Advances in Bioresearch
Adv. Biores., Vol 7 (3) May 2016: 49-55
©2015 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV Value 8.21 [2014]

Advances in Bioresearch

ORIGINAL ARTICLE

Effects of Polychlorinated biphenyls compounds on the number of bacteria in the rhizosphere of sorghum and *Onobrychis sativa*

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ABSTRACT

Polychlorinated biphenyls are a group of persistent organic pollutants (POP_S). These pollutants (PCB_S) are a great environmental and human health concern. Phytoremediation is a strategy inexpensive and environmentally compatible. This technology reduces various types of pollutants. The aim of the present study was to investigate the effects of polychlorinated biphenyls compounds on the number of bacteria in the rhizosphere of sorghum and Onobrychis sativa. Polychlorinated compounds used in this of the design. Formerly, the methods were using transformer oil, which is stable at high temperatures and has excellent electrical insulating properties. It is used in oil-filled transformers, some types of high-voltage capacitors, fluorescent lamp ballasts, and some types of high-voltage switches and circuit breakers. The concentrations can be 100, 200, 400 and 800 ppm, respectively. Soil bacterial population was measure than successive dilutions (Serial method), and expressed as $CFU_{g^{-1}}$ dry soil. In conclusion, 120 days after planting, plants were separated from the soil.The statically analysis is indicated a significant difference among population of bacteria in soils without plant and rhizosphere. ($p \leq 0.05$) Population of bacteria in rhizosphere of sorghum plant was more significant than onobrychis .s rhizosphere. The number of bacteria in different modes of soil without plant such as natural and surfactant was in range of 10^6 cfu/gr dry soil. Plus, about rhizosphere, the different modes of soil with plant was in range of 10^8 cfu/gr.Overall, regarding to the results we can suggest that the sorghum with surfactant and then onobrychis.s with surfactant as the best form to apply the phytoremediation process in compare with other forms.

Keywords: *Phytoremediation, polycholorine biphenyl, sorghum, Onobrychis sativa*

Received 21/02/2016 Accepted 22/04/2016

How to cite this article:
M Zare, M-Mehdi Baneshi, A Rayegan-Shirazi, S Rezaei, S-Abdolmohammad Sadat and E Randjbaran. Effects of Polychlorinated biphenyls compounds on the number of bacteria in the rhizosphere of sorghum and *Onobrychis sativa*. Adv. Biores. Vol 7 [3] May 2016: 49-55.DOI: 10.15515/abr.0976-4585.7.3.4955

INTRODUCTION

Polychlorinated biphenyls are a group of persistent organic pollutants (POPs). POPs are chemicals that remain intact in the environment for long periods, become widely distributed geographically, accumulate in fatty tissue of living organisms and are toxic to humans and wildlife. POPs circulate globally and can cause damage wherever they travel.

These compounds are containing 1 to 10 chlorine atoms were attached to rings biphenyls [1]. Polychlorinated Biphenyls (PCBs) were widely used during the 1930s and 1970s in transformer oil, lubricant, insulating and dielectric fluids. Since 1970 the production of these materials has been banned in some countries due to transportation, storage and improper disposal have found into the environment, especially water and soil. Now, PCBs are still defined as a global problem and a threat to the environment that has a high absorption capacity of the soil, high stability and low solubility in water and in human tissue known. [2, 3]

These compounds are contaminants in the environment unique. According to the applications in several places, they can be achieved that researchers do not to use them, such as Polar Regions and Deep Ocean.

Due to the expensive and destructive of traditional methods of these compounds, simultaneously, it is a need reach the highest effective and efficient at the same time being compatible with the environment. [4, 5].

Moreover, evidence from a large number of academic studies shows that, the amount of soil decomposition of PCBs in the soil is increasing, with or without plants. Phytoremediation is an applicable method for plants to remove contaminants from soil, surface water and ground water. The main processes of the Phytoremediation are including: degradation in the rhizosphere with collaboration the root zone bacteria for the compounds remove organic contaminants from soil and transfer to the roots and shoots [6, 7, and 8]. For reason that the solubility of these compounds in water (K_{OW} = 5-7) is low so Transfer of pollutants to air and root organs is less important than the rhizosphere. It is the role of plants in phytoremediation and in the rhizosphere that injected air into the soil through the roots and it is increase the activity of aerobic bacteria indigenous lead to the secretion of substances such as sugar and organic acid. It is provides energy source for the bacteria (aerobic metabolism). The use of herbs for treatment of soil contamination is how received much success in treating a variety of organic compounds such as polychlorinated [9].

Phytoremediation is a strategy inexpensive and environmentally compatible. There are several mechanisms in Phytoremediation, but the basic mechanism of phytoremediation is stimulated soil microbial activity and the decomposition of pollutants [10].

One notable benefit is proved prevent soil erosion and reduce the movement of the groundwater pollution. It also maintains soil structure and increase soil organic matter. Therefore, modified soil, would be suitable for agricultural purposes. [11] Using indigenous plants that require little care can be destroyed large amounts of pollution. Because of the indigenous plants have adapted to the climate and conditions Hydro Geological, they are better growth and development of their region And also the possibility that the area will become a pest for plants less than non-indigenous plants are preferable. [12]

Since the effectiveness of phytoremediation depends on greatly plant species, soil conditions, weather conditions, physical properties and chemical contaminants it is necessary that grow plants in the range of meadows and be identified a suitable species for phytoremediation to remove any contaminants.

The two plant types used in the present study were: one type of Poaceae (i.e. Sorghum) and one type of legumes (i.e. *Onobrychis sativa*) with a good potential for phytoremediation and the ability of these two plants were evaluated for phytoremediation compounds polychlorinated biphenyls, also The effects of adding non-ionic surfactant Tween80 were evaluated on their performance efficiency.

MATERIALS AND METHODS

Polychlorinated compounds used in this of the design that were Transformer oil using previous studies, the concentrations of 100, 200, 400 and 800 ppm, respectively.

Soil samples were spiked with no history of pollution that were collected in the depth of 0 to 20 cm from the edge of the old road Yasuj -Shiraz meadows. Soil samples were dried by sieving 4 mm sieve. The samples were contaminated with concentrations above the transformer oil, acetone was used as solvent for dispersion of pollutants in the soil, the soil weighed in a cylinder of acetone were added and were allowed to Stan to end penetrate the container lid closed and then stirred in different directions, then the container was under the hood. Stir every half hour operation for 3 hours and then for 6 hours was repeated once every two hours.

Soil contaminants to ensure Uniform so they dig and sift again with our 4 mm sieve. Then the soil let for a week at room temperature until it evaporates completely Stan .1.5 kg of prepared soil in the pot with a concentration of 15 cm width 20 cm height pass. [13]. a surfactant added with the surfactant Tween80 in distilled water and then thoroughly player potting soil by spraying and poke the volume of solution to be added to concentration of 45 mg per kg of soil is dry. Match 3 should be repeated with 60 pots [Table 1]:

5(concentration of polychlorinated biphenyls) ×2(Plant) ×2(Surfactant) ×3(repeat) =60 Pots

In each 60 pot, put 1.5 kg of soil preparation plus 10 seeds of sorghum and 10 seeds of onobrychis in each pot. Supplementary, onobrychis, the sainfoins, are Eurasian perennial herbs of the legume family (Fabaceae). Including doubtfully distinct species and provisionally accepted taxa, about 150 species are presently known. The Flora Europaea lists 23 species of Onobrychis; the main center of diversity extends from Central Asia to Iran, with 56 species – 27 of which are endemic – in the latter country alone. O. viciifolia is naturalized throughout many countries in Europe and North America grasslands on calcareous soil.

By remaining six plants, the rest of them were eliminated. Watering process was done for every other day. Soil bacterial population was measure than successive dilutions (Serial method), first, 1 gram of rhizosphere soil mixed with 100 ml of sterile water to reach the 1 mL dilution of 10^{-2} to 9 ml of distilled water were added consecutively the dilution of 10^{-3}, 10^{-4}, $10^{-5}$of each of these three concentrations

achieved ml on nutrient agar plates containing medium is used then the plates at 37 ° C for 1 day with the number of colonies was counted and expressed as $CFU_{g^{-1}}$ dry soil.

At the end of the study (120 days after planting), plants were separated from the soil slowly. After separating the roots to the shoots, the shoots were measured during each of these components is then removed by water washing was , rinse with distilled water and the water was taken by soft tissue paper and dried at 80 ° C for 48 hours strata end weight was determined by digital scale. [14]

Data analysis

All statistical analyses were performed with SPSS version 16 (SPSS Inc., Chicago, Ill.), the level of statistical significance of p≤0.05 was made in the charts. Graphs were produced using Microsoft Excel software.

Table 1 Treatment of experimental design

□	T_0 □	T_1 □	T_2 □	T_3 □	T_4 □	T_5 □
Natural attenuation	+□	□	□	□	□	□
Surfactant	□	+□	□	□	+□	+□
Sorghum	□	□	+□	□	+□	□
Onobrychis	□	□	□	+□	□	+□

RESULT AND DISCUSSION

The population of microbial in zone of plant's rhizosphere is as an important factor in doing phytoremediation and removing the organic contamination of soil. By increasing the population of bacteria will provide an appropriate situation for plant in rhizosphere zone. There are many studies that have proved the different effects of plant specious on the population of microbial. [13] Root exudates in the zone of rhizosphere causes stimulates soil microorganism.

In other hand, the microbial activity in root zone causes a change in pattern and root characteristic and also it provides food productions for plant. Coexistence between plant and microbe can be considered as an important factor in decomposition of resistant contaminates. [14]

The lowest population is in mode natural condition to rate 6.4 CFU/gr and the most populations of bacterial is in mode sorghum and in concentration 200 ppm to rate 8.5 CFU/gr.

According to average, microbial log removal rate lowest in natural condition is 7.07 CFU/gr and the maximum is in sorghum to rate 8.10 CFU/gr

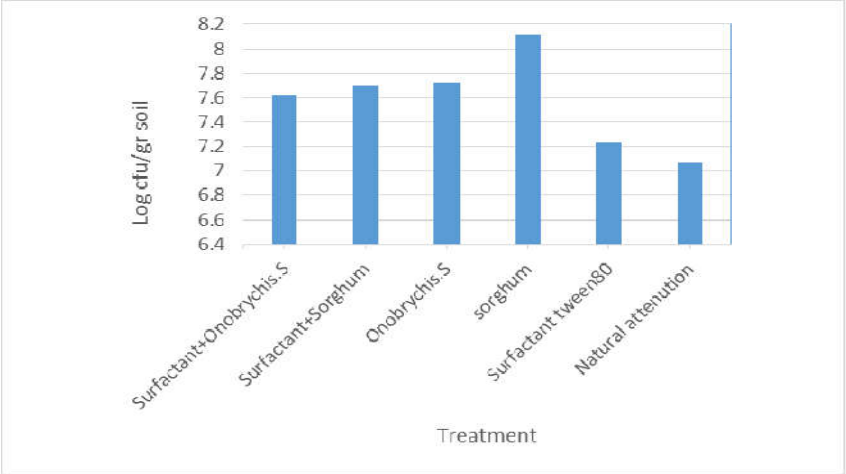

Figure 1:The log average of bacteria number in different modes

The number of bacteria in different modes of soil without plant such as natural and surfactant was in range of 10^6 cfu/gr soil dry. And about rhizosphere, the different modes of soil with plant was in range of 10^8 cfu/gr . (figure1)

By planting both of plant's it significantly shows and increase in number of soil bacteria. This grow in population of bacteria is due to grow of bacteria in presence of plants root.

This case can be due to secretion of materials such as amino acids, organic acids, enzymes, sugars and secretion compounds such as surfactants in zone of plant root. These materials can be used as a carbonate

and energy source and they cause increase in population of bacterial in soil, especially in rhizosphere zone. [15].

Statistical analysis is a component of data analytics, so it involves collecting and scrutinizing every data sample in a set of items from which samples can be drawn. A sample, in statistics, is a representative selection drawn from a total population. Therefore, the statistical analysis also indicated a significant difference among population of bacteria in soils without plant and rhizosphere ($p \leq 0.05$).

Population of bacteria in rhizosphere of sorghum plant was more significant than onobrychis .s rhizosphere.

This case can be result in the stronger root system and also network system of sorghum in compare to onobrychis.s plant [16]

In natural situation there was not a significant relationship between add surfactants and grow of microbial population.

Reversely in presence of plant there was a significant relationship between add surfactant and increase of microbial population. this case is for this reason that by adding surfactant to contaminate soil it increase a bioavailability to pollutant through plant and bacteria.in this way bacteria can use these as an energy and carbonate source and as well as addition to the gradation of this compounds they cause increase in population in zone of rhizosphere .[17]

In two plants, the effect of surfactant on sorghum plant was more significant than onobrychis.s .primary concentrations of polycholorine biphenyl there was not any significant effect on population of soil microbial. Regarding to the toxicity of PCBs in on soil natural bacteria, it seems that the high efficient of phytoremediation may in decrease initiate concentration and or convert and decompose pollutants and also the times that waste for compatibility bacteria with circumstances it lead to lack of concentration effect on bacteria population .previous studies confirms the consequences this research.[18,19]

The total mass of plant

The lowest mass of sorghum in form of sorghum without any surfactant is in concentration 800 ppm and at rate 9.30 gr per pot. The most mass of sorghum in combination form of sorghum with surfactant is in concentration 100 and at rate 15.73g pot. About onobrychis.s we have the lowest mass in form of onobrychis.s without surfactant and concentration 800 ppm and at rate 6.66 gr per pot and, the most of mass at rate of 8.7 in form of onobrychis.s without surfactant is with concentration 0 ppm.

The total mass of both plants have been shown in figure 2 in different initiate concentration of pollutant.

About sorghum plant there is a significant difference in combination with surfactants in compared to sorghum without surfactant? ($p \leq 0.05$)

It seems that surfactant tween80 reduce the total mass of the sorghum, because it can be argued that surfactant causes the release of contaminants from soil and increases the bioavailability for pant. [20]

And according to the toxic effects by these pollutants on plant, they could have an effect of decreasing on plant mass in high concentrations.

There wasn't a significant difference between onobrychis without surfactant and onobrychis in combination with surfactant. And it seems that onobrychis plant makes a lower mass due to its less growth than sorghum .therefore, onobrychis plant has more tolerance than sorghum plant. Thus, this reduce can be resolved by bio augmentation action. [21]

The effect of pollutant concentration on the total mass of plant shows that the maximum mass has been in a state that there are no pollutants in the soil .according to statically analysis it wasn't observed a significant difference between the total of mass plant in vary concentrations of pollutant. [22]

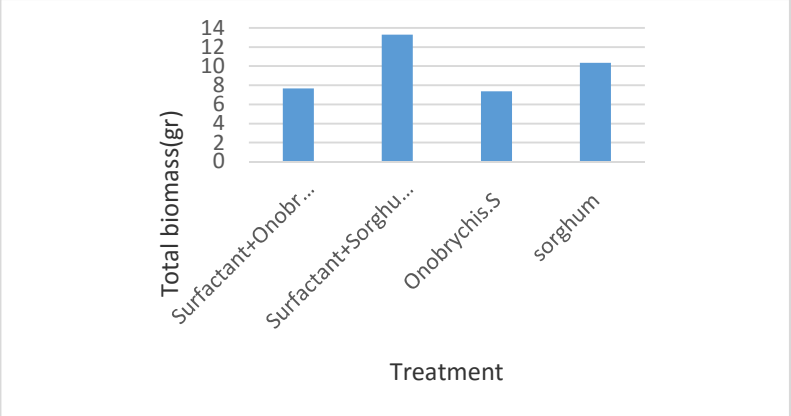

Figure 2 :The total mass of both plants'

The mass of root:

The average root dry mass of sorghum and onobrychis root is about 4.58 and 3.3 gr per pot, relatively. The lowest dry mass of sorghum root in from of sorghum without surfactant is in concentration 800 ppm and the most dry mass in from of concentration with surfactant is in concentration 100 ppm .

In case of onobrychis plant, the lowest dry mass of root in form of onobrychis without surfactant is at rate 2.9 gram and the driest mass of root is about 3.9 gr, in form of onobrychis without surfactant.

Whereas it was observed, the maximum root mass was in a position that there was no pollutant in soil and also at least of root mass was in form of maximum pollutant. Influence of different pollutant concentrations on root mass have been significant, instead, there was no significant the effect of concentration on the total mass plant.

This case shows that concentrations on root mass affected more than total mass of plant and also increase in concentration significantly, have been affected on root mass.

Otherwise some root, of plants are sensitive to PCBs .so, this result can be accurate about above discussion.

In case of sorghum plant, there was no significant relationship between increase of concentration and root mass by adding the surfactant had no so much impact on root mass.

Mass of shoot

Mass of shoot for sorghum and onobrychis.s is approximately 7 gr and 4.06 gr per pot relatively. The most mass of sorghum shoot is related to sorghum with surfactant and the lowest mass of it is about plant without surfactant regarding to statically analysis it shows that the form of combination of sorghum with surfactant has a significant reduce in mass of shoot in compare to sorghum without surfactant and another forms haven't a significant difference with together. About onobrychis.s among different forms of shoots wasn't observed an important difference. Whereas in this indicate both of two plants have good stability but, onobrychis.s has become more stable. Therefore, the different concentrations of pollutant hadn't any more influence on mass of shoot and it seems that the main effect of PCBs is on plant roots and it hasn't a significant effect on shoots. [16]

In high concentration of PCB about 800 it was observed a pale leaves among the few plants of sorghum that it can be due to the effect of PCB on growth of plant.

Table 2 : Root and shoot weights (g) of sorghum and onobrychis.s grown in control and PCB-amended soils

modes	Root weight(gr)					Shoot weight(gr)				
	control	1 00	2 00	4 00	800	control	1 00	2 00	4 00	800
sorghum	4.7	4.9	4.78	4.37	4.15	5.72	7.12	5.82	5	5.25
Onobrychis	3.88	3.35	3.27	3.18	3.02	4.79	3.83	3.93	3.83	3.8
Sorghum+ surfactant	5.5	5.42	4.87	4.87	4.75	9.8	9.54	6.88	7.57	7.31
Onobrychis +surfactant	3.8	3.59	3.47	3.53	3.44	4.82	4.28	3.82	3.77	3.81

ppm*

The ration of root mass to mass of shoots:

The ratio of root mass to mass of shoots can be considered as an important indicate in determine appropriate plant for phytoremediation because whatever this ratio be more the favorable conditions for activity of bacteria the average of this ratio was 0.69 and 0.84 for sorghum and onobrychis.s plants relatively.

Therefore, onobrychis despite having less mass (because it was more the ratio of root to shoot) it can be a good select for phytoremediation of PCB compounds that onobrychis.s can do decomposition of pollutant same as sorghum and even better than it.

The high concentration of PCB had a significantly effect that its reason can be the released more pollutants from soil compounds and also it provide more toxicity for plant. Table 3 shows the correlation between microbial count and plant biomass.

Whereas, there was a significant relationship for both plants in high concentrations of PCB. (P≤0.05)[13]

Table 3 Correlations (R2) of microbial counts and plant biomass

	Bacterial counts	Shoot weight	Root weight
Bacterial counts			
Shoot weight	0.69		
Root weight	0.70	0.80	
Soil PCB level	-0.55	-0.57	-0.90

Length of plant:
According to statically analysis there was no significant relationship between concentrations of PCB and length of both of plants. And these consequences can emphasis on shoot of plant.

CONCLUSION

the conclusion of this study shows that the plant has a positive effects the increase in microbial population from rhizosphere zone and also, decomposition of organic pollutant, whereas the population of microbial in culture treatment with plant was more significantly than average of populations of microbial in treatment without plant .this result indicates a relationship between plant and the microbial of rhizosphere zone, as well. Therefore, it can be concept able that the plant is a provoke factor on increase of population of microbial of pollutant decomposition, in this way, it led to an increase in pollutant remove from soil.
According to study on variants Tween 80 surfactant to prove in activity of microbial population, the increase of surfactant can have positive effects on pollution removal by increasing in bioavailability.
Except several cases about high concentration of PCB, the different concentrations of this pollutant didn't have any effect on biomass. Therefore, this case shows a high tolerance of plant.
In fact as a total result about study of all variants it can explain that both of plant (onobrychis and sorghum) with having characteristics such as power in nitrogen stabilizing and prevent of soil erosion and also having an appropriate root system, they have a high potential in phytoremediation of PCBs compound. Generally regarding to the results we can suggest that the sorghum with surfactant and then onobrychis with surfactant as the best form to apply the phytoremediation process in compare with other forms.

ACKNOWLEDGEMENTS

Authors highly appreciated of Yasuj University of Medical Sciences for their financially supports of the study.

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