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PROGRAMA DE POSGRADO EN CIENCIAS DE LA TIERRA

INSTITUTO DE GEOFISICA

IDENTIFICACIÓN DE PLANTAS ACUMULADORAS DE ELEMENTOS POTENCIALMENTE TOXICOS QUE PUEDEN SER UTILIZADAS EN LA FITOESTABILIZACIÓN EN ZONAS MINERAS DE TAXCO, GUERRERO

TESIS

QUE PARA OPTAR POR EL GRADO DE:
DOCTOR EN CIENCIAS DE LA TIERRA

PRESENTA

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JUAN MIGUEL GÓMEZ BERNAL
ESTUDIANTE DE DOCTORADO
PRESENTE.

El Comité Académico del Posgrado, reunido en sesión ordinaria el 23 de junio del año en curso, aprobó el tema de tesis titulado "Identificación de plantas acumuladoras de elementos potencialmente tóxicos que pueden ser utilizadas en la fitoestabilización en zonas mineras de Taxco, Guerrero". Así mismo ratificó a la Dra. Ofelia Morton Bermea, como asesora del mismo y designó a los siguientes investigadores como jurado para su examen de grado.

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Atentamente,
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LISTA DE ARTÍCULOS

Esta tesis esta basada principalmente en los siguientes artículos, que están referidos en el texto:

- Gómez Bernal Juan Miguel, Santana Carillo Jorge, Romero Martin Francisco, Armienta Hernández María Aurora, Morton Bermea Ofelia, Ruiz Huerta Esther Aurora. 2010. **“Inventario florístico de especies encontradas en dos sitios contaminados con desechos mineros en Taxco, Guerrero, México”**. *Boletín de la Sociedad Botánica de México*. 87: 131-133.
- Ofelia Morton-Bermea, Juan Miguel Gómez-Bernal, María Aurora Armienta, Rufino Lozano, Elizabeth Hernández-Álvarez, Francisco Romero, Javier Castro-Larragoitia. 2014**. Metal accumulation by plants species growing on a mine contaminated site in Mexico**. Environmental Earth Sciences. 71: 5207-5213.
- Gómez-Bernal Juan Miguel, Morton Bermea Ofelia, Ruiz Huerta Esther Aurora, Armienta Hernández María Aurora, González Dávila Osiel. 2014. **Microscopic evidences of heavy metals distribution and anatomic alterations in breaching-leaves of *Cupresses lindleyi* growing around mining wastes**. Microscopy Research and Technique. Aceptado en prensa. DOI 10.1002/jemt.22392

Contenido

RESUMEN

Desde el siglo XVI se ha llevado a cabo la extracción de metales como la plata y el oro en la región de Taxco de Alarcón, Guerrero y sus alrededores. Esta actividad ha liberado a través del tiempo una gran cantidad de desechos mineros que se han depositado a cielo abierto y que contienen metales pesados potencialmente tóxicos, los cuales afectan el entorno ecológico en el que se dispusieron. Por lo cual, este estudio tiene como proposito 1) Realizar un inventario florístico en una zona minera para determinar cuales especies vegetales se desarrollan en estos sitios, 2) Seleccionar las principales especies vegetales para su análisis, 3) Evaluar la eficiencia de la acumulación de metales pesados en especies seleccionadas, crecidas en suelos contaminados en el distrito minero de Taxco, Guerrero, México, 4) Identificar los cambios estructurales en las ramillas de *Cupressus lindleyi* causadas por metales pesados, 5) Determinar la microlocalización de metales pesados en los tejidos de las ramillas de *Cupressus lindleyi*. Para dicho estudio se consideraron dos localidades de influencia minera: el jal “La Concha” y el jal “El Fraile”, adicionalmente una zona control. Para esto se recolectaron ejemplares botánicos durante el 2008 en los tres sitios. El material recolectado se incorporó al Herbario Metropolitano UAMIZ. En total se colectaron plantas correspondientes a 32 especies en los tres sitios. Las plantas colectadas en el jal “La Concha” pertenecen a 25 especies de 17 familias. Las mejor representadas fueron: Asteraceae, Cupressaceae, Amaranthaceae, Commelinaceae y Fabaceae. Las colectadas en el jal “El Fraile”, corresponden a 13 especies de 8 familias. En la zona control fueron 8 especies de 7 familias. Por lo que, las familias mejor representadas de son: Fabaceae y Cupressaceae. Asi mismo se colectaron muestras de suelo y jales en los tres sitios para su análisis químico y determinación de metales potencialmente tóxicos. Se trataron junto con las muestras los siguientes materiales de referencia NIST 2586 para suelos, obteniendo 1.8% de precisión (N=6) y un estándar certificado de hojas de durazno para las plantas. Las localidades presentaron diversas concentraciones de metales con diferencias significativas en sus proporciones en fracciones geoquímicas. Los resultados muestran que las especies *Cupressus lindleyi* y *Juniperus deppeana* acumulan Zn y Mn (totales) en concentraciones anómalas en La Concha, donde el Zn se presenta en la fracción soluble (F1) del suelo. El Mn no se detectó en la fracción soluble del suelo y jal, sin embargo, se encontró acumulación de Mn en los tejidos de las plantas. Por otro lado, las mismas especies colectadas en

El Fraile y en la zona control, no muestran una acumulación de Zn y Mn. Otras especies analizadas creciendo bajo las mismas condiciones de contaminación en La Concha (*Jacaranda mimosifolia* y *Psidium guajava*) no mostraron concentraciones anómalas lo que confirma la tolerancia de estas especies a los metales pesados potencialmente tóxicos. A su vez, estos hechos confirman la capacidad de acumulación Zn y Mn de *C. lindleyi* y *Ju. deppeana*, que dependen en su habilidad de acumulación y en la concentración de estos elementos en la fracción soluble. Entre otros resultados el estudio de microlocalización de los metales pesados en las ramillas de *C. lindleyi* se efectuó mediante la microsonda electrónica de barrido acoplado a un microanalizador WDS/EDS, para entender los cambios estructurales provocados por los desechos mineros en esta especie. Los contaminantes más abundantes en los suelos, jales y órganos de la planta (raíz, tallo y ramillas) fueron Zn, Mn y Pb. Sin embargo, el As fue el más acumulado en la raíz, tallo y ramillas en La Concha. El factor de translocación y de bioconcentración fue menos de 1. Los cambios estructurales observados fueron: gran acumulación de granos de almidón y compuestos fenólicos en el parénquima en empalizada. La distribución de metales pesados en los tejidos de las ramillas fue homogénea en la mayoría de los elementos. Estos resultados muestran que *C. lindleyi* es una especie que se puede emplear en fitoestabilización de zonas contaminadas con desechos mineros debido a que es una planta nativa que no requiere muchas condiciones para su desarrollo. La metodología utilizada puede ser aplicada para la determinación de especies potencialmente aptas para su uso en fitoestabilización en zonas impactadas por residuos mineros, contribuyendo asi a una alternativa de mitigación o contención de la dispersión de los contaminantes.

1. INTRODUCCIÓN

La minería es una de las actividades económicas de mayor tradición en México, la cual ha contribuido desde la época prehispánica hasta nuestros tiempos al desarrollo del país (Contreras-Díaz et al., 2000; González et al., 2005; Hernández-Acosta et al., 2009). Pero esta industria minera ha generado por décadas una gran cantidad de desechos mineros o jales, los cuales pueden ser definidos como lodos residuales originados durante el proceso de beneficio del mineral económicamente aprovechable (González *et al*., 2005). Estos desechos son depositados en las cercanías provocando contaminación por metales pesados y metaloides, que afectan el agua, aire, suelo y plantas (Dudka y Adriano, 1997). Taxco de Alarcón, es una de las principales zonas mineras de plata desde tiempos prehispánicos, localizado en la parte norte del estado de Guerrero. Sin embargo, a partir de las dos primeras décadas del siglo XX con la introducción del proceso de flotación, se convierte en un importante productor de Zn y Pb (Romero-Martin, Com. Pers.). De esta zona minera se localizan varios jales siendo los mas estudiados los jales “La Concha” y “El Fraile” los cuales se localizan aproximadamente a 12 Km del Suroeste de la ciudad de Taxco y contienen desechos mineros originados entre 1940 y 1970 (Talavera, 2006). Para los jales “El Fraile”, Romero *et al*., (2007) reportaron concentraciones totales de elementos potencialmente tóxicos en concentraciones de 585 mg/kg As, 1479 mg/kg Pb, 460 mg/kg Zn, 72 mg/kg Cu y 9.4% de Fe.

La interacción de los desechos mineros con la vegetación causa diferentes respuestas en las plantas donde crecen (Baker, 1981; Barcelo, 2003; Juárez-Santillán *et al*., 2010). Así los metales pesados en los suelos contaminados pueden ser acumulados en las plantas (Siedlecka, 1995), y se translocan desde el suelo hacia las distintas partes de las plantas (Marschner, 1995) y se almacenan en diferentes células y tejidos (Verkleij y Schat, 1990; Ernst, 2006). Estas plantas pueden absorber metales pesados del suelo en diferentes niveles, dependiendo de las especies y el contenido de los metales en el mismo. Algunas de ellas toleran los metales usando una variedad de mecanismos. Por ejemplo, la producción de exudados en las raíces para prevenir la entrada de los metales pesados, o el desplazamiento de los metales de las raíces hacia las partes aéreas y la formación de compuestos estables en las células. Estas plantas son capaces de hacer inofensivos a los metales pesados en varias formas como: fusionarse a la pared celular, la compartimentalización celular o formando complejos metálicos con ácidos orgánicos o proteínas (Raskin, 1997). La exclusión es una característica de las especies sensibles y tolerantes, mientras la acumulación es común entre especies que aparecen en suelos contaminados o metalíferos.

Basados en la capacidad de las plantas para tolerar o acumular altas cantidades de metales pesados, los procesos de fitoremediación son posibles en suelos contaminados. La fitoremediación se refiere a usar plantas para remover, contener o hacer que los contaminantes ambientales sean inofensivos (Robinson *et al*., 2009). Sin embargo, un procedimiento de fitoextracción en suelos muy contaminados podría tomar un largo periodo de tiempo. Teniendo en cuenta esta limitación, algunos autores recomiendan la aplicación de procedimientos de fitoestabilización, que reducen la biodisponibilidad de contaminantes en el suelo, previniendo su lixiviación y absorción por las plantas (Dickinson *et al*., 2009). Con el fin de determinar el potencial de las plantas en los procesos de fitoremediación, se han establecido ciertos criterios: 1) la capacidad de plantas para tolerar o acumular metales. Tales plantas son caracterizadas por un factor de transferencia >1. Por otro lado, las plantas que excluyen la transportación de metales pesados hacia las partes aéreas con un factor de transferencia <1 (Baker, 1981; Baker y Whiting, 2002), 2) las plantas con una alta producción de biomasa; 3) un amplio factor de translocación y 4) un amplio factor de bioconcentracion (Dowdy y McKone, 1997).

Frecuentemente, las especies de plantas silvestres que están en áreas contaminadas han sido identificadas como acumuladoras. Esta capacidad se desarrolla generalmente porque estas plantas están mejor adaptadas a las condiciones locales. La acumulación y distribución de metales en los tejidos de las plantas son aspectos importantes para evaluar su papel en la remediación en sitios contaminados. Subsisten importantes incertidumbres a menudo en relación con los mecanismos de captación de metales en las plantas (Dickinson *et al*., 2009). La viabilidad de acumulación depende más de la función de los nutrientes del elemento que de la concentración del metal pesado en el suelo. El contenido de metales en el suelo no es una buena medida para evaluar su biodisponibilidad o su potencial de riesgo en la contaminación de suelos y sedimentos. Los procedimientos de extracción secuencial se han utilizado en estos casos para evaluar la fracción de la fase sólida que es la fuente potencial de metales disponibles para el medio ambiente (Moral *et al*., 2004; Menzies *et al*., 2007).

La captación de metales a través de las plantas depende, en gran parte, del papel nutricional de los metales. Esta captación de metales se realiza en la rizosfera a través de los tejidos de las raíces hacia los tallos y de estos hacia las hojas. La transportación de metales hacia las partes aéreas de las plantas requiere su entrada por el xilema de la raíz. La mayoría de ellos penetran a las células. Otros metales pueden solamente atravesar la membrana a través de transportadores de proteínas embebidas. Por otro lado, los canales iónicos permiten que iones con un tamaño especifico y una carga se muevan a través de la membrana celular bajo un gradiente de concentración (Robinson *et al*., 2009). Los transportadores son conocidos para Mn, Zn, Cu, Fe, Ni, Co y Cd (Reid y Hayes 2003). Los elementos no esenciales con un tamaño similar a los nutrientes pueden ser captados en el simplasto y ser translocados hacia los brotes (Robinson *et al*., 2009).

Asi mismo, Gupta y Sinha (2007), en un estudio relacionado con el potencial de fitoremediación de plantas creciendo en un sitio contaminado en la India, encontraron que la acumulación de los micronutrientes esenciales se incrementó en las partes superiores de las plantas ensayadas. Comúnmente, la habilidad para acumular metales de plantas con frecuencia ha sido evaluada en hojas (Unterbrunner *et al*., 2007; Gupta y Sinha, 2007). En México, la presencia de especies tolerantes a los metales que crecen directamente en jales mineros en la región semiárida de Chihuahua fue reportada por Carrillo-González y González-Chávez (2005, 2006). Ellos identificaron a las especies *Polygonum aviculare* y *Jatropha dioica* como acumuladoras de Zn. *Prosopis laevigata* y *Acacia farnesiana* también se han identificado por tolerar altas concentraciones de As en Zimapán (Armienta *et al*., 2008).

En este sentido, algunos árboles tienen la habilidad para reaccionar contra niveles tóxicos de metales en suelos por exudación de ácidos orgánicos de las raíces que previenen la captación de metales (abeto, pino y álamo) (Heim *et al*., 1999; Ahonen-Jonnarth *et al*., 2000; Qin *et al*., 2007). Es una forma más común para detoxificar el metal, sin embargo, su inmovilización en los tejidos finos de la raíz conduce a la acumulación hacia una carga máxima. El aluminio se unen principalmente a las pectinas en la pared celular y a las superficies citoplasmáticas de las membranas cargados negativamente, debido a su fuerte potencial electroquímico (Rengel y Zhang, 2003; Kochian *et al*., 2005), en la unión del Al con las pectinas se desplazan cationes tales como Ca^{2+} y Mg^{2+} de la pared celular y membranas. Dentro de las células vegetales, los metales pesados también se unen a proteínas específicas, tales como metalotioneínas (Kohler *et al*., 2004). Los complejos formados son transportados hacia las vacuolas dentro o entre las células. Muchas otras biomoléculas, tales como fitoquelatinas, ácidos orgánicos, nicotianamina o glutatión (Pilon-Smits, 2005), podrían también ser relevantes para la acumulación de metales en especies arboreas.

En general, en México existen pocos estudios que evalúen la concentración de metales pesados en los sustratos y en las plantas creciendo en los desechos mineros (Flores-tavison *et al*., 2003; Carrillo-González, 2005; Prieto-García *et al*., 2005; Carrillo-González y González-Chávez, 2006; Díaz-Villaseñor, 2006; Puga *et al*., 2006; Armienta *et al*., 2008; Franco-Hernández *et al*., 2010; Juárez-Santillan *et al*., 2010; Ruiz y Armienta, 2012; Santos-Jallath *et al*., 2012), así como la identificación de metales pesados dentro de los tejidos vegetales. Estos estudios permiten observar la presencia de metales, llegando a ser evidente la acumulación y translocación hacia diferentes órganos de las plantas, usando herramientas como microscopia óptica, Electrónica y espectrofotometría de energía dispersa de rayos X (Tung y Temple, 1996; Vollenweider y Günthardt-Goerg, 2006; Günthardt-Goerg y Vollenweider, 2007; Meyers *et al*., 2008; Vieira *et al*., 2008; Arias *et al*., 2010; Vollenweider *et al*., 2011).

El presente estudio tiene como propósito 1) realizar un inventario florístico en una zona minera para determinar que especie vegetales se desarrollan en estos sitios, 2) seleccionar las principales especies vegetales para su análisis, 3) evaluar la eficiencia de la acumulación de metales pesados en especies seleccionadas, crecidas en suelos contaminados en el distrito minero de Taxco, Guerrero, México, 4) identificar los cambios estructurales en las ramillas de *Cupressus lindleyi* causadas por metales pesados, 5) determinar la microlocalización de metales pesados en los tejidos de las ramillas de *Cupressus lindleyi*. Con base a los objetivos planteados y el estudio realizado, se generaron los artículos que sustentan este estudio.

En el primer artículo se realizó un inventario florístico para determinar la cantidad de especies vegetales presentes en los dos jales mineros y la zona control, los cuales se identificaaron taxonómicamente e incorporaron al Herbario Metropolitano UAMIZ. Como resultado se colectaron 31 especies vegetales en las tres localidades siendo las familias taxonómicas Fabaceae y Cupressaceae las más abundantes. Se observó que las especies que se encontraron en las tres localidades fueron. *Cupressus lindleyi*, *Juniperus deppeana*, *Jacaranda mimoseafolia* y *Psidium guajava*.

En el segundo artículo se realizaron análisis químicos a *Cupressus lindleyi*, *Juniperus deppeana*, *Jacaranda mimoseafolia* y *Psidium guajava* para determinar su eficiencia en la acumulación de metales pesados. Para esto se cuantificaronlos metales pesados (Cu, Fe, Mn, Pb y Zn) en las hojas de las especies vegetales, así como en los suelos y jales por medio de espectrometría de fluorescencia de rayos X. También se realizaron extracciones secuenciales a los suelos y jales (Mossop y Davison, 2003). Los resultados mostraron que las especies *Cupressus lindleyi* y *Juniperus deppeana* acumulan Zn y Mn en concentraciones anómalas en “La Concha”, donde el Zn se presenta en la fracción intercambiable. El manganeso a pesar de no estar presente en su mayoría en la fracción soluble en el suelo y jales, parece haber aumentado en la fracción soluble después del crecimiento de las plantas. En contraste, las muestras de las mismas especies colectadas en “El Fraile” y en la zona control, donde el Zn y el Mn están principalmente contenidas en la fracción residual, no muestran un enriquecimiento anormal. Otras especies analizadas creciendo bajo las mismas condiciones de contaminación en “La Concha” (*Jacaranda mimosifolia* y *Psidium guajava*) no mostraron concentraciones anómalas. Estos hechos confirman la capacidad de acumulación de Zn y Mn de *C. lindleyi* y *Ju. deppeana*, que dependen de su habilidad de acumulación y de la concentración de estos elementos en la fracción soluble.

En el tercer artículo se seleccionó a *C. lindleyi* en base a su capacidad de acumular Zn y Mn para identificar los cambios estructurales en las ramillas causadas por metales pesados y realizar su microlocalización en estos órganospara observar su acumulación en los tejidos. Se realizaron análisis a los órganos de las plantas (raíz, tallo y hojas), suelos y jales por medio de espectrometría de fluorescencia de rayos X por el método EPA 6200 para determinar el factor de translocación y de bioconcentración de Pb, As, Zn, Cu, Ni, Fe, Mn, Cd y Ag (Tu *et al*., 2003; Rizzi *et al*., 2004; Audet y Charest, 2007). A su vez se realizaron análisis por microscopía óptica (Johansen, 1940) y mapeo elemental (microlocalizacion) en las ramillas para identificar en que tejidos se acumula y puede provocar alteraciones celulares. Los resultados mostraron que los metales pesados mas acumulados en los órganos fueron Zn, Mn y Pb. Sin embargo, el As se acumuló de manera representativa en los órganos de las plantas. El factor de translocación y de bioconcentración fue menor a 1. Los cambios estructurales observados fueron: gran acumulación de granos de almidón y compuestos fenólicos en el parénquima en empalizada cambios en la pared celular hipodérmica, desorganización celular en la epidermis, hipodermis y en el parénquima en empalizada.La distribución de metales pesados en los tejidos de las ramillas fue homogénea en la mayoría de los elementos. Estos resultados muestran que *C. lindleyi* es una planta que puede desarrollarse en zonas mineras dado que no necesitan condiciones edafológicas especiales, ya que esta especie se encontró en mayor abundancia en los sitios de muestreo, e indican la factibilidad derevegetar estos lugares con *C. lindleyi* con el fin de evitar la dispersión de los contaminantes.

2. RESULTADOS

Los resultados de esta investigación están reportados en los siguientes artículos:

- Gómez Bernal Juan Miguel, Santana Carillo Jorge, Romero Martin Francisco, Armienta Hernández María Aurora, Morton Bermea Ofelia, Ruiz Huerta Esther Aurora. 2010. **"Inventario florístico de especies encontradas en dos sitios contaminados con desechos mineros en Taxco, Guerrero, México"**. *Boletín de la Sociedad Botánica de México*. 87: 131-133.
- Ofelia Morton-Bermea, Juan Miguel Gómez-Bernal, María Aurora Armienta, Rufino Lozano, Elizabeth Hernández-Álvarez, Francisco Romero, Javier Castro-Larragoitia. 2014**. Metal accumulation by plants species growing on a mine contaminated site in Mexico**. Environmental Earth Sciences. 71: 5207-5213.
- Gómez-Bernal Juan Miguel, Morton Bermea Ofelia, Ruiz Huerta Esther Aurora, Armienta Hernández María Aurora, González Dávila Osiel. 2014. **Microscopic evidences of heavy metals distribution and anatomic alterations in breaching-leaves of *Cupresses lindleyi* growing around mining wastes**. Microscopy Research and Technique. Aceptado en prensa. DOI 10.1002/jemt.22392

2.1 ARTÍCULO I

- Gómez Bernal Juan Miguel, Santana Carillo Jorge, Romero Martin Francisco, Armienta Hernández María Aurora, Morton Bermea Ofelia, Ruiz Huerta Esther Aurora. 2010. **"Inventario florístico de especies encontradas en dos sitios contaminados con desechos mineros en Taxco, Guerrero, México"**. *Boletín de la Sociedad Botánica de México*. 87: 131-133.

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NOTA BOTÁNICA

PLANTAS DE SITIOS CONTAMINADOS CON DESECHOS MINEROS EN TAXCO, GUERRERO, MÉXICO

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La minería es una de las actividades económicas de mayor tradición en México, la cual ha contribuido desde la época prehispánica hasta nuestros tiempos al desarrollo del país (Contreras-Díaz *et al*., 2000; González, *et al*., 2005; Hernández-Acosta *et al*., 2009). Esta producción minera en México, se concentra en trece entidades: Chihuahua, Jalisco, Michoacán, Zacatecas, Durango, Sonora, Coahuila, Guanajuato, San Luis Potosí, Hidalgo, Sinaloa, Colima y Guerrero (Carrillo-González, 2005). La Unidad Minera de Taxco (UMT) se ha caracterizado históricamente por su producción de plata. Sin embargo, a partir de las dos primeras décadas del siglo XX con la introducción del proceso de flotación, se convierte en un importante productor de Zn y Pb (Romero-Martin, Com. Pers.). Pero esta industria minera ha generado por décadas una gran cantidad de desechos mineros o jales, los cuales pueden ser definidos como lodos residuales originados durante el proceso de beneficio del mineral económicamente aprovechable (González *et al*., 2005). Los jales "La Concha" y "El Fraile" son localizados aproximadamente a 12 Km del Suroeste de la ciudad de Taxco y contienen desechos mineros originados entre 1940 y 1970 (Talavera, 2006).

Uno de los costos ambientales de la actividad minero metalúrgica es la contaminación del medio abiótico (agua, suelo, subsuelo, aire, sedimentos). Son escasos los estudios sobre las especies vegetales que pueden crecer en suelos contaminados por residuos de minas (Olguin *et al*., 2003; Flores-Tavison *et al*., 2003; Carrillo-González, 2005; Armienta *et al*., 2008; Hernández-Acosta *et al*., 2009). También es mínima la información sobre las concentraciones de elementos que pueden acumular las especies (Carrillo-González, 2005; Prieto-García *et al*., 2005; Armienta *et al*., 2008; Hernández-Acosta *et al*., 2009) así como sus mecanismos de adaptación (González, 2005), y si los acumulan en sus estructuras o evitan que los contaminantes penetren la raíz. Flores-Tavison *et al*. (2003) reportaron algunas especies que crecen en suelos contaminados por arsénico. Bailleres (2003) reportó *Pluchea symphytifolia* (Mill.) Gillis creciendo exitosamente en un matorral sobre residuos de minas. Díaz-Garduño *et al*. (2005) recolectó 44 especies de vegetales en cuatro jales de Zacatecas y 29 especies vegetales en tres sitios de Temascaltepec, Estado de México. Carrillo y González-Chávez (2005) reportaron a *Polygonum aviculare* L. y *Jatropha dioica* Sessé como acumuladoras de zinc. Armienta *et al*. (2008) registró *Prosopis laevigata* L. y *Acacia farnesiana* (L.) Willd. en suelos ricos con arsénico en Zimapán, Hidalgo. Hernández-Acosta *et al*., (2009) identificaron 25 especies de plantas, donde *Haplopappus venetus* (Kunth) S.F. Blake fue la más dominante en un jal de mina en Pachuca, Hidalgo. Así mismo, se han detectado varias zonas mineras contaminadas en el país que pueden causar problemas de salud hu-

Figura 1. Localización del municipio de Taxco de Alarcón, Guerrero.

Cuadro 1. Especies recolectadas en los jales "La Concha" (LC) y "El Fraile" (EF) en el municipio de Taxco de Alarcón, Guerrero. P (Planicie del jal), B (Base del jal), Hm (Humedal). Forma de vida: H (Hierba), A (Árbol), Ar (Arbusto), E (Epífita), F (Fanerófita), Hi (Hidrófita).

1. *Acacia cochliacantha* Humb. & Bonpl. ex Willd. (Leguminosae) **A LC B**
2. *Annona squamosa* L. (Annonaceae) **A EF B**
3. *Bouteloua repens* (Kunth) Scribn. & Merr. (Poaceae) **H LC P, Hm**
4. *Buddleja cordata* Kunth (Loganiaceae) **Ar EF B**
5. *Cupressus lindleyi* Klotzsch ex Endl. (Cupressaceae) **A LC P,B EF P, B**
6. *Dodonaea viscosa* Jacq. (Sapindaceae) **H LC B**
7. *Gibasis pulchella* (Kunth) Raf. (Commelinaceae) **H LC B**
8. *Gnaphalium canescens* DC. (Asteraceae) **H LC P**
9. *Gomphena decumbens* Jacq. (Amaranthaceae) **H LC P**
10. *Hypericum philonotis* Schltdl. & Cham. (Hypericaceae) **H LC B**
11. *Ipomoea nil* (L.) Roth (Convolvulaceae) **H LC B EF P**
12. *Iresine celosia* L. (Amaranthaceae) **H LC B**
13. *Jacaranda mimosifolia* D. Don (Bignoniaceae) **A LC P,B EF P**
14. *Juniperus deppeana* Steud. (Cupressaceae) **Ar LC P,B EF P,B**
15. *Juniperus flaccida* Schltdl. (Cupressaceae) **Ar LC P EF P**
16. *Leucena aff. diversifolia* (Scld.) Benth. (Leguminosae) **A LC B**
17. *Lysiloma acapulcense* (Kunth) Benth. (Leguminosae) **Ar EF B**
18. *Lysiloma acapulcensis* (Kunth) Benth. (Leguminosae) **A EF B**
19. *Mimosa aculeaticarpa* Ortega (Leguminosae) **A LC B**
20. *Opuntia* sp. (Cactaceae) **F LC P**
21. *Paspalum* sp. (Poaceae) **H LC P, Hm**
22. *Pithecellobium dulce* (Roxb) Benth. (Leguminosae) **A EF B**
23. *Pityrogramma calomelanos* (L.) Link (Pteridaceae) **H LC B**
24. *Prosopis leavigata* (Humb. & Bonpl. Ex Willd.) M.C. Johnst. (Leguminosae) **A EF B**
25. *Psidium guajava* L. (Myrtaceae) **A LC B EF B**
26. *Salvia polystachya* Cav. (Lamiaceae) **H LC B**
27. *Tagetes lunulata* Ortega (Asteraceae) **H LC B**
28. *Thypa dominguensis* Pers.(Typhaceae) **Hi LC Hm**
29. *Tillandsia achyrostachys* E. Morren ex Baker (Bromeliaceae) **E LC P**
30. *Tripogandra amplexicaulis* (Klotzsch ex C.B. Clarke) Woodson (Commelinaceae) **H LC B**
31. *Verbesina allophylla* S.F. Blake (Asteraceae) **H LC B**
32. *Xanthosoma sagittifolium* (L.) Schott (Araceae) **H EF B**

mana y ecotoxicológicos a mediano y largo plazo, como los observados en Gómez Palacio, Durango, en la presa "La Zacatecana" en Zacatecas, o en San Luis Potosí.

Algunos casos de restauración de sitios contaminados incluyen el recubrimiento de los residuos para reducir la dispersión de contaminantes. Por ejemplo en Zimapán, Hidalgo se cubrieron con suelo dos presas de jales drenados y secos, y se sembraron algunas especies de árboles (eucaliptos y casuarinas) y pastos, para reducir la dispersión de los residuos contaminantes por erosión. A su vez, también se realizaron trabajos similares en Taxco, Guerrero, donde se sembró *Prosopis leavigata* L. en los jales "El Fraile" para amortiguar la dispersión de los contaminantes (Obs. Pers.). El objetivo de este trabajo es identificar las especies vegetales que colonizan las áreas perturbadas por la minería en dos jales de Taxco, Guerrero. Los resultados de este inventario se podrán utilizar para medidas de fitoremediación.

Taxco de Alarcón se localiza al norte de la capital en el estado de Guerrero a 1,752 m s.n.m. (Figura 1). El clima de la zona es de tipo ACw2´´ (w)ig, semicálido, subhúmedo con lluvias en verano, con canícula y poca oscilación térmica (García, 2004). La vegetación que prevalece es el bosque tropical caducifolio (Martínez, *et al.*, 2004; Hinojosa-Espinosa y Cruz-Durán, 2010).

Se recolectaron ejemplares botánicos durante el 2008, utilizando el método de cuadrados. Se estudiaron dos áreas de influencia minera: el jal "La Concha" y el jal "El Fraile". El material recolectado se incorporó al Herbario Metropolitano UAMIZ.

En total se colectaron plantas correspondientes a 31 especies. Las familias mejor representadas de plantas son Fabaceae y Cupressaceae. Las plantas colectadas en el jal "La Concha" pertenecen a 23 especies de 16 familias (Cuadro 1). Las mejor representadas fueron: Asteraceae, Cupressaceae, Amaranthaceae, Commelinaceae y Fabaceae. Las colectadas en el jal "El Fraile", corresponden a 15 especies (Cuadro 1). La vegetación circundante consta de seis tipos de comunidades vegetales, de los cuales el bosque tropical caducifolio es el más abundante, seguido del bosque de coníferas, el bosque mesófilo de montaña, el bosque de galería y finalmente los pastizales con humedales semipermanentes.

Los desechos mineros favorecen la presencia de ciertas plantas de asociaciones vegetales secundarias (e.g. *Bouteloua repens* Kunth, *Gomphena decumbens* Jacq) y también especies del bosque primario, como por ejemplo las especies pioneras *Juniperus deppeana* Steud, *Juniperus flaccida* Schltdl y *Cupressus lindleyi* Klotzsch ex Endl. De modo que para lograr una restauración adecuada es conveniente conocer los procesos de sucesión vegetal. Sugerimos que en la restauración de estos sitios mineros en la zona de Taxco, se consideren las especies que encontramos en este estudio.

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2.2 ARTÍCULO II

- Ofelia Morton-Bermea, Juan Miguel Gómez-Bernal, María Aurora Armienta, Rufino Lozano, Elizabeth Hernández-Álvarez, Francisco Romero, Javier Castro-Larragoitia. 2014**. Metal accumulation by plants species growing on a mine contaminated site in Mexico**. Environmental Earth Sciences. 71: 5207-5213.

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ORIGINAL ARTICLE

Metal accumulation by plant species growing on a mine contaminated site in Mexico

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Abstract The present work aims to assess the efficiency of heavy metal accumulation of native species growing in contaminated soils in the mining district of Taxco, Mexico. Soil and tailing sampling was conducted in three study sites: La Concha, El Fraile, and a control site. The study localities present diverse metal concentrations with significant differences in their proportion in the geochemical fractions. Results show that species *Cupressus lindleyi* and *Juniperus deppeana* accumulate Zn and Mn in anomalous concentrations at La Concha, where Zn is present in soluble fractions. Manganese, despite not being present mostly in the soluble fraction in soils and tailings, seems to have been increased in the soluble fraction after the plant growth. In contrast, samples of the same species taken at El Fraile and in the control site, where Zn and Mn are mainly contained in the residual fraction, do not show an anomalous enrichment. Other analyzed species growing under the same contamination conditions in La Concha (*Jacaranda mimosifolia* and *Psidium guajava*) do not show anomalous concentrations. These facts confirm the Zn and Mn accumulation capacity of *C. lindleyi* and *Ju. deppeana*, which depends on their accumulation ability and on the concentration of these elements in the soluble fraction.

Keywords Metal accumulation · Native plants · Mine pollution · Mexico

Introduction

Mining activities have a considerable impact on the environment since they generate large amounts of waste rocks and tailings, which may become sources of heavy metals to the environment (Armienta et al. 2003; Romero et al. 2007). Highly contaminated sites can support the growth of specific plant species called metallophytes, which can potentially be used to control the fluxes of trace elements in the environment (Robinson et al. 2009). Phytoremediation refers to the use of plants to remove, contain or render harmless environmental pollutants (Robinson et al. 2009). However, a successful phytoextraction procedure in heavily contaminated soils would take a long period of time. Taking into account this limitation, some authors recommended applying phytostabilization procedures, which reduce the bioavailability of contaminants in the soil, preventing their leaching and absorption by plants (Dickinson et al. 2009). In spite of the speculation related to the effectiveness of phytoextraction of metals and metalloids of contaminated soils, the use of accumulator plants has been reported in many recent works as a cost-effective tool for phytoremediation (Conesa et al. 2006, 2009; Kidd and Monterroso 2004).

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Frequently, wild plant species occurring in contaminated areas have been identified as accumulators. This ability is generally developed by these species because these plants are often better adapted to local conditions. The accumulation and distribution of metals in the plant tissues are important aspects to evaluate their role on the remediation at contaminated sites. Considerable uncertainties often remain regarding metal uptake mechanisms in the plant (Dickinson et al. 2009). The accumulation feasibility depends more on the nutrient function of the element than on the heavy metal concentration in the soil. However, total content of metals in soil is not a good measure for assessing their bioavailability or their potential risks from soil and sediment contamination. Sequential extraction procedures have been used in these cases to evaluate the fraction of the solid phase which is the potential source of metal available to the environment (Moral et al. 2004; Menzies et al. 2007).

Metal uptake through the plant depends, in great part, on the nutritional role of the metals. Roots take metals from the rhizosphere to the plant root tissues and then via the stems to the leaves. The transportation of metals to the aerial parts of the plant requires its entry into the root xylem. Most of them must penetrate a cell. Other metals can only traverse the membrane via embedded protein transporters. On the other side, "ion channels" permit ions with a specific size and charge to move across the cell membrane down the concentration gradient (Robinson et al. 2009). Transporters are known for Mn, Zn, Cu, Fe, Ni, Co and Cd (Reid and Hayes 2003). Nonessential elements with a similar size to nutrients may be taken up into the symplast and be translocated to the shoots (Robinson et al. 2009).

Gupta and Sinha (2007), in a study related to the phytoremediation potential of plants growing in a contaminated site in India, found that the accumulation of essential micronutrients was higher in upper parts of the tested plants, whereas toxic metal accumulation was restricted in lower parts of the plants. Commonly, metal accumulation ability of plants is frequently been assessed in leaves (Unterbrunner et al. 2007; Gupta and Sinha 2007). In Mexico, the presence of metal tolerant species that grow directly on mine tailings in the semiarid region of Chihuahua was reported by Carrillo-González and González-Chavez (2006). They identified the species *Polygonum aviculare* and *Jatropha dioica* as Zn accumulators.

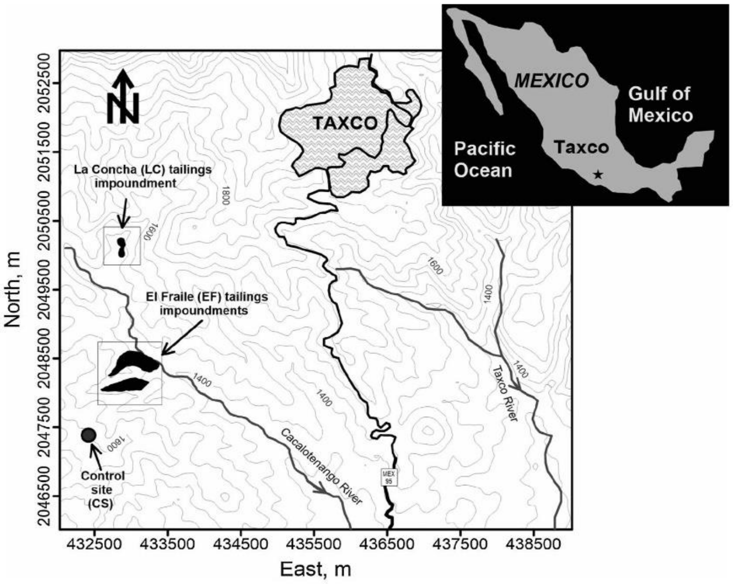
Prosopis laevigata and *Acacia farnesiana* have also been identified to tolerate high As concentrations at Zimapán, Central México (Armienta et al. 2008). Taxco, in the center of Mexico, is one of the main silver mining zones since prehispanic times. This mining district is located in the northern part of Guerrero State in southern Mexico. Large volumes of mining wastes have been produced and accumulated mainly in two important tailing dumpsites: El Fraile and La Concha tailing ponds. For the El Fraile tailings, Romero et al. (2004) reported Pb concentrations of 3,340–7,760 mg/kg. The objective of the present study was to identify the efficiency of the native wild species (*C. lindleyi*, *Ja. mimosifolia*, *Ju. deppeana*, *Ps. guajava*) to accumulate heavy metals to evaluate their possible use to reduce the environmental impact of mining wastes in Taxco, Mexico.

Experimental procedure

Sampling was conducted in three study sites in the Taxco Mining District: La Concha (LC), El Fraile (EF), and a control site (CS), located about 10 km away from tailing dumpsites (Fig. 1). At sites La Concha and El Fraile, nine soil and tailing samples were collected, as well as three soil samples at the control site. In addition, leave samples were collected from three healthy species of *C. lindleyi*, *Ja. mimosifolia*, *Ju. deppeana*, and *Ps. guajava* growing in the selected sites. As was mentioned above, metal translocation from roots throughout the plant commonly lead to achieve the highest concentration in the leaves. This fact and the possibility of comparing the data obtained in this research with results of similar work were decisive to conduct this study on plant leaves. Collected species were identified by the Departamento of Biología, Universidad Autónoma Metropolitana. Soil and tailing samples were air dried, sieved to 2 mm, homogenized and stored in plastic bags prior to laboratory analysis. Plants were washed with tap and distilled water, dried at 45 °C for 48 h. All the cleaned and dried plant samples were ground and homogenized using a plastic mill.

Total metal concentrations (Cu, Fe, Mn, Pb and Zn) in plants were analyzed using X-ray fluorescence spectrometry. About 5 g of each sample was mixed with wax-C using an agate mortar, and pressed to form a pellet to be introduced to Siemens SRS-3000 X-ray fluorescence spectrometer equipped with a Rh anode tube as X-ray source at the Instituto de Geología, UNAM. The accuracy of the procedure was determined by analyzing the certified reference material NIST 2586 (National Institute of Standards and Technology, USA). Results were in excellent agreement with certified concentrations (better than 2.6 % RSD). Precision was better than 1.8 % RSD ($N = 6$). To determine the amount of metals in different chemical fractions in soils and tailing samples, a metal fractionation scheme was carried out following the sequential extraction procedure reported by Mossop and Davison (2003). Sequential extraction procedures were applied for the fractionation of metal content in soils, sediments, tailing and other solid materials. Metals were extracted using reagents possessing diverse chemical properties (acidity,

Fig. 1 Map from the study area showing the location of the sampling sites: La Concha, El Fraile and control site



redox potential, or complexing properties). With such procedures, metals and metalloids are divided into water soluble and acid (Fraction 1), reducible (Fraction 2), oxidizable (Fraction 3) and residual (Fraction 4) fractions. In this case, the extraction procedure was applied as described briefly below: Fraction 1: 1 g of air-dried soil sampled and 40 ml acetic acid (0.11 mol l^{-1}) were shaken overnight. Mixture was centrifuged to separate the extract from the residue. Fraction 2: the residue from 1 was leached with 0.5 mol l^{-1} hydroxyl ammonium and adjusted to pH 1.5 with HNO_3. The extract separation procedure was performed as for Fraction 1. Fraction 3: to the residue from step 2, 8.8 mol^{-1} 1 H_2O_2 was added twice and the mixture taken near to dryness. Then 50 ml of ammonium acetate was added, adjusted to pH 2 with HNO_3. The separation procedure was performed as above. Residual fraction: material remaining from the extraction of Fraction 3 was digested in 20 ml aqua regia with microwave assistance. Concentrations in each fraction were measured using flame AAS (Perkin-Elmer AAnalyst 100) at the Instituto de Geofísica UNAM.

Results and discussion

Total concentrations of Cu, Fe, Mn, Pb and Zn of the analyzed soil and tailing samples as well as relative amounts of each metal obtained by sequential extraction are reported in Table 1 and Fig. 2. An overall comparison between the mean concentrations of all metals, as well metals as min–max 25–75 %, is shown in Fig. 3. It can be seen that total metal concentrations in both study localities varied widely and that concentration of all analyzed elements is higher at La Concha than at El Fraile. Zinc presents a significant proportion in Fraction 1 (water and acid soluble) in soil and tailing samples from La Concha (up to 39 %). Manganese presents low proportion of water and soluble fraction (until 11 %). Particularly noticeable is the fact that, despite the high Fe concentration reported in soil and in tailings, the majority of its concentration is found in the residual fraction (up to 99 %). Copper concentrations are lower (Table 1; Figs. 2, 3), and in both localities residual fractions reach up to 90.4 %. Lead concentration is higher in La Concha than in El Fraile. In both locations, a high proportion of Pb is contained in the residual fraction. Table 2 shows the mean metal concentration in leaves of the analyzed plants. *C. lindleyi* and *Ju. deppeana* leaf samples taken at La Concha showed much higher concentrations of Zn (454 and 469 ppm, respectively) with respect to the other analyzed species (*Ja. mimosifolia* and *Ps. guajava*). This fact could make us consider *C. lindleyi* and *Ju. deppeana* as Zn phytoaccumulator; however, leaves of this species taken at El Fraile and in the control site, where Zn is not always in the soluble fraction, do not show an enrichment of this element. Table 2 shows high concentrations of Zn in the leaves of *C. lindleyi* and in *Ju. deppeana* taken at La Concha, where soils and tailing samples present a

Table 1 Total metal concentration and chemical fractionation % of the analyzed metals in soils in tailings of the study area

Samples	La Concha					El Fraile					Control site		
	Soils			Tailing		Soils		Tailing			Soils		
	LC1	LC2	LC3	LC4	LC5	EF1	EF2	EF3	EF4	EF5	CS1	CS2	CS3
Cu (total) (mg kg^{-1})	591.4	581	419.2	722.5	551.9	104	121	117.2	142.4	397.6	60	42.5	55
F1	3.2	5.6	7.9	1.4	1.0	bdl	1.0	4.4	15.2	20.1	bdl	bdl	bdl
F2	1.6	2.3	8.1	18.0	18.9	bdl	13.7	bdl	9.0	20.7	bdl	bdl	bdl
F3	47.3	**37.8**	**60.9**	14.1	15.1	34.6	47.2	42.7	14.0	28.9	16.7	29.4	19.2
F4	**47.9**	**54.2**	23.1	**66.4**	**65.0**	**65.4**	**38.1**	**53.0**	**61.8**	**30.0**	**83.3**	**70.8**	**81.8**
Fe (total) (mg kg^{-1})	71,060	89,808	84,688	68,126	84,587	54,298	38,920	40,868	49,520	54,028	38,168	27,414	38,108
F1	0.1	bdl	bdl	bdl	bdl	0.1	bdl	bdl	bdl	0.1	0.1	bdl	bdl
F2	2.9	2.0	2.0	0.2	1.9	5.5	4.3	4.3	5.9	13.0	1.7	0.6	2.4
F3	9.1	3.3	3.5	0.7	0.6	6.9	4.2	3.9	3.2	3.7	6.5	17.3	5.8
F4	**88.0**	**94.7**	**94.5**	**99.0**	**97.5**	**87.5**	**91.5**	**91.8**	**90.9**	**83.3**	**91.7**	**82.1**	**91.8**
Mn (total) (mg kg^{-1})	6,715	5,960	7,300	16,765	17,265	411.6	5,032	6,495	461.8	7,030	642	517	773.5
F1	11.3	7.0	9.6	5.1	5.3	19.4	10.7	5.2	12.6	5.1	25.5	23.2	16.0
F2	**37.2**	**38.6**	**38.4**	**64.1**	**46.3**	9.1	22.8	**44.6**	18.4	**44.7**	17.4	19.3	23.8
F3	3.0	2.3	2.7	0.6	0.6	3.4	1.9	1.6	1.9	1.1	4.0	7.2	3.3
F4	**48.4**	**52.0**	**47.3**	**30.2**	**47.8**	**68.0**	**54.6**	**48.5**	**67.1**	**49.1**	**52.0**	**50.3**	**56.9**
Pb (total) (mg kg^{-1})	15,650	19,450	20,600	12,060	18,245	3,088	1,724	482	2,391	2,174	55	101	50
F1	14.72	16.5	23.3	4.6	3.3	bdl	bdl	bdl	bdl	2.0	bdl	bdl	bdl
F2	22.4	24.2	23.3	**38.1**	**43.8**	2.8	7.1	14.9	14.0	**57.0**	**36.4**	15.8	**40.0**
F3	**36.8**	27.0	19.4	18.2	15.8	bdl	bdl	6.3	2.3	14.7	63.6	**84.2**	**60.0**
F4	26.2	**32.4**	**34.0**	**39.0**	**37.0**	**97.1**	**92.9**	**78.8**	**83.6**	26.2	bdl	bdl	bdl
Zn (total) (mg kg^{-1})	40,250	23,625	39,050	24,650	23,650	1,015	7,293	30,975	1,036	4,445	298.1	384	312.1
F1	**39.8**	**32.2**	**33.8**	15.8	13.1	**31.5**	**30.0**	22.6	23.2	**49.5**	7.8	10.4	5.6
F2	22.4	27.0	**30.7**	20.3	**32.5**	25.6	**39.3**	**74.3**	9.26	20.7	14.1	18.2	10.3
F3	25.7	22.8	16.0	**41.6**	26.4	13.3	11.7	1.9	9.7	10.7	27.7	**39.1**	23.2
F4	12.4	18.0	20.5	22.3	27.5	29.6	19.0	1.2	**57.9**	19.1	**50.3**	21.4	**60.9**

Considerable fraction (>30 %) are in bold

bdl below detection limit

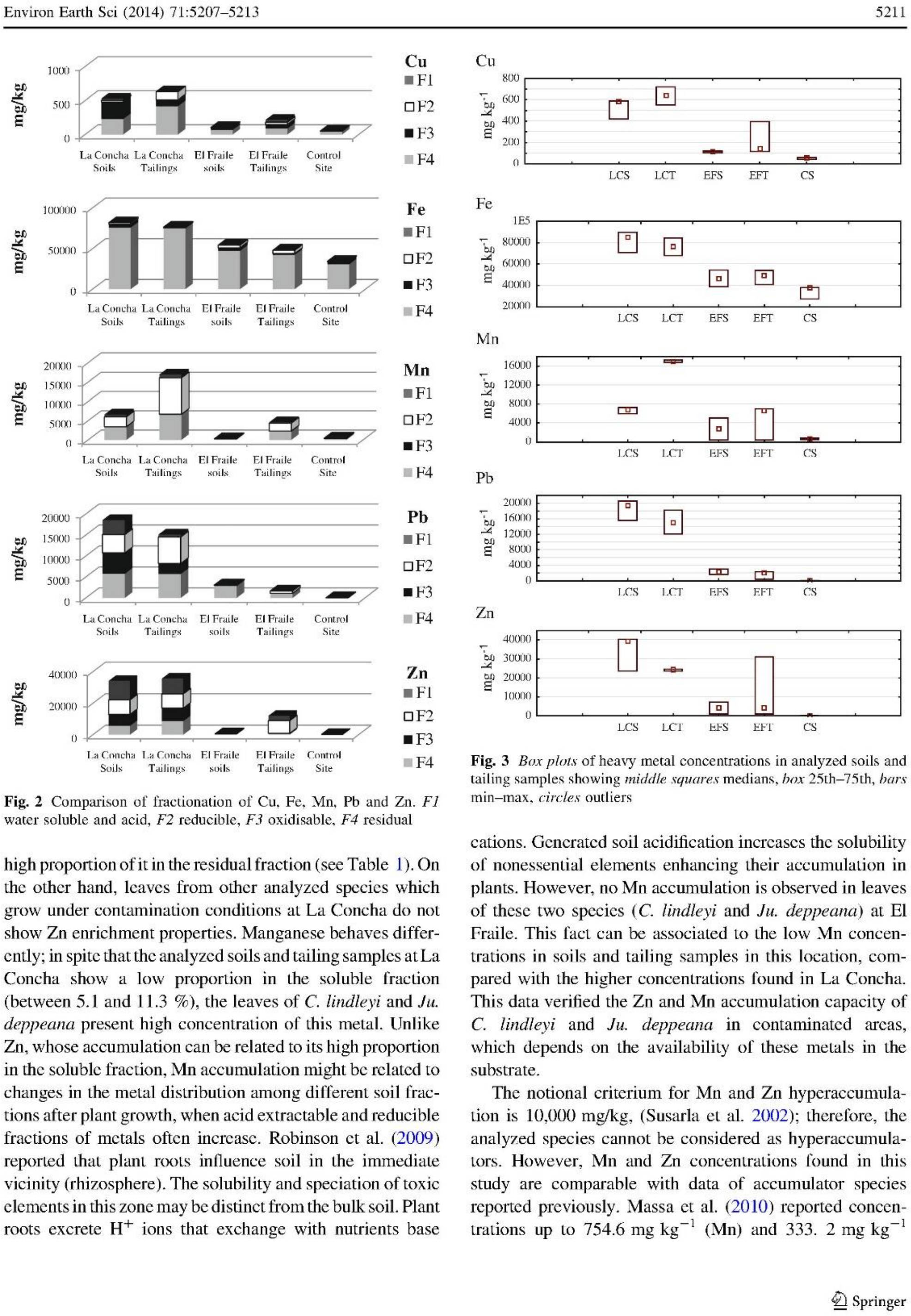


Fig. 2 Comparison of fractionation of Cu, Fe, Mn, Pb and Zn. *F1* water soluble and acid, *F2* reducible, *F3* oxidisable, *F4* residual

Fig. 3 *Box plots* of heavy metal concentrations in analyzed soils and tailing samples showing *middle squares* medians, *box* 25th–75th, *bars* min–max, *circles* outliers

high proportion of it in the residual fraction (see Table 1). On the other hand, leaves from other analyzed species which grow under contamination conditions at La Concha do not show Zn enrichment properties. Manganese behaves differently; in spite that the analyzed soils and tailing samples at La Concha show a low proportion in the soluble fraction (between 5.1 and 11.3 %), the leaves of *C. lindleyi* and *Ju. deppeana* present high concentration of this metal. Unlike Zn, whose accumulation can be related to its high proportion in the soluble fraction, Mn accumulation might be related to changes in the metal distribution among different soil fractions after plant growth, when acid extractable and reducible fractions of metals often increase. Robinson et al. (2009) reported that plant roots influence soil in the immediate vicinity (rhizosphere). The solubility and speciation of toxic elements in this zone may be distinct from the bulk soil. Plant roots excrete H^+ ions that exchange with nutrients base cations. Generated soil acidification increases the solubility of nonessential elements enhancing their accumulation in plants. However, no Mn accumulation is observed in leaves of these two species (*C. lindleyi* and *Ju. deppeana*) at El Fraile. This fact can be associated to the low Mn concentrations in soils and tailing samples in this location, compared with the higher concentrations found in La Concha. This data verified the Zn and Mn accumulation capacity of *C. lindleyi* and *Ju. deppeana* in contaminated areas, which depends on the availability of these metals in the substrate.

The notional criterium for Mn and Zn hyperaccumulation is 10,000 mg/kg, (Susarla et al. 2002); therefore, the analyzed species cannot be considered as hyperaccumulators. However, Mn and Zn concentrations found in this study are comparable with data of accumulator species reported previously. Massa et al. (2010) reported concentrations up to 754.6 mg kg^{-1} (Mn) and 333. 2 mg kg^{-1}

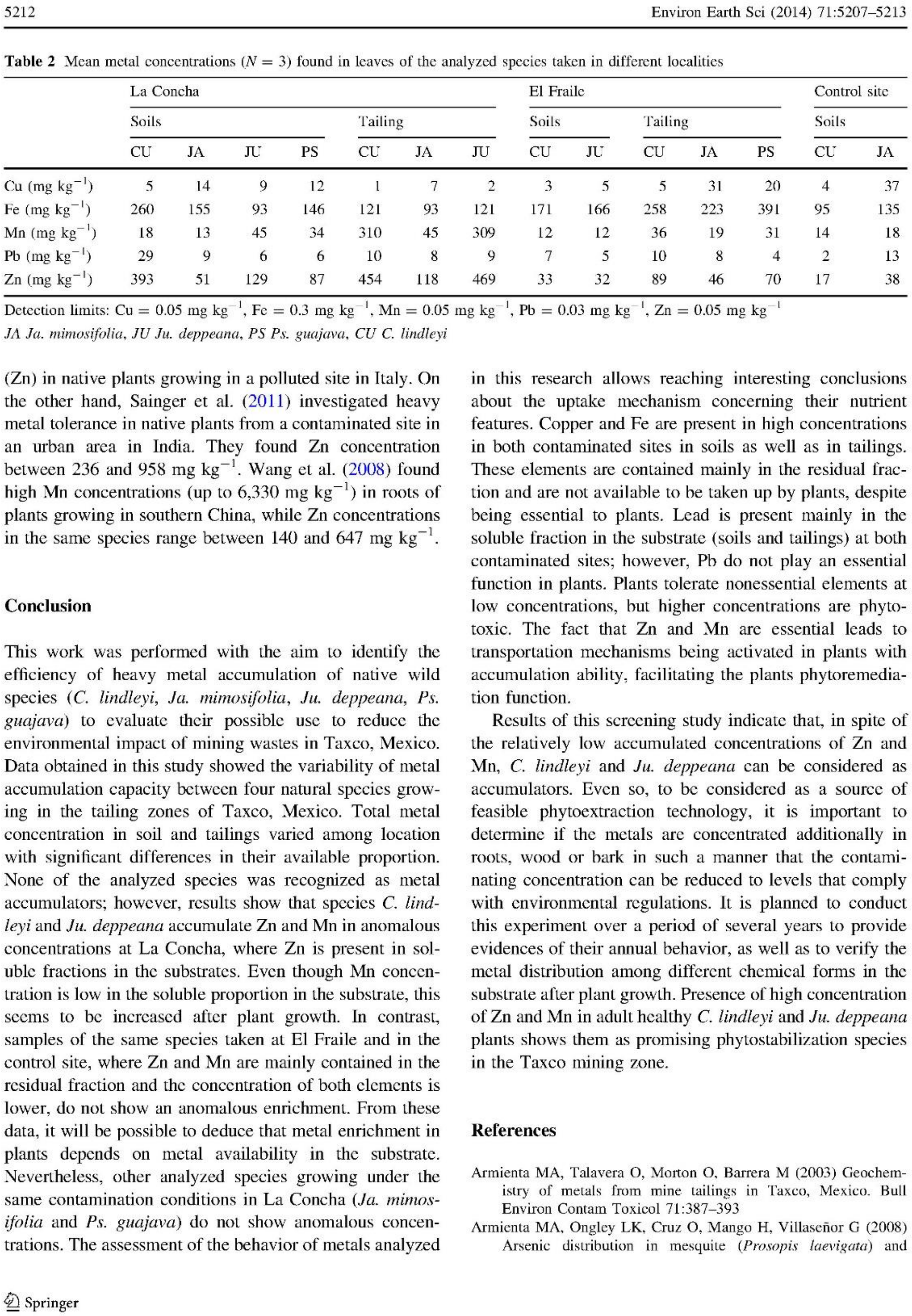


Table 2 Mean metal concentrations ($N = 3$) found in leaves of the analyzed species taken in different localities

	La Concha							El Fraile					Control site	
	Soils				Tailing			Soils		Tailing			Soils	
	CU	JA	JU	PS	CU	JA	JU	CU	JU	CU	JA	PS	CU	JA
Cu (mg kg^{-1})	5	14	9	12	1	7	2	3	5	5	31	20	4	37
Fe (mg kg^{-1})	260	155	93	146	121	93	121	171	166	258	223	391	95	135
Mn (mg kg^{-1})	18	13	45	34	310	45	309	12	12	36	19	31	14	18
Pb (mg kg^{-1})	29	9	6	6	10	8	9	7	5	10	8	4	2	13
Zn (mg kg^{-1})	393	51	129	87	454	118	469	33	32	89	46	70	17	38

Detection limits: Cu = 0.05 mg kg^{-1}, Fe = 0.3 mg kg^{-1}, Mn = 0.05 mg kg^{-1}, Pb = 0.03 mg kg^{-1}, Zn = 0.05 mg kg^{-1}

JA Ja. mimosifolia, JU Ju. deppeana, PS Ps. guajava, CU C. lindleyi

(Zn) in native plants growing in a polluted site in Italy. On the other hand, Sainger et al. (2011) investigated heavy metal tolerance in native plants from a contaminated site in an urban area in India. They found Zn concentration between 236 and 958 mg kg^{-1}. Wang et al. (2008) found high Mn concentrations (up to 6,330 mg kg^{-1}) in roots of plants growing in southern China, while Zn concentrations in the same species range between 140 and 647 mg kg^{-1}.

Conclusion

This work was performed with the aim to identify the efficiency of heavy metal accumulation of native wild species (*C. lindleyi*, *Ja. mimosifolia*, *Ju. deppeana*, *Ps. guajava*) to evaluate their possible use to reduce the environmental impact of mining wastes in Taxco, Mexico. Data obtained in this study showed the variability of metal accumulation capacity between four natural species growing in the tailing zones of Taxco, Mexico. Total metal concentration in soil and tailings varied among location with significant differences in their available proportion. None of the analyzed species was recognized as metal accumulators; however, results show that species *C. lindleyi* and *Ju. deppeana* accumulate Zn and Mn in anomalous concentrations at La Concha, where Zn is present in soluble fractions in the substrates. Even though Mn concentration is low in the soluble proportion in the substrate, this seems to be increased after plant growth. In contrast, samples of the same species taken at El Fraile and in the control site, where Zn and Mn are mainly contained in the residual fraction and the concentration of both elements is lower, do not show an anomalous enrichment. From these data, it will be possible to deduce that metal enrichment in plants depends on metal availability in the substrate. Nevertheless, other analyzed species growing under the same contamination conditions in La Concha (*Ja. mimosifolia* and *Ps. guajava*) do not show anomalous concentrations. The assessment of the behavior of metals analyzed in this research allows reaching interesting conclusions about the uptake mechanism concerning their nutrient features. Copper and Fe are present in high concentrations in both contaminated sites in soils as well as in tailings. These elements are contained mainly in the residual fraction and are not available to be taken up by plants, despite being essential to plants. Lead is present mainly in the soluble fraction in the substrate (soils and tailings) at both contaminated sites; however, Pb do not play an essential function in plants. Plants tolerate nonessential elements at low concentrations, but higher concentrations are phytotoxic. The fact that Zn and Mn are essential leads to transportation mechanisms being activated in plants with accumulation ability, facilitating the plants phytoremediation function.

Results of this screening study indicate that, in spite of the relatively low accumulated concentrations of Zn and Mn, *C. lindleyi* and *Ju. deppeana* can be considered as accumulators. Even so, to be considered as a source of feasible phytoextraction technology, it is important to determine if the metals are concentrated additionally in roots, wood or bark in such a manner that the contaminating concentration can be reduced to levels that comply with environmental regulations. It is planned to conduct this experiment over a period of several years to provide evidences of their annual behavior, as well as to verify the metal distribution among different chemical forms in the substrate after plant growth. Presence of high concentration of Zn and Mn in adult healthy *C. lindleyi* and *Ju. deppeana* plants shows them as promising phytostabilization species in the Taxco mining zone.

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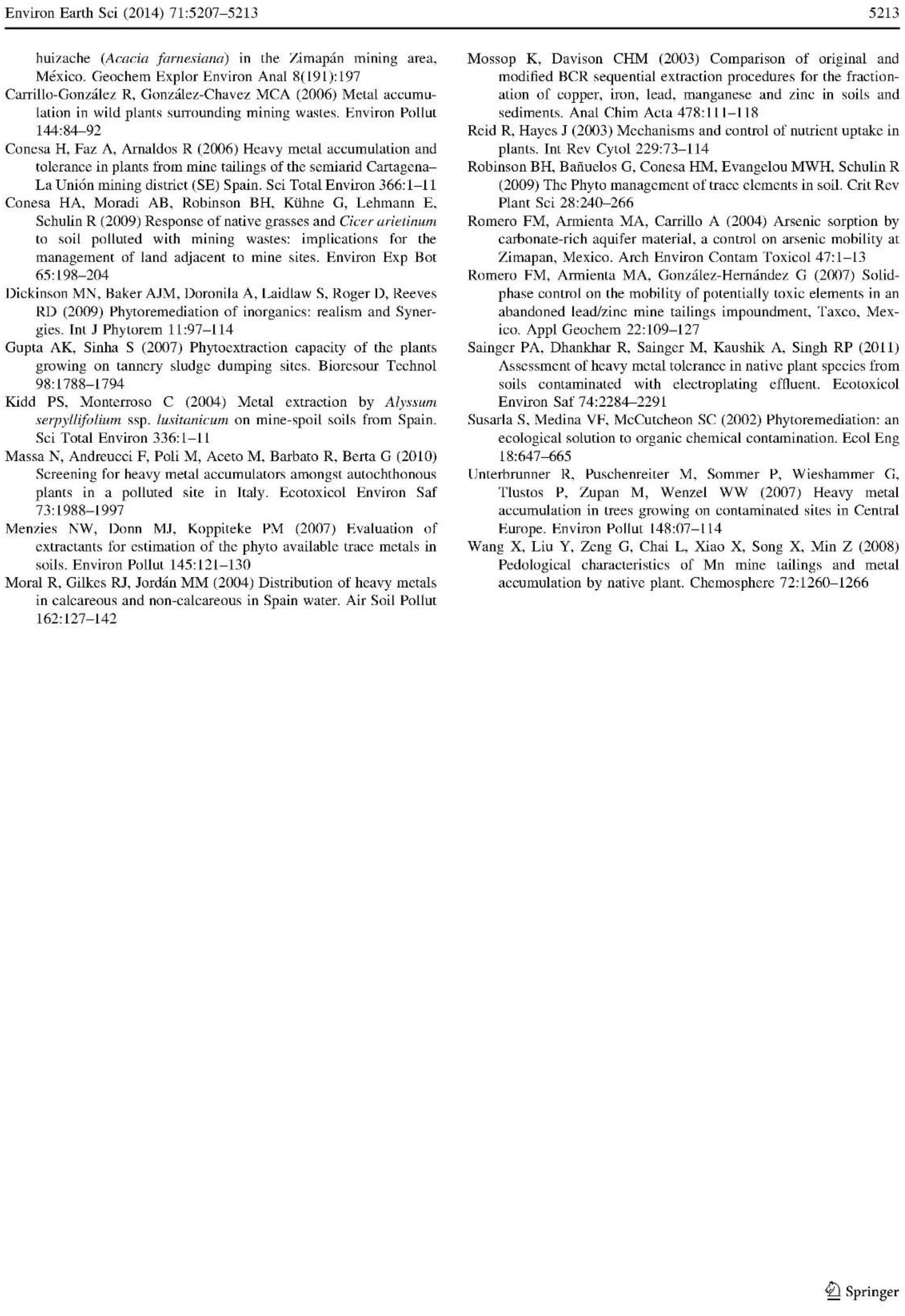

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2.3 ARTÍCULO III

- Gómez-Bernal Juan Miguel, Morton Bermea Ofelia, Ruiz Huerta Esther Aurora, Armienta Hernández María Aurora, González Dávila Osiel. 2014. **Microscopic evidences of heavy metals dsitribution and anatomic alterations in breaching-leaves of Cupresses lindleyi growing around mining wastes**. Microscopy Research and Technique. Aceptado en prensa. DOI 10.1002/jemt.22392

MICROSCOPY RESEARCH AND TECHNIQUE 00:00–00 (2014)

“Microscopic Evidences of Heavy Metals Distribution and Anatomic Alterations in Breaching-Leaves of *Cupressus lindleyi* Growing Around Mining Wastes”

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KEY WORDS phytostabilization; structural changes; translocation factor; contaminants; Taxco

ABSTRACT In this article a study of the distribution of heavy metals in *Cupressus lindleyi* breaching-leaves was done in Taxco, Guerrero. At the same, heavy metals micro-localization was conducted in the breaching-leaves to understand the structural changes provoked by mining waste on plants. The most abundant contaminants in soils, tailings and different plant organs (roots, stems, and leaves) were Zn, Mn, and Pb. Nevertheless, As was more accumulated in the stem and breaching-leaves. The translocation factor and the bio-concentration factor were less than 1. The structural changes observed were the great accumulation of starch grains and phenolic compounds in the palisade parenchyma, changes in the hypodermis cell wall and necrotic zones in the palisade parenchyma. The distribution of heavy metals in breaching-leaves tissues was homogeneous in most of the elements. These results showed that *C. lindleyi* is a species that can be employed in phytostabilization of contaminated zones with mining waste because it is a native plant that does not require a lot of conditions for its development. *Microsc. Res. Tech. 00:000–000, 2014.* © 2014 Wiley Periodicals, Inc.

INTRODUCTION

Mining activities and the smelting of metal ores are among the main sources of heavy metals contamination. Mining activities create large amounts of tailings and waste rock that are disposed of on land surfaces provoking heavy metal contamination and affecting water, air, soil, and plants (Dudka and Adriano, 1997).

Interaction of mining waste with vegetation causes different responses in plants where they grow (Baker, 1981; Barcelo, 2003; Juárez-Santillán et al., 2010). Plants can absorb heavy metals from the soil at different levels, depending on the species, and the heavy metal contents in soil. Some of them tolerate metals using a variety of mechanisms. For example, the production of exudates on the roots to prevent the entry of heavy metals, or the displacement of the heavy metals from roots towards the aerial tissues and the formation of stable compounds in the cells. Plants that accumulate heavy metals capture high amounts of metals that are transferred to the aerial parts where they are accumulated. These plants are capable of making heavy metals harmless in several ways like merging them to the cell wall, vacuolar compartmentalization or forming metal complexes with organic acids or proteins (Raskin, 1997). Exclusion is a feature of sensitive and tolerant to heavy metals species, while accumulation is common among species that appear in contaminated or metalliferous soils.

On the basis of plants capacity to tolerate or accumulate high quantities of heavy metals, phytoremediation processes are possible in contaminated soils. In order to determine the plants potential in phytoremediation processes, certain criteria have been established: (1) plants capacity to tolerate or accumulate metals. Such plants are characterized by a transference factor >1. On the other hand, plants that exclude heavy metal transportation to their aerial parts with a transference factor <1 (Baker, 1981; Baker and Whiting, 2002), (2) plants with a high biomass production; (3) a wide translocation factor; and (4) a wide bioconcentration factor (Dowdy and McKone, 1997).

Some trees have the ability to react against toxic levels of metals in soils by root exudation of organic acids which prevents metal uptake (spruce, pine, and poplar) (Ahonen-Jonnarth et al., 2000; Heim et al., 1999; Qin et al., 2007). A more common form of metal detoxification, however, is immobilization in the fine root tissue leading to accumulation up to a maximum load. Heavy metals and Al primarily bind to pectins in the

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cell walls and to the negatively charged cytoplasm-membrane surfaces due to their strong electrochemical potential (Kochian et al., 2005; Rengel and Zhang, 2003). On binding, they displace cations such as Ca^{2+} and Mg^{2+} from the cell walls and membranes. Within the plant cells, heavy metals also bind to specific proteins, such as metallothioneins (Kohler et al., 2004). The formed complexes are then transported to vacuoles inside or between cells. Many other biomolecules, such as phytochelatins, organic acids, nicotianamine, or glutathione (Pilon-Smits, 2005), could also be relevant to metal accumulation in trees.

Several heavy metals in contaminated soils are accumulated in plants (Siedlecka, 1995), translocated from soil to the plant parts (Marschner, 1995) and stored in different cells and tissues (Ernst, 2006; Verkleij and Schat, 1990). The information that can be obtained about the metal, its compartmentalization and speciation inside phytoextractor trees is important for the phytoextraction process. However, there is a need of studies on micro-localization. Such studies are needed to understand metals intake, to determine mechanisms of metal accumulation in trees and for the design of innovative remediation solutions (Vollenweider et al., 2011a).

In general, in Mexico there are few studies that assess the concentration of heavy metals in substrates and plants growing in mining waste (Armienta et al., 2008; Carrillo-Gonzalez, 2005; Carrillo-González and González-Chávez, 2006; Díaz-Villaseñor, 2006; Flores-Tavison et al., 2003; Franco-Hernández et al., 2010; Juárez-Santillan et al., 2010; Prieto-García et al., 2005; Puga et al., 2006; Ruiz and Armienta, 2012; Santos-Jallath et al., 2012), as well as the identification of heavy metals inside plants tissues. These studies allow to observe the presence of metals, becoming evident the accumulation and translocation to different organs of the plant, using tools as optic and scanning microscopy or energy dispersive X-ray spectrometry (Arias et al., 2010; Günthardt-Goerg and Vollenweider, 2007; Meyers et al., 2008; Tung and Temple, 1996; Vieira et al., 2008; Vollenweider and Günthardt-Goerg, 2006; Vollenweider et al., 2011b).

C. lindleyi was selected based on previous studies of plants species that grow around and in mining tailings (Gómez-Bernal et al., 2010). It was observed that *C. lindelyi* is a tree that grows in Taxco region, Guerrero in environments with and without mining waste impacts. The importance of the study of this species is the localization and distribution of heavy metals in tissues for its possible use in phytostabilization at other regions with similar environments. Such regions are located in the Mexican states of Chihuahua, Guerrero, Hidalgo, Estado de Mexico, San Luis Potosi and Zacatecas. All of them have a very important mining activity. It should be mentioned that *C. lindelyi* is frequently used in reforestation activities due to its resistance to unusual geographical and weather conditions. *C. lindelyi* is also used in the elaboration of paper and furniture and is also found in streets and public gardens (Zamudio et al., 1992:5).

The objectives of this article were (1) to determine heavy metal accumulation in dominant plant species naturally grown around the tailings (2) to identify structural changes in *C. lindleyi* breaching-leaves caused by heavy metals (3) to perform the micro-localization of heavy metals in *C. lindleyi* breaching-leaves in the tissue.

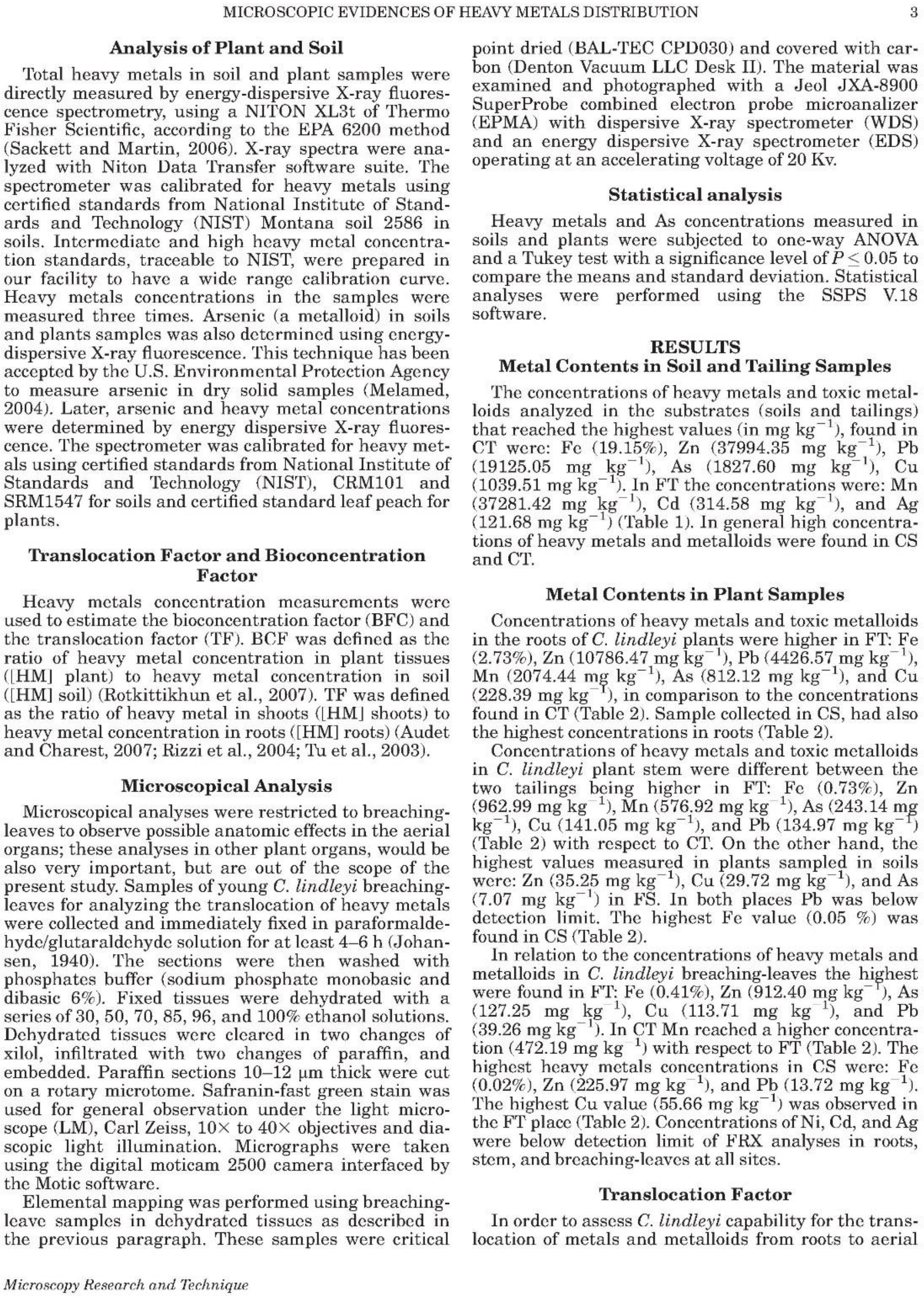
MATERIALS AND METHODS

Sampling Site

Sampling site is located in Taxco de Alarcon in the state of Guerrero, Mexico (north latitude 18.3, wets longitude 99.3). This zone has an altitude ranging from 1,700 to 2,000 m above sea level with a mean annual temperature of 26–30°C and historical precipitation registry indicating an annual average of 1,000 mm (INEGI, 1999). Plants were collected at two locations with heavy metal contamination and one control zone. Tailing pond "La Concha" is located to the north of the San Antonio-"La Concha" mine. Its UTM coordinates are 14Q 432894 E and 2049996 N. It is a deposit of irregular shape with the following average dimensions: 140 m long, 50 m wide, and 10 m average thickness. The factor used as specific weight for the calculation of this pond is 2.2 g/cm^3 giving as result 154,000 tons with the following average metal contents: 0.15 g/t Au, 150 g/t Ag, 2.06% Pb, 5.0% Zn, and 15.3% Fe (CRM, 2003). "El Fraile" dam is located in the village of the same name. Its UTM coordinates are 14Q 433056 E and 2048294 N. This is the second biggest dam in the district. It is a deposit of irregular shape with the following average dimensions: 340 m long, 227 m wide, and 32 m average thickness. The factor used as specific weight for the calculation of this pond is 2.2 g/cm^3 giving 5,433,472 tons with the following average metal contents: 0.2 g/t Au, 35 g/t Ag, 0.33% Pb, 1.4% Zn, and 11.5% Fe (CRM, 2003). At each of these two locations, one plot (20 m^2) was defined. Soils were collected at each plot from 0 to 25 cm depth in October 2008. Two sites were sampled at each of the two polluted locations and the control zone; they are: "La Concha" Tailing (CT), "La Concha" Soil contaminated with mining waste (CS), "El Fraile" Tailing (FT), "El Fraile" Soil contaminated with mining waste (FS), and control zone (CZ). Three soil samples were collected at each site.

Plant Sampling

Three and if possible four individual plants were collected at each sampled site. All plants were mature and appeared healthy and did not show the presence of parasites. Species identification was conducted at the metropolitan herbarium at the UAM-I (Universidad Autónoma Metropolitana-Iztapalapa). Shoots (breaching-leaves and stem) and roots were washed with water and rinsed with deionized water (18 $M\Omega\ cm^{-1}$, Milli-Q Millipore). The roots, stem, and breaching-leaves were analyzed for total metals (Pb, Zn, Cu, Ni, Fe, Mn, Cd, and Ag) and metalloids (As). These plant samples were collected from the top layer (0–30 cm). They contained a mix of mining waste and soil. Thus, samples were washed thoroughly in the laboratory with running tap water, followed by three rinses with deionized water (18 $M\Omega\ cm^{-1}$, Milli-Q Millipore) and a rinse of tri-distilled water. All plant samples were carefully divided into stem, breaching-leaves and roots. They were dried at 60°C for 75 h. The oven-dried plant samples were then crushed, sieved ($<325\ \mu m$), homogenized, and weighed.



Analysis of Plant and Soil

Total heavy metals in soil and plant samples were directly measured by energy-dispersive X-ray fluorescence spectrometry, using a NITON XL3t of Thermo Fisher Scientific, according to the EPA 6200 method (Sackett and Martin, 2006). X-ray spectra were analyzed with Niton Data Transfer software suite. The spectrometer was calibrated for heavy metals using certified standards from National Institute of Standards and Technology (NIST) Montana soil 2586 in soils. Intermediate and high heavy metal concentration standards, traceable to NIST, were prepared in our facility to have a wide range calibration curve. Heavy metals concentrations in the samples were measured three times. Arsenic (a metalloid) in soils and plants samples was also determined using energy-dispersive X-ray fluorescence. This technique has been accepted by the U.S. Environmental Protection Agency to measure arsenic in dry solid samples (Melamed, 2004). Later, arsenic and heavy metal concentrations were determined by energy dispersive X-ray fluorescence. The spectrometer was calibrated for heavy metals using certified standards from National Institute of Standards and Technology (NIST), CRM101 and SRM1547 for soils and certified standard leaf peach for plants.

Translocation Factor and Bioconcentration Factor

Heavy metals concentration measurements were used to estimate the bioconcentration factor (BFC) and the translocation factor (TF). BCF was defined as the ratio of heavy metal concentration in plant tissues ([HM] plant) to heavy metal concentration in soil ([HM] soil) (Rotkittikhun et al., 2007). TF was defined as the ratio of heavy metal in shoots ([HM] shoots) to heavy metal concentration in roots ([HM] roots) (Audet and Charest, 2007; Rizzi et al., 2004; Tu et al., 2003).

Microscopical Analysis

Microscopical analyses were restricted to breaching-leaves to observe possible anatomic effects in the aerial organs; these analyses in other plant organs, would be also very important, but are out of the scope of the present study. Samples of young *C. lindleyi* breaching-leaves for analyzing the translocation of heavy metals were collected and immediately fixed in paraformaldehyde/glutaraldehyde solution for at least 4–6 h (Johansen, 1940). The sections were then washed with phosphates buffer (sodium phosphate monobasic and dibasic 6%). Fixed tissues were dehydrated with a series of 30, 50, 70, 85, 96, and 100% ethanol solutions. Dehydrated tissues were cleared in two changes of xilol, infiltrated with two changes of paraffin, and embedded. Paraffin sections 10–12 μm thick were cut on a rotary microtome. Safranin-fast green stain was used for general observation under the light microscope (LM), Carl Zeiss, 10× to 40× objectives and diascopic light illumination. Micrographs were taken using the digital moticam 2500 camera interfaced by the Motic software.

Elemental mapping was performed using breaching-leave samples in dehydrated tissues as described in the previous paragraph. These samples were critical point dried (BAL-TEC CPD030) and covered with carbon (Denton Vacuum LLC Desk II). The material was examined and photographed with a Jeol JXA-8900 SuperProbe combined electron probe microanalizer (EPMA) with dispersive X-ray spectrometer (WDS) and an energy dispersive X-ray spectrometer (EDS) operating at an accelerating voltage of 20 Kv.

Statistical analysis

Heavy metals and As concentrations measured in soils and plants were subjected to one-way ANOVA and a Tukey test with a significance level of $P \leq 0.05$ to compare the means and standard deviation. Statistical analyses were performed using the SSPS V.18 software.

RESULTS

Metal Contents in Soil and Tailing Samples

The concentrations of heavy metals and toxic metalloids analyzed in the substrates (soils and tailings) that reached the highest values (in mg kg^{-1}), found in CT were: Fe (19.15%), Zn (37994.35 mg kg^{-1}), Pb (19125.05 mg kg^{-1}), As (1827.60 mg kg^{-1}), Cu (1039.51 mg kg^{-1}). In FT the concentrations were: Mn (37281.42 mg kg^{-1}), Cd (314.58 mg kg^{-1}), and Ag (121.68 mg kg^{-1}) (Table 1). In general high concentrations of heavy metals and metalloids were found in CS and CT.

Metal Contents in Plant Samples

Concentrations of heavy metals and toxic metalloids in the roots of *C. lindleyi* plants were higher in FT: Fe (2.73%), Zn (10786.47 mg kg^{-1}), Pb (4426.57 mg kg^{-1}), Mn (2074.44 mg kg^{-1}), As (812.12 mg kg^{-1}), and Cu (228.39 mg kg^{-1}), in comparison to the concentrations found in CT (Table 2). Sample collected in CS, had also the highest concentrations in roots (Table 2).

Concentrations of heavy metals and toxic metalloids in *C. lindleyi* plant stem were different between the two tailings being higher in FT: Fe (0.73%), Zn (962.99 mg kg^{-1}), Mn (576.92 mg kg^{-1}), As (243.14 mg kg^{-1}), Cu (141.05 mg kg^{-1}), and Pb (134.97 mg kg^{-1}) (Table 2) with respect to CT. On the other hand, the highest values measured in plants sampled in soils were: Zn (35.25 mg kg^{-1}), Cu (29.72 mg kg^{-1}), and As (7.07 mg kg^{-1}) in FS. In both places Pb was below detection limit. The highest Fe value (0.05 %) was found in CS (Table 2).

In relation to the concentrations of heavy metals and metalloids in *C. lindleyi* breaching-leaves the highest were found in FT: Fe (0.41%), Zn (912.40 mg kg^{-1}), As (127.25 mg kg^{-1}), Cu (113.71 mg kg^{-1}), and Pb (39.26 mg kg^{-1}). In CT Mn reached a higher concentration (472.19 mg kg^{-1}) with respect to FT (Table 2). The highest heavy metals concentrations in CS were: Fe (0.02%), Zn (225.97 mg kg^{-1}), and Pb (13.72 mg kg^{-1}). The highest Cu value (55.66 mg kg^{-1}) was observed in the FT place (Table 2). Concentrations of Ni, Cd, and Ag were below detection limit of FRX analyses in roots, stem, and breaching-leaves at all sites.

Translocation Factor

In order to assess *C. lindleyi* capability for the translocation of metals and metalloids from roots to aerial

TABLE 1. Average total concentrations (mg kg^{-1}) of metals and As, in soils and tailings collected in different points analyzed using FRX

Site	Zone	Pb 400^{a}	As 22^{a}	Zn	Cu	Ni 1600*	Fe (%)	Mn	Cd 37^{a}	**Ag**
La Concha	Tailing (CT)	13679.49(272.70)	1513.67(154.18)	32684.74(827.70)	768.28(65.15)	**< LD**	16.57(1.721)	9406.80(165.15)	221.82(.92)	85.94(2.4)
	Soil (CS)	**19125.05(2032.30)**	**1827.60(376.80)**	**37994.35(5360.74)**	**1039.51(268.39)**	**< LD**	**19.15(2.435)**	15847.65(1407.20)	224.81(1.88)	74.98(1.39)
El Fraile	Tailing (FT)	9600.21(6627.62)	1561.43(119.76)	24970.41(18923.80)	679.23(309.49)	**< LD**	19.02(6.754)	**37281.42(48995.03)**	**314.58(9.77)**	**121.68(2.78)**
	Soil (FS)	2572.66(105.29)	707.35(25.12)	6322.33(58.48)	207.48(12.90)	**< LD**	5.98(0.010)	1702.06(78.60)	200.79(14.18)	**< LD**
Control Zone	Soil (CZ)	35.25(4.90)	29.92(4.26)	141.00(7.33)	85.36(6.01)	**< LD**	3.67(0.025)	468.80(48.08)	**< LD**	**< LD**

Standard deviations in parenthesis.

[a]The Mexican Official Norm NOM-147-SEMARNAT/SSA1–2004 (SEMARNAT 2007) established the following guideline values for arsenic and heavy metals in agricultural soil in 2007.

TABLE 2. Average total concentrations (mg/kg) of metals and metalloids in roots, stem and breaching-leaves of *C. lindleyi* from Taxco, Guerrero

Site	Zone	Organ	Pb	As	Zn	Cu	Ni	Fe (%)	Mn	Cd	Ag
La Concha	Tailing (CT)	breaching-leaves	15.79(1.31)^{a}	**< LD**	597.44(9.11)^{a}	31.52(4.14)^{a}	**< LD**	0.02(0.001)^{a}	**472.19(30.55)**^{b}	**< LD**	**< LD**
		Stem	23.26(.59)^{b}	**< LD**	650.85(12.70)^{b}	48.59(1.79)^{b}	**< LD**	0.04(0.005)^{b}	103.87(21.25)^{a}	**< LD**	**< LD**
		Root	364.45(4.60)^{c}	79.43(7.77)	1754.07(12.13)^{c}	48.72(7.05)^{b}	**< LD**	0.28(0.002)^{c}	815.05(54.23)^{c}	**< LD**	**< LD**
	Soil (CS)	breaching-leaves	13.72(1.55)^{a}	**< LD**	225.97(11.56)^{b}	42.10(6.319)^{b}	**< LD**	0.02(0.002)^{a}	**< LD**	**< LD**	**< LD**
		Stem	**< LD**	**< LD**	32.35(6.09)^{a}	27.89(1.23)^{a}	**< LD**	0.05(0.002)^{b}	**< LD**	**< LD**	**< LD**
		Root	257.62(4.25)^{b}	51.74(6.82)	1756.30(19.83)^{c}	46.96(14.99)^{b}	**< LD**	0.17(0.00)^{c}	99.84	**< LD**	**< LD**
El Fraile	Tailing (FT)	breaching-leaves	**39.26**	**127.25**	**912.40(652)**^{a}	**113.71**	**< LD**	**0.41(0.340)**^{a}	352.78(88.86)	**< LD**	**< LD**
		Stem	**134.97**	**243.14**	**962.99(714)**^{a}	**141.05**	**< LD**	**0.73(1.096)**^{a}	**576.92**	**< LD**	**< LD**
		Root	**4426.57(416.450)**	**812.12(20.54)**	**10786.47(1102.03)**^{b}	**228.39(16.02)**	**< LD**	**2.73(0.138)**^{b}	**2074.44(51.06)**	**< LD**	**< LD**
	Soil (FS)	breaching-leaves	**< LD**	**< LD**	149.07(9.90)^{b}	55.66(13.09)	**< LD**	0.01(0.001)^{a}	**< LD**	**< LD**	**< LD**
		Stem	**< LD**	7.07	35.25(3.39)^{a}	29.72	**< LD**	0.02(0.002)^{b}	**< LD**	**< LD**	**< LD**
		Root	**< LD**	**< LD**	373.49(9.91)^{c}	58.44(8.50)	**< LD**	0.04(0.003)^{c}	103.63	**< LD**	**< LD**
Control zone	Soil (CZ)	breaching-leaves	**< LD**	**< LD**	10.33(2.97)^{a}	17.21(4.89)	**< LD**	0.01(0.002)^{a}	60.47(14.82)	**< LD**	**< LD**
		Stem	**< LD**	**< LD**	31.88(8.15)^{b}	28.08	**< LD**	0.02(0.001)^{ab}	**< LD**	**< LD**	**< LD**
		Root	**< LD**	**< LD**	54.26(6.78)^{c}	35.68(5.66)	**< LD**	0.01(0.003)^{b}	110.84(8.85)	**< LD**	**< LD**

Standard deviations in parentheses.

[a,b,c]Means followed by the same letters are not significantly different by Tukey Test at $P < 0.05$. Lower detection (<LD).

TABLE 3. Translocation Factor in *C. lindleyi* collected in the study areas in Taxco, Guerrero

Site	Zone	Pb	As	Zn	Cu	Ni	Fe	Mn	Cd	Ag
La Concha	Tailings (CT)	0.043	—	0.341	0.647	—	0.062	**0.579**	—	—
	Soils (CS)	**0.053**	—	0.129	0.897	—	0.102	—	—	—
El Fraile	Tailings (FT)	0.009	**0.157**	0.085	0.498	—	0.151	0.170	—	—
	Soils (FS)	—	—	**0.399**	**0.952**	—	0.291	—	—	—
Control Zone	Soils (CZ)	—	—	0.190	0.482	—	**0.704**	0.546	—	—

TABLE 4. Bioconcentration factor of *C. lindleyi* collected in the study sites in Taxco, Guerrero

Site	Zone	Pb	As	Zn	Cu	Ni	Fe	Mn	Cd	Ag
La Concha	Tailings (CT)	0.010	0.018	0.031	0.051	—	0.007	0.048	—	—
	Soils (CS)	0.005	0.009	0.018	0.032	—	0.004	0.001	—	—
El Fraile	Tailings (FT)	**0.156**	**0.200**	0.169	0.154	—	**0.068**	0.023	—	—
	Soils (FS)	—	0.001	0.029	**0.199**	—	0.004	0.014	—	—
Control zone	Soils (CS)	—	—	**0.228**	0.174	—	0.004	**0.122**	—	—

parts, the translocation factor (TF) was calculated as an absolute ratio (see Table 3). A value very close to 1 means that the metals are translocated almost completely from the roots to the breaching-leaves. In FS, it can be observed that the highest value was for Cu (0.95). This was one of the metals that were more translocated into the aerial parts of the plant. In the same way, the TF of Zn (0.399) was high. In the CZ site the TF for Fe (0.704) was high. The TF of Mn (0.579) was the highest in CT and of Pb (0.053) in CS. Finally, As with a TF of 0.157 was only registered in FT. The Ni, Cd, As, and Ag TF were below detection limit in CT, as well as in CS. Further, Mn was detected only in plants growing in tailings (Table 3). In the CZ sample, Pb, As, Ni, Cd, and Ag were below detection limit.

Bioconcentration Factor

Bioconcentration factors (BF) for *C. lindleyi* plants were high in Zn (0.228) and Mn (0.122) in the CZ site. In FT the results highest BF values were: As (0.200), Pb (0.156), and Fe (0.068). In FS Cu had a BF value of 0.199. Ni, Cd, and Ag were below detection limit in the three places, besides in CZ and FS sites As and Pb were below detection limits (Table 4).

Anatomical Alterations in Breaching-Leaves

C. lindleyi breaching-leaves that grow in CT, present unistratified epidermis and a thin cuticle (Fig. 1A1). There are numerous stomas (please note that in the Fig. 1A1 no stoma are observed) and could be present in both sides of the basal region of the breaching-leaves. In the stoma guard cells a lack of ergastric content was observed. The hypodermis is in abaxial position, sclerosed, and unistratified. In one part of the leaf it is bistratified and close to the resin canal (Fig. 1A1). The mesophyll is dorsiventral inverse. It presents one or two layers of palisade parenchyma and spongy layer (Fig. 1A2). We can observe a superficial squizogen structure (resin canal) with round outline that is very compact (Fig. 1A1).

The most typical changes in *C. lindleyi* breaching-leaves in response to the heavy metal stress occurred in the lower leaf blade, especially in the abaxial epidermis. Groups of dead and collapsed palisade parenchyma sometimes are included in the epidermis and/or hypodermis (Fig. 1A3). The cell wall chemistry was modified, especially in the palisade parenchyma (Figs. 1A3 and C3) and in the hypodermis (Fig. 1A3), with deposits of lignin-like material (Figs. 1A3 and C3 versus E1 and E3). Similar changes were observed in spongy parenchyma close to necrotic sections of lower hypodermis (Fig. 1A3 and C3). These necrotic zones in lower epidermis sections were irregularly scattered throughout the breaching-leaves blade, but preferentially found next to a palisade parenchyma. These necrotic cells, as shown by their cellular destruction (Figs. 1A3 and C3), were possibly responsible for points in breaching-leaves visible on the breaching-leaves abaxial leaf (not shown). Cells with the most severe symptoms (Günthardt-Goerg and Vollenweider, 2007) often belonged to the lower palisade layer and showed features characteristic of the hypersensitive-response-like (HR-like) processes.

Light microscopy revealed the disruption of cell contents, condensation of cell remnants, thickening of cell walls and partial cell collapse (Figs. 1A3 and C3) and occasional accumulation of vacuolar phenolics (Figs. 1A2, A3, B1, B3, C1–C3, D1, and D3). As it is noted in the mesophyll, cells showed tannic and resin contents that took on an intense red colour mainly close to the vascular bundle (Fig. 1A1).

In *C. lindleyi* breaching-leaves collected in CS, a unistratified epidermis with thickening in its wall was observed (Fig. 1B2). The cuticle is thin and the hypodermis is unistratified and sclerosed (Fig. 1B2). The palisade parenchyma presented a high accumulation of starch grains in the chloroplasts but without presenting cell disintegration as was the case of *C. lindleyi* grown in CT (Figs. 1A2 and A3 versus B1 and B3). The vascular bundle presented a conformation without structural changes (not shown). The accumulation of starch grains in zones close to it was present (Figs. 1B1 and B3). The leaves fissures did not present cell alterations (not shown). However, in Figure 1B1 it was noted accumulation of starch grains. The resin canal did not show a cell compression in its structure (not shown).

In the *C. lindleyi* breaching-leaves grown in FS, it was observed that the resin canal was compact and the

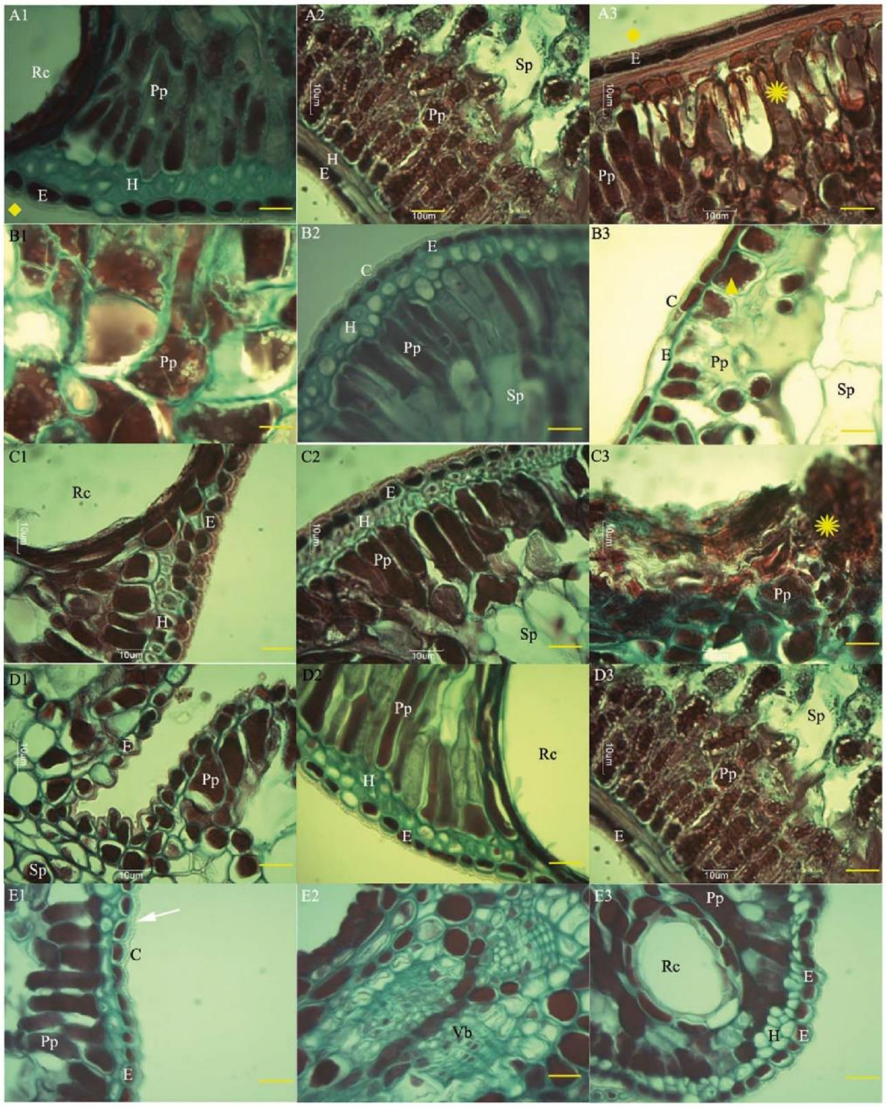


Fig. 1. *C. lindleyi* breaching-leaves. **A**: *C. lindleyi* breaching-leaves collected at CT, yellow star indicates side abaxial, (A1) the accumulation of vacuolar phenolics in Figure 1 are the black cells shown in Figure A1-A3, 10X; (A2) starch grains in the wall cell, 10X; (A3) groups of dead cell and collapsed palisade parenchyma, ×10; (**B**) *C. lindleyi* breaching-leaves collected in CS, B1) accumulation of vacuolar phenolics, ×40; B2) thickening of the cell wall of the palisade parenchyma, ×10; B3) a yellow triangle indicates the starch grains, ×10; (**C**) *C. lindleyi* breaching-leaves collected at FT, C1) thickening of the resin canal, ×10; (C2) thickening of the hypodermis and epidermis, ×10; C3) disruption of cell contents and partial cell collapse, ×40; (**D**) *C. lindleyi* breaching-leaves collected in FS, D1) cuticle is irregularly shaped in the epidermis, ×10; (D2) thickening of the resin canal, hypodermis and epidermis, ×10; (D3) accumumaltion of vacuolar phenolics and presence of the starch grains, ×10, and (**E**) samples collected in CZ, (E1) cuticle without strange particles, ×10; (E2). It presents a vascular bundle surrounded by transfusion tissue, ×10; (E3) no changes were noted in the cell tissues, ×10. Resin canal (Rc), palisade parenchyma (Pp), hipodermis (H), epidermis (E), spongy parenchyma (Sp), cuticle (C), vascular bundle (Vb). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

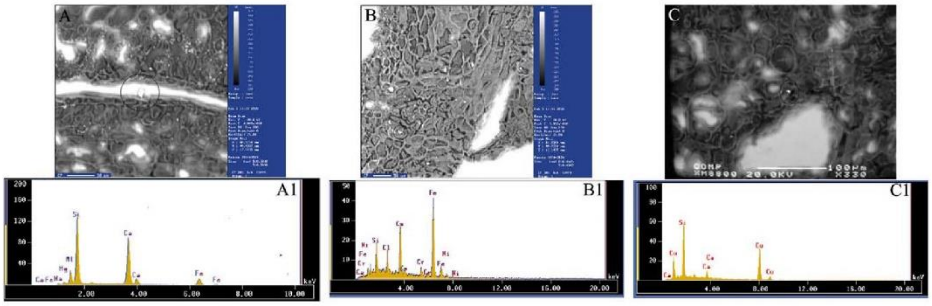


Fig. 2. Longitudinal cut of the *C. lindleyi* breaching-leaves collected in FT. (**A**) Observation of a glandular trichome in SEM, ×10; (A1) EDS analysis showing the chemical elements found in the glandular trichome. (**B**) Sample showing a particle analyzed by EDS, ×10; (B1) EDS analysis showing the chemical elements found in the spongy parenchyma; (**C**) Observation of a particle in the base of de breaching-leaves, ×10; (C1) EDS analysis showing the chemical elements found in the palisade parenchyma. Epidermis (E), spongy parenchyma (Sp). Elemental analysis EDX performed by 20 kV at magnification ×3,900 using a 60 μm condenser diaphragm, 6 mm working distance, take-off angle of 35° with EDX detector; element data scanned during 220-μs dwell time; measurements cumulated over 128 frames, respectively, 90 frames and mapped at 256 × 200 ppi resolution (each point in map represents a total peak count). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

hypodermis presents wall cell thickening as well as a cuticle with the presence of strange bodies different to the control zone (Figs. 1C1 and C2). The presence of phenolic compounds was observed in the palisade parenchyma but without the great accumulation of starch grains as observed in CT (Fig. 1C2 versus 1A1 and A2). In the leaf base the following damages were observed: cell alterations (epidermis and hypodermis) and presence of phenolic compounds (tannin) (Fig. 1C3).

In *C. lindleyi* breaching-leaves grown in FS, a cuticle with incrustations is observed. The epidermis showed a loss of cell and ergastric content (Fig. 1D1). The hypodermis also presented changes in the cell wall (Figs. 1D1–D3). The palisade parenchyma did not present high accumulation of starch grains in the cells as in the samples collected in CT (Fig. 1A2 versus 1D3). A glandular trichoma was observed in a leaves fissure. This trichoma showed ergastric content, the absence of heavy metals shown by EDS analysis (Fig. 2A1) leads to discard the idea that heavy metals could be accumulated in this structure (Fig. 2A1). The resin canal was distinguished with cell compression that was also evident in samples of CT (Fig. 1C1).

In CZ *C. lindleyi* breaching-leaves presented a cuticle without strange particles (see Fig. 1E1 shown by the arrow). The epidermis and hypodermis did not show thickening of the cell wall (Fig. 1E1 and E3). Besides, the palisade parenchyma did not present a great accumulation of starch grains in the chloroplasts (Fig. 1E1 and E3). The resin canal did not show cell compression not structural changes as in CT breaching-leaves (Fig. 1E3 versus D2, C1 and A1). The vascular bundle did not present cell compression close to it (Fig. 1E2).

EDS was conducted in order to find the exact location of metals in the glandular trachoma in a *C. lindleyi* leaf basal (Fig. 2A). Silicon and Ca were abundant. Therefore, the idea of storage in trichomas as a storage mechanism was discared (Fig. 2A1).

In a longitudinal cut of *C. lindleyi* breaching-leaves collected at FT, through EDS analysis a particle with a composition mainly of Fe and Ca and in a fewer proportion with Ni and Cr was found (Fig. 2B1). The particle was located in the mesophyll tissue of the leaf (Fig. 2B).

Close to the base of the *C. lindleyi* breaching-leaves a Cu particle was identified (Fig. 2C1). This particle is located between the hypodermis and the epidermis. This location is important because it could be related with structural alterations observed in optical microscopy (Fig. 2C).

Elemental Mapping

Lead presence, observed through X-ray scan in *C. lindleyi* breaching-leaves grown in CT was detected in the vascular bundle and its periphery, as well as in the spongy and palisade parenchyma (Fig. 3A1). Arsenic was present in a uniform way both in the internal part (epidermis, hypodermis, spongy and palisade parenchyma and vascular bundle) and in the external part (cuticle) (Fig. 3A2). Zinc was mainly found in the breaching-leaves internal part (mesophyll) in a uniform way. In the external part it was also found in a uniform way but in lower quantities than in the internal part (Fig. 3A3).

Copper was found mainly in the breaching-leaves cuticle. It was also observed in the vascular bundle and its periphery. It was present in lower quantity in the palisade parenchyma (Fig. 3A4). Nickel had a very notorious distribution in the external part of the breaching-leaves and in some places of the vascular bundle and its periphery (Fig. 3A5). Iron showed a uniform distribution both in the internal and external parts of the leaves. A wide distribution of Mn in all breaching-leaves was observed. This is due to its importance in the metabolic process (Fig. 3A7). Cadmium was mainly distributed in the internal tissue being more evident in the palisade parenchyma, the vascular bundle and its periphery (Fig. 3A8). Silver

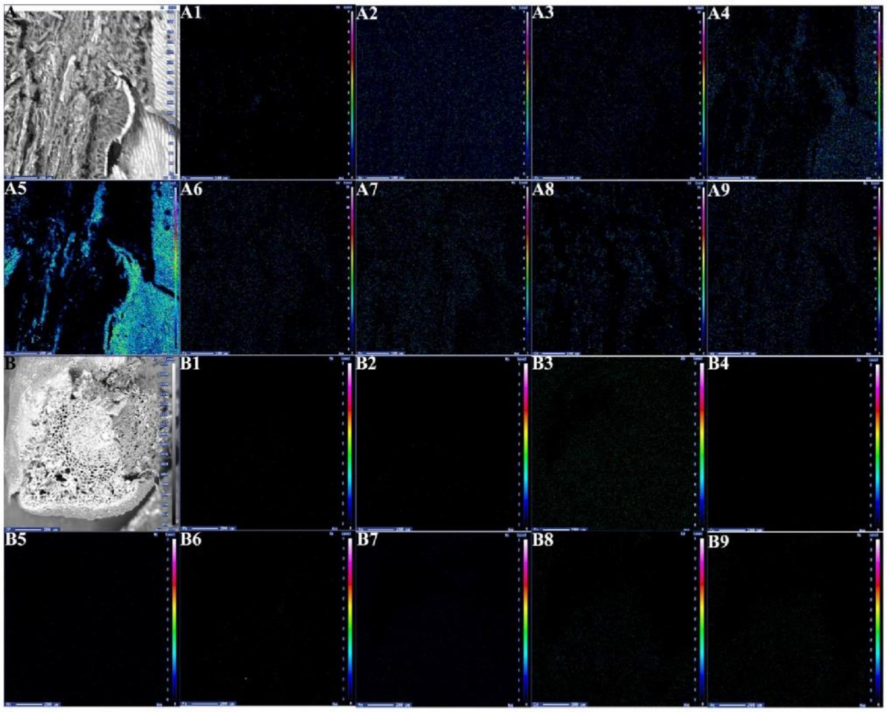


Fig. 3. Distribution of heavy metals deposits at tissue of *C. lindleyi*. (**A**) breaching-leaves collected at CT and (**B**) FT. Symbol of Number: (1) Pb, (2) As, (3) Zn, (4) Cu, (5) Ni, (6) Fe, (7) Mn, (8) Cd, and (9) Ag. Vascular bundle (Vb), palisade parenchyma (Pp), epidermis (E), spongy parenchyma (Sp), cuticle (C). Technical specifications: Pictures taken by a Jeol JXA-8900 SuperProbe combined electron probe microanalyzer (EPMA) with dispersive X-ray spectrometer (WDS) and an energy dispersive X-ray spectrometer (EDS) at acceleration voltages of 20 kV, using 60-μm condenser diaphragm, 7 mm working distance take-off angle of 35° with SE or RBSD detectors. element data scanned during 220-μs dwell time; measurements cumulated over 128 frames, respectively, 90 frames and mapped at 256 × 200 ppi resolution (each point in map represents a total peak count). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

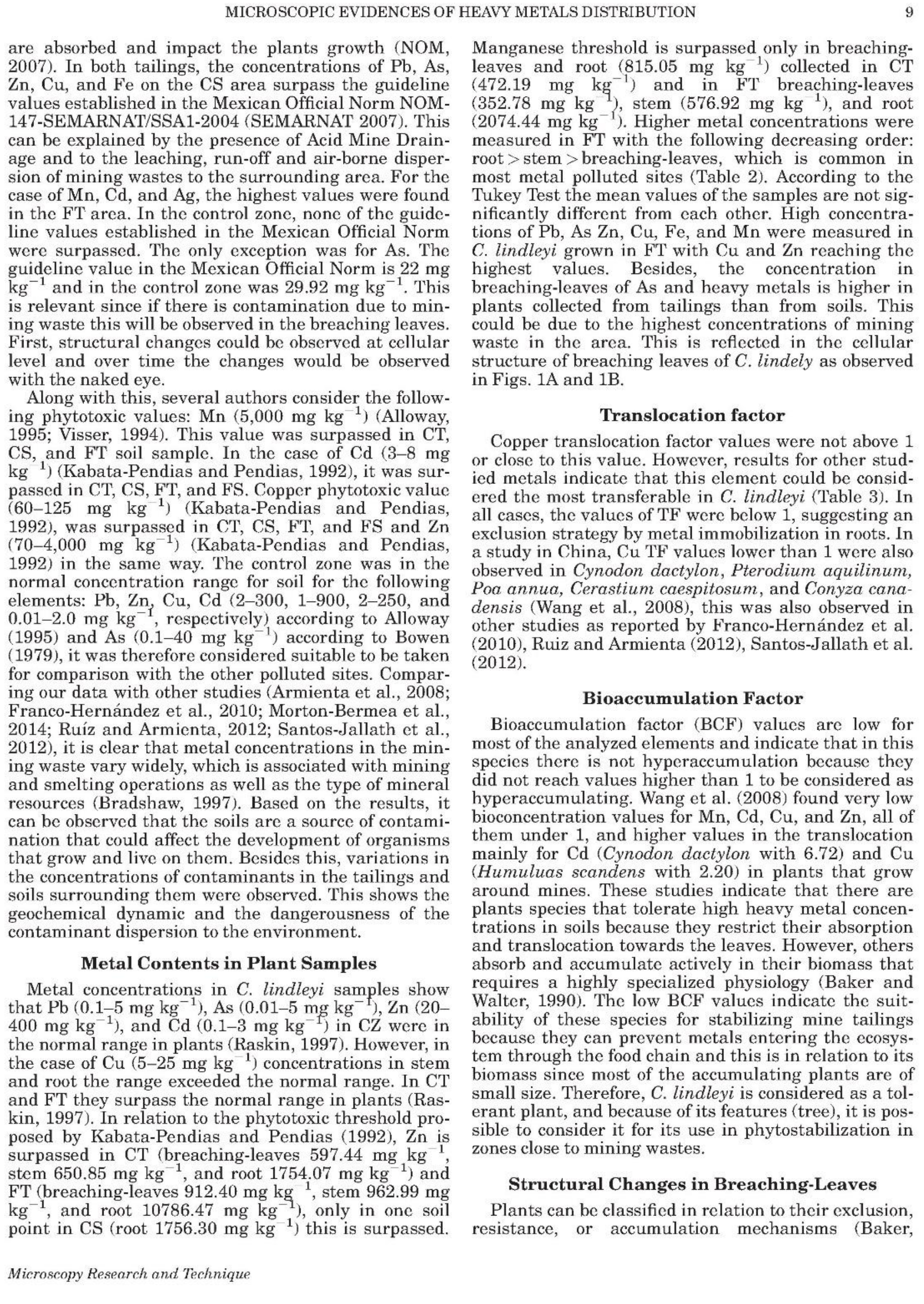
distribution was uniform in the breaching-leaves internal tissue (Fig. 3A9).

In the *C. lindleyi* breaching-leaves grown in FT, Pb showed a uniform distribution in internal and external tissues (Fig. 3B1). A uniform distribution of As was observed in the internal tissues: vascular bundle, palisade parenchyma, epidermis and hypodermis. However, it was less observed in the palisade parenchyma (Fig. 3B2). Zinc showed a uniform distribution in all the studied leaves (Fig. 3B3). Copper was not detected in internal and external tissues (Fig. 3B4). Nickel was distributed in a uniform way in the tissues, as well as Fe, Mn, Cd, and Ag (Figs. 3B5–B9, respectively).

DISCUSSION

Metal Contents in Soil and Tailing Samples

Metal contents in CS and CT show that there is an influence of mining waste on nearby soils since high concentrations have been measured in close-by tailings. Talavera et al. (2008) reported in Taxco region (mg kg^{-1}): Cd (1.0–780), Cu (71.8–1320), Fe (2.49-25.1%), Pb (780-43,700), and Zn (380-10,000). However, in this study Zn was found in higher concentrations. On the other hand, Romero (2008) found the following concentrations Pb (1,479 mg kg^{-1}), Zn (469 mg kg^{-1}), Cu (72 mg kg^{-1}), Fe (9.4%), and As (585 mg kg^{-1}) in tailings in the same zone. Ruiz and Armienta (2012) found the following values in FT: Fe (17.4%), Zn (1731.2 mg kg^{-1}), Cu (153.88 mg kg^{-1}), Cd (5.8 mg kg^{-1}), Pb (6166 mg kg^{-1}), and As (781.66 mg kg^{-1}), they also analyzed FS determining: Fe (5.4%), Zn (16,193 mg kg^{-1}), Cu (125.2 mg kg^{-1}), Cd (152.2 mg kg^{-1}), Pb (1026.6 mg kg^{-1}), and As (456 mg kg^{-1}). Díaz-Villaseñor (2006) reported the following values in agricultural land in “El Fraile” area: Pb (1334.83 mg kg^{-1}), Zn (2620.0 mg kg^{-1}), Cu (122.78 mg kg^{-1}), Fe (3.80%), and As (89.02 mg/kg). It is thus very likely that metallic contaminants in soils



are absorbed and impact the plants growth (NOM, 2007). In both tailings, the concentrations of Pb, As, Zn, Cu, and Fe on the CS area surpass the guideline values established in the Mexican Official Norm NOM-147-SEMARNAT/SSA1-2004 (SEMARNAT 2007). This can be explained by the presence of Acid Mine Drainage and to the leaching, run-off and air-borne dispersion of mining wastes to the surrounding area. For the case of Mn, Cd, and Ag, the highest values were found in the FT area. In the control zone, none of the guideline values established in the Mexican Official Norm were surpassed. The only exception was for As. The guideline value in the Mexican Official Norm is 22 mg kg^{-1} and in the control zone was 29.92 mg kg^{-1}. This is relevant since if there is contamination due to mining waste this will be observed in the breaching leaves. First, structural changes could be observed at cellular level and over time the changes would be observed with the naked eye.

Along with this, several authors consider the following phytotoxic values: Mn (5,000 mg kg^{-1}) (Alloway, 1995; Visser, 1994). This value was surpassed in CT, CS, and FT soil sample. In the case of Cd (3–8 mg kg^{-1}) (Kabata-Pendias and Pendias, 1992), it was surpassed in CT, CS, FT, and FS. Copper phytotoxic value (60–125 mg kg^{-1}) (Kabata-Pendias and Pendias, 1992), was surpassed in CT, CS, FT, and FS and Zn (70–4,000 mg kg^{-1}) (Kabata-Pendias and Pendias, 1992) in the same way. The control zone was in the normal concentration range for soil for the following elements: Pb, Zn, Cu, Cd (2–300, 1–900, 2–250, and 0.01–2.0 mg kg^{-1}, respectively) according to Alloway (1995) and As (0.1–40 mg kg^{-1}) according to Bowen (1979), it was therefore considered suitable to be taken for comparison with the other polluted sites. Comparing our data with other studies (Armienta et al., 2008; Franco-Hernández et al., 2010; Morton-Bermea et al., 2014; Ruíz and Armienta, 2012; Santos-Jallath et al., 2012), it is clear that metal concentrations in the mining waste vary widely, which is associated with mining and smelting operations as well as the type of mineral resources (Bradshaw, 1997). Based on the results, it can be observed that the soils are a source of contamination that could affect the development of organisms that grow and live on them. Besides this, variations in the concentrations of contaminants in the tailings and soils surrounding them were observed. This shows the geochemical dynamic and the dangerousness of the contaminant dispersion to the environment.

Metal Contents in Plant Samples

Metal concentrations in *C. lindleyi* samples show that Pb (0.1–5 mg kg^{-1}), As (0.01–5 mg kg^{-1}), Zn (20–400 mg kg^{-1}), and Cd (0.1–3 mg kg^{-1}) in CZ were in the normal range in plants (Raskin, 1997). However, in the case of Cu (5–25 mg kg^{-1}) concentrations in stem and root the range exceeded the normal range. In CT and FT they surpass the normal range in plants (Raskin, 1997). In relation to the phytotoxic threshold proposed by Kabata-Pendias and Pendias (1992), Zn is surpassed in CT (breaching-leaves 597.44 mg kg^{-1}, stem 650.85 mg kg^{-1}, and root 1754.07 mg kg^{-1}) and FT (breaching-leaves 912.40 mg kg^{-1}, stem 962.99 mg kg^{-1}, and root 10786.47 mg kg^{-1}), only in one soil point in CS (root 1756.30 mg kg^{-1}) this is surpassed. Manganese threshold is surpassed only in breaching-leaves and root (815.05 mg kg^{-1}) collected in CT (472.19 mg kg^{-1}) and in FT breaching-leaves (352.78 mg kg^{-1}), stem (576.92 mg kg^{-1}), and root (2074.44 mg kg^{-1}). Higher metal concentrations were measured in FT with the following decreasing order: root > stem > breaching-leaves, which is common in most metal polluted sites (Table 2). According to the Tukey Test the mean values of the samples are not significantly different from each other. High concentrations of Pb, As Zn, Cu, Fe, and Mn were measured in *C. lindleyi* grown in FT with Cu and Zn reaching the highest values. Besides, the concentration in breaching-leaves of As and heavy metals is higher in plants collected from tailings than from soils. This could be due to the highest concentrations of mining waste in the area. This is reflected in the cellular structure of breaching leaves of *C. lindely* as observed in Figs. 1A and 1B.

Translocation factor

Copper translocation factor values were not above 1 or close to this value. However, results for other studied metals indicate that this element could be considered the most transferable in *C. lindleyi* (Table 3). In all cases, the values of TF were below 1, suggesting an exclusion strategy by metal immobilization in roots. In a study in China, Cu TF values lower than 1 were also observed in *Cynodon dactylon*, *Pterodium aquilinum*, *Poa annua*, *Cerastium caespitosum*, and *Conyza canadensis* (Wang et al., 2008), this was also observed in other studies as reported by Franco-Hernández et al. (2010), Ruiz and Armienta (2012), Santos-Jallath et al. (2012).

Bioaccumulation Factor

Bioaccumulation factor (BCF) values are low for most of the analyzed elements and indicate that in this species there is not hyperaccumulation because they did not reach values higher than 1 to be considered as hyperaccumulating. Wang et al. (2008) found very low bioconcentration values for Mn, Cd, Cu, and Zn, all of them under 1, and higher values in the translocation mainly for Cd (*Cynodon dactylon* with 6.72) and Cu (*Humuluas scandens* with 2.20) in plants that grow around mines. These studies indicate that there are plants species that tolerate high heavy metal concentrations in soils because they restrict their absorption and translocation towards the leaves. However, others absorb and accumulate actively in their biomass that requires a highly specialized physiology (Baker and Walter, 1990). The low BCF values indicate the suitability of these species for stabilizing mine tailings because they can prevent metals entering the ecosystem through the food chain and this is in relation to its biomass since most of the accumulating plants are of small size. Therefore, *C. lindleyi* is considered as a tolerant plant, and because of its features (tree), it is possible to consider it for its use in phytostabilization in zones close to mining wastes.

Structural Changes in Breaching-Leaves

Plants can be classified in relation to their exclusion, resistance, or accumulation mechanisms (Baker,

1981). Exclusion includes strategies to limit the absorption of metals and the restriction of metals transport towards the stems (De vos et al., 1991; MacFarlane and Burchett, 2000). There are several accumulation mechanisms such as phytochelatins accumulation, transport from roots to leaves, leaves vacuole compartamentalization, and epidermis hair accumulation (Hirata et al., 2005; Krämer et al., 2007; Küpper et al., 2001; Lavid et al., 2001; Pilon-Smits and Pilon, 2002; Pilon-Smits, 2005; Perrier et al., 2004; Psaras et al., 2000; Sagner et al., 1998).

At the level of the whole plant, tissue, or cell, a metal surplus, in general, is allocated to metabolically less active organs, tissues, or cell compartments (Ernst, 2006). In Ni and Zn hyperaccumulating plants, more than 60% of the heavy metals are localized in the apoplast cell walls (Yang et al., 2006) as it was observed in CT (Fig. 1A2). Zinc tolerance in plants is considered to be principally achieved by sequestration inside vacuoles (Marschner, 1995). However, in some species, Zn accumulation has also been observed in other symplastic (cytosol, chloroplast) and apoplastic (cell wall) compartments, as observed in CT and FT (Figs. 1A2 and 1C2).

Although there are extensive investigations about the performance of hyperaccumulating plants of heavy metals; the main focus of this study is the determination of their concentrations and distribution among stems and leaves, and their distribution in specific tissues (Figs. 1A2 and D3) (Rabier et al., 2008; Vollenweider et al., 2011a).

Zinc was accumulated mainly inside the vacuoles in zinc tolerant plants (Marschner, 1995). However, in some species, Zn accumulation has also been observed in symplast compartments (cytosol and chloroplasts) and apoplast (cell wall) (Vollenweider et al., 2011a). In this study, alterations were also observed in the leaf base (Fig. 1C3). This could be explained because in that zone, contaminant particles that alter the epidermis cell structure and neighbouring areas may accumulate. The observed cell alterations could be the effect of metals accumulation in the palisade parenchyma since structural alterations in the epidermis were identified (Figs. 1A2, A3, C3, and D3). The accumulation of starch grains in zones close to it was present (Figs. 1A2 and D3). The leaves fissures did not present cell alterations. Accumulation of starch grains was not observed in Figure 1E. The resin canal showed a cell compression in its structure.

These alterations could be caused by H_2O_2 superproduction that is probably induced by heavy metals that provoke changes in the oxidation state. In the palisade parenchyma a great accumulation of starch grains in the chloroplasts was observed (Fig. 1A2). Cellular injury by this type of mechanism is well-documented for iron (Halliwell and Gutteride, 1986; Imlay et al., 1988), copper (Li and Trush, 1993a,b) as well as other metals (Jones et al., 1991; Lund et al., 1991; Shi and Dalal, 1993; Shi et al., 1993).

The cell alterations coincided with the samples collected in CT (being these in the leaves base) (Fig. 1C3 versus A3). Such alterations consist in cell disorganization in the epidermis, hypodermis and part of the palisade parenchyma (Hermle et al., 2007) (Fig. 1C3). However, in some fissures this was not observed, but the loss of ergastric and cell content. In the leaf base a cell alteration consisting in a disintegration of the cell wall of the epidermis and hypodermis and affecting part of the palisade parenchyma was observed (Fig. 1C3). This could be due to the accumulation of the substrate in that area that is provoking such effects.

The compartmentalization and formation of metals complexes absorbed by the plants roots and their translocation by the sap flux towards aerial parts determine the plants tolerance towards heavy metals (Ernst, 2006; Haydon and Cobbett, 2007; Marschner, 1995; Rauser, 1999). This is regulated by the phytoextraction easiness of heavy metals by the plants (Siedlecka, 1995). That is why it is important to know the distribution of these metals in the aerial parts in order to understand the physiological responses of the plants to grow in a determined environment.

Elemental Mapping

As soon as the elemental mapping was conducted, it was found that this could be caused by differences in sample preparations as well as the cultivation conditions and the age of development of the analyzed tissue (Grovenor et al., 2006; Smart et al., 2007).

Zinc was found in abundance in all the study sites as well as in different plants organs (root, stem and breaching-leaves) (Fig. 3A3). Zinc is an essential micronutrient for plants specially located in the cells symplast (it goes from the cytoplast of one cell to the next through plasmodesmata) (Richter, 1993; Marschner, 1995). Zinc has a functional role (Marschner, 1995), previous research suggests a compartamentalization that includes cytoplasm and the nucleus (Rathore et al., 1972). Zinc traces are found in the form of: (a) free divalent cations in the xylem sap, (b) complexes with organic acids or other small organic molecules in the xylema and phloem, (c) functional elements or structural inside the proteins, and (d) structural components ribosome and cell membrane (contributing to its stability) (Marschner, 1995). Knowledge about the forms of unions of Zn in the plants organs is still poor; however, it strongly interacts with other mineral nutrients, mainly phosphorus (Boardman and McGuire, 1990; Marschner, 1995). The increase of Zn availability in contaminated soils (excess of Zn) could provoke damages (Ernst, 2006; Hermle et al., 2007) or even diminish the establishment of plants in the place (Boardman and McGuire, 1990; Ernst, 2006; Marschner, 1995). Besides, the excess of Zn is generally observed in the vacuole (Ernst, 2006; Marschner, 1995).

Furthermore, it is still little known about the effect of the excess of Zn in the cell wall or in trees. The high compartmentalization of Zn in the roots apoplast or driving tissues in the foliage could also affect the kind and frequency of its union. The compartmentalization and complexity of Zn excess are commonly singular features for each phytoextraction (Vollenweider et al., 2011a).

In most of the land ecosystems Cd has not a known function as a nutrient. Besides, it is toxic in very low concentrations (Schützendübel and Polle, 2002). Accumulation of Cd in plants affects the genetic expression (Kovalchuk et al., 2005), inhibiting DNA reparation (Banerjee and Flores-Rozas, 2005), it causes a reduction in photosynthesis, diminishes water and nutrients intake (Di Toppi and Gabbrielli, 1999) and results in visible symptoms such as chlorosis, plant stunt, spots in the tips of the roots and finally death (Kahle, 1993). The cellular deaths observed in Figure 1A3 could be correlated with the characteristics mentioned above. Schlegel et al. (1987) studied the response of spruce (*Picea abies*) seedling to Cd and found that this element diminishes the contents of water and chlorophyll as well as CO_2 fixation.

The distribution of the analyzed elements is in general uniform inside the tissues. It should be highlighted that Ni was widely present in the external part. However, inside the tissues its presence was minimal. This suggests that the accumulation of this element could be due to deposition factors (air) (Fig. 3B5 versus A5). Although by FRX chemical analysis Ni was not detected, possibly due to the detection limit of the technique, its observation by EDS shows its traslocation to the tissues.

It has to be remembered that the collected plants naturally colonized the mine spoils, so they were well adapted to extreme heavy metal concentrations in soil. Previous studies indicated that it was more difficult to introduce seedling or young plants to a highly toxic system than it was for older and better acclimated plants (Landberg and Greger, 1996; Mertens et al., 2004).

CONCLUSIONS

In the evaluated substrates, heavy metals that could be potentially dangerous and present in high concentrations were Zn, Mn, and Pb. They could limit the development of *C. lindleyi* plants that grow in the study sites. This heavy metal pattern was also present in the roots of *C. lindleyi*. However, high concentrations of Zn, Mn, and As were found in stem and breaching-leaves of *C. lindleyi*, demonstrating that elements that are translocated to the aerial parts are Zn and Mn, while translocation of Pb is restricted.

On the other hand, the tissues that showed more alterations or plant responses to the mining waste were observed in samples located in “La Concha” tailing. Most important structural changes, provoked by heavy metals in *C. lindley* breaching-leaves were: a high accumulation of starch grains and phenolic compounds, changes in the hypodermis cell wall, cell disorganization in the epidermis, hypodermis, and the palisade parenchyma. Trichomas showed ergastric content, discarding the idea that heavy metals could be accumulated in this structure. This is important because this allowed to determine that *C. lindleyi* showed a serious harm in the tissues, although it grow without visible damage in the polluted sites.

The distribution of Zn, Mn, and Pb observed in the internal and external tissues of the breaching-leaves could be related with the soil and tailings data determined by FRX analysis. Thus, *C. lindleyi* is a plant that can be adapted to environments with mining waste and that can accumulate heavy metals. *C. lindleyi* could be used as a tailings coverage aiding in this way to limit the dispersion of tailings to the surroundings. Because of its features it can be considered for its use in phytostabilization.

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3. CONCLUSIONES

1. Los desechos mineros favorecen la presencia de ciertas plantas que pueden tolerar y/o acumular metales pesados potencialmente tóxicos en sus diferentes órganos.
2. Se colectaron 32 especies vegetales que pertenecen a 30 géneros de 19 familias botánicas en las tres localidades. Las familias mejor representadas de estas tres localidades son: Fabaceae y Cupressaceae.
3. La concentración de Cu, Fe, Mn, Pb y Zn en los suelos y jal variaron entre las localidades con diferencias significativas en la fracción disponible.
4. Ninguna de las especies analizadas fue reconocida como hiperacumuladora de metales pesados potencialmente tóxicos.
5. Las especies *C. lindleyi* y *Ju. deppeana* acumularon Zn y Mn en concentraciones anormales en La Concha, donde el Zn se encuentra presente en la fracción soluble de los sustratos.
6. *Jacaranda mimosifolia* y *Psidium guajava* no mostraron concentraciones anormales en La Concha.
7. En las localidades de El Fraile y la zona control, las mayores concentraciones de Zn y Mn se encontraron en la fracción residual, siendo poco acumuladas en las especies vegetales.
8. La presencia de altas concentraciones de Zn y Mn en plantas adultas de *C. lindleyi* y *Ju. deppeana* mostraron que son especies interesantes para fitoestabilización en la zona minera de Taxco.
9. Se encontraron altas concentraciones de Zn, Mn y As en los tallos y ramillas de *C. lindleyi*. El Zn y Mn son translocados a las partes aéreas, mientras que el Pb es restringido.
10. Los tejidos que mostraron mas alteraciones en las plantas de *C. lindleyi* fueron los colectados en el jal de La Concha.
11. Los cambios estructurales mas importantes provocados por metales pesados en las ramillas de *C. lindleyi* fueron: alta acumulación de granos de almidón y compuestos

fenólicos, cambios en la pared celular hipodérmica, desorganización celular en la epidermis, hipodermis y en el parénquima en empalizada.

12. Los tricomas mostraron contenido ergástrico descartando la idea que los metales pesados podrían ser acumulados en esta estructura.
13. La distribución de los metales pesados en los tejidos de las ramillas fue homogénea para la mayoría de los elementos.
14. Estos resultados muestran que *C. lindleyi* es una especie que se puede emplear en fitoestabilización de zonas contaminadas con desechos mineros debido a que es una planta nativa que no requiere muchas condiciones para su desarrollo y que se puede adaptar a las prevalecientes en la zona.
15. Finalmente la importancia de este tipo de estudios radica en que constituyen una opción para la búsqueda de especies que eviten la dispersión de contaminantes en sitios con problemáticas similares.

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