Remediation of Arsenic-contaminated Soil in Northwestern China by Pseudomonas Taiwanensis

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Remediation of arsenic-contaminated soil in northwestern China by Pseudomonas taiwanensis

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Abstract

The physiological resistance of *Pseudomonas taiwanensis* to heavy metals was studied and used to repair heavy metal contaminated water and soil. The results showed that the suitable pH conditions for the growth of P. taiwanensis were 5-9, and the salt tolerance was 6%. The tolerance concentrations for heavy metals As(V) and Mn(II) were 500 mg L^{-1} and 120 mg L^{-1}, respectively. The strains were enriched by nutrient broth(NB) medium. After logarithmic phase, the bacteria liquid was mixed with ATCC^{TM}#279 medium in proportion, and a certain amount of Mn(II) was added. The results of removing heavy metals As, Pb and Cd in the composite polluted water phase were 22.09%, 30.75% and 35.33%. The molar ratio of manganese and iron will affect the removal efficiency of single pollution heavy arsenic. When the ratio of iron and manganese is 1:5, the highest removal efficiency is 68%. However, when the remediation method is added to the soil, it cannot fix all metals, such as Cu and Zn. This remediation method provides a reference for the practical engineering application of microbial remediation.

Keyword: Soil pollution; heavy metal; microbial remediation; manganese-oxidizing bacteria; pseudomonas taiwanensis; biogenic manganese oxides

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors made an indispensable contribution to this study. The technical guidance and methods were provided by Shengli Wang. The writing and analysis of the article were completed by Mengbo Liu. The data processing and image production were carried out by Xiang Ning. The experimental scheme and operation were carried out by Meng Yang. The experimental materials and fonds were carried out by Zhongren Nan.

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1. Introduction

Arsenic is ubiquitous in the natural environment and causes worldwide concern because of its health problems(Zhu Y et al, 2014). Arsenic generally exists in the atmosphere, soil and rock, natural water and organisms, and migrates through natural processes (such as weathering, biological activities and volcanic emissions) and a series of human activities (such as mining and pesticides)(Smedley P& Kinniburgh D, 2002; Yun S et al, 2018). Arsenic in soil environment enters the human body through the food chain under the action of bioconcentration. Long-term consumption of contaminated food will increase the risk of carcinogenic and non-carcinogenic health hazards(Qu C et al, 2012; Zhang J et al, 2015; Mao X-L, Li D, Zhu X-H, 2016).

Arsenic affects biological safety in the form of trivalent arsenate and pentavalent arsenate as its main oxidation state(Garbinski LD, Rosen BP& Chen J, 2019). Biologically, the biological activity of trivalent arsenic is higher than that of pentavalent arsenic, so it is easy to migrate to plants and affect their normal growth and development(Liu SX et al, 2001). When applied to organisms, arsenic binds to specific tissue proteins and phosphates with similar structures, thereby interfering ATP synthesis(Ali W et al, 2020). Therefore, reducing the biological activity of arsenic in soil has become the focus of many scholars.

Arsenic fixation in can be fixed by adding additives such as biochar(Wang Y et al, 2020)、Phytoremediation(Lombi E et al, 2001)、or the remediation methods of compost and super accumulator can be used to reduce their active(Cao X, Ma LQ& Shiralipour A, 2003), but these methods have poor applicability, high price, low efficiency, long repair time and may cause secondary pollution, which can not be effectively used in practical engineering. Studies have shown that iron oxide (iron spots) forms on the root surface of plant roots (rice as an example), which can prevent arsenic from further migration to the upper part of the plant(Liu W et al, 2004). Some iron-manganese oxides with poor crystallinity provide major reaction sites for arsenic oxidation and adsorption, which have been used for arsenic immobilization in soils(Wang Y et al, 2020). Similarly, the presence of manganese oxides can also achieve effective results for arsenic fixation(Yun S et al, 2018; Zhang J et al, 2015). Therefore, remediation of arsenic contaminated soil by iron and manganese oxides is a promising method to improve soil environmental quality.

Microbes play a key role in soil element cycling. For arsenic, it has been found that many bacteria can migrate and transform arsenic in the environment directly or indirectly(Kumari N, Rana A& Jagadevan S, 2019). Trivalent arsenic can be converted into pentavalent arsenic in a certain pH range. Some scholars have studied the biotransformation, bioaccumulation, and biosorption of arsenic by D.arsenite, which can adapt to a large pH range and can be repaired in a high arsenic environment(Zhang Z et al, 2016). However, this method can not be guaranteed to be effective for a long time, and it does not take the situation of compound pollution into account. There is a great hidden danger of desorption. Sundman A et al(Sundman A et al, 2020) showed that the magnetite produced by iron oxide bacteria could remove arsenic and chromium efficiently by surface adsorption. However, compared with primary magnetite, the results of biological modification were not satisfactory, and the adsorption effect on mineral surface was greatly affected by the environment and was not stable enough. Study on Pseudomonas Taiwan by Satapute P et al(Satapute P et al, 2019), metal stress promoted the synthesis of cellular proteins, the surface of pseudomonas taiwanensis cells interacts with metal cations (hydroxyl, carboxyl, phosphate, amino) to complete the adsorption of heavy metal ions.

The results by Bao L L et al(Bao L-L, 2020) show that Pseudomonas taiwanensis can produce ferromanganese minerals under ATCC™#279 culture and can wrap heavy metal ions in lattice, which greatly reduces the mobility of heavy metal ions in soil. This method of indirect in situ remediation by microorganisms is cheap and the removal rate of heavy metals is very high, which is a feasible method.

In order to improve the removal rate of heavy metals and reduce the toxicity of heavy metals in the environment, the optimum growth conditions of Pseudomonas taiwanensis, the ability to resist heavy metals and the role of iron-manganese ratio in the mineralization process were discussed. Then, under the condition of combined pollution, the effect of iron-manganese ratio on the repair of Pseudomonas taiwanensis was discussed.

2. Experimental materials and instruments

2.1 Materials and Methods

The soil in this experiment was collected from the surface soil (0-20 cm) of Luojiatan Village (104.24E, 36.54N) (Fig. 1). Baiyin City is located inland, perennial drought and rain. Since the 1960s, sewage has been used to irrigate farmland soil. Luojiatan Village is located in the upstream of the sewage irrigation area, and the sewage irrigation has not been stopped until 2000. For 40 years, heavy metals continue to accumulate and migrate in farmland soil, causing pollution(Liu W-J, 2012). Remove impurities from soil samples by air drying, grinding and sieving (2 mm and 0.149 mm), standby.

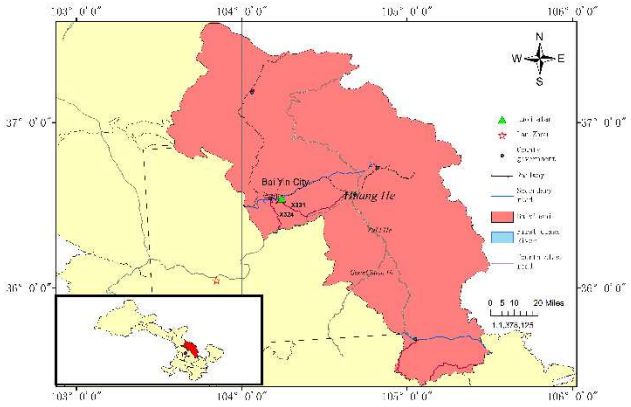

Fig. 1 sampling point location

Soil physical and chemical properties (pH, EC, organic matter, carbonate) were determined according to the method in soil agrochemical analysis by Bao et al. Electric conductivity (EC) and pH were measured with EC electrode (HI98311) and pH meter (pHS-3E) in deionized water (1∶5, m/v), respectively. EC reflects the salinity of the sample(Liu J et al, 2019). Organic matter (SOM) content was determined by potassium dichromate volumetric method -dilute heat method and carbonate ($CO3^{2-}$) content by neutralization titration method(Bao S-D, 2000).

Atomic fluorescence spectrometer (AFS-2880) was used to determine the concentration of total as, and atomic absorption spectrometer (Analytikjena, ZEEnit 700p) was used to determine the concentration of Pb, Cd, Cu, Zn, etc.

2.2 Analysis of pollution degree

The single factor index method is used to analyze the parity of single metal (metal-like) index one by one, so as to determine the pollution degree of main heavy metals (metal-like) in soil. The

calculation formula is as follows:

$$P_i = C_i / S_i$$

Where: P_i is the single pollution index of metal (metalloid) I; C_i is the measured concentration of soil heavy metal (metalloid) I, mg kg^{-1}; S_i is the evaluation standard value of soil heavy metal (metalloid) I, mg kg^{-1}.

The Nemerow index method is used to reflect the degree of heavy metal (metalloid) compound pollution by taking into account the average value and maximum value of single factor index. The calculation formula is as follows(Guan Y et al, 2014):

$$P_z = \sqrt{\frac{P_{i-\max}{}^2 + P_{i-ave}{}^2}{2}}$$

Where: P_Z is Nemerow's comprehensive pollution index; $P_{i\text{-}max}$ is the maximum value of each single pollution index in soil; $P_{i\text{-}ave}$ is the average value of each single pollution index in soil.

2.3 Manganese Oxidizing Bacteria Strains

Pseudomonas taiwanensis (P4) was provided by Pan research group of Zhejiang University of Technology. The strain was inoculated into Nutritional Broth (NB) medium (peptone 10g L^{-1}, beef extract 3 g L^{-1}, sodium chloride 5 g L^{-1}, pH=7.0) for expansion culture. After 12 hours of growth, the solid ATCC™#279 medium containing 5ppm Mn(II) (ammonium ferrous sulfate 0.15 g L^{-1}, yeast extract 0.075 g L^{-1}, sodium pyrophosphate 0.05 g L^{-1}, sodium citrate 0.15 g L^{-1}, pH=6.8, agar 1.5%)(He Z et al, 2019) was coated on the plate. The plate was placed in a constant temperature incubator at 30°C for 4-5 days. The growth of the surface strain was observed, and the well-growing plaque was picked, then expanded and stored in the solid NB medium (agar 1.5%).

Preserved strains with universal primers 27F: 5'AGAGTTTGATCCTGGCTCAG3'; 1492R: 5'TACGGCTACCTTGTTACGACTT3' was used as the primers for PCR amplification of16sRNA (primers were synthesized by Beijing Qingke Biotechnology Co. Ltd Xi'an Branch). Genomic DNA was extracted from by plant genomic DNA extraction kit of Beijing Qingke Biotechnology Co. Ltd. Xi'an Branch. PCR reaction system (50 ul): MIX 46 ul, 27F primer (10 pmol ul^{-1}) 1 ul, 1492R primer (10 pmol ul^{-1}) 1 ul, genomic DNA 2 ul, PCR procedure: 98°C, 30 min; 98°C, 10s; 55 °C, 10s; 72℃, 20s; Cycle 30 times; 72℃, 2min. The amplified fragment was about 1400 bp-1700 bp, and the sequencing was completed by Beijing Qingke Biotechnology Co. Ltd. Xi'an Branch. The splicing results were compared on NCBI (https://www.ncbi.nlm.nih.gov/), and then the phylogenetic tree of Pseudomonas Taiwan was established by Neighbor-Joining method in MEGA (6.06), and phylogenetic analysis was carried out. Bootstraping method was used to evaluate the phylogenetic tree.

2.4 Tolerance experiments

In order to find the best growth conditions, acid and alkali resistance and salt tolerance were tested. First, prepare the NB medium and adjust the pH to be 3, 5, 7, 9, 11, the other group, using sodium chloride as salt, set the salinity gradient to 2%, 4%, 6%, 8%, 10%(the amount of salt added in this experiment is based on the configured medium, NB the original salinity of the medium is 0.5%), sterilize the two groups of culture medium and inoculate 100 ul bacteria solution under relatively sterile conditions, set two groups parallel and sample at 0, 6, 12, 24, 48, 72, 96, 120, 144, 168h. OD600(600 nm ultraviolet spectrum analysis) was determined in time to show the growth of bacteria.

Cadmium nitrate and sodium arsenate as stress sources. The arsenic tolerance experiment was set

to 0, 100, 200, 300, 400, 450 mg L^{-1}, add arsenic source to NB medium, adjust pH=7, inoculate 100 bacteria solution after sterilizing in high pressure sterilizer. In Cd experiment, the Cd gradient was set to 0, 8, 15, 30, 50, 80 mg L^{-1}, after the same treatment, both groups were set parallel, sampling 2 ml, at 0, 6, 12, 24, 48, 72, 96, 120 hours, respectively spectrophotometry.

2.5 Heavy Metal Removal Experiment in Water Solution

In order to study the removal effect of P4 bacteria on Pb, As and Cd in aqueous solution, heavy metal removal experiments were carried out. P4 strain was inoculated in NB medium and cultured to logarithmic growth phase (12-16 h). The following groups were established: 1) group (Pb-P4); 2) group (As-P4); 3) group (Cd-P4); 4) group (composite-P4) and blank control (without adding microorganisms). The 25 ml P4 bacterial solution was transferred to 250 ml conical flask containing Mn(II), target heavy metal with initial concentration of 5 ppm and 225 ml ATCC™#279 medium. At 0, 6, 12, 24, 36, 48, 72, 96, 120, 144, 168h, 1 ml sample was taken, and 9 ml deionized water was added to measure the concentration of heavy metals and pH of culture medium.

2.6 Soil testing

After the P4 strain was cultured for 14h, the mixed solution was prepared according to the ratio of bacterial solution to ATCC™#279=1:10. Adding6ml of the mixture in 10 g soil, shaking 30 min at 25°C, 150 rpm, placed in the ventilation, 14 days later, crushed 0.149 mm sieve, weighed 1.0 g for Tessier continuous extraction(Tessier A, Campbell PGC& Bisson M, 1979). Finally, the contents of heavy metals in the extracted solution were tested respectively

2.7 Research on the Optimal Ferromanganese Ratio

Using the characteristics of microbial mineral passivation of heavy metals, mineral production will undoubtedly affect the passivation effect of heavy metals. In this study, iron and manganese as a mineral element. The addition and proportion are the key factors determining the ore production. According to the tolerance of bacteria to manganese, we keep the content of iron in ATCC™#279 medium unchanged, by changing the amount of manganese added to prepare different ratios of iron and manganese culture medium. The specific method is to prepare Fe/Mn molar ratio of 5:1, 3:1, 1:1, 1:3, 1:5, add 1000 mg L^{-1} Mn(II) to manganese ion concentration of 4.21 mg L^{-1}, 7.02 mg L^{-1}, 20.98 mg L^{-1}, 63.13 mg L^{-1}, 105.26 mg L^{-1}, each group to add the configured arsenic solution to arsenic concentration of 50 mg L^{-1}. After inoculation of Pseudomonas Taiwan, at 30 °C, 180rpm shaking culture for 14 days, take 2 ml sample solution, with 0.22 um water phase filter head filter out supernatant, determination of arsenic concentration. The same method of soil remediation, weighing 30.0 g soil, solid-liquid ratio set to 3:1, every 7 days take a sample, a total of 5 times.

2.8 Representation analysis

The resulting precipitation was cooled and dried for 24h by a cold dryer (Gansu Jimei Instruments & Equipment Technology Co., Ltd., LGJ-12A), and then ground into powder by a mortar. X-ray diffraction (XRD) was performed by X-ray diffractometer (PANalytical, Netherlands, X'PertProMPD) to analyze the change of solid crystal phase before and after the addition of heavy metals. Scanning electron microscopy-X-ray energy spectrum (SEM-EDS) (Hitachi, Japan, S-3400N) was used to analyze the mineral morphology characteristics, element types and contents. Fourier transform infrared spectrometer (FTIR) (NICOLET, NEXUS 670) was used to determine the chemical bonds and

functional groups in the molecules.

2.9 Statistical analysis

Statistical analysis of experimental data using Microsoft Excel 2016 software, drawing using Origin 8.0 and ArcGIS 10.2.

3. Results and discussion

3.1 Soil condition

The physicochemical properties and heavy metal content of soil were determined, and the results are shown in Table 1.

The criteria shown in Table 3 are the Agricultural Land Pollution Risk Control Standards (GB 15618-2018) issued by the Ministry of Ecological Environment of China(Ministry of Ecological Environment, 2018). It can be seen from the data that the soil is neutral soil. According to the risk screening value given by this standard, the single factor pollution index and the comprehensive pollution index are calculated, and the results are shown in Table 4.

Combining Table 2, we know that the soil pollution was mainly heavy pollution caused by Cd and Pb, and slight pollution caused by As and slight pollution caused by Zn and Cu. Among them, As, Cd, Pb, Zn, Cu, Ni, Cr and Hg were 1.59, 284.68, 6.59, 2.24, 2.46, 0.42, 0.23 and 0.41 times of the standard risk screening values, respectively.

3.2 Sequencing of strains

According to the splicing results, BLAST and similarity analysis were performed using its 16S rRNA partial sequence (1433 bp). The results showed that the strain was identified as *Pseudomonas taiwanensis* (similarity 99%, accession number : MT757966) and named as *Pseudomonas taiwanensis* strain ZM11. Its characteristics were Gram-negative, rod-shaped, Athletic, and non-sporogenous, and it was classified as G-protein bacteria(Wang L et al, 2010) and could grow at 5-42 °C, but could not grow at higher temperature (45-60 °C). The optimal growth temperature was 30-37 °C, the strain could grow at pH=4-9, and the optimum pH was 6-8(Li L-L et al, 2019). According to the development tree(Fig. 2), P4-1 strain had the closest relationship with Taiwan Pseudomonas, which was consistent with the identification results.

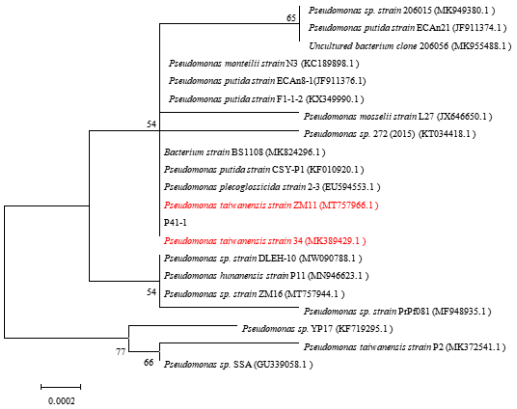

Fig. 2 Phylogenetic tree of P4 strain

3.3 Tolerance of strains

In order to study the environmental adaptability of bacteria and better understand the toxicity

mechanism of heavy metals, the concentration of heavy metals related to the environment and the physical and chemical properties were treated, and the OD value was used to describe the growth of bacteria. The results are shown in the Fig. 3.

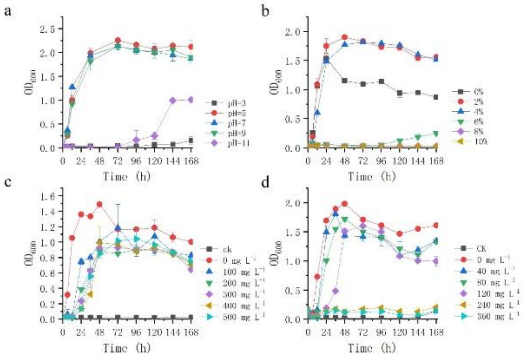

Fig. 3 Growth curve of bacteria under different factors(A: pH; B: salt; C: As(Ⅴ); D: Mn(Ⅱ))

The Fig. 3a showed that the strain could not grow normally under the condition of strong acid (pH < 3) and strong alkali (pH > 11). Moreover, under the condition of weak acid, the strain grew vigorously, and the optimum pH was 5. When the initial pH was 11, the growth of the strain increased after 84 hours, and the growth speed was accelerated. In contrast, when the initial pH was 3, the growth of the strain was always inhibited. Soil pH value is the main factor affecting the structure and composition of bacterial community(Wan W et al, 2020). The pH range of optimal growth of bacteria may directly affect its proportion in soil microbial community(Rousk J et al, 2010), thereby affecting its role in soil. In terms of salinity, when the added NaCl content was more than 4%, the growth of the strain was almost completely inhibited, and when 2% NaCl was added, the growth of the strain was more than that without NaCl (Fig. 3b). Similar to pH, salinity will change the abundance of microorganisms in the soil and affect their role in the soil(Zhang W et al, 2019). So that bacteria on pH and salinity tolerance determines the size of its role in the soil. The pH and EC values of the soil selected in this study were 7.38 and 1922, respectively, which were all within the tolerance range of P4 indicating that the soil physical and chemical conditions were suitable for the growth of this strain.

As the key point of the tolerance of bacteria, the resistance of bacteria to As, Cd and Pb is the key to their survival in contaminated soil. We used trisodium arsenate as the stress source, with 0-500 mg L^{-1} different gradient added to the bacterial liquid and observed its growth. Results as shown in the Fig. 3c, under arsenic stress, the growth of Pseudomonas Taiwan was significantly inhibited, and with the increase of arsenic concentration, the growth rate of bacteria in the initial stage decreased. When the concentration of arsenic reached 500 mg L^{-1} , in 60 h, the growth of bacteria and arsenic concentration in 200, 300, 400 mg L^{-1} at the same time. After 100 h, the highest concentration of bacteria growth began to decline. In contrast, in the blank condition and under the condition of low concentration of arsenic, the decline time of strain growth was estimated after 48 h and 60 h, respectively. It can be seen that arsenic, as a poison, delayed the growth of P. taiwanensis to a certain extent(Halan B et al, 2017). In fact, arsenic and cadmium stress on the cell membrane interaction, may be with the membrane hydroxyl, amino, phosphate. etc(Raja CE, Anbazhagan K& Selvam GS, 2006), and will promote cells to produce proteins such as superoxide dismutase (SOD), catalase (CAT), glutathione transferase (GST)(Satapute P et al, 2019), these feedbacks from microbes enable them to adsorb heavy metals in soil (Cavalcanti luna MA et al, 2015). Manganese as a resource of microbial mineralization, excessive manganese will play a toxic role on microorganisms. Therefore, we explored the manganese tolerance of microorganisms. The results (Fig. 3d) showed that when the concentration of manganese was in the

range of 0-120 mg L^{-1}, the growth of microorganisms was not strongly affected, and when the concentration of manganese reached 240 mg L^{-1}, the bacteria almost did not grow. Compared with that, under the condition of 120 mg L^{-1} manganese concentration, the bacteria reached the logarithmic phase after 36 hours, which was relatively delayed by 1 day compared with the blank. The toxic effect is relatively small, and if the manganese content is more than 120 mg L^{-1}, it can not guarantee the high survival rate of P. taiwanensis, let alone the ore production under this condition.

3.4 Removal of heavy metals in aqueous solution

The water system is relatively simple to the soil, adding heavy metal sources in water, and adding it to the mixed bacterial liquid for interaction. In order to study the performance of microorganisms in the removal of heavy metals in water, and then predict its remediation of soil. According to the data(Fig. 4), the removal rate of complex heavy metals by bacteria seems to be lower than that by single metal source in liquid phase. When bacteria were added to the culture medium containing heavy metals, the pH of the culture medium gradually increased with time. Within 60 hours, the pH of the blank culture medium increased from 6.8 to 7.82. When heavy metals were added to the culture medium, the pH of the culture medium remained the same. The difference was that when arsenic and lead were added respectively, the pH of the culture medium seemed to increase faster, after 60 hours, the pH of the culture medium increased by 1.13 and 1.23 units respectively. When the heavy metals were added to cadmium, the pH of the culture medium changed slowly before 36 hours. When the time came to 60 hours, the pH of the culture medium reached 8.24. It is worth noting that when the three heavy metals were added at the same time, the pH of the culture medium increased by 1.44 units after 60 hours.

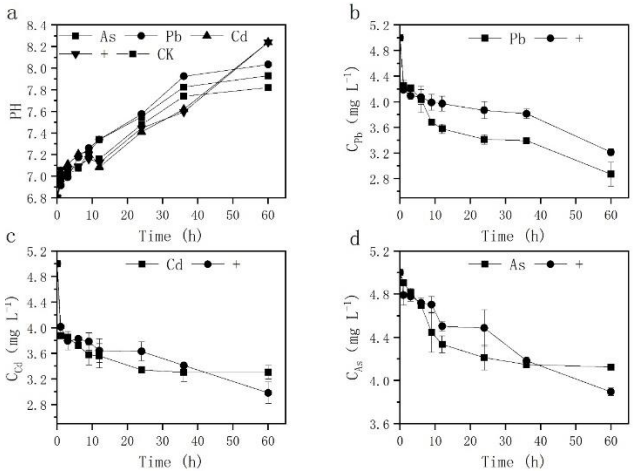

Fig. 4 Concentration and pH changes of heavy metals in single or combined polluted aqueous solution (A: pH; B: Pb; C: Cd; D: As;)

The increase of pH value will affect the growth of bacteria and the precipitation of heavy metal ions. Acidic conditions are not conducive to the oxidation of Mn(II), while Fe(III) is easy to form $Fe(OH)_3$ precipitation under alkaline conditions(Akob DM et al, 2014; Emerson D, Fleming EJ& Mcbeth JM, 2010), which is consistent with the results of Hou et al(Hou D et al, 2020),and Pacini VA et al(Pacini VA, María ingallinella A& Sanguinetti G, 2005). Whether it is due to the consumption of CO_2 by FeOB metabolism(Pacini VA, María ingallinella A& Sanguinetti G, 2005)or the environmental changes caused by MnOB(Akob DM et al, 2014), it is beneficial for bacterial mineral production.

The contents of heavy metals in the four media were tested at different time points to obtain the variation curve shown in the Fig. 4b, c, d. It can be seen from the image that when three kinds of heavy

metal sources are added alone, the decline rate of its concentration in the early stage is generally faster than that when three kinds of heavy metal sources are added at the same time. The Fig. 5 shows us the removal rates of three heavy metals after 60 hours. For arsenic and cadmium, it seems that the treatment rate of microorganisms in the compound pollution is higher, reaching 22.09% and 40.34%, respectively. On the contrary, when lead is added to the culture medium alone, the effect of microorganisms is relatively large, and in a short period of 60 h, the treatment efficiency can reach 42.61%. When it is added to the mixed liquid with the other two metals, the treatment efficiency decreases to 35.75%. Among the three heavy metals, cadmium has the highest treatment efficiency under combined pollution, and lead has the highest treatment efficiency when added alone. In a complex environment, the proteins produced by cells are related to external interference factors, and the stress of heavy metals promotes the production of proteins. When proteins bind to a variety of heavy metals, the affinity of heavy metals to these products and their own properties determine the order of their binding. On the other hand, the properties of Fe-Mn oxides, such as specific surface area, formation process, morphological structure and interaction with different heavy metals are also the key factors to determine the removal efficiency(Nelson YM et al, 2002; Xiao A, Li WC& Ye Z, 2020). The homogeneous chemical adsorption of Pb by BMO is faster than that of Cd, so that the remediation efficiency of Pb is higher(Zhou D, Kim D& Ko S, 2015). The presence of Cd and As reduces the adsorption of Pb by BMO.

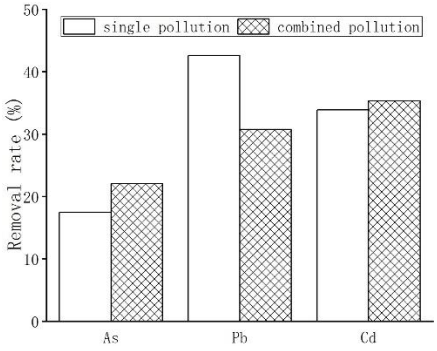

Fig. 5 Removal rate of heavy metals in aqueous solution after 60 hours

This may be due to the greater retention tendency of Cd at the exchange site. Under this pH condition, Cd interacts with the exchange site, competes with Pb, and the adsorption amount is large(Serrano S et al, 2009). The existence of Fe(II) may play a certain role in the reduction of As(V). Studies have shown that the effect of BMO on As is relatively slow, and the adsorption of As(V) is carried out after oxidation(Watanabe J et al, 2013). In the presence of a variety of metals, the adsorption of divalent metal ions is considered to inhibit the charge repulsion between the oxide surface and As(V), thereby promoting the adsorption of As(V)(Tani Y et al, 2004; Watanabe J et al, 2012). In any case, under the simultaneous stress of composite metals, biological ferromanganese ore has a certain adsorption effect on various metals in the formation process.

3.5 Effects of different iron-manganese ratios

The experiment is based on ATCC^{TM}#279 medium formula, by changing the content of Mn(II), so as to explore the conditions of different manganese iron ratio, microbial production and, at the same time, adding the same amount of arsenic stress in the medium, determine the removal of arsenic in the process of microbial production in different iron manganese ratio. The results showed that the addition amount of manganese ions was significantly positively correlated with the remediation efficiency of

microorganisms for arsenic. With the increase in the addition amount of manganese, the concentration of arsenic in the mixture after 14 days of culture decreased. When the concentration of manganese reached 105 mg L^{-1}, the remediation efficiency reached more than 68%, and the iron-manganese ratio was 1:5. Origin Pro2018 is used to fit the data and the result is shown(Fig. 6).

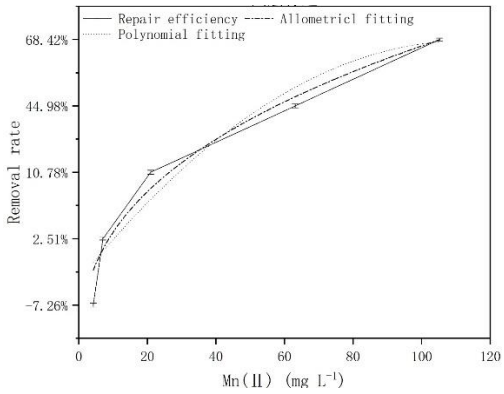

Fig. 6 Removal rate of arsenic under different manganese additions

According to the comparison of the obtained fitting curve data, the power index function is more suitable for the curve, so it can be roughly considered that the addition amount of manganese in this experiment is positively correlated with the removal efficiency of arsenic. In any case, the results showed that the removal efficiency of arsenic was the highest when the iron-manganese ratio was 1:5, which was not consistent with the results of Bai et al(Bai Y et al, 2016), because of the different forms of arsenic and the total amount of iron and manganese. During the formation of Fe-Mn oxides, As(V) and Mn(II) are adsorbed at the same time, and the adsorption mode of As(V) on the generated Fe-Mn oxides changes with time(Watanabe J et al, 2012). The increase in Mn content first increases the production of Mn oxides in the solution, so the adsorption amount of heavy metals is increased.

3.6 Morphological analysis

XRD

As shown in Fig. 7, under different heavy metal stress conditions, the diffraction characteristics of bio-manganese oxide combined with heavy metals are basically the same, with low peak and wide peak width, which is consistent with the results obtained in many studies(Hou D et al, 2020; Zhou D, Kim D& Ko S, 2015). It is reported(Zhang Z et al, 2014), compared with the chemical synthesis of manganese oxide materials, biological manganese oxide materials have poor crystallization and cannot be arranged in an orderly manner. This characteristic may be beneficial to the formation of adsorption conditions for heavy metals. Irregular crystals have larger specific surface area, which increases the surface effective binding sites and exchange sites. Under the stress of different heavy metals, the diffraction peak changed slightly, but did not show a stable peak.

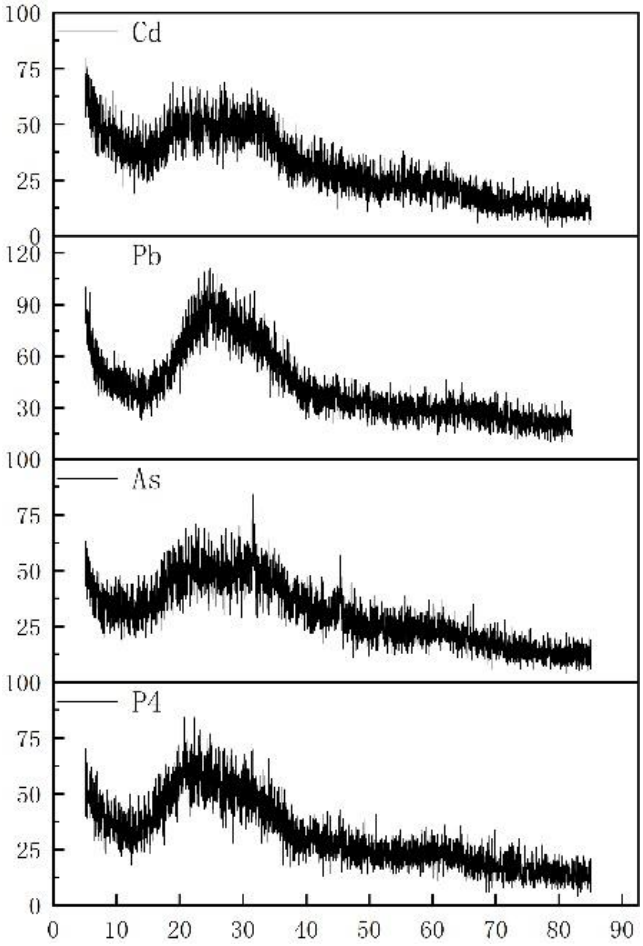

Fig. 7 XRD images of precipitates under different heavy metal treatments

FTIR

The minerals produced by P. taiwanensis were analyzed by Fourier transform infrared spectroscopy(FTIR), and the results are shown in the Fig. 8. The band in the range of 450-800 cm^{-1} can be attributed to the Mn-O vibration of manganese oxides(Feng Q et al, 1999). The absorption bands of 1059-1070 cm^{-1}, 1115-1124 cm^{-1} and 1165-1171 cm^{-1} belong to the inner Mn(III)-OH vibration. Therefore, the peaks at 696.188 cm^{-1} and 615.191 cm^{-1} are the Mn-O lattice vibration in the mineral, and the peaks at 1118.529 cm^{-1} and 1384.662 cm^{-1} are the inner Mn(III) -OH vibration(Chen X et al, 2017; Kang L et al, 2007). The peaks at 3399.943 cm^{-1}, 2929.389 cm^{-1} and 2345.054 cm^{-1} correspond to the vibration of hydroxyl and crystal water(Chen X et al, 2017). In summary, the mineral is consistent with the characterization of biological manganese oxides.

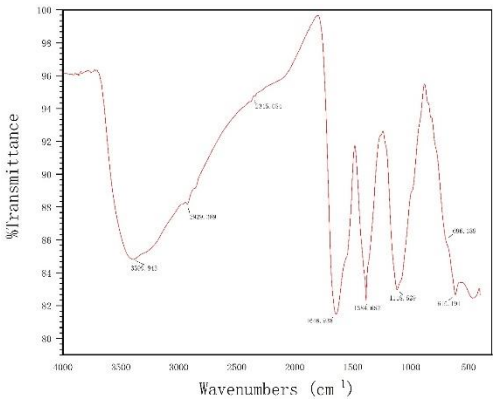

Fig. 8 FTIR spectra of minerals produced by pseudomonas taiwanensis

SEM

The scanning electron microscope images of heavy metals adsorbed by bio-manganese oxide produced during the growth of *Pseudomonas taiwanensis* were shown in Fig. 9. The irregular mineral surface generated by bacteria is uneven, and relatively flat surface can be formed under the stress of heavy metals. From the energy spectrum(Fig. 9f), it can be seen that the mineral surface contains a large number of carbon(C), oxygen(O), nitrogen(N), phosphorus(P), iron(Fe) and manganese(Mn) elements, which indicates that bio-manganese oxide is closely integrated with the organic groups generated by cells. Some scholars(Cömert S& Tepe O, 2020) proposed that bio-manganese oxide exists in the form of complex between manganese oxide and bacterial cells, which can increase the availability of organic nutrients and protect microorganisms from potential toxic substances, reactive oxygen species and ultraviolet radiation(Moura HM& Unterlass MM, 2020). From the mapping image(Fig. 9e), it can be seen that heavy metals adsorbed on its surface, and some studies have shown that(Zhang Y et al, 2019), the removal of heavy metals may also be due to the intracellular complex detoxification. In addition, it is worth noting that the surface phosphorus content is very high, phosphate and arsenate show similar physical and chemical behavior, which will replace arsenic and increase the mobility of arsenic in soil(Cao X, Ma LQ& Shiralipour A, 2003). We believe that phosphorus-containing organic matter in the mixture will compete with heavy metal ions for mineral binding sites, and the use of phosphate by bacteria may reduce this effect(Wang J et al, 2020).

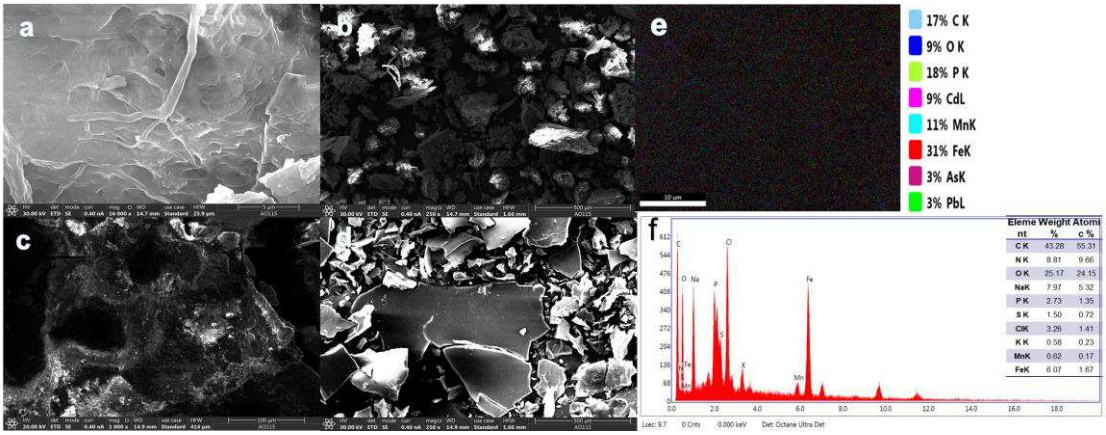

Fig. 9 SEM images and analysis results of minerals produced under different stresses
a: blank; b: Pb; c: As; d: Cd; e/f: Mapping image and eZAF Smart Quant Results under combined

pollution.

3.7 Soil remediation

The remediation efficiency of P. taiwanensis on arsenic in Luojiatan farmland soil is shown in the Fig. 10. The morphology of single heavy metal is analyzed. Before and after remediation, the changes of heavy metal morphology are different under different iron-manganese ratios. The contents of As and Pb Fe-Mn oxides are increased, and the forms of Cd and Cu are mostly decreased. For As, the content of As bioavailable fraction changed little at low concentration, and the organic fraction increased more, which may be due to the role of microorganisms. Pb mainly existed in Fe-Mn oxidation state, while its total content of bioavailable fraction was low, and the Pb in organic fraction was the largest after remediation, which could be doubled. For the most polluted Cd, the addition of microorganisms seems to have failed to make a great change in its pollution status. The metal mainly changes with the increase of carbonate state and the decrease of Fe-Mn oxide state. Similarly, under microbial remediation, the increase of Fe-Mn oxide Cu content cannot avoid the overall activation, which is mainly manifested in the maximum increase of its exchangeable state to twice. In a variety of high concentrations of heavy metals combined pollution, the role of microorganisms is not large in the aqueous phase, the different iron-manganese ratio will affect the change of iron-manganese oxidation state content, for the biologically effective state, when the iron-manganese ratio is 1:5, the removal of heavy metals is the best, respectively, 42% ; Pb, 6% ; Cu, 1% ; However, the maximum removal efficiency of bioavailable Cd was 1.78%, and the Fe/Mn ratio was 5:1.

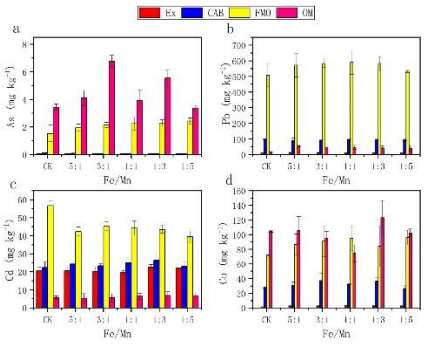

Fig. 10 Morphological changes of heavy metals under different Fe / Mn ratios

According to the data in the water phase experiment, the microbial remediation effect becomes worse under the combined stress of heavy metals. However, from the results of soil remediation, the combined stress of microorganisms on a variety of heavy metals does not seem to play a certain role, and heavy metals such as copper are activated instead. The migration and transformation of heavy metals in the soil are the results of various mechanisms. The detoxification effect of bacteria on heavy metals can only play a limited role, and the role of the medium should be considered when the bacterial solution is added to the soil. Although the phosphorus-containing medium is the growth demand of microorganisms, it may react on the passivation of heavy metals. Some studies have shown that some phosphates can promote the dissolution of minerals(Yan DY& Lo IM, 2012) and reduce the adsorption density of heavy metals on minerals(Qin W et al, 2012), which is undoubtedly contrary to our purpose. Therefore, the utilization rate of phosphate by microorganisms is also crucial.

4. Conclusion

All the results above indicate that Pseudomonas ZM11 is tolerant to heavy metals such as arsenic,

lead and cadmium, and the bio-manganese oxide produced by Pseudomonas ZM11 has a certain removal effect on heavy metals in aqueous phase, which is related to the proportion of iron and manganese added in the culture of microorganisms. The strain can be used as an effective strain for remediation of heavy metal pollution and can be used as a basis for remediation of heavy metal contaminated soil. However, the technology of bioremediation of heavy metal contaminated soil is limited. Considering the co-contamination of multiple heavy metals, its toxicity may increase. The interaction between heavy metals and their interaction with microorganisms may reduce the activity of some heavy metals and may also activate other heavy metals.

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Figures

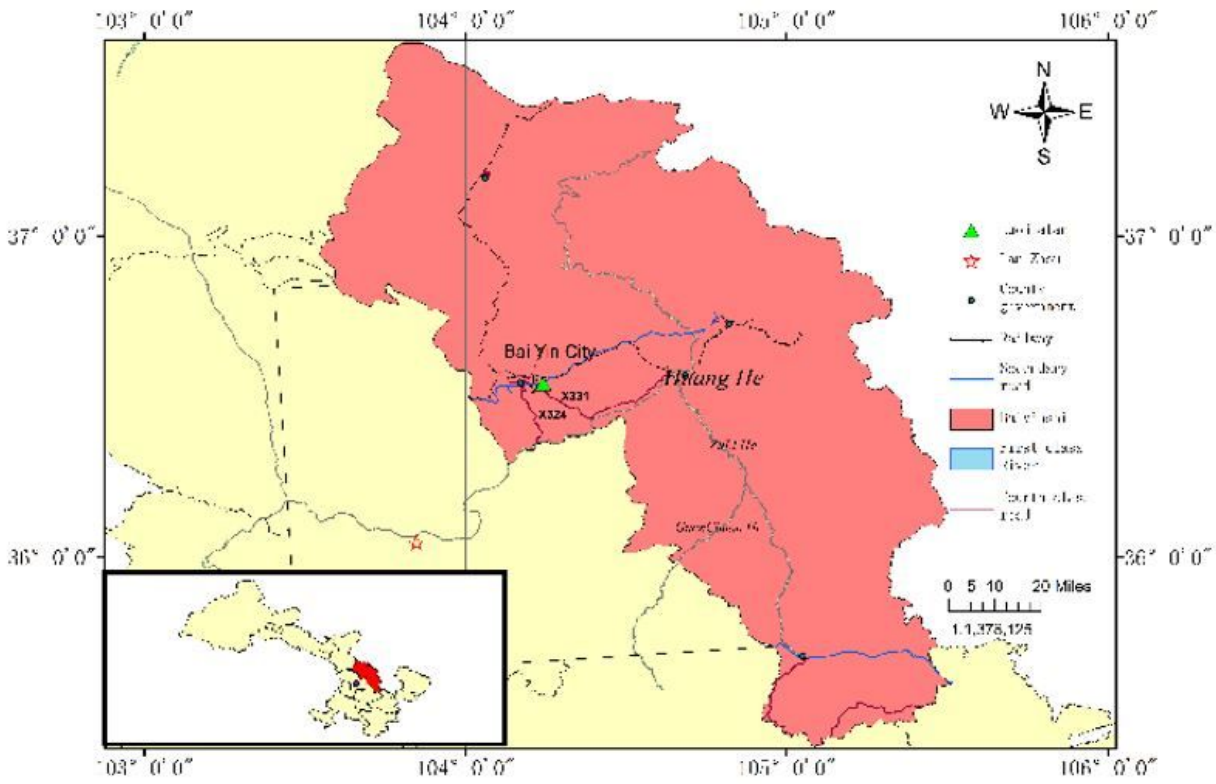

Figure 1

sampling point location Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

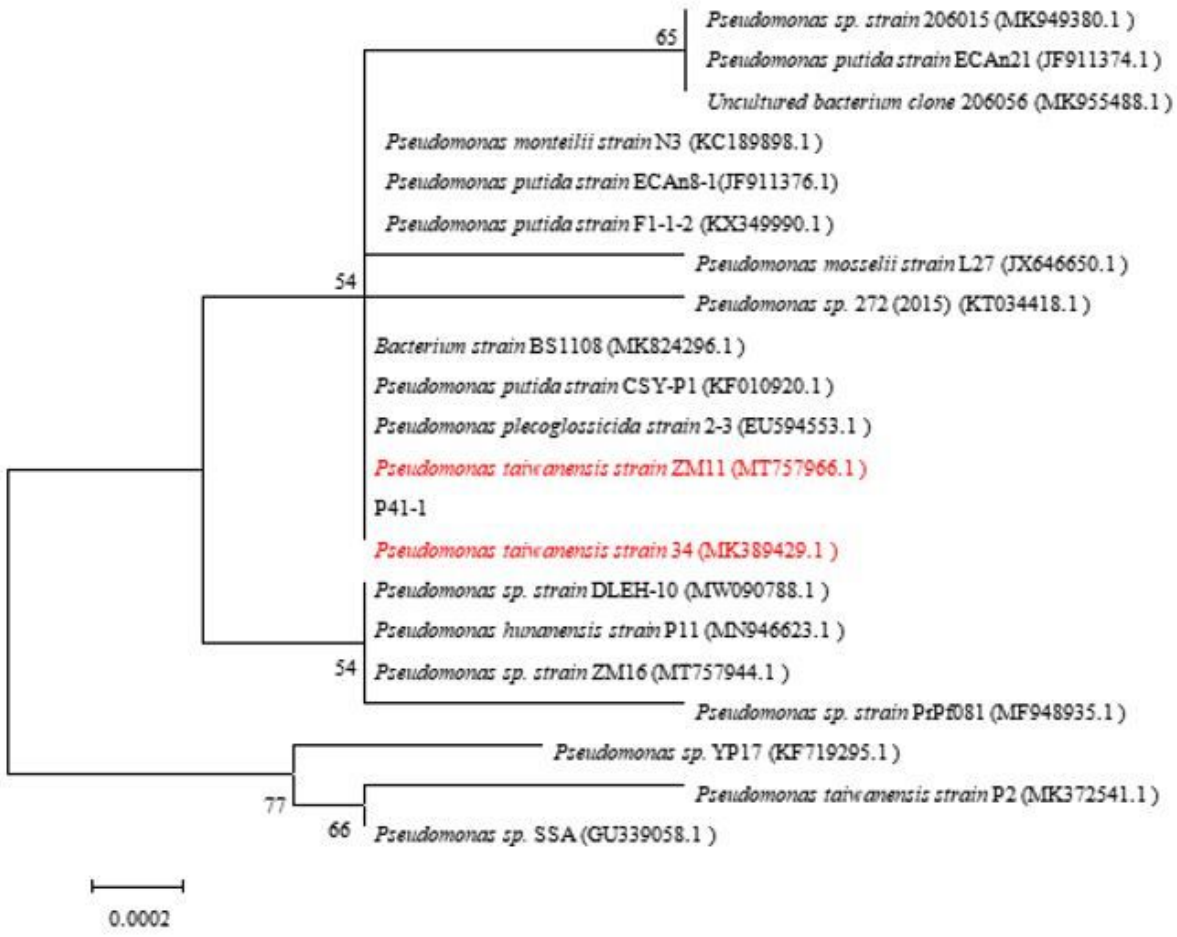

Figure 2

Phylogenetic tree of P4 strain

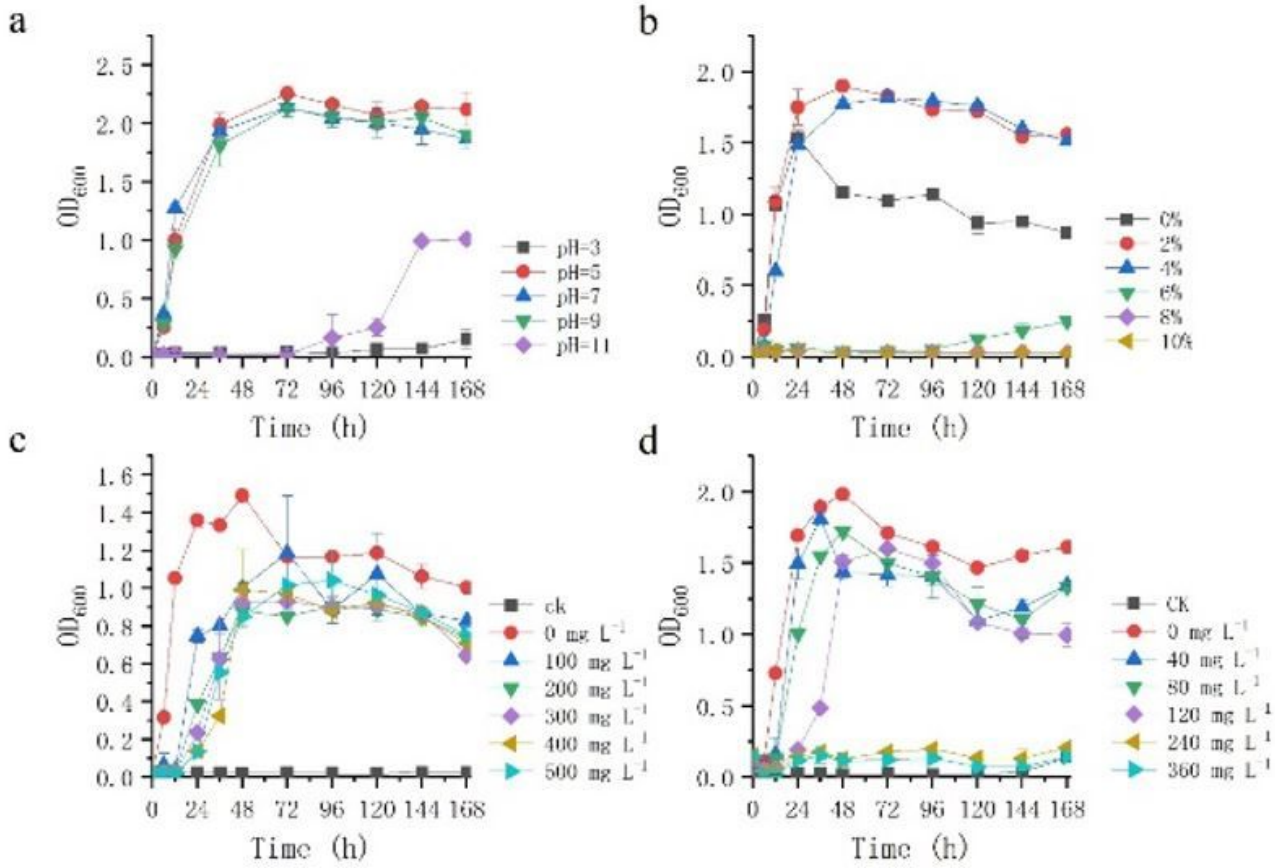

Figure 3

Growth curve of bacteria under different factors(A: pH; B: salt; C: As(Ⅲ); D: Mn(Ⅱ))

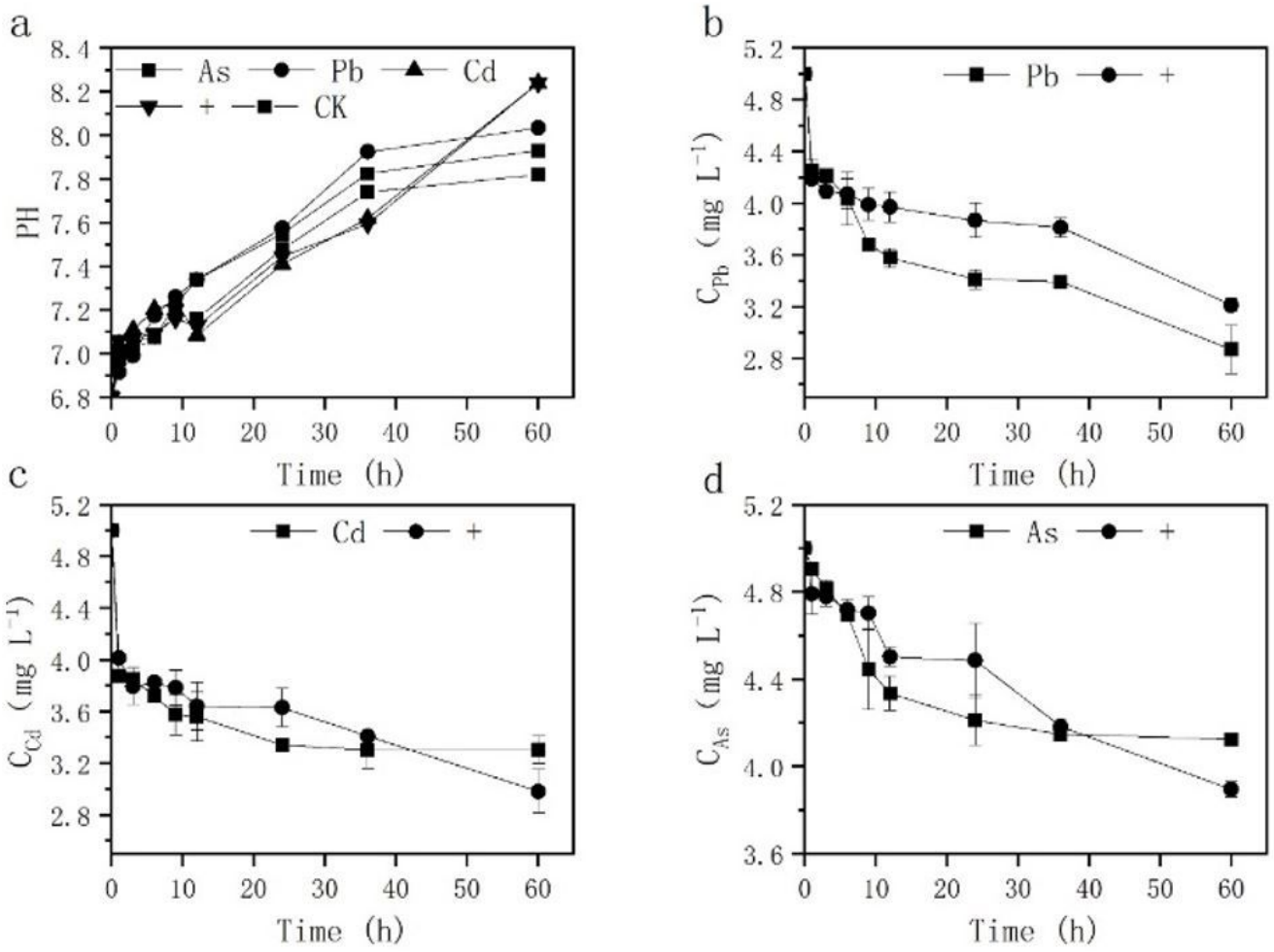

Figure 4

Concentration and pH changes of heavy metals in single or combined polluted aqueous solution (A: pH; B: Pb; C: Cd; D: As;)

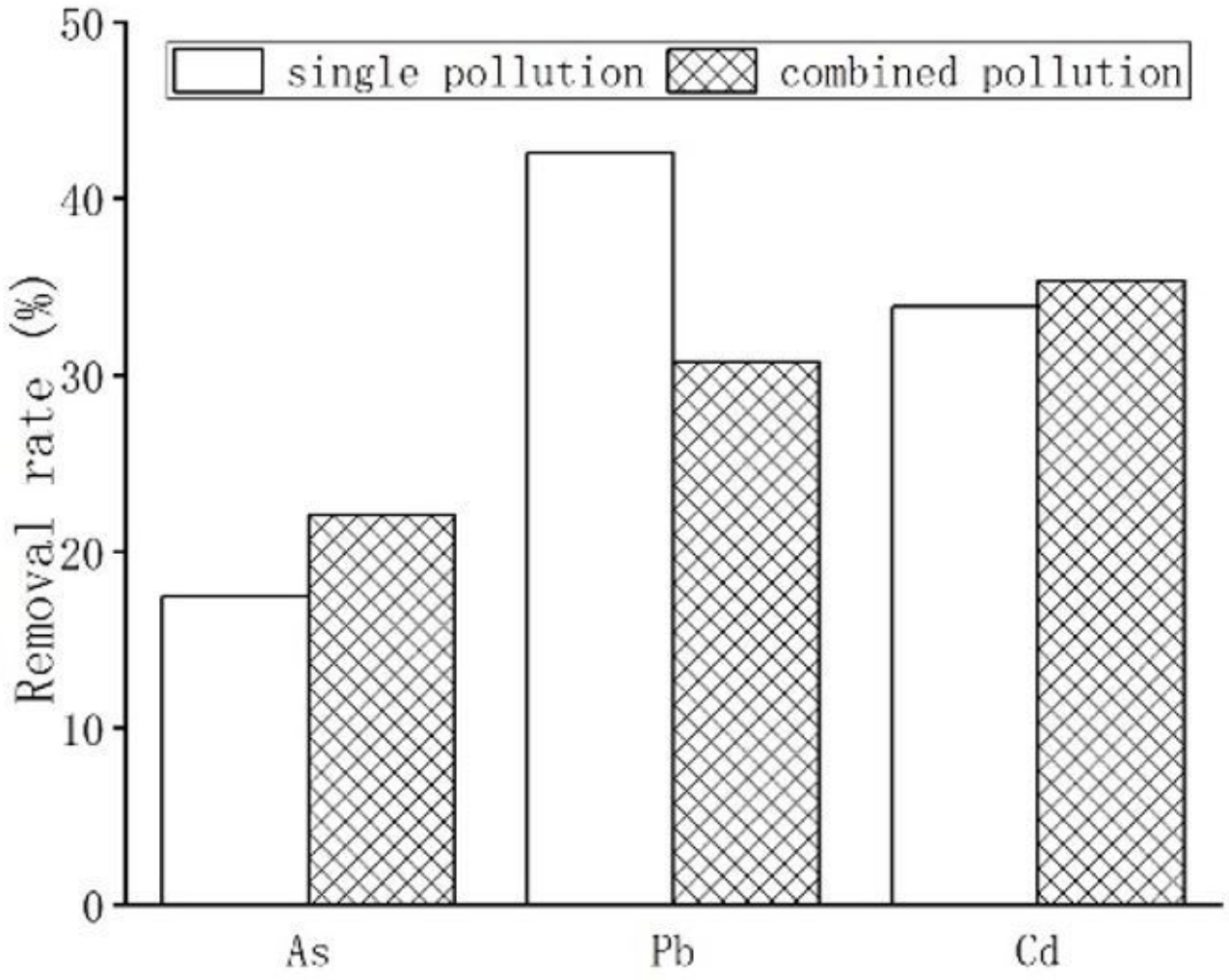

Figure 5

Removal rate of heavy metals in aqueous solution after 60 hours

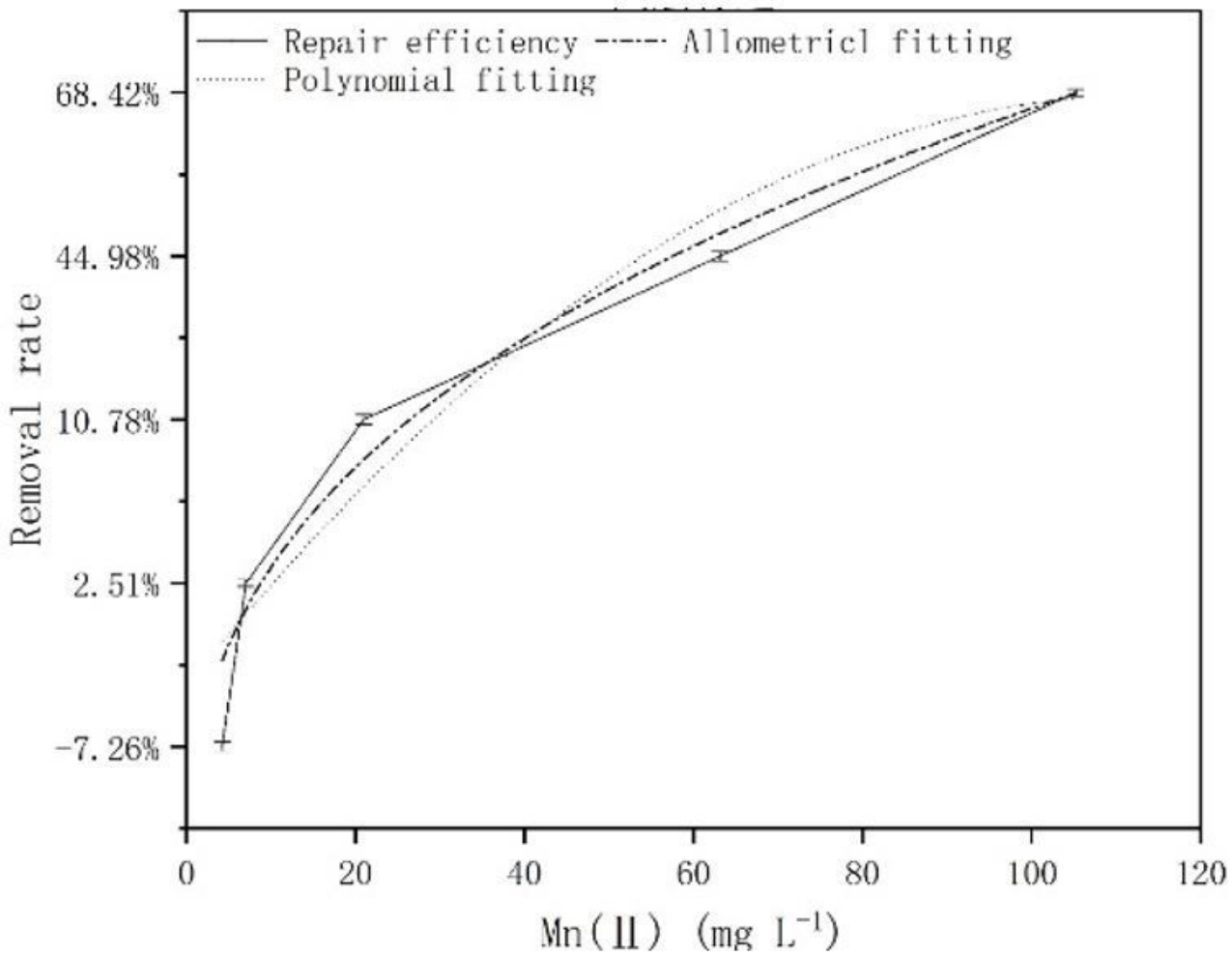

Figure 6

Removal rate of arsenic under different manganese additions

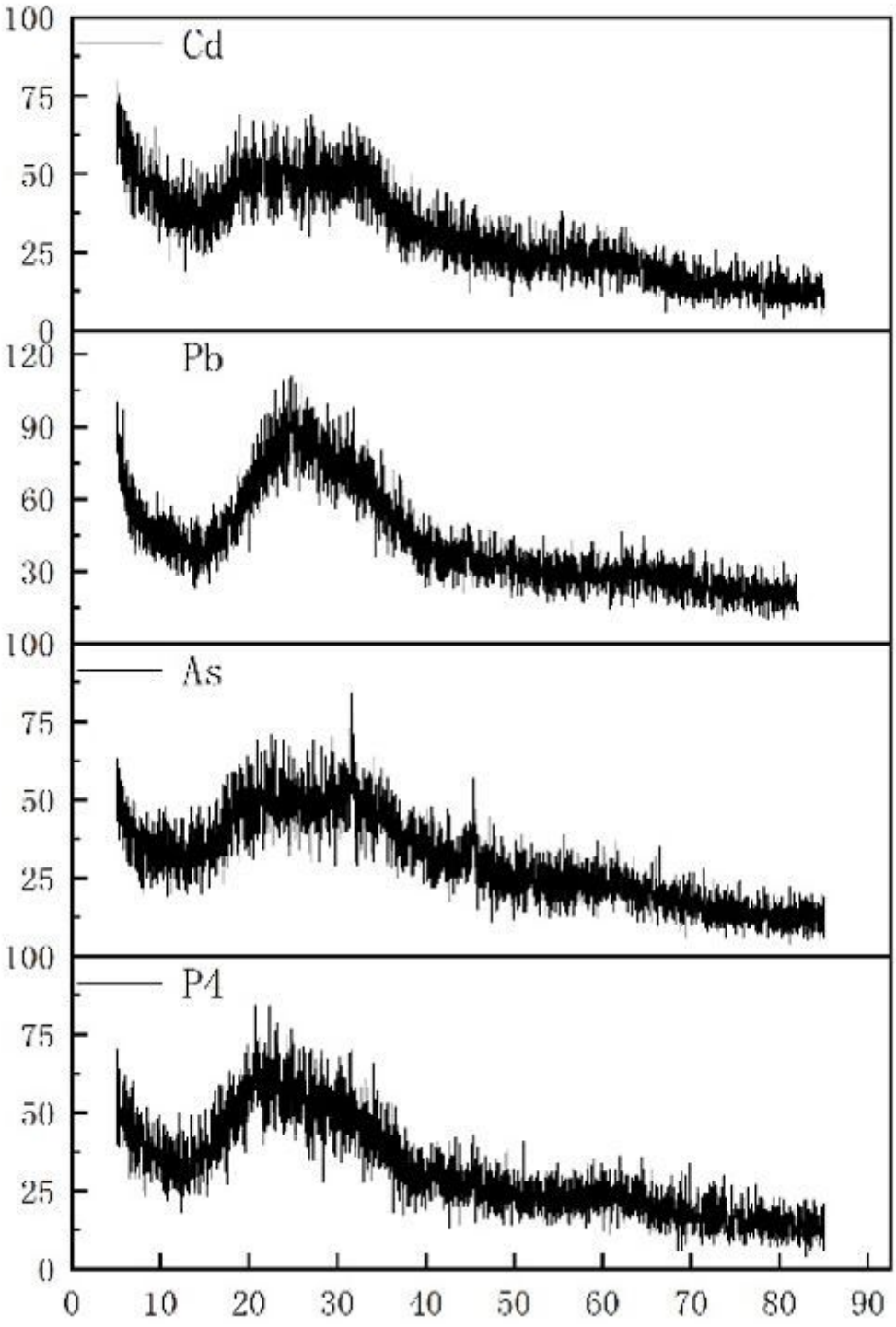

Figure 7

XRD images of precipitates under different heavy metal treatments

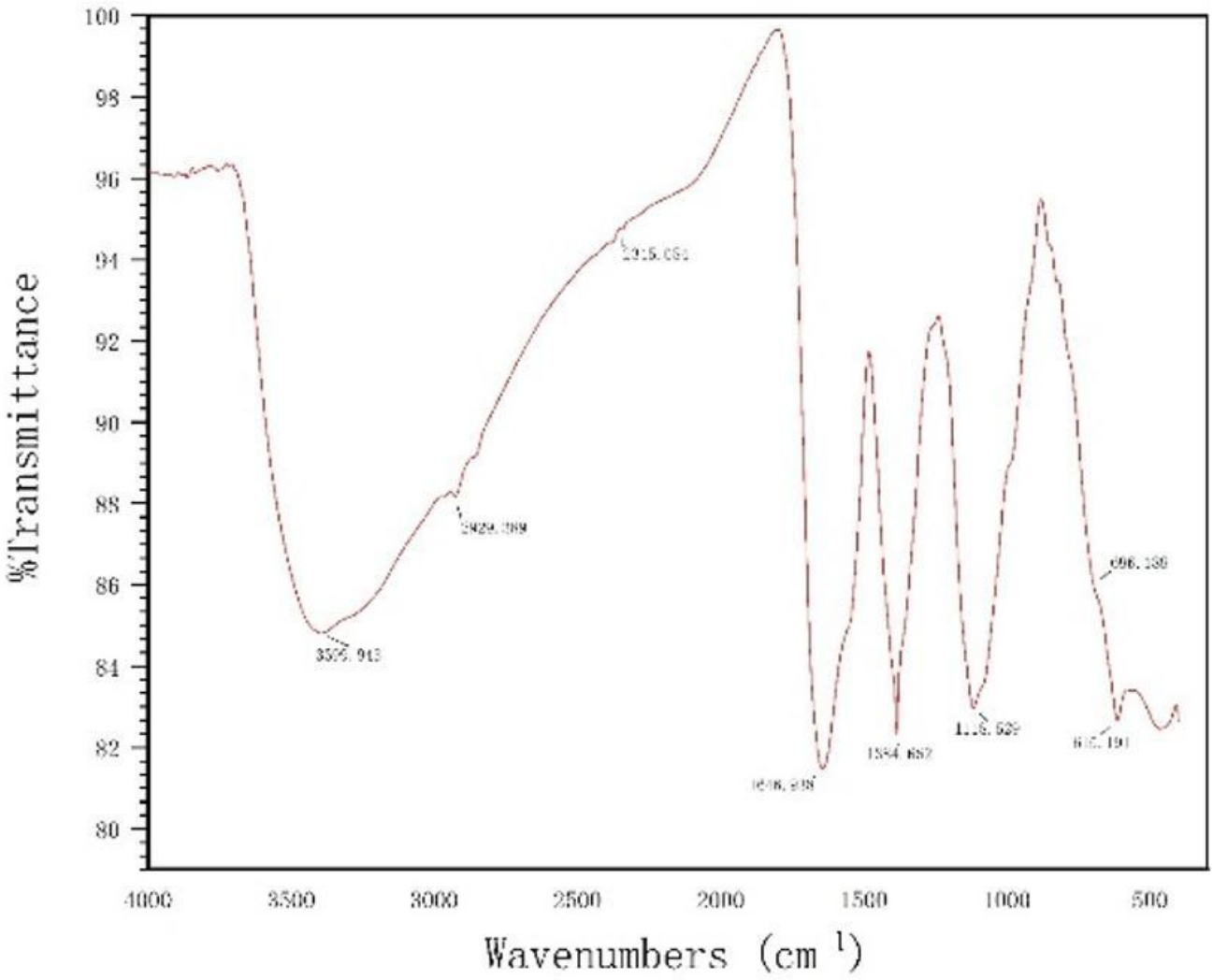

Figure 8

FTIR spectra of minerals produced by pseudomonas taiwanensis

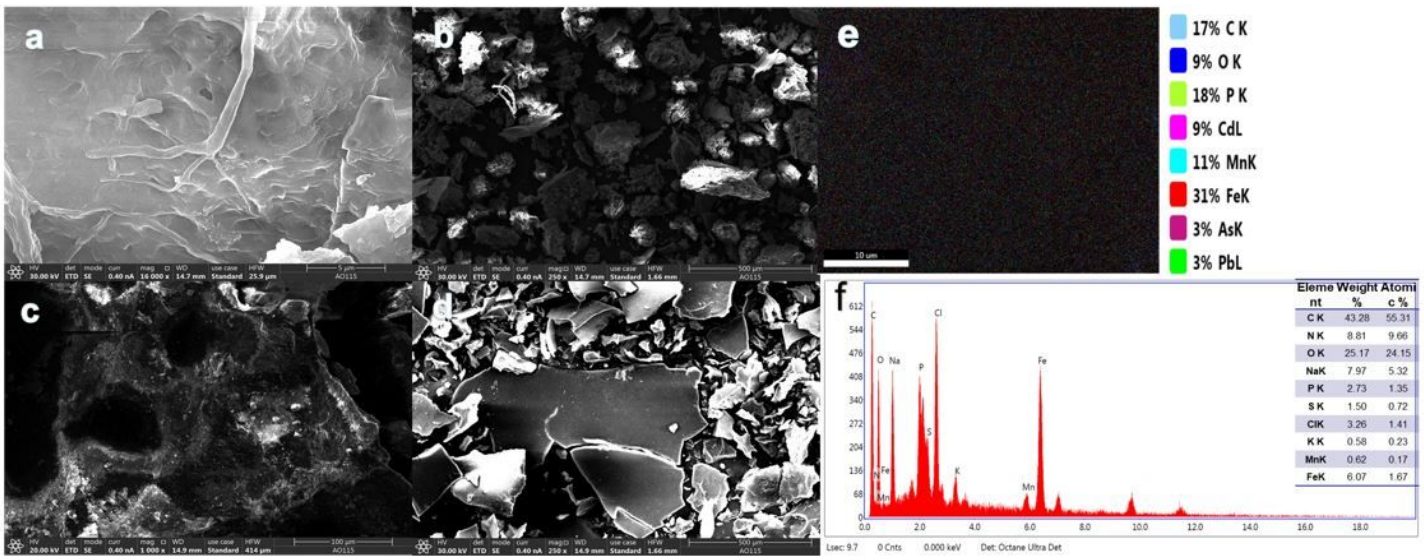

Figure 9

SEM images and analysis results of minerals produced under different stresses a: blank; b: Pb; c: As; d: Cd; e/f: Mapping image and eZAF Smart Quant Results under combined pollution.

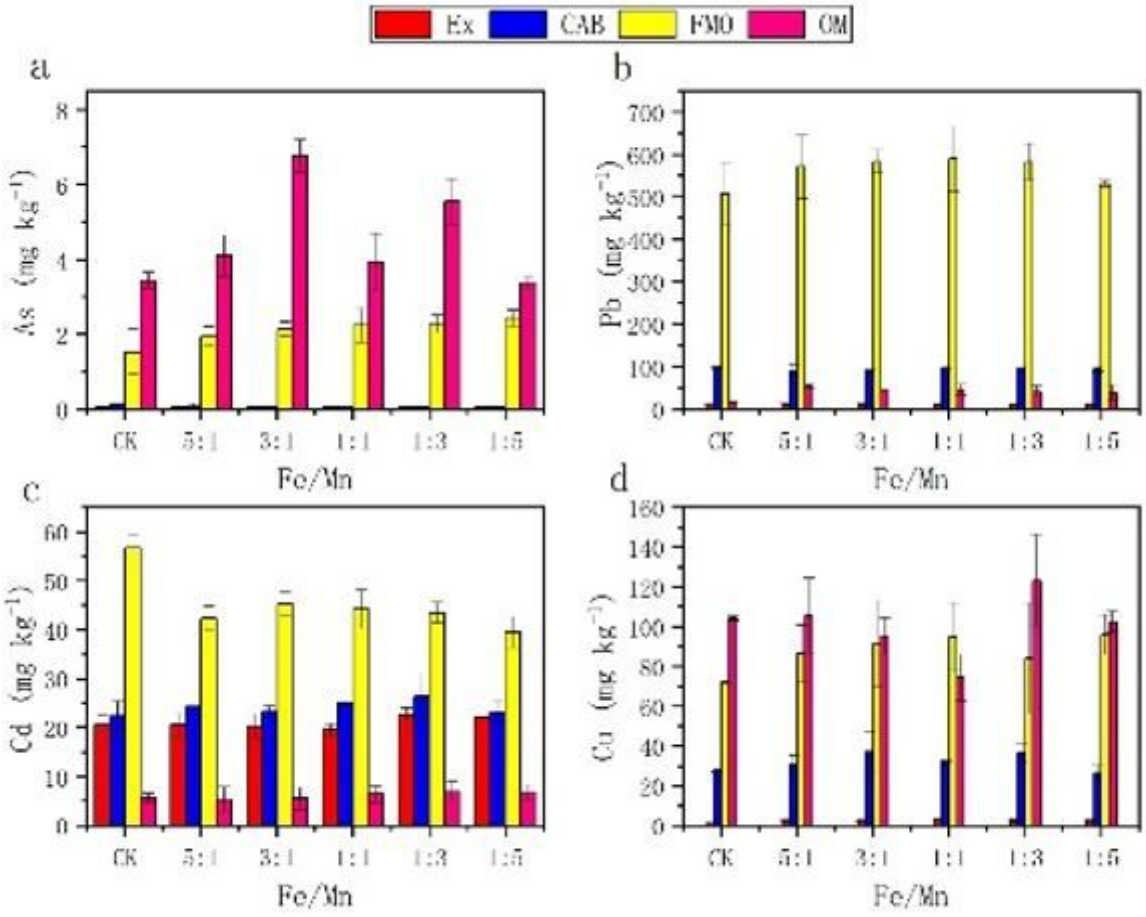

Figure 10

Morphological changes of heavy metals under different Fe / Mn ratios