Assessment of the post-pyrogenic water toxicity in the north-eastern part of the Great Vasyugan Mire (Western Siberia)

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Abstract. This paper devoted the analysis of the post-pyrogenic water toxicity in the north-eastern part of the Great Vasyugan Mire with a test culture of Daphnia magna Straus. It was found that the water samples of pine-shrub sphagnum bogs have a negative impact on the test organisms D. magna Str. The most toxic effect on test organisms provided by pH value. The chemical composition of waters is determined by the high content of NH₄⁺, Fe_total, organic matter at all points of the study. The MPC exceeded for NH₄⁺, Fe_total and COD. The studied post-pyrogenic areas are characterised by an increase in the concentration of heavy metals (Zn, Pb) and Fe_total in waters.

1. Introduction and Background
Water pollution is a global environmental problem [1-3]. During fires, the accumulation of combustion products occurs, and the content of substances increases, the toxicity of which, for most aquatic organisms, manifests itself already in small concentrations. Heavy metals are the greatest environmental hazard. It is known that they are capable of disrupting the integrity of physiological and biochemical processes, causing serious changes in the metabolic reactions of hydrobionts [3-5]. To assess the toxicity of water, along with methods of chemical analysis, biotesting methods are used. The biotesting procedure establishes the toxicity of the environment with the help of test objects. Test objects often signal danger regardless of which substances and in what combination they cause changes in vital functions. One of the standard test objects used in the study of pollution of the aquatic environment are the lower crustaceans Daphnia magna Straus. Thus, the aim of the study is to assess the post-pyrogenic toxic effects of waters on test objects of Daphnia magna Straus.

2. Study Area
The study area is located within the southeast West Siberian Plain, which is the alluvial plain formed as a result of extensive meandering of large rivers [6]. The territory is composed of Mesozoic (Triassic, Jurassic, Cretaceous) and Cenozoic (Paleogene, Neogene, Quaternary) sedimentary deposits, covering the Palaeozoic crystalline basement. Quaternary deposits are represented by carbonate loams and clays, which cover almost all river interfluves. The thickness of the Quaternary deposits on the interfluve of the Bakchar River and the Iksa River reaches 40–60 m [7]. The climate of the territory is characterised as continental, with a short warm summer and a long, harsh and snowy winter. The average annual temperature of the territory is -0.91°C to +1.22°C. The annual amount of precipitation is 473 mm, according to meteorological station near Bakchar village (RIHMI-WDC http://meteo.ru/).

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The average annual evapotranspiration reaches as high as 332 mm. Positive atmospheric water balance allows for the formation and sustainable evolution of bogs (about 40–60% of area). The territory belongs biogeographically to the south taiga zone.

To assess the toxicity, water samples were collected in October 2017 within 4 pine-shrub-sphagnum raised bogs (north-eastern part of the Great Vasyugan mire) and in the Gavrilovka River: 2 samples were taken within the drainage area of the Bakchar bog (BB) in the Gavrilovka River basin and after a fire in 2016, 2 samples were taken selected within the forest drainage area of the Iksa bog (IB) in the interfluve of the Shegarka and Iksa Rivers and after the fire in 1998 (figure 1, table 1). The thickness of the peat deposit is 1.7–3 m, and the moss peat type prevails. Raised bogs were drained in 1980–1985 for forestry. The distance between the drainage channels is 150–160 m, the planned canals width is 1–2 m, and the depth is up to 1 m. At present there is a decrease in the culvert capacity of the channels due to their overgrowing.
Table 1. Description of the study sites.

| Sample | Site  | Description                                                                 |
|--------|-------|-----------------------------------------------------------------------------|
| 1      | D     | Bakchar bog (BB1) Drained pine-shrub-sphagnum raised bog, Gavrilovka River   |
|        |       | basin N56°53′25.8″ E82°40′50.5″                                               |
| 2      | E     | Bakchar bog (BB2) Drained pine-shrub-sphagnum raised bog, burnt in 2016,    |
|        |       | Gavrilovka River basin N56°53′20.3″ E82°40′35.9″                               |
| 3      | A     | Iksa bog (IB3) Drained pine-shrub-sphagnum raised bog, interfluve of the     |
|        |       | Shegarka and Iksa Rivers N56°52′06.3″E83°17′58.2″                            |
| 4      | B     | Iksa bog (IB4) Drained pine-shrub-sphagnum raised bog burnt in 1998,        |
|        |       | interfluve of the Shegarka and Iksa Rivers 56°51′39.4″E83°17′48.3″           |
| 5      | C     | Gavrilovka River N56°55′14.6″E82°45′14.0″                                    |

3. Material and Methods

3.1 Water sampling

Samples of water taken from depths of 30–50 cm were transferred into a specially prepared glass and plastic bottles. Previously wells 1 m deep were drilled in the peat deposit. Before sampling, pumping of water (about 5 l) from the water wells was carried out to avoid dilution due to atmospheric precipitation. Ph (PH200, South Korea) and the water temperature were determined immediately after sampling. After the selection the samples were preserved. Preservation of the samples for the determination of NO$_3^-$ and NH$_4^+$ ions was carried out by the addition of CHCl$_3$. To determine the Fe$^{\text{total}}$, the samples were preserved with HCl to a pH of less than 2. To determine Pb, Cu and Zn, the samples were HNO$_3$ assayed. Before the analysis the samples were stored at a temperature of +4 ... + 6 ºC and filtered through a filter with a pore diameter of 1–2.5 μm. Chemical analysis of the samples was carried out in the accredited analytical laboratory of SibRIAP (Accreditation Certificate No. POCC RU.0001.10ПФ01).

3.2 Analytical methods

The concentrations of Ca$^{2+}$, Mg$^{2+}$, CO$_2$, HCO$_3^-$, and Cl$^-$ in the water were determined using the titrimetric method; Fe$_{\text{total}}$, NH$_4^+$, NO$_3^-$, SO$_4^{2-}$ and DOC were determined using the spectrophotometry method (Specol-1300, Analytik Jena, Germany); and the chemical oxygen demand (COD), humic (HA) and fulvic acids (FA) were estimated with potassium dichromate. The determination of the concentrations of K$^+$ and Na$^+$ ions was carried out using the method of flame photometry (PFA-378, Russia); Pb, Cu and Zn concentrations were determined using the method of inversion voltammetry (STA, Russia).

3.3 Biotesting technique

The toxicity of samples of waters was determined by certified biotesting methods at the Centre for Biotesting Safety of Nanotechnology and Nanomaterials "Biotest-Nano" at the NU TSU. A monoculture of filter cages Daphnia magna Straus was used as a test object. The toxicity index (survival rate of individuals) was evaluated. For control, we took 3 parallel series with cultivation water. The experiments were carried out in test tubes with a volume of 100 cm$^3$, which are filled with 50 cm$^3$ of the test water. Test tubes were placed 10 Daphnia magna Straus at the age of 6-24 hours. Test tubes with water samples and test organisms were placed in a rotating cassette of the device for crustaceans exposing UER-03 (figure 2). Due to the rotation of the cassette, continuous and uniform
aeration occurs for all tested samples. The rotational speed of 6-8 revolutions/min does not create a stressful situation for the crustaceans. To determine the harmless dilution ratio (HDR), the samples were diluted 2, 4, 8 and 16 times (figure 3).

Accounting for Daphnia magna Str. mortality in the experiment and control is carried out every 24 hours. The test is terminated if within 24 hours, in all dilutions of the test water, there is 50% death of the test organisms. To determine the acute toxicity of the studied waters, the percentage of Daphnia magna Str. killed in the test water (A,%) is calculated in comparison with the control:

\[
A = \frac{X_k - X_t}{X_k} \times 100\% ,
\]

where \( X_k \) is the number of D. magna Str. surviving in the control; \( X_t \) is the number of Daphnia magna Str. surviving in the test water. When \( A \leq 10\% \), the test water has no acute toxic effect. This percent of dead crustaceans is used to calculate the harmless dilution ratio of the test waters. When \( A \geq 50\% \), a water sample has an acute toxic effect.

4. Theory
4.1 Features of the test organism Daphnia magna Straus

The biological features of D. magna Str. make these crustaceans valuable test organisms with clear advantages over other species. This species characterised by convenient and relatively simple cultivation conditions (culture content in pure natural water, daily separation of young from adult females and feeding). Young individuals are genetically homogeneous, which is ensured by parthenogenetic reproduction and maintenance of a synchronised culture, which is considered to be a group of individuals that are at one stage of development. D. magna Str. is characterised by rapid ripening of crustaceans of 5–8 days at an optimum temperature of ±20±2°C and good nutrition, with a duration of embryonic development of 3–4 days; regular (every 3-4 days) and the numerous appearances of fry (10-15 in young females and up to 40 individuals in mature females).

D. magna Str. has a fairly high level of organisation and sensitivity to most pollutants, is characterised by the presence of the circulatory and nervous systems, which allows extrapolating
toxicological results to other multicellular representatives of ecosystems and even humans; large dimensions, due to which it is possible to conduct visual observations of many responses without the use of specialised measuring instruments [8]. D. magna Str. is a unique test organism that allows you to evaluate a variety of test functions. Test function—the reaction of the test organism, which serves to determine the toxicity of the test environment. In D. magna Str., test functions of different levels are tested and used: behavioural, morphological, physiological, biochemical and genetic. Of practical interest is not only the search for new test functions of lower crustaceans, but also the determination of the sensitivity of known responses [8].

One of the most common test functions in biotesting is the death of the organism. Despite the fact that the test function of death is a “gross” and the most extreme degree of toxic effect manifestation, it is often used in environmental regulation and environmental practice. For example, for wastewater and aqueous extracts of waste, the most important characteristic is a harmless dilution ratio (HDR), that is, the degree of dilution required to the absence of acute lethal toxicity. Depending on the HDR of an aqueous extract of the waste, it is referred to one of the hazard classes. HDR is traditionally determined using D. magna, although other biotesting techniques can also be used for such an analysis [8].

4.2 Sensitivity of Daphnia magna Straus to pollutants

The representatives of the lower crustaceans are sensitive to many pollutants in the aquatic environment, in dissolved form, with a longer exposure in the form of colloids (particle size up to 100 nm) and unstable organic-mineral complexes [9]. There is evidence that D. magna react more strongly to organometallic compounds than to cations of the corresponding metals [10]. Much attention is paid to the study of the sensitivity of D. magna to mineral compounds, in particular heavy metals. The sensitivity of crustaceans to silver, mercury, cadmium, chromate, dichromate, cyanide and iodide ions was studied, where the greatest effect on D. magna was noted [11-13].

Many heavy metals are necessary for the vital activity of hydrobionts as trace elements involved in metabolism, but an increase in concentration leads to various toxic effects. A concentration of 0.03 mg/l of Cu (II) in copper acetate causes the death of crustaceans, reduces their reproductive and filtration activity, while the MPC of Cu is 0.1 mg/l [14]. However, it was found that Zn and Ni do not have a similar effect in concentrations not exceeding the established standards. According to other data, lethal concentrations of Cu for this group of organisms start from 0.01–0.02 mg/l, Zn from 0.48–0.68 mg/l, Cd from 0.038–0.055 μg/l [15]. In chronic experiments with Daphnia magna, cobalt chloride in concentrations up to 0.1 mg/l, in terms of active ion, affects survival and linear growth of D. magna. Concentrations of cobalt chloride up to 0.3 mg/l have an impact on embryogenesis and its synchronicity with oogenesis and up to 0.16 mg/l effect on intestinal filling and fat accumulation; up to 0.01 mg/l influence on the amount of swept fry. With chronic effects on D. magna, Cu (II), in terms of ion at a concentration of up to 0.05 mg/l, affects the survival and linear growth of Daphnia and up to 0.01 mg/l impact on the total number of juveniles and average fertility [16].

The results of studies by different authors may not coincide when testing the same substances, which is explained by the following circumstances: cultures are found in water from different regions; various conditions of the experiment, for example, the duration of the experiment and the number of test objects in the test environment and unequal model substances are used in assessing the effect of a potentially toxic element.

The toxic effect of heavy metal compounds and many other substances depends on the content of organic substances in waters, on pH, hardness and other physical factors [17]. Changes in pH to the acidic or alkaline side significantly affects the stability of hydrobionts to metal compounds. This refers to the interval of optimal values for Daphnia. The acidification of the environment increases the toxicity of water-soluble metal compounds, and the alkaline reaction reduces toxic effects [18]. This is due to the fact that the pH level effects on the degree of substances dissociation, as a result of which their toxicity changes [19]. Increasing the temperature of water, as a rule, enhances the toxic effect of metals on hydrobionts, and this is more clearly manifested in acute experiments. In the temperature range of 25–30 °C, the toxicity of Cd increases by 3–4 orders of magnitude or more and the toxicity Cu and Zn increases by 2 orders of magnitude, while Hg and Mn remain almost unchanged [20]. From the physiology point
of view, this is explained by the fact that with increasing water temperature, the permeability of tissues of hydrobionts increases, the metabolic rate and oxygen consumption increase [18].

The presence of suspended particles and dissolved organic matter in waters creates conditions for the transition of labile forms of metals into bound, which leads to a decrease in their toxicity to D. magna and other hydrobionts. The complexing ability of dissolved organic matter is a determining factor for maintaining the buffer capacity of freshwater ecosystems to metals [21]. For example, by calculation, it was shown that in fresh surface waters, up to 99% of Hg is combined with organic substances [22]. In this case, the complexation of metals with organic acids depends on the molecular weight of organic compounds, on the concentration of metals and ligands and on pH.

Non-specific organic compounds, such as acids, esters, phenols, amines, amino acids, carbohydrates, free reducing sugars and protein-like substances [23], as well as specific high-molecular organic acids – humic and fulvic acids – are capable of complexing. Complexation involving metals increases with increasing pH [24]. One of the factors affecting toxicity is water hardness due to the presence of Ca\(^{2+}\) and Mg\(^{2+}\) ions. In the presence of Ca\(^{2+}\) ions, the permeability of biological membranes decreases [17, 21]. However, according to other data, the mobility of crustaceans increases with increasing temperature and Cd concentration [25].

The presence of the nervous system in D. magna makes them highly sensitive to organophosphorus and organochlorine compounds inhibiting acetylcholinesterase [26]. The sensitivity of D. magna to organic substances depends significantly on their nature. Phenol at a content of 1–2 mg/l stimulates the reproduction of crustaceans [27]. The lethal effect of surfactants on Daphnia occurs at concentrations of 0.8–30 mg/l [28–29].

5. Results and discussion

5.1 Water chemistry

The chemical composition of waters (table 2, figure 4) is determined by the high content of NH\(^{+}\)\(_4\), Fe\(_{\text{total}}\), organic matter at all points of the study. The MPC excesses for NH\(^{+}\)\(_4\) range from 2.2 to 3.7 times, for Fe\(_{\text{total}}\), 6.6–51 times and for COD, 11.9–17.8 times.

The highest NH\(^{+}\)\(_4\) content is 7.49 and 7.45 mg/l in the drained BB1 and IB3, respectively, and the lowest is 4.39 mg/l in the Gavrilovka river. IB4 is characterised by a maximum concentration of Fe\(_{\text{total}}\) (15.3 mg/l) and COD (266.5 mg/l). At the remaining points of the study, the concentration of Fe\(_{\text{total}}\) varies from 1.98 to 3.63 mg/l. Also, high COD is marked at the IB3 (246 mg/l), points BB1 and BB2 are estimated at 217.8 mg/l and in the Gavrilovka river, the estimation is 178.2 mg/l. The content of NO\(_3\) in the samples does not exceed the MPC values. The maximum amount 3.32 mg/l is estimated in sample No. 3; the minimum 1.89 mg/l in sample No. 5.

| Site | Sample | HCO\(_3\) | Ca\(^{2+}\) | Mg\(^{2+}\) | Cl\(^-\) | NH\(^{+}\)\(_4\) | SO\(_4\)\(^{2-}\) | Fe\(_{\text{total}}\) | NO\(_3\) | FA | HA | COD |
|------|--------|-----------|----------|---------|-------|----------|----------|---------|-------|-----|-----|-----|
| BB1  | 1      | 11.7      | 4.41     | 1.17    | 3.77  | 7.49     | 2.94     | 2.14    | 2.57  | 115 | 12.6 | 217.8 |
| BB2  | 2      | 6.25      | 2.00     | 0.44    | 3.47  | 5.06     | 4.36     | 1.98    | 2.58  | 110 | 6.47 | 217.8 |
| IB3  | 3      | 4.58      | 4.37     | 1.29    | 3.69  | 7.45     | 4.55     | 3.63    | 3.32  | 148 | 6.50 | 246  |
| IB4  | 4      | 11.4      | 7.41     | 1.82    | 4.19  | 6.76     | 5.11     | 15.3    | 2.41  | 151 | 12.6 | 266.5 |
| GR   | 5      | 96.4      | 24.3     | 7.17    | 3.69  | 4.39     | 1.86     | 3.39    | 1.89  | 87.5 | 7.90 | 178.2 |
| MPC\(^1\) | 200 | 50        | 350      | 1.5     | 500   | 0.30     | 45     | 15     |       |     |     |     |

Note: MPC for domestic water supply according to Hygienic standards 2.1.5.1315-03

The Gavrilovka river (sample No. 5) is characterised by the richest ion-salt composition in comparison with other samples (figure 4). This sample is characterised by a maximum content of HCO\(_3\) (96.4 mg/l), Ca\(^{2+}\) (24.3 mg/l) and Mg\(^{2+}\) (7.17 mg/l), but also a minimum concentration of SO\(_4\)\(^{2-}\) (1.86 mg/l). Within IB4, in sample number 4, the highest concentration of Cl\(^-\) and SO\(_4\)\(^{2-}\) (5.11 mg/l) was estimated (4.19 mg/l). IB3 (sample No. 3) is characterised by a minimum value of HCO\(_3\) (4.58
mg/l) in water. The lowest Ca\(^{2+}\) concentration (2.0 mg/l), Mg\(^{2+}\) (0.44 mg/l) and Cl\(^-\) (3.47 mg/l) is in sample No. 2 within BB2.

![Figure 4. Water chemical compositions.](image)

The content of heavy metals in waters (table 3) does not exceed the MPC values, except sample No. 2 in terms of Pb. The concentration of Pb in it is 36 µg/l. Sample No. 2 is also characterised by the highest Zn content of 222 µg/l. In sample number 4, the highest Cu content is 2.89 µg/l, but Zn content is low.

| Site | Sample | Zn  | Cd  | Pb   | Cu  |
|------|--------|-----|-----|------|-----|
| BB1  | 1      | 28.0| <0.20| 1.96 | 0.76|
| BB2  | 2      | 222 | <0.20| 36.0 | 1.24|
| IB3  | 3      | 31.6| <0.20| 0.92 | 1.18|
| IB4  | 4      | 9.83| <0.20| 4.75 | 2.89|
| GR   | 5      | 17.4| <0.20| 0.85 | 0.64|
|       | MPC\(^1\)| 1000| 1   | 10   | 1000|

Note: MPC for domestic water supply according to Hygienic standards 2.1.5.1315-03

The Gavrilovka River (sample No. 5) is characterised by the lowest Pb and Cu content, which is 0.85 µg/l and 0.64 µg/l, respectively. Water analysis showed in all water samples that Cd content is less than 0.2 µg/l. In general, the water of GR (sample No. 5) differs from other samples by the presence of a richer ion-salt composition, exceeding the MPC norms for Fe\(_{\text{total}}\) and for COD. BB1 and IB3 (samples No. 1 and No. 3) have a similar mineral composition, excess of MPC for NH\(_4\)\(^+\), Fe\(_{\text{total}}\) and COD. BB2 and IB4 (samples No. 2 and No. 4) are also characterised by an excess of MPC for NH\(_4\)\(^+\), Fe\(_{\text{total}}\) and COD. However, BB2 water (sample No. 2) is more polluted with heavy metals, while IB4 with Fe\(_{\text{total}}\) in general, is much higher than the MPC standards of Russia.

5.2 Biotesting results

In the process of biotesting (table 4), it was found that the test samples of pine-shrub sphagnum bogs (No. 1–4) have a negative impact on test organisms D. magna Str. The degree of toxicity was defined as toxic and the toxicity index was 100%, hazard class IV.

Upon dilution of the initial sample No. 1 (BB1, pH = 4.56), the HDR of 4.4 was set; when the pH of the sample was normalised to 8.61 (addition of NaOH solution) and the toxicity index decreased to 16.7, hazard class V, the degree of toxicity is non-toxic. Lower mobility was observed in individuals.
Table 4. Biotesting results.

| Sample                                           | Toxicity index, % | Degree of toxicity | Harmless dilution ratio | Hazard Class |
|--------------------------------------------------|-------------------|--------------------|-------------------------|--------------|
| Sample 1. Pine-shrub sphagnum bog in the Gavrilovka river basin (drained) (pH = 4.56) | 100               | toxic              | 4.4                     | IV           |
| Sample 1. Pine-shrub sphagnum bog in the Gavrilovka river basin (drained) (normalized to pH = 8.61) | 16.7              | non-toxic          | 1.0                     | V            |
| Sample 2. Pine-shrub sphagnum bog in the Gavrilovka river basin after a fire (pH = 4.17) | 100               | toxic              | 4.1                     | IV           |
| Sample 2. Pine-shrub sphagnum bog in the Gavrilovka river basin after a fire (normalized to pH = 7.92) | 13.3              | non-toxic          | 1.0                     | V            |
| Sample 3. Pine-shrub sphagnum bog on the interfluve of the Shegar and Iksa rivers (drained) (pH = 4.29) | 100               | toxic              | 1.6                     | IV           |
| Sample 3. Pine-shrub sphagnum bog on the interfluve of the Shegar and Iksa rivers (drained) (normalized to pH = 8.25) | 0                 | non-toxic          | 1.0                     | V            |
| Sample 4. Pine-shrub sphagnum bog on the interfluve of the Shegar and Iksa rivers after a fire (pH = 4.41) | 100               | toxic              | 2.4                     | IV           |
| Sample 4. Pine-shrub sphagnum bog on the interfluve of the Shegar and Iksa rivers after a fire (normalized to pH = 8.46) | 6.7               | non-toxic          | 1.0                     | V            |
| Sample 5. Gavrilovka River (pH = 7.23)           | 16.7              | non-toxic          | 1.0                     | V            |

The HDR of the initial sample No. 2 (BB2, pH = 4.17) was 4.1, with a normalisation of pH to 7.92, the toxicity index decreased to 13.3%, hazard class V, and the degree of toxicity is non-toxic. The *D. magna* Str. was observed to change the colour of the intestine from brown to almost colourless, indicating a violation of the physiological state of crustaceans [30].

Dilution of sample No. 3 (IB3, pH = 4.29) established HDR 1.6 and with normalisation pH = 8.25, the toxicity index was 0%, hazard class V, and the degree of toxicity is non-toxic. There was a visual increase in the mass of *D. magna* Str., high mobility.

The HDR of the original sample No. 4 (IB4, pH = 4.41) was 2.4 and with a normalisation of pH to 8.46, the toxicity index decreased to 6.7%, hazard class V, and the degree of toxicity is non-toxic. Lower mobility was observed, as well as the stay of individuals near the surface.

In the sample number 5 (river Gavrilovka pH = 7.23), the degree of toxicity is characterised as non-toxic, hazard class V, with a toxicity index of 16.7% and HAD is 1.

6. Summary and Conclusion

Drained BB1 and IB3 have a similar mineral composition, exceeding the maximum allowable concentration for NH₄⁺, Fe_total, COD. BB2 and IB4 after the fire events are also characterised by exceeding the MPC for NH₄⁺, Fe_total and COD. However, BB2 (fire event 2016) is more polluted with heavy metals, when IB4 (fire event 1998) with Fe_total, in general, is much higher than the MPC standards. In general, the sample of the Gavrilovka River differs from other samples by the presence of a richer ion-salt composition, exceeding the MPC standards for Fe_total and for the COD. In the process of biotesting, it was found that the test samples of pine-shrub sphagnum bogs (No. 1–4) have a negative impact on test organisms *D. magna* Str. The degree of toxicity was defined as toxic and the toxicity index was 100%, hazard class IV. In the sample number 5 (river Gavrilovka), the degree of toxicity is characterised as non-toxic, hazard class V, with toxicity index 16.7%. The most toxic effect
on test organisms *D. magna* Str. provided by pH value. It was established that with the normalisation of pH the toxicity index decreased. To establish the physiological abnormalities of *D. magna* Str. and their fecundity, it is necessary to conduct an experiment of a long exposure time, and possibly low concentrations of elements affect subsequent generations.

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