Effect of fertilization practice on biological properties of crude oil-polluted soil

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Abstract. Hydrocarbon soil pollution is widespread problem in many regions of the world, where oil is extracted and transported. Pollution with crude oil has numerous adverse impacts on the soil-microorganism-plant system. Although adding fertilizers has been shown to be essential to enhance remediation rate, excessive nutrients may exert toxicity for microorganisms and plants. Field test was conducted in the area previously subjected to several accidental oil spills in the central European Russia. The effects of fresh pig manure and/or conventional NPK fertilizers on plant growth, hydrocarbon content and activities of catalase, dehydrogenase, urease, sucrose, and phosphatase were evaluated depending on an application rate. Fertilizer application resulted in restoration of plant cover and reduction of hydrocarbons by 13 to 46%. Oil pollution reduced activities of catalase, dehydrogenase, sucrose and phosphatase and increased urease activity. Manure application was more effective in regard to plant growth, hydrocarbon removal and enzymatic activity in comparison with mineral fertilizers. The maximum positive effect on contaminated soil-microorganism-plant system was obtained under combined application of manure and NPK.

1. Introduction

The annual world oil extraction is about 15.8 billion liters per day and keeps rising. Increasing oil production and construction of new oil pipelines and refineries lead to increased environmental pollution [1]. Summarizing land being negatively affected by oil and gas extraction and transportation was increased from 0.2 million ha in 2000 to about 3 million ha in 2012 [2]. Soil degradation is one of the most deleterious consequences of leakage of pipelines and storage tanks and accidental spills [3]. As reported by numerous studies, the presence of hydrocarbons disturbs physical, chemical and biological soil properties, including structure, water-holding capacity, redox conditions, nutrient availability, thus leading to deterioration of soil fertility and health [4; 5; 6]. In this regard, the elaboration of environmentally friendly and economically feasible approaches aimed at intensifying hydrocarbon degradation and restoration of soil fertility is an acute issue.

Because of varying physical and chemical properties of multiple components that compose oil, both the migration and transformation modes in soil and response to the remediation approaches differ depending on
composition. Numerous clean-up technologies have been suggested such as bioremediation, bioaugmentation, biostimulation, ventilation, phyto-remediation, phytodegradation, chemical oxidation and others [7; 8]. To successfully develop advanced technologies or enhance the efficiency of conventional strategies for soil remediation, comprehensive understanding of the physical, chemical and biological soil processes under oil contamination is critical.

Microbial-based remediation is considered to be nature-like, relatively cost-effective, and widely used approach. Different microorganisms possess the ability to utilize petroleum hydrocarbons as a source of carbon and energy. Among them, bacteria play a leading role. Numerous bacterial genera indigenous to soil have been reported to decompose crude oil and oil products, for example, Pseudomonas, Bacillus, Phodococcus, and Micrococcus [9]. The efficiency of biodegradation is affected by multiple factors including water regime, temperature, oxygen availability, and many others [10]. Insufficient nutrient supply limits the natural processes of oil degradation [11]. General strategy to enhance the biodegradation is aimed at maintaining optimal conditions for microbial growth.

Traditional mineral and organic fertilizers are widely used to mitigate a negative influence of crude oil on soil and stimulate microbial activity. Mineral fertilizers promote soil fertility and plant growth as well as provide balanced nutrient supply required for hydrocarbon (HC)-degrading microbes [12; 13]. Organic fertilizers improve soil properties, activate the microbial growth, and serve as a source of additional microorganisms and nutrients [14]. Some studies evidence that the combined application of inorganic and organic soil amendments is more efficient than their single application [15]. Excessive amounts of nutrients applied can be deleterious for soil health and microbial activity [10]. The type and application rate of soil amendments depend highly on soil properties.

Any biological process in soil involves the enzyme participation. Soil enzymes play a key role in converting and decomposition of organic pollutants. The main objective of the current investigation was to determine an influence of mineral fertilizers and manure on enzymatic activity and HC removal in crude oil-contaminated soil.

2. Material and Methods

Field test (Gray Forest Soil, Bashkoria, Russia) was conducted in an area previously subjected to several accidental crude oil spills. The upper layer of unpolluted soil had the following characteristic: 2.26±0.10 C%, pH=7.1±0.1, clay content 35.6±0.5%, CEC = 15.8±0.5 cmol(+) kg⁻¹; total phosphorus 1.24±0.15 g kg⁻¹ P; total potassium 28.9±0.3 g kg⁻¹ K; total nitrogen 4.5±0.3 g kg⁻¹ N.

The oil had a high content of sulfur (2.5±0.2%), the content of paraffin 8.0 ±0.2%; naphthenes 6.4±0.1%; aromatic hydrocarbons 15.1±0.4%; density 0.938±0.015. Twenty four plots of 10 m² each were separated and the following treatments were conducted: (1) control without pollution; (2) pollution; (3) pollution + N60P90K90; (4) pollution + N120P180K180; (5) pollution + manure, 70 t ha⁻¹; (6) pollution + manure, 140 t ha⁻¹; (7) pollution + N60P90K90 + manure, 70 t ha⁻¹; and (8) pollution + N120P180K180 + manure, 140 t ha⁻¹. Fresh pig manure with a moisture of 50% was used. Mineral fertilizers were added as ammonium nitrate (Fosagro, Russia), potassium chloride (Uralkali, Russia) and superphosphate (Biomaster, Russia). After application of fertilizers and manure, barley (H. vulgare L., cv. Suzdaletz) was planted with a density of 1000 seeds per m². After the growing season, a percent of survived plants was evaluated.

Soil samples were collected from a 0-20 cm depth in a year. The HC content was analyzed by hexane (C₆H₁₄) and ethanol-benzole (1:1) extraction methods using ST 255 Soxtec system (FOSS). Ten (10) g of soil was placed into Soxtec flask and 100 mL of solvent was added. Samples were extracted at +70°C for 4 hours [16]. Total HC content in an extract was estimated by a gravimetric method [17].

The activities of catalase, dehydrogenase, urease, sucrose, and phosphatase were determined by the methods described by Achuba and Peretiemo-Clarke [19], Tabatabai [20], Guo [21], Ge et al. [22], and Khomutova et al. [23], accordingly. Absorbance of solutions was measured colorimetrically by a
spectrofotometer Shimadzu UV–VIS 160A (Kyoto, Japan). Each treatment and each analysis were conducted in 4 replications. All data obtained was subjected to a statistical analysis based on comparative methods using Duncan’s multiple range tests for mean separation at the 5% level of significance [24].

3. Results and Discussion

Uncontaminated soil was characterized by high content of organic matter and high level of fertility. However, the contamination with oil resulted in death of all barley plants in control soil (Figure 1). The application of mineral fertilizers and/or manure provided a significant increase in the plant survival. Manure had a greater effect as compared to mineral fertilizers.

![Figure 1. Amount of survived plants, %.

The HC content is presented in Table 1. The application of mineral fertilizer or manure considerably decreased the HC content in comparison with untreated soil (by 35.4-46.3%; 20.0-24.6%; and 25.3-46.2% with mineral fertilizers, manure, and combined fertilization, accordingly). Organic C in the polluted soil was slightly reduced under the application of mineral fertilizers, from 7.9 to 7.2 and 7.5% of C, accordingly at a low and high rate of fertilization. The addition of manure significantly increased the soil C up to 12.5%, however, a higher increase was observed at a lesser rate of manure. Manure+mineral fertilizers or manure at 140 t ha⁻¹ provided a smaller raise in the total C, thus evidencing more intensive mineralization of organic compounds due to high microbial activity.

|        | C₆H₁₄-extractable | C₂H₅OH-extractable | C₆H₆-extractable | Total C, % |
|--------|-------------------|--------------------|------------------|------------|
| Control| 0.08              | 0.14               | 2.26             |
| Pollution (P) | 5.20              | 6.29               | 7.94             |
Soil enzymatic activity is one of the most fast and sensitive features reflecting the microbial activity and the HC degradation potential [9; 11]. Different enzymes are related to different types of microbial activity. In our experiment, the oil pollution sharply reduced the catalase, sucrase, urease and phosphatase activities, while dehydrogenase did not change (Table 2). The dehydrogenase activity is considered as a measure of microbial oxidative processes in oil contaminated soil [25]. High level of dehydrogenase evidences the intensive oil degradation. Application of nutrients or organic matter significantly increased dehydrogenase activity, with a maximum effect by manure (an increase by 33-76% as compared with contaminated control).

Catalase is known to promote the decomposition of hydrogen peroxide, thereby preventing poisoning living organisms [26]. Reduced catalase activity is related to decreased soil respiration. In our experiment, the catalase activity reduced 2.3 times in the oil-polluted soil. The manure application benefitted the catalase activity better than mineral fertilizers, probably due to better air penetration to soil.

The sucrase activity reflects the intensity of the sucrose hydrolysis. A sharp reduction in this enzyme observed in the contaminated soil (by 41.8%) may indicate reduced content of sucrose and other sugar-based organic molecules to be released to soil with root exudates. Mineral fertilizers only slightly improved this parameter. The application of manure or manure with NPK enhanced the sucrase activity, but less than that of catalase, dehydrogenase and phosphatase. Perhaps, the restoration of the sucrase activity requires more time.

Urease is the enzyme responsible for the hydrolysis of urea to CO2 and NH3. Most authors report a decrease in soil urease activity under petroleum pollution [21]. In our study, the urease activity sharply increased in polluted soils. Similar significant increase in urease activity has been reported in soil contaminated with crude oil leaked from pipeline [27]. Such contradictory data may be caused by differences in oil composition, contamination level, exposure time and others. Mineral fertilizers had insignificant effect on the urease activity, while manure or manure with NPK considerably increased the urease activity by up to 46% as compared with contaminated control and 4.6 times compared with uncontaminated soil.

The activity of phosphatase is inversely dependent on the availability of mobile phosphorus in soil [28]. The phosphatase activity was reduced 2.4-fold under oil contamination. The application of NPK or/and manure further reduced the phosphatase activity, thereby indicating less P deficiency.

Table 2. Enzymatic activity of crude oil-polluted soil as influenced by organic and mineral fertilizers.

|                        | Catalase, O2 mL | Dehydrogenase, mg of formasana | Sucrase, glucose mg | Urease, NH3 mg | Phosphatase, phenolphthalein mg |
|------------------------|----------------|--------------------------------|---------------------|---------------|--------------------------------|
| Control                | 5.41           | 3.52                           | 20.06               | 0.35          | 7.56                           |
| Pollution (P)          | 2.30           | 3.60                           | 11.98               | 1.10          | 3.10                           |
| P + N60P90K90          | 2.92           | 3.74                           | 12.87               | 0.88          | 2.51                           |
| LSD05                  | 0.10           | 0.12                           | 0.10                |               |                                |
Our data evidences that the contamination with crude oil altered the soil microbial activity. A main negative impact was observed on catalase and sucrase, while urease increased and dehydrogenase changed insignificantly. Reduced phosphatase activity can reflect reduced P deficiency as a result of contamination and fertilization.

Crude-oil pollution adversely affects the soil-microorganism-plant system and alters the microbial activity. Analysis of soil ferment activity demonstrates a reduction in catalase, dehydrogenase, sucrase and phosphatase, whereas urease activity increased. The data obtained evidences that organic fertilizer had more pronounced effect than only mineral fertilizers. A maximum positive effect was observed on contaminated soil-microorganisms-plant system under co-application of manure and NPK fertilizers.

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