## WATER QUALITY AND PROTECTION: ENVIRONMENTAL ASPECTS

# **Ecotoxicological Assessment of After-Effects** of the Volga River Water Contamination

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**Abstract**—Comprehensive assessment of ecotoxicological situation in the Volga River basin is presented. Concentrations of organic and inorganic toxic substances are cited. Basic clinic and postmortem symptoms of intoxication in fish 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. Based on the dose-effect dependences, calculated according to the values of the total concentration of toxic substances, standardized to MPC, and criteria of the physiological state of fish, cases of exceedance of the critical concentrations of pollutants are demonstrated for the investigated river sections.

#### INTRODUCTION

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. Several studies have proved the contamination of water and accumulation of heavy metals, oil products, polycyclic aromatic hydrocarbons, polychlorirozak biphenUls, dioxins, and other chemical compounds in bottom sediments (especially in the places of industrial effluents discharge) [1, 4]. 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.).

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 [2, 6].

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 [20].

In the 1970s, the method of clinic and postmortem examination of fishes, aimed at revealing the reasons of

frequent cases of their mass intoxication within different areas of the Volga-Kama basin, was first used [14]. The attention of numerous researchers was attracted by very frequent incidences of myopathy (muscle exfoliation) and eggshell weakening in Volga-Caspian sturgeon. The first symptoms of these diseases were detected in individual fishes back in the early 1980s, whereas in 1987–1988, these diseases were diagnosed in 70 to 80% of all the fishes examined. Note that the river system pollution is of multi-component character, which makes it difficult to reveal the true reason of different toxicoses and diseases in fish [20].

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.

This work is aimed at identifying the levels of water contamination in individual river sections in the lower course of the Volga River and in its reservoir areas according to a common methodological scheme; reveal main pathological disturbances in fishes caused by their chronic intoxication; present the ecotoxicological assessment of water quality and critical levels of water contamination.

The ecotoxicological approach is aimed at comprehensive assessment of water quality and the state of ecosystem "health" [16]. It is based on establishing dependences between the integral dose of water contamination with toxic substances, on the one hand, and informative criteria of biological disturbances in the ecosystem (effects), allowing the substantiation of the critical levels of water contamination. Different forms of pathology and dysfunction in the fish organisms are

Table 1. Investigated areas in the Volga River basin

No.	Area	Sampling site	Date
I	Shoshinskii Pool, Ivankovo Reservoir	railroad bridge, the automobile bridge, Nizovka-Shosha	August 2001
II	Volzhskii Pool, Ivankovo Reservoir	Melkovo vil., Gorodishche vil., Ploski vil., Konakovo t.	the same
III	Ivan'kovskii Pool, Ivankovo Reservoir	Korovinskii Bay outlet, Fedorovskii Bay outlet, Lipnya vil., Ukhodovo vil., Korcheva vil., near the dam	"
IV	Central part, Gorkii Reservoir	Sokol'skii Site	August 2002
V	Near-dam area, Gorkii Reservoir	Opposite Chkalovsk t.	the same
VI	Lower pool, Gorkii Reservoir	lower pool, Gorkii Reservoir, 3 km downstream	"
VII	Near-dam area, Kuibyshev Reservoir	right bank (near the Usa R.), the left bank	"
VIII	Lower pool, Kuibyshev Reservoir	lower pool, Kuibyshev Reservoir, Fedorovskii Site	"
IX	Volga section from the village of Seroglazka to the pier of Zamyany	Seroglazka vil., Mitinka Site, Mitinka Channel	September 2001
X	Volga section from the pier of Zamyany to the Buzan River	Muzhichiya Fishing Ground, Belyachnaya Fishing Ground	the same
XI	Volga section from the Buzan River to the city of Astrakhan'	1 km upstream of the water divider, Durnoskii Ravine	"
XII	Volga River delta, the Main Bank	4th Ognevka, 10th Ognevka	"
XIII	Volga River delta, the Belinskii Bank	5th Ognevka	"

early and informative manifestations of disturbances in the ecosystem "health", as compared to changes in the communities structure under the conditions of water contamination with toxic agents, which allows us to reveal the effect of individual pollutants, taking into account their long-term and cumulative effect.

#### MATERIALS AND METHODS

In August and September of 2000–2002, comprehensive ecotoxicological studies were carried out in several areas of the Volga reservoirs (in the Ivankovo, Gorkii, and Kuibyshev reservoirs), several sections in the Volga lower course and in its delta. Water was sampled for toxic substance concentrations (metals and toxic organic compounds). At the same time, fishes were examined to study their physiological state in order to reveal different forms of pathology and dysfunction in their organisms, caused by water contamination with toxic substances. All in all, 13 river basin areas were investigated. Their description is presented in Table 1.

All in all, 31 water samples were taken in different river sections and reservoir areas. Observation and sampling points were unevenly distributed; there were 13 points in the Ivankovo Reservoir, 8 points in the midstream of the Volga River, and 10 points in its lower course. Water sampling aimed at determining the concentrations of toxic substances was carried out rarer due to a complicated character of the follow-up sample analysis (all in all, 15 such samples were taken). At the same time, the following condition was strictly observed: the water samples were taken right at the places where fishes were caught for examination.

First, the water samples were filtered through a membrane filter with a pore diameter of 45 µm. After that, microelement concentrations were determined using the atomic-absorption method (GFAAS, "Perkin-Elmer-5000" model, Corp., Norwalk, USA) with flameless atomization (HGA-400). In addition, water elemental analysis was carried out using the method of inductively-coupled plasma with the help of "Plasma Quad 3" mass-spectrometer manufactured by "Fisons Electronic Elemental Analysis" (United Kingdom). This device allows the determination of more than 70 elements. Thirteen toxic elements (Mo, V, Se, Cu, Ni, Zn, Pb, Cd, Co, As, Cr, Mn, and Sr) were singled out in the process of the water samples analysis.

Concentrations of organic microcomponents in previously obtained extracts ("acidic" and "alkaline" extractions of the water samples with methylene chloride) were determined using gas chromatography with the help of "QP-5000" chromate-mass-spectrometer manufactured by "Shimadzu" (Japan). In the water samples, 6 to 72 compounds of the mass varying from 6.75 to 446.8 µg were identified; the amount of compounds of non-identifiable mass did not exceed 10% of the amount of substances whose mass was identified. Toxic substances, most dangerous for ecological systems, were distinguished in the process of the water sample analysis.

Abramis brama bream, the most widespread fish species in the Volga River basin, was used as a bioindicator of water contamination with toxic substances. It is a benthic phagocyte, not making long-distance migrations, which allows us to obtain the material for examination from strictly limited areas of the investigated water bodies. In the time period close to the investi-

gated one (August and early September), 313 fish individuals of one and the same age (from 4+ to 6+) were examined in the investigated reservoir water areas and river sections (Table 1). Only fishes free of internal parasites and with gonads at the third or fourth stage of development were examined.

Hematologic analysis was carried out only for justcaught alive fishes according to the techniques described elsewhere [14]. The fish blood was taken from its tail artery. The values of hemoglobin concentration and erythrocyte sedimentation rate (ESR) were determined in the blood samples; blood smears were made. Visual determination of clinic and postmortem symptoms of organism intoxication (macrodiagnostics) was carried out within the first hour after the fishes were caught, according to the technique presented elsewhere [17]. In order to precise the diagnosis, samples for histologic analysis (microdiagnostics) were taken from organs and tissues having visible disturbances in their morphological structure. The analyzed tissues were immediately fixed to preserve the life-time pattern of the tissue structure. The Buene mixture was used as a retainer. The samples were treated according to generally accepted histologic techniques [8]. The blood smears were dyed in accordance with the Pappenheim technique. The microscopy of histo- and hemathopathologies was carried out under  $10 \times 8$  and  $10 \times 40$ magnification.

The general physiological state of the organism was estimated according to three stages of the disease development. At the first stage, inconsiderable changes in the organism were registered; at the second one, clearly visible disturbances; and at the third stage, significant pathological disturbances in the organism were observed. In the process of expert evaluation of the stage of the disease, it was not only the state of an individual organ, but also the totality of visible clinic symptoms of intoxication that were taken into account. Morbidity in fish Z for each investigated river section was determined as:

$$Z = (1*N_1 + 2*N_2 + 3*N_3)/\Sigma N_{\text{tot}}$$

Here  $N_1$ ,  $N_2$ , and  $N_3$  denote the amount of fishes demonstrating the first, second and third stages of the disease, respectively;  $N_{\text{tot}}$  is the total number of fishes examined for each river section (including healthy individuals). The share of fishes (in per cent) demonstrating different stages of the disease was also determined. In addition to the expert evaluation of the general physiological state of the organism, the rating point system was used to assess the degree of disturbance in vitally important fish organs—liver, kidneys, and branchiae. Each stage of disturbance in any individual organ (especially in the liver) was often higher than the revealed stage of the illness determined according to the totality of symptoms for the whole organism.

### WATER CONTAMINATION WITH TOXIC SUBSTANCES

Microelements make the most widespread group of toxic substances finding their way to aquatic systems. Many of them are very dangerous (Hg, As, Pb, Cd, Zn, Cu, etc). Certain diseases, which are frequently diagnosed in fish, are typical of human beings as well. They are caused by environmental pollution with metals. Thus, Hg causes a neurological effect; Cd and Pb are carcinogenic and toxic for gonads; Sr leads to pathological disturbances in bone tissues; Mo causes gout; and Cu causes anemia [13]. Examples of this kind can be continued.

Numerous studies have shown that, for many years, the Volga River basin has been subject to pollution with heavy metals, among which Cu and Zn are found to be most dangerous. That is why these two microelements are most frequently determined in the system of water quality monitoring [2, 4, 9].

In the investigated areas, microelement concentrations in the water were relatively low: the concentration of Mo, Cd, Co, and Cr was less than 1  $\mu$ g/l, that of Se and Pb varied from less than 1 to 1.7  $\mu$ g/l, that of Ni, V, and Cu varied from less than 1 to 2.8  $\mu$ g/l; the concentration of Zn varied from 1 to 6.2  $\mu$ g/l; that of As, from 1 to 4.2  $\mu$ g/l (Table 2). Relatively high concentrations of Mn (from 20 to 108  $\mu$ g/l) and Sr were observed. The latter accompanies Ca, and that is why Sr concentration in natural water is often high.

The concentration of mercury (one of the most toxic substances) did not exceed the accuracy of its determination using the ICP-MS technique (less than 0.05  $\mu$ g/l). The same is true for Be (its MPC is 0.3  $\mu$ g/l) and W (its MPC is 0.8  $\mu$ g/l). The measured values of Be and W concentrations did not exceed 0.02  $\mu$ g/l. The concentrations of Al and Fe (these metals are found, mostly, in the form of suspensions, which are known to entail no dangerous consequences for the environment) were not studied.

Relatively low concentrations of the investigated elements (especially those of Zn, Ni, Cd, As, 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 the Volga River water contamination, observed at present. The comparison of the element concentrations in the Volga River with the respective "background" values for overland flow in the European Russia [7, 19] showed that the concentration of As was higher than its "background" value in all the investigated areas; those of Ni and Cd exceeded the background ones in the neardam area of the Kuibyshev Reservoir; whereas the "background' concentrations of Cu and Se were exceeded in the central part of the Gorkii Reservoir. It is a well-known fact that zones of atmosphere and land contamination can be found in some places within the

<sup>&</sup>lt;sup>1</sup> Here and below the MPC values are cited according to [3, 18].

Ele- ments	MPC (mg/l)	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Mo	1	0.16	0.22	0.23	0.50	0.43	0.44	0.74	0.82	0.47	0.42	0.42	0.40	0.49
V	1	1.44	1.65	0.98	1.04	0.88	0.94	1.54	1.41	2.33	1.95	2.60	2.27	2.28
Cu	1	1.9	2.1	2.3	5.68	1.13	1.5	2.8	1.1	1.8	1.4	1.7	1.7	2.0
Se	2	0.55	<0.5	<0.5	1.16	1.16	0.50	0.79	0.50	1.14	0.86	0.74	0.62	< 0.5
Cd	5	0.14	0.16	0.10	0.11	0.03	0.04	0.34	0.04	0.15	< 0.02	0.07	0.21	0.11
Pb	6	0.6	0.5	0.4	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	1.7	1.1	1.7	1.6	3.2
Ni	10	1.4	1.7	2.0	0.8	< 0.5	0.5	4.7	1.4	2.2	1.6	2.3	2.1	3.3
Zn	10	1.7	4.6	4.1	<1	<1	<1	4.6	1.0	5.4	3.5	6.3	5.5	6.2
Co	10	0.4	0.3	0.3	0.2	0.1	0.2	0.2	0.2	0.3	0.4	0.7	1.1	0.9
Mn	10	108	83	111	111	110	93.9	34.4	26.4	31.0	23.1	26.4	26.1	35.7
As	50	4.2	2.8	2.0	1.4	1.0	1.1	1.0	0.8	1.7	1.5	2.5	1.4	1.2
Cr	70	0.38	0.95	0.98	0.76	0.57	1.00	0.77	0.58	0.72	0.54	0.63	0.53	0.50
Sr	400	115	87	103	103	97.1	97.3	288	277	514	487	541	542	517
$\Sigma C_i/N$	$^{\prime}$ IPC $_{i}$	15.5	13.4	15.8	23.3	16.9	12.9	11.0	7.3	10.9	8.6	10.6	10.0	11.5

**Table 2.** Concentration of microelements in the water (mg/l) in the investigated sections of the Volga River and the MPC values established for fishery water bodies [18] (here and in Table 3, the values exceeding the MPC are printed in bold)

catchment areas of the Kuibyshev, Saratovand Volgogradskoe reservoirs, as well as in the Lower Volga [2, 12]. Most probably, this fact explains the exceeding of "background" concentrations for such elements as V, Se, Pb, Ni, Co, etc. The concentration of Mn in the Ivankovo and Gorkii reservoirs, as well as the concentrations of V and Cu in the Kuibyshev Reservoir, in the Lower Volga and the Volga River delta were higher than the respective MPC values, established for fishery water bodies.

Thus, the pattern of the element concentration distribution within the investigated areas reflects, primarily, overall diffuse pollution, which is formed against the background of natural geochemical input of microelements—mainly due to pollutant discharge by fuel and energy plants and general economic activity within the catchment area.

Toxic organic compounds. Lately, huge amounts of organic compounds are being synthesized. Many of them are toxic for living organisms [3, 5]. Table 3 presents concentrations of organic substances characterized by the so-called "Toxicological Harmfulness Value," determined according to the established MPC values. The MPC is not established for alkyl derivatives of 1,3-dioxane, but, according to their physicochemical properties, they are identical to dioxalanes and furans; 1,3-compounds are more stable than furans, and, having the properties of acetales, they are similar to 1,3dioxalanes. For furans and alkyl-(2-furil)-1,3-dioxanes, the MPC is 10  $\mu$ g/l. For (2-furil)-1,3-dioxalanes, the MPC is only about 1 µg/l. Note that alkyl derivatives of 1,3-dioxanes, such as, for instance, 4-ethyl-, 4-methyl-, 4,4-dimethyl-, 4,5-dimethyl-1,3-dioxanes, have a narcotic effect, affect liver and kidneys, and can accumulate in the organism [22]. That is why, the value of  $10 \mu g/l$  was adopted as the MPC for such substances.

Dangerous organic substances include oil products, cyclohexane and cyclopentane derivatives, sebaciÒ acid ether, xylene, phthalates, and dioxanes. It is clear from Table 3 that, in all the investigated sections of the Volga River (except the near-dam area of the Gorkii Reservoir, most probably, affected by the effluents of petrochemical enterprises located upstream of the Gorkii Reservoir near Yaroslavl), no considerable exceedance of the MPC for oil products was detected. A high level of water contamination with alkyl derivatives of dioxane was revealed in the Gorkii Reservoir. Owing to large-scale application of polymer products, phthalates (used as softening agents), and xylene (used for phthalic acid production) were observed, actually, in all the investigated sections of the Volga River (especially downstream of Astrakhan) and in the Gorkii Reservoir. Dibutyl phthalate, whose concentration in the water varied from 1.3 to 55.7 µg/l (while its MPC<sub>fish</sub> makes only 1 µg/l), deserves special attention. This substance is not included in the List of Elements To Be Analyzed, complied by the Hydrometeorological Service, although it is very dangerous for living organisms.

Such dangerous substances as chlororganic compounds were not found in the investigated areas, which can be explained both by their absence in the water, and by their low concentrations (not exceeding the "sensitivity threshold" of the applied method). Analysis of scientific papers and data collected by the Hydrometeorological Service has shown that in individual samples of the Volga water, the concentrations of certain dangerous substances (such as DDT, DDE,  $\alpha$ -hexachloran, and  $\gamma$ -hexachloran) exceed the MPC [9]. In 1999–2000,

**Table 3.** Concentrations of organic substances in the water within the investigated section of the Volga River and the MPC values established for fishery water bodies [18] (n.d. means not detected)

Toxic organic substances	MPC (mg/l)	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Hydrocarbons of oil products:														
alkanes		19.3	15.1	1.45	16.2	113.5	12.1	6.2	8.7	0.9	17.7	7.7	15.5	8.4
olefines		0.35	n.d.	n.d.	3.4	4.0	n.d.	n.d.	0.7	n.d.	1.8	n.d.	0.2	0.3
Total	50	19.65	15.1	1.45	19.6	117.5	12.1	6.2	9.4	0.9	19.5	7.7	15.7	8.7
Monatomic saturated alcohols	500	0.4	2.0	n.d.	1.5	5.1	n.d.	n.d.	n.d.	n.d.	1.0	n.d.	4.6	0.1
Ethers of carboxylic acids:														
dioctyl cebacate	1	n.d.	11.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Carbocyclic compounds:														
cyclohexane and its derivatives	10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	n.d.	n.d.	n.d.
cyclopentadiene and its derivatives	10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	0.7	1.0	n.d.
Aromatic compounds:														
xylene	50	n.d.	1.0	n.d.	1.8	n.d.	2.3	2.5	n.d.	n.d.	0.2	1.25	n.d.	1.0
isopropylene benzene	100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	n.d.	n.d.	n.d.
Orthophthalic acids ethers:														
dibutyl phthalate	1	2.7	4.5	1.3	22.4	55.7	18.1	5.7	24.1	40.3	9.6	29.0	44.5	36.9
dioctyl phthalate	10	11.3	17.7	4.0	15.0	47.2	14.3	n.d.	16.3	1.25	0.5	1.6	n.d.	2.3
Heterocyclic compounds:														
derivatives of 1,3-dioxane	10	n.d.	n.d.	n.d.	20.3	81.7	22.3	2.0	11.8	n.d.	n.d.	n.d.	n.d.	n.d.
Sum of chlororganic pesticides (DDT, DDE, α-hexachloran, γ-hexachloran)*	0.01	n.d.	Ì.Ó	n.d.	n.d.	n.d.	n.d.	0.036	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
$\Sigma C_i/\mathrm{MPC}_i$		4.2	18.2	1.7	26.4	71.0	22.1	9.7	27.1	40.4	10.2	29.4	44.9	37.3

<sup>\*</sup> According to the data of Hydrometeoservice for 1999–2000.

these substances were also found in the Kuibyshev Reservoir.

Thus, organic and inorganic pollutants, for which the Toxicological Harmfulness Value have been established, are found in the Volga River water.

### DESCRIPTION OF THE MOST FREQUENT PATHOLOGICAL DISTURBANCES IN FISH

The effect of contaminated reservoir water on fish inhabiting them manifests itself most clearly during the low-flow period, when the degree of pollutant dilution decreases and the hydrochemical state of the water body is deteriorated. The studies were carried out in August and early in September, i.e., exactly when the reservoir water level began to fall and zones affected by toxic pollutants spread for a long distance in the downstream direction. During this period, the number of sick fishes grows [14], and their toxicoses can be clearly diagnosed. Clinic and postmortem symptoms of intoxication in fish allow us to determine the degree of the disturbance in the fish organism and the morbidity in fishes caught in different sections of the river, caused by river water contamination with toxic pollutants.

Epizootology of fish diseases in different river sections was not of a "hotbed" character. Various deviations from the physiological norm were found in all the

fishes in all the investigated river sections, which testifies to the diffuse pattern of toxic agents distribution (unlike the "hotbed" one, typical of places of effluents discharge). The highest morbidity index Z was observed for the Gorkii Reservoir. In the Volzhskii Pool of the Ivankovo Reservoir and in the river section downstream of Astrakhan, this index was sufficiently high (Table 4). A higher percent of fishes with liver troubles (at the second and third stages of the disease), as compared to the estimated overall morbidity in fishes (which was determined for fish in the upper course of the Volga) is explained by the absence of visible changes in other organs in the investigated individuals. The fish organism responds to the cumulative effect of toxic agents within the whole fish lifetime (4 to 6 years). That is why, disturbances in the breams aged 4+ to 6+, described below, result from "chronic" intoxication of the organism within the said period.

Coverlets and eyes of the examined fishes were, actually, normal. In the 1970s, breams caught in the Volga–Kama reservoirs demonstrated such abnormalities as bristling of squama, hemorrhages, and eye nebula [14].

Branchiae are subject to direct contact with water and to the effect of toxic agents. In some cases, the branchiae were pale (while their normal color is scarlet) with a clearly distinct bright limbus (anemic ring) along

Table 4. Characteristics of the physiological state of fishes caught in the investigated sections of the Volga River (here Hb
is the average hemoglobin concentration; dash denotes absence of data)

Characteristic	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Z	1.57	1.71	1.33	2.11	2	_	2.05	1.71	1.14	1.00	1.67	1.74	1.72
Percentage of the fishes dem- onstrating the second and third stages of the disease	42.9	52.4	37.7	85.2	80.0	_	72.7	53.6	22.7	20.0	44.4	57.9	64.1
Percentage of the fihes demonstrating pathological disturbances in the liver	64.3	64.3	41.0	92.6	80.0	_	54.5	46.4	18.2	20.0	44.4	31.6	33.3
Percentage of the fihes demonstrating pathological disturbances in the kidneys	28.6	21.4	26.2	59.3	80.0	_	68.2	32.1	0	0	11.1	10.5	25.6
Hb, mg/l	90.9	108.5	106.3	86.3	97.4	_	98.1	98.5	99.3	98.8	89.4	75.2	80.6
Percentage of the fishes with Hb not exceeding 90 mg/l	50	0	16.7	40.0	20.0	_	22.2	11.8	9.1	40.0	62.5	80.0	76.9
Leukogram, in percent:													
lymphocytes	_	87.5	_	90.4	84.3	_	83.0	87.2	72.6	_	74.3	_	45.9
monocytes	_	1.0	_	1.0	0.8	_	1.8	1.6	4.0	_	3.0	_	4.1
neutrophiles	_	11.3	_	8.6	14.5	_	15.3	11.2	23.4	_	22.7	_	50.0
including foamy	_	7.7	_	4.0	7.0	_	8.9	4.0	11.8	_	12.4	_	24.9
Erythrocyte composition, in percent:													
mature forms	_	94.1	_	94.0	94.5	_	94.3	93.8	94.0	_	94.8	_	91.0
young cells	_	5.9	_	6.0	5.5	_	5.7	6.2	6.0	_	5.2	-	9.0

the branchial arc. Rarely, fishes with swollen branchiae of a cyanotic tint were caught. The largest number of fishes with an anemic ring was caught in the Gorkii Reservoir and in the Lower Volga (downstream of Astrakhan). Discomplexation of the branchial apparatus was diagnosed in fishes inhabiting the Volga River basin back in the 1970s [14].

Liver plays a very important role in the functioning of the organism, participating in detoxification of poisons that penetrated therein. That is why liver is the "target function" for toxic substances. In the process of fish lancing, changes in the liver color, dimensions, and texture were observed. Normally, fish liver is brown, with smooth trochal edges. Bream liver has three excrescencies (blades). Actually, all the breams caught in different river sections had increased loose liver of the color varying from a mosaic light-brown to pale yellow. In some cases, the liver consistence was washy, it had clearly distinct zones of necrosis or pronounced symptoms of atrophy. These clinic symptoms are usually typical of liver cirrhosis (connective-tissue accretions inside the parenchyma) or lipoid degeneration of the parenchyma. Actually, all the examined fishes demonstrated symptoms of liver diseases of different degree of severity. Frequent visible disturbances of this organ were typical of fishes caught in the Gorkii (up to 92.6% of the fishes) and Kuibyshev (up to 54.5% of the fishes) reservoirs, as well as in certain sections of the Lower Volga (Table 4). Disturbances in the liver of fishes inhabiting the Volga River basin are also described elsewhere [20].

Kidneys play an important role in excreting toxic substances from the organism. Like liver, they are "target functions" for toxic substances. The kidneys of the examined fishes were abnormal (having dense darkbrown bands along the dorsal spine, loose structure, increased dimensions). Their color was brighter than that of the normal kidneys, with thin bright veins, owing to which the kidneys acquired visible granular-fibrous texture. Very often, the kidneys were of different size with uneven thickenings inside. Such clinic symptoms are typical of interstitial nephritis (fibroelastosis). The largest number of fishes with pathological disturbances in the kidneys were caught in certain areas of the Gorkii and Kuibyshev reservoirs (Table 4).

Gonads (at the time of the examination, they were at the third or fourth stages of the development). In some cases, their form and texture were abnormal. Their growth in length was uneven, and the gonads bore connective-tissue strangulations. Twisting of the gonads was typical, mostly, of males. As concerns females, uneven development of fish eggs and sometimes, insufficient saturation of egg-bearing plates with eggs were observed.

Thus, fishes caught in the Volga basin had visible clinic and postmortem symptoms of intoxication, testi-

fying to unfavorable water quality and the impact of toxic substances on the organism. Symptoms of disturbances in all the vital organs (branchiae, liver, and kidneys) were revealed, actually, in all the fishes examined. The degree of disturbances in these organs varied from hardly visible to pronounced deep degenerative ones, testifying to the risk of the death of the individual.

#### HISTOPATHOLOGY OF FISH ORGANS AND TISSUES

Histologic analysis of the cell structure disturbance in different organs and tissues allows us to exactly diagnose pathological processes occurring in the organism affected by toxic agents. The histologic analysis was carried out for vitally important organs of fishes with most pronounced pathological disturbances, typical of the third stage of the disease. The results presented below are characteristic of the final stage of the disease. They testify to the main negative processes occurring in the fishes caught in the Volga River basin.

Branchiae. Destructive processes in the branchial apparatus manifested themselves in changes in the branchial epithelium and disturbances in the status of blood circulatory system, causing degradation of filaments and respiratory lamellas. Branchial epithelium desquamation (Fig. 1c), swelling of distal zones of branchial filaments, shortening, curvature, and fusion of respiratory lamellas (Fig. 1b) were observed, which entailed the transformation of the rigidly structured branchial apparatus into an unstructured mass, with the distal filament alone still functioning. Congestive phenomena (stases) were found in most of the respiratory lamellas, which is related to the violation of capillary conductivity. Vast hemorrhages were observed between filaments and respiratory lamellas (Fig. 1d). In certain filaments, the respiratory lamellas were completely destroyed. Few filaments with undisturbed structure demonstrated extraordinary hypertrophy of the branchial epithelium.

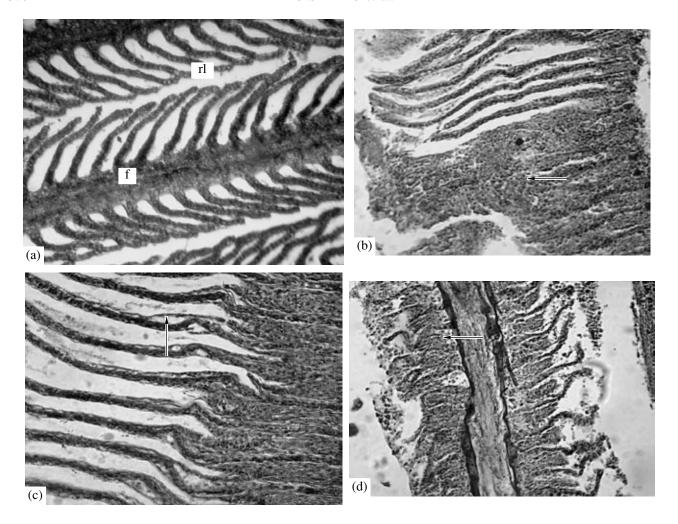
*Kidneys.* Pathological disturbances in the kidney tissue manifested themselves in fibrosis, when vast connective-tissue accretions substituted zones of necrosis in the canaliculi and interstitial tissue. In medicine, similar pathological histology is typical of interstitial nephritis (fibroelastosis). In the connective tissue between the kidney canaliculi, pronounced interstitial inflammation (diffuse infiltrate composed of blood cells) was observed (Fig. 2f). Symptoms of congestive hyperemia in veins were revealed. At the same time, serious degeneration of adipose tissue was diagnosed (Fig. 2d). In this case, the adipose tissue had clearly formed structure; lipocytes were organized in groups (the so-called "lobules" separated from each other by membranes with blood vessels). The following disturbances were found: destruction of lymphoid tissue (Fig. 2b); proliferative inflammation (Fig. 2c), with zones of necrosis surrounded by thick connective-tissue capsules, separating the disturbed zone from normally functioning tissue and preventing the proliferation of pathology; the occurrence of interstitial substance in the kidney parenchyma (Fig. 2e), entailing the compression of the healthy tissue, which, in the long run, can lead to the organ atrophy.

Liver. Morphological and functional changes in the liver manifested themselves in the form of lipoid dystrophy (Fig. 3b) and hydropic dystrophy (Fig. 3c), which are symptoms of progressive hepatopathy. In 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 in protein and water exchange. In this case, the permeability of cell membranes increases, vacuoles appear in the cytoplasm due to water input therein, and the cell's own organoids destroy, while the cell itself becomes filled with water and dies. In case of lipoid dystrophy, considerable amount of occlusions of fat, almost completely filling all the cells, appear in hepatocytes. Diffuse discomplexation of liver beams, 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 (Fig. 3d). Interstitial proliferative inflammation related to hepatocyte necrosis and appearance of inflammation infiltrates were also diagnosed. In the process of their development, the cells of the aforementioned infiltrates transform into collagenic fibres of connective tissue. As a result, a thick connective-tissue capsule can appear around the zone of necrosis (Fig. 3f). Such progressive necrosis and structural reorganization of the tissue can entail liver post-necrosis cirrhosis, leading, in its turn, to hepatic failure. Vast zones of hemorrhages into the parenchyma, destruction of blood corpuscles and blood vessel walls, as well as proliferation of connective tissue around the blood vessels were revealed.

Symptoms of chronic congestive hyperemia in veins were revealed. They testify to varicose veins and capillaries, decrease in intravascular blood pressure, and blood flow deceleration. As a result, the supply of the tissues with blood becomes disturbed, and hypoxia of the tissues 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.

Thus, the revealed serious disturbances in the cell structure of vitally important organs in certain fishes testify to unfavorable conditions of their habitat in the Volga River basin, caused, primarily, by contamination of the water bodies with toxic substances.

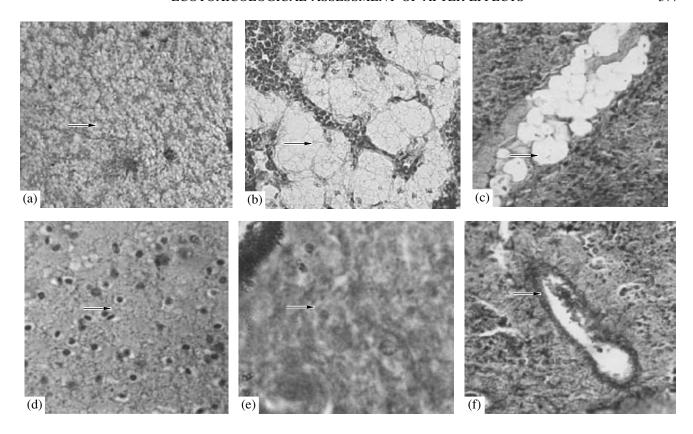


**Fig. 1.** Pathological disturbances in the bream branchiae (here and in Figs. 2–4, arrows). (a) Norm (f is filament, rl is respiratory lamella), 160× magnification; (b) accretion of respiratory lamellas (320× magnification); (c) desquamation of epithelium (320× magnification); (d) hemorrhages between the respiratory lamellas (160× magnification);

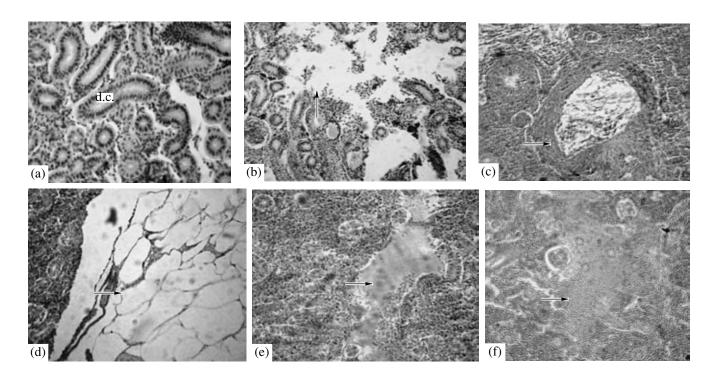
### HEMATHOLOGICAL PARAMETERS OF FISHES USED TO ASSESS THEIR TOXICOSES

The hemogenesis system of fish is sensitive to any environmental changes and changes in the physiological state of the fish organism. At the first stages of toxicosis caused by the effect of toxic agents, protective forces of the organism are mobilized. Such mobilization manifests itself in the intensification of erythropoiesis and leukopoiesiss; young reserve cells and leukocytes of the protective line (monocytes, neutrophiles, and segmentonuclear cells) find their way to the blood channel; the blood becomes thicker. At the stage of destabilization, destroyed cells appear, partly compensated for by reserve young cells. At the stage of degradation, when protective forces of the fish organism are exhausted, blood cells destroy, numerous pathological forms of cells appear, which, in the long run, leads to anemia and death of the fish [15]. Pathologic disturbances in vitally important fish organs (branchiae, liver, and kidneys) develop simultaneously with the destruction of the hemogenesis system of fish, which, in turn, manifests itself in the development of anemia and appearance of pathological blood corpuscles.

The "norm" of hemathological indices is different for each fish species. For most of fish, the concentration of hemoglobin under environmentally optimum conditions varies from 80 to 130 g/l [15]. According to the data presented by L.D. Zhiteneva, the concentration of hemoglobin in the blood of healthy breams varies from 92.0 to 101.0 g/l [10]. A 15% to 30% decrease in hemoglobin concentration is a signal of the fish disease, which can be caused by both invasive and toxic agents. For breams inhabiting the Volga River basin, the value of 90 g/l is adopted as the lower boundary of the "norm" of natural variability in hemoglobin concentration in the blood of these fishes. The largest number of fishes, whose hemoglobin concentration was lower than the said norm, were caught in certain sections of the Lower Volga and in the Gorkii Reservoir.

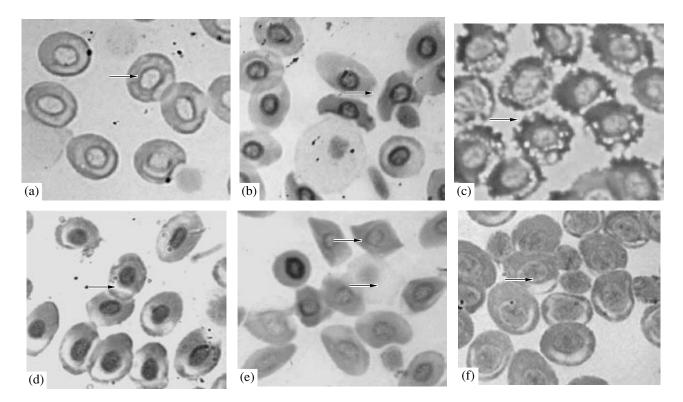


**Fig. 2.** Pathological disturbances in the bream liver (160x magnification). (a) Norm; (b) lipoid dystrophy; (c) hydropic dystrophy; (d) necrosis as symplast; (e) necrosis of the liver parenchyma (almost total absence of cell nuclei, loss of the organ structure); (f) destruction of the liver collagen, drops of cyrrhoid (non-dissolved fat).



**Fig. 3.** Pathological disturbances in the bream kidneys (320× magnification). (a) Norm (d.c. is distal canaliculi); (b) lymphoid tissue degradation; (c) proliferative inflammation; (d) lipoid degeneration; (e) interstitial substance; (f) hemorrhage.

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**Fig. 4.** Pathology in blood corpuscles (160x magnification). (a) Complete necrosis of blood cell nuclei; (b) intussusception of cytoplasm; (c) pronounced vacuolization of the cytoplasm on the blood smears; (d) hypochromasia of the cytoplasm in erythrocytes; (e) rhomboid erythrocytes, lysis of erythrocytes; (f) exfoliation and hypochromasia of the cytoplasm.

The system of hemogenesis of fish is sensitive to the effect of toxic substances. It responds to the latter by different forms of pathological manifestations. Toxic substances affect not only hemoglobin concentration, but change the leukogram and red blood composition as well [10, 11, 21]. The studies have shown that, in different sections of the Volga River, the ratio between different forms of bream cells change (Table 4). The highest percentage of immature forms of erythrocytes was found in the blood smears of the fishes caught in the Lower Volga, which agrees with the low hemoglobin concentration in the blood of the breams mentioned above. Changes in the leukogram of the bream manifested themselves in the increase in the relative amount of neutrophiles and monocytes, especially in certain sections of the Lower Volga and in the lower pool of the Gorkii Reservoir. The main "task" of neutrophiles is to protect the organism against infection and effect of toxic agents. Foam cells were also detected. In the opinion of certain researchers, these cells are transformed neutrophiles, which can be found in peripheral blood of fishes, when their physiological state deteriorates [10, 21]. In some blood specimens, no leukocytes at all were discovered (leukopenia), which testified to disturbed leukopoiesis. In spite of the fact that white blood is a very sensitive indicator of changes occurring in the fish organism, its response is not specific of concrete diseases—it only reflects the disease itself and characterizes its severity.

In the blood smears, different pathological forms of erythrocytes were found. Single instances of pathological forms were reported for the Upper Volga (in the Ivankovo Reservoir), whereas in individual areas of the Kuibyshev Reservoir, the share of pathological blood cell forms revealed in individual fishes reached 8%, and the share of pathological cell forms revealed in fishes caught in the Lower Volga reached 70%. The greatest diversity of pathological forms of red blood cells and leukocytes were observed in the Middle and Lower Volga. Numerous disturbances in the blood cells testify to the fact that the examined fish individuals were at the stage of the organism degradation.

Typical pathology of blood corpuscles included poikilocythemia, anisocytosis, shift of the cell nucleus (resulting even in its occurrence outside the cytoplasm), polymorphism of cell nuclei, opacification of erythrocytes, mitosis of cells and their nuclei, vacuolization of erythrocytes in the cytoplasm, weakening of their shells, different forms of neutrophiles, vacuolization of neutrophiles, eosinophiles, and monocytes in the cytoplasm. Figure 4 presents certain typical pathologic forms of blood cells, observed in the breams caught in the Volga River basin. Poikilocythemia accompanied anemia and manifested itself in the transformation of the form of erythrocytes. Poikilocythemia occurs as a result of decreased elasticity of the blood cells (as a rule, under the impact of chemical factors). Plasmolysis

(disturbances in osmotic resistance) and hemolysis of erythrocytes often occur under the conditions of the fish organism intoxication. These pathological disturbances in the blood corpuscles were typical of the fishes caught in the Gorkii Reservoir and in certain sections of the Lower Volga downstream of Astrakhan.

Thus, the changes revealed in hemathologic parameters of the examined fishes confirm the development of toxicoses in fishes inhabiting the Volga River basin.

### CRITICAL LEVELS OF WATER CONTAMINATION

Water contamination is assessed, as a rule, based on the analysis of the factor of MPC exceedance for individual elements, or the number of cases of MPC exceedance. The governmental report on the environmental state and certain scientific papers consist, mostly, of depressing statement of facts of MPC exceedance in aquatic ecosystems. The following question suggests itself: "How much can such exceedance be dangerous for hydrobionts and human beings?" A complicated character of comprehensive assessment of critical levels of water contamination is explained by the discontinuous character of studies, which does not allow us to reveal the dose-effect dependences between the toxic substance and its biological impact. At the same time, numerous papers substantiate the necessity of determining environmentally substantiated standards or maximum permissible harmful impacts (MPHI). In spite of criticism of the exiting MPC system, it is important for the assessment of the relative danger of different pollutants for aquatic organisms and plants and for determining the role of different substances in the formation of the general ecotoxicological situation. For a long time, MPC values have been playing an important role of "benchmarks" in water protection against contamination.

The MPC values are rather arbitrary. Such conclusion can be made based on the results of comparing the values of critical levels of water contamination adopted in different countries. These values largely differ, in spite of the fact that experimental research techniques of establishing the MPC are universal.

As compared to other countries (Canada, the USA, and member states of the European Union [23, 24]), in Russia, the MPC values for Cu, V, Mn and some other elements are unreasonably underestimated, whereas the MPCs for Cd, As, Pb, and Al are overestimated. The Russian and foreign MPCs for phthalic acid ethers do not coincide (for instance, in Canada, the MPC for dibutyl phthalates is 4  $\mu$ g/l, and for dioctyl phthalates, 0.6  $\mu$ g/l. At the same time, in Russia, the respective values are 1.0 and 10  $\mu$ g/l). Establishing the MPC values for Cu deserves special attention. As a rule, its total concentration in natural waters turns out to exceed the respective MPC value. Cu is found mostly in the form of organic—metal ligands. The "threshold" of the impact

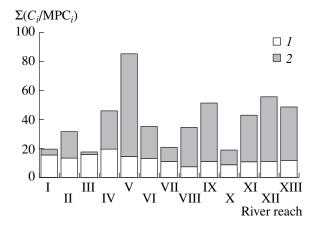
of Cu on aquatic animals and plants is 1.5 µg/l [24]. The value of MPC for Cu is unsubstantiated, and this is the reason why all scientific papers devoted to water body pollution in Russia emphasize the exceedance of the MPC for this metal. The situation with Zn is somewhat similar. Its known acting concentration is 15 µg/l, which is higher than the MPC established for it, and higher than the standards set in Canada and EU (making 30 ug/l). The toxic effect of different elements largely differs depending on the water pH and calcium concentration therein. For instance, in Canada [24], for waters with pH not exceeding 6.5, the MPC for aluminum is 5 µg/l, but where the water pH exceeds 6.5, the MPC for this metal is as high as 100 µg/l. Standards for certain elements are established depending on the concentration of calcium. Thus, if the concentration of CaCO<sub>3</sub> is within the interval of 0 to 60 mg/l, the MPC for Cd will be 0.2 µg/l. If the concentration of CaCO<sub>3</sub> is from 60 to 80 mg/l, the respective MPC for Cd will be 0.8 µg/l. For CaCO<sub>3</sub> concentrations varying from 120 to 180 mg/l and exceeding 180 mg/l, the respective values of the maximum permissible concentration for cadmium will be 1.3 and 1.8 µg/l. Given the same values of CaCO<sub>3</sub> concentration, the respective MPC values for Cu will equal 2, 2, 3, and 4 µg/l; for Pb, they will equal 1, 2, 4, and 7  $\mu$ g/l.

The analysis described above demonstrates the importance of taking into account specific physicochemical parameters of the investigated water in the process of setting MPCs for various pollutants. In the greater part of Russia (from the Arctic zone to the arid one), one and the same MPC values are used, which hampers reliable representation of the actual ecotoxicological situation.

The results of studies analyzed in this paper testify to the fact that the Volga River basin is contaminated with several toxic pollutants. Living organisms are affected by an integral dose of toxic substances, which should be numerically expressed. Different elements and their compounds have different toxicity, which, as was mentioned above, can be arbitrarily characterized by way of standardizing actual pollutant concentration values to their respective MPCs established for fishery water bodies. The integral dose  $(I_z)$  is expressed as the sum of the ratios of the actual concentration  $C_i$  of every element for which the "Toxicological Harmfulness Value" was established, to the respective MPC value  $(MPC_i)$  [3]. This characteristic can be presented as:

$$I_z = \Sigma(C_i/MPC_i)$$
.

For the investigated areas of the Volga basin, Fig. 5 presents the total exceedance of the actual concentrations of toxic elements over their respective MPC values. In different countries, different values, representing the danger of toxic substances occurrence in the aquatic environment, are adopted. Despite of this fact, we used the respective values published in the latest edition of the List of Fishery Water Bodies in Russia [18]. Most probably, it would be more correct to stan-

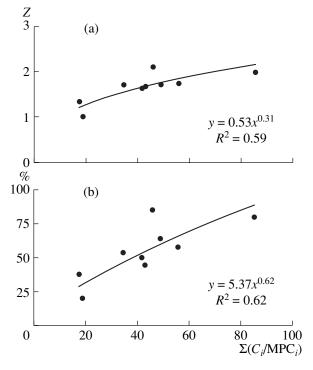


**Fig. 5.** Sum of the actual concentrations of toxic substances divided by the respective  $MPC_{fish}$  values  $(C_i/MPC_i)$  for the investigated sections of the Volga River; (I) microelements; (2) organic compounds.

dardize pollutant concentration values to their respective "threshold values", but no such values have been established for any of the toxic substances found in the Volga River basin. As concerns the whole set of toxic elements, the most heavily contaminated areas were found in the Gorkii Reservoir and in the Lower Volga. Water contamination with metals (from 13 to 20 units) is typical of the Upper Volga. As for inorganic compounds, the maximum concentration values, standardized to the respective MPCs, are typical of Mn, V, and Cu. However, as was mentioned above, this can be explained not so much by the high concentration of these metals in the water, as by the unreasonably underestimated MPC values established for these metals. The concentration of Ca in the Volga River water varied from 24.1 to 39.8 mg/l. 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.

The environmental factors become pathogenic, where the degree of their impact on a living organism exceeds the capacity of its adaptive and protective mechanisms. Under the conditions of "acute" toxicity, the organism quickly dies. If the impact of the toxic factor acquires "chronic" character, the response of the organism turns into a degenerative process. As a result, the disturbed organ or tissue of the affected organism loses its ability to cope with its special functions. Thus, different types of pathology and dysfunction diagnosed in the breams result from comprehensive chronic impact of numerous toxic substances, found in the Volga River water, on the fish organisms.

Determining the critical levels of water contamination requires the calculation of not only numeric parameters characterizing the integral contamination dose but numerical biological criteria, adequately reflecting the effect of toxic substances occurrence in the water as



**Fig. 6.** Dependences of Z (a) on the percentage of fishes, demonstrating the second and third stages of diseases, (b) on the total concentration of toxic substances standardized to MPC ( $C_i/MPC_i$ ).

well. The dose–effect dependences were plotted for several biological parameters, average-weighted for individual river sections: the percentage of fishes in which the second or third stages of diseases were diagnosed; Z index; the percentage of fishes with hemoglobin concentration not reaching 90 g/l; the percentage of neutrophiles in the blood, etc.

The following factors are assumed to have affected the calculation results:

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);

the underestimation of synergetic effects and the presence of other presumably toxic substances in the water, which could have a negative impact on fish organisms;

the persistent effect of toxic substances within the whole lifespan of fishes, the range and concentration of which could be different in different years and seasons;

the subjective character of expert evaluation; measurement errors; small samples of the data obtained, etc.

Despite the complexity of synchronous studies that were carried out and the necessity of accounting for numerous factors, reliable dependences were obtained. These dependences confirm that the morbidity in fishes inhabiting the Volga River basin is related to the occur-

**Table 5.** 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	Blood characteristics								
and compounds $x$	average Hb	Hb not exceeding 90 g/l	/l neutrophiles, percent						
Dibutyl phthalate	y = -5.6Ln( $x$ ) + 107.2 r = 0.65*	-	_						
V	$y = 112.0e^{-0.12x}$ $r = 0.58*$	y = 52.0Ln( $x$ ) + 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 y in								
	the whole organism	the liver	the kidneys						
Hydrocarbons of oil products	y = 8.63 Ln(x) + 32.4 r = 0.53*	y = 10.1Ln( $x$ ) + 24.6 r = 0.56*	y = 0.53x + 16.4 $r = 0.74**$						
Dioctyl phthalate	y = 0.63x + 38.5 $r = 0.61*$	-	_						
Dibutyl 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 Ln(x) - 47.2 r = 0.76 **	y = 0.38x + 5.08 r = 0.68**						

<sup>\*</sup> p < 0.05.

rence of various toxic substances in the water (Fig. 6). Approximation of the dependencies, presented in Fig. 6, into the area of low values to the water quality standard (according to [3], the sum of standardized values for substances with one and the same Toxicological Harmfulness Value should not exceed a unity) yields  $Z \approx 1$ , and the percentage of the fishes, in which the second or third stages of the disease were diagnosed, equal to about 10%. Table 5 presents dependences between the morbidity in fish and the concentrations of certain 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 caused by the negative effect of dioctylphthalate, derivatives of dioxane, and oil products, as well as to that of Cu and Mn. Histologic analysis of fish organs and tissues revealed serious disturbances in the morphological structure and function of the liver and kidneys, as well as in the hemogenesis system; many of such disturbances are irreversible. All this testifies to the disturbance in the organism homeostasis and degradation of its detoxication system. Pathological disturbances in the organs and tissues revealed in the examined fishes could develop under the conditions of water contamination with specific toxic substances, such as organic xenobiotics or heavy metals [5].

The problem of establishing critical levels of water contamination remains open for discussion. The studies carried out by the authors of this paper have shown that the state of the ecosystem "health" in all the investigated river sections is unsatisfactory, and that critical levels of water contamination are exceeded. Different forms of pathology and dysfunction develop in living organisms, which testify to their intoxication. It should be emphasized that, in many aspects, physiological systems of fish are similar to those of hematothermal animals. It means that, based on the investigation of fish diseases, we can predict the consequences of water body contamination with toxic substances for human beings as well. Is the 10% level of "chronic" intoxication in fishes, which approximately corresponds to the standard of the total exceedance of the MPC for toxic elements, allowed? The authors of this paper say: "No." The ecotoxicological studies carried out for certain sections of the Volga River pinpoint the problem of water quality assessment and necessitate further research aimed at correcting water quality standards for the purpose of ecosystem protection against degradation.

#### CONCLUSION

Numerous elements and their compounds that have a toxic effect on living organisms were found in the

<sup>\*\*</sup> p < 0.01.

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 Gorkii 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 con-

vincing for substantiating the information content of methodological solutions and the necessity of continuation of large-scale studies in this field in the future.

#### ACKNOWLEDGMENTS

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