



UNIVERSIDAD AUTÓNOMA
DE AGUASCALIENTES

Centro de Ciencias Básicas
Departamento de Fisiología y Farmacología

Tesis

**Stabilisation of Metals in Soils and Sediments Contaminated by
Mining Activity Using Biomass**

*Estabilización de Metales en Suelos y Sedimentos Contaminados por Actividad
Minera Empleando Biomasa*

Presenta:

Kerry Nigel Mitchell

para obtener el grado de
Doctorado en Ciencias Biológicas

Tutores

Dr. Francisco Javier Avelar González
Dra. Alma Lilian Guerrero Barrera

Asesor

Dr. Frederic Thalasso Siret

*Aguascalientes, Ags, México
Agosto 2016*



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PRESENTE:

Por este conducto hago constar que el L.C.A. Kerry Nigel Mitchell, alumno del Doctorado en Ciencias Biológicas, cumplió satisfactoriamente con todos los requisitos de su trabajo del proyecto de tesis "STABILISATION OF METALS IN SOILS AND SEDIMENTS CONTAMINATED BY MINING ACTIVITY USING BIOMASS".

Su documento de tesis ha sido revisado y avalado como versión final por los miembros del Comité Tutorial, por lo cual puede procederse a la programación de su Examen de Grado.

Sin mas por el momento, agradezco su amable atención a la presente.

Quedo de usted.

ATENTAMENTE

Aguascalientes, Ags, 28 de julio de 2016
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Dr. Francisco Javier Avelar González
TUTOR DE TESIS



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Aguascalientes, Ags, 28 de julio de 2016

"SE LUMEN PROFERRE"

Dra. Alma Lilian Guerrero Barrera
TUTORA DE TESIS



**CENTRO DE INVESTIGACIÓN Y DE ESTUDIOS AVANZADOS
DEL INSTITUTO POLITÉCNICO NACIONAL**

México DF, 21 de julio del 2016

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Decano del Centro de Ciencias Básicas

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Quedo de usted.

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Dr. Frederic Thalasso Siret
Investigador Titular 3C



UNIVERSIDAD AUTÓNOMA
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KERRY NIGEL MITCHELL
DOCTORADO EN CIENCIAS BIOLÓGICAS
PRESENTE.

Estimado alumno:

Por medio de este conducto me permito comunicar a Usted que habiendo recibido los votos aprobatorios de los revisores de su trabajo de tesis y/o caso práctico titulado: **“Stabilisation of metals in soils and sediments contaminated by mining activity using biomass”**, hago de su conocimiento que puede imprimir dicho documento y continuar con los trámites para la presentación de su examen de grado.

Sin otro particular me permito saludarle muy afectuosamente.

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“Se lumen proferre”

EL DECANO

M. en C. JOSÉ DE JESÚS RUÍZ GALLEGOS

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Date: May 05, 2016
To: "Francisco Javier Avelar González" fjavelar@correo.uaa.mx
From: "Bulletin of Environmental Contamination & Toxicology (BECT)" Ashi.Asokan@springer.com
Subject: Decision on your manuscript #BECT-D-15-01166R3

Dear Dr Avelar González:

I am pleased to inform you that your manuscript, "Evaluation of environmental risk of metal contaminated soils and sediments near mining sites in Aguascalientes, Mexico" has been accepted for publication in Bulletin of Environmental Contamination and Toxicology. Your manuscript will be assigned to a current issue as soon as possible.

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Congratulations and best regards,

Erin Bennett
Editor-in-Chief
Bulletin of Environmental Contamination and Toxicology



Evaluation of Environmental Risk of Metal Contaminated Soils and Sediments Near Mining Sites in Aguascalientes, Mexico

Kerry Nigel Mitchell, Magdalena Samanta Ramos Gómez, Alma Lilian Guerrero Barrera, Laura Yamamoto Flores, Juan Armando Flores de la Torre,

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“SE LUMEN PROFERRE”

Dedication

to my beautiful, sweet, loving wife, life partner and best friend,

Sandra Quintana Lemus



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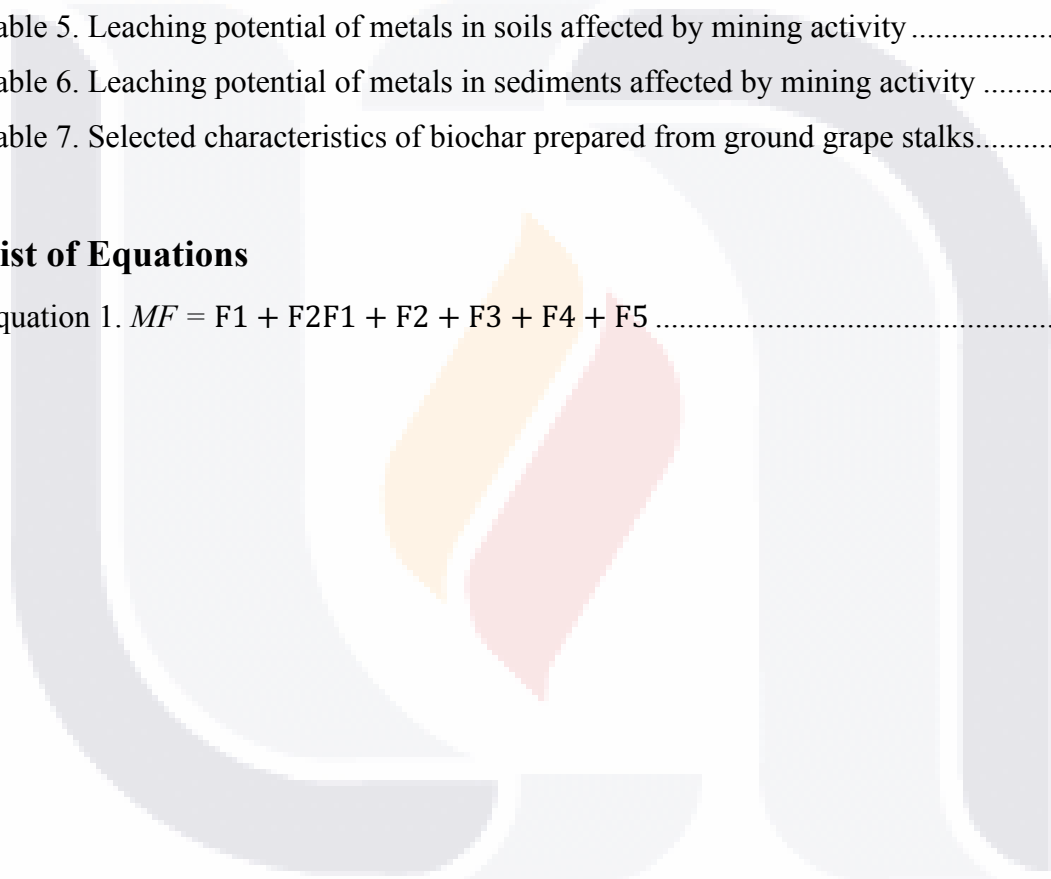
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Abbreviations

AAS – Atomic Absorption Spectroscopy

APHA – American Public Health Association

AWWA – American Water Works Association

BC – Biochar

BS – Biosolids

CCA – Chromated Copper Arsenate

CCME – Canadian Council of Ministers of the Environment

IBI – International Biochar Initiative

ICP-OES – Inductively Coupled Plasma Optical Emission Spectrometry

MF – Mobility Factor

NIST – National Institute of Standards and Technology

NMX – Norma Mexicana (Official Standard)

NOM – Norma Mexicana (Official Standard)

S-CA – Soil with CCA treated wood ash

SEMARNAT – Secretaría del Medio Ambiente y Recursos Naturales de Mexico (Mexican Secretariat of Environment and Natural Resources)

SM – Biochar’s Source Material

S-NCA – Soil with untreated wood ash

TAPPI – Technical Association of the Pulp and Paper Industry

USDA – The United States Department of Agriculture

USEPA – The United States Environmental Protection Agency

WPCF – Water Pollution Control Facility

1 Resumen (español)

De manera natural en los suelos y sedimentos, se encuentran metales en concentraciones bajas o trazas lo cual no representa un riesgo toxicológico significativo. Sin embargo, las actividades antropogénicas incluyendo la fundición y minería han sido la causa de un aumento progresivo en la concentración de metales en el medio ambiente. A concentraciones elevadas, los metales pueden ser muy tóxicos para los organismos, tal es el caso del arsénico, cadmio, cromo, plomo y mercurio los cuales se encuentran entre los compuestos prioritarios para su estudio en materia de salud pública. Estos elementos metálicos se consideran tóxicos sistémicos, persistentes y bio-acumulables incluso en los niveles más bajos de exposición.

Los metales no se pueden degradar ni destruir. Sin embargo, son sujetos a cambios en su especiación química bajo ciertas condiciones fisicoquímicas, como la degradación de materia orgánica, cambios de pH, potencial redox o composición de la fase líquida. Aunque los suelos son amortiguadores naturales de contaminación por metales en bajas concentraciones cuando las concentraciones aumentan, esa capacidad es limitada e incluso, los suelos y sedimentos pueden transformarse en una fuente de contaminación. Cuando esta capacidad de amortiguación se ve rebasada, los contaminantes pueden ser movilizados, lo que está directamente relacionado con su biodisponibilidad y toxicidad; por lo que se vuelve necesario llevar a cabo estrategias de remediación.

La remediación de los sustratos contaminados es una actividad importante para la protección ambiental y la salud de los organismos expuestos a dichos sustratos. En el caso de suelos y sedimentos contaminados por metales las estrategias se han enfocado en el aislamiento, solidificación y/o estabilización. En ese sentido, se han probado diferentes materiales que mejoran dichos procesos. Tal es el caso, de componentes ricos en materia orgánica como son los biosólidos y el biochar. Los biosólidos; material residual semisólido proveniente de procesos de tratamiento de aguas residuales, se caracterizan por tener un alto contenido de materia orgánica y arcillas. Estos componentes están constituidos por grupos funcionales de tipo hidroxilo, carboxilo, amino y fenólico que proporcionan la capacidad de absorber y retener metales, por lo que han sido utilizados con éxito en la

estabilización de metales en suelos y sedimentos contaminados por metales. El biochar; un producto de descomposición térmica de materia orgánica bajo un aporte limitado de oxígeno a temperaturas relativamente bajas. Puede aumentar la capacidad retención de metales del material a partir del cual fue producido, debido a la formación de micro y meso poros que modifican algunas propiedades fisicoquímicas relacionadas con interacciones electrostáticas, grupos funcionales, etc.

En el presente proyecto se pretende implementar una metodología de aplicación de biosólidos y biochar a sedimentos y suelos aledaños al río Piedras Negras ubicado en la zona de explotación minera del municipio de Asientos, Aguascalientes, con la finalidad de disminuir la afectación de los metales presentes en estos sustratos hacia el ambiente. Para ello se realizará una caracterización fisicoquímica de los sustratos, determinando los principales factores relacionados con la movilidad de metales.

Caracterización fisicoquímica mostró que aunque la mayoría de los sitios de muestreo estaban fuertemente contaminados con Pb, Zn, Cu, Cd, superando los valores nacionales e internacionales de referencia, los metales no eran lixiviables en agua. Esto se puede atribuir a las condiciones fisicoquímicas actuales en suelos y sedimentos, incluyendo el pH, el contenido de carbonato y materia orgánica que confieren una alta capacidad amortiguadora a los sustratos. Sin embargo la extracción secuencial de metales mostró que los metales se asociaron en buena medida con fracciones potencialmente móviles tales como la fracción intercambiable la fracción unida a carbonatos. La susceptibilidad de estas fracciones a cambios en las características fisicoquímicas tiene importantes implicaciones en la estabilidad a largo plazo de los metales.

Basado en el contenido total de metales, se utilizaron muestras de sedimentos de la zona 7 obtenido durante el segundo período de muestreo en los experimentos de estabilización. Las muestras fueron modificadas con biosólidos y la biochar y los estudios de lixiviación a pH 5.5 y pH 3.5 durante un período de 4 semanas. Los metales fueron extraídos secuencialmente de todas las muestras al final del período experimental. Los sedimentos tratados con biosólidos y biochar mostraron concentraciones de Pb, Zn, Cu y Cd lixiviables e intercambiables menores. Este efecto fue significativamente mayor en el caso de Cd que

en los otros tres metales. La extracción secuencial mostró que los metales se asociaron a las fracciones recalcitrantes (unidas a óxido de Fe / Mn y materia orgánica) en los sedimentos tratados. Sin embargo, en el caso de Pb, se observó una asociación notable con la fracción unida a carbonatos, destacando reacciones de co-precipitación como fenómenos importantes en la reducción de los metales lixiviables e intercambiables, especialmente en sustratos donde hay una alta capacidad de amortiguación relacionada con carbonatos.

Se obtuvieron resultados similares en un estudio en suelos contaminados con cenizas de madera preservada con arseniato de cobre cromatado. Estos suelos fueron tratados con biomasa vegetal (parras molidas) y biochar producido del mismo. Tratamiento con ambos materiales resultó en una disminución en los metales intercambiables, especialmente Cu. Sin embargo, la biomasa vegetal no alterada fue capaz de reducir el Cr lixiviable en mayor grado que el biochar, probablemente debido a la presencia de más grupos funcionales. La biomasa vegetal no alterada también redujo el pH del sistema, y esto resultó en la reducción de Cr(VI), una especie más móvil y tóxica, a Cr(III), una especie menos móvil y tóxica. Extracciones secuenciales confirmaron esta reducción de metales intercambiables pero mostraron que los metales se asociaron en gran medida a la fracción unida a carbonatos. Como se observó en el experimento anterior, una reducción en movilidad no siempre representa una reducción en movilidad a largo plazo, ya que los metales asociados con la fracción unida a carbonatos todavía se consideran potencialmente móviles.

Los resultados de este proyecto de investigación señalaron la importancia de considerar las características fisicoquímicas durante la evaluación del riesgo ambiental y remediación de sustratos contaminados por metales debido al efecto directo que tiene sobre su movilidad y toxicidad potencial.

2 Abstract

Most metals are found naturally in soils and sediments in low or trace concentrations and as such do not present any significant toxicological risk. However, anthropogenic activities especially smelting and mining have resulted a continuous increase in the concentration of metals in the environment. In elevated concentrations, metals such as arsenic, cadmium, chromium, lead, zinc, copper and mercury can be very toxic and are given high priority in studies related to public health. These toxic metallic compounds are systemic, persistent and bio-accumulative even in lower exposure levels.

Metals cannot be degraded nor destroyed. However, they are subject to changes in chemical speciation under certain physicochemical conditions, such as the degradation of organic matter, pH changes and potential redox. In low concentrations, soils and sediments are natural buffers of pollutants including metals; however, in higher concentrations, this capacity is limited. Under this limited buffer capacity, pollutants can be mobilized, and as such represent an increased risk in bioavailability and toxicity, making it necessary to evaluate remediation strategies.

Remediation of polluted substrates is necessary for the protection of the environment and the health of organisms exposed to mentioned pollutants. In the case of metal polluted soils and sediments, remediation strategies have focused on isolation, solidification and / or stabilisation. Materials with the capacity to improve these processes have been the objective of many scientific studies. Biosolids and biochar, materials rich in organic matter have been the focus of many studies related to metal remediation in soils and sediments. Biosolids are semisolid materials used in the treatment of wastewaters and are characterized as having high organic matter content and clay. These components, rich in functional groups such as hydroxyls, carboxyls, amines and phenols that give them the capacity to adsorb metals, have been used successfully in the stabilisation of metals in polluted soils and sediments. Biochar is a product of the thermal decomposition of organic matter, under a limited supply of oxygen and relatively low temperatures. It has been shown to increase the metal retention capacity of the materials from which it was produced, due to the

formation of nano, micro and mesopores and the modification of certain physicochemical properties related to electrostatic interactions and functional groups' configurations.

The objective of this research project was to determine and characterize the presence of metals in sediments and nearby soils of the Piedras Negras riverbed; due to its proximity to mining operations in Asientos, Aguascalientes, and implement a remediation strategy using biochar and biosolids so as to reduce the environmental effects caused by the presence of toxic metals in the aforementioned substrates.

Physicochemical characterisation showed that though most sample sites were heavily contaminated with Pb, Zn, Cu, Cd surpassing national and international guideline values, metals were not water leachable. This can be attributed in part to current physicochemical conditions in soils and sediments, including pH, carbonate and organic matter content which confer a high buffer capacity to the sampled substrates and limit metal mobility. However sequential extraction of metals showed that metals were associated to a great extent with the potentially mobile exchangeable and carbonate bound fractions. The susceptibility of these fractions to changes in physicochemical characteristics has important implications in the long term stability of metals.

Based on total metal content, sediment samples from zone 7 obtained during the second sample period was used in stabilisation experiments. Samples were amended with biosolids and biochar and leaching studies at pH 5.5 and pH 3.5 were conducted during a period of 4 weeks. Metals were sequentially extracted from all samples at the end of the experimental period. Amendment reduced water leachable and exchangeable Pb, Zn, Cu and Cd, with biochar having a significantly greater effect on Cd than it did on the other three metals. Sequential extraction showed that metals were associated to the recalcitrant Fe/Mn oxide and organic matter fractions in amended sediments. In the case of Pb however, a notable association with the carbonate fraction was observed, highlighting co-precipitation reactions as important phenomena in the reduction of water leachable and exchangeable metals, especially in soils where high carbonate related buffer capacity is present.

Similar results were obtained from a similar study in contaminated soils obtained from the Scottish lowland region. Here, the effect amendment with unaltered grape stalk grounds (source material) and pyrolysed grape stalk grounds (biochar) was observed in soils contaminated with Chromated Copper Arsenate treated wood ash and its characteristically high pH. Amendment with both materials resulted in a decrease in exchangeable metals, especially in the case of Cu. However, the source material was able to reduce water leachable Cr more so than biochar, probably due to the presence of more functional groups. The source material also reduced the pH of the system, and this resulted in the reduction of the more mobile and toxic Cr(VI) to the less mobile and toxic Cr(III). Sequential extractions however confirmed this reduction in exchangeable metals but showed that metals were greatly associated to the carbonate fraction, highlighting as seen in the previous experiment that reduction in current mobility may not always represent a reduction in long term mobility as metals associated with the carbonate fractions are still considered potentially mobile.

The results of this research project highlighted the importance of considering physicochemical characteristics during environmental risk assessment and remediation of metal contaminated substrates due to the direct effect it has on metal mobility and potential toxicity.

3 Introduction

3.1 Mining activity in Aguascalientes

The state of Aguascalientes is located in the central part of the Mexican Republic, bordering with the north, northeast and southeast with the state of Zacatecas and with the state of Jalisco to the south (SGM 2011). It occupies 5,589.2km² of the Mexican territory and its geographic division is made up of 11 municipalities. Tracing back to the colonial era, Aguascalientes has been producer of precious minerals, though this activity became practically non-existent by the end of the 20th century (SGM 2011).

Recently, there has been a resurgence of this activity in the Tepezala-Asientos area; with exploration and exploitation of metals of high economic value being taken up by various large transnational companies. This increase in mining represents a potential increase in material previously associated with metal ores in the environment. If this material is not adequately disposed of, it can cause serious environmental pollution problems (SGM 2011; Mitchell et al. 2016).

3.2 Environmental effect of metals

Environmental pollution by metals and metalloids started with the domestication of fire. It is believed that small quantities of trace metals deposited in caves altered the environment. With the discovery of mining and metallurgic techniques in ancient times, the connection between metals, pollution by metals and human history was formed. Mining and the use minerals of high economic value was an important part of the ancient economies, being the initial step towards technological advancement (Nriagu 1996).

Metals are considered trace elements due to their low concentrations (up to 10ppm) in various environmental matrices. Their bioavailability is influenced by certain physical factors such as temperature, association between different substrate phases, adsorption and sequestration. They are also affected by chemical factors that modify their speciation, complexing kinetics, lipid solubility and octanol/water partition coefficient (Tchounwou et

al. 2012). In trace concentrations, metals in the environment do not represent a toxicological risk (Purushotham et al. 2013).

Chronic metal exposure occurs principally through the trophic network. Acute exposure is not common but not impossible. Metal toxicity depends on various factors, including doses, route of exposure, chemical speciation, as well as age, gender and health of the exposed individuals. Due to their high degree of toxicity, metals such as arsenic, cadmium, chrome, lead and mercury can be very toxic and are given high priority in studies related to public health. These toxic metallic compounds are systemic, persistent and bio-accumulative even in lower exposure levels (Goyer et al. 2004; Tchounwou et al. 2012).

Metals are found naturally in the environment. Cobalt, copper, chromium, iron, magnesium, manganese, molybdenum, nickel, selenium and zinc, for example, are considered essential nutrients in small quantities. In ionic forms, some are essential for several biochemical and physiological functions. Some form part of metallo-enzymes and participate in the stabilisation of active molecules (Williams 1972; Chitturi et al. 2015). But at high concentrations, all metals, including the essential metals, may cause toxic effects (Tchounwou et al. 2012). Increased concentrations may be due to atmospheric deposition, erosion or leaching of metal ions in the soil, sediment resuspension or evaporation from aquatic systems (Nriagu 1996). However, these high concentrations are due primarily to environmental pollution originating from anthropogenic activities such as industrial, agricultural, mining and smelting processes (Bradl 2004; Tchounwou et al. 2012).

The toxic effects of metals are usually the result of the interaction between the metal and the site of action. This effect is often related to the metals' chemical properties and its ability to form stable bonds with various biomolecules or interact with essential metals with electronic similarity (Duruibe et al. 2007). In general terms, metals affect cellular organelles and other cellular components; cell membrane, mitochondria, lysosome, endoplasmic reticulum, nucleus and a number of enzymes involved in metabolism, detoxification and cell damage repairs (Wang and Shi 2001; Tchounwou et al. 2012).

Studies have shown that metallic ions can interact with DNA resulting in conformational changes. Cell cycle modulation and the induction of carcinogenesis or apoptosis have also been seen (Beyersmann and Hartwig 2008), typically affecting the neurological, renal, reproductive and endocrine system (Ambrosi et al. 1991). Evidence suggests that reactive oxygen species play an important role in the induction of cellular response and carcinogenesis, especially in metals such as arsenic, cadmium, chromium, lead and mercury. According to the US Environmental Protection Agency and International Agency for Research on Cancer, as cited by Tchounwou et al. (2012), these metals are classified as “known” or “probable” human carcinogens based on epidemiological and experimental studies that show an association between exposure and cancer incidence in humans and animals (Wang and Shi 2001; Tchounwou et al. 2012). They have potentially systemic toxic effects, meaning that are known to induce multiple organ damage, even at lower levels of exposure (Tchounwou et al. 2012).

Many metals are found in cationic form; mercury, cadmium, lead, nickel, copper, zinc, chromium and manganese. But, there are also metals in anionic form (elements that generally interact with oxygen and become negatively charged, for example, MoO_4^{2-}); arsenic, molybdenum, selenium and boron (USDA 2000). High concentrations of metals in the environment can occur naturally. This form of pollution may be due to metal corrosion, atmospheric deposition, erosion and leaching from soils, re-suspension from sediments, evaporation in its volatile form and volcanic eruptions (Nriagu 1996; Tchounwou et al. 2012).

However, increases in metal concentrations in the environment is frequently attributed to anthropogenic activities, a problem that has been growing over the years especially in urban and industrialized areas (Ziemacki et al. 1989). The principal types of emissions include dust, ash, and prime material spills from petrochemical, chemical, mechanical and metallurgic industries (Landner and Reuther 2005).

Mining and smelting industries are associated with environments polluted with heavy metals and metalloids. Lands near mines are usually not recommended for agricultural use, nevertheless, nearby populations are not always aware of the risks involved and cultivate

the lands (Landner and Reuther 2005). During the processes carried out in mining, wastewater and mine tailings containing toxic metals are discharged into the environment and the metals are transferred to superficial and subterranean water bodies, soils, and from there into the trophic network (Hu et al. 2013).

River sediments are fundamental components of our environment given that they provide nutrients for living organisms and also act as a stabilisation zone for potentially toxic chemical species (Akçay et al. 2003). Unfortunately, large quantities domestic and industrial waste discharges overwhelm this stabilizing capacity, and in so doing adversely affect the ecosystem (Zhou et al. 2007).

3.3 Metal dynamics in soils and sediments

In the context of environmental chemistry, the chemical specie, or the specific form of an element is defined in terms of its isotopic composition, oxidation state, molecular structure or phase. The chemical species of a metal is a very important factor in the study of its behaviour and potential toxicity in the environment (USEPA 2007a).

Metals can be found in one or more forms in the soil including, dissolved in the soil solution (liquid phase), occupying exchange sites or adsorbed on inorganic compounds, associated with insoluble organic matter, precipitated, present in primary or secondary mineral structures (Tessier et al. 1979). Generally, metals originating from anthropogenic activity are found in one or more of the first five phases. The liquid phase, and phases in equilibrium with the liquid phase are key in terms of the migration potential of metals in soils. Metals in the liquid phase are subject to mass transference from the system by means of leaching, uptake by plants and volatilization in the case of metals such as Hg, As or Se. Metals also participate in chemical reactions with the solid phase of the soil. The concentration of metals in either phase of the soil, depends on various interrelating chemical processes such as complex forming, oxidation - reduction, precipitation, dissolution and adsorption – desorption (Mclean and Bledsoe 1992).

3.3.1 Complexing processes

Complex formation, pH and redox reactions are determining factors in the mobility of metals. Complexes are formed between metals and ligands in solution and on the surface of minerals and organisms. From an environmental chemistry perspective, metal cations are considered Lewis acids and ligands Lewis bases. In this context, “weak” species are considered to have easily deformable clouds of polarizable electrons with a preference for covalent bonds and thus be mobilized easily. In contrast, “strong” species are rigid and non-deformable, with low polarizability, firmly retain their electrons and prefer ionic bonds (Langmuir et al. 2004). “Strong” metals (strong acids) are considered less toxic, forming bonds with “strong” bases that contain oxygen and in certain situations weaker bonds with “weak” nitrogen and sulphur species. The bond between strong metals and strong ligands is generally in function of pH. Weak metals form bonds with nitrogen and sulphur ligands and in some situations even weaker bonds with species such as hydroxides and sulphates. The tendency for metals to form solid phases, as in the case of sulphides in sediments is related to its characteristics in the context of acid/base - strong/weak (Langmuir et al. 2004; USEPA 2007a). This distinction is important because metallic sulphides are extremely insoluble and if precipitated, tend to reduce the concentration of mobile metals. Therefore, the presence of complexes in the soil solution can significantly affect the transport of metals in the soil matrix (Mclean and Bledsoe 1992). In other words, depending on the metals’ oxidation state, they can be weakly or strongly adsorbed to the surface of soil particles (Langmuir et al. 2004).

The aforementioned parameters can be utilized to determine the potential mobility, persistence, toxicity, bioavailability and bioaccumulation of metals in soils and sediments. The free metal ion, in general, may be the most bioavailable and toxic form of the metal. However, several metals in the environment exist in more than one oxidation state. The oxidation state of these metals determine their relative mobility, bioavailability and potential toxicity (Langmuir et al. 2004; Mclean and Bledsoe 1992).

3.3.2 Sorption and desorption processes

Toxic levels of metals are generally limited by their adsorption onto the surfaces of minerals and organic material, including microorganisms. Considering that the retention mechanisms of metals on the soil particle surfaces is often unknown, the use of the term sorption is recommended; which describes the loss of a metal ion from an aqueous solution to a contiguous solid phase comprising of three important processes: adsorption, surface precipitation and fixing (Bradl 2004).

Adsorption is an accumulation of matter in the solid/liquid interface and is principally understood in terms of its intermolecular interactions between the solid/solute phase (Mclean and Bledsoe 1992). Unlike precipitation processes, in adsorption there is no formation of new solid phases, only an association between the present particles' surfaces. These particles form the soil matrix, and include organic matter, clay, iron and manganese oxides and hydroxides, carbonates and aluminosilicates (Bradl 2004). Different interactions are involved in this process, including reactions of surface complexation (inner sphere surface complexes with metal ions), electrostatic interactions (external surface complexes), hydrophobic expulsion and surfactant adsorption of poly-electrolyte metal complexes (Bradl 2004; Mclean and Bledsoe 1992).

With gradual increase of metal concentrations in the soil, adsorption sites are further occupied and there is a structural incorporation of precipitated minerals. In porous substrates, the most important adsorbent solids are clays, organic matter and iron and manganese oxyhydroxides; these components having large surface areas, superficial density and cationic exchange sites (Shao et al. 2010; Mclean and Bledsoe 1992).

3.3.3 Precipitation and dissolution processes

In a closed system, the quantity and composition of a mineral that dissolves or precipitates can be described in terms of kinetics and chemical thermodynamics affected by the surface morphology of the species that are dissolving and precipitating. Thermodynamics determine the possible reactions and kinetics determine the time required for transformations (Shao et al. 2010).

Among the factors that affect precipitation and dissolution processes are the liquid phase properties. Substrate saturation plays a fundamental role in determining a reaction's route, kinetics, velocity and surface mechanisms that control precipitation and dissolution (Nagy and Lasaga 1992). The rate of dissolution and precipitation decreases as equilibrium is approached. pH is also a factor as it acts as a catalyst. Adsorption of H^+ and OH^- can alter the surface exchange capacity of a mineral and reduce the nucleation rate by reducing the interfacial tension (Nagy and Lasaga 1992; Shao et al. 2010).

Solid phase properties are also important factors. Properties such as aggregation and composition of the surface layers of minerals affect the liquid phase solution's composition and as such reaction routes and velocity. Solubility increases with surface area, which in turn is determined by particle size and morphology. In saturated solutions, amorphous materials are more readily precipitated as uneven surfaces provide more nucleation sites (Mclean and Bledsoe 1992).

3.4 Metal mobility in soils

Once deposited in soils, some metals can be considered persistent due to their relatively immobile nature. Other metals though, may present a more mobile nature. Metal mobility in soil is associated with potential toxicity risks, due to its ability to move downwards into the aquifers or upwards into plants and from there the rest the trophic network (Sherene 2010). The mobility of the metals in the soil is influenced in a significant way by several factors including; redox potential, clay mineralogy, organic matter content, salinity, water content and pH (Mclean and Bledsoe 1992; Mitchell et al. 2016).

3.4.1 pH and redox potential

pH directly and indirectly affects metal retention in soils. Adsorption of metal cations increases with an increase in pH (Mclean and Bledsoe 1992). As pH decreases (less than pH 5), more metals can be found in soil solution. This increase in mobility is due to an increase in proton concentration and the resulting competition between said protons and ligands (OH^- , CO_3^{2-} , SO_4^{2-} , Cl^- , S^{2-} , PO_4^{3-}). At pH above 7, some metals form soluble

hydroxide complexes and so though metal retention is closely related to an increase in pH, it is not always proportional (Shi et al. 2014; Mclean and Bledsoe 1992; Sherene 2010).

The presence of adsorption sites is another factor related to metal mobility. The number of available negative sites for the adsorption of cations diminishes with a decrease in pH. Metals in the form of hydroxides, oxides, carbonates and phosphates precipitate in alkaline conditions. In order for these precipitates to dissolve, a reduction in pH is necessary. Metals that form hydroxides frequently associate with iron and manganese hydroxides. At pH less than 6, these iron and manganese hydroxides dissolve, effectively transferring these metal ions to the soil solution (Mclean and Bledsoe 1992).

The redox potential of a soil system is a measure of its electrochemical potential, or the electron availability in the system. In the absence of oxygen, reductive conditions tend to prevail. In well-drained and unpolluted soils, oxidizing conditions prevail (Mclean and Bledsoe 1992). The soil's redox potential varies temporally and spatially. In superficial soils, rainfall, biological activity and land use changes are important influencing factors. A reduction in redox potential can result in changes in the oxidation state of metals, forming new minerals or reducing others and as such free associated metals (Ma and Dong 2004). Redox reactions are thus important factors in the transport of metals, especially in acid or alkaline environments (Mclean and Bledsoe 1992).

3.4.2 Cation exchange capacity

Cation exchange capacity is the measure of the total capacity of the soil to retain exchangeable cations. It indicates the total negative charge present in a unit mass of soil (Mitchell et al. 2016). Clay particles are negatively charged and as such are surrounded by cations (positive charges). In part, clay minerals owe their negative charge to isomorphic substitution (higher charge cations such as Al^{3+} , are replaced with the lesser charge cations such as Mg^{2+}), resulting in an excess of negative charges that easily attract exchangeable cations. Moreover, soil colloids that exhibit functional complexing groups (-OH, -COOH) also contribute to the cation exchange capacity of the soil. It is important to note that the dissociation of -OH and -COOH groups is pH dependent. Consequently, cation exchange

capacity increases with an increase in pH. With an increase in cation exchange capacity, metal cations are attracted to these negative sites on solid particles, effectively reducing metal availability in the soil solution (Attwell et al. 1999).

3.4.3 Salinity

A saline substrate contains an excess of soluble salts. These salts contain cations such as sodium, potassium, calcium, magnesium and anions such as chlorine, sulphates, nitrates, bicarbonates and carbonates (Hohl and Varma 2010). Studies have shown that an increase in salinity, increases the mobility of metals in the soil. Two principal mechanisms regulate this process; the capacity of metals to form complexes with salt derived anions and competition between positively charged metals and salt derived cations for adsorption sites on the solid phase (Acosta et al. 2011).

3.4.4 Organic matter

Organic matter is primarily the result of the decomposition of organic remains, on the surface and upper horizons of the soil. Organic matter in soils and sediments can be classified into two groups. The first category is living organisms that include macrofauna, mesofauna, microfauna and microflora. These organisms are involved in production, consumption and decomposition of organic litter; processes when carried out in equilibrium, contribute significantly to the development of the soil profile. The second category is dead organic matter, which is the result of soil organisms' metabolic action (Mirsal 2004). The term humus is used to refer to organic matter that has stabilized, or is in final stages of decomposition (Ramos-Goméz 2012). On average, humus contains 4-6% organic matter, which, in turn, are made up of 85% dead matter, 8.5% of live roots and rootlets and about 6.5% soil organisms (Mirsal 2004).

The amount and type of organic matter in the substrate can affect the solubility of metals due to processes such as complexing and chelating (Sherene 2010; Mclean and Bledsoe 1992). There is a close relationship between pH and the metal retention capacity of organic matter (Sherene 2010). It has been shown that when metals associate with insoluble organic molecules, complex insoluble compounds are formed. However, the formation of metal

complexes with dissolved organic compounds may increase the mobility of the metals (Peng et al. 2009). Organic matter undergoing decomposition processes produce weak humic and fulvic acids. Functional groups present in these acids include carboxyls and hydroxi-phenols, groups able to interact with metal cations (Kyziol 2002). At high pH, these weak acids ionize, resulting in the solubilisation of associated metals (Carrillo-González et al. 2006). The amount of metals associated with organic matter, and the amount of dissolved complexes is controlled in part by Ca in the soil. Ca can act as a bridge between the negative charge of the dissolved organic matter and negatively charged soil oxides and clay minerals (Nriagu 1996; Sherene 2010).

3.4.5 Soil texture

Soil texture refers to the size and organization of the soil particles and sediments. Individual particles can be described according to its grain size. The classification depends on the proportionate amount of sand, clay and silt (Mirsal 2004). Clay particles in particular are very important in determining the concentration of metals in the liquid and solid phases. Studies have shown that soils with high percentages of clay are better able to retain metals due to specific adsorption and cation exchange processes. This is carried out through the adsorption of hydroxyl ions followed by the binding of the metal ion to the clay particle by binding to the hydroxyl ions adsorbed or directly to the sites created by removing the proton. Highly selective adsorption occurs on mineral edges (Selim 2011; Bradl 2004).

3.5 Leaching potential

In the ground system, leaching is the process whereby organic or inorganic contaminants from the interstitial water move to the deeper layers of soil or groundwater by infiltration. In environmental studies however, the composition of the interstitial water gives only a momentary picture of the mobility of trace elements as their composition may change over time. To evaluate the mobility of heavy metals in the long term (potential mobility) and the effect of changing environmental conditions, a variety of leaching and extraction tests are used (Cappuyens and Swennen 2008).

Most leaching potential tests are based on the method established by the USEPA called TCLP (USEPA 1992b). Modified versions of the method is often used to determine the leaching potential of metal cations in situations where material degradation under anaerobic conditions can result in a decrease in pH due to production of weak acids. Solutions acidified with acetic, nitric or sulfuric acid (usually around pH 5) are used (Wuana and Okieimen 2011). However, metals extracted with the TCLP method are not considered to be associated with a definitive geochemical fraction (Mclean and Bledsoe 1992).

3.6 Metal fractionation in soils and sediments

Metals in soils and sediments are bound to different geochemical fractions (Anju and Banerjee 2010). In order to properly evaluate the potential leachability of these metals, it is necessary to separate them from the fraction to which they are associated (Tessier et al. 1979; Clevenger 1990). Evaluation of metal mobility and bioavailability in soils and sediments using sequential extraction is based on the assumption that the mobility and bioavailability decreases with the order of removal, implying that the metals in the exchangeable fractions are more mobile and bioavailable, while metals in the residual fraction are closely linked and are less mobile in natural environmental conditions (Anju and Banerjee 2010; Clevenger 1990).

One of the most used sequential extraction methods is that of Tessier et al. (1979); which is based on the concept that the solid material of the soil and sediment can be separated into five specific fractions; which can be selectively removed using suitable reagents.

Fraction I. Exchangeable. Based on studies showing that changes in ionic composition of water affects adsorption and desorption processes. The metals are considered specifically adsorbed and interchangeable. Its release is achieved with a solution with an excess of cations such as $MgCl_2$ pH 7.0 or NaOAc pH 8.2 (Tessier et al. 1979).

Fraction II. Bound to Carbonates. Studies have shown that there are significant concentrations of metals bound to the carbonate fraction in soils and sediments. They are very labile bonds, susceptible to changes, particularly reductions in pH. A solution of NaOAc with pH adjusted to 5.0 with HOAc is used in this step (Tessier et al. 1979).

Fraction III. Bound to oxides of Fe and Mn. Nodules of Fe and Mn oxides in soils and sediments act as scavengers of metals in the soil. The bond between these oxides and metals is thermodynamically unstable under reducing conditions. A solution of $\text{NH}_2\text{OH}\cdot\text{HCl}$ 0.04M in HOAc 25% v/v with the additional catalyser of a temperature of 85°C is used during this step (Tessier et al. 1979).

Fraction IV. Bound to organic matter. Metal cations can form complexes with the surface binding sites of organic matter (humic and fulvic acids). Under oxidizing conditions, the organic matter is degraded, releasing the complexed metals. The reagents used are H_2O_2 and HNO_3 , with the additional catalyser of a temperature of 85°C (Tessier et al. 1979)

Fraction V (Residual). The metals found in this fraction originate from the primary and secondary soil minerals that contain them in their crystal structure. The release of these metals due to environmental physicochemical changes in the substrates is unlikely. Using the soil sample remaining after the first four extraction steps, total metal content is quantified after acid digestion (Tessier et al. 1979).

3.7 Remediation of soils and sediments

The overall objective of remediation technologies is to protect the environment and human health. In terms of metal pollution in soils and sediments that goal is achieved through technologies focused on reducing its solubility, mobility, toxicity and potential bioavailability. These characteristics are influenced by the chemical species of the metals. A clear understanding of these factors is needed in order to successfully implement a remediation strategy (Evanko and Dzombak 1997; Wuana and Okieimen 2011). Remediation strategies applied to metal pollution problems generally focus on the isolation, immobilisation, toxicity reduction, physical separation and extraction processes. Generally, the implementation of technologies based on one or more of these principles will depend on the chemical species of the metal or metals and specific characteristics of the polluted substrate (Evanko and Dzombak 1997; Mclean and Bledsoe 1992).

In many countries, especially developing countries, technologies focused on isolation, solidification and stabilisation have been the most common. The goal of these strategies is

to contain the pollutants in a specific area and prevent mobility especially in the form of leaching and the subsequent pollution of nearby ecosystems including freshwater aquifers. These methods are also applied when it is physically or economically prohibitive to apply other technologies such as those that involved extraction or destruction of the pollutant (Evanko and Dzombak 1997; Wuana and Okieimen 2011). For solidification techniques, chelating agents are added, effectively complexing and / or encapsulating the metal contaminant in a monolithic solid of high structural integrity and reducing its mobility (Khan et al. 2004). Pozzolans and cement-based materials are among the materials commonly used for chelation, based on the principle of metal complexation with the hydroxides, carbonates and silicates present in the chelating material (Evanko and Dzombak 1997). Stabilisation requires the use of compounds or reagents in the ground in order to produce more chemically stable constituents (Wuana and Okieimen 2011). The polluted soil is mixed with inorganic agents such as clays (Zheng and Zhang 2011), cement, ash (Yamamoto et al. 2015; Jagupilla et al. 2015), materials that contain calcium carbonate (Wang et al. 2016), Fe and Mn oxides, activated carbon and zeolites (Khan et al. 2004). Organic matter such as manure, composted organic matter and biosolids are also used (Song et al. 2014; Ramos-Goméz 2012; Karami et al. 2011).

These remediating materials follow various important principles (Querol et al. 2006). First, they should contribute to the alkalisation of the medium and thus cause precipitation of insoluble phases. Secondly, increased alkalinity should promote adsorption of metals via complexation processes. Mineral surfaces are positively charged at low pH due to the adsorption of protons, but at high pH, they are negatively charged by deprotonation processes; ideal conditions for the formation of complexes with the metal cations (Peng et al. 2009; Shi et al. 2009). Nevertheless, the ideal material should have a high cation exchange capacity and lessen the importance of a specific pH (Edebali and Pehlivan 2016).

3.7.1 Biosolids

As categorized by The Mexican Secretariat of Environment and Natural Resources (SEMARNAT 2002a), biosolids are sludge materials originating from city or municipal systems drainage, water treatment plants or sewage treatment plant that have been

subjected to stabilisation processes. These biosolids have certain desirable characteristics including high organic matter content and nutrients. The organic matter in biosolids contain stable humic and fulvic acid fractions (Dias et al. 2010; Ramos-Goméz 2012). Humic and fulvic acids are produced by the biodegradation of organic matter and are a combination of carboxylic acids and phenolates which behave as dibasic and tribasic acids. They are considered the sponges of the soil system, thermal dampers and storage zones. Soil and sediment substrates act as primary water filters, absorbent metal binders and also play a critical role in regulating physicochemical conditions (Stevenson 1994; Fataftah et al. 1997).

Humic acids are composed of different polymers; including a polycyclic core, weakly bound polysaccharides, peptides, phenols, and chelated metals. They have 3 major components: humic acids, fulvic acids and humin. They also contain functional groups including hydroxyls, carboxyls, amines and phenols, which can interact in different ways with metal ions in the substrate; ion exchange, complexation, surface adsorption and proton exchange. They possess aromatic rings and aliphatic chains with groups of O, N, S and some nonpolar fragments of humic substances are hydrophobic or hydrophilic in nature (Korodi 2012). These organic molecules or ligands can form one or many links with metal ions. The formation of more than one link between metal and ligand generally results in more complex stability (Chen et al. 2008). Studies have shown that reactions between the humic materials and the affected soils and sediments affect the solubility, mobility and potential bioavailability of the metal (Stevenson 1994; Soler-Rovira et al. 2010). Therefore, the content and nature of humic substances play an important role in determining the adequacy of materials used in the remediation of soils and sediments contaminated with heavy metals (Soler-Rovira et al. 2010). However, due to their potentially polluted nature, before being used in the remediation processes biosolids need to be subjected to stabilisation processes; biologically and/or chemically, to minimize complications that can occur during degradation of organic compounds (Idris et al. 2010), and by the presence of pathogenic microorganisms (Shammas and Wang 2008). It is also important to determine the total metal content so as to assure that safety parameters as set out by Federal Regulations are met (SEMARNAT 2002a).

3.7.2 Pyrolytic conversion of biomass (biochar)

Biochar is the product of thermal decomposition of organic matter from varying sources under a limited supply of oxygen and relatively low temperatures (Nartey and Zhao 2014; Tang et al. 2013). Biochars have potentially high adsorption capacities (Beesley et al. 2011). The microscopic physical structure of biochars is one of the most important factors, and during the pyrolysis process, the surface area of the original material is increased significantly, and thus increasing adsorption capacity (Beesley et al. 2011). Other metal retention mechanisms as found in organic matter are also considered such as its cation exchange capacity, indicating that by applying biochar to substrate there is an increase in net negative charge; a condition suitable for the attraction of metal cations (Jiang et al. 2012; Tang et al. 2013).

The pH of the substrate is affected with the addition of biochar, with a tendency to increase, resulting in the precipitation of the metals in the form of oxalates, phosphates and carbonates (Peng et al. 2009; Tang et al. 2013). Additionally, biochar materials contain numerous functional groups including carboxyls, hydroxyls and alcohols, forming complexes with metals (Tang et al. 2013). A combination of the aforementioned mechanisms diminishes solubility, mobility and potential bioavailability of metals in substrate amended with biochar (Beesley et al. 2011). Biochar is also an important source of phosphorus, nitrogen, micronutrients and organic matter than can positively affect soil properties (Agrafioti et al. 2013).

Preparation of biochar for use in remediation is a very important step, with pyrolysis temperature being the most important factor. In a study by Agrafioti et al. (2013) on the production of biochar from biosolids, product yield was shown to decrease significantly with temperatures above 300°C. This decrease may be related to initial primary degradation due to temperature or the initiation of secondary degradation reactions (Onay 2007; Agrafioti et al. 2013). The surface is also affected by the temperature and duration of the pyrolysis process. Surface area and number of micro and mesopores increases with an increase in temperature. According to Schimmelpfennig and Glaser (2012) as cited by Agrafioti et al. (2013), the increase in temperature improves material aromaticity. Humic

and fulvic acids from organic matter play an important role in the retention of metals (Ramos-Gómez 2012), but at higher pH some of these fractions can be solubilized (Kipton et al. 1992). However, studies have shown that the pyrolysis process has the effect of converting the humic acid fractions to a more insoluble form, thus avoiding the solubilisation of adsorbed metals (Varrault and Bermond 2010). Thus, the pyrolytic conversion at ideal temperatures of organic materials such as biosolids is a promising method for the remediation of metal pollution in soils and sediments (Wen et al. 2013; Agrafioti et al. 2013).

4 Justification

Metals found in trace concentrations naturally in environmental matrices do not pose an environmental risk. However, anthropogenic activities including industry, agriculture, smelting and mining, have resulted in an increase in metal concentration in environment.

Mining has been a historically important industry in many areas in Mexico, including Aguascalientes. However, there has been a recent significant increase in this activity in Aguascalientes, due to the discovery of new mineral veins that contain large quantities of metals of high commercial value. Mining processes generate large amounts of waste with high concentrations of metals whose potential toxicity makes adequate disposal a significant challenge. In many cases, soils and sediments in nearby areas are first receptors of this waste, resulting in severe environmental alterations.

The chemical species involved play an important role in the behaviour and potential toxicity of the metals in the soils and sediments. Said metals can be found dissolved in the liquid phase, occupying exchange sites, adsorbed onto inorganic compounds, associated with organic matter or precipitated. The forms in which metals can be found depend on several interrelated processes including complexation, oxidation-reduction, precipitation-dissolution and desorption-adsorption. These pH-dependent processes and other substrate characteristics such as salinity, texture and organic matter of the substrate determine the mobility, bioavailability and toxicity of metals in the soil, thus highlighting the importance of including a physicochemical evaluation of potentially contaminated areas.

In areas with high potential environmental risk, it is necessary to implement remediation techniques to reduce the solubility, mobility, toxicity and bioavailability of the metals in the environment. In Mexico, the removal and / or destruction of pollutants present very significant economic and technical challenges that hamper its viability. As such, more attention has been paid to remediation strategies that focus on isolation, solidification / stabilisation of metals to reduce their effect as much as possible on the environment.

Based on the aforementioned, in order to study the environmental risk posed by the pollution of soils and sediments by metals from mining activities, methodologies to determine the physicochemical characteristics related to the mobility of metals in the polluted substrates will be established. Additionally, experimentation in the application of biomass and pyrolysed biomass to polluted substrates will be carried out, with the objective of studying the effect of these remediating materials on metal mobility in the studied substrates.

5 Hypothesis

1. Physicochemical characteristics influence the stabilisation of metals in affected soils and sediments.
2. Pyrolysed biomass is better able to immobilise metals than non-pyrolysed biomass due to structural and functional changes of their physicochemical properties.

6 Objectives

General Objectives

1. Study the physicochemical characteristics related to mobility of metals in soils and sediments contaminated with mining waste.
2. Study the immobilisation of metals in contaminated solid substrates amended with pyrolysed and non-pyrolysed biomass.
3. Study the changes in organic matter speciation related to the immobilisation of metals in contaminated solid substrates with pyrolysed and non-pyrolysed biomass.

Specific Objectives

- Characterise the physical, chemical and mineralogical properties of sediments and nearby soils of the Piedras Negras River located in Asientos, Aguascalientes.
- Determine the leaching potential of metals present in the sampled sediments and soils.
- Determine the fractionation of metals in sampled sediments and soils.
- Produce biochar from biomass.
- Determine the structural changes resulting from the production of biochar.
- Determine the functional changes relevant in the immobilisation of metals, from the production of biochar by organic matter speciation in both materials.
- Study the immobilisation of metals by amending contaminated substrates with biochar and non-pyrolysed biomass.
- Determine the fractionation of metals in amended substrates.
- Determine the changes organic matter speciation in contaminated substrates amended with biochar and non-pyrolysed biomass.
- Determine the relative abundance of the metals on the surface of non-pyrolysed biomass and biochar before and after application tests using scanning electron microscopy.

7 Materials & Methods

Soil and sediment samples were obtained from the Piedras Negras River in Asientos, Aguascalientes, Mexico, due to their proximity to important mining sites. Physicochemical characterisation, total metal content and potential metal mobility were determined in all samples. Samples were selected based on total metal content for use in subsequent immobilisation studies.

7.1 Sampling

The study area (22°140'N, 102°05'W) is located in Asientos a north-western municipality in the State of Aguascalientes, Mexico. Under the Köppen Geiger climate classification, the State of Aguascalientes has a semi-arid climate (Köppen BSh) with a mean annual temperature of 17.4°C and mean annual precipitation between 400 and 600mm (Peel et al. 2007; SGM 2011). The municipality of Asientos, though with rural characteristics, is a historically important mining zone in this state, with the year 2008 marking an increase in the mining of metals of high economic value including, gold, silver, lead, copper and zinc (Peel et al. 2007; SGM 2011). Representative composite soil and sediment samples were obtained after quartering and homogenization of four cross sectional sub-samples (at a depth of 15 cm) from ten sample zone, with approximately 800m separation, following the guidelines established in USEPA sampling protocols and the Mexican Regulation NMX-AA-132-SCFI-2006 (USEPA 1992a; SEMARNAT 2003).

7.2 Physicochemical characterisation

The following physicochemical characteristics were determined: humidity, using the thermo-gravimetric method (Fernandez Linares et al. 2006), drying time 24 h at 105°C. Organic matter content, Walkley–Black metric titration method (Fernandez Linares et al. 2006). Cation-exchange capacity, the ammonium acetate extraction method (Fernandez Linares et al. 2006). The %CaCO₃ equivalent, acid neutralization method (SEMARNAT 2002b). Finally, a liquid extract obtained after shaking particulate samples in deionized water 1:10 m/v for 24h according to the standardized techniques used by Fernandez Linares et al. (2006) was used in the potentiometric determination of pH, electrical conductivity

(EC) and redox potential. Soluble sulphates and sulphides were determined in the same liquid extract using the USEPA compliant Hach (1999) sulfaver 4 #8051 and methylene blue #8131 method respectively (Hach DR 4000U Spectrophotometer, 1999). In preparation for total metal quantification using atomic absorption spectrophotometry, samples were digested using a two stage system of microwave digestion. Firstly, 1ml of hydrofluoric acid (HF), 2ml of nitric acid (HNO₃) and 2ml of hydrochloric acid (HCl) was added to 10ml of sample. Digestion was carried out for 30 min at 100% power. In the second stage 30ml of boric acid (H₃BO₃) 4% v/v was added and digestion was continued for 10 min at 100% power. The solution was transferred to a volumetric flask of 50ml, completed to 50ml with distilled water (Ramos-Goméz 2012). Total metal concentration was then quantified using flame atomic absorption spectrophotometry (Perkin Elmer AAnalyst 100, USA) after microwave accelerated acid digestion (CEM Corporation Mars 907600, USA) according to standard analytic method 7000B (USEPA 2007b). Acid digestions and atomic absorption spectrophotometry analyses were done using the quality controls established by the Standard Methods (APHA-AWWA-WPCF 1999), including fortified samples (recuperation percentages greater than 97.4 %) and random duplicates (variation coefficient less than 15 %). NIST SRM-8704 (Buffalo River Sediment) were used as standard reference material (National Institute of Standards and Technology, Gaithersburg, MD, USA)

7.3 Leaching potential tests

Leaching tests were carried out in two stages. Samples were placed in agitation in a solution of distilled water with pH adjusted to 5 - 5.5 with CH₃COOH in a 1:10 ratio for 18 hours. The mixture was then completely transferred to a humidity cell and allowed to stand for 12h . The leachate was obtained through vacuum filtration (Ramos-Goméz 2012). Metals in leachate were quantified using flame atomic absorption spectrophotometry (Perkin Elmer AAnalyst 100, USA) after microwave accelerated acid digestion (CEM Corporation Mars 907600, USA) using the aforementioned technique (Ramos-Goméz 2012; USEPA 2007b).

7.4 Metal fractionation

Metal fractionation in samples was done using the Tessier sequential extraction method (Tessier et al. 1979; Clevenger 1990; Ramos-Gómez et al. 2012). 1g of well-homogenized sample was processed as follows;

- Exchangeable (F1): 1g of sample was leached with 8ml of 1M $MgCl_2$ pH 7.0 with continuous agitation for 1 hour.
- Bound to carbonates (F2): The residue from the previous step was leached with 8ml NaOAc 1M pH adjusted to 5.0 with HOAc, with continuous agitation for 5 hours.
- Bound to Fe and Mn oxides and hydroxides (F3): The residue from the previous step was leached with 20ml of $NH_2OH.HCL$ in 25% (v/v) HOAc, with continuous agitation for 6 hours at a temperature of 85°C.
- Bound to Organic Matter and Secondary Sulphides (F4): 3ml of HNO_3 0.02M, 5ml of H_2O_2 at 30% adjusted to pH 2.0 with HNO_3 was added to the residue from the previous step, with continuous agitation for 2 hours at a temperature of 90°C. After cooling, 3ml of H_2O_2 30% adjusted to pH 2.0 with HNO_3 was added. Agitation with temperature at 90°C was continued for 3 hours. After cooling, 5ml of 3.2M NH_4OAc in 20% v/v HNO_3 was added and agitation was continued for 30 minutes.
- Residual (F5): Total remaining metal concentration was quantified in residual fraction.

Once extraction was completed in each extraction step, Samples were centrifuged at 5000 rpm for 15 min and the supernatant was collected and residue was washed with 8ml deionized water and used in the next extraction step. Metal content in each extraction step and total metal content in the residual fraction were quantified using flame atomic absorption spectrophotometry (Perkin Elmer AAnalyst 100, USA) after microwave accelerated acid digestion (CEM Corporation Mars 907600, USA) according to standard analytic method 7000B (Ramos-Goméz 2012; USEPA 2007b).

7.5 Composted biosolids preparation

Sludge from the domestic water treatment plant at the Autonomous University of Aguascalientes was used for the preparation of composted biosolids. These residues are separated from the water at the end of its life, and subjected to a stabilisation process for a period of three weeks, whose final product (stabilized biosolids) is a material with a high content of organic matter and nutrients. A mixture of soil, leaves and grass was added and the left for 3 months with periodic turnover so as to obtain composted biosolids.

The following characteristics were determined in biosolids. Organic matter content, Walkley–Black metric titration method (Fernandez Linares et al. 2006). Cation-exchange capacity, the ammonium acetate extraction method (Fernandez Linares et al. 2006). Additionally, a liquid extract obtained after shaking particulate samples in deionized water 1:10 m/v for 24 h according to the standardized techniques used by Fernandez Linares et al. (2006) was used in the potentiometric determination of pH and electrical conductivity (EC). Total metal content in the amendment was quantified using flame atomic absorption spectrophotometry (Perkin Elmer AAnalyst 100, USA) after acid digestion according to standard analytic method 7000B (Ramos-Gómez 2012; USEPA 2007b) and compared to limits established for amendments produced from wastewater biosolids from international and federal regulatory bodies (IBI 2013; SEMARNAT 2002a).

7.6 Biochar preparation from biosolids

300g of previously composted biosolids were placed in a low-thermal-expansion borosilicate glass container. Pyrolysis was performed in a 24L adjusted capacity box furnace (Thermo Fisher Scientific Lindberg/Blue BF51287C, USA) at 350°C. Oxygen was limited with a continuous nitrogen flow of 400 ml min⁻¹ at atmospheric pressure, initially for 104 min (estimated saturation point) and then throughout the pyrolysis time of 20 minutes. The material was left in muffle furnace to cool for 5h, under the same nitrogen flow of 400 ml min⁻¹. The obtained material was weighed in order to calculate % yield, the quotient between the weight of biochar and weight of biosolids, then ground and sieved (<0.50mm) for further analysis (Agrafioti et al. 2013; Zhang et al. 2013). The following characteristics were determined in produced biochars. Organic matter content, Walkley–

Black metric titration method (Fernandez Linares et al. 2006). Cation-exchange capacity, the ammonium acetate extraction method (Fernandez Linares et al. 2006). Additionally, a liquid extract obtained after shaking particulate samples in deionized water 1:10 m/v for 24h according to the standardized techniques used by Fernandez Linares et al. (2006) was used in the potentiometric determination of pH and electrical conductivity (EC). Total metal content in the amendment was quantified using flame atomic absorption spectrophotometry (Perkin Elmer AAnalyst 100, USA) after microwave accelerated acid digestion (CEM Corporation Mars 907600, USA) according to standard analytic method 7000B (Ramos-Gómez 2012; USEPA 2007b) and compared to limits established for amendments produced from wastewater biosolids from international and federal regulatory bodies (IBI 2013; SEMARNAT 2002a).

7.7 Organic carbon speciation

Speciation of organic matter was carried out using the Dabin technique (Stevenson 1994), where biomass was mixed with $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$ 0.1M pH 10.3 in a 1:10 (m/v). Samples were then shaken for 24h and centrifuged at 4000rpm for 20 minutes. The supernatant was then filtered ($< 0.45 \mu\text{m}$).

7.7.1 Humic acid extraction

The humic acid was then obtained by acidifying the filtrate with concentrated HCl and left for 24h at 4°C , in order for the humic acid to precipitate. The precipitate was then purified with NaOH 0.1M then acidified once more with HCl 1M to precipitate the humic acid. The solution was then centrifuged at 4000 rpm for 20 minutes and the precipitated humic acid dissolved in water and left to dry. The obtained sample was used in the determination of total acidity, carboxylic and phenolic groups.

7.7.2 Total acidity

20ml of $\text{Ba}(\text{OH})_2$ 0.5M was added to 80mg of sample. The mixture was then shaken for 24 hours then filtered and the residue washed with 10ml CO_2 free H_2O . HCl 0.5M was then used to potentiometrically titrate until pH 8.4. Total acidity (cmol/kg) was calculated

considering the mass of sample used and the volume differences between HCl used in the titration of the sample and a prepared blank (Canellas et al. 2002).

7.7.3 Carboxyl groups

10ml of $C_4H_6CaO_4$ 1M, 40ml H_2O was added to 80mg of sample. The mixture was then shaken for 24 hours then filtered, and the residue washed with 10ml H_2O . NaOH 0.05M was then used to potentiometrically titrate until pH 9.8. Carboxyl groups (cmol/kg) was calculated considering the mass of sample used and the volume differences between NaOH used in the titration of the sample and a prepared blank (Schnitzer and Gupta 1965)

7.7.4 Phenolic groups

Calculated as the difference between total acidity and carboxyl groups.

7.8 Biochar preparation from ground grape stalks

The biochar source material used was principally ground grape stalks, which were homogenised, air dried overnight and analysed (before biochar preparation) to determine the ash content and material composition (Appendix A) according to methods established by the Technical Association of the Pulp and Paper Industry (TAPPI 1993; TAPPI 1997). The grape stalks were then pyrolysed at $600^\circ C$ in a muffle furnace under 16.7 ml min^{-1} nitrogen flow rate at atmospheric pressure and retention time of 30 min. Additionally, the percentage yield (Appendix A) of biochar was calculated as the quotient between the weight of biochar and weight of grape stalks. The resulting biochars were then cooled overnight under the same nitrogen flow rate 16.7 ml min^{-1} . Pyrolysed products were then ground, homogenised, sieved ($<0.50\text{mm}$ in size), washed by ultra-clean water MilliQ Integral (Merck Millipore Corp., USA) and dried at $60^\circ C$ for 24h until constant weight. All prepared biochar samples were analysed to determine the yield, bulk density, moisture and ash content (Trakal et al. 2014).

7.9 Application of biosolids and biochar to contaminated sediments

Sediment samples from zone 7 obtained during the second sample period were selected to carry out stabilisation experiments due to high metal content and physicochemical

characteristics that strongly favour temporarily low metal mobility. The experiment was carried out with three main groups.

- 100g un-amended sediment samples (S)
- 100g sediment samples amended with 10g biosolids (S + BS)
- 100g sediment samples amended with 10g biochar (S + BC)

Samples were saturated to 75% with distilled water acidified to pH 5.5 with HOAc. The mixture was left to stand for 1 hour. After 1 hour, 4g of sample was taken and leached in 40ml of CaCl₂ 0.01M acidified to pH 5.5 and agitated for 2 hours. An additional 4g of sample was leached with CaCl₂ 0.01M acidified to pH 3.5 (Houben, Evrard, and Sonnet 2013a; Beesley et al. 2010). The mixture was then centrifuged; supernatant subjected to physicochemical characterisation (pH, Electrical Conductivity) and finally microwave accelerated acid digestion (CEM Corporation Mars 907600, USA; USEPA, 2007b) for the determination of leached metal content using flame atomic absorption spectrometry (Perkin Elmer 900H, USA). This procedure was repeated on day 7, 14, 21 and 28. On day 28, samples were dried and 1g was used for the five step batch metal fractionation procedure (Tessier et al. 1979). Based on fractionation results, the mobility factor which represents the metals that can potentially mobilize and enter the biota (Gasparatos et al. 2015; Shaheen and Rinklebe 2014), was calculated for each treatment with the equation;

$$\text{Equation 1. } MF = \frac{F1+F2}{F1+F2+F3+F4+F5}$$

7.10 Application of ground grape stalks and biochar to contaminated soils

Due to high organic matter and low pH, soils in Aberdeenshire, UK, tend to leach nutrients. As such, wood ash, obtained from the combustion of waste wood for energy production is added to soils to elevate pH and prevent nutrient leaching (Karoline 2012). Soil and ash were obtained from Glensaugh experimental research station and farm near Laurencekirk, Aberdeenshire (UK); 56°53'24.44" N; 2° 32'55.24" W. The soil collected for this experiment was from part of the farm designated for experimental work having received no prior amendment of manure or ash and being lightly grazed by cattle and sheep seasonally (organic carbon (OC) contents of ~7% and nitrogen (N) contents of ~0.6%). After sward

removal a horizontal soil pit was excavated and the top 30 cm of soil bagged and returned to the laboratory (mixed A and B horizons). The soil was then air dried at 25°C for 1 week before it was homogenised by hand and sieved using a 10 mm sieve. Ash was sampled from a 70 kW wood fired biomass burner which provides heat for the farm buildings. The feedstock for the biomass boiler consisted of mixed virgin timber and CCA (Chromated Copper Arsenate) treated waste wood from fencing, pallets and doors. Ash was obtained directly from the boiler's combustion chamber, bagged and returned to the laboratory (organic carbon (OC) contents of ~2.5% and nitrogen (N) contents of < 0.1%).

A three week experiment was setup to evaluate metal(loid) leaching from contaminated soils and the potential leaching reduction exchangeable metals due to biochar (BC) and biochar source material (SM) amendment, and a possible association with lesser mobile carbonate or organic matter fractions using different extracting solutions (Clemente et al. 2006). Four 20 x 6cm acrylic glass columns were filled with:

- 400g of soil + 40g non contaminated ash (NCA)
- 400g of soil + 40g contaminated ash (CA)
- 400g soil + 40g contaminated ash + 4g source material (CA + SM)
- 400g soil + 40g contaminated ash + 4g biochar (CA + BC)

During week 1, columns were gravitationally leached at a constant top flow rate of 0.5 mL min⁻¹ maintained with a PCD 82.4 peristaltic pump (Kouril, Czech Republic) with CaCl₂ 0.01M for 96 hours. Each column head was designed to allow the leaching solution to be dispersedly introduced ensuring homogenous irrigation. After saturation, initial duplicate accumulative initial samples were collected. Additional duplicate samples were collected at 24, 48, 72 and 96 hours. Conductivity and pH were analysed potentiometrically, total metal(load) concentration was determined using ICP-OES (Agilent Technologies 700 series, USA) and DOC concentration was measured using TOC-L Analyser (Shimadzu, Japan) after filtering (<0.45 µm) in all collected samples. The columns were then irrigated for 24 hours with ultra-clean water MilliQ Integral (Merck Millipore Corp., USA). After irrigation, the same experimental technique was used, varying only the extractant. NaOH 0.5M was used during the second week in order to evaluate metals associated with organic

matter, and Na₂EDTA during the third week in order to evaluate metals associated with the carbonate fraction. At the end of the experiment, soils were removed from columns, dried and used for total metal content determination using ICP-OES (Agilent Technologies 700 series, USA) after acid digestion with aqua regia (Fernandez Linares et al. 2006).

In order to confirm exchangeable metals, metals bound to carbonates and metals bound to organic matter, a batch sequential extraction (Clemente et al. 2006) experiment was also carried out using fresh material, based on 4g soil, and respecting the proportion of amendments used in the column experiment. Metals in leachate was quantified using ICP-OES (Agilent Technologies 700 series, USA) after filtering (<0.45 µm). Metals in residual fraction were quantified using ICP-OES (Agilent Technologies 700 series, USA) after acid digestion with aqua regia (Fernandez Linares et al. 2006). Due to high Cr concentrations in all aforementioned experiments, an additional experiment was carried out using fresh material, based on 4g soil and respecting the proportion of amendments used in the column experiment. Samples were agitated in 40ml ultra-clean water MilliQ Integral (Merck Millipore Corp., USA), centrifuged and filtered (<0.45 µm). The leachate was used for ion exchange Cr speciation (Kocaoba and Akcin 2002).

7.11 Statistical analyses

Data obtained from amendment experiments were analysed by means of two-way ANOVA analyses and where differences were found between treatments, a Tukey's multiple comparisons test (p<0.05) was performed using GraphPad Prism version 7.0.

8 Results & Discussion

8.1 Physicochemical characterisation of substrates affected by mining activity

In both soil and sediments samples (Figure 1) there were differences in moisture percentages between the means of the first three sample periods and the last. These differences did not follow the typical pattern of changes in the hydrological conditions of the different seasonal periods, wet season and dry season. According to the Department of Urban Planning and Land Management of the State of Aguascalientes (2014), rainfall in recent years have occurred irregularly, both in frequency and intensity, with respect to the historical behaviour.

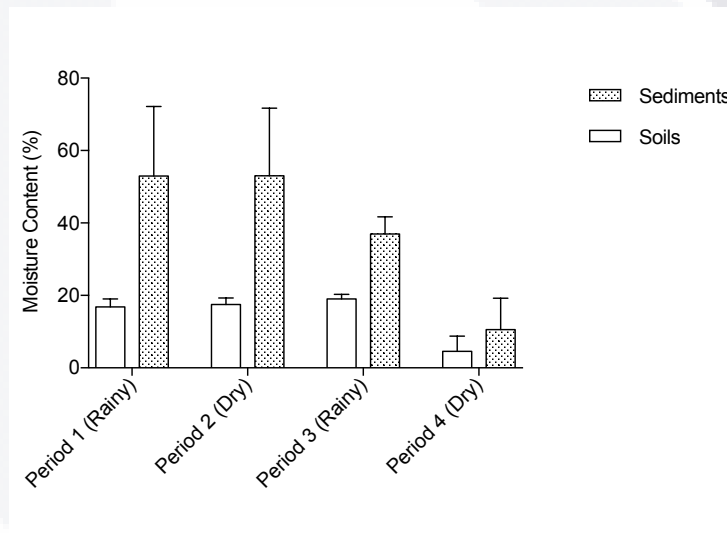


Figure 1. Moisture content (%) in soil and sediment samples during the 4 sample periods; $n = 10$

Another factor to consider is that the Piedras Negras River has an irregular flow fed by domestic and industrial discharges. These conditions are reflected in the atypical pattern deviation of moisture content in the substrates with respect to the seasons. The variability in moisture content is an important factor in the study of physical, chemical and biological properties of soils and sediments as it directly and indirectly influences the movement and distribution of metals (Van Den Berg and Loch 2000; Zheng and Zhang 2011). Studies have shown that moisture conditions affect the pH; under conditions of saturation, there is a tendency to neutrality (Bollinger and Bril 2003; Hans-eike et al. 1997).

Figure 2a shows that 81% of the sampled soils are classified as neutral (between 6.5 and 7.5), 17% mildly acidic (< 6.5) and 2% moderately alkaline (> 7.5). No significant differences between the values obtained from the different seasonal periods were observed

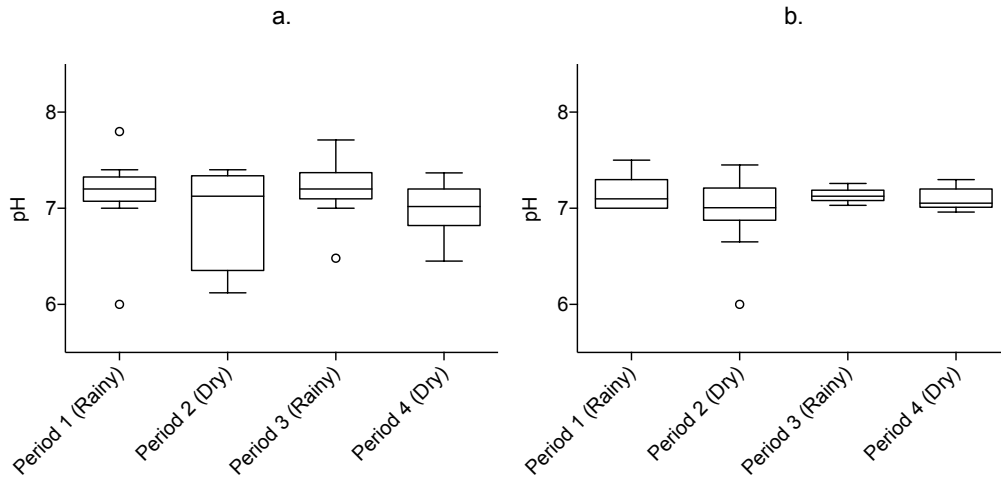


Figure 2. Mean pH of substrates during the 4 sample periods (a) soils; (b) sediments; $n = 10$

According to the classification used by Fernandez Linares (2006) 96% of the sediments sampled (Figure 2b) in both dry season and rainfall had a neutral pH, 2% were classified as moderately acid and the remaining 2% were moderately alkaline. Although no significant differences were found, the median during the dry season was lower than the median obtained during the rainy season.

pH is an important factor in the mobilization of metals in soils and sediments (Fijałkowski et al. 2012) due to the hydrolysis reactions of cations caused by an increase in the concentration of protons due to the competition between them and ligands (OH^- , SO_4^{2-} etc.) resulting in a decrease of adsorption sites (Mclean and Bledsoe 1992). This results in an increase in solubility and therefore the mobility of the metals in inverse proportion to the pH; such that an alkaline pH is not conducive to high metal mobility (Ramos-Goméz 2012). Predominantly neutral conditions of the sampled sites in this study may indicate a temporary reduction in mobility. pH however is highly dependent on soil moisture content. In this study, higher percentages were observed in sediment samples, both sample groups

being affected by the prevalent hydrological conditions. As moisture content increases towards saturation, the pH tends to neutrality.

The redox potential of the substrate is another factor that must be evaluated as it also plays a major role in the mobility of metals. Studies have shown that there can be an increase in mobility due to reduction reactions even under neutral conditions (Bleam 2012; Bollinger and Bril 2003).

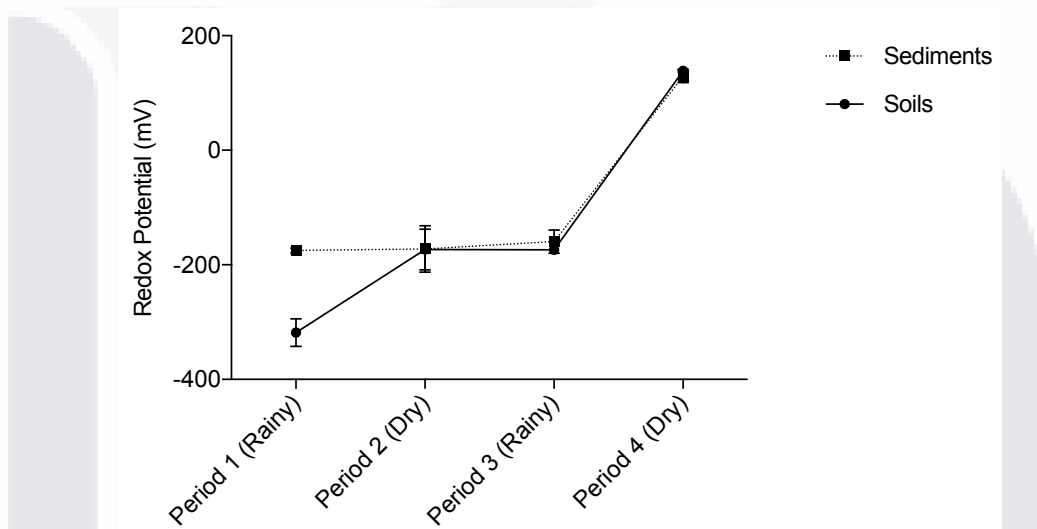


Figure 3. Mean redox potential (mV) of soil and sediment samples during the 4 sample periods; $n = 10$

According to Patrick and Mahapatra (1968) as cited by Bledsoe and Mclean (1992) four general ranges of redox potential measured in mV can be encountered in soils and sediments; oxidized soils $> +400$ millivolts (mV); moderately reduced soils, from $+400$ to $+100$ mV; reduced soils, from $+100$ to -100 mV; highly reduced soils, -100 to -300 mV. Using this classification, Figure 3 shows that 65% of the soils sampled showed highly reducing conditions, 10% of these were classified as reduced and 25% presented moderately reduced conditions. There were significant differences between means from samples obtained in the 4th sampling period as compared to the former 3. In a similar fashion, Figure 3 shows that 64% of the sediments sampled showed highly reducing conditions, 13% of these were classified as reduced and 23% presented moderately reduced conditions. In both cases, less reductive conditions were prevalent during the dry season especially in samples obtained during the 4th sampling period as compared to the former 3.

These differences can be attributed to moisture gradients of study sites as a result of the prevailing hydrological conditions (minimal precipitation) of the seasonal period (Yu et al. 2006). Nevertheless, this prevalent reductive condition in the area studied can greatly affect metal transport possibly contributing to an accelerated migration (McLean and Bledsoe 1992).

Using the classification established by SEMARNAT (2002b), 77% of soils (Figure 4) presented normal conditions of salinity (<1000 $\mu\text{S}/\text{cm}$) and 13% presented slightly saline conditions (between 1000 and 2000 $\mu\text{S}/\text{cm}$). There was an increase in variance and mean values with time. In case of sediments, 47% of samples (Figure 7.4) presented normal saline conditions, 23% were considered slightly saline and 20% moderately saline. As was the case with soil samples, there was an increase in variance and mean values with time.

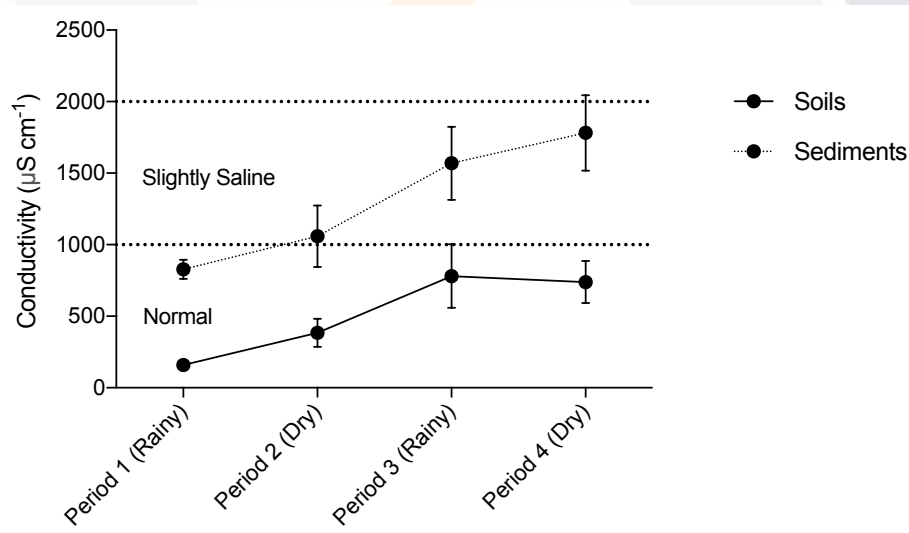


Figure 4. Mean electrical conductivity ($\mu\text{S cm}^{-1}$) of soils and sediments during the 4 sample periods; $n = 10$

This increased salinity has important implications given that an increase in salinity results in an increase in mobility of metals in the soil due to the complex formation capacity of anions derived from metal salts and competition of salts derived from metal cations with positively charged species for the adsorption sites (Acosta et al. 2011; Paalman et al. 1994).

The distribution of sulphates in mg L^{-1} is shown in Figure 5 a and b in both soils and sediments. Sediment samples contained concentrations up to 7 times more than in soils.

These values indicate the presence of contamination by soluble salts, in this case sulphates, a common product of oxidation-neutralization of metal ores or waste reagents from flotation processes used in the mining industry (Gutiérrez-Ruiz et al. 2007). Sulphide concentrations were also higher in sediment samples (Figure 5 c and d), up to 15 times higher in some cases.

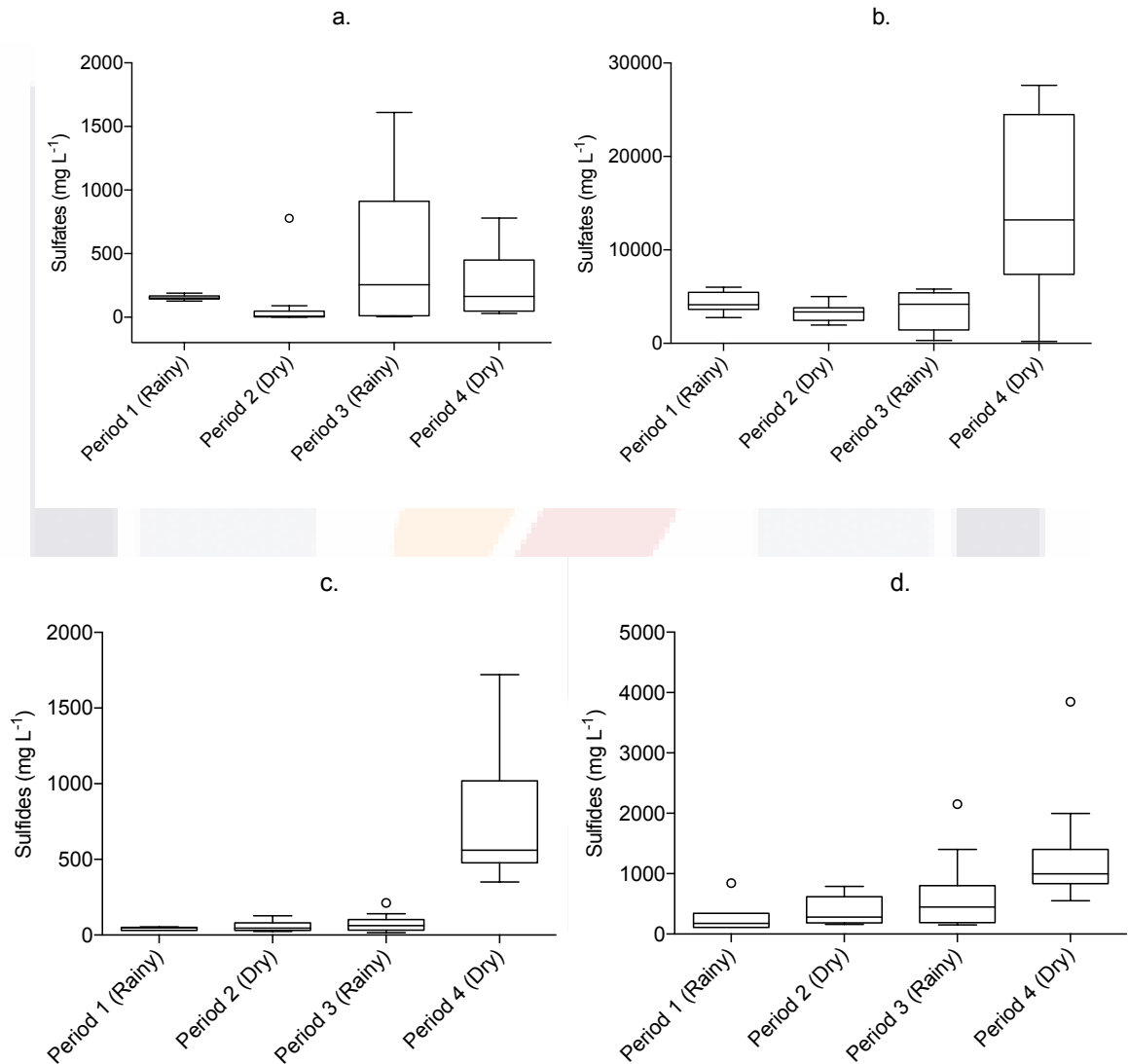


Figure 5. Distribution of mean sulphates and sulphides (mg L⁻¹) values during four sample periods. (a) sulphates in soils, (b) sulphates in sediments, (c) sulphides in soils, (d) sulphides in sediments; *n* = 10

The presence of higher than typical concentrations of sulphides in soils and sediments may indicate constant reception of mine wastes, at a higher rate than that of the oxidation

process sulphides normally undergo. The oxidation process is also important given that it produce sulphates resulting in a decrease in pH of the substrate, an important factor in the mobility and availability of metals (Clemente et al. 2003). Additionally, in substrates where specific adsorption sites become saturated with cations and anions already present, exchange reactions predominate, resulting in competition for these sites between metals and soil ions (McClean and Bledsoe 1992). In the presence of these inorganic ligands, the adsorption of certain metals on soil and soil constituents can be inhibited (Ahmed et al. 2013), as such contribute to an increase in the concentration for the free metal in the liquid phase of the soil.

Certain mining activities requires the use of great quantities of alkaline solutions containing high concentrations of sodium cyanide, free cyanide and metal-cyanide complexes (Korte and Coulston 1998). On average, sediment samples (Figure 6) presented higher concentrations than soil samples.

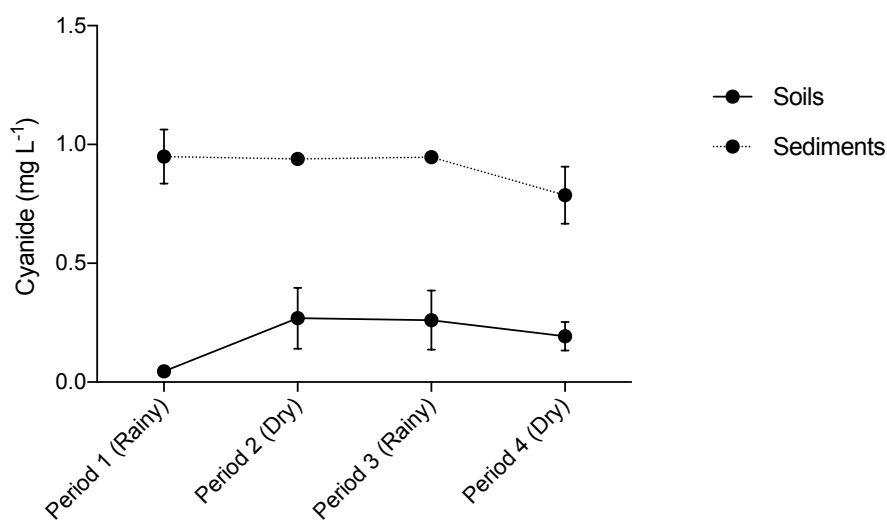


Figure 6. Mean cyanide concentration (mg L⁻¹) in soil and sediment samples obtained during 4 sampling periods; *n* = 10

It is important to note that cyanide biodegrades under aerobic conditions with the initial formation of ammonia, which is converted to nitrite and nitrate in presence of nitrifying bacteria (Eisler and Wiemeyer 2004). Using the soil values to represent typical geological

characteristics, the high cyanide concentrations in sediment samples is indicative of direct and constant pollution from mining activities.

Metal mobility or lack thereof is also affected by the organic matter content of the studied substrate. Organic matter content had little variation during the four sampling periods. According to the classification used by Fernandez Linares et al. (2006), 15% of sampled sediments (Figure 7) presented very high organic matter content (>6.0%), 63% high organic matter content (3.6 - 6.0 %) and 22% moderate organic matter content (1.6 - 3.5%). In the case of soils, 2% of sampled sediments presented very high organic matter content (>6.0%), 80% high organic matter content (3.6 - 6.0 %) and 18% moderate organic matter content (1.6 - 3.5%).

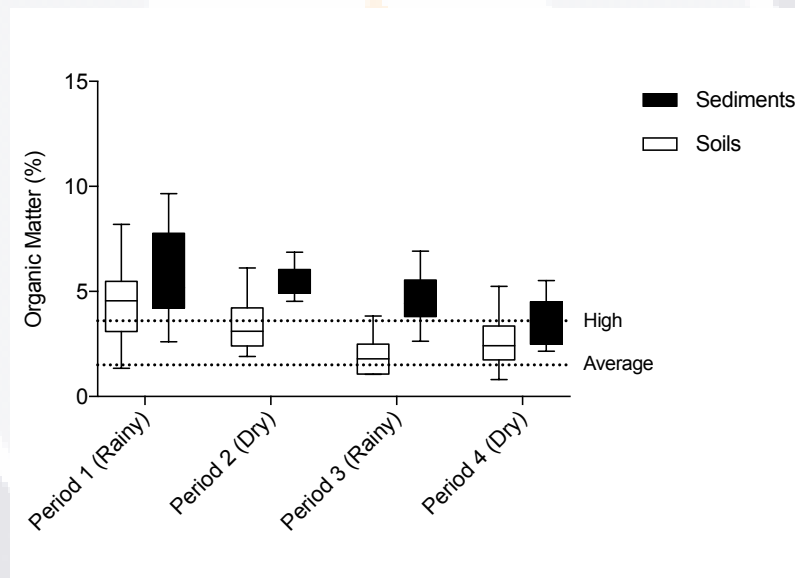


Figure 7. Mean organic matter content (%) in soil and sediment samples obtained during 4 sampling periods; *n* = 10

As with other physicochemical characteristics, organic matter has a direct impact on the behaviour of metals, affecting solubility with complexation and chelation reactions (Kashem and Singh 2001). Studies have shown that during decay, humic and fulvic acids whose functional groups are capable of associating with metal cations (Kyziol 2002) are produced. However, under acidic conditions such groups are ionized resulting in the remobilization of these cations (Carrillo-González et al. 2006), further demonstrating the

close relationship between the pH and other physicochemical characteristics (Sherene 2010), including organic matter content.

As a measure of the soil's capacity to hold exchangeable cations, cation exchange capacity is used to indicate the net negative charge present on a mass unit of soil (Ali 2010). The higher the cation exchange capacity, more easily metal cations are attracted to the negative sites on soil and sediment particles (Attwell et al. 1999).

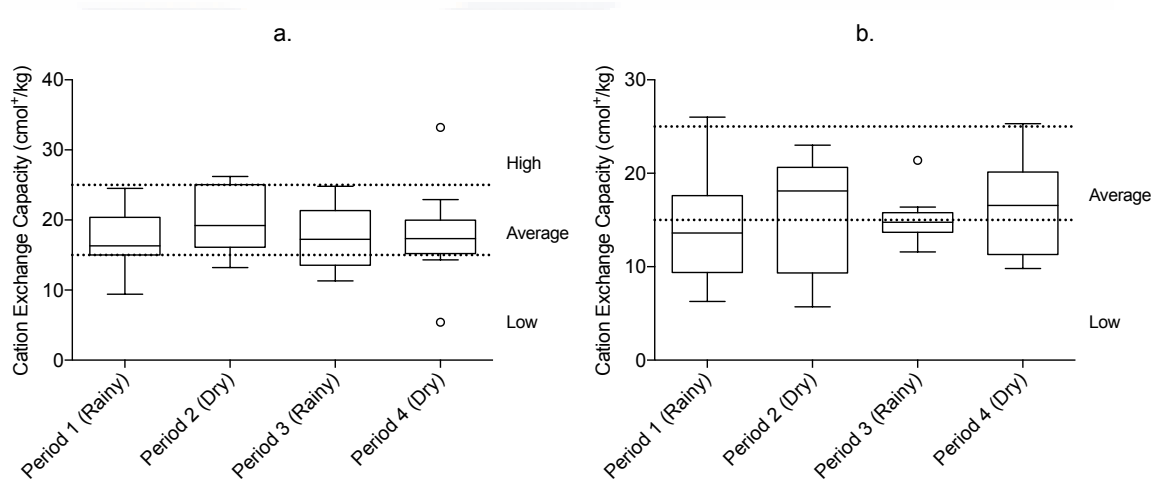


Figure 8. Mean cation exchange capacity (cmol⁺/kg) in (a) soil and (b) sediment samples obtained during 4 sampling periods; $n = 10$;

According to the classification used by SEMARNAT (2000), 85% of soils (Figure 8a) and 60% of sediment samples (Figure 8b) were classified as having average cation exchange capacity (15 - 25 cmol⁺/kg). 15% and 40% respectively presented low cation exchange capacity (5 - 15 cmol⁺/kg), conditions that may contribute to a less immobilisation (Attwell, Kriedemann, and Turnbull 1999).

The majority of soil samples (72.5%) were classified as having average CaCO₃ content with the rest being classified as high, low or very low. Sediment samples were classified as having either average (72.5%) or high (27.5%) CaCO₃ content. A decrease in %CaCO₃ equivalent with respect to time can be observed in soil samples (Figure 9a). The %CaCO₃ in sediment samples (Figure 9b) show neither variances nor tendencies during the four sample periods. Negligible water leachable metals from both substrates can be attributed in

part to the presence of these carbonates, which temporarily reduce metal mobility due to precipitation reactions and the buffer effect (Nazif et al. 2015).

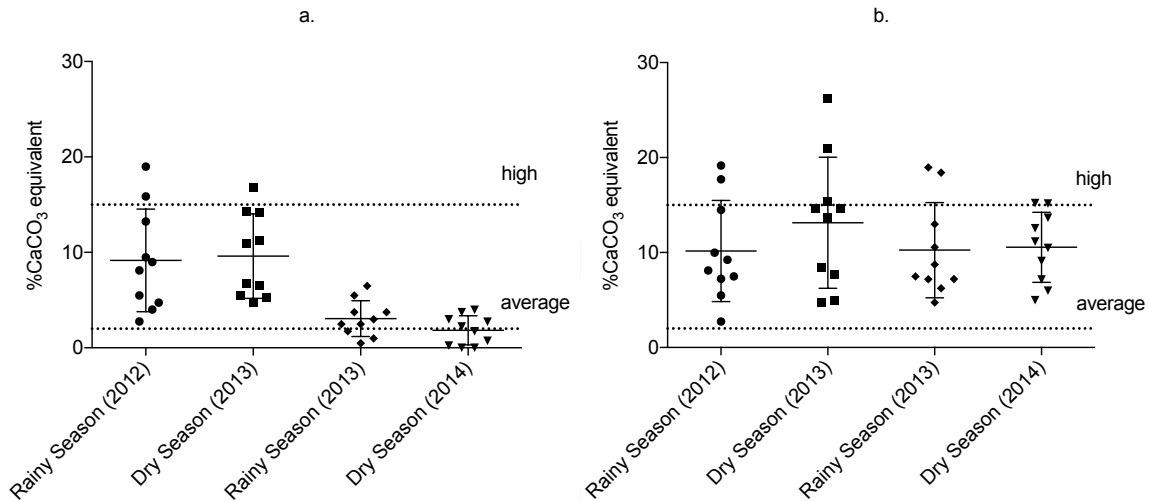


Figure 9. Mean CaCO₃ equivalent (%) in (a) soil and (b) sediment samples obtained during 4 sampling periods; $n = 10$

A reduction in carbonate presence was observed in the rainy period, probably due to variations in pH, redox potential and saturation conditions which directly affect carbonate presence. Continuing reduction would implicate an eventual release of fixed metals to the liquid phase.

8.2 Total metal content and leaching potential

Total copper concentration (Figure 10) in samples collected during the four sampling periods. 63% of sampled soil sites and 95% of sediment sites presented substrates with copper concentrations above the CCME quality guidelines limit (CCME 1993). It is important to note that physicochemical conditions did not favour leaching (Appendix A). Higher concentrations were observed in sediment samples as compared to soil samples, indicating that riverbed may be a direct receptor of polluted material.

Chromium is considered a hazardous metal due to its high toxicity and non-biodegradability (Anwar et al. 2011). Total chromium concentrations were therefore measured in soil and sediment samples during the 4 sample periods.

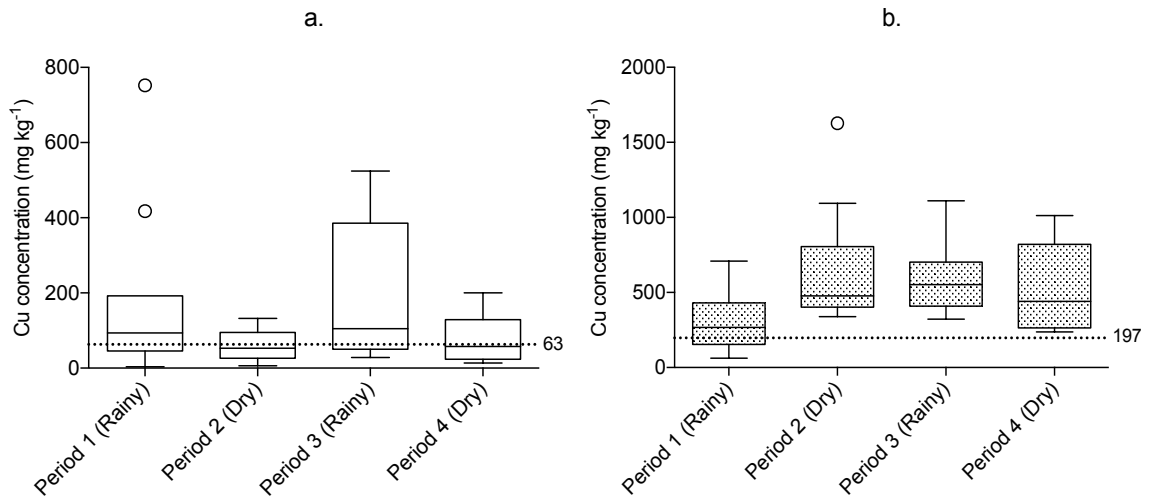


Figure 10. Mean total copper concentration (mg kg^{-1}) in (a) soils and (b) sediments from samples collected during 4 sampling periods. *Black dotted line represents CCME guidelines limit in mg kg^{-1} ; $n = 10$*

Though 37% of samples presented leaching (Appendix A), neither leached concentrations nor total metal concentration (Figure 11) surpassed the guideline limits established by the CCME or SEMARNAT (CCME 1993; SEMARNAT 2004)

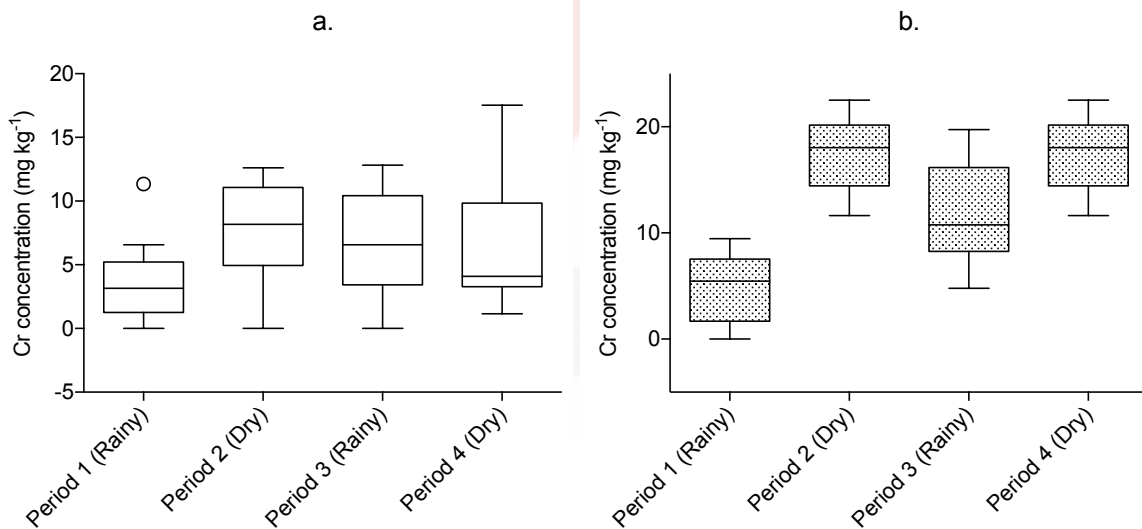


Figure 11. Mean total chromium concentration (mg kg^{-1}) in (a) soils and (b) sediments samples collected during 4 sampling periods; $n = 10$

Zinc is a naturally occurring metal in topsoil as a result of soil weathering (Liu et al. 2013), though in high concentrations it has the potential to be phytotoxic (Beyer et al. 2013).

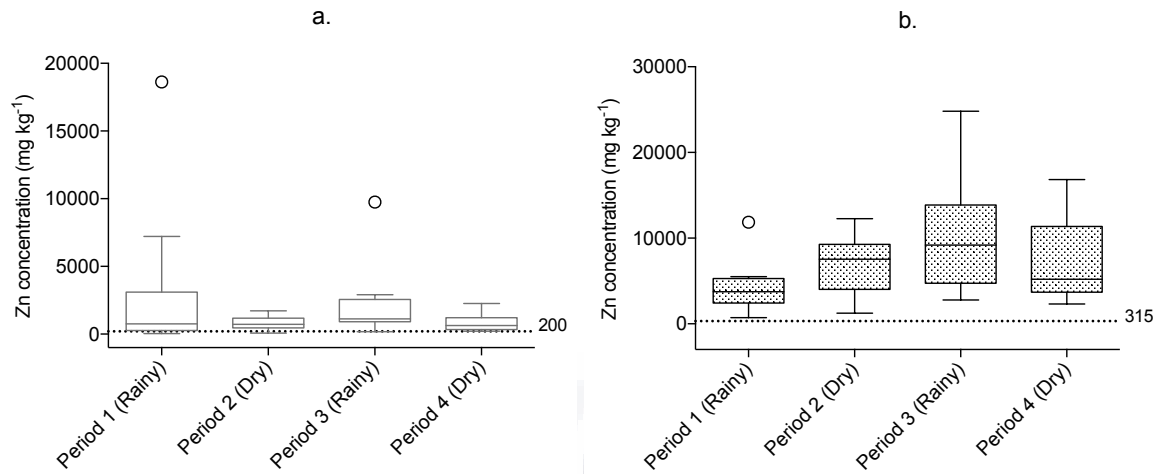


Figure 12. Mean total zinc concentration (mg kg^{-1}) in (a) soils and (b) sediments samples collected during 4 sampling periods. *Black dotted line represents CCME guidelines limit in mg kg^{-1} ; $n = 10$*

As such, concentrations above background levels merits further study due to its potential environmental risk (Marwa et al. 2012). 90% of soil samples (Figure 12) were found to surpass the established guideline CCME value of 200 mg kg^{-1} 100% of sediment samples were found to surpass the guideline value of 91.3 mg kg^{-1} (CCME 1993).

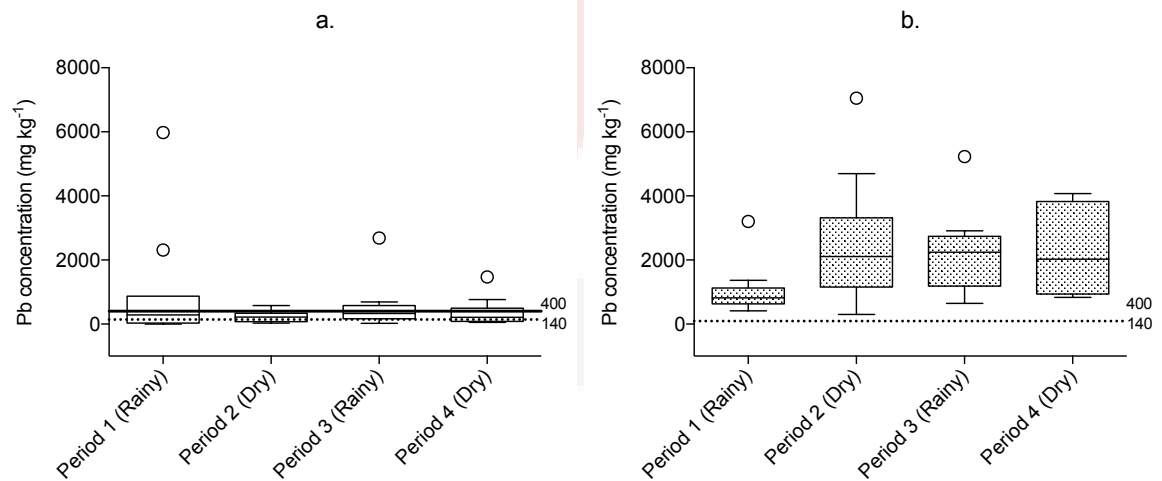


Figure 13. Mean total lead concentration (mg kg^{-1}) in (a) soils and (b) sediments samples collected during 4 sampling periods. *Black dotted line represents CCME guidelines limit in mg kg^{-1} . Solid line represents NOM-147 guideline limit in mg kg^{-1} ; $n = 10$*

Values for Pb concentrations can be observed in Figure 13. All sediment samples surpassed guideline values established by the CCME. However, 77% of soil samples surpassed limit (149 mg kg^{-1}) established by the same regulatory body, whereas if compared to limit

established by the SEMARNAT (400 mg kg^{-1}), the value reduces to 30% (CCME 1993; SEMARNAT 2004).

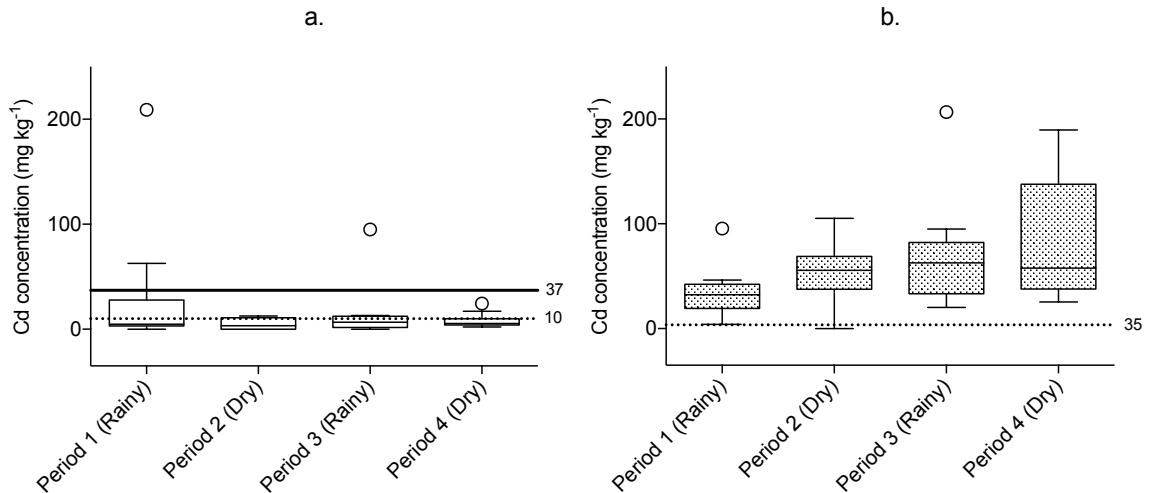


Figure 14. Mean total cadmium concentration (mg kg^{-1}) in (a) soils and (b) sediments samples collected during 4 sampling periods. *Black dotted line represents CCME guidelines limit in mg kg^{-1} . Solid line represents NOM-147 guideline limit in mg kg^{-1} ; $n = 10$*

Figure 14a shows values for Cd concentrations in soils and their comparison with CCME and SEMARNAT guideline values. 43% of the sampled sites surpassed limits established by the former, whereas only 7% surpassed limits established by the latter. 75% of sample sites surpassed CCME guideline sediment values (CCME 1993; SEMARNAT 2004).

Concentrations of these metals above established limits implies grave environmental risk. According to the US Environmental Protection Agency (USEPA) and the International Agency for Research on Cancer (IARC), as cited by Tchounwou et al. (2012), Pb and Cd are classified as either “known” or “probable” human carcinogens based on epidemiological and experimental studies. They have no established biological functions and are considered as nonessential metals and systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure.

8.3 Metal fractionation in soils and sediments affected by mining activity

Total metal concentration does not accurately reflect the potential risk metal pollution represent in the environment (Okoro et al. 2012). The study of a metal's distribution in separate soil fractions is key to understanding their potential mobility and bioavailability (Vaněk et al. 2005; Dalmacija et al. 2010).

Changes in physicochemical characteristics may result in the movement of metals associated to different geochemical fractions (Anju and Banerjee 2010). The study of the distribution of metals in these fractions has been useful in the determination of potential mobility and the prediction of long term behaviour of metals in polluted substrates (Zimmerman and Weindorf 2010; Arenas-Lago et al. 2014).

Metals distribution was studied in five different stages using the Tessier sequential extraction method. The first step extracts exchangeable metal cations, the second step extracts metals associated to carbonates, the third step extracted metals associated to Fe and Mn oxides, and the fourth step extracted metals associated to organic matter and sulphides. (Tessier et al. 1979; Violante et al. 2010).

Potential metal mobility is estimated using combined results obtained from the exchangeable and carbonate fractions, as leachability of metals in these fractions depends principally on pH (Anju and Banerjee 2010). Significant changes in potential redox are needed to mobilize metals associated with Fe–Mn oxide and organic matter fractions, therefore rendering them potentially less mobile (Clevenger 1990). Metals associated with the residual fraction indicates lithogenic origin and are considered immobile, thus representing negligible toxicological risk (Ramos-Gómez 2012).

Distribution of copper in soil fractions is represented in Figure 15. Though there were significant Cu association to the potentially mobile exchangeable and carbonate bound fractions, in most zones, there was an increased prevalence of Cu association to the organic matter in subsequent sample periods. In soils obtained during the 4th sample period, only residual Cu was found. In the case of sediments (Figure 16), in both sample periods, metals were also generally found associated to the organic matter and residual fraction indicating,

a reduced potential for mobility in the sediment system. As was the case of soil samples, there was a tendency for reduced potential mobility in each subsequent sample period, with less than 10% of Cu being potentially mobile in samples obtained during the 4th sample period. It is important to remember that these samples, presented average to high organic matter content, and in comparison to other cations, Cu has a preferential high affinity for soil organic matter (Clemente et al. 2006). As such the increased stabilisation of Cu in affected soils was expected.

Up to 80% of total zinc concentrations were found in the first two fractions (Figure 18 and 19). Generally, mobility was higher in samples obtained during rainy season than in samples obtained during the dry season. The increase in soil moisture can influence the transformation and repartition of heavy metals in soil, as components such as Fe and Mn oxides are reduced under anaerobic conditions, which are typical in conditions of saturation, resulting in an increase in the release of metals and a redistribution among the solid-phase components, and in many cases increasing their availabilities to plants (Zheng and Zhang, 2011). This increase in moisture and its resulting redox change, also affects precipitated forms of zinc, a common form found in soils, facilitating its dissolution and thus increasing its mobility (Clemente et al. 2006; Schweiker et al. 2014).

Figure 19 and 20 shows the distribution of chromium in the five soil and sediment fractions, in which association was primarily with the exchangeable and residual fraction. In it is important to note that Cr concentrations were relatively low in all cases, and the residual fraction may be considered lithogenic Cr, whereas, the Cr found in the exchangeable fraction is of anthropogenic origin. In both substrate cases, only residual Cr was found by the 3rd and 4th sample period. This reduction in mobility can be associated with changes in Cr species. Lithogenic and immobile Cr is usually in the form of the less toxic and immobile Cr(III) species, whereas mobile Cr is usually in the form of the toxic Cr(VI) species. In soils and sediments, especially in reductive conditions, there is a tendency for the Cr(VI) to be reduced to Cr(III), resulting in significant decreases in its mobility (Hellerich and Nikolaidis 2005).

Between 60% of Pb (Figure 21 and 22) and up to 100% of Cd (Figure 23 and 24) were found in the potentially mobile exchangeable and carbonate bound fractions. In the case of Pb, there was an observed increase in organic matter association in samples from the 3rd and 4th sample period, however up to 30% of Pb continued to be potentially mobile. In sediments, potential mobility was primarily related to association with the carbonate fraction, whereas in soils, it was related to the exchangeable fraction. Though Cd in sediments were generally as mobile as Cd in soils, there was an observed increase in association with the more stable organic matter fraction.

The distribution of these two metals in the soil gives some indication as to their origins. Significant concentrations in the mobile fractions may indicate contamination from anthropogenic sources (Vaněk et al. 2005), in this case, nearby mining activities. In the case of lead and cadmium in soils, higher concentrations in these mobile fractions can be observed in the dry season as compared to the rainy season. While this may be due to a remobilization of previously stable complexed metals, it is important to note that climatological (dry and windy) conditions during the sample period tend to favour aerial dispersal, effectively increasing the concentration of “new” mobile metal species.

These results show that despite very high metal concentrations in soils and sediments, in many cases significantly surpassing the national and international guideline values, physicochemical conditions in substrates reduced water leachability. However, the sequential extraction of these metals show that these metals are not completely immobile. Though some (Cu and Zn) were significantly associated with recalcitrant soil fractions, more toxic metals (Pb and Cd) were associated in part with potentially mobile fractions. As such, though currently not water leachable, changes in physicochemical conditions especially reduction in carbonate related buffer capacity or decrease in pH can affect substrate fractions and result in a release of these metals (Ashraf et al. 2012).

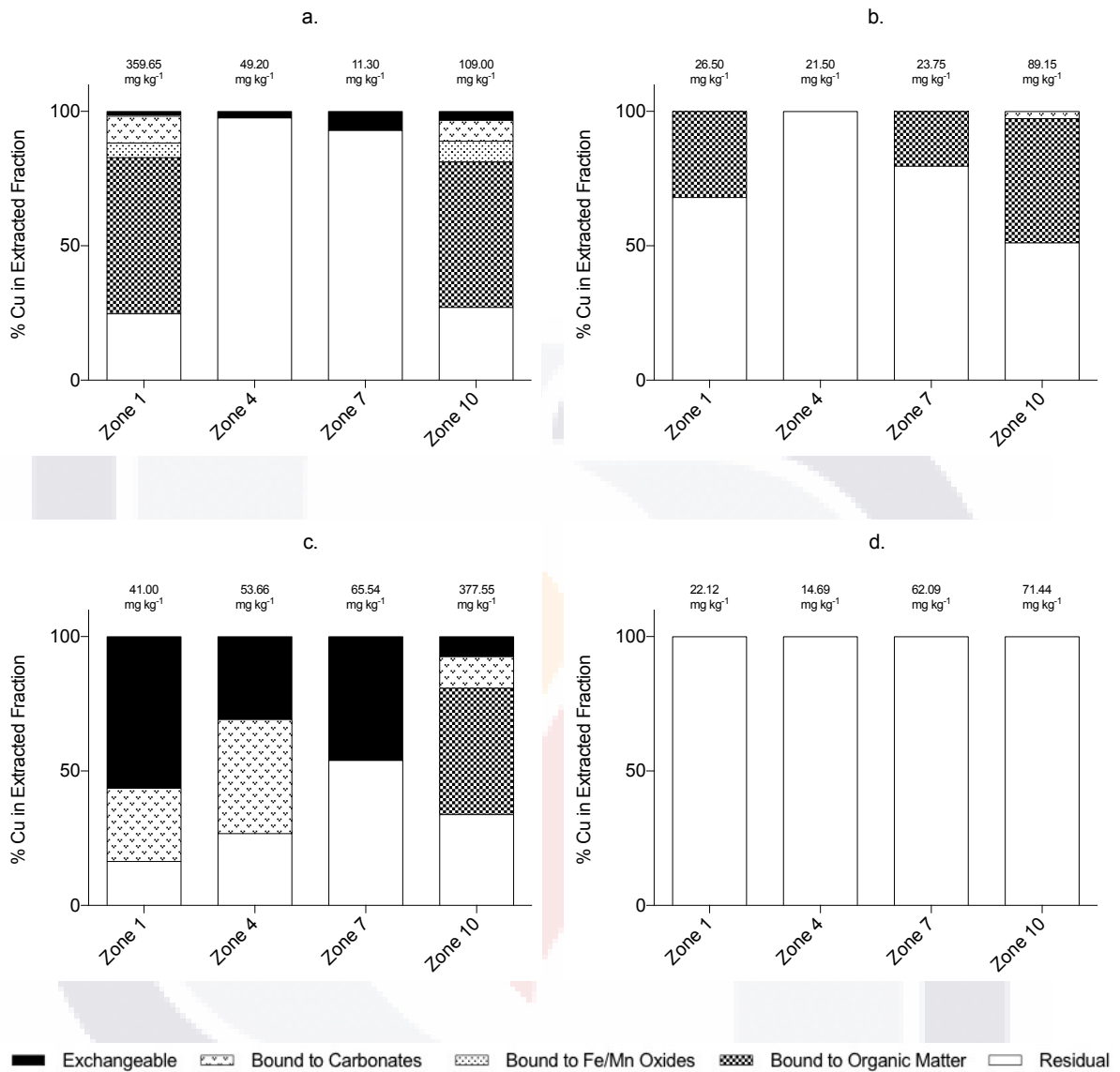


Figure 15. Fractionation of copper in soil from 4 sample zones (a) period 1 rainy, (b) period 2 dry, (c) period 3 rainy, (4) period 4 dry at 4 sample sites; $n=2$

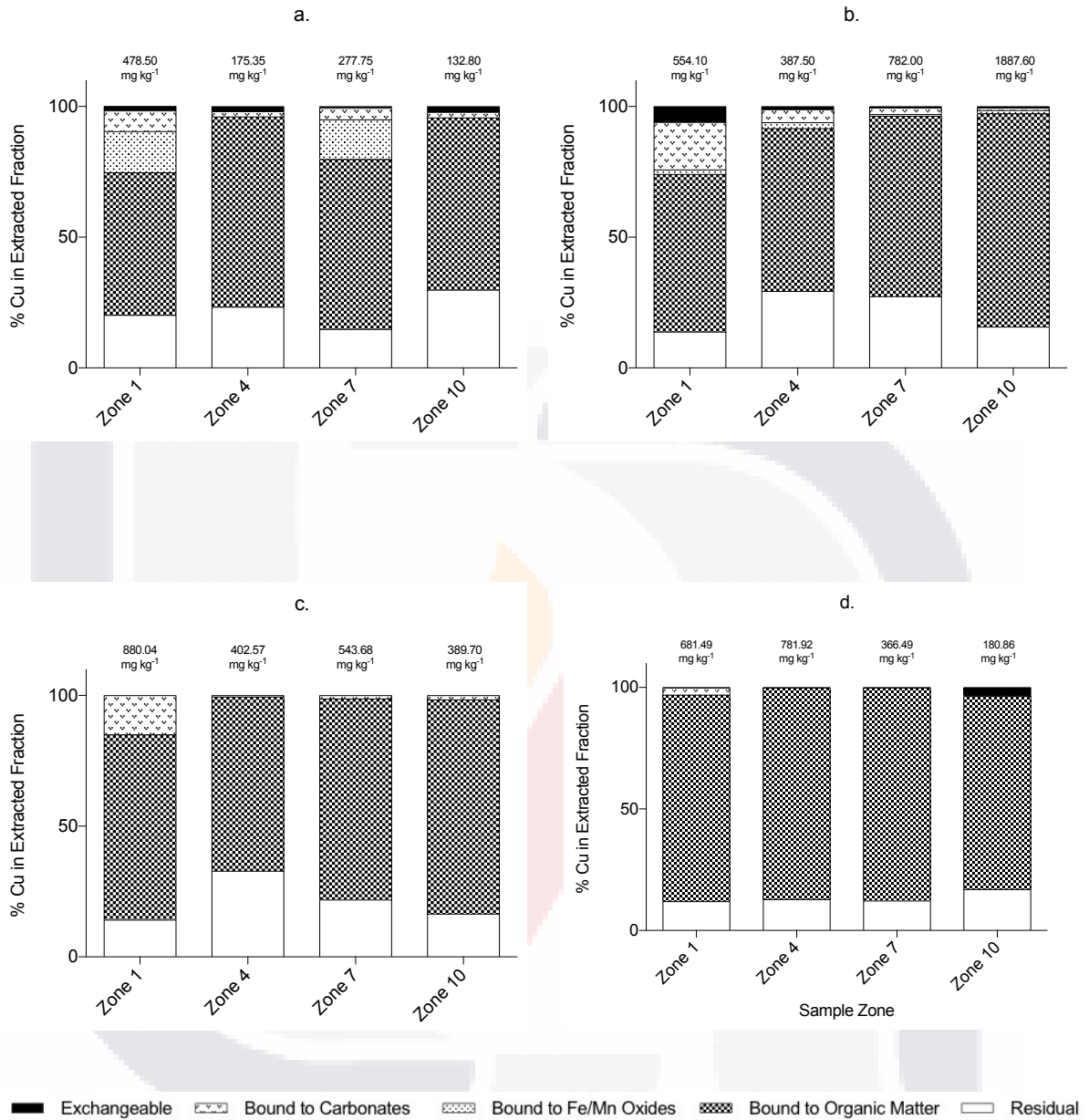


Figure 16. Fractionation of copper in sediments from 4 sample zones (a) period 1 rainy, (b) period 2 dry, (c) period 3 rainy, (4) period 4 dry at 4 sample sites; $n=2$

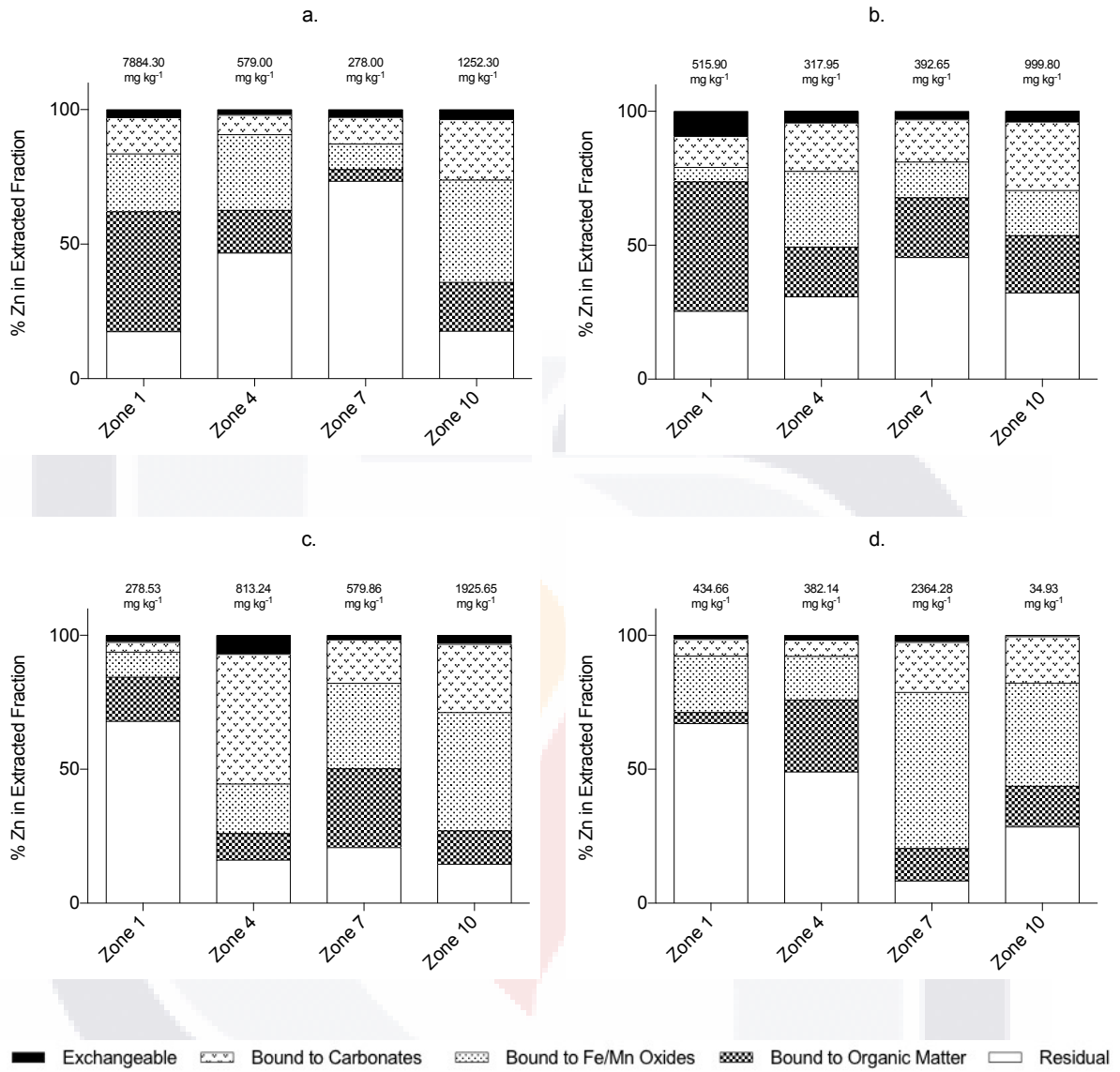


Figure 17. Fractionation of zinc in soil from 4 sample zones (a) period 1 rainy, (b) period 2 dry, (c) period 3 rainy, (4) period 4 dry at 4 sample sites; $n=2$

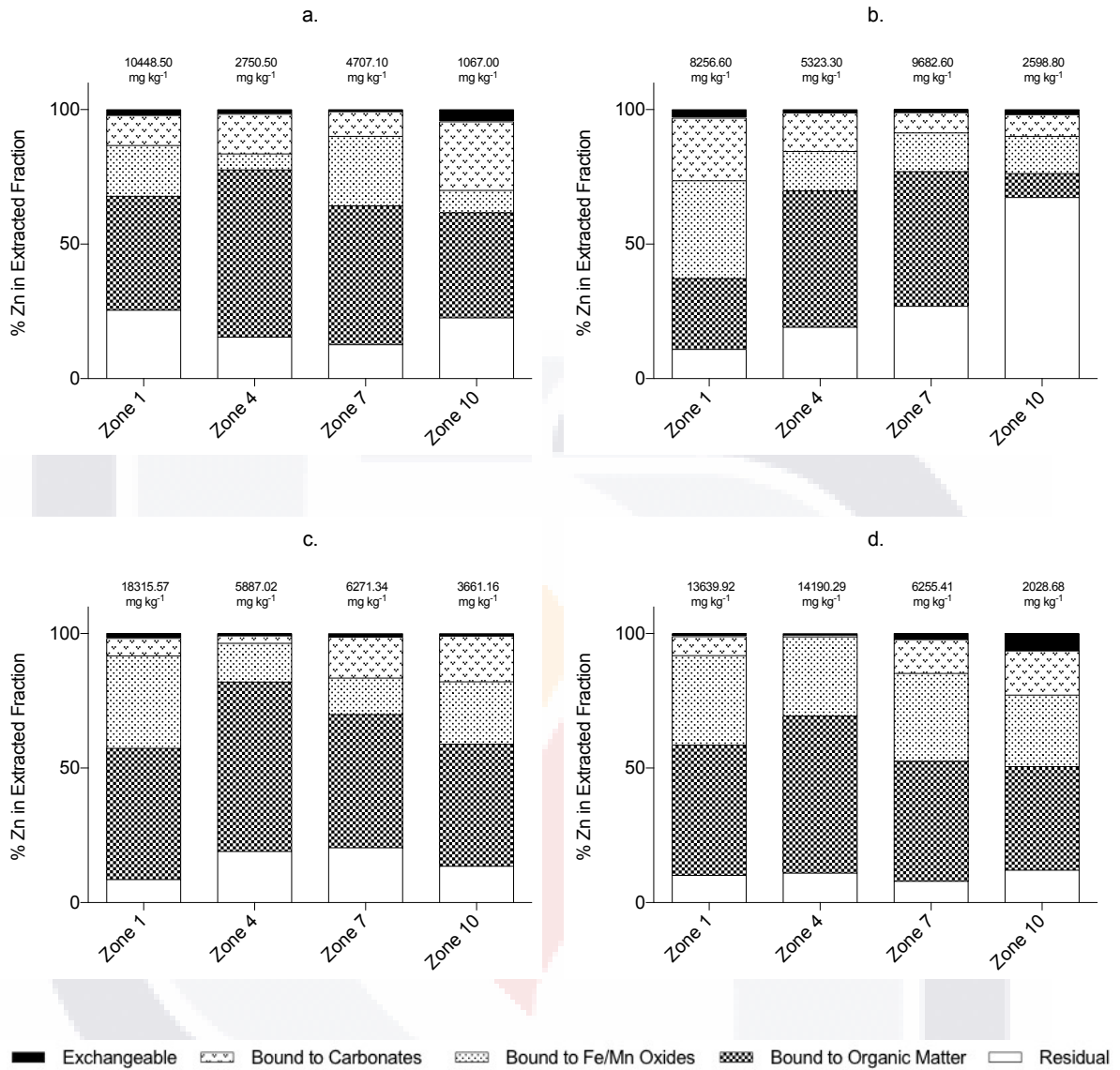


Figure 18. Fractionation of zinc in sediments from 4 sample zones (a) period 1 rainy, (b) period 2 dry, (c) period 3 rainy, (4) period 4 dry at 4 sample sites; $n=2$

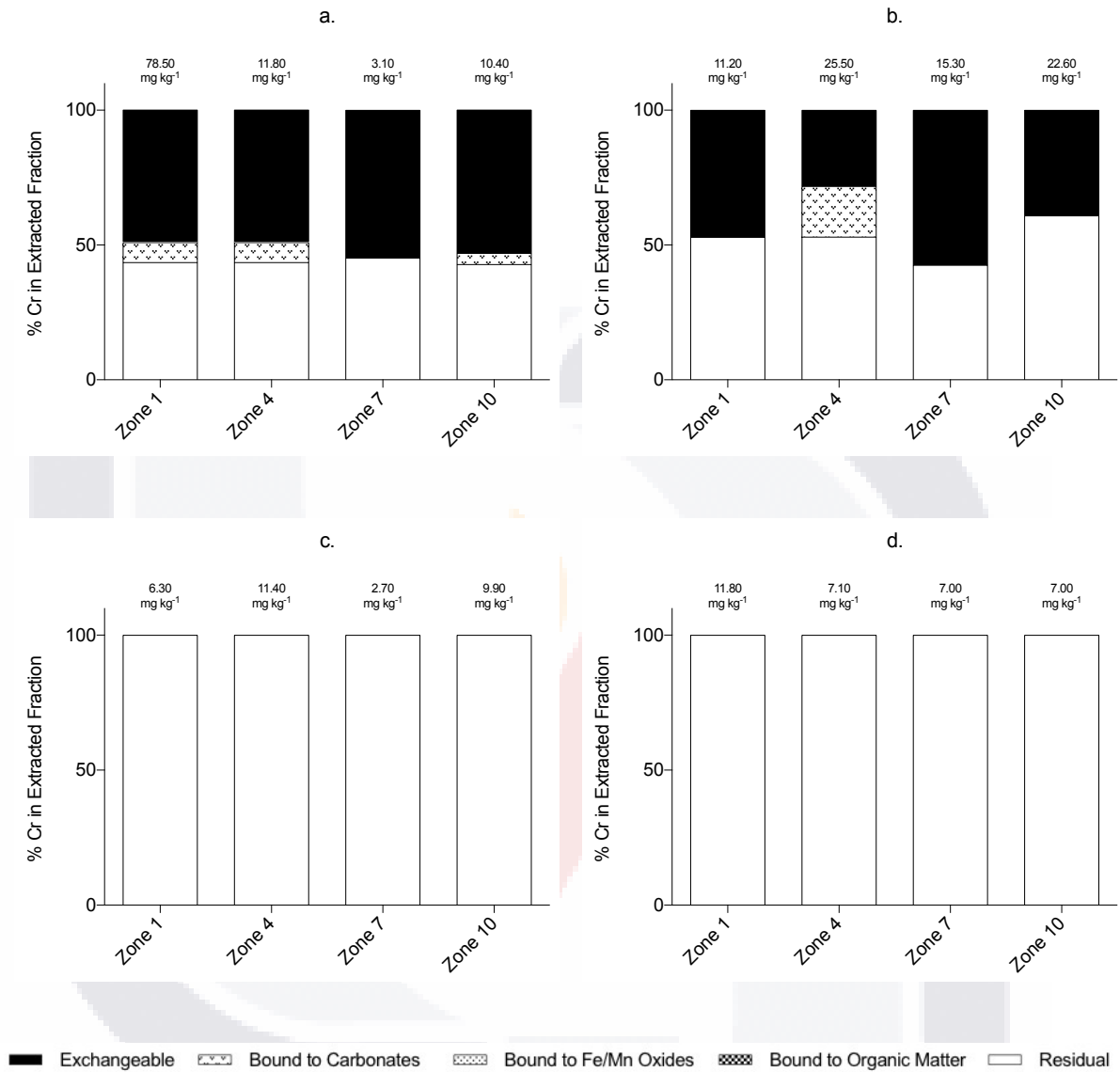


Figure 19. Fractionation of chromium in soil from 4 sample zones (a) period 1 rainy, (b) period 2 dry, (c) period 3 rainy, (4) period 4 dry at 4 sample sites; $n=2$

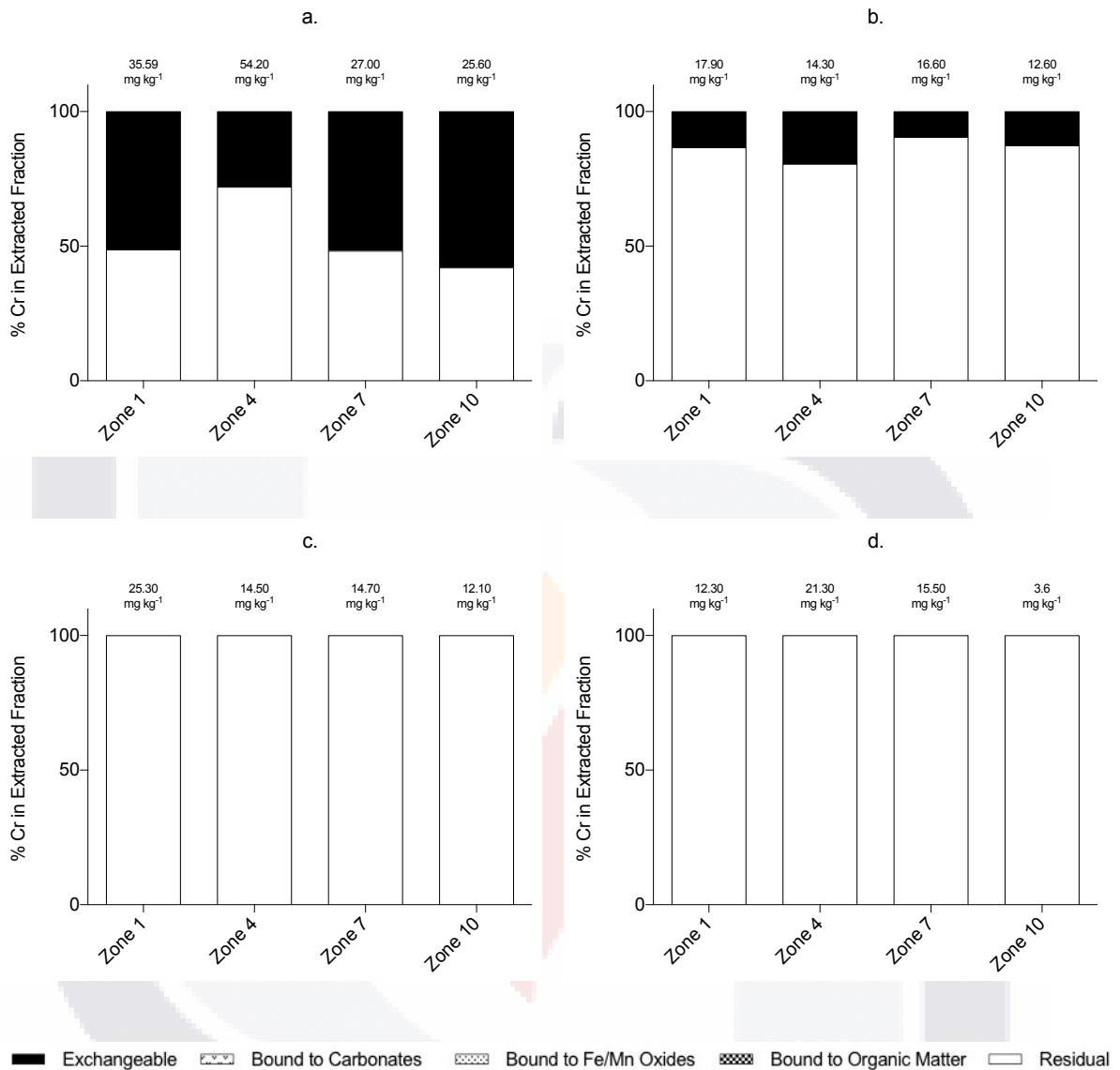


Figure 20. Fractionation of chromium in sediments from 4 sample zones (a) period 1 rainy, (b) period 2 dry, (c) period 3 rainy, (4) period 4 dry at 4 sample sites; $n=2$

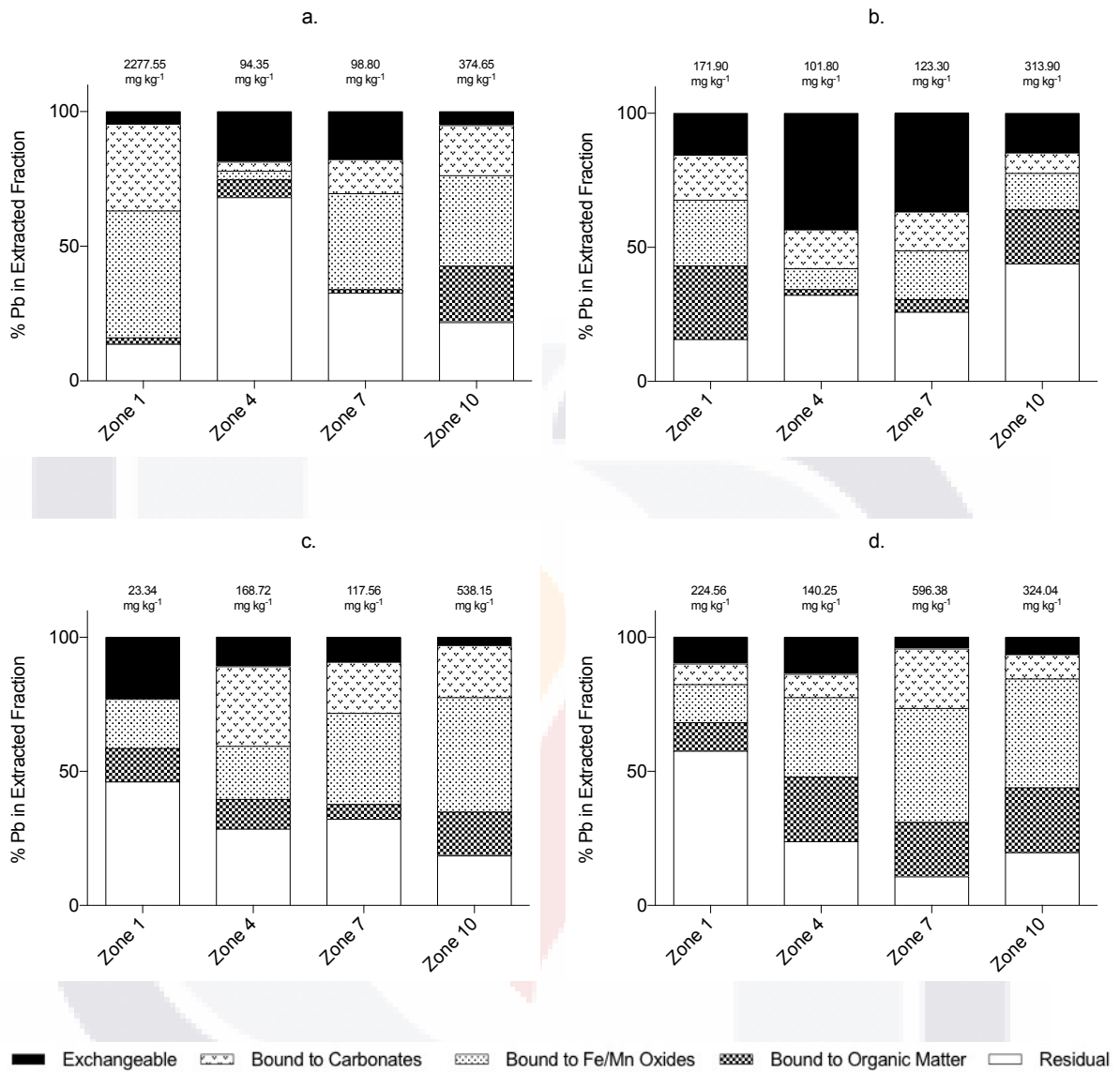


Figure 21. Fractionation of lead in soil from 4 sample zones (a) period 1 rainy, (b) period 2 dry, (c) period 3 rainy, (4) period 4 dry at 4 sample sites; $n=2$

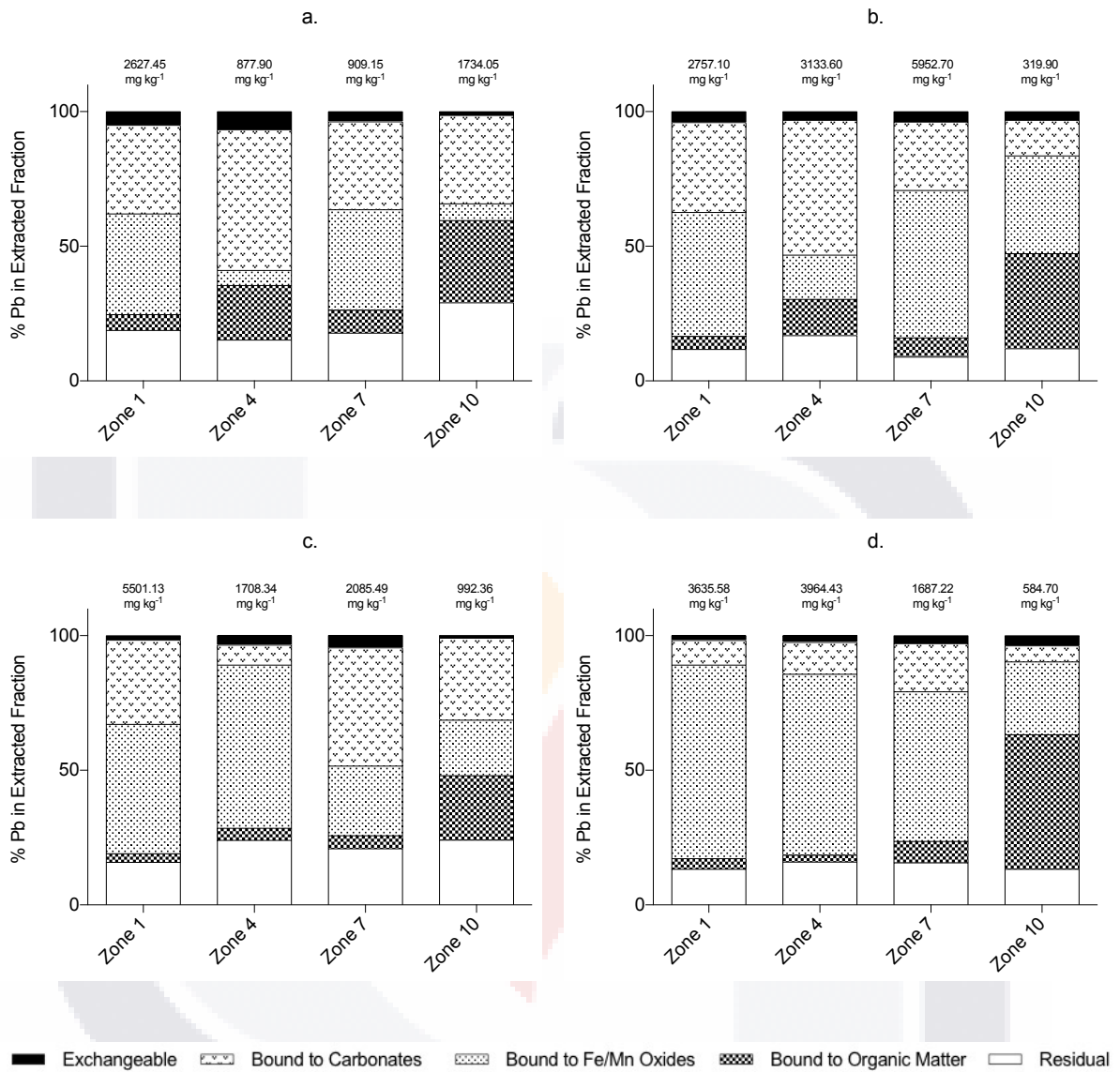


Figure 22. Fractionation of lead in sediments from 4 sample zones (a) period 1 rainy, (b) period 2 dry, (c) period 3 rainy, (4) period 4 dry at 4 sample sites; $n=2$

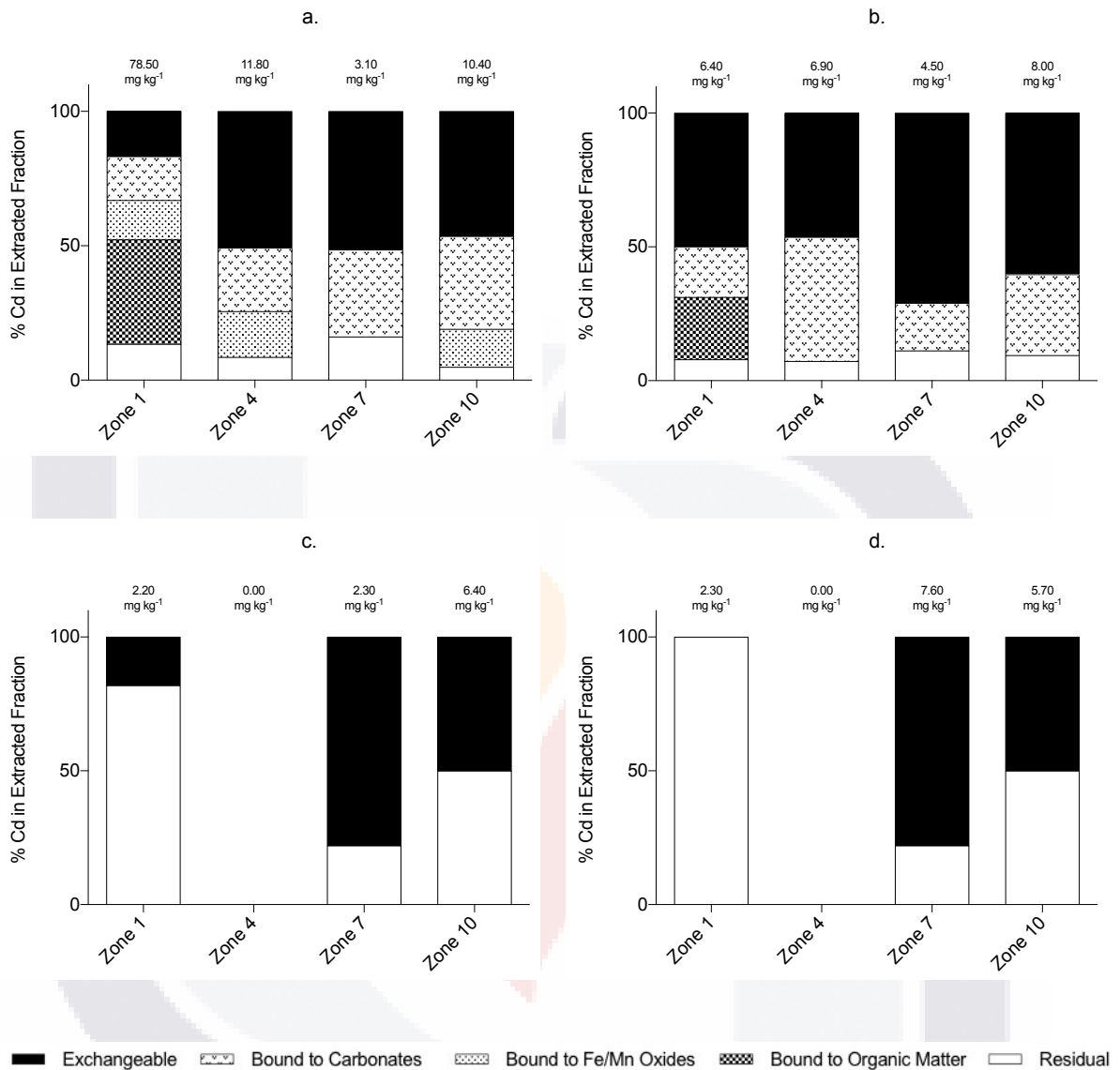


Figure 23. Fractionation of cadmium in soil from 4 sample zones (a) period 1 rainy, (b) period 2 dry, (c) period 3 rainy, (4) period 4 dry at 4 sample sites; $n=2$

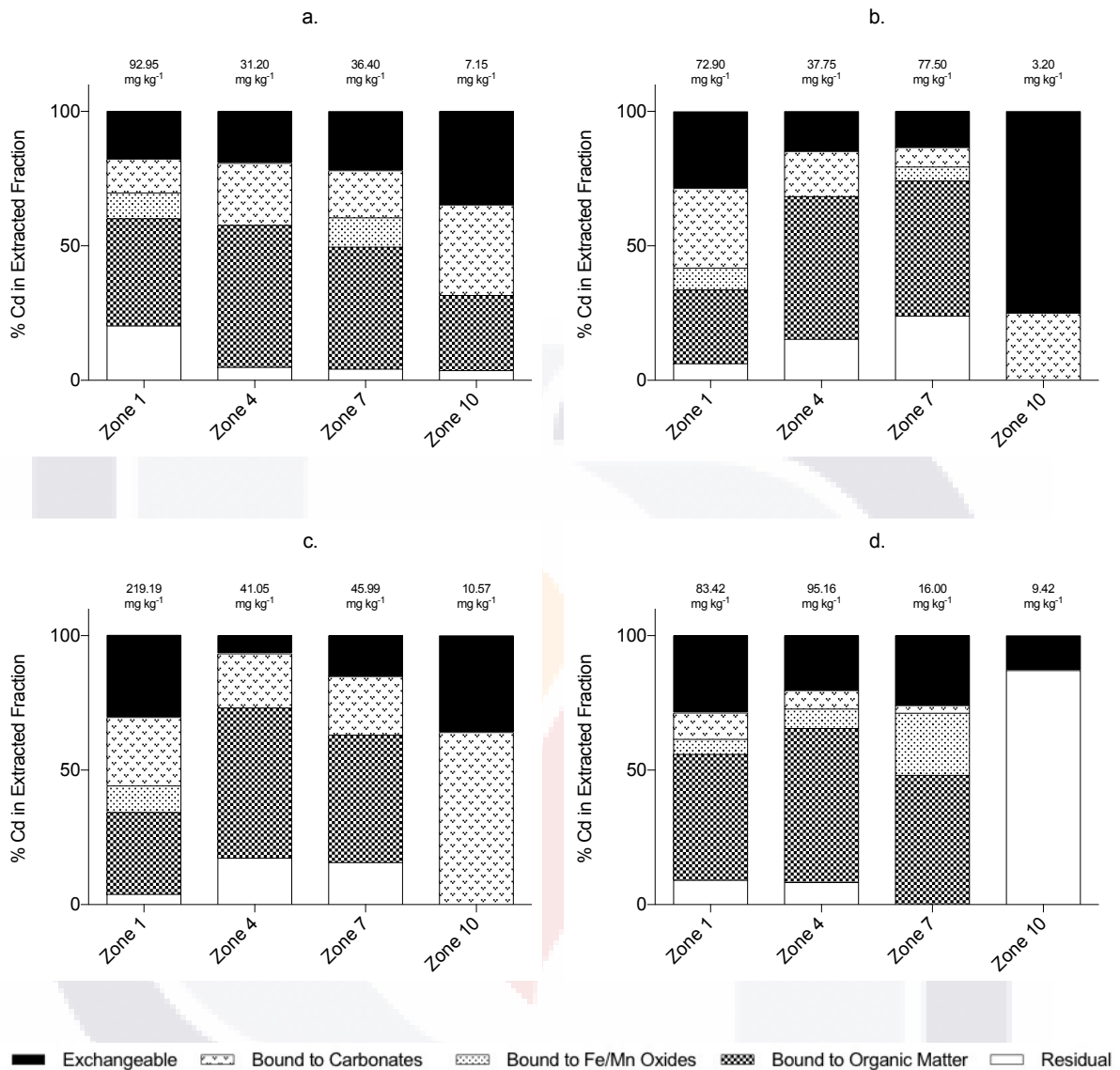


Figure 24. Fractionation of cadmium in sediments from 4 sample zones (a) period 1 rainy, (b) period 2 dry, (c) period 3 rainy, (4) period 4 dry at 4 sample sites; $n=2$

These results highlighted the importance of physicochemical characterisation and its integration with data obtained from single and sequential extraction protocols in the evaluation of the current and potential environmental risk associated with metal contamination in these soil and sediment samples. Continuous seasonal monitoring of the affected area, including metal content in the liquid phase of soils and sediments, will provide valuable information on the evolution of this environmental risk.

8.4 Biochar preparation and characterisation

Biochar was prepared from composted biosolids; material obtained from the wastewater treatment plant of the Autonomous University of Aguascalientes. Sustained temperature and pyrolysis time was based on pre established methodologies (Agrafioti et al. 2013; Méndez et al. 2013), where it was shown that temperature and residence time key factors that affect the physicochemical properties of the final product. An increase in temperature results in a decrease in percentage yield, total organic carbon content and cation exchange capacity, though it increases pH and stable carbon content. It is therefore necessary to standardize the production of biochar taking into account its application and importantly, the physicochemical characteristics of the source material from which it will be produced.

Table 1. Initial biochar production characteristics

Biochar	Quantity (g)	Oven Volume (cm ³)	Initial Nitrogen Flow (ml/min)	Sustained Nitrogen Flow (ml/min)	Saturation Time (minutes)	Temperature Ramp (°C/min)	Pyrolysis Temperature	Pyrolysis Time (minutes)	Yield (%)
A	500	42512.43	441.82	203.40	105.00	11.40	350.00	30.00	85.20
B	500	42512.43	441.82	203.40	105.00	11.40	450.00	30.00	NA
C	300	42512.43	441.82	203.40	105.00	11.40	350.00	30.00	78.00
D	300	34088.43	441.82	441.82	105.00	11.40	350.00	30.00	80.00
E	300	25664.43	441.82	441.82	105.00	11.40	350.00	30.00	88.00

Table 1 summarizes the characteristics of biochars prepared with variations of the established methods (Méndez et al. 2013; Agrafioti et al. 2013) using a muffle furnace (Thermo Fisher Scientific Lindberg/Blue BF51287C, USA). Oven volume was based on calculations obtained from the operator's manual, and adjusted using solid temperature resistant clay aggregates. Nitrogen was used to limit oxygen, essentially reducing the occurrence of combustion reactions during pyrolysis. Final nitrogen saturation time and sustained nitrogen flow was based on calculations factoring in oven volume.

Based on visual cues, it was established that Biochar A was not sufficiently charred in the pyrolysis process, due to the quantity of the material used, and the resulting diminished exposure to pyrolysis temperature. Visual cues established that Biochar C, prepared at a maximum sustained temperature of 450°C, was over-charred, resulting in carbonization of

the material. As such, a maximum sustained temperature of 350°C for the following production assays.

Table 2. Initial biochar physicochemical characterisation, $n = 3$

Material	pH	Conductivity (µS/cm)	Cation Exchange Capacity	% Organic Carbon	Classification (IBI)
Stabilised Biosolids	6.32 ± 0.02	0.73 ± 0.004	57.8 ± 1.75	17.33 ± 0.45	NA
Composted Biosolids	7.51 ± 0.11	2.96 ± 0.05	77.9 ± 0.85	19.5 ± 0.55	NA
Biochar - A	6.86 ± 0.07	3.00 ± 0.06	34.46 ± 1.29	10.88 ± 0.23	3
Biochar - B	NA	NA	NA	4.6 ± 0.14	NA
Biochar - C	6.84 ± 0.03	3.04 ± 0.06	30.79 ± 1.81	9.8 ± 0.07	NA
Biochar - D	7.09 ± 0.06	2.96 ± 0.07	30.68 ± 0.96	7.51 ± 0.68	NA
Biochar - E	7.06 ± 0.04	3.55 ± 0.05	41.1 ± 1.57	18.6 ± 0.26	3

Final product yield increased with a reduction in oven volume. Though temperature is the primary aggressor on biochar yield, at low temperatures, the presence of oxygen and ensuing combustion reactions result in carbonization of material and reduction in final product yield (Zailani et al. 2013). Reducing oven volume effectively increased the impact of nitrogen flow in maintaining a predominantly anoxic atmosphere. Biochar E, produced at a maximum sustained temperature of 350°C, with oven volume 25,664.43cm³ and continuous nitrogen flow of 441.82 ml min⁻¹, had a yield of 88%.

Physicochemical properties, as recommended by the International Biochar Initiative (IBI 2013), were measured, summarized and compared to un-pyrolysed biosolids in Table 2. Biochar metal immobilizing mechanisms have been attributed to the increase of pH and the subsequent tendency to form precipitates, as amendment with materials of a neutral or alkaline pH is preferable (Houben et al. 2013a; Gomez-Eyles et al. 2013). All biochars had neutral pH, with Biochar E having a pH of 7.06. Electric conductivity was low in all biochars. Conductivity, a factor related to the presence of salts, is an important consideration. High values for electric conductivity may affect metal mobility due to complexing reactions between metals and soluble salts (Mclean and Bledsoe 1992; Tofighy and Mohammadi 2011). Biochar E had the highest cation exchange capacity, 35.87 cmol⁺/kg.

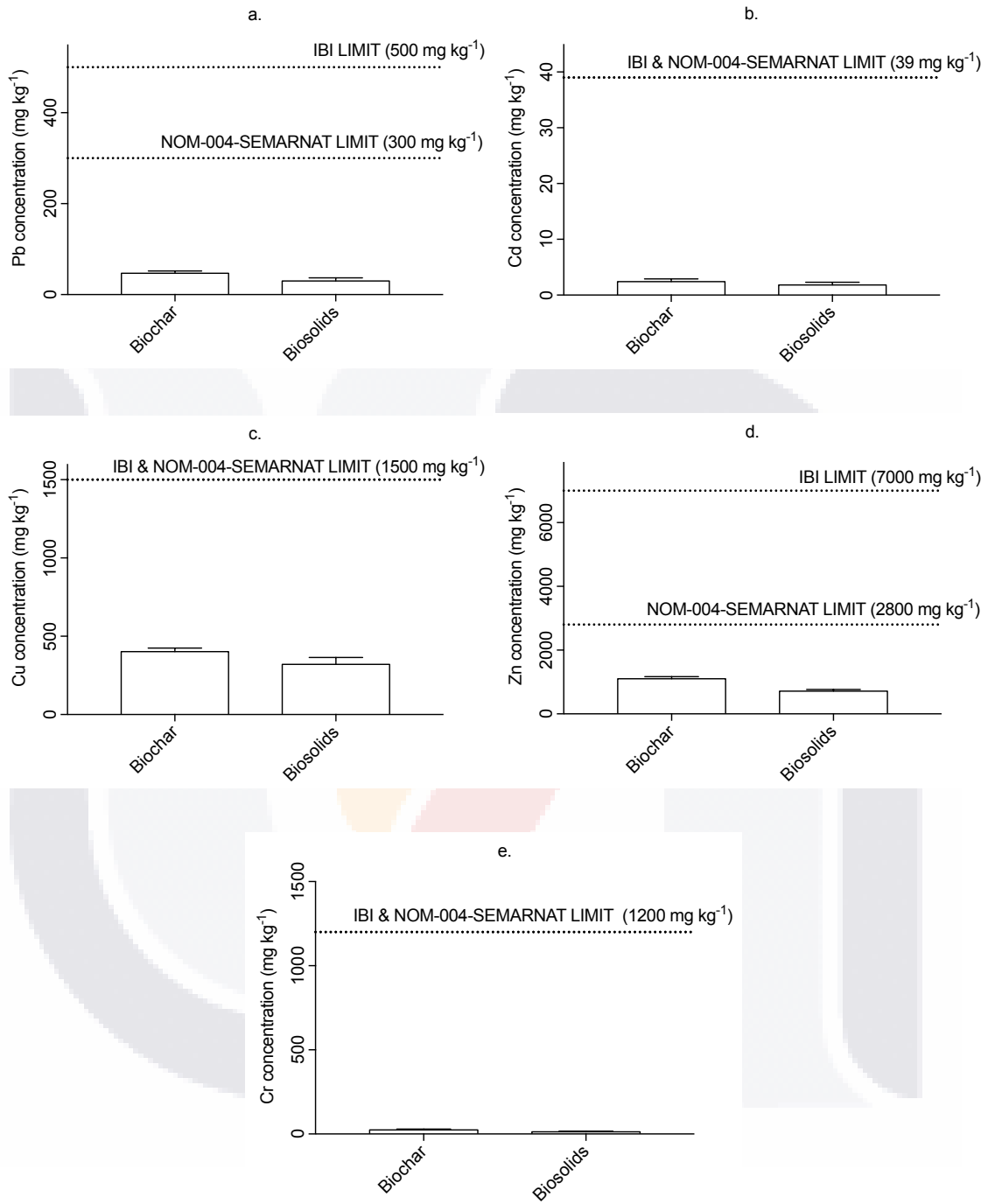


Figure 25. (a - e) Total metal concentration in amendments, and maximum allowed limits established by SEMARNAT (NOM-004) and the International Biochar Initiative (IBI) endorsed by the US Department of Agriculture (USDA); *n*=2

As the measure of the total capacity of the soil to retain exchangeable cations, high cation exchange capacity in biochar may imply an increase in the total negative charge present in

the amended soil, and as such, cations would be better retained (Jiang et al. 2012). Finally, the total organic carbon content was calculated for classification according to the standards established by the IBI (2013). Biochar E, presented the highest organic carbon content, and was classified as Type 3.

The amelioration of the physicochemical properties, can be attributed to more ideal production conditions, including, limited presence of oxygen and adequate temperature. Total metal content and leaching potential (Figure 25) was carried out on Biochar E and composted biosolids to ensure they meet established requirements. Reference values from IBI (2013) and SEMARNAT (2002) were used. Biochar E, stabilized and composted biosolids presented negligible leachability and met established requirements for total Pb, Cd, Cr, Zn and Cu content.

Having established basic production characteristics such as temperature, oven volume, nitrogen saturation and flow, resulting in the selection of Biochar E production conditions, final residence time was varied. Agrafioti et al. (2013), found that residence times more than 30 minutes did not affect the yield, suggesting that pyrolytic conversion was completed before that and thus made unnecessary to continue the process. However extended residence time may result in the loss of physicochemical properties that favour metal retention, especially % organic carbon (Agrafioti et al. 2013; Kim and Parker 2008). Therefore, ideal production conditions would be partly based on the selection of a residence time that doesn't degrade required physicochemical properties, but sufficiently modifies functional and structural properties that favour metal retention.

Figure 26 shows the effect residence time had on four selected physicochemical characteristics. As expected, total organic carbon (Figure 26a) and its related cation exchange capacity (Figure 26b) are reduced with time due to thermal decomposition. However, a plateau can be observed between 20 and 30 minutes, after which, the decomposition continues. It should be noted that the pyrolysis process occurs in various stages, the first of which is water evaporation at lower temperatures, then pyrolytic volatiles are progressively released (Neves et al. 2011), which results in a decrease in organic carbon.

The following stage acts on the plant based material, where there a temperature / time dependant alteration of organic compounds primarily due to the pyrolysis of cellulose and lignin (Mohan et al. 2006), without a net loss in the resulting complex combination of products. However, as temperatures or residence time increase, the decomposition of these products ensue (Mohan et al. 2006; Novotny et al. 2015).

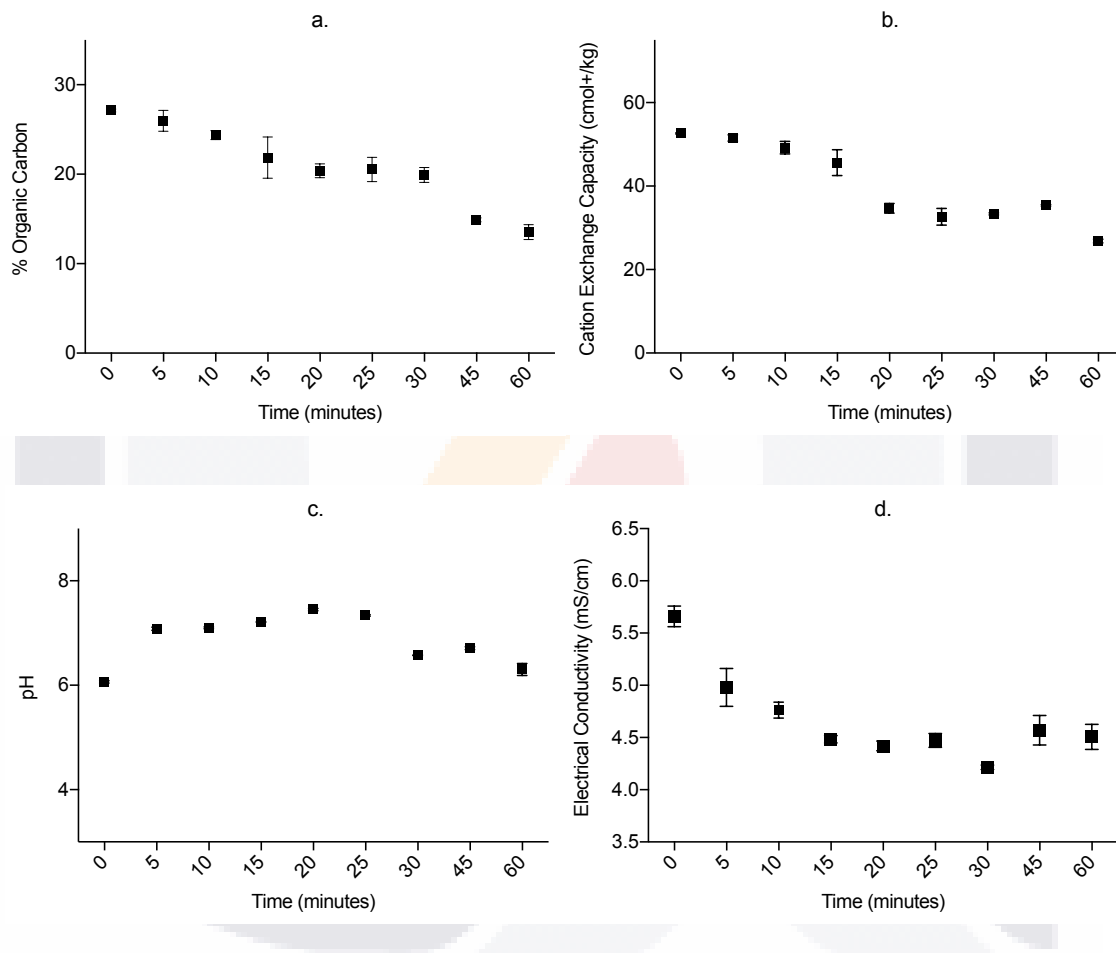


Figure 26. Physicochemical characteristics of biochar with different pyrolysis times (a) organic carbon, (b) cation exchange capacity, (c) pH, (d) electrical conductivity; $n=3$

This incomplete decomposition at low temperatures (less than 500°C) and low residence time (less than 30 minutes) results in a highly functional biochar, as part of the original structure of carbohydrates and phenols are preserved, thus maintaining a relative high content of hydroxyls and carboxyl's groups and the potential to enhance the cationic exchange capacity of the substrate to be amended (Novotny et al. 2015).

Pyrolysis of biomass also results in changes in pH (Figure 26c) and electrical conductivity (Figure 26d) of the product in solution. After 5 minutes of pyrolysis, pH of the material produced oscillated around neutral (7.0) with little variation (<10%), whereas electrical conductivity (EC) continual decreased. Some studies have been carried out with biochars with very alkaline pH > 8.0 and considerably higher EC (Puga et al. 2015; Trakal et al. 2014; Houben et al. 2013b), but these biochars were produced at temperatures in excess of 500°C and residence times in excess of 30 minutes. The relatively neutral pH and low EC of biochars produced for this experiment can be attributed to lower temperatures and relatively short residence times. The pH of biochar is related to the the intensity of the thermochemical conversion process, where at higher temperatures, or extended residence times, a deprotonation of carboxyl and other acidic groups to conjugate bases occurs, resulting in an alkaline pH (Ronsse et al. 2013). At low temperatures and short residence times, the thermal decomposition of functional groups in turn result in a decrease in EC. However studies have shown that as temperatures and residence times increase, the relative ash content increase would result in an increase in EC (Li et al. 2013; Neves et al. 2011). Based on the changes observed, biochars produced at 20 minutes were selected for use, due to its slight increase in pH, and minimum decrease in physicochemical characteristics that would favour metal retention upon application to contaminated samples.

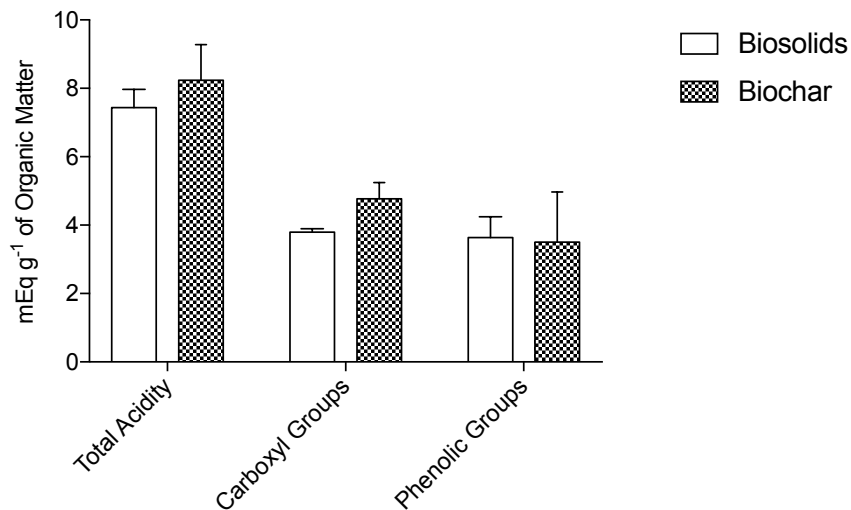


Figure 27. Organic matter speciation of biosolids and biochar prepared from biosolids; *n*=3

Pyrolysis increased total acidity in biochar when compared to the source material, biosolids. This is due to the loss of functional groups by means of progressive depolymerisation and dehydrogenation reactions resulting in more condensed structures (Mukherjee et al. 2014) such as carboxyls and other oxygen containing surface functional groups (Figure 27). These condensed structures play an important role in increasing the adsorption capacity and the recalcitrant character of biochar when compared to its unaltered source material. One such process that results in the increase in recalcitrance, is the dehydrogenation of CH_3 (Jindo et al. 2014) which occurs at low temperatures ($<500^\circ\text{C}$). At higher temperatures, though an even more recalcitrant material is produced, decarboxylation may occur which may in turn affect total cation adsorption capacity.

As observed in these results with biochar produced at 350°C and in experiments with biochar produced at temperatures up to 650°C , there are no significant changes or trends in phenolic groups, and changes in total acidity, has been primarily attributed to carboxylic groups (Mukherjee et al. 2011).

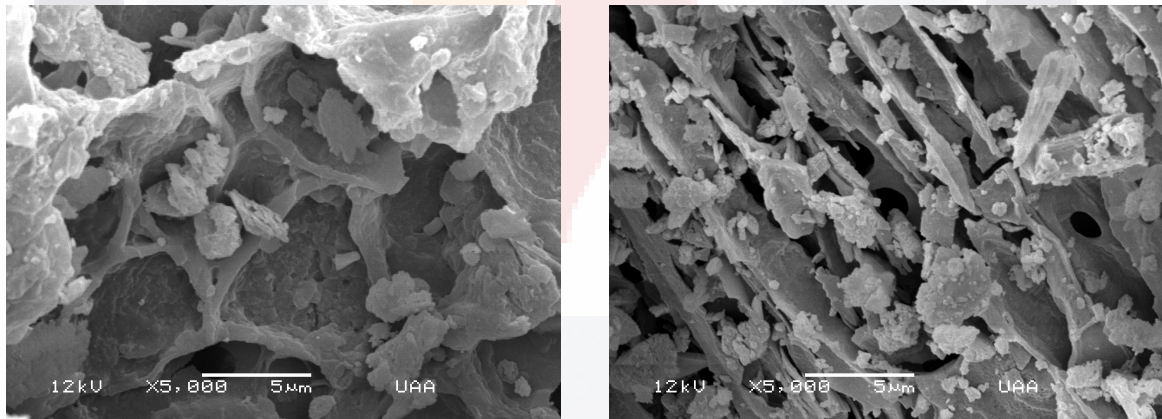


Figure 28. Scanning Electron Microscope (SEM) image of the surface of biosolids (left) and biochar (right) at x5000 magnification showing structural differences including the formation of meso and micropores on biochar samples.

Biosolids and biochar samples were analysed using SEM (Figure 28 and 29), and though relative abundance tests were inconclusive, significant structural differences between amendments were observed. The pyrolysis process enlarges the natural crystallites found in biomass resulting in the formation of voids (Beesley and Marmiroli 2011; Downie et al.

2009), (macro- (>50nm), meso-(>2<50nm) and micro-pores (<2nm), as seen in the micrographs.

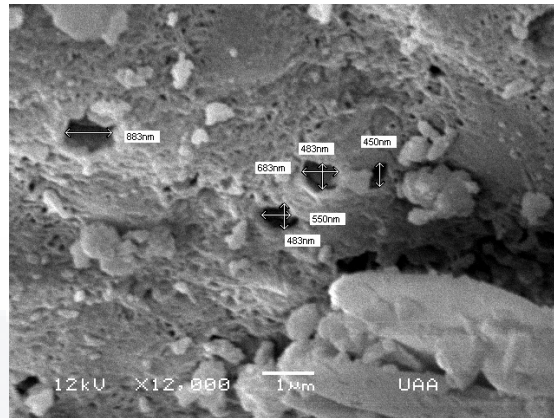


Figure 29. Scanning Electron Microscope (SEM) image of the surface of a biochar sample at x12000 magnification showing the diameters of macropores

This increases the surface area of the amendment and the overall porosity of the substrate to be amended (Fellet et al. 2011), thus playing an important role in many liquid-solid adsorption processes (Downie et al. 2009) due to an increase in adsorption sites.

8.5 Biomass application to sediments contaminated by mining activity

Sediments used for biomass application were notable for the predominance of physicochemical characteristics (Table 3), including neutral pH, high organic matter content and very calcareous conditions, that have a net effect of reducing metal mobility (Mclean and Bledsoe 1992; Mitchell et al. 2016). The impact each of these characteristics have on metal mobility depend principally on pH, but a general prevalence of metals associated to the carbonate fraction can be expected in soils with neutral to alkaline pH, which was then confirmed with the fractionation of metals (Figure 16b to 24b).

It is important to remember that association with the exchangeable or carbonate fraction may reduce mobility under specific conditions of alkalinity (Nazif et al. 2015), however, reduction in pH, though slight, may cause the release of metals, and as such, metals in these associations are considered potentially mobile (Ashraf et al. 2012).

The amendment of affected substrates with carbon rich, biologically stable, humified organic matter (Puga et al. 2015; Trakal et al. 2014), would have an effect on these associations, facilitating an increased association with the more stable Fe/Mn oxide and organic matter fractions capable of weathering physicochemical changes and thus increasing long term metal retention.

Table 3. Characteristics and interpretation of sediments used in biosolids / biochar amendment experiment.

Parameter	Units	Value	Interpretation
pH	<i>N/A</i>	7.18	Neutral
Electrical Conductivity	<i>mS cm⁻¹</i>	1.77	Normal
Redox Potential	<i>mV</i>	-36.90	Reductive
Organic Matter	<i>%</i>	6.24	High
Sulphates	<i>mg l⁻¹</i>	2310.00	Increases Precipitation
Sulphides	<i>mg l⁻¹</i>	230.00	<i>N/A</i>
Cation Exchange Capacity	<i>Cmol+ kg⁻¹</i>	7.91	Low
Carbonates	<i>% CaCO₃ equivalents</i>	14.72	Very calcareous
Chromium	<i>mg kg⁻¹</i>	20.34	Below GVL
Copper	<i>mg kg⁻¹</i>	1094.72	Above GVL
Lead	<i>mg kg⁻¹</i>	7052.52	Above GVL
Zinc	<i>mg kg⁻¹</i>	12263.21	Above GVL
Cadmium	<i>mg kg⁻¹</i>	105.28	Above GVL

GVL = Guideline Value Limits established by CCME & SEMARNAT (CCME 1993; SEMARNAT 2004)

N/A = Not applicable

Each week, metals were leached with CaCl₂ 0.01M at pH 5.5, simulating typical conditions (Ramos-Gómez et al. 2012; Houben et al. 2013a) and pH 3.5 simulating more extreme conditions (Houben et al. 2013a). The average pH conditions during the 28 day period can be observed in Figure 30 a and b.

Sediments (S) amended with biosolids (BS) had a higher pH when compared to S amended with biochar (BC), and S + BC a lower pH when compared to the other two groups. The slight reduction in pH of S + BC, may be due to the initial fast decomposition of semi-soluble functional groups that were destabilized during pyrolysis (Jiang et al. 2016). However, leachates were generally neutral which can be attributed in part to the very calcareous conditions and high buffer capacity in sediments used (Clemente et al. 2006).

The average salinity measured as electrical conductivity (mS cm^{-1}) is shown in Figure 31 a and b. Both amendments, BS and BC significantly increased electrical conductivity. This is due to the release of weakly bound ions (Chintala et al. 2013) from amendments to the soil in the saturated conditions that prevailed during the experiment, a result that may temporarily reduce metal mobility due to precipitation of metal salts formed (Mugwar and Harbottle 2016).

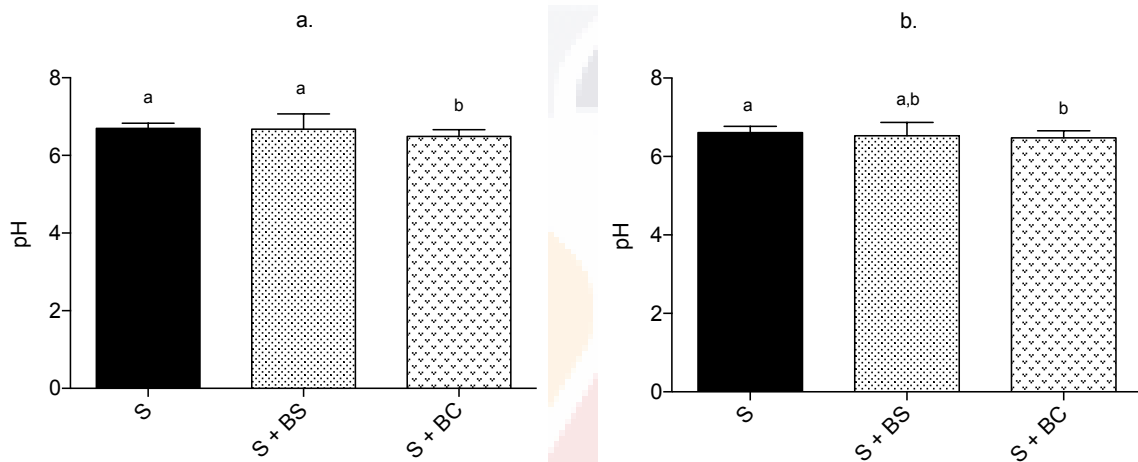


Figure 30. pH of leachate obtained after extraction with (a) 0.01 CaCl_2 pH 5.5 (b) 0.01 CaCl_2 pH 3.5 in sediment (S), sediments and biosolids (S + BS), sediments and biochar (S + BC). Different letters indicate significant differences between groups; $n = 3$, $p < 0.05$.

There was an observable reduction in accumulative Pb leached from both amended sediments with CaCl_2 at pH 5.5 (Figure 32a), though not statistically significant. The main responsible mechanisms for metal immobilisation include precipitation and association with organic carbon, though at neutral to alkaline pH, precipitation is predominant (Houben et al. 2013a; Puga et al. 2015). The observed reduction in Pb leached at 5.5, 37% in case of S + BS and 21% in case of S + BC, can be attributed to precipitation due to the presence of salts, considering the fact that these groups presented high EC values.

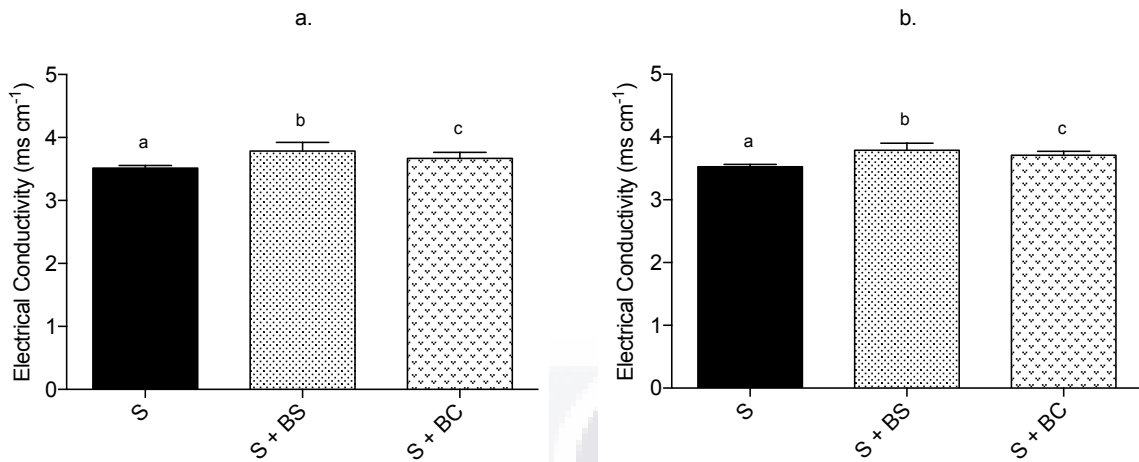


Figure 31. Electrical conductivity (mS cm^{-1}) of leachate obtained after extraction with (a) 0.01 CaCl_2 pH 5.5 (b) 0.01 CaCl_2 pH 3.5 in sediment (S), sediments and biosolids (S + BS), sediments and biochar (S + BC). Different letters indicate significant differences between groups; $n = 3$, $p < 0.05$.

However, this reduction was more pronounced in the case of Pb leached at pH 3.5 (Figure 32b), where S + BC showed the greatest reduction at 49% compared to 20% in the case of S + BS. Studies have shown that exposure to more acidic environments, result in a predominance of metal-organic matter sorption (Puga et al. 2015; Karami et al. 2011) as opposed to precipitation in form of oxides, hydroxides, carbonates and phosphates (Woldetsadik et al. 2016).

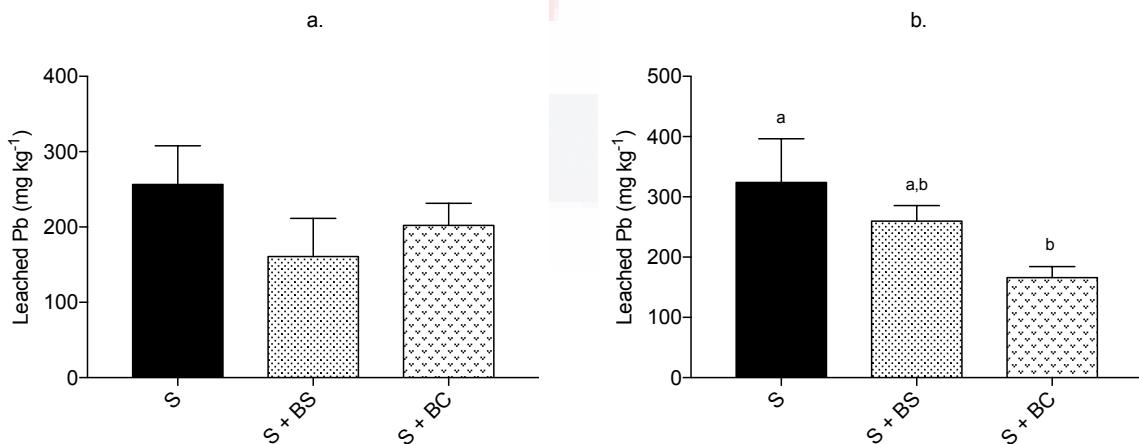


Figure 32. Accumulative (28 day period) leached Pb after extraction with 0.01M CaCl_2 at (a) pH 5.5 and (b) pH 3.5 in sediment (S), sediments and biosolids (S + BS), sediments and biochar (S + BC). Different letters indicate significant differences between groups; $n = 3$, $p < 0.05$.

There is also an increased association of some cations such as lead to the lesser mobile Fe/Mn substrate fraction (Buanuam et al. 2005; Dong et al. 2005). Additionally, due to the stability of functional groups on pyrolysed biomass there is a greater and more long term reduction of metal release (Gomez-Eyles et al. 2013), when compared to non pyrolysed biomass, whose lesser stable functional groups are subject to re-solubilisation when physicochemical conditions change.

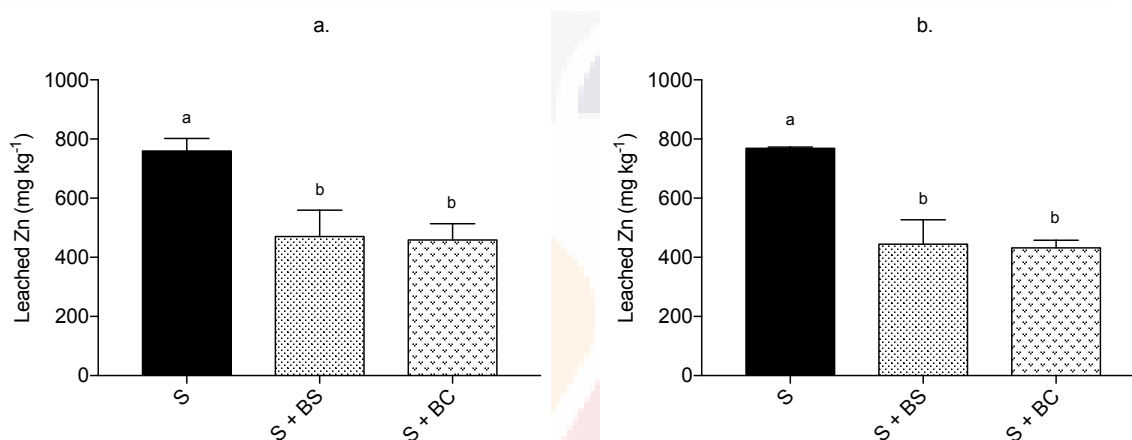


Figure 33. Accumulative (28 day period) leached Zn after extraction with 0.01M CaCl₂ at (a) pH 5.5 and (b) pH 3.5 in sediment (S), sediments and biosolids (S + BS), sediments and biochar (S + BC). *Different letters indicate significant differences between groups; n = 3, p < 0.05.*

As was the case with Pb, amendment of sediments resulted in a significant reduction of mobile Zn (Figure 33). However, there were no significant differences in reduction in Zn mobility when leached at pH 5.5 between the two amendments (38% in the case of S + BS, 39% in the case of S + BC). There was a similar tendency when leached at pH 3.5 (42% in the case of S + BS, 43% in the case of S + BC). Here as was also the case with Pb, there are observable differences between treatments leached at pH 5.5 and pH 3.5. It is reasonable to assume that the extraction at a more acidic pH, results in a predominance of metal-organic matter association instead of precipitation, and though not statistically significant, there was a great reduction in Zn mobility in S + BC, due to its more stable functional groups (Gomez-Eyles et al. 2013; Puga et al. 2015).

Amendment resulted in the greatest reduction in Cd mobility (Figure 34). S + BS had a 74% reduction in Cd mobility and S + BC a 91% reduction with statistical significance

between each group when leached with CaCl_2 0.01M at pH 5.5. The same statistically significant tendency, though more pronounced, was observed when treatments were leached at pH 3.5, S + BS with a 83% reduction and S + BC a 96% reduction in Cd mobility.

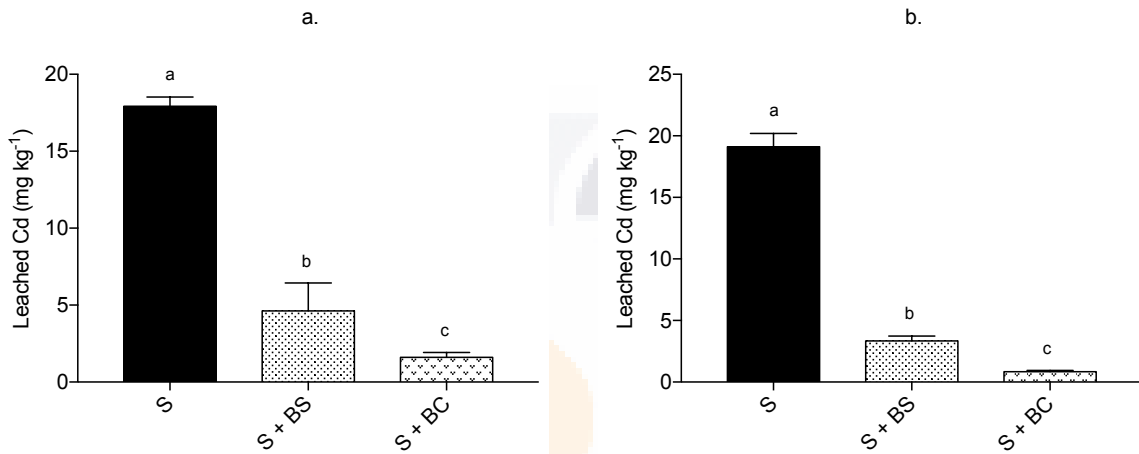


Figure 34. Accumulative (28 day period) leached Cd after extraction with 0.01M CaCl_2 at (a) pH 5.5 and (b) pH 3.5 in sediment (S), sediments and biosolids (S + BS), sediments and biochar (S + BC). *Different letters indicate significant differences between groups; n = 3, p < 0.05.*

Studies have shown that Cd mobility is reduced in alkaline pH due to precipitation in the form of oxides, hydroxides, carbonates and phosphates (Houben, 2013; Méndez et al. 2012). It's important to remember that the biochar amendment had high Mn and Fe content, up to 330 mg kg⁻¹ and 8640 mg kg⁻¹ respectively in biochar, 30% more than in biosolids. Therefore in line with results observed by other research groups (Buanuam et al. 2005; Dong et al. 2005), Cd mobility seems to be particularly affected by ferromanganese oxides in S + BC, resulting in an even greater reduction in mobility when compared to S + BS.

Metal fractionation (Figure 35) using the Tessier scheme (Tessier et al. 1979), showed a reduction of Pb and Zn associated with the potentially mobile exchangeable and carbonate bound fractions. There was an increase in metal association with the Fe/Mn fraction in S + BC, of all three metals, with a more marked increase for Cd than for Pb and Zn. However, for Pb and Zn this association was predominant, whereas organic matter fraction association was predominant in the case of Cd.

Though not the predominant association, amendment resulted in a shift of metals in potentially mobile fractions to lesser mobile fractions. Of particular interest is the 93% increase in organic matter association in S + BS and S + BC, when compared to S.

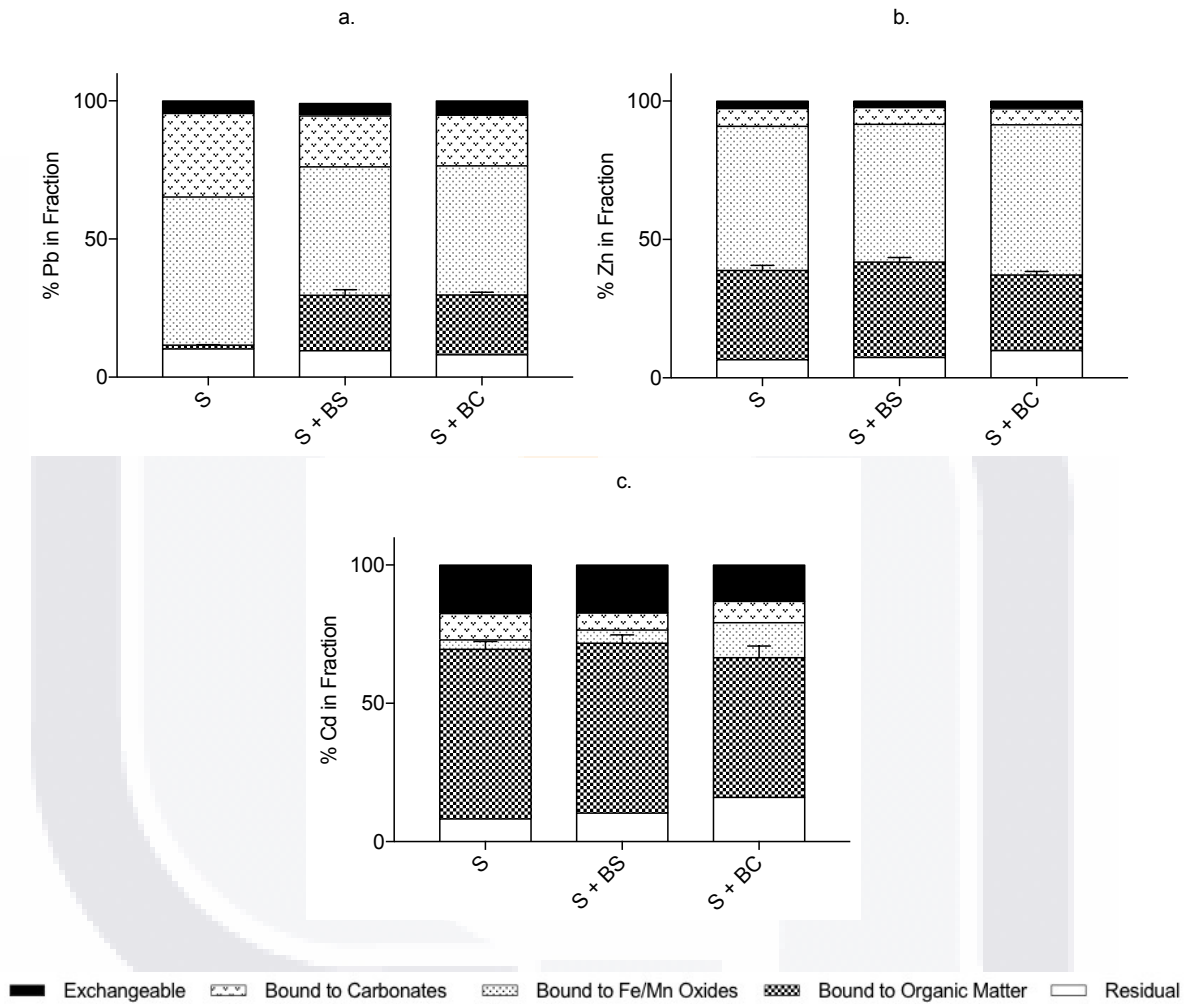


Figure 35. Fractionation of metals in treatments after 28 day experiment in sediment (S), sediments and biosolids (S + BS), sediments and biochar (S + BC); $n=3$.

These results highlight the effect biosolids and biochar amendment has on Cd and Pb in contaminated substrates. Though, different mechanisms prevail, a general reduction in mobility and association with lesser mobile fractions is observed.

The mobility factor (MF), which represents the metals that can potentially easily mobilize and enter the biota (Gasparatos et al. 2015), was calculated and graphed in Figure 36. There is an observable reduction of the MF of Zn in amendment with BS and BC, though not

statistically important. In the case of Pb however, this reduction was significant, 36% and 38% respectively and in Cd, 13% and 23% respectively when compared the MF in unamended sediments (S).

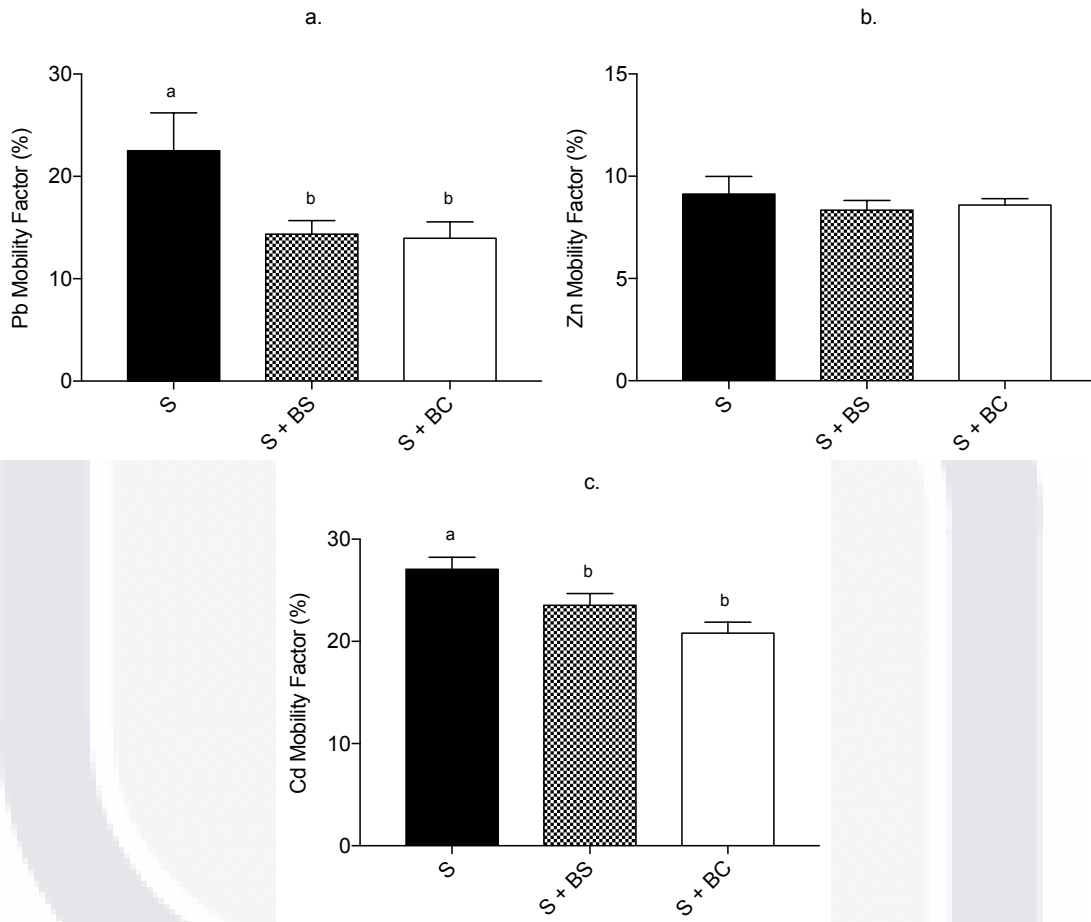


Figure 36. Mobility factor of metals in treatments after 28 day experiment in sediment (S), sediments and biosolids (S + BS), sediments and biochar (S + BC). Different letters indicate significant differences between groups; $n = 3$, $p < 0.05$.

Biochar was able to greater reduce MF in Pb and Cd, but even more so in Cd. These results compliment the CaCl_2 leaching results, showing that amendment with biochar and biosolids, reduce mobile and potentially mobile Pb, Zn and Cd due to the introduction of material that facilitate precipitation and/or association with relatively immobile ferromanganese oxide and organic matter substrate fractions. These results indicate that the use of biomass for the stabilisation of metals in contaminated substrates, even when the said substrate presents physicochemical conditions that temporarily reduce mobility is

effective. Similar results were also observed by other research groups. Though using distinct source materials for biochar production (Clemente et al. 2006; Nazif et al. 2015), the reduction of potentially mobile forms of Zn and Pb was very clear when substrates are amended with carbon rich, biologically stable, humified pyrolysed and non pyrolysed biomass.

8.6 Biomass application to CCA treated wood ash contaminated soils

Average pH and dissolved organic carbon (DOC) were monitored during the column leaching experiment and shown in Table 4 a and b. Ash contaminated soils were alkaline in nature, and the addition of amendments (1%) had little effect on pH.

Table 4. Average characteristics of leachate collected from columns during the 96 hour period (a) pH, (b) total dissolved carbon.

a.

Leachate	pH			
	S-NCA	S-CA	S-CA + SM	S-CA + BC
Exchangeable	8.70 ± 0.52 ^a	9.17 ± 0.26 ^b	8.33 ± 0.89 ^c	8.60 ± 0.25 ^d
Bound to Carbonates	9.91 ± 1.24 ^a	10.11 ± 1.05 ^b	10.27 ± 1.07 ^c	10.24 ± 0.98 ^d
Bound to Organic Matter	10.83 ± 1.96 ^a	10.90 ± 1.78 ^b	11.11 ± 1.77 ^b	11.31 ± 1.94 ^b

b.

Leachate	Total Dissolved Carbon			
	S-NCA	S-CA	S-CA + SM	S-CA + BC
Exchangeable	757.90 ± 42.02	776.72 ± 14.32	885.26 ± 33.58	674.58 ± 19.82
Bound to Carbonates	4321.84 ± 4.24 ^a	4039.04 ± 3.92 ^b	3805.04 ± 42.32 ^b	4617.88 ± 62.20 ^c
Bound to Organic Matter	1874.87 ± 172.81 ^a	1311.75 ± 172.01 ^b	991.68 ± 39.68 ^c	783.11 ± 80.60 ^c

*Brackets indicate standard deviation. S-NCA; Soil and non contaminated ash (negative control), S-CA; Soil and contaminated ash, S-CA + SM; soil, contaminated ash and source material, S-CA + BC; soil, contaminated ash and biochar. Different letters indicate significant differences between groups; n= 2, p < 0.05

During leaching of exchangeable metals with CaCl₂ 0.01M, the pH of the unamended contaminated soils (S-CA) were the highest at 9.17, and pH of S-CA amended with the source material (SM) were the lowest at 8.33, with significant differences (p<0.05) between all groups. When metals bound to organic matter were leached with NaOH 0.5M, the pH of S-CA + SM was higher than the other two groups with contaminated soil. When metals bound to carbonates were leached with Na₂EDTA 0.05M, the pH of S-CA amended with biochar (BC) was higher than the other two groups with contaminated soil.

Due to its implications in potential metal mobility in natural environments, the pH of the leachate obtained during the CaCl₂ extraction in an important consideration. Though there

were differences in groups, and even small changes in pH can affect metal mobility (Houben et al. 2013a), limited mobility was expected in all treatments if pH were taken as a singular factor, as all treatments maintained pH well into the alkaline range. Though statistically significant differences were not found, average DOC results show that DOC was lowest in the S-CA + BC groups in leachate from the exchangeable fraction and bound to organic matter fraction. This is of particular importance in the exchangeable fraction as previously mentioned due to its implications in potential metal mobility.

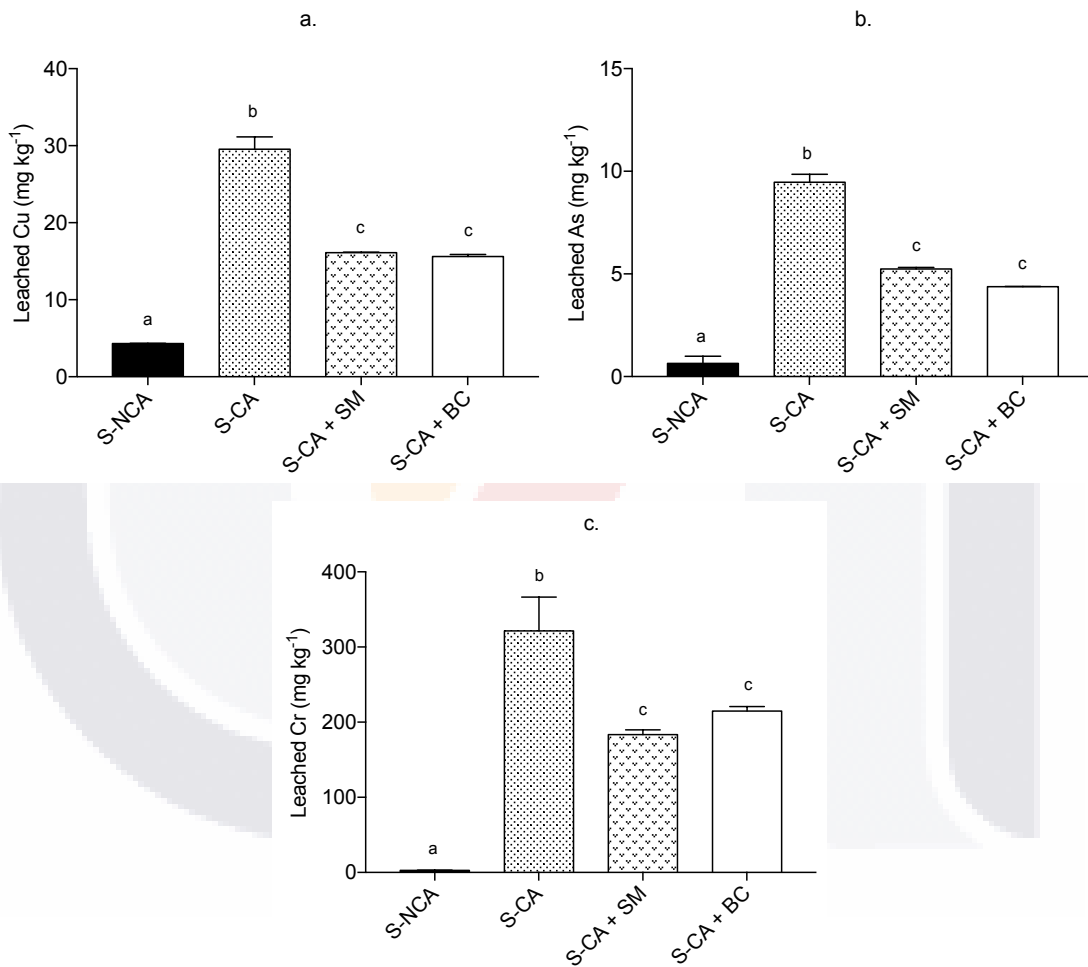


Figure 37. Exchangeable metals leached from columns over 96 hours with 0.01M CaCl₂. S-NCA; Soil and non contaminated ash, S-CA; Soil and contaminated ash, S-CA + SM; soil, contaminated ash and source material, S-CA + BC; soil, contaminated ash and biochar. Different letters indicate significant differences between groups; n=2, p < 0.05

As a singular factor, mobility of some metals may increase when DOC increases (Woldetsadik et al. 2016; Beesley et al. 2010; Schweiker et al. 2014), highlighting an

important consideration when choosing to apply non pyrolysed biomass or pyrolysed biomass. Biochar tends to have more stable forms of carbon, and is not easily affected by typical or even aggressive soil conditions, thus improving the long term stability of its association with metals (Houben et al. 2013b; Méndez et al. 2013).

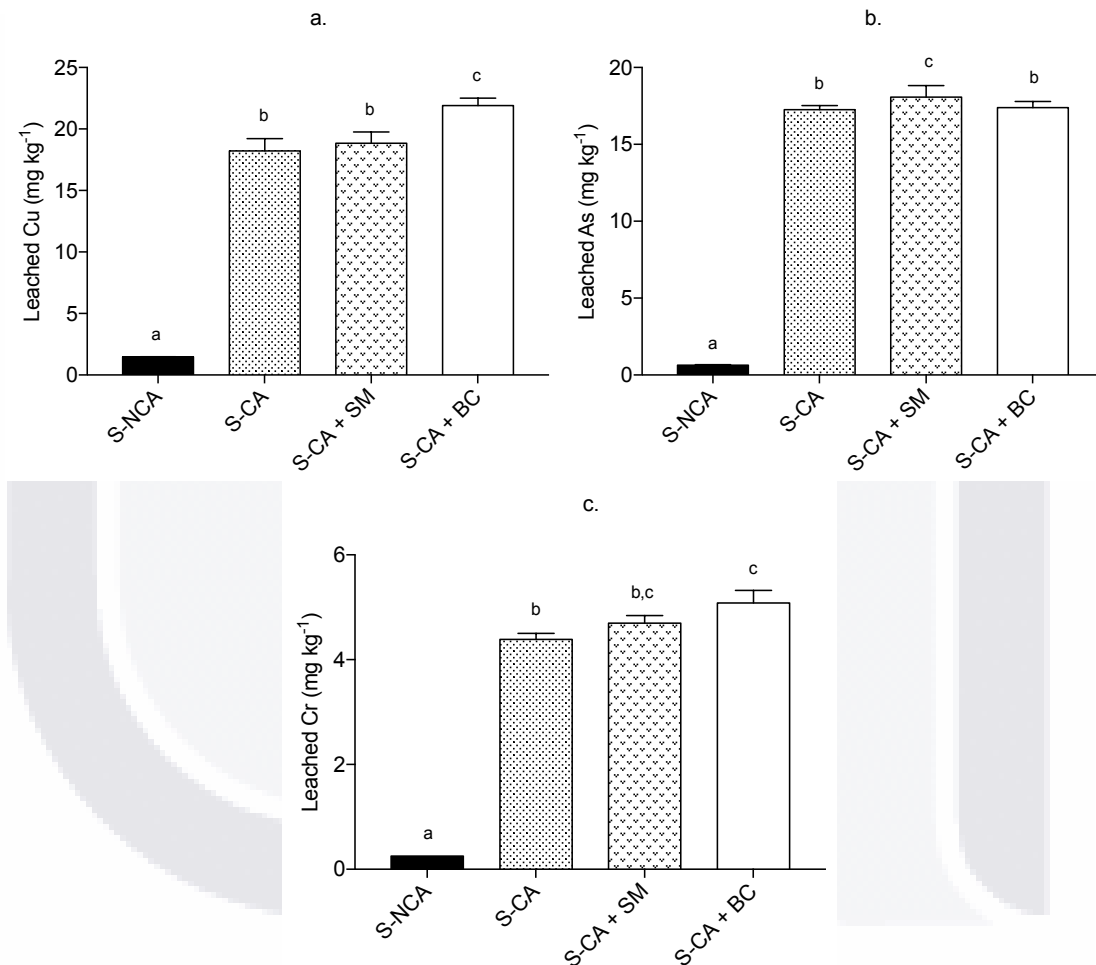


Figure 38. Metals bound to the carbonate fraction leached from columns over 96 hours with 0.05 M Na₂EDTA. *S-NCA*; Soil and non contaminated ash (negative control), *S-CA*; Soil and contaminated ash, *S-CA + SM*; soil, contaminated ash and source material, *S-CA + BC*; soil, contaminated ash and biochar. Different letters indicate significant differences between groups; $n = 2$, $p < 0.05$

Soil amendment reduced exchangeable Cu, As and Cr as observed in Figure 37. Statistically significant metal leaching reductions of around 50% were observed between untreated soils (*S-CA*) and amended soils (*S-CA + SM* and *S-CA + BC*), indicating an

association of these metals to lesser mobile soil fractions. Though not statistically significant, slight differences between S-CA + SM and S-CA + BC can be observed in the case of As and Cr, where biochar treated soils leached less Cr and soils amended with the source material leached less As. This general reduction in exchangeable metals points to phenomena that include metals being precipitated out of solution or association to more recalcitrant soil fractions (Schweiker et al. 2014), as a result of organic amendment.

Cu and Cr in particular were highly associated with the carbonate fraction in BC amended soils at a higher extent than that of SM amended soils and untreated soils (Figure 38 a & c). This is probably due to the co-precipitation effect induced by the expected increase in pH upon biochar application, highlighting an important mechanism in the reduction of exchangeable Cu and Cr as seen in Figure 37 a & c (Nyale et al. 2014). However, it is important to consider long term mobility. Though temporarily immobile, metals associated to the carbonate fraction can be categorized as potentially mobile due to susceptibility to changes in physicochemical conditions (Ashraf et al. 2012). As association with carbonates in BC amended soils was similar to the unamended group and significantly less than the S-CA + SM group, implying a dominance of other metal mobility reduction mechanisms.

A greater association of Cu and Cr with the organic matter fraction was observed in S-CA + SM when compared to S-CA + BC and the unamended group in the case of Cu and Cr. This is likely due to the presence of more oxygen containing functional groups on the unaltered biomass compared to pyrolysed biomass, resulting in a higher cation exchange capacity (Jones et al. 2015). The significantly greater association of Cu to the organic fraction in this case is expected, as the concentration of ionic copper Cu in alkaline mediums tend to be reduced due to metal hydrolysis and metal binding (Kunz and Jardim 2000).

Studies have highlight the fact that Cu has a particularly high affinity for functional groups in organic matter, including carboxylic and amino groups which act as major complexing agents (Trakal et al. 2012; Jones et al. 2015; Kunz and Jardim 2000). Though metals were associated with the organic matter fraction in the source material to a greater extent than that of biochar, it is important to consider the increase capability of the latter in reducing

metal mobility in lower pH environments (Puga et al. 2015; Jones et al. 2015; Houben, Evrard, and Sonnet 2013b), where adsorption stands out as an even more important process than precipitation of solid phases (Rieuwerts et al. 1998).

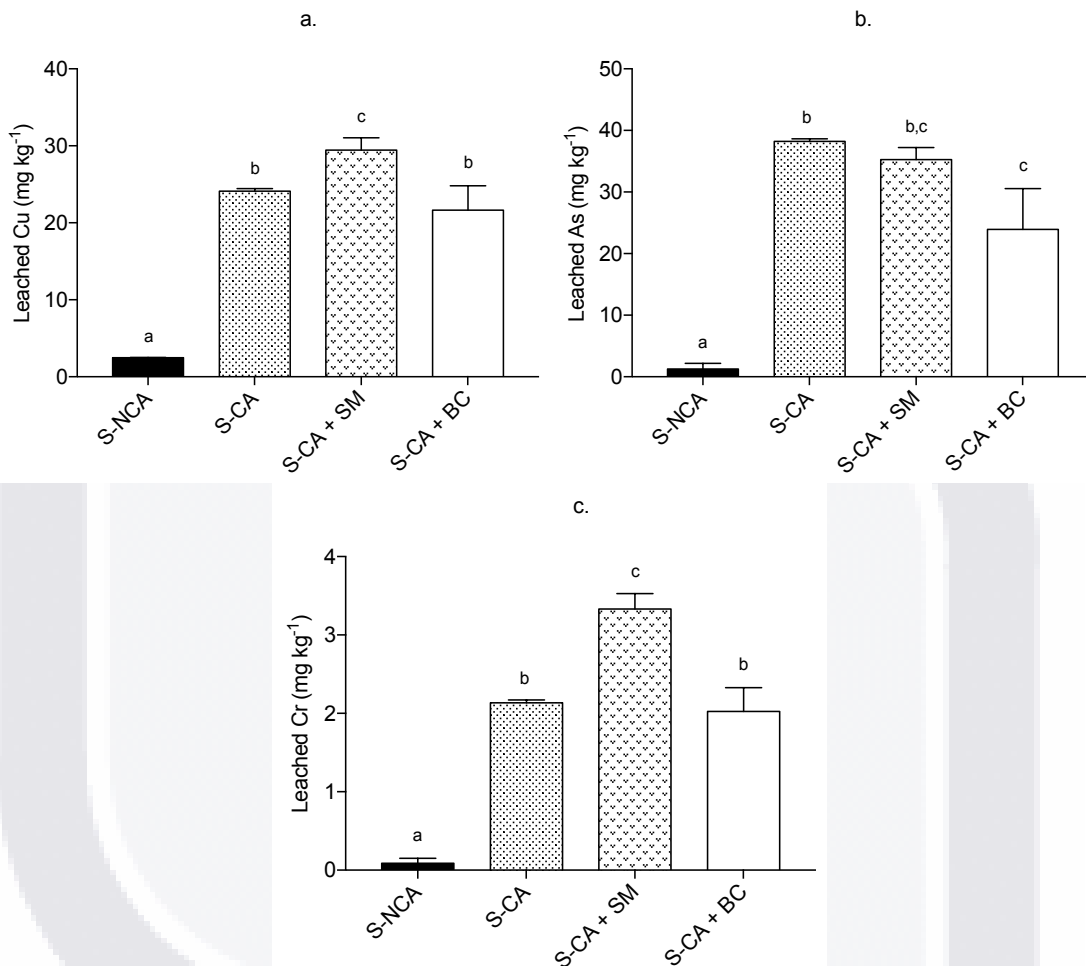


Figure 39. Metals bound to the organic matter fraction leached from columns over 96 hours with 0.5 M NaOH. *S-NCA*; Soil and non contaminated ash (negative control), *S-CA*; Soil and contaminated ash, *S-CA + SM*; soil, contaminated ash and source material, *S-CA + BC*; soil, contaminated ash and biochar. Different letters indicate significant differences between groups; $n=2$, $p < 0.05$.

Here, the increased stability of functional groups on pyrolysed biomass plays an important role in the increase of its metal retention capacity (Houben et al. 2013a). In such environments however, unaltered biomass though with increased presence of functional groups, generally suffers a reduction in stability and metal retention capacity partly due to the increased ionization of some of these functional groups, which in turn may re-mobilise some associated metals (Carrillo-González et al. 2006). As binding with the organic matter

fraction was similar Cu and Cr binding to the same fraction, pointing to similar metal mobility reduction mechanisms, including increased functional groups on source material. Additionally, As binding to the organic matter fraction was found to be significantly greater in unamended soils. Once again, this can be attributed to the alkaline conditions of soils during this experiment, which results in increased precipitation and reduced binding to specific adsorption sites on organic matter. Though As is able to form stable surface complexes over a wide range of pH values, due to similarities in chemical properties, As competes with normally occurring anions in the soils, such as sulphates and carbonates and is considered a more labile analogue of orthophosphates (Casentini 2011; Walsh et al. 1977). Studies have reported increased mobility of As in biochar amended soils citing mechanisms such as competition with and P for adsorption sites (Zheng et al. 2012; Beesley et al. 2013). In this study, soil amendment reduced exchangeable total P (from 149.10 to 105.67 mg kg⁻¹ in the case of SM and 102.54 mg kg⁻¹ in the case of BC) when compared to unamended soils, indicating a fixing of P to more recalcitrant fractions including adsorption sites on the organic matter fraction, effectively displacing As from this fraction.

Results from sequential leaching experiment was compared to results from a batch sequential extraction of groups with the same soil/amendment proportion (Figure 40). There were no statistically significant reduction of Cu and As in the exchangeable fraction of treatment groups. However there were significant differences in Cr in the exchangeable fraction of all groups, where SM amended soils reduced exchangeable Cr by 52%. An increase in carbonate association was observed in all metals in BC and SM amended soils, when compared to untreated soils. Metals in SM and BC amended soils also showed increased association with the organic matter fraction when compared to the untreated group, except in the case of As, where no significant differences were found.

These results are quite similar to results obtained from the sequential leaching experiment. Where amendment clearly results in a reorganization of metal-soil fraction association. In the case of Cu and As, SM amended soils seem to reduce potentially mobile metals at a

greater extent than BC amended soils, possibly to due to predominance of precipitation under frankly alkaline conditions (Nyale et al. 2014).

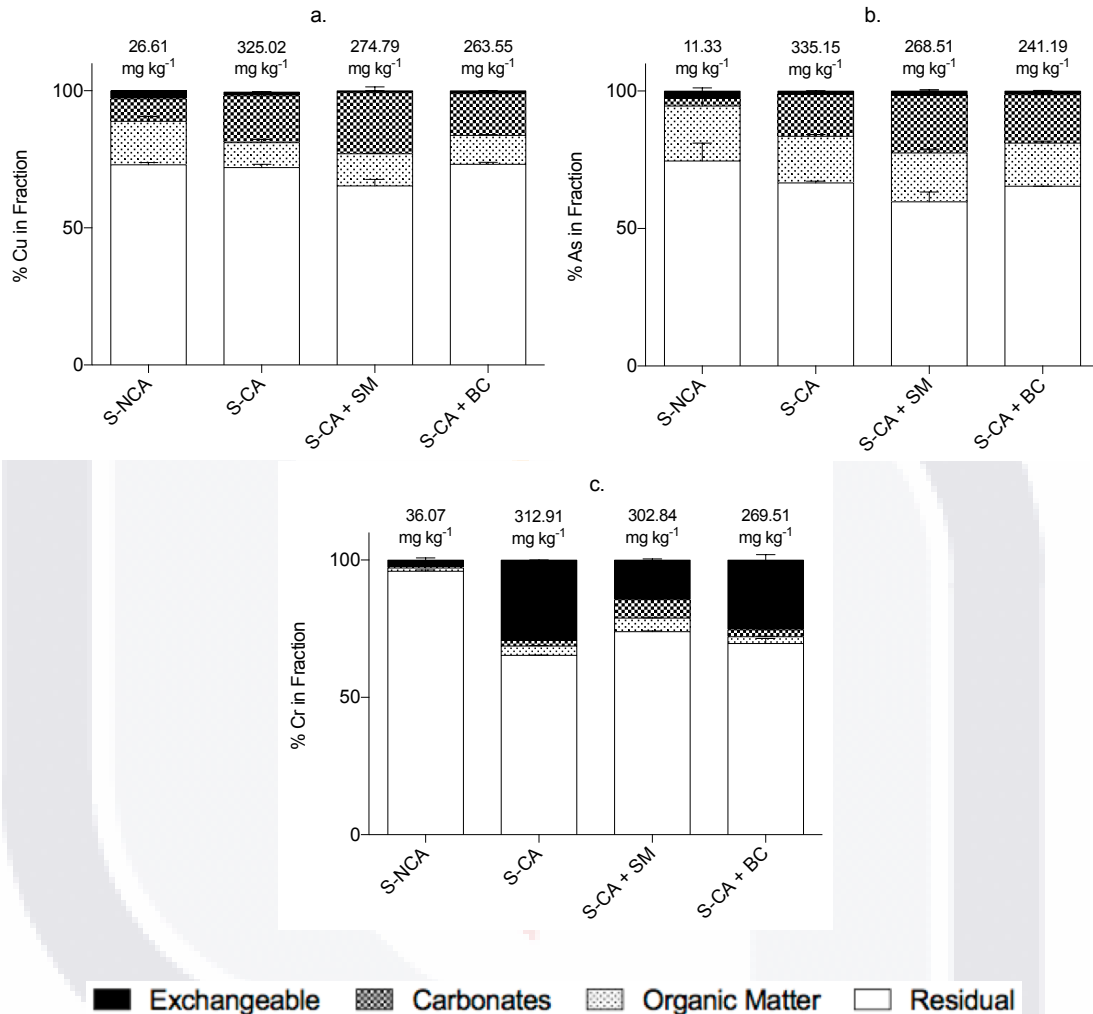


Figure 40. Batch sequential extraction of metals. *S-NCA*; Soil and non contaminated ash (negative control), *S-CA*; Soil and contaminated ash, *S-CA + SM*; soil, contaminated ash and source material, *S-CA + BC*; soil, contaminated ash and biochar; *n* = 3

Though amendments also reduced labile Cr, SM amendment had a greater effect, highlighting the influence of the chemistry of chromium in soil environments. It is well known that the mobility of Cr depends on its oxidation state (Bajaras-Aceves et al. 2007; James 2001), and studies have shown that biochar seems to have a greater effect on Cr(III) than on Cr(VI) (Zhang et al. 2013; Chen et al. 2015).

Results from ion exchange chromium speciation with the same soil/amendment proportion (Figure 41), showed significant differences of Cr(III) / Cr(VI) ratio between groups. There were statistically significant differences in the Cr(III) / Cr(VI) ratio between all groups, with the tendency of proportionally higher Cr(VI), even in the uncontaminated NCA group. However, there was noticeable a increase in Cr(III) in SM amended soils when compared to other groups, suggesting that the introduction of unaltered biomass and the resulting reduction in pH, facilitates an increased reduction of Cr(VI) to Cr(III).

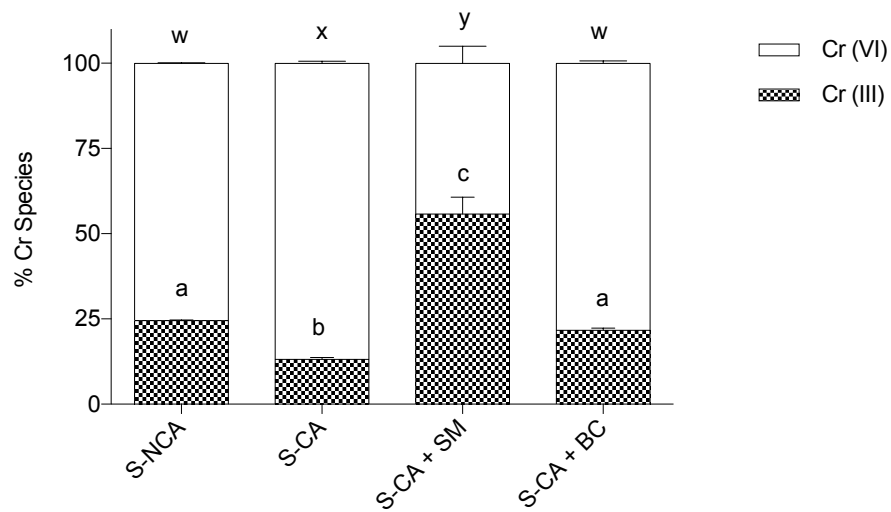


Figure 41. Speciation of Cr; Cr (III) and Cr (VI) in treatments. *Soil and non contaminated ash (negative control), S-CA; Soil and contaminated ash, S-CA + SM; soil, contaminated ash and source material, S-CA + BC; soil, contaminated ash and biochar.* Different letters indicate significant differences between groups; $n=3$, $p < 0.05$

The relationship between pH and Cr species and mobility have been reported in numerous studies, and has important implications. Cr(VI) has been reported to be very mobile especially in alkaline conditions, where mechanisms such as electrostatic repulsion between Cr(VI) particles and negatively charged soil colloids have been cited (Choppala et al. 2013; Chen et al. 2015). However, as pH decreases, not only does Cr(VI) adsorption increase, but so does Cr(VI) reduction to Cr(III), the less mobile form of Cr (James 2001; Lukaski 1999), which in turn complexes with ligands and retains strongly to soil minerals, thus remaining relatively insoluble and thus represents less toxicological risk when compared to the more mobile and toxic Cr(VI) (Choppala et al. 2013). This indeed highlights an important consideration in the selection of remediating materials in metal contaminated alkaline soils.

9 Conclusions

Soils and sediments from 10 zones in Asientos, Aguascalientes affected by mining activity were collected and studied twice during the rainy season and twice during the dry season, spanning a two-year period. High concentrations of copper, zinc, lead and cadmium were found all samples. Metal concentrations were compared to the guideline values established by the Canadian Council of Ministers of Environment (CCME) and the Mexican Secretariat of Environment and Natural Resources (SEMARNAT) where applicable. Copper concentrations surpassed CCME limits in 63% of soil samples and 95% of sediment samples. Zinc concentrations were higher than CCME limits in 90% of soil samples and all sediment samples. Lead and cadmium concentrations surpassed CCME limits in 77% and 43% of soil samples respectively. All samples contained lead and cadmium concentrations above CCME limits. Lead and cadmium concentrations surpassed SEMARNAT limits in 30% and 7% of soil samples respectively. The presence of chemical species specifically related to mining activity such as cyanide, sulphates and sulphides suggests anthropogenic enrichment.

However, once in the environment, the potential environmental risk metals pose, depends not only on their total concentration, but also on physicochemical conditions that play an important role in their solubility, mobility and toxicity. Average to high moisture content and cation exchange capacity coupled with generally neutral pH and organic matter content all contributed to the negligible water leachability of metals. Samples also presented generally high carbonate related buffer capacity during the first two sample periods. This has important implications in the long term stability of metals if this buffer capacity is maintained. However, subsequent sample periods showed a reduction in carbonate content, probably due to variations in pH and saturation conditions. A continuing reduction would implicate an eventual release of fixed metals to the liquid phase.

Sequential extractions were used to evaluate metal association with potentially mobile or recalcitrant substrate fractions. Copper and zinc were found associated primarily with less mobile ferromanganese oxide and organic matter fraction, whereas lead and cadmium were found greatly associated with the potentially mobile exchangeable and carbonate bound

fractions. As such, though currently not water leachable, changes in physicochemical conditions especially reduction in carbonate related buffer capacity or decrease in pH can affect substrate fractions and result in a release of these metals. It was concluded that the studied zone was contaminated with metals of anthropogenic origin, but the current toxicological risk is temporarily buffered due to physicochemical conditions. Close monitoring of physicochemical conditions is required as metal fractionation point to very high potential mobility.

Amendment with biosolids and biochar prepared from biosolids was suggested as means to facilitate metal association with more recalcitrant fractions thus increasing long term stability. Both amendments reduced leachable zinc, copper and lead, though in the case of lead, biosolids was better able to do at pH 5.5 whereas biochar was more effective at pH 3.5. This suggests that the available functional groups on unaltered biomass increase its effectiveness in non aggressive physicochemical conditions. In more aggressive conditions, as simulated by the pH 3.5 extraction, biochar's physical structure coupled with adsorption sites on its more stable surface functional groups is highlighted by the reductions in exchangeable zinc and cadmium 43% and 49% respectively. A similar tendency was observed with cadmium, though, biochar amendment was able to reduce exchangeable cadmium up to 96% compared to 83% in sediments amended with biosolids. Sequential extraction confirmed an increased association of lead and zinc to stable soil organic matter fractions. Cadmium was found associated to the ferromanganese oxide fraction in amended soils. These results confirm that the addition of organic matter, reduced metal leachability even in substrates that are temporarily buffered by the presence of carbonates. The increased stability of functional groups on pyrolysed biomass, result in increased metal retention in aggressive physicochemical conditions.

The effect biochar and the source material from which the biochar was prepared has on metal contaminated soils were also studied in a similar experiment performed on soils from the Scottish Lowlands. These soils though with naturally high organic matter content, were contaminated with Chromated Copper Arsenate wood ash, which increased its pH drastically. Here biochar reduced exchangeable copper, chromium and arsenic pointing to

association with other soil fractions confirmed by sequential leaching and batch sequential extractions. A difference in the prevailing mobility reduction mechanism was observed between the two amendments. Soils amended with the source material had greater associations of copper with the more stable organic matter fraction, whereas the same soils amended with biochar showed greater copper association with the temporarily immobile but considerably less stable carbonate fraction. A similar tendency was observed in the case of chromium, though the decrease in pH in the source material amended soils also facilitated the reduction of Cr(VI) to Cr(III). Though there was a reduction in exchangeable arsenic, the alkaline conditions and high phosphorus content found in soils prevented association with the organic matter fraction in biochar amended soils.

The effect biomass amendment has on metal mobility in contaminated substrates has been discussed at length, and the benefits of stabilizing functional groups by means of pyrolysis has been reported extensively. However, this research project also highlighted the importance of determining the prevailing mechanism involved in metal reduction as this has important implications in the long term stability and viability of amended substrates.

10 Glossary

Adsorption - the adhesion of atoms, ions, or molecules from a gas, liquid, or dissolved solid to a surface.

Amendment - any material mixed into a soil.

Amine - derivatives of ammonia, where in one or more hydrogen atoms have been replaced by a substituent such as an alkyl or aryl group.

Anthropogenic - chiefly of environmental pollution and pollutants; originating in human activity.

Bio-accumulative - the accumulation of substances or other chemicals in an organism.

Bioavailability - the degree and rate at which a substance is absorbed into a living system or is made available at the site of physiological activity.

Carboxyl - consists of a carbon double-bonded to an oxygen and also bonded to a -OH group.

Chemical speciation - the distribution of an element amongst chemical species in a system.

Environmental remediation - the removal of pollution or contaminants from environmental media such as soil, groundwater, sediment, or surface water.

Hydroxyl - a chemical functional group containing one oxygen atom connected by a covalent bonding to one hydrogen atom (-OH).

Leaching - the loss or extraction of certain materials from a carrier into a liquid (usually, but not always a solvent).

Mining - is the extraction of valuable minerals or other geological materials from the earth from an orebody, lode, vein, seam, reef or placer deposits which forms the mineralized package of economic interest to the mine.

Persistent - describing a compound that is not readily broken down and can persist for long periods, especially in the context of environmental pollution.

Phenol - an aromatic organic compound with the molecular formula C_6H_5OH .

Physicochemical - of or relating to physics and chemistry or to physical chemistry.

Pollutant - a substance or energy introduced into the environment that has undesired effects, or adversely affects the usefulness of a resource.

Precipitation - formation of a separable solid substance from a solution, either by converting the substance into an insoluble form or by changing the composition of the solvent to diminish the solubility of the substance in it.

Pyrolysis - thermochemical decomposition of organic material at elevated temperatures in the absence of oxygen.

Recalcitrant - substance that is not biodegradable or is only biodegradable with difficulty.

Smelting - the process of separating a metal from its ore by heating the ore to a high temperature in a suitable furnace in the presence of a reducing agent, such as carbon, and a fluxing agent.

Systemic - relating to or affecting the body as a whole, rather than individual parts and organs.

Toxicity - the degree to which a substance can damage an organism.

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12 Appendix A

Table 5. Leaching potential of metals in soils affected by mining activity

Sample Period	Sample Zone	% Metal Leached				
		Pb	Cd	Zn	Cu	Cr
1	1	0.0%	2.4%	0.3%	0.0%	0.0%
1	2	0.0%	1.4%	0.3%	0.0%	0.0%
1	3	0.0%	0.0%	30.1%	0.0%	0.0%
1	4	0.0%	0.0%	0.3%	0.0%	0.0%
1	5	0.0%	0.0%	4.2%	0.0%	0.0%
1	6	0.0%	0.0%	0.3%	0.0%	0.0%
1	7	0.0%	0.0%	100.7%	0.0%	0.0%
1	8	0.0%	0.0%	1.9%	0.0%	0.0%
1	9	0.0%	0.0%	0.3%	0.0%	0.0%
1	10	0.0%	0.0%	0.1%	0.0%	0.0%
2	1	0.3%	0.0%	0.2%	0.0%	0.0%
2	2	0.0%	0.0%	0.2%	0.0%	0.0%
2	3	0.0%	0.0%	0.3%	0.0%	0.0%
2	4	0.0%	0.0%	0.9%	0.0%	0.0%
2	5	0.0%	0.0%	16.0%	0.0%	0.0%
2	6	0.0%	0.0%	0.5%	0.0%	0.0%
2	7	2.4%	0.0%	14.1%	0.0%	0.0%
2	8	0.0%	0.0%	46.5%	0.0%	0.0%
2	9	0.0%	0.0%	1.3%	0.0%	0.0%
2	10	0.0%	0.0%	0.7%	0.0%	0.0%
3	1	0.0%	0.0%	2.9%	0.0%	0.0%
3	2	0.0%	0.0%	0.4%	0.0%	0.0%
3	3	0.0%	0.0%	0.0%	0.0%	0.0%
3	4	0.0%	0.0%	0.1%	0.0%	0.0%
3	5	0.0%	0.0%	0.0%	0.0%	0.0%
3	6	0.0%	0.0%	0.0%	0.0%	0.0%
3	7	0.0%	0.0%	0.5%	0.0%	0.0%
3	8	0.0%	0.0%	0.5%	0.0%	0.0%
3	9	0.0%	0.0%	0.1%	0.0%	0.0%
3	10	0.0%	0.0%	0.2%	0.0%	0.0%
4	1	0.0%	0.0%	0.7%	0.0%	0.0%

4	2	0.0%	0.0%	0.7%	0.0%	4.9%
4	3	0.0%	0.0%	0.1%	0.0%	0.0%
4	4	0.0%	0.0%	0.3%	0.0%	13.9%
4	5	0.0%	0.0%	2.3%	0.0%	0.0%
4	6	0.0%	0.0%	0.3%	0.0%	0.0%
4	7	0.0%	0.0%	1.0%	0.0%	0.0%
4	8	0.0%	0.0%	1.0%	0.0%	43.5%
4	9	0.0%	0.0%	4.6%	1.0%	10.8%
4	10	0.0%	0.0%	3.3%	0.0%	8.7%

Table 6. Leaching potential of metals in sediments affected by mining activity

Sample Period	Sample Zone	% Metal Leached				
		Pb	Cd	Zn	Cu	Cr
1	1	0.0%	0.5%	0.4%	0.0%	0.0%
1	2	0.0%	0.0%	0.3%	0.0%	0.0%
1	3	0.0%	0.0%	0.1%	0.0%	0.0%
1	4	0.0%	0.0%	0.0%	0.0%	0.0%
1	5	0.0%	0.0%	1.6%	0.0%	0.0%
1	6	0.0%	0.0%	0.1%	0.0%	0.0%
1	7	0.2%	0.0%	0.4%	0.0%	0.0%
1	8	0.0%	0.0%	0.7%	0.0%	0.0%
1	9	0.0%	0.0%	0.8%	0.0%	0.0%
1	10	0.0%	0.0%	0.3%	0.0%	0.0%
2	1	3.2%	100.4%	2.0%	2.6%	0.0%
2	2	0.0%	0.0%	0.1%	0.0%	0.0%
2	3	0.0%	0.0%	0.2%	0.0%	0.0%
2	4	0.0%	0.0%	0.1%	0.0%	0.0%
2	5	0.0%	0.0%	0.1%	0.0%	0.0%
2	6	0.0%	0.0%	0.2%	0.0%	0.0%
2	7	0.0%	0.0%	0.1%	0.0%	0.0%
2	8	0.0%	0.0%	0.1%	0.0%	0.0%
2	9	0.0%	0.0%	0.1%	0.0%	0.0%
2	10	0.0%	0.0%	1.6%	0.0%	0.0%
3	1	0.0%	0.0%	0.3%	0.0%	0.0%
3	2	0.0%	0.0%	0.1%	0.0%	0.0%

3	3	0.0%	0.0%	0.0%	0.0%	0.0%
3	4	0.0%	0.0%	0.1%	0.0%	0.0%
3	5	0.0%	0.0%	0.1%	0.0%	0.0%
3	6	0.0%	0.0%	0.1%	0.0%	0.0%
3	7	0.0%	0.0%	0.1%	0.0%	0.0%
3	8	11.0%	100.0%	5.9%	4.4%	0.0%
3	9	0.0%	0.0%	0.2%	0.0%	0.0%
3	10	0.0%	0.0%	0.1%	0.0%	0.0%
4	1	0.0%	0.0%	0.3%	0.0%	6.5%
4	2	0.0%	0.0%	0.2%	0.0%	6.4%
4	3	0.0%	0.0%	0.3%	0.0%	5.0%
4	4	0.0%	0.0%	0.2%	0.0%	4.4%
4	5	0.0%	0.0%	0.2%	0.0%	5.5%
4	6	0.0%	0.0%	0.2%	0.0%	17.2%
4	7	0.0%	0.0%	0.7%	0.0%	9.8%
4	8	0.0%	0.0%	0.2%	0.0%	8.4%
4	9	0.0%	0.0%	0.8%	0.0%	10.7%
4	10	0.0%	0.0%	1.6%	0.8%	29.3%

Table 7. Selected characteristics of biochar prepared from ground grape stalks

Characteristic	Unit	Value
Bulk Density	%	30.6
Ash Content	%	16.1
Moisture	%	10.25
pH	N/A	10.0
CEC	mmol kg ⁻¹	402.0
N	% weight	1.5
C	% weight	70.2
H	% weight	1.7
O	% weight	12.5

13 Appendix B

13.1 Peer reviewed publications

- Mitchell, K. N., Ramos Gómez, M. S., Guerrero Barrera, A. L., Yamamoto Flores, L., Flores de la Torre, J. A., & Avelar González, F. J. (2016). Evaluation of Environmental Risk of Metal Contaminated Soils and Sediments Near Mining Sites in Aguascalientes, Mexico. *Bulletin of Environmental Contamination and Toxicology*, 97(2), 216–224. <http://doi.org/10.1007/s00128-016-1820-9>
- Mitchell, K. N., et. al. Mobility of metals in high pH CCA wood ash contaminated soils amended with herbaceous agricultural biomass and biochar. *In Manuscript*
- Mitchell, K. N., et. al. Stabilisation of metals in metal contaminated calcareous soils and sediments amended with composted biosolids and biochar. *In Manuscript*

13.2 Conferences and symposiums

- *Mobility and toxicity of heavy metal(loid)s arising from contaminated wood ash application to a pasture grassland soil.* Life Cycle Assessment, EPDs and modified wood, Cost Action FP1407 1st Conference - 2015, Koper, Slovenia
- *Characterization of river sediments polluted by mining activities in Asientos, Aguascalientes.* 5th Congreso Internacional – La Investigación en el Posgrado. 2014. Aguascalientes, Mexico.
 - *Award for best oral presentation*
- *Physicochemical Evaluation of Soils and Sediments Near Mining Operations in Asientos, Aguascalientes.* 4th International Symposium on Environmental Biotechnology and Engineering. 2014. Mexico City, Mexico.
 - *Professor Salvador Ayanegui-Jaritz award for best poster presentation*

13.3 Research visits

- *Mobility and bioavailability of metals in metal contaminated soils amended with biochar, unprocessed organic matter and iron oxides, using pot experiments.* Under

the direction of Dr. Luke Beesley, The James Hutton Institute, Scotland; funded by the National Council of Science and Technology (Mexico)

- *Sequential column extraction of metals in metal contaminated soils amended with biochar and unprocessed organic matter.* Under the direction of Dr. Lukáš Trakal, The Czech University of Life Sciences, Czech Republic; funded by the National Council of Science and Technology (Mexico)

