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Bioremediation Prospects for Removing Pollutants from River Waters

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Abstract. *The present study addresses the problem of pollution in the Sumgayit River, which has been exposed to technogenic impacts for many years, and proposes new approaches to enhance its self-purification capacity. To purify river water from organic and inorganic pollutants, the feasibility of selecting active strains from the native microbiota and applying them in bioremediation was investigated. According to the research results, river waters subjected to anthropogenic pollution exceeded the maximum permissible concentrations (MPCs): the phenol concentration was 0.50 mg/L (MPC = 0.001 mg/L), while the copper concentration was 0.96 mg/L (MPC = 0.01–0.1 mg/L). It was established that the active microbial strains isolated from these water samples belonged to the genera Rhodococcus, Penicillium, and Aspergillus. The obtained results demonstrated that the fungal strains were more effective than the bacterial strain. Under the influence of Rhodococcus sp., the phenol concentration decreased by 62%; Penicillium sp. achieved a 100% reduction, while Aspergillus sp. reduced the phenol concentration by 86%. The copper concentration decreased by 58.4% with Rhodococcus sp., 64.8% with Penicillium sp., and 68.7% with Aspergillus sp. These results indicate that these microorganisms are effective candidates for application in bioaugmentation.*

Keywords: *bioremediation, phenol-absorbing microorganisms, heavy metals, ecotoxicity, bioindication*

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Çay sularının çirkləndiricilərdən təmizlənməsində bioremediasiya üsulunun perspektivləri

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Xülasə. *Təqdim olunan işdə uzun illərdir texnogen çirklənməyə məruz qalmış Sumqayıt çayının çirklənməsi problemi və onun özünü təmizləmə qabiliyyətinin artırılması üçün yeni yanaşmalar irəli sürülmüşdür. Çay sularının üzvi və qeyri-üzvi çirkləndiricilərdən təmizlənməsi üçün aborigen mikrobiotadan aktiv ştamların seçilməsi və onlardan bioremediasiya prosesində istifadə etmə imkanları araşdırılmışdır. Tədqiqat nəticələrinə əsasən, texnogen çirklənməyə məruz qalmış çay sularında fenolun miqdarının 0,50 mq/l (YVH – 0,001 mq/l), misin miqdarının isə 0,96 mq/l (YVH – 0,01–0,1 mq/l) olduğu və hər iki göstəricinin YVH-ni aşdığı müəyyən edilmişdir. Bu su nümunələrindən ayrılan aktiv mikroorqanizm ştamlarının Rhodococcus sp., Penicillium sp. və Aspergillus sp. cinslərinə aid olduğu müəyyən edilmişdir. Nəticələrə əsasən, istifadə edilən göbələk ştamlarının bakteriya ştamı ilə müqayisədə üstünlük təşkil etdiyi aşkar edilmişdir: fenolun miqdarı Rhodococcus sp. təsiri ilə 62%, Penicillium sp. ilə 100%, Aspergillus sp. ilə 86%, misin miqdarı isə Rhodococcus sp. ilə 58,4%, Penicillium sp. ilə 64,8%, Aspergillus sp. ilə 68,7% azalmışdır. Bu nəticələr onları bioaqmentasiya prosesində istifadə etmək üçün səmərəli hesab etməyə imkan verir.*

Açar sözlər: *bioremediasiya, fenolmənimsəyən mikroorqanizmlər, ağır metallar, ekotoksiklik, bioindikasiya*

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Introduction

One of the important problems of the modern era is the ecological pollution of water bodies. The discharge of various industrial, agricultural, and domestic wastes, wastewater, and oil products into water bodies leads to the pollution of seas, lakes, and rivers. One such water body is the Sumgayit River, which is one of the water bodies most severely exposed to anthropogenic and technogenic pollution in Azerbaijan. This river has become a serious ecological hazard due to the increased discharge of domestic sewage directly into the river, resulting from intensive industrialization and population growth in Sumgayit over many years. Pollution of water bodies by various toxic substances poses a serious risk to ecosystems and can negatively affect all organisms interconnected through the food chain, as well as human health.

To prevent this, the use of environmentally friendly and safe methods is a priority. The use of bioremediation makes it possible to reduce the environmental burden by converting pollutants in water bodies into less harmful substances or by completely neutralizing them (Kalitina, 2015; Kuznetsov, 1989). This process relies on harnessing the metabolic capabilities of living organisms to reduce the toxicity of a wide range of contaminants, including heavy metals, pesticides, and organic matter. To address this challenge, adopting environmentally sustainable and safe remediation strategies has become a priority. Among these strategies, self-purification is defined as a biological response to environmental stress (Dasgupta, 2024; Iskuzhina, 2023). The natural ability of microorganisms to adsorb and degrade pollutants makes their use effective in cleaning contaminated environments (Babaev, 2024). In this process, both bacteria and microscopic fungi play a crucial role.

Literature review

Recent studies demonstrate the high efficacy of biopreparations containing specialized bacterial strains in degrading petroleum hydrocarbons. The most commonly used microorganisms include members of the *Pseudomonas*, *Rhodococcus*, and *Bacillus* genera, which can metabolize various oil components (Kuzhamberdieva, 2019; Nadzhafova, 2024). Mycoremediation may be used as an independent or complementary approach to restoring the quality of aquatic biomes currently under serious threat from pollution and degradation (Fedoseeva, 2023). Several authors have suggested that sustained growth, strong regenerative potential, and the ability to form extensive hyphal networks make fungi ideal agents for the bioremediation of soils and aquatic environments contaminated with a wide range of pollutants (Abu Qamar, 2023; Akhtara, 2020). Researchers have also found that complex microbial communities oxidize petroleum hydrocarbons more completely and rapidly in contaminated aquatic environments and soils, as the microorganisms in such communities interact and benefit from a coordinated "metabolic attack" on the hydrocarbons (Shubakov, 2012). Polybacterial preparations have broader adaptive and ecological potential for use. The selection of methods for degrading various hydrocarbons, including those found in oils, does not present any particular problems (Artyukh, 2014).

An advantage of using bacteria for the cleanup and restoration of environments contaminated with heavy metals is their high survival capacity across a wide range of conditions, including extreme environments, owing to their rapid reproduction, as well as their ability to degrade a broad spectrum of toxic compounds through specialized enzyme systems (Abo-Alkasem, 2023). Due to their small size, the biomass of these microorganisms provides a large surface area for capturing pollutants. Strategies developed by bacteria to survive in environments contaminated with heavy metals include mechanisms such as biosorption, bioaccumulation, biomineralization, biotransformation, and methylation (Iskuzhina, 2023).

Purpose and objectives of the study

The aim of the study was to isolate active microorganism strains from river waters exposed to anthropogenic pollution and to restore the ecological safety of river waters through bioremediation using these strains.

To achieve this goal, the physicochemical properties of the water were investigated; aquatic microorganisms (bacteria and micromycetes) were isolated and identified from the collected samples; and the roles of the identified aquatic micromycetes and bacteria in the decomposition of organic matter and the sorption of heavy metals in the aquatic environment were examined.

Materials and Methods

During the study, eight water samples were collected from the coastal zone of the Sumgayit River. Water temperature (°C) was measured *in situ* using a mercury thermometer, pH using a pH Meter 200, and dissolved oxygen (mg/L) using an MW600 dissolved oxygen analyzer. The concentrations of nitrate, nitrite, chloride, and sulfate ions, as well as phenol and copper (Cu), were determined using a Palintest Photometer 7100. Microbiological analyses were conducted according to universally accepted methods (Egorov, 1983; Kuznetsov, 1989). For microbiological analysis, water samples were collected in sterile glass containers. Under laboratory conditions, they were inoculated onto

Nutrient Agar (NA) and Raymond's agarized mineral medium (supplemented with phenol) and then incubated for 3–7 days at 28 ± 2 °C.

Phenol-oxidizing microorganisms were cultured in Egorova's liquid medium to obtain microbial biomass using the following medium composition (g/L): K_2HPO_4 – 1; $(NH_4)_2SO_4$ – 0.1; $MnSO_4 \times 5H_2O$ – 0.016; $FeCl_3 \times 6H_2O$ – 0.034; $CaCl_2$ – 0.1; NaCl – 0.2; $(NH_4)_2HPO_4$ – 0.5; $MgSO_4 \times 7H_2O$ – 0.2; phenol – 1 (Sokolova, 2012; Zaytseva, 2011). Phytotoxicity was assessed by evaluating the effects of chemicals present in the water (phenols and copper) on seed germination rates and plant development parameters of edible lentil (*Lens culinaris*) and common wheat (*Triticum aestivum*) (Terekhova, 2014).

Results and Discussion

The physicochemical properties of the water samples collected from the Sumgayit River are presented in Table 1.

Table 1
Physical and chemical parameters of water samples

Parameters	Max.Permissible Concentration	R. Sumgaitchay
pH	6,5-8,5	7,1-8,5
BOD_5 (mg O_2/L)	≤4	6-11
Nitrite NO_2 (mg O_2/L)	0.08	0,49
Nitrate NO_3 (mg O_2/L)	45	2,1
Chlorides, mg/L	300	340-600
Sulfate ion, SO4 2- mg/L	500	600-800
Cu, mg/L (free)	0,0003-0,046	0,72
Cu, mg/L (total)	0,001	0,96
Phenol mg/L	0,001	0,50
Temperature, ^{0}C	12-17	

Seasonal water samples were collected to determine the total number of microorganisms and the number of phenol-absorbing microorganisms (Table 2).

Table 2
Microbiological analysis of water samples by season

Seasons of the year	Total amount of heterotrophic microorganisms, CFU/ml	Amount of Phenol-oxidizing microorganisms, CFU/ml
Spring	$2{,}8 \times 10^4$	$1{,}7 \times 10^3$
Summer	$3{,}7 \times 10^4$	$2{,}3 \times 10^3$
Autumn	$3{,}2 \times 10^3$	$2{,}2 \times 10^2$
Winter	$2{,}5 \times 10^2$	$1{,}5 \times 10^2$

By screening, active strains of microorganisms capable of assimilating phenol and copper were selected; they belonged to the genera Rhodococcus sp., Penicillium sp., and Aspergillus sp.

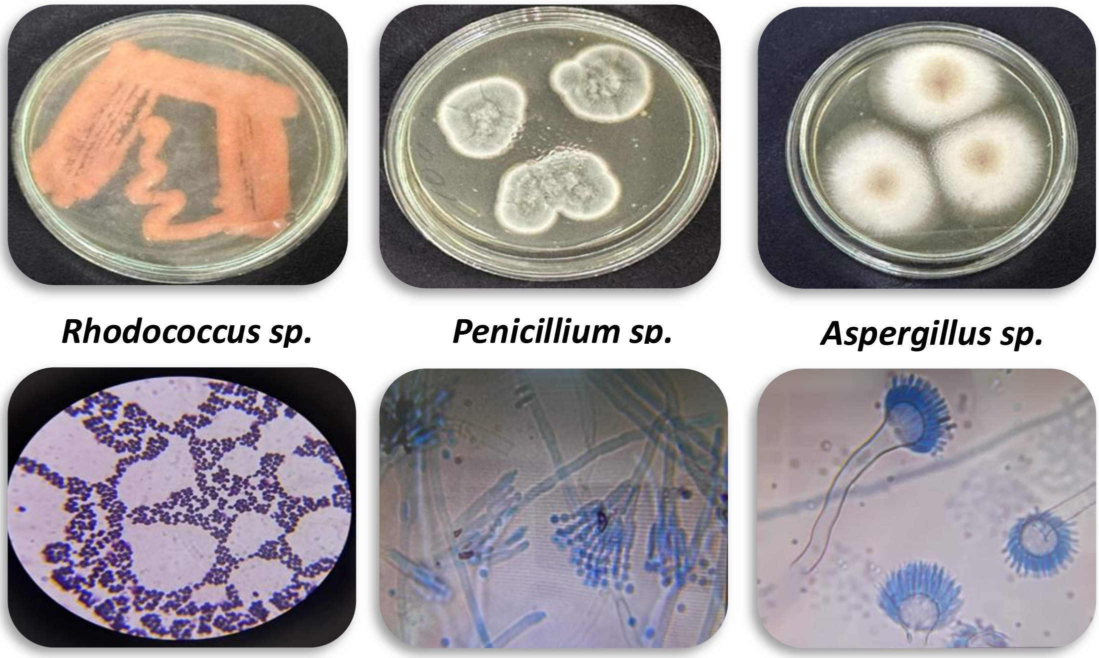

Figure 1
Selection of active microorganisms for bioremediation

Next, a series of experiments tested the effectiveness of active microbial strains for bioremediating water from the Sumgaitchay River. Three sterile flasks were filled with 50 ml of the river water to be tested, and each was individually spiked with cultures of fungi *(Penicillium sp., Aspergillus sp.)* and bacteria (*Rhodococcus sp.)* (1 mg). The flasks were then placed in a shaking incubator at 25°C and 180 rpm for cultivation. Observations were made over a period of 5 days. The residual concentration of phenol and copper in the water was measured every 24 hours. The initial concentrations of phenol and copper in the water sample were 0.50 mg/L and 0.96 mg/L, respectively.
The experimental results are presented in Figures 2 and 3.

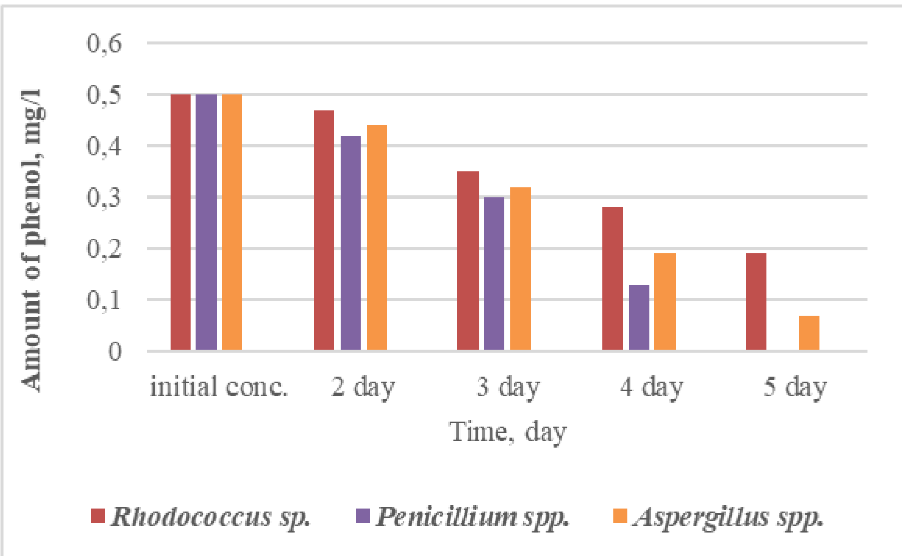

Figure 2
Purification of water from phenol by microorganisms

The results showed that both bacterial strains (Rhodococcus sp.) and fungal strains *(Penicillium sp., Aspergillus sp.)* were effective at removing pollutants from water. However, it was established that fungal strains are more destructive than bacterial strains. Thus, when adding biomass of microorganisms to polluted water, the degree of destruction of phenol by the strain of *Rhodococcus sp*. was 62% (residual quantity - 0,19 mg/l), while the strains of fungi demonstrated higher efficiency — *Penicillium sp.* degree of assimilation of phenol was 100%, and in *Aspergillus sp*.—86% (residual quantity - 0.07 mg/l).

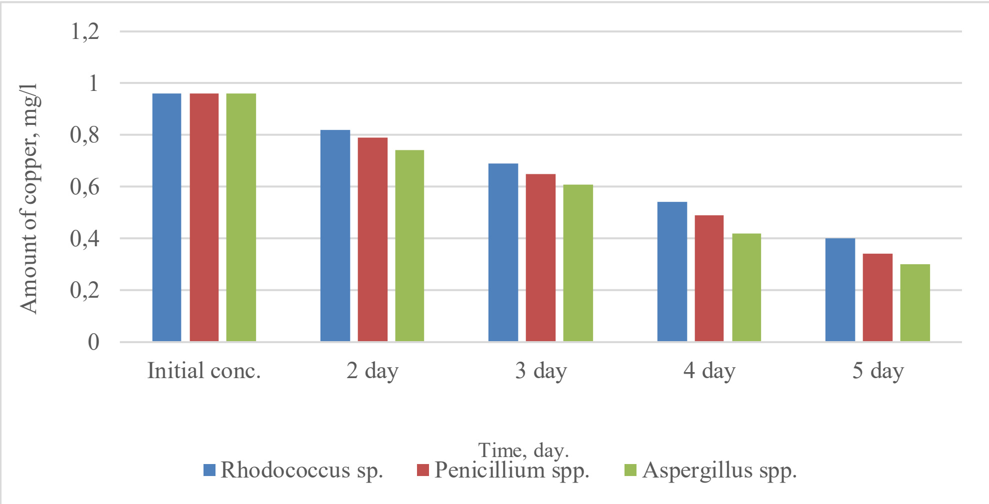

Figure 3

Copper uptake from water by microorganisms (Rhodococcus sp., Pencillium sp., Aspergillus sp.)

The experimental results also showed a significant decrease in the concentration of copper in the water samples (Fig. 3). Furthermore, differences were observed in the copper sorption capacities of the tested microbial strains: *Rhodococcus sp.* (58.4%), *Penicillium sp.* (64.8%), and *Aspergillus sp.* (68.7%). Other studies have also demonstrated that fungi have a high tolerance to various toxicants, making them a powerful tool for wastewater treatment. Furthermore, reports indicate that different fungal species vary both in their mechanisms of action and their remediation efficiency (Abu Qamar, 2023; Dasgupta, 2024; Magan, 2022). Bacterial and fungal bioremediation strategies In addition to basic analytical methods (chemical and physicochemical), bioindication and biotest methods are essential for identifying all pollutants present in the environment and assessing their ecotoxicity (Kalitina, 2019). Plants are among the main indicators in bioindication (phytobionts) and play an important role in environmental monitoring. For this purpose, the ecotoxicity of water samples was also studied using plant seeds. Lentil *(Lens culinaris)* and wheat *(Triticum aestivum)* seeds were used as experimental plants. The toxicity of chemicals (phenols, copper) in water was assessed based on their effects on seed germination and growth parameters (Fig. 4). The influence of these pollutants on seeds was studied in a comparative manner: both before the experiment (the influence of the initial pollutant concentration) and after it (following decomposition and sorption of pollutants by microorganisms).

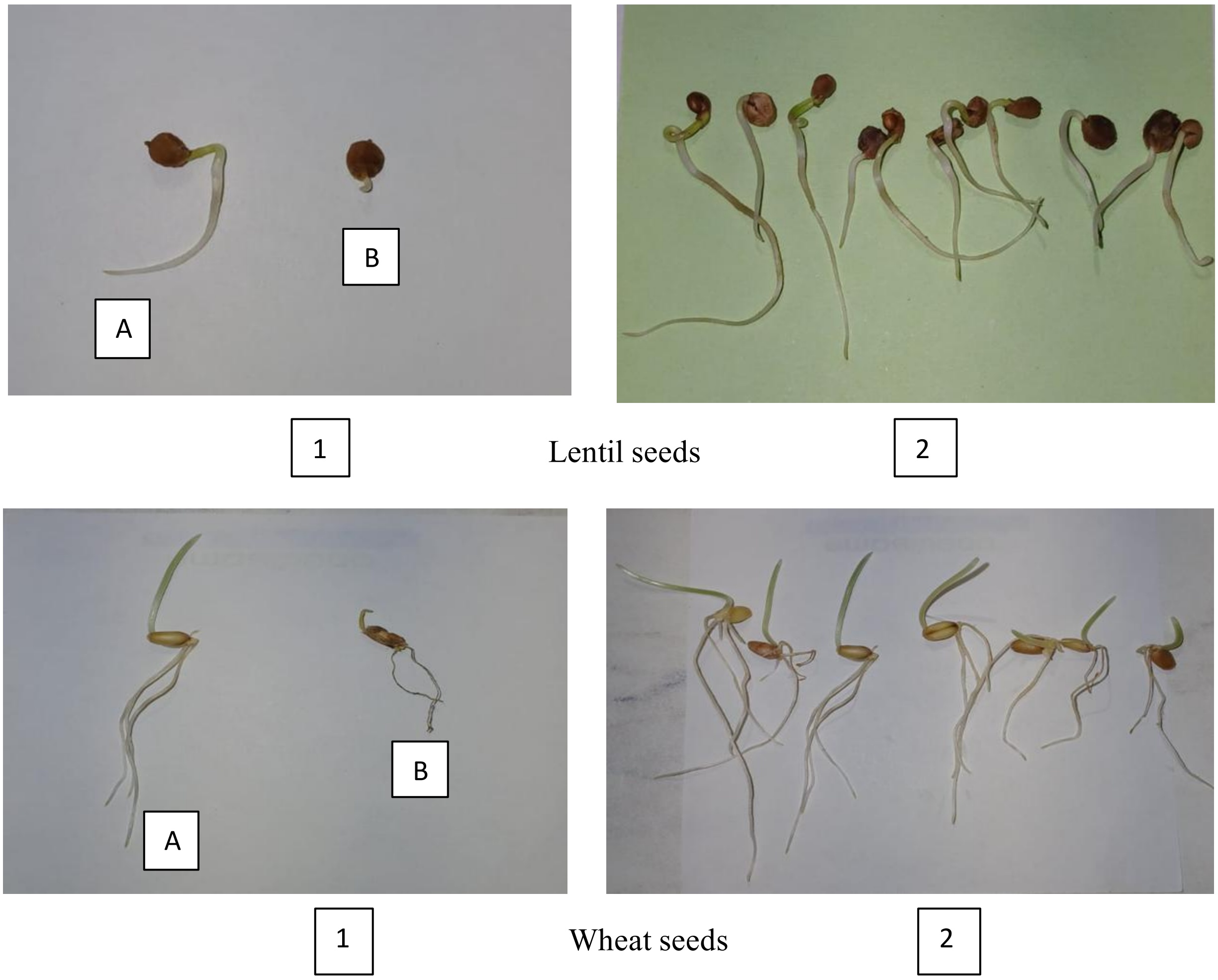

Figure 4
Biotest plants for studying the ecotoxicity of water samples
(A-control, B-dirt water, 1-before the experiment, 2-after the experiment)

The bioassay results were comparatively evaluated using seed germination and root length as indicators before and after the remediation process. The findings demonstrated improved growth and development of plant seedlings in bioremediated water samples. An increase of 80-98 % was observed in both the seed germination rate and the length of the seedlings. Seed germination and length of plant sprouts increased by 80-98%. The findings indicate that microorganisms positively affect plant growth and development by reducing the toxicity of river water, thereby making the ecosystem safer.

Conclusion

Overall, the findings of this study demonstrate the potential of bacteria and fungi, as representatives of the aquatic microbiota, to remove pollutants via biosorption and biodegradation, thereby supporting their use in bioremediation. Moreover, the ability of fungi to degrade toxic compounds through diverse enzymatic systems suggests that mycoremediation is one of the most effective approaches to environmental cleanup.

This is further supported by the fact that, in the present study, fungi belonging to the Penicillium sp. and Aspergillus sp. genera demonstrated greater remediation efficiency than the bacterial genus *Rhodococcus sp.*

The observed enhancement in the growth and development of plant seedlings indicates the high biodegradation and biosorption capacities of these microorganisms for reducing water ecotoxicity and highlights their potential as a promising tool for advancing bioremediation technologies.

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