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Fish toxicology

**CHANGES IN THE IRON LEVEL IN THE ORGANS AND TISSUES
OF WELS CATFISH, *SILURUS GLANIS* L. CAUSED BY NICKEL
ZMIANY ILOŚCI ŻELAZA W NARZĄDACH I TKANKACH SUMA
EUROPEJSKIEGO (*SILURUS GLANIS* L.) POD WPŁYWEM
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Bioaccumulation of nickel and the effect of nickel on the iron level in the tissues and organs of wels catfish, *Silurus glanis* L. were studied in the course of the present work. A number of blood parameters were determined e.g. erythrocyte count (RBC), leukocyte count (WBC), haemoglobin concentration (Hb), haematocrit value (Ht), and erythrocyte indices (MCH, MCV, MCHC) were calculated. On stained blood smears erythrocytes and leukocytes were differentiated. Effect of nickel on the activity of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in the blood serum was studied.

INTRODUCTION

In animal organs and tissues nickel occurs in concentrations of 0.X–X µg/g. Its content in the body fluids, however, is lower (Kabata-Pendias and Pendias 1979). Physiological function of this element consists in activation of arginase and carboxylase and in hormonal activity increase. It also plays a significant role: in the processes of organic compound oxidation in the cell membranes, in stabilisation of the nucleic acid structure, and also in metabolism (Harper et al. 1983). It has been suggested that this element binds with plasmin, thus regulating its functions.

All previous studies on nickel, focused mainly on its accumulation in fish organs (Tsoukali-Papadopoulou et al. 1989; Ray et al. 1990; Sreedevi et al. 1992b). The cognitive aim of the present work was to assess the rate of intake of this toxicant, its distribution during both short and prolonged exposure of a fish, and its effect on the iron content in the organs and tissues. Also, quantitative and qualitative changes of the blood were studied, as

well as the dynamics of the activity of alanine aminotransferase and alkaline phosphatase, inducible enzyme and excretion enzyme. The outlined above scope of the present study can be viewed as a practical one contributing to the general knowledge on the Wels catfish—increasingly more popular in aquaculture and praised for its economic importance.

MATERIAL AND METHODS

The material for the present study were wels catfish, *Silurus glanis* L., reared in the experimental station of the Agricultural University of Szczecin. The station is located at Nowe Czarnowo on a discharged canal of the cooling water of the Dolna Odra power plant. The fish weighing 195 g and measuring 28 cm on average were kept in 100-dm³ aquaria, 5 in each, at 19°C. During two weeks of acclimation they were fed the same pellet feed as they received under the rearing conditions. The water in the tanks was changed daily and the catfish were in a good condition during the acclimation. Water hardness was 30°, pH was 6.9 to 7.2, and the oxygen content—9.9 mg O₂/dm³. Water-soluble nickel nitrate served as the toxicant.

In the short experiment the catfish were exposed to nickel nitrate for 24 hours in the concentrations of 0.03, 0.06, 1, 10, 50, and 100 mg/dm³ of water. The lowest concentration of nickel used, represents the highest permissible concentration of this element in surface waters of the first, second, and third class of water purity (Anonymous 1991). During the prolonged exposure of the fish to the toxicant, the nickel concentration in the water was 0.06 mg/dm³. The exposure times were: 1, 3, 7, 14, 30, 45, and 60 days. The concentration at the above-mentioned level has been selected because of the most distinct changes of the blood parameters studied. During both experimental treatments, the effects of the toxicant on the behaviour of the fish were monitored and the appearance and consistence of the internal organs assessed after the end of the experiments. The skin, gills, muscles, intestine, liver, spleen, kidney and the blood were sampled for toxicological study. The blood served also for haematological study. The samples were subjected to dry mineralisation and to mineralisation with addition of concentrated HNO₃. Nickel and iron contents were determined with flame atomic absorption spectrophotometer (FAAS) using Varian Techtron AA1200.

Element contents in the tissues and organs were determined based on the calibration curve and they were expressed in µg/g wet weight.

The blood was collected from caudal vessels following the methods described by Blaxhall and Daisley (1973). The following parameters were determined: erythrocyte count (RBC), leukocyte count (WBC), haemoglobin concentration (Hb), haematocrit value (Ht), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC). Blood smears stained with May-Grünwald

and Giemsa method were prepared from each fish and the adult leukocytes and their juvenile forms were identified (Ivanova 1983; Sopińska 1985; Sobecka 1986). Activity of alanine aminotransferase (ATL) and alkaline phosphatase (ALP) were determined in the blood serum using a Technicon apparatus.

The results obtained were subjected to single- and multi-variate analyses on the confidence level of 95% and to regression analysis (Parker 1978; Podgórski 1995).

RESULTS

During the short-time exposure of the fish to the toxicant the catfish showed distinctly visible changes in their behaviour in three highest concentrations of nickel in the water. The fish were immobile, they gathered near the bottom and their reactions to light and sound were delayed. The skin was lighter than that on the fish in the remaining tanks. Petechiae occurred on their fins and gills. Anatomico-pathological examination of all fish except for control group and fish from the tank with $0.03 \text{ mg of Ni/dm}^3$, revealed congestion of the liver, particularly on its marginal parts. The fish exposed to three highest concentrations of nickel in water showed also uneven coloration of the kidney and petechiae of the parietal peritoneum.

The catfish exposed to nickel for 60 days showed in the last 10 days loss of appetite. Anatomico-pathological examination of the fish kept in water with nickel for more than 30 days revealed excessively filled gall bladder and a minor congestion of the liver. After 60 days of the exposure the kidney of the catfish were grey with occasional hyperaemic foci, while the structure of the organ was spongy, with liquefied spots.

After 24 hours of exposure, all organs and tissues studied showed increased nickel content (Fig. 1). The highest content was observed in the gills and it was statistically different from control sample in all concentrations of the toxicant. The concentrations of nickel in the spleen were statistically significant for all its exposure concentrations except the lowest. Blood, intestine, liver, skin, and kidney accumulated nickel in smaller extent and they differed statistically from control group in three highest concentrations. The lowest level of the toxicant was in the muscles and the correlation between its variable concentration in water and its accumulation in the muscles was relatively low.

The highest levels of nickel, during its action in stable concentration and variable exposure time, were observed in the kidney and the content of this element increased until the end of the experiment. The second highest accumulation rate of nickel was observed in the gills. The toxicant content in this organ constantly grew until day 45 of the experiment and then it systematically declined. The intestine, blood, spleen, and skin cumulated smaller amounts of nickel and differences between its content in all samples and control were statistically significant. Smaller amounts of the toxicant were detected in the muscles of cat-

fish, and the correlation between time and nickel accumulation was—as it was the case for the 24-hour experiment—relatively low (Fig. 2).

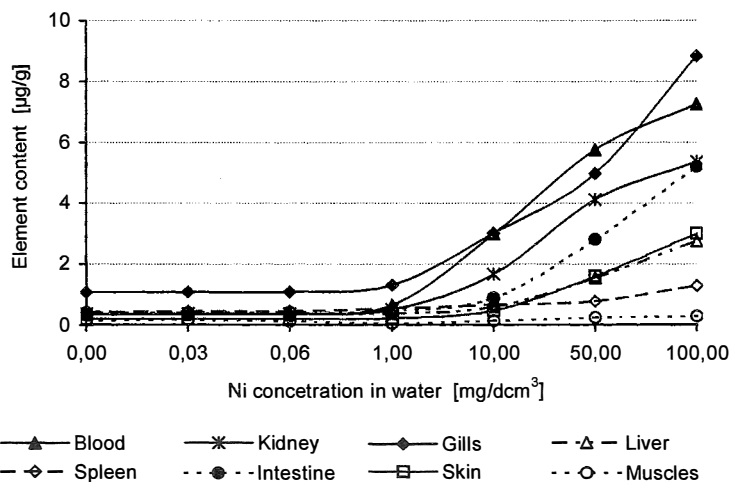


Fig. 1. Changes in the nickel content in tissues and organs of catfish in relation to the nickel concentration in the water (regression functions)

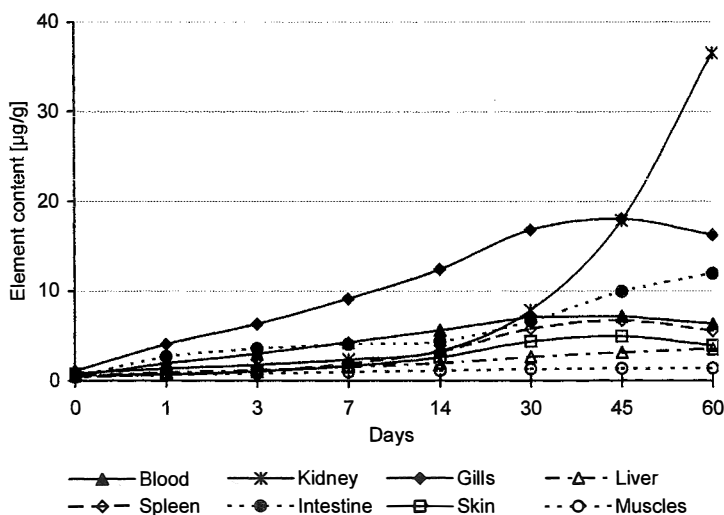


Fig. 2. Changes in the nickel content in the tissues and organs of catfish in relation to the exposure time (regression functions)

The result of short-time exposure of fish to the action of nickel were changes in the iron content in the tissues and organs of catfish (Table 1). The highest level of this microelement was detected in the blood, where significant increase of its level in relation to control group, was visible in four highest levels of the toxicant in water. Iron content in the gills showed also a steady increase and the differences between all samples and the control were statistically significant. In physiological state, iron is present in high amounts in spleen. The increase of the toxicant levels in the water was associated with its content decrease in the latter organ. The same tendency was observed in relation to the iron present in the intestine and all nickel concentrations were statistically significant in relation to control group. In the liver and kidney under lower exposure concentrations of the toxicant the iron content increased, whereas the increase of nickel content was adversely correlated with the iron content in both organs. Changes in the content of the discussed microelement in the skin and muscles were statistically insignificant, with exception for its decrease in the muscles with the highest exposure concentration of the toxicant.

Prolonged period of exposure to nickel in the concentration of 0.06 mg/dm^3 of water had effect on the iron content in the tissues and organs of the fish studied (Table 2). The highest level of this microelement was recorded in the blood after three days of the experiment. Initial increase, followed by a decrease in iron content was observed also in the spleen and kidney and statistically significant differences were demonstrated on each level of the experiment. A small decrease in iron content was noted in the liver, gills, and the intestine of catfish and statistically significant differences in relation to control sample occurred in the two former organs. The iron content in the skin ranged from 2 ± 1.29 to $13 \pm 1.44 \text{ } \mu\text{g/g}$, and in the muscles—from 2 ± 0.7 to $3.8 \pm 0.4 \text{ } \mu\text{g/g}$ of wet weight. No correlation was stated between the exposure time to nickel and the iron content in the muscles and skin.

A small decrease in the haemoglobin level (from 5.2 to 4.8 mmol/l) occurred in the blood of fish following a 24-hour exposure to nickel in the concentration of 100 mg/dm^3 . A visible haematocrit value drop accompanied the following nickel concentrations in water: 0.03, 1, 10, and 100 mg/dm^3 . The lowest value of this parameter was $25\% \pm 2$, while the lowest— $20\% \pm 2$. The erythrocyte count ranged from $(0.96 \div 1.72 \cdot 10^{12}/\text{l})$. Initial increase in the erythrocyte count followed by a decrease was statistically significant in relation to control for the following concentrations of nickel in water: 0.03, 50, and 100 mg/dm^3 . No significant differences in mean values of corpuscular haemoglobin concentration were observed ($\text{MCHC } 20 \pm 2.2$ to $23 \pm 2.0 \text{ mmol/l}$). Differences in mean corpuscular haemoglobin ($\text{MCH } 28 \pm 4$ to $47 \pm 1.3 \cdot 10^{-12} \text{ g}$) and mean values of corpuscular volume ($\text{MCV } 143 \pm 1.5$ to $210 \pm 6.5 \text{ fl}$) were statistically significant for blood of catfish kept in water representing the two lowest concentrations of nickel.

Table 1

Changes in the iron content [mg/kg of wet weight] in organs and tissues of catfish subjected to different concentration of nickel from the range of 0–100 mg/dm³

Ni [mg·dm ⁻³]	Blood [mg·kg ⁻¹]		Kidney mg·kg ⁻¹		Gills mg·kg ⁻¹		Liver mg·kg ⁻¹		Spleen mg·kg ⁻¹		Intestine mg·kg ⁻¹		Skin mg·kg ⁻¹		Muscles mg·kg ⁻¹	
	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
0	458.45	19.5	97.83	2.35	239.18	10.75	274.56	5.62	519.61	10.95	89.43	0.83	6.07	0.24	2.68	0.36
0.03	465.97	26.14	96.7	6.15	341.71	12.91	269.9	2.31	495.27	1.43	92.85	1.06	5.8	0.08	2.54	0.19
0.06	501.22	27.88	85.52	2.77	358.14	6.54	273.95	4.16	495.64	2.38	79.83	0.55	4.68	0.08	2.22	0.2
1	584.41	24.16	104.71	2.63	349.98	6.01	276.03	4.08	498.5	2.01	77.51	0.89	4.83	0.08	2.39	0.1
10	563.57	17.36	100.17	5.98	412.79	10.93	291.11	2.77	492.1	1.41	75.42	0.76	5.49	0.17	2.55	0.18
50	624.31	31.35	85.52	1.44	448.32	9.8	260.62	4.31	474.19	4.44	73.22	0.99	5.72	0.11	2.41	0.17
100	734.39	14.11	84.93	1.73	496.51	10.06	260.65	2.57	473.21	0.91	71.44	0.66	4.87	0.06	1.69	0.17

Table 2

Changes in the iron content [mg/kg of wet weight] in organs and tissues of catfish subjected to a constant concentration of nickel (0.06 mg/dm^3) in time, within the range of 0–60 days

Day	Blood		Kidney		Gills		Liver		Spleen
	$\text{mg}\cdot\text{kg}^{-1}$		$\text{mg}\cdot\text{kg}^{-1}$		$\text{mg}\cdot\text{kg}^{-1}$		$\text{mg}\cdot\text{kg}^{-1}$		$\text{mg}\cdot\text{kg}^{-1}$
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean
0	494	13.38	109	2.43	220.88	0.3	224.82	1.59	421
1	527	7.39	107	3.01	217	0.45	213.02	0.66	431
3	555	13	125	0.41	220.98	1.22	214	1.09	436
7	531	9.66	128	1.55	228.29	0.77	221.54	1.03	441
14	533	8.61	127	1.04	224.2	0.76	214.49	0.89	422
30	539	8.11	131	1.15	219.34	1.23	216.64	0.45	426
45	526	5.34	133	0.95	217.36	1.18	216.4	0.83	418
60	527	2.95	124	1.3	210.7	0.76	214.48	0.36	417

In this experimental treatment a distinct decrease in leukocyte count from 17.66 to $4.29\cdot 10^9/\text{l}$ was observed. The highest difference in relation to control was recorded for the concentration of 0.06 mg Ni/dm^3 in water. The differences between individual values and control sample were statistically significant for all experimental treatments.

Levels of alanine aminotransferase (ALT) in the blood serum of catfish ranged from 42 ± 0.5 to $27 \pm 3 \text{ IU/dm}^3$, and it was statistically different from control sample in the following water concentrations: 0.06 , 1 , 50 , and 100 mg Ni/dm^3 . Differences in the toxicant concentrations did not change significantly the values of alkaline phosphatase. They ranged from 11 ± 1.6 to $18 \pm 1.2 \text{ IU/dm}^3$.

Presence of myeloblasts, promyelocytes, and myelocytes was recorded in the blood of catfish kept in water of the highest concentration of the toxicant. Metamyelocytes and rod-like neutrophils were present in the blood of all fish and they constituted $0.2 \div 2.2\%$ and $1.2 \div 8.8\%$, respectively. Two highest concentrations of the toxicant were associated with vacuoles found in the cytoplasm and nucleus of segmented neutrophils. Pseudo-eosinophiles occurred in the blood of catfish kept in water containing 1 mg/dm^3 of nickel. The number of the latter cells grew significantly in the blood of fish exposed to nickel in two highest concentrations. It amounted to $2.2 \pm 0.3\%$. The number of monocytes also grew and the differences between the numbers of those cells in control sample (4.4% on the average) and other samples studied were statistically significant for each sample. The highest number of monocytes (26.8%) was observed in the blood of catfish placed in water containing 0.06 mg/dm^3 of nickel. In the remaining samples the number of those cells 3–4 times exceeded the number of monocytes in control sample. The number of lymphocytes decreased along with increasing concentrations of the toxicant in water. Single lymphoblasts and prolymphocytes (1.8%) occurred in the blood of fish above the water concentra-

tions of 0.06 mg Ni/dm^3 . The content of those forms associated with the highest nickel concentrations in water reached the level of 5.6%. Erythrocytes of fish kept in water of the highest nickel concentrations exhibited abnormalities in their shape and size. A high number of erythrocytes with hypochromatic cytoplasm and weakly stained nucleus with loose structure of chromatin occurred. Two highest nickel concentrations in water were associated with numerous shadows of disintegrating blood cells. Stain edges occurred around the erythrocytes with very bright cytoplasm.

Changes in the haemoglobin levels occurred in the blood of fish exposed for a long time to nickel in the concentration of 0.06 mg/dm^3 . Increase in the haemoglobin levels become visible since third day of the experiment. The highest values amounting to $15.2 \pm 0.6 \text{ mmol/l}$ were observed after 45 days. Haematocrit values fluctuated and the changes followed the tendencies exhibited by the haemoglobin value changes. The differences between control and experimental samples were statistically significant after 7, 14, 30, 45, and 60 days of catfish exposure to nickel. A distinct increase in the number of erythrocytes was observed after 30 days of experiment ($1.67 \pm 0.15 \cdot 10^{12}/\text{l}$). The highest values of this parameter ($0.79 \pm 0.02 \cdot 10^{12}/\text{l}$) occurred in the blood of fish exposed to the toxicant for the longest time. The mean concentration of haemoglobin in a red blood cell (MCHC) was the highest in the blood sampled after 45 days ($37 \pm 2 \text{ mmol/l}$), while the lowest—after 60 days of exposure to a constant concentration of nickel in water ($19 \pm 2.6 \text{ mmol/l}$). Mean corpuscular haemoglobin (MCH) and the mean corpuscular volume (MCV) were the highest past days 45 and 60 of the experiment, respectively. The above parameters reached the lowest values after 30 days of exposure ($66 \pm 5.6 \cdot 10^{-12} \text{ g}$ and $208 \pm 19 \text{ fl}$, respectively). As early as in the first day of the experiment, a sudden decrease in the leukocyte count was noted. Only in the terminal phase of the experiment it reached a level similar to control ($15 \pm 0.5 \cdot 10^9/\text{l}$).

The highest activity of alanine aminotransferase was recorded in the serum of catfish after one-day exposure to nickel dissolved in water. It amounted to $53 \pm 5.4 \text{ IU/dm}^3$. In time ALT values decreased to drop below the value of control, which was $19.6 \pm 1.1 \text{ IU/dm}^3$. In samples collected after 1, 3, and 7 days of experiment a decrease in alkaline phosphatase content was noted in the blood serum of fish. The enzyme reached the highest value of its activity in day 30 ($21.6 \pm 1.9 \text{ IU/dm}^3$) gradually decreasing towards the end of the experiment to the level of $19.2 \pm 0.9 \text{ IU/dm}^3$. Statistically significant differences in relation to control sample were observed after 30, 34, and 60 days of fish organism exposure to nickel.

In the blood picture—myeloblasts and promyelocytes were found in selected smears of the fish exposed to nickel for the longest time. The same tendency was observed in relation to myelocytes (19.6%), metamyelocytes (12.6%), and rod-like neutrophils (5%).

A distinct drop in the number of segmented-nucleus neutrophils was observed on blood smears of all fish. The differences were statistically significant in relation to control sample. The share of these cells in control was 9% on average, while only 1%—after 30 and 45 days. Their cytoplasm was abundant in vacuoles and their nuclei did not undergo sufficient divisions. The monocyte numbers slightly increased in consecutive days of the experiment. Lymphocytes constituted the most numerous group in the blood picture assessed. Their number was significantly different in relation to control sample at all levels of the experiment. Presence of activated lymphocytes as well as small cells was stated. After prolonged exposure of the toxicant the nuclei of lymphocytes divided. The percentage of the immature forms of these cells, similarly as eosinophils and basophils was low and statistically insignificant.

Red blood cells showed a tendency for a rouleau formation, especially after 45 days of experiment. Basophilic erythroblasts were also observed in the blood of this group of fish—some 3 ÷ 5 in a single view field. The blood picture of fish exposed for nickel for 60 days featured a distinct drop in erythrocyte count, anisocytosis, uneven outer edge of cells with the stain halo, and toxic granulations, present in the cytoplasm of cells. Similarly as during a short exposure to nickel, shadows of disintegrating blood cells were visible, although they occurred in smaller numbers.

DISCUSSION

Element content in the tissues and organs of fishes is positively correlated with their amounts added to the water. The relative content was as follows: gills > blood > kidney > intestine > skin > liver > spleen > muscles. Sreedevi et al. (1992b) after four days of carp exposure to nickel dissolved in water, found out that the highest accumulation of this element occurred in the gills followed by the liver, muscles, and the kidney of fish. On the other hand Ray et al. (1990) studying for 4 days the dynamics of nickel accumulation in tissues of *Clarias batrachus* noticed that the highest amounts of this element were found in the kidney, followed by the liver, gills, and the intestine. The reason behind the discrepancies between the cited above authors, were probably different exposure times of nickel assumed in the experiments.

During the prolonged exposure of fish to nickel present in water (0.06 mg/dm³) the most of this toxicant accumulated in the kidney, with a distinct increase after 14 days. Taking into account the appearance of this organ, assessed during the anatomo-pathological study—it can be concluded that the excretory potential of the kidney decreased in the course of prolonged nickel exposure (Gardner and Yevich 1970). Ray et al. (1990) reached a similar conclusion studying results of a 30-day exposure to nickel on *Clarias batrachus*.

The above authors determined that the organs most vulnerable to toxicants are kidneys and gills. The same sequence of nickel accumulation was recorded in the presently studied catfish. Stagg and Shuttelworth (1982), based on their study results on effects of a prolonged copper exposure on the organism of a flounder, also found out that the strongest action of the toxicant was observed on the gills and kidney, where processes of osmotic and ionic exchange take place. The associated stress causes increased permeability of the epithelium while the toxicant itself destroys the structure of the soft tissue, exposed on the direct way to its action.

Relatively high amounts of nickel were accumulated in the intestine where the nickel was delivered in two ways: with the blood, through ionic exchange and through water and food. The latter way does not allow forgetting about a significant role of the food chain in transmission of pollutants to its higher levels (Pujin et al. 1990).

It has been demonstrated that the amounts of heavy metals accumulated in the liver, many times exceeded the amounts accumulated in the muscles (Hanna 1989; Hellou et al. 1992). The liver binds the toxicants with thiol groups creating metallo-thioneins and thus removing them from organismal fluids. The above process contributes to the lowering of the concentrations of the toxic substance circulating in the organism. Roch and McCarter (1984) determined a direct proportion between the concentration of a toxic compound and the amounts of metallo-thioneins in the liver. The amounts of nickel in the blood of catfish studied increased gradually towards day 30 of the experiment to stabilise for the next thirty days. The graph illustrating the accumulation pace of this compound shows a picture similar to a plateau. A similar course shows a curve illustrating the pace of nickel accumulation in the spleen, which is associated with the structure and function of this organ.

The skin and muscles gathered the smallest amounts of nickel after 60 days of exposure. During a comparative study on metal content in tissues of catfish, reared in water bodies of the Czech Republic Svobodova et al. (1995) noticed relatively low concentrations of cadmium, copper, chromium, and zinc in the muscles. On the other hand, lead and mercury attained sometimes higher values than in the liver and gonads. Therefore the accumulation levels of metals in the muscles are not equal. Windom et al. (1987) suggested that the accumulation level of metals in the muscles depends on the size and age of fish, and also on the toxicity level of the chemical compounds affection the organism.

The effect of a toxicant on an organism can be determined through changes of iron content in the organs and tissues of fish studied. In the first experimental treatment the iron content in the blood and gills grew constantly, declining at the same time in the spleen, liver, and the skin. The content of nickel in the kidney and liver decreased also with two lowest concentrations of this element in the water. In a healthy organism the pool of serum iron is constantly supplemented by the iron supplied by intestinal absorption, stored in the

liver and spleen. The principal source, however, is the iron provided by physiological disintegration of erythrocytes. Iron release from erythrocytes takes place in the liver and spleen, where old blood cells are phagocyted by macrophages. Macrophages have ability to decompose hem and to rely iron to transferin (Williams et al. 1990). Transferin provides iron in the process of erythropoiesis (Stratil et al. 1985) and it better binds iron from mucous cells of the intestine. Whanger (1973) suggests that cadmium hinders intestinal absorption blocking iron binding potential in mucous cells. Similar conclusions were reached studying lead poisoning cases in humans (Krechniak 1994). It is therefore possible, that other heavy metals, in this number also nickel, may play a similar role. Transferin molecule, instead of iron ions, can bind to bivalent copper, zinc, magnesium, and cobalt ions (Malkiewicz 1983). It is therefore very likely that it can bind also to nickel ions. The above process would be a good explanation for the declining content of iron in the organs of the catfish studied.

Substantial numbers of disintegrating blood cells were visible in the blood picture of fish in both experimental treatments. The reason behind such a state was probably a destructive influence of nickel on cell membranes of erythrocytes through binding of the toxicant with immunoglobulins or through disturbance of the activity of erythrocyte enzymes, especially those responsible for reduction of glutathione and thiol groups of proteins (Sun et al. 1985). According to Kleczkowski et al. (1998) the excessive loss of glutathione, increased release of iron to intracellular spaces, peroxidation, destruction of cell membranes, and release of metal ions to the surrounding tissues should be attributed to free oxygen radicals. The latter are formed in an organism under influence of iron and copper ions, to name just a few causes. The effect of the described processes is instability of haemoglobin, structural changes in erythrocytes and increased susceptibility to haemolysis. Consequently the pool of the serum iron from disintegrating erythrocytes increases, while the iron content in the spleen decreases, especially during a short exposure of fish to nickel in high concentrations.

In the present study a significant decrease in erythrocyte count, haematocrit, and the leukocyte count was observed in the blood of catfish as a response to 24-h exposure to increasing concentrations of nickel in the water. The haemoglobin values fluctuated with the prevailing declining tendency. A similar effect was described by Areechton and Plumb (1990) and Singh et al. (1992) who subjected fish to pesticides. The observed symptoms, suggest onset of anaemia caused by a disturbance of haemopoiesis through the presence of toxicant or by balance disturbance in cell membranes through acetylcholine hydrolysis in body fluids by erythrocytic cholinesterase. The decrease of the numbers of circulating erythrocytes can be explained by a response of fish organism to ACTH increase as a result of stress caused by the presence of nickel. People and animals poisoned with chemical sub-

stances, in this number heavy metals, typically show a decreased production of erythrocytes, their shortened viability, and less effective erythropoiesis. In vitro exposure of isolated erythrocytes to lead demonstrated that this element disturbs the action of cation pump, and probably also limits the activity of ATP-ase of cell membranes, leading to their destruction (Williams et al. 1990). The above authors mentioned, that a decrease in the number of erythrocytes in the circulating blood not necessarily has to be associated with decreasing iron level. Even in cases of anaemia caused by iron deficiency, the level of this element, in the initial phase of the disorder does not change and even sometimes rises. Mean corpuscular haemoglobin (MCH) increases proportionally to the nickel content in the water, similarly as mean corpuscular haemoglobin (MCV). Considering the shortened life span of erythrocytes and the associated disorders in oxygen transport—it is a natural, defensive reaction of the organism. Decrease in haemoglobin content, erythrocyte count and increase of MCH and MCV accompanied intoxication of carp, exposed for 24 hours to cypermethrin (Reddy and Bashamohideen 1989). The authors compared the obtained results with symptoms of hypochromic microcytic anaemia, associated with iron deficiency, which in turn leads to a decrease in haemoglobin synthesis. Microcytic anaemia in fish coincides usually with haemolytic anaemia (Stoskopf 1993). Cypermethrin caused also an increase of MCHC in the blood of carp, which in humans, according to Aleksandrowicz (1969), occurs only in congenital sphaerocytosis.

Effects of nickel are the most distinct during prolonged exposure of an organism to its action. A distinct decrease in erythrocyte count after 60-day exposure of catfish to nickel was accompanied by a decrease in haemoglobin concentration, following its initial increase. The same reaction was described by Niimi and Lowe-Jinde (1984) who studied effects of mercury on rainbow trout and Dawson (1979)—on American flounder. The exposure caused a substantial increase of MCV factor, and no changes in MCH. A similar effect was observed for the above coefficients in the blood of catfish studied. The MCHC value decreased, but this parameter was confined to the limits determined for healthy catfish (Mares 1996, Svobodova et al. 1997). Chmielnicka (1994) describing mechanism of toxic action of lead, determined that anaemia and disorders of alimentary tract and kidney function were associated with latter stages of this disease. The above-mentioned element inhibits activity of enzymes involved in synthesis on hem. Inhibiting activity of ferrochelatase causes an increase of free porphyrines in the erythrocytes and an increase of iron in the blood serum of catfish. It is worth to mention, that changes in kidneys occurred, especially in the catfish exposed to the toxicant for the longest time.

A distinct decrease in the number of white blood cells (WBC) was observed in both experimental treatments, while a decrease in percentage share in—blood smears. According to Tomaszewski (1997) the cause of leukopenia can be hindering of granulopoiesis or

lymphopoiesis, induced by primary or secondary changes in haematopoietic organs. Another cause can be inhibition of white blood cell maturation and their release from tissue reservoirs. Srivastava and Sahai (1987) explain occurrence of leukopenia by an organismal response to a stress caused by toxic compounds. The authors suggest that both processes can be associated with occurrence of allergic reactions.

An increased number of juvenile erythrocytes along with intensified haemolytic changes were visible in stained blood smears, which was an effect of regenerative mechanism. A toxic granulation occurred in the erythrocyte cytoplasm of fish exposed for the longest time for the toxic compound. Eiras (1990) studying pathologic variability of red blood cells in different fish species, suggested that such changes are effect of antropogenic water pollutants. In addition, the catfish studied shown hypochromatic anaemia, anisocytosis and poikilocytosis. Very similar changes in eels, as a reaction to pollutants, described Orecka-Grabda (1986).

An increase in the number of young leukocytes and their degenerative changes were observed in the white-blood-cell system, especially during long exposure to the action of nickel. Vacuolisation occurring in the nuclei and the cytoplasm of these cells is a result of oxygen management disorders (Begeman and Rasteter 1979), while degenerative changes in the nuclei of granulocytes and agranulocytes are explained by an effect of a toxicant to haematopoietic tissue.

Per se examination of liver functions is based on three types of clinical tests, performed on humans. The first one consists in measuring time needed for removal of a toxic substance from the blood. The second method covers monitoring of the concentration changes of thioneins, produced by an organism or substances catalysed by liver e.g. bilirubins. The third approach consists in examining the blood serum for activity of the enzymes, which are usually present in higher concentrations in the liver parenchyma. Their elevated activity in the blood indicates an irritation or dysfunction of this organ.

The lowest activity of ALT was observed with the concentration of 0.06 mgNi/dm^3 of water and this level was maintained for the other concentrations of the toxic compound. The highest activity of this enzyme, almost three times higher, than that of control sample, was observed in second experimental treatment after one day of exposure for nickel. ALT activity, with small fluctuations, gradually decreased, to retain after 60 days the initial value. After 30 days of the experiment, the highest ALP activity, almost two times higher in relation to control sample, was observed.

A low activity of aminotransferases is associated with renal failure (Murray et al. 1995). An increase in enzyme activity occurs usually along with increased activation of compensative mechanisms in the liver, understood as a form of adaptation to prolonged stress caused by the presence of a toxicant (Sredevi et al. 1992a). Singh and Reddy (1990)

noticed that along with prolonged exposure time of *Heteropneustes fossilis* to copper the activity of ALP and ALT increased. The growth curve for both enzymes assumes a similar course to the results observed in wels catfish.

CONCLUSIONS

1. The results obtained, justify the statement, that both factors nickel concentration in the water and prolonged exposure time had effect on organism of wels catfish. The amount of the toxicant in tissues and organs of fish was in direct proportion to its amount in the water, but the pace of its build-up in organs and tissues was variable.
2. Nickel accumulation in tissues and organs caused disorders in distribution of iron, particularly in the blood, gills, intestine, and skin.
3. Short exposure to nickel caused symptoms of developing anaemia in catfish, while prolonged exposure—a toxic poisoning.
4. Changes in activity of alanine aminotransferase indicated worsening renal failure. Increase in alkaline phosphatase indicated a liver damage, resulting from active detoxification function and nickel accumulation in this organ.
5. A minimal nickel accumulation in the muscles is caused probably by their least extensive blood supply and different structure of the blood vessels. It is particularly significant from a point of view of a consumer.

ACKNOWLEDGMENTS

I would like to thank Prof. Dr hab. Mikołaj Protasowicki for his valuable comments in the course of the present work. I appreciate help of Dr Michał Sobecki in the statistical aspects of this paper.

REFERENCES

- Aleksandrowicz J., 1969: Choroby krwi i układu krwiotwórczego [Diseases of blood and haematopoietic system]. PZWL, Warszawa. (In Polish).
- Anonymous, 1991: O czystości wód powierzchniowych [On the purity of surface waters]. Dziennik Ustaw RP, 116, poz. 503.
- Areechton N., J.A. Plumb, 1990: Sublethal effect of malathion on channel catfish, *Ictalurus punctatus*. Bull. Environ. Contam. Toxicol., **44**: 435–442.
- Begeman H., J. Rasteter, 1979: Atlas of clinical hematology. Springer Verlag, Berlin.
- Blaxhall P.C., K.W. Daisley, 1973: Routine haematological methods for use with fish blood. J. Fish Biol., **5**, 771–781.

- Chmielnicka J.**, 1994: Metale i metaloidy [Metals and metaloids]. In: Toksykologia [Toxicology] [Seńczuk W. (ed.)]. PZWL, Warszawa. (In Polish).
- Dawson M.**, 1979: Hematological effect of long-term mercury exposure and subsequent periods of recovery on the winter flounder, *Pseudopleuronectes americanus*. Marine pollution: functional responses. Academic Press Inc.: 171–182.
- Eiras J.C.**, 1990: Observation on erythrocyte abnormalities in fish. Bull. Eur. Ass. Fish Pathol., **10**: 64–68.
- Gardner G.R., P.P. Yevich**, 1970: Histological and hematological responses of an estuarine teleost to cadmium. J. Fish. Res. Bd Can., **27**: 2185–2196.
- Hanna Rifaat G.M.**, 1989: Levels of heavy metals in Red Sea fish before hot brine pools mining. Mar. Pollut. Bull., **20** (12): 631–635.
- Harper H.A., V.W. Rodwell, P.A. Mayes**, 1983: Zarys chemii fizjologicznej [Outline of physiological chemistry]. PZWL, Warszawa. (In Polish).
- Hellou J., W.G. Warren, J.F. Payne, S. Belkhode, P. Lobel**, 1992: Heavy metals and other elements in three tissues of cod, *Gadus morhua* from the Northwest Atlantic. Mar. Pollut. Bull., **24** (9): 452–458.
- Ivanova N.T.**, 1983: Atlas krotek krovi ryb [Atlas of fish blood cells]. Legkaja i piščevaja promyšlennost, Moskva. (In Russian).
- Kabata-Pendias A., H. Pendias**, 1979: Pierwiastki śladowe w środowisku biologicznym [Trace elements in biological environment]. Wydawnictwa Geologiczne, Warszawa. (In Polish).
- Kleczkowski M., W. Kluciński, E. Sitarska, J. Sikora**, 1998: Stres oksydacyjny i wybrane wskaźniki stanu antyoksydacyjnego zwierząt [Oxidative stress and selected factors of anti-oxidative state of animals]. Med. Wet. **54** (3): 166–171. (In Polish).
- Krechniak J.**, 1994: Losy trucizn w organizmie [Faith of toxins in an organism]. In: Toksykologia [Toxicology] [Seńczuk W. (ed.)]. PZWL, Warszawa. (In Polish).
- Malkiewicz B.**, 1983: Biochemia żelaza i pierwiastków śladowych oraz ich rola w ustroju [Biochemistry of iron and trace elements and their role in an organism]. In: Biochemia kliniczna w praktyce lekarskiej [Clinical Biochemistry in medical practice] [Sznajda J. (ed.)]. PZWL, Warszawa. (In Polish).
- Mareš J.**, 1996: Biological and technological aspect of intensive breeding of European wels (*Silurus glanis* L.). Dissertation. Mendel Univ. Agricult. Forestr. Brno.
- Murray R.K., D.K. Granner, P.A. Mayes, V.W. Rodwell**, 1995: Biochemia Harpera [Harpers's biochemistry]. PZWL, Warszawa. (In Polish).
- Niimi A.J., L. Lowe-Jinde**, 1984: Differential blood cell ratios of rainbow trout (*Salmo gairdneri*) exposed to methylmercury and chlorobenzen. Arch. Environ. Contam. Toxicol., **13**: 303–311.
- Orecka-Grabda T.**, 1986: Haematological, clinical and anatomical pathology of the European eel (*Anguilla anguilla* (L.)) from polluted waters of northwestern Poland. Acta Ichthyol. Piscat., **16**, 1: 107–127.
- Parker R.E.**, 1978: Wprowadzenie do statystyki dla biologów [Introduction to statistics for biologists]. PWN, Warszawa. (In Polish).
- Podgórski J.**, 1995: Statystyka z komputerem [Microcomputer statistics]. MIKOM, Warszawa. (In Polish).
- Pujin V., N. Djukić, S. Maletin, S. Obradović, D. Kostić**, 1990: Content of heavy metals in some fish species in the section of the Danube flowing through Vojvodina. Wat. Sci. Tech., **22**, 5: 79–86.
- Ray D., S.K. Banerjee, M. Chatterje**, 1990: Bioacumulation of nickel and vanadium in tissues of the catfish *Clarias batrachus*. J. Inorg. Biochem., **38**: 16–173.
- Reddy P.M., M.D. Bashamohideen**, 1989: Fenvalerate and cypermethrin induced changes in the haematological parameters of *Cyprinus carpio*. Acta Hydrochim. Hydrobiol. **17**: 101–107.
- Roch M., J.A. McCarter**, 1984: Hepatic metallothionein production and resistance to heavy metals by rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol., **77C**, 1: 77–82.

- Singh N.N., A.K. Srivastava**, 1992: Blood discrasia in the freshwater indian catfish *Heteropneustes fossilis* after acute exposure to a sublethal concentration of propoxur. *Acta Hydrobiol.*, **34**, 1/2: 189–195.
- Sobecka E.**, 1986: Wpływ poziomu białka w paszy na wybrane parametry krwi obwodowej karpia (*Cyprinus carpio* L.) [Effect of protein level in the feed on selected parameters of peripheral blood of carp (*Cyprinus carpio* L.)]. *Zesz. Nauk. Akad. Rol. Szczec.*, **121**: 3–10. (In Polish).
- Sopińska A.**, 1985: Effect of physiological factors, stress and disease on hematologic parameters of carp, with a particular reference to the leucocyte patterns. III. Changes in blood accompanying branchionecrosis and bothriocephalosis. *Acta Ichthyol. Piscat.*, **15**, 2: 141–170.
- Sreedevi P., B. Sivaramakrishna, A. Suresh, K. Radhakrishnaiah**, 1992a: Effect of nickel on some aspect of the gill and kidney of the freshwater fish, *Cyprinus carpio* L. *Environ. Pollution*, **77**: 59–63.
- Sreedevi P., A. Sures, B. Sivaramakrishna, B. Prabhavathi, K. Radhakrishnaiah**, 1992b: Bioaccumulation of nickel in the organs of the freshwater fish, *Cyprinus carpio*, and the freshwater mussel, *Lamellidens marginalis*, under lethal and sublethal nickel stress. *Chemosphere*, **24**, 1: 29–36.
- Srivastava A.K., L. Sahai**, 1987: Effect of loading density on carbohydrate metabolism and haematology in the freshwater catfish, *Heteropneustes fossilis*. *Aquaculture*, **66**: 275–286.
- Stagg R.M., T.J. Shuttleworth**, 1982: The accumulation of copper in *Platichthys flesus* L. and its effects on plasma electrolyte concentration. *J. Fish Biol.*, **20**: 491–500.
- Stoskopf M.**, 1993: Fish medicine. W.B. Saunders Company, Philadelphia.
- Stratil A., V. Tomasek, J.R. Clamb, J. Williams**, 1985: Partial characterization of transferrin of catfish (*Silurus glanis* L.) and pike (*Esox lucius* L.). *Comp. Biochem. Physiol.*, **80 B**, 4: 909–911.
- Sun T., L. Chin-Yang, L.T. Yam**, 1985: Atlas of cytochemistry and immunochemistry of hematologic neoplasm. Am. Soc. Clin. Pathol. Press, Chicago.
- Svobodova Z., V. Piacka, B. Vykusova, J. Machova, M. Hejtmanek, M. Hrbkova, J. Bastl**, 1995: Residues of pollutants in siluriformes from various localities of the Czech Republic. *Acta Vet.*, **64**: 195–208.
- Svobodova Z., J. Kolarova, J. Kouril, J. Hamakova, B. Vykusova, P. Kalab**, 1997: Hematological investigation in *Silurus glanis* L. females during pre- and postspawning period. *Pol. Arch. Hydrobiol.*, **44**, 1–2: 67–81.
- Tomaszewski J.J.**, 1997. Diagnostyka laboratoryjna [Laboratory diagnostics]. PZWL, Warszawa. (In Polish).
- Tsoukali-Papadopoulou H., I. Kaniou-Gregoriades, P. Epivatianos, J.A. Stratis**, 1989: Heavy metals in marine organisms of Geras Gulf. Lesvos, Greece. *J. Environ. Sci. Health*, **24**, 1: 39–47.
- Whanger P.D.**, 1973: Effect of dietary cadmium upon intracellular distribution of hepatic iron in rat. *Res. Commun. Chem. Pathol.*, **5**: 733–738.
- Williams W.J., E. Beutler, A.J. Erslev, M.A. Lichtman**, 1990: Hematology. McGraw-Hill bl. Comp., New York.
- Windom H., D. Stein, R. Sheldon., J.R. Smith**, 1987: Comparison of trace metal concentrations in muscle tissue of a benthopelagic fish (*Coryphaenoides armatus*) from the Atlantic and Pacific ocean. *Deep-Sea Research*, **34**, 2: 213–220.

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ZMIANY ILOŚCI ŻELAZA W NARZĄDACH I TKANKACH SUMA EUROPEJSKIEGO
SILURUS GLANIS L. POD WPLYWEM DZIAŁANIA NIKLU

STRESZCZENIE

Badano bioakumulację niklu w skórze, skrzelach, mięśniach, wątrobie, nerce, śledzionie, jelicie i krwi sumy europejskiego *Silurus glanis* L. Badano także wpływ niklu na poziom żelaza w narządach oraz oznaczano we krwi liczbę erytrocytów (RBC), leukocytów (WBC), ilość hemoglobiny (Hb), hematokryt (Ht), obliczano wskaźniki czerwonych krwinek (MCH, MCV, MCHC). W barwionych rozmazach różnicowano krwinki białe i czerwone. W osoczu badano wpływ niklu na aktywność aminotransferazy alaninowej (ALT) i fosfatazy zasadowej (ALP). Po 24 godz. ekspozycji na działanie niklu, ilość jego wzrosła we wszystkich badanych narządach i tkankach w następującym porządku: jelito > skrzel > krew > nerka > skóra > wątroba > mięśnie. Najwięcej zmian zaobserwowano przy stężeniu 0,06 mgNi/dm³ w wodzie. Po 60-dniowej ekspozycji na działanie toksykantu kumulacja niklu przebiegała w sposób następujący: nerka > skrzel > jelito > krew > śledziona > skóra > wątroba > mięśnie. W tkankach i narządach sumów w obu wariantach doświadczenia zauważono zmiany zawartości żelaza, a także wybranych parametrów krwi i aktywności ALT i ALP.

Received: 31 October 2000

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