



**ELECTROBIOREMEDIATION AS A HYBRID TECHNOLOGY TO TREAT SOIL CONTAMINATED WITH TOTAL PETROLEUM HYDROCARBONS**

**LA ELECTROBIORREMEDIACIÓN COMO UNA TECNOLOGÍA HÍBRIDA EN EL TRATAMIENTO DE SUELO CONTAMINADO CON HIDROCARBUROS TOTALES DE PETRÓLEO**

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**Abstract**

Contaminated soil with Total Petroleum Hydrocarbons (TPH) was subjected to treatment in an experimental prototype with effluent recirculation. A  $2^k$  experimental design was used, with two factors (electric field and microorganisms' growth) and two levels (presence and absence of the factors), resulting in 4 treatments (triplicate) designated as: leaching, bioremediation, electroremediation, and electrobioremediation. Total petroleum hydrocarbons attached to the soil surface showed a higher migration to the aqueous phase due to the application of low intensity current. Growth of native microorganisms present in the soil was stimulated with the addition of micro and macronutrients; TPH were degraded used as carbon source and electron donor, using oxygen (aeration) as electron acceptor. There were statistical significant differences between treatments supporting EBR as the best alternative. Results showed  $24.5 \pm 3.1\%$  of TPH removal from the soil into the aqueous phase for lixiviation and  $32.1 \pm 1.4\%$  for bioremediation, both at 360 hours. For electroremediation a  $54.8 \pm 2.2\%$  removal (360 h) and 68% (700 h) and  $68.6 \pm 3.8\%$  (360 h) and 89% (700 h) for electrobioremediation. Remediated soil in electrobioremediation reached 2,596 mg TPH/kg soil, such concentration is lower than the maximum permissible limit (MPL) set by Mexican regulation.

*Keywords:* electrobioremediation, leaching, TPH, nutrients, contaminated soils.

**Resumen**

Suelo contaminado con Hidrocarburos Totales de Petróleo (HTP) fue sometido a tratamiento en una celda experimental con recirculación. Se utilizó un diseño experimental  $2^k$ , (factores: campo eléctrico y crecimiento de microorganismos) y (niveles: presencia y ausencia de los factores), resultando en 4 tratamientos (triplicado) denominados como: lixiviación, biorremediación, electrorremediación y electrobiorremediación. Los hidrocarburos totales de petróleo adheridos al suelo mostraron una mayor migración a la fase acuosa por la aplicación de corriente de baja intensidad. El crecimiento de microorganismos nativos del suelo se estimuló con la adición de micro y macronutrientes; los HTP fueron degradados como fuente de carbono y donador de electrones empleando oxígeno (aireación) como aceptor de electrones. Existieron diferencias significativas entre los tratamientos siendo la EBR la mejor alternativa de tratamiento. La remoción de HTP del suelo a la fase acuosa fue de  $24.5 \pm 3.1\%$  (lixiviación) y  $32.1 \pm 1.4\%$  (biorremediación), ambos a 360 horas. Para electrorremediación hubo una remoción de  $54.8 \pm 2.2\%$  (360 h) y 68% (700 h) y  $68.6 \pm 3.8\%$  (360 h) y 89% (700 h) en electrobiorremediación. El suelo remediado con electrobiorremediación alcanzó 2,596 mg HTP/kg de suelo, concentración inferior al límite máximo permisible (LMP) establecida por la regulación Mexicana.

*Palabras clave:* electrobiorremediación, lixiviación, HTP, nutrientes, suelos contaminados.

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## 1 Introduction

Nowadays there are many areas with hydrocarbon contaminated soils, which represents a more serious problem than those present in air and water. Soil is the surface of the earth and has a relevant importance in human beings, animals and plants because it provides food and sustenance for survival. The properties of a soil contaminated with hydrocarbons present harmful variations to humans and animals, generally in pH, texture and organic matter content, among others, which makes the soil to become toxic and unproductive. The use of natural resources (timber, minerals, water, etc.) has been fundamental to the development of humanity, but its exploitation and irrational use has caused an obvious imbalance to the environment. In addition, as a result of the automation of productive processes since the industrial revolution (1750 DC), the use of these resources increased and consequently generated large volumes of industrial and urban waste. These wastes have generated a lot of contaminated sites with heavy metals, organic compounds and other hazardous materials sites (De la Rosa-Pérez *et al.*, 2007).

Contamination by total petroleum hydrocarbons (TPH) in the soil is mainly due to accidental spills of crude oil, gasoline, oil and lubricants. A typical example of soil contamination occurs in industries because of maintenance or impairment of the machinery and the company's own vehicles cause spillage of fuels and lubricating oils accidentally. Mining industry is not the exception; because of its many activities (operation and maintenance), causes soil contamination with diesel, fuel oil and residual oil. So the implementation of a flexible and low cost method is needed to achieve levels established by Mexican regulations (NOM-138-SEMARNAT/SS-2003) (Nápoles *et al.*, 2005; SEMARNAT, 2003; Unzueta-Medina, 2010).

The company First Mining Corp. (formerly Goldcorp Inc.) is located in Tayoltita, municipality of San Dimas in the state of Durango, Mexico and for years has faced this problem. At the time of conducting this research, the company had approximately 800 tons of TPH contaminated soil, confined to prevent the contamination from spreading to other areas. Due to this situation the company decided to undergo the "Clean Industry" program in which they must comply with existing environmental regulations, therefore it was imperative to support research to find a suitable technology to remediate the contaminated soil and meet the maximum permissible limits (MPL) of

TPH in soil according to NOM-138-SEMARNAT/SS-2003 (Soto-Álvarez, 2007; Gurrola-Nevárez, 2008; Unzueta-Medina *et al.*, 2007; Unzueta-Medina, 2010; Osorio-Rodríguez, 2010; Martínez-Prado *et al.*, 2011a; Martínez-Prado *et al.*, 2011b).

## 2 Background

There are different techniques (Fig. 1) to carry out remediation of a contaminated soil such as biological, physicochemical, and thermal (Volke and Velasco, 2002; Volke and Velasco, 2003; Gentry *et al.*, 2004; Mediana-Valtierra *et al.*, 2005; De la Rosa-Pérez *et al.*, 2007; Medina-Moreno *et al.*, 2009; Gomes *et al.*, 2012; Martínez-Trujillo and García-Rivero, 2012). In this research a hybrid remedial option was used, combining a physicochemical and a biological technique to treat the TPH contaminated soil via electrobioremediation. Such alternative takes advantage of mechanisms of each of the two methods, electroremediation and bioremediation, to make more efficient the soil-effluent remediation (Megharaj *et al.*, 2011; Unzueta-Medina *et al.*, 2007; Unzueta-Medina, 2010).

### 2.1 Electroremediation

Is a technology that uses the conductive properties of the soil, which aims to separate and remove organic and inorganic (metals) contaminants of saturated and unsaturated soils, sludges and sediments, using an electric field that allows removing charged species (ions). This technology involves the application of a direct current of low intensity between a positive and a negative electrode (Pellini, 2006; Yeung *et al.*, 2011). Electroremediation can be performed *in-situ* and it is effective for fine-grained soils and low hydraulic permeability which are difficult to treat by other methods. Its advantages include control over the direction of movement of water and dissolved contaminants, even across heterogeneous soil, contaminant holding in a confined area, and low energy consumption (Page & Page, 2002). Similarly combined electrokinetic biodegradation has been documented with the addition of nutrients and donor/acceptor electrons (Reinout Lageman *et al.*, 2005; Unzueta-Medina *et al.*, 2007); treatment of contaminated soil with organochlorine compounds (Gomes *et al.*, 2012), polycyclic aromatic hydrocarbons (Pazos *et al.*, 2010), and heavy metals (Virukyte *et al.*, 2002), among others.

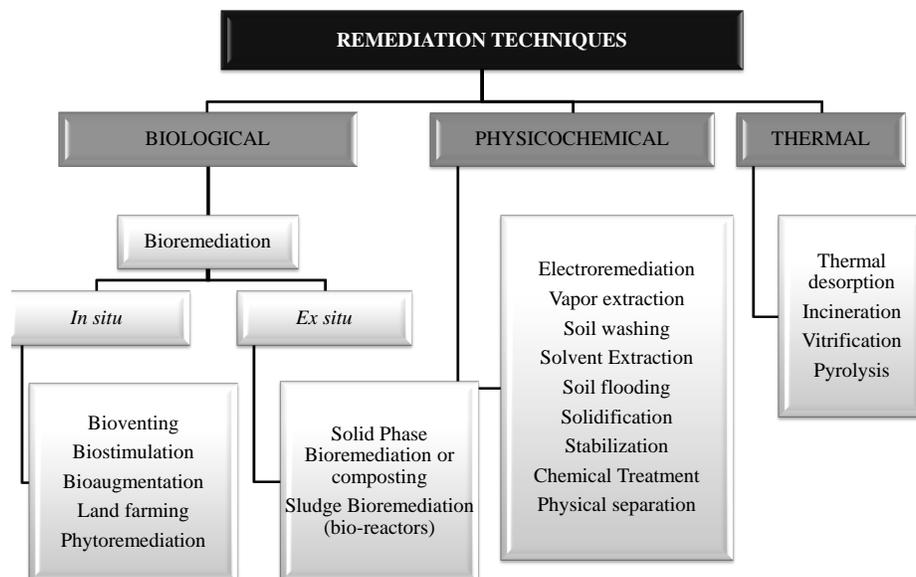


Fig. 1. Remediation techniques for contaminated soils

## 2.2 Bioremediation

Meanwhile, bioremediation uses living organisms, cellular components and free enzymes, in order to start a process of accelerating the rate of natural degradation of organic compounds or contaminants in different sites such as mangrove soil, agricultural and industrial sludges and soils (Wan *et al.*, 2002; Gómez-Contreras, 2007; Megharaj *et al.*, 2011; García Frutos *et al.*, 2012; Martínez-Prado *et al.*, 2011a; Ruiz-Marín *et al.*, 2013). The bioremediation process may be divided into three phases. The first phase is natural attenuation, where contaminants are degraded by microorganisms without stimulation induced by human action. The second is biostimulation, where nutrients and oxygen are supplied to the system to improve its effectiveness and accelerate biodegradation. Last phase is bioaugmentation, where more microorganisms are added to the system. These added microorganisms must be more efficient than the natives and capable of degrading contaminants in the system.

The main objective of this research was to evaluate four different treatments: leaching also known as lixiviation (LIX), bioremediation (BR), electroremediation (ER), and electrobioremediation (EBR); to remediate TPH contaminated soil from a mining company, in order to prove that the hybrid option offers soil-effluent treatment with a higher efficiency. Experiments were conducted in a

polycarbonate experimental unit with recirculation to significantly reduce the amount of effluent used in the system.

## 3 Materials and methods

### 3.1 Experimental design

To test the removal efficiency of TPH, due to the application of electric current and/or action of microorganisms, a factorial design  $2^2$  with three replicates was established. Table 1 shows the experimental design considering biological and electrical requirements as factors with its presence (+) or absence (-) as levels of treatment. That combination led to four treatments: Leaching (LIX), Bioremediation (BR), Electroremediation (ER) and electrobioremediation (EBR); described in Table 2.

Table 1. Experimental Design. Combination of presence (+) or absence (-) of electric field and microorganisms was used to establish the treatments

		<i>Microorganisms</i>			
		-	-	-	+
<i>Electric Field</i>	-	(LIX)		(BR)	
	+	(ER)		(EBR)	

### 3.2 Experimental unit

A polycarbonate experimental unit was constructed to treat 27 kg (9 kg per section) of contaminated soil (bulk density of 1.4 kg/L) flooded with 30 L of tap water or mineral salts solution, depending on the treatment; the effluent was recirculated to the system at a flow of 19 mL/min. Table 3 summarizes design characteristics.

#### 3.2.1 Description of experimental unit

Experimental unit was built with solid polycarbonate. It consisted of nine inner compartments; three were to contain the soil to be treated and the remaining six for the liquid (water or MSS, based on the experiment to be carried out). The unit consisted of three sections of equal size and volume (Fig. 2), each section has two internal divisions perforated in a triangular arrangement to ensure a homogeneous distribution of the liquid passing through the soil.

Table 2. Description and factors considered in all 4 treatments evaluated

Treatments	Factors		Description
	MO	EF	
Leaching (LIX)	-	-	It is also known as lixiviation, both factors are absent. This treatment evaluates the desorption process of the contaminant (TPH) attached to the soil matrix, due to the action of water.
Electroremediation (ER)	-	+	Presence of only one factor, electric field. Contaminant desorption (TPH), from the solid phase into the aqueous phase, was evaluated; electric current (4.8 V) by the 3 pairs of stainless steel electrodes was applied with a total intensity of 0.21 A.
Bioremediation (BR)	+	-	It corresponds to the action of microorganisms. Native microorganisms of the soil were stimulated, and measured through the increase in biomass, with the addition of micro and macronutrients present in the mineral salt solution (described in section 3.3.2) and the use of TPH as substrate/electron donor ( $ed$ ) coupled with oxygen (aeration) as the electron acceptor ( $ea^-$ ).
Electrobioremediation (EBR)	+	+	Presence of both factors, combination of ER and BR treatments as discussed above.

MO = Microorganisms

EF = Electric Field

Table 3. Experimental unit characteristics

Material	Solid Polycarbonate
Sections for treatment	3
Mass of soil to treat	27 kg
Effluent volume	30 L
Flow direction	Horizontal
Flow rate	19 mL/min
Volumetric flow density	0.036 mL/cm <sup>2</sup> min
Cross section of stainless steel electrode	525 cm <sup>2</sup>
Distance between electrodes	10 cm

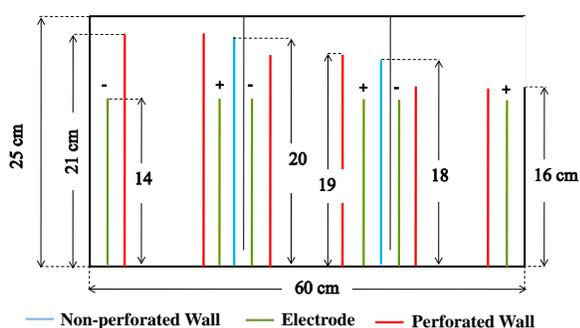


Fig. 2. Design and dimensions of experimental unit: Lateral view.

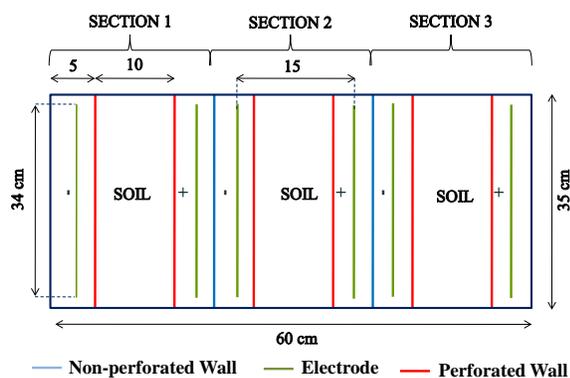


Fig. 3. Design and dimensions of experimental unit: Plant view.

The inner walls of the spaces intended to contain the soil were covered with plastic mesh (2 mm diameter aperture) to prevent the soil from spreading throughout the experimental unit.

Each of the three sections were identical in almost all measures, except height (Fig. 3); both the walls that divide them and the internal divisions, in order to force the liquid to flow in a stepwise manner through

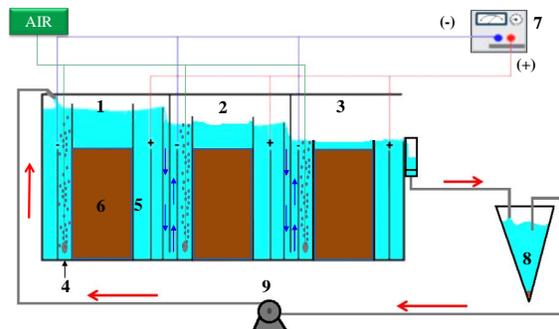


Fig. 4. Experimental unit. Three treatment compartments 1, 2 and 3, cathode 4, anode 5, contaminated soil 6, generator of direct current 7, separation cone with effluent 8, and peristaltic pump for recirculation 9. Red arrows show the direction of flow.

the system that way each section would receive the effluent from the preceding section. Inside the unit, extra internal walls were placed (2 cm from the bottom of the unit) in the cathode compartments (where the liquid from the previous section falls into the following; sections 1 and 2), to force the fluid through the soil both horizontally and vertically (Fig. 4).

### 3.2.2 Operation of experimental unit

The electrodes, perforated stainless steel plates with dimensions of 14 cm high by 34 cm long, (positive and negative) adapted to the experimental unit were activated during electroremediation and electrobioremediation experiments. The unit had six electrodes, three positive and three negative which were connected in parallel to a source of direct current (DC power supply DS-304M Zurich) for supplying a direct current of low intensity (0.07 A, for each pair). The arrangement of each pair of electrodes within the compartments was cathode-anode-soil, with a total of three soil treatment sections (Fig. 3). The electrodes were used to generate the necessary electric field to separate the hydrocarbons present in the soil, which once in the liquid phase mostly migrated towards the anode compartment, mainly by the direction of the volume flow and the direction of electroosmotic flow generated by the electric potential difference (De la Rosa-Pérez *et al.*, 2007).

The effluent was collected at the exit of the last section of the unit to a separating cone to sediment all solids in it, before returning back to the first compartment. The effluent was recirculated by a peristaltic pump (Mity-Flex 913 series dual head)

calibrated at 19 mL/min, sending it from the conic container to the cathode compartment of section 1, promoting the recirculation of the effluent through the system (Fig. 4). During the treatment time it was necessary to take control of the liquid level in the whole system to keep it constant, adding fresh solution and account for evaporation losses.

To ensure aerobic conditions in the systems of BR and EBR, an aeration system was implemented along the entire system to increase the amount of dissolved oxygen in the liquid phase and thus ensure that microorganisms will develop favorably and would be able to degrade the maximum amount of contaminant available. This system consisted of a dual pump air (Optima: Elite Plus), which by means of flexible hoses coupled nozzles distributing an equal amount of air along the system to a pressure of about 3-4 psi (Fig. 4).

### 3.3 Experimental phase

Soil was homogenized for at least 30 minutes in a mixer prior to treatment. Each treatment was performed in triplicate for a period of 360 hours (15 days), except for the last extra pair of ER and EBR run for 700 h (almost one month). During this time period samples were taken to monitor the behavior of different parameters according to the current Mexican environmental legislation and standard methods.

#### 3.3.1 Soil to be treated

Hydrocarbon contaminated soil was obtained from the mining company First Mining Corp. (formerly Goldcorp Inc.), located in Tayoltita, municipality of San Dimas in the state of Durango, Mexico, and treated *ex-situ* in Durango City at ITD facilities. The soil was contaminated with diesel, fuel oil and waste oils as a result of multiple operations and maintenance activities, and confined in an area built for this purpose to prevent the pollution spreading to other areas.

#### 3.3.2 Mineral salts solution (MSS)

The MSS was used as a source of micro and macronutrients to stimulate microorganisms present in the soil, in the process of bioremediation, with (EBR) and without electric field (BR). The MSS was prepared by adding the following nutrients:  $K_2HPO_4$  (1.5 ppm),  $KH_2PO_4$  (1.5 mg/L),  $NH_4NO_3$  (2.0 mg/L),  $MgSO_4 \cdot 7H_2O$  (0.5 mg/L), NaCl (1.0 mg/L) and

1 mL of  $FeSO_4 + EDTA$  ( $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ ) (Martínez-Prado, 2002).

#### 3.3.3 Evaluation and measurement of parameters

The parameters analyzed were: pH, oxidation-reduction potential (ORP), electrical conductivity (EC), total dissolved solids (TDS), concentration of total petroleum hydrocarbons [TPH] and colony forming units (CFU) along the experiments, to both the effluent and the soil. All analyzes were performed according to the procedure established by Standard Methods of Analysis (APHA, 2005).

All parameters were analyzed to the liquid phase every 72 hours; for the solid phase, the first four parameters were measured every 72 hours and the last two parameters were measured at the beginning, at the middle and at the end of each replicate.

**Solid samples.**- Soil samples were sampled (15 to 20 grams) in each of the three sections of the experimental unit, were perfectly mixed and placed to dry in a forced convection oven (ShellLab ) for 24 hours at 40-60°C to remove excess moisture and allowed to cool down to room temperature afterwards. Dried samples were triturated in a porcelain mortar, sieved and then 40 g of soil was weighed into a glass vial of 60 mL to which 40 mL of distilled water were added, stirred well and allowed to sit for at least 24 hours. Then, the liquid was used to determine EC, TDS, ORP, pH and viable count of CFU.

**Liquid samples.**- About 30 to 40 mL of effluent of the anode compartment, point where the fluid from each section overflows to enter the next or to be recirculated, were collected in a glass vial of 60 mL. EC, pH, ORP, SDT and CFU's were directly measured as previously described. For the case of ER and EBR, where electric current is applied, the parameters were monitored in both the anode and the cathode compartment of each of the three sections of the unit. The main reason was because electricity produces water electrolysis yielding an acid front in the positive electrode and a basic front in the negative electrode, altering the fluid properties, so it is important to record these changes.

**Quantification of Total Petroleum Hydrocarbons (TPH).**- A gas chromatograph with a flame ionization detector (GC-FID) was used to quantify [TPH] following the 1005 method established by the Texas Natural Resource Conservation Commission (TNRCC, 2001; Unzueta-Medina, 2010).

**Colony Forming Units (CFU).**- The presence of aerobic mesophilic microorganisms, in both solid and

liquid samples, was carried out by determining the number of colony forming units present on standard agar method. To estimate viable cells, counting was conducted according to the technique of pour plate, at  $35 \pm 2^\circ\text{C}$  for 24 hours.

### 3.4 Analysis of results

To validate the differences between treatments STATISTICA 7<sup>®</sup> software was used and a factorial analysis  $2^k$  was run with standardized values (z). Z indicates the direction and extent to which an individual value is away from the mean, on a scale of standard deviation units.

## 4 Results and discussion

Leaching (LIX), bioremediation (BR), electro-remediation (ER) and electrobioremediation (EBR), were evaluated according to the experimental design established for the treatment of TPH contaminated soil. Results are discussed by pairs (LIX & BR) and (ER & EBR), to be addressed by the absence or presence of electric field. Each replicate ( $n = 3$ ) was carried out using soil from the same batch; during the same period of time (360 h y 700 h) and with a hydraulic retention time of 1.1 days. Same volume of water (30L) was recirculated through the system, so for 360 hours effluent passed through the experimental unit 15 times.

### 4.1 LIX and BR effluent: pH, ORP, EC, and TDS

The behavior of pH was relatively constant, obtaining average values of  $8.11 \pm 0.7$  for LIX and  $7.15 \pm 0.2$  for BR. Tap water used in LIX had a pH of about 8, revealing that the effluent passed through the system by simply washing the soil without change. For BR, where MSS was used, the pH value decreased from 8 reaching around neutrality, although some changes took place since the salt concentration varied due to the consumption by microorganisms.

The ORP changes were quite similar to that observed in the pH data as they have a direct relationship. For pH values below 7 the ORP showed positive values, oxidation was taking place in the medium. Conversely, when pH values are above 7; negative values are obtained for ORP, meaning a reduction.

A clear difference between the two experiments was observed for EC and TDS, due to the presence of mineral salts versus tap water and that both parameters measure dissolved ionic compounds. EC values in both treatments remained almost constant throughout time. Average values for LIX was  $1,575 \pm 172 \mu\text{S}/\text{cm}$ , whereas in BR this value was increased about fourfold  $6,762 \pm 183 \mu\text{S}/\text{cm}$ . Although salts were not added in LIX, the EC measured was due to the presence of salts naturally contained in tap water and those desorbing from the soil during the leaching process. The average values for TDS were  $793 \pm 95 \text{ mg}/\text{L}$  (ppm) for LIX and  $3,200 \pm 85 \text{ ppm}$  in the BR experiment.

### 4.2 ER and EBR effluent: pH, ORP, EC, and TDS

In both treatments electric current was passed between the submerged electrodes to generate an electric field and encourage the removal of TPH attached to the soil. pH was measured in each of the three compartments; an acid front was created in the anode and a basic front in the cathode, due to electrolysis that occurs in the effluent under the action of the electric field (De la Rosa-Pérez *et al.*, 2007). According to data pH behavior was as expected, with average values for ER of  $8.6 \pm 0.4$  at the cathode and  $4.7 \pm 0.7$  at the anode, with similar readings for EBR.

ORP is an important selective factor in all environments, and probably influences microorganisms and their metabolism. ORP indicates the ratios of oxygen (respiration) of living organisms and can be used to specify the environment from which a microorganism is capable of generating energy and synthesize new cells without use of molecular oxygen. Aerobic microorganisms require positive values of ORP to grow while negative values for anaerobes. The oxidation-reduction processes are defined in terms of electronic migrations between chemicals. Oxidation is the loss of electrons while reduction is the gain of electrons. As already mentioned, electrolysis occurs in the effluent due to the electric field. Oxidation occurred at the anode (+) due to low pH values with ORP average values of  $119 \pm 39 \text{ mV}$  and  $108 \pm 44 \text{ mV}$ , for ER and EBR, respectively; while at the cathode (-) a reduction took place because of the basic front generated, with average values of  $-84 \pm 28 \text{ mV}$  and  $-86 \pm 21 \text{ mV}$ .

In EBR experiments MSS was used as effluent; the EC average values were  $6,100 \pm 208 \mu\text{S}/\text{cm}$ , for both the anode and cathode compartments, with TDS average values of  $3,000 \pm 170 \text{ ppm}$ .

Table 4. Parameters evaluated in the soil for each treatment, values are given as Average of 3 replicates  $\pm$  Std. Dev.

Time (h)	pH	ORP (mV)	EC ( $\mu$ S/cm)	TDS (ppm)
Leaching (LIX)				
0	7.43 $\pm$ 0.4	-33 $\pm$ 4	213 $\pm$ 11	409 $\pm$ 19
180	8.13 $\pm$ 0.4	-59 $\pm$ 5	141 $\pm$ 9	70 $\pm$ 14
360	7.98 $\pm$ 0.3	-50 $\pm$ 4	167 $\pm$ 15	82 $\pm$ 21
Bioremediation (BR)				
0	7.43 $\pm$ 0.2	-33 $\pm$ 2	213 $\pm$ 7	409 $\pm$ 24
180	7.52 $\pm$ 0.6	-25 $\pm$ 7	511 $\pm$ 10	258 $\pm$ 16
360	7.4 $\pm$ 0.3	-28 $\pm$ 4	388 $\pm$ 15	193 $\pm$ 20
Electroremediation (ER)				
0	7.59 $\pm$ 0.3	-31 $\pm$ 6	367 $\pm$ 13	181 $\pm$ 9
180	8.4 $\pm$ 0.3	-73 $\pm$ 4	204 $\pm$ 9	99 $\pm$ 13
360	7.91 $\pm$ 0.5	-49 $\pm$ 7	170 $\pm$ 4	84 $\pm$ 5
Electrobioremediation (EBR)				
0	7.59 $\pm$ 0.4	-31 $\pm$ 5	367 $\pm$ 8	181 $\pm$ 7
180	7.82 $\pm$ 0.6	-42 $\pm$ 6	468 $\pm$ 16	233 $\pm$ 14
360	7.38 $\pm$ 0.1	-21 $\pm$ 2	449 $\pm$ 14	224 $\pm$ 18

The high electrical conductivity in EBR allowed reduction of the resistance in the soil and the fluid to the passage of electric current generated between the electrodes, causing a more efficient process with a lower voltage. When tap water (ER) was used as an electrolyte, the values for EC and TDS ranged around 1,150  $\pm$  145  $\mu$ S/cm and 550  $\pm$  98 ppm, respectively; remarking that the presence or absence of electric field hardly affected the concentration of salts in the liquid.

### 4.3 Soil properties

Soil samples were taken at time 0, 180 and 360 h, in each of the three compartments for all 4 treatments; pH, ORP, EC and SDT were measured to observe changes in soil properties.

In all experiments pH values remained above 7; but in those where the effluent was tap water (LIX and ER) maintained a more basic pH than those where MSS was used (BR and EBR). The ORP showed the same tendency as for pH, with negative values. The values for EC and TDS increased in the BR and EBR experiments due to the presence of minerals, the opposite occurred in the case of LIX and ER. Table 4 shows the average values of all replicates for each parameter.

### 4.4 Presence of microorganisms (CFU) in effluent and soil

Numerous microorganisms are present in soil naturally; these microorganisms are adapted to the

compounds present in the soil and develop the ability to degrade them, as long as they have energy source (carbon), electron donor ( $ed^-$ ), electron acceptor ( $ea^-$ ), and the macro and micro nutrients needed for metabolism. The native microorganisms (MO), present in the contaminated soil, were responsible for the degradation of TPH requiring them to be available in the aqueous phase. The presence and growth of MO was quantified through the viable count by expressing it in terms of CFU. It was noted that when MSS (macro and micro nutrients) was used CFU were greater than in cases where tap water was used.

Fig. 5 shows the microorganism's growth rate in the effluent for all 4 treatments. According to the results of LIX and BR, the amount of MO was lower compared to ER and EBR; because the amount of hydrocarbons available in the effluent was smaller, limiting the carbon source, energy and donor electron (substrate) necessary for their growth and reproduction. However, if LIX and BR are compared, the latter maintains higher values of MO in the effluent due to the presence of mineral salts and oxygen supply which serve as nutrients and electron acceptor, respectively; having the same tendency for ER and EBR experiments.

The EBR experiment showed a greater presence of MO in the effluent, desorption of TPH from the soil increased significantly when using an electrical gradient (as in ER), increasing its concentration in the aqueous phase. A greater amount of substrate in the medium, the use of MSS, presence of oxygen in the

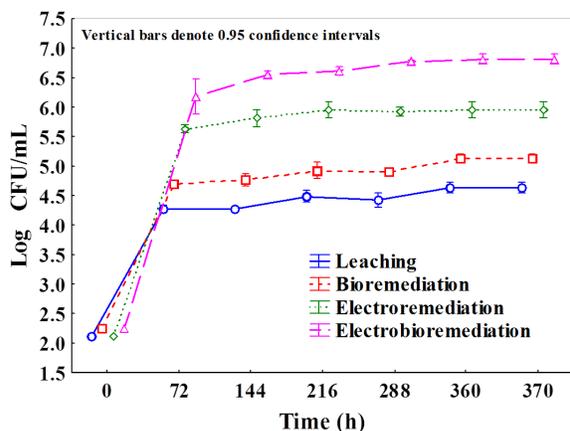


Fig. 5. Microbial growth rate in CFU/mL in effluent for treatments tested: Leaching (LIX), Bioremediation (BR), Electroremediation (ER), and Electrobioremediation (EBR).

effluent, and the actual movement of fluid provides the most appropriate conditions of the four treatments to encourage an extensive microbial growth in the medium.

#### 4.5 Degradation of TPH

For a better interpretation of the results it is useful to recall the principles of each of the evaluated treatments. In the case of LIX soil washing occurred using tap water as effluent; it was observed that  $24.5 \pm 3.1\%$  of the soil TPH migrated into the aqueous phase. Soil washing also took place in the ER with greater desorption when the electric field was applied across the three pairs of electrodes, resulting in a greater removal of hydrocarbons from the soil to the aqueous phase, corresponding to  $54.8 \pm 2.2\%$ . The percentage difference between LIX and ER was mainly due to the application of low current improving desorption of contaminant attached to the soil surface. Also noteworthy that in both cases, LIX and ER, there was not a noticeable degradation of TPH already available in the aqueous phase by microorganisms, as they lacked the necessary nutrients to metabolize them.

In BR and EBR treatments MSS and oxygen (aeration) was provided, as a source of nutrients and electron acceptor, respectively. Results revealed that microorganisms were able to degrade TPH available in the aqueous phase. In BR treatment hydrocarbons were removed from the soil in a  $32.1 \pm 1.4\%$ ; higher than the results obtained in LIX. EBR offered a more efficient treatment of the soil due to the action of MO

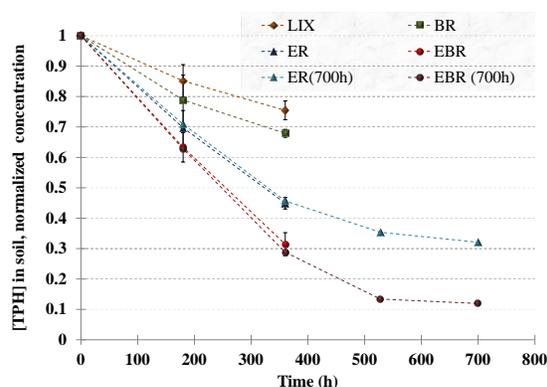


Fig. 6. Tendency of TPH normalized concentration in soil. Leaching (LIX), Bioremediation (BR), Electroremediation (ER), and Electrobioremediation (EBR).

and electric field applied; reaching a  $68.6 \pm 3.8\%$  removal from the soil but with a relatively medium concentration of TPH in the aqueous phase, while in the ER treatment TPH only migrated to the aqueous phase. Fig 6 shows the comparison of both pairs of treatments.

It was noted that in ER and EBR treatments the trend of concentration continued downwards; so a last replica of both was ran up to 700 h, monitoring the concentration of the TPH until maximum removal was obtained. A removal of 68% for ER and 89% for EBR was achieved (Fig. 6), corresponding to a concentration that meets the maximum permissible limit (MLP) for TPH according to Mexican regulations. In order to support the results random samples were sent to a certified laboratory, results were very similar to those quantified in this research. Table 5 summarizes results of TPH removal for all four experiments evaluated.

Table 5. Soil TPH removal for all treatments evaluated at 360 h (n = 3) and 700 h (maximum removal was reached).

Treatments	Removal (Average $\pm$ Std. Dev.)
Leaching (LIX)	$24.5 \pm 3.1\%$
Electroremediation (ER)	$54.8 \pm 2.2\%$
Bioremediation (BR)	$32.1\% \pm 1.4\%$ (68% @ 700 h)
Electrobioremediation (EBR)	$68.6\% \pm 3.8\%$ (89% @ 700 h)

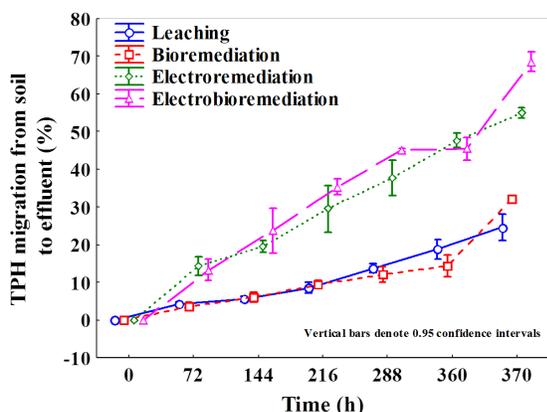
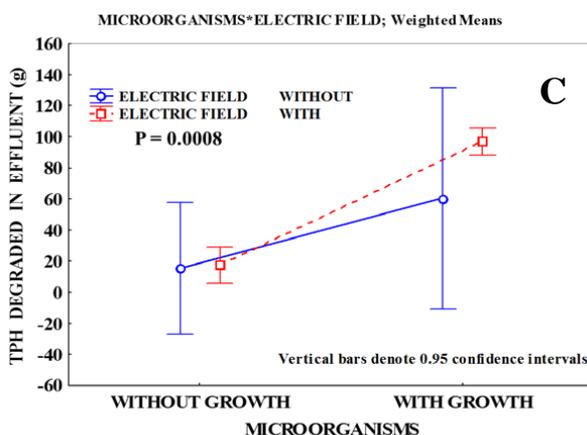
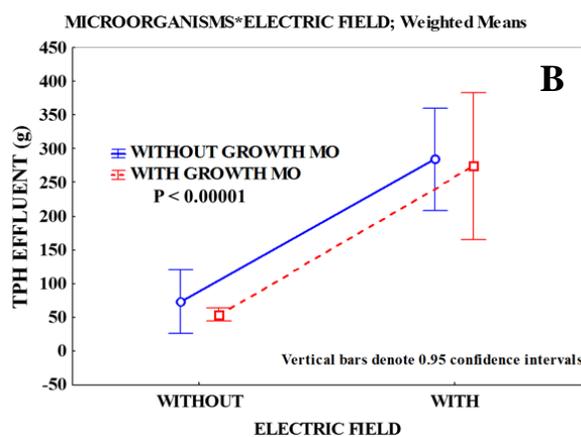
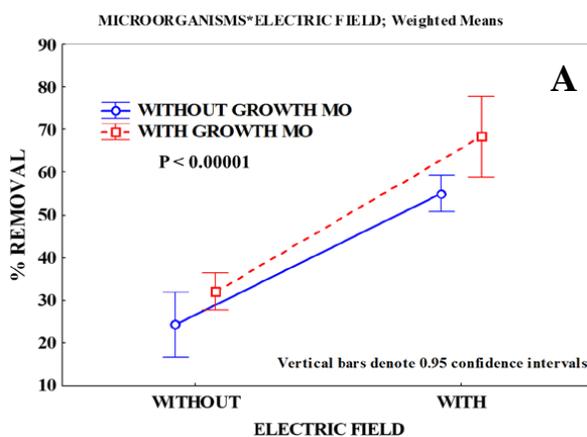


Fig. 7. Removal of TPH from soil migrating to the liquid phase. Leaching (LIX), Bioremediation (BR), Electroremediation (ER), and Electrobioremediation (EBR).

Standardized data was subjected to statistical analysis showing there were significant differences between treatments. A comparison of migration of TPH from soil to the effluent among each treatment is shown in Fig. 7. It is observed that in experiments where electric current was applied (ER and EBR) removal of contaminant (TPH), from soil to liquid phase, was higher than in LIX and BR when the effluent (water and MSS) was only recirculated through the unit. Fig. 8 supports the influence of the factors evaluated in the 2<sup>2</sup> ANOVA for the behavior of the treatments conducted. Fig. 8A indicates how microorganisms (MO) were stimulated by MSS (growth of MO), versus less growth when tap water was used, and electric field applied influenced in the TPH % removal.



Dependent Variable	F values of the effects		p values of the effects	
	Electric Field	Micro-organisms	Electric Field	Micro-organisms
% Removal of Soil	50.17	510.67	> 0.00001	0.00006
TPH in the Effluent (g)	193.2	0.92	> 0.00001	0.36
TPH degraded in Effluent (g)	4.49	45.69	0.063	0.00008

Fig. 8. Statistical analysis of results supports how microorganisms (MO), mineral salts solution (MSS), and electric field influence in the TPH % removal (A). Interestingly, in the TPH effluent, it is clearly observed that the electric field helps in flushing or migration of hydrocarbons from soil to liquid phase (B). Whereas, microorganisms were the responsible for the degradation of TPH present in the effluent (C).

Table 6. Mass balance of TPH in each replicate in treatments evaluated

TPH (g)		Removal (%)	TPH (g)		
In	Out		Removal soil	Effluent	Degraded and losses
Leaching (LIX)					
466.4	350.2	24.9	116.2	56.4	59.8
266.4	209.7	21.3	56.7	51.9	4.7
343.0	248.9	27.4	94.1	87.8	6.2
Bioremediation (BR)					
466.4	320.1	31.4	146.3	56.4	89.9
266.4	176.8	33.6	89.5	56.7	32.8
343.0	235.7	31.3	107.3	49.5	57.8
Electroremediation (ER)					
533.3	247.0	53.7	286.3	263.3	23.0
501.2	214.2	57.3	287.0	271.0	16.0
587.9	268.6	54.3	333.0	319.3	13.7
Electrobioremediation (EBR)					
533.3	191.0	64.2	342.3	241.0	101.3
501.2	148.5	70.4	352.7	257.0	95.7
587.9	169.3	71.2	418.6	324.0	94.6

Fig. 8B shows, when evaluating the TPH present in the effluent, that the electric field helped in flushing (migration) hydrocarbons attached to the soil matrix. Lastly, Fig. 8C shows that microorganisms were the responsible for the degradation of TPH present in the effluent, which is supported by the CFU counts (Fig. 5).

#### 4.6 Mass balance of TPH

A mass balance in the system for all four treatments was conducted using Eq. 1. Data was analyzed considering TPH changes in soil and effluent: hydrocarbons present in the soil at the beginning and the end of each treatment and the mass degraded, lost and present in the liquid phase at the end of the experiments.

$$[TPH's]_{In}^{Soil} - [TPH's]_{Out}^{Soil} = [TPH's]_{Present}^{Effluent} + ([TPH's]_{Degraded}^{Effluent} + [TPH's]_{Losses}^{Effluent}) \quad (1)$$

It was not possible to establish the amount of TPH degraded by the MO and losses in the system; so both

were considered in the same category. An increase was clearly observed in BR and EBR with respect to LIX and ER. Mass balance (g) is shown in Table 6, keep in mind that the approximate volume of effluent (tap water or MSS) was 30 L and 27 kg of the contaminated soil.

#### 4.7 Comparison with other authors

Research conducted by She *et al.* (2006) reported experimental results with *Enterobacter dissolvens* and suggested that microorganisms should be under a current of about 10 mA for optimal metabolic activity, since for values from 20 to 100 mA can arise a bactericidal effect in the medium. In EBR treatment evaluated in this study there was presence of microorganisms, on the soil and in the effluent, at an average value of 70 mA. Although a specific identification of the microbial population capable of withstanding these conditions was not completed, in experiments conducted in a previous stage of this research *Aspergillus* (fungus) and *Flavobacterium* (bacteria) were isolated from soil and found to be capable of degrading TPH present

in the contaminated soil under these conditions (Soto-Álvarez, 2007). Meanwhile, (Olszanowski and Piechowiak, 2006) found that the best conditions for *Klebsiella pneumoniae* and *Pseudomonas putida* microorganisms were at a current density of  $6 \times 10^{-3}$  mA/cm<sup>2</sup>, quite low as compared to the 0.14 mA/cm<sup>2</sup> applied in these experiments. Based on the above it can be concluded that native soil microorganisms developed in EBR treatments had a higher tolerance to electric current than those reported in the literature for similar technology.

Hybrid technology evaluated in this study gave positive results, providing a solution to treat both contaminated soil and the effluent produced. Compared with She *et al.* (2006), experiments were conducted with the same type of pollutant and achieved an 82% removal in 1,008 hours. Garcia-Frutos *et al.*, (2012) reported 57% efficiency in the treatment of sludge samples contaminated with 2,243 mg TPH/kg in 28-day experiments at laboratory level. In another experiments reported by Luo *et al.* (2006) a 58% removal of phenol contaminated soil (as a model for other hydrocarbons) was achieved at 240 h. It is noteworthy to mention that in this project 27 kg of soil were treated; which is a much higher mass compared to many scientific publications reviewed. In addition, the volume of solution used in this research was only 30 L, which represents a relatively small volume compared to treatments reported by Turer and Genc (2005) where 1 kg of heavy metals contaminated soil was treated using 20 L of solution.

## Conclusions

Remediation of a contaminated soil with TPH from the mining corporate *Primero Mining* (formerly Goldcorp Inc.) in the state of Durango, Mexico was achieved. Four different treatments (LIX, BR, ER, and EBR) were evaluated in an experimental prototype with recirculation of the effluent. The experimental unit allowed to establish a treatment to remediate a greater amount of soil with low voltage, electrode distance remained the same (10 cm) as the predecessor research (Soto-Álvarez, 2007) helping to reduce the electrical resistance of the medium. A combination of biological (BR) and physicochemical (ER) remediation techniques was implemented with favorable results, offering a solution to treat both the effluent and contaminated soil. In EBR treatment  $68.6 \pm 3.8\%$  (360 h) and 89% (700 h) removals were achieved. Statistical analysis showed significant

differences between the four experiments; supporting the importance that microorganisms have in the degradation of TPH and the role of electric field in TPH desorption from the soil. Replicates kept the same patterns of behavior, concluding that the EBR treatment was the most effective alternative for the remediation of the contaminated soil. The remediated soil in the EBR treatment reached 2,596 mg TPH/kg soil, concentration lower than the maximum permissible limit (MPL) set by Mexican regulation; ranging between 5,000 to 6,000 mg TPH/kg soil for medium and heavy TPH fraction, respectively. After EBR treatment, soil presented a heavy load of microorganisms and nutrients; so, remaining TPH could be further degraded by natural attenuation or bioaugmentation under aerobic conditions (Gentry *et al.*, 2004; Martínez-Prado *et al.*, 2011a; Martínez-Prado *et al.*, 2011b; Ruiz-Marín *et al.*, 2013). Since TPH were not completely degraded in the resulting EBR effluent; alternate preliminary experiments were conducted using biocatalytic particles as a bioaugmentation process. Biocatalytic particles were produced with isolated microorganisms from the same contaminated soil and immobilized in sodium alginate; experiments were conducted with effluent and slurry in batch reactors obtaining promising results (Roldán-Meza, 2010; Martínez-Prado *et al.*, 2011b). Use of immobilized microorganisms is well documented and has been explored by several authors, under very different experimental conditions and proven to remove a wide variety of pollutants (Gentry *et al.*, 2004; Mediana-Valtierra *et al.*, 2005; Quek *et al.*, 2006; Medina-Moreno *et al.*, 2009; Roldán-Meza, 2010; Martínez-Prado *et al.*, 2011b; Martínez-Trujillo and García-Rivero, 2012); however, scale-up and field test experiments must be conducted to establish the technical and economic feasibility of this technology. It is recommended to conduct further experiments combining EBR and bioaugmentation with biocatalytic particles at a larger scale, in order to support findings in this research to remove TPH from soil and degrade those present in the effluent.

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## Nomenclature

$[TPH's]_{In}^{Soil}$	Total Petroleum Hydrocarbons in the contaminated soil entering the system, g.
$[TPH's]_{Out}^{Soil}$	Total Petroleum Hydrocarbons in the contaminated soil at the end of the treatment, g.
$[TPH's]_{Present}^{Effluent}$	Total Petroleum Hydrocarbons present in the effluent at time = t, g.
$[TPH's]_{Present}^{Degraded}$	Total Petroleum Hydrocarbons degraded in the effluent, g.
$[TPH's]_{Present}^{Losses}$	Losses of Total Petroleum Hydrocarbons in the effluent, g.

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