Mg and Cd biosorption by native bacteria form Djebel Onk mine (Algeria)

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Abstract

Mining and processing of phosphate ore are one of the most important branches of the economy in developing countries, including Algeria. However, this activity can negatively influence the environment, as huge amounts of waste, which contains dangerous metals, are released during ore processing. Therefore, in line with environmental needs, conventional methods of ore beneficiation should be gradually replaced with safe, biotechnological methods that involve (among others) the biosorption phenomenon. This work aimed at the investigation of biosorption abilities of native microorganisms, isolated from Djebel Onk ore (Kef Essnoun region, Algeria). Examined bacterial strains differed in their efficiency of metal accumulation. In the vast majority of native bacteria, the content of Cd or Mg found was higher than in a reference *B. subtilis* strain. Incubation of phosphate ore with selected bacterial strains (for 20 minutes and at different pH) significantly increased the recovery of Mg and Cd. Thus, we showed that biosorption could be much more effective for native bacteria isolated from the specific substrate. We have shown that *Bacillus* sp. HK4 strain can be used to remove various metals over a wide pH range, and it can be considered in the development of eco-friendly measures to clean ore and post-flotation waste.

1. Introduction

Mining, including extraction and processing of phosphate ores, is one of the most important branches of the economy in developing countries, including Algeria. Although it is associated with the production of a considerable amount of waste dangerous for humans and the environment (due to the accumulation of pollutants, which are released during processing), they can be potentially reused (as long as more efficient and cheaper methods will be developed). One of the challenges of the modern mining industry is to develop the technology of ore processing and waste reuse, which will be environmentally friendly and based on natural processes.

A burning issue of modern civilization is the production of a large amount of waste, which contains metals, including cadmium. Burning fossil fuels, industry, agriculture, as well as mining and ore processing contribute to increasing pollution of the environment with metals. Considering that they are highly persistent in the environment, metals can be accumulated in water and soil ecosystems, posing a potential threat to any organisms[1]. According to EU policy, cadmium content in soil, phosphate fertilizers, and raw material had to be limited[2]. Consequently, countries where phosphorene deposits are rich in cadmium and other metal impurities have to gradually limit sales, as demand for their raw material will systematically decrease. Such consequences may impact Nauru, Senegal, USA (North Carolina deposits), Tunisia, Morocco, Israel, Egypt, Syria, and Algeria. According to Mar and Okazaki[3]. Cadmium content in phosphate ores from these countries can reach up to 243 mg Cd per kg of phosphate rocks. A reasonable and profitable solution for countries possessing raw material rich in Cd is further ore beneficiation, including decadmiation. In line with environmental needs, conventional ore beneficiation methods should be gradually replaced with safe, biotechnological methods, which involve microorganisms, such as

bioflotation, bioleaching of metals, or others involving the biosorption phenomenon[4–7]. Microorganisms might also be successfully used for treating post-flotation wastes, all wastewater after ore processing, and for metal recovery after appropriate biomass processing. Therefore, the need arises to select and characterize bacterial strains of high biosorption capabilities.

Biosorption is the ability of biomolecules or biomass to bind certain ions, removing them from aqueous solutions. Biosorption has to be distinguished from bioaccumulation, which involves active transport through cell membranes and the storage of pollutants inside cells[1,8,9]. Biosorption is currently a fundamental phenomenon and is studied intensively due to its vast potential in the purification of water, sewage, industrial waste, and by-products of ore processing. Its advantages include a relatively low cost, high efficiency, as well as possibility to recover and reuse metals[1]. The biosorption process involves chemical and physical mechanisms which act separately or (more often) simultaneously. Physical processes include mainly Van der Waals' attraction forces, while chemical processes include creating stronger chemical bonds between the functional groups of a biosorbent and the adsorbed substance. In general, the processes of coordination complex formation, chelation, and ion exchange have to be considered when describing the mechanism of biosorption[1,10]. Due to a very beneficial surface to mass ratio and the presence of various functional groups on the surface of cells, microorganisms seem to be perfect biosorbents. Therefore, it is not surprising that they have caught the attention of scientists working on ecological methods of environmental remediation. Due to the different structures of their cell wall, bacteria are characterized as Gram-positive and Gram-negative. Gram-positive bacteria have a thick layer of peptidoglycan with teichoic acids taking on a chelating role. Gram-negative bacteria have a thinner layer of peptidoglycan and an outer membrane rich in lipopolysaccharides[11]. The composition of these layers can be vastly different in various species, making them exhibit different biosorption properties. To date, many species of bacteria have been studied for their biosorption capabilities. Among the commonly studied species with a high application potential, some of the most prospective are Gram-positive bacteria from the genus *Bacillus*. Species such as *B. subtilis*, *B. cereus*,

B. megaterium, *B. thuringiensis*, *B. circulans, B. licheniformis*, *B. safensis*, and others have been described as capable of efficient biosorption of Zn, Au, Pb, Cd, and other metals. Most of these species, which exhibit outstanding biosorption capabilities, were isolated from specific, highly contaminated environments. Long-term exposure to strong stress factors stimulates those bacteria to develop and reinforce their unique properties, which enable them to bind substantial amounts of metals[1,12–24].

This study is a continuation of our earlier work, where we analyzed the metal biosorption abilities of five bacterial species from the collection of the University of Silesia in Katowice after incubation with phosphate ore from Djebel Onk[15]. These organisms did not originate from heavy metal contaminated sites and did not exhibit any exceptional capabilities of biosorption. Therefore, in this study, we have focused on native species isolated from phosphate ores in Djebel Onk. We isolated over 160 species of microorganisms and selected four bacterial strains of different morphology to further test their biosorption-related properties. The main aim of our study was to look for heavy metal-resistant species

capable of efficient biosorption of Cd and Mg. Moreover, we assessed the relationship between the pH of the solution during incubation and the scale of microbial biosorption. We have noted significant differences in biosorption capabilities, which depended on the strain and pH of the solution. The two most promising bacterial strains belonging to the genus *Bacillus* were selected.

2. Results

2.1. Cadmium biosorption by native bacteria

Cadmium biosorption differed between the experimental groups, and both the type of microorganism and the pH during incubation influenced this differentiation (Fig. 1, Table 1). *Pseudarthrobacter* sp. HK2, *Bacillus mycoides* HK3, and *Bacillus* sp. HK4 efficiently accumulated cadmium at pH 7 and when the pH of the incubation solution was lowered to 4. The average concentration of Cd in biomass of these strains after incubation at pH 4 was, respectively: 43.83, 60.84, and 85.01 μg g^{-1}. An increase of pH to 10 created the least beneficial biosorption conditions for the studied bacterial strains. High Cd accumulation was achieved in these conditions by two *Bacillus* strains, *B. mycoides* HK3 and *Bacillus sp*. HK4. Average Cd accumulation by these strains at pH 10 reached 38.11 and 84.99 μg g^{-1}, respectively. Statistical analysis revealed that the ability of Cd accumulation by *Bacillus sp*. HK4 and the reference strain *Bacillus subtilis* USK1 was independent of pH; however, a high difference in Cd accumulation between these strains was reported. Cd concentration in the biomass of *B. subtilis* USK1 was higher than in the control group (bacteria incubated without ore), but average values never exceeded 20 μg Cd g^{-1}. The same trend was observed for *Bacillus* sp. HK4, but the average Cd accumulation was above 80 μg g^{-1}, indicating this strain as a very good biosorbent of Cd, active in a wide range of pH. Post-hoc analysis showed that *Bacillus* sp. HK4 had the highest variance in Cd accumulation at pH 4, compared to other strains (Table 1). Contrary, Cd biosorption by the other studied strains, *Pseudarthrobacter* sp. HK2 and *B. mycoides* HK3 highly depended on pH, reaching the lowest values at pH 4 and the highest at pH 10 (Fig. 1). To sum up, Cd accumulation by native strains, especially *Bacillus* sp. HK4 was higher than by the reference strain *B. subtilis* USK 1, which originated from the uncontaminated environment.

2.2. Magnesium biosorption by native bacteria

Bacillus sp. HK4 also revealed very high efficiency in Mg accumulation. Mg content in its biomass (mean: 8147 μg Mg g^{-1}) was 272 times higher than in the control, where bacteria were incubated without ore. At pH 7, a relatively good Mg biosorption was also shown by *Lysinibacillus* sp. HK1 and *B. mycoides* HK3. The same strains also demonstrated high Mg biosorption at pH 10. *Bacillus* sp. HK4 and *Pseudarthrobacter* HK2 have shown to be very efficient in Mg accumulation at pH 4 (5741 and 4103 μg Mg g^{-1}) (Fig. 2, Table 2). As in the case of Cd, *Bacillus* sp. HK4 revealed a high Mg biosorption capacity over a wide pH range (Fig. 2). Besides that strain, *B. mycoides* HK3 has not changed its capability of Mg accumulation in different pH. In the case of the reference strain, *B. subtilis* USK1, and *Pseudarthrobacter* sp. HK2, a negative correlation between Mg accumulation and pH (accumulation was the highest at low

pH) was reported. Post-hoc testing revealed the highest variance of Mg accumulation at pH 10 for *Bacillus* sp. HK4 and *B. mycoides* HK3, while at pH 7, for *Bacillus* sp. HK4 only (Table 2).

3. Discussion

In 2019, we conducted the first attempts of using bacteria to purify phosphate ores from Djebel Onk[15]. In that research, we selected five species from the collection of the University of Silesia in Katowice: *B. subtilis*, *Rhodococcus erythropolis* CD 130, *Pseudomonas fluorescens*, *Escherichia coli* and *Candida albicans*. Cd accumulation in the biomass of most of the studied microorganisms did not exceed 3 μg g^{-1}. In two cases, it reached values close or slightly above 7 μg Cd g^{-1} (*C. albicans* at pH 7 and *R. erythropolis* at pH 3), and in one case, it reached the value of 13.6 μg Cd g^{-1}. This result was disappointing, as efficient biosorption was not observed, despite previous encouraging reports on the capabilities of *B. subtilis* in this area[1,12,13,18,22]. Even *R. erythropolis* CD 130, which was isolated from heavily contaminated soil and displayed the presence of siderophores[25], did not accumulate large amounts of Cd. It seemed that *B. subtilis* and *C. albicans* were the most promising microorganisms in the context of biosorption; therefore, we decided to focus on them. We also suspected that Cd concentration in the studied ore samples (and consequently in the incubating solution) was not very high, and the biomass used was enough to remove the vast majority, if not all of Cd washed out during incubation[15]. Recent studies conducted with native bacteria do not fully confirm our earlier suspicions and shed new light on designing new, environmentally friendly methods of treating phosphate ores.

Compared to the bacteria used in our previous study[15], Cd (and Mg) concentrations in native bacteria were much higher. The most effective strain, *Bacillus* sp. HK4, isolated from raw ore, accumulated over 80 μg Cd g^{-1}, irrespective of the pH of incubation solution. Therefore, the potential application of this strain would not require pH adjustment, which simplifies and decreases the costs of the technological procedure Our research points out and confirms the enormous value and potential of the genus *Bacillus* in biotechnological applications in line with other studies.

In the recent study of Roşca et al.[13], a detailed analysis on removing Cd ions from liquid effluents by two bacterial species was conducted. *Bacillus megaterium* isolated from food products was one of them. Biosorption measurements were conducted in the function of pH, incubation time, temperature, initial metal concentration, and the amount of biosorbent. Authors reported the highest Cd accumulation by *B. megaterium* (15.1 μg Cd g^{-1}) at pH 4, 35°C, biosorbent dose of 3 g L^{-1}, and 20 min of incubation. Although we used a lower amount of biosorbent (about 0.2-0.3 g·L^{-1}), these findings are comparable to our results for *B. subtilis* USK1 from the collection of the University of Silesia in Katowice. Still, Cd accumulation by *B. megaterium* and *B. subtilis* USK1 was are much lower than by the native strain, *Bacillus* sp. HK4 (Fig. 1). In this context, results obtained by Yilmaz and Ensari[18] have shown the biosorption capabilities of native, HM-resistant strain of *Bacillus circulans* EB1 seems very interesting. In a solution containing 28.1 mg Cd L^{-1}, accumulation as high as 5.8 mg Cd g^{-1} of dry biomass was

observed after the first 8 hours of incubation (for cells growing in the presence of Cd). However, Cd biosorption by the resting cells (biomass production in the absence of Cd), for fresh and dry biomass, was significantly higher and reached, respectively: 9.8 and 26.5 mg Cd g^{-1}. These exceptional qualities of EB1 undoubtedly place it very high among metal biosorbents with a potentially great biotechnological application. Another research on strains isolated from industrial waste activated sludge included 37 native strains[22]. Two of them, *Bacillus* sp. C13 and *Bacillus* sp. C16, had a high resistance and high capability of accumulating metals such as Cd, Cr, Mn, and Pb. In that study, biosorption in the alkaline environment turned out to be higher than in the acidic one[22] . The phenomenon of biosorption is extraordinarily complex and depends on many variables. Therefore, Ahmad et al.[20] used an artificial neural network (ANN) to predict the biosorption capabilities of *B. subtilis* to remove Cd ions from an aqueous solution. Their investigation led to a conclusion that the biosorption capability of *B. subtilis* can be as high as 251.91 mg Cd g^{-1} in the following optimal conditions: pH 5.91, temperature 45 °C, time of contact 3 h, initial Cd concentration of 496.23 mg L. In that case, the optimal conditions for biosorption shifted more towards acidic pH, which can be related to interactions between the negatively charged surface of the biosorbent and a positive charge of Cd ions[1,20]. Boyanov et al.[12] conducted X-ray absorption fine structure (XAFS) spectroscopy, where they tested the capability of *B. subtilis* to bind Cd ions to its cell walls. The research was done in the pH range of 3.4 – 7.8 and proved that various functional groups might be engaged in binding Cd ions, depending on pH. When pH was below 4.4, Cd was bound mainly by phosphoryl ligands, and when pH reached higher values, a significant role of carboxyl groups in binding Cd was described. The importance of phosphoryl sites was also demonstrated at pH 7.8; however, with different binding characteristics than at pH 4.4[12]. In the context of results quoted above, our strain, *Bacillus* sp. HK4, seems to be very perspective in further research, as it shows the capability of binding Cd in the wide range of pH. Moreover, it might be applicable in high phosphate concentrations (such as in soil and communal waste), as it was isolated from phosphate ore.

Native bacteria isolated from phosphate ore from Djebel Onk have also exhibited high capabilities of magnesium sorption. Isolated strains accumulated Mg in concentrations exceeding even 2000 µg g^{-1}, and for *Bacillus* sp. HK4, the biosorption exceeded 8000 µg Mg g^{-1} at pH 7 (Fig. 2). Compared to the values obtained for B. subtilis USK1 from the collection of the University of Silesia in Katowice[15] , these values are much higher. In that study, Mg accumulation in the biomass of microorganisms stayed in a range from 324 to 2698 µg Mg g^{-1} and was higher at pH 7 than at pH 3[15], emphasizing the high potential of native species for biotechnology. Accumulation of significant amounts of Mg is highly important because it helps removing unwanted Mg from phosphate ores, and it could be useful in reclaiming this element from waste and recycling it. Yasue et al.[26] reached interesting conclusions when studying the influence of heated dolomite powder on the formation of spores by *B. subtilis*. Treating dolomite with high temperature (a known procedure of treating phosphate ores, which aims to reduce Mg concentration) leads to the production of CaO and MgO, which have antibacterial properties. It turned out that dolomite powder, heated at 1000 °C for one hour, killed *B. subtilis* spores (it is notable, especially that spores are highly resistant to extreme pH values). However, the authors did not relate this effect to the direct

influence of MgO. Instead, they pointed to an increased generation of active oxygen species by heated dolomite powder[26]. Oknin et al.[27] has been found that Mg can affect biofilm formation by *Bacillus* species. 50 mM or higher concentration of Mg ions can inhibit biofilm formation but not the growth of bacterial cells. The authors concluded that Mg ions specifically decrease the expression of genes involved in biofilm formation, inhibiting the synthesis of extracellular matrix[27]. Notably, the authors mentioned that other bivalent ions (such as Ca^{2+}) did not inhibit biofilm synthesis by *Bacillus* species. Moreover, adding Ca^{2+} ions might contribute to biofilm formation[27–30]. Contrary, Mhatre et al.[31] proved that Ca^{2+} ions inhibit the expansion of *B. subtilis* colonies and biofilm development. Nonetheless, considering that dolomite contains magnesium, we can assume that after a sufficiently long incubation of *Bacillus* sp. with phosphate ore, such biofilm can develop easier on the surface of apatite. However, its development on the surface of dolomites might be slowed down/stopped (by Mg ions). This quality could be potentially useful when designing further procedures aimed at creating an ecological method of ore treatment because it is a potential factor differentiating both minerals. However, this issue requires further detailed testing.

Acquired results are also a good background for considering the adaptability of microorganisms inhabiting Djebel Onk. Adaptability is directed by a number of environmental variables, with time, the intensity of selective factor, as well as the species undergoing selection (its biology and lifespan) being the key ones. For organisms with a fast development cycle, when the lifespan of a single generation is short, adaptation is very important, in contrast to phenotypic plasticity, which becomes more influential for species with long lifespans[32,33]. Statistical analysis (Tables 1 and 2) has shown that studied strains differed significantly in their ability to accumulate metals – even though selection conditions remained the same. Undoubtedly, further studies are required to determine mechanisms that enable certain species to survive and develop in the extreme conditions of phosphate deposits in Djebel Onk.

Incubation of phosphate ore with selected native bacterial strains (for 20 minutes at different pH) can significantly increase the recovery of metals such as Mg and Cd, and perhaps others. *Bacillus* species are especially promising and worthy of recommendation. They can be potentially useful in designing an eco-friendly ways to clean ore and post-flotation wastes and recycle metals.

4. Materials And Methods

The phosphate ore originated from Djebel Onk (Kef Essnoun region) was acquired courtesy of the National Company of Iron and Phosphate FERPHOS. Ore samples were collected from a site where no excavation or any other human activity was conducted. The material was collected into aseptic containers under sterile procedures, stored in air-tight conditions, and transported to the laboratory to isolate native microorganisms. The second part of the collected ore was used for physicochemical analysis and biosorption tests after proper mechanical treatment.

4.1. Characteristics of the raw ore (before mechanical preparation).

After transporting ore samples to the laboratory, a granulometric study was conducted first. The particle size analysis of the sample revealed that 3% of the particles were less than 80 µm (Fig. S1, Table S1). Seventeen granulometric classes were distinguished, which were characterized by their metal contents and mineralogical composition.

4.2. Measurements of metal concentration in phosphate ore.

In order to measure metal concentration, ore samples of each granulometric class were first mineralized in wet conditions, using a Multiwave 3000 Microwave Digestion of Perkin Elmer. After drying the material (temp 30°C, 0.5 h), 0.200 g samples were placed directly into Teflon vessels and covered with concentrated acids in the following volumes: 6 mL HNO_3 (65%), 2 mL HCl (35%), and 4 mL HF (40%). The samples in the vessels were placed in a microwave oven and mineralized according to the program: the first step (raising the temperature to 220°C) – power: 1800 W, time: 25 min, temperature: 220°C; the second step (maintaining the temperature of 220°C) – power: 1800 W, time 20 min. After this step, samples were cooled, and 24 mL 4% H_3BO_3 was added to each dish to neutralize the remaining HF. Then, the vessels were placed again in the microwave oven according to the program: the first step (raising temperature to 120 °C) – power 1200 W, time: 10 min; the second step (maintaining the temperature of 120 °C) – power 1200 W, time 5 min. After mineralization, the samples were cooled and filled to 50 mL with deionized water. Metal contents in the samples were measured using iCE™3500 AAS atomic absorption spectrometer (ThermoFisher Scientific). Each sample was measured in two repetitions. The results of these analyses were collected in table 3. Following that, the mineralogical composition was assessed in each fraction, using the XRD method.

4.3. Mineralogical composition assessment: X-ray diffraction (XRD).

XRD analyses were performed on powdered samples using a PANalytical X'Pert Pro MPD (multipurpose diffractometer) powered by a Philips PW3040/60 X-ray generator and fitted with a 1D silicon strip detector (X'Celerator) with a 2.122° 2θ active length. The measurements were performed using Cu Kα-radiation with a wavelength of 0.1540598 nm, an acceleration voltage of 40 kV, a current of 40 mA, and with 0.02 °2θ step sizes between the angles of 5° and 70° 2θ and a 200 s measurement time per step. Powder diffraction analysis parameters are gathered in Table S2. The data obtained were processed using HighScore+ software (version 4.1), linked to the ICSD database (2015) and the PDF4+ ICDD database (2018).

For the standardless, quantitative phase analysis, the Rietveld method was used. Rietveld structure fit module is a part of the HighScore Plus program suite[34]. Quantitative phase analysis can be performed on multi-phase samples using the formalism described by Hill and Howard[35]. The Rietveld method is a full-pattern fit method. The measured profile and a profile calculated from crystal structure data are compared. By variation of many parameters, the difference between the two profiles is minimized. In order to obtain quantitative calculations, the semi-automatic Rietveld mode in HS+ was used. The refinement was carried out until good statistical parameters were obtained: R expected = 4,30; R profile =

6,50; Weighted R profile= 8,61; Goodness of Fit =4,01. The results of XRD analyses for each ore fraction are presented in table 4.

4.4. Mechanical preparation of ore for testing.

In the next stage, ore was prepared for biosorption tests. In order to achieve a homogenous fraction, mechanical procedures (crushing, grinding, and sifting) were conducted according to the algorithm presented in fig S2.

Homogenous fraction (80-160 μm) was subjected to the assessment of mineralogical composition using the XRD method. For this fraction the results were as follows: carbonate fluoroapatite (CFA; Ca5(PO4,CO3)3F) 87%, dolomite (CaMg(CO3)2) 10%, calcite (CaCO3) 1.5%, clinoptilolite (Ca2-3[Al3(Al,Si)2Si13O36]·12H2O) 1.0%, and quartz (SiO2) 0.5%. (Fig. 3). Before biosorption tests, ore was sterilized to eliminate any microorganisms interfering with the experiment. Samples of 1 g of processed ore were weighed out, autoclaved (121 °C, 2 h), and stored in sterile conditions for further use.

4.5. Isolation and identification of microorganisms from raw ore.

In order to isolate native bacteria from the phosphate ore from Djebel Onk mine (Algeria) 10 g of ore was suspended in 90 mL of sterile NaCl, shaken for 1 hour at 120 rpm, and serially diluted. 0.1 mL of each ore dilution (from 10^{-1} to 10^{-6}) was spread on the solid culture media LB and R2A (BTL, Łódź, Polska). The cultures were incubated for 96 h at 28 °C. Single bacterial colonies were passaged on fresh media to obtain pure cultures. Among the isolated bacteria, four morphologically different strains were selected for identification and biosorption testing. Molecular identification of bacterial strains was based on the sequence of 16S rDNA gene fragment. PCR with primers 8F and 1492R[36] was performed as described by Pacwa-Płociniczak et al.[37]. 1484 bp PCR products were cloned with the use of pGEM®-T Easy Vector System (Promega, Madison, Wisconsin, USA) and sequenced at Genomed S.A. (Warsaw, Poland). The edition of sequences was conducted manually using Chromas Lite 2.01 (Technelysium Pty Ltd, Brisbane, Queensland, Australia), and chimera detection was performed using Decipher 2.19.2[38]. Obtained sequences were aligned to the reference sequences of 16S rRNA gene available in the GenBank database (National Centre for Biotechnological Information) using BlastN. The 16S rDNA sequences of strain HK1 showed 99.80% of identity to the sequence of *Lysinibacillus sphaericus* (CP026120.1) and 99.74% of identity to the sequences of *L. sphaericus* (KF228905.1) and *Lysinibacillus fusiformis* (CP010820.1, EU545408.1). The sequence of strain HK2 showed 99.12% of identity to the sequences of *Pseudarthrobacter oxydans* (KR085945.1, KR085776.1) and *Pseudarthrobacter scleromae* (KR085778.1), and 99.06% of identity to the sequence of *Pseudarthrobacter psychrotolerans* (CP047898.1). The sequence of strain HK3 showed 99.93% identity to the sequences of *Bacillus mycoides* (MT827167.1, CP031071.1). The sequence of strain HK4 was 99.93% identical to the sequences of *Bacillus subtilis* (KX281166.1, KU551251.1), *Bacillus amyloliquefaciens* (KU551122.1) and *Bacillus velezensis* (CP053764.1). The sequences were deposited in GenBank with accession numbers: MZ046078 (USK1), MZ046079 (HK1), MZ046080 (HK2), MZ046081 (HK3), MZ046082 (HK4).

4.6. Biosorption testing.

Native microorganism strains chosen for tests and *Bacillus subtilis* USK1 from the collection of the University of Silesia in Katowice (as a reference strain) were cultured in LB medium for 24 hours. After incubation, bacterial cultures were washed and suspended in 0.98% NaCl to get the concentration of 3 g of biomass L^{-1} for each strain. Suspensions were portioned into 50 mL aliquots for biosorption analysis. Sterilized 1 g ore samples were incubated (28°C with constant shaking) with microorganisms (50 ml suspension) for 20 minutes at different pH values (4, 7, and 10). After incubation, samples were set aside for 5 min to facilitate sedimentation of ore particles. Next, the solution containing microorganisms was gently poured into sterile 50 ml centrifuge tubes, and metal concentrations in biomass were assessed according to a procedure described elsewhere[15]. In short, the suspensions of microorganisms were centrifuged (Ultracentrifuge Beckman Optima LE-80K) at 8000 rpm (4 °C; 15 min). Biomass was frozen at -70°C and lyophilized (Freeze dryer Alpha 1–4; Christ, Germany) at −35°C and 0.2 mBar for 24 h. Portions of ~0.02 g of dried microorganisms were mineralized with 0.5 mL of ~65% HNO_3 at 110°C for 48 h. After mineralization, the samples were diluted with deionized water to a total volume of 5 mL. Cd and Mg contents were measured by AAS methods with an iCE™ 3500 AAS atomic absorption spectrometer (Thermo Fisher Scientific). The quality of the analytical procedure was confirmed using standard solutions from Merck at the initial concentration of 1 g of metal L^{-1} of water. Metal content was expressed as μg g^{-1} of biomass. The metal analyses were done in two technical replications. All tests in each experimental group were done in triplicates. Results were expressed as mean ± SD. The analysis of variance, followed by the Fishers Least Significant Difference test (LSD, ANOVA; $p < 0.05$) was performed to assess the significance of differences in metal biosorption among the studied bacterial strains and different pH. Statistical analysis was conducted using Statistica 13.1 software.

Declarations

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Author Contributions

H. R – Conceptualization, methodology, formal analysis, writing–original draft

M.O. H – Conceptualization, supervision, project administration, funding acquisition, writing - review & editing

K. K – Conceptualization, methodology, formal analysis, writing–original draft, visualization, project administration, funding acquisition

T. K – Methodology, investigation, writing–original draft, funding acquisition

M. M – Conceptualization, methodology, formal analysis, writing–original draft, visualization, project administration, funding acquisition

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Additional Information

Competing Interests: The authors declare no competing interests.

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Tables

Table 1. The results of post-hoc test (ANOVA, LSD, $p < 0.05$) for Cd concentration in the biomass of bacterial strains isolated from Djebel Onk ore.

Abbreviations: x - a given pair of means differ significantly in term of Cd accumulation. (A) – control, (B) – pH 4, (C) – pH 7 and (D) – pH 10.

		HK1	HK2	HK3	HK4		
control	HK1			x	x	HK1	pH4
	HK2				x	HK2	
	HK3				x	HK3	
	HK3					HK3	

		HK1	HK2	HK3	HK4		
pH7	HK1			x	x	HK1	pH10
	HK2	x		x	x	HK2	
	HK3				x	HK3	
	HK3	x	x			HK3	

Table 2. The results of post-hoc test (ANOVA, LSD, $p < 0.05$) for Mg concentration in the biomass of bacterial strains isolated from Djebel Onk ore. *Abbreviations: x - a given pair of means differ significantly in term of Mg accumulation. (A) – control, (B) – pH 4, (C) – pH 7 and (D) – pH 10.*

		HK1	HK2	HK3	HK4		
control	HK1				x	HK1	pH4
	HK2					HK2	
	HK3		x		x	HK3	
	HK3		x			HK3	

		HK1	HK2	HK3	HK4		
pH7	HK1			x	x	HK1	pH10
	HK2	x		x	x	HK2	
	HK3				x	HK3	
	HK3	x	x			HK3	

Table 3. Metals concentration ($\mu g \cdot g^{-1}$) in different fractions of phosphate ore from Djebel Onk.

Fractions	Metals						
	Cd	Cu	Mn	Fe	Mg	Ni	Zn
≥ 5 mm	23.7	7.9	17.3	1210.0	3893.9	1.5	166.6
4-5 mm	23.6	8.8	20.2	1577.2	4681.8	1.7	144.5
2.5-4 mm	34.7	9.9	23.1	1527.3	4102.6	1.7	163.6
2-2.5 mm	42.0	10.2	22.6	1626.0	4336.5	1.6	167.2
1.6-2 mm	44.3	9.8	23.9	1692.3	4280.8	1.7	172.1
1-1.6 mm	45.8	10.6	21.9	1627.4	5213.4	1.7	174.4
800 µm-1 mm	42.7	9.1	20.6	1533.4	3635.7	1.8	164.1
500-800 µm	30.2	10.7	14.8	1533.3	4395.9	1.5	161.8
315-500 µm	19.9	11.5	12.6	1516.6	3350.0	1.5	146.0
250-315 µm	19.8	10.5	10.8	1423.6	2747.0	1.5	147.7
160-250 µm	25.3	9.9	14.5	1296.0	4172.2	1.7	179.8
125-160 µm	32.4	13.4	18.5	1530.5	3969.6	2.0	206.3
80-125 µm	36.8	13.1	23.4	1629.0	5369.1	1.8	217.5
63-80 µm	-	-	-	-	-	-	-
40-63 µm	49.1	9.5	30.5	2072.9	5217.8	17.4	234.3
≤ 40 µm	49.6	10.9	29.3	2144.5	4391.0	6.4	256.6

Table 4. Mineralogical composition of different fractions of phosphate ore from Djebel Onk.

Fractions	Mineralogical composition (%)				
	Calcite	CFA	Clinoptilolite	Dolomite	Quartz
≥ 5 mm	5	64	2	29	1
4-5 mm	5	66	1	27	1
2.5-4 mm	7	58	1	33	1
2-2.5 mm	4	69	2	23	2
1.6-2 mm	9	69	1	18	2
1-1.6 mm	4	69	1	25	2
800 µm-1 mm	4	73	0	18	4
500-800 µm	2	85	0	13	1
315-500 µm	0	97	0	2	0
250-315 µm	0	93	2	5	0
160-250 µm	1	89	0	6	4
125-160 µm	3	73	1	21	2
80-125 µm	1	62	2	29	6
63-80 µm	3	60	2	35	0
40-63 µm	4	59	7	30	0
≤40 µm	5	54	13	28	0

Figures

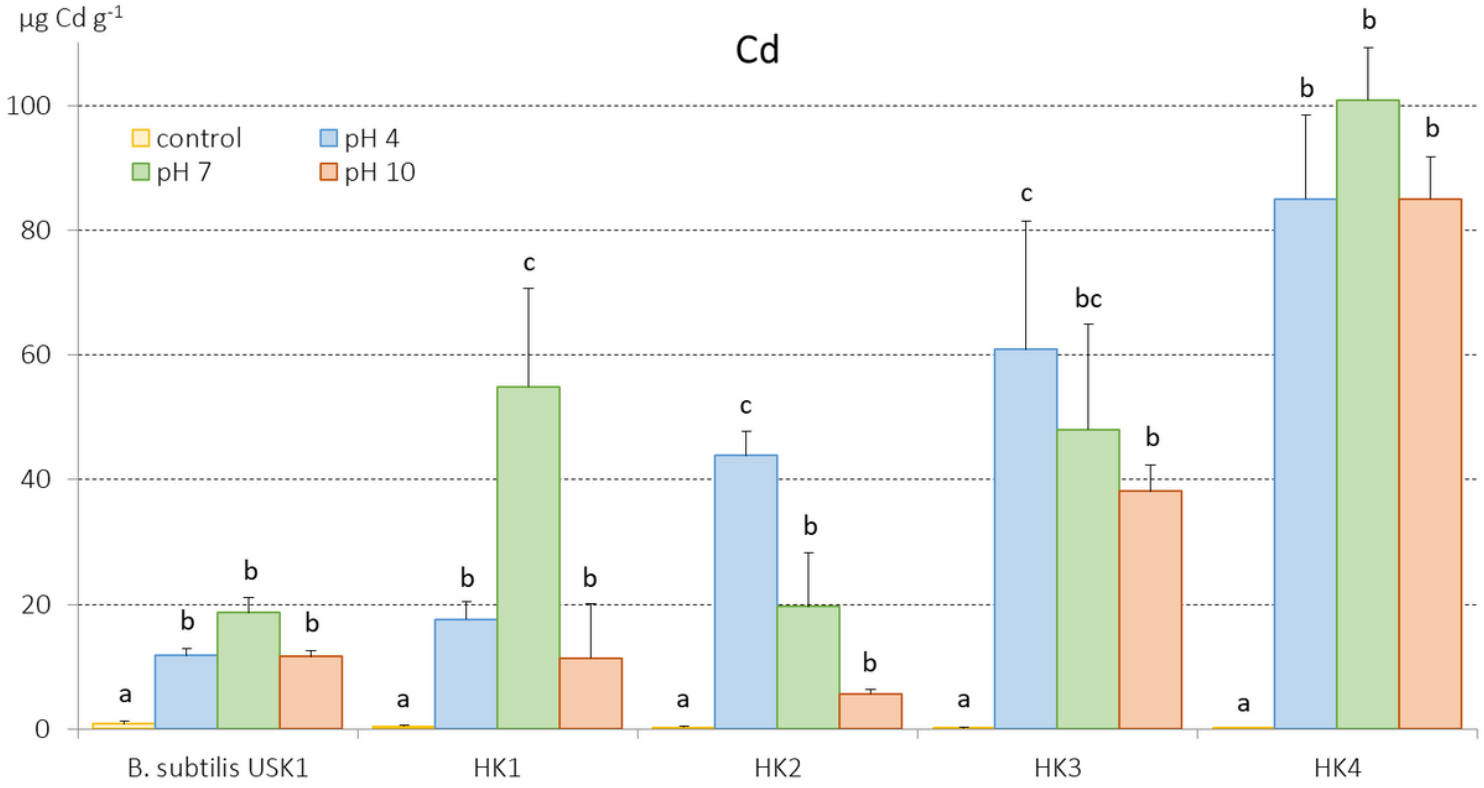

Figure 1

Cd accumulation (mean ± SD; µg g-1) in the biomass of bacterial strains isolated from the phosphate ore (HK1 – HK4 strains) and B. subtilis USK1 reference strain, after incubation with the ore from Djebel Onk at different pH. The same letter in particular strain denotes no significant differences among pH groups (ANOVA, LSD; $p<0.05$).

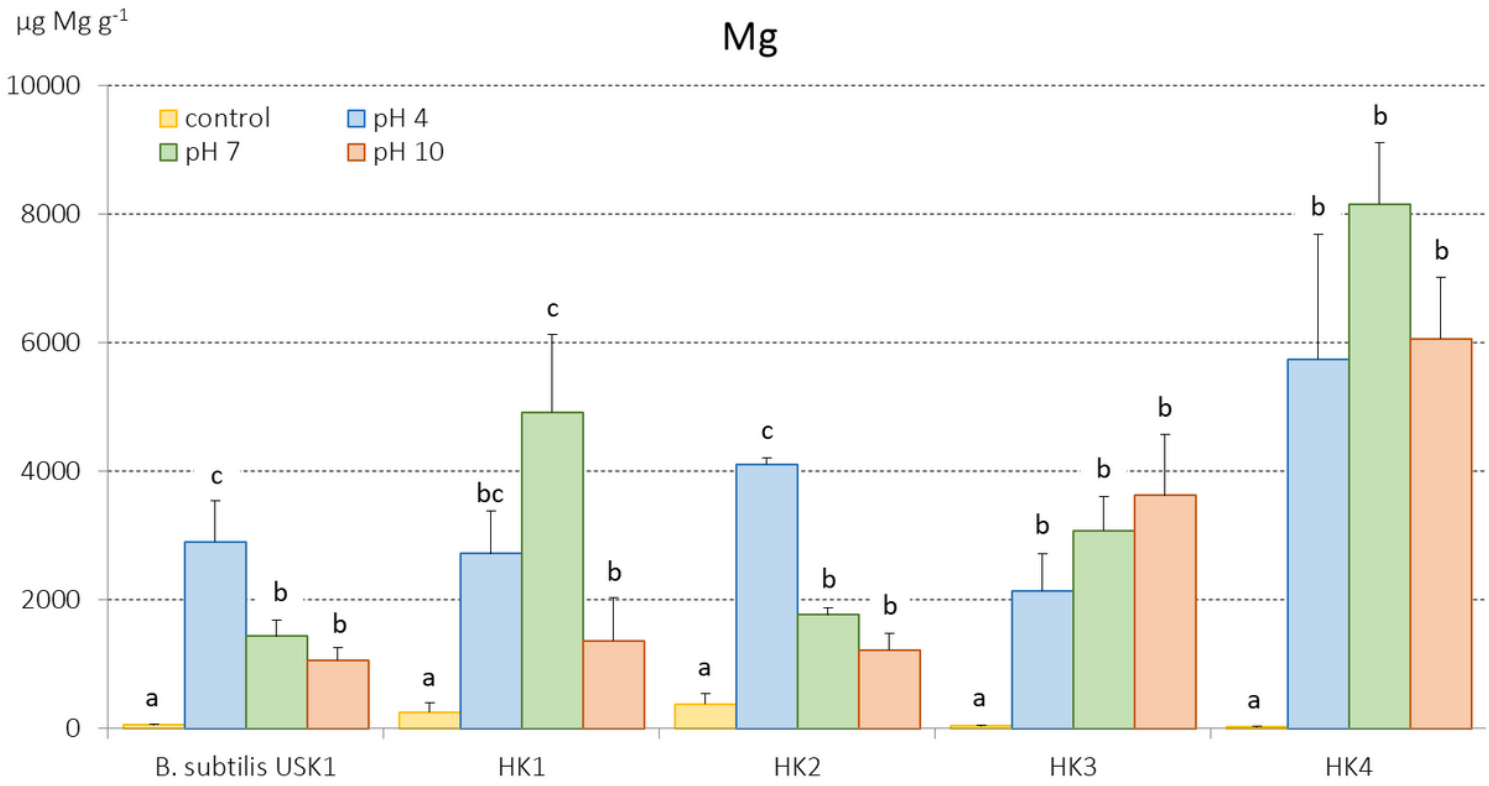

Figure 2

Mg accumulation (mean ± SD; µg·g-1 in the biomass of bacterial strains isolated from the phosphate ore (HK1 – HK4 strains) and B. subtilis USK1 reference strain, after incubation with the ore from Djebel Onk at different pH. The same letter in particular strain denotes no significant differences among pH groups (ANOVA, LSD; $p<0.05$).

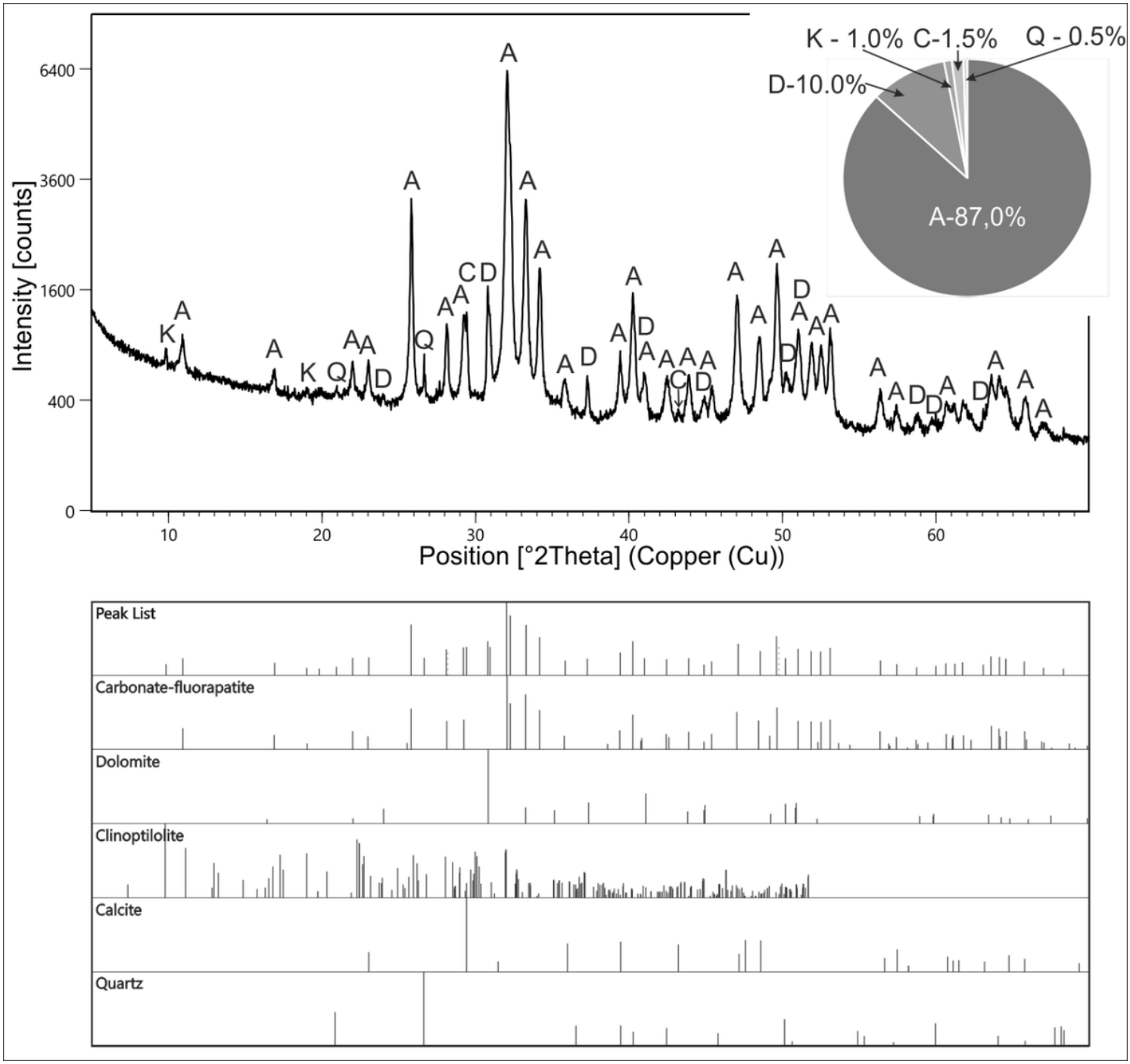

Figure 3

XRD pattern of a Djebel Onk sample after mechanical preparation (fraction 80–160 µm), with the result of qualitative analysis (table showing the matched patterns) and the semi-quantitative calculations (pie chart, all values are weight)

Supplementary Files

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