Toxicity of Uranium and Lead to B.subtilis

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Abstract
B.subtilis has the broad foreground in dealing with the heavy metals waste water. It is necessary to study the toxicity of heavy metals to B.subtilis and get “the Maximum No-Effect Level ” and “the Lethal Concentration”.The toxicity of different concentration Uranium and Lead to B.subtilis in its growth was studied by three different methods in terms of measuring the diameter of the circle of bacteriostasis, counting the bacterial population by Heamacytometer and observing the change of the shape and the density of the bacteria through microscope. The experimental results have indicated that these two heavy metals had great inhibition effect to the growth of B.subtilis. The Maximum No-Effect Level of Uranium and Lead to B.subtilis are below 1mg/L and the Lethal Concentration of Uranium and Lead to B.subtilis are 500mg/L and 1000mg/L respectively. In these methods used for the toxicity of heavy metals to microorganism，counting the bacterial population are more sensitive.
Keywords：toxicity；B.subtilis；Uranium；Lead；

1. Introduction

There are two important field in the microbiological application , one is the removing of radioactive and toxic heavy metals from industry waste solid and waste water by biosorption, the other is the extracting of heavy metals from ores by microbiological leaching. It was found that bacteria, epiphyte, algae are able to absorb metal ions. For example, *B.subtilis* can absorb metal ions such as Ag^{+}、La^{3+}、Cu^{2+}、Cd^{3+}.The effect of Selenium to absorb Uranium by 67 kinds of microbe had been done by A.Nakajima(17 kinds of bacteria ,19 kinds of actinomyces, 18 kinds of algae).The results of experiment showed that the capacity of absorbing Uranium by *B.subtilis* attained *to* 48.1mg by every gram microbe. Murray and Beveridge have been found that the cell wall separating from *B.subtilis* can unite large numbers of Mg^{2+}、Fe^{3+}、Cu^{2+}、Na^{+} and K^{+}, medium numbers of Mn^{2+}、Zn^{2+}、Ca^{2+}、Au^{3+} and $Ni^{2+}$and a small quantity of Hg^{2+}、Sr^{2+}、Pb^{2+} and Ag^{+} from water, but Li^{+}、Ba^{2+}、Co^{2+}、Al^{3+}which can not be united can form the microcosmic-gold-crystal when put the cell wall to the water which contains AuCl. It is obvious that B.subtilis has the broad foreground in dealing with the heavy metals waste water. The premiss of application of microbe is that microbe is not killed by heavy metals. So it is necessary for us to study the toxicity of heavy metals to *B.subtilis* and get “the Maximum No-Effect Level ” and “the Lethal Concentration”.

The toxicity of Uranium which is radioactive metal to *B.subtilis* has been studied for the first time. At the same time, the toxicity of Lead to *B.subtilis* has been compared with that of Uranium and the different methods has also been compared. The study of this experiment offers the basic data to the toxicity of Uranium to microbe, the appraisement and detection of microbiology.

2. Experimental

2.1 Materials

The strain of Bacillus subtilis was obtained from the microbe staff room of School of Architectural Engineering, Resources and Environment in Nanhua University. Before the toxicity experiments, the bacterial had been purified and activated by streak inoculation and repeating

culture.

A standard 1000mg/L Uranium concentration solution was confected according to the method given by EJ 267.3-84.The U_3O_8 used was standard pure. The series of different Uranium concentration solutions were put up by diluting or concentrating the above standard solution. the Uranium concentration of these solution is1、100、250、500、1000、2000mg/L respectively. Similarly, the different lead concentration solutions were prepared by dissolving the analytic reagent $Pb(NO_3)_2$ in distilled water in proportion. The lead concentration of these solution is1、100、250、500、1000、2000mg/L respectively.

The basal culture medium in the experiment was beef-proteose peptone culture medium. The experimental culture medium which contained different U and Pb concentration was prepared by fixing the heavy metal solution into the basal culture medium in proportion. All these culture medium were sterilized before inoculation.

2.2 Experimental Procedure

All the following operational work was performed according to the aseptic manipulation.

2.2.1The method of the antibacterial ring

The strain of B.subtilis was cultured in basal liquid medium for 24h at 37℃. The 0.25 mL culture fluid was coated onto the surface of the plate medium evenly for inoculation. Each plate medium was divided into three parts. Each part was stuck one piece of sterilized filter paper with a diameter of 10mm. 0.1 mL heavy metal solution was dropped onto the filter paper for the toxicity test. The plate medium was placed upside down into the incubator at 37℃.After 24h, the antibacterial ring appeared. The diameter of antibacterial ring was measured by ruler. The sketch map of the antibacterial ring was showed in Fig 1.

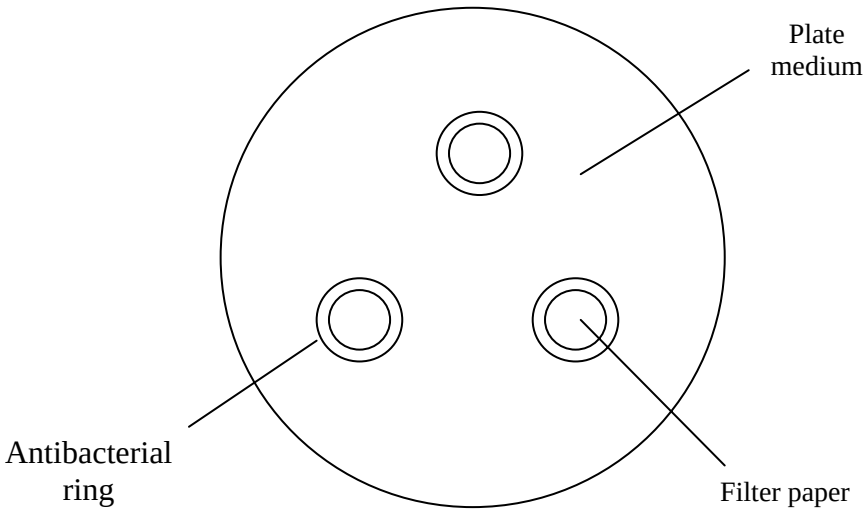


Fig 1 The sketch map of the antibacterial ring

2.2.2The method of the bacterial population

The bacteria were inoculated into the experimental liquid medium. The amount of the bacteria in the medium was taken count by Heamacytometer after the bacteria was cultured for 24h at 37℃.

2.2.3 The method of the shape of the bacteria

The bacteria which were cultured in experimental culture for 24h were stained by crystal violet oxalate. Under the photomicroscope, the pictures of the bacteria were taken in order to observe the change of the shape and the density of the bacteria.

3. Results

3.1The toxicity of Uranium to *B.subtilis*

3.1.1The method of the antibacterial ring

If a kind of solution dropped into the filter paper is toxic to bacteria, the bacteria at the edge of the filter paper will die and an antibacterial ring comes into being. So the diameter of the antibacterial ring can reflect the inhibitor effectiveness of the solution to the growth of bacteria. The diameter of the antibacterial ring under the different Uranium concentration solution was showed in the table 1. From table 1, it can be seen that the antibacterial ring didn't appear, when the Uranium concentration was 1 mg/L and 100 mg/L. The inhibitor effectiveness of Uranium to *B.subtilis* on the solid medium is not obvious under these two concentrations. When the concentration of U increased from 250 mg/L to 2000 mg/L, the antibacterial ring appeared. The growth of the bacterial was restrained by Uranium. The toxicity of Uranium to *B.subtilis* had enhanced with the increasing of Uranium concentration.

Observing the growth of *B.subtilis* on the solid medium, it can be found that the colonies of bacteria near the edge of the filter paper was much smaller than those far away from the filter paper. The diameter of the former was about 0.1mm, while the diameter of the latter was approximately 2~3mm which was the normal size. It is clear that the heavy metal of Uranium can restrain the growth of *B.subtilis*, even lead the death of the bacteria.

Table1 The diameter of antibacterial ring of B.subtilis under different U concentrations

U concentration /（mg·L^{-1}）	0	1	100	250	500	1000	2000
The diameter of antibacterial ring /mm	10	10	10	10.50	11.00	12.00	12.33

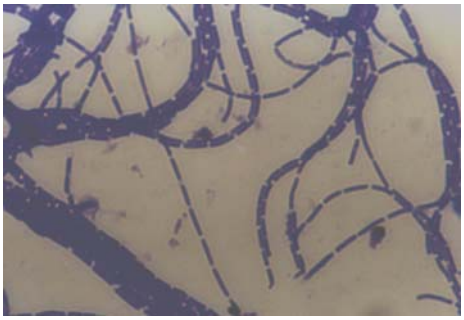
3.1.2The method of the bacteria population

The bacteria population of *B.subtilis* under different concentration of Uranium was showed in Table 2. From the Table 2, it can be seen that the bacteria population decreased rapidly with the increasing of the Uranium concentration.

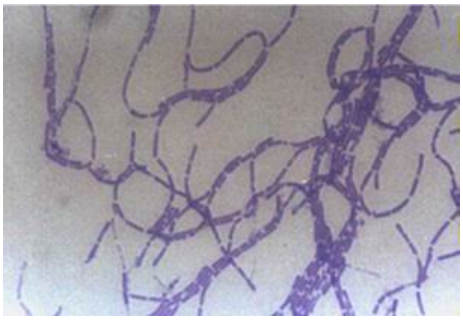
Table2 The bacterial population of *B.subtilis* under different U concentrations

U Concentration /（mg·L^{-1}）	Bacterial population/（unit·mL^{-1}）	Percentage of decrement /%
0	12.825×10^{9}	
1	4.325×10^{9}	66.28
100	5.926×10^{8}	95.38
250	9.778×10^{7}	99.24
500	4.125×10^{5}	99.997
1000	0	100
2000	0	100

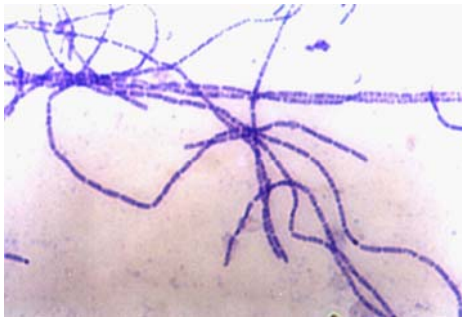
3.1.3The method of the shape of the bacteria



A U concentration=0mg/L



B U concentration =1mg/L



C U concentration =250mg/L

Fig 2 the shape and the density of *B.subtilis* under different U concentrations

Fig 2 included three photos of the bacteria in different Uranium concentration culture medium. Comparing A and B, it is obvious that the quantity of the bacteria dropped off. When the Uranium concentration was 250mg/L, the bacteria became shorter and more slender gradually. The higher concentration of Uranium can restrain the growth of bacteria completely.

3.2the toxicity of Lead to *B.subtilis*

3.2.1the method of the antibacterial ring

The antibacterial ring appeared when the concentration of Lead reached 500 mg/L. Compare with another two methods, the sensitivity of this method is lower for determination the toxicity of heavy metals.

Table 3 The diameter of antibacterial ring of *B.subtilis* under different Lead concentrations

Lead Concentration / （$mg \cdot L^{-1}$）	0	1	100	250	500	1000	2000
The diameter of antibacterial ring /mm	10	10	10	10	10.83	11.53	12.00

3.2.2the method of the bacteria population

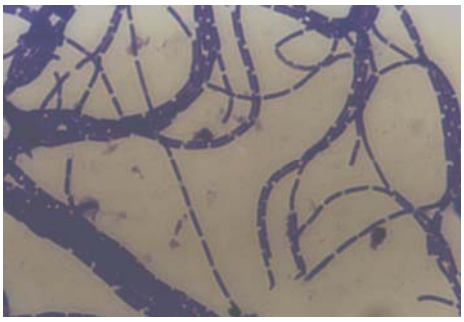
From Table 4, it can be seen that the amount of the bacteria reduced obviously with increasing of Lead concentration. When the concentration of Lead was 1mg/L, the population of the bacteria had almost reduced by half.

Table4 The bacterial population of B.subtilis under different Pb concentrations

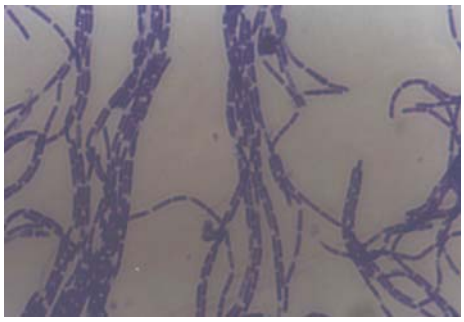
Lead Concentration /（mg·L^{-1}）	Bacterial population/（unit·mL^{-1}）	Percentage of decrement /%
0	12.825×10^{9}	
1	7.100×10^{9}	44.64
100	8.575×10^{8}	81.87
250	6.925×10^{7}	93.31
500	4.000×10^{2}	99.995
1000	0	100
2000	0	100

3.2.3 The method of the shape of the bacteria

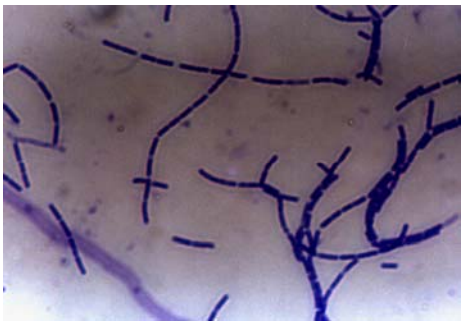
The photos in Fig 3 showed the change of the shape and the density of *B.subtilis* under different Lead concentration. In Photos B, the quantity of the bacteria decreased and the shape of the bacteria had no obvious change. At this concentration, the heavy metal of Lead can inhibit the growth of *B.subtilis*, but it had no effect to the shape of the bacteria. When the concentration reached 250 mg/L, the amount of the bacteria reduced rapidly. At the same time, the chain of the bacteria became much shorter. In Photos D, the brood cell of *B.subtilis* appeared. The brood cell is an especial state of bacteria to resist the bad growth conditions. The bacteria was not able to connect into a line, just became a dot. In Photos E, there were almost no bacteria. Hence, the heavy metal of Lead can badly influence the growth and the the growth and the shape of *B.subtilis*, even lead the death of the bacteria.



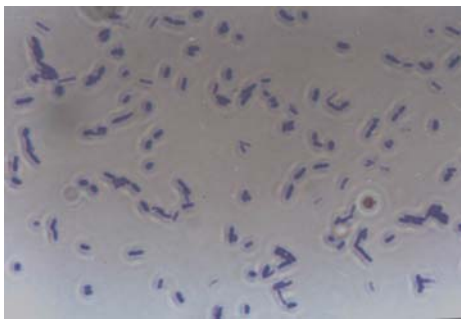
A Lead concentration=0mg/L



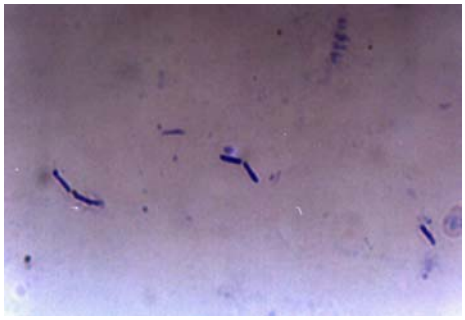
B Lead concentration=1mg/L



C Lead concentration=250mg/L



D Lead concentration=500mg/L



E Lead concentration=1000mg/L

Fig3 The shape and the density of *B.subtilis* under different Lead concentrations

3.3 Comparing the toxicity of Uranium with Lead to *B.subtilis*

At the same concentration the toxicity of Uranium was larger than that of Lead. When the antibacterial ring appeared, the concentration of Uranium was 250mg/L, while the concentration of Lead was 500mg/L. In the Fig 4, the tangent slope of the Uranium curve was larger than that of the Lead curve. It can draw the same conclusion by Comparing the Fig 2 with Fig 3

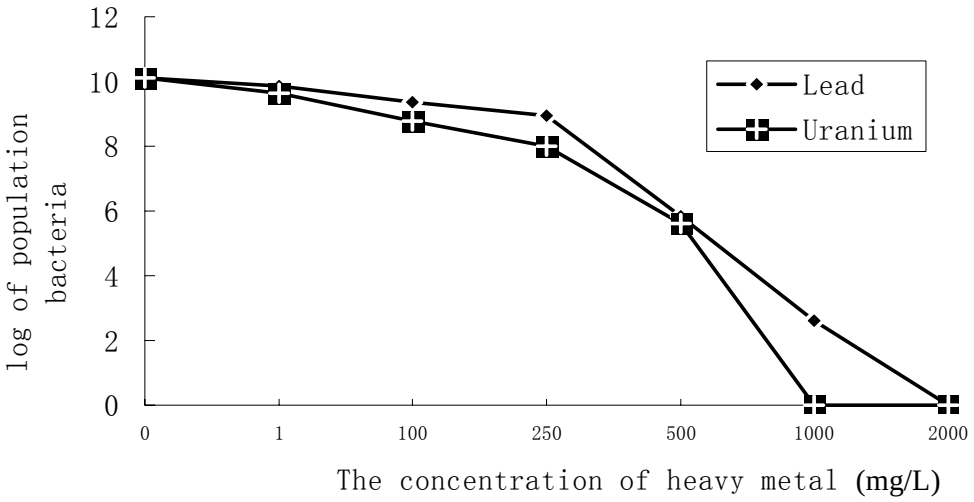


Fig4 Log of population bacteria of *B.subtilis* under different Lead and Uranium concentrations

4. Conclusion

(1)Uranium and Lead have distinct toxicity to *B.subtilis*. The Maximum No-Effect Level of Uranium and Lead to *B.subtilis* are below 1mg/L and the Lethal Concentration of Uranium and Lead to *B.subtilis* are 500mg/L and 1000mg/L respectively. When the concentration of Uranium and Lead was 1mg/L,the amount of *B.subtilis* had reduced greatly. So the Maximum No-Effect Level of Uranium and Lead to *B.subtilis* should be below 1mg/L.The farther research should be carried out to confirm the determinate concentration. When the concentration of Uranium was 500mg/L,the bacteria of *B.subtilis* can not growth, so the Lethal Concentration of Uranium and Lead t to *B.subtilis* is confirmed 500mg/L and 1000mg/L respectively.

(2) In these three methods used for the toxicity of heavy metals to microorganism，counting the bacterial population are more sensitive than the method of bacteriostasis ring and observing the change of the shape and the density of the bacteria through microscope. The reason of that is the bacteria can contact the heavy metals more direct in the liquid medium than that in the plate medium. This experiment has provided the reference to choose the appropriate method for

studying the toxicity of heavy metals to microorganism. The method of counting the bacterial population is better for studying the effect of heavy metals to the growth and the propagation of the microorganism. If the influence of heavy metals to the shape of the bacteria is studied, the method of obverting bacteria through microscope should be chosen. While if the effect of heavy metals to the genome of the microorganism need to be research, the technique of molecular biology such as SSH(Suppression Subtractive Hybridization) should be applied.

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