytes; e – inflammation; f – breakdown of blood cells.

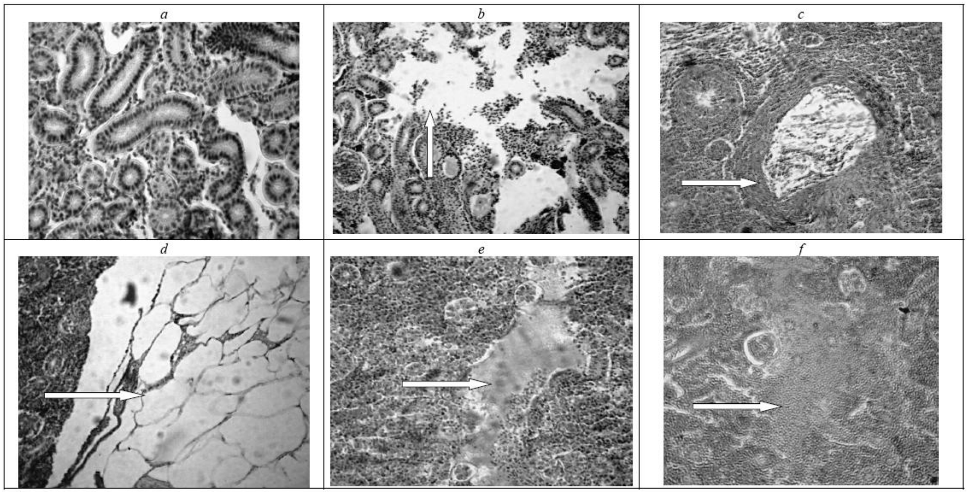

Figure 5. Pathological changes in kidneys of bream (arrowed), x320: a – normal structure; b – necrosis of the hematopoietic tissue; c – proliferative inflammation with fibrosis of the hematopoietic tissue; d – lipoid degeneration; e – interstitial inflammation; f – hemorrhage.

Gonads. At the time of the examination, gonads were at stage II–III of development. In some cases, their form and texture were abnormal. Their growth was uneven. Some parts of gonads were replaced by nonfunctional connective tissue. Twisting of the gonads was typical, mostly in males. In females, uneven development of fish eggs was observed.

Hematology. Pathology developed simultaneously with disturbances in vitally important organs of the fish. The "norm" of hematological indices is different for each fish species. According to the data presented by Zhiteneva *et al.* (1989), the concentration of hemoglobin in the blood of healthy bream varies from 92.0 to 101.0 g/l. A 15% to 30% decrease in hemoglobin concentration is a signal of fish disease, which can be caused by both invasive and toxic agents. For bream inhabiting the Volga River basin, a value of 90 g/l is adopted as the lower boundary of the "norm" of natural variability in hemoglobin concentration. The largest number of fish whose hemoglobin concentration was lower than the norm, was caught in a certain site of the Lower Volga and in the Gorkii Reservoir.Toxic substances affect not only hemoglobin concentration but also change the leukogram and red blood cell composition (Ivanova, 1976; Zhiteneva *et al.*, 1989). Studies have shown that, in different sites of the Volga River, the ratio between different forms of blood cells of bream changes. The highest percentage of immature forms of erythrocytes was found in blood smears of fish caught in the Lower Volga, which is in agreement with the low hemoglobin concentration in the blood. Changes in the leukogram of the bream manifested themselves in an increase in the relative amount of neutrophils and monocytes, especially in fish from certain sections of the Lower Volga and the Gorkovskoe Reservoir (Table 6). In the blood smears, different pathological forms of erythrocytes (lacy erythrocytes, poikilocythemia, vacuolization of the cytoplasm, pycnosis of the cell nuclei, amitosis of the cell nuclei, etc.) were found. The changes revealed in hematological parameters of the examined fish confirm the development of toxicoses in fish inhabiting the Volga River basin.Thus, fishes caught in the Volga basin had visible clinic and postmortem symptoms of intoxication. The degree of disturbances in their organs varied from hardly visible to pronounced deep degenerative changes, increasing the risk of death of the individual.

Table 6. Characteristics of the physiological state of fishes caught in the Volga river

Parameter	Upper Volga		Middle Volga		Lower Volga	
	X	Min-Max	X	Min-Max	X	Min-Max
Z	1.54	1.33-1.71	1.97	1.71-2.11	1.45	1.00-1.74
Percentage of the fishes demonstrating second and third stages of the disease	44.3	37.7-52.4	72.9	53.6-85.2	41.8	20.0-64.1
Percentage of the fishes demonstrating pathological disturbances in the liver	56.5	41.0-64.3	68.4	46.4-92.6	29.5	18.2-44.4
Percentage of the fishes demonstrating pathological disturbances in the kidneys	25.4	21.4-28.6	59.9	32.1-80.0	9.4	0-25.6
Hemoglobin (Hb), mg/l	103	81-124	96	52-126	88	56-122
Percentage of the fishes with Hb not exceeding 90 mg/l	15.2	0-37.5	22.0	11.8-40.0	54.3	9.1-80.0
Leucocytes:						
lymphocytes, %	87.5	82-93	85.8	68-94	64.7	29-93
monocytes, %	1.0	0-2	1.5	0-5	3.7	0-10
neutrophiles, %	11.3	5-14	12.6	5-29	31.6	6-66
including foamy, %	7.7	2-11	6.0	1-20	16.2	3-34
Erythrocytes:						
mature forms, %	94.1	89.9-96.6	94.1	85.5-99.9	92.6	76.1-99.8
young cells, %	5.9	3.4-10.1	5.9	0.1-14.5	7.4	0.2-23.9

ECOTOXICOLOGICAL ASSESSMENT WATER QUALITY OF VOLGA RIVER

The most common approach to setting environmental regulations, in Russia as well and other country has been based largely on the assessment of chemical attributes of anthropogenic pollution. The system of water quality assessment is based on the concept of Maximum Permissible Concentration (MPC) or Guideline Concentration (GC) of pollutants in the water. At present, the ecotoxicological approach to estimating of water quality gradually meets the approval of more and more researchers. Ecotoxicological assessment of water quality is aimed at obtaining an integrated assessment of water quality, based on symptoms of disturbance in the ecosystem (in situ). The term “ecosystem health” is increasingly used in scientific literature of the past decades. Aquatic ecosystems are stressed in all levels, ranging from individual and up to the population and community levels. For ecosystem health assessment the following four definitions have been used: i) cellular health, which describes the structural integrity of cellular organels and the maintenance of biochemical processes; ii) individual health, which presents structural and morphological health and functioning in terms of physiology of the entire organism; iii) population health, which measures the sustainability and maintenance of a population of a particular species; iv) community health, which describes a group of organisms and the relationships between species in that group. Each method has its limitations and advantages, and the type of method used defines how we interpret the effect of a stressor on ecosystem health (Cairns, 1990; Rapport, 1992, 1995; Calow, 1992; Cash, 1995; Arttril, Depledge, 1997; Elliott et al., 2003). In general, indicators at the biochemical and physiological levels provide information on the functional status of individual organisms, while intermediate-level responses, such as histopathological condition, are indicative of the structural integrity of tissues and organs. Community and population level measurements integrate the responses to a variety of environmental conditions, and therefore may be less reflective of contaminant-induced stress in comparison to the level of organisms (Hinton, Lauren, 1990; Fober, Fober, 1994; Newman, Jagoe, 1996). Many groups of organisms can be used as indicators of environmental and ecological change. But numerous publications attest that fish (in situ) is a good indicator of environmental change and ecosystem health, especially in case of toxic water pollution (Cash, 1995; Wrona, Cash, 1996; Wong, Dixon, 1995; Simon, 2000; Whitfield, Elliott, 2002; Moiseenko, 2005). Fish occupy the top level in the trophic system of aquatic ecosystems. Pathological changes in fish organ enable us to determine the toxicity of water and the potential danger of man-entering substances in water. Fish, in comparison with invertebrate, are more sensitive to many toxicants and are the convenient test-object for indication of ecosystem health. Our results show that water quality and living conditions for aquatic species in the Volga River are unsatisfactory. Based on the prevalence of signs of intoxication in test-organism fish (*Abramis brama* (L.), we can conclude that the ecosystem health conditions are quite dramatic and give a clear signal of the need to decrease toxic pollution. The main question for environmental management is the level to which pollution loading must be reduced to achieve reference conditions and to preserve ecosystem health.

To answer this question, we need to accomplish three tasks (Moisenko *et al.*, 2006):

1. Determinate how hydro-chemical information on water quality can be interpreted in terms of a unified parameter, which could reflect the real impacts of the dose taking into account contaminant complexes (multi-pollution);
2. Assign criteria for ecosystem health that informatively reflect the impacts of pollution;
3. Determine critical levels of water pollution and required load reductions based on a dose–effect relationship.

An integrated impact dose. In rivers and reservoirs, aquatic organisms are exposed to a mixture of all toxicants. It is important to find a numerical parameter describing the total toxic impact on fish. The integrated impact dose of contaminants is determined by their number, concentration, toxic properties of each and aquatic medium – pH, Ca, TOC (Forstner, Wittman, 1983). The values of Maximum Permeation Concentrations (MPC) largely differ by country, in spite of the fact that experimental research techniques to establish the MPCs are universal. In Russia, the MPC values for Cu, V and some other elements are possibly underestimated, whereas the MPCs for Cd, As and some other elements are possibly overestimated. For example, in Canada for Cu and Cd guideline values are 2-4 and 0.01-0.06 μg/l accordingly in dependence of $CaCO_3$, for As it is equal 5 μg/l; in the Netherlands the

MPC value for Cu, V and Cd are 3.8, 5.1 and 2 μg/l accordingly. (Can. Water Qual. Guidelines, 1994; Env. Quality Obj., 2001; Bioassey meth. aquatic org., 1985; Methodological recommendations., 1998). Because the Volga River is in Russia we used data on the toxicological properties of each toxicant based on the MPC adopted in Russia (see Tables 3 and 4). For the investigated areas of the Volga basin Figure 6. presents the total exceedance of the actual concentrations of toxic elements over their respective MPC values. For inorganic compounds, the maximum concentration values, standardized to the respective MPCs, are typical of Mn, V, and Cu. Water contamination with metals (from 13 to 20 units) is typical of the Upper Volga (I, II, III). For the whole set of toxic elements, the most heavily contaminated areas were found in the Gorkovskoe Reservoir (sites IV, V, VI) and the Lower Volga (IX, X, XI, XII, XIII). In the middle and lower courses of the Volga, the toxic properties of water were due to its contamination with organic compounds (mainly with phthalic acid ethers), the sum of the exceedance factors of which reaches 70.

Criteria of ecosystem health. The different types of pathology and dysfunction diagnosed in the bream result from comprehensive chronic impact of numerous toxic substances, found in the Volga River water, on the fish organisms. Histological analysis of fish organs and tissues revealed serious disturbances in the morphology and function of the liver and kidneys, as well as in the hematopoietic system; many of these disturbances are irreversible. These pathologies are based on the physiological reactions through disturbing the homeostasis and proper functioning of vital biological processes.

Determination of the critical levels of water contamination requires numerical biological criteria, which also adequately reflect the effect of toxic substances in the water. Thus, the following biological parameters were used as criteria for fish and ecosystem health (the average weighted for individual river sections):

i) the percentage of fish in which the second or third stages of diseases were diagnosed;
ii) the Z-index defined above;
iii) the percentage of fish with hemoglobin concentration below 90 g/l ;
iv) the low levels of neutrophils in the blood, etc.

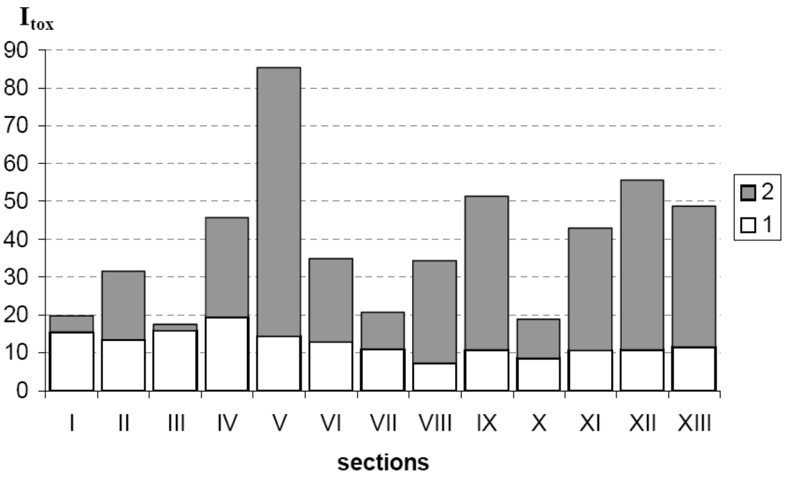

Figure 6. Sum of the concentrations of toxic substances divided by the respective MPC values for the investigated sections of the Volga River (1 - microelements; 2 - organic compounds).

Dose–effect dependencies and critical levels. Basing on dose–effect dependencies (between numerical indices of fish health and the chemical parameters of water quality, in particular the total concentration of toxic substances in the water standardized to MPC), the critical levels of water contamination can be determined. The dose–effect dependencies were plotted for the above biological parameters.

The following factors are assumed to have affected the results: i) the biased nature of the values of MPC, to which the pollutant concentrations were standardized in the process of the integral dose determination (especially for toxic organic compounds); ii) the underestimation of synergetic effects and the presence of other presumably toxic substances in the water, which could also have a negative impact on fish organisms; iii) the persistent effect of toxic substances over the whole lifespan of the fish, the range and concentration of which could be different in different years and seasons; iv) the subjective character of expert evaluation; measurement errors; small samples obtained, etc. However, despite the complexity of the synchronous studies that were carried out and the necessity of accounting for numerous factors, reliable dependencies were obtained. These dependencies confirm that the morbidity in fishes inhabiting the Volga River basin is related to the occurrence of various toxic substances in the water (Figure 7).

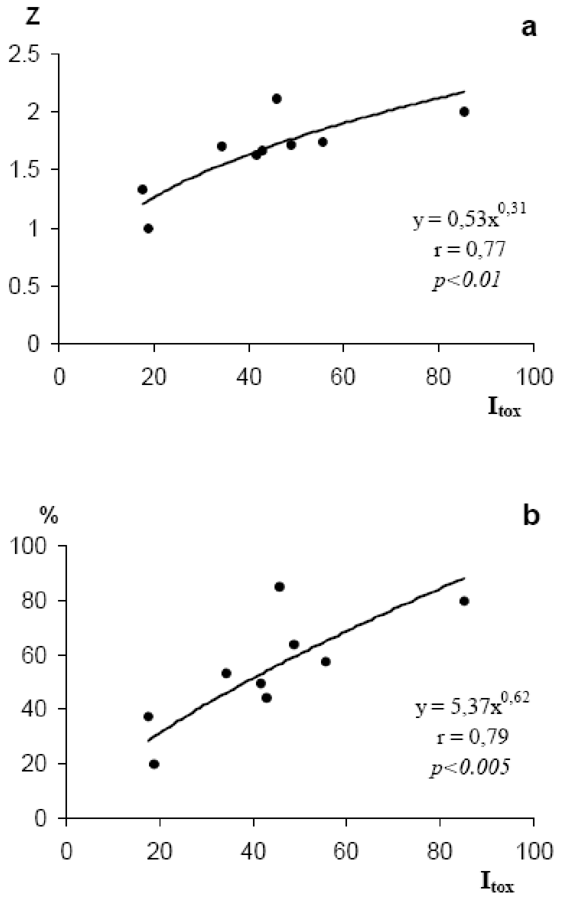

Figure 7. Dependencies of Z (a) and the percentage of fish demonstrating the second and third stages of disease (b) on the total concentration of toxic substances standardized to MPC values.

Table 7. Dependence of characteristics of the physiological state of fish on the concentration of hazardous substances in the water (dash denotes absence of reliable data)

Toxic elements and compounds (x)	Blood characteristics (y)		
	Average Hb	Hb not exceeding 90 g/l	Neutrophiles, %
Dibutyl phthalate	$y = -5.6\mathrm{Ln}(x) + 107.2$ $r=0.65$**	-	-
V	$y = 112.0e^{-0.12x}$ $r=0.58$*	$y = 52.0\mathrm{Ln}(x) + 16.9$ $r=0.68$***	$y = 6.2e^{0.58x}$ $r=0.58$*
Pb	$y = 98.2e^{-0.07x}$ $r=0.64$**	$y = 21.7x + 21.9$ $r=0.79$****	$y = 10.3x + 9.48$ $r=0.88$***
	Percentage of the fishes demonstrating different pathologic disturbances in: (y)		
	The whole organism	The liver	The kidneys
Hydrocarbons of oil products	$y = 8.63\mathrm{Ln}(x) + 32.4$ $r=0.53$*	$y = 10.1\mathrm{Ln}(x) + 24.6$ $r=0.56$*	$y = 0.53x + 16.4$ $r=0.74$***
Dibutyl phthalate	$y = 0.63x + 38.5$ $r=0.61$*	-	-
Dioctyl phthalate	$y = 0.78x + 45.3$ $r=0.59$*	$y = 1.17x + 37.9$ $r=0.70$***	$y = 1.41x + 13.6$ $r=0.87$****
Derivatives of 1,3-dioxane	$y = 0.47x + 48.7$ $r=0.62$**	$y = 0.53x + 45.0$ $r=0.59$*	$y = 0.81x + 20.2$ $r=0.87$****
Cu	$y = 8.73x + 34.3$ $r=0.52$*	$y = 10.7x + 25.9$ $r=0.57$*	-
Mn	-	$y = 24.9\mathrm{Ln}(x) - 47.2$ $r=0.76$***	$y = 0.38x + 5.08$ $r=0.68$***

*-$p<0.05$, **-$p<0.01$, ***-$p<0.005$, ****-$p<0.001$

Among the various negative ambient factors that cause pathologic disturbances in fish organs and tissues, it is very difficult to single out the most important factors. Table 7. presents the dependencies between the parameters of fish morbidity and the concentrations of toxic substances in the water. Depletion of certain blood parameters is most significantly related to the impact of V and Pb, whereas pathological disturbances in the fish liver and kidneys are associated with the negative effects of dioctylphthalate, derivatives of dioxane, and oil products, as well as those of Cu and Mn.

The accumulation of toxic metals can also enhance (and, in certain cases, even directly cause) pathologies in fish. Therefore, the relationship between the accumulation of microelements in fish and pathological disturbances in the organs and tissues of bream in the Volga River basin was analyzed. The increase of metals in the water medium may bring adverse effects on fish health. The surplus of trace elements in the organism initiates some specific diseases: Hg causes neurological effects, Cd and Pb have carcinogenic properties, Sr leads to pathology of bone tissues, Cu to anemia, etc. (Conto Cinier *et al.*, 1997; Patriarca *et al.*, 1998; Vatras *et al.*, 1998; Musibono, Day, 1999).

Organisms have mechanisms of metal detoxification by induction of metallothionein synthesis. These proteins bind specifically to neutral essential trace elements, such as Zn and Cu, as well as to potentially toxic metals such as Cd and Hg (Phillips, 1995; Linde *et al.*, 2001). The effects of metal accumulation on fish and their pathologies, without the necessity of explaining the internal metabolism of metals, is the key purpose for our data.

Notwithstanding the low sensitivity of the method applied, which prevented determination of the concentration of Hg in the water, the accumulation of this metal in fish was observed, especially in the Middle Volga. A reliable correlation was established between Hg accumulation in fish kidneys ($Hg_{kidneys}$) and pathologic disturbances in this organ (Pat., %), as well as Z:

$$Pat._{kidneys} = 210\ Hg_{kidneys} - 9.68,\ r = 0.81,\ p<0.005;$$
$$Z = 53.8\ Hg_{kidneys} + 0.029,\ r = 0.85,\ p<0.005.$$

Thus, irrespective of the fact that the concentration of Hg in the investigated water was lower than the analytical detection limit (less than 0.05 μg/l), its accumulation in the organism can cause pathogenic disturbances in fish.

A reliable correlation was also established between the accumulation of Cd in fish gills (Cd_{gills}) and hematologic parameters of fish –such as the concentration of hemoglobin in the blood (Hb), and neutrophils (N) in the leukocyte count.

$$Hb = -104\ Cd_{gills} + 103,\ r = 0.87,\ p<0.001;$$
$$N = 34.5\ Cd_{gills} + 3.35,\ r = 0.88,\ p<0.001.$$

As mentioned above, the accumulation of Cd in the organism is accompanied by an increase in the concentrations of some other elements. Most probably, the joint accumulation of several toxic elements in the fish organism entails a decrease in the concentration of hemoglobin in the blood and the development of anemia, accompanied by an increased percentage of neutrophils in the leukocyte count. In addition, a correlation between the concentration of Pb in fish kidneys (Pb_{kidhey}) and pathological disturbances in this organ (Pat_{kidney}) was established.

$$Pat._{kidneys} = 53.2\ Pb_{kidneys} + 17.2,\ r = 0.54,\ p<0.1.$$

All this testifies to the fact that accumulation of metals (especially Hg and Cd) leads to pathological conditions in fish. Thus, the increase in the metal concentrations in the Volga River basin results in their accumulation in fishes, leading to the development of microelementoses and pathologic disturbances in fish organs and tissues.

The established critical levels of water contamination remain open for discussion. The studies carried out by the authors have shown that the water quality and ecosystem health in all the investigated river sections are unsatisfactory, and that critical levels of water contamination are exceeded. Approximation of the dependencies into the area of low values of the water quality standard (less than 1 unit) shows that the percentage of fish in which the second or third stage of disease was diagnosed was equal to about 10% (Figure 7).

The dose–effect dependencies clearly show that total pollution of the Volga River must be significantly decreased, by at least 5–7 times, first for toxic contaminants. These studies have confirmed the high information value of the ecotoxicological approach to the assessment of water quality and ecosystem health. Note that ecotoxicological studies were carried out for the Volga River basin for the first time, and many important river sections or reservoir areas were not investigated. In this respect, our studies could be considered "screening analysis of the ecotoxicological situation," but at the same time, they substantiate the information content of methodological solutions and the necessity of the continuation of large-scale studies in this field in the future.

CONCLUSION

The Volga is the longest river of Europe. Large-scale contamination of the Volga River basin is caused by its geographical position within the most economically developed region of Russia. Domestic and industrial wastewaters, air-borne pollution of the catchment area, as well as non-sewerage effluents from settlement areas find their way to this water basin.

Numerous elements and their compounds that have a toxic effect on living organisms were found in the water samples taken within the investigated sections of the Volga River. Among inorganic substances, V, Cu, and Mn play the most important role in the formation of the general ecotoxicological situation. As for organic compounds, a high level of water contamination with phthalic acid ethers and dioxane derivatives was first recorded. In the investigated sections of the Upper Volga, water contamination with metals prevails; in the Middle and Lower Volga, contamination with organic xenobiotics prevails. The highest levels of the total exceedance of the actual substances concentration over the respective MPC values were observed for the Gorkovskoe Reservoir and certain sections of the Lower Volga. Morphological and functional disturbances in the organs and tissues of fishes testify to their intoxication. Most of the fishes with different forms of pathology and dysfunction were caught in the Gorkii Reservoir and in certain sections of the Lower Volga (downstream of Astrakhan).

Results of the research testify to the fact that the examined fish individuals are subject to the effect of multicomponent “chronic” water contamination. Numerous registered disturbances (necroses, neoplasms) are referred to as irreversible. However, hypertrophy, hyperplasia, and encapsulation, accompanying the above disturbances, are structural and functional bases of adaptive reactions aimed at surviving of fish under the conditions of subtoxic aquatic environment.

Hemathologic characteristics of the examined fishes confirm the fact of their intoxication. Symptoms of anemia and increased concentration of neutrophiles and monocytes were found. All this is the response of the organism to unfavorable habitat conditions. On certain blood smears, numerous pathological forms of blood cells (laky erythrocytes, poikilocythemia, vacuolization of the cytoplasm, pycnosis of the cell nuclei, amitosis of the cell nuclei, etc.) were found. They testify to disturbances in the system of hemogenesis of fish caused by toxic substances.

Based on the dose–effect dependences, it has been found that diseases of fish are caused by water contamination with toxic substances. The negative impact of organic xenobiotics on the fish liver and kidneys has been demonstrated, in addition to the negative impact of certain microelements (e.g. vanadium, lead and some other ones) on the hemogenesis system. The studies that were carried out confirm the high information value of the ecotoxicological approach to the assessment of the ecological state of water bodies, as well as the necessity of establishing more reliable MPC values and maximum permissible "Toxicological Harmfulness Value". Note that ecotoxicological studies were carried out for the Volga River basin for the first time, and many important river sections or reservoir areas were not investigated. In this respect, our studies can rather be called "screening analysis of the ecotoxicological situation," but at the same time, they are convincing for substantiating the information content of methodological solutions and the necessity of continuation of large-scale studies in this field in the future.

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REFERENCE

Adams, S.M, Ryon, M.G.A., 1994. Comparison of health assessment approaches for evaluating the effects of contaminant-related stress on fish populations. *J. Aquat. Ecosys. Health*. 3, 15-25

Anthropogenic Impact on Water Resources of Russia and Neighboring Countries in the late 20th Century. 2003. (Eds.) Koronkevich, N.I. and Zaitseva, I.S. Nauka, Moscow (in Russian with English abstract and conclusion).

Attrill, M.J, Depledge, M.H. 1997. Community and population indicators of ecosystem health: targeting links between levels of biological organization. *Aquat. Toxicol*. 38, 183 - 197

Bioassey methods for aquatic organisms. 1985. Standart Methods for the Examination of Water and Wastewater (sixteenth edition). American Public Health Association. Washington.

Bucke, D. 1994. Histology. In: Austin B., Austin D.A. (eds.). Methods for the Microbiological Examination of Fish and Shellfish. Ellis Horwood Ltd, Chichester. 69-97.

Burtseva, L.V., Lapenko, L.A., Kononov, E.Ya. 1991. Background Concentrations of Lead, Mercury, Arsenic, and Cadmium in Natural Environments (Based on Data on the World). In: Izrael Yu.A. (eds.), *Monitoring the Background Pollution of Natural Environments*, 7. Gidrometeoizdat, Leningrad, 23—56 (in Russian with English abstract).

Cairns, J.Jr. 1990. The predict, validation, monitoring and migration of anthropogenic effect upon natural systems. *Environ. aud*. 2, 19-25

Calow, P. 1992. Can ecosystems be healthy? Critical consideration of concepts. *J. Aquat. Ecosystem Health* 1, 1-15.

Canadian Water Quality Guidelines. 1994. Published by Canadian Council of Ministry of Environment, Ottawa.

Cash, K.J., 1995. Assessing and monitoring aquatic ecosystem health - approaches using individual, population, and community/ecosystem measurements. Northern River Basins Study Project, Report No 45, 68p.

Chowdhuury, M., Van Ginneken, L., Blust, R. 2000. Kinetics of waterborne strontium uptake in common carp, *Ciprinus carpio* (L) et different calcium level. *Environ. Toxicol. Chem*. 19, 622-630.

Conto-Cinier, C., Petit-Ramel, M., Faure, R., Garin, D. 1997. Cadmium bioaccumulation in carp (*Cyprino carpio*) tissues during long-term exposure: analysis by inductively coupled plasma-mass Spectrometry // *Ecotox. Environ. Safety*. 38, 137-143.

Dirilgen N., Doğan F. 2002. Speciation of chromium in the presence of copper and zinc and their combined toxicity // *Ecotox. Environ. Safety*. 53. 397-403.

Edelstein K.K., 1998. Reservoirs of Russia: ecological problems and ways of their solution. Moscow: GEOS. 277 p. (in Russian)

Elliott, M., Hemingway, K.l., Krueger, D., Thiel R., Hylland, K., Arukwe, A., Forlin, l., Sayer, M. 2003. From the individual to the Population an Community Responses to Pollution. In: (eds.) Lawrence A.J., Hemingway K.L. Effects of Pollution on Fish. Blackwell Science Ltd.

Environmental Quality Objectives for Hazardous Substances in Aquatic Enviroment. 2001. UMWELTBUNDESAMT, Berlin.

Farkas, A., Salanki, J., and Specziar, A. 2003. Age- and Size-Specific Patterns of Heavy Metals in the Organs of Freshwater Fish *Abramis Brama* L. Populating a Low-Contaminated Site. *Water Res*. 37, 183-208

Förstner, U., Wittman, G.T.W. 1983. *Metal Pollution in the Aquatic Environment*. Springer-Verlag, Berlin.

Friedmann, A.S., Kimble Costain E., Deborah L., MacLatchy, Stansley W., Washuta E.J. 2002. Effect of Mercury on general and reproductive health of largemouth bass (*Micropterus salmoides*) from Three Lakes in New Jersey. *Ecotox. Environ. Safety*. 52, 117-122.

Gochfeld, M. 2003. Case of mercury exposure, bioavailability and absorption. Ecotoxicology and Environ. *Safety*. 56, 174 – 179.

Hovind, H. 2000, 2002. Intercomparison 0014, 0216, 2000, 2002. pH, K_{25}, HCO_3, NO_3+NO_2, Cl, SO_4, Ca, Mg, Na, K, total aluminium, aluminium – reactive and nonlabile, TOC, COD-Mn, Fe, Mn, Cd, Pb, Cu, Ni, and Zn. ICP *Water Report* 55, 68. Oslo.

Ivanova, N.T., 1976. Method of Morphological Analysis of Blood in Ichthyopathological Studies. Inst. Ozerov. Rybn. Khoz. 105, 114-117 (in Russian).

Krylov, O.N., 1980. Textbook on Preventive Measures and Diagnostics of Fish Intoxication by Hazardous Substances, Moscow: Pishch. Prom. 117p (in Russian)

Linde, A.R., Sanchez-Galan, S., Valles-Mota, P., Garcia-Vasquez, E. 2001. Metallothionein as bioindicator of freshwater metal pollution: European eel and brown trout. *Ecotox. Environ. Safety*. 49, 60-63.

List of Fishery Standards: Maximum Permissible Concentrations (MPC) and Safe Reference Levels of Impact (SRLI) of Hazardous Substances for Water Bodies Used for Fishery). 1999. Moscow, VNIRO (in Russian).

Makinen, J., Lattunen, H., Vanni, T. 2002. Laboratorioiden valinen vetailukoe 1/97. Helsinki, Suomen Ymparistokeskus (In Finish)

Maximum Permissible Concentrations (MPC) and Safe Reference Levels of Impact (SRLI) of Hazardous Substances for Water Bodies Used for Fishery. 1999. VNIRO, Moscow (in Russian).

McGeer, J.C., Szebedinsky, C., McDonald, D.G., Wood, C.M. 2000. Effects of chronic sublethal exposure to water-borne Cu, Cd or Zn in *Rainbow trout*: tissue specific metal accumulation. *Aquat. Toxicol*. 50, 245-256.

Moiseenko T.I., Voinov A.A, Megorsky V.V., Gashkina N.A., Kudriavtseva L. P., Vandish O.I., Sharov, A.N., Sharova Y.N., Koroleva I.N. 2006. Ecosystem and human health assessment to define environmental management strategies: the case of long-term human impacts on an arctic lake. *Science Tot. Environ*.369, 1-20.

Moiseenko, T.I. 2005. Ecotoxicological approach to an estimation of water quality. *Water resour*. 4, 410-424.

Moiseenko, T.I., Kudryavtseva, L.P. 2002. Trace metals accumulation and fish pathologies in areas affected by mining and metallurgical enterprises. *Environ Pollut*. 114, 285-297.

Moiseenko, T.I., Kudryavtseva, L.P., Gashkina, N.A. Trace Elements in Surface Water: Technophility, Bioaccumulation and Ecotoxicology. Nauka, Moscow. (in Russian with English abstract and conclusion)

Moore, J.W., Rammamoorthty, S. 1983. Heavy Metals in Natural Waters. Springer-Verlag.

Musibono, D.E. and Day, J.A. 1999. The Effect of Mn on mortality and growth in the freshwater Amphipod *Paramelita Nigroculus* (Barnard) exposed to a mixture of Al and Cu in acidic waters. *Water Res*. 33, 207-213.

Patriarca, M., Menditto A., Di Felice G., Petrucci F., Caroli, S., Merli, M., Valente, C. 1998. Recent developments in trace element analysis in the prevention, diagnosis, and treatment of diseases. *Microchem. J*. 59, 194-202.

Petrukhin, V.A., Burtseva, L.V., Lapenko, L.A. 1989. Background Concentrations of Microelements in Natural Media (Based on Data on the World), Gidrometeoizdat, Leningrad. (in Russian with English abstract).

Phillips, D.J.H. 1995. The Chemistries and Environmental Fates of Trace Metals and Organochlorines in Aquatic Ecosystems. *Marine Poll. Bull.* 31, 193-200.

Rapport, D. J., 1995. Ecosystem health: and emerging integrative science. In: Rapport, D. J., Gaudt, C. Calow, P. (eds). Evaluating and Monitoring the Health of Large-Scale Ecosystems. Proceedings of the NATO advanced research workshop. Springer-Verlag, Berlin. 5-31.

Rosseland, B.O., Eldhuset T.O., Staurnes M. 1990. Environmental Effects of Aluminum. *Environ. Geochem. Health*. 12, 17-27.

Rozenberg G.S., 2009. The Volga River basin: on the way to sustainable development. Tol'yati: Kassandra. 478 p. (in Russian).

Rozenberg, G.S., Krasnoshchekov, G.P.1996. Volga Basin: Environmental Situation and Methods of Rational Nature Management. Published by Inst. Volga Basin ecology. Tol'yati, No. 249 (in Russian).

Simon TP. 2000. The use of biology criteria as tool for water resource management. *Environ Sci. Policy*. 3, 43-49.

Spry, D.J., Wiener, J.G. 1991. Metal Bioavailability and toxicity to fish in low-alkalinity lakes: a critical review. *Environ. Pollut*. 71, 243-304.

State Report on the Environmental Conditions in the Russian Federation in 2002. 2003. Published by Ministry of Nature Resources. Moscow 479 p. (In Russian).

Svobodova, Z., Dusek, L., Hejtmanek, M., Vykusova, B., and Smid, R. 1999. Bioaccumulation of Mercury in Various Fish Species from Orlik and Kamyk Water Reservoirs in the Czech Republic. *Ecotoxicol. Environ. Safety*. 43, 231-240.

Watras, C.J., Back, R.C., Halvorsen, S., Hudson, R.J.M., Morrison, K.A. 1998. Bioaccumulation of Mercury in pelagic freshwater food webs. *Sci. Total Environ*. 219, 183-208.

Whitfield, AK, Elliott, M. 2002. Fish as indicator of environmental and ecological changes within estuaries: a review of progress and suggestions for the future. *J. Fish Biol*. 61, 229-250.

Wong, P.T.S, Dixon, D.G. 1995. Bioassessment of water quality. *Environ. Toxicol. Water Qual*. 10, 9–7.

Wrona, F.J., Cash, K.J. 1996. The ecosystem approach to environment assessment: moving from theory to practice. *J. Aquatic. Ecosys.health*. 5, 89-97.

Zhiteneva, L.D., Poltavtseva, T.G., Rudnetskaya, O.A. 1989. Atlas of Normal and Pathologically Changed Cells of Fish Blood. Rostovskoe knizhn. izd., Rostov-on-Don (In Russian).