Journal of Chemical Health Risks 3(3): 69-76, 2013
ISSN:2251-6719

Investigation of the Possibility of Phytoremediating a Soil Contaminated with Anthracene

M. Ahmadi[1], Z. T. Alipour[1], A. Farrokhian Firuzi[2]*

[1] Department of Soil Science, Damghan Branch, Islamic Azad University, Damghan, Iran
[2] Department of Soil Science, Shahid Chamran University, Ahvaz, Iran

(Received: 30 July 2013 Accepted: 20 August 2013)

Abstract. Polycyclic Aromatic Hydrocarbons (PAHs) are one of the most important organic pollutants frequently found in the environment. In this experiment, the effect of phytoremediation as a cost effective method was studied on the concentration of anthracene ($C_{14}H_{10}$) which is one of PAHs. The effect of sorghum (V_1), hairy vetch (V_2) and oat (V_3) was studied under four concentrations of anthracene (S_1, S_2, S_3 and S_4) in soil. In S_1 level which pollution was the lowest, the three plants had the highest reduction rate. The reduction rate was decreased by increasing the pollution level (S_2 and S_3),; the lowest reduction rate was observed in S_4 level which had the highest pollution level. There was significant difference between the three plants and the fallow. Generally, hairy vetch had the highest phytoremediating capacity and resistance compared with the other plants.

Keywords: hairy vetch, oat, Polycyclic Aromatic Hydrocarbons, sorghum.

INTRODUCTION

In oil producing countries, oil is an important source of environmental pollutions. A large volume of soil is polluted around the oil refineries and oil storage stations [1]. Polycyclic Aromatic Hydrocarbons (PAHs) are one of the most important organic pollutants frequently found in the environment and are poisonous, cancerous, mutagenic and became bioaccumulated in organisms body [2]. PAHs enter soil from various sources. They may originate from natural events such as forests fires or volcanic activity and human activities such as combustion of fossil fuels or oil refinery activities [3-4].

Different physical, chemical and biological methods are suggested to reduce oil pollution in soil. The physical and chemical methods are expensive and have some side effects and are rarely used; however, the biological methods are more safe and cost effective [5-6].

Phytoremediation is one of the most commonly used biological methods. Phytoremediation is an emerging green technology, in which plants are used to recover soils, surface and underground water sources, and sediments, polluted with heavy metals, nuclear pollutants and organic pollutants [7].

In most studies, it was indicated that legumes and grasses are the most suitable plant species to be used in phytoremediation because of their special potential. Grasses have highly developed and extensive root system with high absorption surface [8]. Legumes are also suitable for phytoremediation purposes because of nitrogen fixing ability [9-10]. Curl and Truelore [11] found the reduction of PAHs in soils under plants cultivation and suggested that mechanisms occur in soil and plant roots release compounds which decompose PAHs. There are reports about the

Corresponding Author: M. Ahmadi, Department of Soil Science, Damghan Branch, Islamic Azad University, Damghan, Iran. Email: ahmadi_6441@yahoo.com

ability of plants to decompose PAHs. Edwards [12] reported that Anthracene and Benzo Anthracene were decomposed when beans were grown in the nutrient solution. Rezek and his colleagues [13] studied the effect of ryegrass on 15 types of PAHs in a 12-18 month experiment. They reported that PAHs were reduced by 50% after one year cultivation period. In another experiment, it was indicated that grasses and legumes reduced PAHs content in soil [14]. In this experiment, separately cultivated alfalfa, switch grass, tall fescue and sudangrass significantly reduced Anthracene compared with the non-cultivated treatment. Regarding the benefits of phytoremediation, the objective of this experiment was to evaluate the ability of sorghum, hairy vetch and oat in reducing Anthracene content in a polluted soil.

MATERIALS AND METHODS

Soil preparation

In this research, a soil polluted with petroleum hydrocarbons was obtained from the area around the oil well no. 27 in Masjed Soleiman, Iran, and a non polluted soil was also obtained from the same area. Soil samples were dried in open air and were passed through a 4 mm sieve. The pollution level of the main soil sample was 11.7%, and to create different levels of pollution, the fully polluted soil sample was mixed with the non polluted one at the ratio of 1:1 (S_1), 3:1 (S_2), 5:1 (S_3) and 1:0 (fully polluted soil; S_4). Soil samples were held at the field capacity and were mixed to reach a homogenous sample. After 30 days, soil samples were ready for physico-chemical properties and Anthracene content (Table 1) measurement and plants cultivation.

Physico-chemical properties measurements

After passing the soil through a 2 mm sieve, some physico-chemical properties were measured: soil texture was measured by hydrometery, EC waste measured in saturated soil, available Na and K were measured by extraction with ammonium acetate using flame photometry, organic carbon was measured by Walkley-Black method [15], To determine lime amount, the total neutralizing value (TNV) on the basis of calcium carbonate was measured using acid acetic volume consumed to neutralizing carbonates. Acidity was measured using a pH Meter [16], and the content of some heavy metals in soil were evaluated AB-DTPA extract through atomic absorption spectroscopy [17] (Table 2 and Table 3).

Table 1. Anthracene concentration in different levels of pollution.

Pollution level	Anthracene concentration (ppm)
S_1	1414.382
S_2	2681.8
S_3	3473.073
S_4	4010.306

S_1, 1:1; S_2, 3:1; S_3, 5:1; S_4, 1:0 (the ratio of polluted to non-polluted soil).

Table 2. Some of the physico-chemical properties of the soil samples.

Soil sample	Texture	EC (dsm^{-1})	pH	K (ppm)	Organic matter (%)	Ca (meq/l)	TNV (%)	CEC (cmol+/kg)
Polluted	Loam	5.1	7.34	0.08	5.37	4.61	21	7.98
Non polluted	Loam	5.03	7.54	0.09	0.4	4.60	24	7.98

Table 3. The concentration of some heavy metals in the soil samples.

Soil sample	Fe (ppb)	Pb (ppb)	Cd (ppb)	Zn (ppb)	Cr (ppb)
Polluted	48140	<10	<6	102	75
Non polluted	50900	<10	<6	102	66

Extraction of oil from soil

After mixing the soil samples, in order to measure Anthracene concentration, 50 g of the soil was poured in Meyer Flask along with 50 cc dichloromethane, was packed and shook on a shaker for 1 h. Then, the mixture was passed through a filter paper, and the obtained solution was injected to HPLC.

Plants cultivation

For the phytoremediation studies, 5-3 kg pots were filled with 3 kg of the soils with three repetitions and the seeds were planted 1-2 cm below the soil surface. A non cultivated control was also considered for each level of soil pollution to estimate and remove the effect of environmental conditions on the reduction of pollutants. This experiment took three months; soil samples were taken from the area around the roots to evaluate Anthracene concentration reduction.

Experimental design and statistical analysis

This experiment was conducted in factorial in the form of a completely randomized design with three replications. Data were analyzed using SAS at $P \leq 0.05$ and means were compared according to the Duncan's multiple range test.

RESULTS AND DISCUSSION

Biomass

Analysis of variance of the effect of treatments on plant dry matter yield indicated that pollution level and the interaction of pollution level × plant type significantly affected plant dry matter yield (Table 4). Mean comparison showed the reduction of plant growth as the result of Anthracene presence in soil; the reduction was more obvious in higher pollution levels (Table 5). Hairy vetch produced the highest dry weight compared with the other plants (Table 5).

According to the results, all plants had higher biomass in S_1 pollution level which had the lowest Anthracene concentration; the lowest biomass was observed in S_4 which had the highest Anthracene concentration. All pollution levels had significant differences with the control, and 64, 78, 82 and 89% reduction of biomass was occurred in S_1, S_2, S_3 and S_4 levels, respectively, compared with the control (Table 5). Sorghum was the most sensitive plant species to the enhancement of Anthracene concentration and its biomass production reduced more severely when pollution level increased (Table 5). In another experiment [18] it was observed that grasses and legumes biomass production was reduced by 43 and 64%, respectively, as the result of PAHs pollution. Fan and his colleagues [19] also reported that alfalfa root and shoot growth and dry weight were reduced in soil polluted with Pyrene; the reduction was more drastic in higher pollution levels

Table 4. Analysis of variance of the effect of treatments on the measured traits.

SOV	df	Mean Square (MS)	SOV	df	Mean Square (MS)
		Plant dry weight			**Anthracene reduction**
Concentration (A)	4	248.173*	Concentration (A)	3	1150.401*
Plant (B)	2	2.921ns	Plant (B)	3	2567.096*
A × B	8	13.038*	A × B	9	61.138ns
Error	30	1.92	Error	32	42.901
Total	44	-	Total	47	-

Ns, non significant; *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$.

Table 5. Effect of Anthracene level, plant species and their interaction on the measured traits.

Treatments	Plant dry weight (gr)	Anthracene reduction (%)
S_0 (non polluted)	14.446a	-
S_1	5.085b	41.228a
S_2	3.152c	33.263b
S_3	2.580cd	25.586c
S_4 (fully polluted)	1.480d	18.515d
V_1	4.842a	28.563c
V_2	5.652a	44.506a
V_3	5.551a	35.509b
V_4 (fallow)	-	10.014d
S_0V_1	17.303a	-
S_0V_2	10.867b	-
S_0V_3	15.170a	-
S_0V_4	-	-
S_1V_1	3.650cdef	41.597bcd
S_1V_2	6.207c	59.923a
S_1V_3	5.400cd	49.393ab
S_1V_4	-	13.997fg
S_2V_1	1.407fg	35.653cd
S_2V_2	4.773cde	46.937bc
S_2V_3	3.277defg	39.170bcd
S_2V_4	-	11.293fg
S_3V_1	0.960g	22.847ef
S_3V_2	3.873cdef	40.660bcd
S_3V_3	2.907defg	30.237de
S_3V_4	-	8.600g
S_4V_1	0.893g	14.153fg
S_4V_2	2.543efg	30.503de
S_4V_3	1.003g	23.237ef
S_4V_4	-	6.167g

S0, 0:1; S_1, 1:1; S_2, 3:1; S_3, 5:1; S_4, 1:0 (the ratio of polluted to non polluted soil).
V_1, sorghum; V_2, hairy vetch; V3, oat.

Phytoremediation studies

Analysis of variance indicated the significant effect of pollutants concentration and plant species on the reduction of Anthracene concentration at P0.05 (Table 4); proving the effect of vegetations on the decomposition of petroleum pollutants in soil. The interaction of the two factors had no significant effect on the reduction of Anthracene concentration.

Mean comparison of the plant species indicated the significant variation among the plants. Results indicated that all plant species showed resistance to the pollution and reduced the concentration of Anthracene. Anthracene reduction was 28.563% in sorghum cultivation, 44.506% in hairy vetch, 35.509% in oat and 10.014% in fallow (Table 5). Mean comparison also indicated that the levels of pollution had significant differences. In other words, the reduction of Anthracene concentration varied in different pollution levels. The highest reduction was observed in S_1 level (41.22%) and the lowest reduction was observed in S_4 (18.515%). The reduction of Anthracene concentration was 33.26% in S_2 and 25.85% in S_3 levels (Table 5). So, it can be concluded that the reduction of Anthracene concentration occurs more slowly in soils with higher pollution rate. On the other hand, although plants were more effective in soils with lower pollution rate; however, they could tolerate high pollution rates well.

Among the three plants, in S_1 pollution level, hairy vetch was the most effective one, reducing Anthracene content by 59.923% compared with the non cultivated control. The reduction was 41.597% in sorghum and 49.393% in oat. In S_1, hairy vetch, sorghum and fallow were significantly different but there were no significant differences between the hairy vetch and oat. In S_4, S_3, S_2 and S_1 concentrations of Anthracene, sorghum showed 14.15%, 22.84%, 35.65% and 41.59% reduction, hairy vetch showed 30.50%, 40.66%, 46.93% and 59.92% reduction and oat showed 23.23%, 30.23%, 39.17% and 49.39% reduction, respectively, compared with the control (fallow). In all pollution levels, hairy vetch had the highest reduction and sorghum had the lowest. It seems that sorghum was not effective at all in high Anthracene concentration; it had no significant differences with the fallow in S_4 level.

Although the phytoremediation capacity of the three plant species was different; however, they were all effective on the reduction of Anthracene content in soil compared with the non cultivated control which was designed to evaluate the effect of environmental factors on soil pollution reduction. This may be attributed to high microbial activity in the rhizosphere compared with the non cultivated soil. Plant roots release some nutrients and substances to soil and increase soil aeration; promoting the activity of soil microbial population which decomposes the petroleum pollutants in soil [20]. Ebbs and Leon [21] tested the possibility of phytoremediating zinc by oat (*Avena sativa*), barley (*Hordeum vulgare*) and Indian mustard (*Brassica juncea*) and reported that oat was more resistant to high concentrations of cupper, cadmium and zinc. These metals accumulated at high concentrations in the buds. They found that Zn concentration was higher in Indian mustard but the two other species were more resistant. The resistance of oat to Total Petroleum Hydrocarbons (TPHs) was also reported by Banuelos and his colleagues [22]. They concluded that various plants such as canola (*Brassica napus*), oat and barley are resistant to the accumulation of different metals such as selenium, cupper, cadmium and zinc. In their experiment, oat had resistance to the organic pollutants.

In our experiments, different plant species from grasses and legumes were tested; all showed resistance to the petroleum pollutants and reduced Anthracene concentration in soil. These findings were in agreement with those of Aprill and Sims [8], Gunther and his colleagues [23] and Reilley and his colleagues [14]. In these experiments, the phytoremediation ability of various plant species from grasses and legumes was proved. Smith and his colleagues [24] tested seven plant species from grasses and legumes in soils polluted with PAHs and reported that the pollutions had no effect on the germination of plants. Wang and his colleagues [25] found the main reduction in soil PAHs concentration occurs within 30 days from plants cultivation. Schwab et al. [26] observed the mineralization of Phenanthrene in a soil under cultivation of sorghum, bermuda grass and alfalfa. They reported that within 14 days, the highest biodegradation was related to sorghum (46%) and bermuda grass (31%); the biodegradation rate was only 11% in the non cultivated control.

CONCLUSION

Results of this experiment indicated that different concentrations of Anthracene in soil reduced plants growth and dry matter yield. Sorghum, hairy vetch and oat reduced Anthracene content in soil with significant differences from the reduction occurred in the non cultivated control. Moreover, the phytoremediation ability of the three plant species was lower in high Anthracene concentration. It seems that the legumes are more suitable for phytoremediation purposes, probably because of their nitrogen fixation ability. On the other hand, grasses with extensive root system may be really effective in phytoremediation because absorption of the pollutants mainly occurs in direct contact with root surface. In our experiment, the three plants were formed grasses or legumes; hairy vetch had the highest resistance and biodegradation capability and sorghum had the lowest.

REFERENCES

1. Malayeri B., Ghodratzadeh H., Yousefi N., 2008. In: Detection of the plant species resistance to petroleum pollutants. Proceedings of the 2nd

Scholar Conference on Biology and the Modern World, Gorgan Univrsity of Agriculture and Natural Resources, Iran.

2. Denton G.R.W., Concepcii L.P., Wood H.R., 1999. Heavy metals, PCBs and PAHs in marine organisms from four harbor locations on Guam, WERI Technical Report, 81, 120 p.

3. Piccardo M.T., Coradeghini R., Valerio F., 2001. Polycyclic Aromatic hydrocarbon pollution in native and caged mussels. *Mar. Pollut. Bull*. *42*, 951-956.

4. Tolosa I., de Mora S., Sheikholeslami M.R., Villeneuve J. P., Bartocci J., Cattini S., 2004. Aliphatic and aromatic hydrocarbons in coastal Caspian Sea sediment. *Mar. Pollut. Bull*. *48*, 44-60.

5. Atlas R.M., 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiol. Rev. 45*, 180-209.

6. Zhou Q.X., Song Y.F., Principles and Methods of Contaminated Soil Remediation, Science Press: Beijing, 2004.

7. Pradhan S.P., Conrad J.R., Paterek J.R., Srivastava V.J., 1998. Potential of phytoremediation for treatment of PAHs in soil at MGP sites. *J. Soil Contam*. *7*, 467-480.

8. Aprill W., Sims R.C., 1990. Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. *Chemosphere*. *20*, 253-265.

9. Gudin C., Syratt W.J., 1975. Biological aspects of land rehabilitation following hydrocarbon contamination. *Environ. Pollut*. *8*, 107-112.

10. Lin Q., Mendelssohn I.A., Suidan M.T., Lee K., Venosa A.D., 2002. The dose-response relationship between No. 2 fuel oil and the growth of the salt marsh grass, *Spartina alterniflora*. *Mar. Pollut. Bull*. *44*, 897- 902.

11. Curl E.A., Truelore B., The Rhizosphere. Springer-verlag: Berlin, 1986.

12. Edwards N.T., 1988. Assimilation and metabolism of polycyclic aromatic hydrocarbons by vegetation-an approach to this controversial issue and suggestions for future research. National Technical Information Service, AC05-84OR21400.

13. Rezek J., Wiesche G., Machova M., Zadrazil F., Macek T., 2008. The effect of ryegrass (*Lolium perenne*) on decrease of PAH content in log term contaminated soil. *Chemosphere*, *70*, 1603-1608.

14. Reilley K.A., Banks M.K., Schwab A.P., 1996. Organic chemicals in the environment; dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. *J. Environ. Qual*. *25*, 212-219.

15. Manteghi N., *Explanation of Laboratory Methods and Studies on Water and Soil Sample*. Bulletin no. 168. Soil and Water Research Institute: Iran, 1977.

16. Black C.A., Evans D.D., White J.L., Ensminger L.E., Clark F.E., *Methods of Soil Analysis*: Part 2. Agronomy Monograph: ASA, Madison, Wisconsin, 1965.

17. Lindsay W.L., Norvell W.A., 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Sci. Soc. Am. J. 42*, 421-428.

18. Palmroth, M.R.T., Pichtel, J., Puhakka, J.A. 2002. Phytoremediation of subarctic soil contaminated with diesel fuel. *Bioresour. Technol*. *84*, 221- 28.

19. Fan S., Li P., Gong Z., Ren W., He N., 2008. Promotion of pyrene degradation in rhizosphere of alfalfa. *Chemosphere*, *71*, 1593-1598.

20. Li C.H., Ma B.L., Zhang T.O., 2002. Soil bulk density effects on soil microbial population and enzyme activities during the growth of maize (*Zea mays*) planted in large pots under field exposure. *J. Plant. Sci. 82*, 147-154.

21. Ebbs D.S., Leon V.K., 1998. Phytoremediation of zinc by oat (*Avena sativa*), barley (*Hordeum vulgar*), and Indian mustard (*Brassica juncea*). *Environ. Sci.Technol. 32*(6), 802-806.

22. Banuelos G.S., Ajwa H.A., Machey B., Wu L., Cook C., Akohoue S., Zambruzuski S., 1997. Evalution of different plant species used for phytoremediation of high soil selenium. *J. Environ. Qual. 26*, 639-646.

23. Gunther T., Dornberger U., Fritsche W., 1996. Effects of ryegrass on biodegradation of hydrocarbons in soil. *Chemosphere*, *33*, 203-215.

24. Smith M.J., Flowers T.H., Duncan H.J., Alder J., 2005. Effects of PAHs on germination and subsequent growth of grasses and legumes in freshly contaminated soil and soil with aged PAH residues. *J. Environ. Pollute*. *101*, 1-7.

25. Wang Z., Gao D., Li F., Zhao J., Xin Y., Simkins S., Xing B., 2008. Petroleum hydrocarbon degradation potential of soil bacteria native to the Yellow River Delta. *Pedosphere*. *18*(6), 707-716.

26. Schwab A.P., Banks M.K., Arunachalam M., Biodegradation of Polycyclic Aromatic Hydrocarbons in Rhizosphere Soil. In: *Bioremediation of Recalcitrant Organics*, Hinchee, R.E., Anderson, D.B., Hoeppel, R.E. Eds., Batfelle Press: Columbus, 1995. pp. 23-29.