SOME HAEMATOLOGICAL AND PHYSIOLOGICAL INDICES MODIFICATIONS ON FISH UNDER THE ACTION OF CHEMICAL POLLUTANTS

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Abstract. Pollution represents one of the main causes inducing modifications of the structures and physical functions of the aquatic ecosystems. The toxic substances harmful action works upon the living material, first at the individual level, and then, like a chain reaction, at the other levels: species and biocoenosis. The toxicity of a chemical substance varies very much from a species to another and even from an organism group to another. From our experiments, one has noticed that copper sulphate has an inhibitory effect on the oxygen consumption at all observed fish. Realizing the survival curves, one has realized that the toxicity of the copper sulphate grows with the concentration, being stronger at bigger fish. The red cells number is smaller, both in the copper sulphate case and the rest of the used fungicides (Champion and Funguran contain copper hydroxide, Ridomil and Curzat contain copper oxychloride). The red cells low number determines the low oxygen consumption. The experiments show the fact that the used substances are insignificantly toxic for the fish.

Keywords: fish, pollution, connotative elements, oxygen, pesticides, toxic substances.

Rezumat. Modificări ale unor indici hematologici şi fiziologici la peşti sub acţiunea unor agenţi chimici poluanţi. Poluarea reprezintă una din cauzele principale ale modificării structurilor şi funcţiilor naturale ale ecosistemelor acvatice. Acţiunea nocivă a substanţelor toxice se manifestă asupra materiei vii mai întâi la nivelul individului şi apoi, asemenea unei reacţii în lanţ, şi asupra celorlalte niveluri: specie şi biocenoză. Toxicitatea unei substanţe chimice variază foarte mult de la o specie la alta şi chiar de la o grupă la alta de organisme. Din experienţele efectuate de noi s-a constatat că sulfatul de cupru are efect inhibitor asupra consumului de oxigen pentru toate exemplarele de peşti cercetate. Realizând curbele de supravieţuire, s-a constatat că acţiunea toxică a sulfatului de cupru creşte odată cu creşterea concentraţiei, fiind mai puternică asupra exemplarelor de talie mai mare. Numărul de eritrocite a scăzut atât în cazul sulfatului de cupru cât şi la restul fungicidelor folosite (Championul şi Funguranul conţin hidroxid de cupru, Ridomilul şi Curzatul, oxiclorură de cupru). Scăderea numărului de eritrocite determină scăderea consumului de oxigen). Experienţele efectuate ilustrează faptul că substanţele folosite sunt toxice pentru peşti în concentraţii foarte mici.

Cuvinte cheie: peşti, poluare, elemente figurate, oxigen, pesticide, substanţe toxice.

INTRODUCTION

Pollution represents one of the main causes inducing modifications of the structures and physical functions of the aquatic ecosystems (BOTNARIUC & VĂDINEANU, 1982; BREZEANU & SIMON-GRUIŢĂ, 2002). The most important consequence of the water chemical pollution is the entire functional and structural changing, which the polluted waters bring upon the biological systems present in the aquatic ecosystems. The inherent complexity of the ecological effects often leads at the sudden quality change as a result of some progressive quantity accumulation. In order to characterize, ecotoxicologically speaking, the dynamics of such an ecosystem, in its interaction with the pollutant factors, and to exactly understand the ecological significance of the toxic effects both on the living organisms community and on the a biotic component, where these are structurally and functionally integrated, one must analyse all the phases of the pollution process. Numerous research in the environment pollution biological effects field are oriented towards the functional changes at animals, in the presence of some chemicals obtained in the industrial technology processes or in agriculture, which are further collected by internal waters (DRĂGHICI, 1979). The paper presents the results of the investigations of the haematological indices changings at fish, under the action of some pollutants (fungicides). The harmful action of the toxic substances works upon the living material, first at the individual level, and then, like a chain reaction, at the other levels: species and biocoenosis. The toxic action of a substance, its intensity and speed vary according to certain factors. These factors influence the substance toxic action in two ways, which normally act together:

- a direct action upon the organism physiological activity, changing the metabolic processes;
- an action upon that toxic substance, which can get its physic-chemical properties or its concentration modified.

The most important external factors are: the toxic substances interaction, temperature, the water content from the dissolved oxygen, the content from the carbon dioxide, Ph, the mineral salts content, light, the water current speed and turbidity. Among the internal factors with a bigger significance concerning the toxic action intensity are: species, the organism weight, its physical state, season, previous life conditions. The chemical substance toxicity varies a lot from one species to another and even from one group to another. For example, it is known the fact that the gastropods and crustaceans are more sensitive to copper than fish, which, in turn, are more sensitive than the chironomid larvae (GAVRILESCU, 2008; MĂLĂCEA, 1969).

The fish exposure to a copper sulphate bearable concentration (0.36 mg/l) in different periods showed a significant decrease in haemoglobin (Hb) 10.73 - 6.60%, in the red cells from the blood (RBC) 2.86 - 1.84 x $10^6/mm^3$ and haematocrit (PCV) 31.00 - 23.33%. The number of the white cells (WBC) grew 60.00 - 92.48 x $10^3/mm^3$, the coagulation time (TC) from 27.66 to 43.00 seconds, the erythrocyte sedimentation rate 5.0 - 13.66 mm/hr (SINGH et al., 2008). The oxygen transport in blood depends on the amount of haemoglobin. One has noticed significant growth on the levels of cortisol in plasma at the Rainbow Trout after the exposure at bearable copper concentration (0.25 mg/l) for 24 hours (CARBALLO et al., 1995).

At *Cyprinus carpio gibelio* (L.) newborns exposed at bearable copper and cadmium concentrations (0.1, 0.25, 0.4 ppm), one noticed a low survival rate together with the growth of each metal. The copper is more toxic than the cadmium, the survival rate decreases from 50% to 10% for copper, and from 70% to 20% for cadmium, during 15 days. The oxygen consumption decreases while the concentration increases and there is a reverse correlation between the oxygen consumption and the metals concentration. The results indicated the epithelium separation from the secondary lamellae, hyperplasia, fusion of the secondary lamellae and necrosis, the main death cause for the hard metals exposed fish being hypoxia (HASSAN, 2011). The copper toxicity at fish has been reported by others authors (TAYLOR et. al., 2000; ERIKSEN et al., 2001; TRUFAȘ, 2003; LODHI et al., 2006).

Both essential metals (for example Cu, Mn, Zn) and those unessential (i.e. Cd, Pb, Hg) are important in aquatic toxicology because high dose exposure has toxic effects on living organisms. These effects are generally due to the unspecific binding of the reactive cation of the metal to the important cell macromolecules, determining a change in its functions (CHRISTIE & COSTA, 1884). The adjustment mechanisms of the intracellular concentration of metal distribution between different macromolecules have the main role of metal homeostasis maintaining and of optimum cell function (MARINESCU, 2000; MARINESCU et al., 1997).

MATERIAL AND METHODS

The research have been realized on the *Carassius auratus gibelio* because it adjusts well to the captivity conditions from the aquarium, it tolerates huge temperature loggers and it is resistant to a low oxygen level, possessing a low hypoxic lethal limit. It is a species of economic interest, spread in Romanian internal waters. The representatives of *Carassius auratus gibelio* were from the Olt river, Slatina region, considering the physiological state, the body integrity and the size. The aquarium used during the intoxication experiment had 20-30 litres and possessed shaking and aeration installations. These recipients have been cleaned and sanitized. The fish have been introduced in the aquarium a month before the start of the experiment, in order to adjust themselves to the new conditions.

From the three general methods of estimating the quantity of the oxygen present in the water (iodometric, gasometrical, polar graphic methods), in this experiment, we used the Winkler iodometric method (unmodified) as described at the end of the last century (WINKLER, 1888), being considered the safest way of estimating the quantity of the oxygen present in the water. In order to establish the oxygen consumption, we used the confined space technique (explained by STROGANOV, 1962), which allows us to exactly establish the right quantity used by the fish in a previously determined time, by the difference obtained between the oxygen from a witness-boll ant that achieved at the end of the experiment.

To carry out the experiments rendering the variation in the number of erythrocytes under the fungicides action, we drew fish blood and used counting chambers. All the determinations made on fish have been rigorously controlled. The long-time experiments or those needing different day series have been always realized in a narrow nyctemer limits (the same hours of the day), avoiding thus a possible circadian variation influence on the energetic metabolism. The representatives used in different experiments have been chosen according to weight, in order to avoid or, in the contrary, emphasize the individual body weight factor. Following the achievement of some experimental conditions as close as possible to those existing in nature, we kept and used in the experiment natural photoperiodism fish. Although, a direct relationship between the energetic metabolism and the light has not been proved, we took into account the light importance while determining some functional conduct (MOTELICĂ et al., 1965).

A big interest had the fish manipulation effect in the pre-experimental phase, which can modify the determination results (FRY, 1957; MARINESCU, 1972). In the case of the experiments made with the confined space technique, where the fish disturbance by manipulation could not be avoided, we tried its limitation. Thus, we chose for the experiment low mobility species of fish (Prussian carp) and "blind" trials before the experiment have been made.

Experimental variants. Eight experimental variants have been conceived. In the first three, we followed the copper sulphate action on the oxygen consumption. Two groups of fish were used: the small weight group and the big weight group, each with three variants. For the rest of the variants, we observed the copper sulphate and other fungicides action (Champion 50 WP - contains copper hydroxide 50% metallic copper, Funguran 50 WP-contains copper hydroxide 50% metallic copper, Ridomil gold plus 42.5 WP-contains oxychloride copper with 40% metallic copper, Curzate manox-contains oxychloride copper 50% metallic copper),on some haematological indices (the number of red cells in the blood of fish). We used eight-fish groups, with a 30-50 g weight. The determinations were made after seven days of treatment.

RESULTS AND DISCUSSIONS

In the first experimental variant, we observed the copper sulphate influence on the oxygen consumption at the Prussian carp, using 0.8 mg copper sulphate concentration/l water (Fig. 1). For both fish groups (having as average weight 10.56 g and 58.74 g), it was observed a progressive growth of the oxygen consumption in the first hours (after 48 hours for the small weight group the growth was 22.80% compared to the witness - 0:00 a.m., while for the large weight group the growth was 24.14% compared to the witness - 0:00 a.m.), followed by a progressive deduction of this index value.

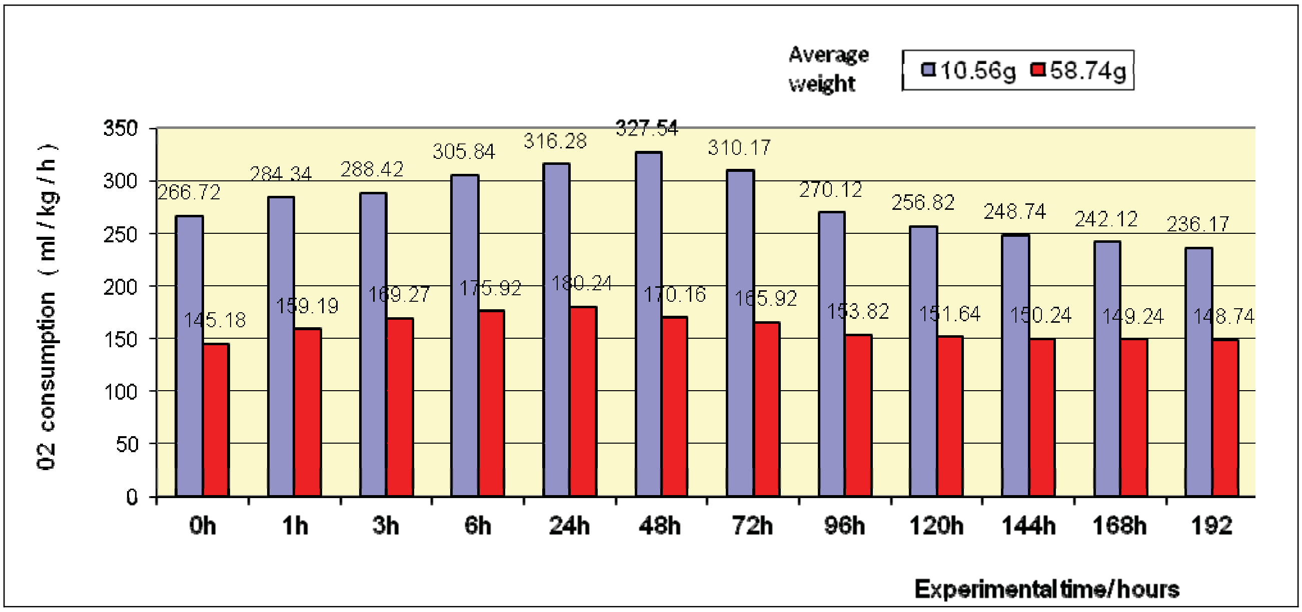

Figure 1. The oxygen consumption variation at the *Carassius auratus gibelio* under the copper sulphate influence, at 0.8 mg/l concentration.

One might conclude that at this concentration the copper sulphate has a little toxic action in the first experimental hours, yet the fish completely recover after the eighth day in the big weight group (the growth is 2.45% compared to the witness) and almost completely for the small weight individuals (the oxygen consumption representing 88.54% from the witness consumption). Thus, the small weight fish proved to be more sensitive to the copper sulphate action, in comparison with the big weight fish at the same concentration.

In the second experimental variant, we have used 1.6 mg/l water copper sulphate concentration (Fig. 2). There has been a progressive growth of the O_2 consumption in the first 24 hours for the 14.98 g medium weight fish, the growth being 44.52% compared to the witness, followed by a progressive decrease of this index value and a progressive O_2 consumption increase in the first 6 hours for the 40.38 g weight fish, the growth being 42.03% compared to the witness, followed by a progressive decrease. At both groups, we registered mortality, the average tolerance limit (TLm) being 192 h for the 14.98 g medium weight fish and 118 h for the 40.38 g weight fish.

The first fish from the small size group died after 106 h, while the last one after 226 hours. The first fish from the large size group died after 88 h, and the last one died after 132 hours. One may observe that large size fish are more sensitive at this concentration than the small size ones.

In the third experimental variant, we used 3.2 mg/l water copper sulphate concentration (Fig. 3). There is a progressive decrease of the oxygen consumption of 70.04 % compared to the witness at 15.02 g medium weight group, while at 58.14 g weight group, there is a progressive decrease of the oxygen consumption of 60.81 % compared to the witness.

At both groups, we registered mortality, the average tolerance limit (TLm) being 48 h at big size fish and 96 h at small size fish. The first big size fish died after 26 h, and the last one after 74 h. The first small size fish died after 72 h, and the last one after 110 h. One may observe that the big size fish are more sensitive at this concentration, as well.

Drawing survival curves (Fig. 4), one notices that the 3.2 mg/l copper sulphate has the biggest toxicity at big size fish, while the 1.6 mg/l copper sulphate has the lowest toxicity at small size fish. The toxicity of the 0.8 mg/l substance did not cause death, the fish recovering after 8 days of experiment. The fact that the 3.2 mg/l copper sulphate and the 1.6 mg/l copper sulphate toxicity is bigger at big size fish is probably due to their size, the concentration growth decreasing the survival time.

The average tolerance limit (TLm) is in descending order as it follows: TLm for the 1.6 mg/ l concentration, small size fish (192 h) ≥ 1.6 mg/l concentration, big size fish (118 h) ≥ 3.2 mg/l concentration, small size fish (96 h) ≥ 3.2 mg/l concentration, big size fish (48 h). The closest to the OY axis the survival curve is, the smallest the fish survival is.

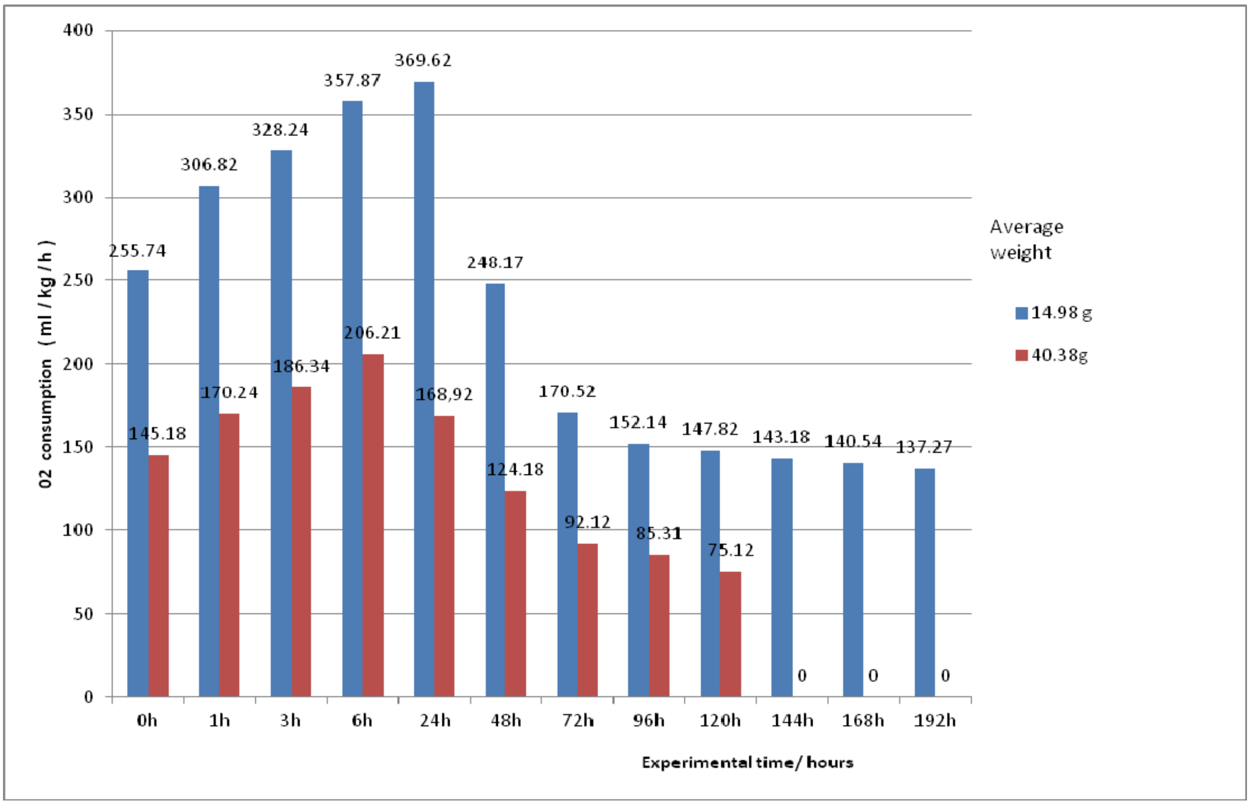

Figure 2. The oxygen consumption variation at the *Carassius* under the copper sulphate influence, at 1.86 mg/l concentration.

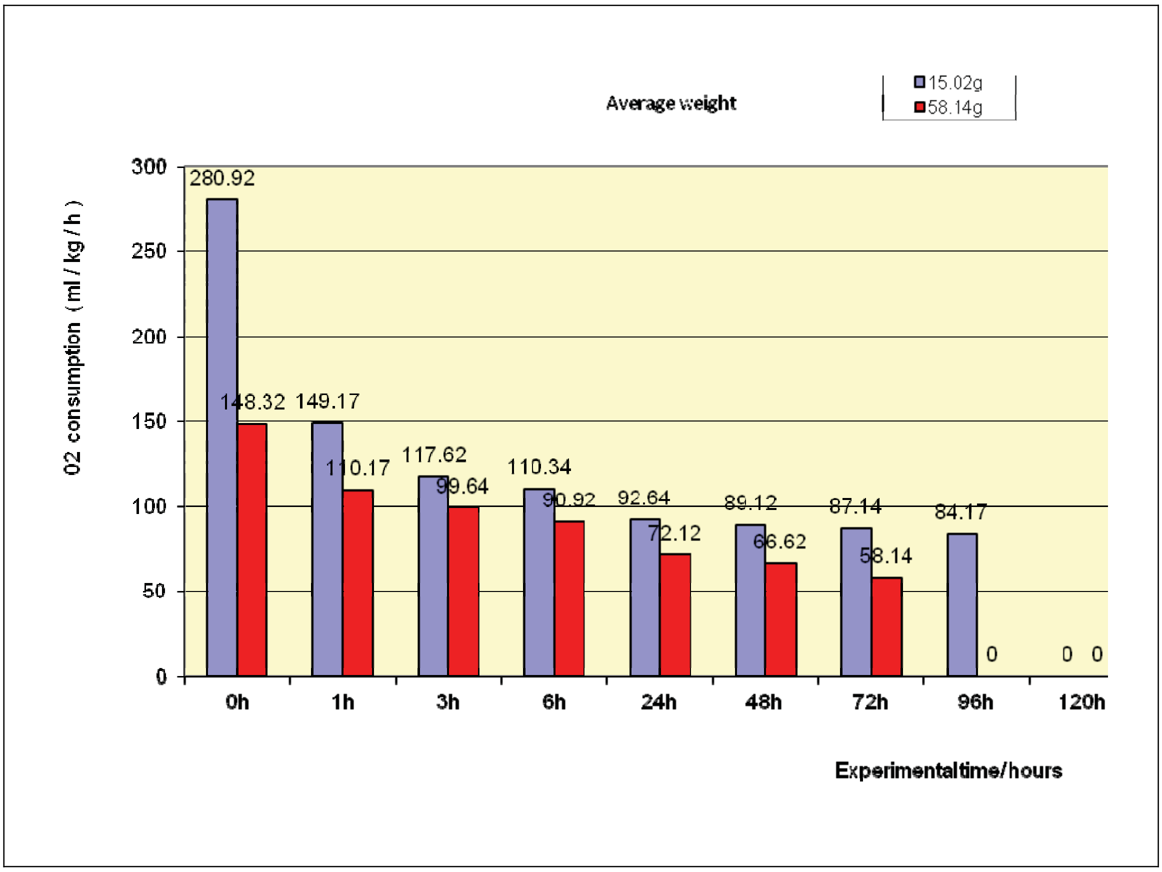

Figure 3. The oxygen consumption variation at the *Carassius* under the copper sulphate influence, at 3.2mg/l concentration.

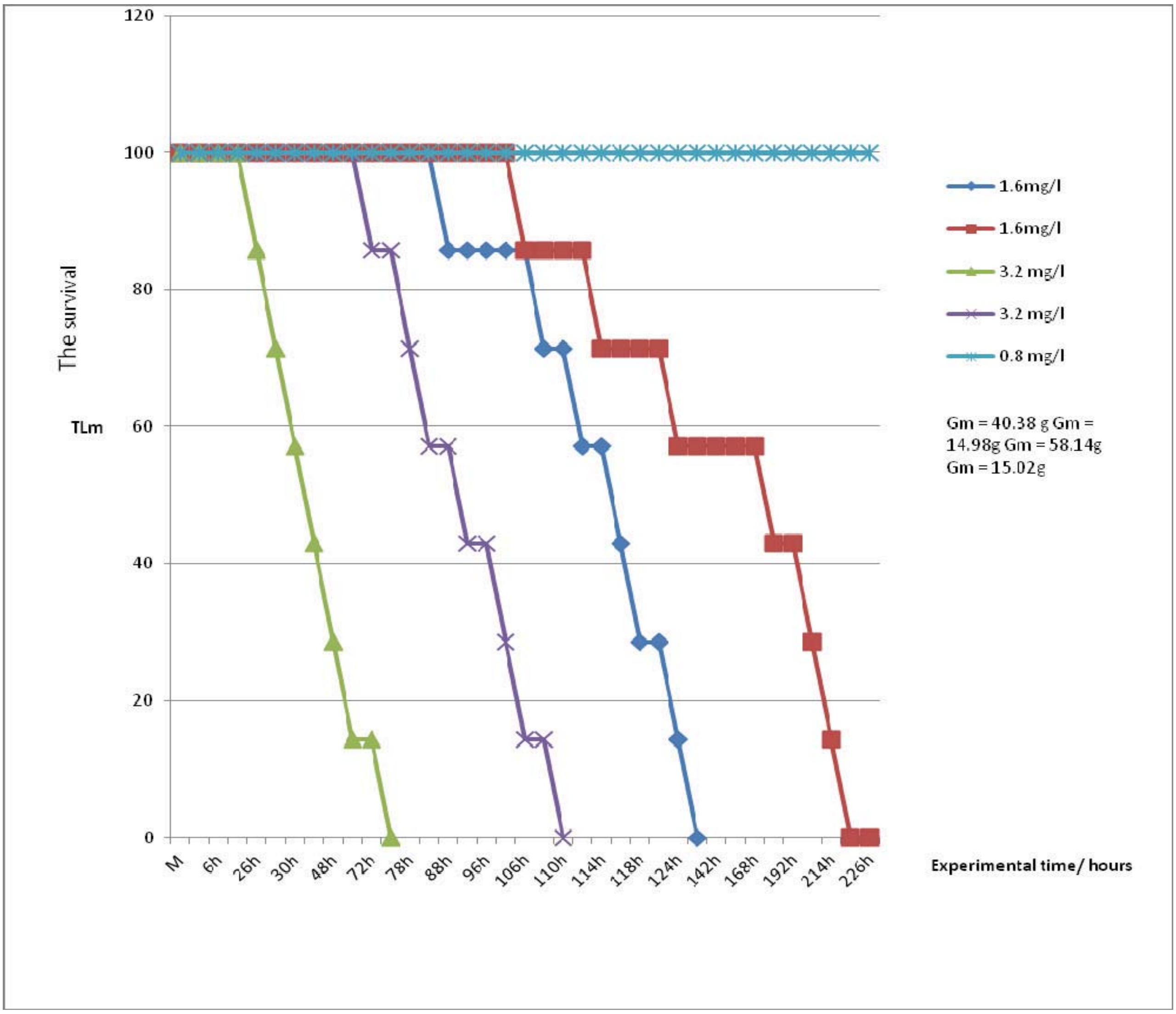

Figure 4. The fish survival curve under the copper sulphate action, at different concentration.

In the figures 5-9, there are shown the variations of the number of red blood cells in the carp under the copper sulphate action and under the Champion, Funguran, Ridomil şi Curzate fungicides in 0.5 mg/ l concentrations.

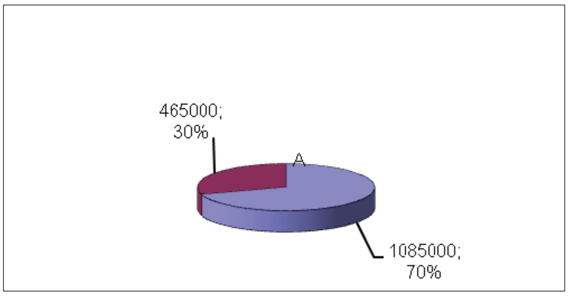

Figure 5. The 0.5 mg/ l copper sulphate effect on the number of red blood cells in *Carassius auratus gibelio* BLOCH.

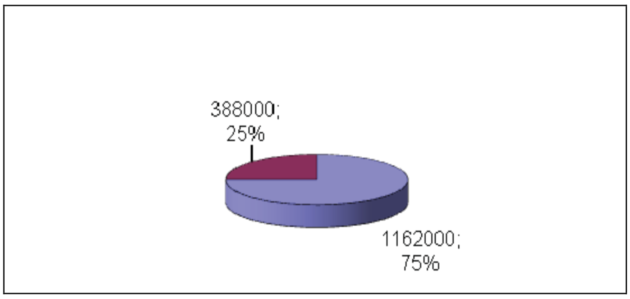

Figure 6. The influence of 50 WP Champion fungicide in a 0.5 mg/l concentration on the number of red blood cells in *Carassius auratus gibelio* BLOCH.

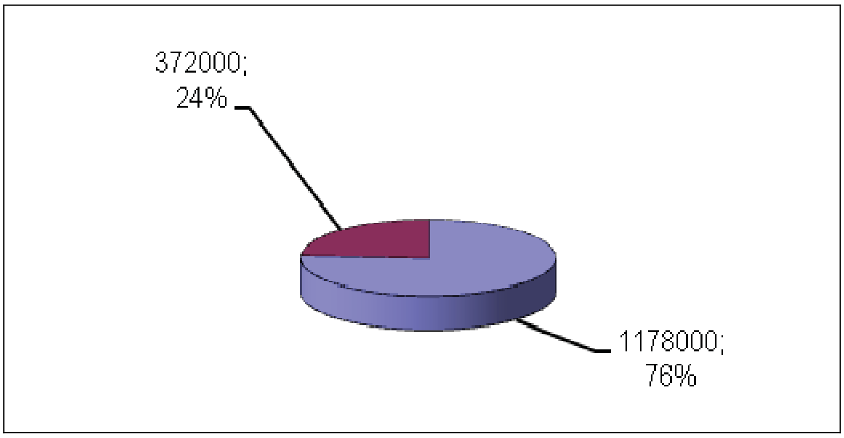

Figure 7. The influence of 50WP Funguran fungicide in a 0.5 mg/l concentration on the number of red blood cells in *Carassius auratus gibelio* BLOCH.

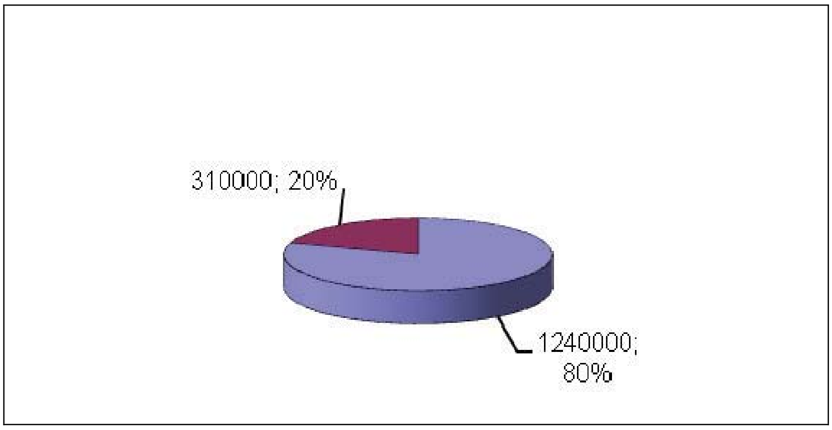

Figure 8. The influence of 42.5WP Ridomil fungicide in a 0.5 mg/l concentration on the number of red blood cells in *Carassius auratus gibelio* BLOCH.

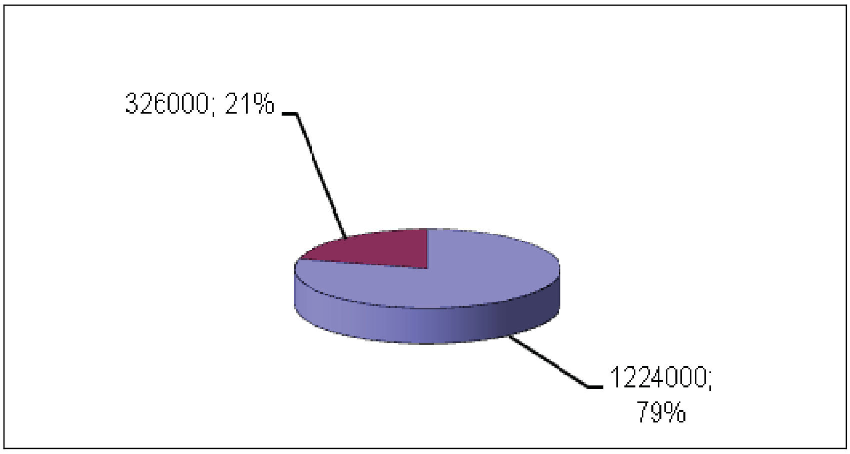

Figure 9. The influence of Curzate manox fungicide in a 0.5 mg/l concentration on the number of red blood cells in *Carassius auratus gibelio* BLOCH.

In the red section there is the difference between the initial red blood cells (witness group) and the final one (the experimental group, for 7 days at that specific concentration). It is obvious that the number of the red blood cells decreased in all 5 experiments. As it is shown, the biggest regression of the number of red blood cells is in the copper sulphate case (Fig. 5), representing 30% from the witness value. The rest of the fungicides had a similar action, the single difference being the percentage: the decrease was 25% from the witness value in Champion case (Fig. 6), 24% in Funguran case (Fig. 7), 20% in Ridomil case (Fig. 8) and 21% in Curzate case (Fig. 9).

The decrease of the number of red blood cells may be produced not necessary by erythrocytes haemolysis but through reductions of the capacity of education and free circulation of erythrocytes. There might appear the reduction of the haematopoietic tissue and its replacement with fat tissue. The liver and kidney damage, organs with haematopoietic tissue, might also cause the decrease of the moving red blood cells.

The toxic copper action on fish manifests through whitish mucus secretion, first on the gills, and then on the entire body, the growth of the breathing movements being caused by the gill damage, which has as consequence the reduction of their functional capacity, and furthermore the decrease of oxygen intake; it can be noticed the swelling or bleeding of the brachial epithelium and the mouth cavity, the equilibrium loss and finally death.

CONCLUSIONS

From the experiments, it is obvious the copper sulphate inhibitory effect on the oxygen consumption for all the observed specimens. At the 0.8 mg/ l copper sulphate, small size fish have proved more sensitive, in comparison to big size fish, both groups recovering after the eighth day of the experiment. At 1.6 mg/l copper sulphate and 3.2 mg/l copper sulphate, big size fish have been more sensitive than small ones. At these concentrations, death has been registered. Achieving survival curves, it has been noticed that the copper sulphate toxicity increases with the concentration growth, being stronger at big size fish.

The results obtained in all 5 experiments of dilutions of tested substances and a control test show that all used substances present negative action on the haematological indices. The number of red blood cells decreased both in the copper sulphate case and in other used fungicides case (Championul and Funguranul contain copper hydroxide, Ridomil and Curzat contain copper oxychloride). The number of red blood cells decrease determines a low oxygen consumption (a weaker oxygen intake of the blood can be caused among others by a moving red blood cells decrease). The experiments show the fact that the used substances are toxic for fish even in small concentration, thus it is recommended to avoid their use in a river or other water sources.

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