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Bioremediation of Chlorinated Hydrocarbons in a Sewaged Soil by Certain Remediative Amendments Followed By Phytoremediation

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ABSTRACT

Due to the limited water resources in Egypt, It is quite obvious that management and reuse of sewage effluent is one of the challenges that Egypt will have to deal with in the coming decades. Under regular water irrigation in a column experiment and treated sewage effluent irrigation in a field experiment, the key chlorinated hydrocarbon compounds were bio-remediated in a high contaminated sewaged soil ecosystem using varied single and/or combined remediative amendments followed by phytoremediation. Out of nine investigated chlorinated hydrocarbons in the high contaminated sewaged soil only two compounds were detected, i.e. PP-DDE and PP-DDT. Results showed that PP-DDE entirely disappeared from the soil ecosystem after two month bioremediation with all the tested remediative amendments. PP-DDT tented to persistently disappear from the sewaged soil ecosystem under the action of both indigenous biomass and root exudates and reached a non-detectable level at the maturity stage of the experimented hyperacculator plants in both column and field experiments.

Keywords: bioremediation, phytoremediation, persistent organic pollutants, chlorinated hydrocarbons

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INTRODUCTION

Chlorinated hydrocarbons include a variety of compounds that are characterized with their strong chlorine-carbon bonds. The overwhelming majority of them had been universally banned because of their unacceptable slow and persistent degradation together with their unforeseen adverse impacts on health and environment. Yet they are still found at detectable levels in many ecosystems. The chemical and microbiological characterization of soils irrigated with sewage effluent in Egypt for extending periods confirmed their contamination with chlorinated hydrocarbons at levels confronting sustainable management [18]. In most cases they are toxic chemicals adversely affect human health and environment. There are few natural sources of aliphatic hydrocarbons, yet the majority of which are manufactured and released to the environment either intentionally or as byproducts, e.g., as pesticides. The chemical and microbiological characterization of soils irrigated with sewage effluent for extending periods ranging from 2.5 to 82 years under various landscapes confirmed their contamination with POPs at levels confronting sustainable management [21]. The existence of contaminated soils poses a risk to the environment, and it is thus necessary to eliminate such pollutants. There are several approaches for this purpose. Methods such as direct engineering or natural cleanup (without human interference) are very effective. One of these methods, bioremediation, uses biological activity *in situ* to decrease or eliminate chlorinated hydrocarbon pollution. This method relies on microbes that use hydrocarbons as an energy resource and converts them to simple non-toxic materials such as water and carbon dioxide [11]. Other method, let us to use plants for rehabilitation of polluted environments is known as phytoremediation. This technology was developed after the identification of certain plants, POP's "hyperaccumulators", that are able to accumulate and tolerate extremely high concentrations of these pollutants in their shoots [12]. The main goal of the present work is to decontaminate chlorinated hydrocarbons 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 [6,17]. *Thiobacillus ferrooxidans* were isolated and grown in DSMZ medium 882 [3]. *Thiobacillus thiooxidans* were isolated and grown in modified Waksman medium [8, 19, 10]. 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 chlorinated hydrocarbons

The most important aliphatic hydrocarbon individuals were estimated in the sewaged soil samples according to Nasr et al (2009) and Jian-gang et al. (2011). 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 chlorinated hydrocarbons. Chemistation software was used for instrument control and data analysis.

RESULTS AND DISCUSSION

Results

The studied members of chlorinated hydrocarbons included a-HCH, g-HCH, heptachlore, aldrin, Hepta-epoxide, dieldrin, PP-DDE, PP-DDD and PP-DDT. Results showed that not all these members were detectable in the studied contaminated sewaged soil ecosystem. Data given in Table (1) revealed that a-HCH, g-HCH, heptachlore, aldrin, hepta-epoxide, PP-DDT and dieldrin were not detectable in the contaminated sewaged soil ecosystem. On the other hand, the members PP-DDE and PP-DDD were initially detected in the contaminated sewaged soil ecosystem, yet, at different intensities.

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Table 1: Existence and concentration of chlorinated hydrocarbon in the contaminated sewaged soil ecosystem

Chlorinated hydrocarbon	ng/g dry weight	Chlorinated hydrocarbon	ng/g dry weight
a-HCH	ND	Dieldrin	ND
g-HCH	ND	PP-DDE	1.05
Aldrin	ND	PP-DDT	6.53
Heptachlore	ND	Hept-epoxide	ND
PP-DDD	ND		

ND=not detected

Results evidenced noticeable degradation of the two detected 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. A similar pattern of the two tested 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 also obvious.

PP-DDE

Table 2 Degradation of the chlorinated hydrocarbon PP-DDE in a sewaged soil ecosystem irrigated with treated sewage effluent after bioremediation and phytoremediation canola, Indian mustard or black nightshade

Treatments						
Initial content	1.05					
1st Stage (Bioremediation period extended from 0 to 60 days)						
	Ng/g soil	% of initial				
Indigenous Biomass (IB)	ND	0				
IB +*Thiobacillus* mixture*	ND	0				
IB +Probentonite	ND	0				
IB + Combined mixture of all remediative amendments	ND	0				
2nd Stage (Phytoremediation period extended from 61 to 120 days)						
IB +Un-cultivated	ND	0				
Hyperaccumulator plant	Canola		Indian mustard		Back nightshade	
Units	Ng/g soil	% of initial	Ng/g soil	% of initial	Ng/g soil	% of initial
IB+Cultivated Control	ND	0	ND	0	ND	0
IB + AM inoculation	ND	0	ND	0	ND	0
IB + Thiobacillus mixture*	ND	0	ND	0	ND	0
IB + Probentonite	ND	0	ND	0	ND	0
IB + Combined mixture of all remediative amendments	ND	0	ND	0	ND	0

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

Results clarified in Table (2) specified that the sole action of indigenous soil biomass as well as their action in association with *Thiobacillus* sp. inoculation, probentonite enhancement or a combined mixture of all irrigation water was highly operative in

degrading PP-DDE in the sewaged soil ecosystem. After a bioremediation period extended for 60 days, PP-DDE completely disappeared from the sewaged soil ecosystem under treated sewage effluent or regular water irrigation after being initially 1.05 ng/g dry weight soil.

At 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, PP-DDE entirely disappeared from the sewaged soil ecosystem under all treatments.

PP-DDD

Table 3: Degradation of the chlorinated hydrocarbon PP-DDD in a sewaged soil ecosystem irrigated with treated sewage effluent after bioremediation and phytoremediation canola, Indian mustard or black nightshade

					Treatments	
Initial content	6.53					
1st Stage (Bioremediation period extended from 0 to 60 days)						
	Ng/g soil	% of initial				
Indigenous Biomass (IB)	4.66	71				
IB+*Thiobacillus* mixture*	4.28	66				
IB +Probentonite	4.30	65				
IB + Combined mixture of all remediative amendments	4.13	63				
2nd Stage (Phytoremediation period extended from 61 to 120 days)						
IB +Un-cultivated	**1.26**	19				
Hyperaccumulator plant	Canola		Indian mustard		Back nightshade	
Units	Ng/g soil	% of initial	Ng/g soil	% of initial	Ng/g soil	% of initial
IB+Cultivated Control	ND	0	ND	0	ND	0
IB + AM inoculation	ND	0	ND	0	ND	0
IB + Thiobacillus mixture*	ND	0	ND	0	ND	0
IB + Probentonite	ND	0	ND	0	ND	0
IB + Combined mixture of all remediative amendments	ND	0	ND	0	ND	0

**Thiobacillus thiooxidans*&*Thiobacillus ferrooxidans*

Results given in Table (3) showed that bioremediation by the sole action of indigenous soil biomass was operative in decomposing PP-DDD in the sewaged soil ecosystem. After a bioremediation period extended for 60 days, PP-DDD decreased in the uncultivated soil from 6.53 to 4.66 ng/g dry weight soil under irrigation with treated sewage effluent and to 4.53 ng/g dry weight soil under irrigation with regular water, and decreased to 4.28, 4.30 or 4.13 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 effluent. The same trends were obvious under irrigation with regular water, yet at slightly higher rates. The chlorinated hydrocarbon PP-DDD decreased to 4.18, 4.14 or 4.03 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 PP-DDD to 18 and 19% of their initial value (1.16 and 1.26 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 an entire removal of PP-DDD from the sewaged soil ecosystem in both field and column experiments.

DISCUSSION

The main problem with chlorinated hydrocarbons strength is that once they are applied they could be around for a long time. Clarke et al (2010) and Jian-gang et al. 2011 in a time series analysis (1995–2006) detected lindane, aldrin HCB, heptachlor, DDT, DDD in sewage sludge samples collected in Australia. They found a correlation between dieldrin and chlordane levels ($P < 0.05$) which provides evidence of similar environmental mechanisms facilitating movement of dieldrin and chlordane through environment compartments. It has taken more than 10 years for dieldrin and chlordane to be reduced to less than detectable concentrations in freshly generated sewage sludge in Australia. They added that internationally, reported concentrations of chlorinated hydrocarbons in sewage sludge were consistently low and often less than detection limits. They concluded that chlorinated hydrocarbons are not considered to be a contaminant of regulatory concern for countries that phased out chlorinated hydrocarbons use several decades ago. Estimates of the total POPs content in the studied sewaged soil (672 ppm) are open to a certain amount of uncertainty and further work is required to improve the reliability of these estimates. However, it is worthy to mention that, in the current work, the content of estimated chlorinated hydrocarbons in the experimented contaminated sewaged soil did not reach a hazard level [7,12,21]. In current work the response the two detected chlorinated hydrocarbons in the sewaged soil ecosystem to the experimented bioremediation treatments was followed. Results showed varied responses of PP-DDE and PP-DDD to the experimented remediative amendments followed by phytoremediation. PP-DDE completely degraded in the sewaged soil ecosystem even by the sole action of soil biomass in the uncultivated treatment. PP-DDD, on the other hand, exhibited a serious persistent diminish in the sewaged soil ecosystem in response to the different experimental treatments and entirely disappeared at the maturity stage of the hyperaccumulator plants. It was always noticed that the action of the combined mixture of all the remediative amendments on the degradation of PP-DDD far exceeded the effects of sole application. It is well evidenced that the degradation of chlorinated hydrocarbons continuously occurs by indigenous soil biomass simply because microorganisms use them in their own growth and reproduction as a source of nutrients and energy.

The Agency for Toxic Substances and Disease Registry (2005) stated that over time, most POPs including chlorinated hydrocarbons 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. Nester et al (2001) also mentioned that the white-rot fungus, *Phanerochaete chrysosporium*, could bind to, and in some instances, mineralize a wide array of POPs in the presence of oxygen through aerobic respiration with the release of CO_2 and H_2O. Parallel to microbial bioremediation of POPs, phytoremediation had largely focused on the use of certain hyperaccumulator plants that expressed some capacities to uptake and convert POPs quickly to less toxic metabolites and/or stimulate their degradation in the rhizosphere through their root exudates and enzymes particularly those compounds strongly bound to root surface and soil colloids' and are not easily translocate to plant [5,19].

Therefore, chlorinated hydrocarbons, besides being detected in the studied sewaged soil ecosystem in amounts less than the permissible level, many of them were not initially detected in the sewaged soils ecosystem. The two found chlorinated hydrocarbons 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 under both irrigation with water in the column experiment or with treated sewage effluent in the field experiment.

In general, sewage farming should be applied with caution and if it is proposed to be applied, soil characteristics 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. The aesthetical quality is an important criterion for the successful sales management and advertisement of the sewaged soils products.

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