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# Behavioral and Lethal Effects of La Salt and a Mixture of Cu and La Salt on *Daphnia magna* Straus

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**ABSTRACT**

The study of joint effects of REE and HM is relevant, since they are often satellite deposits, their areas of application are similar, and an increase in concentrations of elements of these groups in the areas that are not places of their extraction and enrichment is proven. The purpose of this work was to find out the pre-lethal and lethal effects of La, Cu and their equimolar mixtures in tests for *Daphnia magna* Straus. Bioassays of artificially polluted natural waters, initially free of toxic elements, was carried out. In bioassays on the mortality of *D. magna* in the space of 96 hours it was found that acute toxicity of copper sulfate solutions is observed at the calculated concentration of $Cu^{2+}$ 0.1 mg/L (0.0016 mmol/L), and the acute toxicity of lanthanum sulfate is when the dose of $La^{3+}$ is equal to 50 mg/L (0.36 mmol/L). In the solutions comprising mixtures of Cu and La salts (1:1 calculated using metals), the concentrations of which are equimolar to the investigated solutions of copper sulfate, the mortality of *D. magna* begins in the solution containing 10 times less toxic elements. It was found that 25% of individuals died in the variant "0.00016 mmol/L", the mortality of 100% of individuals was at the total metal concentration of 0.0008 mmol/L. The solutions containing La (0.072–0.72 mmol/L) and Cu (0.00016–0.0016 mmol/L) naturally inhibit the motor activity of *D. magna* by 1.3–5.3 times and 1.2–1.9 times in 1 hour and 1.7–2.8 and 1.4–2.2 times in 24 hours, respectively. The solutions containing mixtures of Cu and La salts inhibited the motor activity of *D. magna* in the same way as copper sulfate solutions with the $Cu^{2+}$ concentrations equimolar "$Cu^{2+}$ + $La^{3+}$". Therefore, when testing the solutions with the same molar concentrations of $Cu^{2+}$ and the mixture of "$Cu^{2+}$ + $La^{3+}$" it was shown that La potentiates the pre-lethal effect of Cu to the level of individual effects of Cu. The additions of La salt to the solutions containing pre-lethal doses of Cu lead to lethal effects of such mixtures for *D. magna*.

**Keywords**: lanthanum, copper, bioassay, *Daphnia magna*, motor activity, joint effect, synergism, potentiation.

## INTRODUCTION

Until the 21st century, the harmful effects of metals were associated mainly with the effects of heavy metals (HM). Indeed, the result of their direct use, as well as the migration of HM from metal alloys, coal ash, "waste" rock of sludge dumps and other wastes, was the accumulation of lead, copper, mercury, cadmium in soil and bottom sediments of water bodies (Cooke and Bindler, 2015). There is an increase in the content of HM in biomass, for example, in the bark of trees growing in urban transport zones (Olkova et al., 2016).

In the 21st century, the environmental problem of environmental pollution with metals has expanded its boundaries, and now it includes pollution with rare earth elements (REE), platinum group metals, and radioactive metals. Among the listed elements, REE are becoming a strategic material for the development of advanced technologies and transformation of traditional industries into high-tech ones (Cheng et al., 2019). The production of computers, modern telephones, the latest optical devices, and medical equipment is impossible today without REE and their compounds.

Many rare earth elements are found in the Earth's crust together with each other, as well as in combination with other substances that are dangerous for biota even at low concentrations in the environment. For example, florencite contains Ce and Al ($CeAl_3(PO_4)_2(OH)_6$), Ba and Ce can be mined from cordylite ($BaCe_2F_2[CO_3]_3$), davidite is enriched in Fe, Ti and La ($Fe_5LaFe_3Ti_2O_{35}$) (Jordens et al., 2013). In placer deposits, REE are often found in titanium-zirconium sands (Vlasov, 1964). As a result, REE mining areas tend to be contaminated not only with target elements, but also with other elements, including HM, for example, Mn, Zn, Co, Ni (He et al. 2021). Confirmation of this environmental problem can be seen in the example of the Mine Tailings Kola Subarctic, where the finely dispersed material of the tailings of loparite and complex ores is 1.5–3.0 times enriched in heavy and rare earth metals, compared to the total material of the tailings (Krasavtseva et al., 2021). In this regard, it is very important to study the effect of various REE on biota, as well as to determine the effects of the combined action of HM and REE.

The published data is ambiguous. It is known that REE, in particular, lanthanum, samarium, and neodymium, are used in China as components of mineral fertilizers for plants (Li et al., 2013). It turned out that these elements help to reduce the growing season of plants, improve seed germination, increase yields and atmospheric nitrogen fixation in leguminous crops (Yanjun et al., 2016). However, REE-based fertilizers were subsequently shown to change the bioavailability of HM in soils (Wang et al., 2003).

The danger of increasing of the content of REE in the environment is explained by the mechanism of their action, which is similar to the molecular effects of HM (Pagano et al., 2015). In the work (Qiu et al., 2005), REE were characterized by the presence of "biological intelligence", when regulation of cellular processes due to antagonism or replacement of main biometals in target cells was discussed.

More and more scientists report on the ability of various representatives of the biota to accumulate REE. Microalgae can extract yttrium from lighting waste (Charalampous et al., 2019). In fish, REE are accumulated to a greater extent in internal organs and bones, and to a less extent in muscles (Mayfield and Fairbrother, 2015). The tree fungus Ganoderma applanatum accumulates up to 15 times more REE than platinum group elements (Mleczek et al., 2016). Consequently, there is enough scientific evidence that there is an increase in the bioaccumulation of REE by the modern biota, but there are very few works devoted to consequences of this process.

The information about the joint effect of REE and HM is even poorer. Thus, richness and diversity of bacterial communities turned out to be lower in jointly polluted soils (Luo et al., 2021). In (Zhang et al., 2003), the authors state that La at the concentration of 10 mg/L reduces the toxic effect of Ni for *Hydrocharis dubia* B. L. Baker, but at doses of Ni above 10 mg/L, the toxic effect may be aggravated. Various combinations of REE (La, Ce, Nd) with Cd led to a decrease in the accumulation of Cd by rapeseed roots, but at the same time increased the transfer of HM to the aerial parts of the plant (Ma et al., 2004).

Thus, determination of individual effects of REE and combined effects of HM and REE using classical test organisms will bring clarity to this question. Therefore, the aim of this work was to experimentally establish the pre-lethal and lethal effects of La on *Daphnia magna* Straus, as well as to establish the joint effect of La and Cu on *D. magna*.

## MATERIAL AND METHODS

### Preparation of model solutions

The object of the study was artesian water, to which $La_2(SO_4)_3 \cdot 8H_2O$, $CuSO_4 \cdot 5H_2O$ additives as well as mixtures of La and Cu salts were added. The effect of lethal and non-lethal doses of substances established in preliminary experiments was evaluated. The solutions with mixtures of salts contained the same molar concentrations of $La^{3+}$ and $Cu^{2+}$, and they were also equimolar to the solutions containing only copper sulfate calculated using the $Cu^{2+}$ ion.

### *D. magna* bioassays

Bioassays of freshly prepared solutions of rare earth elements, copper and solutions containing mixtures of salts of REE and copper were conducted. For experiments, juvenile of *D. magna* was obtained by parthenogenesis from a group of females born from one maternal individual in one offspring. The age of the juvenile *D. magna* did not exceed 24 hours.

Three individuals of Daphnia were added in 100 ml of the test solution, the repetition was three times. The motor activity and mortality of individuals were determined in 1 and 24 hours of the experiment. The mortality of individuals was found by complete immobilization (Federal Register

1.39.2007.03222, 2007). The motor activity of each individual was determined by using the direct visual method (Olkova and Zimonina, 2020). The bottom of a glass chemical beaker with a diameter of 3 cm, placed on a palette with cells 0.5x0.5 cm, was used as a field of view. The number of intersections of the palette lines within 5 minutes (PLI) was considered as an indicator of motor activity.

### Mathematical processing of the results and their presentation

The mortality of individuals was presented as a proportion of dead individuals compared to the control variant. The result of measuring the motor activity of *D. magna* was presented as M±δ, where M is the arithmetic mean of 9 determinations of the indicator, δ is the standard deviation of the indicator from the mean. The significance of differences between the values from the control data and with one another was determined by the t-test using Excel algorithms.

## RESULTS

### Comparison of lethal concentrations of La and Cu

Lethal concentrations of La and Cu differed by 3 orders. The acute toxicity of lanthanum sulfate solutions was observed at the calculated $La^{3+}$ concentration of 100 mg/L (0.72 mmol/L) and 50 mg/L (0.36 mmol/L), and in the solution of copper sulfate all daphnia died at the $Cu^{2+}$ concentration of only 0.05 mg/L (0.0031 mmol/L) (table 1). For copper, this result is quite natural, since the approved environmental standard of the Russian Federation for fishery water bodies is 0.001 mg/L (Order No. 552, 2020). In the EU countries, copper is defined as a hazardous substance and its concentration for salmon and carp waters is set at 0.04 mg/L (Directive 2006/11/EC, 2006). The standards for the content of REE, including lanthanum, are not established.

Thus, a mixture of La and Cu salts is lethal at the dose of 0.0008. The comparison of the results of determining the acute toxicity of salts La, Cu and their mixtures showed that La enhances the effect of Cu, if it is in solution at a non-lethal concentration while a solution containing $Cu^{2+}$ at an equimolar dose does not lead to the death of *D. magna*. When the dose toxicants (calculated for metals – 0.00016 mmol/L) is halved, the effect is similar, but it is weaker: in the solution containing only $Cu^{2+}$, no death of daphnia is observed, while in the solution with a mixture of $La^{3+}$+$Cu^{2+}$ 33% of individuals die in 96 hours.

Testing solutions with lethal doses of $Cu^{2+}$ and equimolar solutions of mixtures of Cu and La salts showed very similar results at first glance.

**Table 1.** The effect of La, Cu salts and mixtures of their salts on the mortality of *D. magna*

| Version | | Concentration | | Mortality *D. magna*, % | | |
|---|---|---|---|---|---|---|
| | | mmol/L | mg/L | 1 hour | 24 hours | 96 hours |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Individual effect | $La^{3+}$ | 0.072 | 10 | 0 | 0 | 40 |
| | | 0.36 | 50 | 0 | 0 | 85 |
| | | 0.72 | 100 | 0 | 0 | 90 |
| | Non-lethal and pre-lethal doses of $Cu^{2+}$ | 0.00016 | 0.01 | 0 | 0 | 0 |
| | | 0.0008 | 0.05 | 0 | 0 | 0 |
| | | 0.0016 | 0.1 | 0 | 0 | 100 |
| | Lethal doses of $Cu^{2+}$ | 0.00062 | 0.04 | 0 | 100 | - |
| | | 0.0031 | 0.2 | 100 | - | - |
| | | 0.0062 | 0.4 | 100 | - | - |
| Joint effect | Doses of $La^{3+}$+$Cu^{2+}$ (1:1)* equimolar to non-lethal solutions with $Cu^{2+}$ | 0.00016 | 0.01 ($La^{3+}$) + 0.005 ($Cu^{2+}$) | 0 | 0 | 33 |
| | | 0.0008 | 0.056 ($La^{3+}$) + 0.025 ($Cu^{2+}$) | 0 | 100 | - |
| | | 0.0016 | 0.1 ($La^{3+}$) + 0.05 ($Cu^{2+}$) | 0 | 0 | 100 |
| | Doses $La^{3+}$+$Cu^{2+}$ (1:1)* equimolar to ethal solutions with Cu | 0.00062 | 0.04 ($La^{3+}$) + 0.02 ($Cu^{2+}$) | 0 | 0 | 100 |
| | | 0.0031 | 0.2 ($La^{3+}$) + 0.1 ($Cu^{2+}$) | 100 | - | - |
| | | 0.0062 | 0.4 ($La^{3+}$) + 0.2 ($Cu^{2+}$) | 100 | - | - |

**Note:** The same calculated molar ratio of metals; "-" means that the experiment was completed earlier.

However, it should be kept in mind that the dose of copper, the most toxic element of the mixture, in the combined solution is 2 times less than in the solution containing only $Cu^{2+}$. It was also discussed above that the lethal doses of $La^{3+}$ upon individual exposure are extremely high. Consequently, the mortality of the solutions containing a mixture of Cu and La salts for *D. magna* is achieved by enhancing the action of copper in the presence of lanthanum.

Thus, rather high lethal concentrations of $La^{3+}$ are shown in comparison with $Cu^{2+}$: the death of 90% of individuals in the La salt solution was observed at 0.72 mmol/L, and in the Cu salt solution a similar effect was already achieved at 0.0031 mmol/L. The combined presence of $La^{3+}$ and $Cu^{2+}$: in solution led to an increase in the acute lethal effect of copper.

The obtained results confirm the concern of the scientific community with the increasing amount of HM (Bradl, 2005) and REE in the environment (Chen et al., 2020; Balaram, 2019). The results of the experiments showed that the REE concentrations that are very far from lethal doses can enhance the toxic effect of HM up to lethal effects. For natural ecosystems, any changes in the vital activity of organisms are important; therefore, the pre-lethal effects of La, Cu salts and their mixtures are shown below.

## Effect of La, Cu salts and their mixtures on the motor activity of *D. magna*

The motor activity of *D. magna* in pure control water varied from 139.0±19.6 to 204.1±25.0 PLI/5 min. In the water containing metal salts and their mixtures in the ranges according to Table 1, the motor activity of crustaceans decreased. All the obtained results were expressed in relative values, taking control data as 100% (Fig. 1).

Under the influence of $La^{3+}$, already in 1 hour of exposure, a regular inhibition of the motor activity of *D. magna* occurs in response to an increase in the dose of the toxicant ($r$ = -0.98). Differences from control are significant, but close to critical levels (p from 0.002 to 0.02). In a day, no strict "dose-effect" pattern was observed ($r$ = 0.08). However, the inhibition of the test function remains at a significant level (from 1.7 to 2.8 times), individual fluctuations in the reactions of organisms are reduced, as a result of which all the studied solutions have a significant inhibition of the mobility of *D. magna* ($p$<0.05).

Non-lethal mass concentrations of $Cu^{2+}$ were 1000 times lesser than similar doses of $La^{3+}$ (Table 1). Except for this fact, the toxicological picture was repeated. In 1 hour of exposure, a regular inhibition of the intensity of *D. magna* movements was observed with a high degree of "dose-effect" relationship ($r$ = -0.98), and in a day the dependence becomes weaker ($r$ = -0.63). Weakening of the "dose-effect" relationship over time indicates the activation of the internal defense mechanisms of organisms, which leads to smoothing of the effects in 24 hours, compared to acute reactions in 1 hour.

The difference between effects of lanthanum in the range of 10–100 mg/L (0.072–0.72 mmol/L) and the negative effect of copper in doses of 0.01–0.1 mg/L (0.00062–0.0062 mmol/L) was that a decrease in the Daphnia activity in copper solutions in 1 hour of exposure was significant only at the highest dose of HM (0.1 mg/L, $p$ = 0.001). However, in 24 hours, the differences from control indicators become significant ($p$<0.05).

The effects of combined action of $La^{3+}$ and $Cu^{2+}$ are the most interesting (Figs. 2 and 3).

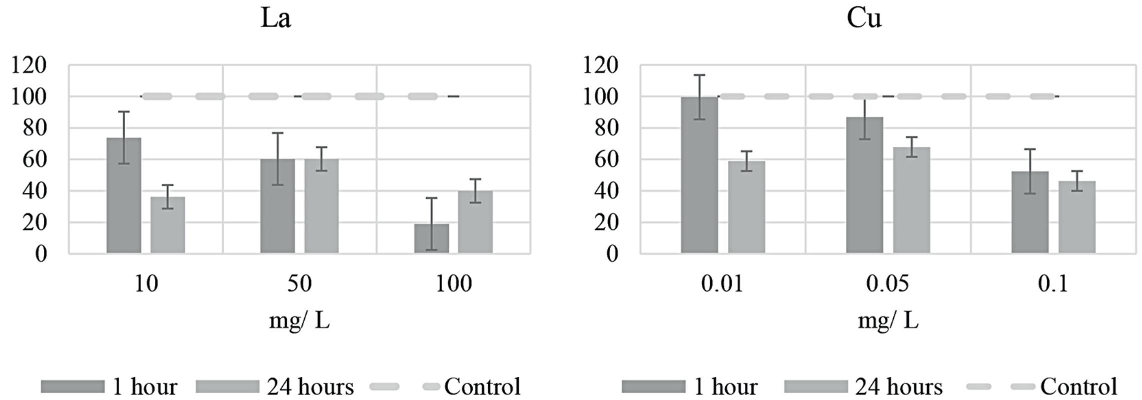


**Figure 1.** The motor activity of *D. magna* under the influence of La and Cu salts outside the mixture
Note: the abscissa shows the relative values of the motor activity of test organisms (%)

In 1 hour of the experiment, the solutions containing non-lethal doses of $Cu^{2+}$ and an equimolar mixture of $Cu^{2+}$ and $La^{3+}$ salts (calculated as ions) had the same effect on *D. magna*: with increasing concentration the motor activity was naturally inhibited, but there were no differences between the variants "Cu" and "Cu+La" ($p>0.05$). In a day, there was an increase in effects in all experimental variants. When testing the solutions with the maximum (0.0016 mmol/L) and minimum (0.00016 mmol/L) concentrations of $Cu^{2+}$ and a mixture of Cu and La salts, no significant difference between the options was observed. However, as in the case of lethal effects, it must be taken into account that Cu is a more active toxicant than La. Thus, the addition of only 50% Cu compared to the concentration contained in "individual" solutions of copper, mixed with an equivalent amount of La, leads to the effects similar to those of "individual" solutions of copper. Therefore, it can be assumed that $La^{3+}$ enhances the effect of $Cu^{2+}$ on the behavioral effects of *D. magna*. The death of all *D. magna* individuals in the "Cu + La – 0.00016 mmol/L" variant, although it is out of the general pattern of effects, confirms the assumption about the potentiation of $Cu^{2+}$ in the presence of $La^{3+}$.

With increasing doses, it becomes even more difficult to distinguish the difference between the individual action of the Cu salt and the mixture of Cu and La salts, since many individuals begin to die in 1 hour (Table 1). Figure 3 shows the effects of a Cu salt and a mixture of Cu and La salts in the same final concentrations equal to 0.00062 mmol/L.

Probably, with an increase in the concentration of toxicants, the effects of copper begin to prevail. In one hour of the experiment, the inhibition of the motor activity of *D. magna* under the influence of copper was 1.3 times ($p<0.05$), and under the influence of a mixture of Cu + La – 1.1 times ($p>0.05$). In 24 hours of the experiment, in the solutions containing only $Cu^{2+}$, all daphnia died, while in the solutions with a mixture of $Cu^{2+}$+$La^{3+}$ the experimental individuals were

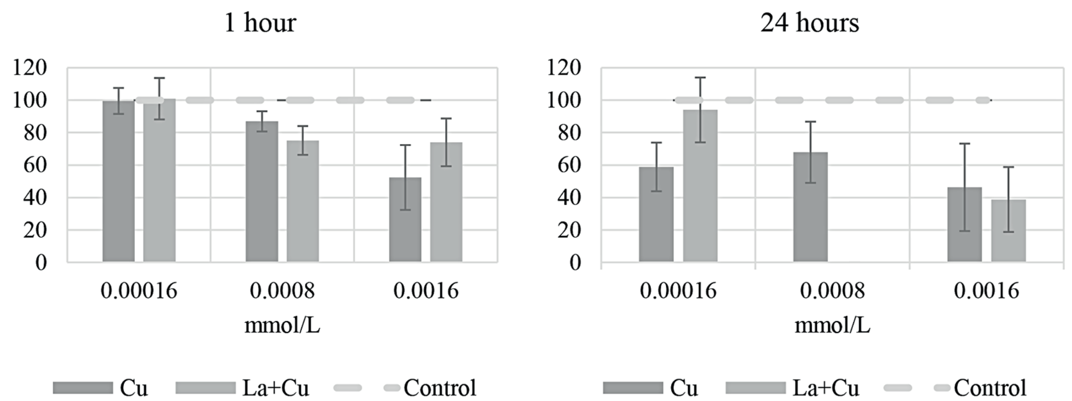


**Figure 2.** Behavioral effects of Cu salt and a mixture of Cu and La salts (1:1) containing non-lethal doses of toxicants
Note: the abscissa shows the relative values of the motor activity of test organisms (%)

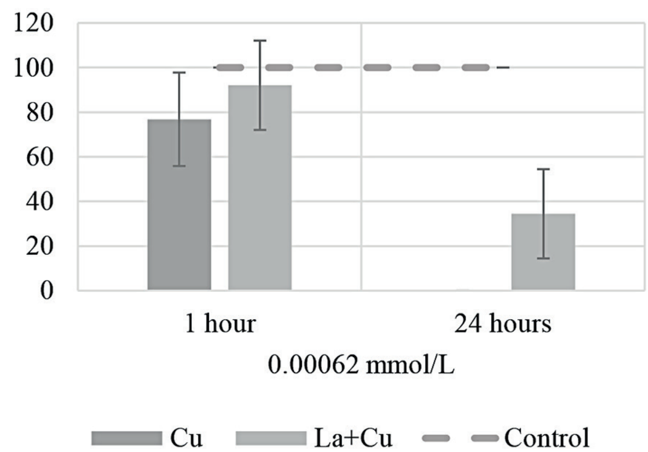


**Figure 3.** Behavioral effects of Cu salt and a mixture of Cu and La salts (1:1) containing lethal doses of toxicants
Note: the abscissa shows the relative values of the motor activity of test organisms (%)

alive, although their motor activity was significantly inhibited (by 2.9 times, $p<0.05$). The solutions containing a mixture of $Cu^{2+}+La^{3+}$ at the concentration of 0.00062 mmol/L also proved to be lethal for *D. magna* not in 24 hours but in 96 hours (Table 1).

Thus, it was shown that the solutions of La and Cu salts containing the doses of toxicants equivalent to "individual" solutions of copper sulfate have a similar effect. Accordingly, it can be assumed that the action of Cu is potentiated at the expense of La.

## DISCUSSION

The lethal concentrations of $La^{3+}$ for *D. magna* turned out to be quite high. In the bioassays on the mortality of *D. magna* during 96 hours it was found that the acute toxicity of copper sulfate solutions is observed at the calculated $Cu^{2+}$ concentration of 0.1 mg/L (0.0016 mmol/L), and the acute toxicity of lanthanum sulfate is at the dose of $La^{3+}$ equal to 50 mg/L (0.36 mmol/L). There is evidence of the negative effect of lanthanum on living organisms at lower doses. For example, in the work (Koval, Olkova, 2022) it was found that at the $La^{3+}$ concentration of 0.001 mg/L, the amount of chlorophyll *a* in *Nostoc linckia* decreased by 4.2 times compared to the control, while the intensity of lipid peroxidation processes increased by 2.2 times. Such differences in effective concentrations are associated, firstly, with the assessment of different effects – non-lethal and lethal effects. If we compare the $La^{3+}$ concentrations that cause significant non-lethal effects in *N. linckia* and *D. magna*, then the effective doses will be more comparable: 0.001 and 0.01 mg/L, respectively. Secondly, *D. magna* is quite highly organized animals in terms of evolution and formed toxicological barriers, so it is quite natural that the inhibition of their vital activity will be caused by relatively high concentrations of the element.

As mentioned above, there are conflicting data on the combined effect of REE and HM compounds on living organisms. In general, when analyzing the joint effect of metals on representatives of the biota, various joint influences can be found. The effects of Hg are known to be enhanced in the presence of Pb (Yao at al., 2014) or Pb and Mn (Papp at al., 2006). There are data on the antagonistic effect of metals. The combined treatment of Co and Cu reduced the toxicity of both metals in comparison with each metal treatment taken separately (Lwalaba et al., 2019) One of the results of the presented work was the proof of the enhancement of the copper action in the presence of lanthanum. Additions of La salt to the solutions containing pre-lethal doses of $Cu^{2+}$ lead to lethal effects of such mixtures for *D. magna*.

A limitation of the study results is that the findings were obtained under standard laboratory conditions on the specific test organism, *D. magna*. It is known that many environmental factors modify the responses of organisms to toxic stress (Olkova, Ashikhmina, 2021). For example, with regard to La, it is known that its negative effect on wheat is reduced in the presence of Ca and nitrilotriacetic acid (Li et al., 2020). Therefore, the joint effect of REE and HM compounds under different conditions and for a more diverse list of test organisms remains to be clarified.

## CONCLUSIONS

The present work shows the lethal and non-lethal effects of La and Cu salts and their mixtures. The death of 100% of *D. magna* occurs at the calculated $Cu^{2+}$ concentration of 0.05 mg/L (0.0031 mmol/L) and a similar effect of $La^{3+}$ – 90% – is found at its dose of 100 mg/L (0.72 mmol/L). Lanthanum sulfate solutions (10–100 mg/L calculated as $La^{3+}$) naturally inhibit the motor activity of *D. magna* 1.3–5.3 times in one hour of the experiment and 1.7–2.8 times in 24 hours. The inhibition of the motor activity of *D. magna* in copper solutions had the same regularities, but the mass concentrations of $Cu^{2+}$ in the solution were 1000 times lesser. Despite such a significant difference between the effective lethal and non-lethal concentrations of metals, it was possible to conclude that synergistic effects are observed under the combined action of equimolar doses of $La^{3+}$ and $Cu^{2+}$ Since copper salts are more toxic than lanthanum salts, the potentiation of the action of copper in the presence of lanthanum can be observed.

In further work, it is necessary to find out the effect of mixtures of La and Cu salts at low non-lethal concentrations on the chronic toxicity for *D. magna*. In particular, from the ecological point of view, it is always important to assess the effect of toxicants on the ability of individuals to reproduce.

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