

Parameters Regulating Microorganism Properties and Influencing Soil Remediation Potential

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Abstract – Scientists state that in recent years, the level of soil contamination worldwide has significantly increased, due to atmospheric deposits, floods, agricultural, industrial activities, and mining. Soil contamination is a widespread issue that negatively impacts the functions and processes of soil microorganisms. Heavy metals are significant factors of soil pollution, posing a threat to ecosystem functions due to their toxicity, persistence, and ability to bioaccumulate. The soil hosts diverse communities of microorganisms with specific metabolic capacities. Some microorganisms contribute to the decomposition of organic matter by interacting with toxic metals, while others participate in the formation of natural nanoparticles, thereby reducing the toxicity of heavy metals. Microorganisms can be used to immobilize heavy metals, but their effectiveness depends on various parameters such as soil type, chemical composition, pH, temperature, and moisture. Although microorganisms can be used in bioremediation, their activity is influenced by soil parameters. Understanding microorganisms' properties and operational parameters is essential for their successful application in soil bioremediation processes. To support further experimental studies, the Visual MINTEQ computer program was applied as a modelling tool to evaluate the behaviour and distribution of cadmium, lead, and copper in soil under different environmental conditions such as pH and temperature. These simulations do not replace microbial research but provide essential baseline information, helping to identify potentially hazardous soil conditions and to design more effective experiments on microorganisms in bioremediation could become one of the main solutions for combating soil contamination and achieving environmental sustainability.

Keywords – Bioremediation; heavy metal; immobilization; microorganisms; soil pollution.

1. INTRODUCTION

Soil contamination poses a risk to water quality and food safety, as heavy metals can contaminate crops and enter the human body through the food chain [1]. The toxic effects of metals on living organisms occur even at low concentrations, necessitating the removal of these metals from the environment [2]. To remediate and restore soil quality while reducing the bioavailability of heavy metals to plants and humans, it is crucial to implement environmentally friendly technologies [3], [4].

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Recent studies published in *Environmental and Climate Technologies* highlight eco-friendly biotechnological and modelling approaches for environmental remediation. Recent studies have assessed the life-cycle impacts of microalgae-derived pigments, highlighting microbial applications in sustainable technologies [5]. Other research has reviewed technological strategies in climate change mitigation, which are relevant to environmental modelling and remediation [6].

The use of microorganisms for heavy metal removal is a promising approach to mitigating soil contamination caused by human activities [7], [8]. Biological remediation methods can be effective and yield long-term results by utilizing living systems that adapt to various soil conditions [9]. These biological approaches can be applied across different geographical regions, contributing to international efforts to address this pollution issue [10].

Soil immobilization is an effective large-scale method for restoring heavy metal-contaminated soil [11], [12]. Immobilization via ion exchange reactions alters the adsorption, complexation, and precipitation of heavy metals, transforming them from active to stable phases and reducing their bioavailability in soil [13], [14]. The efficiency of microorganisms in this process depends on soil type, chemical composition, and environmental conditions, with factors such as pH, temperature, and moisture influencing their activity. Different microorganisms exhibit varying effectiveness in immobilizing specific heavy metals [15].

In this research, three heavy metals were chosen because of their toxicity. Cadmium is easily absorbed by plant roots, particularly in acidic soils, and accumulates in organisms because it undergoes minimal biotransformation [16]–[17]. Pb is highly toxic to living organisms, causing neurological disorders, anaemia, and long-term accumulation in tissues. In soils, Pb mobility is strongly dependent on pH, with higher mobility in acidic conditions and reduced availability in neutral to alkaline conditions [18]–[19]. Copper is an essential micronutrient for plants and microorganisms, but at elevated concentrations it becomes toxic, reducing crop growth and disturbing metabolism. It tends to accumulate in upper soil layers and binds strongly to soil particles, which limits mobility but not necessarily biological availability [20], [21]. These toxicological characteristics highlight the need for effective and environmentally friendly remediation methods. The use of microorganisms has emerged as a promising approach for reducing heavy metal mobility and bioavailability [7], [14]. To support further microbial studies, computational modelling (Visual MINTEQ) was used in the research to evaluate the behaviour of Cd, Pb, and Cu under varying soil conditions [22]. The model provides baseline information for identifying potentially hazardous soil conditions and designing microbial immobilization strategies.

2. POTENTIAL APPLICATIONS OF MICROORGANISMS

The use of microorganisms for heavy metal removal is a promising approach to mitigating soil contamination caused by human activities [7], [8]. Biological remediation methods can be effective and yield long-term results by utilizing living systems that adapt to various soil conditions [9]. These biological approaches can be applied across different geographical regions, contributing to international efforts to address this pollution issue [10].

Technological advancements have promoted bioremediation as a viable alternative to conventional methods. Certain microorganisms have developed effective detoxification mechanisms to neutralize toxic metal effects [23]. In addition to immobilizing heavy metals in soil, microorganisms can promote crop growth and improve soil and vegetable quality [24]. However, some soil microorganisms are sensitive to heavy metal toxicity, which can suppress or eliminate them [25].

Microbial cell surfaces exhibit properties that prevent metal ions from entering cells, adsorbing them onto the surface and acting as barriers. Isolates of fungi and bacteria can effectively biosorb heavy metals such as copper, lead, and chromium [26]. Microbial

biofilms, composed of extracellular polymers, can accumulate metal ions and protect the cells within from toxic effects. Enzymes also contribute to microbial metal resistance by biologically transforming or chemically modifying metal ions into less toxic forms [27].

Microbial cell membranes contain various proteins and metabolic products capable of forming complex structures with metal ions. Extracellular sequestration is a process in which organisms (fungi and bacteria) remove heavy metals from their cells by binding them to external components. The ability of bacterial cells to accumulate metals is utilized in waste treatment processes [28].

Microorganisms play a crucial role in essential biogeochemical cycles, transforming metals between soluble and insoluble forms. These processes are significant due to the natural abundance of microorganisms in the environment and their ability to efficiently participate in these cycles [29]. Environmental parameters such as temperature, pH, and nutrient availability are critical for microbial activity. Temperature affects microbial metabolism and viability: low temperatures slow microbial growth, optimal temperatures enhance growth and metabolism, and excessively high temperatures can damage cells and denature enzymes. Bioremediation is typically conducted at temperatures between 20 °C and 30 °C [22].

Cadmium, lead, and copper exhibit different solubility and mobility depending on soil conditions, which directly influences the efficiency of microbial immobilization. Therefore, understanding the environmental behaviour of these metals is crucial for designing microbial-based remediation strategies. Nevertheless, the application of microorganisms in heavy metal immobilization has certain drawbacks. Their efficiency can be reduced by extreme environmental conditions such as very low or high pH, temperature fluctuations, or nutrient limitations [30], [31]. In addition, competition with native microorganisms communities may suppress the introduced strains [24]. Another major limitation is the difficulty in scaling up laboratory findings to field conditions, which often raises challenges related to stability, reproducibility, and regulatory acceptance. Finally, high concentrations of toxic metals themselves may inhibit or even eliminate sensitive microorganisms populations [23].

3. METHODOLOGY

Computer modelling programs can be used to establish the relationship between soil physical and chemical properties and heavy metals. Scientific studies often utilize the Visual MINTEQ program [32]–[35], which considers various heavy metal reactions and interactions with soil components. To enhance understanding, these processes must be modelled. To determine the chemical speciation of cadmium, copper, and lead was used the Visual MINTEQ program. The Visual MINTEQ database contains equilibrium constants for different complex-forming ligands, allowing for the determination of heavy metal speciation. This model requires specific soil properties as input variables to assess heavy metal speciation. Working with the Visual MINTEQ program requires soil pH, temperature, and heavy metal concentration as input parameters (Table 1).

TABLE 1. PARAMETERS USED IN THE VISUAL MINTEQ PROGRAM

Parameters	Mean
Cu ²⁺	128.8 mg/L
Pb ²⁺	143.6 mg/L
Cd ²⁺	3.65 mg/L
Cu ²⁺ , Pb ²⁺ , Cd ²⁺	127.7 mg/L; 121.4 mg/L; 3.4 mg/L
Temperature	from 0 °C to +35 °C
pH	from 4 to 8

The temperature and pH were selected differently to determine whether there are variations in heavy metal activity. The temperature was chosen starting from 0 °C because the computational program does not model data at negative temperatures. Additionally, at subzero temperatures, microorganisms capable of immobilizing heavy metals are not active.

The heavy metal data were obtained from a study in which uncontaminated soil was artificially contaminated with cadmium, copper, and lead. The contamination level exceeded the permissible limit in Lithuania by approximately 1.5 times. Four soil samples were contaminated: three with a single heavy metal each, and one with all three heavy metals. The contaminated soil was left to dry naturally for two weeks.

The heavy metal contamination in the soil samples was measured using an XRF device (model: *Olympus Vanta* C-series). The obtained heavy metal concentrations, initially expressed in mg/kg, were converted to mg/L. For this conversion, the bulk density of the soil, which was 1.14 g/cm³, was required. The experimentally obtained concentrations (mg/L) were converted to millimoles per litre (mmol/L) using the molar mass of each element, according to the formula ($\text{mmol/L} = (\text{mg/L} \div \text{molar mass [g/mol]})$). The measurements followed ISO 13196:2013 standard (or its modifications) for XRF analysis of soils. The soil type used was sandy loam (ISO 14688-1).

The data collected were processed using Microsoft Excel 2019.

4. RESEARCH RESULTS

The study determined that both pH and temperature influence changes in the concentrations of heavy metals in all soil samples contaminated with cadmium, copper, and lead.

According to the data presented in Fig. 1, copper concentration was low (1.058–2.553 mmol/L) at high soil temperatures (+30 °C, +35 °C). At pH 8, the lowest concentration was observed at +35 °C (1.058 mmol/L). At 0 °C, the highest concentration occurs at pH 7 (9.980 mmol/L) (Fig. 1).

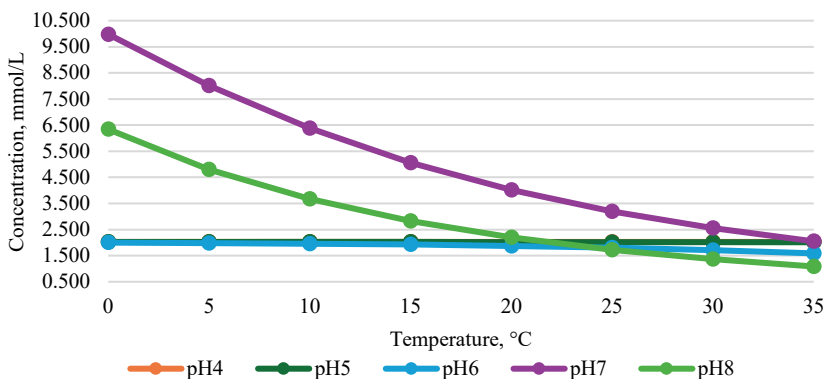


Fig. 1. Copper concentration depending on pH and temperature, °C.

According to Fig. 2, lead concentration was lowest at high soil temperatures (+30 °C, +35 °C) at pH 7 and pH 8. At 0 °C, the highest concentration occurs at pH 4 (6.931 mmol/L) and pH 5 (6.930 mmol/L). Lead concentration remains nearly identical at pH 4 and pH 5 (Fig. 2).

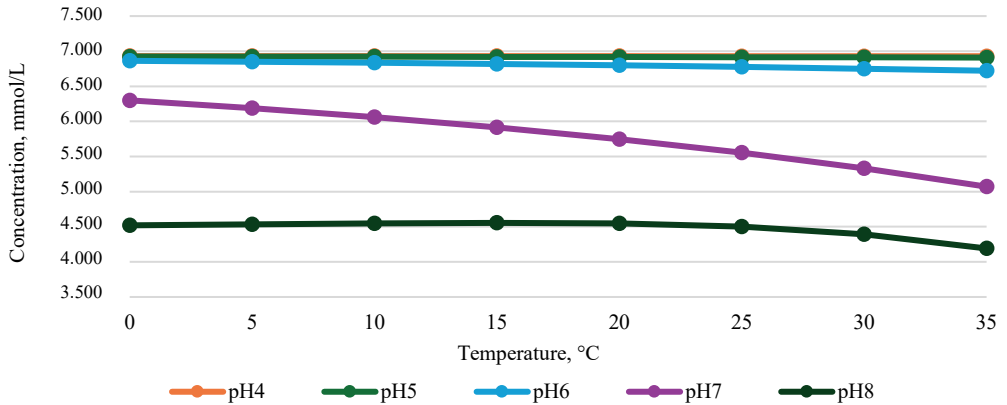


Fig. 2. Lead concentration depending on pH and temperature °C.

According to Fig. 3, cadmium concentration was lowest at the highest soil temperature (+35 °C) at pH 8 (3.196 mmol/L). From 0 °C to +35 °C, this heavy metal's concentration remains within the pH 4 to pH 6 range (2.247 mmol/L) (Fig. 3).

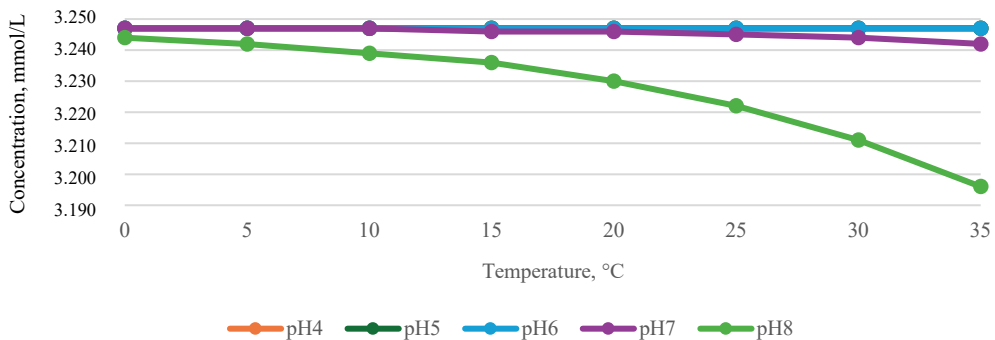


Fig. 3. Cadmium concentration depending on pH and temperature, °C.

Fig. 4 illustrates the lowest copper, cadmium, and lead concentration and activity at pH 8. Copper concentration was directly proportional to its activity. The activity of this heavy metal decreases with the increasing soil temperature. Cadmium and lead concentration were directly proportional to their activity. As shown in Fig. 4, the activity of this heavy metal decreases as soil temperature increases, with the most significant reduction occurring at +35 °C.

Fig. 5 illustrates cadmium, copper, and lead concentrations and activity at 0 °C. Copper and lead concentrations decrease at pH 7, while cadmium concentration remains stable. Copper concentration was highest at pH 8, whereas lead concentration was highest at pH 4 (Fig. 5).

Since the results at +5 °C, +10 °C, and +15 °C showed no significant differences in cadmium, lead, and copper concentration, only the data at +0 °C was presented in the Fig. 5 for clarity.

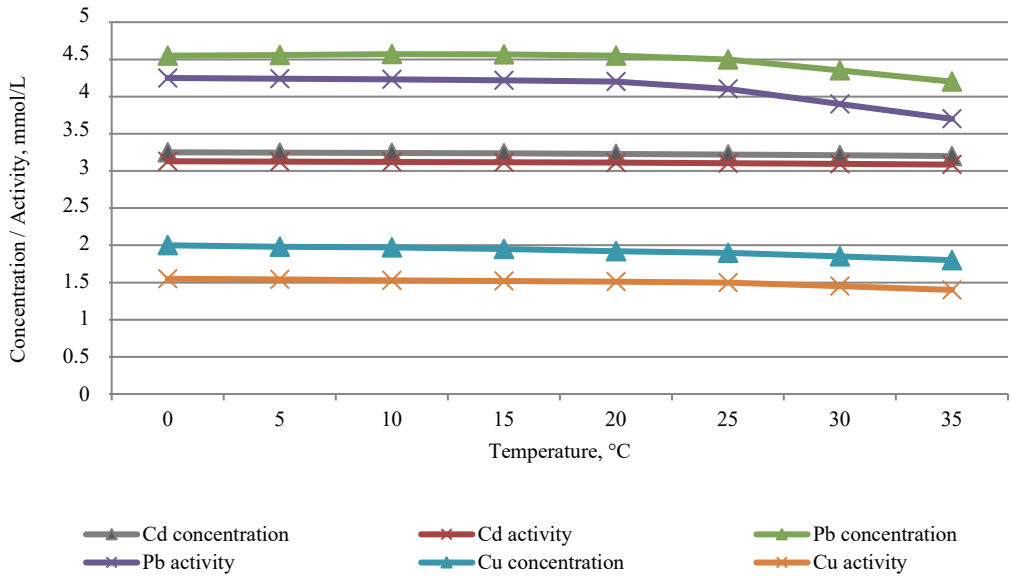


Fig. 4. Effect of temperature on concentration and activity of lead, cadmium, and copper at pH 8.

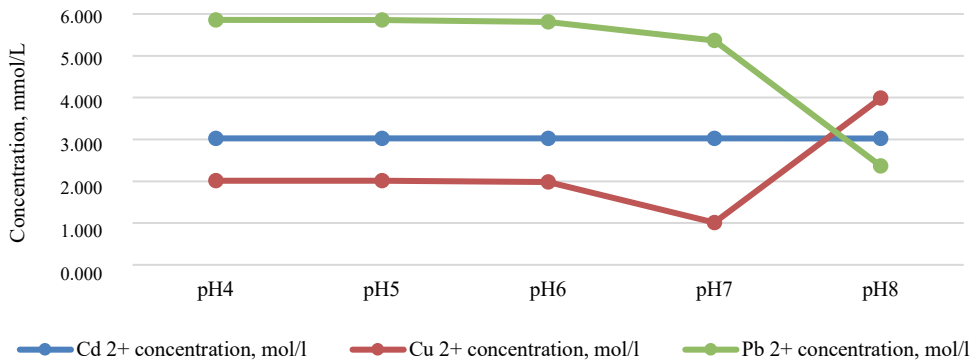


Fig. 5. Cadmium, copper, lead concentration depending on pH at 0 °C.

Fig. 6 shows cadmium, copper, and lead concentrations and activity at +15 °C. Lead concentration decreases at pH 7, cadmium concentration slightly decreases at pH 8, copper concentration was highest at pH 7, and lead concentration was highest at pH 4, as seen in Fig. 6.

Fig. 7 presents cadmium, copper, and lead concentrations and activity at +20 °C. Lead concentration decreases at pH 7 and is lowest at pH 8. Cadmium concentration slightly decreases at pH 8. Copper concentration was highest at pH 7, and lead concentration was highest at pH 4, as seen in Fig. 7.

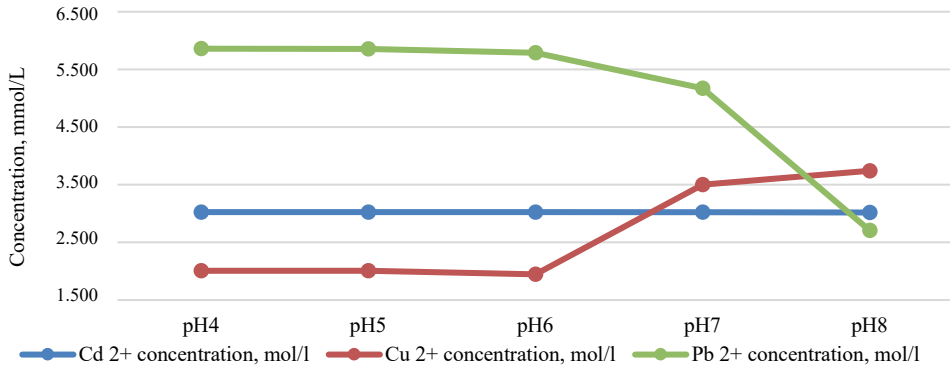


Fig. 6. Cadmium, copper, lead concentration depending on pH at +15 °C.

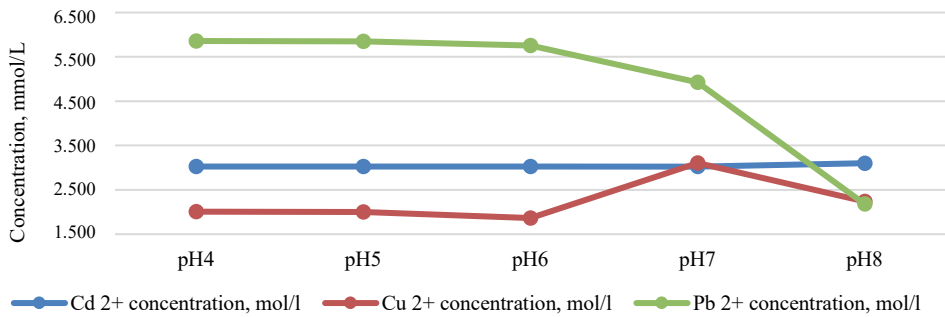


Fig. 7. Cadmium, copper, lead concentration depending on pH at +20 °C.

Fig. 8 presents cadmium, copper, and lead concentrations and activity at +30 °C. Lead concentration was lowest at pH 7 and highest at pH 8. Cadmium concentration slightly decreases at pH 8. Copper concentration was highest at pH 7 and lowest at pH 8 (Fig. 8).

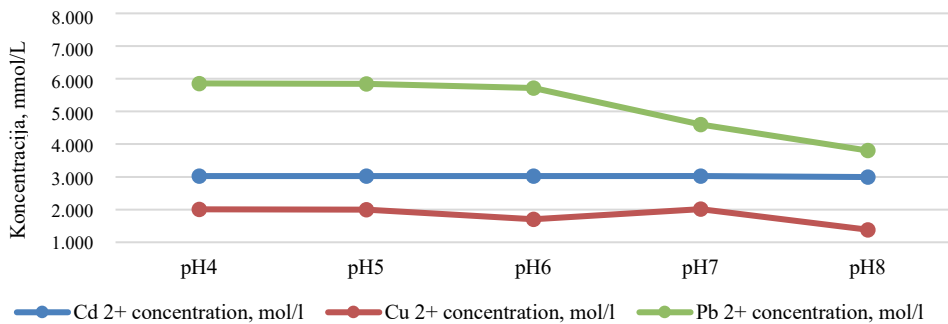


Fig. 8. Cadmium, copper, lead concentration depending on pH at +30 °C.

Since the results at +25 °C, and +35 °C showed no significant differences in cadmium, lead, and copper concentration, only the data at +35 °C was presented in the figure for clarity. Fig. 9 illustrates cadmium, copper, and lead concentrations and activity at +35 °C.

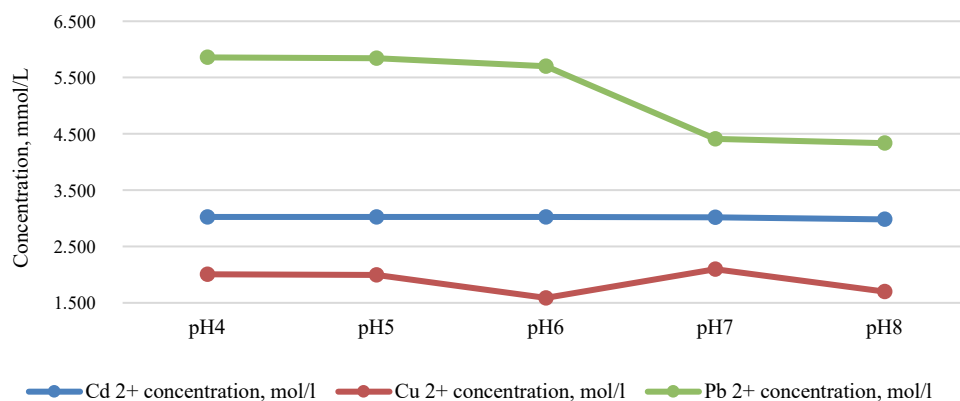


Fig. 9. Cadmium, copper, lead concentration depending on pH at +35 °C.

The concentration of lead was the lowest at pH 7 and the highest at pH 8. The concentration of cadmium decreases slightly at pH 7. The concentration of copper was the highest at pH 7 and the lowest at pH 8 (Fig. 9).

5. DISCUSSION

In our study, metal activity followed concentration trends but remained slightly lower due to ionic interactions in solution, which is consistent with the findings of Karmakar et al. (2024) [36]. Previous research also confirms that pH influences the activity of heavy metals [37], [38], which agrees with our modelling results.

Our results showed that cadmium maintained high mobility under acidic conditions, while its availability decreased in alkaline soils. This behaviour was explained by its binding with carbonates and adsorption onto soil organic matter in neutral to alkaline conditions. In contrast, organic acids increase cadmium mobility by forming soluble complexes. Literature confirms that cadmium was biologically active in both terrestrial and aquatic environments [17], and cadmium accumulation in plant tissues significantly alters plant biochemical and physiological processes [16]. These findings highlight why cadmium is one of the most toxic metals in soil ecosystems.

Lead was more mobile in acidic soils, whereas at neutral to alkaline pH (7–8). Its activity significantly decreased, especially at elevated temperatures. This is consistent with the tendency of lead to precipitate as hydroxide and carbonate complexes in alkaline conditions, reducing its solubility and biological availability. Lead contamination disrupts microbial activity and crop productivity [18]. Our study supports these observations, showing that Pb mobility is strongly reduced at neutral to alkaline pH. Microorganisms contribute to Pb immobilization by forming insoluble compounds, thereby reducing mobility and bioavailability [39].

Copper concentrations decreased with increasing pH and temperature, with the lowest activity observed at pH 8 and 35 °C. Copper is less mobile in soils due to its strong binding to particles, but it remains biologically available. Excess Cu negatively affects plant growth and metabolism [20], [40]. Organic matter reduces Cu availability, while organic acids such as humic acids enhance its solubility. Microorganisms influence copper dynamics through biosorption and redox reactions (e.g., Cu^{2+} reduction to Cu^+), thereby reducing toxicity [17].

Our findings confirm that copper mobility is highly dependent on pH, temperature, and soil organic composition.

These modelling results are particularly relevant for planning microbial immobilization studies. Neutral to alkaline pH and moderate temperatures are favourable not only for reducing Cd, Pb, and Cu mobility but also for microorganisms' growth and activity. For example, *Bacillus* sp., and other soil bacteria are most effective under such conditions, immobilizing heavy metals through biosorption, bioprecipitation, and redox transformations. Therefore, the Visual MINTEQ model provides valuable baseline information for designing experiments that integrate microbial processes into soil remediation strategies. For further research, microorganisms will be cultivated in soil contaminated with these heavy metals, at a temperature of +30 °C and pH not exceeding 7.

6. CONCLUSION

The Visual MINTEQ modelling results confirmed that soil pH and temperature significantly affect the behaviour of cadmium, lead, and copper. Cadmium remained highly mobile under acidic conditions, while its availability decreased in alkaline soils. Lead was more mobile in acidic soils, whereas its activity markedly decreased at neutral to alkaline pH, particularly at higher temperatures. Copper concentrations decreased with increasing pH and temperature, with the lowest activity observed at pH 8 and +35 °C.

These findings provide baseline information for planning further microbial immobilization experiments, indicating that neutral to alkaline pH and moderate temperatures are the most favourable conditions for reducing heavy metal mobility.

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