The Effect of Bacteria on Seed Germination in Sorghum and Rape Under Cadmium and Petroleum Conditions

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Abstract: A large amount of oil hydrocarbons and heavy metals have been discharged into the environment and caused soil polluted. Petroleum and cadmium in soil accumulated in crops and lead to threaten human healthy through the food chain. In this experiment, seeds of sorghum and rape were germinated in deferent concentrations of petroleum and cadmium, and the effect of *Peptococcus activus* sp. SH3-3-9 on the germination was studied. The results showed that petroleum and cadmium inhibited seeds germination, and the effects were stronger as their concentrations increased. *Peptococcus activus* sp.SH3-3-9 had the role of enhancing seed germination, which indicates it has high potential in plant-microbial remediation of petroleum and cadmium in soil.

Keywords: Bacteria, cadmium, petroleum, sorghum, rape.

INTRODUCTION

In current, the exploitation and demand of oil is increasing with the acceleration of industrialization. The world energy mainly relies on oil. In the United States, 30% of the energy provided by the oil, this ratio in Britain reached 50% [1]. The organization of the Petroleum Exporting Countries estimates that about 100 millions barrels of oil per day will be consumed in the globe by 2020 [2]. Petroleum is mainly composed of hydrocarbon and non hydrocarbon compounds [3]. There are many kinds of non hydrocarbon compounds in crude oil [4], including many pollutions such as oxygen containing hydrocarbon derivatives, sulfur containing hydrocarbon derivatives, nitrogen containing hydrocarbon derivatives, colloid in the oil, asphalt, etc. [5, 6]. The crude oil entering the soil in various forms adheres to the soil particles directly affecting the permeability of the soil, so as to reduce soil fertility [7, 8], on the other hand, it destroys the balance between soil and plant water, hinder the normal respiration of root, and affect the growth of plants [9, 10]. Which caused the changes of soil physical and chemical properties, groundwater pollution, crop growth inhibition, microbial community structure changes, etc. [11] Some constituents of petroleum in soil accumulated in crops, and can threaten human healthy through the food chain. Those toxic and harmful substances were harmful to the human nervous system, urinary system, respiratory system, circulatory system, blood system, etc., and have the effects of carcinogenesis, teratogenesis and mutagenesis to the mammals and human beings.

In the process of petroleum exploration, production and transportation, due to natural and man-made reasons, a large amount of oil hydrocarbons and heavy metals are discharged into the environment, which caused a large amount of soil has been polluted by oil as well as heavy metals [12], Heavy metals in soil were high toxicity and persistence. cadmium is one of the most toxic element of those [13, 14]. Cadmium in the soil is accumulated in the edible part of the plant through the absorption and translocation of the plant roots. Because the half-time of Cd in human body is usually 20 - 40a, if intaking Cd for a long time and Cd content exceeded the amount of tolerance intake (PTW I; 7 u g/kg body weight per week) , human will accumulate Cd in body and threaten healthy severely [15, 16].

Microorganism has the effect of degrading crude oil, and bioremediation has become an important method to repair contaminated soil because of its low cost and in situ remediation [17]. The main cause of Cd pollution in soil is the industrial wastes emissions, sewage irrigation, pesticide and fertilizer application. Cd pollution has more and more damaged to farming soil, plants and microorganisms, and may lead to accumulation in human body [18]. Plant roots can absorb Cd, but the important limiting factor for phytoremediation of heavy metal is the inhibition of plant growth, and the small biomass. In this study, Sorghum and rape seeds were used, considering their sensitive characteristics to Cd and crude oil, to investigate the response of seeds to crude oil, Cd pollution and microorganism.

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MATERIALS AND METHODS

Microorganisms

The strain of *Peptococcus activus* sp.SH3-3-9, screened from the oil contaminated soil from the Shenyang economic and Technological Development Zone, was applied as petroleum degrading bacterium. The Culture medium is: $CaCl_2$ 0.02g , K_2HPO_4 1.5g , NaCl 0.5g, $MgSO_4 \cdot 7H_2O$ 0.2g , $NaNO_3$ 2g , $FeSO_4 \cdot 7H_2O$ 0.01g , crude oil 5g , water 1000ml , pH7, steam sterilization (121°C, 30min).The colony of bacteria was gray or cream colour, raised, with complete edge. Anaerobic culturing for 5-7 days, the number of viable bacteria was about $5 \times l0^9$ / ml, the cultrue was diluted to one tenth with water for the suspension of the bacteria.

Methods

The full seeds of sorghum and rape were soaked in the greenhouse at about 25°C for 3 hours and then these seeds covered with filter paper were placed in Petri dishes in a controlled growth chamber under the conditions of 28°C. Solutions of 10, 30, 50, 100, 200mg/mL crude oil content and 100, 200, 300, 400mmol/l $CdCl_2$ content were prepared respectively. And the above solution concentrations were also doubled and then mixed with the same amount of bacteria suspension. These solutions were used to moisten the filter paper. The treatments to sorghum seeds were also include: 30/100, 50/100, 30/300, 50/300 crude oil (mg/ml)/ $CdCl_2$ (mmol/l), and the treatments to rape seeds were also include: 30/50, 30/100, 50/50, 50/100 crude oil (mg/ml)/ $CdCl_2$ (mmol/l), which compare with the treatments with the same level of crude oil/ $CdCl_2$ concentration just mixed with bacteria suspension, that is a total of 64 treatments were set up in this experiment, and the water treatment was used as control. Each treatment was with three relicates. Seed germination rate was measured after 6 days treatment. Seed germination rate=(the number of germination seeds /the total number of seeds)×100%. The SSR method was adopted for sample multiple comparison.

RESULTS AND ANALYSIS

Effects of Crude Oil and Bacteria on the Seed Germination Rate of Sorghum and Rape

Data are means of 3 replicates (n=3). Data within same column with different letters indicated significant difference ($P<0.05$).

Figures **1** and **2** show crude oil significantly inhibited the seed germination of sorghum and rape, and the inhibition effect increased with the increase of the concentration of crude oil. *Peptococcus activus* sp.SH3-3-9 can relieve the inhibition effect. The inhibition of 10mg/ml crude oil on the germination of rape seeds was not significant, while the 30mg/ml crude oil has significant inhibition effect, and the germination rate was less than 50% when the concentration of crude oil was higher than 50mg/ml. The inhibition effect of crude oil on sorghum was stronger than rape. the germination rate of rape was less than 50% at 10mg/ml crude oil. Under the various concentration of crude oils, *Peptococcus activus* sp.SH3-3-9 can promote the germination of seeds. Except at the lower crude oil concentration (10 and 30mg/ml), the bacteria performance significant promotion effect on the germination of sorghum and rape seeds at any other concentration of crude oil.

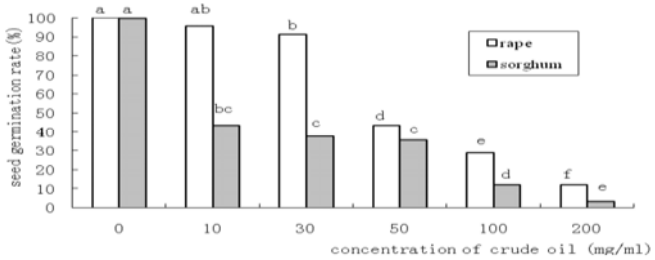

Figure 1: Effect of crude oil on seed germination in sorghum and rape.

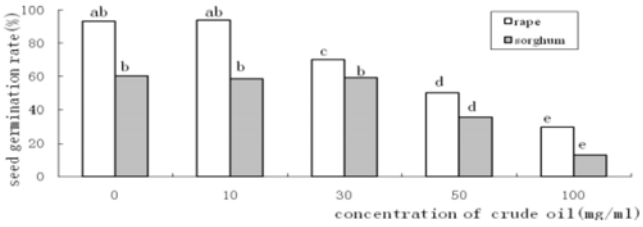

Figure 2: Effect of crude oil on seed germination in sorghum and rape as bacteria were applied.

Effects of Cd and Bacteria on the Seed Germination Rate of Sorghum and Rape

Figures **3** and **4** show Cd significantly inhibited the seed germination of sorghum and rape, and the inhibition effect increased with the increase of the concentration of Cd. The inhibition effect of $CdCl_2$ on the germination of seeds was weakest at 100 mmol/l, the germination rate of rape decreased to 50% at 200mmol/l $CdCl_2$, while that of sorghum decreased to 50% at 300mmol/l $CdCl_2$, which indicates that the adaptability of sorghum to Cd is higher than that of

rape. the microorganisms suplied in this experiment release this inhibition effectively, the effect is most pronounced at 200mmol/l $CdCl_2$.

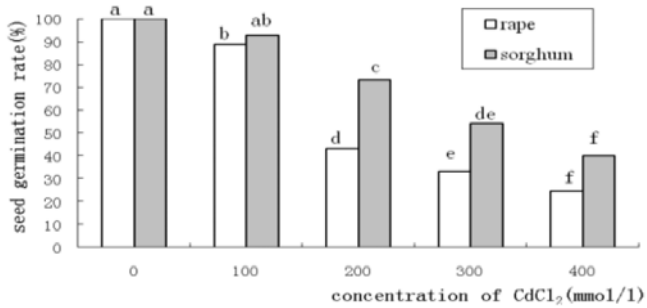

Figure 3: Effect of Cd on seed germination in sorghum and rape.

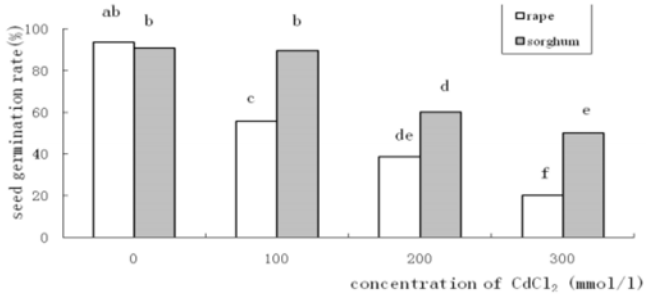

Figure 4: Effect of Cd on seed germination in sorghum and rape as bacteria were applied.

Effects of Crude Oil-Cd and Bacteria on the Seed Germination Rate of Sorghum and Rape

Figures **5** and **6** show the effects of crude oil-Cd compound pollution on seed germination rate of sorghum and rape were significant. When at the lowest crude oil(mg/ml) or $CdCl_2$ (mmol/l) concentration of 30/100 or 30/200, the seed germination rate of rape or sorghum decreased to 50%. *Peptococcus activus* sp.SH3-3-9 can effectively improve the germination rate of seeds. Except at 30/200(for rape seeds) and 50/300(for sorghum seeds) crude oil(mg/ml)/$CdCl_2$ (mmol/l), the effect of the microorganisms on promoting seed germination rate had reached a significant level.

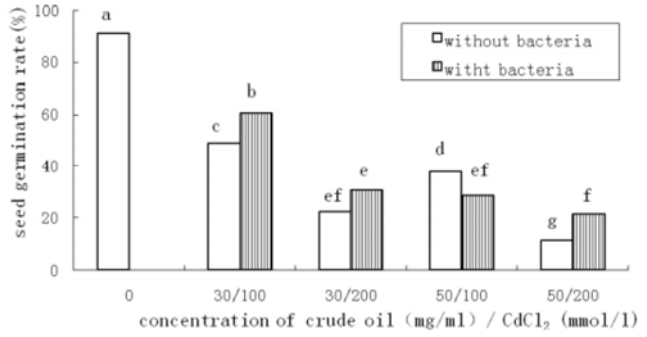

Figure 5: Effect of crude oil-Cd and bacteria on seed germination in rape.

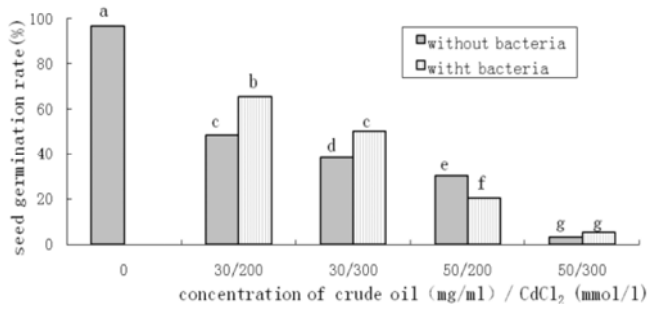

Figure 6: Effect of crude oil-Cd and bacteria on seed germination in sorghum.

CONCLUSION AND DISCUSSION

The experiment shows crude oil and Cd significantly inhibited the seed germination of sorghum and rape, and the inhibition effect increased with the increase of the concentration of those. The supplied bacteria can effectively improve the seed germination rate of the two crops under cadmium and petroleum conditions. The effect of crude oil on the germination of sorghum seed was greater than that of rape, and the response of rape seeds to Cd was stronger than that of sorghum seeds, and rape seed germination and sorghum seed germination were both severely inhibited under crude oil-Cd compound pollution. It is also indicated that the ability of *Peptococcus activus* sp.SH3-3-9 to improve the germination rate in crude oil is better than in Cd, which probably because *Peptococcus activus* sp.SH3-3-9 is a petroleum degrading bacteria which uses crude oil as the only carbon source when selecting and cultivating it. Microbial degradation of crude oil pollution is an effective method [19, 20]. Plant-microbial remediation of crude oil and Cd pollution is a new way to study [21]. This technique is to combine the plant remediation with microbial remediation. This process has the advantages of low cost, better treatment effect, less damage to soil, no secondary pollution [22, 23]. The application of Plant-microbial remediation technology is increasing [24, 25], to promote the degradation of pollutants by the interaction between plants, microorganisms and the rhizosphere environment [26, 27]. When plants grow, their roots provide the best place for the growth of microorganisms [28]. Conversely, the increase of the quantity of microorganisms enhanced the reduction and mineralization of organic pollutants, which provide better growth conditions for plants. Microbial metabolites can also improve ecological environment of

soil [29, 30]. In addition, microorganisms also be able to secrete plant hormones, siderophore and other active substrates to promote the growth of plants [31]. In bioremediation, it is necessary for plant and microbe to has a high biomass, in order to ensure the efficiency of remediation [32, 33]. In this experiment, the effect of *Peptococcus activus* sp.SH3-3-9 to improve the inverse resistance in crops was studied. It showed that the microorganism had a strong ability to degrade crude oil and promote the growth of crops.

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