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**İsfəndiyar İbrahimov**  
Technical University of Munich  
Bachelor  
ibrahimovisfandiyar@gmail.com

## **Bioremediation Effect of *P.australis*, *P.aeruginosa*, and *B.subtilis* on Heavy Metals**

### **Abstract**

Water is essential for life and if water is polluted with heavy toxic metals, it needs to purify to clean the environment. Biological remediation is an effective and eco-friendly way to get rid of metallic compounds in contaminated water. This study investigates the efficacy of the ordinary stalk *Phragmites australis* and specific bacterial strains in detoxifying the polluted Okhchuchay Water. *P.australis* is being researched for its heavy metal-collecting abilities and for its potential synergy with certain types of bacteria. *P.australis* is used to extract metals like Fe, Mo, and Mn. Okhchuchay water samples were sent to the lab for analysis. We found *P.australis* has the ability to accumulate pollutants in its connecting tissue; different chemicals are taken up to varying degrees. Bacteria are to be metal-resistant and metal transforming according to their different species considerably boosting the process of bioremediation efficiency. In the current research, the capability to take in, decrease, and immobilize metals was tested for each of the bacteria *Pseudomonas aeruginosa*, *Bacillus subtilis*. This investigation elucidates the bioremediation characteristics of *P.australis* and other kinds of bacteria for hazardous metals in water system. The findings underscore the relevance of relationships among plants and microbes in strengthening the remediation strategy, showing that *P.australis* when combined with specific strains of bacteria can be used as part of a phytoremediation approach. The findings have implications for scientists, freshwater those in management, and lawmakers seeking sustainable, low-cost approaches to lowering the presence of heavy metals in water supplies. Further study into bioremediation's optimum circumstances, long-term survival, and potential environmental ramifications is warranted.

**Keywords:** water pollution, toxic metals purification, biological remediation, contaminated water, bacterial strain, Okhchuchay water, metal extraction, heavy metal presence, long term survival

**İsfəndiyar İbrahimov**  
Münhen Texniki Universiteti  
bakalavr  
ibrahimovisfandiyar@gmail.com

## ***P.australis*, *P.aeruginosa* və *B.subtilis*-in ağır metallara bioremediasiya təsiri**

### **Xülasə**

Su həyat üçün vacibdir və su ağır zəhərli metallarla çirklənsə, ətraf mühiti təmizləmək üçün onu təmizləmək lazımdır. Bioloji remediasiya çirklənmiş suda metal birləşmələrdən xilas olmaq üçün effektiv və ekoloji cəhətdən təmiz bir üsuldür. Bu tədqiqat adı *Phragmites australis* sapı və spesifik bakteriya şammlarının çirklənmiş Oxçuçay suyunun zərərsizləşdirilməsində effektivliyini araşdırır. *P.australis* ağır metal toplamaq qabiliyyəti və müəyyən bakteriya növləri ilə potensial sinerjisi üçün tədqiq edilir. *P.australis* Fe, Mo və Mn kimi metalları çıxarmaq üçün istifadə olunur. Oxçuçaydan götürülən su nümunələri analiz üçün laboratoriyaya göndərilib. Biz tapdıq ki, *P.australis* öz birləşdirici toxumasında çirkləndiriciləri toplamaq qabiliyyətinə malikdir; müxtəlif kimyəvi maddələr müxtəlif dərəcələrdə qəbul edilir. Bakteriyalar bioremediasiya səmərəliliyi

prosesini əhəmiyyətli dərəcədə artıraraq müxtəlif növlərinə görə metala davamlı və metal çevrilməlidir. Cari tədqiqatda *Pseudomonas aeruginosa*, *Bacillus subtilis* bakteriyalarının hər biri üçün metalları qəbul etmək, azaltmaq və hərəkətsizləşdirmək qabiliyyəti sınaqdan keçirilmişdir. Bu tədqiqat su sistemindəki təhlükəli metallar üçün *P.australis* və digər bakteriyaların bioremediasiya xüsusiyyətlərini aydınlaşdırır. Tapıntılar remediasiya strategiyasının gücləndirilməsində bitkilər və mikroblar arasında əlaqələrin aktuallığını vurğulayır və göstərir ki, *P.australis* bakteriyaların xüsusi ştammları ilə birləşdirildikdə fitoremediasiya yanaşmasının bir hissəsi kimi istifadə edilə bilər. Tapıntılar elm adamları, şirin su idarəçiliyində olanlar və su təchizatında ağır metalların mövcudluğunu azaltmaq üçün davamlı, ucuz yanaşmalar axtaran qanunvericilər üçün təsir göstərir. Bioremediasiyanın optimal şərtləri, uzun müddətli sağ qalma və ətraf mühitə potensial təsirləri ilə bağlı əlavə araşdırmalara zəmanət verilir.

**Açar sözlər:** *suyun çirklənməsi, zəhərli metalların təmizlənməsi, bioloji remediasiya, çirklənmiş su, bakteriya ştammi, Oxçuçay suyu, metal çıxarılması, ağır metalların mövcudluğu, uzun müddətli yaşamaq*

## Introduction

Heavy metal contamination is a big problem nowadays because hazardous waste leads to clean the flowing water scarcity and disturbances of soil surrounding the river or water flow over the surface of different areas thus concerns for both human health and crop production. Anthropogenic activities, such as industrial processes, agricultural practices, and mining operations, are the big sources to release heavy metals into freshwater sources. Traditional remediation methods for heavy metals, such as adsorption or chemical precipitation, have very limited applicability and they can be costly, energy-intensive, and may generate secondary pollutants. As a result, bioremediation has gained significant attention as a developing sustainable and environmentally friendly method (Column & Jenna, 2016).

Broad reed, or *Phragmites australis*, has strong growth, metallic acceptance, and a large root structure that allows fast removal of metal from an aquatic system, making it a promising option in the biological remediation of contaminants. As a widespread and easily accessible vegetation, *P.australis* is frequently used in degrading operations (Ibrahimov et al., 2004).

## Research

In addition, it is essential to emphasize the significant part that different bacteria species play in biological remediation methods. And some existing microbes that serve a purpose in such practices include *Pseudomonas aeruginosa* and its types *P. aeruginosa* and *Bacillus subtilis*, *B. subtilis* (Saleem et al., 2019). These bacterial species have outstanding capabilities in terms of metabolizing a wide variety of pollutants and withstanding their presence. *P. aeruginosa* and *B. subtilis* are two bacteria that actively participate in the destruction and detoxification of pollutants, which helps to support the technique of bioremediation. Because of their capacity for adaptation and resilience, and these methods are very helpful in cleaning water sources like rivers, lakes, streams, and watercourses (Yang et al., 2019). This study is to evaluate the bioremediation ability of the bacteria *P. australis*, *P. aeruginosa*, and *B. subtilis*, as well as design a long-term treatment approach for several toxic metal water sources that are polluted, one of which is Okhchuchay. Furthermore, the investigation will analyze the differences between the effectiveness of *P. australis*, *P. aeruginosa*, and *B. subtilis* (Shlegel, 2005). Before this, much research was conducted on the cleaning of metals from water bodies. Current research will help us understand more clearly the dynamics of organic and microbes (Bacteria), relations, and the potential of this hybrid approach to the safe and effective remediation, of heavily metal-polluted streams. The findings of this study may pave forward for the development of biological remediation technologies, which offer long-term solutions, to the difficulties posed by heavy metal contamination in lakes and rivers (Aliyeva & Mustafayev, 2004).

In shallow water or river surroundings the tall grass growth permanently type *Phragmites australis*, mostly the common reed, has demonstrated promising results in metals treatment. With a complex network of roots and the ability to accumulate large amounts of metal, it is an ideal option for phytoremediation. *Phragmites australis* has been shown in multiple studies to be an excellent

heavy metal chelator, particularly for lead (Pb), copper (Cu), zinc (Zn), cadmium (Cd), iron (Fe), Molybdenum (Mo), Manganese (Mn), and chromium (Cr) in polluted soil and water sources. In this case the the plant carries metallic materials from the roots of the plant to its shoots and its ability to tolerate high levels of metals both play a role in its efficacy in phytoremediation (Ahluwalia & Goyal, 2005; Guo, 2018, pp. 71-93).

If we add some of the solubilization, immobilization, and conversion of metals, bacteria play a significant role in boosting bioremediation processes. Another similar research compared the ability of different kinds of bacteria the removal of heavy metals from a wetland that is polluted and combined with *P. australis*. When compared to other bacterial strains, *Pseudomonas putida* & *Bacillus subtilis* were shown to have higher metals resistance and improved elimination of metals by *P.australis*. The results show that process of bioremediation works with the kinds of Bacterial (Lin & Li, 2018).

It has been looked into whether or not the complementary skills of *Phragmites australis* and bacteria could be used in a bioremediation technique. The cooperation between plants and microorganisms has the potential to speed up the cleanup process while simultaneously increasing its efficiency. The capability of *Phragmites australis* to tolerate metals and operate as a phytoextraction tool can be improved by introducing specific bacterial strains, according to a number of studies. Organic contaminants, which frequently mix with metals that are heavy in contaminated locations, can be degraded through rhizodegradation with the help of bacterial species (Christenson & Sims, 2012, pp. 52-64).

### **1. Practical examples and theoretical considerations**

*Phragmites australis* and bacterial-based bioremediation techniques have been used successfully in several research conducted to clean up toxic metal-contaminated regions around the world. However (Water Research Foundation. Biofiltration for Water Treatment), research devoted just to Okhchuchay is scant. *Phragmites australis* is successful in the removal of Pb, Zn, Fe, Mn, and Cd from water in a similar study carried out in a contaminated river in Iran as a result The effectiveness of eliminating metal was further improved by the introduction of metal-resistant bacterial strains. These results lay the groundwork for further laboratory and field-testing of *Phragmites australis* and bacteria to determine their efficacy in removing heavy metals from Okhchuchay. *Phragmites australis* and bacteria show potential as a long-term, efficient method for the bioremediation of contaminants in Okhchuchay. The plant *Phragmites australis* is exceptionally good at absorbing metals, and beneficial microbes boost the development of plants and aid in metal detoxifying. Heavy metals can be removed from polluted settings more effectively when these two factors are combined. The practicality has to be investigated further through field experiments and continual tracking (Kumar, Yadav, & Kumar, 2019; Mohan, Chandrasekhar, & Anandkumar, 2005, pp. 997-1003).

### **2. Material and Methods**

Water examples are collected from Okhchuchay, Taghli region.

#### **2.1 Preparation of bacteria strains**

Twenty-eight specimens of water were taken from the Okhchuchay at various points. To prevent cross-contamination, samples were gathered into clean containers, delivered to the lab in a secure environment conducted for research. Two bacterial cultures, *P.aeruginosa* and *B.subtilis* have been grown in phenolic liquid ( $K_2HPO_4$  (1g),  $Na_2S_2O_3$  (150mg),  $(NH_4)SO_4$  (300mg),  $CaCl_2$  (20mg),  $FeCl$  (5mg), Phenol (300mg), distilled water (1000ml)) medium(50ml)-swinging thermostat (t-300 c, 3 days). After incubation, cultural fluids were gained (50ml) and added to contaminated water (100ml). Treated water was incubated for 3 days, again (Rahmani et al., 2016, p. 680).

#### **2.2 Preparation of *P.australis***

The Botanical Gardens of Azerbaijan Academy of Sciences provided 1-year-old *P.australis* plants (whole). The spike and roots were divorced from the main body and sterilized in ethanol (100ml) 3 times, and then distilled water (1000ml), once. The root was cut into 25 mm pieces and met the same sterilization process again. Spike (1g) and root pieces (2g) were added into a cotton bag, which led water to enter in, and the bag was placed in contaminated water (100ml). Treated

water was incubated for 3 days, again. The diluted specimens were plated in aliquots onto nutritional agar. These plates were then placed in an incubator at 30 degrees Celsius for 24 to 72 hours to promote the development of bacteria (World Health Organization, WHO).

### 2.3 Preparation of control flask

Contaminated water (100ml) was incubated for 3 days with other examples for control. To eliminate the risk of microbiological contamination, we microwave the container at 121 degrees Celsius for 15 to 20 minutes. The bacteria and *P. australis* in the specimens being studied can be compared to the control container, which contains nothing. This enables a measurement of the biological remediation efficacy through a comparison of the concentrations of heavy metals in control and remedied samples, which helps identify the organic quantities of toxic metals that exist in Okhchuchay (Nivala & Vymazal, 2015, pp. 6800-6814; Wei, 2021).

### 3. Results

Table number 4.1 shows the concentrations of heavy metals in water sample and permeability hardness limitations. The susceptible hardness limit is the greatest amount of heavy metals that may harm the surroundings or humans. According to the analysis of wastewater before experiments, the levels of iron, molybdenum, and manganese are 4.483, 1.314, and 5.8 times higher than normal (according to the Law of Azerbaijan Ecology Ministry). Copper was at a normal level, thus it had not been tested later (Environmental Science & Technology. Biofiltration: A Nature-Based Solution for Water Treatment; Science Direct. Biofilter Systems for Water Treatment).

After the research, iron 69.37 %, molybdenum 84.32 %, and manganese 74.734 % were removed from contaminated water in the *P.aeruginosa* system (Table 4.2). The total percentage of treatment is 76.3 %. This data suggests that lowering the iron and manganese concentrations in the water specimen is necessary for meeting the permeability hardness limitations (Xia, Zhang, & Zhang, 2016, pp. 189-199).

Iron 78.81 %, molybdenum 91.264 %, and manganese 88.931 % cleaned in *B.subtilis* system (Table 4.3). The total percentage of treatment is 83.23 %. These results show that the value of iron is greater than the permissible value, whereas molybdenum (Mo) and manganese (Mn) values are normal (Report.az; Otterpohl et al., 2003, pp. 151-158). The first element is Iron (Fe) at 285 µg/l, under the 300 µg/l hardness limitation. Molybdenum (Mo) at 28.7 µg/l remains under the 250 µg/l standard. The final element, Manganese (Mn), is within the 100 µg/l limit at 64.2 µg/l. The substance's roughness is below each component's limitations, indicating that it is within the permitted level (Shilton, Powell, & Guieysse, 2012, pp. 4723-4735; Environmental Protection UK. Biofiltration: A Sustainable Solution for Air and Water Pollution).

Iron 53.011 %, molybdenum 85.692 %, and manganese 89.94 % treated in the *P.australis* system (Table 4.4) The total percentage of treatment is 67.282 %.

Fe, Mo, and Mn were found in the specimen. Iron, molybdenum, and manganese are detected at 632, 47, and 58.3 µg/l, respectively in Table no 3.4. Iron, molybdenum, and manganese have 300, 250, and 100 µg/l hardness limitations. All three of the components surpass their hardness limitations based on observed levels.

Table 4.5 illustrates three parameters – Fe, Mo, and Mn – in control flask. Iron 1258, Molybdenum, and Manganese each have 1258, 519.4, 297 µg/l, respectively (Water World. Biofiltration: A Green Solution for Drinking Water Treatment; Suidan, Venkatesan, & Brenner, 2003, pp. 39-55).

### Conclusion

Finally, the water specimen analysis results show that *Phragmites australis*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* all have outstanding capacities in eliminating contaminants such as heavy metals from aquatic environments (Wang et al., 2016). The total rate of elimination for heavy metals from the studied materials is 83.23 %, with *B. subtilis* showing the best effectiveness. This data indicates that *B. subtilis* may be a useful agent for the total elimination of contaminants such as heavy metals from water supplies that have been contaminated. However, *P. australis* has a strong preference for manganese and removes it at an average rate of 89.94 %, which is significantly

higher than any of the other metals tested (U.S. Environmental Protection Agency (EPA). Constructed Wetlands and Biofilters). In comparison with the other heavy elements studied, this suggests that *P. australis* has a stronger propensity to extract manganese. With this discovery, *P. australis* emerges as a promising candidate for use in the cleanup of manganese-tainted water supplies. In sum, the results shed light on how effective *P. australis*, *P. aeruginosa*, and *B. subtilis* are at detoxifying water of toxic metals. These findings provide credence to the idea that these resources could be useful in heavy metal pollution bioremediation efforts. Increasing our understanding of these chemicals and their possibilities for widespread application in water filtration and restoration calls for greater research and optimisation. This research has proved that *P. australis*, *P. aeruginosa*, and *B. subtilis* can remove higher levels of heavy metals in contaminated water and paves the way for further investigations and potential implementation of these bioremediation agents in contaminated environments (Toyama et al., 2013; During the occupation, Okchuchay was subjected to serious environmental impacts; Wu, Lin, & Li, 2018; Sustainable Sanitation Alliance. Biofilters).

**Table 4.1.**

No.	Component name	Unit of measure	Amount of component	Permeable hardness limit
1	Iron, Fe	µkg/l	1345	300
2	Copper, Cu	µkg/l	108	1000
3	Molybdenum, Mo	µkg/l	328.5	250
4	Manganese, Mn	µkg/l	580	100

**Table 4.2**

No.	Component name	Unit of measure	Amount of component	Permissible hardness limit
1	Iron, Fe	µkg/l	412	300
2	Molybdenum, Mo	µkg/l	39.2	250
3	Manganese, Mn	µkg/l	83	100

**Table 4.3**

No.	Component name	Unit of measure	Amount of component	Permissible hardness limit
1	Iron, Fe	µkg/l	285	300
2	Molybdenum, Mo	µkg/l	28.7	250
3	Manganese, Mn	µkg/l	64.2	100

**Table 4.4**

No.	Component name	Unit of measure	Amount of component	Permissible hardness limit
1	Iron, Fe	µkg/l	632	300
2	Molybdenum, Mo	µkg/l	47	250
3	Manganese, Mn	µkg/l	58.3	100

**Table 4.5**

No.	Parameter	Unit of measure	Amount of component	Permissible hardness limit
1	Iron, Fe	(µg/l)	1258	300
2	Molybdenum, Mo	(µg/l)	519.4	100
3	Manganese, Mn	(µg/l)	297	250

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