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Biochemical Approaches for Mitigating heavy metals in Contaminated Soil Ecosystems: Kinetic Evaluation and Phytostablization Strategies

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Abstract

Soil, a vital component in biosphere is frequently endangered to superfluity of various pollutants, particularly heavy metals. Unlike organic pollutants, heavy metals are persistent and cause environmental, health and socio-economic adverse impacts, Consequently, heavy metals removal from soil ecosystem is not only mandatory but also a topic of interest nowadays. This study assessed four biochemical treatments from a kinetic perspective to evaluate their efficacy in reducing heavy metals concentrations and risks in soils cultivated with tomatoes. The processing materials included: (T1) bentonite + rock phosphate+ phosphate dissolving bacteria (*Bacillus megatherium*), (T2) bentonite + elemental sulfur + *Thiobacillus thiooxidans* , (T3) bentonite + elemental sulfur + rock phosphate + *Thiobacillus thiooxidans* + *Bacillus megatherium*, and (T4) bentonite + kaolinite clay minerals + rock phosphate+ *Bacillus megatherium* + elemental sulfur +*Thiobacillus thiooxidans* . Furthermore, two untreated controls were represented by cultivated control and uncultivated control soil. The results showed that all kinetic models described the rate of pollutants desorption from the treated soil were succeeded to describe the rate of Zn, Cu and Ni. However, Modified Freundlich equation (MFE) was the best. All treatments significantly reduced heavy metals desorption rates, with T4 emerging as the most effective management practice. Despite, T1 enhanced Zn phytoextraction by tomatoes; However T4 reduced all tested heavy metals accumulation in tomatoes fruits, making it a promising phytostabilization strategy for safe vegetable production. Overall, T4 represents a viable solution for alleviating heavy metals hazard in contaminated agricultural soils.

Keywords: Heavy metals, Clay minerals, Remediation, Kinetic studies, Soil contamination, *Thiobacillus thiooxidans , Bacillus megatherium*.

1. Introduction

Soil is a fundamental component of Earth's biosphere; it sustains life on Earth and tools up goods and services for the well-being of humans and environment thanks to the natural processes that occur within its matrix and the interaction of biotic and abiotic components [1]. However, soil pollution threatens these functions, leading to biodiversity loss and ecosystem impairment. Pollutants, particularly inorganic pollutants such as heavy metals(HMs), disrupt soil microbial community levels, enhancing resistant organisms over sensitive ones and may even induce antimicrobial resistance in microorganisms [2]. In addition, soil contaminants have cascading effects on the primary productivity of natural and agricultural ecosystems and lead to loss of biodiversity and services of soil ecosystem due to these contaminants enter the food chain and associated with a set of adverse impacts on natural ecosystems and economy [3]. The loss of biodiversity and biomass therefore leads to a decrease in organic matter and changes in nutrient inputs and cycling [4]. Various developmental activities such as mining, smelting, chemical farming, waste disposal and industrial activities and the use of low-quality irrigation water discharge, avariety of inorganic pollutants mainly arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) in many terrestrial and aquatic ecosystems [5]. These elements adversely affect plants growth, reducing seed germination, biomass accumulation, protein metabolism, chlorophyll content, and enzyme activity [6].

Various physical, chemical and biological technologies are common in remediation of heavy metals contaminated ecosystems. Physical and chemical methods, such as in situ verification, washing, flushing, solidification, and stabilization, often require intensive labour, are cost-prohibitive, and may irreversibly alter natural ecosystem properties or harm indigenous microorganisms [7]. In contrast, bioremediation using plants, bacteria, or their partnerships has spouted as a sustainable alternative for remediation of polluted soils [8].

Plant-microbialpartnerships bioremediation leverages plant growth-promoting rhizobacteria (PGPR) to enhance plant growth and alleviate heavy metals toxicity. Plant growth promoting rhizobacteria facilitate phytoremediation through different mechanisms such as phytohormone production, nutrient supply, siderophore release, in addition to specific enzymatic activity and nitrogen fixation [9] and [6]. Studies demonstrated the effectiveness of PGPR in reducing plant stress in heavy metal-polluted soils, while accelerating pollutant remediation [10]. For instance, soil

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inoculation with *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* decreases soil pH, increasing the solubility and bioavailability of inorganic pollutants [11]. Similarly, inoculation with *Thiobacillus thiooxidans* and *Arbuscular mycorrhizae* (AM) improved hyperaccumulator plants' ability to uptake inorganic pollutants [12]. Multi-metal resistance (Pb, Cd, Cu, and Zn) strain, B. cereus KMS3-1, was able to produce extracellular polymeric substances, which is considered an important mechanism for bioremediation of heavy metals for alleviation of heavy metals toxicity [13].

This study aims to evaluate the potential of synergistic plant-bacteria partnerships and localized biotechnologies for remediating heavy metal contamination in the soil ecosystem of El-Rahawy village. The remediation efficacy was assessed through a kinetic perspective, focusing on the reduction of heavy metals and their risks in contaminated soils.

Materials and Methods

Soil Samples

Surface soil samples (0–30 cm) were collected from both uncultivated (UC) and cultivated (C) areas in El-Rahawy village, Giza Governorate, Egypt (Figure 1). These soils had been subjected to prolonged irrigation with low-quality water comprising a mixture of raw sewage effluent, agricultural runoff, and industrial effluents discharged into the El-Rahawy drain (Table 2). Collected soil samples were air-dried at room temperature, crushed, sieved through a 2-mm sieve, and packed into experimental pots for subsequent treatments.

Clay minerals used in remediation

Bentonite, kaolinite and rock phosphate(RP) were purchased from El-Nasr Company for minerals.

Microbiological Methods

Growth Media

Microorganisms used in this study were cultivated in a Bioflo & Celligen fermentor/bioreactor, each in its specific growth medium, to achieve a concentration of 106 cfu /ml

Bacillus megatherium and *Thiobacillus thiooxidans* strains were obtained from National Research Centre, Cairo, Egypt. Phosphate-dissolving bacteria (*Bacillus megatherium*) were isolated and grown on phosphate-dissolving bacteria medium [14]. *Thiobacillus thiooxidans* was cultivated on modified Waksman medium [15].

Treatments

The study included the following treatments:

- UC: Un-cultivated control soil.
- CC: Control cultivated soil with tomato plants.
- T1: Bentonite + RP + *Bacillus megatherium*.
- T2: Bentonite + elemental sulfur + *Thiobacillus thiooxidans.*
- T3: Bentonite + elemental sulfur + RP + *Thiobacillus thiooxidans* + *Bacillus megatherium* .
- T4: Bentonite + kaolinite + *Bacillus megatherium* + RP + elemental sulfur + *Thiobacillus thiooxidans* .
- Specification of Natural Clay Minerals
- The physical and chemical properties of the natural clay minerals used in the treatments are detailed in table(1)

Soil Chemical Characterization

Soil chemical properties were determined following the protocol described by Sparks., [16]:

- pH: Measured using a glass electrode in a 1:2.5 soil-water suspension.
- Electrical Conductivity (EC): Measured in dS m-1 at 25°C in 1:5 soil water extract.
- Total inorganic pollutants (Inorganic pollutants): Determined using Atomic Absorption Spectrophotometry (AAS) on a Perkin-Elmer Model-2380 instrument as described by Cottenie *et al.*, [17].

Plant Analyses

Dried plant materials were wet-digested with 5 ml concentrated nitric acid and 2 ml 30% hydrogen peroxide at 125°C for 1 hour. This process was repeated three times to ensure clear digestion. The digests were filtered through cellulose filters (pore size 2.5 µm) and nitrocellulose syringe filters (pore size 0.45 µm), then diluted to a final volume of 20 ml. Samples were stored at 4°C and analyzed for Cu, Pb, and Zn using Atomic Absorption Spectrophotometry (AAS) with a Perkin-Elmer Model-2380 instrument. Analysis wavelengths were 324.752 nm for Cu, 220.353 nm for Pb, and 213.857 nm for Zn [17].

Soil Quality Criterion

The Zn Equivalent Model was used to quantify HMs toxicity levels based on the following equation:

Zn Equivalent (ppm) = (1×Zn) + (2×Cu) + (8×Ni). A quality criterion index exceeding 250 units indicated a high-risk scenario requiring remediation for sustainable farming management [18].

Table (1) Specification of natural clay minerals used in remediation of both drainage water and soils

Eelement	Results %	
	Kaolin	Natural Bentonite
Silicon dioxide(SiO_2)	50-56max	49-55%
Aluminum oxide(Al_2O_3)	30-33min	20-24%
Iron oxide (Fe_2O_3)	1.0-1.3	2.5-6%
Titanium dioxide (TiO_2)	1.3-1.8	-
Calcium oxide (CaO)	0.10-0.25	2-4%
Magnesium oxide(MgO)	0.05 -0.10	0.5-2%
Sodium oxide (Na_2O)	0.07-0.15	0-2.4%
Potassium oxide (K_2O)	0.03-0.06	1.2-14%
Chlorine(Cl)	<0.05	-
Loss in ignition(105°C-1000°C)	11-12	9-10%

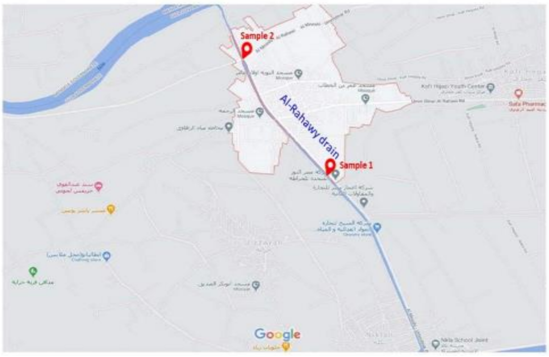

Figure (1) Locations of soil sample collected from El-Rahawy region(El-hadar Site)

Table (2) Inorganic pollutants content in El-Rahawy agricultural drainage and River Nile water compared to the safe levels

Water samples	Cd	Cu	Mn	Zn	Ni
	mg l^{-1}				
Safe Level	0.01	0.2	0.2	2.0	0.20
River Nile	0.00	0.01	0.00	0.01	0.00
El- Rahawy Drain	0.09	0.45	0.93	4.14	0.33

Table (3) Some physical, chemical and soil water relationship characterization of selected soil samples (Oven dry basis)

Land use	pH	EC dS.C m^{-1}	OM %	Depth Cm	Particle size distribution %						Water content (cm^3/cm^3)			HC cm/h
					Coarse sand	Fine sand	Total Sand	Silt	Clay	Text.	FC	WP	AW	
Common beans	8.16	2.1	1.96	0-30	1.3	13.4	14.7	34.1	51.2	Clay	0.44	0.3	0.15	0.25
	8.44	2.6	1.21	30-60	1.2	11.9	13.1	33.1	53.8	Clay	0.48	0.3	0.17	0.24

Table (4) Chemical characteristics of Rahawy soils as affected by low quality applied * (Oven dry basis)

Soil Depth cm	Period of sewage farming Years	Exchangeable Cations				ESP %	S. area	CEC	SAR
		Ca	Mg	Na	K				
0-30	>80	3.55	4.91	13.82	0.72	60.09	256	23	4.75
30-60		4.31	6.22	24.55	1.32	39.86	269	36.5	4.48

Table (5) Chemical characterization of El-Rahawy soil samples for their total Inorganic pollutants content (Oven dry basis).

Soil No.	Soil Depth cm	Period of farming Years	pervious land use	Cd	Cu	Fe	Mn	Pb	Zn	Ni	Zn equivalent Model
1	0-30	80	Tomato	6.40	13.00	163	6.00	16.00	180	9.50	282
	30-60			11.90	19.20	92	19.58	18.70	185	6.00	272

Statistical analyses

Each treatment was taken in three replicates; Standard deviation (SD) among the three replicates was calculated [19].

Results

Kinetics of Zn Desorption from tested Soil

Desorption of inorganic pollutants from soils is a critical process influencing their bioavailability and subsequent uptake by biota, which can lead to growth retardation and toxicity. Kinetic analysis of soil-Inorganic pollutants interactions provide valuable insights into sorption and desorption dynamics, aiding the understanding of pollutant behaviour in remediated soil ecosystems.

As shown in Figure (2), the results confirmed a variable desorption rate of Zn from soil, at three reaction periods, the 1st period, the 1st 30 min of starting reaction time was rapid, followed by the 2nd period, characterized by a decline in inorganic pollutants adsorption from clay minerals treated soils followed by 3rd stage characterized by almost steady-state conditions of pollutants desorption from soil ecosystem. Kinetic of Zn desorption was significantly influenced by the different tested remediation amendments applied. Growing tomato plants in polluted soil ecosystems significantly reduced Zn desorption rates by approximately 43% compared to the untreated control (UC), a statistically significant reduction according to standard deviation analysis. While this decrease mitigates Zn hazards in the soil ecosystem, it concurrently increases Zn accumulation in plant tissues, highlighting the importance of selecting effective remediation strategies.

Among the tested treatments, the application of bentonite and kaolinite clay minerals inoculated with *Bacillus megatherium* and *Thiobacillus thiooxidans* (T4), was the most effective, reducing Zn desorption by 94.5% compared to the untreated control (UC) (Figure 3). Treatments T2 (bentonite + elemental sulfur + *Thiobacillus thiooxidans*) and T3 (bentonite + elemental sulfur + RP + *Thiobacillus thiooxidans* + *Bacillus megatherium*) achieved Zn desorption reductions of 79.5% and 75.5%, respectively, relative to the UC treatment.

The hierarchy of treatment efficacy in minimizing Zn desorption from the amended soils was as follows:

T4 > T1 > T2 > T3.

Rate constants of the best fitted models describe Zn desorption from polluted soils as affected by remediation amendments

Results given in table (2) indicated that the values of coefficient of determination R2 were highly significant (at 0.01 level) for all the four kinetic equations, yet those for Elovich and modified Freundlich were higher (mostly 0.87**-0.99**) compared to (0.71**-0.96**) for the first-order and the diffusion equations. The SE values, yet, were in the order of modified Freundlich < first-order <Elovich< diffusion equations. This result emphasized that MFE was the best fitted model to describe the kinetic data followed by Elovich and for less extent 1st order and Diffusion model.

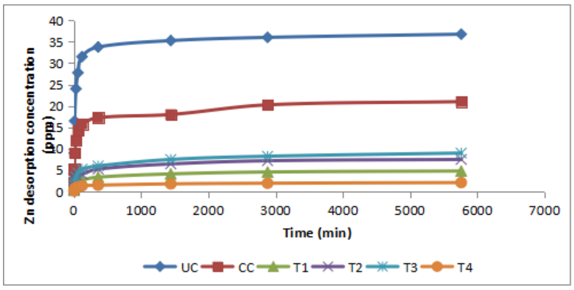

Figure (2) Kinetics of Zn desorption from polluted soil as affected by remediation materials applied

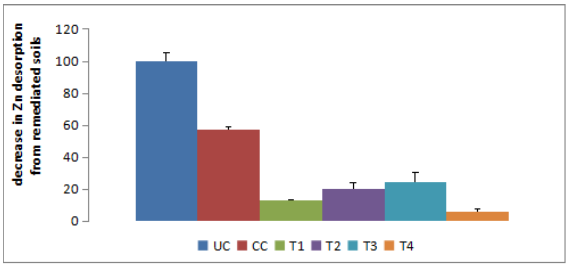

Figure (3) Percentage in Zn desorption from polluted soil as affected by remediation materials applied

The power function (modified Freundlich) equation in the linear form is:

Ln Ct = ln kd + b\ ln t. The integrated form is qt = kd t b\, where qt the amount of Zn release at time t, a and b are constants. Taking the derivation of integrated form:

$$dq / dt = a\ b\ t\ b^{-1} .$$

Where kd is directly proportional to the rate of Zn release and was considered as the apparent desorption rate coefficient. The effect of b\ on Zn release is more complex. The reaction rate is proportional to a only at t = 1 in which case:

$$\mathbf{dq /dt = ba.}$$

The b value is convenient to estimate of the initial release rate when comparisons are made between power function equations. It is, however, designated as the reversibly adsorbed Zinc.

The apparent desorption rate coefficient a and the initial release rate b, the slop and the intercept of the data plotted according to the linear form of the modified Freundlich equation used for evaluation of soil remediation indicated that remediation treatments applied significant influenced the desorption constant values. The a constant given in Table (6) is convenient to describe the amount of pollutant(s) desorption from treated soil [19] & [20]. Results in Table (6) imply that a constant decreased from 0.32 in UC to 0.28 mgkg^{-1}min^{-1}, this decrease represents the effect of the tomato plant on absorbing pollutant from polluted soil.

Table (6) Rate constants of best fitted models describe Zn release from polluted soil as affected by trailed remediation amendments

Modified Freundlich equation				
Treatments	a	b	R2	SE
UC	0.32	0.88	0.87**	0.16
CC	0.28	0.61	0.90**	0.14
T1	0.20	-0.01	0.99**	0.03
T2	0.24	0.04	0.98**	0.07
T3	0.25	0.09	0.97**	0.08
T4	0.10	-0.06	0.96**	0.04
Elovich equation				
UC	3.91	7.48	0.95**	4.03
CC	2.15	3.61	0.98**	1.48
T1	0.55	0.14	0.99**	0.11
T2	0.87	0.07	0.99**	0.21
T3	1.00	0.23	0.98**	0.27
T4	0.23	0.10	0.99**	0.05
Parabolic Diffusion equation				
UC	0.33	18.38	0.80**	8.83
CC	0.19	9.30	0.79**	4.13
T1	0.05	1.44	0.89**	0.75
T2	0.09	2.16	0.90**	1.16
T3	0.10	2.65	0.90**	1.34
T4	0.02	0.72	0.87**	0.35
1st order equation				
UC	4.9*	1.11	0.85**	0.32
CC	3.9	0.99	0.90**	0.21
T1	4.1	0.48	0.96**	0.12
T2	4.4	0.69	0.97**	0.11
T3	3.3	0.45	0.94**	0.13
T4	3.4	0.81	0.93**	0.14

Application of Bentonite, RP, Bacillus megatherium (PDB) (T1) significantly decreased the rate of Zn desorption from 0.32 in CC to 0.20 mgkg^{-1}min^{-1}. This decrease might relate to the two reactions that take place; the first related to the sorption of Zn by bentonite and the second related to the absorbing of Zn ions by RP mixed with PDB. Likely, the application of KB + MCC (T4) significantly decreased the rate constant to 0.10 mgkg^{-1}min^{-1}. However, application of Bentonite + Sulfur + MCC (T3) decreased the rate constant values but not higher than T1 or T4, reaching 0.24 and 0.25 mgkg^{-1}min^{-1}, respectively.

The applied remediation amendments influenced the capacity factor represented by the b constant of MFE and other kinetic models tested in the polluted soil ecosystem. Results in the table (6), for example, indicated that soil cultivated with tomato plants significantly decreased the capacity factor from 0.88 to 0.61 mg kg^{-1}. Yet, the application of bentonite incorporated with rock phosphate and Bacillus megatherium (T1), decreased the b constant value to -0.01 mg kg^{-1}, exhibiting the effect of remediation with modified clay minerals on Zn retention in the soil ecosystem. Nevertheless, the capacity factor highly decreased to -0.06 mg kg^{-1} in the soil treated with modified clay minerals amended with the MCC (T4). It should also be mention that the same constant had negative values for the same treatment in MFE model,which confirm that the treatment was the best in decreasing the rate of pollutant desorption from polluted soils.

Diffusion mechanism take place in pollutants desorption from the amended soil.

The rate of Zn release was studied through the determination of the diffusion rate coefficient a and the intercept parameter b which is presumably the quantity of pollutant in solution at the time of starting work [21] the slope and the intercept of the data plotted according to parabolic diffusion equation in the form:

$$q = b + at\ 0.5$$

Where q = the amount of ion desorbed in the time t; a = apparent diffusion rate coefficient a and b = constant.

The kinetic parameters a and b are represented for the remediated soil Table (6), indicated that the b value representing the quantity of Zn at zero time and the diffusion rate coefficient of Zn release a were affected by the tested remediation agents. Both the rate of Zn release a and the intensity factor b consistently decreased with application of remediation agents or even cultivation the polluted soil with tomato plants. Rate of Zn release a value decreased from 0.33 to 0.19 by cultivation of tomato and to 0.05 and 0.02 by application of T1 and T4, the best treatments applied. The same trend, however, was also reached in the 1st order model.

Kinetic of Cu desorption from polluted soil as affected by remediation additives

Copper is one of the most imperative pollutants existing in agricultural soil ecosystems exposed to industrial effluents and adversely impact human health.

Minimizing of such inorganic pollutant and its adverse hazard on soil ecosystems is now a commitment. Results drawn in figures (4and5) verify that growing tomato plants significantly decreased Cu release by about 23% from control soil. The application of T1 in polluted soil, led to decrease Cu from amended soils reaching 59% compared to UC soil. The combined treatment with a mixture of bentonite , sulfur and *Thiobacillus thiooxidans* mixture (T2), significantly increased the retention of Cu or decreased pollutant availability in treated soil up to 76.5% compared to UC treatment. T3 represents the worth of modified clay minerals in decreasing pollutants desorption from polluted soil ecosystems, Compared to all trailed treatments, the maximum decreasing of Cu desorption from polluted soil was attained in T4 reaching 77% under control.

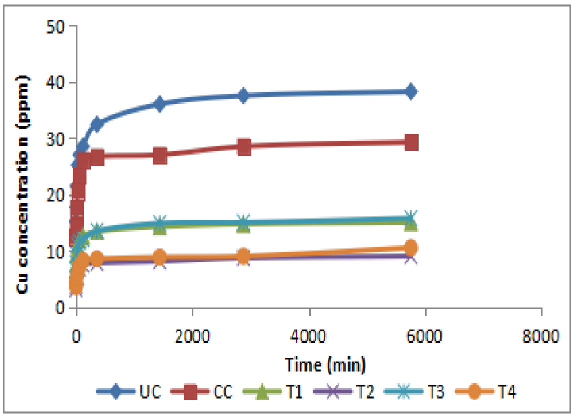

Figure (4) Kinetics of Cu Desorption from remediated soils as affected by remediation materials applied

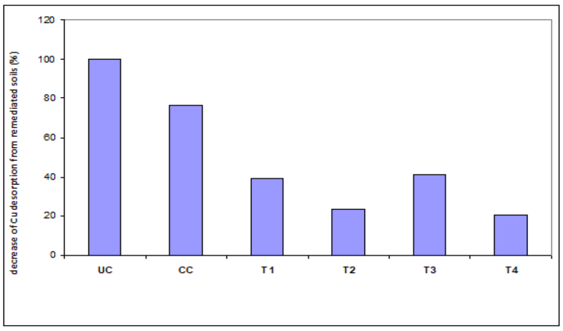

Figure (5) Percentage in Cu desorption from polluted soils as affected by different remediation materials applied

Kinetic parameters of Cu desorption from polluted soils as affected by trailed remediation amendments

According to the coefficient of determination R2values in table (7), again MFE, Elovich were the best fitted equations described the kinetic results, the numerical values ranged between 0.94**, 0.98** and 0.96**0.99** in above mentioned models, respectively. According to the standard error SE values, the decreasing order arranged as MFE (0.11-0.18), Elovich (0.98-2.71), means the priority of MFE compared to other models to be used in comparison study. Nevertheless, it should be mention that other models used also contribute in explaining the different mechanisms-controlled release phenomena. The rate constants of MFE represented the effect of low-quality water and treatments applied on the rate of release of Cu.

Table (7) Rate constants of best fitted models describe Cu release from polluted soil as affected by trailed remediation amendments

Modified Freundlich equation				
Treatments	a	b	R2	SE
UC	0.25	1.21	0.98**	0.03
CC	0.20	1.14	0.94**	0.05
T1	0.10	0.86	0.95**	0.04
T2	0.13	0.59	0.95**	0.05
T3	0.15	0.86	0.97**	0.03
T4	0.08	0.03	0.96**	0.05
Elovich equation				
UC	3.91	14.84	0.99**	0.88
CC	2.15	13.27	0.96**	1.78
T1	0.55	3.87	0.98**	0.66
T2	0.87	6.20	0.97**	0.51
T3	0.62	6.74	0.99**	0.42
T4	0.23	3.53	0.97**	0.56
Parabolic Diffusion equation				
UC	0.33	22.01	0.86**	4.49
CC	0.19	18.74	0.76**	4.19
T1	0.05	9.60	0.79**	1.97
T2	0.09	5.06	0.80**	1.34
T3	0.10	9.51	0.84**	1.80
T4	0.02	5.52	0.83**	1.37
1st order equation				
UC	4.90*	1.15	0.86**	3.15
CC	3.90	0.91	0.84**	2.26
T1	2.60	0.64	0.91**	2.21
T2	3.00	0.51	0.88**	2.21
T3	3.30	0.72	0.88**	3.20
T4	2.40	0.61	0.79**	2.19

Reading equal to a-10^-4

The constant a that is convenient to describe the rate of Cu desorption showed significant variations in cultivated CC and uncultivated UC soils. For example, the a values of Cu desorption decreased from 0.25 to 0.20 mg kg^{-1} soil min^{-1} in both El-Rahway uncultivated and cultivated soils, respectively. This result represents the uptake and retention of this pollutant in tomato fruits produced from these soils. Although the same trend was observed in other treatments, results showed that the rate of Cu desorption and uptake in soils treated with T1 and T4 was lower than CC value reached to 0.10 and 0.08 mg kg^{-1} min^{-1}. However, the treatments of polluted soil with T2 and T3 decreased the pollutant concentrations but less than T4 with numerical values 0.13 and 0.15 mg kg^{-1} min^{-1}, respectively.
The variations between treatments applied to minimize the release of Cu from the studied soils were more pronounced through the Elovich model. The same table showed an increase in a constant reaching 14.84 mg $kg^{-1}min^{-1}$ in uncultivated soil UC, decreased to 13.27 mg $kg^{-1}min^{-1}$ in the cultivated soil CC. The treatments T1 and T4 applied reduced the rate of Cu desorption to 3.87 and 3.53 mg $kg^{-1}min^{-1}$ to record the lowest values compared to other treatments. Although the capacity factor b given in the same table showed an increase in the soils treated with T2 and T3 compared to T4, the uncultivated soil UC and cultivated one CC were significantly higher than those treatments, i.e.T2 and T3. The other models used to describe the kinetics of Cu almost showed the same trend in increasing the release of Cu in uncultivated soil over other trailed treatments. Also, T4 was the best treatment in minimizing Cu desorption from remediated soil.

Kinetics of Ni desorption from polluted soil as affected by remediation amendments

The results plotted in Figures (6 and 7) show rapid desorption of Ni from the treated soil ecosystem. The rate of desorption of pollutant is divided into three periods of chemical reaction: The first period is characterized by a rapid reaction rate spanning 30 minutes, and the second period is characterized by a decrease in the desorption of contaminant from treated and untreated soil. However, the third period was characterized by steady-state conditions for nickel desorption and persisted during the rest of the reaction time [19].
The kinetic of Ni desorption was significantly influenced by different applied remediation amendments. Cultivation of tomato plants in the polluted soil ecosystem decreased Ni desorption by about 26% compared to UC. According to standard deviation, this result significantly minimizes the hazard of Ni in soil. Here lies the source of danger in growing crops whose fruits are edible because they absorb inorganic pollutants without any detention for those harmful metals.
Concerning the treatments applied, in general, all tested treatments or even cultivating the soil with tomatoes significantly decreased the rate of pollutants desorption from polluted soil.
The application of Bentonite and RP mixed with PDB (T1) decreased Ni desorption 76% compared to UC. However, (T4) was the best treatment in decreasing Ni availability in remediated soil with percentage equal to 82% under uncultivated control.

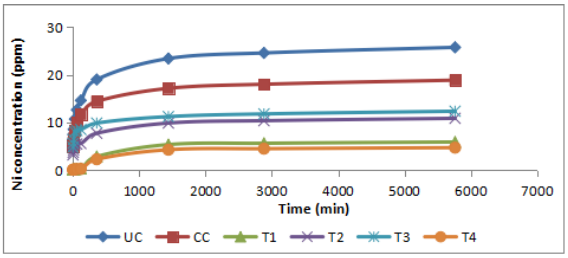

Figure (6) Kinetics of Ni desorption from remediated soils as affected by applied remediation materials

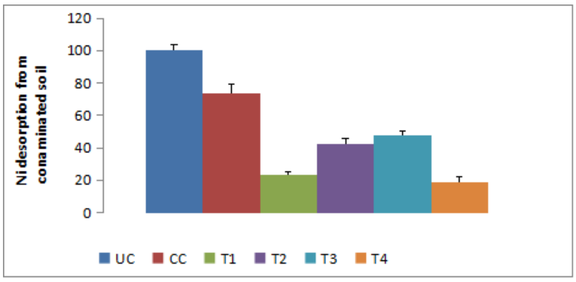

Figure (7) percentage in Ni desorption from polluted soils as affected by different applied remediation materials applied.

The coefficient of determination R2 represented in Table (8) showed that MFE was the best-fitted model to describe the kinetic values compared to other models used. The numerical values calculated for MFE ranged between 0.90** and 0.99** for different treatments applied in the El-Rahawy soil ecosystem. Although the same trend was reached in Elovich and 1st order, MFE is still the best since it has the lowest SE values compared to other models. The values of SE in MFE ranged between 0.03-0.97. Meanwhile, it was 1.14-3.16 in the 1st order. The Elovich kinetic model becomes the 2nd priority in describing the kinetic data.
The Elovich model will be compared to different treatments applied since it gives a significant coefficient of determination R2. The succession of more than one model in describing the kinetic results means that the present mechanisms in the sorption of contaminants found in the soil system and the type of completion between the different pollutants are located in the studied soils. The rate constant of MFE represented in the same Table showed wide variations between cultivated and uncultivated soils in the release of the studied pollutant. The values of a constant that is convenient to describe the rate of Ni release were 4.18 decreased o 2.17 mg kg^{-1}soi $lmin^{-1}$ in both uncultivated and cultivated soils, respectively.
The increasing intensity factor of Al-Rahawy soils might be related to the fact that these soils were exposed to low-quality irrigation for over 80 years. Besides that, these soils have hardpans at about 40-50 cm depth, increasing the capacity and intensity factors for the study of different contaminants; results of the capacity factor emphasize this fact.
Results given in the same Table (8) showed that the capacity factor b constant values were 5.78 mg kg-1soil min-1in uncultivated soil. Applying T1 and T4 decreased rate constant values to -1.54 and -1.23 mg $kg^{-1}min^{-1}$, respectively.

Elovich equation values calculated for the same pollutant in remediated soil gave a significant trend in describing decrease of Ni bioavailability in the studied soils. Results in the same table showed that the constant a values decreased from 2.53 to 1.80 mg $kg^{-1}min^{-1}$ by cultivation tomatoes in polluted soil, this narrow decrease represents the decreasing of bioavailable form in soil.This result perhaps is emphasized in T1 and T4 for having minus values i.e. -1.54 and -1.23 in these treatments respectively.

Table (8) Rate constants of best fitted models describe Ni release from polluted soil as affected by trailed remediation amendments

Modified Freundlich equation				
Treatments	a	b	R2	SE
UC	4.18	5.78	0.99**	0.03
CC	2.17	4.69	0.99**	0.03
T1	1.85	-1.54	0.90**	0.21
T2	2.23	2.23	0.97**	0.69
T3	2.08	3.59	0.99**	0.27
T4	1.48	-1.23	0.90**	0.97
Elovich equation				
UC	2.53	3.71	0.98**	1.44
CC	1.80	3.33	0.99**	0.88
T1	0.80	-1.54	0.90**	1.21
T2	0.97	1.23	0.97**	0.69
T3	0.90	2.59	0.99**	0.27
T4	0.64	-1.23	0.90**	0.97
Parabolic Diffusion equation				
UC	0.26	9.64	0.92**	3.11
CC	0.18	7.64	0.90**	2.37
T1	0.09	0.11	0.94**	0.93
T2	0.10	4.44	0.93**	1.08
T3	0.09	6.79	0.89**	1.26
T4	0.08	0.09	0.94**	0.74
1st order equation				
UC	4.2*	1.16	0.96**	2.13
CC	4.1	1.00	0.95**	1.14
T1	4.00	0.74	0.97**	3.16
T2	3.90	0.78	0.97**	2.11
T3	3.65	0.69	0.94**	3.13
T4	4.10	0.64	0.97**	1.18

* Reading equal to a*-10^4 **: significant at 0.01 levels

Furthermore, increasing the rate constant a values of bentonite and sulfure incorporated with the *Acidithiobacillus,* T2 or modified bentonite mixed with the *Bacillus megatherium* + RP and *Thiobacillus thiooxidans* + sulfure (T3) could be due to the ability of these materials to increase bioavailability of Ni in such soil. This result could be trusted through the increasing of capacity factor vales of these treatments.

FOOD SAFETY

A greenhouse experiment was done to investigate inorganic pollutants concentrations in the edible parts of fresh tomatoes as a common indicator for food safety. The concentrations of inorganic pollutants in editable parts are directly proportional to their uptake by grown plants that introduce to humans (Table 9). All tested treatments exhibited high potential in reduction of tested elements and reduced their translocation to tomatoes fruit except for T1, in case of Zn, it improved their translocation from soil (9.75, 9.85 mg kg^{-1}), compared to cultivated plant control. It was noted that the best treatment was T4, it exhibited the highest reduction in uptake of the tested elements from soil. Worthy to state that cadmium concentration was undetectable in the tested soil.

Table (9) Inorganic pollutants concentration in tomato fruits grown in El-Rahawy soil under different remediated and non-remediated inputs in a green-house experiment

Treatments	Metals (mg kg^{-1})			
	Zinc (Zn)	Nickel (Ni)	Copper (Cu)	Cadmium (Cd)
UC	-	-	-	< d.l.
CC	9.75	2.23	1.48	< d.l.
T1	9.85	1.20	0.69	< d.l.
T2	8.82	0.78	0.511	< d.l.
T3	5.06	0.14	0.297	< d.l.
T4	5.54	nd	0.25	< d.l.

< d.l.: less than detected level nd: not detected

Discussion

The persistence and co-existence of heavy metals (HMs) in both soil and aquatic ecosystems represent a significant environmental challenge, as these pollutants can undergo biomagnification and accumulate in the biomass, leading to serious ecological and health risks.

Zinc equivalent represented by the equation "Zn concentration×1 + Cu concentration×2 + Ni concentration×8". Zinc equivalent values more than 250, the critical value, reached to 282 and 272 in surface and subsurface layers, which represent actual hazard situation of HMs on selected soil ecosystem [18].Cultivation of tomatoes in untreated contaminated soil decreased the rate constants of the intensity factor a. Results in Table (8) showed that the rate of Ni desorption decreased from 4.18 in UC to 2.17 mg kg^{-1} min^{-1} in CC, meaning that toxic pollutants can easily uptake by cultivated plants in polluted soils, the same trend was observed in other studied pollutants which was confirmed by decreasing the capacity factor b in MFE model.

The application of T1 (Bentonite+ RP + *Bacillus megatherium*) to contaminated soil cultivated with tomatoes significantly decreased the rate of pollutants desorption. According to MFE, the best fitted model, results showed that the rate of Zn desorption decreased from 0.32 to 0.20 mg kg^{-1} min^{-1} for Zn, from 0.25 to 0.10 mg kg^{-1} min^{-1} for Cu and from 4.18 to 1.85 mg kg^{-1} min^{-1} for Ni compared to UC soil. Asaad *et al.,* [22] stated that natural and modified clay minerals had been investigated as adsorbents for alleviating toxicity of various toxic and hazardous contaminants of major concern to the environment. Immobilization of inorganic pollutants ions by RP reduced their solubility in soil ecosystems as well as their availability to plant uptake and consequently minimized their hazards on soil biomass [23].

The application of Bentonite or kaolinite clay minerals for inorganic pollutants remediation are common approaches [18]. Modified clay minerals applied in polluted soils can significantly decreased inorganic pollutants by different mechanisms i.e. sorption which involving adsorption, ion exchange etc… In addition, decreasing of soil pH enhances inorganic pollutants availability to be sorbed by applied clay minerals. Moreover, it is well known that specific adsorption brings about strong and irreversible binding of inorganic pollutants ions to organic matter or variable charged materials, while non-specific adsorption is an electrostatic phenomenon in which cations from the pore water are exchanged for cations near the surface. Cation exchange is a form of outer-sphere complexation with only weak covalent bonding between inorganic pollutants and charged soil surfaces. It is a reversible process in nature and occurs rather quickly, as is typical for reactions that are diffusion-controlled and electrostatic [24].

Bentonite is an aluminium Phyllosilicate mineral. It is basically composed of Montmorillonite (Smectite) with other clays and inorganic minerals. Types of bentonite depend on their dominant cations (K, Na, Ca and Al) (Table 1). In this work, the commercial bentonite was modified by RP and PDB to strengthening the ability of the compound to retain the studied inorganic pollutants, [24] and [25]. In addition, results showed that the application of T1 also significantly decrease the capacity factor (b) as shown in different models tested for different tested elements. According to parabolic diffusion model, as an example, the rate of diffusion for Cu significantly decreased from 0.55 to 0.33 mg kg^{-1} min^{-1}, the same trend was observed for other tested elements. Consistent with our findings Rajkumar *et al*., [26] proved that inoculation various plants with Bacillus megatherium SR28C reduced translocation of Ni from roots to shoots compared with the control

Park *et al*., [27] mentioned that different scenarios had been proposed for the phosphate-induced immobilization of inorganic pollutants including direct adsorption by P compounds, phosphate anion-induced metal adsorption, direct precipitation of inorganic pollutants with P in solution as metal phosphates. The precipitation through the liming action of RP led to a heavy metal fixation in the soil ecosystem .Other mechanism by solubilization of insoluble and biologically unavailable inorganic pollutants by secretion of low molecular weight organic acids with chelation properties. Such acids have chelating cations such as Ca, bound to phosphate in case of RP addition through their carboxyl and hydroxyl groups or via solubilize them though proton liberation, leading to their transformation from insoluble phosphate to a soluble one. Schalk *et al*., [28] mentioned that phosphate solubilizing bacteria chelated several inorganic pollutants such as As, Cd, Ni and Zn with variable affinities.

Bacillus megatherium & *Thiobacillus thiooxidans* are habitually used microbial tools for mitigation inorganic pollutants toxicity and are well known as plant growth promoting bacteria (PGPR) [10] and [11].

Microbial reduction of inorganic pollutants might be linked with their transformation to become either less toxic, easily volatilized, more water soluble and thus could be removed by leaching, less water soluble that allows them to precipitate and become easily removed from the soil ecosystem or being less bioavailable [29].

Application of Bentonite enhanced with elemental sulfur and *Thiobacillus thiooxidans* (T2) was also tested to evaluate the decreasing of media pH by S on increasing the efficiency of bentonite to retain inorganic pollutants. In MFE, the application of T2 significantly decreased the rate of Zn desorption from 0.32 in UC to 0.24 mg kg^{-1} min^{-1}, however, this decrease was higher than T1 (0.20 mg kg^{-1} min^{-1}), this result may due to increasing the rate of pollutant desorption with decreasing of soil pH.

Increasing the rate of pollutants release in T2 and T3 compared to T1 could be mainly due to decrease the pH of the soil media for using elemental S. Shaheen *et al*., [30] mentioned that Sulfur is of great agro-environmental concern, acidifying elemental sulfur significantly reduces plant growth-restricting alkalinity, and decreases soil pH considerably. Application of S in T2 plus Thiobacillus thiooxidans in polluted soil amended with modified bentonite increased the rate of pollutants found in soils compared to T1, but still significantly less than UC or even CC.

The 4^{th} treatment T4 represents the mixture of all above treatments beside Kaolinite clay minerals. Results indicated that T4 is the best treatment and it could be the best management practice in minimizing the hazards of studied inorganic pollutants. Coles and Yong [31] mentioned that treated polluted soils with Kaolinite achieved 100% adsorption.

Zinc is an important element in regulating the immune system in the human body. Alexander *et al*., [32] stated that zinc deficiency in the human diet has many devastating effects. However, the very high concentration of zinc in vegetables may lead to vomiting, convulsions, and kidney disorders. Shehata *et al*., [33] evaluated the concentration of HMs pollutants in some vegetables irrigated with wastewater in Morocco and demonstrated that despite the absence of cadmium in tomato fruits, zinc, copper and nickel are present at concentrations of 26.07, 7.97 and 56.93 mg/kg respectively, which are relatively higher than those in our findings. Also, Elbagermi, *et al*., [34] monitored the content of HMs in some fruits and vegetables collected from different market locations in Libya; they found that tomatoes contained 8.427, 2.245, 0.20 and 0.250 mg/kg of zinc, copper, nickel and cadmium, consistent with our findings except for cadmium. Chowdhury *et al*., [35] studied the accumulation of inorganic pollutants in tomatoes and cabbage fruits grown in some industrially contaminated soil ecosystems in Bangladesh; they recorded different concentrations of inorganic pollutants in tomatoes and found them varied depending on the sample location with the highest value of 8.91, 7.22, 2.88, 3.38 and 419.6 mg/kg for Ni, Cr, Cd, Cu and Fe respectively. It should be mentioned that these values are relatively higher than our findings about Ni, Cu and Cd. Wang *et al*., [36] stated that remediation of a given soil from inorganic pollutants provides safe food production and evading the health problems associated with their high contamination. Vegetables are one of the most important edible crops and are indispensable in the human diet.

Unuabonah *et al*., [25] investigated the amount of time for inorganic pollutants to be adsorbed by modified kaolinite and indicated that adsorption improved with increased contact time (residence time) and pH between 4 and 7. Modified kaolinite was found to adsorb up to eight times more than unmodified kaolinite. Madhaiyan *et al*., [37] reported an increase in tomato plant growth after reducing the accumulation level of Cd and Ni in their shoot and root tissues following inoculation with Methylobacterium oryzae and Burkholderia sp. The combined use of both microorganisms and plants in the bioremediation of polluted soil ecosystems resulted in a faster and more efficient clean-up of the polluted soil ecosystems [38].

Conclusion

The findings of this work demonstrate the significant potential of combining modified bentonite, elemental sulfur, rock phosphate, and phosphate-dissolving bacteria (PDB) in reducing the bioavailability and desorption rates of inorganic pollutants in contaminated soils. Treatments such as T1 (bentonite, rock phosphate, and PDB) and T4 (a combination of all treatments plus kaolinite) effectively minimized the hazards of heavy metals, as evidenced by the substantial decrease in pollutant desorption and bioavailability, especially for zinc, copper, and nickel. The inclusion of microbial tools like *Thiobacillus thiooxidans* and *Bacillus megatherium* further enhanced the efficiency of the treatments, promoting the transformation of inorganic pollutants into less toxic forms.

The study highlights the importance of soil pH adjustment, through the application of elemental sulfur, in influencing the release and desorption of contaminants, although it also indicates that excessive acidification may lead to increased pollutant release under certain conditions. Overall, the results suggest that the combination of mineral amendments and biological agents offers a promising, cost-effective approach to the remediation of contaminated soils, contributing to improved environmental health and safer agricultural practices. Further studies should explore the long-term effectiveness and environmental impact of these treatments, as well as their applicability to other types of contaminated ecosystems.

Abbreviations:

CC: Control cultivated soil
UC: uncultivated Control soil
KB: Kaolinite & Bentonite
RP: Rock Phosphate
PDB: Phosphate Dissolving Bacteria
PGRP: Plant Growth-promoting Rhizobacteria
MFE: Modified Freundlich Equation
**: Statistically significant at 0.01 level
*: Statistically significant at 0.05 level or, in other cases, indicates a multiplication sign according to its position in equation(s).
MCC: Mixed Culture Consortium (*Bacillus megatherium &Thiobacillus thiooxidans)*

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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