Silicon substances for restoration of oil-contaminated areas

P Zhang¹, V V Matichenkov², E A Bocharknova², S M Sevostianov²

¹Hunan University of Finance and Economics, Changsha 410205, China
²Institute of Basic Biological Problems RAS, 142290, Pushchino, Russia

Abstract. Numerous investigations demonstrate that active forms of silicon (Si) enhance the plant tolerance against abiotic stresses by several mechanisms, including increasing the antioxidant activities and minimizing oxidative damage. Soil contamination with oil and oil products relates to abiotic stress that detrimentally affects soil microbial population and plant growth. Considering the crucial role of microorganisms and plants in bioremediation of oil-polluted areas, Si substances can be beneficial to acceleration of soil reclamation. In greenhouse experiment, wheat was grown in Grey Forest Soil contaminated with used motor oil. The effect of fumed silica and monosilicic acid on soil enzymatic activity and plant growth was studied. Both Si substances provided increasing the plant biomass and the activities of catalase and dehydrogenase. As regards the plant growth, the effect of Si was more pronounced in polluted soil, while the enzyme activity was higher affected in unpolluted soil. The activities of catalase and dehydrogenase were closely correlated to the water-soluble Si in soil (R=0.91-0.92). Silicon substances with high content of plant- and microorganism-available Si might be promising for involvement in bioremediation technology for oil-contaminated soil.

1. Introduction

Soil pollution with petroleum and petroleum products is a worldwide problem that causes many detrimental consequences for the environment and human health [1]. Bioremediation is the most widely used approach to restore petroleum polluted soil due to its advantages such as low cost, environment safety, easy operation, and good performance [2; 3]. Bioremediation is based on using microorganisms and plants to destroy or neutralize petroleum compounds [4; 5]. Plants contribute to soil decontamination by direct impact on pollutants and by altering soil properties [6].

Silicon is the second most abundant element in the Earth. The role and functions of Si in plant physiology, veterinary and medicine have been investigated for more than 200 years [7]. Improved Si nutrition of plants and microorganisms has a multi-effect on biomass and cell functioning [8; 9]. Silicon-mediated benefits like sugar formation, DNA stability, transport regulation (activated transport of Fe, B, K, Ca and reduced that of Cd, Hg, As, Na) have been reported as a result of additional Si nutrition [10; 11].

The results of numerous current investigations demonstrate that active forms of Si have a positive influence on plant growth and protection against biotic and abiotic stresses [8; 12; 13]. Plants and microorganisms exposed to abiotic stresses suffer from oxidative destruction induced by reactive oxygen species (ROS), leading to severe damage to cell structure, organelles, and metabolically essential molecules. To alleviate this damage, plants or microorganisms have developed the complex defense system to maintain homeostasis through non-enzymatic and enzymatic antioxidants [14]. Silicon-rich substances are able to contribute to regulating the ROS accumulation via increasing the activities of antioxidant enzymes - superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase and others [15;
As a result, Si substances mitigate the negative impact of stress and enhance the viability of plants and microorganisms under unfavorable conditions. There is data demonstrating the Si benefits in remediation of oil-contaminated soil [17; 18].

The direct positive effect of Si substances on soil microbial activity is reported in the recent investigations [19; 20]. However, the underlying mechanisms remain poorly understood.

The aim of the current study was to determine the effect of Si-rich substances on soil microbial activity and plant growth with regard to Si involvement in oil-contaminated soil restoration strategy.

2. Materials and Methods

Greenhouse experiment was conducted with upper layer of Grey Forest Soil at the Institute Basic Biological Problems Russian Academy of Sciences. The selected soil properties are presented in Table 1. Spent motor oil (SMO) (5W-40, Shell Helix Ultra with viscosity 41.2 mm² s⁻¹) was used as a source of hydrocarbon contamination. Before experiment, 15 mL of SMO was mixed with 1 kg of soil and kept for 1 week with daily agitation.

The following solid and liquid Si substances were used.
1) Chemically pure fumed silica (SiO₂) (Sigma-Aldrich, CAS 112945-52-5), 0.007-0.030 µm particle sizes, pH 6.9, average surface area including pores – 395 m² g⁻¹.
2) Monosilicic acid (MA) (Fisher Scientific, CAS-No 7699-41-4).

Before planting, seeds of wheat (Triticum aestivum L cv Novosibirskaya) were treated with 3% H₂O₂ and washed in distilled water (DW). Ten seeds were planted in each 1-L plastic pot. Plants were grown for 25 days at the following conditions: air temperature 26 ± 4°C during the day and 22 ± 2°C during the night; the light period was 12 h at intensity of 200 lmol photons m⁻² s⁻¹ by UV/Vis lighting, and the relative air humidity was 85±5% during the day and 78 ± 5% during the night. Soil was irrigated with DW to maintain soil moisture between 20 and 40%. Solid silica was applied before seeding at rates of 50, 100 and 200 kg ha⁻¹, which are equal to 0.05; 0.1 and 0.2 g pot⁻¹, respectively. Monosilicic acid was applied together with irrigation solution at concentrations of 0.1; 0.5 and 1 mM Si.

Biomass of wheat plants was measured 4 weeks after seeding. Soil samples were analyzed for water- and acid-extractable Si by the elaborated methods [21]. The soil microbial activity was evaluated by testing the activities of catalase and dehydrogenase. The catalase and dehydrogenase activities were determined using the methods described by F I Achuba and B O Peretiemo-Clarke [22] and M A Tabatai [23]. Absorbance of solutions was read spectrophotometrically using 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

The application of both Si substances significantly increased the biomass of roots and shoots (Table 2). The plant growth enhanced with increasing an application rate of MA. Fumed silica was more efficient at a rate of 100 kg ha⁻¹ (increases by 63.6 and 39.1% for roots and shoots, respectively, in control soil and by 140 and 50% in contaminated soil). When applied at 200 kg ha⁻¹ SiO₂ demonstrated diminishing 

| Table 1. Selected properties of experimental soil. |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| pH               | Total C, g kg⁻¹ | Total N, g kg⁻¹ | Total Fe, g kg⁻¹ | Total P, g kg⁻¹ | Total K, g kg⁻¹ |
| Grey Forest Soil | 6.5             | 25.3            | 12.1             | 0.94            | 21.1             | 450.3            |
effect, perhaps due to reduced water-soluble Si in the soil (Table 3). Previously we found that high application rates of amorphous Si can induce the transformation of monomers of silicic acid into polymers [25]. The soil water-soluble Si gradually increased with increasing the MA concentration in irrigation water, whereas in the case of SiO$_2$ it reached higher values at a rate of 100 kg ha$^{-1}$. The soil acid-extractable Si increased with increasing an application rate of both solid and liquid Si substances.

The soil contamination with SMO significantly (by 54.5 and 47.8\% for roots and shoots, respectively) reduced the plant biomass. Fumed silica and MA provided the enhancement of plant growth. The benefits of Si substances were more pronounced for SMO-stressed plants as compared to unstressed ones. The contents of water- and acid-extractable Si were slightly reduced in the contaminated soil.

Table 2. The effect of Si-rich substances on dry weight of wheat, g plant$^{-1}$.

| Treatment          | Non-contaminated soil | SMO-contaminated soil |
|--------------------|-----------------------|-----------------------|
|                    | Roots     | Shoots  | Roots | Shoots  |
| Control            | 0.11      | 0.23    | 0.05  | 0.12    |
| SiO$_2$ 50 kg ha$^{-1}$ | 0.15    | 0.29    | 0.08  | 0.14    |
| SiO$_2$ 100 kg ha$^{-1}$ | 0.18     | 0.32    | 0.12  | 0.18    |
| SiO$_2$ 200 kg ha$^{-1}$ | 0.16     | 0.3    | 0.10  | 0.11    |
| MA 0.1 mM Si       | 0.14      | 0.25    | 0.09  | 0.15    |
| MA 0.5 mM Si       | 0.19      | 0.31    | 0.11  | 0.18    |
| MA 1.0 mM Si       | 0.21      | 0.34    | 0.13  | 0.19    |
| LSD$_{05}$         | 0.02      | 0.02    | 0.01  | 0.02    |

Table 3. The water- and acid-extractable Si in the soil after greenhouse test, mg kg$^{-1}$.

| Treatment          | Non-contaminated soil | SMO-contaminated soil |
|--------------------|-----------------------|-----------------------|
|                    | Water-extractable Si | Acid-extractable Si | Water-extractable Si | Acid-extractable Si |
|                    | mg kg$^{-1}$          | mg kg$^{-1}$          | mg kg$^{-1}$          | mg kg$^{-1}$          |
| Control            | 5.6                   | 323                   | 5.2                | 301                  |
| SiO$_2$ 50 kg ha$^{-1}$ | 15.8               | 378                   | 15.1               | 321                  |
| SiO$_2$ 100 kg ha$^{-1}$ | 20.4               | 412                   | 20.0               | 400                  |
| SiO$_2$ 200 kg ha$^{-1}$ | 14.5               | 457                   | 14.1               | 415                  |
| MA 0.1 mM Si       | 16.7                  | 332                   | 16.9               | 308                  |
| MA 0.5 mM Si       | 23.4                  | 357                   | 23.0               | 323                  |
| MA 1.0 mM Si       | 30.4                  | 371                   | 30.1               | 343                  |
| LSD$_{05}$         | 0.5                   | 20                    | 0.6                | 20                   |

The application of SiO$_2$ or MA resulted in significantly increased the activity of both enzymes in polluted and unpolluted soils (Table 4). Monosilicic acid at a higher rate was the most efficient, providing increases by 38.1 and 28.7\% for catalase and dehydrogenase, respectively, in non-contaminated soil and by 86.5 and 29.2\% in SMO-contaminated soil. Fumed silica demonstrated better efficacy at a rate of 100 kg ha$^{-1}$ (increases by 33.2 and 24.3\% for catalase and dehydrogenase, respectively, in non-contaminated soil and by 68.2 and 14.1\% in contaminated soil). It should be noted that in contaminated soil both Si substances had greater influence on catalase, while dehydrogenase was more influenced by Si in non-contaminated soil.
Table 4. The activity of catalase and dehydrogenase in the soil after greenhouse test.

| Treatment          | Non-contaminated soil | Contaminated soil |
|--------------------|------------------------|-------------------|
|                    | Catalase, mL O₂        | Dehydrogenase, mg of formasana | Catalase, mL O₂ | Dehydrogenase, mg of formasana |
| Control            | 6.12                   | 4.56              | 2.45            | 3.12          |
| SiO₂ 50 kg ha⁻¹    | 7.23                   | 4.94              | 3.56            | 3.33          |
| SiO₂ 100 kg ha⁻¹   | 8.15                   | 5.67              | 4.12            | 3.56          |
| SiO₂ 200 kg ha⁻¹   | 6.98                   | 4.87              | 3.12            | 3.21          |
| MA 0.1 mM Si       | 6.99                   | 4.85              | 3.22            | 3.28          |
| MA 0.5 mM Si       | 7.45                   | 5.32              | 4.18            | 3.59          |
| MA 1.0 mM Si       | 8.45                   | 5.87              | 4.57            | 4.03          |
| LSD₀.₀₅            | 0.05                   | 0.03              | 0.03            | 0.02          |

The data obtained have demonstrated that solid or liquid forms of Si can significantly enhance plant growth and soil microbial activity in unpolluted and oil-polluted soils. Considering a key role of soil microbial population in the oil and oil product degradation, the Si application could accelerate the remediation of contaminated soil [26].

The correlation coefficients between the water- and acid-extractable Si in the soil and the activity of catalase and dehydrogenase evidence that both soil enzymes are strongly affected by the soil water-extractable Si (Table 5). The correlation between the enzyme activity and the acid-extractable Si was very low. The soil water-extractable Si is mostly presented in the form of MA.

Table 5. Correlation coefficients between the enzyme activity and the water- and acid-extractable Si in the soil and between the enzyme activity and plant weight.

|                     | Si-water-extractable | Si-acid-extractable | Biomass Roots | Biomass Shoots |
|---------------------|----------------------|---------------------|---------------|---------------|
| Catalase            | 0.92                 | 0.33                | 0.92          | 0.91          |
| Dehydrogenase       | 0.91                 | 0.25                | 0.92          | 0.89          |
| SMO-contaminated soil |                     |                     |               |               |
| Catalase            | 0.91                 | 0.91                | 0.96          | 0.23          |
| Dehydrogenase       | 0.83                 | 0.88                | 0.94          | 0.10          |

As evident from the current study, Si substances able to release MA, the only form of Si available to plants and probably to microorganisms, could enhance the efficacy and lower the cost of oil-polluted soil bioremediation.

Acknowledgements
This work was financially supported by Hunan Provincial Base for Scientific and Technological Innovation Cooperation, China (2018WK4013); the Key Research and Development Program of Hunan Province, China (2019WK2031); the Ministry of Science and Higher Education of Russian Federation, theme AAAA-A17-117030110137-5 and AAAA-A17-117030110139-9.

Reference
[1] Thapa B and Ghimire A 2012 Kathmandu university journal of science, engineering and technology 8(1) 164-170
[2] Tsai T T et al. 2009 Environmental engineering science 26(3) 651-659
[3] Stupin D J 2009 Soil pollution and new technologies for its restoration (St. Peterburg Lan)
[4] Dos Santos J J and Maranho L T 2018 Journal of Environmental Management 210 104-113
[5] Wu M et al. 2016. International Biodeterioration & Biodegradation 107 158-164
[6] Wang J et al. 2008 Petroleum Science 5(2) 167-171
[7] Snyder G H et al. 2016 In: Handbook of plant nutrition. CRC Press, p. 567
[8] Ma J F and Takahashi E 2002 Soil, fertilizer, and plant silicon research in Japan Elsevier
[9] Leynaert A et al. 2009 Limnol Oceanogr 54(2) 571-576
[10] Adrees M et al. 2015 Ecotox Environ Safe 119 186-197
[11] Bocharnikova EA et al. 2014 Mosc Univ Soil Sci Bull 69(2) 84-87
[12] Verma KK et al. 2020 Sugar Technologies 22 741-749
[13] Vivancos J et al. 2015 Molecular Plant Pathology 16(6) 572-582
[14] Cakmak I 2005 Journal of Plant Nutrition and Soil Science 168(4) 521-530
[15] Balakhnina T I et al. 2015 Plant growth regulation 75(2) 557-565
[16] Kim YH et al. 2017 Frontiers in Plant Science 8 510
[17] Izobil M et al. 2013 Science of the Total Environment 521 37-45
[18] Wei X et al. 2020 IOP Conference Series: Materials Science and Engineering 921(1) 012029
[19] Wang L et al. 2013 Biological trace element research 152(2) 275-283
[20] Włodarczyk et al. 2019 Science of the Total Environment 685 1-9
[21] Ji X et al. 2017 Environmental Science and Pollution Research 24(11) 10740-10748
[22] Achuba F I and Peretiemo-Clarke B O 2008 Int Agrophys 22(1) 1
[23] Tabatabai M A 1982 In: Methods of Soil Analysis. Part 2. Chemical and Microbiotlogical Properties (ASA and SSSA, Madison, WI)
[24] Duncan D B 1957 Biometrics 13(2) 164
[25] Matichenkov V V and Bocharnikova E A 2001 Studies in Plant Science 8 209-219
[26] Das M and & Adholeya A 2012 Microorganisms in environmental management Springer, Dordrecht p.81