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Esquema de fitorremediación de un sitio contaminado con elementos potencialmente tóxicos

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RESUMEN

Los residuos del Reciclaje de Baterías Ácidas de Pb (RBAP) contienen Pb, SO_4^{2-} y PO_4^{3-}; éstos pueden contaminar el ambiente, lo que representa un riesgo para la salud pública. Los métodos normalmente usados para remediar suelo se basan en procesos físicos y químicos, son de implementación cara y complicada. Ante ello, la fitorremediación es una tecnología de utilidad. Existen especies arbóreas capaces de crecer en concentraciones elevadas de sales y metales pesados, las cuales forman biomasa masiva y por ello son útiles en la dendro-remediación. En el presente trabajo se caracterizó el suelo de una recicladora abandonada de baterías ácidas de Pb con el objetivo de establecer un sistema de dendro-remediación. Además, se analizaron las condiciones del sitio y sus implicaciones en relación a la legislación ambiental Mexicana. Esto incluyó una evaluación de impacto ambiental, en la que se observó que una planta de RBAP puede afectar severamente el ambiente si durante su operación y manejo de residuos, no se llevan medidas de control adecuadas. Se encontraron altas concentraciones de Pb total (444,105 mg kg^{-1}) y extractable (12,458 mg kg^{-1}), PO_4^{3-} (1,321 mg kg^{-1}) y SO_4^{2-} (34,086 mg kg^{-1}) disponibles para plantas, así como de Na soluble en agua (26,106 mg L^{-1}). El pH y la conductividad eléctrica alcanzaron hasta 9.4 y 65 dS m^{-1}, respectivamente. Las concentraciones de Pb total superaron 1,110 el límite permisible de lo propuesto por la legislación Mexicana. Con base en la distribución espacial de los factores mencionados, se estableció un diseño de tratamientos factorial completo con *Acacia farnesiana*, *Casuarina equisetifolia*, *Cupressus lusitanica*, *Ecualyptus obliqua*, *Fraxinus excelsior*, *Prosopis laevigata* and *Pinus greggii* y dos enmiendas (vermicompost y vermicompost + aserrín). La supervivencia de las plantas varió de 83% a 100% y el crecimiento de cada especie fue afectado de forma diferente por las sales y metales pesados, así como por las enmiendas. Se encontró mayor crecimiento debido a vermicompost en *C. equisetifolia*, *C. lusitanica, E. obliqua* y *P. greggii* y debido a vermicompost + aserrín en *A. farnesiana, F. excelsior y P. laevigata. S*olo se observó efecto negativo de Pb disponible en *C. lusitanica.* El costo total de la presente investigación fue de MX $443,895. Además, debido a la supervivencia y crecimiento de las plantas, a pesar de la concentración de Pb en sus tejidos, se sugiere que todas las especies estudiadas podrían usarse en suelos seriamente afectados por residuos del reciclaje de baterías usadas de automóviles. Se concluye que se ha iniciado un proceso de bajo costo de dendro-remediación en el sitio de estudio.

Palabras clave: Dendro-remediación, enmiendas orgánicas, metales pesados, salinidad.

ABSTRACT

Lead acid Battery Recycling (LABR) wastes contain Pb, SO_4^{2-} and PO_4^{3} which can contaminate the environment and represent a risk for public health. Common methods to remediate contamination are based on chemical and physical process; however, they are expensive and their implementation is complicated. A useful technology is phytoremediation. Some tree species are able to growth on soils with elevated concentrations of heavy metals and salts, they produce a massive biomass, and then can be used for dendro-remediation. In the present work, a defunct lead acid battery recycling site was characterized in order to establish a dendro-remediation system. In addition, the site conditions were analyzed taking into account environmental Mexican legislation. This included an environmental impact assessment. It was observed that a LABR facility could affect severely the environment if control measures are not carried out. High total Pb (444,105 mg kg^{-1}) and extractable (12,458 mg kg^{-1}) concentrations were found, likewise available PO_4^{3-} (1,321 mg kg^{-1}), SO_4^{2} (34,086 mg kg^{-1}) and water soluble Na (26,106 mg L^{-1}). pH and electrical conductivity were 9.4 and 65 dS m^{-1}, respectively. Total Pb concentrations were up to 1,110 times above the maximum allowable limit set by Mexican environmental legislation. Based on the spatial distribution of factors mentioned, a factorial treatment design was established. Factor one was trees (*Acacia farnesiana*, *Casuarina equisetifolia*, *Cupressus lusitanica*, *Ecualyptus obliqua*, *Fraxinus excelsior*, *Prosopis laevigata* and *Pinus greggii*) and factor two, organic amendments (vermicompost y vermicompost + sawdust). Survival was from 83% to 100% and heavy metals and salts affected each species growth in different way. Nevertheless, only in *C. lusitanica* was observed a negative effect due to available Pb in soil. The highest growth was observed due to vermicompost in *C. equisetifolia*, *C. lusitanica, E. obliqua* and *P. greggii* and due to vermicompost + sawdust in *A. farnesiana, F. excelsior* and *P. laevigata*. Total cost of dendro-remediation was US $29,593. In spite of Pb accumulation in tissues, all tested species had high survival, good plant growth. Therefore, these tree species could be proposed as candidates to remediate extreme polluted soils under similar chemical and environmental conditions. Based on these results, it is possible to conclude that a successful low cost dendro-remediation process has started.

Keywords: Dendro-remediation, organic amendments, heavy metals, salinity.

GENERAL INTRODUCTION

Nowadays, the use of automobiles has incremented and proportionally the demand of energy to impulse them. A common battery in a vehicle is composed by Pb submerged in an electrolytic solution of H_2SO_4 (Table 1) and contains elements as Cu, Cd or Ni to foster oxidation-reduction reactions.

Table 1. Components of a typical lead acid battery.

Component	Percentage of weight
Pb (alloy) components (grid, poles)	25% - 30%
Electrode paste (fine particles of Pb and $PbSO_4$)	35% -45%
Sulfuric acid (10% - 20%)	10% - 15%
Polypropylene	5% - 8%
Other plastics (polycarbonate, polystyrene, PVC)	4% - 7%
Ebonite	1% - 3%
Other materials	< 0.5%

From van den Berg (2009)

When the device is aged, all the materials in it are reproached through lead acid battery recycling (LABR). In US, 99% of the LAB are recycled (EPA, 2015). This activity is highly efficient, up to 95% of Pb recovery has been reported and 80% of a common battery comes from recycling (Kreusch *et al.*, 2007). In Mexico these percentages have not been documented or the information is not available. In addition, 80% of the worldwide produced Pb is destined for LAB manufacturing and 95% of used LAB in Europe and US come from recycling (Kreusch *et al.*, 2007) and the same happens with 60-80% of the plastic in the device (EPA, 2015). LABR involves the recycling of the following components:

Plastic: The battery is dismantled by broken apart the plastic box. All the fragments go into a vat, where Pb and heavy materials sediment go down to the bottom and the light materials rise to the top. Plastics are washed, dried and sent to a plastic recycler plant to form small plastic pellets. This material is ready for manufacturing new battery cases (Guthrie, 1994).

Metal: Pb grids and electrodes are cleaned and melted in a furnace. Pb is poured into ingot molds. The impurities float in the ingot and are scraped away. Part of the Pb ($PbSO_4$, PbO_2 and $PbO.PbSO_4$) remains in the sludge.

Electrolyte: this solution is treated with sodium compounds to form sodium sulfate. The acid turns into water, which is treated, cleaned (removing salts) and released. Sulfuric acid can be reclaimed and reused in new batteries (Battery Council International, 2016). Previous steps can change depending of the available technology. In countries with low environmental standards and poor inversion in technology, the procedures for LABR are very risky because manual manage. This happens in many countries as Mexico and Senegal (Haefliger *et al*., 2009; Suplido and Ong, 2000; van der Kuijp *et al.*, 2013).

A LABR process also involves generation of materials and residues during recycling. The different stages can be impact negatively the environmental as the next list shows (Basel Convention/UNEP, Kreusch *et al*., 2007):

1. Grinding of the battery to separate plastic, electrolyte and Pb grid.
    a. Dust emission
    b. Leak of acid electrolyte containing Pb
    c. Particulate Pb
    d. Contaminated waste
    e. Salts deposition from the electrolyte and dust of poles
2. Pb reduction in a furnace
    a. Pb contaminated scraps
    b. Dust from filters containing Pb
    c. Emission of SO_2
    d. Chlorinated compounds emission
3. Separation of metallic Pb from slag, then refining
    a. Emission of Pb gases
    b. Emission of SO_2
    c. Fine dust containing Pb and other metals
    d. Chlorine gas

Due to these impacts, in some LABR sites, soil Pb concentrations reach up to 400,000 mg kg^{-1} (Blacksmith Institute and Green Cross Switzerland, 2013). The risk of pollute soils and water increases when the technology used for Pb recycling is obsolete and the waste management are inadequate. Hence, it is possible to identify two problems about a LABR polluted soil. The first one is the high Pb concentrations and the second is the presence of salts that comes from the solution of the battery. Pb is a heavy metal with toxic known effects to life. In humans, Pb can affect negatively every organ in the body, but the nervous system is the most susceptible (ATSDR, 2012). Therefore, it is necessary to avoid human exposition to residues generated by LABR. For this reason, it is essential to take actions to reduce Pb emission to the environment and to clean up already polluted sites. Nevertheless, heavy metals as Pb cannot be decomposed, only is possible to change its chemical form in order to reduce toxicity and its risk within the food chain (González-Chávez, 2005). Common methods to clean up a heavy metal polluted soil consist in chemical or physical treatment, which are expensive, complicated and in most cases soil needs to be transported from the polluted site to another place where it can be treated. Only transport itself involves exposition risk to people and environment. Moreover, salts affect plants modifying characteristics of soil as pH and increasing osmotic pressure outside the plant (Parida and Das, 2005)

In a salt and heavy metal polluted soil, common remediation should deal first with salt content to avoid chemical interferences. Only after this action, treatment to metals will be possible. Salt remediation increments the remediation cost of LABR sites, especially in developing countries where efficient technologies and economical resources are serious limitations. One rising alternative is the use of plants (phytoremediation) capable to growth under elevated concentrations of metal and salts, likewise the chemical conditions generated by them. An alternative to remediate using trees (dendro-remediation) is useful to cope with this problem. Trees have advantages in relation to other plants: the massive biomass can accumulate a great amount of heavy metals, the abundant root system is capable to explore a huge volume of soil (González-Oreja *et al*., 2008) and their perennial cycle makes cropping every year unnecessary.

However, establishment of plants on a polluted site is difficult because the soil toxicity. Pb exposure reduces germination (Thakkar, 2014), root elongation and growth of shoots (Islam *et al*., 2007). Nutritional and specific climate regimes requirements of plants are other difficulty. Hence, it is essential to select properly the species to use in dendro-remediation. A support technology is the use of organic amendments to help plants to growth in a polluted site. These materials can increase soil fertility and improve soil physical characteristics. In addition, many investigations show that organic matter stabilizes heavy metals and reduces their soil availability (Paz-Ferreiro *et al*., 2014). Other factor to take into account for any type of remediation is the legal regulation of the country. Because pollution and potential risk are public health and environmental problems, the government and population have an important function. In Mexico, there is a complex system of laws, bylaws and norms that regulate all the stages of contamination: generation of toxic residues, their management, remediation of a polluted environments and the liability of the person or industry that generates the problem.

The present investigation addresses the dendro-remediation of a defunct LABR site in Mexico. Twenty years ago, the recycling factory was operating, but the population of the municipality present complains against this industry because respiratory problems such as cough, headache and eye irritation were observed and were related to emissions of polluted air. Population forced closing of the factory and then, the situation was presented to the Federal Agency of Environmental Protection (PROFEPA by its abbreviation in Spanish). This agency found high Pb concentrations in soil and registered the site as a highly polluted site. In addition, PROFEPA ordered intervention to decontaminate the site and reduce the risk for human population. The agency asked some scientists from Colegio de Postgraduados to research a cheap option to remediate the site. The scientists explained to the people from the agency that none of the *in situ* techniques is a panacea for remediating contaminated soils, and it was necessary to test and optimize some procedures to clean-up the soil.

This thesis addresses the mentioned problem in order to perform a phyto-remediation strategy. This document consists of three chapters; the first one provides information of lead acid battery recycling regulation in the international context. It also makes a detailed description of the situation of the Mexican environmental legislation that has effect in LABR activity. For legal implications, a description of the site of study from the law perspective is presented. In addition, an environmental impact assessment of a hypothetical LABR factory in operation was performed. The analysis also included a comparison of a situation in where environmental care measures are applied in that LABR facility. This information was useful to determinate if a recycling plant establishment could be environmental friendly.

The second chapter is a detailed physico-chemical characterization of soil of the site. Data about electro-chemistry, soil solution heavy metal speciation, heavy metals fractionation, salts concentration and the spatial distribution maps of these variables through geostatistics. From this information, it was possible to determinate the degree of toxicity for plants and the risk for humans. The third chapter used the information from chapter II to design and establish a dendro-remediation experiment with seven tree species and two organic amendments. Additionally, the response of each tree species to heavy metals in soils and their effects in growth, as well as the metal accumulated in plant tissues are presented.

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CHAPTER I. MEXICAN ENVIRONMENTAL LEGISLATION IN RELATION TO LEAD ACID BATTERY RECYCLING POLLUTION

1.1 ABSTRACT

Recycling of lead acid batteries could be an environmental friendly source of lead (Pb) and plastic for industry, because it saves time and energy for mining Pb and hydrocarbons. However, there is lack of good practices for optimized recycling operations, which may pollute soil and water. Environmental legislation should protect natural resources and population rights to live in a healthy environment. In this chapter addressed Mexican environmental legislation system related to recycling of Pb acid batteries. It analyzed a case study in which a recycling factory at central Mexico closed and left a soil severely polluted mainly with Pb. This case study included an environmental impact assessment based on a hypothetical recycling facility in operation in order to determinate the environmental factors affected by LABR. In addition, it presents a brief revision of environmental legislation of Unites States of America and European Union on this topic.

Keywords: Hazardous wastes, lead pollution, environmental impact.

1.2 INTRODUCTION

Lead acid battery recycling (LABR) has advantages as low cost to produce basic supplies for the industry and reduction of wasted materials. Almost 90% of the used lead acid batteries (ULAB) components are recyclable and represent cost effective sources of Pb and plastic. Nevertheless, Pb may deposit in the environment during recycling. In addition, in minor proportion, ULABs are source of other elements as copper, iron, sodium and chloride and salts as sulfates (SO_4^{2-}) and phosphates (PO_4^{3-}) due to the ULAB composition (Chen and Dutrizac, 1996). Mexican laws consider that ULABs are hazardous materials. Therefore, LABR produces residues potentially harmful to the environment and population. The aim of environmental legislation is to reduce environmental impacts of risky activities like LABR. Once the environment is negatively affected, legislation points out liability to prevent and remediate damages. This chapter focused on discussion of Mexican environmental legislation and made a comparison to the American and European regulations. This thesis also presents a case study of a defunct LABR site as reference of the potential damages to environment from a soil polluted by ULABs recycling. The studied site is one of the most Pb polluted in the world as Chapter 2 shows. The site conditions were probably resulting of inadequate LABR waste management and poor environmental care measures.

1.3 MEXICAN ENVIRONMENTAL REGULATIONS

Mexican Federal Constitution is the basis for all environmental legislation in Mexico, which guarantees human rights (Article 1°) and the right of people in Mexico to live in a healthy environment (Article 4°). These articles pointed out that the government has to guarantee the respect for these rights and to affirm that damages to environment will generate charges for anyone who causes the injury.

Besides Constitution, there are laws that have recommendations to ensure protection to Mexicans rights. Laws are hierarchically followed by Regulations; which are administrative processes that allow execution of laws purposes. Then, Regulations are followed by Federal Official Mexican Norms (Norma Oficial Mexicana, NOM). The NOMs are technical regulations containing procedures and methodologies; which allow government departments to measure parameters to assess risks to people and environment. NOMs also set maximum permissible limits or thresholds of pollutants in natural resources (soil, water or materials as residues). Above that limits, remediation is required. All the laws, regulations and NOMs cited in this paper are related to LABR environmental impacts. Mexican Environmental Legislative framework was formulated for preventing environmental and health damages. Secretariat of Environmental and Natural Resources (Secretaría de Medio Ambiente y Recursos Naturales, SEMARNAT) is the institution in charge to generate and administrate this legislation.

Environmental legislation concerning LABR is: General Law of Ecological Equilibrium and Environmental Protection (Ley General de Equilibrio Ecológico y Protección al Ambiente, LGEEPA). This gives the directives for all environmental issues and the sustainable resources to approach in the national territory. LGEEPA and other laws support environmental Mexican legislation. For example: General Law for the Integral Management and Prevention of Wastes (Ley General para la Prevención y Gestión Integral de los Residuos, LGPGIR); which regulates the management and risk of hazardous wastes. It includes transportation, disposal, importation and exportation of wastes. Management of residues from LABR is not explicitly included in LGPGIR, but ULAB are listed in Article 31 of LGPGIR as a hazardous residue. ULAB also could be considered as wastes from mining and metal industry (Table 2, Hazardous waste definition/identification). Another law concerning LABR is the Federal Law of Environmental Liability (Ley Federal de Responsabilidad Ambiental, LFRA), it includes economic sanctions and actions that the damagers (physical or moral person that generates a damage the environment) have to carry out to reverse an environmental injury. Another important law related with LABR activity is Law of National Waters (Ley de Aguas Nacionales, LAN). It is involved because water could be polluted with Pb from LABR and generate adverse environment effects.

Regulations regarding LABR are: 1) regulations of LGPGIR (Reglamento de la Ley General para la Prevención y Gestión Integral de los Residuos; R-LGPGIR); which addresses management of residues (identification, disposal, storage, treatment), 2) regulations of LGEEPA for Prevention and Control of Atmospheric Pollution (Reglamento de la Ley General del Equilibrio Ecológico y la Protección al Ambiente en Materia de Prevención y Control de la Contaminación de la Atmósfera, R-LGEEPA-MPCCA); which regards prevention of atmospheric pollution from particles release, 3) regulations of LGEEPA about Environmental Impact (Reglamento de la Ley General del Equilibrio Ecológico y Protección al Ambiente en Materia de Evaluación de Impacto Ambiental, R-LGEEPA-MEIA); which regulates residues identification, storage and remediation of polluted sites, and 4) terrestrial transport of materials and hazardous residues (Reglamento para el Transporte Terrestre de Materiales y Residuos Peligrosos, R-TTMRP); which regulates residues transport outside from sites where these were generated.

Table 2. Environmental Mexican Legal System regarding LABR. Laws or regulations, chapters are presented in brackets.

Legal issue	LGEEPA	LGPGIR	R-LGPGIR	R-LGEEPA-MPCCA	R-LGEEPA-MEIA	R-TTMRP
Hazardous waste definition/identification	3 (XXXII)	5 (XXXII), 16, 31 (IV), 53	35		7	
Hazardous residues (generation and management)	150	21	24			
Federal jurisdiction	5 (VI)	7 (XVII)				
Registry of person who produces hazardous wastes		36	43	21		
Recycling		17, 28 (I)	49 (III), 87			
Environmental impact statement / risk study	30, 142, 28 (IV and XIII)		140			
Waste management federal authorization: imports and exports	153	1 (XI), 50 (X)	32			
Remediation responsibilities	152	62, 68, 69			56	
Types of management planning		28	16, 24			
Imports	142, 153	86	107, 108,110, 111, 115, 117			
Imports: remediation responsibilities		91	126			
Procedures for remediation of polluted sites/ residues treatment		42	132, 137, 143	17 BIS		
Illegal imports		92				
Hazardous waste tracking system		88				
Gathering of hazardous wastes			85			
Emissions to atmosphere	111			17		
Discharges of polluted residual waters to water bodies	121, 123					
Discharge of residues in soil	134					
Residue storage and registration of inputs and outputs of residues		65, 66, 67, 46	71		5	
Present informs of waste generation		46	72			
Environmental insurance		46				
Containers of hazardous residues, packing / identification						19, 23, 32
Isolation from other substances						23, 24
Requirements of vehicles which transport hazardous residues						33, 34, 46
Permission to transport						50
Information required to transport hazardous substances in disposition to the carrier						51, 52
Sanctions						136

ULAB or LABR wastes are not included in the NOM that list materials classified as hazardous residues (NOM-052-SEMARNAT-2005, NOM-052). NOM-053-SEMARNAT-1993 can be used to fill this gap; it describes procedures to identify a residue as hazardous when it is not listed in NOM-052. Hence, a material identified as hazardous must be treated according the legislation for this kind of materials. All industries of LABR operating in Mexico must satisfy requirements established by laws, regulations and NOMs of Mexican environmental legislation:

First, hazardous residues generators must identify the residues (Article 53 LGPGIR) and register the company as polluter to SEMARNAT (Article 36 LGPGIR). The company must have a management plan to be evaluated by SEMARNAT (Article 43 R-LGPGIR) and must present an environmental impact statement and a risk study (Articles 30, 142 and 28 of LGEEPA).

Second, the company should use proper residue containers (Article 46, section V, R-LGPGIR). In this way, R-LGPGIR establishes the following characteristics that all residue storage area must have (Article 82 R-LGPGIR): 1) Operation areas should be separated from areas of production, services, offices and, storage of final products and raw material, 2) the residue containers must be located in areas with minimal exposition risk was , 3) each factory must have devices such as walls, parapet or pits of contention to capture any possible leak, 4) in case of management of liquid residues (for example water from ULAB washing up process), the residue area must have channels or ducts which guide leaks to retention pits, 5) the area must have paths that allow transit of electrical or mechanical machinery and movements of security groups, 6) areas must have fire extinction systems and security emergency equipment, 7) areas must have signals that clearly identify the hazardous of the residue, 8) residue storage must be carried out in adequate containers considering physico-chemical characteristics of the residue and 9) the maximum height of containers is three barrels. Third, records of input/outputs of residues must be generated (Article 46 LGPGIR, Article 71 Fraction RLGPGIR). Fourth, a residue generator must remediate polluted soils as result of their industrial activity (Article 69 y 72 LGPGIR).

In order to remediation of polluted sites, NOM-147-SEMARNAT/SSA1-2004 (NOM-147) establishes maximum concentrations of As, Ba, Be, Cd, $Cr_{(VI)}$, Hg, Ni, Pb, Se, Tl and V in soil. In NOM-147, there are two thresholds (maximum permissible limits) useful to determinate if a soil need remediation. The first one based on total metal concentration in soil; it is called Total Concentration of Reference. This metal concentration does not consider if the metal is water-soluble, available for plants nor immobilized in non-soluble compounds. This value bases on health risk evaluation (NOM-147, paragraph 9). The second threshold bases on water-soluble metal concentration, which is an approximation to the concentration potentially carried or transported to groundwater. Total Pb concentrations mentioned in NOM-147 are 400 and 800 mg kg^{-1} for residential/agricultural or industrial use, respectively. The water-soluble threshold is 0.5 mg kg^{-1} for all uses. NOM-021-RECNAT-2000 (NOM-021) also refers a threshold concentration of Pb considered as dangerous for crops (>100-300 mg kg^{-1} extracted with a DTPA solution). NOM-147 declares that when a metal soil concentration exceeds one or more of the mentioned thresholds, the recommendation is to treat the soil. The goal of this treatment is to reduce metal availability and total concentration in the mentioned soil. After a treatment process, one of the following points must be reached (Page 5 of NOM-147, points 1-4): 1) total metal concentrations must be below the threshold value, 2) total metal concentrations must be below background total concentrations (natural metal concentration of soil), 3) specific concentrations (relation between total concentrations and toxic proportion based on availability for humans) must be low or 4) soluble concentrations must be below the sum of soluble concentrations of background samples plus the soluble threshold concentration.

LABR companies also manage NaOH and $NaCO_3$ compounds that are harmful substances. These chemicals are not listed in NOM-052, but they are corrosive and very reactive. NOM-052 mentions that these characteristics represent a risk for ecological equilibrium, environment and human health. For this reason, it is necessary to manage these compounds as hazardous materials and legislation must apply to them as residues.

In addition, it must be taken into account LAN regarding impact to water. Articles 87 of LAN mentions that Mexican authority will determinate parameters of pollutants in water bodies and Article 92 points out suspension of activities of a factory when their discharges to water bodies do not satisfy NOMs parameters. For that reason, any industry that discharges wastewater to rivers of body water should analyze their effluents (Article 94 BIS, LAN). This is supported by NOM-001-ECOL; which is related with maximum allowable limits for pollutants in residual waters to be discharged in national waters. This NOM refers 0.4 mg kg^{-1} as Pb threshold concentrations for river water used for agricultural irrigation. It becomes very important because water could carry Pb from LABR.

Based on Article 29, section II, wastewater from LABR process must be treated before discharge them in receiving bodies. NOM-001 (Table 2) mentions that 1 mg L^{-1} is the maximum allowable Pb concentrations in waters to be discharged in national waters. Permissible concentration is higher when discharges go to body waters used for irrigate agricultural soil (10 mg L^{-1}).

Other important NOMs to take into account are the related to security equipment for workers who manage hazardous materials. NOM-017-STPS-1994 specifies security equipment for personal safety in labor activities and includes specification for management of chemical substances to protect the whole body. Other NOMs specific for each protection equipment should be considered (NOM-030-STPS-1993 related to protection to respiratory system, NOM-113-STPS-1994 for foot protection, NOM-115-STPS- 1994 for head protection, NOM-116-STPS-1994 about respirator to filter harmful particles and NOM-117-STPS-1995 about protection gloves). All LABR factories should consider all these specifications to protect workers.

1.3.1 Disposal of hazardous wastes

Mexican environmental legislation gives technical specification about mitigation measures for hazardous residue storage areas. The first specification is the minimum distance to human settlement (5 km) stated in Article 65 of LGPGIR. In LABR site, the nearest distance is to 280 m. NOM-055-SEMARNAT-2003 mentioned requirements of a site to construct a disposal area. NOM-056-SEMARNAT-1993 gives specifications about design and construction of complementary works of a disposal site. NOM-057-SEMARNAT-1993 establishes the requirements to design, build and operate disposal cells and NOM-058-ECOL-1993 sets the requirements for operation of a disposal site.

1.4 REGULATIONS IN OTHER COUNTRIES

National pollution problems concerning LABR are important to other nations due to collaborations, trades or any activity regarding transportation of hazardous materials. One of the most developed environmental legislations for LABR comes from the United States of America Environmental Protection Agency (EPA). Since many years ago, this country has tried to promote ULAB recycling. Legislation is based on a general scheme suggested by the Battery International Council; which proposes no disposal of ULABS in municipal wastes. An inadequate management of these devices is considered as law violation. Moreover, batteries are classified as hazardous materials and should be managed under the Code of Federal Regulation 40 CFR, section 273.9 (EPA, 2016).

In Europe, ULAB are classified as dangerous goods, and any activity that creates, moves, or disposes them is subject to legislation. The region has adopted the 2005/64/EC directive of the European Parliament and of the Council Directive; which sets rules about recycling of vehicles and their parts when finishing service life. In relation to soil pollution, the European Union recommends threshold soil metal concentrations through the Joint Research Program to ensure crop production. In the case of Pb, that program consider as normal, a total soil concentration of 50-300 mg kg^{-1} (Gawlik and Bidoglio, 2006). The wide interval is proposed because the soil composition is very heterogeneous. In the same way, according to Kabata-Pendias (2011), threshold total metal concentrations proposed for agricultural soils in some countries are: Pb 50-300 mg kg^{-1}; Cu 60-500 mg kg^{-1}; Cd 2-20 mg kg^{-1}; Zn 200-1500 mg kg^{-1}, therefore a single threshold value for comparisons cannot be defined.

1.5 MULTINATIONAL AGREEMENTS

International trades have implications for environmental protection; they could promote the exportation and importation of used batteries. However, the excessive transportation of ULAB enhances the exposition risk to pollutants. For this reason, preventive agreements exist. One example is the Basel Convention (Secretariat for the Basel Convention/UNEP, 2002): its aim is to minimize transboundary movement and generation of wastes. This protocol sets a three steps strategy: 1) minimize the generation of wastes, 2) treat wastes as near as possible from the site they were produced and, 3) reduce transboundary movements of hazardous wastes. However, the amount of used batteries exported from the US to Mexico has increased in the last years. This is because in Mexico the cost of batteries recycling by melting down is lower than in the US as a result of restriction of airborne Pb emissions in the US and considerable differences in environmental and occupational regulations between these countries (OKI, 2011). These exportations are governed by the Organization for Economic Cooperation and Development (OECD). However, the Basel Convention and La Paz Agreement mentions that batteries should be managed as hazardous wastes; which is in accordance to the Mexican environmental legislation. OECD restricts exportation of hazardous materials to non-OECD countries unless the exporter demonstrates that the importer has disposal facility to do this. In the case of Mexico, used batteries are destined for recovery, hence batteries exportation procedures have been performed much more easily, without providing regular transportation documents or tracking information (Gottesfeld and Durand, 2010). Currently, from 30% to 60% of all batteries recycled in Mexico were imported from the US (CEC, 2013). However, this amount may be even higher according to one report from EPA and US Census Bureau. One report mentioned that used batteries exportation was carried out without applying the proper harmonized tariff code. In this report, it was recommended that Canada and Mexico governments should commit to achieve equivalent levels of environmental and health protections regarding Pb industry to those in the United States (CEC, 2013). Laborer health standards should also be reviewed and harmonized between these two countries.

ULAB recyclers take advantage of differences in environmental standards where Mexico has the less strict. Table 3 includes the main phases for LABR. In some cases, the difference is outstanding. Weak laws allow recycling batteries with low expenses without care on environmental and health security. During Pb recycling, factories emit Pb into the air, which is dispersed long distances to reach house areas and schoolyards. Johnson (2013) mentioned that people and workers usually are not able to organize or complain for this dangerous contamination condition.

There are also negative implications when shipping this hazardous waste to developing countries which are ill equipped to manage these materials in an environmentally friendly manner and there are few resources to mitigate health impacts arising out of the handling the wastes (Sonak *et al*., 2008).

Table 3. Comparison of standards for secondary Pb smelters in US, Mexico and Canada.

Standard	US	Mexico	Canada
End for stack (Pb)	10 μg m^{-1} max/stack 2 g μg m^{-3} facility wide	No standards	Ontario: point of impingement standards: 0.5 μg m^{-3} for 24 h, 0.2 μg m^{-3} for 30 day average
Fugitive	Enclosure, negative pressure	No required	Enclosure, negative pressure
Ambient air	0.15 μg m^{-3} over 3 month rolling period	1.5 μg m^{-3} (average over 3 month period[1]	0.5 μg m^{-3} for 24 h average and 0.2 μg m^{-3} for 30 day average
Permissible airborne exposure	50 μg m^{-3} average over 8 h period	50 μg m^{-3} average over year[2]	0.05 mg m^{-3} average over 8 h period
Blood Pb levels	60 μg m^{-3} or 50 μg dL^{-3} over an extended time period: industry voluntary standard for removal at 0.4 μg m^{-3}	10-70 ug dL^{-1} depending on age, occupation or pregnancy stage in women[3]	69.9 μg dL^{-3}
Transport	Sealed package and low pressure	Pealed package and low pressure system	No specified

Modified from (CEC, 2013). Mexico standards: [1] NOM-026-SSA1-1993, [2] NOM-025-SSA1-1993, [3] NOM-199-SSA1-2000.

ULABS or the wastes generated during recycling can be included in the following sections of Basel Convention: as Pb-acid batteries (Annex VIII, A1160: hazardous wastes) as toxic (Annex III, H11) or eco-toxic substances (Annex III, H12: Eco-toxic) and wastes having Pb (Annex VIII, A1020). This is an evidence of an international risk concern to environment and human health that LABR and ULAB management can produce.

However, Basel Convention needs to be accepted by the countries involved to give positive results. 183 parties form this convention, being Mexico one participant, signed the agreement in 1989 and ratified in 1991. Regulations began in 1992 (Basel Convention, 2015). Canada and all countries in Europe also signed Basel Convention. Nevertheless, US, a country which exports great amounts of used batteries (68% of the disposed batteries; CEC, 2013) to Mexico, has not even signed it. In 2011, US exported 389,539,360 kg of used batteries to Mexico (CEC, 2013). According to the RLGPGIR, the amount of imported wastes should not be higher than the amount produced in the country. However, in Mexico there is no available information of the amount of used batteries destined to recycling. A simple question should be answered: is this amount of used batteries imported from the US lower than the amount produced in the country?

It is possible to say that Basel Convention is only a guide, which does not force the members involved in this convention to specific actions. However, faculties of each country can do it through its own legislation in accordance to the mentioned convention objectives. For example, Article 93° of the LGPGIR mentioned that when hazardous wastes are generated in Mexico from imported products, machinery or any material to be remanufactured, recycled or re-processed must be sent to the country from which the imported material comes. But, only the material entered to Mexico in a modality called temporary importation regime must be returned to the exporter country (SAT, 2015). Otherwise, environmental liability derived from waste generation corresponds to Mexico and more specifically, to the company that generates the residue in accordance with the LFRA.

1.6 CASE STUDY

1.6.1 Site description and environmental vulnerability

The defunct LABR was located in central Mexico and its surface was 1041 m^2. The climate regime is C (w1) (w) with an annual average rainfall of 683 mm. The dominant winds go to the southwest (225°) at 8 km h^{-1}. The site is a fragmented ecosystem with ravine vegetation around it. Plant community is very disturbed. Some vegetal species found near the site were: *Ardea alba*, *Cynodon plectostachium*, *Buddleia cordata*, *Ligustrum vulgare* and, trees belonging to *Quercus*, *Eucalyptus* and *Opuntia* species.

At the East, there is vegetation such as: *Schinus molle* L., *Rynchelytrum repens* and *Bidens alba*, which are covering the soil in a 45° slope along 20 m. There is also a ravine and a permanent creek known as Río Viejo; which is fed by runoff from the highway (Figure 1).

At rain season (August), there is a water runoff from the site, which ends in the mentioned creek. It is probable that this runoff carries pollutants. To the East, there is a creek which flows to an established recreational park where fishing activities are performed. At the North, human settlements are approximately at 350 m (San Andrés de las Peras) and there is a vehicular bridge to the North. To the West, there is a human settlement to 280 m (Santa Inés), and to the South, there is a successful reforestation area.

The management performed by the former factory in this LABR site left the soil severely polluted with Pb and salts. Polluted soil in site is weathering and pollutants are continuously dispersing and negatively impacting the surrounding resources and human settlements. In the rain season (May to August), leachates flow to the creek in the West area. During dry season (November, December, January, March, April), wind carries dust from the site and mainly persons who use footprints are exposed. For that reasons, the present study discusses the LABR site condition that should be addressed from a legal perspective.

1.6.2 Legal implications of site conditions

In this site, soil was polluted with salts and Pb. Analysis showed that total Pb soil concentration ranged from 10,792 to 444,105 mg kg^{-1} (Chapter 2). This probably was due to inadequate waste management and poor control of process. Therefore, total Pb ranged from 27 to 1,110 times above the reference limit given by NOM-147 for residential use; which implies high risk for human health. These concentrations are 215 to 8,882 times above of the proposed permissible concentrations established by some countries for agricultural soil use (Kabata-Pendias, 2011). Hence, it means this site contains a soil that cannot support plant growth. In addition, maximum value for water-soluble Pb concentration (0.5 mg kg^{-1}, NOM-147) was exceeded 9.3 times in LABR site as Chapter 2 shows.

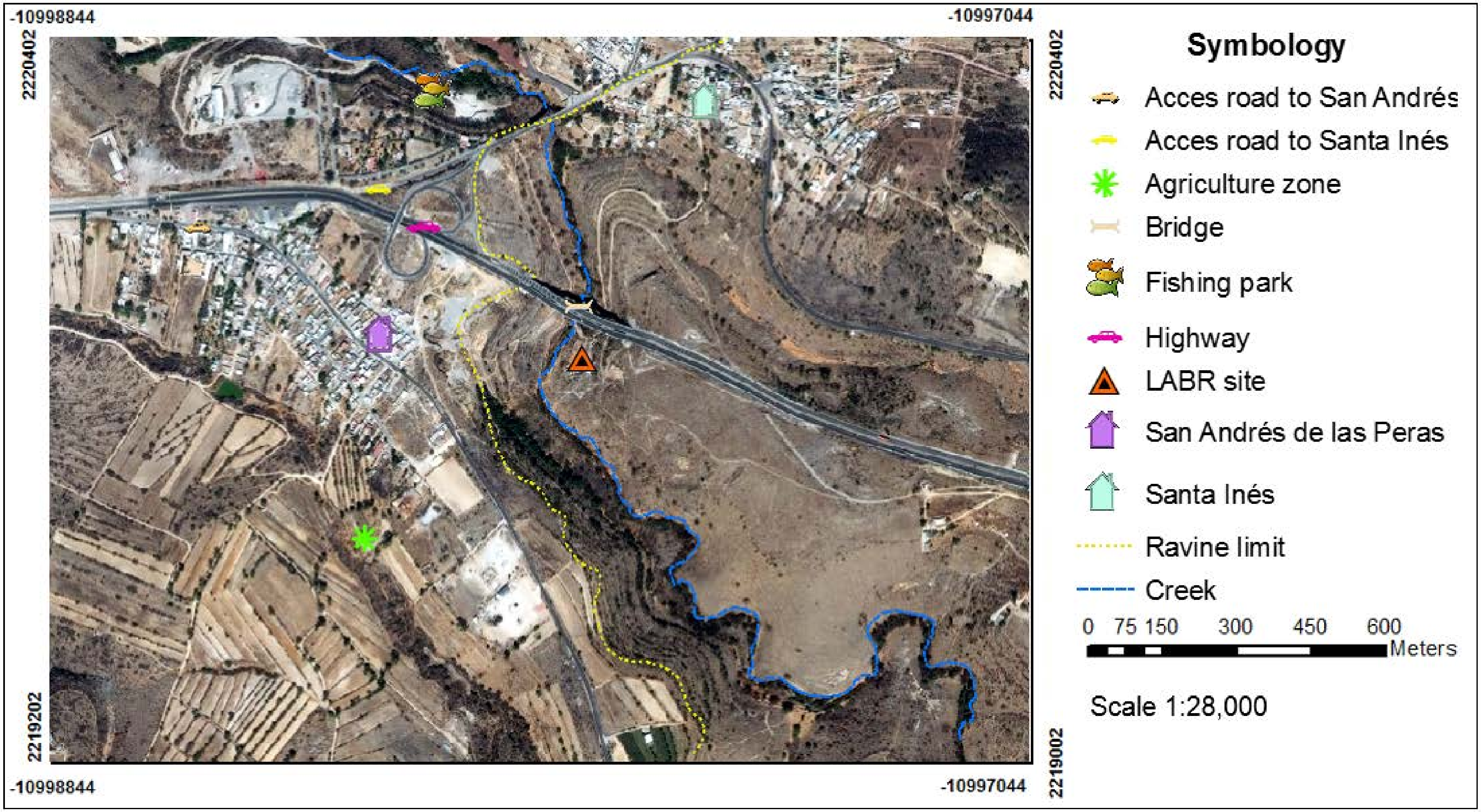

Figure 1. Identified vulnerabilities from a severely polluted soil by LABR.

NOM-147 is also supported by Article 117 from LGEEPA; which mentions that in order to avoid risk to ecosystems, the reduction of water pollution is fundamental. Articles 121 and 122 mention that discharges to water bodies, which contain pollutants, must be previously treated and Article 123 says that leachates must satisfied NOMs values.

Pb water-soluble concentrations in LABR site (Chapter 2) were up to 11.6 times above the maximum concentration set by NOM-001-ECOL-1996 for waters to be discharged in national waters as the creek near to the LABR site. In LABR soil, it is an empirical evidence of a leachate is dispersed. Based on the LGEEPA mentioned articles, this situation should be changed. Article 151 of LGEEPA refers that management, disposal and liability of hazard residues correspond to generators of these residues. In the same way, Article 152 BIS of LGEEPA forces to polluters to remediate polluted soils. Article 66 of LGPGIR; which forces to pollution generator to repair the damage caused also supports this.

However, there is no available information of the operators in LABR site. In this situation, Article 73 mentions that federation and municipalities will formulate programs to remediate. In LABR site, Pb concentration reached up 40-120 times (up to 12,000 mg kg^{-1}) the threshold mentioned by NOM-021 as dangerous for crops. Therefore, under three criteria for Pb concentrations included in Mexican regulations (total, water-soluble and dangerous for crops), the soil must be remediated. In addition, total Pb soil concentration, in the non-polluted areas near of the site (15 m far), was 306 mg kg^{-1}; which evidences an inadequate management of processes and residues disposal. It is important to mention that NOM-021 does not consider Cu, Fe, Zn and Mn as dangerous. pH, sodium and nitrogen concentrations found in LABR site are considered by that NOM as non-adequate for plants development (Chapter 2).

The site we studied can be described as an environmental contingency and an ecological emergency under the definition given by LGEEPA. This law defines an environmental risk as a situation which generates a negative impact to environment, ecosystems or any factors in them. Article 56 of R-LGEEPA-MEIA mentions that when an environment risk exists, SEMARNAT can apply the Article 170 of the LGEEPA, it is isolation of residues or their neutralization to avoid negative effects.

Due to the high Pb concentration in LABR site, it is highly recommendable to avoid any activity (agriculture or recreation) near of the site. There is no available information to define a secure distance. Eolic dispersion of soil particles containing Pb makes necessary to be careful, especially during dry season when particles can be carried easily by the wind.

High Pb concentration makes also necessary to manage the soil as hazardous residue; which should be treated. Chemical methods for remediation are constrained due to high salts and chloride content found in LABR soil (Chapter 2). They interfere with substances used in remediation processes and it is possible to emit toxic substances if chloride is heated.

An option to treat the LABR soil is its isolation as NOM-057-SEMARNAT-1993 specifies. To manage the soil in that way, the mentioned NOM sets the requirements to design, construct, and operate confinement cells for hazardous residues. However, this kind of construction can imply elevated costs. Chapter 3 gives a low cost alternative, which covers the soil with tree species to avoid exposition to pollutants. Moreover, this alternative provides the environmental services of (Chapter 3). However, NOM-147 does not consider the establishment of biological alternatives as a remediation criterion. In contrast, NOM-147 refers only to reduction of heavy metal soil concentration. Several scientific information has demonstrated that biological remediation alternatives have the following advantages: 1) Low cost or implementation, 2) it can be applied *in situ*, 3) it does not require highly specialized technicians and 4) it is applicable to various metals.

1.6.3 Environmental impact assessment of a hypothetical the LABR facility

This section presents an environmental impact assessment of a hypothetical LABR facility, which operated without any environmental care measure in the site studied. The hypothetical factory was based on the conditions and elements found in the soil site (metals and salt concentrations; Chapter 2), where total soil Pb concentrations left the soil as one of the most Pb polluted site in the world.

The aim of this analysis is to show how a LABR could damage environment. From the information generated, measures to protect environmental factors can be proposed and their application can be evaluated in terms of effectiveness to reduce or avoid the mentioned environmental damages. For this, a weighted matrix based on Leopold method was used (Leopold *et. al,* 1971; Conesa-Fernández-Vitoria, 1997).

Factors evaluated were: soil, water, air, vegetation, fauna, infrastructure, landscape (visual effect on ecosystem) and socioeconomic. Environmental impact assessment also allows detecting what factor is the most affected by each processes of a LABR operation. The analysis was conducted taking into account intensity, extension, persistence, effect, periodicity and recoverability. A positive (+) valuation is considered as beneficial and a negative (-) as damaging. A scale in multiples of 2 was used. Sum with high negative values means high negative effect on environment in relation to an optimal (Estado Ambiental del Factor con Respecto a su Óptimo, EAFRO). Comparison between EAFROs allows to determinate influence of each factor in environment and expresses it in comparable values (Unidades de Calidad Ambiental, UCAs).

Then, it is possible to determinate Absolute Impact (AI), where high values mean high negative impacts and hence, environmental care measures are very necessary (Table 4). The process included all stages of a LABR: ULABs, uses of energy for recycling process, separation of ULABs components, treatment of electrolyte for phosphorus and sulfur compounds, metal smelting, generation of residues and the obtained LABR final products (Pb^0, Cu, Ni, Fe, phosphorus and sulfur compound and plastic pellets):

1) Gathering
    - ULAB storage
    - ULAB transport (vial infrastructure for vehicles)
2) Inputs used
    - Na_2CO_3
    - NaOH
    - Source of carbon
    - Water
3) Energy consumption
    - Hammermill operation
    - Electrical
    - Fossil (gas, oil)

4) Separation of ULAB components by liquid immersion
    - Plastics
    - Electrolyte
    - Metal compounds of Pb, Cu, Fe, Ni, Zn
5) Electrolyte treatment which generates H_2SO_4, 2NaOH, Na_2SO_4
6) Plastic smelting
    - Polypropylene
    - Ebonite
7) Two process of metal smelting
    - First smelting: to carry out the reaction: $PbSO_4 + 2NaOH \leftrightarrow Na_2SO_4 + PbO + H_2O$
    - Second smelting: to perform the reaction: $PbO + C \leftrightarrow Pb^0 + CO_2$. In this process, also $PbSO_4$ is present. Not all of this compound reacts in first smelting because process is not completely efficient.
6) Residues generation
    - Sludge containing PbO_2, $PbSO_4$, $PbO.PbSO_4$, SO_4^{2-}, PO_4^{3-}, Na^+, Cl^-
    - Residual waters containing SO_4^{2-}, PO_4^{3-} , Na^+, Cl^-, Pb^{2+}
    - Gas emission ($PbSO_4$, PbO, SH_2, SO_X)
7) Generation of recycling products
    - Pb and other metals (Cu, Fe, Ni, Zn)
    - Pellets of polypropylene
    - Pellets of ebonite
    - Phosphorus and sulfur compounds

However, LABR also has positive effects in environment. ULABs could be disposed in dumps polluting soil and water. LABR generates pollution, but probably, it is not comparable if ULAB would not recycle due to recycling efficiency (95%, EPA, 1991). Other important benefit is the reduction of Pb mining with the consequent decrease of wastes from that industry which also could be a serious environmental problem (Dudka and Adriano, 1997). Reduction in Pb mining also represents a reduction in energy consumption and ecosystem alteration.

Factors that have positive environmental (services provide by LABR) were included in the impact matrix:

1) Pb mining reduction
    - Less residues generation
    - Less energy consumption
    - Less water consumption
    - Lower ecosystem alteration
2) ULAB recycling
    - Reduction of exposition to ULABs as residues
    - Less Pb, Cu, Fe, Ni, Zn, Na, Cl, Cd, Mn, Zn, SO_4^{2-} and PO_4^{3-} pollution

1.6.3.1 Environmental impacts

LABR process has negative interlinked impacts on the environmental factors. Even the recycling final recycling could damage environment (Table 4). Major negative effects are due to the residues generated (sludge, wastewaters and toxic gasses) which directly influence negatively soil, water and air with AI of -303, -188 and -249 respectively generating direct and residual impacts (effects that remains after an alteration). The incorporation of heavy metals and salts to soil by sludge and wastewaters and even depositions from air, limits directly vegetation establishment, which have an AI of -299. Chapters 2 and 3 discuss how LABR soil conditions constitute a very hostile environment to plants development. In addition, fauna near the facility are exposed to the pollutants in soil, water and air (AI: -254) and they are vulnerable because they could drink water from the creek near the site, which is an indirect effect on fauna due to water pollution.

Exposition to sludge and wastewater as well as the possible inhalation of toxic gases emitted during recycling may affect near population in similar way to fauna. These gases could reach greater distances than sludge and their impacts have greater extension. However, LABR workers had the most direct negative environmental direct impact (AI for socioeconomic factor: -361) because they are exposed closely to pollutants. They also manage all the harmful materials during recycling (ULABs, corrosive substances and residues). Also, economic benefits are only for workers and population does not obtain any economic benefit from LABR activity.

Another potential situation that impacts negatively population is consumption of plants exposed to heavy metals by animals. This incorporates that elements to the food chain, therefore fauna and people could be indirectly affected by vegetation, which absorbed metals. A residual adverse impact of this situation is that heavy metals are accumulated in organisms and their effects could be permanent. This may be an indirect impact on socioeconomic and fauna factors due to vegetation affectation.

Another indirect effect is on the fishing park at the north of the site. The wastewater and sludge affects directly the creek at the west of the LABR site (Figure 1), which flows to the north, making the park vulnerable to potential adverse impacts. Other adverse direct impacts are due to ULAB transport roads establishment because they damage the soil. That avoids plants growth and fragments ecosystems, which affects directly fauna. Animals paths are truncated by roads to communicate LABR facility. In addition, gas emissions influenced directly landscape (AI: -233), where toxic gasses had the higher negative impact due to extension that they reach.

Another important effect of LABR is on infrastructure (AI:-285). Recycling process as smelting and storage of residues, as well as ULABs and Na compounds management have a direct impact deteriorating installations that prevent pollutants dispersion. This constitutes a risk for environment and human health

Finally, environmental synergic impacts could be present in LABR operation. They are referred to interaction of impacts on environment that generates not only accumulated damages in the system, but the final effect of all impacts is stronger than a single sum of their effects. In any management of ULABs and their recycling residues, this kind of impacts exists due to the simultaneous pollution of soil, water and air, which constitutes a combination of impacts on environmental system, generating severe effects. Movement of ULABs to recycle them in other regions, such as the situation of Mexico, where massive amounts of these materials are imported (Section 1.5), also enhances this.

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Table 4 Weighted impact matrix of the hypothetical LABR factory established at central Mexico.

			Gathering		Inputs				Energy			Separation				Smelting				Residue			Products					Services							
																							Metal recycling					Pb mining, reduction in				LABR, reduction in			
ENVIRONMENTAL FACTOR		UCA	ULAB storage	Transport	Na_2CO_3	NaOH	Carbon	Water	Hammermill	Fossil	Gas Furnaces	Plastics	Electrolyte	Metallic component	Electrolyte treatment	Propylene	Ebonite	First smelting	Second smelting	Sludge	Wastewaters	Gas emission	Pb^0	Cu, Fe, Ni, Zn	Pellets polypropylene	Pellets ebonite	P and S compounds	Residues	Energy consumption	Water consumption	Ecosystem alteration	Residue exposition	Pollution	ABSOLUTE IMPACT	RELATIVE IMPACT
Physical	Soil	18	-58	-17	-17	-23	-9	-9	-9	-11	-9	-11	-16	-16	-17	-9	-9	-14	-14	-42	-34	-22	-11	-11	-9	-9	-11	23	15	15	15	23	23	-303	-55
	Water	11	-9	-9	-13	-13	-9	-9	-9	-9	-9	-9	-19	-19	-19	-9	-9	-15	-15	-9	-48	-9	-10	-10	-9	-9	-18	26	15	24	24	24	24	-188	-20
	Air	16	-7	-19	-9	-9	-9	-9	-9	-12	-21	-9	-9	-9	-12	-9	-9	-30	-30	-10	-10	-48	-9	-9	-9	-9	-20	17	15	15	15	17	17	-249	-40
	Landscape	10	-24	-24	-9	-9	-9	-9	-9	-9	-9	-9	-9	-9	-9	-13	-13	-24	-24	-24	-24	-28	-11	-10	-9	-9	-15	18	20	20	20	20	20	-233	-24
	SUBTOTAL	**55**	**-98**	**-69**	**-48**	**-54**	**-36**	**-36**	**-36**	**-41**	**-48**	**-38**	**-53**	**-53**	**-57**	**-40**	**-40**	**-83**	**-83**	**-85**	**-116**	**-107**	**-41**	**-40**	**-36**	**-36**	**-64**	**84**	**65**	**74**	**74**	**84**	**84**	**-973**	**-139**
Biotic	Vegetation	10	-30	-32	-14	-14	-9	-9	-9	-9	-9	-9	-14	-14	-14	-9	-9	-11	-11	-42	-44	-27	-14	-14	-9	-9	-16	19	15	15	15	19	19	-299	-29
	Fauna	10	-10	-26	-9	-9	-9	-9	-12	-9	-12	-9	-10	-11	-10	-9	-9	-24	-24	-26	-32	-36	-14	-14	-9	-9	-19	20	18	18	18	21	21	-254	-25
	SUBTOTAL	**20**	**-40**	**-58**	**-23**	**-23**	**-18**	**-18**	**-21**	**-18**	**-21**	**-18**	**-24**	**-25**	**-24**	**-18**	**-18**	**-35**	**-35**	**-68**	**-76**	**-63**	**-28**	**-28**	**-18**	**-18**	**-35**	**39**	**33**	**33**	**33**	**40**	**40**	**-553**	**-54**
	Infrastructure	9	-21	-23	-19	-19	-9	-9	-19	-10	-12	-10	-11	-11	-17	-9	-9	-30	-30	-26	-28	-28	-9	-9	-9	-9	-9	18	18	18	18	19	19	-285	-25
	Socio-economic	17	-14	-23	-16	-18	-9	-9	-9	-9	-11	-9	-14	-14	-22	-12	-12	-30	-30	-44	-32	-48	-20	-13	-9	-9	-20	16	15	15	15	16	18	-361	-60
	SUBTOTAL	**25**	**-35**	**-46**	**-35**	**-37**	**-18**	**-18**	**-28**	**-19**	**-23**	**-19**	**-25**	**-25**	**-39**	**-21**	**-21**	**-60**	**-60**	**-70**	**-60**	**-76**	**-29**	**-22**	**-18**	**-18**	**-29**	**34**	**33**	**33**	**33**	**35**	**37**	**-646**	**-85**
	TOTAL	**100**	**-173**	**-173**	**-106**	**-114**	**-72**	**-72**	**-85**	**-78**	**-92**	**-75**	**-102**	**-103**	**-120**	**-79**	**-79**	**-178**	**-178**	**-223**	**-252**	**-246**	**-98**	**-90**	**-72**	**-72**	**-128**	**157**	**131**	**140**	**140**	**159**	**161**	**-2,172**	**-278**

1.6.3.2 Environmental care measures

To reduce negative environmental impacts mentioned before, preventive (to avoid damages), corrective (to repair a negative affected condition) and mitigation (to palliate a generated injury) measures are always necessary to establish and operate a LABR facility. For this reason, a complementary matrix of these measures was included in the impact assessment. The aim is to show how LABR could be an environmental friendly activity if the mentioned measures are rigorously carried out according to Mexican environmental legislation. The following points are the measures introduced and the legislation that regulates them:

1. Gathering and transport
    - Infrastructure for ULAB storage (Article 82 R-LGPGIR)
    - Identification of vehicles as hazardous materials carriers (Article 31 R-TTMRP, NOM-004-SCT/2008)
    - Containers for hazardous materials during transport (Article 19 R-TTMRP, NOM-003-SCT/2008)
    - Security equipment for workers for raw ULAB management (transport and management, NOM-017-STPS-1994)

2. Inputs
    - Containers for corrosive substances (NOM-003-SCT/2008)
    - Infrastructure for corrosive substances (Article 82 R-LGPGIR)
    - Labeling of corrosive substances containers (NOM-018-STPS-2015)
    - Water containers to avoid leaks
    - Security equipment for inputs management (NOM-017-STPS-1994)

3. Energy
    - Infrastructure for noise reduction (NOM-081-SEMARNAT-1994)
    - Infrastructure for gas storage (NOM-009-SESH-2011)
    - Infrastructure for oil storage (NOM-EM-001-ASEA-2015)
    - Supervision of oil and gas services (NOM-EM-001-ASEA-2015)
    - Labels of gas, water and electricity conducts (NOM-026-STPS-2008)

4. Component separation (immersion)
    - Operative design to prevent accidents (NOM-001-STPS-2008)
    - Leachate collector to water treatment plant (Article 82 R-LGPGIR)

5. Electrolyte treatment
    - Security equipment for workers while electrolyte treatment (NOM-017-STPS-1994)

6. Smelting
    - Operative construction of furnaces to reduce heat (NOM-001-STPS-2008)
    - Extinguishers installation (NOM-002-STPS-2010)
    - Evacuation routes (NOM-002-STPS-2010, NOM-028-STPS-2012)
    - Suits against burning (NOM-017-STPS-2000)
    - Scrubbers to depurate air (maximum Pb emissions: NOM-026-SSA1-1993; maximum PM (10) particles emissions: NOM-025-SSA1-1993)

7. Residue
    - Security equipment for workers that manage hazardous wastes (NOM-017-STPS-1994)
    - Sludge treatment (Article 42 LGPGIR, Articles. 132, 137, 143 R-LGPGIR, Art. 17 LGPGIR)
    - Identification of residues in storage containers (Article 46, section IV, R-LGPGIR, NOM-003-SCT/2008)
    - Hazardous residues storage area (Article 82 R-LGPGIR, NOM-057-SEMARNAT-1993, NOM-055-SEMARNAT-2003, NOM-056-SEMARNAT-1993)
    - Treatment of residual water and leachates (Article 135 section II of Regulation of LAN, NOM-001- SEMARNAT-1996)
    - Temporary residues storage to prevent exposition (NOM-056-SEMARNAT-1993)
    - Scrubber filters supervision

8. Products
    - Storage of Pb^0, Cu, Ni and Fe in containers for hazardous materials with detailed labels (NOM-003-SCT/2008)
    - Sending of hazardous residues to confinement centers
    - Plastics packing
    - Sending plastics to recycling centers
    - Packing of phosphorus and sulfur compounds in containers for corrosive substances (NOM-003-SCT/2008)
    - Sending of phosphorus and sulfur compounds to chemical industry

Table 5. Weighted impacts matrix of mitigation measures of the hypothetical LABR factory established at central Mexico.

			Preventive																					Corrective				Mitigation					Mitigation/Corrective						
ENVIRONMENTAL FACTOR		UCA	Infrastructure for ULAB storage	Identification of vehicles which transport ULAB	Suits against burning	Containers for hazardous materials during transport	Security equipment for workers for ULAB management	Containers for corrosive substances	Infrastructure for corrosive substances	Labeling of corrosive substances	Water containers to avoid leaks	Security equipment for inputs management	Infrastructure for noise reduction	Infrastructure for gas storage	Infrastructure for oil storage	Supervision of oil and gas services	Labels of gas, water and electricity conducts	Operative design to prevent accidents (Facility)	Security equipment for workers (electrolyte treatment)	Operative gas furnaces construction to reduce heat exposition	Extinguishers installation	Evacuation routes	Identification of residues in storage containers	Leachate collector to treatment.	Hazardous residues storage area	Scrubber filters supervision	Storage of Pb_0, Cu, Ni, Fe in containers	Scrubbers to clean air	Security equipment for workers that manage hazardous wastes	Sludge treatment	Treatment of wastewater	Temporary residue storage	Sending of hazardous residues to disposal centers	Plastics packing	Sending plastics to recycling centers	Packing of phosphorus and sulfur compounds in containers	Sending phosphorus and sulfur compounds to chemical industry	ABSOLUTE IMPACT	RELATIVE IMPACT
Physical	Soil	18	19	15	18	21	15	21	21	15	18	15	16	19	19	16	15	18	18	19	16	15	18	19	21	13	21	15	15	27	27	21	14	16	14	16	14	620	109
	Water	10	19	15	15	17	15	19	19	15	19	15	15	16	16	13	15	18	15	15	15	15	15	21	21	13	21	15	15	27	27	21	14	16	14	19	13	593	58
	Air	10	16	15	18	19	15	19	16	15	15	15	16	19	19	16	18	18	15	21	19	15	15	19	19	23	19	27	15	21	19	19	14	14	13	17	13	606	58
	Landscape	9	19	15	15	16	15	19	19	15	18	15	19	18	18	16	15	19	15	19	15	18	15	19	19	19	19	25	15	25	22	19	16	17	16	17	16	617	58
	SUBTOTAL	**46**	**73**	**60**	**66**	**73**	**60**	**78**	**75**	**60**	**70**	**60**	**66**	**72**	**72**	**61**	**63**	**73**	**63**	**74**	**65**	**63**	**63**	**78**	**80**	**68**	**80**	**82**	**60**	**100**	**95**	**80**	**58**	**63**	**57**	**69**	**56**	**2,436**	**283**
Biotic	Vegetation	10	19	15	15	19	15	19	19	15	16	15	15	16	16	13	15	18	15	16	15	15	15	21	19	13	19	15	15	27	27	19	13	17	13	17	13	584	58
	Fauna	18	19	15	15	19	15	19	19	15	18	15	21	19	19	13	15	15	15	18	15	15	15	21	21	23	19	27	18	27	27	19	13	17	13	17	13	624	110
	SUBTOTAL	**28**	**38**	**30**	**30**	**38**	**30**	**38**	**38**	**30**	**34**	**30**	**36**	**35**	**35**	**26**	**30**	**33**	**30**	**34**	**30**	**30**	**30**	**42**	**40**	**36**	**38**	**42**	**33**	**54**	**54**	**38**	**26**	**34**	**26**	**34**	**26**	**1,208**	**168**
	Infrastructure	9	19	15	15	19	15	19	19	15	18	15	21	19	19	13	15	15	15	18	15	15	15	21	21	23	19	27	18	27	27	19	13	17	13	17	13	624	57
	Socio-economic	17	19	19	19	19	19	19	19	18	18	19	19	19	19	16	19	19	18	19	19	19	19	18	21	23	19	25	19	27	27	19	16	17	16	17	16	673	114
	SUBTOTAL	**26**	**38**	**34**	**34**	**38**	**34**	**38**	**38**	**33**	**36**	**34**	**40**	**38**	**38**	**29**	**34**	**34**	**33**	**37**	**34**	**34**	**34**	**39**	**42**	**46**	**38**	**52**	**37**	**54**	**54**	**38**	**29**	**34**	**29**	**34**	**29**	**1,297**	**171**
	TOTAL	**100**	**149**	**124**	**130**	**149**	**124**	**154**	**151**	**123**	**140**	**124**	**142**	**145**	**145**	**116**	**127**	**140**	**126**	**145**	**129**	**127**	**127**	**159**	**162**	**150**	**156**	**176**	**130**	**208**	**203**	**156**	**113**	**131**	**112**	**137**	**111**	**4,941**	**622**

The application of the measures mentioned in accordance to NOMs resulted in positive influence on environmental valuation of the LABR hypothesized facility (Table 5), where some of them had high positive impacts on specific environmental factors. Nevertheless, it is necessary to consider all measures because they are interlinked and must be applied together during ULAB recycling process.

First, infrastructure for storage hazardous materials (ULABs and inputs) as well as the use of adequate containers and equipped vehicles during transport, likewise in the storage in LABR facility, prevent accidents and exposition of pollutants to soil (IA: +620) and avoid indirect impacts to water. In addition, roads to access to the LABR facility promote communication of population with the ravine (socioeconomic AI: +673).

During LABR operation, preventive measures as labeling of containers which storage harmful substances avoid contact with persons. The operative design of the facility and its equipment with extinguishers and evacuation routes minimizes the risk of accidents. Similarly, security equipment for workers along LABR process, mainly during electrolyte treatment and the management of hazardous wastes, prevents injuries due to the contact with those hazardous materials.

Furthermore, the use of scrubbers to prevent air pollution in a local extension is a mitigation measure with positive impact on environment valuation because it focuses on reduction of residues toxicity; making air less vulnerable (air AI: +606). In addition, sludge and wastewater treatment in a plant, as well as sending them once treated to disposal centers, is a corrective measure that limits contact of pollutants with soil and water (water AI: +593). Similarly, in a residues treatment plant, environmental measures prevent not predicted impacts as incorrect functioning of plants elements and unexpected situations as floods that could cause spillovers. Those measures are included in the infrastructure construction in where the plant and residues containers are located.

As a result of hazardous residues management, vegetation, fauna and population are less exposed to pollutants. In addition, the adverse indirect effects mentioned in the previous section (1.6.3.1) on fauna due to vegetation negative impact will be minimized because plants consumed by animals will be less adversely affected by

LABR processes. Therefore, a positive consequence is the reduction of heavy metals introduced in food chain because soil and plants are not exposed to these pollutants.

At the final of LABR process, packing plastics, sulfur and phosphorus compounds, as well as sending LABR products to chemical industry had a positive impact on environment. The reason is the prevention of accidents that could exposes that compounds to environment, making mainly soil and water vulnerable. Toxic compounds will not be disposed in dumps or exposed in environment.

It is important to note the necessity to include periodic supervisions of installations as preventive measures. For example, constant check for leaks, damages in containers or change filters in air scrubbers to ensure an adequate operation of LABR process.

Finally, a LABR factory will be environment friendly if and only if all preventive, corrective and mitigation measures mentioned are applied and supervised in all their stages (planning, construction, operation and abandonment). In the hypothesized LABR facility, the absolute environmental impact is positive in 4,941 of AI and compared with the total absolute impact (-2,172), there is a difference of +2,769. This gives an environmental feasibility of 43% (100*environmental impacts AI /environmental impact measures AI) which means negative impacts could have a strong effect if environmental measures are not applied.

Moreover, another benefit of the measures mentioned is the synergic impacts minimization, prevention and control, because that measures work together on all factors of the environmental system. This information is helpful to design of plans and programs for hazardous residues management as an element of a Strategic Environmental Evaluation. This is an instrument aimed to integrate environmental issues in programs, plans and policies, likewise their functioning in long term. This includes considerations, not only in a site, but also at regional level including countries. Hence, it takes into account internal and international policy as well as boundaries. The importance of this instrument is also the incorporation of an environmental approach of all sectors implicated.

1.7 RECOMMENDATIONS FOR MEXICAN ENVIRONMENTAL LEGISLATION

First, biological based techniques as phytoremediation could be included as an option to remediate polluted soils. Based on NOM-147 aim is to reduce the risk of exposition to heavy metals to ecosystems and people; a novel option is covering the soil with plants. These techniques involve use to immobilize toxic elements in the same site (phytostabilisation) as Chapter 3 referred and could be more appropriated than common remediation. This option has advantages compared with commonly used technologies (chemical and physical alternatives) because is low cost, low time consuming and does not require highly trained technicians. An example of phytoremediation was carried out in Chapter 3 where tree species were introduced in the LABR site. However, there is a conflict with Mexican legislation that should be resolved. Section 2 of Article 149 of R-LGPGIR prohibits the use of biological agents to treat leachates *in situ*. It argues an uncontrolled transference of pollutants from one media to another. This regulation should be reconsidered because biological remediation can be modeled and controlled for *in situ* treatment. Therefore, to prove the effectiveness of biological organisms use to reduce pollution, it will be necessary to evaluate their impacts constantly.

Another recommendation is the harmonization of NOMs thresholds for heavy metal pollution assessment with European Union and US; they have stricter standards that imply minor risk to public health and environment. In addition, the LABR activity should be included in joined plans and programs between the countries, supported through a strategic environmental evaluation.

Other point is that Mexican NOMs should develop thresholds values for plants because natural ecosystems and agricultural systems react in different way to pollutants than humans. NOM-021 sets dangerous metal concentrations to crop plants; which also could be included as thresholds in NOM-147; which proposes a single reference of total concentrations for residential and agricultural land use in as maximum allowable value to proceed to remediation. Nevertheless, natural ecosystems are not discussed in this NOM, therefore pollution of these ecosystems should be included in land use planning to proceed to their management. In the same way, potential negative effects of LABR makes necessary to plan the location of recycling facilities. It is preferable to set facilities in industrial zones far from human settlements, where ecosystems are not in risk in accordance with the aim of LFRA.

Another recommendation is that Mexican regulations concerning heavy metals must include Cu, Mn and Zn in NOM-147 and NOM-021 as risky elements and develop maximum allowable limits in soil to prevent public health and crop plant affectation.

Mexican environmental legislation should generate a system or a productive chain to promote LABR, from gather ULABs by retailers and wholesalers as well as collection center establishment to carry ULABs to LABR facilities. In order to avoid dump of these devices and to prevent human exposition. Regulation of LABR process is compulsory.

1.8 CONCLUSIONS

In Mexico, there is an environmental legislation system to prevent and remediate environmental negative impacts generated by LABR in soil, water and air mainly due to waste disposal. When that system is not applied properly and preventive, corrective and mitigation measures are not carried out, LABR will have strong negative impacts on environment as the case study showed in this study. Mexican environmental legislation should be enriched with the recommendations given such as consideration of phyto-remediation technologies to prevent pollutants dispersion especially in rural areas where economic resources are limited.

In the international context, there are differences in legislation between US, Canada and Mexico. Canadian and US have much stricter environmental standards than these from Mexico. As a result, there is an opportunity to these two developed countries to process massive amounts of ULABs in Mexico and reduce costs in environmental care despite as Basel Convention. Therefore, standards for secondary Pb smelters should be harmonized in the three countries in order to avoid that Mexico becomes the unique and common deposition country of the wastes produced by the tree mentioned countries.

All the mentioned points must be performed in accordance with the Mexican environmental legislation in order to achieve the goal established by the Mexican Federal Constitution; where each person in Mexico has the right to live in a healthy environment.

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CHAPTER II POLLUTION ASSESSMENT OF A DEFUNCT LEAD ACID BATTERY RECYCLING SITE IN MEXICO

2.1 ABSTRACT

Unsuitable waste management from lead acid battery recycling (LABR) industry can severely pollute soil and water. The aim of this work was to assess heavy metals contamination of a defunct LABR site in Mexico as a basis for designing a phytoremediation strategy. Total, DTPA-extractable, water soluble-metal concentrations and fractionation were analyzed. Physical and chemical soil characterization was also performed. Speciation of Na and Pb, main contaminants in recycling residues, was calculated based on soil solution analysis. Spatial distribution maps of the measured variables were drawn through Kriging. High variability in all chemical properties was found on the terrain. Pb and Cu were the riskiest metals in the site. Total Pb and Cu concentrations reached up to 474,105 mg kg^{-1} and 3,911 mg kg^{-1}, respectively. While, the highest DTPA-extractable Pb concentration was 12,000 mg kg^{-1} and water soluble Pb was 4.5 mg kg^{-1}. High available concentrations of PO_4^{3-} (1,321 mg kg^{-1}), SO_4^{2-} (34,086 mg kg^{-1}) and Na (33,101 mg kg^{-1}) were also found. Pb fraction concentrations decreased in the following order: exchangeable>bond onto sulfides> weakly bound onto organic matter>bound onto Fe and Mn oxides> residual phase> strongly bound to organic matter. The maximum exchangeable Pb value was 284,580 mg kg^{-1}. Predominant chemical species in soil solution were Pb^{2+} (up to 86%) and Na^+ (up to 99%). Soil available nitrogen content was low, especially on the south of the terrain. The phytoremediation strategy should consider vegetal species capable to establish and growth under Pb and Cu pollution conditions, poor soil fertility levels and salinity.

KEYWORDS: Pb spatial variation, heavy metals, salinity, Pb speciation.

2.2 INTRODUCTION

Lead acid batteries (LAB) are widely used for many industries as source of electric energy in every single vehicle. Used batteries are considered as hazard waste due to their metal content. Lead acid battery recycling (LABR) generates vapors during Pb melting that can be emitted to the atmosphere and increase exposition risk (van der Kuijp *et al.*, 2013). LAB are built with metal grids (25-30% of weigh), electrode paste (35- 45%), sulfuric acid solution (10- 15%), connectors and poles of Pb alloy, grid separators made up of PVC (5-8%), ebonite (1-3%) and a plastic case. LAB cells are composed of a Pb electrode (anode) and a Pb oxide electrode (cathode) immersed in a solution of sulfuric acid; metallic grids and connections (Zhang, 2013). Continuous processes as corrosion or sulfation age the batteries (Ruetschi, 2004). LABR tries to recover Pb and plastic from batteries. In US ninety-nine percent of LAB are recycled (EPA, 2015), and the efficiency of Pb recovery in 1990 was up to 95% (EPA, 1991). In Mexico, this information is not documented or available. In the world, 80% of the produced Pb is destined for LAB manufacturing and 95% of Pb used in batteries comes from recycling. LABR involves the following steps:

The batteries are dismantled by broken apart the plastic box, usually in a hammer mill. Once milled, the pieces are separated in a vat: heavy materials sediment to the bottom and the light materials rises to the top. Pb grids and poles, and Pb oxides are cleaned and latter melted in a furnace. Pb is poured into ingot molds and after few minutes, the impurities float in the ingot and are scraped away (Volpe *et al*., 2009). Part of the Pb ($PbSO_4$, PbO_2 and $PbO.PbSO_4$) remains in the sludge. The electrolytic solution of the battery can be recycled as sodium sulfate crystals or sulfuric acid can be reclaimed and reused in new batteries (Kreusch *et al*., 2007).

Because LABR manages hazardous substances, good control of the processes and wastes disposal is required (Nedwed and Clifford, 1998) and when are not correctly performed, Pb can seriously pollute soil and groundwater (Mohammed *et al*., 1996). Some polluted soils are associated to Pb recycling industry (Palacios *et al*., 2002). In developing countries, LABR involves inadequate process and residues disposal. Hence, workers involved on LABR are highly exposed to contaminants because they manipulate the material by hand without safety equipment.

Pb is the second priority risk substance by the Agency for Toxic Substances & Disease Registry at sites in the National Priorities List of United States (ATSDR, 2015). Blacksmith Institute and Green Cross Switzerland reported the world worst polluted sites, these sites are commonly related with LABR industries and Pb deposition. Places contaminated by LABR were the first problem of the 2012 and 2015 lists (Blacksmith Institute and Green Cross Switzerland, 2013; Pure Earth/Green Cross Switzerland, 2015).

The present research involves a LABR plant, which was closed by the Mexican Environmental Agency due to complaints from people living in the vicinity. This agency suspected of high Pb concentrations in LABR soil. Nevertheless, there were no files or available information about the procedure used for recycling batteries or the protocol for waste management. Hence, Mexican Environmental Agency asked to evaluate no conventional procedures to remediate this defunct LABR site. To clean up polluted soils, physico-chemically methods, which involve high cost, workforce and trained technicians (Ruiz-Olivares *et al*., 2013) are commonly used. As alternative, it has been proposed the use of plants (phytoremediation) tolerant to heavy metals to remediate contaminated soils. Vegetal species can cover the soil and hence prevent pollutants dispersion. In order to define a phytoremediation strategy, it is necessary to provide precise information of the conditions of the soil to be treated. For this reason, single (total and extractable metal concentrations) and sequential extractions were used to determinate plant available metal, and metal distribution on soil fractions. Hence, the aims of this study was to assess concentrations of metals and salts and their spatial variability on the soil of a defunct LABR factory located at Mexico State in the municipality of Tepetlaoxtoc. The present research may provide specific information of the site conditions as a basis to design a phytoremediation strategy which consists in plant species selection, structure and maintenance of planting as well as generation of experimental design and experiment monitoring for research purposes.

2.3 MATERIALS AND METHODS

2.3.1 Soil sampling

LABR soil was sampled systematically (Gilbert, 1987). A grid of 5 m x 5 m was made and 54 soil composed samples were taken on each interception (5-20 cm depth). Moreover, six soil samples near of LABR site were taken to determinate natural soil heavy metal concentrations.

2.3.2 Physico-chemical analysis of soil

2.3.2.1 Fertility

Immediately after soil collection, oxide-reduction potential (ORP) was measured in soil samples in a 1:2.5 soil:water ratio slurry with a pH-meter (ThermoOrion 420A) using ORP electrode. The equipment was calibrated using the Light’s solution. After ORP determination, the soil was air dried and sieved (2 mm) prior to other analyses. Plant available nutrients: phosphorus (PO_4^{3-}; Olsen *et al*., 1954), sulfur (SO_4^{2-}; turbidimetric procedure, Walker, 1972) and nitrogen (KCl extraction and Kjeldahl distillation; Bremner, 1965) were measured. In addition, total carbonate was determined by calcimeter method (Loeppert and Suarez, 1996) and organic matter (OM) by loss on ignition method (Rowell, 1994).

2.3.2.2 Soil solution analysis

Liquid extract from saturation paste (USDA, 1954) was obtained in order to determinate soil solution composition and characteristics. pH was measured with an Orion Research 420A pH-meter, electrical conductivity (EC) with a Fisher Brand 09-325-360 conductivity-meter and ORP with a pH-meter (ThermoOrion 420A with an ORP electrode). The following water soluble salts were measured in the extract: SO_4^{2-} (turbidimetric; modified 9038 method; EPA, 1986), PO_4^{3-} (colorimetric), HCO_3^- and CO_3^{2-} (H_2SO_4 titration), Cl (argentometric), Ca and Mg (EDTA-Titration), Na and K (flame photometric). These PO_4^{3-} and SO_4^{2-} differ from the determined in in the section 2.2.2, because soil solution is related with soluble compounds which react in the liquid phase of the soil (soil solution) and PO_4^{3-} and SO_4^{2-} from previous section represent the plant available concentration. In addition, heavy metal concentrations were also determined in the extract from saturation paste prior to chemical speciation simulation, mimicking the soil solution reactions. Other important parameters measured were sodium adsorption (SAR) and potassium adsorption (PAR) ratios. These parameters were calculated in order to determinate the relationship between Na^+, K^+, Ca^{2+} and Mg^{2+} as follows:

$$(1)\ SAR: \frac{Na^+}{\sqrt{\frac{Ca^{2+}+Mg^{2+}}{2}}} \qquad (2)\ PAR: \frac{K^+}{\sqrt{\frac{Ca^{2+}+Mg^{2+}}{2}}}$$

SAR is the sodium adsorption ratio, PAR is the potassium adsorption ratio and Na+, K^+, Ca^{2+} and Mg^{2+} are the concentration of these cations in miliequivalents L^{-1}.

2.3.3 Soil heavy metals concentrations

Total metal concentration was determined through acid digestion in 0.5 g of soil with 5 mL of HNO_3-$HClO_4$-H_2O_2 (3:1:1) mixture (modified 3050B method, EPA, 1996). The extractable metal was determined with a buffered (pH 7.3) solution of DTPA-TEA-$CaCl_2$ (DTPA-extractable) in a 1:5 soil:solution ratio (Lindsay and Norvell, 1978).

Based on these variables, bioavailability index (Chen *et al*., 1996) of metals was calculated as follows: [(DTPA-TEA-$CaCl_2$ extractable concentration/total metal concentration)*100]. Fractionation of metals was analyzed by sequential extraction through Pagnanelli *et al*. (2004) modified method. The obtained fractions were: exchangeable ($_{EX}$); bound onto Fe and Mn oxides ($_{FeMn}$); weakly bound to organic matter ($_{WOM}$); strongly bound onto organic matter ($_{SOM}$) and bound onto sulfide phase ($_{S}$). After the last stage of the fractionation (metals bound to the sulfide phase), an acid digestion was performed in the samples with 5 mL of HNO_3-$HClO_4$-H_2O_2 (3:1:1) mixture in order to determinate concentration of metals in residual fraction ($_{R}$). Metals were quantified using flame atomic absorption spectrometry (Perkin Elmer model 3110). Reagents used in this study were prepared in deionized water. Qa/Qc control analysis was followed using certified standard solution for instrument calibration, running blanks and control reference samples.

2.3.4 Geostatistical analysis

A graphic representation of Pb and Cu (metals in highest concentration in the site) concentrations in the terrain was obtained through prediction maps in order to determine the values in non-sampled soil and recognize distribution patterns of the variables in the space. Kriging techniques were used and their validations were performed with semivariograms. The statistical package used was R 3.1.3 with the libraries gstat, sp, maptools, grDevices and raster (R Core Team, 2015).

2.3.5 Data analysis

Speciation in saturation paste solution was carried out simulating the soil solution using Geochemist's Workbench 10 software. Correlations were performed with Spearman or Pearson method depending on the assumptions satisfied by the variables. To validate these correlations, p-values were used. The statistical software used was R 3.1.3 (R Core Team, 2015).

2.4 RESULTS

2.4.1 Physico-chemical properties of soil

In general, soil pH was high (Table 6), where 68% of the samples were alkaline (pH>7) and 9% strongly alkaline (pH>8.5). All soil samples were in oxidizing conditions (ORP: 300-600 mV; Figure 4a). Together with pH, EC is a reference for species that can be formed in soil solution. The soil was saline (EC>4 dS m^{-1}) in 55% of the samples with presence of high concentrations of PO_4^{3-}, SO_4^{2-}, Cl^- and Na^+ contributing to EC increment. In addition, SAR and PAR were out of recommended values (Table 6). Concerning soil fertility features, OM content varied from 0.8% to 13.4%. In 8% of the samples, OM was under value that implies poor soil structure (1.7%, Hazelton and Murphy, 2007). The CO_3^{2-} content was detected between 0.2%-3.6% and the highest concentrations were found on the east part of the terrain. Plant-available PO_4^{3-} and SO_4^{2-} were detected in high concentrations ranging from 19 to 1,354 mg kg^{-1} and from 3.8 to 34,086 mg kg^{-1}, respectively. Concentration of available nitrogen was low, form 0 to 5 mg kg^{-1} of NH_4^+ and from 0 to 5 mg kg^{-1} of NO_2^-+NO_3^-. However, NH_4^+ was detected only in the north part of the field. Hence, the trend of the site was to be alkaline and poor in fertility in almost whole site.

Table 6. Salt concentrations and electrochemical properties in saturation paste extracts (soil solution) from a defunct LABR site. Percentage of samples out of recommended values is presented in brackets.

Variable	Recommended	Value in soil	Variable	Recommended	Value in soil (mg L^{-1})
pH	6.5-7.0	6.0-9.4 (68%)	Mg^{2+}	SAR, PAR	0-119
CE (dS m^{-1})	<4	0.3-65.3 (56%)	Cl^-	<10 mg L^{-1}	44.3-8,050 (47%[a])
ORP (mV)	-	212-374	CO_3^{2-}	-	0-552
PO_4^{3-} (mg L^{-1})	-	4.1-7.2	HCO_3^-	-	0-420
SO_4^{2-} (mg L^{-1})	57	88-47,130 (100%[a])	Na^+	SAR<12	74-33,101 (94%[b])
Ca^{2+} (mg L^{-1})	SAR, PAR	1-178	K^+	PAR: 6-8	2-256 (100%[c])

n = 34.

SAR: Sodium adsorption ratio, PAR: Potassium adsorption ratio.

[a] Percentage of samples above average in soil solution (Marschner and Rengel, 2012),

[b] Percentage of samples above maximum recommended SAR value (Hazelton and Murphy, 2007). [c] Percentage of samples out of recommended PAR value (Smiles and Smith, 2004)

2.4.2 Heavy metal soil concentrations

2.4.2.1 Total concentrations

Total metal concentration in the site decreased as follows Pb>Fe>Cu>Zn>Mn>Ni>Cd (Table 7). Concentration of all metals was above of the value found in the non-polluted site, near of the LABR site, up to 14,803, 602, 19 and 16 times for Pb, Cu, Cd and Ni, respectively. Interpolation shows higher concentrations of total Pb and Cu were found at the southeast of the terrain (Figures 2a and 3a).

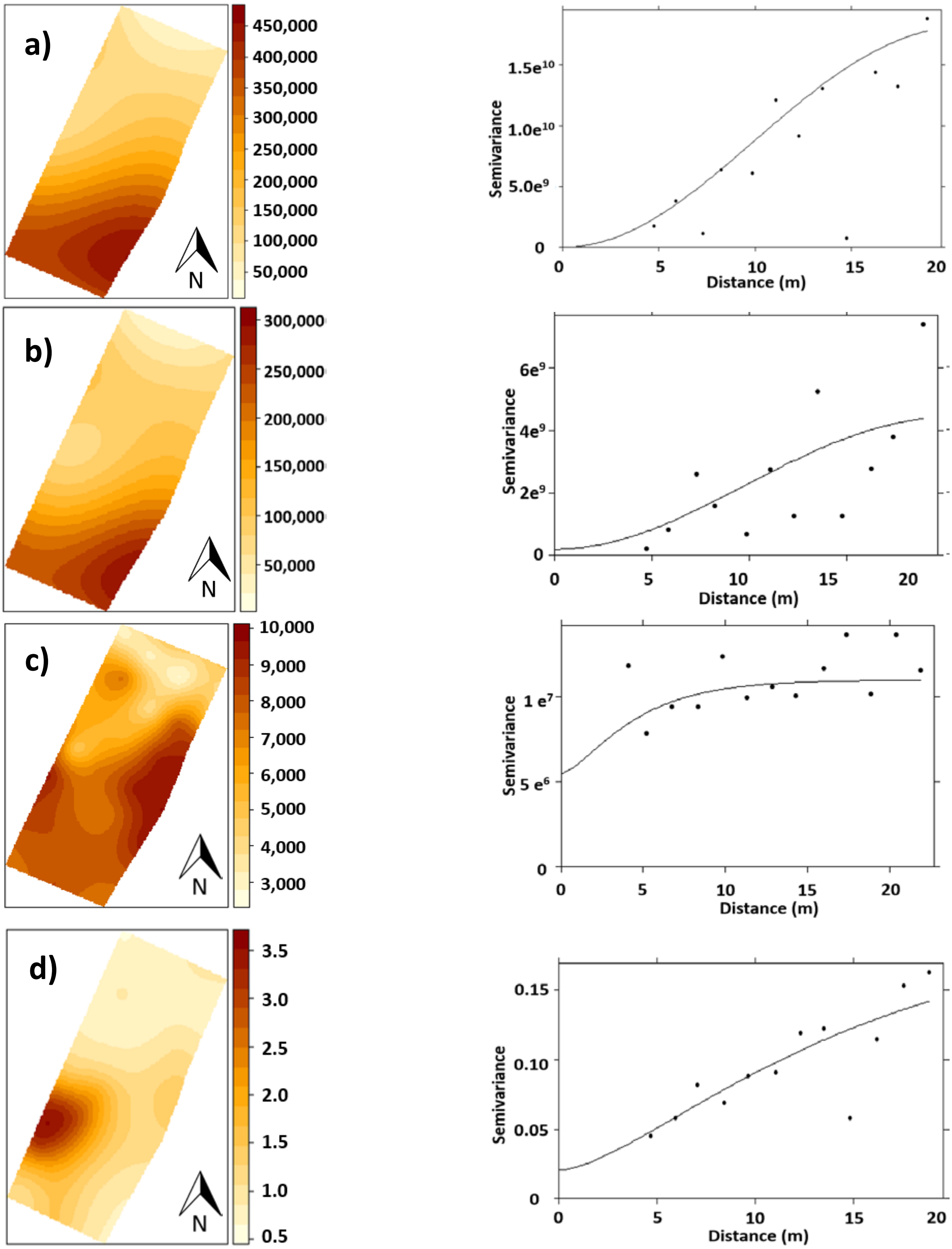

Figure 2. Spatial distribution of Pb a) total, b) exchangeable, c) DTPA-TEA-$CaCl_2$ extractable and d) water soluble. Colors in right bars represent the concentration in mg kg^{-1}. The semivariograms are shown at the right of the maps.

2.4.2.2 DTPA- extractable concentrations

High variability in DTPA-extractable concentrations was found for all metals. Pb concentrations varied from 154 to 12,000 mg kg^{-1} and Cu ranged from 0 to 1,936 mg kg^{-1}, both elements had similar distribution patterns (Figures 2c and 3b) and the highest values were at the south of the site. A negative correlation was observed between DTPA-extractable Cu concentration and OM content (-0.722, p=0.005).

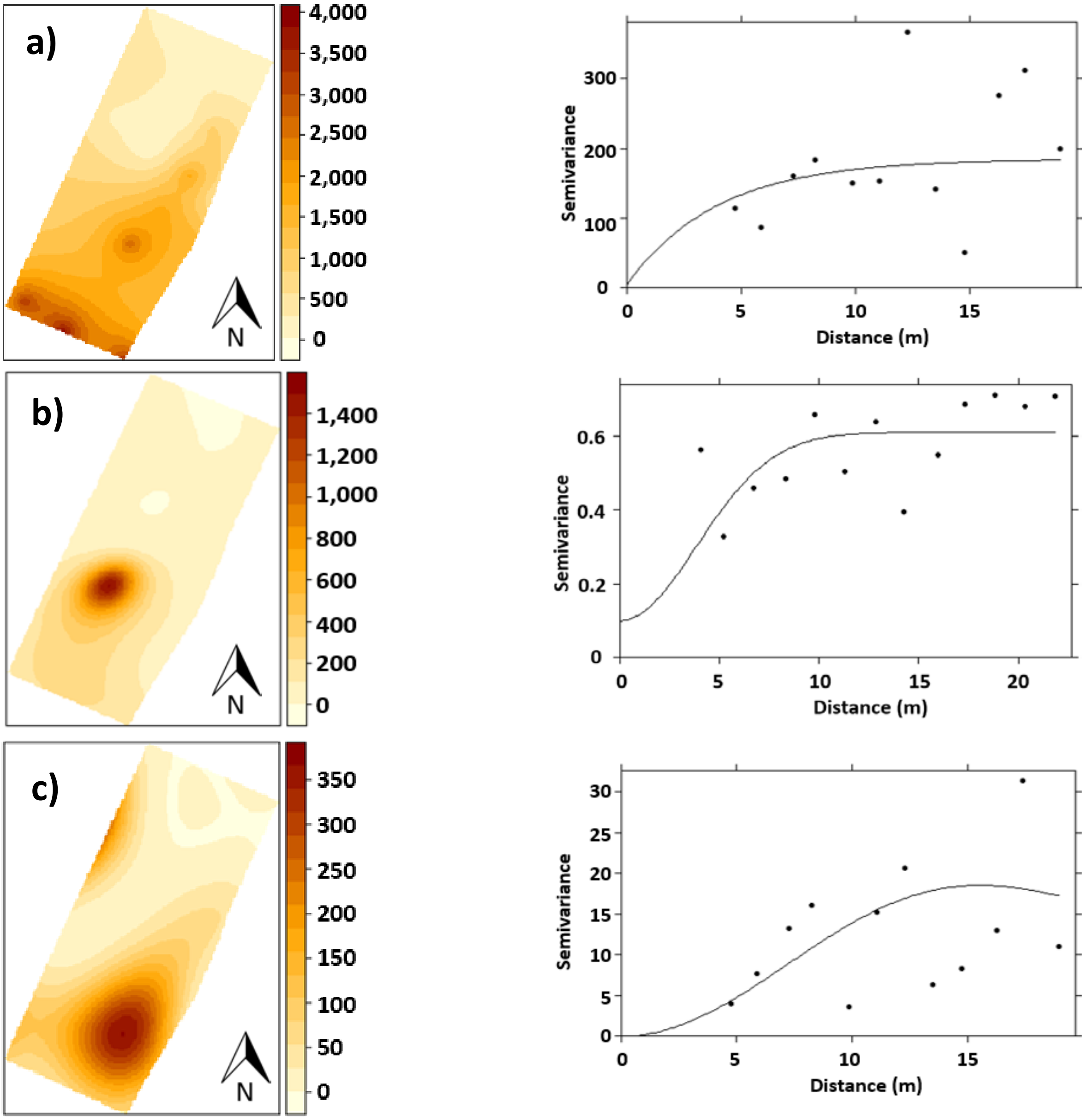

Figure 3. Spatial distribution of Cu a) total, b) DTPA-extractable and c) Exchangeable. Colors in right bars represent the concentration in mg kg^{-1}. The semivariogram is shown at the right of the map, (in order to achieve normality, semivariances correspond to square root transformed values in total and exchangeable maps and to log$_{10}$ transformed values in DTPA-extractable map).

Bioavailability index ranged 1%-66% for Pb, 1%-54% for Cu, 1%-25% for Zn, 1%-8% for Ni, 0%-10% for Mn, 0%-25% for Cd and 0%-0.4% for Fe. In addition, bioavailability index of Pb was negatively correlated with OM (-0.570, p=0.021). Low concentrations of DTPA-extractable Fe, Mn, Zn, Ni and Cd were found. In fact, soluble concentrations of Mn, Zn, Fe and Cu in some samples were lower than detection limits.

2.4.2.3 Heavy metal fractionation

2.4.2.3.1 Pb

The highest Pb concentrations were observed in the exchangeable fraction (5666-284580 mg kg^{-1}) and general sequence in soil fractions decreased in following the order $Pb_{EX}>Pb_{S}>Pb_{WOM}>Pb_{FeMn}>Pb_{R}>Pb_{SOM}$. Pb_{EX} was spatially distributed with a pattern similar to total and DTPA-extractable Pb, and had a descendent gradient from the south to the north side of the site (Figure 2c). The percentage of Pb_{EX} in relation to total Pb (100*Pb_{EX} / sum of Pb concentrations in all fractions) was above 50% in most samples. Positive correlations of Pb_{EX} with SO_4^{2-} (0.528, p=0.024), PO_4^{3-} (0.749, p<0.001) and OM (0.641, p=0.004) were found. Pb_{SOM} was correlated positively with pH (0.492, p=0.039) and similarly Pb_{FeMn} with SO_4^{2-} (0.510, p=0.024). The proportion of Pb_{WOM} ranged from 0.1% to 31.6%. In contrast low concentration of Pb_{SOM} was found (< 2% of total Pb).

2.4.2.3.2 Cu

Cu was the second element with high total concentration (up to 3,911 mg kg^{-1}). Fraction concentrations followed the next trend: $Cu_{S}>Cu_{WOM}>Cu_{FeMn}>Cu_{R}>Cu_{EX}>Cu_{SOM}$. A positive correlation was found between Cu_{EX} and soil OM content (0.71, p<0.001); Cu_{FeMn} with OM (0.72, p<0.001) and with SO_4^{2-} (0.79, p<0.001). Similarly, Cu_{SOM} was correlated with OM content (0.67, p=0.002) and Cu_{S} with SO_4^{2-} concentrations (0.68, p=0.001). In addition, Cu_{EX} spatial distribution was similar to the pattern of total and DTPA-extractable Cu concentrations.

2.4.2.3.3 Cd, Fe, Mn, Ni and Zn

Maximum concentrations of Cd followed the next trend: $Cd_{S}>Cd_{FeMn}>Cd_{EX}>Cd_{WOM}=Cd_{R}>Cd_{SOM}$ where Cd_{EX} was up to 63% of the total Cd. Concentrations Fe and Mn forming oxides (Fe_{FeMn} and Mn_{FeMn}) varied from 74 to

30,130 mg kg^{-1} for Fe, and 30 to 221 mg kg^{-1} for Mn. Up to 21% and 63% of total Pb and total Cu was bound to Fe and Mn oxides (Table 7).

Maximum Ni concentration in soil fractions has the following trend: $Ni_{FeMn}>Ni_{S}>Ni_{EX}>Ni_{WOM}>Ni_{R}>Ni_{SOM}$ and concentration as Ni_{EX} was up to 63 mg kg^{-1}. The maximum Zn concentration followed this order: $Zn_{EX}>Zn_{FeMn}>Zn_{WOM}>Zn_{S}>Zn_{R}>Zn_{SOM}$. Where Zn_{EX} ranged from 24 to 2,337 mg kg^{-1}. High percentages of this metal were found as Zn_{R} (up to 73%) and Zn_{WOM} (up to 70%).

2.4.3 Chemical speciation of Pb and Na in soil solution

Soluble Pb concentrations ranged from 0.024 to 4.650 mg kg^{-1} (Table 8). Pb^{2+} ion was present in all pH values tested and was the predominant species at low pH. SO_4^{2-}, CO_3^{2-} and Cl^- had strong influence on Pb species in solution. Formation of Pb-Cl complexes can be observed at the highest Pb concentration and in Figure 4b. Lead was associated with SO_4^{2-} at the lowest pH value. In the sample with the lowest Pb concentration and highest pH, CO_3^{2-} influence can be outstanding (Table 8).

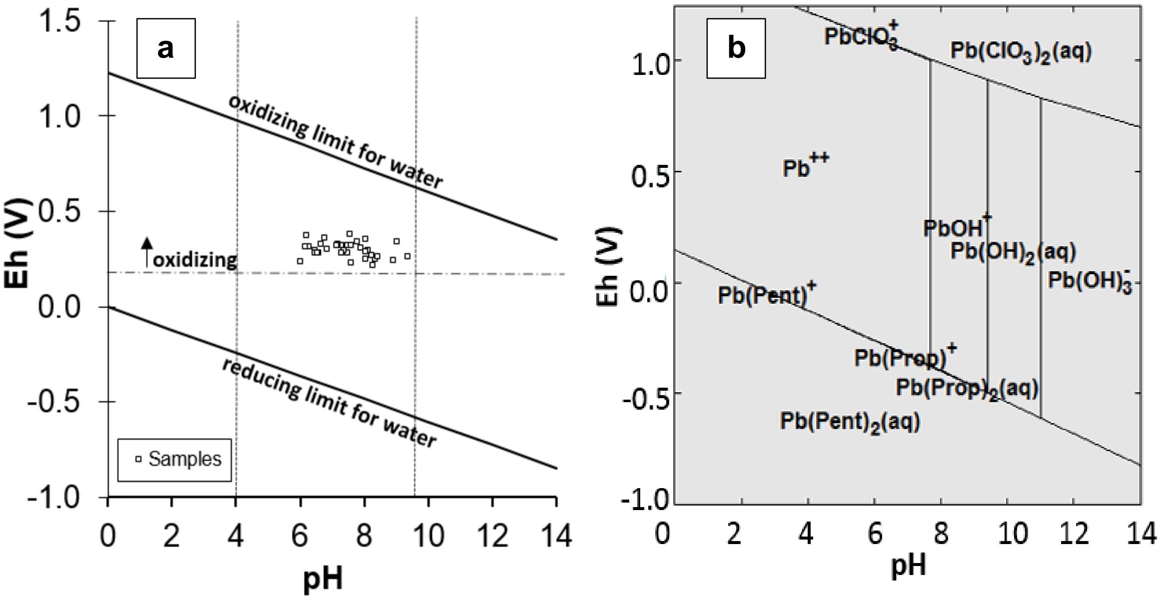

Figure 4. a) Oxide-reduction curves and values of ORP and pH and b) pH-Eh diagram determined in saturation paste extract (soil solution) from a defunct LABR site. Vertical dotted lines represent the range of pH commonly found in soils (Radojević and Bashkin, 1999).

Table 7. Range of metal concentrations (mg kg^{-1}) and percentage (in brackets) of soil metal fractions of the defunct LABR site in Mexico.

Fraction	Pb	Cu	Mn	Zn	Cd	Fe	Ni
Exchangeable	5,666-284,580 (43-97)	0-248 (0-35)	11-156 (6-31)	1-1,984 (0-85)	0-11 (0-63)	1-268 (0-3)	2-63 (8-44)
Bound onto Fe and Mn oxides	59-46,278 (0-21)	0-15 (0-63)	30-221 (27-69)	2-339 (2-46)	0-16 (0-40)	74-30,130 (2-51)	7-181 (17-46)
Weakly bound to organic matter	45-73,642 (0-30)	2-18 (5-90)	3-95 (1-17)	1-132 (3-70)	0-5 (2-37)	6-21,335 (0-53)	1-62 (3-44)
Strongly bound to organic matter	33-7,506 (0-0.2)	0-1,063 (0-25)	0-1 (0-1)	0-24 (0-4)	0-3 (3-29)	1-13 (0-0)	2-7 (1-16)
Bound onto sulfides	1,494-147,690 (2-39)	5-1,757 (2-74)	8-168 (8-34)	7-194 (3-42)	0-19 (8-34)	1,442-19,554 (7-94)	1-90 (3-30)
Residual	64-30,555 (0-22)	0-1,063 (1-13)	25-250 (7-36)	9-65 (1-73)	1-5 (8-35)	27-10,629 (1-62)	2-29 (4-21)
Total	10,792-444,105	9-3,911	94-696	24-2,337	5-57	2,133-59,606	24-408
Total NP[1]	306±30	5±2	33±9	18±1	3±2	1,000±374	28±12
Kabata [2]	27	14-109	411-550	60-89	0.41	35,000	13-37
Kabata [3]	50-300	60-500	-	200-1,500	2-20	-	75-150

[1] Total soil heavy metal concentrations in the non-polluted surrounding area of the LABR site.

[2] Normal total concentrations of elements (Kabata-Pendias, 2011).

[3] Maximum allowable total concentrations for heavy metals in agricultural soils (Kabata-Pendias, 2011).

The highest Na concentration (33,101 mg kg^{-1}) corresponded to the lowest pH (6.0) in the site (Table 8). In this condition, Na was present mainly as free ion (Na^+=97%) and in minor proportion was associated with Cl^-, CO_3^- and OH^-. Lowest concentration of Na was associated with a high pH (8.5) and also, the major proportion was found as Na^+ (98%). The remaining Na concentration was present bound to Cl^-, CO_3^{2-} and PO_4^{3-} species.

Table 8. Pb and Na speciation at four contrasting conditions of paste saturation extracts from defunct LABR soil samples.

Species	Concentration in solution (mg L^{-1})	Proportion of the total in solution (%)
Highest Pb concentration 4.65 mg L^{-1}, pH=6.18		
Pb^{2+}	4.00	85.97
$PbCl^+$	0.62	13.34
$PbCl_2$(aq)	0.03	0.70
Lowest Pb concentration 0.024 mg L^{-1}, pH= 8.25		
$PbCO_3$(aq)	$2.14\ e^{-2}$	90.2
$Pb(CO_3)_2^{2-}$	$2.32\ e^{-3}$	9.8
Pb in the highest pH 2.65 mg L^{-1}, pH=9.38		
$Pb(CO_3)_2^{2-}$	1.39	52.41
$PbCO_3$(aq)	1.09	41.10
$PbOH^+$	$9.31\ e^{-2}$	3.51
$Pb(OH)_2$(aq)	$7.310\ e^{-2}$	2.76
Pb^{2+}	$3.02\ e^{-3}$	0.11
$PbHCO_3^+$	$2.81\ e^{-3}$	0.10
Pb in the lowest pH 2.274 mg L^{-1}, pH= 6.05		
$Pb(SO_4)^{2-}$	1.27	55.79
$PbSO_4$(aq)	$8.93\ e^{-1}$	39.27
Pb^{2+}	$9.16\ e^{-2}$	4.03
$PbCl^+$	$7.88\ e^{-3}$	0.35
$PbHCO_3^+$	$1.27\ e^{-2}$	0.56
At the highest Na concentration 33,101 mg L^{-1}, pH=6.05		
Na^+	32,730	97.18
NaCl(aq)	943.8	2.80
$NaHCO_3$(aq)	3.8	0.01
$NaCO_3^-$	$1.7\ e^{-3}$	<0.01
NaOH(aq)	$1.3\ e^{-4}$	<0.01
At the lowest Na concentration 74 mg L^{-1}, pH=8.5		
Na^+	73.8	98.98
$NaHCO_3$(aq)	0.4	0.53
$NaHPO_4^-$	$1.70\ e^{-1}$	0.22
NaCl(aq)	0.1	0.13
$NaCO_3^-$	$8.20\ e^{-2}$	0.11
NaH_2PO_4(aq)	$3.80\ e^{-3}$	<0.01

(aq): aqueous species.

2.5 DISCUSSION

2.5.1 Soil alkalinity and salinity

Alkaline conditions (pH up to 9.4) were found in the site in spite of rock that originates the soil of the area (welded tuff and volcanic ashes; Cortés, 1970); which may reduce pH due to sulfur content in volcanic ashes. However, high concentrations of Cl^- in the site (up to 8,050 mg kg^{-1}) increase pH because Cl^- replace OH^- ions in positive charged sites of soil particles, and OH^- remains free contributing to pH increment (Xu *et al.*, 2000). High Na concentration in soil solution (up to 33,101 mg kg^{-1}) can also contribute to raise pH due to the formation of OH^- when this Na^+ reacts with water. Hence, Na and Cl are in concentrations enough to neutralize acid reactions in LABR soil. These alkaline conditions have strong negative effect on vegetal species (Hazelton and Murphy, 2007) and this is a limiting factor for plant establishment. In addition, Na and SO_4^2 are the principal contributors to EC in the soil solution (Table 8).

Source of Na in the site is probably the use of compounds as NaOH to convert H_2SO_4 in the battery electrolyte to Na_2SO_4 during sulfate recuperation process (Ramus and Hawkins, 1993) and Na_2CO_3 to transform $PbSO_4$ from battery electrodes to $PbCO_3$ (Stevenson, 2004). These parts of recycling procedure also explain the high SO_4^{2-} concentrations in soil. Other possible source of Na is the NaCl in batteries, which is a compound added in a mixture with a polysaccharide to increment battery lifetime (Larin, 2014). This also explains the high Cl^- concentrations found in soil. Most likely, poor control of residues and raw material (used batteries and Na compounds) containing Na and SO_4^{2-} was performed in this LABR site.

Presence of high concentrations of SO_4^{2-} and Na in soils can affect negatively plants development. This is because excess of SO_4^{2-} induces severe changes in plant sulfur metabolisms (Brouwer *et al.*, 1981). 100% of the soil samples from LABR had sulfate values above the average in soil solutions (Table 8). High concentration of Na induces K deficiency in plants (Schachtman and Liu, 1999), increase the wilting point of the soil and plants suffer water deficiency (Pessarakli and Szabolcs, 2016). Na also promotes clay dispersion, which can increase Pb mobilization by environmental factors as wind, or rain that may easily carry these dispersed particles and the pollutants with them. In addition, Na^+, Ca^{2+} and SO_4^{2-} have the following complex relations that may affect their activity in soil solution: On one hand, SO_4^{2-} behaves as counter-ion during the mobility of Na in soils, because precipitation of Ca as Ca_2SO_4.

Therefore, Ca ions are removed from exchange surface and Na^+ remains instead (Sivapullaiah, *et al.*, 2010). On the other hand, Na_2SO_4 can be formed, which is more water soluble (Ksp=10.3 mol L^{-1}) than Ca_2SO_4 (Ksp=9.1 x 10^{-6} mol L^{-1}). Hence, SO_4^{2-} may increase Na in exchange sites or increase its mobility.

Other important ionic relations are PAR and SAR. On one hand, PAR gives reference of structural stability and K retention in soil. In the LABR site, PAR was under acceptable limits which may imply leaching of this nutrient. Moreover, high SAR values mean no enough Ca and Mg concentration to compete with Na by the exchange surface in soil and hence, sodium excess causes Ca deficiency (Manahan, 2004). In LABR site, SAR ranged from 5 to 1,973 and 85% of the samples had SAR>102, which is the maximum value proposed for very tolerant crops (Hazelton and Murphy, 2007). In addition, taking into account SAR and EC values, in LABR site, 57% of the soil is saline-sodic; 34% is sodic and only 9% was not affected by salts or Na. Therefore, a remediation strategy should use plants capable to cope with stress promoted by salts, especially with Na.

PO_4^{3-} was also present in high concentrations in the site (Table 6). These were above of normal soil concentrations (200-500 mg kg^{-1}; Rowell, 1994) and its presence may be due to H_3PO_4 used as electrolyte in some batteries. A possible method to reduce concentrations of salts and heavy metals from solution is leaching the soil. However, the process might spend large amounts of water and the obtained leachate will represent a hazard which must be treated properly.

Moreover, results show (Table 8) that PO_4^{3-}, SO_4^{2-}, Cl^- and Na^+ strongly influenced metal species. These salts increase ionic strength (IS), as a result higher mobility of metals like Cd and Pb may occur (Acosta *et al.*, 2011). In the LABR site IS ranged from 0.005 to 1.185 M; which are values mainly due to Na^+ and SO_4^{2-}. Usually, IS in soil solution interval is from 0 to 0.45 M (Liu and Huang, 1999) and in non-polluted soils the range is 0.003-0.016 M (Edmeades *et al.*, 1985). IS increases metal solubility because two reasons: 1) salts content with metals for exchange sites in soil particles and 2) formation of metal-salt species with no charge or negative charge such as metal-Cl_2, metal-Cl_3^- and metal-Cl_4^- (Petruzzelli *et al.*, 1985), while soils particles are predominantly negative charged. On the contrary, low concentrations of nitrogen were found (0-5 mg kg^{-1}). This represents poor soil fertility and in practical terms the need of fertilizer or soil amendment application to support plant growth.

2.5.2 Pb and Na chemical speciation in soil solution

An oxidizing (ORP: 300-600 mV; Rowell, 1994) and alkaline (pH>7.4) conditions was generally observed in soil samples. ORP and pH has direct influence in the metal compounds formation which have different mobility in soils. Based on these values, it was expected the presence of $[PbOH]^+$ and $[PbO_2]$ species (Takeno, 2005). Values of pH-Eh in dry and wet samples are shown in the Figure 4, no change due to soil manage is observed. In addition, it is important to mention that metals species in soil solution can be carried by water. In this study, Pb and Na speciation was conducted due to the high concentrations of these elements.

High concentration of Pb in soil solution remained as a free divalent ion (Pb^{2+}). Other species are present in minor concentrations (Table 8). Pb species are adsorbed on soils and have different mobility. Weng, (2004) found that Pb^{2+} is less adsorbed on soils than $Pb(OH)^+$. In addition, other Pb species have low solubility, which is expressed in their Ksp values ($PbCO_3$: 7.4 x 10^{-14} M, $Pb(OH)_2$: 1.2 x 10^{-15} M and $PbSO_4$: 1.6 x 10^{-8} M). In the same way, Nedwed and Clifford (1998) pointed out that Pb^0, $PbSO_4$ and PbO are the predominant species found in LABR soils. They mentioned that the presence of CO_3^{2-} or CaO can lead to the formation of $Pb_3(CO_3)_2(OH)_2$, $PbCO_3$ or $Pb_4SO_4(CO_3)_2(OH)_2$ that reduce Pb migration. Other Pb species found in the LABR site is $Pb(SO_4)$ which was present in low concentration despite of high SO_4^{2-} content in soil solution (up to 47,130 mg kg^{-1}). Pb associations with SO_4^{2-} were present only at low pH. $Pb(SO_4)^{2-}$. It might due to the following reasons: 1) low solubility of compounds, which once formed, low quantity is water soluble, 2) high pH maintain SO_4^{2-} free and 3) Pb competition with cations with more affinity for SO_4^{2-} such as Ca^{2+}, Na^+ and K^+. This higher affinity can be explained by the hard soft acid base Lewis principle that describes metal complex stability. Hard acids as Ca^{2+}, Na^+ and K^+ have higher affinity for hard bases as SO_4^{2-} than border acids as Pb^{2+} for those hard bases. In the LABR site $CaSO_4$ was in 286.8 mg kg^{-1} at pH=6.05, in 4.7 mg kg^{-1} at pH=9.38, and in 220.3 mg kg^{-1} and 1.5 in mg kg^{-1} in the samples with the highest and the lowest Pb concentrations respectively. Furthermore, it is important to note that compounds of Pb^{2+} and SO_4^{2-} that can be formed in soil, are probably precipitated in solid phase and hence, they are not in soil solution. In fact, adsorption and precipitation of Pb on SO_4^{2-} and PO_4^{3-} compounds are important buffer mechanism for controlling the migration and fixation of Pb on the environment (Jensen *et al*., 2006). Other possible reaction of SO_4^{2-} is the reduction as sulfides, substances which can bind metals. However, inverse reaction is also possible in which sulfides oxidation contribute with SO_4^{2-} presence in soil. In the results, Pb_S are in high proportion (up to 39% of the total Pb). This makes important to consider interactions between solid and liquid phases where Pb compounds in soil solution may precipitate in solid forms and Pb minerals may solubilize into soil solution.

Sodium ion (Na^+) was the predominant species, but others species were also present (Table 8). There are few studies regarding Na speciation in soil and its relation with metal mobility and plant bioavailability. One of these studies was conducted by Zhang *et al*. (2012), they found that $NaHCO_3$ and NaCl had negative effects in some cellular variables of *Nicotiana tabacum.* However, $NaHCO_3$ has higher negative impact than NaCl. In the LABR site, these two Na species were found. In addition, in other investigation conducted by Ghallab and Usman (2007), the effect of NaCl on Cd speciation and bioavailability for plants was studied. The authors found increment in Cd concentration in solution due to NaCl addition. Na in exchange surface of soil particles displaces Cd. In addition, the authors observed that Cd accumulated in tissues of *Zea mays* was correlated with total Cd in solution and Cd-Cl complexes concentration.

2.5.3 Total metal concentrations

Total Pb concentration in this site (up to 444,105 mg kg^{-1} equivalent to 40%) is comparable with soils polluted by LABR in other regions. Wasay *et al*. (2001) observed 24,600 mg kg^{-1} (2.46%) of Pb in a soil polluted by disposal of wastes of a LABR in Canada. Blacksmith Institute and Green Cross Switzerland (2013) refer places polluted by LABR activity with 400,000 mg kg^{-1} (40%) of Pb. Similarly, Mohammed *et al*. (1996) found a site affected with up to 70% of Pb. In the same way, Yeh *et al*. (1996) reported up to 3,590 of mg kg^{-1} Pb in a soil near of a LABR factory in Taiwan. Rodríguez *et al.* (2014) found near to a LABR plant in Argentina, 1,050 mg kg^{-1} and 51 mg kg^{-1} of Pb and Zn *cuasi*-total concentrations (digestion with $HCl:HNO_3$ mixture) respectively. They found that these concentrations were high compared to a control soil from agricultural plots.

Total concentrations of Pb, Cu, Cd and Ni in soil of the present study were also above the referenced by Kabata-Pendias (2011) for agricultural soils: Up to 8,882, 50, 29 and 5 times higher for Pb, Cu, Cd and Ni, respectively (Table 7). In addition, total Pb concentrations (10,792-444,105 mg kg^{-1}) exceeded the limits of many regulations. For example, the thresholds for residential land use in US is 500-1000 mg kg^{-1}, for Canada is 375 mg kg^{-1}, for Netherlands is 50-600 mg kg^{-1} and for England is 500 mg kg^{-1} (Nedwed and Clifford, 1998).

Hence, total Pb concentrations make necessary intervention to soil remediation in order to reduce the risk for public health and avoid environmental damages. In addition, Pb concentrations in LABR soil compared with the non-polluted soil near the site (Table 7) demonstrate the impact of inadequate waste management from this LABR factory Spatial distribution of total Pb and Cu (Figure 2a and 3a) show that south zone in the terrain was the most affected by pollution.

2.5.4 DTPA- extractable metals

DTPA-extractable Pb concentration was high in the whole terrain. Results show negative correlation between bioavailability Pb index and OM in soil. This might be due to Pb sequestration in OM. This metal can be strongly adsorbed by OM and fixed as not extractable forms (Wang *et al*., 2015) or complexed with humic substances. Nevertheless, high concentration remains extractable despite of OM presence, probably due to the saturation of sites on OM and the high IS (Impellitteri *et al*., 2001), which is increased by salts concentration. Therefore, a possible strategy to reduce the DTPA-extractable Pb is the addition of amendments based on organic materials, which may fix Pb. This negative correlation between DTPA-extractable Pb and OM was observed (-0.570, p=0.021). Other important property of soil is the CO_3^{2-} content, which in site was from 0.2% to 3.6%. Alloway (2013) pointed out that these compounds are linked to soil ability to absorb cations. Therefore, availability of metals in site can be influenced by CO_3^{2-}.

The high Pb, Cu and salt concentrations is the struggling condition for these soils, a remediation strategy should consider plant species capable to survive under high concentrations of both heavy metals and salts, especially sodium. In addition, the geostatistical interpolations show that there is a spatial pattern in not only Pb concentrations, but also DTPA-extractable Cu. The highest Cu concentrations were found also at the south part of the site (Figure 3). Hence, plants growth will be probably more limited in that zone than in the north area of the LABR soil. This spatial pattern should be considered especially in treatment evaluation for research purposes under phytoremediation strategy.

2.5.5 Soil heavy metals fractionation

The present investigation is the first report of metal fractionation directly in a LABR polluted soil, Pb was the riskiest element due to its total and exchangeable concentrations. The major proportion of Pb (up to 97%) was found as exchangeable form (Table 7). Apparently, Pb concentration is high enough to compete with cations such as Ca and Mg by the exchange surface of soil particles. In other soil affected by LABR, Rodríguez *et al*. (2014), found up to 640 mg kg^{-1} of Pb_{EX} at a distance of approximately 100 m near of a LABR facility.

Concentrations of SO_4^{2-}, PO_4^{3-} and OM content are correlated positively with $Pb_{EX.}$ This can indicate the presence of available forms of Pb bound to the mentioned anions. The correlation with OM probably means Pb-OM association keeps Pb in exchangeable fraction. The ability of OM to sequestrate heavy metals has been widely studied (Yin *et al*., 2002; Zhou *et al*., 2012) and can also explain the Pb concentration observed as Pb_{SOM} and Pb_{WOM}. The negative correlation found between OM and Cu_{EX} can be explained in terms of high affinity of Cu by OM and it suggests that OM contributes with reduction of Cu_{EX} concentrations.

Zn_{EX}, Cu_{EX}, Ni_{EX} and Cd_{EX} were also found at high concentrations (Table 7) where Cd is a risky element due to its toxicity. An important fraction that ameliorate potential effects of Cd are OM likewise Fe and Mn oxides. Royer et al., (1992) mentioned capacity of these oxides for metal binding. This is supported by the concentrations of Cd as Cd_{SOM} (up to 37%), Cd_{WOM} (up to 29%) and Cd_{FeMn} (up to 40%). Other hazardous element is Ni, and similarly to Cd, concentrations in OM and Fe and Mn oxides may contribute to the diminution of Ni_{EX} concentrations.

Evidently, LABR had an impact on soil by increasing Zn, Cu, Ni and Cd concentrations. This is confirmed by the higher total concentrations of these metals found in the LABR soil compared with the concentration in the non-polluted soil (Table 7). A possible source of Zn, Cu, Ni and Cd are the Pb alloys from batteries grids and poles, as well as, wires in these devices.

Regarding to Pb complexation onto Fe and Mn oxides, an important proportion was found in this fraction (Pb_{FeMn} up to 21% of the total Pb). Some authors stated that behavior of heavy metal with stable oxidation status depends indirectly on Fe compounds (Xu *et al.*, 2006; Vodyanitskii, 2010). Suda and Makino (2016) referenced that Pb, followed by Cu is in general the heavy metal which is bound on the Fe and Mn oxides surface stronger than other metals. Clearly, Pb fixation on Fe and Mn oxides is an important mechanism which reduces Pb_{EX} concentration in the LABR soil.

2.6 CONCLUSIONS

The LABR activities in this site left a heavily polluted soil with three kind of hazards: 1) Very high total Pb concentrations (up to 444,107 mg kg^{-1}); which makes this site comparable with other ones reported as the most polluted by this metal in the world; 2) elevated total concentrations of other metals as Cu and (up to 3,911 mg kg^{-1}) and 3) high concentration of both plant available salts (SO_4^{2-}, PO_4^{3-}), salts in soil solution (SO_4^{2-}, PO_4^{3-}, Cl^- and Na^+), as well as alkaline conditions promoted mainly by Na^+ and Cl^-. This kind of hazards has not been reported in any other LABR site and represents a potential risk for human health and environment, therefore limiting conditions for plant development. This research provides the basis for designing a phytoremediation strategy to establish in this LABR site and gives a baseline assessment of this polluted soil before any remediation action.

Based on generated information, selection of plants should consider species capable to growth under both heavy metal concentration and saline condition. In addition, low nitrogen content makes necessary to provide an input of this nutrient. An option for that is the addition of organic amendments to the soil, since these materials may improve the extreme hostile soil conditions adding nutrient to soil. Additionally, because their capacity to reduce heavy metal availability and mobility in soils.

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CHAPTER III. DENDRO-REMEDIATION OF A HEAVY METAL AND SALT POLLUTED DEFUNCT LEAD ACID BATTERY RECYCLING SITE AT CENTRAL MEXICO

3.1 ABSTRACT

Inadequate disposal of lead acid battery recycling (LABR) wastes left soil severely polluted with metals (Pb, Cu) and salts (Na_+, SO_4^{2-}, PO_4^{3-}). The use of tree species (dendro-remediation) may be a useful option to cope with the concentrations of these pollutants. Organic amendments may contribute with essential nutrients and sequester metals to allow plant establishment. Seven tree species (*Acacia farnesiana*, *Casuarina equisetifolia*, *Cupressus lusitanica*, *Ecualyptus obliqua*, *Fraxinus excelsior*, *Prosopis laevigata* and *Pinus greggii*) and two organic amendments (vermicompost and vermicompost + sawdust mixture) were tested for phytoremediation of a defunct LABR site. Available metals and salts concentrations, as well as their spatial pattern in soil were analyzed. Plant species and amendments were compared through analysis of covariance and longitudinal analysis. High concentrations of DTPA-extractable metals (up to 15,685 mg kg^{-1} and 478 mg kg^{-1} for Pb and Cu) and salts (292 mg kg^{-1} and 23,578 mg kg^{-1} for PO_4^{3-} and SO_4^{2-}) were found in soil after three and six months of setting up the experiment. Spatial pattern of PO_4^{3-}, $SO_4^{2-}$and DTPA-extractable Pb and Cu changed slightly through time. In spite of extreme soil conditions, *A. farnesiana, E. obliqua, C. equisetifolia* and *F. excelsior* had 100% of survival. Available metals and salts in the soil differently affected each tested species. In addition, negative effect on growth due to Pb accumulated in shoots was observed only in *C. lusitanica*. In many individuals of all species Pb hyperaccumulation was detected (>1000 mg kg^{-1} Pb in shoots). Based on the results, all the evaluated plant species may be useful for dendro-remediation.

Key words: Hyperaccumulation, organic amendments, re-vegetation, metal stabilization.

3.2 INTRODUCTION

Lead acid battery recycling (LABR) is an activity which may pollute soil with heavy metals (Pb, Cu) and salt ions (Na^+, PO_4^{3-}, SO_4^{2-}) through waste generation or inadequate disposal of raw materials (Espinosa *et al*., 2004). Commonly, a polluted soil is treated using physical or chemical methods. The most frequently used procedures are: vitrification, pyrometallurgical separation (Bitler and Baranski, 1994), solidification/stabilization, electro-kinetic treatment, and metal extraction with chelates (Jankaite and Vasarevičius, 2005). However, all these methods involve high cost, workforce and trained technicians (Ruiz-Olivares *et al*., 2013). As alternative to these methods, the use of tolerant plant species capable to cope with toxic elements and grow under polluted soil conditions as been proposed to soil remediation.

No experimental information has been published in relation to the use of phytoremediation (*in situ*) in polluted soils by LABR. A pot experiment using corn was conducted with LABR soil (De-Araújo and Do-Nascimento, 2010). Nevertheless, experimental conditions are different from field in LABR sites. Hence; it is necessary to conduct phytoremediation experiments directly in the field, where environmental conditions as soil heterogeneity and water scarcity represent different challenges that are not observable in pot experiments.

Plant species to be used in phytoremediation should have some characteristics: to grow in nutrient poor soils, to develop a strong root system, to have fast growth rate, to tolerate high heavy metals concentration, to have low propagation and maintenance costs. Many tree species seem to have these characteristics; therefore, there is a possibility for their use in phytoremediation.

These species have many advantages (Komives and Gullner, 2006). Trees have massive biomass and root systems that can penetrate in soils much more than herbaceous species (González-Oreja *et al*., 2008). They also may prevent dispersion of particles that can be carried through wind (Sánchez-López *et al*., 2015), and contribute with organic matter to the soil. Moreover, the use of trees provides other advantages such as CO_2 sequestration (Resh *et al*., 2002) and participation on restoration of degraded lands (Sadio and Negreros-Castillo, 2016). This could be considered with economic benefit through payment for environmental services. In addition, the wood with the accumulated pollutant could be used in structures that have no contact with humans. Furthermore, trees are perennial and there is no necessity to harvest every single year. However, the use of trees in remediation (Dendro-remediation) is a few explored research topic. Table 9 shows a list of experiments with tree species which have been used in phytoremediation of metal polluted sites, whereas Table 10 mentions the use of trees to remediate soils with high concentrations of salts. It is relevant to mention that apparently no one experiment has been carried out using trees for phytoremediation of polluted soils by LABR.

Table 9. Tree species used for phytoremediation of soils affected by heavy metals.

Plant species	Heavy metals	Experiment conditions and reference
Acer pseudoplatanus	Cd, Cu, Pb, Zn	Field; Mertens *et al*., 2007
Alnus glutinosa	Cd, Cu, Pb, Zn	Field; Mertens *et al*., 2004
Betula spp.	Cd, Zn	Pot; Rosselli *et al*., 2003
Celtis australis	Cd, Cu, Pb, Zn	Field; Mancuso *et al*., 2006
Eucalyptus camaldulensis	Cd, Cr, Cu, Mn, Ni, Pb, Zn	Field, Fine *et al*., 2014
Fraxinus excelsior	Cd, Cu, Pb, Zn,	Field; Mertens *et al*., 2007
Koelreuteria paniculata, Elaeocarpus decipens	Cd, Mn, Ni, Zn	Field, Tian *et al*., 2009
Paulownia tomentosa	Cd, Cu, Pb, Zn	Pot; Doumett *et al*., 2011
Populus alba	Cd, Cu, Pb, Zn	Field; Martens *et al*., 2007
Populus robusta	As, Cd, Cr, Cu, Ni, Pb, Zn	Field; Pilipović *et al*., 2005
Quercus ilex	Cd, Zn	Field; Mancuso *et al*., 2006
Salix spp.	Cd, Zn	Field; Dos Santos Utmazian *et al*., 2007; Robinson *et al*., 2007

Table 10. Tree species used for phytoremediation of soils affected by salts.

Plant species	Salt or soil condition	Experiment conditions/references
Acacia sp.	Na^+, Cl^-	Pot, Morais *et al*., 2012; Rehman *et al*., 2000
Betula pendula, B. kirghisorum, B. pubescens	pH 7.22 - 8.22, EC 5 - 20 dS cm^{-1}	Pot, Zhao *et al*., 2014
Casuarina sp.	Na^+	Pot, Batra and Dikshit, 1994
Casuarina glauca	pH 8.6 - 10.5	Field, Goel and Behl, 2005
Eucalyptus spp.	Na^+, Cl^-	Pot, Field; Adams *et al*., 2005
Pinus pinea	Na^+, Cl^-	Pot, Khaldi *et al*., 2011
Prosopis juniflora, Acacia nilotica	pH 8.6 - 10.5	Field, Goel and Behl, 2001
Prosopis laevigata	Na^+, Cl^-, SO_4^{2-}	Pot, Rios-Gomez *et al*., 2010
Prunus sp.	Na^+, Cl^-	Field, Catlin *et al*., 1993
Populus sp.	Na^+, Cl^-	Pot, Chen *et al*., 2003; Ma *et al*., 2015
Salix sp.	Na^+, Cl^-, K^+, SO_4^{2-}, NO_3^-	Pot, Hangs *et al*., 2011; Mirck and Volk, 2012

EC: Electrical conductivity

For vegetation establishment in polluted soils, it is necessary to take into account not only chemical properties of the contaminants and the resistance of the plants to toxic effects, but also their nutrient requirements. Regarding to this point, the addition of organic amendments can contribute to establish plant species. It has been observed that these materials provide nutrients and also can modify metal availability and improve soil physical characteristics (Farrell *et al*., 2010).

The aim of this research was to evaluate the ability of seven species (*Acacia farnesiana, Casuarina equisetifolia, Cupressus lusitanica, Eucalyptus obliqua, Fraxinus excelsior, Pinus greggii and Prosopis laevigata*) to survive and growth on a LABR defunct site strongly polluted with heavy metals (Pb and Cu) and salts (SO_4^{2-}, PO_4^{3-} and Na), likewise the accumulation of metals in biomass. This site was previously characterized (Chapter II) in order to provide detailed information for plant establishment from a phytoremediation strategy.

3.3 MATERIALS AND METHODS

3.3.1 Soil sampling and physicochemical analysis

Soil heavy metal and salts concentrations as well as Pb and Cu spatial patterns described in Chapter II were the key factors to define the experimental design, set up the plants in the field and statistical analysis of results. In order to detect changes of metal and salts concentration in soil, two samplings were performed in intervals of three months after the experiment establishment. DTPA-TEA-$CaCl_2$ extractable (DTPA-extractable) metals was determined with the method proposed by Lindsay and Norvell (1978). Extractable phosphates concentration was measured with the bicarbonate method (Olsen *et al*., 1954) and sulfates through turbidimetric method (Chesnin and Yien, 1951).

3.3.2 Experiment establishment

Plant species were obtained from the National Commissions for Water (CNA for its abbreviation in Spanish). Seventy plants of approximately 40 cm were donated by the CNA: twelve individuals of *Casuarina equisetifolia, Fraxinus excelsior, Cupressus lusitanica, Pinus greggii* and *Eucalyptus obliqua*. In addition, ten individuals of *Acacia farnesiana* and *Prosopis laevigata* were raised from seeds collected from a multi heavy metal polluted zone in Zimapan, Hidalgo.

The treatments to evaluate were 1) soil + vermicompost (80%, 20%) and 2) soil + vermicompost + sawdust (60%, 20%, 20%). Soil was obtained from the LABR site. In the field, each experimental unit consists in holes of 40 x 40 x 40 cm filled with the respective treatment. The trees were transplanted and watered at the time of experimental units preparation. Plant height was measured weekly in order to perform longitudinal analysis in which measure of a response variable through time in the same experimental units is called response profile (growth profile in this case).

3.3.3 Statistical analysis and experimental design

3.3.3.1 Plant growth

Based on the spatial distribution of the concentrations of heavy metals and salts, a generalized randomized block design was established. Two blocks were defined in function of two partially homogeneous zones of DTPA-extractable concentrations of Pb and Cu in soil (Chapter II). Three repetitions for treatment were established. For the discussion, type 3 errors were used, which are independent of order of introduction of variables in models.

The variables analyzed were heavy metal concentration in shoots and tree height. Both vertical and longitudinal analyses were carried out to determinate the plant growth effect of amendments applied. Due to variability between experimental units, statistical models were adjusted by the following covariates (non-controllable, but measurable variables): initial height of trees at the moment of transplanting and concentrations of soil heavy metals and salts as well as metal accumulated in shoots.

Estimate of these covariates provided information about their effect on growth profile, where higher estimate values implies higher effect of the covariate. For hypothesis test in this analysis, p-values are presented based on F, T and chi-square tables. In addition, interpretation of differences for amendments and species was based on interaction of these factors with time (amendment*time, species*time)

Regarding metal accumulation in shoots, analysis of covariance (ANCOVA) which also included the mentioned covariates was performed. Taking into account the mentioned variability of experimental units, lsmeans were used instead of simple means in order to covariate adjustment. Therefore, the intervals presented in Tables 11 to 15 are a reference of the variability in metal accumulation by plants. Nevertheless, it should not be used for comparisons between species. The software used was R 3.1.3 with the packages lsmeans and car for ANCOVA and correlations. For longitudinal analysis, SAS 9.4 was used with optimization of the mean and the covariance structure based on Bayesian information criterion (BIC) to achieve the best model for the data description, where smaller BIC is better for the model. Normality and homoscedasticity of variances were checked in all parametric analysis.

3.3.3.2 Geostatistical analysis

Prediction maps were generated in order to determinate the changes in the spatial pattern of physico-chemical variables in relation to the initial conditions reported in Chapter II. The software used was R 3.1.3 with the packages gstat, sp, maptools, grDevices and raster (R Core Team, 2015).

3.3.4 Costs

Costs of the whole investigation including materials, field work, laboratory analysis, elaboration of design and data analysis were considered. In addition, in order to compare the dendro-remediation of LABR site with other methods for polluted soil treatment, separation between costs of this dendro-remediation process and cost for research purposes was performed.

3.3.5 Metal concentration in plants

Metals concentration in plants shoots (leaves and steams) was determined at the six month of setting up the experiment as follows: vegetal material was rinsed consecutively with distilled water, EDTA 0.01% and deionized water after shoots sampling to remove soil particles from the tissue surface. Then, the samples were dried at 65 °C for 72 h. Acid digestion was performed with 0.5 g of plant tissue with 5 mL of HNO_3-$HClO_4$-H_2O_2 (3:1:1) mixture at 115 °C for 24 h (Jones and Case, 1990).

Metals concentration was determined by FAAS with a Perkin-Elmer 3110 equipment. Na concentration was also measured (Jenway flame photometer) in the acid digestions. In addition, in order to determinate the magnitude in which trees to accumulate available metals from soil, bio-concentration factors (BCF) were calculated with the next ratio: [shoots metal concentration/DTPA-extractable metal concentration]. A $BCF>1$ means metal concentration in shoots higher than DTPA-extractable metal concentration in soil.

3.4 RESULTS

3.4.1 Physico-chemical properties of rhizospheres

Soil properties varied widely as it was showed in Chapter II. However, all plant species grew in these adverse conditions with high metal and salt concentrations. 41% of the rhizospheres were very strongly alkaline (pH>9, Hazelton and Murphy, 2007) and 6.5% saline (EC>4 dS m^{-1}). All rhizospheres had oxidant soil conditions (ORP>200; Jackson and Barak, 2005) with high concentrations of PO_4^{3-} and SO_4^{2-}. Nevertheless, there was a general diminution in the concentration of both anions after six month of the treatment in relation to the concentration after three months (Table 11).

Table 11. Some physico-chemical characteristics of the rhizosphere from seven plant species established on LABR site.

Tree	pH	EC (dS m^{-1})	ORP (mV)	PO_4^{3-} (mg kg^{-1})		SO_4^{2-} (mg kg^{-1})	
				Three month sampling	Six month sampling	Three month sampling	Six month sampling
A. farnesiana	7.3-9.7	0.39-4.4	324-390	28 - 201	9-168	1,118-28,078	1,281-17,442
C. equisetifolia	7.3-10.2	0.2-11.2	290-520	7 - 169	12-117	1,054-10,513	1,309-14,170
E. obliqua	7.9-9.9	0.2-7.6	277-390	9 - 201	1-209	901-23,578	1,064-12,674
P. laevigata	7.4-9.2	0.3-3.8	315-377	31 - 240	12-125	870-11,864	1,128-20,819
C. lusitanica	7.5-9.5	0.3-1.9	293-410	19 - 216	7-103	955-7,360	935-6,176
P. greggii	6.5-9.1	0.3-2.0	327-392	24 - 292	6-112	455-19,580	1,297-14,511
F. excelsior	7.9-9.9	0.2-4.2	295-406	19-167	5-129	1,476-12,785	1,119-7,666

EC=electrical conductivity, ORP: oxidation-reduction potential, PO_4^{3-}: available phosphates, SO_4^{2-}: available sulfates.

The spatial pattern of PO_4^{3-} concentrations varied slightly between the third month and the six month samplings (Figures 5a and 5b). The highest concentration was located in the west side of the site in both samplings. Regarding SO_4^{2-} concentration and spatial pattern do not varied between samplings and the highest concentration was located in the southwest zone (Figure 5c and 5d).

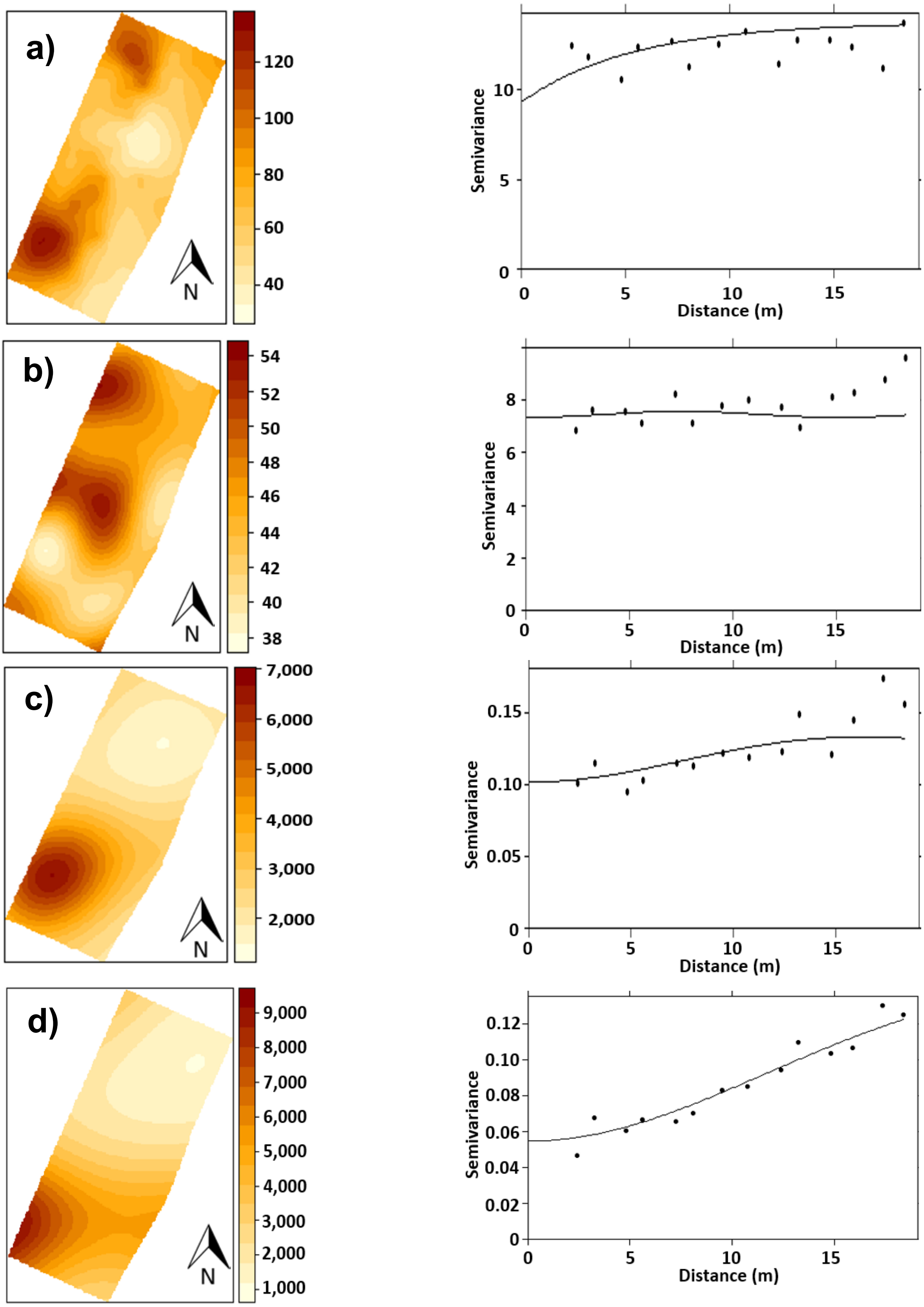

Figure 5. Maps of anions distribution in the soil and their semi-variograms: PO_4^{3-} after three months (a); six months (b); SO_4^{2-} after three months (c), and six months of set up the experiment. Right color bars represent concentration mg kg^{-1}.

3.4.2 DTPA-extractable metals

Lead and Cu concentrations were high in the rhizospheres at the two samplings: up to 156 and 96 times for Pb and Cu above the recommended value by Mexican Standard (Table 12). In addition, higher concentrations of DTPA-extractable Pb concentration were observed at the six-month sampling compared to the three-month sampling (Tables 12 and 13). Cd was not found in none experimental units.

Spatial patterns of Pb varied from initial conditions found in Chapter II (Figures 2 and 6). However, between three and six moth samplings, it was not possible to compare because validation of first sampling map was not achieved. Concerning Cu spatial pattern, highest concentrations were found at the south of the terrain.

In 45% of the samples, extractable Mn was under the recommended values for soil fertility (< 1 mg kg^{-1}) by Mexican regulations for crop plants. In 12% of the samples Mn was not detected. Similarly, 45% of the samples had low Fe concentration. Ni concentrations were into the normal threshold for soil (Tables 12 and 13).

Table 12. Intervals of DTPA-extractable metal concentrations in the rhizospheres (mg kg^{-1}) after three months of setting the experiment up.

Tree	Pb	Cu	Mn	Zn	Fe	Ni
A. farnesiana	334-15,685	3-478	0.0-12.5	3.4-12.3	3-69	0.4-9.2
C. equisetifolia	3,320-10,101	13-150	0.0 4.2	2.8-42.1	1-74	0.0-4.2
E. obliqua	1,559-14,216	10-424	0.0-7.3	1.7-43.1	14-76	1.7-7.1
P. laevigata	1,287-5,474	2-151	0.1- 9.9	2.2-10.7	6-47	0.0-5.0
C. lusitanica	918-11,101	11-161	0.3-7.9	2.0-10.3	7-75	1.4-3.7
P. greggii	2,344-9,895	10-194	0.0-5.4	4.0-12.6	10-72	0.0-3.7
F. excelsior	1,325-13,383	30-257	0.0-5.5	2.8-24.3	5-68	0.1-5.2
Adequate concentration	-	>0.2	>1	>0.2	>4.5	-
Toxic concentration	100- 300	5	-	-	-	50

[a] NOM-021-SEMARNAT-2000

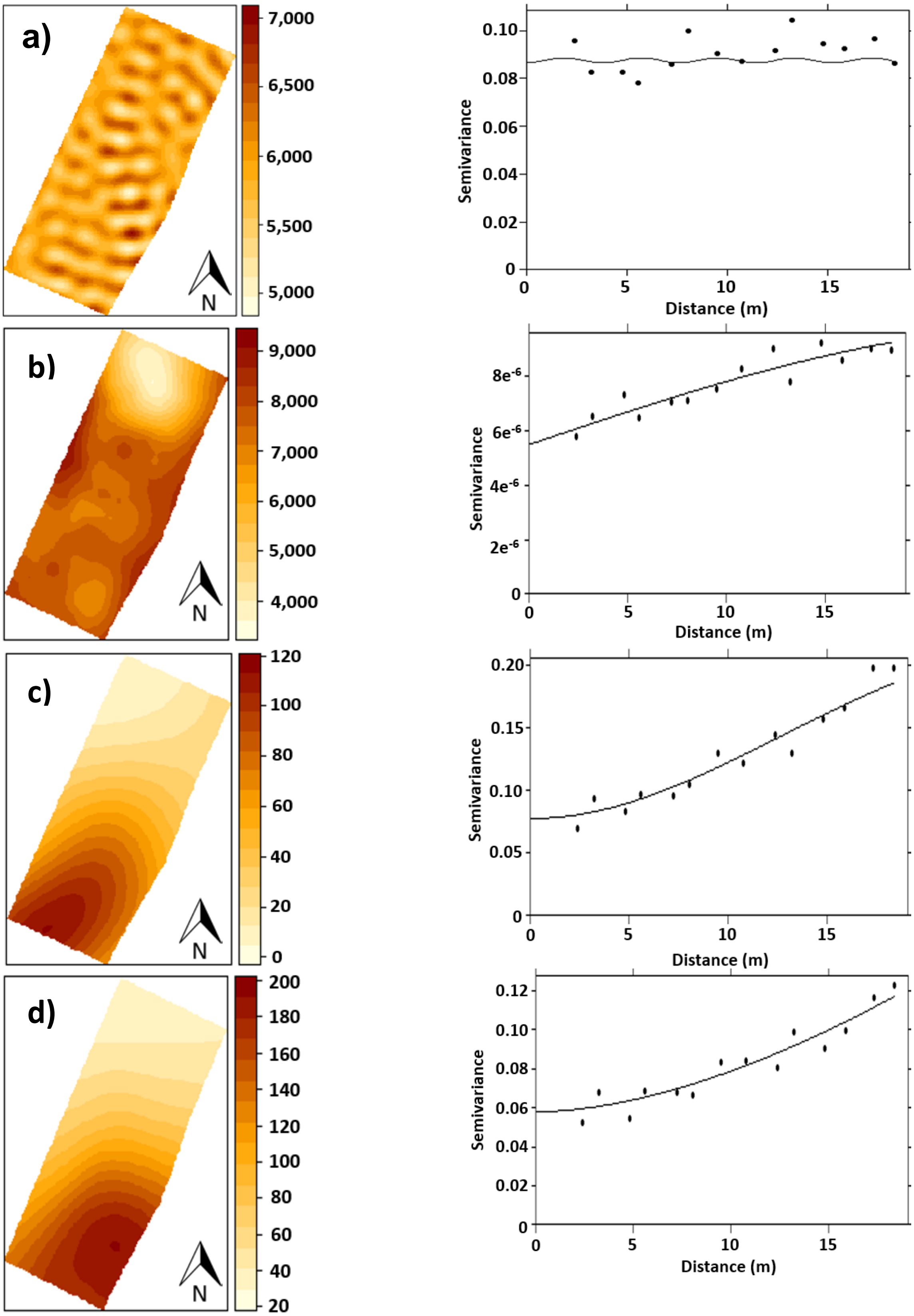

Figure 6. Distribution of DTPA-extractable metals from soil and their semi-variograms after set up the experiment: Pb three months (a), six months (b); Cu three months (c) and six months (d).

Table 13. Intervals of DTPA-extractable metal concentration in the rhizospheres (mg kg^{-1}) after six months of setting the experiment up.

Tree	Pb	Cu	Mn	Zn	Fe	Ni
A. farnesiana	459-11,182	2-633	0.2-5.2	3.6-13.3	1.0-10.2	0.0-10.6
C. equisetifolia	976-12,924	42-343	0.6-4.6	3.5-22.1	1.2-86.7	0.0-3.3
E. obliqua	933-11,967	36-580	0.4-5.7	0.8-10.8	0.0-22.0	0.0-11.8
P. laevigata	3,473-12,474	27-237	0.2-7.5	3.70-10.33	0.5-19.2	0.0-2.1
C. lusitanica	1,985-11,527	35-198	0.3-5.8	1.7-11.6	2.1-15.4	0.0-4.9
P. greggii	3,555-11,546	38-319	0.0-4.1	3.0-12.3	0.1-7.6	0.0-1.8
F. excelsior	1,363-12,249	31-364	0.2-5.4	3.4-20.6	0.2-13.1	0.0-3.6
Adequate concentration	-	>0.2	>1	>0.2	>4.5	-
Toxic concentration	300	5	-	-	-	50

[a] NOM-021-SEMARNAT-2000

3.4.3 Metal plant accumulation

Even though the high heavy metals and Na concentrations in tissues, survival of species was 100%. Only in *C. lusitanica, P. greggii* and *P. laevigata*, survival was 91%, 83% and 83% respectively. However, each species accumulated different concentration of heavy metals and responded differently to this accumulation.

3.4.3.1 Pb

All plant species accumulated this metal in their shoots (Table 14). The maximum Pb uptake by plant species was decreasing in the following order: *E. obliqua* > *P. greggii* > *C. equisetifolia* > P*. laevigata* > *F. excelsior* > *A. farnesiana* > *C. lusitanica.* However, high variability was observed even in the individuals of the same species. The lowest and the highest concentration of Pb were measured in *E. obliqua*. Correlation between DTPA-extractable Pb and Pb accumulated in the shoots was observed in *A. farnesiana* (0.567, Pr=0.085). In all species Pb accumulation reached toxic values proposed by Kabata-Pendias (2011).

The BCF for Pb varied widely (Table 15), the highest value was observed in *E. obliqua* with up to 190 and the minor was observed in the same species with 0.5. This matches with the observed for Pb accumulation in the same species. Regarding amendments, the major Pb accumulation was detected in experimental units with addition of vermicompost + sawdust (Pr>t: 0.038).

Table 14. Metal accumulated in trees shoots after six months of setting up the experiment (mg kg^{-1}).

Tree	Pb	Cu	Mn	Zn	Fe	Ni	Na
A. farnesiana	82-1,280	11-308	14-31	18-51	188-647	5.4-21.8	353-7,191
C. equisetifolia	132-2,416	4-20	26-75	12-51	91-516	1.3-15.0	201-12,587
E. obliqua	19-4,263	3-125	10-2,112	18-57	74-1,100	5.6-16.8	352-11,209
P. laevigata	269-1,616	4-21	15-46	18-53	175-422	0.4-21.7	155-5,039
C. lusitanica	108-1,262	4-43	10-60	15-44	213-989	4.4-13.4	50-8,334
P. greggii	374-2,719	12-80	14-67	20-568	476-1,132	1.5-15.4	2,363-19,546
F. excelsior	167-1,526	5-32	20-110	5-54	62-452	1.8-14.5	428-4,618
Toxic[1]	30-300	20-100	400-1,000	100-400	-	10-100	-

[1] Concentration considered as toxic or excessive for plants (Kabata-Pendias, 2011).

Table 15. Bio-concentration factors of trees after six months of setting up the experiment.

Tree	Pb	Cu	Mn	Zn	Fe	Ni
A. farnesiana	1 - 51	0.1 - 20.6	0.0 - 0.88	0.09 - 0.33	0.00 - 0.06	0.04 - 1.70
C. equisetifolia	2.8 - 66	2.2 - 9.9	0.0 - 0.11	0.09 - 0.90	0.00 - 0.04	0.00 - 1.79
E. obliqua	1 - 190	0.1 - 10.0	0.0 - 0.16	0.04 - 1.06	0.01 - 0.20	0.14 - 1.11
P. laevigata	3 - 16	0.4 - 6.4	0.0 - 0.29	0.07 - 0.51	0.01 - 0.09	0.00 - 1.98
C. lusitanica	3 - 16	0.5 - 10.3	0.0 - 0.42	0.10 - 0.47	0.00 - 0.06	0.10 - 0.69
P. greggii	1 - 23	0.2 - 7.7	0.00 - 0.27	0.01 - 0.64	0.00 - 0.02	0.00 - 1.84
F. excelsior	3 - 50	0.4 - 6.4	0.00 - 0.17	0.05 - 1.45	0.00 - 0.12	0.01 - 1.94

Concerning salts and Pb accumulation, positive correlation was observed between Pb in *E. obliqua* tissues and PO_4^{3-} in soil (0.633, Pr=0.026). Similar behavior was found in *C. lusitanica*, where positive correlation between Pb in shoots and available SO_4^{2-} in soil was found (0.598, Pr=0.05). In addition, partial correlations between DTPA-extractable Pb and the concentration of Pb accumulated in shoots were obtained in order to adjust any possible influence of covariates (SO_4^{2-}, PO_4^{3-}, pH and initial plant height) on the coefficients. This kind of correlation was positive for *C. equisetifolia* (0.692, Pr=0.018) and C. *lusitanica* (0.670, Pr=0.043) and negative for *P. laevigata* (-0.895, Pr=0.004). LS-means from ANCOVA (Table 16) shows differences between species for Pb accumulation where *P. greggii* had the highest value and *A. farnesiana* the lowest. In addition, ANCOVA showed positive effect on Pb accumulation by trees (estimate: 1.3 x 10^{-3}, Pr>F: 0.0005).

Table 16. LS-means for Pb accumulated in shoots

Species	Accumulated Pb
P. greggii	2.97 A
C. equisetifolia	2.86 AB
F. excelsior	2.75 AB
C. lusitanica	2.74 AB
E. obliqua	2.73 AB
P. laevigata	2.62 AB
A. farnesiana	2.45 B

Amendment Pr>t: 0.038, Tree species Pr>F: 0.041.

3.4.3.2 Cu

Cu concentration in plant tissues ranged from 4 to 308 mg kg^{-1}. Sequence of maximum Cu accumulated was *A. farnesiana* > *E. obliqua* > *P. gregii* > *C. lusitanica* > *F. excelsior* > *P. laevigata* > *C. equisetifolia*. ANCOVA performed showed no differences between species. However, individuals growing in the vermicompost + sawdust treatment accumulated higher concentration of Cu than trees with the vermicompost (Pr>T: 0.036). A correlation between DTPA-extractable Cu and the accumulated Cu in shoots was detected for *C. equisetifolia* (0.514, Pr=0.086) and *P. greggii* (0.557, Pr=0.093). Also, the Cu accumulated by *C. equisetifolia* was positively correlated with available SO_4^{2-} (0.548, Pr=0.06). Cu was the second metal with the highest BCF with an average over 1 in all plant species. *A. farnesiana* had a value up to 20 for this factor.

3.4.3.3 Fe, Mn, Ni and Zn

In *C. equisetifolia*, a positive relation between DTPA-extractable Zn and Zn accumulated in shoot was observed (0.694 Pr=0.012). In *P. greggii*, positive correlation was detected between DTPA-extractable Ni and the concentration of this metal accumulated in their shoots. In *P. laevigata*, it was observed a positive correlation between DTPA-extractable Mn and Mn accumulated in shoots (0.651, Pr=0.08). Regarding salt effects on metal accumulation, positive correlation were found between soil plant available SO_4^{2-} and Mn in shoots of *P. greggii,* (0.831, Pr=0.002) and between plant available PO_4^{3-} and Mn in shoots of *F. excelsior* (0.602, Pr=0.038). Fe in tissues was not correlated with DTPA-extractable Fe or salts in any species.

3.4.3.4 Na

Sequence of maximum Na accumulation was: *P. greggi* > *C. equisetifolia* > *E. obliqua* > *C.* lusitanica > *A. farnesiana* > *P. laevigata* > *F. excelsior* (Table 14). In general, major concentrations were observed in *P. greggii* (2,363-19,546 mg kg^{-1}).

3.4.4 Plant growth and longitudinal analysis

According to the longitudinal analysis, it was found that the effect of the amendments on plant growth profile was significantly different (Pr>F: 0.0125, Table 17). Vermicompost + sawdust mixture was the best treatment. In addition, significant differences among the growth profiles of the studied species (Pr>F and Pr>Chisq: 0.0001) were observed.

Table 17. Longitudinal analysis of plant growth as response of treatment applied to the soil.

Effects	Pr>Chisq	Pr>F	Estimate	Degrees of freedom (numerator)	Degrees of freedom (denominator)
Intercept	-	-	5.627	-	-
Species	0.0013	0.0044	-	6	51
Amendment	0.0728	0.0728	-	1	51
Vermicompost	-	-	-0.173	-	-
Vermicompost + sawdust	-	-	0	-	-
Time	0.0001	0.0001	-	19	1121
Species*time	0.0001	0.0001	-	114	1121
Amendment*time	0.0116	0.0125	-	19	1121
Species*amendment*time	0.0001	0.0003	-	114	51
Initial tree height	0.0001	0.0001	0.970	1	51
		Soil			
Pb	0.5734	0.5758	-0.00002	1	51
Mn	0.0685	0.0743	-0.09905	1	51
SO_4^{2-}	0.5167	0.5196	-0.00002	1	51
		Metals in shoots			
Cu	0.195	0.200	0.00482		51
Mn	0.495	0.498	0.00308	1	51
Pb	0.272	0.277	-0.00023	1	51

BIC: 3725.5
BIC without covariates: 4122.8

Regarding species, it was found significant differences, based on pair comparisons, the series of the highest growth response was: *C. equisetifolia* > *C. lusitanica, P. laevigata, E. obliqua, A. farnesiana* > *F. excelsior* > *P. greggii.* Longitudinal analysis also showed negative effect due to extractable Pb, Mn and SO_4^{2-} in soil, and Pb shoot concentrations. On the contrary, Cu and Mn in shoots had positive effect (Table 17). Specific response of the trees in relation to covariates and amendments applied to the soil was performed by analyzing data subsets by species (Tables 18 to 24). That is marginal evaluation, but provides useful information of species behavior. The following paragraphs describe the effects found. In addition, comparisons of BIC between models with and without covariates are presented.

3.4.4.1 *Acacia farnesiana*

DTPA-extractable Pb concentrations, Na concentration in tissues and initial height had positive effect on the growth of *A. farnesiana* (Table 18). In contrast, negative influence on growth profile was observed due to SO_4^{2-} in soil Regarding of amendments, vermicompost + sawdust was the best treatment (Pr>Chisq: 0.0347, Pr>F: 0.0505).

Table 18. Longitudinal analysis of *A. farnesiana* growth and the effect of fixed factors and covariates.

Effects	Pr>Chisq	Pf>F	Pr>T	Estimate	Degrees of freedom (numerator)	Degrees of freedom (denominator)
Intercept	-	-	-	-2.2663	-	-
Amendment	-				1	2
Vermicompost	-	-	0.8977	-1.2821	-	-
Vermicompost +Sawdust			-	0	-	-
Time	0.0001	0.0001	-	-	19	133
Amendment*time	0.0347	0.0505	0.0008	1.2659	19	133
Initial tree height	0.0001	0.0008			1	2
			Soil			
Pb			0.0048	0.00126	1	2
SO_4^{2-}			0.0301	-0.00021	1	2
			Metals in shoots			
Na	0.0001	0.0608	0.0608	0.00096	-	-

BIC: 418.7
BIC without covariates: 563.9

3.4.4.2 *Casuarina equisetifolia*

No effect of the amendment on the growth of *C. equisetifolia* was detected (Table 19). Initial height of the trees has positive effect on this variable. On the contrary, PO_4^{3-} had negative effect on plant growth (Pr>Chisq: 0.0854, Pr>T: 0.0867). No differences between amendments were observed.

Table 19. Longitudinal analysis of *C. equisetifolia* growth and the effect of fixed factors and covariates.

Effects	Pr>Chisq	Pf>F	Pr>T	Estimate	Degrees of freedom (numerator)	Degrees of freedom (denominator)
Intercept	-	-	0.8798	-3.068	1	226
Amendment	0.3254	0.3264	-	-	1	226
Vermicompost	0.0001	0.0001	-	4.857	-	-
Vermicompost + Sawdust				0	-	-
Time	0.0001	0.0001	0.0001	-	1	226
Amendment*time	0.0001	0.0001			1	226
Initial tree height	0.1989	0.2002	-	1.072	1	226
			Soil			
PO_4^{3-}	0.0854	0.0867	0.0867	-0.077	1	226

BIC: 1448.4
BIC without covariates: 1448.8

3.4.4.3 *Cupressus lusitanica*

Pb accumulated in tissues of *C. lusitanica* had negative effect on growth. In contrast, initial height had positive effect on plant growth (Table 20). Vermicompost application was the amendment with the best effect on *C. lusitanica* growth (Pr>F: 0.0001).

Table 20. Longitudinal analysis of *C. lusitanica* growth and the effect of fixed factors and covariates.

Effects	Pr>Chisq	Pr>F	Pr>T	Estimate	Degrees of freedom (numerator)	Degrees of freedom (denominator)
Intercept	-	-	0.47	4.5	-	-
Amendment	-	-	-	--	1	3
Vermicompost	-	-	0.093	16.9	-	-
Vermicompost + Sawdust	-	-	-	0	-	-
Time	0.0001	0.0001	-	-	19	171
Amendment * time	0.0001	0.0001	-	-	19	171
Initial tree height	0.0001	0.0005	0.0005	1.19	1	3
Metals in shoots						
Pb	-	-	0.040	-0.01	1	3

BIC: 696.9
BIC without covariates: 708.0

3.4.4.4 *Eucalyptus obliqua*

Cu in shoots had negative effect on *E. obliqua* growth profile (Table 21). This species responded better to the addition of vermicompost than the addition of vermicompost + sawdust (Pr>Chisq: 0.0805, Pf>F: 0.0956).

Table 21. Longitudinal analysis of *E. obliqua* growth and the effect of fixed factors and covariates.

EFFECTS	Pr>Chisq	Pf>F	Pr>T	Estimate	Degrees of freedom (numerator)	Degrees of freedom (denominator)
Intercept	-	-	-	-	-	-
Amendment	0.5613	0.5775	-	-	1	8
Vermicompost	-	-	-	0.658	-	-
Vermicompost + sawdust	-	-	-	0	-	-
Time	0.0001	0.0001	-	-	19	190
Amendment*time	0.0805	0.0956	-	-	19	190
Initial tree height	0.0001	0.0001	0.0001	-	1	8
Metals in shoots						
Cu	0.0033	0.0187	0.0187	-0.025	1	8

BIC: 991.7
BIC without covariates: 1093.7

3.4.4.5 *Fraxinus excelsior*

Positive effect of DTPA-extractable Pb on *F. excelsior* growth was observed (Table 22). In contrast, negative effects of Cu on soil and available SO_4^{2-} were detected. Likewise, there were significant differences between amendments where vermicompost + sawdust had better result than vermicompost (Pr>F: 0.0011).

Table 22. Longitudinal analysis of *F. excelsior* growth and the effect of fixed factors and covariates.

Effects	Pr>Chisq	Pf>F	Pr>T	Estimate	Degrees of freedom (numerator)	Degrees of freedom (denominator)
Intercept	-	-	0.0017	25.179	-	-
Amendment	0.0446	0.1150	-	-	1	4
Vermicompost	-	-	0.6275	-0.994	-	-
Vermicompost + Sawdust	-	-	-	0	-	-
Time	0.0001	0.0001	-	-	19	190
Amendment*time	0.0004	0.0011	-	-	19	190
Initial tree height	-	-	0.0001	0.701	1	4
				Soil		
Pb	-	-	0.0356	0.0003	1	4
Cu	-	-	0.0349	-0.0221	1	4
SO_4^{2-}	-	-	0.0054	-0.0006	1	4

BIC: 495.9.
BIC without covariates: 478.8

3.4.4.6 *Pinus greggii*

Pb in shoots, available SO_4^{2-} and initial tree height had positive effect on *P. greggii* growth (Table 23). In contrast, Na and Cu accumulated in tissues had negative effects. There were no significant differences between amendments.

Table 23. Longitudinal analysis of *P. greggii* growth and the effect of fixed factors and covariates.

Effects	Pr>Chisq	Pf>F	Pf>T	Estimate	Degrees of freedom (numerator)	Degrees of freedom (denominator)
Intercept	-	-	0.0209	7.8	1	3
Amendment	0.1117	0.2152	-		-	-
Vermicompost	-	-	-	2.00	-	-
Vermicompost + Sawdust	-	-	-	0	-	-
Time	-	-	-	-	19	152
Amendment*time	-	-	-	-	19	152
Initial tree height	-	-	0.0022	0.756	1	3
			Soil			
SO_4^{2-}	0.0133	0.0896	0.0896	0.00008	1	3
		Metals in shoots				
Pb	0.0001	0.0147	0.0147	0.0046	1	3
Na	0.0078	0.0022	0.0763	-0.0001	1	3
Cu	0.0001	0.0234	0.0234	-0.1437	1	3

BIC: 220.3
BIC without covariates: 233.0

3.4.4.7 *Prosopis laevigata*

Vermicompost + sawdust mixture had the best result on *P. laevigata* growth (Pr>F: 0.0001). In addition, positive effect of Na in tissues and initial tree height were observed (Table 24).

Table 24. Longitudinal analysis of *P. laevigata* growth and the effect of fixed factors and covariates.

Effects	Pr>Chisq	Pr>F	Pr>T	Estimate	Degrees of freedom (numerator)	Degrees of freedom (denominator)
Intercept	-	-	.1128	-9.3003	-	-
Amendment	0.0001	0.0001	0.0001	-	1	131
Vermicompost	-	-	-	-8.3030	-	-
Vermicompost + Sawdust	-	-	-	0	-	-
Time	0.0001	0.0001	0.0001	0.0671	-	-
Amendment*time	0.0001	0.0001	0.0001	-	1	131
Initial tree height	-	-	0.0001	1.7558	1	131
Metals in shoots						
Na	-		0.0006	0.0014	1	131

BIC: 834.5

BIC without covariates: 923.4

3.4.5 Costs

Costs were calculated as an inversion project and were divided in two areas, dendro-remediation and dendro-remediation + research. The first includes acquisition of fixed assets as vehicle for transportation and materials for field work. Also includes analysis of pH, Pb and Cu (total and DTPA-extractable), available nitrogen, soluble salts in Chapter II, likewise trees establishment with amendments. Hence, dendro-remediation has a total cost of US $29,593 assigned as follows: US $22,220 for field expenses, $673 for office, $300 for laboratory and $6,400 for salary of the people involved in the work.

Dendro-remediation + research includes the fixed assets in dendro-remediation and all chemical analysis in Chapters II and III assigned as follows: US $23,906 for field, $673 for office, $27,367 for laboratory and $85,867 for salary of the people involved in the work (currency exchange rate: 1 US$ =15 MX$). Table 25 shows the costs of dendro-remediation and dendro-remediation + research by parts.

Table 25. Costs of dendro-remediation and dendro-remediation + research of a field work in a defunct LABR site.

	Materials	Dendro-remediation	Dendro-remediation + research
Field	Ford Ranger XL	$ 21,613	$ 21,613
	Compost	$ 80	$ 80
	Sawdust	$ 33	$ 33
	Stakes	$ 33	$ 33
	Number of wages	$ 15	$ 70
	Price/wage	$ 13	$ 13
	Total for wages	$ 200	$ 200
Field operative costs	Gasoline ($/L)	1	1
	Visits	15	70
	Litters per visit	20	20
Total		$ 260	$ 1,213
Field total		$ 22,220	$ 23,907

	Material	Dendro-remediation	Dendro-remediation + research
Office	Computer	$ 667	$ 667
	Notebooks	$ 3	$ 3
	Pencils	$ 3	$ 3
Total		$ 673	$ 673
Laboratory	Initial sampling	$ 300	$ 20,327
	Third month sampling	-	$ 3,520
	Sixth month sampling	-	$ 3,520
Total		$ 300	$ 27,367
Salary	Fortnights cost	$ 800	$ 800
	Number of fortnights	4	52
	People	2	2
	Design elaboration	-	$ 2,667
Total		$ 6,400.0	$ 85,867

Some quantities are rounded.

3.5 DISCUSSION

3.5.1 Salts and trees

Taking into account the experimental data obtained from soil sampling, it is possible to consider most rhizospheres as moderately alkaline (pH 79 - 8.4) or strongly alkaline (pH 8.5 - 9.0) and some as extremely saline (EC>16 dS m^{-1}), that means only very salt-tolerant crops can yield satisfactorily (Hazelton and Murphy, 2007).

Salinity in LABR site is promoted by PO_4^{3-}, SO_4^{2-}, which were found in high concentrations on the soil (Section 3.4.1). These anions have corrosive effects on plant roots and accumulation in tissues depletes plants yield (Chapman and Pratt, 1991) because leaf growth and photosynthesis rates are reduced. Salts have osmotic effect on plant tissues and water homeostasis potential (Munns, 1993; Parida and Das, 2005; Rameeh *et al.*, 2012; Parida and Das, 2005).

Longitudinal analysis showed that *E. obliqua*, *C. lusitanica*, *P. greggii* and *P. laevigata* were not influenced negatively by PO_4^{3-} or SO_4^{2-} in soil (Tables 20 21 and 24). An investigation conducted by Rios-Gomez *et al*. (2010) support the capability to *P. laevigata* seedlings to growth under salt stress. Ability of *Eucalyptus* species to growth in fields affected by salts also has been investigated by Bush *et al.*, 2013; who showed that *E. camaldulensis* grew well at EC from 2 to 9 dS m^{-1}. Feikema and Baker (2011) evaluated the growth of three species of *Eucalyptus* on soils with EC up to 10 dS m^{-1}. In the LABR site, *E. obliqua* growth under EC ranged from 7.9 to 9.9 dS m^{-1}; which means highly saline conditions (EC 8-16 dS m^{-1}; Hazelton and Murphy, 2007). In the same way, *P. greggi* growth on LABR site was influenced positively by SO_4^{2-}. Hence, a recommendation is that this species can be introduced in soils with salinity problems due to this anion. This is supported by studies that describe species of *Pinus* growing under soil salinity conditions (Croser *et al.*, 2001; Khaldi *et al.*, 2011).

In contrast, longitudinal analysis of *C. equisetifolia* growth showed negative effect of PO_4^{3-} concentrations. However, the results showed 128.9% of net growth in *C. equisetifolia* (100*final height / initial height) and final survival of 100% at one year of trees establishment. *F. excelsior* was also affected negatively by SO_4^{2-} and this species also achieved 100% of survival. There are no previous studies related to SO_4^{2-} effect on *Fraxinus* species.

3.5.1.1 Na in shoots

Although high shoot Na concentration was observed, all tested species survived and grew without any damage or Na toxicity symptoms during the experimental period (1 year). This is a remarkable result because in general plants are not tolerant to Na. This is due mainly to its competition with K and Ca (Mäser *et al.*, 2002; Stassart *et al.*, 1981). In fact, longitudinal analysis showed no effect of Na on growth profile of *C. equisetifolia, C. lusitanica, E. obliqua, F. excelsior* (Tables 19 and 22). Other studies showed that *C. equisetifolia* trees growing under sodic conditions survived for seven years (time of sampling) in conditions of pH ranging from 8.5 to 9.5 (Rana *et al*., 2001). In other experiment, Singh *et al.*, (2010) observed that *C. equisetifolia* had 95% of survival after 10 years growing in an alkali and sodic soil with pH values up to 10.6 and exchangeable sodium percentage of 60-89. Batra and Dikshit (1994) conducted an experiment with four species of *Casuarina* and found that *C. equisetifolia* accumulated approximately 4.6 mg kg^{-1} of Na in steams growing under 70% of exchangeable sodium. Bryson and Barker (2002) reported *Fraxinus* trees growing in soil with up to 101 mg kg^{-1} of Na, this plant accumulated up to 193 mg of Na kg^{-1} in leaves (dry weight bases) and no visible signs of salt injury were observed.

No effects of Na toxicity (necrosis or leaf abscission) was observed. Moreover, *A. farnesiana* and *P. laevigata* showed the best response to Na. Longitudinal analysis of these species showed positive influence of this cation accumulation on growth profile. Remarkable high Na concentration was observed in their shoots (up to 7,191 mg kg^{-1} and 5,039 mg kg^{-1} for *A. farnesiana* and *P. laevigata*, respectively). In addition, in LABR site, there was no effect of SO_4^{2-} or any salt in soil on *P. laevigata* growth profile (Table 24). This suggests that these species can be support soils severely affected by Na. Salinity tolerance by chloride and sulfate salts has been observed in *P. laevigata* under greenhouse conditions (Rios-Gomez *et al*. 2010). These authors concluded that *P. laevigata* can be used for rehabilitation of soils affected by salts when EC<5 dS m^{-1} (Maximum EC In the present study: 3.8 dS m^{-1}). Goel and Behl (1995) also observed ability of six species of *Prosopis* to grow on alkali soils with pH between 8.5 and 10.6. Aref *et al*. (2004) also observed Na tolerance in *Acacia* species. However, other authors have observed negative Influence of NaCl on germination and growth of *Acacia* species (Morais *et al*., 2012; Rashid *et al*., 2004). Nevertheless, in both investigations, it is important to consider that *Acacia species* achieve high percentage of germination and height in spite of the high NaCl concentrations and salinity conditions of the experiments (up to 4,600 mg kg^{-1} of NaCl and 22.5 dS m^{-1}). In Aref *et al.* (2004) study, *Acacia* species germination was up to 69% in 4 dS m^{-1}, which EC is similar to this observed at LABR site. Morais *et al.* (2012) showed that *Acacia* plants had 30% height reduction under NaCl 200 mM compared with plants growing in 0 mM. In the LABR site, the only salt which affected negatively plant growth profile of *A. farnesiana* was SO_4^{2-}. However, this plant species had 100% of survival even the high salt concentrations and EC in the soil (up to 22 dS m^{-1}).

FAO (2002) recommended species of *Acacia*, *Casuarina* and *Eucalyptus* to grow in soils with salinity problems in the United States. In addition, the Joint Genome Institute on United States of America pointed out that species from Casuarinaceae family are able to tolerate salts and heavy metals (USDE, 2015). The multipurpose capabilities of these species regarding fertility management and rehabilitation of lands affected by salts (Barthwal *et al.*, 2005) have been observed. These capabilities can improve soil conditions in the LABR site, where very low nitrogen content was observed (Chapter II, section 2.4.1). These results suggest the possibility of use of these plant species for dendro-remediation of this kind of polluted soils and in consequence decrease dispersion of pollutants. However, further evaluations must include assessment trees behavior for longer periods. A possible strategy to ameliorate possible negative Na effects on trees is the addition of amendments with Ca. As results from Chapter II (section 2.5.1), Ca concentrations are not enough to cope with Na concentrations in soil. Some studies support ability of Ca to reduce negative Na effects in plants (Al-Khateeb, 2006; Guimaȓes *et al.*, 2012).

Other factor to take into account in relation to salts effect in trees growth is the spatial variation in time of soil salt concentrations. This variation can be observed in in LABR site, where spatial patterns changes between samplings were observed. For SO_4^{2-} concentrations spatial pattern, slight differences were observed (Figures 5c and 5d). In contrast in PO_4^{3-} pattern an evident change was observed (Figures 5a and 5b). The mentioned changes make necessary to perform samplings in the future to assess the effect of salts influence in tree growth and survival.

3.5.2 Metals and trees

The relationship between trees and metals has been poorly studied mainly due to the long growth period of these species. In order to understand how metals can affect dendro-remediation of the site, this research focused on uptake and accumulation of Pb and Cu, which were in the highest concentrations.

3.5.2.1 Pb

Pb accumulation for individuals of the same species was different, probably due to the patchiness of the soil conditions and the intrinsic variability of the species. Significant higher Pb shoot concentrations were found in plants growing under vermicompost + sawdust treatment than the plants growing under vermicompost. Sawdust mineralization might desorb Pb previously adsorbed at the beginning of the experiment. DTPA-extractable Pb was positively influenced by pH (Pr>F: 0.0001). Soluble chemical species could be present at high pH values (for instance some Pb hydroxides) and contribute to available Pb. This is supported by results from Chapter II, where water-extracted Pb was detected bound to OH^- (Table 8 and Figure 4). It is important to note that there are no acidic rhizospheres. So, evidently the mentioned correlation may be negative under low pH values, where increment of soil acidity promotes heavy metal availability.

SO_4^{2-} concentrations also were correlated with DTPA-extractable Pb (Pr>F: 0.0001). Influence of SO_4^{2-} in shoots concentration may be due to an association of Pb and SO_4^{2-} in soil compounds. This influence is supported by ANCOVA performed for Pb accumulation which showed positive effect of SO_4^{2-} on Pb accumulation (estimate: 1.34×10^{-3}, Pr>F: 0.0005). Gaurina-Medjimurec (2014) mentioned that Pb^{2+} is reduced to $PbSO_4$ and $PbCO_3$ and therefore stabilized in the soil. These compounds have low water solubility (0.00425 g 100 mL^{-1} and 0.00011 g 100 mL^{-1} for $PbSO_4$ and $PbCO_3$ respectively). Moreover, Han (2007) pointed out that at pH>8, $PbOH^+$ contributes significantly to total inorganic Pb, while at pH>9 $Pb(OH)_2^0$ and $Pb(OH)_3^-$ may be formed. The same authors mentioned that other forms of Pb as halides, sulfates and nitrates become important when concentrations of these ligands are major to 10^{-2} M. In the rhizospheres from LABR site, concentrations of sulfate were around 2.9×10^{-1} M (28,078 mg kg^{-1}, Table 11).

Positive correlations were found between shoot accumulated Pb and available PO_4^{3-} for *E. obliqua* and between Pb accumulated in shoots of *C. lusitanica* and SO_4^{2-} (section 3.4.3.1). It may be due to an association of these anions with Pb. PO_4^{3-} and SO_4^{2-} can form compounds with Pb; which are one of the less mobile Pb forms in soil (Wuana and Okieimen, 2011). Phosphate treatment reduces Pb solubility (Stanforth and Qiu, 2001) and $PbSO_4$ water solubility is very low (Ksp= 1.8 x10^{-6}). Hence, despite of the alkalinity produced by these salts, their presence in the LABR site is a factor to reduce Pb mobility. However, *C. lusitanica* and *E. obliqua* are apparently capable to break these compounds bonds or solubilization processes are occurring in their rhizospheres; but this hypothesis requires deeper studies.

Pb accumulated in shoots affected trees growth differently depending on the species. A negative effect of plant Pb concentration on growth profile was observed only in *C. lusitanica* (Table 20). General longitudinal analysis showed no effects of DTPA extractable Pb or Pb accumulated in shoots on trees growth profile (Table 17). In fact, DTPA-extractable Pb had positive influence on *A. farnesiana* and *F. excelsior* growth. Similarly, positive effect was observed for *P. greggii* growth due to Pb in tissues of this species (Tables 18, 22 and 23). Aissa and Kéloufi (2012) observed that *Casuarina* trees accumulated 23-60 mg kg^{-1} of Pb in their needles in an urban zone. In LABR site under this study, higher Pb accumulation in all species was observed (ranging from 19 to 4,263 mg kg^{-1}). Concentrations are considered as toxic or excessive for plants (Kabata-Pendias, 2011) with up to 4.3, 8.1, 14.2, 5.4, 4.2, 9.1 and 5.1 times for *A. farnesiana, C. equisetifolia, E. obliqua, C. laevigata, C. lusitanica, P. greggii* and *F. excelsior* respectively (Table 14). 28% of the trees established accumulate Pb in the range of hyperaccumulation (>1000 mg kg^{-1} Pb; van der Ent *et al.*, 2013). BCF for Pb were up to 190 and 66 for *E. obliqua* and *C. equisetifolia*, respectively (Table 15).

In order to consider that these plants are Pb hyperaccumulators, besides Pb accumulation and BCF, it is necessary to take into account trees biomass; which should be high. With these three data, it will be possible to assess the amount of metal that plants extract from soil through a ratio of metal stabilized per area per time. In addition, hyperaccumulation does not give much useful information itself for dendro-remediation. However, the amount of metal stabilized in plant tissues per area or volume of soil can be estimated and will be valuable.

3.5.2.2 Cu

In spite of DTPA-extractable Cu concentrations (Tables 12 and 13), trees accumulated low concentrations of this metal in shoots (Table 14) which is reflected in low BCFs (Table 15). These factors were in many cases less than one. However, in some trees of *A. farnesiana,* BFC were up to 20. In another study, Rosselli *et al*. (2003) found BCF in *F. excelsior* of 0.01 for Cu in leaves in an experiment with metal contaminated compost. Regarding Cu in shoots growth effect, general longitudinal analysis (Table 17) showed positive influence of Cu on trees growth profile. However, different response was observed between species. For instance, negative effect of this metal was detected on growth profile of *E. obliqua, P. greggii* and *F. excelsior*. In *E. obliqua*, maximum shoots Cu concentration was above the considered as toxic for plants (Table 14). In other study, Mertens *et al*. (2007) found Cu accumulation of 1.2 mg kg^{-1} of Cu in wood of *F. excelsior* after 33 years of growing in sediments with pseudo-total concentrations (acid digestion with HCl and HNO_3) of approximately of 186 mg kg^{-1} of Cu and. In the other species tested in LABR site, there was no effect due to Cu in shoots on growth profile. This is a remarkable fact because these species can be recommended in sites with high concentrations of Cu.

Concerning amendments effect on Cu accumulation in shoots, plants growing under influence of vermicompost + sawdust accumulated higher Cu concentration than plants growing with only vermicompost. Cu could be bound in OM in sawdust which is supported by ability of OM to sequestrate this metal in soil (Mohamed *et al.*, 2010). However, OM mineralizes into the soil and probably liberates the sequestered Cu. This makes necessary to replace mineralized OM. In this way, trees are able to supply constantly OM to soil which replaces the mineralized OM (Bot and Benites, 2005).

3.5.2.3 Fe, Mn, Ni and Zn

In general, concentrations of Mn, Ni and Fe were low in all species, except for *E. obliqua;* which accumulated 42 times more Mn concentration considered as excessive for plants (Table 14). However, longitudinal analysis of plant growth profile showed no effect due to Mn in tissues of this species. Longitudinal analysis showed general negative tendency of DTPA-extractable Mn in soil on growth profile (Pr>Chisq: 0.0685, Pr>F:0.0743). In the soil of this study, DTPA-extractable Mn was above the recommended value in trees of all species in the two soil samplings (Tables 12 and 13). This evidenced the inadequate waste management carried out in the LABR; which had negative influence on plant growth. This was also observed in Chapter 2 where Mn concentrations were above the concentration of non-polluted soil near the site (Chapter 2, Table 7). This is related with the correlation found between DTPA extractable Mn concentrations and Mn accumulated in (0.694 Pr=0.012). Remarkably, there was no effect on *P. greggii* growth profile due to Mn in shoots. This means ability to this species to growth in spite of excessive Mn accumulation. Negative effects due to Mn plant concentration are low Fe plant absorption (Alam *et al.*, 2000). This is supported by the low Fe BCF found in P. greggi (Table 15).

3.5.3 Costs

It is not possible to perform a direct comparison between dendro-remediation and physico-chemical methods for metal polluted soils remediation, but it can be referenced. Cost approximate to remediate a metric ton of soil polluted with metals is US$ 10-90 for containment (covers, barriers), US$ 60-290 for solidification/stabilization, US$ 400 - 870 for vitrification, US$ 60-245 for soil washing, US$ 60-163 for soil flushing and US$ 250-560 for pyrometallurgical methods (EPA, 1997). If a density of 1.5 g m^{-3} in the LABR site with an area of 1041 m^2, it would be necessary to remediate 468 ton of superficial soil (0-30 cm). This would generate a cost from US$ 4,680 for the cheapest physico-chemical method (containment) to 262,080 for the most expensive physico-chemical method (pyrometallurgical) assuming applicability of the methods. This because methods which apply heat should be avoided because in LABR site chloride concentration is very high and heating chloride could generate toxic gas, also salts could interfere in methods as soil washing (EPA/OSWER/ORD, 1991). However, costs would increment when using physico-chemical methods mainly due to salts content and pH, and the necessary previous physical rock removing and or crushing. In this dendro-remediation work, total cost was US $29,593; which meant US $28 per square meter. In addition, it is important to point out the different costs of dendro-remediation process itself and dendro-remediation and research. The cost increases when profuse laboratory analyses are performed for research purposes as the determination of metals concentration in soil fractions or soil solution (Table 25).

3.5.4 Potential environmental benefits of dendro-remediation

Dendro-remediation in the LABR site provides a vegetal covering that reduces dispersion of particles that contain heavy metals and then trees accumulate high concentrations of metals in shoots (Table 14). Nevertheless, there are other potential benefits of trees establishment that has been investigated by other authors. One of them benefits is carbon sequestration. It has observed that *C. equisetifolia* sequesters up to 2.9 ton C ha^{-1} year^{-1} (Wang *et al*., 2013), *Eucalyptus* 8.8 ton C ha^{-1} year^{-1} (Du et al. 2015), *F. excelsior* 1.8 ton C ha^{-1} year $^{-1}$ (Wellock et al. 2013), *C. lusitanica* 192 kg C tree^{-1} (24 year old individuals; Berhe et al. 2013) and *P. greggii* 8 kg C tree^{-1} (6 year old individuals; Pacheco-Escalona et al. 2007). Other benefit is related to trees positive influence in nutrient cycling through addition of nutrients to soil when litter decomposes in soil. In addition, some trees as *C. equisetifolia* contribute with nitrogen fixation (Buck et al., 1999). Nutrient recycling could increment nitrogen concentration in the site which was low in whole LABR soil terrain (section 2.4.1).

3.6 CONCLUSION

The species used in this experiment were able to survive in the very hostile conditions found in LABR soil and the establishment of trees was successful. *C. equisetifolia* and *C. lusitanica* seem to be the species with the best response because they had the high percentage of survival and major growth than the other species in spite of metal accumulation in shoots. Nevertheless, all trees species were able to cope with both salinity and metal pollution in the soil. They responded in different way to these factors and amendments through the period of the experiment. In general, vermicompost + sawdust gave the best plant growth response compared with vermicompost as single amendment. Moreover, trees species established in LABR site may be useful for remediation of severely affected by metals due to their high survival percentages and the growth achieved in spite of high heavy metal concentrations in soils and the accumulated in trees shoots. Hence, based on results obtained, it is possible to conclude that a low cost dendro-remediation process has been started in the defunct LABR site. Further investigation should include long term growth evaluation, dynamics of heavy metal plant accumulation and asses of environmental benefits.

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GENERAL CONCLUSIONS

Lead Acid Battery Recycling (LABR) soil studied in Tepetlaoxtoc is one of the most polluted LABR sites in the world. Total soil Pb concentration exceeds recommended thresholds by some nations (up to 444,105 mg kg^{-1}; 44%). This constitutes a potential high potential risk for human health and it makes necessary to remediate the site. In addition, high salt concentrations and scarce nutrients content observed in the site makes LABR site a very hostile environment to plant growth. This situation represents a difficulty for soil chemical remediation because it must necessary to remove salts before treatment to address Pb pollution. Regarding dendro-remediation, the challenge is to found trees species resistant to both Pb and salts concentration.

No experiment or previous research has been conducted to remediate sites polluted by Pb from recycled acid batteries using plants. Dendro-remediation seems to be good option to decrease Pb and Cu risks in the site under study. Trees cover the surface and avoid pollutant dispersion. In addition, their roots have huge exploration area and massive biomass, which can stabilize great amounts of pollutants in the site.

For this reason, seven species were tested for remediation. All of them were capable to establish in the LABR site and survive (100% in some cases) and in many individuals Pb hyperaccumulation was observed. Although hostile soil physico-chemical conditions were observed, tree growth was no interrupted and none visible effect of toxicity due to Pb, SO_4^{2-}, PO_4^{3-}, Na were observed. However, each species responded differently to the soil variables and the amendment used. *C. equisetifolia* seems to be the most successful species, Pb has not negative effects during its development, and only PO_4^{3-} affected negatively its growth. In contrast, *P. greggii* was the species with the minor growth rate; however, *P. greggii* still reached high survival (83%).

Based on the obtained results, it is possible to recommend all the tested species for dendro-remediation of lead polluted soils. They were useful to deal with the harsh conditions found in the site. This research represents a starting point in a remediation process where plant establishment was successful. This likely will prevent dispersion of pollutants and hence, reduction of risk exposition to living organism.