



Existence and Decontamination of HVC, Infectious Enteric Bacteria and Parasites in Sewaged Soils

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ABSTRACT

A surface sample representing a high contaminated loamy sand soil irrigated with sewage effluent since 30 years and was cultivated with artichoke was collected from Abu-Rawash sewage farm. The existence of HVC, enteric infectious bacteria and parasites in sewaged soil found to be negative for the forward and positive for the latter's. Out of the 30 samples separated from the sewaged soil sample, only 3 samples contained parasitic fauna of developed and undeveloped *Ascaris* (10%) and five samples contained *Entamoeba coli*. Results showed that the number of *Ascaris* eggs/gm soil was 0.017 and the number of *E. coli*/gm was 0.26. Decontamination of soil parasites was effective using either calcium hypochlorite or potassium permanganate. *Salmonella*, *Vibrio* and *Campylobacter* were detected in the high contaminated sewaged soil and survived for 120 days in the sewaged soil under all control and bioremediated treatments irrigated with either sewage effluent or water.



Council for Innovative Research

Peer Review Research Publishing System

Journal: JOURNAL OF ADVANCES IN AGRICULTURE

Vol. 3, No. 1

jaaeditor@gmail.com

www.cirjaa.com



INTRODUCTION

The possible broadcast of pathogens, predominantly HVC and infectious enteric bacteria and parasites from sewage soils to humans is of undeniable anxious under Egyptian conditions due to the commonness of a widespread assortment of enteric pathogens in sewage soils and the customary use of manual labor in sewage farms who work in close contact with the contaminated sewage soils, and somewhat low standards of hygiene. In addition to that it is worthy to mention that great volumes of sewage effluent is disposed raw in canals and drains all over Egypt, and hence reaches the soil causing severe undesirable consequences. Certainly, current sewage effluent treatment does not remove all pathogens, and in many cases pathogen re-growth is significant. Remediation and sustainable sewage effluent management in combination with high-efficiency sewage treatment are the only way to meet this confront. Our main concern in this work is detecting the existence and decontamination of HVC and infectious enteric bacteria and parasites from sewage soils to reduce their intensities to levels that would not give rise to risk of infection.

MATERIALS AND METHODS

Sampling: A surface sample representing a high contaminated loamy sand soil irrigated with sewage effluent since 30 years and was collected from Abu-Rawash sewage farm.

Existence of Hepatitis C virus in Sewaged Soils: The existence of hepatitis C Virus (HCV) was qualitatively tested in the sewage soil according to the method described by Pawlotsky et al. (2002) on the basis of principle target amplification using either "classic" polymerase chain reaction (PCR), "real-time" PCR or TMA (Roche molecular systems with HCV amplicor monitor® v2.0). The RNA in hepatitis C virus was extracted and reverse transcribed into a double stranded complementary DNA (cDNA), before being subsequently processed into a cyclic enzymatic reaction leading to the generation of a large number of detectable copies according to RTPCR Manual. Double-stranded DNA copies of HCV genome were synthesized in PCR-based assays, whereas single-stranded RNA copies were generated in TMA.

Existence and Decontamination of Infectious Enteric Parasites in Sewaged Soils: Three kg soil portion was divided to three equal parts, one Kg each, before being subdivided into ten portions (100 gm each). Each subdivision was subjected to flotation technique for the detection of enteric parasites eggs and larvae as well as oocytes by staining with eosin and Zeil-Nelson stain according to the method described by Hassanain and Tawfik (1985).

To decontaminate the sewage soils from enteric parasites, two types of chemical remediators were tried, calcium hypochlorite and potassium permanganate. The first was added at a rate of 500 ppm and the second at the rates of 500, 1000, 2000 or 3000 ppm. The existence of enteric parasites was detected initially and after one week incubation at room temperature for calcium hypochlorite and 24 hours incubation for potassium permanganate.

Survival and Decontamination of Infectious Pathogenic Bacteria in Sewaged Soils: At Abu-Rawash sewage farm and the NRC greenhouse, two completely randomized field and pot experiments with four replicates were carried out using canola hyper-accumulator plants to decontaminate a high contaminated sewage soil. The field experiment was irrigated with sewage effluent and the column experiment was irrigated with water. The contaminated sewage soil was chemically and biological bioremediated for two months before sowing canola by six treatments including uncultivated control, cultivated control, inoculated with AM, inoculated with a mixture of *Thiobacillus thiooxidans* & *Thiobacillus ferrooxidans*, soil treated with 1% pentonite plus 1% rock phosphate and inoculated with phosphate dissolving bacteria and soil treated with all the aforementioned remedial amendments. Soil samples were collected initially and after 60, 90 and 120 days to follow the survival of certain infectious enteric bacteria. *Salmonella*, *Shigella*, *Vibrio* and *Campylobacter* species were respectively detected on SS agar, TCBS agar and charcoal cefoperazone desoxycholate agar plates according to the scheme described by Fox (2011) for *Salmonella* and *Vibrio* and by Murray et al. (2003) for *Campylobacter*.

RESULTS AND DISCUSSIONS

Diverse types of infectious enteric pathogens including bacteria, virus and parasitic fauna, are always found in sewage soils and to a lesser extent on plant phyllosphere. The main infectious enteric viruses, parasites and bacteria associated with sewage farming are hepatitis, enteric adenoviruses, poliovirus, multiple strains of echoviruses, coxsackievirus, *Entamoeba histolytica*, *Giardia intestinalis* and *Cryptosporidium parvum* and *Salmonella*, *Shigella*, *Vibrio* and *Campylobacter* species. (Santamaría and Toranzos, 2002).

Existence of HVC in Sewaged Soils: Although many decades had been passed since the first studies on the presence of human enteric viruses in soil were begun, the public health significance of such contamination had yet to be evaluated (WHO 1979). Monitoring the existence and survival of infectious virus in sewage soils is of high importance for risk assessment which is still a relatively new discipline as a tool to identify, assess, and manage risks (Sobsey et al., 1995). Extensive practical knowledge of the monitoring of infectious bacterial pathogens in sewage farms are available, but there is only a very limited experience with regard to viral contamination (WHO 1979).

However, It is becoming more possible to qualitatively evaluate the prevalence of pathogenic viruses in the environment, through the application of new molecular tools such as real-time polymerase chain reaction (PCR) and nucleic acid sequence-based amplification (NASBA). Sequencing of viral genes facilitates the uncovering of relationships between strains and enables tracing of the origins of outbreaks.

Present sewage treatment procedures might not always be sufficient to prevent viruses from reaching soils. The possible deposition of significant concentrations of viruses on the soil might result in several health hazards, e.g. direct virus



infection of farm workers and their contacts, virus contamination of crops used for human consumption; virus contamination of drinking-water sources as a result of surface run-off or infiltration into groundwater; dissemination of viruses by insect or animal vectors in contact with contaminated soil. When sewage effluent is practiced by sprinkler-irrigation, virus dissemination by aerosol might occur, with consequent risks of infection through the respiratory tracts of farm workers, residents of adjacent areas or travelers in the vicinity. Abd-el-naim and El-houseini (2002) found that 20% of 50 persons representing sewage workers, handlers and those living close to El-Gabal El-Asfer sewage farm in Cairo were HBC and HVC hepatitis carriers and could infect their families. Also (Bidawid et al., 2009) stated that infectious pathogenic viruses causing a variety of diseases such as gastroenteritis and hepatitis could survive and be transmitted through various environments

In the current work, the existence of hepatitis C virus (HCV) was qualitatively checked in five soil samples (solarized and desiccated sewage soil sample, bioremediated and non-bioremediated sewage soil samples irrigated with either water or sewage effluent). Results given in Table (1) showed that HCV human hepatitis C viremia was found in all the investigated soil at intensities ranged between <600 and <300 IU/ml indicating a non-detectable negative existence in the sewage soils at Abu-Rawash sewage farm.

Table (1) Existence of HVC in remediated and non-remediated contaminated sewage soils (IU/ml)

Soil treatment	Existence of HVC (IU/ml)
Solarized and desiccated	<600
Non-bioremediated and irrigated with water	<300
Bioremediated and irrigated with water	<300
Non-bioremediated and irrigated with sewage effluent	<300
Bioremediated and irrigated with sewage effluent	<300

Vantarakis and Papapetropoulou (1999) used Nested-PCR approach to confirm the existence of enteroviruses and adenoviruses and the absence of HAV in raw sewage effluent; however all were not detected in the effluent after biological treatment. They concluded that the frequent isolation of adenoviruses from raw sewage indicated their stability as virological indicators of soil contamination. In contrast with raw sewage, they were unable to detect enteroviruses or adenoviruses in samples collected after biological sewage treatment indicating the effectiveness of secondary purification. In some cases, indicators are not detected when infectious pathogenic viruses are present in soil and could initiate an outbreak; this argues in favor of the proposal of new tools to estimate viral pollution in sewage soils

There are many factors that affect the survival of enteric viruses in soil including pH level, ionic concentration, moisture content, temperature, exposure to sunlight and organic matter. Sewage soils ecosystem is for sure unsuitable for virus survival as Gilbert et al. (1976) emphasized that human viral infectious pathogens are apparently absorbed and degraded by soil colloids and hence their intensities were reduced by 99.99%. Sagar et al. (1979) studied the comparative adsorption of a number of different types and strains of human enteroviruses and bacteriophages in nine different soil types and observed a great deal of variability between adsorption of different strains of echovirus type1, indicating that viral adsorption to soils is highly strain dependent. Enteric viruses in loamy and sandy-loamy soil had considerable stability, with survival times of up to 170 days (WHO 1979). Poliovirus had been detected in sewage soils after 96 days of irrigation in winter and 11 days in summer and on the surface of mature vegetables 23 days after irrigation had ceased. Poliovirus had also been recovered in soil and on the surface of crops 8 days after irrigation with experimentally infected sewage. These reported periods of virus survival are not necessarily maximum values since other enteric virus types might be even more resistant. In addition, some viruses might be difficult to recover by usual methods (WHO 1979). Parashar et al. (2011) analyzed the survival of hepatitis A virus (HAV) and hepatitis E virus (HEV) in soil samples spiked with respective viruses using real-time PCR. They found that both HAV and HEV were less stable at fluctuating environmental temperature than at 37°C. Of the 403 soil samples collected in the vicinity of Mutha River, India, 19.1% and 4.9% were found to be contaminated with HAV and HEV, respectively.

It was repeatedly noticed that virus survival on crops is shorter than in soil since viruses on crop surfaces are directly exposed to detrimental environmental factors such as sunlight and desiccation. However, more prolonged survival could be expected in moist or more protected parts of plants, such as within the folds of leafy vegetables, in deep stem area and on rough cracked surfaces of edible roots. Other studies indicated that human infectious viruses could penetrate damaged roots and, under certain conditions, enter the stem and leafy parts of edible plants. While evidence of this phenomenon is still tenuous, its possible role in crop contamination should not be overlooked. Once crops are harvested, enteric viruses could survive for prolonged periods during commercial and household storage at low temperature (WHO 1979). Disinfection of sewage effluent prior to land disposal, particularly in the case of sprinkler-irrigation could be an effective preventive measure.

Despite, HVC was not detected in the sewage soil samples in the current work, however, the persistence of viruses might be challenged by various disinfection techniques, comprehensively on all enteric virus types. No survival or disinfection



study had comprehensively included all enteric virus types, and there is no hard information on such important viral agents as hepatitis. Hence, periodical monitoring of pathogenic viruses is an important public health consideration for land disposal of sewage effluent.

Existence and Decontamination Infectious Enteric Parasites in Sewaged Soils: For the sake of sustainable sewage farming the risk associated with the existence of infectious enteric parasites in soil and harvests should be cared about. Many researchers confirmed in the vast literature the existence of infectious enteric parasites in sewaged soils (Blumenthal et al.1996; Rais and Xanthoulis 1999; Carlander et al.2009;Nikaido et al. 2010). They detected varied infectious parasites including *Ascaris lumbricoides*, *Trichuris*, *Ascaridia galli*, *Hymenolepis nana*, *Enterobius vermicularis* eggs, nematode larvae and *Giardia*, *Cryptosporidium* and *Entamoeba coli* oocysts in sewaged soils. Amahmid et al. (1999) in field trials confirmed that irrigating crops with raw sewage effluent led to contamination with *Giardia* cysts reaching 155 cysts/Kg and 0.7 eggs/Kg in carrots and 59.1 cysts/Kg and 1.64 eggs/Kg in radish. Gupta et al. (2009) revealed that the predominant infectious parasites existed with sewage farming were represented by 68.6% in soil and 44.2% in vegetables for *Ascaris lumbricoides* (36%), *Trichuris trichura* (1.7%) and hook worms (6.4%).

The major types of parasitizes include species of protozoa, helminthes or worms, and arthropods. More than 45,000 species of protozoa are known, many of which are parasitic that had historically been the cause of more suffering and death. Intestinal protozoa are especially common in areas where food and water sources are subject to contamination from sewage effluent. Typically, protozoa that infect their host through water or food do so while in an inactive state, called a cyst consists of a protozoan encased in a protective outer membrane. The most famous infectious protozoa are amebic dysentery caused by *Entamoeba histolytica* and *Giardia lamblia*.

Helminthes are wormlike organisms including nematodes (roundworms), cestodes (tapeworms), and trematodes (flukes). The largest parasitic roundworm, common among humans living in tropics are *Ascaris lumbricoides* and *Schistosoma*.

In the current work it was found that out of the 30 samples separated from the solarized sewaged soil sample that was exposed to irrigation with sewage effluent for 30 years at Abu-Rawash sewage farm, only 3 samples contained parasitic fauna of developed and undeveloped *Ascaris* (10%) and five samples contained *Entamoeba coli*. Results showed that the number of *Ascaris* eggs/gm soil was 0.017 and the number of *Entamoeba coli* oocysts/gm was 0.260. Results confirmed that *Ascaris* egg was the prevalent at Abu-Rawash sewages soil as previously monitored in sewaged soils by other authors (Carlander et al., 2009 and Gupta et al.,2009). It is worthy that the concentration of eggs/gm or/Kg soil found in the current study was lower than that recorded other authors (Nikaido et al., 2010).

The survival of developed and undeveloped *Ascaris sp* eggs, *E. histolytica* oocysts and *Entamoeba coli* oocysts in a high contaminated sewaged soil sampled from Abu-Rawash sewage farm was followed for 120 days under irrigation with either sewage effluent or water (Tables 2 & 3 & 4). Results showed that all the three tested infectious enteric parasites existed and survived in the sewaged soil in all control and bioremediated testaments. They all existed and survived in the sewaged soil, except *E. histolytica* oocysts which was not detected in certain instances, both under sewage effluent or water irrigation in both control and soils receiving different bioremediative treatment.

In the current work, data given in Table (5) show that decontaminating infectious soil parasites with either calcium hypochlorite or potassium permanganate was effective against *Ascaris* egg, *Entamoeba coli* oocysts and *E.histolytica* oocysts and resulted in a complete degeneration of eggs and oocysts.

Table (2) Survival of developed and undeveloped *Ascaris sp* eggs (per g soil) in sewaged soil irrigated with water or sewage effluent

Treatment	0 day		60 day		90 day		120 day	
	Source of irrigation							
	Water	Sewage	Water	Sewage	Water	Sewage	Water	Sewage
Uncultivated control	0.017	0.017	0.111	0.017	0.301	0.770	0.430	0.017
Cultivated with canola	0.017	0.017	0.111	0.017	0.301	0.770	0.430	0.017
AM	0.017	0.017	0.111	0.017	0.301	0.770	0.430	0.017
Thiobacillus	0.017	0.017	0.111	0.017	0.341	0.111	0.430	0.017
Bentonite	0.017	0.017	0.111	0.017	0.311	0.235	0.430	0.017
All	0.017	0.017	0.111	0.017	0.43	0.133	0.430	0.017

AM: inoculated with Arbuscular Mycorrhizae

Thiobacillus: Inoculated with *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*

Bentonite: treated with bentonite and rock phosphate and PDB

All: treated with all chemical and biological treatments



Table (3) Survival *E. histolytica* oocysts (per g soil) in sewage soil irrigated with water or sewage effluent

Treatment	0 day		60 day		90 day		120 day	
	Source of irrigation							
	Water	Sewage	Water	Sewage	Water	Sewage	Water	Sewage
Uncultivated control	0.319	0.319	0.190	ND	0.319	0.331	ND	ND
Cultivated with canola	0.319	0.319	0.190	ND	0.319	0.331	ND	ND
AM	0.319	0.319	0.190	ND	0.319	0.331	ND	ND
Thiobacillus	0.319	0.319	ND	ND	0.297	0.211	ND	ND
Bentonite	0.319	0.319	ND	ND	0.281	0.091	ND	ND
All	0.319	0.319	ND	ND	0.31	0.011	ND	ND

AM: inoculated with Arbuscular Mycorrhizae

Thiobacillus: Inoculated with *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*

Bentonite: treated with bentonite and rock phosphate and PDB

All: treated with all chemical and biological treatments

Table (4) Survival of *Entamoeba coli* oocysts (per g soil) in sewage soil irrigated with water or sewage effluent

Treatment	0 day		60 day		90 day		120 day	
	Source of irrigation							
	Water	Sewage	Water	Sewage	Water	Sewage	Water	Sewage
Uncultivated control	0.260	0.260	0.210	0.290	0.202	0.312	0.111	0.320
Cultivated with canola	0.260	0.260	0.210	0.290	0.202	0.312	0.111	0.320
AM	0.260	0.260	0.210	0.290	0.202	0.312	0.111	0.320
Thiobacillus	0.260	0.260	0.210	0.290	0.211	0.312	0.111	0.320
Bentonite	0.260	0.260	0.210	0.290	0.211	0.312	0.111	0.320
All	0.260	0.260	0.210	0.290	0.211	0.312	0.111	0.320

AM: inoculated with Arbuscular Mycorrhizae

Thiobacillus: Inoculated with *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*

Bentonite: treated with bentonite and rock phosphate and PDB

All: treated with all chemical and biological treatments

In harmony with that, Gaspard and Schwartzbrod (1993) tried different eluting solutions of detergents, including distilled water, formaldehyde, sodium hydroxide and sodium hypochlorite and found that sodium hypochlorite resulted in 60% recovery.

**Table (5) Decontamination of enteric parasites in the sewage soil by either calcium hypochlorite or potassium permanganate**

Treatments	<i>Ascaris sp.</i> eggs		<i>E. coli</i>		<i>E.histolytica</i> oocysts	
	Counts per g soil	%	Counts per g soil	%	Counts per g soil	%
Control	0.017	10	-	-	0.26	16.6
Chlorinated 500 ppm	Degenerated		Degenerated		Degenerated	
Potassium permanganate 500 ppm	viable		viable		viable	
Potassium permanganate 1000 ppm	viable		viable		viable	
Potassium permanganate 2000 ppm	Degenerated		Degenerated		Degenerated	
Potassium permanganate 3000 ppm	Degenerated		Degenerated		Degenerated	

Feachem et al. (1983) and Pescod (1992) stated that the possible levels of *Ascaris lumbricoides* in sewage effluent per liter should not exceed 600. The level reached after decontamination with either calcium hypochlorite or potassium permanganate were less than the aforementioned recommended safe levels.

It seems reasonable to conclude that control measures of infectious enteric parasite aim at protecting agricultural field workers and crop handlers from infection. Following several meetings of environmental specialists and epidemiologist, a WHO Scientific Group on Health Aspects of Use of treated sewage effluent in agriculture and aquaculture arrived at guidelines for sewage effluent use in farming. These norms stated that intestinal nematodes per liter should be less than 1 and be less than 1000 geometric means. Their guidelines were based on the consensus view that the actual risk associated with irrigation with treated sewage effluent is much lower than previously thought and that earlier standards and guidelines for effluent quality, such as WHO (1973) recommended standards were un-justifying restrictive. These guidelines are stricter than the previous standards in respect of the requirements to reduce the numbers of helminthes eggs (*Ascaris* and *Trichuris* species and hookworms) in the effluents to a level of not more than one per liter. Also implied by the guidelines is the expectation that protozoan cysts will be reduced to the same level as helminthes eggs.

The WHO Scientific Group considered effluent quality would increase public health protection for large numbers of people who were being infected in areas where crops eaten uncooked are being irrigated in unregulated, and often illegal, manner with raw sewage effluent. It was felt that the recommended guidelines, if adopted, would achieve this improvement and set targets which are both technologically and economically feasible. However, the need to interpret the guidelines carefully and modify them in the right local epidemiological, socio-cultural and environmental factors was also pointed out. However, as given in Table 4-1 in the report of the permanent committee on reuse of treated sewage effluent in farming part one: Code (2004) entitled norms of sewage effluent, the permitted limit number of viable cells or eggs of parasitic nematode per liter must be less than 1, according to article no 66 in the law number 48 (1982) .

Certainly, health risks on human and animals associated with sewage farming are of great concern. The needs to definite acceptable risk criteria, more accurate dose-response modeling, information regarding pathogen survival in soil and additional data related to the passage of pathogens into and in the plants are urgent for sustainable farming with sewage effluent.

Survival of certain Infectious Enteric Bacteria in Swaged Soils: The survival of *Salmonella*, *Campelobacter* (zoonotic microorganisms) and *Vibrio* in a high contaminated sewage soil was followed for 120 days under irrigation with sewage effluent or water (Tables 6 & 7 & 8). Results showed that all the three tested enteric infectious pathogens existed and survived in the sewage soil under all control and bioremediated treatments. They all existed and survived in the sewage soil both under sewage effluent or water irrigation. Shuval et al. (1986) reached the same finding when detected the existence of *V. cholerae* in the irrigated soils.



Table (6) Existence of *Salmonella* in sewage soil irrigated with water or sewage effluent

Treatment	0 day		60 day		90 day		120 day	
	Source of irrigation							
	Water	Sewage	Water	Sewage	Water	Sewage	Water	Sewage
Uncultivated control	+	+	+	+	+	+	+	+
Cultivated with canola	+	+	+	+	+	+	+	+
AM	+	+	+	+	+	+	+	+
<i>Thiobacillus</i>	-	+	-	+	-	+	-	+
Bentonite	+	+	+	+	+	+	+	+
All	-	+	-	+	-	+	-	+

AM: inoculated with Arbuscular Mycorrhiza

Thiobacillus: Inoculated with *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*

Bentonite: treated with bentonite and rock phosphate and PDB

All: treated with all chemical and biological treatments

Table (7) Existence of *Vibrio* in sewage soil irrigated with water or sewage effluent

Treatment	0 day		60 day		90 day		120 day	
	Source of irrigation							
	Water	Sewage	Water	Sewage	Water	Sewage	Water	Sewage
Uncultivated control	-	-	-	-	-	-	-	-
Cultivated with canola	-	-	-	-	-	-	-	-
AM	-	-	-	-	-	-	-	-
<i>Thiobacillus</i>	-	-	-	-	-	-	-	-
Bentonite	-	-	-	-	-	-	-	-
All	-	-	-	-	-	-	-	-

AM: inoculated with Arbuscular Mycorrhiza

Thiobacillus: Inoculated with *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*

Bentonite: treated with bentonite and rock phosphate and PDB

All: treated with all chemical and biological treatments

**Table (8) Existence of *Campylobacter* in sewage soil irrigated with water or sewage effluent**

Treatment	0 day		60 day		90 day		120 day	
	Source of irrigation							
	Water	Sewage	Water	Sewage	Water	Sewage	Water	Sewage
Uncultivated control	+	+	+	+	+	+	+	+
Cultivated canola	+	+	+	+	+	+	+	+
AM	+	+	+	+	+	+	+	+
<i>Thiobacillus</i>	-	-	-	-	-	-	-	-
Bentonite	-	-	-	-	-	-	-	-
All	+	+	+	+	+	+	+	+

AM: inoculated with Arbuscular Mycorrhizae

Thiobacillus: Inoculated with *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*

Bentonite: treated with bentonite and rock phosphate and PDB

All: treated with all chemical and biological treatments

It is worthy that present sewage effluent treatment procedures might not always be sufficient to prevent HVC and/or other infectious pathogenic bacteria and parasites from reaching soils. The possible deposition of significant concentrations of these infectious microorganisms to soil will result in several health hazards.

On the basis of these findings, a periodical mentoring programme should be scheduled and the proper decontamination of infectious enteric bacteria. applied

ACKNOWLEDGMENTS

The authors would like to express their appreciations and gratitude to the authorities of Science and Technology Development Fund (STDF) for financing the present work through the project number 1425 contracted with the National Research Center on Bioremediation of Sewaged Soils.

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