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UNIVERSIDAD AUTÓNOMA DEL ESTADO DE MORELOS

FACULTAD DE CIENCIAS BIOLÓGICAS

ESTUDIO ECOTOXICOLÓGICO SOBRE LA BIOACUMULACIÓN DE METALES PESADOS EN DOS ESPECIES VEGETALES ASOCIADA A LOS JALES DE HUAUTLA, MORELOS

TESIS

PARA OBTENER EL GRADO DE

DOCTOR EN CIENCIAS NATURALES
PRESENTA:

MIGUEL SANTOYO MARTÍNEZ

DIRECTOR DE TESIS: DR. EFRAÍN TOVAR SÁNCHEZ

CUERNAVACA, MORELOS AGOSTO 2020

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– A mis padres –

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Santoyo-Martínez, M. 2020. Estudio ecotoxicológico sobre la bioacumulación de metales pesados en dos especies vegetales asociada a los jales de Huautla, Morelos. Tesis de Doctorado. Doctorado en Ciencias Naturales, Universidad Autónoma del Estado de Morelos. México.

RESUMEN

La minera es una actividad económica primaria, sin embargo, genera residuos denominados jales, los cuales pueden contener elementos potencialmente tóxicos (EPT), como metales pesados (MP). En México existen diversos sitios contaminados por estos elementos, un ejemplo de ello se localiza en el poblado de Huautla, municipio de Tlaquiltenango en el estado de Morelos, donde prevaleció la actividad minera hasta 1988 generando 780 mil toneladas de residuos mineros, donde se han registrado biodisponibilidad de metales y metaloides como el As, Cd, Cu, Fe, Mn, Pb y Zn, representando un riesgo potencial para el ambiente, por su capacidad de bioacumularse en la biota circundante. En este sitio se han establecido de forma natural diversas especies de plantas, desconociendo su capacidad de acumulación de MP. Por lo que, este estudio evaluó la capacidad de bioacumulación y el efecto del tiempo de exposición a MP sobre los cambios macro y micro morfológicos en *Vachellia campechiana* y *Crotalaria pumila* bajo condiciones de invernadero. Inicialmente se evaluó la germinación de semillas de las dos especies de estudio provenientes de sitios expuestos a MP (jales) y sitios control, los resultados obtenidos no mostraron efecto significativo del sitio, alcanzando una germinación >90% para ambos sitios de estudio. Se establecieron plantas en invernadero con las semillas provenientes de sitios control para ambas especies de estudio, las cuales crecieron en sustrato proveniente de la zona contaminada (jale) y sustrato testigo. Se detectaron cinco metales en tejido de raíz y tejido foliar en *V. campechiana* con un patrón de acumulación similar en ambos sustratos: Pb>Fe>Cr>Cu>Zn. Siendo el Cr, Cu y Pb los elementos que mayormente se translocaron a el tejido foliar. Por su parte *C. pumila* se detectaron cuatro metales con el siguiente patrón de bioacumulación en ambos tejidos: Fe>Pb>Cu>Zn, en donde el Cu fue el elemento que mayor se translocó hacia el tejido foliar. Así mismo, se evaluó el efecto de la morfología, observando que en los individuos de *V. campechiana* crecidos en sustrato expuesto a MP mostraron una disminución del 94% de los caracteres evaluados a través del tiempo en comparación con individuos en sustrato testigo. Por su parte en *C. pumila* el 75% de los caracteres evaluados mostraron una disminución significativa en individuos crecidos en sustrato expuesto respecto a los individuos creciendo en sustrato testigo.

Los resultados sugieren que *V. campechiana* es una especie con potencial para fitorremediar ambientes contaminados, debido a su capacidad de bioacumulación Cr, Cu y Pb en tejido de raíz (0.83 mg/kg, 0.37 mg/kg, 4.23 mg/kg, respectivamente) y tejido foliar (2.75 mg/kg, 0.38 mg/kg, 4.75 mg/kg, respectivamente). Mientras que *C. pumila* bioacumula Cu en ambos tejidos evaluados (0.46 mg/kg, 0.45 mg/kg, respectivamente), aunado a lo anterior, son especies eficientes para translocar estos elementos hacia la parte aérea de la planta, la cual no se ve comprometida la supervivencia en individuos crecidos en un sustrato expuesto a MP, ambas especies presentan una amplia distribución geográfica, presentan un alto porcentaje de germinación en semillas para su propagación y se establecen en ambientes alterados por actividades antropogénicas. Este estudio demuestra que la capacidad de bioacumulación de MP entre especies difiere, por lo que pueden ser consideradas para tratamientos que involucren múltiples especies para fitorremediar sitios contaminados por MP.

ABSTRACT

Mining is a primary economic activity, however, it generates waste called tailings, which may contain potentially toxic elements como metales pesados (HMs). In Mexico there are many sites contaminated by these elements. An example of this is located in Huautla, in the state of Morelos, where mining activity prevailed until 1988. It is estimated that there are approximately 780,000 tons of waste where, bioavailability of metals and metalloids such as As, Cd, Cu, Fe, Mn, Pb y Zn have been recorded, representing a potential risk to the environment, due to its ability to bioaccumulate in the surrounding biota. In this site, various species of plants have been established in a natural way, without knowing their ability to accumulate HMs. This study evaluated the bioaccumulation capacity and the effect of the exposure time to HMs on changes macro and micro-morphological in *Vachellia campechiana* y *Crotalaria pumila* under greenhouse conditions. Initially, the germination of seeds of the two study species from sites exposed to HMs (tailings) was evaluated and control sites, the results obtained did not show a significant effect of the site, with a germination of >90% for both study sites. Plants were established in a greenhouse with the seeds from control sites for both study species, which grew in substrate from the contaminated área (tailings) and control substrate. Five metals were detected in root tissue and leaf tissue of *V. campechiana* with a similar accumulation pattern in both substrates. Pb>Fe>Cr>Cu>Zn. Where Cr, Cu and Pb were the elements that most translocated to the leaf tissue. In C. pumila four metals with the following bioaccumulation pattern were detected in both tissues: Fe>Pb>Cu>Zn, where Cu was the element that most translocated towards the leaf tissue. Likewise, the effect of morphology was evaluated, observing that in the individuals of *V. campechiana* grown in substrate exposed to HMs, they showed a decrease of 94% of the characters evaluated over time in comparison with individuals in control substrate. On the other hand, in *C. pumila*, 75% of the evaluated characters showed a significant decrease in individuals grown in exposed substrate compared to individuals growing in control substrate.

The results suggest that V. campechiana is a species with potential to phytoremediate contaminated environments, due to its ability to bioaccumulate Cr, Cu and Pb in root tissue ((0.83 mg/kg, 0.37 mg/kg, 4.23 mg/kg, respectively) and leaf tissue (2.75 mg/kg, 0.38 mg/kg, 4.75 mg/kg, respectively). While C. pumila bioaccumulates Cu in both tissues evaluated (0.46 mg/kg, 0.45 mg/kg, respectively), In addition to the above, they are efficient species to translocate these elements towards the aerial part of the plant, which is not compromised survival in individuals grown in a substrate exposed to HMs, in addition to the above, they are efficient species to translocate these elements towards the aerial part of the plant, which is not compromised survival in individuals grown in a substrate exposed to HMs, both species have a wide geographic distribution, They present a high percentage of germination in seeds for their propagation and are established in environments altered by anthropogenic activities. This study demonstrates that the bioaccumulation capacity of HMs between species differs, so they can be considered for treatments that involve multiple for phytoremediation of environments contaminated by HMs.

INTRODUCCIÓN GENERAL

La minería y su desarrollo en México

La minería ha sido una actividad fundamental en el desarrollo económico en diversas partes del mundo. El origen de la minería se remota a los inicios de la humanidad, donde el hombre teniendo la capacidad de construir herramientas, ha ido perfeccionando las técnicas de extracción de minerales, además de reconocer las propiedades fisicoquímicas de los mismos como el oro y la plata (Coll-Hurtado *et al*., 2002). En la actualidad esta actividad sigue siendo de gran relevancia debido a la utilización de metales como insumos empleados en diferentes industrias (Osorio, 2012).

México, se caracteriza por presentar una alta riqueza minera debido a sus yacimientos y depósitos minerales, cuyo origen se remonta a su historia geológica, que dieron lugar a una gran riqueza mineral conformada por elementos metálicos como: plata, oro, plomo, cobre y zinc (Coll-Hurtado *et al*., 2002; Aguirre, 2012). Por lo anterior, la minería es una de las actividades con mayor tradición y contribución al desarrollo económico del país (Delgado de Cantú, 2003). Esta actividad, data de la época prehispánica en el siglo XI con la producción de oro y cobre, siendo los principales distritos mineros, los ubicados en Taxco, Pachuca, Guanajuato y Querétaro (Sariego, 1994; Canet *et al*., 2012). A partir del siglo XVIII, la actividad minera aumentó su intensidad, promoviendo el surgimiento de la mayoría de las ciudades coloniales (Barbosa-Ramírez, 1981).

En el 2016, el sector minero en México alcanzó un valor de producción de 272.3 mil millones de pesos, contribuyendo con el 4% del Producto Interno Bruto (PIB) (SE, 2016). Los principales minerales de producción fueron: oro (23.4%), cobre (17.2%), plata (17.2%), zinc (7.0%) y fierro (3.6%), que en conjunto representaron el 68% del valor total. Los principales estados productores del sector fueron: Sonora, Zacatecas, Chihuahua y Coahuila. Estas cuatro entidades aportaron en conjunto el 67.7% del valor total de la producción minera (SE, 2015; INEGI, 2015). Sin embargo, México destaca a nivel mundial en la producción de plata, oro, cobre, bismuto, arsénico, plomo, molibdeno y zinc, destacando en primer lugar la producción de plata, seguido por cadmio y plomo en cuarto lugar y molibdeno en quinto lugar (INEGI, 2015; SE, 2016).

Impacto de la minería

La minería a pesar de ser una actividad económica primaria genera una gran cantidad de desechos, principalmente en forma de gases, humos, partículas, aguas residuales y los denominados jales, también llamados relaves, colas o tailings, que se han generado en grandes cantidades como consecuencia de varios siglos de actividad minera (Gutiérrez-Ruiz *et al*., 2007).

Los residuos mineros son generados mediante un proceso de trituración y molienda de la roca que contiene los minerales de interés, son de consistencia fina con un tamaño de partícula ≤50 µm, los cuales contienen sulfuros metálicos como la arsenopirita (FeAsS), calcopirita ($CuFeS_2$), esfalerita (ZnS), galena (PbS), pirita (FeS_2) y pirrotita (FeS), fuente principal de los elementos potencialmente tóxicos (EPT) como los metales pesados (MP) o metaloides. Estos elementos presentan una densidad mayor a 5 g/cm^3, como el arsénico (As), cadmio (Cd), cromo (Cr), cobre (Cu), hierro (Fe), mercurio (Hg), manganeso (Mn), níquel (Ni), plomo (Pb), zinc (Zn) entre otros, que suelen ser tóxicos de acuerdo al metal y su concentración en el ambiente (Duffus, 2002; Cruz *et al*., 2004; Volke y Velasco, 2004; Armienta *et al*., 2005; Romero *et al*., 2007).

La peligrosidad de los jales radica en la disponibilidad de los MP hacia los sistemas biológicos, que está en función de factores fisicoquímicos, como el pH, índice de intercambio catiónico, y materia orgánica, que facilita su bioacumulación en el tejido y transferencia a través de las cadenas tróficas (biomagnificación), aunado a que tienen la característica de no biodegradarse, provocando efectos tóxicos en los organismos (Velasco *et al*., 2004; Velasco *et al*., 2005; Kim *et al*., 2015). Sin embargo, hay algunos MP esenciales como el Cu, Fe, y Zn que en elevadas concentraciones tiene la capacidad de provocar daños a los organismos, provocando efectos a nivel molecular o celular, hasta afectar escalas más grandes de organización biológica, como los ecosistemas (Gutiérrez y Moreno, 1995; Mussali-Galante *et al*., 2013). Por otro lado, algunos MP en bajas concentraciones como el As, Cr y Pb provocan efectos tóxicos en los organismos, debido a que no cumplen funciones biológicas (Furini, 2012).

No obstante, los jales son vertidos al medio ambiente sin ningún tratamiento previo que inactive o inmovilice los metales, los cuales son susceptibles a ser dispersados por acción del viento o en algunas ocasiones son colocados a un costado de cuerpos de agua como ríos o arroyos, lixiviándose, contaminado el agua y generando un riesgo latente tanto para la biota circundante como para la salud humana (Gutiérrez y Moreno, 1995; Marin-Guirao *et al*., 2005).

A pesar de que en México existe una gran cantidad de sitios contaminados por MP y residuos provenientes de la industria minera, no existe un inventario de la cantidad y situación de estos sitios (Romero y Gutiérrez-Ruiz, 2010). Se estima que el 65% de los residuos industriales en México provienen de esta industria (Carrizales *et al*., 2005).

Metales pesados y su toxicidad en plantas

Los elementos minerales se dividen en dos grupos: los nutrientes esenciales y los no esenciales o tóxicos (Loué, 1988). Los minerales esenciales incluyen a los macronutrientes como el nitrógeno (N), potasio (K), calcio (Ca), magnesio (Mg), fósforo (P), azufre (S) y silicio (Si), y los micronutrientes como el cloro (Cl), hierro (Fe), boro (B), manganeso (Mn), sodio (Na), zinc (Zn), cobre (Cu), níquel (Ni) y molibdeno (Mo). Éstos son los componentes esenciales del metabolismo y la estructura de la planta, su ausencia o deficiencia reduce e inhibe su crecimiento y funcionalidad (DalCorso, 2012).

Por otra parte, existen los minerales tóxicos, como el cadmio (Cd), mercurio (Hg), plomo (Pb), cromo (Cr), arsénico (As), plata (Ag) y antimonio (Sb), que afectan a los organismos, incluso en bajas concentraciones. El impacto de los MP en los organismos se debe a que una vez que éstos son absorbidos no se pueden eliminar, por lo que, el ingreso genera afectaciones en diferentes niveles de organización, como célula, tejido, órgano, sistema, etc. (Sanità di toppi y Gabbrielli, 1999; Furini, 2012).

Arsénico (As)

El As es un elemento que se distribuye ampliamente en la naturaleza (cerca de 0.0005% de la corteza terrestre). En el ambiente, se une a otros elementos como el oxígeno, cloro y azufre para formar compuestos inorgánicos, y en los organismos se combina con carbono e hidrógeno para originar compuestos orgánicos (ATSDR, 2007; Nordberg, 2014). El As puede ser liberado al ambiente por actividades naturales como el vulcanismo y la erosión de los depósitos minerales, y por actividades antropogénicas como: plantas generadoras de energía, utilización de pesticidas, herbicidas y por desechos mineros (Vahter, 1994). Los límites máximos permisibles de As de acuerdo con la NOM-147-SEMARNAT/SSA1-2004 en suelo no deben exceder los 22 mg/kg en suelo residencial y 260 mg/kg en suelo industrial.

El As es un elemento no esencial para las plantas, y en altas concentraciones interviene en los procesos metabólicos, inhibiendo el crecimiento y frecuentemente llevando a la muerte de la planta (Tu y Ma, 2005).

Cadmio (Cd)

El Cd es un MP considerado como uno de los elementos más tóxicos. Se encuentra ampliamente distribuido en la naturaleza asociado a plomo y zinc. A su vez, las actividades antropogénicas han contribuido en gran medida a su generación y dispersión, como el caso de la minería (Nordberg, 2014). Es uno de los metales más fitotóxicos, debido a que es altamente soluble en agua y rápidamente absorbido por las plantas (Balestrasse *et al.*, 2003). Por lo anterior, la norma mexicana NOM-147-SEMARNAT/SSA1-2004 establece que los niveles de Cd en suelo no deben exceder los 37mg/kg en suelo residencial y 450 mg/kg en suelo industrial.

A bajas concentraciones, el Cd es absorbido por las raíces de las plantas y transportado a las partes aéreas, teniendo un efecto negativo en la nutrición mineral, la homeostasis, el crecimiento y el desarrollo de la planta (DalCorso *et al*., 2010).

Cromo (Cr)

El Cr es un elemento poco común en la corteza terrestre, encontrándose naturalmente como un mineral denominado cromita. Las fuentes naturales de contaminación por cromo se deben a erupciones volcánicas, incendios forestales y restos vegetales, mientras que las fuentes antropogénicas se deben a su uso como insumo primario en diferentes industrias. Por ejemplo, para producir pigmentos, en el tratamiento del cuero, la conservación de madera, fabricación de acero, cromados, catalizador, incineración de basura, refinado de cromita, elaboración de cemento, desgaste de neumáticos y revestimiento de frenos, entre otros (Pazos-Capeáns, 2007; Nordberg, 2014). La norma mexicana NOM-147-SEMARNAT/SSA1-2004 establece que los niveles de Cr en suelo no deben excéder los 280 mg/kg en suelo residencial y 510 mg/kg en suelo industrial.

En plantas adultas, la toxicidad por Cr inhibe el crecimiento de brotes, reduce el número de hojas, así como el área de la hoja y su biomasa, causa quemaduras en los bordes de las hojas e induce clorosis y necrosis, así como inhibe el crecimiento de la raíz primaria (Prasad *et al*., 2001).

Cobre (Cu)

El Cu es un metal que se encuentra principalmente en forma de compuestos minerales ampliamente distribuido en el planeta, de importancia en la industria como la eléctrica debido a sus propiedades de conducción de electricidad (Nordberg, 2014). El Cu es un nutriente esencial para las plantas, actúa como un componente estructural en la regulación de proteínas y un cofactor en enzimas como citocromo c oxidasa y lacasa, participando en una variedad de procesos metabólicos, tales como señalización hormonal, el metabolismo de la pared celular y respuesta al estrés (DalCorso, 2012). Sin embargo, en elevadas concentraciones tiene un efecto tóxico, en donde la norma establece un límite máximo permisibles de 200mg/kg de Cu en suelo (Cleanup Standards for Contaminanted New Yersey Departament of Environmental Protection, 2007).

La toxicidad de Cu en plantas afecta su crecimiento, así como la reducción y desarrollo de raíces laterales, inhibe el funcionamiento del oxígeno, afectando el rendimiento fotosintético (Sabat, 1996; Maksymiec y Baszynski, 1999).

Hierro (Fe)

El Fe es el segundo metal más abundante y el cuarto de todos los elementos, superado únicamente por el oxígeno, el silicio y el aluminio. Este metal es utilizado para la fabricación de piezas de hierro y acero fundidos, así como en aleaciones con otros metales (Nordberg, 2014). Este elemento es un micronutriente en las plantas, con funciones en el transporte de electrones fotosintéticos, tolerancia al estrés oxidativo, respiración mitocondrial, fijación de nitrógeno, síntesis de hormonas y mantenimiento de organelos (Hänsch and Mendel, 2009). Sin embargo, resulta ser tóxico en elevadas concentraciones.

El Fe es un metal redox altamente reactivo que produce grandes cantidades de peróxido de hidrógeno y superóxido durante la reducción del oxígeno molecular. Por lo tanto, el exceso de Fe induce la formación de radicales hidroxilos que pueden provocar daños al ADN, proteínas, lípidos y azúcares. Un síntoma visual típico de toxicidad por hierro es el bronceado de las hojas debido a la acumulación de polifenoles (Becker y Asch, 2005). Además, se ve reducida la transpiración del agua y actividad fotosintética, viéndose afectado el contenido de clorofila en hojas (Chatterjee *et al*., 2006; Adamski *et al*., 2011).

Manganeso (Mn)

El Mn es uno de los elementos más abundantes de la corteza terrestre. Se encuentra en los sedimentos, las rocas, el agua y los productos biológicos (Nordberg, 2014). La utilización de Mn se ha incrementado en la industria química, en la producción de acero, así como en la utilización de sales de Mn como fertilizantes, además de que se genera como un desecho de las actividades mineras (Ducic y Polle, 2005). Sin embargo, en México no existen lineamientos que regulen los límites máximos permisibles para este elemento, tomando como referencia a la norma establecida por “Cleanup Standards for Contaminanted New Yersey Departament of Environmental Protection 2007)” establece un límite máximo permisible de 3,000 mg/kg de Mn en suelo.

El Mn es un elemento esencial en las plantas, actuando como un cofactor de diversas enzimas (Hänsch y Mendel, 2009). Sin embargo, el exceso de Mn, en plantas suele ser tóxico, induciendo efectos negativos como retraso del crecimiento, clorosis, hojas arrugadas, lesiones necróticas marrones y muerte en los casos más severos (Ducic y Polle, 2005).

Níquel (Ni)

El Ni es un elemento abundante en rocas, se encuentra en forma de minerales, combinado con azufre, oxígeno, antimonio, arsénico y sílice. Tienen diversos usos, en los que destacan la utilización para la fabricación de cintas magnéticas y componentes informáticos, prótesis quirúrgicas y dentales, baterías de níquel-cadmio (Nordberg, 2014). Al igual que otros metales pesados, las actividades antropogénicas como la minería, quema de combustibles fósiles, emisiones de gases vehiculares, y la fabricación y eliminación de baterías contribuyen a la liberación de elemento al ambiente. La norma mexicana NOM-147-SEMARNAT/SSA1-2004, establece que los límites máximos permisibles de Ni en suelo no deben exceder los 1600 mg/kg en suelo residencial y 20,000 mg/kg en suelo industrial.

Aunque el Ni es un nutriente esencial en plantas, las cantidades en exceso son tóxicas, los efectos se evidencian en la germinación de semillas, así mismo, se ve afectado el desarrollo de la raíz, el nivel de nitrógeno y fósforo y se ve afectado el proceso de la fotosíntesis. (Rao y Sresty, 2000; Bhardwaj *et al*., 2007; Gajewska y Sklodowska, 2008; Sharma *et al*., 2008).

Plomo (Pb)

El Pb es un metal que se encuentra en diversos lugares del mundo, siendo uno de los elementos más tóxicos, ya que puede contener otros metales tóxicos como el cadmio (Nordberg, 2014). La presencia de Pb en el ambiente se debe principalmente a actividades antropogénicas, como la industria minera y desechos industriales (Shannon, 1980; McLaughlin *et al*., 1999; Volke Sepúlveda *et al*., 2005). Por lo que, la norma mexicana NOM-147-SEMARNAT/SSA1-2004, establece que el Pb en suelo no deben exceder los 400 mg/kg en suelo residencial y 800 mg/kg en suelo industrial.

El efecto de la contaminación de Pb en plantas se ve reflejado en su tasa de crecimiento, mediante la inhibición de las células radiculares, además de que está implicado en el bloqueo de elementos esenciales (K, Ca, Mg, Mn, Zn, Cu y Fe), afectando la absorción de nutrientes y su productividad, que se ve reflejado en una disminución en su biomasa, talla y longitud en la raíz (Godbold y Kettner, 1991; DalCorso, 2012). Aunado a lo anterior, elevadas concentraciones de plomo afectan el estado hídrico de la planta, así como una reducción en su transpiración, inhibición en la síntesis de proteínas, daña e inhibe la fotosíntesis e inhibe el proceso de germinación de las semillas (Godbold y Kettner, 1991).

Zinc (Zn)

El zinc (Zn) se encuentra ampliamente distribuido en la naturaleza y constituye aproximadamente un 0.02 % de la corteza terrestre, la esfalerita, el principal mineral de zinc y fuente de al menos el 90% del zinc metálico, contiene otros metales como el hierro y cadmio (Broadley *et al.*, 2007; Nordberg, 2014).

La concentración de Zn en el ambiente se ha incrementado a través de actividades humanas como la minería, la fundición, revestimiento de piedra caliza, quema de combustibles fósiles y el uso de fertilizantes a base de fosfato (Nriagu, 1996). La norma establecida por “Cleanup Standards for Contaminanted New Yersey Departament of Environmental Protection 2007)” establece un límite máximo permisibles de 150mg/kg de Zn en suelo. Esta norma se toma como referencia debido a que en México no existen lineamientos que regulen los límites máximos permisibles para este elemento.

El Zn, es un elemento esencial que participa en diversos procesos de las plantas, como la activación enzimática, el metabolismo de proteínas, carbohidratos, lípidos y ácidos nucleicos, así mismo el Zn es un cofactor en diversas enzimas vegetales con funciones importantes en su metabolismo (por ejemplo, glutamato deshidrogenasa, anhidrasa carbónica, enzimas involucradas en el transporte de electrones y enzimas antioxidantes) (Chang *et al*., 2005; Sharma, 2006). Sin embargo, el exceso de Zn provoca toxicidad, la cual se ve reflejada en el enrojecimiento de las hojas, debido a la producción de antocianinas, inhibe el crecimiento de la raíz primaria y la aparición de raíces laterales, en casos severos se presenta clorosis y necrosis en las hojas (Harmens *et al.*, 1993; Fontes y Cox, 1995; Broadley *et al.*, 2007).

Movilidad y efectos de los metales pesados en plantas

El primer paso de entrada de los MP al ecosistema es mediante las plantas, debido a que están en contacto directo con suelo contaminado (Wenzel *et al*., 2003). Las plantas tienen la capacidad de adquirir elementos minerales del suelo, principalmente en forma de iones inorgánicos, siendo la raíz la estructura vegetal que tiene la capacidad para absorber estos compuestos, debido a que este órgano posee carga negativas en sus células (rizodermis), por la presencia de grupos carboxilos del ácido péctico, que interactúan con las cargas positivas de los metales, lo que facilita la entrada mediante un proceso de difusión en el medio y por intercambio catiónico (Wang y Chen, 2009). Una vez unidos los MP en la pared celular, se transportan en parte por vía apoplástica y en parte por la vía simplástica (Sanità di toppi y Gabbrielli, 1999; Gupta *et al.*, 2013).

La membrana plasmática constituye la frontera para el ingreso de los MP al interior de la célula, en donde existen diversos transportadores (ZIP, NRAMP, C_{tr}, de tipo primario ABC, CDF, y las ATPasas de tipo P) encargados de controlar el ingreso y salida de diversos metales, con un alto grado de especificidad (Manara, 2012). Por ejemplo, los transportadores ZIP son los principales encargados del ingreso del Zn al interior de la célula, así como del Co, Mn, Fe y Cd en menor proporción (Colanguelo y Guerinot, 2006), mientras que los transportadores NRAMP son los encargados del ingreso a través de la membrana de una amplia gama de iones metálicos como el Mn, Fe, Cd, Ni y Co (Nevo y Nelson 2006), mientras que el principal transportador de Cu es el C_{tr} (Puig y Thiele 2002). Por su parte los transportadores primarios ABC y CDF están implicados en la translocación de diversos MP como Zn, Co, Mn y Cd, desde el citoplasma al interior de organelos celulares como la vacuola y retículo endoplásmico o al exterior celular (Colanguelo y Guerinot, 2006; Krämer *et al*., 2007; Manara, 2012). Mientras que el transportador ATPasa de tipo *p* son los responsables del transporte interno principalmente del Cd y Zn hacia el xilema (Vögeli-Lange y Wagner, 1990; Colanguelo y Guerinot, 2006; Manara, 2012).

Una vez que los MP son translocados a la parte aérea de las plantas y se acumulan, utilizan un mecanismo al interior de la célula para detoxificar los MP, uniendo el metal con un ligando (quelación) como grupos sulfhidrilo, radicales amino, fosfato, carboxilo e hidroxilo, básicamente son aminoácidos, ácidos orgánicos, y más específicamente, dos clases de péptidos: fitoquelatinas y metalotioneinas (Rauser, 1995; Cobbett, 2000). Para esto el MP se rodea de los ligandos formando un complejo, de esta manera, el metal queda inmerso en una interacción química que mantiene en equilibrio electrónico, pero que no lo deja fuera del metabolismo, debido a que son trasladados a compartimentos celulares inactivos principalmente vacuolas, por lo que sigue siendo potencialmente tóxico (Yong-Eui *et al*., 2004; Yang *et al*., 2005; Rodríguez-Serrano *et al*., 2008; Lin y Aarts, 2012).

El resultado de estas uniones, ligando-metal, puede ser perjudicial para la célula causando diversas afectaciones como alteraciones a nivel proteico por inhibición o cambios en su estructura, desplazamiento de elementos esenciales de su metabolismo, la catálisis de reacciones de generación de especies reactivas de oxígeno (EROs) o radicales libres que provocan estrés oxidativo, los cuales pueden afectar al ADN provocando mutaciones, aberraciones cromosómicas, alteraciones en la síntesis y reparación de ácidos nucleicos, intercambios de cromátidas hermanas, formación de micronúcleos, oxidación y alquilación de bases nitrogenadas (Navarro-Aviño *et al.*, 2007; Valavanidis *et al*., 2009).

La raíz al ser el primer órgano vegetal que interactúa con los MP, responde disminuyendo su elongación, debido a que las células radiculares pueden presentar daño en sus organelos y pueden afectar la división celular, se ha documentado que el Cr y Pb inhiben el proceso de mitosis en las células de la raíz, reduciendo la extensión de este tejido (Bini *et al*., 2012; Furini, 2012). Así mismo, el exceso de metales esenciales como el Cu, Fe y Zn afectan el crecimiento de la raíz (Cuypers *et al.*, 2013). Por otro lado, la translocación de los MP hacia la parte aérea afecta diferentes estructuras de la planta que deriva en cambios macro-morfológicos, como la disminución de tallo y hojas, así como cambios a niveles micro-morfológicos como cambios en la cantidad de estomas y tricomas (Lukovic *et al*., 2012). Aunado a esto, se ha reportado que la presencia de MP inhibe el proceso de germinación de semillas, principalmente por MP no esenciales como el Cr y Pb, ya que estos MP al translocarse al interior de las semillas alteran una serie de procesos bioquímicos, como la disminución de las enzimas α-amilasa y β-amilasa, implicadas en el suministro de azúcar a los embriones en desarrollo, afectando la germinación, impidiendo se active este proceso (Seregin y Kozhevnikova, 2005; Singh *et al.,* 2013).

Con relación a los efectos en plantas por exposición a MP en zonas mineras se han realizado varios estudios. Salas-Salmerón (2007); Hernández-Lorenzo (2015); Santoyo-Martínez (2016); Castañeda-Bautista (2016); Rosas-Ramírez (2018) y Tovar-Sánchez *et al.* (2018), reportaron una disminución del 50-90% en caracteres macro y micro-morfológicos de *Zea mays* L. (Poaceae), *Sanvitalia procumbens* Lam. (Asteraceae), *Vachellia farnesiana* (L.) Wight & Arn. (Fabaceae), *Dodonea viscosa* Jacq. (Sapindaceae), *Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M.C. Johnst. (Fabaceae) y *Pithecellobium dulce* (Roxb.) Benth. Fabaceae, expuestas a MP como el Cd, Cr, Cu, Fe, Mn, Ni, Pb y Zn.

Especies acumuladoras de metales pesados, una alternativa para fitorremediar ambientes contaminados

Las plantas al ser expuestas a MP pueden presentar diferentes respuestas fisiológicas, las cuales varían dependiendo de la especie vegetal, el metal específico al que es expuesta y la concentración de éste en el suelo. En general, las plantas responden mediante dos estrategias: 1) la exclusión de MP, lo que permite el ingreso controlado de estos metales a la planta y su baja translocación hacia la parte aérea. 2) la acumulación de MP, donde se absorben los MP en mayor proporción translocándolos activamente hacia las partes aéreas (Brooks *et al.,* 1977; Baker, 1981; Capo, 2007). Actualmente, se han descrito 721 especies con un alto grado de acumulación de MP, destacando especies acumuladoras de Ni, Cu, Cd, Co, Cr, Mn, As, Pb y Zn, incluidas en 52 familias, las más representadas: Brassicaceae, Phyllanthaceae, Caryophyllaceae, Flacourtaceae, Asteraceae, Lamiaceae, Poaceae (Mahar *et al.*, 2016; Reeves *et al*., 2018).

La caracterización de plantas acumuladoras de MP ha sido una estrategia para implementarlas en procesos de fitorremediación de sitios contaminados, utilizando especies para la inmovilización y almacenamiento de MP en los tejidos vegetales como raíz y hoja (Volke-Sepúlveda *et al.,* 2005; Shiqi *et al.,* 2018). En este sentido, se han propuesto medidas para evaluar el potencial de diversas especies vegetales para almacenar MP, por ejemplo, el factor de bioconcentración (FBC), que determina la eficiencia de la planta para acumular el metal proveniente del suelo en su tejido y el factor de translocación (FT) que indica la eficiencia para transportar el metal de la raíz a su parte aérea (Olguín y Sánchez-Galván, 2012; Ali *et al.,* 2013; Covarrubias y Cabriales, 2017). En este sentido, Abhilash *et al.* (2009) reportaron a *Limnocharis flava* (L.) Buchenau (Limnocharitaceae) como una especie con una eficiencia de translocación de Cd, mientras que Yoon *et al.* (2006) documenta a *Cyperus esculentus* L (Cyperaceae), *Phyla nodiflora* (L.) Greene (Verbenaceae), *Rubus fruticosus* L (Rosaceae), *Sesbania herbacea* (Mill.) McVaugh (Fabaceae) como especies útiles para fitorremediar ambientes contaminados por MP por su capacidad de acumulación y translocación de Cu, Pb y Zn.

Debido a lo anterior, estudiar el comportamiento de acumulación de MP en especies que crecen de manera natural en ambientes contaminados por estos elementos, y en función de sus repuestas proponerlas para fitorremediar ambientes contaminados por MP. Por lo que la implementación de estudios *ex*-situ, como la propagación de especies en invernadero, puede ser útil debido a que se controlan las condiciones climáticas como luz y agua, así como depredación, competencia, ya que son un factor limitante en el desarrollo de la planta, con lo que se disminuye la mortalidad de plantas. Además de que se puede evaluar el proceso de la bioacumulación de MP y su efecto en la morfología a través del tiempo (Ghosh y Singh, 2005). En este contexto, Solís-Domínguez *et al*. (2012) y Gil-Loaiza (2016) evaluaron la acumulación de MP bajo condiciones de invernadero durante 40 meses en *Buchloe dactyloides* (Nutt.) Engelm. (Poaceae), quien acumulo Cu y Pb (18.5 mg/kg, 9.79 mg/kg, respectivamente), *Prosopis juliflora* (Sw.) DC. (Fabaceae), quien acumulo Cu, Pb y Zn (12.6 mg/kg, 6.21 mg/kg, 476 mg/kg, respectivamente) y *Acacia greggii* A. Gray (Fabaceae), que acumulo Cu y Zn (9.1 mg/kg, 642 mg/kg, respectivamente) sus resultados sugieren a estas especies útiles para fitorremediar ambientes contaminados por MP por su capacidad de acumulación a través del tiempo.

Por lo tanto, el conocer el comportamiento de bioacumulación de MP y sus efectos en la morfología de especies nativas, mediante su propagación en invernadero es de utilidad en proyectos de fitorremediación.

El caso de Huautla, Morelos

La minería en el estado de Morelos es principalmente no metálica produciendo arcilla, arena, basalto, grava, yeso y tezontle, no obstante, en la entidad también se presentó minería metálica, la cual se concentraba en el poblado de Huautla, perteneciente al municipio de Tlalquiltenango. Durante los siglos XVIII y XIX se explotaron de manera continua las minas: San Francisco, Santa Ana, Plomosa, Reforma, Ánimas y San Esteban (Velasco *et al.*, 2004; Martínez-Pacheco, 2008). Posteriormente, durante la década de 1950 la compañía “Exploradora de Minas, S.A.” explotó las minas de Tlalchichilpa, Santiago, Peregrina y Nueva Peregrina; así mismo, entre 1976 y 1988, la empresa “Rosario de México, S.A.” logró una producción entre 140 y 190 toneladas de Ag, Pb y

Zn diarias (Werre y Ortiz-Hernández, 2000; Velasco *et al.*, 2004). En la actualidad esta zona se encuentra inactiva, debido a la caída del precio de la plata en 1992; posteriormente, en 1999 se decretó a la Sierra de Huautla como área natural protegida federal (Reserva de la Biosfera Sierra de Huautla) (Werre y Ortiz-Hernández, 2000; Velasco *et al.*, 2004; Dorado *et al.*, 2005; SGM, 2006).

Se estima que en la región de Huautla existen 780 mil toneladas de jales abandonados, en donde se han reportado metales biodisponibles como el As, Cd, Fe, Pb, Zn, entre otros (Solís, 2016). Estos materiales se encuentran a la intemperie y al borde del Arroyo Chico, un arroyo de temporal que posteriormente se une con los arroyos Juchitlán, Salitre y Atlipa, para formar el Arroyo Grande que desemboca en el Río Amacuzac. Existe una gran probabilidad de que, durante la temporada de lluvias, estos residuos se lixivien hacia los cuerpos de agua cercanos y se transporten hacia otras regiones (Velasco *et al.*, 2004).

En estudios realizados por la SEMARNAT (2004, 2005) en conjunto con el Instituto Nacional de Ecología, se determinó que los jales de Huautla contenían elevadas concentraciones de Pb (hasta 3,340 mg/kg) y As (hasta 274 mg/kg), que rebasan los límites máximos permisibles propuestos por la NOM-147-SEMARNAT-2004 (SEMARNAT 2004) para As (22 mg/kg en suelo residencial y 260 mg/kg en suelo industrial) y para Pb (400 mg/kg en suelo residencial y 800 mg/kg en suelo industrial). Por otra parte, Solís (2016) reportó una elevada concentración de Fe (hasta 28.3 mg/kg), Zn (hasta 3.2 mg/kg), Pb (hasta 5,265 mg/kg), Mn (hasta 605.4 mg/kg), Cu (hasta 214.8 mg/kg) y Cd (hasta 48.6 mg/kg), en la base, parte media y en la superficie del jal. Asimismo, en los jales de Huautla se han registrado metales biodisponibles como el As, Cd, Cu, Zn, Pb, Fe y Mn, lo que facilita su bioacumulación en la biota circundante, lo que provoca afectaciones en diferentes niveles de organización biológica (SEMARNAT, 2004; SEMARNAT, 2005; Solís, 2016).

En los jales de Huautla se han realizado estudios donde se ha empleado el uso de multi-biomarcadores (exposición, efecto, susceptibilidad, permanentes y de efectos evolutivos) evaluando diferentes niveles de organización biológica para tener estudios ecotoxicológicos eficientes. Por ejemplo, Tovar-Sánchez *et al*. (2012) mediante el uso de biomarcadores de exposición y de efecto, encontraron que en mamíferos pequeños como *Peromyscus melanophrys* y *Biomys musculus* se bioacumulan elevadas concentraciones de As, Pb, Cd, Hg, Ni, Cu, y Al, provocando elevados niveles de daño genético (rompimientos de cadena sencilla). Por su parte, Mussali-Galante *et al.* (2013) con el uso de biomarcadores permanentes, utilizando microsatélites (marcadores moleculares), reportan pérdida de diversidad genética en las poblaciones de roedores de la especie *P*. *melanophrys* asociadas a los jales.

Además, se han realizado estudios utilizando biomarcadores que tiene efectos en la comunidad de invertebrados, donde han documentado que la diversidad y densidad de artrópodos y microartrópodos se ve reducida en un gradiente de distancia en dirección a un jal (González-Brito, 2015; Hernández-Gómez, 2015).

Por otro lado, Rebollo-Salinas (2019) realizó un estudio evaluando con un biomarcador de efecto en el ecosistema, la transferencia de MP en una cadena trófica, documentando que el productor primario *Z. mays* bioacumula Cu, Fe, Pb y Zn, transfiere estos elementos al consumidor primario (*Sphenarium purpurascens*) quien acumuló mayor cantidad Pb y Cu, mientras que el Fe y Zn se bioacumularon en bajas concentraciones. A su vez, estos elementos se transfirieron al consumidor secundario (*Neoscona oaxacensis*) observando un proceso de biomagnificación de MP con el siguiente patrón: Zn>Pb=Fe>Cu.

Con respecto a estudios en especies vegetales, Martínez-Becerril (2009) mediante biomarcadores con efecto en la comunidad vegetal asociada a los jales, encontró 19 especies contenidas en 10 familias, siendo la familia Fabaceae la más representativa. Asimismo, evaluó la riqueza de especies asociada a los jales, donde encontró un patrón en las formas de vida estudiadas (hierbas>arbustos>árboles). Por otra parte, se han realizado estudios por Hernández-Lorenzo (2014), Castañeda-Bautista (2106), Santoyo-Martínez (2016), Rosas-Ramírez (2018) utilizando biomarcadores de exposición, reportando bioacumulación de Cu, Pb y Zn en tejido foliar de *Prosopis leavigata*, *Pithecellobium dulce*, *Vachellia farnesiana* y *Sanvitalia procumbens*. Así mismo, observaron una reducción en los caracteres morfológicos en individuos asociados a los jales. Por su parte, Murillo (2015), Castañeda-Bautista (2106), Santoyo-Martínez (2016), con la utilización de un biomarcador de efecto (ensayo cometa), registraron un elevado nivel de daño genético en los mismos individuos de *P. leavigata*, *P. dulce*, y *V. farnesiana* que habitan en los jales de Huautla.

Especies de estudio

Se seleccionaron como especies de estudio a *Crotalaria pumila* y *Vachellia campechiana*, ambas pertenecientes a la familia *fabaceae*, con diferente forma de vida: *C. pumila* (herbácea) y *V. campechiana* (arbusto). Ambas especies presentan un amplio rango de distribución en el país, principalmente en el bosque tropical caducifolio, por lo que son especies que toleran la sequía y habitan ambientes alterados por actividades antropogénicas, como las producidas por la minería. Estos atributos sugieren que ambas especies pueden ser utilizadas como modelos para evaluar su capacidad acumuladora de MP, sus posibles efectos a nivel macro y micromorfológico y su potencial para ser utilizadas en propuestas de fitorremediación en zonas mineras.

Vachellia campechiana (Mill.) Seigler & Ebinger

V. campechiana es una especie conocida comúnmente como cubata. Son arbustos pequeños de hasta 4.5 m de altura, con espinas cóncavas, generalmente rojizas cuando jóvenes y pardas cuando maduras, en forma de cuchara de hasta 3.5 cm de longitud y 1.3 cm de ancho (Arce, 2000). Su fruto es una legumbre de 6.0-8.5 cm de largo, 0.7-1.1 cm de ancho, 2.5-5 mm de grosor, aplanada, indehiscente, sin márgenes evidentes, con septos de un tejido rígido, las valvas coriáceas, casi leñosas, de color pardo-rojizas. Las semillas miden 4-5 mm de largo, 3.5-4.5 mm de ancho y 2-3 mm de grosor, forma elipsoidal, de color pardo claro o amarillentas, sin presencia de un arilo. Las semillas presentan latencia física, sin embargo, cuando se rompe esta latencia el porcentaje de germinación llega a ser del 90% (Figura 1).

Es una especie de vegetación secundaria del bosque tropical caducifolio que se distribuye en lugares de clima cálido subhúmedo, a altitudes que van de 1,150 a 1,450 m s.n.m. Es una especie perennifolia, florece de mayo-agosto, fructifica de septiembre-mayo, obteniendo frutos maduros de marzo-mayo. Se aprovecha como combustible, postes para cercos y construcción; también sobresale por su uso como tutores y en la elaboración de implementos agrícolas; además es una especie forrajera y medicinal, usándose en problemas renales y estomacales (Rico, 2001; Cervantes y Sotelo, 2002).

Figura 1. Especie de estudio: *Vachellia campechiana A. Hojas. B. Flores. C. Frutos.*

Crotalaria pumila Ortega.

C. pumila es una hierba anual, conocida comúnmente como tronadora o chipil. Crece de forma erecta o ascendente, con tallos hasta 50 cm de alto. Hojas trifolioladas, folíolos 3.2-5 cm largo, lineares, elípticos u obovados, base cuneada o redondeada, ápice subredondeado. Su fenología de floración y fructificación se da de mayo a diciembre. Las flores están dispuestas en racimos, el fruto es una legumbre inflada de 15 mm de largo por 8 mm de diámetro, con semillas asimétricas reniformes, de 1.5-2.6 mm de largo y 1.7-2.8 mm de ancho de color café verdoso, verde amarillento o café (Figura 2). Es una especie originaria de Mesoamérica, que se distribuye desde el sur de los Estados Unidos hasta Sudamérica, en México está ampliamente distribuida, habitando bosques tropicales caducifolios, matorral xerófilo, encontrándose también en bosques de pino-encino y bosques perennifolios. Es una especie que tiene un uso principalmente alimenticio, sus cultivos se promueven con la finalidad de autoconsumo, comercializándose en ocasiones de manera regional (Martínez, 1979; Espinosa y Sarukhán, 1997; Soto-Estrada, 2004).

Figura 2. Especie de estudio: *Crotalaria pumila A. Hojas. B. Flores. C. Frutos.*

JUSTIFICACIÓN

La industria minera metalúrgica es una de las actividades económicas de mayor tradición en México y de gran aporte económico. Sin embargo, y como consecuencia de varios siglos de actividad, el procesamiento de los metales ha generado una gran cantidad de residuos (jales) que ejercen un efecto negativo sobre el ecosistema; debido principalmente, al alto contenido de EPT, como los MP. En particular, Huautla, Morelos es una entidad que se encuentra dentro de la Reserva de la Biosfera Sierra de Huautla (REBIOSH), que registró actividad minera y como consecuencia de ésta se generarón 780 mil toneladas de desechos con altos contenidos MP. Estos residuos permanecen a la intemperie sin tratamiento alguno, documentando la presencia de metales y metaloides biodisponibles como el As, Cd, Cu, Fe, Mn, Pb y Zn, reportando bioacumulación de estos elementos en diferentes organismos, como invertebrado, mamíferos pequeños, plantas e incluso la población humana. Con lo que se incrementa el riesgo de contaminar los recursos naturales de la zona, así como promover afectaciones a la flora y fauna circundante. *V. campechiana* y *C. pumila* son dos especies que presentan una forma de vida contrastante, habitan los jales de Huautla de forma natural, ambas especies presentan un amplio rango de distribución en el país y desempeñan un papel ecológico relevante en la estructura y funcionamiento de este ecosistema. Sin embargo, se desconoce el potencial de acumulación de MP de dichas especies y sus consecuencias a nivel macro y micromorfológico. En este estudio se pretende evaluar la germinación de semillas procedentes de sitios expuestos a MP, su bioacumulación de MP a través del tiempo, así como sus efectos en la morfología en individuos creciendo bajo condiciones de invernadero, con la finalidad de conocer su potencial como especies fitorremediadoras, lo que ofrecería una herramienta para mitigar problemas de contaminación por metales en suelo, aunado a generar información sobre los efectos en su germinación y crecimiento (a través del tiempo), siendo que son importantes en términos de adecuación.

HIPÓTESIS

Se hipotetiza que, si los jales de Huautla contienen metales pesados biodisponibles, entonces se espera que los individuos de *Vachellia campechiana* y *Crotalaria pumila* bioacumulen metales pesados en sus tejidos, que provocarán afectaciones en la repuesta germinativa de sus semillas, así como una reducción de sus caracteres morfológicos foliares.

OBJETIVOS

Objetivo general

El objetivo general de esta tesis es conocer la capacidad de *Vachellia campechiana* y *Crotalaria pumila* en condiciones de invernadero, para bioacumular metales pesados de muestras de jales.

Por otra parte, los objetivos particulares derivados del anterior son los siguientes:

1) Conocer el efecto de la procedencia (jales mineros y sitios testigos) de los individuos de *V. campechiana* y *C. pumila* sobre el porcentaje de germinación.
2) Determinar la concentración de metales pesados en raíz y tejido foliar de individuos de *V. campechiana* y *C. pumila* creciendo en tratamientos contrastantes (jal *vs*. suelo) en condiciones de invernadero.
3) Determinar el efecto de los metales pesados sobre la morfología foliar de *V. campechiana* y *C. pumila* creciendo en tratamientos contrastantes (jal *vs*. suelo) en condiciones de invernadero.
4) Conocer los niveles de bioconcentración y translocación de metales en tejido foliar de *V. campechiana* y *C. pumila* en individuos asociados a jales.

5) Con base en los niveles de bioconcentración y translocación de metales pesados y biomasa vegetal, proponer las especies que pueden ser utilizadas para fitorremediar los jales de Huautla.

De acuerdo con lo anterior, este trabajo de tesis incluye tres capítulos, en cada uno de los cuales se aborda una línea de investigación como se describe a continuación:

Capítulo 1

La bioacumulación de metales pesados y cambios morfológicos en *Vachellia campechiana* (Fabaceae) revelan su potencial para fitoextraxción de Cr, Cu y Pb en jales mineros

El objetivo de este capítulo fue conocer la capacidad de *V. campechiana* para bioacumular metales bajo condiciones de invernadero. Las preguntas que se plantaron para este capítulo fueron: 1) ¿Los individuos de *V. campechiana* creciendo en sustrato jale, biocumularán MP en tejido de la raíz y foliar?; 2) ¿El tiempo de exposición a MP es un factor que favorece los niveles de bioacumulación en el tejido (raíz y hoja) de *V. campechiana*?; 3) ¿La exposición a MP promueve cambios en los caracteres macro y micromorfológicos en individuos de esta especie?; 4) ¿Hay un efecto del sitio de procedencia de los individuos (testigo, expuesto) sobre los niveles de biocumulación de MP y germinación en semillas? y, 5) ¿La respuesta morfológica y de bioacumulación (raíz, hoja) de MP en individuos de *V. campechiana* por exposición crónica a jales mineros la hacen útil para fitorremediar ambientes contaminados?

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RESEARCH ARTICLE

Heavy metal bioaccumulation and morphological changes in *Vachellia campechiana* (Fabaceae) reveal its potential for phytoextraction of Cr, Cu, and Pb in mine tailings

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Abstract

Vachellia campechiana (Mill Seigler & Ebinger) is widely distributed in Mexico and is a dominant species of tailings in Huautla, in the state of Morelos, Mexico. Mining activities carried out in this region generated about 780 thousand tons of bioavailable heavy metal waste (HMs) that were deposited in the environment without any treatment. This study evaluates the bioaccumulation capacity and morphological changes of *V. campechiana* growing during 1 year in control or tailing substrates (treatments) under greenhouse conditions. The concentration of six HMs was also measured in roots, leaves, and seeds by atomic absorption spectrophotometry. Five metals showed a similar bioaccumulation pattern in the roots and leaves of *V. campechiana* grown in both substrates: Pb > Fe > Cr > Cu > Zn. The concentrations of Cr, Cu, and Pb were significantly higher in the roots and leaves of individuals growing on the exposed substrate. The presence of essential metals (Cu, Fe, Zn) was only recorded in the seeds, with similar concentrations in both treatments. Seventeen of 18 morphological characters evaluated in *V. campechiana* decreased in plants exposed to metals. Pb, Cu, and Fe showed a bioconcentration factor greater than one in roots and leaves. The translocation factor showed the following pattern: Cr > Cu = Pb. In conclusion, *V. campechiana* is a candidate species to phytoremediate environments contaminated with Pb, Cr, and Cu due to its ability to establish itself and turn into the dominant plant species in polluted sites, its ability to bioaccumulate non-essential metals in roots and leaves, and its high rate of HMs translocation.

Keywords Heavy metals · Mine tailings · Phytoremediation · Accumulator species · Translocation

Introduction

Mining generates waste in the form of gases, sewage, and/or tailings (Gutiérrez-Ruiz et al. 2007). As a result of the improper management of the waste derived from this activity, tailings have created environmental and health problems in Mexico (Mireles et al. 2012; Cortés-Jiménez et al. 2012; Mussali-Galante et al. 2013) due to their content of potentially

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toxic elements such as heavy metals (HMs), which harm living organisms by bioaccumulating in their tissues and damaging their genes and their ecosystems (Gutiérrez and Moreno 1995; Mussali-Galante et al. 2013).

The existence of plant species that tolerate high concentrations of metals in the soil has been reported before. These plants restrict the absorption of heavy metals and/or translocate them to the leaves, which allows them to maintain constant and relatively low concentrations of these pollutants in the aerial biomass, regardless of the concentration of metals in the soil (exclusion strategy). Other plant species actively absorb metals from the soil and accumulate them in non-toxic forms in the aerial biomass (accumulation strategy) (Brooks et al. 1977; Furini 2012; Marrero-Coto et al. 2012).

This differential response in the absorption of HMs present in the environment depends on the bioavailability of the metals, the retention capacity of the metals, the interaction between plants, roots, and metals, the plant metabolism, and the physicochemical properties of the soil, such as pH, organic matter content, and electrical conductivity (Kabata-Pendias 2000; Barceló and Poschenrieder 2003; Prieto et al. 2009; Rascio and Navari-Izzo 2011). It has been reported that the bioaccumulation of HMs by plants varies considerably across different taxonomic groups (Calow 1993).

Exposure to HMs can have various effects on plants, such as inhibiting seed germination, inhibiting root growth, causing alterations in the root biomass, hindering seedling development, causing micro-morphological alterations (stomata and trichomes), reducing the plant biomass (roots, stem, and leaves), and altering biochemical processes such as protein inhibition (Yadav 2010; Rengel et al. 2011; Tovar-Sánchez et al. 2019). Despite these adverse effects, some plant species have the ability to deal with the presence of HMs in the environment where they grow (Rascio and Navari-Izzo 2011). These species are known as accumulators and have the ability to establish themselves naturally in environments contaminated with HMs. They are fast growing species, with abundant aerial biomass, high capacity to absorb HMs from the soil, efficient translocation of HMs from root to leaf tissue, and high capacity to detoxify and retain large amounts of HMs in their leaf tissue (Rascio and Navari-Izzo 2011; Cappa and Pilon-Smits 2014; Shiqi et al. 2018). To determine if a species has these characteristics, it is necessary to evaluate how it responds to the effects of HMs when exposed to them.

The use of heavy metal accumulator plants has been a phytoremediation strategy in contaminated sites. These plants absorb the elements and remove them from mining waste, preventing their leaching to the water table. Phytoremediation uses plant species to immobilize and store HMs in different plant structures such as roots, stems, and leaves (Shiqi et al. 2018). Some indexes have been proposed in the literature that measure the capacity of plants to store HMs. For example, Yoon et al. (2006) proposed the bioconcentration factor (BCF) as a parameter that measures the efficiency of a plant species to accumulate metals from the soil in leaf tissue. They also proposed the translocation factor (FT), which indicates the efficiency with which metals are transported from the roots to the aerial parts of the plant. Using the latter index, Abhilash et al. (2009) reported that *Limnocharis flava* (L.) Buchenau (Limnocharitaceae) is an accumulator of cadmium (Cd).

About 450 species of plants have been recognized as accumulators of HMs, mainly Cd, nickel (Ni), copper (Cu), lead (Pb), and zinc (Zn) (Macnair 2003; Van der Ent et al. 2013). It is still important to characterize new plant species with the potential to bioaccumulate HMs and to be used in the phytoremediation of contaminated environments (Mendez and Maier 2008).

In Mexico, Huautla, located in the state of Morelos, was a mining area until 1988. It is estimated that there are in the area approximately 780,000 tons of waste in the form of tailings. These are rich in Pb, manganese (Mn), Cd, arsenic (As), Zn, Cu, iron (Fe), and chromium (Cr), all of which are bioavailable (Velasco et al. 2005; Mussali-Galante et al. 2013). Despite this, some species of plants, such as *V. campechiana* (Fabaceae), a shrubby species with different uses that is widely distributed in Mexico, mainly in arid and semi-arid areas, have established themselves in the Huautla area (Arce 2001; Rico 2001; Cervantes and Sotelo 2002; Armienta et al. 2008). Since it inhabits areas contaminated with HMs, *V. campechiana* could have characteristics that allow it to be considered as a plant with potential for phytoremediation; it has a high seed germination percentage, is fast growing, and has abundant leaf biomass. However, its possible role in the bioremediation of environments contaminated with HMs is still unknown. Therefore, the present study evaluated, under greenhouse conditions, the accumulation of HMs in the leaf and root tissue of *V. campechiana* individuals growing on a tailing substrate and a control substrate and the effect of this accumulation on seed germination and on the macro- and micromorphological leaf characters of the plants. The questions that guided this study were as follows: 1) Do individuals of *V. campechiana* growing on tailing substrate bioaccumulate HMs in root and leaf tissue? 2) Is the exposure time to HMs a factor that favors the bioaccumulation in root and leaf tissue of *V. campechiana*? 3) Does exposure to HMs promote changes in the macro- and micromorphological characters of this species? 4) Does the growing substrate (control, exposed) have an effect on the bioaccumulation levels of HMs and on seed germination? 5) Does the morphological and HMs bioaccumulation response (root, leaf, seeds) of *V. campechiana* plants chronically exposed to mine tailings make them useful for the phytoremediation of contaminated environments?

Methods

Study sites

This study was conducted in the municipality of Tlaquiltenango, in the state of Morelos. Mining activities were carried out in this area until 1988, mainly extracting Pb, Zn, and Ag. For decades, the waste generated from mining was deposited in the open, without any type of treatment. Three main tailings were formed, both of which are located in the town of Huautla, south of the municipality of Tlaquiltenango, Morelos, within the Sierra de Huautla Biosphere Reserve (REBIOSH). Tailing mine 1 (T1) is the largest and is located 500 m from the town (8° 26′ 36.37″ N and 99° 01′ 26.71″ W). Tailing mine 2 (T2) is located 1000 m from the town (18° 26′ 22.62″ N and 99° 01′ 51.71″ W). Tailings are rich in metals such as Pb, Mn, Cd, As, Zn, Cu, Fe, and Cr (Velasco et al. 2005). The following control sites were chosen: Quilamula (C1 site) located at 18° 30′ 52″ N and 98° 59′ 59″ W, at an altitude of 1100 m, and Ajuchitlán (C2 site), located at 18° 27′ 52″ N and 98° 58′ 53″ W, at an altitude of 1050 m. Both sites have very similar ecological and geographical characteristics to the exposed sites (Martínez-Pacheco 2008), but have no records of mining activity or anthropogenic metal contamination (Mussali-Galante et al. 2013). Both sites are more than 6 km in linear distance from the exposed sites. In general, Tlaquiltenango presents a natural richness of mineral soils (mainly sulfur minerals) of silver and lead. The most commonly found minerals are as follows: arsenopyrite (FeAsS), galena (PbS), acantite (Ag2S), and calclacita (Cu2S) (Volke et al. 2004, 2005; Secretaria de Economía 2011). Therefore, the soils of the region are naturally rich in minerals.

Study species

V. campechiana is a shrub-like species (Fabaceae) commonly known as "cubata," reaching up to 4 m in height, with concave spines, usually reddish when young and brown when mature, spoon-shaped, and up to 3.5 cm long and 1.3 cm wide (Arce 2001). Its fruit is white, flattened, indehiscent, and brown-reddish, with no obvious margins. Seeds of ellipsoidal shape were light brown or yellowish, without aryl (Arce 2001). The seeds show physical latency; however, when this latency is broken, the germination percentage reaches 90% (Baskin and Baskin 2004). It is a kind of secondary vegetation of tropical deciduous forests that inhabits places with sub-humid or dry climates, at altitudes ranging from 1150 to 1450 m. It is an evergreen species that blooms in May–August and produces fruit in September–May. It is used as fuel, as material for fence posts and construction, and as a growing tutor for other plants to make agricultural tools. It is also a forage and medicinal species, used for kidney and stomach problems (Rico 2001; Cervantes and Sotelo 2002).

Seed collection

Seeds of *V. campechiana* were collected from individuals established in the two control sites (C1 and C2) and in the two exposed sites (T1 and T2) (Fig. 1). So that the germination analysis encompassed the genetic variability of the species, the seeds from sites C1 and C2 were considered as control seeds, while the seeds from T1 and T2 were considered exposed seeds. At each site, 20 individuals were randomly selected and 20% of the seeds were collected from them (Gold et al. 2004). The seeds were transported to the laboratory, where they were cleaned and selected, removing the seeds parasitized by insects.

Germination of *V. campechiana* and plant growing

To evaluate the germination percentage, *V. campechiana* seeds from the control and the exposed sites were subjected to mechanical scarification due to the presence of physical latency. Twenty-five seeds were sown in a Petri dish with agar (1%), using six replicates per treatment. This assay was monitored for 20 days.

After the seeds germinated, 72 seeds were transplanted into individual nursery polyethylene bags (15 L) with the treatment substrates (36 in tailing substrate and 36 in control substrate). Soil from Quilamula was used as control substrate; it was sieved with a stainless steel sieve number 35 (Fiicsa), with a 0.5 mm mesh, in order to obtain a particle size similar to that of the mine tailing. The mixture of residues from tailings 1 and 2 was considered the exposed substrate. All the plants were kept under greenhouse conditions; they were watered twice a day, three times a week, and the temperature ranged from 32 to 35 °C. The plants obtained were used to evaluate the bioaccumulation of HMs and measure the macro- and micromorphological characters of interest.

Evaluation of morphological characters

To assess the effect of the time of exposure to HMs on the morphological characters of *V. campechiana*, six individual plants were randomly selected per treatment (control and tailing substrates). Six leaves were randomly chosen from each individual, and the foliar macro- and micromorphological characters shown in Table 1 were measured every 2 months of exposition to treatments, over 1 year. The macromorphological characters were measured with a digital vernier (Stainless Hardened) and a digital scale (Acculab). For the micromorphological characteristics, a foliar epidermal impression was done using a replication technique with cyanoacrylate glue. Three slides with epidermal impressions of the abaxial part of the leaf were made per each individual plant. The slides were observed with an optical microscope (Leica) at 40X with bright field illumination (CC) and differential interference contrast (CDI).

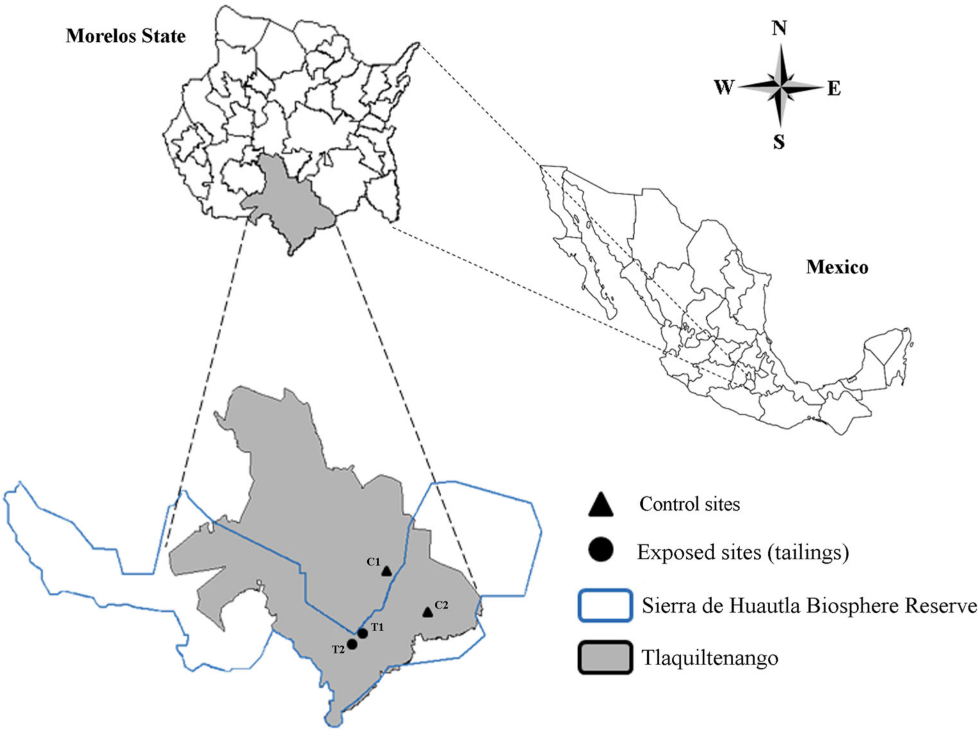

Fig. 1 Geographical distribution of the two study sites at the Sierra de Huautla Biosphere Reserve, Morelos, Mexico. Control site (black-filled up-pointing triangle). Exposed site (black-filled circle)

Three photomicrographs were taken at random from each slide. From the nine photomicrographs, an average of the number of stomata (E), epidermal cells (CE), and stomatal index (IE) was obtained for each individual plant. The stomatal index was calculated according to Salisbury (1968).

Table 1 Size and macro- and micro-morphological characters analyzed in *Vachelia campechiana*

Abbreviation	Character	Units
Size characters		
RL	Root length	cm
SL	Stem length	cm
FRB	Fresh root biomass	g
DRB	Dry root biomass	g
FLB	Fresh leaf biomass	g
DLB	Dry leaf biomass	g
Macro-morphological characters		
LBL	Leaf blade length	mm
WLB	Width of the leaf blade	mm
LP	Length of the petiole	mm
PD	Petiole diameter	mm
LIV	Length of the intermediate vein	mm
WIV	Width of the intermediate vein	mm
1/3AW	1/3 Apical width	mm
1/3 BW	1/3 Basal width	mm
LIL	Length of the intermediate leaflet	mm
WIL	Width of the intermediate leaflet	mm
CLB	Coverage of leaf blade	mm^2
Micro-morphological characters		
SI	Stomatal index	mm^2

Concentration of heavy metals in the substrate and plant tissue of *V. campechiana*

A total of 10 samples of the exposed substrate were analyzed to determine the concentration of metals (Cd, Cr, Cu, Fe, Mn, Pb, and Zn). The samples were dried and sieved following the method established by the Mexican standard NMX-AA-132-SCFI-2006. This process consists in adding 50 mL of CaCl2 (0.01 M) to 10 g of substrate. The sample is kept under stirring for 24 h and centrifuged at 1500 rpm for 15 min, recovering the supernatant by filtration. The concentration of metals in the substrate samples was determined by atomic absorption spectrophotometry using the flame method (GBC 908 A).

To evaluate the concentration of metals (Cd, Cr, Cu, Fe, Mn, Pb, and Zn) in the root and leaf tissue of *V. campechiana*, three samples were taken from six individuals per substrate (tailing and control), every 2 months of exposure to treatments over 1 year. To evaluate the concentration of metals in seeds of *V. campechiana*, three samples were taken from six individuals per substrate (tailing and control). An amount of 0.25 g of each plant structure were pulverized in containers previously washed with HNO_3.The samples were subjected to acid digestion in an Accelerated Reaction System Microwave (CEM® MARS-5) using 10 mL of HNO_3 (70%) in Teflon pumps. The samples were dissolved and filtered in distilled water to a final volume of 50 mL until further analysis. A sample without tissue was processed simultaneously and was used as a control. The metals were analyzed by atomic absorption spectrophotometry using the flame method (GBC 908 A). The spectrophotometer was calibrated using standard solutions and known concentrations for each metal analyzed. The minimum detection limits (mg/L) of Cd, Cr, Cu, Fe, Mn, Pb, and Zn were 0.0004, 0.003, 0.001, 0.0015, 0.0015, 0.01, and 0.0005, respectively. The samples from the exposed and the control sites were processed simultaneously and in triplicate.

Statistical analysis

A two-factor analysis of variance (model I fixed effects, Zar 2010) was performed to assess the effect of the site (control or exposed), the treatment (mechanical scarification or no treatment), and interaction site × treatment on the germination of seeds of *V. campechiana*. A Tukey test was performed to find significant mean differences between sites and between treatments (Zar 2010). Students' *t* tests were performed to evaluate the effect of the site of origin of the seeds (control and exposed) on their biomass and cover.

Two-factor analysis of variance was used to determine the effect of exposure time (60, 120, 180, 240, 300, 360 days), treatment (control and exposed), and the interaction time × treatment on the variations of 18 morphological characters (17 macro and one micro). Afterwards, a Tukey test was carried out to determine significant differences between pairs of average values of morphological characters in both treatments (Zar 2010).

The same analysis was carried out to evaluate the effect of exposure time, treatment (control and exposure), and interaction time × treatment on the accumulation of Cr, Cu, Fe, Pb, and Zn (except for Cd, which was not detected) in the roots and leaves of individuals of *V. campechiana*. A Tukey test was also used to determine significant differences in the average concentration of each metal over time by plant structure analyzed, for both treatments (Zar 2010).

The Mann Whitney *U* analysis was used to assess the effect of seed origin (control or exposed substrate) on the accumulation of HMs in the testa and embryo. All analyses were performed using the STATISTICA 8 program (StatSoft 2004).

The capacity of *V. campechiana* to phytoextract HMs was evaluated using two indices: the bioconcentration factor (BCF), which determines the efficiency of the plant in the accumulation of substrate metals in its tissue (Yoon et al. 2006), and the translocation factor (FT), which measures the efficiency of the plant in the transportation of metals from the root to the aerial parts (Yoon et al. 2006). These indices are calculated as follows:

$$\mathrm{FBC} = \mathrm{Cfoliar}/\mathrm{Ctailing}$$

$$\mathrm{FT} = \mathrm{Cfoliar}/\mathrm{Croot}$$

where Cfoliar is the concentration of the metal in the leaf tissue, Ctailing is the bioavailable concentration in the tailing, and Croot is the concentration of the metal in the root tissue. It has been reported that if a plant has FT values > 1, the species is considered an accumulator of the analyzed metal (Yoon et al. 2006; Covarrubias and Cabriales 2017).

Results

Germination and morphological characteristics of *V. campechiana* seeds from the control site and the exposed site (tailing mine)

The germination experiment showed that the site of origin of the individual plants (control vs exposed) did not influence the germination percentage of the seeds. In contrast, the pre-germinative treatment did significantly change germination percentages. There was no significant effect of the interaction site × treatment on germination percentages (Table 2). The results indicate that, considering both sites of origin (control and exposed), only 12% of the seeds germinated when they did not receive pre-germinative treatment. In contrast, when the seeds were subjected to mechanical scarification, the percentage of seed germination increased to more than 90% in both sites (Table 2).

The site of origin of the individual plants did have an effect on seed biomass and surface area. The results indicate that *V. campechiana* seeds from the site exposed to heavy metals showed statistically lower values for both characters analyzed compared with seeds from the control site (Table 3).

Table 2 Seedling percentage of *Vachelia campechiana* from the control and the exposed site, under pregerminative treatments

Site	Treatment	Seedling (%)	ANOVA	
Control	No scarification	12.00 a	Site (S)	$F_{1,20} = 0.57$, ns
	Mechanical scarification	98.67 b	Treatment (t)	$F_{1,20} = 530.03$***
Exposed	No scarification	12.67 a	S × t	$F_{1,20} = 0.57$ ns
	Mechanical scarification	99.33 b		

Different lower case letters denote significant differences between treatments (Tukey $p < 0.05$). Average ± e.e. *** = $p < 0.001$, ns = not significant differences

Table 3 Biomass (g) and coverage (mm^2) average (± e.e.) of *Vachellia campechiana* seeds from the control and the exposed sites

	Control		Exposed		Student *T*-test
Biomass	0.036 ± 0.0005	a	0.023 ± 0.0004	b	19.03***
Covarage	42.81 ± 0.40	a	37.26 ± 0.32	b	10.96***

Different lower case letters denote significant differences between sites*** = $p < 0.001$

Morphological and size changes in individuals of *V. campechiana* growing on tailing substrate or control substrate

In general, the results show that the time of exposure (t), the treatment (T), and the interaction between these two factors (t × T) had a significant effect on all the size and macro- and micromorphological characters analyzed during 360 days in individual plants of *V. campechiana* growing on a greenhouse under two different treatments (tailing substrate and control substrate). The treatment had no significant effect on the diameter of the petiole (DP), while the interaction between factors (t × T) had no significant effect on length (RL), dry biomass, and fresh root biomass (Table 4).

Size characters

All the evaluated characters of *V. campechiana* individuals grown on the control substrate showed a significant increase over time (360 days): root length (RL), stem length (SL), fresh root biomass (FRB), dry root biomass (DRB), fresh leaf biomass (FLB), dry leaf biomass (DLB). In contrast, in the plants grown on the tailing substrate, 50% of the characters (FRB, DRB, and DLB) showed a significant increase, while the remaining 50% of the characters (RL, SL, and FLB) showed no changes over time (Table 4).

The fresh and dried root biomass did not change significantly between both treatments, remaining unchanged in both treatments at the beginning and the end of the experiment. In contrast, the dry and fresh leaf biomass was significantly lower in plants grown on tailing substrate. In general, the RL did not change between treatments over time, while the SL was significantly lower in tailing substrate individuals (Table 4).

Macro-morphological characters

In the individuals of *V. campechiana* grown on the control substrate treatment, 54.5% of the evaluated characters had a significant increase over time (360 days): leaf blade length (LBL), petiole diameter (PD), length of the intermediate vein (LIV), leaf-shape characters such as the 1/3 apical width (1/3AW) and the 1/3 basal width (1/3BW), as well as coverage of leaf blade (CLB). Moreover, 27.3% of the characters decreased in size over time: width of the intermediate vein (WIV), length of the intermediate leaflet (LIL), and width of the intermediate leaflet WIL. The width of the leaf blade (WLB) remained more or less constant over time, while the length of the petiole (LP) showed oscillatory changes (Table 4). In the individuals established in the tailing substrate, 45.4% of the characters did not show changes over time: WLB, LP, WIV, WIL, and CLB. In contrast, 54.6% of the characters showed oscillatory changes over time: LBL, PD, LIV, 1/3BW, 1/3AW, and LIL (Table 4).

When comparing individuals from *V. campechiana* between treatments, the values of 63.6% of the analyzed characters decreased over time in the plants grown on the tailing substrate compared with the plants grown on the control substrate. Moreover, 27.3% of the characters showed an inverse pattern (WIV, LIL, and WIL), while the PD character did not change between treatments (Table 4).

Micro-morphological characters

With respect to micromorphology, the stomatal index (SI) decreased over time in individuals growing on the control substrate. Individuals growing on tailing substrate showed an inverse pattern. However, at the end of the treatment, there were no statistically significant differences in SI values between substrates (Table 4).

Heavy metal concentration in roots, leaves and seeds of *V. campechiana*

Roots

In general, the presence of Cr, Cu, Fe, Pb, and Zn was detected in the roots of individuals of *V. campechiana*, but Cd and Mn were not detected. The analysis of variance showed a significant effect of time (t), treatment (T), and interaction (t × T) on the bioaccumulation of four metals (Cr, Cu, Pb, and Zn) in the root of individuals of *V. campechiana*. In contrast, the aforementioned variables did not have a significant effect on the concentration of Fe in the roots (Table 5).

The bioaccumulation of Cr was only recorded in the roots of plants grown on tailing substrate, with a significant increase in the concentration with the time of exposure. The concentration of Cu in the roots was statistically higher in individuals growing in the control substrate, compared with those grown in tailing substrate. The concentration of Cu in the control substrate individuals remained constant over time, while plants growing in tailing substrate showed a significant increase in the concentration of Cu with the time of exposure. In general, there was no significant effect of time, treatment, and interaction (t × T) on the bioaccumulation of Fe. With respect to Pb, its concentration was significantly higher in

Table 4 Average (± e.e.) of macro- and micro-morphological characters from roots, stem and leaves from *Vachellia campechiana* growing for a year in greenhouse conditions on tailing substrate and reference substrate

Character	Time (days)	Treatment					ANOVA	
		Control		Exposed	SDT			
Size characters								
Root length								
	60	40.2 ± 2.7	a	35.9 ± 3.1	AB	ns	Time (t)	$F_{5,60} = 5.72$ ***
	120	40.7 ± 1.9	a	28.6 ± 1.2	A	***	Treatment (T)	$F_{1,60} = 30.89$ ***
	180	54.7 ± 3.7	ab	44.7 ± 5.4	B	ns	t × T	$F_{5,60} = 1.76$ ns
	240	50.9 ± 4.0	ab	39.4 ± 2.8	AB	ns		
	300	42.4 ± 3.0	a	36.4 ± 4.6	AB	ns		
	360	59.6 ± 4.7	b	36.2 ± 3.6	AB	**		
Stem lengh								
	60	42.2 ± 2.7	a	34.3 ± 3.9	A	ns	Time (t)	$F_{5,60} = 6.58$ ***
	120	59.5 ± 9.2	a	40.5 ± 5.6	A	ns	Treatment (T)	$F_{1,60} = 60.74$ ***
	180	55.2 ± 5.7	a	38.5 ± 3.8	A	*	t × T	$F_{5,60} = 4.48$ ***
	240	85.0 ± 8.2	b	45.2 ± 5.5	A	**		
	300	105.1 ± 6.9	c	42.7 ± 7.9	A	***		
	360	101.1 ± 17.3	c	36.8 ± 5.2	A	**		
Fresh root biomass								
	60	11.50 ± 9.29	a	5.86 ± 2.80	A	ns	Time (t)	$F_{5,60} = 16.80$ ***
	120	9.53 ± 3.99	a	6.15 ± 2.54	A	ns	Treatment (T)	$F_{1,60} = 13.67$ ***
	180	13.04 ± 7.78	a	5.46 ± 2.45	A	*	t × T	$F_{5,60} = 1.14$ ns
	240	40.3 ± 23.72	b	18.01 ± 4.65	B	*		
	300	35.19 ± 11.85	b	25.52 ± 13.69	B	ns		
	360	33.61 ± 8.19	b	29.37 ± 7.72	B	ns		
Dry root biomass								
	60	3.67 ± 2.35	a	2.21 ± 0.82	A	ns	Time (t)	$F_{5,60} = 20.05$ ***
	120	4.83 ± 2.07	a	3.08 ± 0.86	A	ns	Treatment (T)	$F_{1,60} = 21.95$ ***
	180	6.57 ± 3.87	a	3.05 ± 1.53	A	ns	t × T	$F_{5,60} = 2.48$ ns
	240	21.19 ± 11.39	b	8.03 ± 2.27	B	*		
	300	19.86 ± 6.13	b	12.56 ± 7.16	B	ns		
	360	17.80 ± 4.19	b	12.94 ± 3.26	B	ns		
Fresh leaf biomass								
	60	4.91 ± 1.46	a	3.20 ± 2.00	A	ns	Time (t)	$F_{5,60} = 6.51$ ***
	120	3.72 ± 1.46	a	1.19 ± 0.59	A	***	Treatment (T)	$F_{1,60} = 14.88$ ***
	180	3.55 ± 1.85	a	1.59 ± 1.35	A	ns	t × T	$F_{5,60} = 5.17$ ***
	240	12.83 ± 4.65	b	9.24 ± 3.14	B	**		
	300	39.64 ± 3.81	c	4.08 ± 1.47	A	**		
	360	14.04 ± 5.25	b	4.28 ± 0.94	A	**		
Dry leaf biomass								
	60	2.26 ± 1.13	a	1.27 ± 0.40	A	ns	Time (t)	$F_{5,60} = 5.43$ ***
	120	1.76 ± 0.50	a	0.64 ± 0.27	A	***	Treatment (T)	$F_{1,60} = 49.10$ ***
	180	1.53 ± 0.80	a	0.73 ± 0.62	A	ns	t × T	$F_{5,60} = 4.52$ ***
	240	5.84 ± 2.30	b	2.66 ± 0.67	B	**		
	300	7.84 ± 2.84	b	2.84 ± 2.33	B	**		
	360	6.75 ± 3.05	b	2.77 ± 0.43	B	**		
Macro-morphological characters								
Leaf blade lenght								
	60	47.38 ± 1.51	a	50.47 ± 2.59	A	ns	Time (t)	$F_{5,414} = 94.77$ ***
	120	42.34 ± 2.35	a	27.74 ± 2.10	B	***	Treatment (T)	$F_{1,414} = 258.79$ ***
	180	41.86 ± 2.71	a	19.64 ± 1.40	B	***	t × T	$F_{5,414} = 24.302$ ***
	240	75.72 ± 3.10	b	59.47 ± 2.33	C	***		
	300	79.59 ± 2.22	b	45.05 ± 1.33	D	***		
	360	80.54 ± 2.54	b	42.59 ± 1.89	D	***		
Width of the leaf blade								
	60	30.31 ± 0.47	ab	31.18 ± 1.63	A	ns	Time (t)	$F_{5,414} = 23.87$ ***
	120	27.25 ± 0.94	b	20.36 ± 0.98	AB	***	Treatment (T)	$F_{1,414} = 174.79$ ***
	180	31.35 ± 0.83	a	16.95 ± 0.61	AB	***	t × T	$F_{5,414} = 15.13$ ***
	240	36.51 ± 0.89	c	25.95 ± 0.58	C	***		
	300	32.88 ± 1.78	ac	27.60 ± 0.72	AC	***		
	360	31.98 ± 1.41	a	21.11 ± 0.73	B	***		
Length of the petiole								
	60	7.28 ± 0.26	a	8.95 ± 0.35	A	***	Time (t)	$F_{5,414} = 33.13$ ***
	120	6.27 ± 0.20	b	5.48 ± 0.38	B	ns	Treatment (T)	$F_{1,414} = 35.01$ ***
	180	7.63 ± 0.82	ad	4.91 ± 0.16	B	***	t × T	$F_{5,414} = 20.40$ ***

Table 4 (continued)

Character	Time (days)	Treatment					ANOVA	
		Control		Exposed	SDT			
	240	6.69 ± 0.18	d	6.02 ± 0.13	B	*		
	300	6.47 ± 0.21	d	5.70 ± 0.17	B	**		
	360	6.23 ± 0.23	b	4.69 ± 0.15	B	***		
Petiole diameter								
	60	0.54 ± 0.01	a	0.66 ± 0.03	A	ns	Time (t)	$F_{5,414} = 56.17$ ***
	120	0.43 ± 0.02	b	0.48 ± 0.03	B	ns	Treatment (T)	$F_{1,414} = 3.69$ ns
	180	0.49 ± 0.02	b	0.54 ± 0.03	BC	ns	t × T	$F_{5,414} = 6.36$ ***
	240	0.73 ± 0.02	c	0.70 ± 0.03	A	ns		
	300	0.61 ± 0.02	ac	0.58 ± 0.03	C	ns		
	360	0.63 ± 0.03	ac	0.57 ± 0.03	C	ns		
Length of the intermediate vein								
	60	15.73 ± 0.39	a	18.09 ± 0.57	A	**	Time (t)	$F_{5,414} = 53.79$ ***
	120	12.57 ± 0.43	b	10.12 ± 0.53	B	***	Treatment (T)	$F_{1,414} = 124.27$ ***
	180	14.57 ± 0.40	c	7.99 ± 0.24	C	***	t × T	$F_{5,414} = 23.79$***
	240	17.47 ± 0.40	d	14.48 ± 0.34	D	***		
	300	16.23 ± 0.54	d	13.87 ± 0.33	D	***		
	360	16.26 ± 0.71	d	10.95 ± 0.34	B	***		
Width of the intermediate vein								
	60	4.81 ± 0.12	a	4.95 ± 0.35	A	ns	Time (t)	$F_{5,414} = 14.88$ ***
	120	3.61 ± 0.12	b	5.48 ± 0.38	B	***	Treatment (T)	$F_{1,414} = 120.96$ ***
	180	4.16 ± 0.12	c	4.91 ± 0.16	B	ns	t × T	$F_{5,414} = 9.42$ ***
	240	3.67 ± 0.14	b	6.20 ± 0.13	A	***		
	300	3.48 ± 0.18	b	5.70 ± 0.17	B	***		
	360	3.30 ± 0.15	b	4.69 ± 0.15	B	***		
1/3 apical width								
	60	28.23 ± 0.70	a	28.54 ± 1.54	A	ns	Time (t)	$F_{5,60} = 24.32$ ***
	120	27.64 ± 0.88	b	18.83 ± 0.99	B	***	Treatment (T)	$F_{1,60} = 212.06$ ***
	180	29.64 ± 0.83	c	16.49 ± 0.57	B	***	t × T	$F_{5,60} = 16.17$***
	240	36.06 ± 0.90	d	25.02 ± 0.46	C	***		
	300	31.08 ± 1.03	c	27.97 ± 0.74	A	*		
	360	31.25 ± 1.23	c	21.03 ± 0.77	C	***		
1/3 basal width								
	60	27.09 ± 0.73	a	27.23 ± 1.63	A	ns	Time (t)	$F_{5,60} = 31.75$ ***
	120	22.83 ± 0.79	b	17.20 ± 0.99	B	***	Treatment (T)	$F_{1,60} = 225.14$ ***
	180	27.46 ± 0.76	ac	14.40 ± 0.47	C	***	t × T	$F_{5,60} = 14.37$ ***
	240	33.51 ± 0.77	d	23.61 ± 0.49	A	***		
	300	30.89 ± 1.09	cd	24.17 ± 0.50	A	***		
	360	30.13 ± 1.23	cd	18.48 ± 0.58	B	***		
Length of the intermediate leaflet								
	60	3.04 ± 0.08	a	3.28 ± 0.21	A	***	Time (t)	$F_{5,414} = 46.39$ ***
	120	2.39 ± 0.05	bc	2.74 ± 0.99	B	***	Treatment (T)	$F_{1,414} = 28.41$ ***
	180	2.68 ± 0.14	ab	2.18 ± 0.07	C	**	t × T	$F_{5,414} = 13.69$ ***
	240	2.14 ± 0.08	c	2.69 ± 0.08	B	***		
	300	2.19 ± 0.11	c	2.69 ± 0.09	B	***		
	360	2.25 ± 0.06	c	2.23 ± 0.04	C	ns		
Width of the intermediate leaflet								
	60	0.66 ± 0.03	a	1.46 ± 0.17	A	***	Time (t)	$F_{5,414} = 53.04$ ***
	120	0.51 ± 0.02	b	0.83 ± 0.02	B	***	Treatment (T)	$F_{1,414} = 387.34$ ***
	180	0.49 ± 0.02	b	0.59 ± 0.02	B	***	t × T	$F_{5,414} = 7.86$ ***
	240	0.47 ± 0.02	bc	0.76 ± 0.02	B	***		
	300	0.42 ± 0.02	bc	0.73 ± 0.03	B	***		
	360	0.39 ± 0.02	c	0.60 ± 0.03	B	***		
Coverage of leaf blade								
	60	383.38 ± 8.62	a	402.92 ± 17.6	A	ns	Time (t)	$F_{5,414} = 77.64$ ***
	120	343.39 ± 13.4	a	237.39 ± 14.5	B	***	Treatment (T)	$F_{1,414} = 302.93$ ***
	180	361.29 ± 16.5	a	180.56 ± 7.37	B	***	t × T	$F_{5,414} = 23.77$ ***
	240	553.85 ± 18.5	b	421.48 ± 13.2	B	***		
	300	555.00 ± 14.6	b	367.39 ± 8.86	B	***		
	360	555.25 ± 18.2	b	285.27 ± 13.1	B	***		
Micro-morphological characters								
Stomatal index								
	60	36.72 ± 0.61	a	23.66 ± 0.44	A	***	Time (t)	$F_{5,414} = 13.55$ ***
	120	34.19 ± 0.58	b	33.45 ± 0.69	B	ns	Treatment (T)	$F_{1,414} = 95.42$ ***

Table 4 (continued)

Character	Time (days)	Treatment					ANOVA	
		Control		Exposed	SDT			
	180	30.01 ± 0.53	c	32.59 ± 0.44	BC	***	t × T	$F_{5,414} = 47.25$ ***
	240	33.08 ± 0.53	b	29.21 ± 0.56	C	***		
	300	32.01 ± 0.62	bc	27.87 ± 0.43	C	***		
	360	30.31 ± 0.67	c	31.05 ± 0.67	C	ns		

Different lower case letters denote significant differences between control individuals during treatment time (Tukey $p < 0.05$)

Different upper case letters denote significant differences between exposed individuals during treatment time (Tukey $p < 0.05$)

SDT = statistical differences between treatments, *nd* = not detected, *ns* = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

the roots of the plants growing in tailing substrate compared with the plants growing in the control substrate. The concentration of Pb in individuals growing in the control substrate did not change significantly over time, while the roots of the plants growing in tailing substrate showed an increase in the concentration of Pb over time. Similarly, the concentration of Zn in the roots of the plants growing in tailing substrate was significantly higher than in the plants growing in the control substrate. The plants growing in the control substrate showed a significant reduction in the concentration of Zn over time; in the plants growing in the tailing substrate, the concentration remained constant (Table 5).

Leaves

In general, the presence of Cr, Cu, Fe, Pb, and Zn was detected in the leaves of individuals of *V. campechiana*, but the presence of Cd was not. The analysis of variance showed a significant effect of time, treatment, and interaction of factors on the concentration of Cr and Zn in the leaves of individuals of *V. campechiana*. Cu did not respond to the interaction of factors (t × T), while Fe did not respond to time of exposure or interaction (t × T), and Pb did not respond to time of exposure (Table 5).

The results indicate that the bioaccumulation Cr occurred only in the leaves of plants grown on the exposed substrate, with a significant increase in the concentration with the time of exposure. The concentration of Cu in leaf tissue was statistically higher in individuals growing in the control substrate compared with those grown in the tailing substrate. The concentration of Cu in the control substrate plants remained constant over time, while plants growing in tailing substrate showed a significant increase in Cu with time of exposure. Moreover, there was no significant effect of time and interaction of factors (t × T) on the bioaccumulation of Fe in leaf tissue. In contrast, there was a significant effect of the treatment on the bioaccumulation of Fe. The Tukey test ($P < 0.05$) showed significant differences in the concentration of Fe at 300 and 360 days, with higher concentrations in the plants growing in the control substrate.

With respect to Pb, its concentration was significantly higher in the leaves of plants growing in exposed substrate compared with plants growing in the control substrate. However, the concentration of this metal remained constant over time in plants growing in the control substrate. Moreover, the concentration of Pb showed a tendency to increase over time in the tailing substrate plants, it being significantly higher at 360 days.

Similarly, the concentrations of Zn in the leaves of plants growing in exposed substrate were significantly higher than in those growing in the control substrate. The concentration of Zn showed a significant decrease over time in the plants growing in the control substrate, while concentration remained constant (Table 5) in the plants growing in exposed substrate.

Seeds

Regarding the concentration of heavy metals in the seeds of *V. campechiana*, there was bioaccumulation of Cu, Fe, and Zn in the testa and embryo, but no bioaccumulation of Cr, Cd, and Pb.

There were no significant differences in the concentration of Cu and Fe between the testa and the embryo of the seeds from control sites, but there were significant differences in the concentration of Zn between these seed structures, with the highest concentration of this metal found in the embryo. The seeds from exposed sites did not show significant differences in the concentration of the three metals evaluated between the testa and the embryo. There were significant differences in the concentration of Cu and Zn in the seed testa between sites (control vs exposed), with Cu showing the highest concentration values in the testa of seeds from control sites. In the case of Zn, the highest values were found in the testa of seeds from the exposed sites. In the case of the embryo, no significant differences were found in the concentration of the metals evaluated in the seeds from both sites. Finally, no significant

Table 5 Average values (± e.e) of heavy metal concentration (mg/Kg) in roots and leaves of *Vachelia campechiana,* growing in reference substrate and tailing substrate during 1 year treatment

	Treatment									
	Root					Leaf				
Time (days)	Control		Exposed			Control		Exposed		
Chrome (Cr) 0.003 DL (mg/L)										
60	nd		0.55 ± 0.003	A	*	nd		0.92 ± 0.031	A	*
120	nd		0.54 ± 0.036	A	*	nd		1.01 ± 0.033	A	*
180	nd		0.68 ± 0.033	B	*	nd		1.24 ± 0.07	AB	*
240	nd		0.67 ± 0.097	B	*	nd		1.55 ± 0.027	B	*
300	nd		0.81 ± 0.030	C	*	nd		1.99 ± 0.083	C	*
360	nd		0.83 ± 0.032	C	*	nd		2.75 ± 0.156	D	*
ANOVA	Time (t)		$F_{5,60} = 4.87$ ***			$F_{5,60} = 23.40$ ***				
	Treatment (T)		$F_{1,60} = 880.11$ ***			$F_{1,60} = 726.21$ ***				
	t × T		$F_{5,60} = 4.89$ ***			$F_{5,60} = 23.40$ ***				
Cooper (Cu) 0.001 DL (mg/L)										
60	0.55 ± 0.010	a	0.25 ± 0.008	A	*	0.51 ± 0.011	a	0.34 ± 0.008	A	*
120	0.54 ± 0.011	a	0.26 ± 0.008	A	*	0.54 ± 0.004	a	0.35 ± 0.011	A	*
180	0.50 ± 0.030	a	0.29 ± 0.009	AB	*	0.55 ± 0.016	a	0.39 ± 0.008	B	*
240	0.51 ± 0.017	a	0.32 ± 0.009	BC	*	0.52 ± 0.005	a	0.35 ± 0.007	A	*
300	0.56 ± 0.012	a	0.34 ± 0.012	CD	*	0.52 ± 0.013	a	0.39 ± 0.006	B	*
360	0.54 ± 0.024	a	0.37 ± 0.012	D	*	0.53 ± 0.006	a	0.38 ± 0.003	B	*
ANOVA	Time (t)		$F_{5,60} = 7.26$ ***			$F_{5,60} = 4.54$ ***				
	Treatment (T)		$F_{1,60} = 853.62$ ***			$F_{1,60} = 734.84$ ***				
	t × T		$F_{5,60} = 2.73$ ***			$F_{5,60} = 2.06$ ns				
Iron (Fe) 0.005 DL (mg/L)										
60	2.62 ± 0.049	a	2.06 ± 0.252	A	ns	2.33 ± 0.141	a	1.37 ± 0.341	A	ns
120	2.68 ± 0.099	a	1.92 ± 0.162	A	ns	2.58 ± 0.189	a	1.90 ± 0.266	A	ns
180	2.65 ± 0.136	a	3.17 ± 0.800	A	ns	2.55 ± 0.187	a	1.96 ± 0.155	A	ns
240	2.75 ± 0.046	a	1.52 ± 0.379	A	ns	2.40 ± 0.052	a	1.68 ± 0.251	A	ns
300	2.38 ± 0.137	a	1.96 ± 0.195	A	ns	2.59 ± 0.126	a	1.68 ± 0.160	A	*
360	2.66 ± 0.088	a	2.00 ± 0.211	A	sn	2.47 ± 0.141	a	1.46 ± 0.218	A	*
ANOVA	Time (t)		$F_{5,60} = 0.62$ ns			$F_{5,60} = 0.53$ ns				
	Treatment (T)		$F_{1,60} = 3.22$ ns			$F_{1,60} = 20.80$ ***				
	t × T		$F_{5,60} = 0.66$ ns			$F_{5,60} = 0.15$ ns				
Lead (Pb) 0.01 DL (mg/L)										
60	1.49 ± 0.102	a	2.44 ± 0.132	A	*	1.49 ± 0.078	ab	3.74 ± 0.139	A	*
120	1.37 ± 0.176	a	3.22 ± 0.252	BC	*	1.47 ± 0.043	ab	3.53 ± 0.098	A	*
180	1.60 ± 0.022	a	2.83 ± 0.121	AB	*	1.44 ± 0.105	ab	3.80 ± 0.111	A	*
240	1.57 ± 0.035	a	3.32 ± 0.054	BC	*	1.45 ± 0.085	ab	3.88 ± 0.176	A	*
300	1.41 ± 0.137	a	3.64 ± 0.236	CD	*	1.72 ± 0.079	b	4.06 ± 0.117	A	*
360	1.44 ± 0.113	a	4.25 ± 0.146	D	*	1.25 ± 0.075	a	4.75 ± 0.185	B	*
ANOVA	Time (t)		$F_{5,60} = 3.60$ ***			$F_{5,60} = 2.23$ ns				
	Treatment (T)		$F_{1,60} = 199.92$ ***			$F_{1,60} = 564.25$ ***				
	t × T		$F_{5,60} = 4.70$ ***			$F_{5,60} = 3.97$ ***				
Zinc (Zn) 0.0005 DL (mg/L)										
60	0.13 ± 0.003	a	0.48 ± 0.038	A	***	0.28 ± 0.001	a	0.19 ± 0.001	A	***
120	0.19 ± 0.003	b	0.63 ± 0.094	B	***	0.16 ± 0.001	b	0.16 ± 0.013	A	ns
180	0.13 ± 0.003	a	0.68 ± 0.014	B	***	0.08 ± 0.0003	c	0.21 ± 0.020	A	***
240	0.09 ± 0.001	c	0.76 ± 0.056	C	***	0.12 ± 0.0009	d	0.15 ± 0.006	A	**

Table 5 (continued)

	Treatment									
	Root					Leaf				
Time (days)	Control		Exposed			Control		Exposed		
300	0.06 ± 0.002	c	0.47 ± 0.022	A	***	0.13 ± 0.002	d	0.19 ± 0.024	A	*
360	0.10 ± 0.0006	c	0.47 ± 0.015	A	***	0.11 ± 0.0009	d	0.21 ± 0.011	A	***
ANOVA	Time (t)		$F_{5,60} = 2.54$ *			$F_{5,60} = 7.29$ ***				
	Treatment (T)		$F_{1,60} = 166.52$ ***			$F_{1,60} = 12.51$ ***				
	t × T		$F_{5,60} = 2.00$ ns			$F_{5,60} = 8.37$ ***				

Different lower case letters denote significant differences between control individuals during treatment time

Different upper case letters denote significant differences between exposed individuals during treatment time

LD = detection limit, *nd* = not detected, *ns* = not significant

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

differences were found between sites in the concentration of Fe in the testa and embryo of seeds (Table 6).

The translocation factor and heavy metal enrichment in the roots and leaves of *V. campechiana* plants growing in substrate exposed to metals

Enrichment (BCF) of Cu, Fe, and Pb was recorded in the roots and leaves of *V. campechiana* plants growing in tailing substrate. In contrast, no enrichment of Cr and Zn was detected in any of the plant structures analyzed (Table 7). In roots, the metal enrichment factor showed the following pattern: Pb > Cu > Fe. In leaf tissue, the pattern of the enrichment factor was as follows: Cu > Pb > Fe (Table 7). The pattern of the translocation factor (FT) was as follows: Cr > Pb = Cu > Fe > Zn (Table 7).

Discussion

Bioaccumulation of heavy metals in *V. campechiana*

There are few studies that evaluate the transport and accumulation of metals in the seeds of wild plant species for ecotoxicological purposes. The present study found that *V. campechiana* seeds from both the control and the exposed sites accumulated only essential metals (Cu, Fe, and Zn) and that the concentration of metals in the embryo of seeds did not differ between sites. In contrast, the bioaccumulation of metals in the testa of seeds from the control site had a higher concentration of Cu and Zn. Our results are similar to those reported by Tyler and Zohlen (1998) for several herbaceous species, *Achillea millefolium* L. (Asteraceae), *Arctium tomentosum* Mill. (Asteraceae), *Arenaria serpyllifolia* L. (Caryophyllaceae), *Cerastium semidecandrum* L. (Caryophyllaceae), *Filipendula ulmaria* (L.) Maxim. (Rosaceae), *Hypericum maculatum* Crantz. (Hypericaceae), and *Laserpitium latifolium* (L.) (Apiaceae), the seeds of which bioaccumulate high concentrations of essential metals such as Mn, Cu, Fe, and Zn. Furthermore, the present study agrees with the study by Waters and Sankaran (2011), who reported an increase in the transport of micronutrients, mainly Fe and Zn, to the seeds of plants with nutritional potential.

It has been documented that the transport of minerals to the reproductive parts and to the seeds is carried out through the phloem (Zhang et al. 2007); in the leaves, it is carried out through the xylem. Recent studies have shown that the nicotianamine (NA) molecule transports various metals

Table 6 Average values (± e.e) of heavy metal concentration (mg/Kg) in testa and embryos of *Vachelia campechiana* seeds from the exposed and control sites

	Control		Exposed		*U* Mann Whitney Test	
	Testa	Embryo	Testa	Embryo	Testa	Embryo
Cu	0.40 ± 0.16	0.20 ± 0.10	0.0005 ± 0.00	0.06 ± 0.02	2.38 *	1.19 ns
Fe	0.66 ± 0.05	0.89 ± 0.15	0.56 ± 0.09	0.61 ± 0.03	0.57 ns	1.19 ns
Zn	0.30 ± 0.05	0.58 ± 0.03	0.80 ± 0.18	0.55 ± 0.13	− 2.78 **	− 0.66 ns

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, *ns* not significant

Table 7 Heavy metal enrichment (FBC) and translocation values (FT) of Cr, Cu, Fe, Pb, and Zn in roots and leaves of *Vachelia campechiana* from individuals growing on tailing substrate during treatment

	Concentration (mg/Kg)					
Time (days)	Tailing	Root	Leaf	FBC root	FBC leaf	FT
Chrome (Cr)						
	–	0.55	0.92	–	–	1.67
	–	0.54	1.01	–	–	1.87
	–	0.68	1.24	–	–	1.82
	–	0.67	1.55	–	–	2.31
	–	0.81	1.99	–	–	2.46
	–	0.83	2.75	–	–	3.31
x̄ ± d.e						2.24 ± 0.61
Copper (Cu)						
	0.04	0.25	0.92	6.25	23.00	1.36
	0.04	0.26	1.01	6.50	25.25	1.35
	0.04	0.29	1.24	7.25	31.00	1.34
	0.04	0.32	1.55	8.00	38.75	1.09
	0.04	0.34	1.99	8.50	49.75	1.14
	0.04	0.37	2.75	9.25	68.75	1.03
x̄ ± d.e				7.62 ± 1.16	39.42 ± 17.37	1.22 ± 0.15
Iron (Fe)						
	0.4	2.06	1.37	5.15	3.43	0.67
	0.4	1.92	1.90	4.80	4.75	0.99
	0.4	3.17	1.96	7.93	4.90	0.62
	0.4	1.51	1.68	3.80	4.20	1.11
	0.4	1.96	1.68	4.90	4.20	0.86
	0.4	2.00	1.46	5.00	3.65	0.73
x̄ ± d.e				5.28 ± 1.55	4.34 ± 0.49	0.83 ± 0.19
Lead (Pb)						
	0.23	2.44	3.74	10.61	16.26	1.53
	0.23	3.32	3.53	14.43	15.35	1.06
	0.23	2.83	3.80	12.30	16.52	1.34
	0.23	3.22	3.88	14.00	16.87	1.20
	0.23	3.64	4.06	15.83	17.65	1.12
	0.23	4.25	4.75	18.48	20.65	1.12
x̄ ± d.e				15.08 ± 2.31	17.41 ± 1.99	1.23 ± 0.18
Zinc (Zn)						
	2.14	0.48	0.19	0.22	0.09	0.40
	2.14	0.63	0.16	0.29	0.07	0.25
	2.14	0.68	0.21	0.32	0.10	0.31
	2.14	0.76	0.15	0.36	0.07	0.20
	2.14	0.47	0.19	0.22	0.09	0.40
	2.14	0.47	0.21	0.22	0.10	0.45
x̄ ± d.e				0.27 ± 0.05	0.086 ± 0.01	0.33 ± 0.09

through the phloem, including Cu, Fe, and Zn, towards reproductive structures, including seeds (Grillet et al. 2014). This suggests that *V. campechiana* bioaccumulates and translocates essential metals such as Cu, Fe, and Zn, since these metals participate in the development of seeds and seedlings when germination begins (Stacey et al. 2008; Grillet et al. 2014).

All the analyzed metals (Cu, Fe, Zn, Cr, and Pb) bioaccumulated in the root and leaf tissue of

V. campechiana, except for Cd. Three of these metals are considered essential (Cu, Fe, and Zn); the other two are considered non-essential (Cr and Pb). The accumulation pattern was as follows: Pb > Fe > Cr > Cu > Zn. It was also found that the accumulation of metals, mainly Cr and Pb, was greater in plants growing on exposed substrates than in control plants. This coincides with what has been found in other phylogenetically related plant species, such as *Acacia robeorum* Maslin. (Fabaceae), which has been reported to bioaccumulate metals such as Al, Fe, Mn, Cu, Zn, and Mo. These results suggest that the bioaccumulation of HMs in the leaf tissue of plant species inhabiting sites contaminated with heavy metals could be a detoxification strategy (He et al. 2012).

In the present study, only plants growing in substrate exposed to HMs accumulated Cr in root and leaf tissue, the latter being where Cr accumulated in greater amounts. The absorption of Cr through the roots may be due to the union of organic acids with the insoluble metals found in the soil, making them available for plant absorption (Panda and Choudhury 2005). The accumulation of Cr occurs when it is immobilized in vacuoles in the root cells, which makes it less toxic to the plant (Shanker et al. 2005). Moreover, the high concentration levels of Cr in the leaf tissue of *V. campechiana* is supported by the study conducted by Skeffington et al. (1976) on the *Hordeum vulgare* L. (Poaceae) species. The authors describe that Cr can enter the aerial parts of the plant through molecules that transport essential elements such as Fe, S, and P and that it can be translocated to the leaves through the xylem.

With respect to Pb, the present study showed that its concentration in root and leaf tissue increased over time in plants growing on tailing substrate, with the highest concentration found in the leaves. Similar results have been described for other shrub-like species similar to *V. campechiana*; for example, *Brickellia veronicifolia* (Kunth) A.Gray. (Asteraceae) accumulates Cd, Cu, Ni, and Pb in its leaf tissue, with Pb showing the highest concentration (Hernández-Acosta et al. 2009). Salas-Luévano et al. (2009) found high concentrations of Pb in the leaf tissue of *Buddleja scordioides* Kunth. (Scrophulariaceae), *Mimosa aculeaticarpa* Ortega. (Fabaceae), and *Acacia schaffneri* (S.Watson) F.J.Herm. (Fabaceae). This could be due to the fact that Pb inhibits the transport of essential metals such as Cu, Fe, and Zn (Patra et al. 2004), which increases in concentration over time and thus its translocation to the aerial parts of the plant.

In *V. campechiana*, low concentration levels of Cu, Fe, and Zn were found in the root and leaf tissue. The explanation of these results could be that the absorption of Pb replaces that of Cu, Fe, and Zn, probably modifying the activity and permeability of the membranes, making them unavailable for absorption and transport within the plant (Patra et al. 2004).

Changes in the morphological characters of the roots, stems, and leaves of *V. campechiana* due to exposure to heavy metals

Most ecotoxicological studies analyze the effect of metals on the morphology of herbaceous species, with few studies focusing on shrub- or tree-like plants. In addition, most of these studies focus on three morphological characters: root length, root biomass, and leaf biomass (Prasad et al. 20011; Maldonado-Magaña et al. 2011). The development of effective phytoremediation strategies for contaminated sites requires the study of plant species with different life forms. Herbaceous life forms, for example, have a short life cycle that can last up to 24 months; shrubs and trees, by contrast, have a long life cycle that can last several years, and this influences their efficiency in the accumulation of HMs.

In the present study, seventeen of 18 morphological characters of *V. campechiana* decreased in plants exposed to HMs with respect to control plants. These results show that the HMs interfere in the plant growth, causing a reduction in the *V. campechiana* growth. Similar results were reported by Tovar-Sánchez et al. (2018), who observed a decrease in the morphological values of the roots and leaves of *Zea mays* L. (Poaceae) associated with sites contaminated by tailings in Taxco de Alarcón, GRO, Mexico. Furthermore, Hernández-Lorenzo (2015) found a decrease in the leaf morphological values of *Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M.C.Johnst. (Fabaceae) associated with tailings in Huautla, MOR, Mexico. In other shrub-like species [*Salix viminalis* L. (Salicaceae), *Caesalpinia pulcherrima* (L.) Sw. (Fabaceae), *Albizia lebbeck* (L.) Benth. (Fabaceae), *Acacia holosericea* Cunn. ex G.Don. (Fabaceae), *Leucaena leucocephala* (Lam.) de Wit. (Fabaceae) and *Vachellia farnesiana* (L.) Wight & Arn. (Fabaceae)] exposed to Cr and Pb, there have been reports of a decrease in root length and leaf biomass (Panda and Patra 2000; Prasad et al. 2001; Iqbal et al. 2001; Suseela et al. 2002; Shanker 2003; Shanker et al. 2005; Maldonado-Magaña et al. 2011). Some studies have reported that the reduction in the plant morphological characters may be due to the fact that HMs could interfere with metabolic processes and are potentially toxic. Resulting in growth abnormalities (Bini et al. 2012) as a weak plant growth, yield depression, and may be accompanied by disorders in plant metabolism such as reduction of the meristematic zone (Maleci et al. 2001). In addition, other studies have found that nonessential HMs such as Cr and Pb inhibit the mitosis process in root cells, reducing the extension of this tissue. For example, it has been reported that nonessential HMs such as Cr and Pb inhibit the mitosis process in root cells, reducing the extension of this tissue. Prasad et al. (2001) reported a toxic effect of Cr, which inhibited primary root growth and suppressed the growth of new secondary roots. Studies conducted by Shanker et al. (2005) found that Cr inhibits root growth in *Caesalpinia pulcherrima* (L.) Sw. (Fabaceae), *Triticum aestivum* L. (Poaceae), and *Vigna radiata* (L.) R.Wilczek. (Fabaceae) Furthermore, the absorption of Pb causes a decrease in the rate of root growth and affects the root branching pattern (Maldonado-Magaña et al. 2011). For example, in *Picea abies* (L.) H.Karst. (Pinaceae), the development of secondary roots is particularly sensitive to exposure to Pb (Godbold and Kettner 1991; DalCorso 2012).

Changes in the leaf morphology of plant species that are exposed to pollution with HMs can be explained by the biochemical changes that form part of the adaptive mechanisms developed by plants to tolerate or bioaccumulate these metallic elements in their tissues (Meharg 1994). The bioaccumulation of HMs in leaf tissue triggers a detoxification mechanism in which metals bind to a ligand (chelation) such as sulfhydryl, phosphate, carboxyl, and hydroxyl groups and to peptides such as phytochelatins and metallothioneins (Rauser 1995; Cobbett 2000). The HMs are surrounded by ligands, forming a complex that is immersed in a chemical interaction that keeps it in electronic balance while it is transferred to inactive cell compartments, mainly vacuoles (Yong-Eui et al. 2004; Yang et al. 2005; Rodríguez-Serrano et al. 2008; Lin and Aarts 2012).

In the present study, the stomatal index decreased in individuals of *V. campechiana* grown in substrate exposed to HMs. Similar results were reported for *P. laevigata* exposed to HMs (Hernández-Lorenzo 2015). Thakur (1990) mentioned that the decrease in the number of stomata per unit area (mm^2) prevents excessive perspiration in plants and increases stomatal resistance. Although few studies have focused on the stomata of plants exposed to metals, it has been reported that occlusive cells are sensitive to chemical stress, so that the position and number of stomata can change or a mechanism for fall of leaves could be developed to protect the plant against the effects of metals (Zimmermann 2001).

With respect to the seeds of *V. campechiana*, the size and biomass of the seeds was smaller in the sites exposed to metals. However, germination was not affected, with similar values in both treatments. This could be due to the fact that seeds from sites exposed to HMs only accumulated essential elements (Cu, Fe, and Zn), which are involved in the germination process and the development of seedlings.

Potential use of *V. campechiana* as a phytoremediation species in sites contaminated with metals

V. campechiana is a shrub species that is distributed in disturbed places such as sites contaminated by HMs. This species commonly shares its habitat with *V. farnesiana*, a taxonomically close species that has been reported to accumulate HMs as As, Cu, Mn, Ni, Pb, V, and Zn (Maldonado-Magaña et al. 2011; Alcantara-Martinez et al. 2016; Cervantes-Ramírez et al. 2018). However, the phytoremediation potential of *V. campechiana* is still unknown. In general, it has been documented that the plant species used to phytoremediate environments contaminated by HMs belong to the following families: Asteraceae, Brassicaceae, Caryophyllaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphorbiaceae (Prasad 2003; Mahar et al. 2016), these being mostly herbaceous species. The results obtained in the present study suggest that *V. campechiana* has phytoremediation potential for sites contaminated by Cr, Cu, and Pb. The reasons that justify this suggestion are as follows: 1) *V. campechiana* is an accumulator species of Cr, Cu, and Pb. Olguín and Sánchez-Galván (2012) and Ali et al. (2013) propose that a plant can be considered an accumulator if its translocation factor (TF) is equal to or greater than 1. In the present study, in spite of *V. campechiana* growing under experimental conditions, it showed TF values greater than one for Cr (2.24), Pb (1.23), and Cu (1.22). These levels are similar to the TF found in species considered as accumulators that grow directly in contaminated environments such as *Gentiana pennelliana* Fernald [(Gentianaceae) (TF Zn = 1.2)], *Cyperus esculentus* L [(Cyperaceae) (TF Pb = 1.6, TF Zn = 1.1)], *Phyla nodiflora* (L.) Greene [(Verbenaceae) (TF Cu = 12.0, TF Zn = 1.1)], *Rubus fruticosus* L [(Rosaceae) (TF Cu = 5.6), *Sesbania herbacea* (Mill.) McVaugh [(Fabaceae) (TF Cu = 4.0)], and *Limnocharis flava* (L.) Buchenau [(Alismataceae) (TF Cd = 1.3)] (Yoon et al. 2006; Abhilash et al. 2009). 2) The bioaccumulation factor (BCF) for Cu in roots and leaves was 7.6 and 39.4, respectively. The study by Lin et al. (2003) supports the results obtained in the present work. They reported bioaccumulation of Cu in *Helianthus annuus* L. (Asteraceae), with a concentration that was 2 to 10 times higher than the concentration of Cu in the soil. With respect to Pb, it showed a BCF in roots of 15.1 and of 17.4 in leaf tissue. 3) In *V. campechiana*, the accumulation of Cr and Pb increased over time (12 months) in leaf tissue; thus, long-term studies could record a higher accumulation of Cr and Pb. 4) Seeds do not accumulate nonessential metals such as Cr and Pb, and seed germination is not affected by exposure to heavy metals. Unlike other species such as *Sanvitalia procumbens* Lam. (Asteraceae), the germination percentage of seeds from sites exposed to HMs decreases compared with the germination percentage of seeds from control sites (Rosas-Ramírez 2018). 5) Throughout the study (12 months), no mortality was recorded in the plants exposed to HMs. 6) The root biomass of *V. campechiana* plants was not significantly affected by the bioaccumulation of heavy metals.

Conclusion

In the present study *V. campechiana* chronically exposed to mine tailings in greenhouse/experimental condition showed high levels of HMs translocation, its ability to bioaccumulate non-essential metals in roots and leaves and changes in the macro- and micromorphological characters. These findings suggest that this plant may be a suitable candidate for use in phytoremediation studies in contaminated environments mainly for Cr, Cu, and Pb. Future studies in situ are necessary for complementing the role of *V. campechiana* as a phytoremediation plant. Also, we consider that conducting ecotoxicological studies on new plant species with different life forms provides useful information to arrive at effective phytoremediation strategies for soils contaminated with HMs. A detailed characterization of the effects of these xenobiotics on the exposed plants would allow the selection of the species whose macro- and micro morphological characters are affected the least by exposure to HMs. Furthermore, different species can extract different metals, so it is necessary to combine plant species to develop more effective strategies that can be used in environments polluted with more than one HMs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Capítulo 2

Potencial de fitorremediación de *Crotalaria pumila* (Fabaceae) en suelos contaminados por metales pesados

El objetivo de este capítulo fue conocer la capacidad de bioacumular MP en *C. pumila* bajo condiciones de invernadero, así como determinar su efecto sobre los caracteres macro y micromorfológicos a través del tiempo de exposición. Las preguntas que se plantaron para este capítulo fueron: 1) ¿Los individuos de *C. pumila* creciendo en sustrato con MP, los bioacumularán en su tejido de la raíz y foliar en mayor proporción respecto a los individuos testigo?; 2) ¿El tiempo de exposición a MP es un factor que favorece los niveles de bioacumulación en el tejido (raíz y foliar) de *C. pumila*?; 3) ¿La exposición a MP induce cambios en la morfología de individuos de *C. pumila*?; 4) ¿La respuesta morfológica y de bioacumulación de MP en tejido de raíz y foliar de individuos de *C. pumila* por exposición crónica a MP la hacen útil para fitorremediar ambientes contaminados?

Phytoremediation potential of *Crotalaria pumila* (Fabaceae) in soils polluted with heavy metals

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Abstract

Phytoremediation is a useful, low-cost, and environmentally friendly alternative for the rehabilitation of environments contaminated with heavy metals (HM). This technology takes advantage of the ability of certain plant species to accumulate HM in their tissues. *Crotalaria pumila* is an herbaceous plant with a wide geographical distribution that grows naturally in environments contaminated with HM. In this work, the bioaccumulation capacity of seven HM (Cd, Cr, Cu, Fe, Pb, and Zn) in root and leaf was evaluated, as well as the morphological changes presented in *C. pumila* growing in control substrate (without HM) and mine tailing substrate (with HM) under greenhouse conditions, for a period of 150 days. Four metals with the following concentration pattern were detected in both tissues and substrates: Fe>Pb>Cu>Zn. Fe, Pb, and Zn concentrations were significantly higher in root and leaf of individuals growing on mine tailing substrate compared to the control substrate, while the Cu concentration increased over time in exposed individuals. The bioconcentration factor showed a similar pattern in root and leaf: Cu>Fe>Pb>Zn. Around 60% of the morphological characters evaluated in this species decreased significantly in individuals exposed to HM. The bioconcentration factor shows that *C. pumila* is efficient in absorbing Cu, Fe, and Pb from the mine tailing substrate, at the root and leaf tissue, and the translocation factor shows its efficiency in translocating Cu from the root to the leaves. Therefore, *C. pumila* is a heavy metals accumulator plant species with great potential for phytoremediation of environments contaminated with Cu, Pb, and Fe. This is due to its ability to establish itself naturally in contaminated environments, without altering its germination percentage. It exhibits wide geographical distribution, it has a short life cycle, and rapid growth, and can retain the mine tailing substrate, extracting HM in a short time.

Keywords: Bioaccumulation, phytoextraction, herbaceous plants, mining tailings, heavy metals, morphological effects.

Introduction

Mining is a primary economic activity that generates waste called mine tailing. Their hazardousness lies in the content of potentially toxic elements such as HM, which may be bioavailable and absorbed by biological systems, causing effects at all levels of biological organization (Gutiérrez-Ruiz *et al.,* 2007; Mussali-Galante *et al.,* 2013). Metals are toxic because they are not biodegradable, tending to bioaccumulate in the different tissues of exposed organisms so that they can reach higher concentrations than those registered in the environment (Reeves and Baker 1999).

Plants are the main access route for these elements to the food chain (Gutiérrez-Ruiz *et al.,* 2007; Mussali-Galante *et al.,* 2013). The bioaccumulation capacity of HM in plants varies between taxonomic groups (Calow 1993). However, it is also determined by the retention capacity of the metal, the plant-root-metal interaction, the metabolism of the plant, as well as the bioavailability of the metal, the physicochemical properties of the soil, such as pH, electrical conductivity and the organic matter content (Kabata-Pendias 2001; Barceló and Poschenrieder 2003; Prieto 2009).

Plant species can respond in a differential way to the absorption of HM present in the soil (Rascio and Navari-Izzo 2011). Many species tolerate high concentrations of metals because they restrict their root absorption and translocation to the leaves, allowing them to maintain relatively low concentrations in the aerial biomass, regardless of the concentration of metals in the soil (exclusion strategy). On the other hand, other plant species actively absorb metals from the soil and accumulate them in non-toxic forms in their aerial biomass (accumulative strategy) (Brooks *et al.,* 1977; DalCorso 2012; Marrero-Coto *et al.,* 2012). Of the latter, different degrees of accumulation have been recognized, ranging from those that can only do so in small concentrations to those that can resist exceptionally high concentrations of HM without showing any visible signs of toxicity (Brooks *et al*., 1977; Macnair 2003; DalCorso 2012; Marrero-Coto *et al.,* 2012; Van der Ent *et al.,* 2013). Currently, 721 species of HM accumulating plants have been reported (As, Cd, Co, Cu, Mn, Ni, Pb, Sb, Se, Tl, Zn), included in 52 families and 130 genera (Reeves *et al.,* 2018). The most represented plant families are Brassicaceae, Phyllanthaceae, Fabaceae, Euphorbiaceae, Asteraceae, Lamiaceae, Phyllanthaceae, and Scrophulariaceae (Macnair 2003; Van der Ent *et al.,* 2013; Reeves *et al.,* 2018).

The HM accumulator plants are efficient in detoxification processes, mediated by the translocation of metals to the aerial part, and their storage in cell walls and organelles such as vacuoles, as well as in structures such as the epidermis, trichomes, and even the cuticle, where HM cause less damage to the photosynthesis process (Vögeli-Lange and Wagner 1990; Leea *et al.,* 1997; Rascio and Navari-Izzo 2011; Manara 2012). However, exposure to HM can cause various adverse effects on plants, such as inhibition of germination in seeds, root growth, the reduction of aerial biomass (stem and leaf), as well as micro-morphological alterations such as changes in the stomatal and trichome density, as well as alterations in biochemical processes. All these adverse effects are reflected in a decrease in seedling development (Meharg 1994; Guala 2010; Yadav 2010; Rengel *et al.*, 2011; Lukovic *et al.*, 2012).

For example, it has been documented that HM promote the reduction of foliar macro-morphological and micro-microphological characters in plant species such as: *Prosopis laevigata* (Fabaceae) (Muro-González *et al.*, in press), *Vachellia farnesiana* L. (Fabaceae) (Santoyo-Martínez *et al*., 2020), and *Zea mays* (Poaceae) (Tovar-Sánchez *et al.*, 2018).

Despite the adverse effects mentioned above, there are plant species that establish themselves naturally in contaminated environments, adopting some degree of accumulation. In the phytoremediation processes of soils contaminated by HM, the use of local species is recommended for the preservation of genetic integrity and the conservation of local diversity. Mainly, in mine tailing where there are physical, chemical, and biological limitations for the establishment of plants. Therefore, the search for native HM accumulating plants, capable of storing HM in different structures such as root, stem, and leaf, and able to be implemented in phytoremediation strategies for contaminated environments, is important. In this sense, it has been documented that the Bioconcentration Factor (BCF), that measures the efficiency of a plant species to accumulate metals from the soil in its tissues, and the Translocation Factor (TF) which indicates the efficiency of the plant to transport the metal from the root to its aerial part (Olguín and Sánchez-Galván 2012), are useful for selecting species with phytoremediation potential. For example, TF and BCF have been used to propose the plant species *Phyla nodiflora* (L.) (Verbenaceae), *Rubus fruticosus* L (Rosaceae), *Sesbania herbacea* (Fabaceae), *Gentiana pennelliana* (Gentianaceae) (Yoon *et al.*, 2006), and *Polygonum thunbergii* (Polygonaceae) (Kim *et al.*, 2003) as heavy metal accumulating.

Huautla, Morelos, Mexico is an area of mining activity, active until 1988. It is estimated that there are approximately 780,000 tons of waste in the form of mine tailing rich in As, Cu, Cd, Fe, Pb, Zn (Velasco *et al.*, 2005; Mussali-Galante *et al.*, 2013). Bioavailable metals have been reported in these residues which have contaminated the natural resources of the region, causing adverse effects on the health of the human population (Tovar-Sánchez *et al.*, 2016). In addition, adverse effects have been documented in arthropod and microarthropod diversity (Hernández-Gómez 2014; González-Brito 2015), as well as noting a reduction in the population density of small mammals (Mussali-Galante *et al.*, 2013), also alterations in the morphological, physiological and genetic characters of plant species (Santoyo-Martínez *et al.*, 2020). Therefore, it is essential to characterize species with the potential for phytoremediation in this type of environment.

Crotalaria pumila (Fabaceae) is a species with an herbaceous life form that naturally establishes in contaminated environments. *C. pumila* has high germination percentages, rapid growth, is a dominant plant in the herbaceous stratum and has a wide geographical distribution in Mexico (from temperate zones to arid and semi-arid zones). These characters make it an ideal system to assess for its potential to phytoremediation in environments contaminated by HM. Therefore, this study evaluated: a) the percentage of seed germination of *C. pumila* individuals from exposed sites and control sites; b) the bioaccumulation of Cd, Cr, Cu, Fe, Pb and Zn in root and leaf tissue of individuals growing on mine tailing substrate and control substrate in greenhouse conditions, c) the effect of bioaccumulation of HM on macro- and micro-morphological aspects of *C. pumila* individuals growing in mine tailing substrate and control substrate in greenhouse conditions, and d) to determine the potential of *C. pumila* for phytoremediation in environments contaminated by HM through the use of the FT and BCF.

Materials and Methods

Crotalaria pumila seed collection sites

The seed collection was carried out in the town of Huautla, municipality of Tlaquiltenango, Morelos, currently located within the Sierra de Huautla Biosphere Reserve (REBIOSH, acronym is Spanish). In this town, mining activity prevailed until 1988, mainly exploiting Pb, Zn and Ag, where for decades waste was generated (mine tailings) and deposited outdoors, forming three tailings rich in metals such as Pb, Mn, Cd, As, Zn, Cu, Fe and Cr (Velasco *et al.*, 2005). The exposed sites selected were mine tailing 1 (site MT1), which is located 500 m from town and is the largest in the area (18°26´36.37´´N-99°01´26.71´´O); mine tailing 2 (site MT2), is located 1,000 m from the population (18°2´22.62´´N-99°01´51.71´´W, Fig. 1). Also, two control sites were chosen: Quilamula (site C1) located at coordinates 18°30´52´´N and 98°59´59´´W and Ajuchitlán (site C2) which is located at coordinates 18°27´52´´N-98°58´53´´W (Figure 1). Both sites have a sub-humid temperate climate with summer rains and average annual precipitation of 900 mm. The annual average temperature exceeds 22ºC, with deciduous tropical forest the predominant vegetation. The forest is characterized by presenting a marked seasonality, where the trees do not exceed 15 m in height and lose their leaves in the dry season (Rzedowski 2006). The control sites are more than six kilometers away in a straight line from the exposed sites; they present ecological and geographical characteristics similar to the exposed sites, and do not show a record of mining activity or metallic contamination due to anthropogenic activity. Moreover, the water streams and predominant winds flow in a north-south direction (Mussali-Galante *et al.*, 2013). Hence, neither the winds nor the watercourses can be dispersing the pollutants to the control sites.

Species of study

Crotalaria pumila is an annual herbaceous species of the Fabaceae family. It grows upright or upward, with stems up to 50 cm long. It presents trifoliate leaves, leaflets 3.2-5.0 cm long, linear, elliptical or obovate, cuneate or rounded base, sub-rounded apex. Its phenology of flowering and fruiting occurs from May-December. The flowers are arranged in clusters, the fruit is an inflated legume 15 mm long by 8 mm in diameter, with asymmetric kidney-shaped seeds 1.5-2.6 mm long and 1.7-2.8 mm wide, greenish-brown, yellowish-green or coffee color (Rzedowski and de Rzedowski 2005). It is a species native to Mesoamerica, which is distributed from the southern United States of America to South America. In Mexico, it is widely distributed, both in deciduous tropical forests and also in temperate forests and xerophilous scrubs (Challenger and Soberón 2008). It is a species that has a primary use as food, and its cultivation is promoted for self-consumption (Espinosa and Sarukhán 1997; Challenger and Soberón 2008).

Seed collection and germination

C. pumila seeds were collected from individuals established at the study sites (control and exposed), following the protocol reported by Gold *et al.* (2004). In the study sites, 20 individuals were chosen at random, from which 20% of the ripe fruits were collected. Ripe fruits were transported to the laboratory; the seeds were extracted, cleaned, and selected, eliminating those parasitized by insects. In order to have a homogeneous representation of the genetic variability of the species, seeds collected from both control sites (C1 and C2) were considered as a control site, while collected seeds from both exposed sites (MT1 and MT2) were considered as an exposed site.

To evaluate the germination percentages of *C. pumila*, the seeds - from the control and the exposed sites - were subjected to mechanical scarification due to the physical latency they present. Twenty-five seeds were sown in Petri dishes, using 1% agar-agar as substrate using six replicates per treatment, evaluating this test over 20 days.

After seed germination, 72 seedlings were placed in individual polyethylene bags for nurseries (5 L capacity) with the substrates of the treatments; 36 in the mine tailing substrate and 36 in the control substrate. Soil from Quilamula was used as the control substrate, which was sieved with a stainless steel sieve number 35, with a mesh aperture of 0.5 mm (Fiicsa brand, Mexico City, Mex), in order to obtain a particle size similar to that of mine tailings. As a mine tailing substrate, a mixture of mine tailing from the exposed sites (MT1 and MT2) was used. The plants were kept under greenhouse conditions at a temperature that ranged from 32-35°C, where they were watered twice a day, three times a week. The plants obtained were used to evaluate the bioaccumulation of HM and to measure macro and micro morphological characters.

Evaluation of macro- and micro-morphological characters

To evaluate the HM exposure effects over the morphological characters of *C. pumila*, six individuals were selected at random by treatment (control and mine tailing substrate). Root and stem length were evaluated from each individual. Subsequently, six leaves per individual were randomly chosen to evaluate the macro- and micro-morphological characters shown in Table 1. These evaluations were carried out every 30 days of exposure in the treatments until a 150 day period was accomplished.

Foliar macro-morphological characters were evaluated with a digital Vernier (Stainless Hardened) and a digital scale (Acculab Scales, Titusville, NJ, USA). For micro-morphological characters, a foliar epidermal impression was made using the cyanoacrylate glue replica technique. Per individual, three lamellae were made with epidermal impressions of the abaxial part of the leaf. The lamellae were observed with a 40X light microscope (Leica, Schweiz, CH) with bright field illumination (BF) and differential interference contrast (DIC). From each slide, three photomicrographs were randomly taken. Finally, from nine microphotographs, the number of stomata and the number of epidermal cells were obtained per individual, and with these data, the stomatal index (SI) was determined, which was calculated according to Salisbury (Salisbury 1968).

$SI= (SN/SN+NEC) \times 100$

Where:

SI = Stomatal index

NE = Number of stomatal cells per area unit

NCE = Number of ordinary epidermal cells per area unit

Determination of heavy metals

Ten samples of the mine tailing substrate were analyzed to determine seven metals (Cd, Cr, Cu, Fe, Pb, and Zn). The samples were dried and sieved following the methodology of the Mexican standard NMX-AA-132-SCFI-2006. This process consists of adding 50 mL of 0.01 M $CaCl_2$ to 10 g of substrate. The sample was left stirring for 24 h and centrifuged at 1,500 rpm for 15 min, recovering the supernatant by filtration. The metal concentration in the substrate samples was determined by atomic absorption spectrophotometry using the flame method in a spectrophotometer (GBC 908 A, GBC Scientific Equipment Pty Ltd, Victoria, AU).

For the evaluation of the metals (Cd, Cr, Cu, Fe, Mn, Pb, and Zn) in the root and leaf tissue of *C. pumila*, three samples were taken from six individuals per substrate (mine tailing and control), every 30 days of exposure, taking 0.25 g of each structure, which were then pulverized and poured into containers previously washed with HNO_3. The samples were subjected to acid digestion using a Microwave Accelerated Reaction System (CEM® MARS-5, CEN Matthews, NC, USA), using 10 mL of 70% HNO_3 in closed Teflon pumps. The samples were dissolved and filtered in distilled water to a final volume of 50 mL until analysis was performed. A non-tissue sample was simultaneously processed and used as a control. Finally, the metals were analyzed by atomic absorption spectrophotometry using the flame method in a spectrophotometer (GBC 908 A, GBC Scientific Equipment Pty Ltd, Victoria, AU). The instrument was calibrated with standard solutions and known concentrations for each metal analyzed. The minimum detection limits for Cd, Cr, Cu, Fe, Mn, Pb, and Zn were: 0.0004, 0.003, 0.001, 0.0015, 0.0015, 0.01, 0.0005 mg/L respectively; the samples from the exposed and control site were processed simultaneously by triplicate.

Statistical analysis

A two-factor analysis of variance was performed (Zar 2010) to assess the effect of the site (control and exposed), treatment (mechanical scarification and no treatment), and site per treatment interaction on seed germination of *C. pumila*. In addition, a Tukey test was performed to establish significant differences in the averages between sites and between treatments (Zar 2010).

Likewise, two-factor analysis of variance was performed to determine the effect of the exposure time (30, 60, 90, 120, 150 days), treatment (substrate: control and mine tailing) and time per treatment interaction on the variation in 16 morphological characters (15 macro and one micro morphological). Subsequently, a Tukey test was carried out to determine significant differences between pairs of average morphological character assessed over time in both treatments (Zar 2010).

The same analysis was carried out to evaluate the effect of exposure time, treatment (substrate: control and mine tailing), and time by treatment interaction on the accumulation of Cu, Fe, Pb, and Zn in root and leaf of individuals of *C. pumila*. Likewise, a Tukey test was used to determine the significant differences in the mean concentration of each metal over time by analyzed structure, for both treatments (substrate: control and pull) (Zar 2010). All analyses were done using the STATISTICA 8 program (StatSoft 2004).

The HM phytoextraction capacity of *C. pumila* was evaluated with two indices: the bioconcentration factor (BCF) that determines the efficiency of the plant to accumulate the metal from the substrate in its tissue and the translocation factor (TF) that measures the efficiency to transport the metal from the root to its aerial part (Olguín and Sánchez-Galván 2012; Ali *et al.,* 2013). These indices were calculated as follows:

$BCF_{root} = C_{root}/C_{mine\ tailing}$

$BCF_{leaft} = C_{leaft}/C_{mine\ tailing}$

$TF = C_{leaft}/C_{root}$

Where $C_{mine\ tailing}$ is the concentration in the mine tailing, and C_{leaft} is the concentration of the metal detected in the leaf tissue, and C_{root} is the concentration of the metal detected in the root tissue. It has been reported that if a plant presents values of TF>1, as well as BCF>1, the species is considered an accumulator for the analyzed metal (Yanqun *et al.*, 2005; Covarrubias and Cabriales 2017; Bader *et al.*, 2019).

Results

Germination of *Crotalaria pumila* seeds from control sites and sites exposed to HM

The germination results showed that the place of origin of the seeds (control *vs* exposed) does not influence the percentage of germination of the seeds. However, pregerminative treatment has a significant effect on germination. Likewise, there was no significant effect of the site-per treatment interaction (Table 2). The results show that the seeds from both sites (control, exposed) have less than 10% germination when they do not receive a pregerminative treatment. In contrast, when the seeds from both sites undergo mechanical scarification, their germination percentage is higher than 90% (Table 2).

Bioaccumulation of heavy metals in the root and leaf of *Croralaria pumila*

Of the seven HM analyzed (Cd, Cr, Cu, Fe, Mn, Pb, and Zn), the presence of Cu, Fe, Pb, and Zn was only detected in root and leaf tissue of *C. pumila* individuals, while Cr, Cd, and Mn were not detected. Two-way analysis of variance only showed a significant effect of time (t) on Zn concentration of the leaf tissue. While treatment (T) showed significant differences in the concentration of Fe, Pb, and Zn in root and leaf, the exception was Cu, which only showed significant differences in its concentration in the root; while the interaction (t × T) showed significant differences in the concentration of Zn in roots and leaves and the concentration of Pb in roots (Table 3).

Regarding bioaccumulation, it was found that Cu was significantly higher in root and leaf in individuals growing in control substrate compared to individuals growing in the mine tailings. It remained constant over time, except for the concentration of root in exposed individuals; on day 150 an increase was shown (Table 3).

With respect to the concentration of Fe between treatments, the individuals growing in mine tailing substrate presented a higher accumulation in the evaluated tissues (root and leaf). On the other hand, the concentration of Fe in both evaluated structures (root and leaf) of control and exposed individuals did not change throughout the exposure time. Notably, the concentration in leaf tissue of exposed individuals showed a decreased concentration on day 120 (Table 3).

The concentration of Pb presented significant differences between treatments, observing the highest concentrations in root and leaf of exposed individuals concerning the control individuals, the latter without presenting changes through the exposure time. While the concentration of root and leaf in the exposed individuals increased their concentration through the exposure time, on day 150 the highest concentration was found in both structures (Table 3).

In the case of Zn, the concentration values in between treatments denoted significant differences in both structures evaluated, presenting the highest values in the exposed individuals. However, the root and leaf concentration of exposed individuals decreased over the exposure time while the root concentration of control individuals increased over time. The leaf concentration of control individuals was oscillatory and the highest concentration occurred on day 60 (Table 3).

Bioconcentration factor (BCF) and translocation (TF) of HM in root and leaf from *Crotalaria pumila* individuals growing on mine tailing substrate

The BCF presented the following pattern: Cu>Fe>Pb in root and leaf tissue in *C. pumila* individuals growing on mine tailing substrate. The values of Fe and Pb in the root were higher concerning those found in the leaf. In contrast, the BC of Cu in leaf was greater than in roots (Table 4), and the Zn values were reduced in both structures evaluated. On the other hand, the TF presented the following pattern: Cu>Pb>Fe>Zn, where Cu presented an average value of 1.33, while the Pb, Fe, and Zn values were lower than 1 (Table 4).

Macro and micro morphological changes in *Crotalaria pumila* individuals growing under greenhouse conditions in control substrate and mine tailing substrate over time.

The analysis of variance of two factors indicated that of the 16 morphological characters evaluated, 63% denoted significant differences in time (t), Treatment (T) and interaction (t × T) in individuals of *C. pumila* growing during 150 days under greenhouse conditions in two treatments (control substrate and mine tailing substrate). Except for the fresh root biomass FRB and the dry root biomass DRB, no significant effects were observed in the evaluated factors. While for the characters of the fresh leaf biomass FLB and the dry leaf biomass DLB, a significant effect of treatment (T) was observed, but not of time (t) or interaction (t × T). For the 1/3 apical width (1/3AW) and the 1/3 basal width (1/3BW) characters, treatment (T) did not show a significant effect (Table 5).

Regarding the height characters evaluated, the *C. pumila* individuals grown in mine tailing substrate showed a significant increase in 100% over time (Table 5). In contrast, the individuals growing in the control substrate did not show significant differences over time in the characters mentioned above except for stem length, which showed an increase in character over the exposure time (Table 5).

For the foliar macro-morphological characters evaluated, it was found that 100% of them showed a decrease from day 60 in *C. pumila* individuals grown in the control substrate. On the other hand, individuals growing on mine tailing substrate showed a significant reduction in 89% of the characters evaluated over time. In contrast, the width of the intermediate vein WIV showed oscillatory changes through exposure time (Table 5). The micro-morphology, the results of the stomatal index (SI) in the *C. pumila* individuals growing in the control substrate and the mine tailing substrate showed a significant increase over the exposure time (150 days) (Table 5).

Finally, individuals growing on mine tailing substrate showed a significant reduction (t=-18.007, d.f.= 58, P= 0.003) in the numbers of flowers (mean ± standard deviation) (23.57±5.47) in comparison with individuals growing in the control substrate (59.90±9.60).

Discussion

Bioaccumulation of heavy metals in *C. pumila*

In the present study, we used an herbaceous species due to the characteristics of its life cycle (annual), its rapid growth, and the fact that it can extract the HM in a short time; *C. pumila* can be implemented in phytoremediation strategies. The *C. pumila* individuals bioaccumulated four of the seven HM analyzed (Cu, Fe, Pb, and Zn) in root and leaf tissue, of which only Pb is not essential. The individuals that grew in the exposed substrate concluded their life cycle 60 days after the individuals in the control substrate, and the bioaccumulation of HM can explain this.

Accumulation of heavy metals causes different adverse effects in the physiology and development of plants, including inhibition of the activity of enzymes and coenzymes, alteration in nutrients uptake, reduction in photosynthesis activities, as well as inhibition in seeds germination, plant growth and death (Li *et al.*, 2005; Sarma 2011; Wani *et al*., 2012; Chibuike and Obiora 2014; Asati *et al.,* 2016). Copper is an essential metal for adequate growth and development in plants. However, the presence of this metal in excess interferes with essential physiological processes, such as photosynthesis, causing inhibition in growth (Yruela 2005). Accumulation of Cu causes adverse effects such as reduced root development, root malformations in *Phaseolus vulgaris* (Fabaceae) (Cook *et al*., 1998), as well as the reduction in total plant biomass, seeds production and lifetime in *Polygonum convolvulus* (Polygonaceae) (Kjaer and Elmegaard 1996).

Iron is another essential metal for adequate development and growth of the plants. Fe is implicated in the electron transport in the photosynthetic processes, the development of the chloroplast, the biosynthesis of chlorophyll, and hemeproteins (Balk and Schaedler 2014), due to its importance the uptake and homeostasis of Fe in plants in highly regulated (Morrissey and Guerinot 2009; Connorton *et al.,* 2017). Plant accumulation of the ion Fe^{+3} is not related directly to adverse effects. However, in the soil, the reduction of the Fe^{+3} to Fe^{+2}, mediated by microbial action under flooded conditions, may cause adverse effects in some plants (Kalaivanan and Ganeshamurthy 2016; Küpper and Andresen 2016). The plant Fe^{+2} uptake and bioaccumulation have been related with toxic effects such as free radicals release, reduction in photosynthesis and yield in different aquatic and flooded cultured plants, including *Hydrilla verticillata* L.f. (Hydrocharitaceae) (Sinha *et al.,* 1997), and *Oryza sativa* L. (Poaceae) (Becker and Asch 2005), as well as in *Pisum sativum* L. (Fabaceae) on hydroponic culture (Suh at al., 2002).

Lead is a non-essential metal in plants. It has been documented that Pb can cause an imbalance in the uptake of nutrients such as Ca, K, and P by the roots and their distribution to the different parts of the plant (DalCorso 2012). Moreover, the accumulation of Pb, even of small concentrations, causes adverse effects in photosynthesis, biochemical pathways, as well as inhibiting seed germination and growth in several plant species (Nas and Ali 2018). In *Zea mays* (Poaceae) the accumulation of Pb causes a reduction in germination percentages, growth suppression and reductions in plant biomass (Hussain *et al*., 2013). In the tree species *Thespesia populnea* (Malvaceae) the accumulation of Pb was related to a reduction in the number of leaves and the leaf area (Kabir *et al.* 2009).

Zinc is an essential metal for plant growth, but the bioaccumulation of Zn in different plant tissues causes adverse effects. In *Zea mays* (Poaceae) the exposition to ZnO causes a reduction in net photosynthesis, transpiration rates, and stomatal conductance, especially in early development stages of the plant, Zn was translocated to the cobs. The presence of Zn was related to a reduction in the number of wholly developed cobs and corn yields (Zhao *et al.*, 2015). In this work, the different adverse effects related to the accumulation of heavy metals, Cu, Fe, Pb, and Zn, could generate a delay in the development of the individuals, which was reflected in the observed increment in the lifecycle time of the plants exposed to the mine tailing substrate, in comparison with plants grown in the control substrate.

In this study, the accumulation of Cu in exposed individuals showed small differences over time, presenting root accumulation values of 0.39 to 0.46 mg/g, while in the case of the leaves there were no significant differences in the accumulation of this metal, showing a range of 0.43 to 0.45 mg/kg. The Cu accumulation above the plant requirements causes a reduction in growth and cellular damage (Yruela 2005). Cu is a redox-active heavy metal. In plants, the Cu excess induces the release of reactive oxygen species and free radicals, leading to severe adverse effects, including the disruption of cell homeostasis in different plant tissues, DNA damage, protein degradation, photosynthesis efficiency decline, and cell death. In response to the stress caused by Cu accumulation, the production of different proteins and molecules is induced to cope with the Cu toxicity; phytochelatins and proline are produced at the root level to immobilize the Cu excess and reduce the Cu translocation to aerial tissues. In contrast, at the leaf level, different metallothioneins are expressed, some of them are implicated in the Cu detoxification (Emamverdian *et al.*, 2015), it is possible that some of these molecules are implicated in the maintenance of a stable concentration of Cu in the leaf of *C. pumila*.

Cu is essential for the health of the plant. It is interesting to note that some species have a great tolerance to the increase in the concentration of Cu, even though it affects their growth, causes oxidative damage to cells, interferes with photosynthesis, and accumulates extremely high amounts of this metal in its tissues. As was reported by Jiang *et al.* (2004) the greatest copper accumulation of *Elsholtzia splendens* (Lamiaceae) was found in roots (1,700 mg/kg) compared to what was found in leaf (10 mg/kg). However, the accumulation pattern differs between plant species, as reported by Lange *et al*. (2017) who describe the bioaccumulation of Cu in leaf tissue of various species such as *Agrostis stolonifera* L. (Poaceae) (0.01 mg/kg), *Minuartia hirsuta* (Caryophyllaceae) (0.05 mg/kg), *Calamagrostis epigejos* L. (Poaceae) (0.13 mg/kg), *Anisopappus chinensis* (Asteraceae) (0.5 mg/kg), *Silene burchelli* (Caryophyllaceae) (1 mg/kg), *Aeollanthus subacaulis* (Lamiaceae) (9 mg/kg), *Haumaniastrum robertii* (Lamiaceae) (8.5 mg/kg), and *Elsholtzia splendens* (Lamiaceae) (5.2 mg/kg). These species are included in families that have been reported as accumulators and hyperaccumulators of heavy metals (Vara-Prasad and De Oliveira 2003; Sheoran *et al*., 2010; Parmar and Singh 2015; Mahar *et al.*, 2016). Overall, this study demonstrates that *C. pumila* can accumulate Cu in the leaf tissue at similar concentrations as in other plant species with an herbaceous life form.

The accumulation of Cu has also been reported in plant species with a different life form than herbaceous. In shrub species such as *Aeolanthus biformifolius* (Labiatae), the accumulation of Cu ranges from 2,150 mg/kg in leaf tissue to 13,700 mg/kg in the whole plant (Malaisse *et al.,* 1978; Chaney *et al.,* 2010; Yan *et a.,* 2020). In arboreal species such as *Olea europea* L. (Oleaceae), a Cu accumulation of 5.82 mg/kg in leaves has been reported (Wilson and Pyatt 2007). In another study, the Cu accumulation in *Jatropha curcas* L. (Euforbiaceae) ranges from 5.4 -7.2 mg/kg in whole plant biomass (Ahmadpour *et al.*, 2014). On the other hand, grasses such as *Cenchrus pennisetiformis* L. (Poaceae), and *Cynodon dactylon* L. (Poaceae), showed a root Cu accumulation of 416.9 and 59.4 mg/kg respectively, while the shoot respective concentrations were 64.1 y 8.3 mg/kg (Malik *et al.*, 2010). Bindweeds are also proposed as Cu accumulators; *Convolvulus arvensis* L. (Convolvulaceae) shows Cu accumulation of 560 mg/kg of dry tissue (Gardea-Torresdey *et al.*, 2004), while *Ipomea alpine* L. (Convolvulaceae) showed a maximum Cu accumulation of 12,300 mg/kg in the whole plant (Yan *et al.,* 2020). In addition, aquatic plants have been reported as Cu accumulators; the aquatic macrophyte *Eleocharis acicularis* L. (Cyperaceae) has been reported as a Cu hyperaccumulator, with a Cu accumulation of 20,200 mg/kg determined in shoots (Sakakibara *et al.*, 2011). The accumulation of Cu is differentiated between the different forms of plant life, so that species capable of accumulating or hyperaccumulating Cu can be identified in different structures, roots, leaves or the entire plant, according to the values determined for BCF_{root} (10.2 ± 0.34), BCF_{leaf} (13.5 ± 2.11) and TF (1.33 ± 0.21), *C. Pumila* can be classified as an accumulating species for Cu.

Regarding the accumulation of Fe, it was detected that the concentration in root and leaf was higher in *C. pumila* individuals growing in the exposed substrate. Furthermore, the concentration of this metal in the leaf tissue of exposed individuals decreased over time. However, when comparing structures, it was observed that there is greater bioaccumulation of Fe in the root than in the leaf of the exposed individuals. Swapna *et al.* (2015) report similar results in *Chromolaena odorata* L. (Asteraceae) finding that the highest bioaccumulation of this metal was observed in the root (750 mg/kg) compared to the leaf (80 mg/kg). Similarly, the authors reported that as the concentration of Fe in the root increased, its concentration in the leaf decreased.

In plants, the root system is responsible for the uptake of heavy metals from the soil at the root level. The process of metal uptake is mediated by chelating molecules and transport proteins, as well as soil pH changes induced by plants (Tangahu *et al.,* 2011). The root system may play a key role in limiting the distribution of heavy metals into the aerial parts and avoiding excessive or toxic heavy metal accumulation in the shoot system (Mazhoudi *et al.,* 1997). Fe is an essential metal for plant development and physiology, due to its crucial role in the photosynthesis process, as well as in the biosynthesis of hemeproteins, and important biomolecules such as chlorophyll (Römheld and Marschner 1997; Broadley *et al.,* 2012). However, the Fe high-level accumulation, may cause adverse effects related to reactive oxygen release (Connolly and Guerinot 2002). Fe first enters the root system and then it is transported and distributed to the aerial part of the plant; Fe homeostasis is a highly regulated process to avoid deficiency or toxicity (Briat *et al.,* 2010). The accumulation of Fe in the root is a tolerance mechanism to prevent its excessive accumulation in the aerial part of the plants (Sperotto *et al.,* 2010). Different plants, such as *Centella asiatica* L. (Apiaceae) (Bhat *et al.,* 2016), *Jatropha curcas* L. (Euphorbiaceae) (Ghavri *et al.,* 2010), *Pelarginium hortorum* (Geraniaceae) (Orroño and Lavado 2009), *Typha latifolia* L. (Typhaceae) (Klink *et al*., 2013), can cope with Fe toxicity by accumulation in the roots. Nicotinamide and the proteins implicated in the Fe transport are also related to the transport of other metallic ions such as Cu, Ni, Mn, Zn, Co, and Cd, in soil with the presence of heavy metal mixtures, the transport of Fe into the roots and the subsequent translocation to the aerial parts of the plants could be limited, due to the different affinity of the metallic ions to the transporters (Krämer *et al.,* 2007; Morrissey and Guerinot 2009; Conte and Walker 2011).

In the case of Zn and Pb, a higher concentration was observed in exposed individuals of *C. pumila* for both structures evaluated. For Zn, exposed individuals decreased their concentration over time for both structures, while in Pb, an increase in their concentration was detected in the structures evaluated of the exposed individuals through exposure time. Both metals showed a higher root concentration, 0.99 and 2.02 mg/kg, respectively, in comparison with the concentration determined in leaf, 0.23 and 1.75 mg/kg. Similar results were reported by Rosas-Ramírez (2018) for Zn, who describes a similar accumulation process in *Sanvitalia procumbens* (Asteraceae) (root 1.52 mg/kg and leaf 0.70 mg/kg). Similarly, Maiti and Jaiswal (2008) reported a higher Zn concentration in roots of four herbaceous species: *Typha latifolia* L. (Typhaceae) (root 179.9 mg/kg and leaf 55.6 mg/kg); *Fimbristylis dichotoma* L. (Cyperaceae) (root 115.8 mg/kg and leaf 31.4 mg/kg); *Amaranthus defle*xus L. (Amaranthaceae) (root 48.0 mg/kg and leaf 36.6 mg/kg) and *Saccharum spontaneum* L. (Poaceae) (root 53.6 mg/kg and leaf 32.1 mg/kg).

Zn is an essential micronutrient for plant growth and development. The Zn plays essential roles in photosynthesis, membrane integrity, phytohormones, and nucleic acids synthesis, as well as a cofactor required for the structure and function of numerous enzymatic proteins (Imran *et al.*, 2014; Goyal *et al*., 2020). The Zn concentrations are variable in the different aerial structures according to the development stage of the plant. The Zn is absorbed through the root system, and then is translocated to the aerial part of the plants. Zn generally is stored in the stem tissues, and later mobilized to the growing tissues. In plants, the Zn is redistributed from senescing tissues to young shoots, reproductive organs, and seeds; the seeds are considered reservoirs of this metal (Longnecker and Robson 1993). It has been reported that several plant species can accumulate high Zn levels in their roots, rather than in their leaves and shoots, as a mechanism to avoid the adverse effects of the Zn overload. Also, it has been reported that Zn accumulation in plants is differential between flowering and post-flowering stages; in addition, old leaves show lower Zn concentrations with respect to young leaves (Gupta *et al*., 2016).

With regard to Pb it has been reported that the root structure is where the highest concentration with respect to the leaf has been found. Xiong (1997) reports that *Sonchus oleraceus* L. (Asteraceae) accumulates a higher concentration of Pb in the root (1,113.24 mg/kg) compared to that accumulated in the leaf (65.67 mg/kg). For their part, Rotkittikhun *et al.* (2006) documented a higher concentration of Pb accumulated in the root compared to that accumulated in the leaf; in 26 plant species such as Asteraceae, Cyperaceae, Euphorbiaceae, Fabaceae, Malvaceae, Poaceae. These findings could be explained by the fact that Pb is absorbed by the roots and retained by cell walls of the root cells. Further, the Pb translocation to the aerial part of the plant is limited due to the absence of specific transporters. Illustrating the above, it has been described that in plants in general 3% of the Pb in the root is translocated to leaf since Pb has not displayed any role in the plant development; the small concentrations that translocate to the aerial parts increase over time (Kabata-Pendias 2001).

It is worth mentioning that the individuals in this study that grew on mine tailing substrate survived 150 days, while the control individuals survived only 90 days. This observation could be attributed to the fact that in the exposed individuals, the entrance of nutrients is limited, affecting their life cycle (DalCorso 2012). Taking into account the above, it has been documented that Cr and Pb affect the cell cycle, which induces the inhibition of cell division, causing alterations to the growth of roots, which could have consequences in the growth of shoots, affecting the normal development of the plant (Sundaramoorthy *et al*., 2010).

Lastly, it should be mentioned that the presence of HM in individuals witnessing *C. pumila* growing in the region's substrate, is due to the fact that the municipality of Tlaquiltenango presents a natural wealth of sulphur minerals in soil (mainly silver and lead). Commonly found minerals are: Arsenopyrite (FeAsS), Galena (PbS), Acantite (Ag_2S), Calclacite (Cu_2S) (Volke *et al.*, 2004; Volke *et al.,* 2005; Secretaría de Economía 2011). Therefore, the soils of the region are naturally rich in minerals.

Effect of heavy metals on the root, stem and leaf morphology of *C. pumila*

In the present study, 16 macro and micro morphological characters were evaluated, of which 63% decreased in individuals growing in mine tailing substrate compared to individuals growing in control substrate. Similar results were reported in species with herbaceous life form exposed to HM; Tovar-Sánchez *et al.* (2018) document for *Zea mays* L. (Poaceae) a reduction of 50% of the evaluated leaf traits, while Rosas-Ramírez (2018) and Sidhu *et al.* (1993) document a decrease in the size of the root, leaves and biomass for *S. procumbens* and *Chenopodium murale* L. (Amaranthaceae), respectively.

Pb was one of the metals that bioaccumulated *C. pumila,* increasing its concentration over time in exposed individuals for both structures evaluated (root 2.02 and leaf 1.75). It was in the root where it bioaccumulated in the highest concentration, attributing the effects on the morphological changes in the exposed individuals. Similar results were reported by Rosas-Ramírez (2018) in the herbaceous *S. procumbens.* The bioaccumulation of Pb in root was 4.88 mg/kg while in leaf was 3.765 mg/kg, with morphological characters such as root length, stem, and biomass being affected. Similarly, *S. oleraceus* exposed to different concentrations of Pb (0, 800, 1.600, and 3.200 mg/kg), where the biomass (3.64 g, 2.99 g, 2.50 g, and 2.04 g), and leaf length (73.97 cm, 70.58 cm, 69.46 cm, 63.0 cm), progressively decreased with the increasing in Pb concentration (Xiong 1997). For their part, Venkatachalam *et al.* (2017) report that the growth of *Acalypha indica* L. (Euphorbiaceae) is affected after 12 days of exposure with Pb at concentrations of 500 mg/kg where the size of the leaves significantly decreased (11.6 to 5.92 cm) and root length (8.33 to 4.16 cm) by 50.9% and 49.9% respectively.

The results of the stomatal index in individuals of *C. pumila* growing in mine tailing substrate differ significantly from those growing in control substrate, presenting a lower index in individuals exposed to HM. These results are similar to other species with shrubby life, such as *V. campechiana* (Santoyo-Martínez *et al.*, 2020) and *P. laevigata,* a species of arboreal life, where they have documented a decrease in the number of stomata per unit area. This can be explained by a response of the plant to avoid excess gas exchange, increasing its stomatal resistance (Rajakaruna and Baker 2006).

In this study, the exposed individuals show a decrease in the size of the root, the stem, as well as in the biomass, in the macro and micro morphological characters of the leaves (Table 5). These results coincide with that reported by Tovar-Sánchez *et al.* (2018), where they point out that individuals exposed to heavy metals presented a decrease in size, biomass, and micro-morphological characters in *Zea mays* (Poaceae). The observed effects may be related to the accumulation of Cu, Fe, Pb, and Zn in *C. pumila*. The exposition to heavy metals have been reported to have adverse effects on plant development, as a result of the alteration of important biochemical and physiological processes. These adverse effects are reflected in reduced seed germination percentage, slow growth, a reduced number of leaves, branches, roots, shoots, biomass yield, and inclusively the plant death (Sharma and Agrawal 2005; Chibuike and Obiora 2014; Bezini *et al.,* 2019; Ghori *et al*., 2019; Yu *et al.,* 2020). Specifically, Cu in excessive amounts negatively affects the growth of plants such as *Brassica juncea* L. (Brassicaceae) and causing chlorosis in *Banksia ericifolia* (Proteaceae), while, on the other hand, iron generates production of Reactive Oxygen Species (ROS), which have affect membrane structure and their permeability. The lead causes a reduction in the root length and root dry mass in *Zea mays* (Poaceae), and *Pisum sativum* L. (Fabaceae) (Goyal *et al.,* 2020). Liu *et al.* (2004) reported that Pb might be associated with inhibition of plant and root growth, as well as affecting the photosynthesis and chlorophyll biosynthesis (Mitra *et al.,* 2020). Meanwhile, Xiong *et al*. (2005) demonstrated that Pb has toxic effects on nitrogen assimilation and growth of *Brassica pekinensis* (Brassicaceae). Goyal *et al.* (2020) mention that Zn accumulation causes a reduction in plant biomass and effects in cell division.

The use of *C. pumila* as a species to rehabilitate Cu-contaminated soils

In most of the studies that have evaluated the bioaccumulation of PM in plants, species belonging to the Brasicaceae family have been selected, a herbaceous species that inhabits temperate and cold zones. The present study focused on knowing the ability to accumulate metals in *C. pumila*, an herbaceous species that inhabits diverse types of vegetation such as xerophilous scrub, tropical deciduous forest, and temperate forests (Rzedowski 2006). Since it is distributed in different types of vegetation, it is a species of great importance to phytoremediation of various environments contaminated by HM.

The germination percentages of *C. pumila* seeds were not affected by the origin, reaching values close to 100% for the seeds from both control and exposed sites. Similar findings were reported by Santoyo-Martínez *et al.* (2020) where they observed similar percentages in *V. campechiana*, attributing this to the fact that in some cases the seeds only bioaccumulate essential HM such as Cu, Fe and Zn, essential elements to trigger the germination process. The results obtained in this work propose *C. pumila* as an accumulating species of Cu with the potential to phytoremediate environments contaminated by this metal, due to its ability to translocate Cu to the aerial part of the plant. Those mentioned above, taking into consideration the proposal of Olguín and Sánchez-Galván (2012) and Ali *et al.* (2013), that an accumulating species is one that can efficiently translocate HM to the aerial part, that is, those with a translocation factor (TF) value greater than or equal to 1. In this study, *C. pumila* growing on mine tailing substrate under controlled conditions presented translocation (TF) values greater than 1 (Cu = 1.3).

These levels in TF are similar in herbaceous species reported with Cu accumulating potential as *Taraxacum officinale* (Asteraceae) (FT = 1.2 Cu), *Phyla nodiflora* L. (Verbenaceae) (FT Cu = 12.0, FT Zn = 1.1), *Rubus fruticosus* L. (Rosaceae) (FT Cu = 5.6), *Sesbania herbacea* (Fabaceae) (FT Cu = 4.0) (Yoon *et al.*, 2006; Bini *et al.*, 2012). Additionally, the *C. pumila* bioconcentration factor (BCF) in both structures revealed its ability to accumulate metals in its tissues: Cu (root = 10.2), leaf = 13.5), Fe (root = 9.55), leaf = 6.91), Pb (root = 7.96), leaf = 5.93). Similar results were reported by Yoon *et al.* (2006) in the root of five herbaceous species: *Gentiana pennelliana* (Gentianaceae) (BCF = Pb = 11, Cu = 22 and Zn = 2.6), *Herbaceous sesbania* (Fabaceae) BCF = Pb = 1.1, and Zn = 1.5), *Stenotaphrum secundatum* (Poaceae) (BCB Root Cu = 1.1), *Plantago major* L. (Plantaginaceae) (BCF Cu = 1.2), *Bidens alba* L. (Asteraceae) (FBC Cu = 6.6). Likewise, Ndimele *et al.* (2014) and González and González-Chávez (2006) reported BCF values similar to those found in *C. pumila* leaves in the leaf tissue of: *Pontederia crassipes* (Pontederiaceae) (FBC Fe = 2.31), *Polygonum aviculare* L. (Polygonaceae) (FBC Cu = 2.7 Pb = 1.1), *Teloxys graveolens* (Chenopodiaceae) (BCF Cu = 3.3 Pb = 2.0), *Jatropha dioica* (Euphorbiaceae) (BCF Cu = 6.5 Pb = 1.7), *Andropogon barbinodis* (Gramineae) (FBC Cu = 12.5 Pb = 7.3), *Bahia absinthifolia* (Compositae) (FBC Cu = 37.5 Pb = 3.4), *Solanum elaeagnifolium* (Solanaceae) (FBC Cu = 4.7 Pb = 2.7).

Herbaceous plants are widely distributed in tropical and subtropical environments (Marques *et al.*, 2009). They have been reported as medicinal and ornamental plants but also play important ecological roles in the environments in which they establish, playing a part in increasing biodiversity and reducing erosion (Saghi *et al.*, 2016). Another advantage of this kind of plant is its great phytoremediation potential; many herbaceous species are heavy metal accumulators and hyperaccumulators, and show fast growth and high-level biomass production (Ahmadpour *et al.,* 2012). *C. pumila* is an herbaceous plant of the Fabaceae family, with an annual life cycle (Soto-Estrada 2004), and has been reported as a pioneer species of heavy metal impacted sites (Sánchez-López 2015). Species of the Fabaceae family have characteristics that make them resilient to environmental stress conditions, like those present in heavy metal polluted environments. Different Fabaceae have been proposed for revegetation and phytostabilization of mine soils, since they can produce high biomass, show fast growth rates, and can accumulate different heavy metals in their tissues (Safronova *et al.*, 2011). Different heavy metal phytoremediation studies propose the introduction of non-native species. However, the introduction of foreign species may cause a threat for the ecological dynamics of the impacted site, mainly related to the possibility of an invasive profile in the introduced species (Leguizamo *et al.*, 2017), *C. pumila* has a broadly natural distribution, from the south of the United States to Argentina in South America (Gómez-Sosa 2000). This plant has also been reported in almost all regions of Mexico, and is widely distributed in the Huautla region. *C. pumila* is well adapted to the presence of the heavy metals distributed in this area, capacity probably related to the production of antioxidants in shoots and leaves (Villa-Ruano *et al.*, 2013). This study demonstrates that *C. pumila* is a species with potential for phytoremediation of environments contaminated with Cu in different areas impacted by the mining activities in Mexico, due to its resistance to heavy metal adverse effects, its Cu accumulation character in aerial parts, its high percentage of germination for its propagation, and broad geographical distribution. It should also be noted that although there is a decrease in morphology, survival and germination were not affected.

For all of the above, this study demonstrates *C. pumila* is a species with the potential for phytoremediation of environments contaminated with Cu, due to its high percentage of germination for its propagation, and an efficient translocation of Cu towards the aerial part. Likewise, it should be noted that although there is a decrease in morphology, survival and germination are not affected.

Conclusions

The results obtained in this work allow us to propose *C. pumila* as a phytoremediator species for environments contaminated with Cu, Pb, and Fe. Because it is a species that presents a high percentage of seed germination, it is efficient in translocating Cu from the root to the leaves and presents a high capacity to bioconcentrate Cu, Fe, and Pb in its tissues. Despite presenting changes in most of the morphological characters, the survival of the individuals was not diminished, and their phenological period was longer than the plants in the control substrate. Other characteristics that strengthen it as a phytoremediator species are its wide geographical distribution, encompassing three types of vegetation (deciduous tropical forests, temperate forests, and xerophilous scrubs), its natural establishment in contaminated environments, high recruitment rates, rapid growth, short life cycle, and efficient control of tailings erosion. Therefore, it is important to use native plants with the characteristics of *C. pumila* for the phytoremediation of HM contaminated environments, since they are often efficient in terms of survival, growth, and reproduction in environmental conditions under stress.

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Table 1. Macromorphological characters analyzed in *Crotalaria pumila.*

Abbreviation	Character	Units
	Size characters	
RL	Root length	cm
SL	Stem length	cm
FRB	Fresh root biomass	g
DRB	Dry root biomass	g
FLB	Fresh leaf biomass	g
DLB	Dry leaf biomass	g
	Macro-morphological characters	
LBL	Leaf blade length	mm
WLB	Width of the leaf blade	mm
LP	Length of the petiole	mm
DP	Petiole diameter	mm
LIV	Length of the intermediate vein	mm
WIV	Width of the intermediate vein	mm
1/3AW	1/3 Apical width	mm
1/3BW	1/3 Basal width	mm
CLB	Coverage of leaf blade	mm^2
	Micro-morphological characters	
SI	Stomatal index	mm^2

Table 2. Seedling percentage of *Crotalaria pumila* from the control and the exposed site, under pregerminative treatments

Site	Treatment	Seedling (%)	ANOVA	
Exposed	No scarification	6.00±1.71 a		
	Mechanical scarification	97.33±1.23 b	Site (S)	$F_{1,20}$ = 1.09, ns
			Treatment (t)	$F_{1,20}$ = 525.64 ***
Control	No scarification	4.00±2.07 a	S×t	$F_{1,20}$ = 0.14 ns
	Mechanical scarification	96.67±1.23 b		

Different lower case letters denote significant differences between treatments (Tukey $p<0.05$). Average ± SE ***=$p<0.001$, ns =not significant differences

Table 3. Average values (± SE) of heavy metal concentration (mg/kg) in roots and leaves of *Crotalaria pumila*, growing in reference-substrate and tailing-substrate.

	Treatment									
	Root					Leaf				
Time (days)	Control		Exposed			Control		Exposed		
Cooper (Cu) 0.001 DL (mg/L)										
30	0.49±0.023	a	0.39±0.008	A	*	0.49±0.008	a	0.43±0.003	A	**
60	0.50±0.011	a	0.40±0.003	A	**	0.50±0.001	a	0.48±0.04	A	ns
90	0.50±0.018	a	0.38±0.01	A	**	0.49±0.014	a	0.45±0.007	A	*
120			0.41±0.01	AB				0.49±0.45	A	
150			0.46±0.01	B				0.45±0.01	A	
ANOVA										
Time (t)			$F_{2,15}$=0.60 ns			$F_{2,15}$=0.42 ns				
Treatment (T)			$F_{1,15}$=71.05 ***			$F_{1,15}$=1.76 ns				
t x T			$F_{2.15}$=0.39 ns			$F_{2,15}$=0.16 ns				
Iron (Fe) 0.005 DL (mg/L)										
30	2.75±0.010	a	3.66±0.084	A	***	2.40±0.013	a	3.28±0.106	A	**
60	2.54±0.017	a	3.93±0.089	A	***	2.19±0.061	a	2.98±0.112	A	**
90	2.77±0.017	a	3.48±0.078	A	***	2.21±0.135	a	3.09±0.321	A	ns
120			4.15±0.113	A				2.21±0.064	B	
150			3.88±0.354	A				2.26±0.076	B	
ANOVA										
Time (t)			$F_{2,15}$=1.31 ns			$F_{2,15}$=0.349 ns				
Treatment (T)			$F_{1,15}$=265.55 ***			$F_{1,15}$=11.67 **				
t x T			$F_{2.15}$=11.19 ns			$F_{2,15}$=0.016 ns				
Lead (Pb) 0.01 DL (mg/L)										
30	1.23±0.086	a	1.79±0.061	A	**	1.33±0.135	a	1.32±0.062	A	ns
60	1.52±0.048	a	1.63±0.173	A	ns	1.42±0.046	a	1.25±0.053	A	ns
90	1.22±0.060	a	1.75±0.056	A	***	1.45±0.111	a	1.15±0.073	A	*
120			1.97±0.087	B				1.35±0.077	A	
150			2.02±0.161	B				1.75±0.215	B	
ANOVA										
Time (t)			$F_{2,15}$=0.54 ns			$F_{2,15}$=0.078 ns				
Treatment (T)			$F_{1,15}$=31.194 ***			$F_{1,15}$=0.149 *				
t x T			$F_{2.15}$=3.870 *			$F_{2,15}$=0.038 ns				
Zinc (Zn) 0.0005 DL (mg/L)										
30	0.09±0.001	a	1.30±0.001	A	***	0.13±0.005	a	1.07±0.001	A	***
60	0.04±0.001	b	1.41±0.001	A	***	0.18±0.001	b	0.38±0.026	B	***
90	0.18±0.003	c	1.17±0.019	AB	***	0.11±0.004	c	0.28±0.021	C	***
120			1.33±0.044	A				0.18±0.070	D	
150			0.99±0.112	B				0.23±0.012	CD	
ANOVA										
Time (t)			$F_{2,15}$=5.93, ns			$F_{2,15}$=92.45, ***				
Treatment (T)			$F_{1,15}$=9868.98, ***			$F_{1,15}$=327.83, ***				
t x T			$F_{2.15}$=90.85, ***			$F_{2,15}$=93.04, ***				

Different lower case letters denote significant differences between control individuals during treatment time
Different upper case letters denote significant differences between exposed individuals during treatment time
LD= detection limit, nd = not detected, ns= not significant
*=P<0.05, **=P<0.01, ***=P<0.001

Table 4. Bioconcentration factor (BCF) values and translocation factor (TF) of Cu, Fe, Pb y Zn in roots and leaves of *Crotalaria pumila* from individuals growing on tailing substrate during treatment

	Concentration (mg/kg)					
Time (days)	Tailing	Root	Leaf	BCF Root	BCF Leaf	TF
Copper (Cu)						
30	0.04	0.39	0.43	9.75	10.75	1.1
60	0.04	0.4	0.48	10.0	12.00	1.2
90	0.04	0.38	0.45	9.50	11.25	1.2
120	0.04	0.41	0.89	10.3	22.25	2.2
150	0.04	0.46	0.45	11.5	11.25	1.0
$\bar{x} \pm$ SE				10.2±0.34	13.5±2.11	1.33±0.21
Iron (Fe)						
30	0.4	3.66	3.28	9.15	8.20	0.9
60	0.4	3.93	2.98	9.83	7.45	0.76
90	0.4	3.48	3.09	8.70	7.73	0.89
120	0.4	4.15	2.21	10.38	5.53	0.53
150	0.4	3.88	2.26	9.7	5.65	0.58
$\bar{x} \pm$ SE				9.55±0.29	6.91±0.55	0.73±0.08
Lead (Pb)						
30	0.23	1.79	1.32	7.78	5.74	0.74
60	0.23	1.63	1.25	7.09	5.43	0.77
90	0.23	1.75	1.15	7.61	5.00	0.66
120	0.23	1.97	1.35	8.57	5.87	0.69
150	0.23	2.02	1.75	8.78	7.61	0.87
$\bar{x} \pm$ SE				7.96±0.31	5.93±0.44	0.75±0.04
Zinc (Zn)						
30	2.14	1.30	1.07	0.61	0.50	0.82
60	2.14	1.41	0.38	0.66	0.18	0.27
90	2.14	1.17	0.28	0.55	0.13	0.24
120	2.14	1.33	0.17	0.62	0.08	0.13
150	2.14	0.99	0.23	0.46	0.11	0.23
$\bar{x} \pm$ SE				0.57±0.03	0.20±0.06	0.34±0.13

Table 5. Average values (± SE) of macro and micro-morphological characters from *Crotalaria pumila* growing in greenhouse conditions on tailing-substrate and reference-substrate.

Character	Time (days)	Treatment				SDT	ANOVA	
		Control		Exposed				
Size characters								
Root length								
	30	22.3±5.1	a	8.2±2.8	A	*	Time (t)	$F_{4,50}$ =7.23 ***
	60	23.3±5.7	a	13.2±1.1	B	*	Treatment (T)	$F_{1,50}$ =8.9 **
	90	17.6±7.3	a	13±2.6	B	ns	t x T	$F_{4,50}$ =31.46 ***
	120			18.2±6.2	C			
	150			21±3.9	D			
Stem length								
	30	17.8±4.3	a	7.7±2.1	A	*		
	60	26±2.1	b	15.7±3.1	B	*	Time (t)	$F_{4,50}$ =7.32 ***
	90	26±2.1	b	11.5±1.1	AB	*	Treatment (T)	$F_{1,50}$ =7.80 **
	120			20.2±3.3	C		t x T	$F_{4,50}$ =24.51 ***
	150			21.67±6.7	C			
Fresh root biomass								
	30	1.61±0.7	a	1.04±0.4	A	ns		
	60	0.35±0.08	a	0.20±0.03	A	ns	Time (t)	$F_{2,27}$ =1.48 ns
	90	0.31±0.05	a	0.40±0.07	A	ns	Treatment (T)	$F_{1,27}$ =3.27 ns
	120			0.89±0.12	B		t x T	$F_{2,27}$ =3.17 ns
	150			1.42±0.012	C			
Dry root biomass								
	30	0.25±0.06	a	0.10±0.06	A	ns		
	60	0.12±0.06	a	0.07±0.06	AB	*	Time (t)	$F_{2,27}$ =0.28 ns
	90	0.09±0.08	a	0.09±0.06	AB	ns	Treatment (T)	$F_{1,27}$ =3.39 ns
	120			0.17±0.03	B		t x T	$F_{2,27}$ =1.96 ns
	150			0.30±0.23	C			
Fresh leaf biomass								
	30	7.11±1.35	a	0.30±1.35	A	*		
	60	6.17±1.35	a	1.76±135	B	**	Time (t)	$F_{2,27}$ =0.32 ns
	90	6.51±1.91	a	3.19±1.35	C	*	Treatment (T)	$F_{1,27}$ =16.61 *
	120			4.51±0.30	C		t x T	$F_{2,27}$ =0.76 ns
	150			4.92±1.31	D			
Dry leaf biomass								
	30	2.00±0.46	a	0.09±0.46	A	ns		
	60	1.93±0.46	a	0.55±0.46	AB	*	Time (t)	$F_{2,27}$ =0.342 ns
	90	1.84±0.66	a	1.12±0.47	B	ns	Treatment (T)	$F_{1,27}$ =10.52 *
	120			1.71±0.48	C		t x T	$F_{2,27}$ =0.64 ns
	150			1.68±0.49	C			
Macro-morphological characters								
Leaf blade length								
	30	19.09±060	a	13.45±0.50	A	***		
	60	15.44±0.37	b	13.50±0.51	A	**	Time (t)	$F_{2,191}$ =15.02***
	90	16.22±0.47	b	10.87±0.32	B	***	Treatment (T)	$F_{1,191}$ =109.32 ***
	120			11.87±0.29	BC		t x T	$F_{2,191}$ =9.34 ***
	150			12.90±0.32	C			
Width of the leaf blade								
	30	9.04±0.31	a	6.13±0.22	A	***		
	60	7.22±0.22	b	6.28±0.24	A	**	Time (t)	$F_{2,191}$ =14.59 ***
	90	6.98±0.60	b	4.88±0.29	B	***	Treatment (T)	$F_{1,191}$ =66.34 ***
	120			4.87±0.29	B		t x T	$F_{2,191}$ =6.55 **
	150			4.88±0.13	B			

Table 5 (continued)

Character	Time	Treatment				SDT	ANOVA	
	(days)	Control		Exposed				
Length of the petiole								
	30	15.88±0.61	a	11.04±0.57	A	***		
	60	12.79±0.31	b	11.23±0.60	A	*	Time (t)	$F_{2,191}$ =5.05 **
	90	14.79±0.83	ab	9.22±0.34	B	***	Treatment (T)	$F_{1,191}$ =77.96 ***
	120			9.27±0.30	B		t x T	$F_{2,191}$ =7.85 **
	150			9.31±0.30	B			
Petiole diameter								
	30	0.53±0.02	a	0.54±0.02	A	ns		
	60	0.41±0.02	b	0.52±0.02	AB	***	Time (t)	$F_{2,191}$ =7.63 ***
	90	0.46±0.02	ab	0.47±0.02	B	ns	Treatment (T)	$F_{1,191}$ =7075 ***
	120			0.47±0.02	B		t x T	$F_{2,191}$ =4.30 *
	150			0.46±0.16	B			
Length of the intermediate vein								
	30	12.67±0.42	a	9.66±0.36	AB	***		
	60	9.54±0.33	b	9.76±0.41	A	ns	Time (t)	$F_{2,191}$ =10.28 ***
	90	10.72±0.35	b	8.43±0.42	B	**	Treatment (T)	$F_{1,191}$ =24.98 ***
	120			8.39±0.23	B		t x T	$F_{2,191}$ =9.71 ***
	150			8.38±0.20	B			
Width of the intermediate vein								
	30	6.66±0.22	a	4.93±0.20	A	***		
	60	5.35±0.22	b	5.00±0.22	A	ns	Time (t)	$F_{2,191}$ =4.02 *
	90	5.52±0.42	ab	4.62±0.39	A	ns	Treatment (T)	$F_{1,191}$ =18.14 ***
	120			4.02±0.10	B		t x T	$F_{2,191}$ =3.53 *
	150			4.18±0.10	AB			
1/3 Apical width								
	30	18.31±0.97	a	12.74±0.66	A	***		
	60	11.78±0.44	b	16.82±0.60	B	***	Time (t)	$F_{2,191}$ =4.61 *
	90	13.21±0.50	b	13.52±0.54	A	ns	Treatment (T)	$F_{1,191}$ =0.015 ns
	120			9.43±0.29	C		t x T	$F_{2,191}$ =33.96 ***
	150			9.64±0.29	C			
1/3 Basal width								
	30	16.01±0.79	a	10.46±0.62	A	***		
	60	9.13±0.29	b	10.12±0.43	A	ns	Time (t)	$F_{2,191}$ =31.84 ***
	90	8.37±0.38	b	10.60±0.39	A	***	Treatment (T)	$F_{1,191}$ =2.85 ns
	120			5.49±0.18	B		t x T	$F_{2,191}$ =29.23 ***
	150			5.99±0.18	B			
Coverage of leaf blade								
	30	217.00±6.91	a	151.06±5.95	A	***		
	60	174.91±3.71	b	153.01±0.96	A	**	Time (t)	$F_{2,191}$ =3.23 ***
	90	187.51±6.71	b	123.26±3.40	B	***	Treatment (T)	$F_{1,191}$ =115.73 ***
	120			128.37±2.94	B		t x T	$F_{2,191}$ =10.45 ***
	150			126.28±3.08	B			
Micro-morphological characters								
Stomatal index (SI)								
	30	25.07±0.42	a	22.50±0.47	A	***	Time (t)	$F_{2,191}$ =10.15 ***
	60	26.01±0.42	ab	23.88±0.32	AB	***	Treatment (T)	$F_{1,191}$ =43.41 ***
	90	27.20±0.59	b	24.54±0.47	B	***	t x T	$F_{2,191}$ =0.21 *
	120			24.39±0.35	B			
	150			24.33±0.42	B			

Different lower case letters denote significant differences between control individuals during treatment time (Tukey $p<0.05$)
Different upper case letters denote significant differences between exposed individuals during treatment time (Tukey $p<0.05$)
SDT= Statistical differences between treatments, ns = not significant, *=$P<0.05$, **=$P<0.01$, ***=$P<0.001$

DISCUSIÓN

Los estudios ecotoxicológicos son relevantes para comprender el efecto de la contaminación por MP sobre los organismos vivos y sus consecuencias tras su exposición (Capó, 2007), así como evaluar la capacidad que tienen algunos organismos para establecerse, sobrevivir y desarrollarse en ambientes contaminados con MP. En este contexto, en el presente estudio se analizaron los efectos de los MP, en primera instancia se evaluó la procedencia de las semillas de sitios expuestos a MP y sitios testigo. Posteriormente, se realizó un diseño experimental en condiciones de invernadero que permitiera evaluar la acumulación de MP a través del tiempo de exposición y sus efectos a nivel macro y micro-morfológicos en dos especies (*Vachellia campechiana* y *Crotalaria pumila*) que presentan diferente forma de vida, demostrando que cada especie responde diferente a la bioacumulación de MP. *V. capechiana* es eficiente en translocar Cr, Pb y Cu hacia la parte área, mientras que *C. pumila* fue eficiente en translocar Cu hacia hojas. No obstante, se evidencio que ambas especies presentan disminución en su morfología sin provocar mortalidad. Este trabajo aporta información valiosa en términos de seleccionar especies para fitorremediar ambientes contaminados por Cr, Pb y Cu, aunado a que ambas especies presentan una amplia distribución en el país.

Bioacumulación de MP en *Vachellia campechiana* y *Crotalaria pumila*

Los resultados de este estudio demuestran que la procedencia de las semillas (poblaciones expuestas a MP y poblaciones testigo) no influye en su germinación. Estos resultados se pueden atribuir a que en las semillas no se ven afectadas por la presencia de metales no esenciales. Esto se corroboró con la medición de metales en semillas (testa y embrión) de *V. campechiana* en donde se detectaron los metales Cu, Fe y Zn, resultados similares han sido documentados en diferentes especies de herbáceas como: *Achillea millefolium* L. (Asteraceae), *Arctium tomentosum* Mill. (Asteraceae), *Arenaria serpyllifolia* L. (Caryophyllaceae), *Cerastium semidecandrum* L. (Caryophyllaceae), *Filipendula ulmaria* (L.) Maxim. (Rosaceae), *Hypericum maculatum* Crantz. (Hypericaceae) y *Laserpitium latifolium* L. (Apiaceae), donde las semillas bioacumulan concentraciones altas de metales esenciales como Mn, Cu, Fe y Zn (Tyler y Zohlen, 1998). Esto se debe a que el transporte de minerales hacia las semillas se realiza mediante el floema (Zhang *et al.*, 2007), a diferencia de las hojas que se realiza mediante el xilema. Estudios recientes han evidenciado que la molécula de nicotianamina (NA) transporta diversos metales a través del floema (incluidos el Cu, Fe y Zn) a las estructuras reproductivas incluyendo las semillas, con la finalidad de activar el proceso de la germinación cuando tenga las condiciones adecuadas de humedad, temperatura y luz, además, estos elementos están implicados en el desarrollo inicial de la planta (Stacey *et al*., 2008; Grillet *et al*., 2014).

Por otro lado, se evaluó su capacidad de bioacumulación en individuos creciendo bajo condiciones de invernadero. Para ambas especies se midieron siete metales (Cd, Cr, Cu, Fe, Mn, Pb, Zn) detectando en *V. campechiana* cinco metales (Cr, Cu, Fe, Pb y Zn) con un patrón de acumulación de (Pb>Fe>Cr> Cu>Zn), mientras que en *C. pumila* se detectaron cuatro metales (Cu, Fe, Pb y Zn) con un patrón de acumulación (Fe>Pb>Cu>Zn). Cabe mencionar que los individuos que crecieron en sustrato testigo (sustrato proveniente de los sitios testigo) presentaron cantidades detectables de los metales presentes en los individuos expuestos a excepción del Cr en *V. campechiana*, lo cual se atribuye a que los suelos de Tlaquiltenango presentan de manera natural una riqueza de minerales azufrados, particularmente de plomo y plata. Los minerales comúnmente encontrados en la región son: arsenopirita (FeAsS), galena (PbS), acantita (Ag_2S), y calclacita (Cu_2S) (Volke *et al.*, 2004, 2005; Secretaría de Economía, 2011).

De manera particular, *V. campechiana* bioacumuló elevadas concentraciones de Cr y Pb en individuos expuestos, incrementando su concentración a través del tiempo de exposición en raíz y hoja, siendo esta última estructura donde se observó la mayor concentración para ambos metales (2.75 mg/kg Cr y 4.75 mg/kg Pb). Cabe destacar que en esta especie de estudio se detectó una mayor acumulación en hoja respecto a lo encontrado con raíz. Con relación a lo anterior, He *et al*. (2012), Hernández-Acosta *et al*. (2009) y Salas-Luévano *et al*. (2009) han documentado elevadas concentraciones de Pb en hoja de *Acacia robeorum* Maslin. (Fabaceae), *Brickellia veronicifolia* (Kunth) A. Gray. (Asteraceae), *Buddleja scordioides* Kunth. (Scrophulariaceae), *Mimosa aculeaticarpa* Ortega. (Fabaceae) y *Acacia schaffneri* (S.Watson) F. J. Herm. (Fabaceae).

En particular, la acumulación de Cr en *V. campechiana* contrasta con lo que se ha documentado en otros trabajos, donde han descrito que la mayor acumulación de este elemento se presenta en raíz, lo cual se atribuye al secuestro de Cr en vacuolas de las células de la raíz, quedando limitada su translocación hacia la parte aérea de la planta (Shanker *et al.,* 2004; Singh *et al.,* 2013). No obstante, el incremento del Cr en hojas observado en este estudio se puede atribuir a que el Cr es transportado hacia las hojas por transportadores de elementos esenciales como el Fe y azufre (S) provocando una competencia entre estos elementos (Cervantes *et al.*, 2001). Se ha documentado que especies de la familia Brassicaceae como la especie *Brassica rapa* L. (Brassicaceae) acumula elevadas concentraciones de Cr en su parte aérea, lo que se atribuye a que el Cr es translocado hacia las hojas mediante el mecanismo de captación y translocación Fe y S (Cervantes *et al.*, 2001; Singh *et al.,* 2013). Por otra parte, la baja concentración de Cu, Fe y Zn en tejido de raíz y hoja de *V. campechiana* se puede a tribuir a que el Cr y Pb se bioacumularon en mayor concentración en ambas estructuras evaluadas, sustituyendo la absorción del Cu, Fe y Zn (Patra *et al*., 2004; DalCorso, 2012).

Por otro lado, *C. pumila* bioacumuló Pb en individuos creciendo en sustrato jale incrementando su concentración a través del tiempo de exposición (150 días), su concentración en raíz fue de 2.02 mg/kg mientras que en hoja se observó una concentración de 1.75 mg/kg. Estas concentraciones fueron mayores respecto a los individuos creciendo en sustrato testigo. Es de destacar que las raíces de *C. pumila* acumularon una mayor concentración de Pb respecto a lo encontrado en hojas. Con relación a lo anterior, se han documentado resultados similares en otras especies como: *Sonchus oleraceus* L. (Asteraceae) [Raíz (1,113.24 mg/kg) hoja (65.67 mg/kg)], *Coronopus didymus* L. (Brassicaceae) [Raíz (3,684.3 mg/kg) hoja (862.8 mg/kg)], *Acalypha indica* L. (Euphorbiaceae)[Raíz (121,600 mg/kg) hoja (17,500 mg/kg)], *Chenopodium murale* L. (Amaranthaceae) [Raíz (2,513 mg/kg) hoja (2,301 mg/kg)] (Xiong, 1997, Sidhu *et al*., 2016, Venkatachalam *et al*., 2017 y Sidhu *et al*., 2018). La alta acumulación de Pb en raíces se atribuye a que este elemento queda retenido en las paredes celulares de las raíces (Han *et al*., 2016), reduciendo su translocación hacia las partes aéreas. Otra razón podría estar relacionada con la síntesis de fitoquelatinas que forman complejos con el Pb dentro del tejido vascular de las raíces de la planta (Andra *et al*., 2009). Por otra parte, en *C. pumila* la acumulación de Cu, Fe y Zn en raíz y en tejido foliar por lo general se mantuvo contante a través del tiempo de exposición. Con respecto a lo anterior se ha documentado que el Pb bloquea la absorción de otros cationes como K, Ca, Mg, Mn, Zn, Cu y Fe probablemente modificando la actividad y la permeabilidad de las membranas, haciéndolos no disponibles para su absorción y transporte en la planta (Patra *et al*., 2004; DalCorso, 2012).

A pesar de que *C. pumila* y *V. campechiana* crecieron bajo las mismas características de sustrato, humedad y temperatura respondieron de manera diferente a la bioacumulación de MP. La diferencia en los niveles de acumulación depende principalmente a la función bioquímica y genética de la especie, más allá de la concentración presente en el sustrato (Calow, 1993; Navarro-

Aviño *et al*., 2007). No obstante, las especies de estudio, aunque pertenecen a la misma familia (Fabaceae) tiene forma de vida contrastante, por un lado *C. pumila* con forma de vida herbácea y *V. campechiana* arbustiva. La diferencia en la forma de vida se ve reflejada en su tasa de crecimiento, por ejemplo, las herbáceas crecen de manera más rápida, incrementando su metabolismo, lo que se ve reflejado en mayor captación de nutrientes y agua, en contraste las especies leñosas como los arbustos presentan un crecimiento y metabolismo lento (Villar *et al*., 2004). Con relación a lo anterior, se ha documentado que entre especies responden de manera diferente al proceso de bioacumulación, Lange *et al.* (2017) describen la bioacumulación de Cu en tejido foliar de diversas especies como: *Agrostis stolonifera* (0.01 mg/kg), *Minuartia hirsuta* (0.05 mg/kg), *Calamagrostis epigejos* (0.13 mg/kg), *Anisopappus chinensis* (0.5 mg/kg), *Silene burchelli* (1 mg/kg), *Aeollanthus subacaulis* (9 mg/kg), *Haumaniastrum robertii* (8.5 mg/kg) y *Elsholtzia splendens* (5.2 mg/kg).

Respecto a la baja concentración de Zn en *V. campchiana* y *C. pumila* en tejido de raíz y foliar, se atribuye a la sustituido de este elemento por MP no esenciales como el Cr y Pb, donde Sundaramoorthy *et al.* (2010) han documentado elevadas concentraciones de Cr en *Oryza sativa* Roshev. (Poaceae) con relación a la acumulación de Zn (200 mg/kg, 35 mg/kg, respectivamente), mientras que (Patra, 2004) documenta una concentración de Pb mayor en *Sonchus oleraceus* L. (Asteraceae) respecto al Zn detectado (65.67 mg/Kg, 12.74 mg/kg, respectivamente).

Cambios morfológicos en *Vachellia campechiana* y *Crotalaria pumila* expuesta a metales pesados

Los estudios sobre el efecto de los MP en la morfología se centran en caracteres como longitud de tallo, raíz y su biomasa. Sin embargo, en este estudio se evaluaron caracteres foliares macro y micromorfológicos, debido a que en estas estructuras se almacenan los MP, los cuales están implicados en actividades metabólicas foliares (Furini, 2012).

Con respecto al mayor tiempo de sobrevivencia en individuos de *C. pumila* creciendo en sustrato jale respecto a los individuos creciendo en sustrato testigo, se puede atribuir al incremento de Pb en raíz a través del tiempo de exposición. Lo anterior, se explica debido a que la exposición de Pb disminuye el crecimiento de la planta, esto se debe al déficit de agua causado por la acumulación de este elemento, alterando el equilibrio hídrico, otro factor es la deficiencia en la absorción de nutrientes, por la sustitución del Pb por elementos esenciales (Kabatas-pendia, 2001; Ekmekçi *et al.*, 2009). Por ejemplo, Sundaramoorthy *et al.* (2010) y Bini *et al.* (2012) documentaron que el Pb afecta el ciclo celular, lo que induce la inhibición de la división celular, provocando alteraciones al crecimiento de las raíces, lo que tiene consecuencias en el crecimiento de los brotes, viéndose afectado el desarrollo normal de la planta.

De manera general, los caracteres morfológicos de las especies de estudio se vieron reducidos por la presencia de MP. En particular, en *V. campechiana* los caracteres de los individuos creciendo en sustrato jale mostraron una reducción en el 50% (LT, biomasa en hoja) respecto a los individuos creciendo en sustrato testigo. Por otra parte, en *C. pumila* disminuyó el 67% de los caracteres en individuos creciendo en sustrato jale (Biomasa en hoja) respecto a los individuos creciendo en sustrato testigo. Estos resultados se atribuyen a la acumulación de Cr y Pb en *V. campechiana* y Pb en *C. pumila*, siendo estos elementos no esenciales en las plantas, los que sustituyen a elementos esenciales como el Fe, Mn, Cu y Zn, que son fundamentales en la fisiología de la planta, vinculados a procesos de absorción y función enzimática (Kabatas-pendia, 2001). Por ejemplo, se ha documentado, que la absorción de Pb provoca la inhibición de las actividades enzimáticas, desequilibrio de agua, alteraciones en la permeabilidad de la membrana y altera la nutrición mineral, afectando el crecimiento de las plantas (Sharma y Dubey, 2005). Por su parte, el

Cr en las raíces inhibe la división celular y acorta la longitud total de las raíces, lo que puede conducir a procesos deficientes de absorción de agua y nutrientes, que a su vez conducen a la disminución del crecimiento del brote (Shanker *et al*., 2005). Otros estudios han encontrado que el Cr y el Pb inhiben el proceso de mitosis en las células de la raíz, reduciendo la extensión de este tejido. Lo anterior, es apoyado por Prasad *et al.* (2001) quienes documentaron un efecto tóxico del Cr provocando la inhibición del crecimiento primario de la raíz y la supresión de nuevas raíces secundarias en *Acacia nilotica* L. (Fabaceae). La disminución en la longitud de la raíz y biomasa de tejido foliar en especies con diferente forma de vida expuestas a Pb y Cr ha sido reportado en los arbustos: *Salix viminalis* L. (Salicaceae), *Albizia lebbek* (L.) Benth. (Fabaceae), *Acacia holocerica* Cunn. ex G.Don. (Fabaceae), *Leucaena luecocephala* (Lam.) de Wit. (Fabaceae) y *Vachellia farnesiana* (L.) Wight & Arn. (Fabaceae) (Panda y Patra, 2000; Prasad *et al*., 2001; Shanker *et al*., 2005; Maldonado-Magaña *et al*., 2011) y las herbáceas: *Sanvitalia procumbens* Lam. (Asteraceae), *Chenopodium murale* L. (Amaranthaceae), *Sonchus oleraceus* L. (Asteraceae) (Suseela *et al*., 2002; Shanker, 2003; Rosas-Ramírez, 2018; Sidhu *et al.*, 2018).

Por su parte, los caracteres morfológicos foliares evaluados en los individuos de *V. campechiana* creciendo en sustrato jale mostraron una reducción en el 67% de los caracteres. Por su parte en *C. pumila* los caracteres en individuos creciendo en sustrato jale mostraron una reducción del 80% respecto a los individuos creciendo en sustrato testigo. Estos resultados son apoyados en trabajos donde se ha documentado la reducción en la morfología foliar en especies con diferente forma de vida expuestas a MP, realizados en los sitos de este estudio: Tovar-Sánchez *et al.* (2018) en *Z. mays* una reducción del 50%, Santoyo-Martínez *et al.* (2020) observan una reducción del 63% para *V. campechiana*, mientras que Hernández-Lorenzo (2014) encontraron que para *P. laevigata* presenta una disminución del 82% en individuos expuestos a MP. Los cambios en la morfología foliar de especies vegetales que se exponen a fuentes de contaminación por MP se deben a cambios bioquímicos, en donde desarrollan mecanismos de adaptación para tolerar o bioacumular estos elementos metálicos en sus tejidos (Meharg, 1994). La bioacumulación de MP en el tejido foliar desencadena un mecanismo de detoxificación, en donde el metal se une a un ligando (quelación) como grupos Sulfhidrilo, fosfato, carboxilo e hidroxilo y péptidos como la fitoquelatinas y metalotioneinas (Rauser, 1995; Cobbett, 2000), para esto el MP se rodea de los ligandos formando un complejo, quedando inmerso en una interacción química que mantiene en equilibrio electrónico y que son trasladados a compartimentos celulares inactivos, principalmente vacuolas (Yong-Eui *et al.*, 2004; Yang *et al.*, 2005; Rodríguez-Serrano *et al.*, 2008; Lin y Aarts, 2012).

Por otro lado, en este estudio el índice estomático disminuyó en los individuos de *V. campechiana* y *C. pumila* que crecieron en sustrato jale. Resultados similares fueron documentados en *P. laevigata* expuesta a MP (Hernández-Lorenzo, 2015). Thakur (1990) documenta que la disminución de la cantidad de estomas por unidad de área (mm^2) evita un exceso de intercambio gaseoso de las plantas, aumentando su resistencia estomática. Aunque son pocos los estudios realizados con estomas en plantas expuestas a metales, se ha reportado que las células oclusivas son sensibles al estrés químico, por lo que, se pueden producir cambios de posición y número de estomas (Jordan, 2001; Rajakaruna y Baker, 2006).

En este estudio se evaluó el tamaño y la biomasa en semillas de *V. campechiana*, encontrando que los caracteres evaluados en las semillas fueron menores en los sitios expuestos a metales. Sin embargo, no se vio afectada la germinación, ya que en ambos sitios de estudio los porcentajes de germinación fueron similares. Esto podría deberse a que las semillas provenientes de sitios expuestos a MP sólo acumularon elementos esenciales (Cu, Fe y Zn), los cuales están implicados en el proceso de la germinación y el desarrollo de la plántula (Waters y Sankaran, 2011; Grillet *et al.*, 2014).

En este estudio se observó que para *V. campechiana* y *C. pumila* la bioacumulación de MP influyen en su morfología, sin embargo, a pesar de que por lo general hay una disminución de los caracteres en los individuos expuestos a MP no se ve comprometida su supervivencia.

Potencial de *Vachellia campechiana* y *Crotalaria pumila* para fitorremediar ambientes contaminados por metales pesados

Estudios en especies de plantas acumuladoras de MP con potencial para fitorremediar ambientes contaminados son reducidos, en relación con el número de especies que hay en el mundo, que es de alrededor de 272,000 (Espinosa *et al.,* 2008). El número reportado de especies acumuladoras a nivel mundial es de 271 (Reeves *et al.,* 2018), las especies de plantas utilizadas para fitorremediar ambientes contaminados por MP pertenecen principalmente a las familias: Asteraceae, Brassicaceae, Caryophyllaceae, Flacourtaceae, Lamiaceae, Poaceae, Violaceae y Euphorbiaceae (Prasad, 2003; Mahar *et al.,* 2016). Los resultados obtenidos en este estudio proponen a *C. pumula* con uso potencial para fitorremediar Cu, mientras que *V. campechiana* para fitorremediar Cr, Cu y Pb. Esto antes mencionado, de acuerdo con lo propuesto por Olguín y Sánchez-Galván (2012) y Ali *et al.* (2013) quienes proponen que una planta puede considerarse acumuladora si su Factor de Translocación (FT) es igual o mayor a 1. En el presente estudio, *V. campechiana* mostró valores del FT ≥1 para Cr (2.24), Pb (1.23) y Cu (1.22). Estos resultados son similares a los FT que han sido documentados en especies arbustivas consideradas como acumuladoras: *Gentiana pennelliana* Fernald [(Gentianaceae) (FT Zn=1.2)], *Cyperus esculentus* L [(Cyperaceae) (FT Pb=1.6, FT Zn=1.1)], *Phyla nodiflora* (L.) Greene [(Verbenaceae) (FT Cu=12.0, FT Zn=1.1)], *Rubus fruticosus* L [(Rosaceae) (FT Cu=5.6), *Sesbania herbacea* (Mill.) McVaugh [(Fabaceae) (FT Cu=4.0)], *Limnocharis flava* (L.) Buchenau [(Alismataceae) (FT Cd=1.3)] (Yoon *et al.,* 2006; Abhilash *et al.,* 2009). Por otro lado, *C. pumila* mostró valores del FT mayores a 1 para Cu (1.3).

Resultados similares han sido documentados en especies de herbáceas con potencial acumulador de Cu de acuerdo con los valores de translocación propuestos previamente en: *Taraxacum officinale* aggr. [(Asteraceae) (FT=1.2)], *Phyla nodiflora* (L.) Greene [(Verbenaceae) (FT =12.0, FT Zn=1.1)], *Rubus fruticosus* L. [(Rosaceae) (FT =5.6)], *Sesbania herbacea* (Mill.) McVaugh [(Fabaceae) (FT =4.0)] (Yoon *et al.*, 2006; Bini *et al.*, 2012).

Aunado a esto, resulta útil conocer el potencial que tiene de bioacumular MP del suelo hacia tejidos como la raíz y hoja, por lo que, en este estudio se evaluó el factor de bioconcentracion (FBC) en ambas estructuras. Los resultados obtenidos en *V. campechiana* muestra la eficiencia de acumular metales en sus tejidos provenientes del suelo con FBC de Cu (raíz=7.6, hoja=39.4), Fe (raíz=5.3, hoja=4.3), Pb (raíz=15.1, hoja=17.4), lo que la hace una especie con potencial acumulador (Yanqun *et al.*, 2005; Covarrubias and Cabriales 2017; Bader *et al.*, 2019). Estos resultados son similares a los documentados por Ahmad *et al.* (2011) quienes encontraron un FBC en *Limnanthes alba* Hartw. ex Benth. (Limnanthaceae) de Cu (raíz=54.1, hoja=84.1), Fe (raíz=3.7, hoja=4.5), Pb (raíz=16.9, hoja=22.5), *Sesbania cannabina* (Retz.) Poir. (Fabaceae) Cu (raíz=80.1, hoja=116.8), Fe (raíz=6.25, hoja=6.5), Pb (raíz=7.8, hoja=10.) y *Eclipta alba* (L.) Hassk. (Asteraceae) Cu (raíz=135.8, hoja=186.9), Fe (raíz=7.1, hoja=9.9), Pb (raíz=30.8, hoja=39.3). Mientras que en *C. pumula* los resultados del FBC mostraron una eficiencia de acumulación de Cu en raíz y hoja provenientes del suelo (raíz=10.2), hoja=13.5), Fe (raíz=9.55), hoja=6.95), Pb (raíz=7.96), hoja=5.93), lo que la hace una especie acumuladora de estos metales (Yanqun *et al.*, 2005; Covarrubias and Cabriales 2017; Bader *et al.*, 2019). Estos resultados son apoyados por Yoon *et al.* (2006) quienes reportan cinco especies de herbáceas con un FBC en raíz para *Gentiana pennelliana* Fernald [(Gentianaceae) (Pb=11, Cu=22 y Zn=2.6)], *Sesbania herbácea* (Mill.) McVaugh [(Fabaceae) FBC= Pb=1.1, y Zn=1.5)], *Stenotaphrum secundatum* (Walter) Kuntze [(Poaceae) (FBCraíz Cu=1.1)], *Plantago major* L. [(Plantaginaceae) (FBC Cu=1.2)], *Bidens alba*

(L.) DC. [(Asteraceae) (FBC Cu=6.6)]. Por su parte Ndimele *et al.* (2014) y González y González-chaves (2006) reportaron valores de FBC en hoja para *Pontederia crassipes* Mart. [(Pontederiaceae) (Fe=2.31)], *Polygonum aviculare* L. [(Polygonaceae) (Cu=2.7 Pb=1.1)], *Teloxys graveolens* Willd. [(Chenopodiaceae) (Cu=3.3 Pb=2.0)], *Jatropha dioica* Sessé ex [(Euphorbiaceae) (Cu=6.5 Pb=1.7)], *Andropogon barbinodis* Lag. [(Gramineae) (Cu=12.5 Pb=7.3)], *Bahia absinthifolia* Benth [(Compositae) (Cu=37.5 Pb=3.4)], *Solanum elaeagnifolium* Cav. [(Solanaceae) (Cu=4.7 Pb=2.7)].

Los resultados en este estudio son relevantes ya que las especies estudiadas a pesar de que presentan forma de vida contrastantes y pertenecen a la misma familia (Fabaceae), son las especies de la familia Brassicaceae las mas estudiadas, documentando 83 especies de plantas acumuladoras (Reeves *et al.*, 2018), siendo en su mayoría especies de vida herbácea, que habitan zonas templadas y frías. Lo que hace a estas especies de estudio importantes para fitorremediar ambientes contaminados por MP, principalmente Cr, Pb, Cu, además son especies que crece de forma natural en sitios contaminados por MP derivados de la actividad minera (Martinez-Becerril, 2009), presentan una amplia distribución geográfica, por un lado, *V. campechiana* se distribuye en ambientes semiáridos como el bosque tropical caducifolio (Rico, 2001), mientras que *C. pumila* se distribuye en ambientes áridos y semiáridos como el matorral xerófilo y el bosque tropical caducifolio. Así como ambientes templados como el bosque de pino encino (Soto-Estrada, 2004), lo que las hace potencialmente útiles para fines de fotorremediación en diferentes ambientes.

CONCLUSIONES

El presente estudio demostró que *V. campechiana* y *C. pumila* son especies que bioacumulan metales en individuos expuestos crónicamente a jales mineros, bajo condiciones de invernadero. Derivado de lo anterior, podemos concluir lo siguiente:

1. Las especies responden diferente a la capacidad de bioacumular MP en tejidos de raíz y hoja, por un lado *V. campechiana* bioacumula Cr, Cu, Fe, Pb y Zn, mientras que *C. pumila* tiene la capacidad de bioacumular Cu, Fe, Pb y Zn.

2. El Zn fue el elemento que se bioacumuló en menor concentración en tejido de raíz y tejido foliar para ambas especies de estudio.

3. En el presente estudio, los individuos de *V. campechiana* expuestos crónicamente a jales mineros en condiciones de invernadero, mostraron una eficiencia de translocación de Cr, Pb y Cu, hacia el tejido foliar, de acuerdo su FT>1 reportado para estos metales.

4. Los individuos de *C. pumila* creciendo en sustrato expuesto a MP (jales) son eficientes en la translocación de Cu desde la raíz a las hojas.

5. Los resultados en este estudio muestran que la acumulación de MP influye en caracteres macro y micro-morfológicos, observando una reducción de estos caracteres en ambas especies. A pesar de esto, no se vio comprometida la supervivencia de los individuos creciendo en sustrato expuesto a MP (Jale).

6. Se evidenció que en ambas especies de estudio no influye la procedencia de las semillas en su germinación, evidenciando para *V. campechiana* que acumula metales esenciales (Cu, Fe y Zn) en esta estructura, siendo fundamental para el proceso en la germinación.

7. Las especies en este estudio se consideran útiles para fitorremediar ambientes contaminados por MP por su capacidad de bioacumulación y translocación de MP hacia el tejido foliar, sus semillas presentan un alto porcentaje de germinación para su propagación, tienen una amplia distribución geográfica, habitando diferentes tipos de ambientes.

PERSPECTIVAS

El presente trabajo reveló que las especies de estudio tienen la capacidad de utilizarse en proyectos de fitorremediación principalmente de Cr, Cu, Fe y Pb para *V. campechiana* y Cu, Fe y Pb para *C. pumila*, debido a la capacidad de bioacumulación y translocación hacia el tejido foliar de las plantas. A pesar de que hay una disminución en su morfología no se ve comprometida la supervivencia de los individuos expuestos crónicamente a MP. Estos resultados abren la posibilidad de caracterizar especies que habiten ambientes contaminados por estos elementos y seleccionar las especies en donde los caracteres macro y micro-morfológicos se vean menos afectados por la exposición a MP. Con la finalidad de que diferentes especies vegetales pueden extraer diferentes metales, por lo que es necesario combinar especies de plantas para desarrollar estrategias más efectivas de fitorremediación de ambientes contaminados con más de un MP.

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