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Original Article

The effect of application of electrokinetic and chelating agents on substrate induced respiration and bacterial and fungal populations in a multi-metal contaminated soil

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ABSTRACT

Application of combination of electrokinetic and chelating agents as a method of soil decontamination is an emerging technique recently. However there is not enough study about their effects on soil microbial activity. The current study investigated the effects of this new method on soil microbial activity to brighten its biological aspects. A multiple metal contaminated soil was treated by EDTA as a chemical chelator, cow manure extract (CME) and poultry manure extract (PME) as organic chelators (2 g kg-1) after 30 days irrigation in pots. Two weeks later the soils were treated by four oriented center electrical fields in each pot (0, 10 and 30 volts) for an hour per day during 14 days. Soil bacterial and fungal populations and substrate induced respiration (SIR) were analyzed at the end of the experiment in the soils around the cathodes and anodes separately. Result indicated that application of electrical fields had harmful effects on the bacterial and fungal populations and reduced SIR in both cathodic and anodic soils. Increasing the intensity of electrical field could increase its negative impacts. Application of EDTA catastrophically affected the analyzed indices in both cathodic and anodic soils. But utilization of PME or CME was a friendlier option in soil remediation techniques.

KAYWORDS: Electrokinetic, chelating agents, biological properties, soil

INTRODUCTION

Long-term intensive industrial activity, mining and smelting, certain agricultural activities and inappropriate waste disposal have led to significant soil pollution. An important issue is their increased presence in soil as they have adverse impacts on human and environmental health [1]. Increasing the concentrations of heavy metals in soil can severely reduce the growth and survival of soil microorganisms thereby affecting many ecosystem functions driven by these organisms [2, 3]. Microbial activities are certainly affected by accumulation of pollutants in soil. Although low concentrations of specific transition metals such as Co, Cu, Ni, and Zn are essential for many cellular processes of bacteria, these metals are often cytotoxic in higher concentrations. Not only other heavy metals including Pb, Cd, Hg, Ag and Cr don not have beneficial effects but also have toxic effects even at low concentrations [4].

In order to decrease the negative effects of heavy metal pollution, a variety of technologies have been developed during the last decade. In spite of the number of decontamination technologies, most of the physico-chemical techniques have their own adverse impacts on soil quality. Of the various decontamination technologies, electrokinetic method recently attracted scientists' interest [5].

Briefly when a direct current (DC) electrical field is applied to a polluted soil, migration of charged ions is occurred. Cationic heavy metals migrate toward cathode, and anions migrate toward anode by electromigration. Electro-osmosis flow is responsible for translocation of non-ionic species during an electrokinetic remediation of soil. Changing the soil pH in an electrokinetic process is inevitable whereas H+ and OH- ions are generated near anodes and cathodes respectively [6]. In addition several changes in different soil properties, such as redox potential and electrolyte concentration, are would be occurred as results of applying an electrical field in soil [7].

Operating of some chemical chelating agents is also usual to enhance electrokinetic decontamination's efficiency [8, 9]. One of the most common and effective chelating agents is ethylene-diamine-tetra-acetic acid (EDTA) which is reported to have

shown some undesirable impacts on soil microbial activity [10] attributed to solubilizing the heavy metals in soil [11], as increasing the availability of heavy metals in soil reduces activity of microorganisms [12]. Besides using the chemical chelator, especially EDTA, some of scientist had applied some organic chelating agents to enhance the efficiency of their remediation technique [13]. Considering that fresh residues of manures contain soluble organic compounds, they can increase the availability of metals gradually after application in soil [14]. Organic compounds also provide a significant portion of the beneficial bacteria's energy in soil thus addition of the manures makes a favorable habitat for microorganisms, increases their functions and leads the metals to be bioavailable in different ways [15].
Although some researchers have investigated the biological effects of electrokinetic method and chelating agents [16, 17, 18, 19], they have not represented a bright perspective of these techniques in terms of its biological effects. In order for describing the condition of microbial communities in soil, it is common to analyze soil biological factors such as: soil microbial respiration, microbial biomass, and microbial numeration [20]. In this study an electrokinetic system in collaboration with some organic and chemical chelating agents was investigated. The objective of the current study was to assess and brighten the effects of electrokinetic decontamination on soil biological population and activity.

MATERIALS AND METHODS

PRIMARY ANALYSIS OF SOIL

The study was conducted as a pot experiment. A multi-metal contaminated soil was selected for the experiment. Soil sampled from 0-30 cm layer of the Āhangarān Lead Mine (13˚ 48′ 30" E and 56˚ 34′ 0" N), in the southern-east of Malayer city, Hamadan, Iran. Properties of the selected soil were determined according to "Methods of Soil Analysis" published by SSSA [21, 22]. Soil texture was loamy and soil pH, EC, OC, ECC and CEC were 7.7, 580.65 µs cm-1, 0.59 %, 22% and 14.24 cmolc (+) kg-1 respectively. Total contents of soil Pb were extracted by concentrated and hot nitric acid. The soluble-exchangeable, organic-bound and carbonate-bound fractions of Pb were extracted by the Sposito et al. [23] method. The extracted heavy metals were analyzed by atomic absorption spectrometry on a Varian 220 instrument. Soil total Pb, carbonate-bound, organic-bound and soluble-exchangeable fractions were 1220.72, 621.41, 45.43 and 23.33 mg kg-1 respectively.

SOIL BIOLOGICAL ANALYSIS

Soil bacteria and fungi were measured based on colony counting method on nutrient agar (NA) and potato dextrose agar (PDA) media respectively [24]. Soil substrate induced respiration (SIR) was determined by addition of glucose, KH2PO4 and NH4Cl to the soil at field capacity and measured by alkali (Na(OH)2 + BaCl2) absorption of the CO2 released in 72 h [24], followed by titration of the residual OH- ions with standardized hydrochloric acid after adding three drops of phenolphthalein as an indicator, as reported by Isermayer [25]. Three replicates of each sample were tested. Data are expressed as mg CO2 g-1 dry soil day-1.
Bacterial and fungal populations of the primary soil were 8.11 and 7.55 log of CFUs g soil-1 respectively. Primary substrate induced respiration (SIR) was 0.9 mg CO2 g-1 dry soil day-1.

EXPERIMENTAL SETUP

A multiple anode system was utilized in this study. The polluted soil (7.0 kg dry soil) was put into plastic pots with 22 cm in diameter and 20 cm in height. Four graphite electrodes (1cm×1cm×15cm) were placed around the pots as anodes and a central electrode was used as cathode (figure. 1-A) so that, four oriented center electrical fields were made in each pots (figure. 1-B).

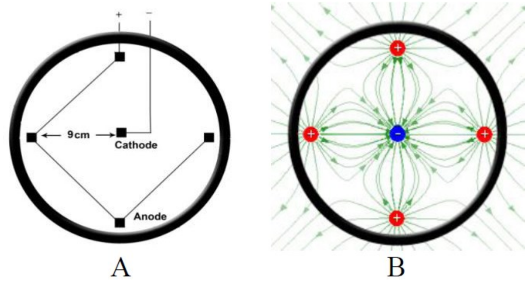

Fig. 1. A- Arrangement multiple anode system used in this study. B- electrical fields' lines.

The arrangement of the electrodes was designed based on an electrokinetic remediation experiment (data not shown) conducted by Tahmasbian Gahfarokhi [9]. After 30 days of irrigation of the pots in glasshouse condition, Ethylene-diamine-tetra-acetic acid (EDTA) as a chemical chelator, cow manure extract (CME) and poultry manure extract (PME) as organic chelators were applied (2g kg-1) to simulate a decontamination system of soil [26, 27]. EDTA was purchased in reagent grade form Sigma Chemical. Cow manure extract (CME) and poultry manure extract (PME) were prepared after shaking (120 rounds per min for 20 min), centrifuging and then filtering of a 1:5 manure/distilled water suspension, [26]. Cow manure extract and poultry manure extract's pH were 7.72 and 8.2 respectively. The total solid of CME and PME were measured for calculating the required amounts of amendment using for soil treatments (2g kg-1 soil). Two weeks after treating with chelating agents, soil was treated with DC electricity (0, 10 and 30 V). Electrical treatments were applied for an hour per day for 14 days [27]. After that, 11 cm diameter (half of pot's diameter) of the soil around the cathode was collected and

labeled as cathodic soil. The remained soil in the pots, around the anodes, was labeled as anodic soil.
Fresh cathodic and anodic soil samples were stored at 4 oC for microbiological analyses. Soil Substrate induced respiration (SIR), heterotrophic bacteria and fungal populations were determined by the mentioned methods.

STATISTICAL ANALYSIS

A completely randomized was designed as factorial for this experiment in three replicates. The factors were chelating agents (no chelator, EDTA, CME and PME) and electrical fields (0, 10 and 30 volts). Analysis of variance (ANOVA) was used to determine the significance of the effects of chelating agents and electrical fields on soil biological factors in cathodic and anodic soils in comparison with control. Duncan's new multiple range tests were performed to assess the effect of treatments on analyzed indices in anodic and cathodic soils. Analyzing of data was done by Ms-Excel and SAS 6.12.

RESULTS AND DISCUSSION

Analysis of variance (table 1) indicated that population of fungi and substrate induced respiration (SIR) significantly affected by application of electrical fields in the vicinity of cathode while bacterial population was not affected. Using the chelating agents and interaction of them with electrical fields were obviously effective to alter all analyzed indices in cathodic soil. However SIR was not affected significantly with the interaction between treatments.
In soil around anodes the population of bacteria was only affected by application of chelating agents. While both of the treatments and also their interaction had significant effects on fungal population and SIR in soil in the vicinity of anodes.

Table 1. Analysis of variance of the effects of electrical fields and chelating agents on the populations of bacteria and fungi and SIR in cathodic and anodic soils

		Cathodic soil			Anodic soil		
	DF	Bacteria	Fungi	SIR	Bacteria	Fungi	SIR
Electricity	2	0.05 ns	0.57 **	0.59 **	0.21 ns	0.22 **	0.92 **
Chelators	3	0.99 **	0.26 **	0.49 **	0.37 **	2.62 **	0.58 **
Interaction	6	0.14 **	0.09 **	0.06 ns	0.07 ns	0.28 **	0.05 **
Error	24	0.04	0.01	0.03	0.07	0.01	0.01

*: significant impacts at the 0.05; **: significant impacts at the 0.01; ns: not significant; SIR: substrate induced respiration.

SOIL BIOLOGICAL PROPERTIES AROUND THE CATHOD

As the interaction effects of electrical fields and chelating agents was significant, means of each treatments were not discussed separately. Results (table 2) showed that the highest value of bacterial population (8.45± 0.15 log of CFUs g-1 soil) was found in no voltage-PME and the lowest one (7.33± 0.09 log of CFUs g-1 soil) was found in 10 V-EDTA without any significant difference with no voltage-EDTA. In general bacterial population was markedly low in application of electrical field compared to that in control. On the other hand, bacterial population was markedly higher in application of PME and CME and it was lower in application of EDTA compared to that obtained in control. Founding the lowest value in EDTA was predictable because of the solubilizing of heavy metals by this chelator and its negative effect on soil microbial activity [11, 10]. However, being in the vicinity of a cathode with a strong electric potential (30 V) might cause the solubilized heavy metals to precipitate and therefore its negative effects was reduced in 30 V-EDTA. PME and CME treated soil have a better condition in comparison with EDTA in all levels of electrical fields; although, the number of bacteria reduced by application of the electrical fields. This implies that cathode might have undesirable effects on bacterial population.
The means of fungal population in cathodic soil were compared (table 2). Decreasing the number of fungi by increasing the electrical potential in all treatments of this experiment could be an acceptable reason for the negative effect of cathode on fungal enumeration which confirmed the obtained results for bacteria. The maximum value of fungal population (7.89±0.02 log of CFUs g-1 soil) was found in no voltage-PME followed by no voltage-control (7.83±0.16 log of CFUs g-1 soil). On the other hand, the minimum value (6.86±0.06 log of CFUs g-1 soil) was obtained in 30 V-EDTA confirming that the present of a cathode could intensify the negative effect of EDTA on fungal population.

Table 2. The comparison of means of bacterial and fungal populations (log of CFUS g-1 soil) in cathodic soil in application of chelating agents and electrical fields.

		No voltage	10 V	30 V
Bacteria	CME	8.35 (±0.26)ab	8.21 (±0.02)abc	7.99 (±0.09)bcd
	PME	8.45 (±0.15) a	8.05 (±0.04)bcd	8.23 (±0.09)abc
	EDTA	7.39 (±0.11) e	7.33 (±0.09) e	7.83 (±0.27) d
	Control	8.07 (±0.43)bcd	8.16 (±0.07)abcd	7.94 (±0.23) cd
Fungi	CME	7.45 (±0.02) b	7.47 (±0.18) b	7.27 (±0.10) c
	PME	7.89 (±0.02) b	7.36 (±0.02) bc	7.27 (±0.04) c
	EDTA	7.26 (±0.04) c	7.18 (±0.11) c	6.86 (±0.06) d
	Control	7.83 (±0.16) a	7.19 (±0.02) c	7.29 (±0.10) bc

CME: cow manure extract. PME: poultry manure extract. Values with different letters show significant differences at the 0.05 probability level.

Although there was not an effective interaction between chelating agents and electrical fields, both of the treatments impacted SIR in cathodic soil (table 1). Existence of cathode in soil markedly decreased SIR from 1.24±0.34 mg CO2 g-1 dry soil in no-voltage to 1.05±0.33 mg CO2 g-1 dry soil in 10 V and 0.81±0.13 mg CO2 g-1 dry soil in 30 V (table 3), which showed that increasing the voltage intensified the negative effects of cathode in this study.

Table 3. The comparison of means of SIR (mg CO2 g-1 dry soil day-1) in cathodic soil in application of chelating agents and electrical fields.

Chelators	SIR	Electrical fields	SIR
CME	1.17 (±0.27) ab	No voltage	1.24 (±0.34) a
PME	1.24 (±0.42) a	10 V	1.05 (±0.33) b
EDTA	0.73 (±0.12) c	30 V	0.81 (±0.13) c
Control	1.02 (±0.23) b		

CME: cow manure extract. PME: poultry manure extract. SIR: substrate induced respiration. Values with different letters show significant differences at the 0.05 probability level.

Same as bacterial and fungal population, soil SIR was markedly higher in application of PME and CME and it was lower in application of EDTA compared to that obtained in control. The amount of SIR reached the maximum level in PME (1.24±0.42 mg CO2 g-1 dry soil day-1) and the minimum level in EDTA (0.73±0.12 mg CO2 g-1 dry soil day-1). There was no significant difference between CME (1.17±0.27 mg CO2 g-1 dry soil day-1) and control (1.02±0.23 mg CO2 g-1 dry soil day-1).

SOIL BIOLOGICAL PROPERTIES AROUND THE ANODES

Analysis of variance (table 1) depicted that existence of the anodes in soil did not have any significant impacts on bacterial population of soil while application of chelators significantly affected the number of bacteria in anodic soil. Application of EDTA significantly reduced the population of bacteria in the anodic soil to 7.59 ± 0.18 log of CFUs g-1 soil in comparison with control (7.93 ± 0.26 log of CFUs g-1 soil). Although bacterial population in CME (7.94 ± 0.29 log of CFUs g-1 soil) and PME (8.06 ± 0.38 log of CFUs g-1 soil) were significantly higher than EDTA, they did not have any significant difference with control (table 4).

Table 4. The comparison of means of bacterial population (log of CFUs g-1 soil) in anodic soil.

Chelator	Bacterial population
CME	7.94 (±0.29) a
PME	8.06 (±0.38) a
EDTA	7.59 (±0.18) b
Control	7.93 (±0.26) a

CME: cow manure extract. PME: poultry manure extract. Values with different letters show significant differences at the 0.05 probability level.

The population of fungi in all treatments markedly decreased in anodic soil compared to that in control (table 5). Like the previous results, lower amounts of fungal population were counted in EDTA treatment and their values decreased with increasing the intensity of electrical field. Likewise, Lear et al. [16] reported that fungal population decreased in the anodic soil. However, in control soil (with no chelating agents), a significant increase was observed in application of the electrical fields.

Table 5. The comparison of means of fungal population (log of CFUs g-1 soil) in anodic soil in application of chelating agents and electrical fields.

	No voltage	10 V	30 V
CME	7.48 (±0.20) c	7.09 (±0.08) ef	7.10 (±0.03) ef
PME	7.75 (±0.02) b	7.31 (±0.03) d	6.98 (±0.02) f
EDTA	7.16 (±0.04) e	6.81 (±0.03) g	6.71 (±0.04) g
Control	7.80 (±0.16) b	8.39 (±0.01) a	8.30 (±0.08) a

CME: cow manure extract. PME: poultry manure extract. Values with different letters show significant differences at the 0.05 probability level.

The maximum value was found in 10 V-control (8.39 log of CFUs g-1 soil) followed by 30 V-control (8.30 log of CFUs g-1 soil). The result obtained in control soil was strange and it needs to be more studied.

Substrate induced respiration (SIR) obviously decreased in anodic soil by application of electrical field. Increasing of the intensity of electrical fields resulted in decreasing the levels of SIR in all anodic soils treated with chelating agents (table 6). Wang et al. [17] reported that the lowest amount of soil respiration was obtained near the anode. The minimum value of SIR was measured in 30 V-EDTA with 0.47±0.02 mg CO2 g-1 dry soil day-1 and the maximum level was measured in no voltage-PME with 1.56±0.04 mg CO2 g-1 dry soil day-1 followed by CME with 1.46±0.05 mg CO2 g-1 dry soil day-1. The application of CME and PME improve SIR in comparison with control when the soil was not treated with electrical fields. Lear et al. [16] reported that microbial respiration had been reduced after applying an electrokinetic system in soil. They explained that minimum amount of respiration had been measured close to the anode.

Table 6. The comparison of means of SIR (mg CO2 g-1 dry soil day-1) in anodic soil in application of chelating agents and electrical fields.

	No voltage	10 V	30 V
CME	1.46 (±0.05) ab	1.07 (±0.05) dc	0.68 (±0.02) f
PME	1.56 (±0.04) a	1.27 (±0.01) bc	0.82 (±0.21) ef
EDTA	0.73 (±0.16) f	0.66 (±0.06) f	0.47 (±0.02) g
Control	1.16 (±0.17) dc	0.97 (±0.12) de	0.74 (±0.17) f

CME: cow manure extract. PME: poultry manure extract. Values with different letters show significant differences at the 0.05 probability level.

CONCLUSION

Effects of the electrical fields along with EDTA as a chemical chelator and CME and PME as organic chelating agents were analyzed. Results illustrated that combination of EDTA and electrical field as a remediation method had devastating impacts on soil microbial activities. In spite of the beneficial effects of EDTA application on the bioavailability of heavy metals in soil for phytoremediation, it reduced significantly the bacterial and fungal populations and SIR in both cathodic and anodic soils. Utilization of organic chelating agents such as CME and PME in this study was friendlier than using EDTA. The application of electrical fields in the most cases had negative and significant effects on the bacterial and fungal populations and reduced SIR in both cathodic and anodic soils.

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