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Bioremediation of Polychlorinated Biphenyl (PCBs) in a Sewaged Soil by Certain Remediative Amendments Followed By Phytoremediation.

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ABSTRACT

Control of water pollution and environmental protection are major issues to preserve living conditions for the future in Egypt. Polychlorinated biphenyl (PCBs) in sewaged soil were decontaminated in two experiments, a column experiment irrigated with regular water and a field experiment irrigated with treated sewage effluent. Decontamination of the key members of PCBs was done in two stages, bioremediation with various single and/or combined remediative amendments followed by phytoremediation with certain hyperaccumulator plants. Out of eight investigated polychlorinated biphenyl in the high contaminated sewaged soil only three PCBs were detected, i.e. 2 3´,4, 4´,5-pentachlorobiphenyl , 2,2´,3,5,6,6´-hexachlorobiphenyl and 2,3,3´,4,5,5´,6-heptachlorobiphenyl. Results indicated that the three detected PCBs tented to persistently disappear from the soil under the action of both indigenous biomass and root exudates in the presence and absence of the experimented remediative amendments. The highest diminish rate was recorded under the action of indigenous biomass and root exudates in association with a combined mixture of all remediative amendments. After bioremediation2 3´, 4, 4´,5-pentachlorobiphenyl PCB decreased to 8% of its initial value and disappeared entirely from the soil ecosystem after phytoremediation. On the other hand, 2,2´,3,5,6,6´-hexachlorobiphenyl PCB decreased to 25 and 23% of its initial value after bioremediation associated respectively with regular water or treated sewage effluent irrigation, and was found in the soil ecosystem after phytoremediation. 2,3,3´,4,5,5´,6-heptachlorobiphenyl PCB completely degraded after microbial remediation and was undetectable in the soil ecosystem after phytoremediation.

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INTRODUCTION

Polychlorinated biphenyls (PCBs) were first introduced into commerce in 1929 and became widely used mostly as pesticides. Because they are very stable compounds [23] with a long half-life between 8 to 10 years [15], degrade very slowly in the environment and build up in the food chain, they had been banned from further production in many countries. Their destruction by chemical, thermal, and biochemical processes is extremely difficult. The environmental transport of PCBs is complex and nearly global in scale. They accumulate primarily in the hydrosphere, in the organic fraction of soil, and in organisms, however, a small fraction had been detected in the atmosphere of the most urbanized areas. Bioremediation is a process of using microorganisms to degrade PCBS in place with the goal of converting harmless chemicals as end products [18]. Chlorinated herbicides (*e.g. s* triazines) and polychlorobiphenyls (PCBs) are persistent organic pollutants (POPs) that are widely distributed in the environment. *s*-Triazine herbicides are used in agriculture and forestry in diverse regions of the world. PCBs were produced worldwide for industrial applications, and an important amount of these compounds have been released into the environment. PCBs and *s*-triazines are toxic compounds that could act as endocrine disrupters and cause cancer. Therefore, environmental pollution with *s*-triazines and PCBs is of increasing concern. Bioremediation is an attractive technology for the decontamination of polluted sites. Microorganisms play a main role in the removal of POPs from the environment [22].

Chekol et al. 2004 stated that PCBs biodegradation in soil seem to be positively influenced by the presence of plants and plant–bacteria interactions. Also, the results suggested that phytoremediation could be an environmentally friendly alternative for PCB-contaminated soils.

The inclusive ambition of the current work is the decontamination of Polychlorinated biphenyl (PCBs) in contaminated sewaged soil ecosystem through bioremediation with certain remediative amendments followed by phytoremediation.

MATERIALS AND METHODS

Experimental

Two experiments were carried out to decontaminate certain aliphatic hydrocarbons in a soil sewaged for 32 years. The first was a field experiment carried out at Abu-Rawash sewage farm, and second was a column experiment carried in the greenhouse at the National Research center. The moisture content of the soil was initially adjusted to 50% of the soil field capacity (35%), and was thereafter kept at this level during the experimental period by eventual irrigation with either treated sewage effluent in the field experiment or regular water in the column experiment. In both experiments, the decontamination process was carried out in two successive stages, bioremediation followed by phytoremediation. Bioremediation extended for 60 days in uncultivated control, cultivated control, soil inoculated with a mixture of *Thiobacillus thiooxidans & Thiobacillus ferrooxidans,* soil enhanced with probentonite (a mixture of 1% bentonite + 1% rock phosphate inoculated with phosphate dissolving bacteria) and soil treated with a combined mixture of all the

aforementioned remediative amendments. After bioremediation stage the sewaged soil was phytoremediated with canola (*Brassica napus*) in the column experiment and with canola, Indian mustard (*Brassica juncea*) and black nightshade (*Solanum nigrum*) hyperaccumulator plants inoculated with arbuscular mycorrhizal conidia (AM) in the field experiment for two months. Composite soils were prepared from the different replicates in each treatment initially, after bioremediation and at the maturity stage of the experimenter hyperaccumulator plants to examine the existence and degradation of certain chlorinated hydrocarbons in the sewaged soil ecosystem.

Culture collection

Phosphate dissolving bacteria (*Bacillus megatherium* var. *phosphaticum*) were isolated and grown on Pikovskyaya's medium [7,19]. *Thiobacillus ferrooxidans* were isolated and grown in DSMZ medium 882 [4]. *Thiobacillus thiooxidans* were isolated and grown in modified Waksman medium [10, 21,12]. Mycorrhizal (AM) conidia were extracted from soil by wet sieving and sucrose density gradient centrifugation according to [1].

All microorganisms used in the remediative amendments except AM were grown in Bioflo & Celligen fermentor/bioreactor, each in its specific growth medium, to reach 10^6 CFU. Each microbial suspension was impregnated on a proper mordant at the rate of 20 ml microbial suspension per 100 gm mordant oven dried basis. Sole or combined mixture of the remediative amendments was used to treat the contaminated sewaged soil at a rate of 100 gm impregnated mordant/400 gm sewaged soil. AM inoculums were prepared by mixing the spores in tap water (about 200 spore $10ml^{-1}$), and the soil at the rate of 20 ml pot^{-1} [1].

Determination of polychlorinated biphenyls (PCBs)

The most important aliphatic hydrocarbon individuals were estimated in the sewaged soil samples according to Nasr et al (2009). A gas liquid chromatogram (Hewlett-Packard Model 5890N series II) with split/splitless injection system, capillary column capability and flam ionization detector was used in estimating the polychlorinated biphenyls (PCBs). Chemistation software was used for instrument control and data analysis.

RESULTS AND DISCUSSION

Results

The studied members of the poly chlorinated biphenyl PCBs included the compounds 70:2 3', 4', 5-tetrachlorobiphynen, 101:2, 2´, 4, 5, 5-pentachlorobiphenyl, 105: 2,3,3´,4,4´-pentachlorobiphenyl, 118: 2 3´,4, 4´,5-pentachlorobiphenyl, 138:2,2´,3,4,4´,5´-hexachlorobiphenyl, 152: 2,2´,3,5,6,6´-hexachlorobiphenyl, 180:2,2´,3,4,4´,5,5´-heptachlorobiphenyl and 192: 2,3,3´,4,5,5´,6-heptachlorobiphenyl. Results given in Table (1) showed that not all these members were detectable in the studied contaminated sewaged soil ecosystem. Data revealed that the compounds 70: 2,3',4',5 -tetrachlorobiphynen, 101: 2,2´,4,5,5-pentachlorobiphenyl, 105: 2,3,3´,4,4´-pentachlorobiphenyl, 138:2,2´,3,4,4´,5´-hexachlorobiphenyl and 180: 2,2´,3,4,4´,5,5´-heptachlorobiphenyl were not detectable in the contaminated sewaged soil ecosystem. On the other hand, the three poly chlorinated biphenyl PCBs 118: 2,3´,4,4´,5-pentachlorobiphenyl, 152: 2,2´,3,5,6,6´-hexachlorobiphenyl

and 192: 2,3,3´,4,5,5´, 6-heptachlorobiphenyl were initially detected in the contaminated sewaged soil ecosystem, yet, at different intensities.

Results evidenced noticeable decreases in the content of the three detected poly chlorinated biphenyl PCBs in the contaminated sewaged soil ecosystem irrigated with treated sewage effluent from their initial values in response to bioremediation with either sole or combined mixture of the experimented remediative amendments followed by phytoremediation with canola, Indian mustard or black nightshade hyperaccumulator in a field experiment. A similar pattern of the three tested poly chlorinated biphenyl PCBs disappearance from the sewaged soil ecosystem irrigated with regular water in the column experiment from their initial values in response to bioremediation with either sole or combined mixture of the experimented remediative amendments followed by phytoremediation with canola was obvious. Varied efficiencies in decontaminating the three studied poly chlorinated biphenyl PCBs from the sewaged soil ecosystem were evident, yet canola was the most efficient followed by Indian mustard and black nightshade despite the differences between them was not that great.

Table (1) Existence and concentration of polychlorinated biphenyl PCBs in the contaminated sewaged soil ecosystem

PCBs POP	ng/g dry weight	PCBs POP	ng/g dry weight
2 3', 4', 5-tetrachlorobiphynen	ND	2,2´,3,4,4´,5´-hexachlorobiphenyl	ND
2, 2´, 4, 5, 5-pentachlorobiphenyl	ND	2,2´,3,5,6,6´-hexachlorobiphenyl	0.87
2,3,3´,4,4´-pentachlorobiphenyl	ND	2,2´,3,4,4´,5,5´-heptachlorobiphenyl	ND
2 3´,4, 4´,5-pentachlorobiphenyl	0.38	2,3,3´,4,5,5´,6-heptachlorobiphenyl	0.35

2, 3´, 4, 4´, 5-pentachlorobiphenyl

Results presented in Table (2) indicated that bioremediation by the sole action of indigenous soil biomass was efficient in decomposing 2, 3´, 4, 4´, 5-pentachlorobiphenyl in the sewaged soil ecosystem. After a bioremediation period extended for 60 days, 2, 3´, 4, 4´, 5-pentachlorobiphenyl decreased in the un-cultivated soil from 0.38 to 0.05 ng/g dry weight soil under irrigation with treated sewage effluent or regular water0.03 ng/g dry soil respectively under the combined action of soil biomass associated with *Thiobacillus* sp. inoculation, probentonite enhancement or a combined mixture of all remediative amendments under irrigation with treated sewage effluent or regular water .
In the un-cultivated treatment, bioremediation with sole indigenous biomass continued till 120 days and resulted in decreasing 2, 3´, 4, 4´, 5-pentachlorobiphenyl to 8% of their initial value (0.03 ng/g dry soil) respectively under irrigation with treated sewage effluent or regular water.

Bioremediation with sole or combined remediative amendments was continued till 120 days in association with phytoremediation with canola, Indian mustard or black nightshade hyperaccumulator plants associated with treated sewage effluent irrigation. At the maturity stage of the tested hyperaccumulator plants, results showed a marked

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diminish in the content of 2, 3′, 4, 4′, 5-pentachlorobiphenyl at varied rates under the various treatments.

The combined action of indigenous soil biomass and root exudates in the cultivated treatment decreased 2, 3′, 4, 4′, 5-pentachlorobiphenyl in the sewaged soil ecosystem from 0.38 to 0.02 ng/g dry soil under canola, Indian mustard or black nightshade irrigated with treated sewage effluent respectively.

Table (2) Degradation of the PCB 2 ,3′, 4, 4′, 5-pentachlorobiphenyl in a sewaged soil ecosystem irrigated with either regular water or treated sewage effluent after bioremediation and phytoremediation canola, Indian mustard or black nightshade

Treatment								
Initial	0.38							
Type of irrigation	Regular water a column experiment		Treated sewage effluent in a field experiment					
Units	Ng/ g soil	% of initial	Ng/ g soil		% of initial			
1st Stage (Bioremediation period extended from 0 to 60 days)								
Indigenous Biomass (IB)	0.05	13	0.05		13			
IB *+Thiobacillus* mixture*	0.04	10	0.04		10			
IB +Probentonite	0.04	10	0.04		10			
IB + Combined mixture of all remediative amendments	0.03	8	0.03		8			
2nd Stage (Phytoremediation period extended from 61 to 120 days)								
IB +Un-cultivated	0.03	8	0.03		8			
Hyperaccumulator plant	Canola		Canola		Indian mustard		Black nightshade	
Units	Ng/ g soil	% of initial	Ng/ g soil	% of initial	Ng/ g soil	% of initial	Ng/ g soil	% of initial
IB+Cultivated Control	0.02	5	0.02	5	0.02	5	0.02	5
IB + AM inoculation	0.02	5	0.02	5	0.02	5	0.02	5
IB + Thiobacillus mixture*	0.02	5	0.02	5	0.02	5	0.02	5
IB + Probentonite	0.02	5	0.02	5	0.02	5	0.02	5
IB + Combined mixture of all remediative amendments	ND	0	ND	0	ND	0	ND	0

ND = not detected **Thiobacillus thiooxidans & Thiobacillus ferrooxidans*

While under irrigation with regular water the combined action of indigenous soil biomass and root exudates decreased 2, 3′, 4, 4′, 5-pentachlorobiphenyl in the sewaged soil ecosystem from 0.38 to 0.02 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with Am inoculation decreased 2, 3′, 4, 4′, 5- pentachlorobiphenyl in the sewaged soil ecosystem from 0.38 to 0.02 ng/g dry soil under canola, Indian mustard or black nightshade irrigated

with treated sewage effluent respectively. While under irrigation with regular water the combined action of indigenous soil biomass and root exudates associated with Am inoculation decreased 2, 3´, 4, 4´, 5-pentachlorobiphenyl in the sewaged soil ecosystem from 0.38 to 0.02 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with *Thiobacillus* sp. inoculation decreased 2, 3´, 4, 4´, 5-pentachlorobiphenyl in the sewaged soil ecosystem from 0.38 to 0.02 ng/g dry soil under canola, Indian mustard or black nightshade irrigated with treated sewage effluent respectively. While under irrigation with regular water the combined action of indigenous soil biomass and root exudates associated with *Thiobacillus* sp. inoculation decreased 2, 3´, 4, 4´, 5-pentachlorobiphenyl in the sewaged soil ecosystem from 0.38 to 0.02 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with probentonite enhancement decreased 2, 3´, 4, 4´, 5-pentachlorobiphenyl in the sewaged soil ecosystem from 0.38 to 0.02 ng/g dry soil under canola, Indian mustard or black nightshade irrigated with treated sewage effluent respectively. While under irrigation with regular water the combined action of indigenous soil biomass and root exudates associated with probentonite enhancement decreased 2, 3´, 4, 4´, 5-pentachlorobiphenyl in the sewaged soil ecosystem from 0.38 to 0.02 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with a combined mixture of all tested remediative amendments totally degraded 2, 3´, 4, 4´, 5-pentachlorobiphenyl in the sewaged soil ecosystem under canola, Indian mustard or black nightshade irrigated with treated sewage effluent respectively. Also under irrigation with regular water the combined action of indigenous soil biomass and root exudates associated with a combined mixture of all tested remediative amendments entirely degraded 2, 3´, 4, 4´, 5-pentachlorobiphenyl in the sewaged soil ecosystem under canola phytoremediation. Therefore, results set the superiority of the combined mixture of all tested remediative amendments in the whole degradation of 2, 3´, 4, 4´, 5-pentachlorobiphenyl in the sewaged soil ecosystem under irrigation with treated sewage effluent or regular water.

2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl

Results given in Table (3) showed that bioremediation by the sole action of indigenous soil biomass was operative in decomposing 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl in the sewaged soil ecosystem. After a bioremediation period extended for 60 days, 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl decreased in the uncultivated soil from 0.87 to 0.49 ng/g dry weight soil under irrigation with treated sewage effluent and to 0.39 ng/g dry weight soil under irrigation with regular water, and diminished to 4.34, 5.10 or 4.12 ng/g dry soil respectively under the combined action of soil indigenous biomass coupled with *Thiobacillus* sp. inoculation, probentonite enhancement or a combined mixture of all remediative amendments under irrigation with treated sewage 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl decreased to 0.42, 0.36 or 0..30 ng/g dry soil respectively under the combined action of indigenous soil biomass associated with *Thiobacillus* sp. inoculation, probentonite enhancement or a combined mixture of all remediative amendments under irrigation with regular water. In the un-cultivated treatment, bioremediation with sole indigenous biomass

continued till 120 days and resulted in decreasing 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl to 25 and 23% of their initial value (0.22 and 0.20 ng/g dry soil) respectively under irrigation with treated sewage effluent or regular water.

Bioremediation with sole or combined remediative amendments was continued till 120 days in association with phytoremediation with canola, Indian mustard or black nightshade hyperaccumulator plants associated with treated sewage effluent irrigation in a field experiment and with canola associated with regular water irrigation in a column experiment. At the maturity sage of three tested hyperaccumulator plants, results showed a marked diminish in the content of 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl at varied rates under the various treatments.

Table (3) Degradation of the PCB 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl in a sewaged soil ecosystem irrigated with either regular water or treated sewage effluent after bioremediation and phytoremediation canola, Indian mustard or black nightshade

Treatment								
Initial	**0.87**							
Type of irrigation	Regular water a column experiment		Treated sewage effluent in a field experiment					
Units	Ng/ g soil	% of initial	Ng/ g soil		% of initial			
1st Stage (Bioremediation period extended from 0 to 60 days)								
Indigenous Biomass (IB)	0.39	45	0.49		56			
IB+*Thiobacillus* mixture*	0.32	37	0.42		48			
IB +Probentonite	0.30	34	0.36		41			
IB + Combined mixture of all remediative amendments	0.28	32	0.30		34			
2nd Stage (Phytoremediation period extended from 61 to 120 days)								
IB +Un-cultivated	0.22	25	0.20		23			
Hyperaccumulator plant	Canola		Canola		Indian mustard		Black nightshade	
Units	Ng/ g soil	% of initial	Ng/ g soil	% of initial	Ng/ g soil	% of initial	Ng/ g soil	% of initial
IB+Cultivated Control	0.18	21	0.22	25	0.24	28	0.24	28
IB + AM inoculation	0.16	18	0.20	23	0.22	25	0.21	24
IB + Thiobacillus mixture*	0.16	18	0.18	21	0.20	23	019	22
IB + Probentonite	0.12	14	0.16	18	0.18	21	0.17	20
IB + Combined mixture of all remediative amendments	0.08	9	0.11	13	0.12	14	0.13	15

**Thiobacillus thiooxidans & Thiobacillus ferrooxida*

The combined action of indigenous soil biomass and root exudates in the cultivated treatment decreased 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl in the sewaged soil ecosystem from 0.87 to 0.22, 0.24 and 0.24 ng/g dry soil under canola, Indian mustard or black nightshade irrigated with treated sewage effluent respectively. While under irrigation with

regular water the combined action of indigenous soil biomass and root exudates decreased 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl in the sewaged soil ecosystem from 0.87 to0.18 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with Am inoculation decreased 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl in the sewaged soil ecosystem from 0.78 to 0.20, 0.22 and 0.21 ng/g dry soil under canola, Indian mustard or black nightshade irrigated with treated sewage effluent respectively. While under irrigation with regular water the combined action of indigenous soil biomass and root exudates associated with Am inoculation decreased 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl in the sewaged soil ecosystem from 0.87 to 0.16 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with *Thiobacillus* sp. inoculation decreased 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl in the sewaged soil ecosystem from 0.87 to 0.18, 0.20 and 0.19 ng/g dry soil under canola, Indian mustard or black nightshade irrigated with treated sewage effluent respectively. While under irrigation with regular water the combined action of indigenous soil biomass and root exudates associated with *Thiobacillus* sp. inoculation decreased 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl in the sewaged soil ecosystem from 0.87 to 0.16 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with probentonite enhancement decreased 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl in the sewaged soil ecosystem from 0.78 to 0.16, 0.18 and 0.17 ng/g dry soil under canola, Indian mustard or black nightshade irrigated with treated sewage effluent respectively. While under irrigation with regular water the combined action of indigenous soil biomass and root exudates associated with probentonite enhancement decreased 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl in the sewaged soil ecosystem from 0.87 to 0.12 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with a combined mixture of all tested remediative amendments decreased 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl in the sewaged soil ecosystem from 0.87 to 0.11, 0.12 and 0.13 ng/g dry soil under canola, Indian mustard or black nightshade irrigated with treated sewage effluent respectively. While under irrigation with regular water the combined action of indigenous soil biomass and root exudates associated with a combined mixture of all tested remediative amendments decreased 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl in the sewaged soil ecosystem from 0.87 to 0.08 ng/g dry soil under canola phytoremediation.

In conclusion, results confirmed the superiority of the combined mixture of all tested remediative amendments in decreasing 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl content in the sewaged soil ecosystem particularly under irrigation with regular water. Under the action of the combined mixture of all remediative amendments associated with phytoremediation with canola, Indian mustard or Black nightshade in a field experiment irrigated with treated sewage effluent, 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl contents were respectively reduced to 13, 14 or 15% from their initial content. In the column experiment irrigated with regular water, 2, 2´, 3, 5, 6, 6´-hexachlorobiphenyl content was reduced to 9% from their initial

content under the combined action of all remediative amendments followed by canola phytoremediation. Although the rates of 2, 2′, 3, 5, 6, 6′-hexachlorobiphenyl content diminish under phytoremediation with any of the three experimented hyperaccumulator plants were more or less the same, yet canola hyperaccumulator action was somewhat distinguishable.

2, 3, 3′, 4, 5, 5′, 6-heptachlorobiphenyl

Results clarified in Table (4) specified that the sole action of indigenous soil biomass was highly operative in degrading 2, 3, 3′, 4, 5, 5′, 6-heptachlorobiphenyl in the sewaged soil ecosystem. After a bioremediation period extended for 60 days, 2, 3, 3′, 4, 5, 5′, 6-heptachlorobiphenyl completely disappeared from the un-cultivated soil under treated sewage effluent or regular water irrigation after being initially 0.35 ng/g dry weight soil. 2, 3, 3′, 4, 5, 5′, 6-heptachlorobiphenyl was also not detectable under the combined action of soil biomass coupled with *Thiobacillus* sp. inoculation, probentonite enhancement or a combined mixture of all remediative amendments under both types of irrigation water. In the un-cultivated treatment, bioremediation with sole indigenous biomass continued till 120 days and resulted in completely degrading 2, 3, 3′, 4, 5, 5′, 6-heptachlorobiphenyl in the sewaged soil ecosystem under irrigation with treated sewage effluent or regular water.

In the 2nd stage that was extended during the period from 61 to 120 days, the bioremediated sewaged soil ecosystem was exposed to phytoremediation with canola, Indian mustard or black nightshade under treated sewage effluent irrigation in field experiment and with canola under regular water irrigation in a column experiment. At the maturity stage of the three experimented hyperaccumulator plants, 2, 3, 3′, 4, 5, 5′, 6-heptachlorobiphenyl totally disappeared from the sewaged soil ecosystem under all treatments.

DISCUSSION

Previous findings confirmed the existence of POPs in the used sewaged soil ecosystem at contamination roughly reaching 672 ppm that confronts their sustainable management [20]. However, this content of POPs did not reach a hazard level [8], [13], [24] and [25]. It was repeatedly mentioned that POPsare realistic to stick with sewaged soil ecosystems, to be competent of long-range transport, be biomagnified in food chains, and had potential significant adverse impacts on health and environment. Despite there are few natural sources of PCBs, yet the majority of which are manmade and are intentionally released to the soil ecosystem. Results showed varied responses of three detected PCBs to the experimented remediative amendments followed by phytoremediation. Some entirely disappeared from the sewaged soil ecosystem after bioremediation followed by phytoremediation, others exhibited a serious persistent diminish in the sewaged soil ecosystem in response to the different experimental treatments at varied rates and did not entirely disappeared till harvesting the hyperaccumulator plants. It was always noticed that the action of the combined mixture of all the remediative amendments far exceeded the effects of sole application.

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Table (4) Degradation of the PCB 2, 3, 3´, 4, 5, 5´, 6-heptachlorobiphenyl in a sewaged soil ecosystem irrigated with either regular water or treated sewage effluent after bioremediation and phytoremediation canola, Indian mustard or black nightshade

Treatment								
Initial	0.35							
Type of irrigation	Regular water a column experiment		Treated sewage effluent in a field experiment					
Units	Ng/ g soil	% of initial	Ng/ g soil	% of initial				
1st Stage (Bioremediation period extended from 0 to 60 days)								
Indigenous Biomass (IB)	ND	0	ND	0				
IB *+Thiobacillus* mixture*	ND	0	ND	0				
IB +Probentonite	ND	0	ND	0				
IB + Combined mixture of all remediative amendments	ND	0	ND	0				
2nd Stage (Phytoremediation period extended from 61 to 120 days)								
IB +Un-cultivated	ND	0	ND		0			
Hyperaccumulator plant	Canola		Canola		Indian mustard		Black nightshade	
Units	Ng/ g soil	% of initial	Ng/ g soil	% of initial	Ng/ g soil	% of initial	Ng/ g soil	% of initial
IB+Cultivated Control	ND	0	ND	0	ND	0	ND	0
IB + AM inoculation	ND	0	ND	0	ND	0	ND	0
IB + Thiobacillus mixture*	ND	0	ND	0	ND	0	ND	0
IB + Probentonite	ND	0	ND	0	ND	0	ND	0
IB + Combined mixture of all remediative amendments	ND	0	ND	0	ND	0	ND	0

**Thiobacillus thiooxidans & Thiobacillus ferrooxidans*

Much recent work had centered on the study of micro-organisms that are able to biodegrade PCBs in one of two ways, using them as a source of carbon, nutrients and energy or replacing their chlorine atom with hydrogen on the biphenyl skeleton. However, there are significant problems associated with this approach as microorganisms work well in laboratory conditions; however, this is not that simple under to a natural soil ecosystem. This is simply because microorganisms could access other sources of carbon, which they decompose in preference to PCBs [22] and [18].

Abramowicz (1990) and Pentyala et al. 2011 mentioned that two distinct classes of bacteria had been identified to biodegrade PCBs by different mechanisms. These two PCB-degradative systems include aerobic bacteria which live in oxygenated environments and anaerobic bacteria which live in oxygen free environments. The aerobes attack PCBs oxidatively, breaking open the carbon ring and destroying the compounds. Anaerobes, on the other hand, leave the biphenyl rings intact while removing the chlorines. This anaerobic de-chlorination degrades highly chlorinated compounds into less chlorinated derivatives. These two naturally occurring processes are complementary, and a two-step treatment may permit the biological destruction of nearly all of the PCB mixtures commonly used.

The Agency for Toxic Substances and Disease Registry (2005) stated that as time goes on, POPs are broken down in sewaged soil ecosystems into less harmful substances by algae, fungi and bacteria; however, the process is relatively slow and dependent on ambient environmental conditions. Also, Nester et al (2001) mentioned that the white-rot fungus, *Phanerochaete chrysosporium*, could bind to, and in some instances, mineralize a wide array of POPs including polychlorinated biphenyls (PCBs). Billingsley et al (2002) and Pentyala et al. 2011 attempted to remediate polychlorinated biphenyl (PCB) in soil through washing with a variety of commercial nonionic or anionic surfactants incubated with *Pseudomonas* sp. LB400. They found that nonionic surfactants washed more PCBs from the soil (up to 89%) but inhibited their biodegradation, while anionic surfactants washed less PCBs from the soil but were more effective in biodegradation tests, removing up to 67% of total PCBs.

In parallel, phytoremediation had largely focused on the use of plants to accelerate degradation of POPs, usually in concert with rhizosphere microorganisms and root exudates. Direct uptake of POPs by plants is a surprisingly efficient removal mechanism from sewaged soil ecosystems moderately contaminated with hydrophobic POPs that are strongly bound to root surface and soil colloids' and are not easily translocate within the plant, as well as POPs that are quite water soluble are not sufficiently sorbet to roots nor actively transported through plant membranes (Briggs et al., 1982). Many hydrophobic POPs are candidates for phytostabilization and / or rhizosphere bioremediation. Many plants had expressed some capacities to uptake and convert POPs quickly to less toxic metabolites. Others might stimulate the degradation of certain POPs in their rhizosphere through their root exudates and enzymes (Chekol, et al. 2004). They suggested that phytoremediation is best suited for removing moderately hydrophobic POPs from soil ecosystem; yet, high levels of POPs are toxic to plants and prevent successful phytoremediation. Once a PCB is translocated, the plant store it and its fragments into new plant structures via lignification or it could be volatilized, metabolized, or mineralized completely to CO_2 and H_2O.

Clarke et al (2010) investigated the temporal trends of PCBs in sewage sludge in Australia. Time series analysis (1995–2006) PCBs sewage sludge concentrations (*n* = 2266) taken from six plants indicated that PCBs were infrequently detected (< 8%). Internationally, concentrations of PCBs in sewage sludge were consistently low and rarely exceeded European contaminant limits and therefore, regulatory limits may warrant review.

In conclusion PCBs, besides existing in the sewaged soil ecosystem in amounts less than the hazardous level, many of them were not initially detected in the sewaged soils ecosystem. Most of the investigated PCBs that were detected in the sewaged soil ecosystem tented to persistently disappear in response to the effect of indigenous soil biomass and plant root exudates particularly in association with the experimented remediative amendments followed by phytoremediation both under irrigation with water in the column experiment or with treated sewage effluent in the field experiment.

If it is intended to apply sewage farming, soil ecosystem should be checked periodically to determine the type and rate of needed remediative amendments. Sustainable management of sewaged soils necessitates continuous evaluation for their hygienic, chemical and physical as well as its aesthetical characteristics.

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