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PETROLEUM POLLUTION AND ITS ECOLOGICAL IMPACT ON *SALSOLA GLAUCA* BUNGE IN THE YELLOW RIVER DELTA NATURE RESERVE, CHINA

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ABSTRACT

With its close proximity to Shengli Oilfield, China's second largest oilfield, the ecosystem of Yellow River Delta Nature Reserve (YRDNR) is at high risk for crude oil contamination. In this study, the total petroleum hydrocarbons (TPH) of soil and its ecological impact on *Salsola glauca* Bunge (*S. glauca*) in YRDNR were studied by in-situ investigation and laboratory experiments to study the petroleum pollution level of YRDNR and evaluate possibilities of *S. glauca* for potentially restoring oil-contaminated soil. Concentrations of TPH in the sediments of YRDNR varied from 77.72 to 4850 mg/kg dry wt, indicating that the pollution level was relatively low or moderate compared to world-wide locations reported to be chronically contaminated by oil. Oil pollution may exert influence on the biological and ecological characteristics of *S. glauca*, and the effect increased with the pollution level. Furthermore, the seed germination time was significantly delayed, and the final germination rate and seedling growth can be restrained when the petroleum pollution concentration in soil was more than 20 g/kg. However, a certain amount of crude oil may stimulate the growth of *S. glauca*. Dissipation of TPH in the vegetated soils ranging from 25.2% to 43.6% indicated the potential of phytoremediation by *S. glauca*. These studies have proven that *S. glauca* has a potential in phytoremediation of oil-contaminated soil of YRDNR at present petroleum pollution level.

KEY WORDS Petroleum pollution, YRDNR, *S. glauca*, Ecological Impact, Phytoremediation.

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1. INTRODUCTION

The Yellow River Delta Nature Reserve (YRDNR, 118°33′–119°20′E, 37°35′–38°12′N), established in 1992 and covering an area of 153 000 ha, is located at the mouth of the Yellow River in the northeast of Shandong Province, China. It is a national nature reserve for protection of newborn wetland ecosystem, and rare and endangered birds. Moreover, YRDNR is one site of the East Asia birds' migration network, a membership of East Asia-Australia wading birds network, and also a biosphere reserve of China MAB. However, the second largest oil field of China-Shengli oilfield is located here, which threats to the ecosystem of YRDNR due to oil well blowouts, leaks and spills from underground tank, pipelines and illegal disposals. Therefore, environmental remediation in this region has considerable applied significance. Phytoremediation may be especially useful in wetland environments because it provides a less intrusive approach than conventionally mechanical clean-up methods. Under petroleum-polluted conditions, plants or plant associated microflora can convert hydrocarbons to nontoxic forms. However, the successful use of phytoremediation depends, in part, on identifying the petroleum hydrocarbon concentration that will allow successful transplant establishment into the contaminated marsh sediment [1]. At present, the unique environmental condition in YRDNR has received great attention for its environmental significance, ecological sensitivity and potential development. However, most contaminant studies are mainly focused on distributions of aromatic hydrocarbons in sediment and sources of identification aspects in Yellow River Delta [2, 3], few studies on hydrocarbon pollutants in soil were reported for YRDNR.

Phytoremediation, a strategy that uses plants to degrade, stabilize, and/or remove soil contaminants can be an alternative green technology method for remediation of hydrocarbon-contaminated soil [4, 5]. Different types of plants have been found useful for phytotreatment of soil contaminated by hydrocarbons. Application of native plants or microorganisms for remediation of hydrocarbon contamination is valuable for locally specific climate and ecological properties. As a matter of fact, *S. glauca* is not only a typical protective and salinity-and-alkali resistant plant, but also a pioneering species spreading from the inland area to the coast one in YRDNR. Fortunately, *S. glauca* can survive in some oil-contaminated areas of YRDNR, which undoubtedly provides some good chances for the restoration and transformation of oil polluted soil. Nevertheless, information regarding tolerance of *S. glauca* to crude oil is still lacking, but is essential for successful restoration and remediation of oil-impacted habitats. During the seed germination process, plants are particularly sensitive to environmental stresses [6].

At present, there are a few reports about the ecological impact of crude oil on *S. glauca*, especially the processes of seed germination. Furthermore, to our knowledge, data describing the dose–response relationship and documenting the tolerance limits of *S. glauca* to petroleum oil rarely exist. There is therefore an urgent need for research on the problems associated with petroleum pollution and its effects on the plant grown on it. Therefore, the objectives of the study were to: (1) analyze the level of petroleum contamination in sediments of YRDNR; (2) assess the ecological impact of crude oil on *S. glauca* and determine the tolerance limits of *S. glauca* to crude oil; and (3) evaluate of possibilities of *S. glauca* for potentially restoring oil-contaminated habitats.

2. MATERIALS AND METHODS

2.1. Collection and analysis of soil samples

The sampling points in Experimental Area, Buffer Area and Core Area of YRDNR, are illustrated in Fig. 1. The samples were collected from top 0-20 cm using a Dutch auger and bulked to form composite samples for analysis. For S1, S2, S5, the samples were collected away from the oil-well followed by 0.1, 20, 100 m, respectively, in order to analyze the horizontal distribution of pollutant in different distance from the oil-well base. Bulked (600-1000 g) composite surface soil samples from each sampling point were put in a sterile polyethylene bag, flame-sealed and transported to the laboratory for analysis. This research was based on the traditional Soxhlet extraction method with chloroform for 72 h and using the weight determination of soil total hydrocarbon content (TPHs).

2.2. Field Study

In each sampling point, three 50cmx50cm quadrats were set up randomly for surveying plant height, main root length, basal diameter and leaf thickness. Moreover, the aboveground biomass and the underground biomass were also measured in the laboratory.

2.3. Seed germination and growth experiment

The seeds of the *S. glauca* collected from polluted areas of YRDNR were subjected to a germination test. Petroleum, collected from Shengli Oilfield, was dissolved

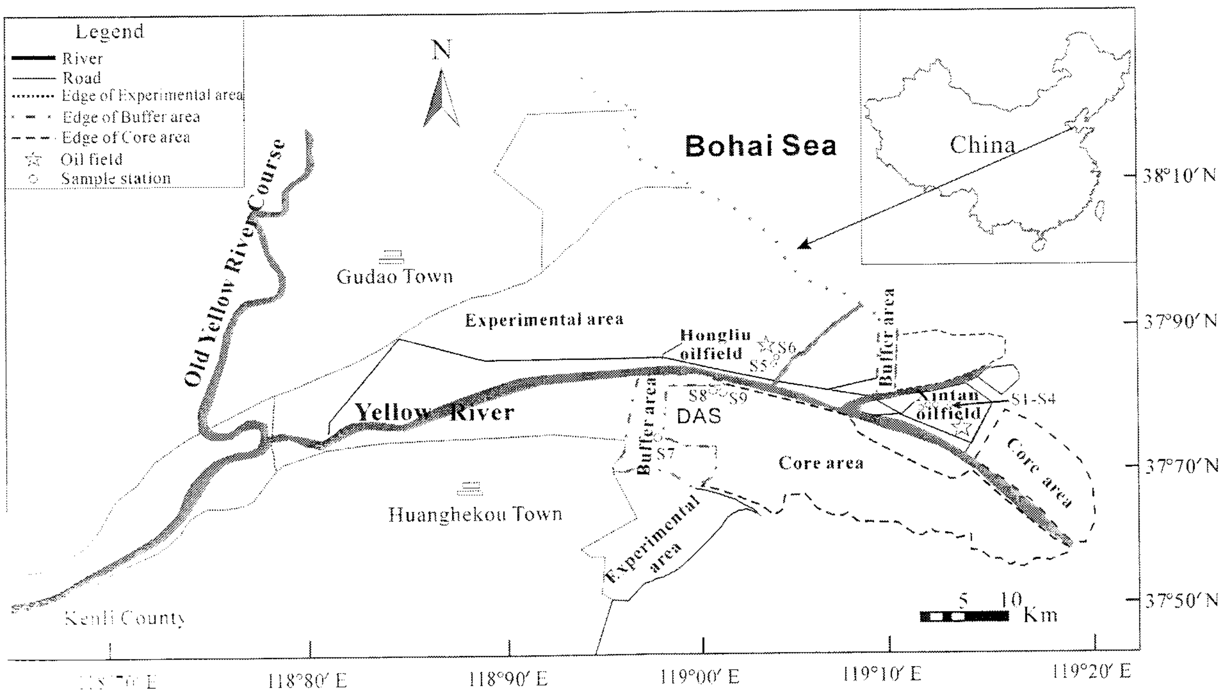

FIGURE 1 -Map of the study area and sampling locations

into petroleum ether. The polluted soils with petroleum concentrations at 5 g/kg, 10 g/kg, 20 g/kg and 40 g/kg were prepared by mixing the solution with soil. The clean soil and soil mixed with petroleum ether were also set to be as control groups. Six treatments totally were assigned randomly to 18 plastic pots (10 cm in diameter, and 8 cm in depth), and each treatment dealt with three replicated pots with 30 seeds buried at a depth of 2 cm below surface. Every 3 days, seedlings were counted to determine germination rate, and height and dry weight of seedlings were examined 30 days later. This experiment was carried out in a glass greenhouse (average daytime temperatures about 20-28℃ and no supplemental illumination), and seeds and the seedlings were watered appropriately. After 30 days' bioremediation using seed-growing *S. glauca*, concentrations of petroleum pollution were determined in the soil of experimental pots and compared with control concentrations.

3. RESULTS

3.1. Assessment of petroleum contamination

Petroleum hydrocarbon concentrations in sediments are typically a few mg/kg in unpolluted coastal areas and from 50 to >1,000 mg/kg in contaminated areas [7]. It has been reported that the free hydrocarbon content in the sediment of Yellow River Delta ranges from 10 to 440 mg/kg [2]. Results of hydrocarbon content determination are shown in Table 1. The higher Total Petroleum Hydrocarbons (TPH) concentration was found at oilfield, which might be a con-sequence of illegal discharge of petroleum wastewater or crude oils. For example, the highest value for the sludge samples occurred at S1 and S2, where a small-scale oil spill accident had taken place. Compared with this, the lower values were found at S7 in the Buffer Area, S8 and S9 of Dawenliu Administrative Station (DAS) in the Core Area, ranging from 77.72 to 170 mg/kg. In general, the concentrations of TPH in Experimental Area and Buffer Area were much lower than those in Experimental Area. Moreover, TPH appears significant descend trend outward from a center of oil-well (Table 1).

TABLE 1 - Total petroleum hydrocarbon (mg/kg dry wt) in surface sediment with different distance from oilwell

Site	Station		TPH (mg/kg)		
No.	Distance from oil well (m)		0	20	100
S1	Experimental area	Xintan Oilfield	1780	360	210
S2			4850	720	240
S3			114.0	-	-
S4			651.16	-	-
S5		Hongliu Oilfield	240	180	100
S6			182.9	-	-
S7	Buffer area		77.72	-	-
S8	Core area	DAS	170	-	-
S9			134.3	-	-

-: Data deficiencies

Whereas total hydrocarbon concentrations >500 mg/kg are generally indicative of significant pollution, values <10 mg/kg are considered to denote unpolluted sediments [8]. Except the sample from sludge samples that was significantly contaminated, the levels of TPH concentration in YRDNR were relatively lower or moderate compared to those at world-wide locations reported to be chronically contaminated by oil, such as 0.05-779 mg/kg in the Gulf of Oman [9], 60–646 mg/kg in highly contaminated sediments from Hong Kong's Victoria Harbor [10] and 11–6900 mg/kg along the oil-impacted coastline of Saudi Arabia after the Gulf War [11].

3.2. Laboratory experiments on seed germination

Effect of petroleum pollution on seedling growth of *S. glauca* was studied in greenhouse with the relatively controlled conditions. Germination time and germination rate were affected significantly at high petroleum concentrations (Fig. 2). The germination time was similar and the final germination rate reached 100% at the end for the 5 g/kg treatment and the two control groups. Compared with the former three treatments, seeds under the 10 g/kg treatment germinated slightly slowly with a nearly equal final germination. On the other hand, germination was significantly delayed and the final germination rates were much lower ($p < 0.05$) in the 20 g/kg and 40 g/kg treatments, and the effects of 40 g/kg concentration on germination were much greater than those of 20 g/kg ($p < 0.05$). However, over half of seeds germinated at the end even under the highest petroleum concentration of 40 g/kg.

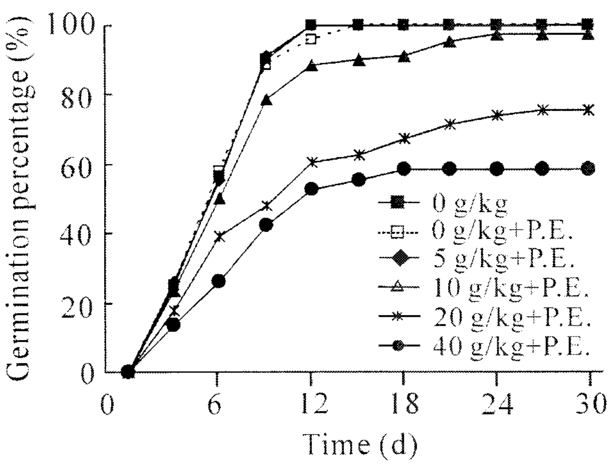

FIGURE 2 -Effects of petroleum pollution on germination percentage *S. glauca*. (P.E. indicating petroleum ether)

Both dry weight (total biomass) and bud length were taken 30 d after seeding. Neither biomass nor shoot height (Figs. 3a and 3b) of seedlings of the treatments of 5 g/kg and 10 g/kg oil were significant different from the two controls. However, the biomass and shoot height of the treatments of 20 g/kg and 40 g/kg oil were significant ($p<0.05$) lower than those of the two controls. And the effects of 40 g/kg concentration were much greater than those of 20 g/kg ($p < 0.05$) on seedling growth in both dry weight (Fig. 3-a) and bud length (Fig. 3-b).

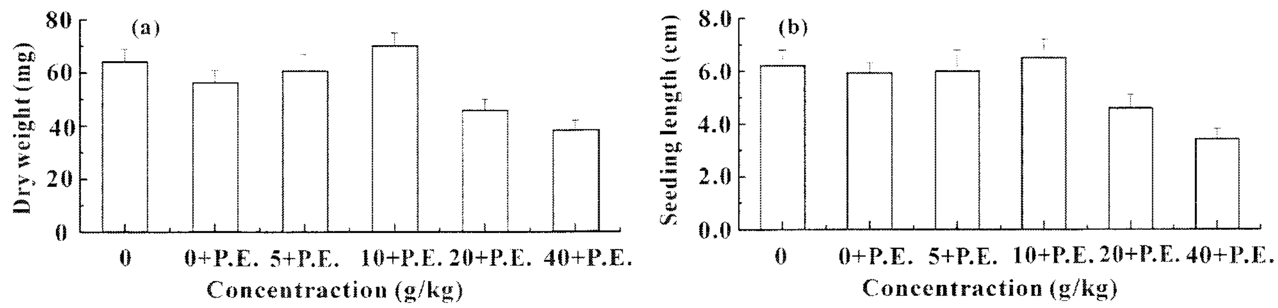

FIGURE 3 -Effects of petroleum pollution on seedling growth of *S. glauca*. (Bars indicate standard errors).

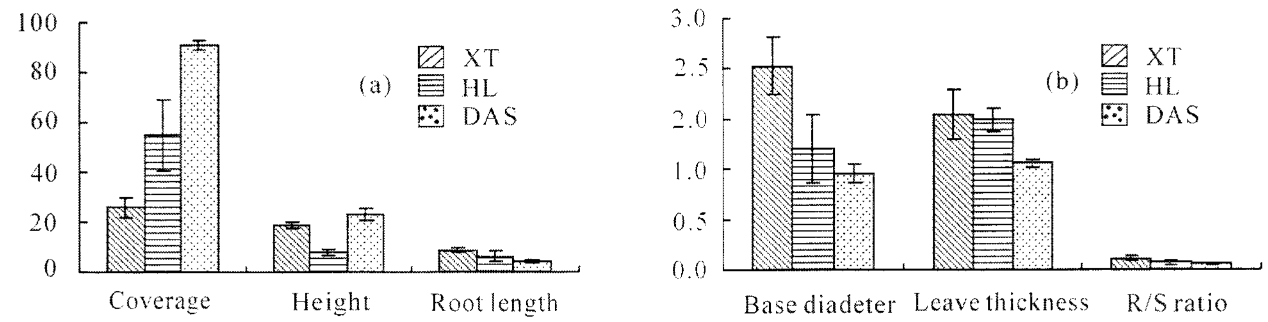

FIGURE 4 - Ecological impact of petroleum pollution on *S. glauca* in the ecological zone of YDR (Bars indicate standard errors)

3.3. Analysis in the field

Soil polluted with crude oil had an adverse effect on the plant growth and the effects increased with increase in level of pollution. Growth parameters included coverage, height, root length, root biomass, leaf thickness, base diameter, and root/shoot ratio (R/S) (Fig.4). Concentrations of the soil TPH decreased systematically from Xintan oilfield to Hongliu and DAS (Table 1). From field observation, a negative correlation was found between the coverage and the TPH and a positive correlation between root length, root biomass, leaf thickness, basal diameter and TPH (Fig. 4). With hindsight, it is interesting to note that a certain amount of crude oil could stimulate plant growth for root length, root biomass, leaf thickness, base diameter. Generally speaking, R/S ratio smaller, the environmental conditions of plant more favorable. It appears to be a positive correlation between R/S and oil concentration, observed from the Fig 4. It may be because that *S. glauca* from the hydrocarbon-contaminated soil has well developed root system in order to absorb nutrients as much as possible in such poor condition.

3.4. Degradation rate of crude oil in the soil with S. glauca

A number of studies have indicated the potential of phytoremediation for reducing the concentrations of petroleum hydrocarbons [1,4,5,12-14]. According to the result from the germination experiment, the petroleum would not adversely affect the height of seedling of *S. glauca* in the soil with oil concentration lower than 10 g/kg while the petroleum would remarkably restrain it when the mass fraction of oil-contaminated soil was at 20-40 g/kg. Thus, the inhibited effect of crude oil on the ecology of *S. glauca* could be limited in studied areas of YRDNR with TPH of 77.72-4850 mg/kg. It has been reported that the degradation rate of oil leaks in the soil with *S. glauca* is 21.7%-37.9% higher than in the control soil [15]. Our results also showed that phytoremediation with *S. glauca* reduced the residual TPH concentration in the soil by 25.2% to 43.6%.

4. DISCUSSION

Petroleum pollution of soils is a major environmental pollution in many countries. Serious risks can occur to public health and the environment when the soil is polluted by crude oil [16,17]. The concentrations of TPH show a sharp decrease with soil layer and most TPH was limited to the first 20 cm of soil. Various studies have reported the adverse effect of crude oil pollution on germination of seeds [18-21]. Crude oil in soil makes the soil condition unsatisfactory for plant growth, due to the reduction in the level of available plant nutrient or a rise in toxic levels of certain elements such as iron and zinc [18,22].

A number of studies [1,22-24] have investigated the effect of petroleum hydrocarbons on wetland plants, especially coastal salt marsh plants. Soil polluted with crude oil had an adverse effect on the plant growth and the effect is proportional to the level or amount as well as the concentration of crude oil. In the present field experiment, a negative correlation was found between the coverage and the TPH. In the greenhouse experiment, petroleum concentration in soil of lower 10 g/kg had no significant effects on the final germination rate, the height and the biomass of *S. glauca* (p >0.05), while more higher concentrations depressed significantly the seed germination and the seedling growth (p<0.05). The highest level of pollution 40 g/kg crude oil soil resulted in the least growth. The oil contaminated soils becomes compact, resulting in reduced aeration, poor wettability and increased amounts of toxic substances, which reduce germination. From field observation, a positive correlation was found between root length, root biomass, leaf thickness, basal diameter and the level of petroleum pollution, which indicates that the growth of plants may be improved by low stresses. The results agree with previous studies [20,26], even though they experimented on different plant species.

Crude oil can exert acute or chronic toxicity or both on soil properties and *S. glauca*. However, petroleum hydrocarbon utilizers can tolerate oil contaminated environments because they may possess the capacity to utilize oil as energy sources. In this study, the seed of *S. glauca* collected from oilfield could be more adaptive to oil pollution than that of *S. glauca* inhabited in unpolluted environment. Furthermore, the concentration of crude oil significantly inhibited the germination and growth of *S. glauca*. obtained from indoor simulation tests was higher than that from the field. The difference could be explained by their different abilities to tolerate the degree of pollution under different conditions. In contrast to the stable and suitable growth conditions in simulation experiment, the abiotic stresses, such as drought and cold, could exert severe influences on growth and development of plant of the wind.

Plant can adjust morphological structure to enhance it's ability of ecological adaptation under the heavy metals stresses [27], drought habitats [28], ultraviolet radiation stress [29]. For example, with increasing of content rations of Zn and Cr, the biomass of rice plant had a trend to reduce, but the ratio of R/S had an increasing trend [27]. The results from field experiment showed that *S. glauca* in the oil polluted area had longer root length, increasing root shoot ratio, thicker basal diameter and leaves, within certain range of concentrations, which may also be due to the self-protecting and self-adjusting mechanisms.

Phytoremediation of petroleum pollution is a cost-effective green technology. As mentioned above, the petroleum pollution level of YRDNR is lower than that of *S. glauca* can survive. Our results are in accordance with the results of an earlier study that showed several legumes and graminoids were found in the petroleum-polluted soils with about 5% pollution [30,31]. It means the potential for simultaneous restoration and remediation by transplanting dominant native salt marsh plants, like *S. glauca*., into oil-contaminated wetlands.

5. CONCLUSION

Except the sludge samples that were significantly contaminated, the pollution levels in YRDNR were relatively low or moderate compared to those at world-wide locations reported to be chronically contaminated by oil. Furthermore, TPH in Core Area and Buffer area of YRDNR were much lower than that of Experimental Area.

The field and laboratory studies demonstrated that oil pollution may exert influence on the biological and ecological characteristics of *S. glauca*, and the effects increased with the pollution level. Oil concentration in the sediment is an important factor controlling the successful transplantation of the coastal salt marsh plant, *S. glauca*., in oil-contaminated wetlands. Seedlings in soils treated with pollution levels of less than 10 g/kg of soil were not affected. However, crude oil may have an adverse effect on the germination and growth of *S. glauca* when pollution level is higher than 10 g/kg. On the other hand, a certain amount of crude oil can stimulate plant growth for root length, root biomass, leaf thickness, base diameter. Furthermore, *S. glauca* had strong adaptability to oil pollution and may be well use for bioremediation of oil-contaminated soil. *S. glauca* may grow very well in soil with certain degree of petroleum contamination and the residue oil in sediment can gradually degraded, indicating the potential of using native dominant salt marsh plant *S. glauca* for restoration and remediation of oil-contaminated wetlands in YRDNR.

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