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Application of *Arthrospira (Spirulina) platensis* against Chemical Pollution of Water

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Abstract: The basis of phytoremediation technology for cleaning chemically polluted water was developed in the framework of the presented work. This technology is based on the ability of blue-green alga *Arthrospira platensis* to eliminate different environmental toxicants from water. This technological approach was conducted for the following pollutants: 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), 2,4,6-trinitrotoluene (TNT), and cesium ions. The effectiveness of the technology was tested in model experiments, which were carried out in glass containers (volume 40 L). In particular, the different concentrations of alga biomass with the aforementioned pollutants were incubated with permanent illumination conditions and air barbotage, at a temperature of 25 °C. The results of the model experiments showed that after two weeks from the start of remediation *Arthrospira* effectively cleaned artificially polluted waters. Particularly in the case of TNT 56 mg/L concentration, the effect of water remediation was 97%. In the case of DDT 10 mg/L concentration, the degree of cleaning was 90%. Similar results were obtained in the case of 100 mg/L concentration of cesium ions. Thus, the model experiments confirmed that the alga *Arthrospira* effectively removed tested pollutants from water. That is the basis of phytoremediation technology.

Keywords: phytoremediation; water pollution; DDT; TNT; heavy metals; cesium ions

1. Introduction

For the last few decades, distribution of chemical pollutants from urban, agricultural, and industrial sources caused the accumulation of toxic substances potentially harmful for plants and animals, becoming a serious concern for the ecosystem survival. In this context, the development of soil and water remediation methods for the removal of chemical contaminants is a widely recognized, challenging problem. Nowadays, the technological implementation of methodologies devoted to the removal of pharmaceutical and waste residues, pesticides, and heavy metals from natural and agricultural ecosystems is urgent for all countries.

Currently, urban environmental pollution comprises hundreds of substances, including aliphatic, aromatic and polycyclic hydrocarbons, phenols, pesticides, organochlorine compounds, explosives based on nitro compounds, heavy metals, radionuclides, etc. [1]. Despite the major efforts that have been made over recent years to clean up the environment, pollution remains a major problem and poses continuous risks to health.

At present, the freshwaters from rivers and lakes of industrialized countries are mainly used to produce tap water for large cities. Proceeding from the information mentioned above, the creation of quick-response, strategic approaches against chemical pollution of numerous precipitates of sewage sludge will resolve the important environmental problem.

The given work is focused on the setup of the technological approach on the bioaccumulation and removal of pollutants from water, based on the integrated assimilation of target toxicants by using modern phytoremediation technology [1–3].

Modern phytoremediation technologies may include different methodological approaches, depending on their purposes. One of the methods of cleaning the chemically polluted waters is phycoremediation, based on the application of algae [4]. There are some examples of using algae for cleaning water polluted with heavy metals, petroleum hydrocarbons, pesticides, etc. [4–11].

The main idea of the presented work is to create the basis for an effective cleaning technology of water that is artificially polluted with cesium ions, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (or 1,1′-(2,2,2-trichloroethane-1,1-diyl)bis(4-chlorobenzene)—DDT) and 2,4,6-trinitrotoluene (TNT) by using blue-green alga *Arthrospira platensis* (formerly *Spirulina platensis*) as a phytoremediator.

Arthrospira, which is currently widely used in the food industry and in pharmacology [9,10], can be applied also as a phytoremediation agent for cleaning the chemically polluted waters. The cells of *Arthrospira* contain large amounts of compounds (enzymes, peptides, amino acids, etc.) capable of binding as ions of heavy metals to organic toxic compounds. The structures inside the cells of *Arthrospira* swell with air bubbles, which promotes their emerging on the water surface. Thus, the algae biomass is easily removed from the testing reservoirs.

It is known that *Arthrospira* is applied for remediation of water that is contaminated mainly with heavy metals [5,11–17]. Additionally, it is known for its use in cases of water polluted with petroleum hydrocarbons [12], pesticides [13], radioactive elements [18], fluoride ions [19], some estrogens [20], etc.

Water pollution caused by hazardous heavy metals and radionuclides is one of the most important ecological problems. Among them, ^{137}Cs is very dangerous. Nonradioactive cesium ions that occur in nature and are released into the environment through mining and milling of ores are only mildly toxic [21]. Both radioactive and stable cesium, when they occur in a human or animal body, act the same way [22]. There are only limited data concerning the use of *Arthrospira* as a phytoremediation agent in cases of water polluted with Cs^+ ions.

TNT that is used as a military high explosive is one of the most toxic compounds. It can accumulate in human organs, stimulating some chronic diseases, and it is considered a hazardous contaminant of the environment [23].

DDT was a widely used insecticide in the 20th century because it was applied to fight malaria-carrying mosquitoes [24]. Despite banning DDT application, this pesticide, as other organochlorine pollutants, stays in the environment in undestroyed form for a long time due to its chemical stability. Its high hydrophobicity allows the insertion of DDT into the food chain. DDT easily penetrates human organisms through the digestive tract or the skin and damages nerve tissue [1].

In our previous works it we showed *Arthrospira* has a high ability to eliminate Cs^+ ions, TNT, and DDT from artificially polluted water via adsorption on the surface of cellular lipopolysaccharides and then by their moving into cells [25–27]. The presented article gives the results of work that was performed to test the phytoremediation potential of *Arthrospira* in the model experiments.

Thus, the goal of the presented work is to investigate opportunities to use *Arthrospira platensis* as a tool for removing pollutants with different chemical natures from water. For this aim, model experiments were conducted to establish the optimal concentration of biomass of *Arthrospira* that allows elimination of Cs^+ ions, TNT, and DDT from artificially contaminated water with maximal effectiveness.

2. Materials and Methods

In the experiments we used the biomass *Arthrospira platensis* obtained via cultivation in standard Zarrouk's medium (pH—8.7; content in g/L: $NaHCO_3$—16.8, K_2HPO_4—0.5, $NaNO_3$—2.5, K_2SO_4—1.0, NaCl—1.0, $MgSO_4 \cdot 7H_2O$—0.2, $CaCl_2 \cdot 2H_2O$—0.04, $FeSO_4 \cdot 7H_2O$—0.01, EDTA—0.08; and microelements kit A5—1 mL). The cultivation was conducted in the following conditions: Permanent air barbotage (rate of air flow 2 L/min); temperature of 25 °C; a photoperiod of lighting of 16 L/8 D (16 h of light:8 h of dark); a total photosynthetic photon flux density (PPFD) of ≈15 μmol·m^{-2}·s^{-1}.

The biomass of *Arthrospira* in the incubation medium was measured spectrophotometrically at 750 nm [28].

For determination of DDT, the incubation medium was centrifuged at 1000 g for 20 min and DDT was extracted from the obtained supernatant by hexane. The extract was concentrated by evaporation, and DDT content was determined by gas chromatographic analysis (Instrument—Agilent 7890A (Beijing, China)) [29]. Limit of detection—0.01 mg/L. Detailed conditions for analysis are described in our previous work [26].

Concentration of residual TNT in the incubation area was measured spectrophotometrically at 447 nm, in a highly alkaline (pH > 12.2) area (spectrophotometer—Shimadzu UV1900 (Kyoto, Japan)) [30]. Limit of detection—0.2 mg/L.

The content of Cs^+ ions in the samples was determined by the method of atomic absorption (flame emission) analysis [31]. Conditions for analysis are the following: Instrument—PerkinElmer HGA900 Graphite Furnace (Waltham, MA, USA); wavelength—852.1 nm; slit—0.2/0.4 nm; flame—air-acetylene; stock standard solution—CESIUM 1000 mg/L; light source—EDL; interface—ionization controlled by addition of 0.1% KCl. Limit of detection—0.5 mg/L.

The model experiments for cleaning water polluted by tested toxicants were carried out in the following conditions: (1) *Arthrospira* was cultivated in Zarrouk's medium (volume 20 L) for 7 days; (2) thereafter, the solution of DDT, TNT, or Cs^+ was added (volume 20 L); (3) the incubation period was finished after 15 days. According to our previous experiments [21–23], the concentrations of pollutants were as follows: 10 mg/L in case of DDT, 22.5 or 56 mg/L in case of TNT, and 100 mg/L in case of Cs^+ ions. The biomass of *Arthrospira* varied from 3.0 to 4.5 g/L (see tables in Section 3). The incubation was carried out in a glass container with sizes 60 × 21 × 40 (in cm, length × width × height), with permanent air barbotage (rate of air flow 2 L/min), at a temperature of 25 °C, under the following illumination conditions: 24 L/0 D, PPFD ≈ 15 μmol·m^{-2}·s^{-1}. In the control variant, instead of the tested contaminant solution, we added 20 L of water. Model experiments were carried out in periods: April–June, 2017; September–October, 2017; April–June, 2018; September–October, 2018.

Presented data are the mean of three replicates ± standard deviation (SD). The replicates represent an assay of a sample from the same source multiple times. The statistical analysis of obtained data was performed using the method of Descriptive Statistics of Excel.

3. Results and Discussion

The model experiments for development of the phytoremediation technology based on the application of *Arthrospira* for cleaning chemically polluted waters were carried out. In the model experiments the initial biomass of *Arthrospira* was changed until we got maximal absorption of the toxicant.

In the case of DDT, *Arthrospira* was cultivated in artificially polluted waters containing different concentrations of the pollutant and *Arthrospira* biomass. The initial biomass of *Arthrospira* was changed in the model experiments to reach maximal elimination of the toxicant from the polluted water. The obtained results are given in Table 1.

The obtained data show that the remediation process was optimal when the biomass content of *Arthrospira* in the incubation medium was 4 g/L (Table 1, Experiment No. DDT-3). In this case the mass ratio of the DDT and the raw biomass of the alga was approximately 1:400. In this experiment alga removes about 90% of the tested pollutant, indicating the effectiveness of the presented method. At an increase of the alga biomass up to 4.5 g/L, a significant decrease of the DDT amount in the polluted water was not observed. On higher concentrations of DDT (15 mg/L), the effectiveness of elimination out of the polluted water decreased up to approximately 77% in spite of an increase of the initial content of the *Arthrospira* biomass (Table 1, Experiment No. DDT-5).

Table 1. Results of model experiments for cleaning water polluted with DDT by using *Arthrospira*. Mean values (n = 3) and ±SD are given.

Number of Model Experiment	Initial Content of *Arthrospira* in Polluted Water, g/L	Content of DDT in Polluted Water, mg/L	
		Initial (before Incubation)	Final (after Incubation)
DDT-1	3.0	10	2.56 ± 0.15
DDT-2	3.5	10	1.42 ± 0.08
DDT-3	4.0	10	0.98 ± 0.06
DDT-4	4.5	10	0.97 ± 0.06
DDT-5	4.5	15	3.49 ± 0.21

The results of the model experiments for the development of phytoremediation technology for cleaning waters polluted by TNT are given in Table 2. As seen from the obtained results, the most efficient cleaning rate could be achieved by a ratio of 1:60 from the contaminant and algal concentration (Table 2, Experiment No. TNT-3). In this case, alga removes about 98% of TNT from the polluted water. These data indicate the high effectiveness of the presented method for cleaning water polluted by TNT.

Table 2. Results of model experiments for cleaning water polluted with TNT by using *Arthrospira*. Mean values (n = 3) and ±SD are given.

Number of Model Experiment	Initial Content of *Arthrospira* in Polluted Water, g/L	Content of TNT in Polluted Water, mg/L	
		Initial (before Incubation)	Final (after Incubation)
TNT-1	3.0	22.5	2.61 ± 0.13
TNT-2	3.3	56.0	4.50 ± 0.25
TNT-3	3.5	56.0	1.80 ± 0.11

The model experiments for testing of *Arthrospira* as a remediator for cleaning water polluted with 100 mg/L of Cs^+ ions show that the best results were obtained in cases of using 3.5 and 4.0 g/L of the *Arthrospira* biomass (Table 3, Experiments Nos. CS-2 and CS-3). As can be seen from the obtained data, in these cases the efficiency of remediation was achieved up to 90%. When both the Cs^+ ions concentration and the *Arthrospira* initial biomass were increased, the effectiveness of uptake was only about 83%. These results indicate that the most effective ratio of heavy metal and raw biomass of alga was approximately 1:35.

Table 3. Results of model experiments for cleaning water polluted with Cs^+ ions by using *Arthrospira*. Mean values (n = 3) and ±SD are given.

Number of Model Experiment	Initial Content of *Arthrospira* in Polluted Water, g/L	Content of Cs^+ Ions in Polluted Water, mg/L	
		Initial (before Incubation)	Final (after Incubation)
CS-1	3.0	100	18.3 ± 0.91
CS-2	3.5	100	10.3 ± 0.06
CS-3	4.0	100	9.90 ± 0.05
CS-4	4.5	150	25.1 ± 2.26

Comparison of the obtained results with the results of the previous publications will corroborate that the cleaning rate was improved by choosing the proper correlation of the biomass of *Arthrospira* and the organic pollutants. In particular, in the case of DDT, cleaning efficiency was improved from 70% [26] to 90%, and in the case of TNT, from 87% [25] to 97%. As for cesium ions, nothing different from the previous experiments [27] was received.

The ability of *Arthrospira* to remove Cs^+ ions from polluted water supposedly might be related to the presence of heavy metal chelating compounds in this alga. This mechanism is still unknown and should be clarified in further studies. As for the organic pollutants, supposedly their uptake should occur via metabolizing by alga, which implies the primary transformations of compounds penetrated in the cells and then the conjugation of formed intermediates with intracellular compounds. In the case of

TNT, the primary transformations should be performed via reduction of nitro groups to amine groups. Such transformations must be catalyzed by enzymes similar to nitroreductases, which participate in TNT transformation in plants and microorganisms [1]. DDT may be undergoing dehalogenation and/or hydroxylation analogous to plants, by the enzymes such as monooxygenases, peroxidases, phenoloxidases, and dehalogenases [1,32]. Intermediates of transformation of DDT and TNT probably conjugate with the following intracellular compounds: Saccharides, amino acids, peptides, proteins, etc., which are in *Arthrospira* in large amounts. Therefore, to determine the mechanism of removing organic pollutants by *Arthrospira*, it is necessary to study the above-mentioned enzymes in this alga.

The above results show that the application of *Arthrospira* is indicated for cleaning chemically polluted waters. Until now, *Arthrospira* was considered effective mainly in cases of pollution with heavy metals [5,7,14–18]; but the presented results corroborate universal phytoremediation features and the high cleaning potential of *Arthrospira*. Further, it is desirable to continue investigations in the following directions:

- Optimization of incubation conditions of *Arthrospira*;
- Investigation of the processes of organic toxicant metabolism and binding heavy metals in cells of *Arthrospira*;
- Revelation of enzymes and enzymatic systems of *Arthrospira*, participating in the transformation of organic toxicants and chelating of heavy metals.

Moreover, it will be possible to refine and improve *Arthrospira*-based phytoremediation technology, due to the development of proper equipment providing, in automatic mode, cultivation of *Arthrospira*, a polluted water supply, and purified water removal.

Although the information and works are often found on remediation of sewage from heavy metals and microorganisms where *Arthrospira* is the key bio-tool, the presented work contains specific novelty. Removal from water and biodegradation of such persistent and toxic organic pollutants as TNT and DDT via the application of ecological potential *Arthrospira* is the first attempt and has no direct analogy.

4. Conclusions

The overall results of completed investigations can become a basis for the development of phytoremediation technology based on the application of *Arthrospira* for cleaning waters polluted with research toxicants.

The main idea of these technologies is to add the effective amount of *Arthrospira* biomass to polluted water and remove it from the artificially contaminated areas consecutive times.

The effectiveness of the technological approach tested in the model experiments was carried out in glass containers (volume 40 L). The results of the model experiments showed that after 15 days from the start of the experiments using *Arthrospira* the following results are obtained:

- Water remediation was occurring by 97%, in the case of 56 mg/L concentration of TNT and 3.5 g/L initial content of *Arthrospira*;
- The degree of cleaning was 90%, in the case of 10 mg/L concentration of DDT and 4.0 g/L initial content of *Arthrospira*;
- The degree of cleaning was 90%, in the case of 100 mg/L concentration of cesium ions and 3.5 g/L initial content of *Arthrospira*.

From the above-mentioned data, it can be supposed that *Arthrospira platensis* has high phytoremediation potential to pollutants with different chemical natures.

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