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Bioremediation of Aliphatic Hydrocarbons in a Sewaged Soil by Certain Remediative Amendments Followed by Phytoremediation.

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ABSTRACT

Nowadays the limited water resources in Egypt lead to use the sewage effluent in agriculture; however, there are concerns about the long-term accumulation and potential effects of aliphatic hydrocarbons contained in the sewage effluent used. In two columns and field experiments respectively irrigated with regular water or treated sewage effluent, the key aliphatic hydrocarbon members were bio-remediated in a high contaminated sewaged soil ecosystem using various single and/or combined remediative amendments included a mixture of *Thiobacillus thiooxidans & Thiobacillus ferrooxidans,* soil enhanced with probentonite and soil treated with a combined mixture of all the aforementioned remediative amendments that followed by phytoremediation with certain hyperaccumulator plants. Out of eleven investigated aliphatic hydrocarbons investigated in the high contaminated sewaged soil only five aliphatic hydrocarbons were detected, i.e. n-hexadecane, n-octadecane, n-eicosan, n-docosane and n-tetracosane. Results indicated that the five detected aliphatic hydrocarbons 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. After bioremediation followed by phytoremediation, n-hexadecane, reached a non-detectable level, while the content of the other four tested POPs were markedly reduced in the soil ecosystem.

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

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INTRODUCTION

Persistent organic pollutants (POPs) include varied compounds related to aliphatic and chlorinated hydrocarbons, PAHs and PCBs. They are characterized with their high resistant to degradation, low water solubility, high lipid solubility, semi-volatility and high molecular masses [22]. 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 [24]. Aliphatic hydrocarbons are realistic to stick with sewaged soil ecosystems, to be competent of long-range transport, to be biomagnified in food chains. 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 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 [9].

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 [11].

The overall goal of the current work is the decontamination of aliphatic 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

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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 aliphatic hydrocarbons in the sewaged soil ecosystem.

Culture collection

Phosphate dissolving bacteria (*Bacillus megatherium* var. *phosphaticum*) were isolated and grown on Pikovskyaya's medium [21]. *Thiobacillus ferrooxidans* were isolated and grown in DSMZ medium 882 [3]. *Thiobacillus thiooxidans* were isolated and grown in modified Waksman medium [23] and [8]. 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 aliphatic hydrocarbons

The most important aliphatic hydrocarbon individuals were estimated in the sewaged soil samples according to [17]. 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 aliphatic hydrocarbon. Chemistation software was used for instrument control and data analysis.

RESULTS AND DISCUSSION

Results

The studied members of the aliphatic hydrocarbon POPs included C12: n-dodecane, C 14: n-tetradecane, C16: n-hexadecane, C18: n-octadecane, C20: n-eicosan, C22: n-docosane, C24: n-tetracosane, C26: n-hexacosane, C2:8 n-octacosane, C30 n-triacontane and C32: 17B (H), 21B (H)-bishomohopane. Results given in Table (1) indicated that not all these aliphatic hydrocarbon POPs were detected in the studied contaminated sewaged soil ecosystem. Data revealed that C12: n-dodecane, C 14: n-tetradecane, C26: n-hexacosane, C28: n-octacosane, C30 n-triacontane and C32: 17B (H), 21B (H)-bishomohopane were not detected in the contaminated sewaged soil ecosystem. On the other hand, the five aliphatic hydrocarbon POPs members C16: n-hexadecane, C18: n-octadecane, C20: n-eicosan, C22: n-docosane and C24: n-tetracosane were initially distinguished in the contaminated sewaged soil ecosystem, yet, at different intensities.

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Results evidenced noticeable decreases in the content of the five detected aliphatic hydrocarbons 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 five tested aliphatic hydrocarbons disappearance from the sewaged soil ecosystem irrigated with regular water 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 five studied aliphatic hydrocarbons 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 aliphatic hydrocarbons in the contaminated sewaged soil ecosystem

	aliphatic hydrocarbon	ng/g dry weight		aliphatic hydrocarbon	ng/g dry weight
C12	n-dodecane	ND	C24	n-tetracosane	8.30
C14	n-tetradecane	ND	C26	n-hexacosane	ND
C16	n-hexadecane	8.58	C28	n-octacosane	ND
C18	n-octadecane	9.49	C30	n-triacontane	ND
C20	n-eicosan	6.82	C32	17B (H), 21B (H)-bishomohopane.	ND
C22	n-docosane	4.42			

ND=not detected

n-hexadecane

Results clarified in Table (2) specified that the sole action of indigenous soil biomass was highly operative in degrading n-hexadecane in the sewaged soil ecosystem. After a bioremediation period extended for 60 days, n-hexadecane decreased in the un-cultivated soil from 8.58 to 5.19 ng/g dry weight soil under treated sewage effluent irrigation and to 4.36 ng/g dry weight soil under regular water irrigation, and reached an undetectable level 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 decreasing n-hexadecane to 42 and 30% of their initial value (3.70 and 2.62 ng/g dry soil) respectively 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, n-hexadecane disappeared from the sewaged soil ecosystem under all treatments.

n-octadecane

Results given in Table (3) showed that bioremediation by the sole action of indigenous soil biomass was operative in decomposing n-octadecane in the sewaged soil ecosystem. After a bioremediation period extended for 60 days, n-octadecane decreased in the uncultivated soil from 9.49 to 5.55 ng/g dry weight soil under irrigation with treated sewage effluent and to 4.01 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 effluent. The same trends were obvious under irrigation with regular water, yet at slightly higher rates. The aliphatic hydrocarbon n-octadecane decreased to 3.14, 3.63 or 2.33 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 n-hexadecane to 45 and 30% of their initial value (4.23 and 2.98 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 n-octadecane at varied rates under the various treatments.

The combined action of indigenous soil biomass and root exudates in the cultivated treatment decreased n-octadecane in the sewaged soil ecosystem from 9.49 to 2.53, 2.78 and 2.81 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 n-octadecane in the sewaged soil ecosystem from 9.49 to 1.74 ng/g dry soil under canola phytoremediation.
The combined action of indigenous soil biomass and root exudates associated with Am inoculation decreased n-octadecane in the sewaged soil ecosystem from 9.49 to 2.32, 2.41 and 2.65 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 n-octadecane in the sewaged soil ecosystem from 9.49 to 1.67 ng/g dry soil under canola phytoremediation.

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 n-eicosan to 48 and 35% of their initial

value (3.29 and 2.39 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 diminish in the content of n-eicosan at varied rates under the various treatments.

The combined action of indigenous soil biomass and root exudates associated with *Thiobacillus* sp. inoculation decreased n-octadecane in the sewaged soil ecosystem from 9.49 to 2.23, 2.33 and 2.57 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 n-octadecane in the sewaged soil ecosystem from 9.49 to 1.78 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with probentonite enhancement decreased n-octadecane in the sewaged soil ecosystem from 9.49 to 2.60, 2.73 and 2.93 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 n-octadecane in the sewaged soil ecosystem from 9.49 to 1.59 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 n-octadecane in the sewaged soil ecosystem from 9.49 to 1.83, 1.93 and 2.03 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 n-octadecane in the sewaged soil ecosystem from 9.49 to 1.33 ng/g dry soil under canola phytoremediation.

In conclusion, results confirmed the superiority of the combined mixture of all tested remediative amendments in decreasing n-octadecane 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, n-octadecane contents were respectively reduced to 19, 20 or 21% from their initial content. In the column experiment irrigated with regular water, n-octadecane content was reduced to 14% from their initial content under the combined action of all remediative amendments followed by canola phytoremediation. Although the rates of n-octadecane 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.

n-eicosan

Results presented in Table (4) indicated that bioremediation by the sole action of indigenous soil biomass was efficient in decomposing n-eicosan in the sewaged soil ecosystem. After a bioremediation period extended for 60 days, n-eicosan decreased in the un-cultivated soil from 6.82 to 4.67 ng/g dry weight soil under irrigation with treated sewage effluent and from to 3.17 ng/g dry weight soil under irrigation with regular water, and diminished from 6.82 to 2.88, 3.26 or 2.65 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. The same trends were obvious under irrigation with regular water, yet at slightly higher rates. The aliphatic hydrocarbon n-eicosan decreased from 6.82 to 2.37, 2.91 or 2.09 ng/g dry soil respectively under the combined action of indigenous soil biomass associated with the combined action of indigenous soil biomass and root exudates in the cultivated treatment decreased n-eicosan in the sewaged soil ecosystem from 6.82 to 2.10, 2.20 and 2.38 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 n-eicosan in the sewaged soil ecosystem from 6.82 to 1.22 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with Am inoculation decreased n-eicosan in the sewaged soil ecosystem from 6.82 to 2.00, 2.10 and 2.22 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 n-eicosan in the sewaged soil ecosystem from 6.82 to 1.41 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with *Thiobacillus* sp. inoculation decreased n-eicosan in the sewaged soil ecosystem from 6.82 to 1.86, 1.90 and 2.01 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 n-eicosan in the sewaged soil ecosystem from 6.82 to 1.83 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with probentonite enhancement decreased n-eicosan in the sewaged soil ecosystem from 6.82 to 2.08, 2.10 and 2.19 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 probentonite enhancement decreased n-eicosan in the sewaged soil ecosystem from 6.82 to 1.89 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 n-eicosan in the sewaged soil ecosystem from 6.82 to 1.52, 1.68 and 1.79 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 n-eicosan in the sewaged soil ecosystem from 6.82 to 1.17 ng/g dry soil under canola phytoremediation.

Therefore, results set the superiority of the combined mixture of all tested remediative amendments in decreasing n-eicosan content in the sewaged soil ecosystem for the most part 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, n-eicosan contents were respectively reduced to 22, 25 or 26% from their initial content. In the column experiment irrigated with regular water, n-eicosan content was reduced to 17% from their initial content under the combined action of all remediative amendments followed by canola phytoremediation. Although the rates of n-eicosan 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.

n-docosane

Results given in Table (5) showed that bioremediation by the sole action of indigenous soil biomass was successful in decomposing n-docosane in the sewaged soil ecosystem. After a bioremediation period extended for 60 days, n-docosane decreased in the uncultivated soil from 4.42 to 2.76 ng/g dry weight soil under treated sewage effluent irrigation and to 2.46 ng/g dry weight soil under regular water irrigation due to sole indigenous biomass activity, and diminished from 4.42 to 2.14, 2.53 or 2.01 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. The same trends were obvious under irrigation with regular water, yet at slightly higher rates. The aliphatic hydrocarbon n-docosane decreased from 4.42 to 1.88, 2.16 or 1.67 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 resulting in decreasing n-docosane to 59 and 46% of their initial value (2.61 and 2.05 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. At the maturity sage of the tested hyperaccumulator plants, results showed a marked diminish in the content of n-docosane at varied rates under the various treatments. The combined action of indigenous soil biomass and root exudates in the cultivated treatment decreased n-docosane in the sewaged soil ecosystem from 4.46 to 1.96, 2.11 and 2.29 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 n-docosane in the sewaged soil ecosystem from 4.42 to 1.46 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with Am inoculation decreased n-docosane in the sewaged soil ecosystem from 4.42 to 1.77, 1.89 and 2.01 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 n-docosane in the sewaged soil ecosystem from 4.42 to 1.37 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with *Thiobacillus* sp. inoculation decreased n-docosane in the sewaged soil ecosystem from 4.42 to 1.47, 1.52 and 1.56 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 n-docosane in the sewaged soil ecosystem from 4.42 to 1.27 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with probentonite enhancement decreased n-docosane in the sewaged soil ecosystem from 4.42 to 1.83, 1.93 and 1.99 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 n-docosane in the sewaged soil ecosystem from 4.42 to 1.33 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 n-docosane in the sewaged soil ecosystem from 4.42 to 1.14, 1.29 and 1.41 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 n-docosane in the sewaged soil ecosystem from 4.42 to 0.95 ng/g dry soil under canola phytoremediation.

Thus, results confirmed the superiority of the combined mixture of all tested remediative amendments in decreasing n-docosane 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, n-docosane contents were respectively reduced to 26, 29 or 32% from their initial content. In the column experiment irrigated with regular water, n-docosane content was reduced to 21% from their initial content under the combined action of all remediative amendments followed by canola phytoremediation. Although the rates of n-docosane content diminish under phytoremediation with any of the three experimented

hyperaccumulator plants were more or less the same, yet canola hyperaccumulator action was somewhat discernible

n-tetracosane

Results presented in Table (6) indicated that bioremediation by the sole action of indigenous soil biomass was valuable in decomposing n-tetracosane in the sewaged soil ecosystem. After a bioremediation period extended for 60 days in the un-cultivated soil, n-tetracosane decreased from 8.32 to 5.71 ng/g dry weight soil under treated sewage effluent irrigation and to 4.73 ng/g dry weight soil under regular water irrigation due to sole indigenous biomass activity, and diminished from 8.32 to 4.38, 4.97 or 4.12 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. The same trends were obvious under irrigation with regular water, yet at slightly higher rates. The aliphatic hydrocarbon n-tetracosane decreased from 8.32 to 4.73, 4.11 or 3.03 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 resulted in decreasing n-tetracosane to 55 and 43% of their initial value (4.56 and 3.57 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. At the maturity stage of the tested hyperaccumulator plants, results showed a marked diminish in the content of n-tetracosane at varied rates under the various treatments.

The combined action of indigenous soil biomass and root exudates in the cultivated treatment decreased n-tetracosane in the sewaged soil ecosystem from 8.32 to 2.61, 2.71 and 2.88 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 n-tetracosane in the sewaged soil ecosystem from 8.32 to 1.92 ng/g dry soil under canola phytoremediation. The combined action of indigenous soil biomass and root exudates associated with Am inoculation decreased n-tetracosane in the sewaged soil ecosystem from 8.32 to 2.39, 2.50 and 2.69 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 n-tetracosane in the sewaged soil ecosystem from 8.32 to 1.75 ng/g dry soil under canola phytoremediation.

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Table (2) Degradation of the aliphatic hydrocarbon n-hexadecane in a sewaged soil ecosystem irrigated with either regular water or treated sewage effluent after bioremediation and phytoremediation canola, Indian mustard or black nightshade (ng/g dry soil)

Treatment								
Initial	8.85							
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)	4.36	41	5.19		59			
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	2.62	30	3.70		42			
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

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

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Table (3) Degradation of the aliphatic hydrocarbon n-octadecane in a sewaged soil ecosystem irrigated with either regular water or treated sewage effluent after bioremediation and phytoremediation canola, Indian mustard or black nightshade (ng/g dry soil)

Treatment								
Initial	9.49							
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)	4.01	42	5.55	58				
IB+*Thiobacillus* mixture*	3.14	33	4.34	46				
IB +Probentonite	3.63	38	5.10	54				
IB + Combined mixture of all remediative amendments	2.33	26	4.12	43				
2nd Stage (Phytoremediation period extended from 61 to 120 days)								
IB +Un-cultivated	2.98	30	4.23	45				
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	1.74	18	2.53	27	2.78	29	2.81	30
IB + AM inoculation	1.67	18	2.32	24	2.41	25	2.65	28
IB + Thiobacillus mixture*	1.78	19	2.23	23	2.33	25	2.57	27
IB + Probentonite	1.59	17	2.60	27	2.73	29	2.93	31
IB + Combined mixture of all remediative amendments	1.33	14	1.83	19	1.93	20	2.03	21

**Thiobacillus thiooxidans & Thiobacillus ferrooxidans*

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Table (4) Degradation of the aliphatic hydrocarbon n-eicosan in a sewaged soil ecosystem irrigated with either regular water or treated sewage effluent after bioremediation and phytoremediation canola, Indian mustard or black nightshade (ng/g dry soil)

Treatment								
Initial	6.82							
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)	3.17	46	4.67		68			
IB *+Thiobacillus* mixture*	2.37	35	2.88		42			
IB +Probentonite	2.91	43	3.26		49			
IB + Combined mixture of all remediative amendments	2.09	31	2.65		39			
2nd Stage (Phytoremediation period extended from 61 to 120 days)								
IB +Un-cultivated	2.39	35	3.29		48			
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	1.22	19	2.10	31	2.20	32	2.38	34
IB + AM inoculation	1.41	21	2.00	29	2.10	30	2.22	32
IB + Thiobacillus mixture*	1.83	27	1.86	27	1.90	28	2.01	29
IB + Probentonite	1.89	28	2.08	30	2.10	31	2.19	32
IB + Combined mixture of all remediative amendments	1.17	17	1.52	22	1.68	25	1.79	26

**Thiobacillus thiooxidans & Thiobacillus ferrooxidans*

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Table (5) Degradation of the aliphatic hydrocarbon n-docosane in a sewaged soil ecosystem irrigated with either regular water or treated sewage effluent after bioremediation and phytoremediation canola, Indian mustard or black nightshade (ng/g dry soil)

Treatment								
Initial	4.42							
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)	2.46	55	2.76		62			
IB *+Thiobacillus* mixture*	1.88	43	2.14		48			
IB +Probentonite	2.16	49	2.53		57			
IB + Combined mixture of all remediative amendments	1.67	38	2.01		45			
2nd Stage (Phytoremediation period extended from 61 to 120 days)								
IB +Un-cultivated	2.02	46	2.61		59			
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	1.46	33	1.96	44	2.11	48	2.29	52
IB + AM inoculation	1.37	31	1.77	40	1.89	43	2.01	45
IB + Thiobacillus mixture*	1.27	29	1.47	33	1.52	34	1.56	35
IB + Probentonite	1.33	30	1.83	42	1.93	43	1.99	45
IB + Combined mixture of all remediative amendments	0.95	21	1.14	26	1.29	29	1.41	32

**Thiobacillus thiooxidans & Thiobacillus ferrooxidans*

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Table (6) Degradation of the aliphatic hydrocarbon n-tetracosane in a sewaged soil ecosystem irrigated with either regular water or treated sewage effluent after bioremediation and phytoremediation canola, Indian mustard or black nightshade (ng/g dry soil)

Treatment								
Initial	8.32							
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)	4.73	57	5.71		69			
IB +*Thiobacillus* mixture*	3.78	45	4.38		58			
IB +Probentonite	4.11	49	4.97		60			
IB + Combined mixture of all remediative amendments	3.03	36	4.12		50			
2nd Stage (Phytoremediation period extended from 61 to 120 days)								
IB +Un-cultivated	3.57	43	4.56		55			
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	1.92	23	2.61	31	2.71	33	2.88	35
IB + AM inoculation	1.75	21	2.39	29	2.50	30	2.69	32
IB + Thiobacillus mixture*	1.62	19	2.11	25	2.34	28	2.53	30
IB + Probentonite	1.70	20	2.36	28	2.40	30	2.49	30
IB + Combined mixture of all remediative amendments	1.02	12	1.98	24	2.07	25	2.19	26

**Thiobacillus thiooxidans & Thiobacillus ferrooxida*

The combined action of indigenous soil biomass and root exudates associated with *Thiobacillus* sp. inoculation decreased n-tetracosane in the sewaged soil ecosystem from 8.32 to 2.11, 2.34 and 2.53 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 n-tetracosane in the sewaged soil ecosystem from 8.32 to 1.62 ng/g dry soil under canola phytoremediation.

The combined action of indigenous soil biomass and root exudates associated with probentonite enhancement decreased n-tetracosane in the sewaged soil ecosystem from 8.32 to 2.36, 2.40 and 2.49 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 n-tetracosane in the sewaged soil ecosystem from 8.32 to 1.70 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 n-tetracosane in the sewaged soil ecosystem from 8.32 to 1.98, 2.07 and 2.19 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 a combined mixture of all tested remediative amendments decreased n-tetracosane in the sewaged soil ecosystem from 8.32 to 1.02 ng/g dry soil under canola phytoremediation.

Consequently, results confirmed the superiority of the combined mixture of all tested remediative amendments in decreasing n-tetracosane 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, n-tetracosane contents were respectively reduced to 24, 25 or 26% from their initial content. In the column experiment irrigated with regular water, n-tetracosane content was reduced to 12% from their initial content under the combined action of all remediative amendments followed by canola phytoremediation. Although the rates of n-tetracosane content diminish under phytoremediation with any of the three experimented hyperaccumulator plants were more or less the same, yet canola hyperaccumulator action was somewhat obvious.

DISCUSSION

POPs are realistic to stick with sewaged soil ecosystems [24], and had a potential significant adverse impacts on health and environment. It is worthy to mention that the content of the studied aliphatic hydrocarbons in the experimented sewaged soil did not reach a hazard level [7], [13]. Results showed varied responses of the five detected aliphatic hydrocarbons to the experimented remediative amendments followed by phytoremediation, some disappeared from the sewaged soil ecosystem after bioremediation followed by phytoremediation, others exhibited a serious persistent diminish 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 their sole application. Destruction of aliphatic hydrocarbons continuously occurs by indigenous soil biomass which is capable to use them in their growth and reproduction as a source of carbon and electrons. Günther et al (1996) grew ryegrass (*Lolium perenne L.*) to biodegrade hydrocarbons in laboratory scale soil columns. In the rhizosphere soil ecosystem, they found that the aliphatic hydrocarbons disappeared faster in uncultivated columns. Elimination of hydrocarbons was accompanied by an increase in microbial numbers and activities as the microbial plate counts and soil respiration rates were substantially higher in the rhizosphere than in the bulk soil. Their results indicated that biodegradation of hydrocarbons in the rhizosphere is stimulated by ryegrass roots. Colombo et al (1996) compared the biodegradation of aliphatic hydrocarbons by natural soil micro-flora and seven fungi species, including imperfect strains and higher level lignolitic species, in a 90-day laboratory experiment using a natural, not-fertilized contaminated soil. Normal alkanes were almost completely degraded in the first 15 days, whereas aromatic compounds exhibited slower kinetics. *Aspergillus terreus* and *Fusarium solani* efficiently attacked of aliphatic hydrocarbons. They found that the imperfect fungi isolated from polluted soils showed a somewhat higher efficiency, but the performance of unadapted, indigenous, lignolitic fungi was comparable, and all three species, *Pleurotus ostreatus, Trametes villosus* and *Coriolopsis rigida*, effectively degraded aliphatic hydrocarbons. Okere and Semple 2012 stated that over time, 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. Nester et al (2001) mentioned that the white-rot fungus, *phaneorochaete chrysosporium*, could bind to, and in some instances, mineralize a wide array of aliphatic hydrocarbon in the presence of oxygen through aerobic respiration with the release of CO_2 and H_2O**.** Ghazali, et al (2004) investigated the bioremediation of hydrocarbon in contaminated soils by mixed cultures of hydrocarbon-degrading bacteria. Their bacterial consortia, denoted as Consortium 1 and Consortium 2 consisted of 3 and 6 bacterial strains, respectively. Bacterial strains used were isolated from hydrocarbon-contaminated soil enriched with either crude oil or individual hydrocarbons as the sole carbon source. They found that Consortium 2, which is predominantly consisted of *Bacillus* and *Pseudomonas* sp., was more efficient at removing the medium- and long-chain alkanes. They added that Consortium 2 could effectively remove the medium- and long-chain alkanes which were undetectable after a 30-day incubation period. Consortium 2 Rates of aliphatic hydrocarbons biodegradation depend greatly on their composition, state and concentration as well as on their dispersion and absorption by soil particulates. Temperature, moisture, oxygen, salinity, pH, biomass and nutrient are also important variables. Adaptation by prior exposure of microbial communities to aliphatic hydrocarbons increases their degradation rates. Adaptation is brought about by selective enrichment of hydrocarbon-utilizing microorganisms. Perfumo et al. (2007) mentioned that POPs could be adsorbed to soil particles thus rendering them unavailable to microbial biodegradation. Hydrophobic POPs like aliphatic hydrocarbon had low solubility in water and tend to adsorb strongly in soil with high organic matter content. In such cases, surfactants are utilized as part of the bioremediation process to increase solubility and mobility of these contaminants.

In parallel, phytoremediation had largely focused on the use of plants to accelerate degradation of POPs, usually with rhizosphere microorganisms and root exudates. Direct

uptake of aliphatic hydrocarbons by higher plants is a surprisingly efficient removal mechanism from sewaged soil ecosystems moderately contaminated with aliphatic hydrocarbons that are strongly bound to root surface and soil colloids' and are not easily translocate within the plant, as well as those that are quite water soluble are not sufficiently sorbet to roots nor actively transported through plant membranes [5]. Many plants had expressed some capacities to uptake and convert aliphatic hydrocarbons quickly to less toxic metabolites. Others might stimulate their degradation in the rhizosphere through root exudates and enzymes [25]. They suggested that phytoremediation is best suited for removing moderately hydrophobic aliphatic hydrocarbons from soil ecosystem; yet, their high levels are toxic to plants and prevent successful phytoremediation. Once an aliphatic hydrocarbon is translocate, the plant store it and its fragments into new plant structures via lignification or it could volatilize, metabolized, or mineralized completely to CO_2 and H_2O.

Bardi et al (2000) and Dindar 2013 confirmed that biodegradation of non-chlorinated aliphatic hydrocarbons was influenced by their bioavailability. They added that hydrocarbons are very poorly soluble in water, easily adsorbed to clay or humus fractions in the soil, and pass very slowly to the aqueous phase, where they are metabolized by biomass. Surfactants that increase their solubility and improve their bioavailability could thereby accelerate their degradation as shown by the decreases of dodecane (C12), tetracosane (C24) anthracene when added individually as the sole carbon source to mineral medium liquid cultures.

In conclusion aliphatic hydrocarbons, besides existing in the sewaged soil ecosystem in amounts less than the permissible levels, many of their key members were not initially detected in the sewaged soils ecosystem. All investigated aliphatic hydrocarbons 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 under both irrigation with regular 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 intended 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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