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ECO-TOXICOLOGICAL RESEARCH OF THE NICKEL INFLUENCE ON SOME PHYSIOLOGICAL PARAMETERS IN FISH (*CARASSIUS AURATUS GIBELIO* BLOCH AND *RHODEUS SERICEUS AMARUS* L.)

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Abstract: This paper studied the effect of nickel derived from nickel acetate (Ni $(NO_3)_2$), in three concentrations of nickel (3, 6 and 9 mg·l^{-1}of water) on some physiological indexes in two species of native fish (*Carassius auratus gibelio* Bloch and *Rhodeus sericeus amarus* L.). The consumption of oxygen in both investigated species decreased significantly after the first 24 hours of exposure to nickel (with more than 30% as compared to control values) for all the three investigated concentrations, the values determined subsequently showing gradual increase from one determination to the other, with the re-establishment of the energetic metabolism being slower in the Prussian carp (*Carassius auratus gibelio*) as compared to the European bitterling (*Rhodeus sericeus amarus*). The respiratory rate of the fish exposed for 7 days to the 3 investigated sub-lethal concentrations of Ni also increased progressively (except for the values measured after 24 hours of exposure), until the 7th day of exposure, the values of this index determined after 336 hours being significantly lower than the control values. To be noted that the for the European bitterling, the frequency of the opercular movements after 14 days of exposure to the three Ni concentrations does not show significant changes as compared to the values registered after 24 hours from the immersion of fish into the Ni solutions. Average glycaemia and blood cell count values in the case of groups treated with Ni increased significantly, the most pronounced increases being recorded at the highest concentration of nickel (9 mg·l^{-1} of water; at this concentration the glycaemia levels exceeded almost four times the values recorded in the control group at both species).In conclusion, the changes observed indicate that investigated physiological parameters can be used as an indicator of Ni related stress in fish on exposure to elevated Ni levels.

Key words: nickel, Prussian carp, European bitterling, oxygen consumption, glycaemia, breathing frequency

1. INTRODUCTION

The impact of xenobiotics of anthropogenic origin on aquatic ecosystems is a major concern on the map in eco-toxicological research. Especially heavy metals are common contaminants and persistent on the aquatic environment; they come as a result of human activities or of natural processes (Garcia-Montelongo et al., 1994; Jordao et al., 2002; Greger, 2004). Contamination of water ecosystems by heavy metals occurs due to their toxicity, their persistence and bioaccumulation and bio-amplification along food chains with long-term risks for the ecological systems (Ma et al., 1997; Adams et al., 2000; Papagiannis et al., 2004, Iordache, 2009).

Adapting the living organism to his surrounding environment is one of the most complex and on the map problems of fundamental and applied biological sciences. Defined as modification of organisms, including of their regulatory processes, under the prolonged or repeated action of one (or more) stimuli from the environment (Adolph, 1964), the adapting process is present in all categories of living creatures.

Adapting to the environment this been recorded at all levels of biological organization (Prosser, 1958; Precht, 1973; Hochachka and Somero, 1973). From a behavioural standpoint, organisms appear acting in ways that lead to improving their chances of survival, as well as of exploitation of their particular habitats. At the physiological level (the so-called “physiological adaptation” (Pora et al., 1952; Marinescu, 1971), the manner in which the functions are realized almost always reflect the conditions of the respective ambient.

Aquatic organisms are able to accumulate heavy metals up to concentrations that are tenths and even thousands of times higher than their concentrations in the environment.

The main natural sources are the rocks and soils (Bradl, 2005). In the upper part of the Argeș River, the geological formations consist of metamorphic rocks of the Upper Getic Nappe and of the Argeș Nappe, (Balintoni 1997). Lithozone of Mioarele from Upper Getic Nappe and lithozone of Vâlsan from Argeș Nappe contain paragneisses, amphibolites, metabases with high nickel content. The amphibolites of the Mioarele lithozone in Vîlsan Valley hosted mineralizations with pyrite Chalcopyrite and pyrrhotite with nickel (Udubasa et al., 1988). Fragments of these formations appear in the sands and gravel of Mio-Pliocene in the Getic Depression. This is the nickel source in the Arges-Vedea Water Basin.

In this case, the nickel pollution in the Arges River Basin illustrated in Table 1 and 2 results especially from the large urban agglomerations, from the industrial and agricultural activities.

Water pollution with heavy metals affects various physiological processes in fish. Heavy metals at high concentrations can cause harmful effects on metabolic, physiological and biochemical systems of fishes (Yang et al., 2003) and it causes long-term eco-toxicological effects (Starmark & Braunbeck, 2000).

The effects of waterborne metalson fish are related to their uptake and accumulation by the organism, resulting in metal-induced disturbances in the structure and function of various tissues and organs (Jezierska & Witeska, 2001). While small amounts of nickel are essential for the aquatic physiological processes, a series of tests have demonstrated that some compounds of this metal ($NiSO_4 \cdot 6H_2O$ and $NiCl_2 \cdot 6H_2O$) can be classified as substances harmful for the aquatic environment, when found in high concentrations, causing aquatic animals homeostasis disorder (Farkas et al., 2002; Javed, 2003).

This study is justified also by the presence of nickel in the Argeş River, as shown in Table 3, which contains data from the Argeş-Vedea Water Basin Administration.

Reduction of oxygen consumption rate in fish exposed to heavy metals indicate the onset of hypoxia under metallic stress (James, 1990), because metals accumulate in gill epithelium and induce lesions like necrosis, thickening and separation of respiratory epithelium (Peuranen et al., 1994; Hassan, 2005), also it may result in an increase of diffusion distance between the water and blood which makes oxygen absorption difficult (Dalzell & MacFurtan, 1994; Aardt & Booysen, 2004).

Goss and Wood (1988) suggested that heavy metals act on gill function resulting in a decrease in oxygen consumption rate because of ion regulatory and acid-base disturbance.

Tabel 1. Discharges of heavy metals into water resources from industrial and agricultural point sources in the Argeş-Vedea hydrographical area (Source: Argeş-Vedea Water Basin Administration 2012).

Categories of agglomerations/Pollutants evacuated	Copper Kg/year	Zinc Kg/year	Cadmium Kg/year	Nickel Kg/year	Lead Kg/year	Mercury Kg/year	Chrome Kg/year
IPPC Industry	19.000	707.396	2.607	43.042	9.026	1.041	13.063
NON IPPC Industry	0.034	28.204	0.310	1.000	1.000	0.000	20.002
Total Industry	19.034	735.600	2.918	44.042	10.026	1.041	33.065
Other Points of Sources	0.318	0.000	0.000	0.127	0.000	0.010	0.000

Tabel 2. Discharges of heavy metals into the water resources from human agglomerations in the Argeş-Vedea hydrographical area (Source: Argeş-Vedea Water Basin Administration 2012).

Categories of agglomerations/Pollutants evacuated	Copper Kg/year	Zinc Kg/year	Cadmium Kg/year	Nickel Kg/year	Lead Kg/year	Mercury Kg/year	Chrome Kg/year
>100000 inhabitants	5521.00	107331.00	782.00	4283.00	3416.00	0.000	4540.00
10000-100000 inhabitants	30.39	1729.00	3.66	9.68	9.60	0.000	46.05
2000-10000 inhabitants	4.0	143.00	1.00	4.00	4.00	0.000	3.02
<2000 inhabitants							
Total	5555.39	109203.00	786.66	4296.68	3429.65	0.000	4589.07

Table 3. Concentration of heavy metals found in the photic zone of the Budeasa Dam in 2012 (Source: Argeş-Vedea Water Basin Administration 2012).

Group	Indicator	Unit of measure	Count	Average	Min	Max	StDev
PS-M	Dissolved cadmium	µg·l	12.000	0.050	0.050	0.050	0.000
SP-M	Total cadmium	µg·l	12.000	0.068	0.050	0.210	0.047
PS-M	Dissolved mercury	µg·l	11.000	0.017	0.005	0.122	0.035
SP-M	Total mercury	µg·l	11.000	0.027	0.005	0.166	0.047
PS-M	Dissolved nickel	µg·l	12.000	1.000	1.000	1.000	0.000
SP-M	Total nickel	µg·l	12.000	1.317	1.000	4.810	1.099
PS-M	Dissolved lead	µg·l	12.000	1.085	1.000	2.020	0.294
SP-M	Total lead	µg·l	12.000	1.403	1.000	3.300	0.773
SP-M	Dissolved copper	µg·l	4.000	0.730	0.250	2.170	0.960
SP-M	Total copper	µg·/l	12.000	1.179	0.250	2.320	0.676
SP-M	Total zinc	µg·/l	12.000	25.000	25.000	25.000	0.000
SP-M	Total chrome (Cr^{3+} + Cr^{6+})	µg·/l	12.000	0.500	0.500	0.500	0.000
SP-M	Total arsenic	µg·/l	11.000	2.112	1.500	6.390	1.488
SP-M	Dissolved barium	µg·/l	1.000	49.580	49.580	49.580	0.000
SP-M	Total barium	µg·/l	11.000	17.988	1.000	35.360	10.064
SP-M	Dissolved beryllium	µg·/l	1.000	0.050	0.050	0.050	0.000
SP-M	Total beryllium	µg·/l	11.000	0.073	0.050	0.310	0.078
SP-M	Dissolved boron	µg·/l	1.000	0.750	0.750	0.750	0.000
SP-M	Total boron	µg·/l	11.000	1.346	0.750	7.313	1.978
SP-M	Dissolved selenium	µg·/l	1.000	0.525	0.525	0.525	0.000
SP-M	Total selenium	µg·/l	11.000	0.570	0.525	1.030	0.152

SP-M – specific pollutants metals, PS-M – Priority substances metals

The low number of data in the specialized publications regarding modification of some physiological indices under the action of nickel in fish, in general, and in the species *Carassius auratus gibelio* and *Rhodeus sericeus amarus,* in particular, has motivated us to conduct these researches that could highlight the effect of this metal on biomarkers such as **oxygen consumption, respiratory rate, number of erythrocytes** and **glycaemia** in fish exposed to various concentrations of Ni.

2. MATERIAL AND METHODS

This study was performed with the approval of the local Committee of Bioethics according to the Romanian law 205/2004 art.7, 18, 22 and regulation number 143/400/2002 for care and use of animals for research purposes.

Measurements were performed in (2012) on specimens of species *Carassius auratus gibelio* and *Rhodeus sericeus amarus* taken from Lake Budeasa, with a weight between 14.2 and 18.45 g for the Prussian Carp and 6.0 and 12.0g for the European bitterling.

Prior to the placing the fish in experiments, an “acclimatization” of each batch in part was carried out (Fry, 1967) at 18–20^{O}C (for 1 week) (AT=ET) (AT = adaptation temperature; ET = experiment temperature). The illumination period was of 8 to 12 hours.

The “negative” influence was avoided (in the sense of a “hypo-metabolic”effect) of the low concentrations of oxygen dissolved in water, with the oxygen consumption provided (in preliminary “optimization” measurements) (Pora et al., 1952) as not to exceed 25–30% of the overall quantity existing at the beginning of the experiment.

The choice of specimens and establishment of experimental groups were made with great attention; only healthy fish with appropriate appearance were used.

Four experimental groups, each consisting of 10 specimens of Prussian carp and 10 specimens of European bitterling were prepared for the researches: Batch 1 (control), where the fish were maintained throughout the experiment in dechlorinated tap water, and lots 2–4, with fish exposed to nickel, at concentrations of 3, 6 and 9 $mg \cdot l^{-1}$of water. In all the experimental versions with nickel, the nickel derived from nickel acetate.

The investigated concentrations were established after research of data in specialized publications and conducting preliminary tests of survival.

Traditional acute and chronic toxicity tests can be static (where the test solution remains the same throughout the test), recirculatory (where the test solution is recirculated through a filter or other apparatus to maintain water quality), renewal (where the test solution is replaced periodically) or through flow (where the test water is continually replaced) in design (Mitchell et al., 2002).

The test was performed by the semistic (renewal) bioassay method in which the exposure medium was exchanged every 24 h to maintain toxicant strength and level of dissolved oxygen as well as minimizing the ammonia excretion levels during this experiment.

For each specimen of the 4 groups, the oxygen consumption and respiratory rate were determined at intervals of 24, 48, 72, 96, 168 and 336 hours. Determination of oxygen consumption was realized through the confined space method (Marinescu, 1971) and the measurement of the respiratory rate was achieved using a process indicated by Pora et al., (1952); successive determinations of this index have been carried out (using a stopwatch) until 3 close values were obtained (their average mean representing the respiratory rate at the time).

Glycaemia and the number of erythrocytes were determined at the end of the experiments, respectively 14 days after installing the experimental versions, when the fish were sacrificed in order to achieve blood uptake in the caudal artery (Picos & Nastasescu, 1988). The glycaemia was determinedwith the help of the Accutrend GCT device, and the number of red blood cells was determined by light microscopy using the Thoma counting chamber.

Three repetitions were performed for the determinations, for each investigated index. The results obtained were interpreted graphically in Microsoft Excel and the statistical interpretation was conducted by using the Duncan test and Pearson correlation in the program Anova LSD.

3. RESULTS AND DISCUSSIONS

Determining and tracking oxygen consumption of aquatic organisms can be considered a better method for assessing the toxicity of a substance (respectively the quality of water) than achieving acute toxicological tests (determine the survival of different species) as it works for low concentrations of toxic substance (Backthavathsalam & Reddy, 1983).

Variations in oxygen consumption in fish exposed to three concentrations of nickel are shown in figure 1. The nickel, in all three investigated concentrations, causes a significant decrease in the consumption of oxygen to Prussian carp after 24 hours of immersion in the toxic solution. Basically, after this period, all specimens exposed to nickel suffer a “metabolic shock”, the values of this physiological index being reduced by almost a half (being lower by 47.91% at the concentration of 9 mg·l^{-1}, with 47.56% at the concentration 6 mg·l^{-1} and 41.1% at the concentration of 3 mg·l^{-1}).

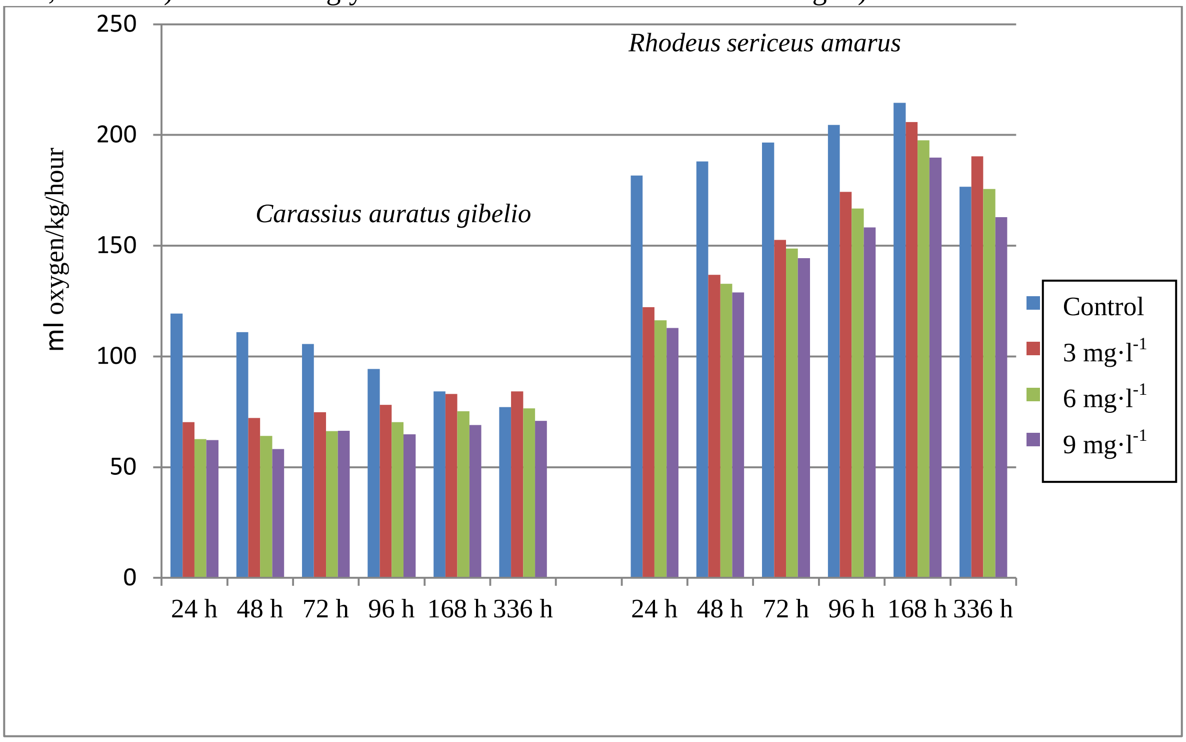

Figure 1. Influence of nickel nitrate over the specimens of *Carassius auratus gibelio* and *Rhodeus sericeus amarus*

The next two days no longer recorded significant changes in oxygen consumption, and after four days of exposure (the acute test duration) values of this parameter registered slight increase, the values measured after 14 days being much lower as compared to those determined in the control group. Although the evolution of the average values of oxygen consumption is similar in both species, the Prussian carp show a slower recovery of energy metabolism as compared to European bitterling.

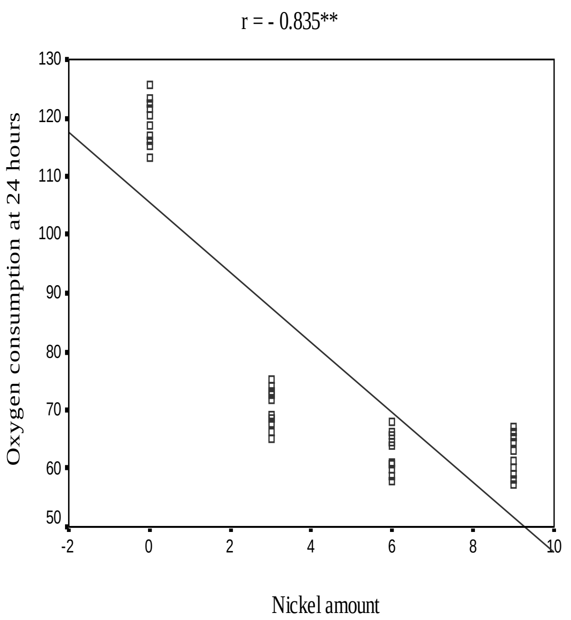

Figure 2. Correlation between the consumption of oxygen (24 hours) and the amount of nickel in *Carassius auratus gibelio* (** the correlation is significant for $p < 0.01$)

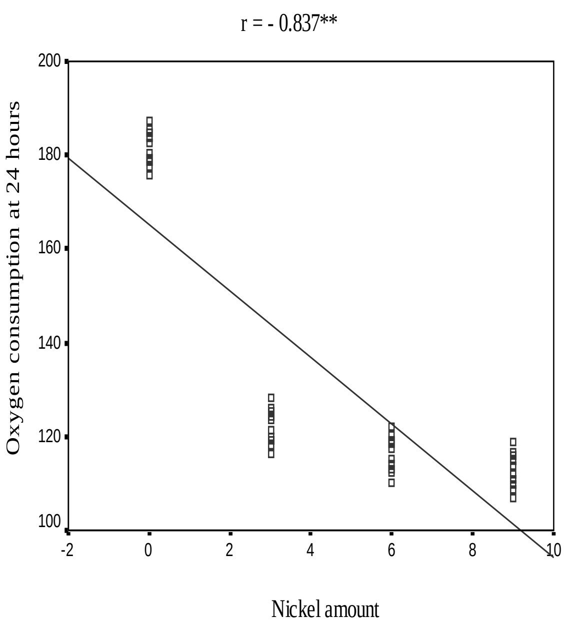

Figure 3. Correlation between the consumption of oxygen (24 hours) and the amount of nickel in *Rhodeus sericeus amarus* (** the correlation is significant for $p < 0.01$)

Between these experimental alternatives, there are significant differences for the threshold of significance $p<0.05$. Increasing the concentration of nickel from 6 mg to 9 $mg \cdot l^{-1}$ does not significantly change the oxygen consumption determined after 24 hours and 72 hours of influence, but the values recorded for oxygen consumption are much lower than those of the control batch.

Figures 2 and 3 represent the correlations between oxygen consumption registered for the Prussian carp and European bitterling after 24 hours of nickel influence. The presence of significant close negative correlations is found between oxygen consumption and the amount of nickel in both species of fish, where the recorded values of Pearson correlation coefficient arer = - 0.835 in Prussian carp and r = - 0.837 in European bitterling for the significance threshold $p < 0.01$.

After a slight decrease in the respiratory rate of Prussian carps exposed to nickel for 24 hours, significant decrease only for nickel concentrations of 6 and 9 $mg \cdot l^{-1}$ of water, the values of this physiological index grow progressively, with a maximum recorded in all three investigated concentrations after 7 days of exposure, so that, at the end of the experiment (14 days of exposure) the frequency of respiratory movements to be significantly lower than that determined in the control batch, at all the nickel concentrations that were studied. In European bitterling, significant decrease of this physiological parameter appears only after 24 hours of exposure to the concentration of 9 $mg \cdot l^{-1}$ Ni of water, further developments in respiratory rate being similar to that seen in Prussian carps, with the following observation: after 14 days of immersion in nickel solutions, no significant differences appear between the three levels, the slowdown of the rhythm being practically the same, regardless of concentration.

Increased respiratory rate within 7 days of exposure to nickel means that the fish is trying to compensate for the low oxygen installed, subsequently submitted. Probably the decrease of this index towards the end of the experiment is due to irreversible conditions produced at gill level, disorders presented in Peuranen et al., (1994) and Hassan (2005), which however were not the subject of this study.

Figure 4 shows the variations in the respiratory rate in the two species exposed to various concentrations of nickel. Fish gills are an important way of uptake of heavy metal compounds into the organism; thus the gills are the very first site where metal-induced lesions may occur which may result in an impaired gas and ion exchange (Witeska et al., 2006). Gill tissue hypoxia is the main factor causing the death of fish exposed to lethal levels of heavy metals (Burton et al., 1972).

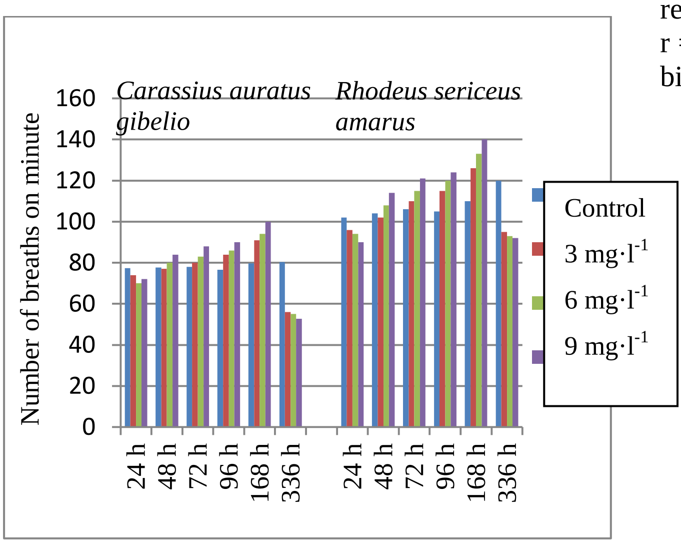

Figure 4. Influence of $Ni(NO_3)_2$ over the respiratory rate in the specimens of *Carassius auratus gibelio* and *Rhodeus sericeus amarus*

Except for the first 24 hours, symptoms of fish intoxicated with ions of different heavy metals that occur after the phase of unrest are similar to those described by Malacea (1969) an increase in the respiratory rate followed by asphyxia and cramps.

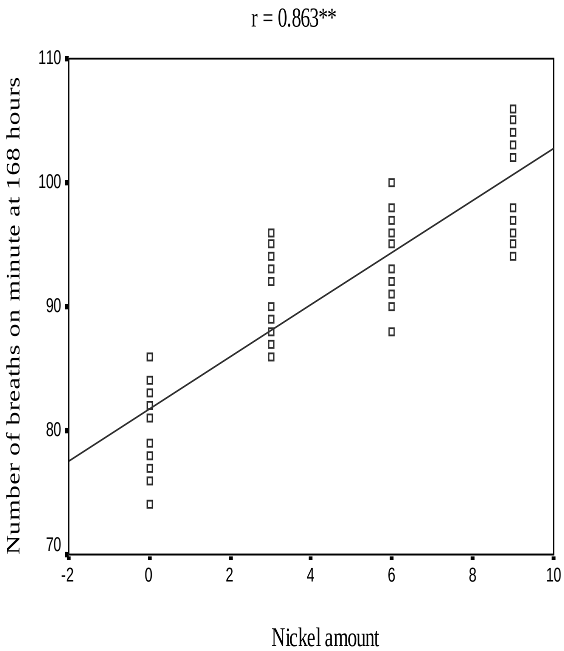

Figure 5. The correlation between the respiratory rate (168 hours) and the amount of nickel in *Carassius auratus gibelio* (**Correlation significant for p< 01)

Figures 5 and 6 graphically represent the correlation between respiratory rates recorded in both species after 168 hours of nickel influence. There are significant positive correlations between the respiratory rate and amount of nickel, where the recorded values of Pearson correlation coefficient r = 0.863 in Prussian carp and r = 0.927 in European bitterling for the significance threshold $p < 0.01$.

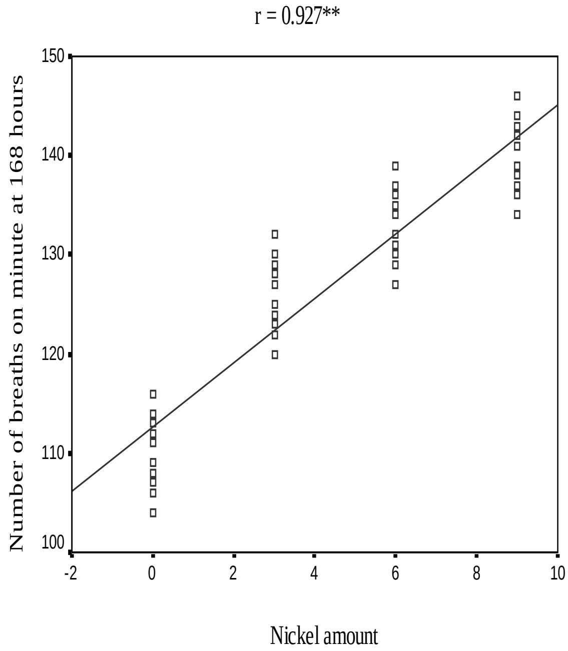

Figure 6. The correlation between the respiratory rate (168 hours) and the amount of nickel in *Rhodeus sericeus amarus*(**Correlation significant for p< 0.01)

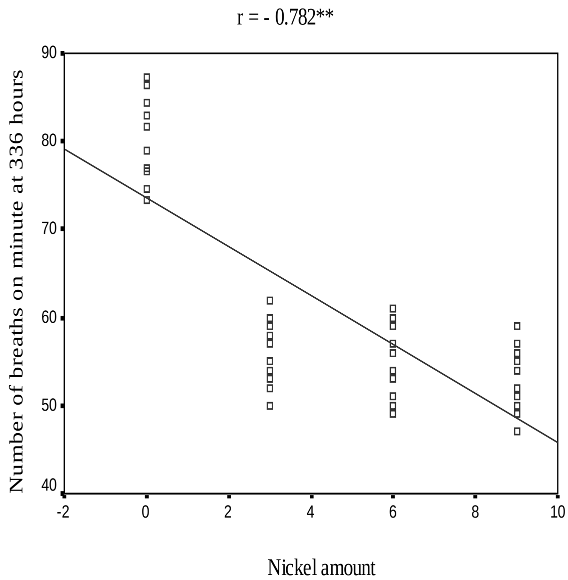

Figure 7. Correlation between the respiratory rate (336 hours) and the amount of nickel in *Carassius auratus gibelio* (**Correlation significant for p < 0.01)

Figures 7 and 8 graphically represent the correlation between respiratory rate registered in Prussian carp and European bitterling after 336 hours of nickel influence. There are significant negative correlations between the respiratory rate and the amount of nickel, where the recorded values of Pearson correlation coefficient is r = - 0.782 in Prussian carp and r = - 0.791 in European bitterling for the significance threshold $p < 0.01$. Haematological variables remain veritable tools in determining the sub-lethal concentration of pollutants such as heavy metals in fish (Witeska et al., 2003).

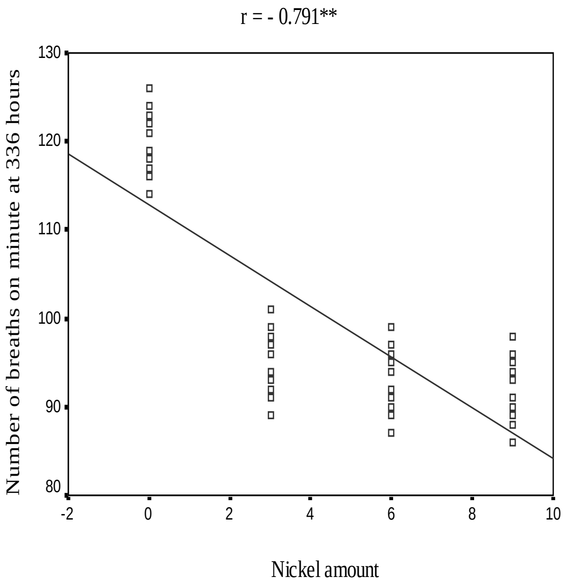

Figure 8. The correlation between the respiratory rate (336 hours) and the amount of nickel in *Rhodeus sericeus amarus* (**Correlation is significant for $p < 0.01$)

Figure 9 graphically represents the average values of the number of red blood cells in Prussian carp and European bitterling after 14 days as of starting procedures. For both species, the exposure for 14 days to the three levels of nickel resulted in an increase of the average number of erythrocytes. It can easily be noticed that the effect was more pronounced in the species *Rhodeus sericeus amarus*.

Although the specialized publications record especially consecutive anaemia of fish exposure to heavy metals, particularly, at a brief exposure, certain blood parameters (hematocrit, RBC, mean corpuscular volume, haemoglobin) may be increased (Vosyliene, 1996; Dethloff et al., 1999).

On the other hand, long-term exposure (30 days) to low concentrations of chromium (1.9 and 2.9 $mg \cdot l^{-1}$ increased the erythrocyte count, haemoglobin concentration, and hematocrit percentage in the blood of freshwater barbus (*Barbus conchonius*), (Schiffman & Fromm, 1959) and rainbow rout (*Oncorhyncus mykiss*), (Morsy & Protasowicki, 1990).

The reported fluctuations in these blood indices, in addition to differences in species and milieu, may also be attributed to a defence reaction against toxicity through the stimulation of erythropoiesis and are also indicative of the toxic effects of Cr on both metabolic and hemopoietic activities of *Cyprinus carpio* (Parvathi et al., 2011).

Figures 10 and 11 represent the correlation between the number of erythrocytes registered in Prussian carp and European bitterling after 336 hours of nickel influence. Are there significant positive correlations between the number of red blood cells and the amount of nickel, where recorded values of Pearson correlation coefficient are r = 0.846 in Prussian carp and r = 0.588 in European bitterling for the significance threshold $p < 0.01$. Average glycaemia values after 14 days of exposure to nickel for Prussian carp and European bitterling are shown in Figure 12.

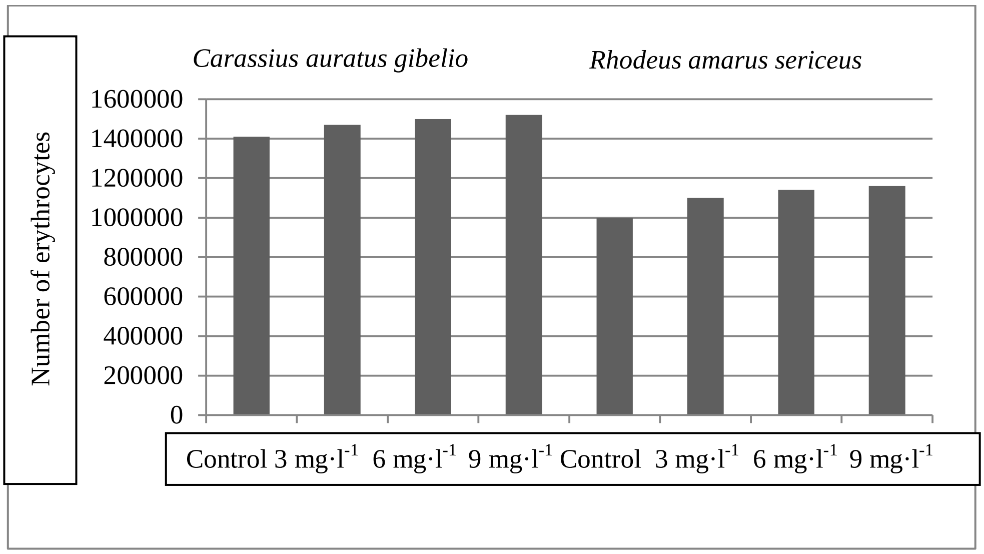

Figure 9. Influence of Ni $(NO_3)_2$ on erythrocytes in the specimens of *Carassius auratus gibelio* and *Rhodeus sericeus amarus*

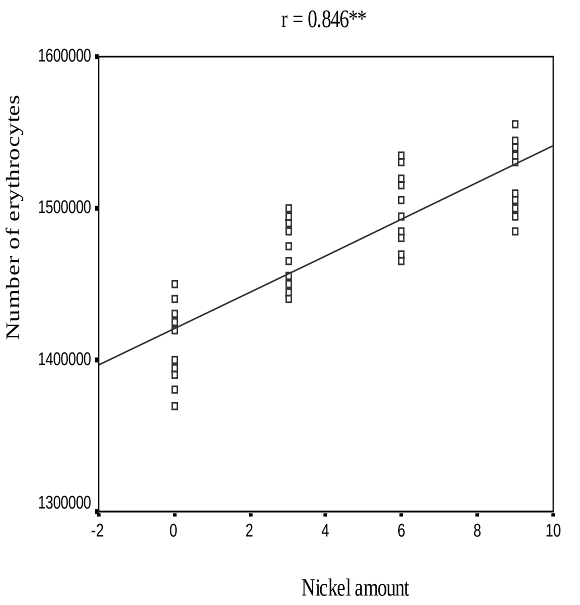

Figure 10. Correlation between the number of erythrocytes (336 hours) and the amount of nickel in *Carassius auratus gibelio* (** Correlation significant for $p < 0.01$)

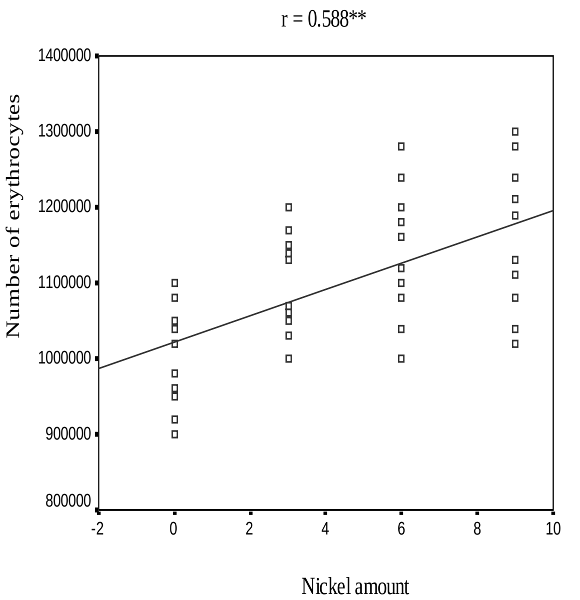

Figure 11. Correlation between the number of erythrocytes (336 hours) and the amount of nickel in *Rhodeus sericeus amarus* (** Correlation significant for $p < 0.01$)

Glycaemia levels increased in both species after exposure to nickel, the growth being very pronounced (with more than 300% at a concentration of 3 mg Ni/l of water and nearly 4 times at the concentration of 9 $mg \cdot l^{-1}$ Ni of water), the results being similar in both species.

Additionally, significant hyperglycaemia was noted by Nath & Kumar (1988) in the catfish (*Heteropneustes fossilis*) exposed to nickel, Radhakrishaniah & Venkataramana et al., (1992) and Van Vuren et al., (1994) in the fish *Labeo rohita* and *Claries gariepinus* subjected to copper and James et al. (1992) in the teleost (*Oreochromis mossambicus*) exposed to lead. Wedemeyer & McLeay (1981) reported that high levels of blood glucose are caused by disorders in carbohydrate metabolism appearing in the condition of physical and chemical stresses.

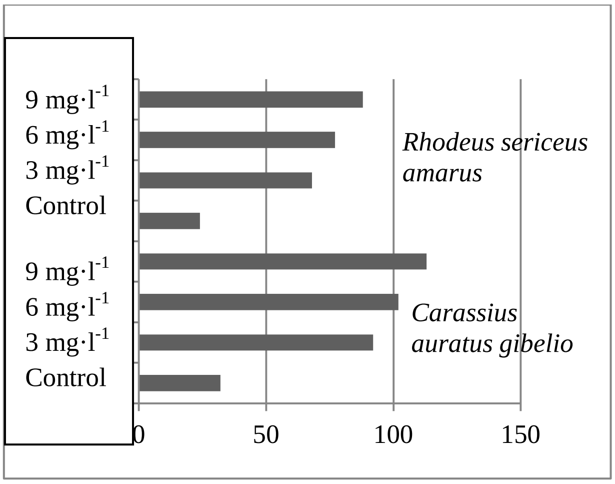

Figure 12. Influence of Ni $(NO_3)_2$ over glycaemia in the specimens of *Carassius auratus gibelio* and *Rhodeus sericeus amarus*

According to statistical interpretation, increasing the concentration of nickel from 6 $mg \cdot l^{-1}$ to 9 $mg \cdot l^{-1}$ does not significantly alter blood glucose determined after 336 hours of influence, the values being fairly close together but higher than blood glucose of the control batch.

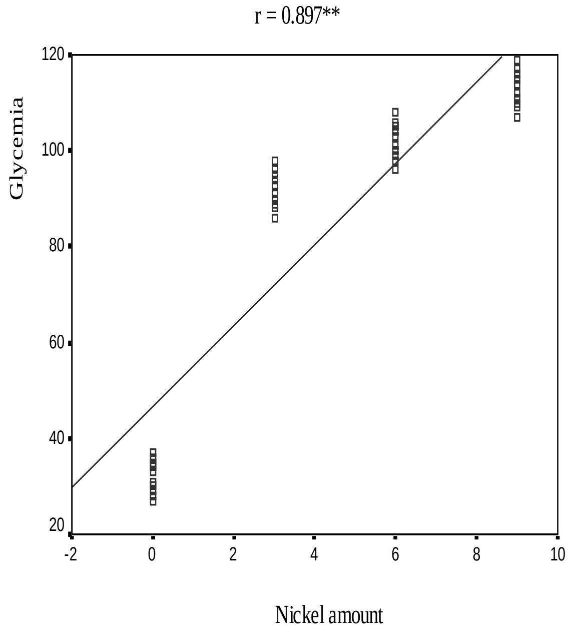

Figure 13. Correlation between glycaemia (336 hours) and the amount of nickel in *Carassius auratus gibelio* (** Correlation significant for $p < 0.01$)

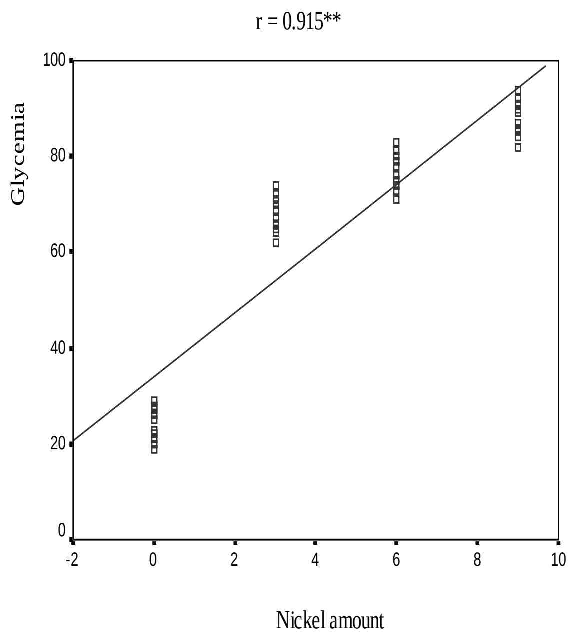

Figure 14. Correlation between glycaemia (336 hours) and the amount of nickel in *Rhodeus sericeus amarus* (** Correlation significant for $p < 0.01$)

Figures 13 and 14 represent the correlation between the glycaemia of the Prussian carp and European bitterling recorded after 336 hours of nickel influence. There is a positive correlation between glucose and a significant amount of nickel, where the values of Pearson correlation coefficient are $r = 0.897$ in Prussian carp and $r = 0.915$ in European bitterling for the significance threshold $p < 0.01$.

4. CONCLUSIONS

1. Under the action of nickel significant modifications in glucose, oxygen consumption and respiratory rate were registered for the species *Carassius auratus gibelio* and *Rhodeus sericeus amarus*, as they were considered responses to the stress caused by the noxious substance of Ni metal.

2. The oxygen consumption in both investigated species decreased significantly after the first 24 hours of exposure to nickel (with more than 30% as compared with control values) for all three investigated concentrations, the values determined subsequently showing gradual growth from one determination to another; restoring the energetic metabolism in Prussian carp was slower as compared to European bitterling.

3. The respiratory rate of the fish exposed for 7 days to 3 studied sub-lethal concentrations of nickel progressively increased from one determination to another (except for values determined after 24 hours of exposure) until the 7th day exposure, the index values determined after 336 hours being significantly lower than control batch values.

4. Average values of blood glucose and red blood cells in the batches treated with nickel increased significantly, the most pronounced increases being recorded at the highest concentration of 9 $mg \cdot l^{-1}$ of water (at this concentration, the blood glucose levels exceeded almost 4 times the values recorded in the control group in both species).

5. The changes in the haematological parameters indicate that they can be used as indicators of Ni related stress in fish on exposure to elevated levels in the water.

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