Study of toxic impact of fly ash bearing construction materials on aquatic organisms

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Abstract. The article studies the toxicological characteristics and behavior of geopolymer samples GB-1 and GB-2 based on fly ash from thermal power plants. Cement paste also was used to provide comprehensive comparative analysis. Aerated tap water was used as a reference medium. The toxicity of the studied materials was determined by applying standard biotesting methods using such aquatic organisms as Scenedesmus quadricauda microalgae and cladocerans Daphnia magna. Based on the obtained experimental and analytical data, it was found that the most favorable environment for algae is sample GB-2. Different behavior is observed in crustaceans. In this case, the most favorable medium for acute and chronic toxicity is a cement paste. The highest toxic effect is observed in the GB-1 sample medium vs. the others studied samples for all considered parameters of toxicity, including chronic one: the decrease in the concentration of algae is 30-35 %; the death of daphnia is 50-80 %. Chronic toxicity, which was determined by the value of the total fertility of daphnia in GB-1 medium, is 5-18 times less compared to the Reference sample.

1. Introduction

In modern conditions of the constantly increasing standard of human life, there is a tendency to tighten the requirements for the quality of the surrounding infrastructure, including, building materials. The eco- and bio-safety of building materials is one of the key quality indicators. According to some scientific studies, minimizing the use of toxic building materials is one of the main principles of the sustainable development of the construction industry. However, despite this, nowadays structures are often built from toxic building materials. This phenomenon can be explained by two reasons:

- poor awareness of builders about the characteristics of the building materials used;
- the economic aspect. Resolving the issue of environmental friendliness, as a rule, requires additional material investments [1].

One of the striking examples of the use of toxic materials can be noted impregnation for wood (fungicides, creosote, copper, chromium, and arsenic, etc.), which are used to protect wooden structures from mold and insects [2]. Another equally common example is the use of toxic mineral industrial waste in structures, for example, road structures, which are liable to leach during operation [3].
In both cases, the negative effect manifests itself when rainwater hits the material, as a result of which a significant proportion of pollutants and hazardous substances enter the environment. Another case of toxicity in building materials is associated with materials that emit toxic fumes during fires. Some studies show that most of the deaths in fires are due to the inhalation of toxic fumes [4]. In addition to chemical pollution, building materials can be characterized by the presence of radioactive elements such as $^{226}$Ra, $^{232}$Th, $^{40}$K, which are also quite dangerous and can be classified as toxic. It is known that the use of wastes with a risk of radiological contamination is dangerous at the level of human health. The danger is that long-term exposure, even at low dosages, can develop cancers. In most cases, building materials are toxic to a much lesser extent than raw materials that are used to synthesize materials and composites [5]. According to the analysis provided by Pacheco-Torgala F. et al. [6], the most toxic types of raw materials are phosphogypsum, some blast furnace slags [7], and some fly ash types [8–10].

One of the most important methods in ecology, allowing to answer many questions about toxicity, is biotesting, which, using certain organisms (test objects), solves a wide range of environmental problems. Most often, this is an assessment of the quality of wastewater treatment, the safety of chemicals for various purposes; an assessment of the composition of bottom soil, and control of the water quality of fishery reservoirs. Biotesting, unlike other, even the most subtle analytical methods, allows the determination of how dangerous the factor under study is for the life of biosystems [11].

Experiments were carried out to study the acute and chronic toxicity of daphnia *Daphnia magna* and *Scenedesmus quadricauda* algae to study the environmental friendliness of fly ash-containing building materials. In toxicological studies, test organisms are represented by planktonic unicellular algae from different zones (*Scenedesmus quadricauda, Chlorella vulgaris, Phaeodactylum tricornutum, etc.*) [12–14]. Crustaceans, most often – the cladocerans *Daphnia magna, D. longispina, Ceriodaphnia affinis*, and others, are used as typical representatives of zooplankton [15–17].

An indicator of the toxicity of substances is a whole range of biological criteria, ranging from the death of an organism to subtle molecular rearrangements of its enzymatic systems [18–20]. The most significant indicators of the biological resistance of any organism are its survival rate, fertility, and the quality of the offspring, which determine its safety as a species [11, 13, 15]. Acute toxicity is determined in short-term experiments lasting from several minutes to 4 days, however, based on these data, it is impossible to predict what will happen to the body in the future, especially when exposed to low concentrations of the toxicant. Long-term experiments, which serve to determine chronic toxicity, allow predicting the state of biological processes in the environment, and recognizing latent toxic effects [15, 22]. Determination of water quality using microalgae is necessary stage in the biotesting procedure in all countries of the world [21].

In Russia, the species *Scenedesmus quadricauda* is recommended as a test object for the standard biotesting procedure (ISO 8692:2004 2012), whereas in Europe and the United States, the alga *P. subcapitata* is used more often (Russian Federal Register.1.39.2007.032232007).

*S. quadricauda* belongs to coenobial chlorococcal algae; 2- and 4-cell coenobia of elongated oval cells with rounded ends (8–36 × 2.1–12 µm) are more common. The marginal cells of one coenobium have two spines bent outward. During reproduction, 2–4 autospores are formed in the mother cell, which, upon exiting, form coenobia [22, 23].

### 2. Materials and Methods

#### 2.1. Materials

Some text. The studied systems were geopolymers samples GB-1 and GB-2 obtained on the basis of fly ash from thermal power plants of various production. For biotesting, the pure culture of *Scenedesmus quadricauda* algae in an exponential growth stage (3–5 days after reseeding) and genetically homogeneous culture of daphnia *Daphnia magna* were used.
2.2. Methods
Biotesting methods were used in the study to determine toxic characteristics of geopolymer samples. The biotesting method using daphnia is widely used in Russia and abroad both for testing waste and natural waters and in order to identify biological effects and mechanisms of negative influence of anthropogenic factors when earlier ecotoxicological research results. Biotesting for *Daphnia magna* is realized in the framework of ISO and Russian standards [OCDE-2011; MU 2.1.4.783-99; PND F T 14.1: 2: 4.12-06]. Experimental and control cultures of algae were grown in the medium "Uspensky 1", which is suitable for growing microalgae. A synchronized culture was taken into the experiment.

For synchronization, the test culture was kept in complete darkness at ambient temperature for 2-3 days. As a result, the old cells settled to the bottom, while the young ones remained in the upper layer of the cylinder. The upper layer was transferred without stirring into a sterile nutrient medium. To obtain a double-synchronized culture, the synchronization procedure was repeated after 5-7 days of the exponential growth of the culture. To maintain the exponential stage of algal growth, reseeding was carried out once every 7 days.

3. Results

3.1. Algae biotesting
Before the start of the experiment, the initial culture of algae (at the age of 3–5 days) was concentrated until a suspension was formed, and the initial number of cells of the suspension of algae in the Goryaev chamber was determined to calculate the required amount of suspension addition (in control and test vessels), which would provide the required cell density when inoculated at the beginning of the experiment. The number of algae at the beginning of biotesting should be 25–35 thousand cells/ml.

After sowing and thorough mixing, the algae in the control and test vessels were counted in the Goryaev chamber before the start of the experiment. The repeated counting of algae in the Goryaev chamber was carried out after 72 hours. The experimental results are presented in the Table. 1.

| Sample      | Number of microalgae (cells / ml) in model media of the studied sample after rotation (days), in brackets – a decrease in the amount of algae relative to the control |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| GB-1        | 3.25·10⁶ (35%)                                                                                                                                  | 3.5·10⁶ (3%)                                                                                          |
| GB-2        | 4.5·10⁶ (10%)                                                                                                                                 | 4.5·10⁶ (10%)                                                                                          |
| Cement paste| 3.75·10⁶ (25%)                                                                                                                                  | 4.0·10⁶ (20%)                                                                                          |
| Reference   | 5.0·10⁶                                                                                                                                            | 5.0·10⁶                                                                                                 |

The experimental results are considered reasonable if the number of algae in the control increased by more than 10 times within 72 hours of exposure. In this case, the concentration of algae increased 200 times. In addition, it is necessary that the change in pH at the end of the experiment should not exceed 1.5. This requirement was also met. In accordance with the procedure for conducting an experiment on biotesting on microscopic algae, non-toxic media are those in which the decrease in the concentration of algae does not exceed 20 %. In all considered variants of the experiment, medium of GB-2 samples lead to a decrease in the concentration of algae by only 10–16 %, therefore, they are the safest for microalgae. As a result of experiments carried out on microscopic algae, it was found that the most unfavorable for the environment are samples GB-1, then - Cement paste. Thus, medium of GB-2 samples are more dangerous for algae.
3.2. Acute toxicity
During the experiments on the study of acute toxicity on daphnia *Daphnia magna*, the prepared solutions of the extract of building materials and the control solution were poured into 100 ml vessels in triplicate for each extract; 10 individuals were seated, whose age did not exceed 24 hours. The number of survived daphnia was recorded after 3, 24, 48, 72, 96 hours. The dissolved oxygen concentration must be above 3 mg/L at the beginning and throughout the test. The pH should be in the range of 6.5–8.5 and should not change more than 1.5 units during the test. The results of a short-term experiment on daphnia are shown in Table 2 and in Figures 1 and 2.

Table 2. Number of survived dafnias in model media of the studied samples.

| Sample       | Sample rotation time, days | Number of survived dafnias (units) under different time period of test (hours) |
|--------------|----------------------------|--------------------------------------------------------------------------------|
| GB-1         | 1                          | 10 10 10 10 6 5                                                             |
|              | 3                          | 10 10 10 10 2 2                                                             |
| GB-2         | 1                          | 10 10 10 10 8 8                                                             |
|              | 3                          | 10 10 10 8 6                                                               |
| Cement paste | 1                          | 10 10 10 10 10 10                                                          |
| Reference    | -                          | 10 10 10 10 10 10                                                          |

As can be seen from the results of an acute toxicity test, the most favorable habitat for daphnia was the cement stone medium. During the observation period of 96 h, no death of the animals was recorded.

In the case of GB-1 samples, the most toxic was the model medium with a long sample rotation time - 3 days. In this case, on the second day of observation, the death of daphnia equal to 80 % was recorded. With the samples that were in contact for a shorter time - 1 and 3 days; the death of animals was observed at a later time - on the 3rd day of observation. At the end of the experiment (after 96 h), all the options considered showed acute toxicity. In the case of GB-2 medium, a long sample rotation time (3 days) was also the most toxic. In this case, on the 3rd day of observation, the death of daphnia was recorded equal to 20 %, and on the next day, the death was 40 %. Thus, at the final stage of the test (after 96 h), these model media did not have such high toxicity as in the case of GB-1 (20 % and 40 %) vs. GB-2 (50 and 80 %).

3.3. Chronic toxicity
During the studies of chronic toxicity, the prepared solutions of the toxicant and the control solution were poured into 0.1 L beakers in 3 replicates, 10 individuals up to 24 hours were placed in each one to register the first litter for each daphnia. The appearance of juveniles, which were removed, was monitored daily. The chronic experiment lasted 21 days.

The crustaceans were fed daily chlorella *Chlorella vulgaris Beyer*, which is cultured in the laboratory. Optimal conditions of detention such as temperature 23 ± 0.5 °C and light regime day-night (16-8 hours) were maintained automatically. In long-term experiments, the medium was completely changed after 5 days. For this, immediately at the start of the experiment, reserve solutions of all model solutions were prepared.

The detection and quantification of the content of pollutants in the environment are not enough to answer the question about the sustainability of building material. It is also necessary to determine their danger to living organisms most sensitive to pollution. Thus, research on lower aquatic organisms (protozoa and crustaceans) can solve many important ecological problems. *Cladocera Daphnia magna Straus* is a ubiquitous species with well-studied biology. Daphnia cultivated in laboratory conditions have a short life cycle, which rarely exceeds 2-3 months.
An important biological effect of toxic exposure is the fertility and reproductive success of the population because the long-term survival of a species depends on the fertility of individuals and the quality of the offspring. Changes in reproductive processes are classified as one of the most significant effects of pollution. For this purpose, a chronic experiment on daphnia was carried out for 21 days. The results of a chronic experiment on daphnia are given in the Table 3.

**Table 3.** Results of a chronic toxicity test on in model medium of the studied samples.

| Sample     | Sample rotation time, days | Total fertility, units | First litter dates, days | Number of litters for 21 days, units. |
|------------|----------------------------|------------------------|--------------------------|----------------------------------------|
| GB-1       | 1                          | 15                     | 10                       | 2                                      |
|            | 3                          | 4                      | 18                       | 1                                      |
| GB-2       | 1                          | 38                     | 7                        | 4                                      |
|            | 3                          | 36                     | 8                        | 4                                      |
| Cement paste| 1                          | 68                     | 7                        | 7                                      |
|            | 3                          | 56                     | 6                        | 6                                      |
| Reference  | -                          | 72                     | 6                        | 7                                      |
The most favorable medium among those considered ones was the medium formed as a result of the interaction of the cement stone. In the considered variant, the aqueous media had the same chronic effect as in the reference medium. Slightly less (by one) the number of litters in the reference medium with a rotation time of 3 days, the total fertility is less by 22%. GB-2 medium, with chronic exposure, also inhibits the development of daphnia in all variants of model media. However, this kind of impact is much less than GB-1 medium. In the case of this sample, the total fertility of daphnia is 5-18 times less than in the Reference sample and 2 times less than in GB-2 medium. The same pattern is observed with the onset of the first litter and with the total number of litters.

4. Discussion
The results of experiments on biotesting showed a different degree of toxicity of the studied media of samples, which increases in the following sequence: Cement paste → GB-2 → GB-1. GB-2 reduces algae concentration by only 10%. It was revealed that from the point of view of acute toxicity the GB-1 medium of the sample is the most dangerous, where the death of Daphnia in a short-term experiment was up to 80%. At the same time, the Cement paste medium is a favorable environment for Daphnia magna, where no animal deaths were recorded.

The results of studies of chronic toxicity, which was assessed by the total fertility of daphnia, demonstrated that GB-1 medium is again the most toxic, then in GB-2 medium. According to this indicator, a medium based on cement paste is also favorable for daphnia.

5. Conclusion
Thus, xenobiotics entering water bodies from leached building materials can have a negative effect on various parameters of the vital activity of aquatic organisms, including survival and fertility. From the point of view of the toxic effect, the medium of the GB-1 sample has a greater negative effect, both with short-term and long-term exposure to the aquatic ecosystem.

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