UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

FACULTAD DE CIENCIAS QUÍMICAS

Posgrado en Ciencias Químicas

Hongos endófitos de *Typha latifolia* que participan en la tolerancia a metales pesados

Tesis que para obtener el grado de:

Doctorado en Ciencias Químicas

Presenta:
Ponce Hernández Amauri

Directora de tesis: **Dra. Candy Carranza Álvarez**

Codirector de tesis: **Dr. Domingo Martínez Soto**

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Asesor: **Dra. Gabriela Navarro Tovar**

SAN LUIS POTOSÍ, S.L.P. **FEBRERO 2026.**

Este proyecto se realizó en el Laboratorio de Investigación en Ciencias Ambientales de la Facultad de Estudios Profesionales Zona Huasteca adscrita a la Universidad Autónoma de San Luis Potosí (UASLP) y en el Departamento de Microbiología de la División de Biología Experimental y Aplicada adscrito al Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), en el periodo comprendido entre febrero de 2022 y enero 2026, bajo la dirección de la Dra. Candy Carranza Álvarez, en codirección con el Dr. Domingo Martínez Soto con recursos del proyecto de ciencia básica y de frontera con clave: CBF-2025-I-1755.

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Facultad de Ciencias Químicas
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Por este medio me permito informarle el porcentaje de similitud obtenido mediante Ithenticate para la tesis titulada: Hongos endófitos de *Typha latifolia* que participan en la tolerancia a metales pesados presentada por el autor M.C. Ponce Hernández Amauri. La tesis es requisito para obtener el grado de Doctorado en el Posgrado en Ciencias Químicas. El análisis reveló un porcentaje de similitud de 0 % excluyendo referencias y metodología.

Agradezco sinceramente su valioso tiempo y dedicación para llevar a cabo una exhaustiva revisión de la tesis. Quedo a su disposición para cualquier consulta o inquietud que pueda surgir en el proceso.

Sin más por el momento, le envío un cordial saludo.

A T E N T A M E N T E

_____

Dr. Fidel Martínez Gutiérrez

Coordinador Académico del Posgrado en Ciencias Químicas

Esta obra está dedicada a mi Dios por estar siempre a mi lado y permitirme alcanzar mis sueños.

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RESUMEN

Las altas concentraciones de metales pesados (HMs) en el ambiente son un problema mundial por los efectos tóxicos sobre la salud humana y los ecosistemas. La fitorremediación es una estrategia eficaz para mitigar la contaminación. *Typha latifolia* es una planta utilizada en procesos de fitorremediación de sitios contaminados por HMs. Para adaptarse a ambientes extremos han establecidos interacciones simbióticas con hongos endófitos (HE), que promueven el crecimiento vegetal y las protege frente a factores bióticos y abióticos. En nuestra investigación se dilucidaron los efectos de los hongos endófitos aislados de *Typha latifolia* sobre la tolerancia y absorción de metales pesados (HMs) en la planta. Los resultados demostraron que *T. latifolia* puede bioacumular concentraciones tóxicas de arsénico (As), cadmio (Cd), cobre (Cu) y plomo (Pb) en las raíces. De estas se aislaron los HE, que se identificaron como *Talaromyces liani*, *Talaromyces trachyspermus*, *Talaromyces austrocalifornicus*, *Talaromyces* sp., *Nesartorya fischeri* y *Aspergillus sydowii*. Estos hongos toleran concentraciones tóxicas de plata (Ag), cobre (Cu) y Cd. Aunque, la exposición a Cd afectó negativamente el crecimiento fúngico y la morfología de las hifas. Además, estos hongos producen sideróforos y solubilizan fosfatos. *Talaromyces* spp. mostraron actividad quitinolítica, que inhibe el crecimiento de fitopatógenos. *N. fischeri* colonizó la corteza de la raíz de *T. latifolia*. *Talaromyces* spp. y *N. fischeri* asociados a *T. latifolia* mejoran el bienestar de la planta y favorecen la acumulación de Cd en los tejidos vegetales. Finalmente, *A. sydowii* pudo crecer en medio contaminado con glifosato y Captan, considerándolo capaz de tolerar pesticidas.

Palabras clave: Metales pesados, Fitorremediación, *Typha latifolia*, Hongos endofíticos, *Talaromyces*, *Neosartorya fischeri*, *Aspergillus sydowii*.

ABSTRACT

High concentrations of heavy metals (HMs) in the environment are a global problem due to their toxic effects on human health and ecosystems. Phytoremediation is an effective strategy for mitigating contamination. *Typha latifolia* is a plant used in phytoremediation processes at sites contaminated by HMs. To adapt to extreme environments, they have established symbiotic interactions with endophytic fungi (EF), which promote plant growth and protect them from biotic and abiotic factors. Our research elucidated the effects of endophytic fungi isolated from *T. latifolia* on the tolerance and absorption of heavy metals (HMs) in the plant. The results showed that *T. latifolia* can bioaccumulate toxic concentrations of arsenic (As), cadmium (Cd), copper (Cu), and lead (Pb) in its roots. The EF were isolated from these roots and identified as *Talaromyces liani*, *Talaromyces trachyspermus*, *Talaromyces austrocalifornicus*, *Talaromyces* sp., *Nesartorya fischeri*, and *Aspergillus sydowii*. These fungi tolerate toxic concentrations of silver (Ag), copper (Cu), and Cd. However, exposure to Cd negatively affected fungal growth and hyphal morphology. In addition, these fungi produce siderophores and solubilize phosphates. *Talaromyces* spp. showed chitinolytic activity, which inhibits the growth of phytopathogens. *N. fischeri* colonized the root cortex of *T. latifolia*. *Talaromyces* spp. and *N. fischeri* associated with *T. latifolia* improve plant health and promote Cd accumulation in plant tissues. Finally, *A. sydowii* was able to grow in media contaminated with glyphosate and captan, suggesting that it can degrade pesticides.

Keyword: Heavy metals, Phytoremediation, *Typha latifolia*, Endophytic fungi, *Talaromyces* spp., *Neosartorya fischeri*, *Aspergillus sydowii*.

INDICE

Introducción

El aumento de las actividades antropogénicas, como la minería, la fundición, la agricultura, la industria y la quema de combustibles (Ponce-Hernández et al., 2025), ha provocado que las concentraciones de metales pesados (MPs) y metaloides en el medio ambiente aumenten de forma constante a lo largo de los años (Briffa et al., 2020). Esto ha creado un problema mundial, ya que los MPs son tóxicos en bajas concentraciones, no son biodegradables y pueden entrar en la cadena alimentaria, causando graves daños a la salud humana y a los ecosistemas (Fediuc y Erdei, 2002).

Para mitigar este problema, se han aplicado diversas estrategias de remediación con el fin de reducir las concentraciones de metales pesados en los sitios contaminados, incluyendo procesos fisicoquímicos y biológicos (Barrera-Díaz, 2017). Los procesos fisicoquímicos son eficaces para eliminar los metales pesados, pero suelen ser muy costosos, consumen grandes cantidades de energía y en ocasiones, generan contaminantes secundarios (Kumar et al., 2021). Por otro lado, la fitorremediación destaca entre los procesos de remediación biológica como una técnica propuesta como alternativa para eliminar los metales de los sitios contaminados (Yan et al., 2020). La fitorremediación se considera una técnica respetuosa con el medio ambiente y de bajo coste basada en el uso de plantas con gran potencial para tolerar, absorber y eliminar los contaminantes presentes en el sitio (Raskin y Ensley, 2000). En esta técnica, las plantas utilizan principalmente mecanismos como la fitoextracción, la fitodegradación, la fitoestabilización, la rizofiltración y la fitovolatilización (Grzegórska et al., 2020), que permiten la captura de metales al actuar como filtros que metabolizan las sustancias de forma natural (Raskin y Ensley, 2000).

Typha latifolia es una planta macrófita que se ha utilizado ampliamente en la fitorremediación debido a su rápido crecimiento, alta producción de biomasa, alto nivel de tolerancia y potencial para acumular metales pesados. Se ha demostrado que acumula cromo (Cr), cadmio (Cd), hierro (Fe), manganeso (Mn) y plomo (Pb) de las

aguas residuales municipales (Carranza-Álvarez et al., 2008). Sin embargo, las concentraciones excesivas de metales pesados pueden causar diversos efectos nocivos en la planta (Maine, 2012). Para adaptarse a entornos extremos y contrarrestar los efectos tóxicos de los metales pesados, las plantas establecen interacciones simbióticas con microorganismos de la rizosfera, incluidos los hongos endófitos (HE) (Alam et al., 2021). Los hongos endófitos benefician a la planta huésped de forma directa o indirecta, desempeñando un papel mutualista que promueve el crecimiento y protege contra factores bióticos y abióticos (Sánchez-Fernández, 2013).

Hasta la fecha, se desconocen los efectos que los hongos endófitos tienen sobre las plantas *Typha latifolia*, que les permit tolerar y acumular altas concentraciones de metales pesados. Por lo tanto, el objetivo de este estudio es dilucidar los efectos que los hongos endófitos tienen sobre la tolerancia y absorción de metales pesados, así como en la promoción del crecimiento en las plantas de *Typha latifolia*.

Antecedentes

Previos estudios han demostrado que las plantas de *T. latifolia* pueden crecer en aguas contaminadas y absorber metales pesados. Hejna et al. (2020) demostraron que *T. latifolia* acumula zinc (Zn) y cobre (Cu) de aguas residuales contaminadas y los trasladaba a sus tejidos sin mostrar signos de toxicidad. Hazra et al. (2015) informaron de que las plantas de *T. latifolia* tienen un alto potencial para acumular Fe y Mn en las raíces y los brotes. Además, Yang y Shen (2020) informaron de que *T. latifolia* tiene un efecto reparador en los suelos contaminados con Cd. La capacidad de eliminación de metales también se ha demostrado en condiciones hidropónicas controladas. El estudio realizado por Alonso-Castro et al. (2009) demostró que *T. latifolia* puede eliminar eficazmente el Cd y el Pb de las soluciones en condiciones hidropónicas axénicas, acumulando estos metales principalmente en las raíces.

Nuestro equipo también demostró que *T. latifolia* elimina metales pesados como Pb, Cd, Cr y Mn de su entorno, acumulando altas concentraciones en sus raíces

(Carranza-Álvarez et al., 2008). Además, nuestro equipo ha tratado de dilucidar el papel de los microorganismos aislados de *T. latifolia* en su tolerancia a metales pesados. En el estudio realizado por Hernández-Morales et al. (2014), se informó del aislamiento de 42 bacterias endófitas de las raíces de *T. latifolia*. Demostraron que algunas de estas bacterias pueden solubilizar fosfatos, degradar pectina y crecer hasta en 250 mg/kg de Pb. Además, Moctezuma-Granados et al. (2017) demostraron que las bacterias aisladas de *T. latifolia*, concretamente la cepa *Pseudomonas rhodesiae* GCR140, pueden aumentar el porcentaje de eliminación de Cd hasta en un 50 % en comparación con las plantas no inoculadas. Observaron que la concentración de Cd era mayor en el tejido radicular. Rolón-Cárdenas et al. (2022) indicaron que *P. rhodesiae* GRC140 aumentaba la translocación de Cd a los brotes, lo que sugiere que la fitoextracción por *T. latifolia* mejora con la inoculación de *P. rhodesiae* GRC140. Lo anterior ha demostrado que algunos microorganismos, como las bacterias aisladas de la rizosfera de *T. latifolia*, mejoran la fitoextracción y la tolerancia a los metales pesados. Sin embargo, hasta ahora se desconocen los efectos que los hongos endofíticos (HE) aislados de *T. latifolia* inducen sobre la tolerancia y la acumulación de metales pesados, así como en la promoción del crecimiento de estas plantas.

Los HE son un grupo de hongos que viven de forma asintomática en el tejido vegetal. Pueden aumentar la tolerancia de la planta huésped al estrés biótico y abiótico (Soleimani et al., 2010). Diversos estudios han demostrado el papel beneficioso de los HE en plantas expuestas a metales pesados. En el estudio realizado por Yamaji et al. (2016), aislaron HE de las raíces de *Clethra barbinervis*, encontrando con alta frecuencia *Phialocephala fortinii*, *Rhizodermea veluwensis* y *Rhizoscyphus* sp. Su estudio demostró que estos hongos mejoraban significativamente el crecimiento de las plántulas y reducían las concentraciones de Cu, níquel (Ni), Zn, Cd y Pb en las raíces. En contraste, la ausencia de HE en las raíces provocó que la planta apenas creciera en condiciones de contaminación por metales pesados, presentando síntomas de toxicidad. Soleimani et al. (2010) demostraron que los hongos endófitos del género *Neotyphodium* promueven una alta producción de biomasa y un alto potencial de

acumulación de Cd en las raíces y brotes de *Festuca arundinacea* y *Festuca pratensis*. Ren et al. (2011) demostraron que la infección por HE en la planta *Lolium arundinaceum* aumentaba significativamente la biomasa bajo estrés por Cd y aumentaba la acumulación y el transporte de Cd desde la raíz hasta el tallo, en comparación con las plantas libres de endófitos. Por otro lado, el endófito *Penicillium foniculosum*, aislado de plantas de soja, mostró tolerancia al Cd y al Cu. La inoculación de este hongo en plantas de soja promovió un aumento significativo de la biomasa de las plantas expuestas al Cu, en comparación con las plantas no inoculadas. Además, el endófito aumentó la tasa de tolerancia al Cu en las plantas de soja (Khan y Lee, 2013). En cuanto a los estudios publicados sobre *T. latifolia*, Sharma et al. (2017) aislaron 20 hongos endófitos de los diferentes tejidos vegetales. De todos los aislados, *Aspergillus niger* A40 fue capaz de adsorber el 100 % del Pb. La interacción entre el biosorbente fúngico y el ion metálico mejoró con un tiempo de contacto de 180 minutos. Esto demostró que los HE tienen el potencial de eliminar los contaminantes del suelo. Sin embargo, este estudio no investigó el efecto de la interacción entre hongos y plantas en la eliminación de metales pesados.

Justificación

La creciente exposición a metales pesados y metaloides ambientales, así como a sus toxicidades asociadas, se ha convertido en una grave amenaza para la salud humana (Haidar et al., 2023). Por lo tanto, la aplicación de técnicas de biorremediación es impredecible, ya que son económicas y respetuosas con el medio ambiente. La literatura científica ha demostrado que las plantas *Typha latifolia* tienen un alto potencial para absorber, translocar, acumular y tolerar concentraciones tóxicas de metales pesados. Para adaptarse al entorno extremo y contrarrestar los efectos tóxicos de los metales pesados, las plantas establecen interacciones simbióticas con microorganismos de la rizosfera, incluidos los hongos endofíticos.

Sin embargo, existe poca información sobre los hongos endófitos asociados a plantas *Typha latifolia* y sus efectos sobre ella. Por lo que, es importante identificar los

hongos endófitos asociados a *T. latifolia* y determinar los efectos fisiológicos y mecanismos que intervienen en la interacción entre los hongos y plantas en respuesta al estrés causado por los metales pesados.

Hipótesis

Los hongos endofíticos asociados a las raíces de *Typha latifolia* aumentan la tolerancia y la absorción de altas concentraciones de metales pesados, así como favorecer el crecimiento de las plantas.

Objetivos

Objetivo general

Identificar los hongos endófitos de raíces de *Typha latifolia* y determinar sus efectos sobre la tolerancia y absorción de metales pesados, así como en la promoción del crecimiento de las plantas.

Objetivos específicos

1. Cuantificar el contenido de metales pesados en los tejidos de plantas de *T. latifolia* recolectadas en un sitio contaminado.
2. Identificar morfológica y molecularmente los hongos endófitos asociados a las raíces de *T. latifolia*.
3. Determinar la capacidad de tolerancia a metales pesados de los hongos endófitos aislados de *T. latifolia*.
4. Evaluar la capacidad de los hongos endófitos para producir sideróforos, solubilizar fosfatos e inhibir el crecimiento de hongos fitopatógenos.
5. Evaluar el efecto de los hongos endófitos en la fitoextracción de metales pesados por plántulas de *T. latifolia*.

Estructura de Tesis

La situación actual de la contaminación ambiental por metales pesados en México se describe en el capítulo uno de este documento. Los cuatro capítulos siguientes describen los efectos que los hongos endofíticos aislados de las raíces tienen en las plantas de *T. latifolia* para mejorar la biorremediación. Además de evidenciar la capacidad de los hongos endófitos para tolerar diversos tipos de estreses abióticos. Cada uno de estos capítulos de la tesis representa un artículo original que serán publicados en revistas de prestigio internacional. El capítulo final incluye una discusión general que destaca la importancia y la contribución de la investigación realizada, así como conclusiones, recomendaciones y perspectivas.

El capítulo I se centra en una revisión que analiza la información recopilada a partir de artículos originales sobre la contaminación por metales pesados (MP) en diversos entornos de México. Identifica los estados mexicanos en los que se ha informado de concentraciones de MP que superan los límites máximos permitidos para diversos tipos de suelos, aguas y sedimentos. El objetivo de este capítulo es documentar sobre la situación de contaminación por metales pesados que existe en México y los riesgos a la salud que se producen.

El capítulo II presenta un estudio que evalúa el potencial de los hongos endófitos del género *Talaromyces* asociados a las raíces de *Typha latifolia* para tolerar y acumular metales pesados, promover el crecimiento de las plantas e inhibir el desarrollo de fitopatógenos, proponiendo una alternativa adecuada para la biorremediación de masas de agua contaminadas con metales pesados.

En el capítulo III se evaluó la capacidad de los hongos endófitos del género *Talaromyces* para tolerar estreses abióticos de tipo osmótico, oxidativo y de pared celular, contribuyendo a conocer las condiciones óptimas para sus aplicaciones biotecnológicas.

En el capítulo IV se estudia el potencial del hongo endófito *Neosartorya fischeri* para tolerar y biosorber metales pesados a través de su biomasa. También se evaluó

su efecto en la promoción y la aptitud de *T. latifolia* bajo estrés por cadmio, lo que reveló la relación colaborativa entre los hongos beneficiosos y las plantas en la remediación.

Por último, en el capítulo V se identifica y caracteriza el hongo endofítico *Aspergillus sydowii*, se evalúa su capacidad para tolerar concentraciones tóxicas de algunos metales pesados y plaguicidas como el glifosato y el captan. Además, se valora su capacidad para producir sideróforos y degradar fosfatos. En conjunto, los resultados de esta investigación resaltan el potencial de *A. sydowii* para su uso como complemento de las tecnologías ecológicas para remediación de entornos contaminados.

Referencias

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CAPÍTULO I

Overview of the heavy metal contamination in Mexico: sources of the contamination and issues in human health

Abstract

In the present review, information was collected from original articles published between 1992 and 2022 that are related to the presence of heavy metals in the different states of Mexico. The objective of this review was to present the states of Mexico in which concentrations of heavy metals that exceed the maximum permissible limits established by national and international standards for environmental media such as water, soil and sediment have been reported. It was found that in 25 states of Mexico at least one heavy metal exceeds the maximum permissible limits in water, in 24 states at least one heavy metal exceeds the maximum permissible limits in soil and in 24 states at least one heavy metal exceeds the maximum permissible limits in sediment. It was found that the main anthropogenic activities that release heavy metals into the environment are industry, urban wastewater, mining, pesticides and fertilizers. Furthermore, it was found that the main diseases caused by exposure to heavy metals are cancer, damage to the CNS, DNA, kidneys, and lungs. Finally, it is proposed that fungal-assisted phytoremediation may be an appropriate application strategy to reduce the problem of heavy metal contamination in the country. However, this strategy needs to be deepened to decipher the mechanisms involved in improving the phytoremediation process.

Keywords: Heavy metals contamination, Diseases, Anthropogenic activities, Mexico, Fungal-assisted phytoremediation

Introduction

Heavy metals (HM) are elements naturally found in the environment and are essential for life. However, they become toxic when they exceed natural concentration or accumulate in living organisms (UNEP, 2023). It is well known that heavy metals can persist for a long period of time in the environment, promoting negative effects on human health. Nowadays HMs are causing severe problems of environmental contamination, their toxicity is affecting ecology, evolution, nutrition, and environmental niches of living organisms (Jaishankar et al., 2014). Interestingly, world industrialization (Gautam et al. 2016) and concentrations of HMs in the environment have increased over the years. The mobilization and transport of metals in the environment have increased considerably since the 1940s (Ali et al. 2019). For example, from 1970 to the 2010 the metals exceeding the international standards in rivers and lakes increased up from two metals (Fe and Mn) to ten (Cd, Pb, Cr, Hg, Zn, Cu, Ni, Al, Mn, Fe and As) (Zhou et al. 2020). This increasing concentration of HMs in the environment has a direct relation with the increasing of the anthropogenic activities looking to satisfy the needs of societies. Anthropogenic activities are considered the main sources of HM release, among them mining, agriculture, metallurgy, industry, and carbon combustion are causing the severest contamination problems worldwide.

In Mexico, official standards establish the permissible limits of HM in soils, waters, and sediments. Also, it has been determined that it is necessary to carry out remediation and management of the hazardous materials generated by industrial activities. All these strategies are looking to prevent environmental contamination, risks to human health, and the deterioration of non-renewable natural resources. However, despite Mexican federal regulations, the presence of HMs has been reported in various environments including rivers, lakes, soils, and air (Pérez-Castresana et al., 2019, Macías et al., 2022, Saha et al., 2022a). For example, several reports have shown that HM contamination has caused various pathologies in Mexico such as cancer, damage to the Central Nervous System (CNS), kidneys, lungs, and DNA (Balali-Mood et al., 2021).

In this review we summarize the current information on HMs in Mexico. We had a deep discussion about heavy metal contamination in Mexican soils, waters, and sediments. Additionally, we update information on the anthropogenic activities responsible for the release of HMs into the environment, and the human diseases reported because of exposure to toxic concentrations of HMs. The objective is to offer an overview of the current situation in Mexico regarding heavy metal contamination, seeking to highlight the seriousness of the issue and promote resolution strategies. Therefore, the issue of phytoremediation assisted by microorganisms is addressed as an ecological alternative to contribute to the restoration of contaminated environments. Finally, the information discussed in this work can be used by the Mexican authorities to handle the HMs contamination in each region of the country, and it can be a reference about the current view of HMs contamination in Latin America.

Generalities of the heavy metal contamination

The term Heavy Metal (HM) has been highly discussed among the scientific community. In this review the HM concept will be used according to Ali and Khan (2018), who indicated that HMs include all natural metals achieving the following requirements: i) have an atomic number greater than 20, and ii) have a density greater than 5 g/cm^3. The term HM includes arsenic and mercury, and it is also used to refer to metals or metalloids with potential to cause toxicity problems (Alloway 2013). HMs are naturally present in the environment; their concentration depends on the type of materials or kind of rocks present in each site. For example, Table 1.1 shows some HMs such as As, Cd, Cr, Hg and Pb that are considered highly toxic. However, in uncontaminated soils they are usually found in small concentrations not causing harm to the environment or humans.

Table 1.1. Typical heavy metals concentrations in soils.

Heavy metal	Typical Concentration Range in Soil (mg kg^{-1})	Reference
As	5–7.5	Matschullat (2000)
Cd	0.1–1.0	Mertens y Smolders (2013)
Ni	20-50	Shacklette y Boerngen (1984)
Cr	40-70	Richard y Bourg (1991)
Mn	950	Levinson (1974)
Cu	2-50	Lide (2009)
Pb	17	Nriagu (1978)
Hg	40	Wedepohl (1995)
Zn	10-100	Mertens y Smolders (2013)

Sources of heavy metals contamination

There are two sources of HM's, natural or anthropogenic (Bu et al., 2020). Natural sources include volcanoes, forest fires, sea salt, metal corrosion, metal evaporation from soil and water, soil erosion, and geological weathering (Briffa et al., 2020). Rocks releasing the highest concentrations of HM into the environment are granitic, basaltic, ultramafic, sandstone, shale, black shale, and limestone (Alloway et al., 2013). However, anthropogenic activities are considered the main sources of releasing and contamination by HM in the environment, due to accelerated industrialization, urbanization, and increasing population. The anthropogenic activities mostly contaminating the water bodies are manufacturing, mining, wastewater discharges, fertilizers, and pesticides (Zhou et al., 2020). The sediments are contaminated mainly by industrial discharges, agriculture and aquaculture that are correlated by releasing V, Al, Co, Ni, Hg and Pb. Also, maritime transport is associated with the release of Cu, Zn, Cr, and Cd on sediments (Tang et al., 2018; Zhang et al., 2022; Fan et al., 2022). The anthropogenic activities contributing to soil pollution include industry, petrochemical production, coal combustion and mining being the main release Hg, Pb

and Cd. The smelting of minerals releases Cu, Zn and Pb into the soil, and agriculture releases As because it is a component of fertilizers and pesticides (Bu et al., 2020; Saha et al., 2022b). For example, Table 1.2 shows minimum and maximum values of HM concentrations reported in water, soil, and sediments by mining and industrial activities. These studies show that wood burning, coal combustion and waste incineration are anthropogenic activities releasing high concentrations of HMs to the environment (50,000 t/year of Cr worldwide) (Ali et al., 2019). Additionally, it has been reported that carbon combustion can release up to 60,000 t/year of Ni (Merian, 1984). In the case of Cd, it has been reported that up to 7000 t/year are emitted by incineration of sewage sludge and carbon combustion (Merian, 1984).

Heavy metals causing contamination problems in Mexico

There are studies describing the contamination of HMs in specific natural environments in Mexico. In the present review we analyze all reports published about the HM concentrations in the states of Mexico. The search was based on articles published between 1992 and 2022, considering that in Mexico the first article of HM in soil, sediment or water was reported in 1992. A total of 361 articles were analyzed, of which 140 report HM in soils, 92 in sediments and 129 in water.

Table 1.2. Concentrations of HM's in water bodies, soils and sediments affected by mining and industrial activities.

Heavy Metals (HM)	HM concentration in water (mg L^{-1})		WHO Standard (mg L^{-1})	HM Geoaccumulation in soils (mg Kg^{-1})		HM Geoaccumulation in sediments (mg Kg^{-1})		Standard (Kabata-Pendias, 2011; Alloway 2013) (mg Kg^{-1})
	Mining	Industry		Mining	Industry	Mining	Industry	
As	0.0078 a 13.6	38	0.01	0.13 a 10,209	3 a 360,000	4.9 a 579	555 a 2,221	4.7
Cd	0.0002 a 2.1	0.011 a 0.9	0.003	0.081 a 2,089	0.012 a 1,407	0.3 a 199	0.07 a 5	0.41
Cr	0.0015 a 0.6	0.03 a 1,779.8	0.05	1.9 a 1,320	1 a 2,160	19.8 a 126	67 a 138,284	42
Cu	0.002 a 222.8	0.042 a 15,560.3	2	0.12 a 175	2 a 27,984	6 a 105,898	2.6 a 86	14
Pb	0.0004 a 1.6	0.0004 a 326	0.01	0.2 a 71,161	0.7 a 52,455	16.6 a 10,322	14 a 38,400	25
Hg	-	3 a 9.9	0.006	5.49 a 55	1 a 123	0.49 a 4	-	0.07
Ni	0.008 a 13.6	0.021 a 245.4	0.007	226.7 a 2,460	1.5 a 570	8.37 a 72,963	3.6 a 54	18
Zn	0.02 a 683.5	0.008 a 505.5	-	10.2 a 63,706	9.5 a 32,298	193.9 a 4,573	50 a 245	62

Data modified and taken from Vereda et al. (2019) and Ponce-Hernández et al. (2025)

Soil

The first reports in Mexico of HM in soil were published in 1992. and the highest number of publications was reached in 2019, with 14 reports. According to the data collected, the HMs that were most reported in the articles regarding soils follow the following order: Pb > Cu, Zn > Cr > Fe, Ni > Cd > Mn > As, Hg.

Water

The first reports published in Mexico about HMs in water were published starting in 1999, and the highest number of reports about it was in 2019, with 14 reports. The HMs that were most reported in the analyzed articles follow the following order: Cu > Cd, Pb > Cr, Zn > As > Mn > Fe > Hg > Ni.

Sediment

For sediment, the first reports of HM in Mexico were published in 1994, reaching the highest number of reports in 2013, with eight reports. The HMs that were most reported in the analyzed articles follow the following order: Ni, Pb, Zn > Cu > Cd, Cr > Mn > Fe > As.

Main anthropogenic sources of heavy metal release into the environment in Mexico

According to the scientific reports analyzed in this work, the main anthropogenic sources releasing heavy metals in Mexico include the industry (ID) mentioned in 455 studies and being the activity responsible for releasing HMs into the environment; urban wastewater (WD) discharges communicated in 325 studies as responsible for the release of HM into the environment; mining (M) mentioned in 323 studies; fertilizers and pesticides (FP) described in 259 studies, carbon combustion (CC) indicated in 95 studies, and smelting (F) reported in 15 studies.

Diseases in humans due to metal contamination in Mexico

Heavy metals have native issues on human health and the environment, causing severe damage to hundreds of millions of people around the world. According to the

studies analyzed in this review, it was found that the main diseases caused by MH are cancer, damage to the Central Nervous System (CNS), kidneys, lungs, genotoxic, cardiac, gastrointestinal, endocrine, skin, liver, in the reproductive system, immunological, poisoning, teratogenic, in bones, hemolytic and in growth development (Balali-Mood et al., 2021).

Curiously, according to the reviewed articles there are 15 HMs causing the greatest number and diversity of pathologies in humans. Of them, 10 HMs are responsible for the worst pathologies affecting humans by HMs. For example, Pb is considered the main cause of health problems, causing 17 different diseases; followed by As, which cause 16 different diseases; Cd, Cr and Zn are the cause of 14 diseases; Fe causes 13 diseases; Mn and Hg 12 diseases; Cu causes 10 diseases; and finally, Ni causes 9 diseases.

Lead (Pb) exposure can cause neurological, respiratory, and urinary disorders, due to its immune modulating, oxidative, and inflammatory effects (Balali-Mood et al., 2021). Chronic exposure to Pb promotes the development of arteriosclerosis and hypertension, thrombosis and pathologies related to the cardiovascular system (Vaziri, 2008). In the liver, Pb generates cellular exhaustion and infiltration, causing liver cirrhosis (Hegazy and Fouad, 2014). Furthermore, Pb is considered a carcinogenic affecting the DNA repair mechanism and the gene regulation causing cellular disorders and tumors (Silbergeld et al., 2000). Exposure to Pb has been related to a high risk of lung, stomach, and bladder cancer (Rousseau et al., 2007). Although, the organs that it most effects are the kidneys, causing acute and chronic nephropathies (Mitra et al., 2022).

Likewise, arsenic (As) is mainly related to diseases such as liver cancer (hepatocellular carcinoma and angiosarcoma), pathologies in the lungs, heart, skin, CNS, kidneys, liver and gastrointestinal. (Liaw et al. 2008; Yu et al., 2006). It has been described that As binds to DNA-binding proteins and slows down the DNA repair process (Singh et al., 2007; García-esquinas et al., 2013). Recent studies indicate that As causes vasodilation and circulatory collapse (Jolliffe et al., 1991). Also, As has been related

changes in synaptic transmission and the balance of neurotransmitters and neurotoxic effects are attributed to it due to the induction of multiple apoptotic mechanisms (Garza-Lombó et al., 2019).

Cadmium (Cd), is mainly related with several cancers, followed by pathologies in the kidney, lung, CNS, and DNA damage. Studies show that exposure to Cd can promote the development of cancers in various organs and tissues such as the kidney, lungs, pancreas, breast, prostate, and digestive tracts (Balali-Mood et al., 2021). Cd is considered carcinogenic to humans by the International Agency for Research on Cancer (IARC), and it affects the renal cortex and liver. In the kidney, excessive Cd exposure cause renal failure, renal tubular acidosis, and excess calcium in the urine (hypercalciuria). In the liver, it causes liver failure (Hyder et al., 2013). Also, Cd severely affects CNS functionalities because Cd induce death of neural cells by apoptosis (Wang and Du, 2013), leading to cognitive impairment and behavioral changes in children and adults (Marchetti, 2014). Other diseases caused by inhalation of industrial dusts contaminated with Cd are lung damage and lung injuries (Nishijo et al., 2017; Batáriová et al., 2006).

In the same way, Zinc (Zn) toxicity can affect the respiratory and gastrointestinal tracts, the brain and produce various side effects (Hussain et al., 2022). Inhalation of industrial fumes containing Zn can cause fever, nausea, muscle fatigue, chest pain, cough, shortness of breath, and fever.

In like manner, Chromium (Cr) has been related to cancer, lung, gastrointestinal and kidney damage. According to the International Agency for Research on Cancer (IARC) (2018), hexavalent chromium (Cr VI) is classified as occupational carcinogen group. For example, several scientific studies mention that exposure to Cr (VI) can cause a variety of cancers including lung, larynx, bladder, kidney, testicle, bone, and thyroid cancer in humans (Deng et al., 2019). Furthermore, it was reported that people exposed to groundwater contaminated with Cr (VI) presented dermatological and gastrointestinal complications (Sharma et al., 2012).

The metalloid mercury (Hg) accumulates preferentially in the kidneys, causing harmful effects on the proximal tubules (Balali-Mood et al., 2021). Also, Hg can generate reactive oxygen species (ROS) that stimulate protumorigenic signaling and cancer cells growth. ROS can contribute to carcinogenesis by damaging cellular proteins, lipids, and DNA (Zefferino et al., 2017). Chronic exposure to Hg toxicity can cause neurological damage including ataxia, muscle weakness, numbness of the extremities, speech, chewing and swallowing disturbances, and fast tendon reflexes (Dos Santos et al., 2018). Besides, occupational exposure to Hg promotes chronic lesions in peripheral nerves (Li et al., 2017).

Finally, accumulation of Cu human cause genetic disorders such as Menkes disease and Wilson disease. Interestingly, Cu accumulation is significantly higher in intestinal mucosa and kidney, inducing a growth retardation, skeletal defects, and progressive neurological degeneration (Valko et al., 2005). For example, Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Cookson and Shaw, 1999) Also, the bioaccumulation of Cu and Ni has been related with reproductive issues (Liang et al., 1999), and the exposure to $CuSO_4$ promotes hepatotoxicity (Feng et al., 2003).

Phytoremediation of heavy metals

Phytoremediation is a biological technology using plants with capacity to absorb high concentrations of contaminants without affecting their normal development and contributing to restore contaminated sites (Schück and Greger, 2020). This technology can remove potentially toxic contaminants such as heavy metals from the environment such as soil, water and/or sediments (Ashraf et al., 2019). Phytoremediation has some advantages over other physicochemical technologies because it has a widely accepted approach for HMs decontamination, non-invasive, less disruptive to the environment, contaminant removal efficiency, less expensive, and the collected biomass can be valorized for producing bioenergy, such as biodiesel production (Al-Thani and Yasseen, 2020; Martínez-Soto et al., 2021). According to Khalid et al. (2021), approximately 500

plant species belonging to 45 families have been reported to have a high potential to remove heavy metals from the environment.

Phytoremediation assisted by bacteria

Phytoremediation can be enhanced by the effect associated with rhizosphere microorganisms. Among these microorganisms are bacteria, which have a presence of approximately 95% in the rhizosphere (Glick, 2012). Microbial diversity plays a key role in heavy metal detoxification (Ponce-Hernández et al., 2023). Various genera of bacterial species have been applied in phytoremediation processes. They facilitate the adaptation of plants to suboptimal soil conditions and improve the efficiency of phytoremediation by promoting growth, alleviating metal toxicity, and altering the bioavailability of metals, and increase absorption into the plant (Kong and Glick, 2017).

Phytoremediation assisted by fungi

Fungi are considered as another important microbial community with benefits for plants under phytoremediation conditions (García-Ortega et al., 2022). The fungus-plant interaction is built by communication processes that use signal molecules such effector proteins, mRNAs, secreted chemical compounds, etc. This interaction includes arbuscular mycorrhizal fungi (AMF), ectomycorrhizal fungi (EMF), endophytic fungi (EF), and dark septate fungi (DSF), which colonize the internal tissue of the roots plants. These fungi facilitate the translocation of nutrients to the host plant, biotransformation of organic substances, and improve plant tolerance to inorganic contaminants (Latef et al., 2015). It is well known that mycorrhizal fungi have a greater adsorption capacity for contaminants, making them potential microorganisms to be used in bioremediation (Singh and Gauba, 2014; Ma et al. 2016; Schneider et al., 2017). Therefore, to identify the impact of the interaction between fungi and plants on the phytoremediation of heavy metals, we carried out a similar search as described above.

Conclusions

This review shows the number of original articles published between 1992 and 2022 related to the presence of heavy metals in water, soil, and sediments in all states of Mexico. Demonstrating that in recent years the number of publications about heavy metals in the environmental media has been increasing. Furthermore, this work shows the states of Mexico with concentrations of heavy metals exceeding the maximum permissible limits established by national and international standards, concluding that in 25 states of the country at least one heavy metal exceeds the maximum permissible limits in soils, in 26 states at least one heavy metal exceeds the maximum permissible limits in sediments, and in 26 states at least one heavy metal exceeds the maximum permissible limits in waters. Furthermore, this work identifies the industry, urban wastewater, mining, and fertilizers/pesticides as the anthropogenic activities being the main sources of heavy metal contamination in the soils, sediments, and waters bodies of Mexico. Similarly, this work pays special attention to cancer, damage to the CNS, DNA, kidneys, lungs, and heart problems as the main diseases caused by exposure to heavy metals. Collectively, all this information demonstrates the serious situation existing in Mexico regarding contamination by heavy metals, and it is a point of reference for the HMs contamination in Latin America. For sure, all this information can be used by official authorities looking for strategies to solve the specific HMs contamination in each area of the country. In addition, this work highlights the importance of implementing ecological and economic strategies to solve this severe contamination problem, where phytoremediation assisted by bacteria or fungi can be biotechnological tool to reduce the problem of heavy metal contamination in Mexico and other countries.

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CAPÍTULO II

Characterization of heavy metals-tolerant endophytic fungi of the genus *Talaromyces* isolated from *Typha latifolia* roots

Abstract

Due to the gradual increase of heavy metals (HMs) in the environment, various cost-effective and efficient strategies have been sought. Among these strategies is bioremediation, in which organisms such as plants and fungi are used to remove HMs from contaminated environments. Therefore, the objective of the present study was to evaluate the potential of endophytic fungi (EF) isolated from *Typha latifolia* roots to tolerate and accumulate HMs, as well as their ability to promote plant growth and limit the development of phytopathogenic fungi. For this purpose, the isolated fungi were morphologically characterized and molecularly identified as *Talaromyces liani*, *Talaromyces trachyspermus*, *Talaromyces austrocalifornicus* and *Talaromyces* sp. Their tolerance to toxic concentrations of HMs: silver (Ag) (1 mg/kg), Cu (60 mg/kg) and Cd (8 mg/kg) were evaluated, and it was found that the isolates presented high tolerance to Ag (92.7 % - 98.8 %) and medium tolerance to Cu (62.7 % - 95.9 %) and Cd (44.6 % - 70.5 %). Except for *T. trachyspermus*, which showed very low tolerance to Cd (5.5 %). In addition, the fungi were grown in liquid medium contaminated with Cd, being able to grow in the medium and accumulate Cd in their biomass (2.0 mg/g - 6.3 mg/g). Confocal microscopy showed that the hyphae of the fungi exposed to toxic concentrations of Cd presented aberrant growth, greater branching and septation, in contrast to the control hyphae that showed polarized growth. Assays to evaluate the ability of the fungi to solubilize phosphates and produce siderophores were carried out on Pikovskaya's and CAS medium, respectively. All fungi, except for *T. austrocalifornicus*, were found to solubilize phosphate from the medium (80 % - 100 %) and produce siderophores (24.7 % - 62.7 %). To evaluate the chitinolytic activity, semi-

solid medium with colloidal chitin was used. The assay revealed that all fungi have chitinolytic activity, although mostly *T. liani* and *T.* sp. (100 %). The confrontation test against phytopathogenic fungi showed that *Talaromyces* has a high percentage inhibition of *Botrytis cinerea*, and a medium to low percentage inhibition of *Fusarium* species and *Alternaria alternata*. Finally, in the fungus-plant interaction assay, it was observed that the *Talaromyces* consortium significantly increased the dry biomass of *T. latifolia* plants exposed to Cd with respect to plants not inoculated with the consortium. In addition, the consortium increased the concentration of Cd in the shoot of *T. latifolia*. Therefore, in the present study we propose that *T. liani*, *T. trachyspermus*, *T. austrocalifornicus* and *T*. sp. can be used in bioremediation of environments contaminated with HMs, in addition to serving as a biofertilizing and biocontrol agent of phytopathogenic fungi.

Keywords: Heavy metals, *Typha latifolia, Talaromyces*, Endophytic fungi

Introduction

Since the 1940s, the mobilization and transport of heavy metals (HMs) in the environment have been accelerated (Ali et al., 2019). Moreover, it has been observed that the concentrations of HMs in the environment have gradually increased over the years (Zhou et al., 2020). So, HMs have been considered as an important category of globally distributed pollutants (Gautam et al., 2016). This has been derived from increased anthropogenic activities, mainly mining, industry, urbanization and excessive use of agrochemicals (Dagdag et al., 2023). Concern arises because HMs are non-biodegradable elements, persistent in the environment, and toxic to organisms when they exceed the maximum acceptable limits (Briffa et al., 2020). Among the HMs classified as the most toxic pollutants are cadmium (Cd), mercury (Hg), chromium (Cr), copper (Cu), arsenic (As), lead (Pb), nickel (Ni), zinc (Zn), and silver (Ag) (Mansouri et al., 2012; Azfaal et al., 2022; Padhye et al., 2023; Angon et al., 2024). Specifically, Cd in plants affects nitrogen and carbohydrate metabolisms, impairs photosynthesis, disrupts chlorophyll synthesis, and causes stomata closure (Hocaoglu-Ozyigit and Genc, 2020). In humans it causes cancer in various organs, hypercalcemia, nephrotoxicity, DNA damage and lipid peroxidation (Tchounwou et al., 2012; Hocaoglu-Ozyigit and Genc, 2020; Chen et al., 2022). Cu, on the other hand, affects plant growth, affects photosynthesis and pigment content, induces the production of Reactive Oxygen Species (ROS), index lipid peroxidation and genotoxicity (Shabbir et al., 2020). In humans, Cu has been reported to cause gastrointestinal, respiratory and hepatic effects (ATSDR, 2024). In the case of Ag, it is toxic to soil microorganisms, birds and mammalian species. Causing adverse effects such as reduced growth and reproduction, and increased mortality (Padhye et al., 2023). Mansouri et al. 2012, reported that Ag is more toxic than Hg to freshwater fish. Multiple physicochemical treatments such as adsorption, membrane filtration, electrodialysis, chemical precipitation and photocatalysis have been applied to reduce the toxic concentrations of HMs present in the environment (Sodhi et al., 2022). Although they are effective for that purpose, they unfortunately have the disadvantage of high energy consumption and high operational and management costs (Dhingra et al., 2020). In contrast,

phytoremediation is a biological technique employed to restore sites contaminated with HMs, and is considered as an environmentally friendly, easy to apply, sustainable and economically viable alternative (Tan et al. 2023). For several decades plants of the genus *Typha* have been used in the remediation of environmental matrices contaminated by HMs (Carranza-Álvarez et al., 2008; Chandra and Yadav, 2010; Taufikurahman et al., 2018; Putri and Moersidik, 2021; Lei et al., 2023; Ebrahimbabaie et al., 2023). However, many plants considered as hyperaccumulators have some limitations for phytoremediation, such as their low biomass production and slow growth in HMs contaminated sites (Li et al., 2012). Therefore, it is necessary to develop strategies to promote plant growth and increase their biomass, which will improve their capacity for bioremediation of HMs.

Endophytic fungi (EF) enhance multiple host plant characteristics, such as growth, biomass, and phytoremediation potential (Khan et al., 2017). EF can supply nutrients, produce siderophores and growth-promoting hormones to their hosts, as well as absorb, complex, and biotransform HMs (Ali et al., 2019; El-Mahdy et al., 2021). Many studies have shown that EF belonging to the genus *Talaromyces* can biosorb HMs in their biomass, as well as promote growth, uptake and accumulation of HMs in plants (Bengtsson et al., 1995; Romero et al., 2006; Nam et al., 2019; El-Shahir et al., 2021; Guerra Sierra et al., 2022; Taha et al., 2023). Therefore, the present study evaluates the potential of *Typha latifolia* root-associated EF to tolerate and accumulate HMs, as well as their ability to promote plant growth and limit the development of phytopathogenic fungi.

Materials and methods

Sampling site and sample collection

Healthy whole plants (without apparent disease symptoms) of *Typha latifolia* were collected from the sampling site located in San Luis Potosí, State of San Luis Potosí,

Mexico (coordinates: 22° 09' 10” N and 101° 02’ 12” W) (Fig. 2.1). The plants were transported to the laboratory in sterile plastic bags for further processing.

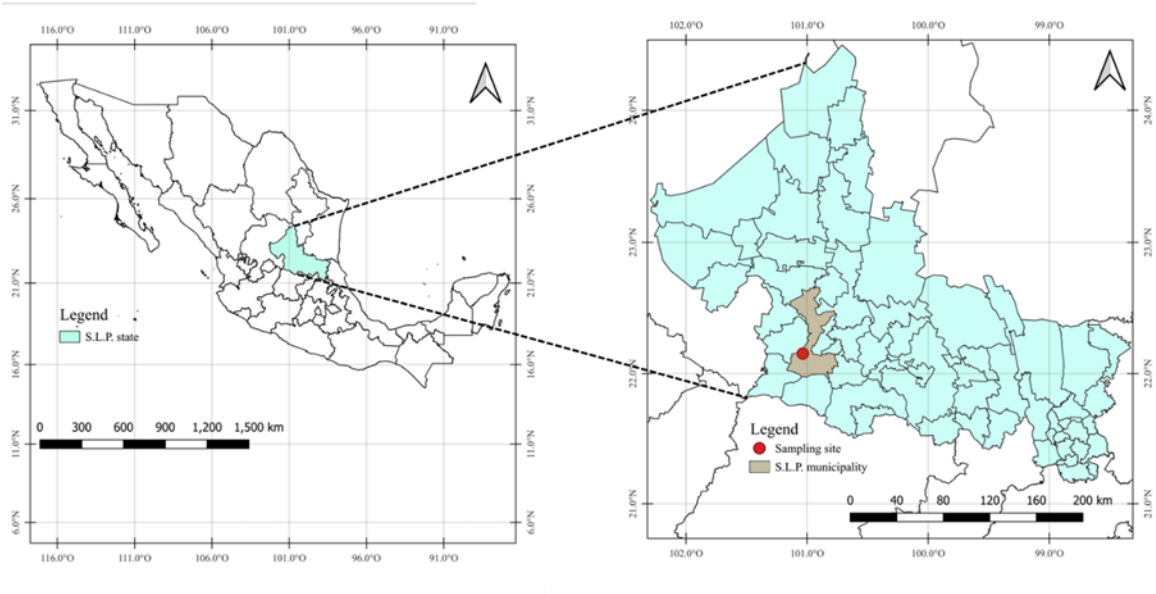

Fig. 2.1. Location map showing the sampling site.

Plant sample processing and heavy metals determination

T. latifolia plants were carefully washed with sterile deionized water to remove soil residues and organic matter. Plants were sectioned into leaves, stem, rhizome and root. The plant material was dried separately at 70 ℃ for 48 h and then ground in a universal mill (IKA M 20). The pulverized plant tissues were digested separately. For this purpose, 0.1 mL of concentrated nitric acid (HNO_3) (reactive grade) was added per 2 mg of plant material for four days. The acid digestion was carried out at room temperature in closed Teflon containers. To complete the oxidation of organic matter, the acid digestion was continued by adding 0.5 mL of hydrogen peroxide (H_2O_2) 30 % (v/v) for 24 h (Carranza-Alvarez et al., 2008). The determination of HMs in plant tissues was performed in triplicate using the Inductively Coupled Plasma Optical Emission Spectophotometry (ICP-OES) technique.

Isolation of root endophytic fungi

On the other hand, the plants were washed with sterile distilled water (SDW). For isolation of EF, 1 cm^2 portions of the plant root were taken. Under sterile conditions, the root portions were rinsed with SDW, followed by washing with 70 % ethanol for 2 min, 1 % $NaClO_2$ for 2 min and three final washes with SDW. Then, the portions were cut longitudinally and placed from the front side of the cut on Petri dishes containing Potato Dextrose Agar (PDA) medium. Seeding was performed in triplicate. All plates were incubated in a culture room at 30 ℃. Fungal growth was monitored daily. Once fungal colony growth was observed, each colony was sampled and transferred to a new PDA plate to obtain pure cultures.

Identification of fungal strain

The EF were identified by macroscopic observations of colonies, microscopic observations of conidia and hyphae, and by molecular identification. For macroscopic identification the EF were grown on PDA medium, Minimal Medium Agar (MMA), Yeast Extract Sucrose Agar (YES), Czapek's Yeast Extract Agar (CYA), Malt Extract Agar (MEA), Oatmeal Agar (OA). Plates were incubated at 30 ℃ in darkness. Fungal colonies were photographed at nine days of growth and the degree of sporulation, colony color on the obverse and soluble pigment production were recorded. Preparations to identify microscopic characteristics were made using seven-day-old colonies grown on PDA. Features were captured on an Olympus FluoView FV100 confocal microscope (Olympus, Japan) using the inverted agar block method described by Hickey et al. (2004). Reproductive structures and hyphae were stained with 0.1 g/L Calcofluor white (Thermo Fischer Scientific™). For molecular identification, genomic DNA was extracted from two-week-old strains grown in Papa Dextrose Broth (PDB) using the DNeasy® Plant Mini Kit and evaluated for quality using NanoDrop (Thermo Fischer Scientific™). Polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS) region was performed using the primer set ITS-5 (5′-GGAAGTAAAAGTCAGTCGTAACAAGG-3′) and ITS-4 (5′-TCCTCCGCTTATTGATATATGC-3′) (Bellemain et al., 2010). The amplification steps

consisted of preheating for 3 min at 94 ℃ followed by 30 cycles of 30 s at 94 ℃, 30 s at 56 ℃ and 1 min at 72 ℃ with a final extension of 5 min at 72 ℃. For the amplification of beta-tubulin (β-tubulin), the primer set Bt2a (5′-GGTAACCAAATCGGTGCTGCTGCTTTC-3′) and Bt2b (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′) was used (Glass and Donaldson, 1995). The amplification steps consisted of preheating for 3 min at 94 ℃ followed by 35 cycles of 30 s at 94 ℃, 30 s at 58 ℃ and 1 min at 72 ℃ with a final extension of 5 min at 72 ℃. TaKaRa LA Taq® DNA polymerase was used for both amplifications. The size of the amplified PCR products was verified with electrophoresis using a 1 % agarose gel. The PCR products were purified using the QIAquick® PCR & Gel Cleanup Kit method and sequenced. The best matches of the raw sequences were searched for by BLAST using ntBLAST in NCBI GenBank. The homologous sequences of the ITS and β-tubulin region of fungi were retrieved from NCBI and a concatenated phylogenetic tree was constructed with the sequences from both regions using the MAFFT alignment option in Jalview software version 2.11.3.3. The aligned data sets were analyzed using maximum likelihood (ML). The best model for ML was selected based on the Akaike Information Criterion (AIC). subsequent heuristic search performed using the Nearest-Neighbour-Interchange (NNI) method. The support at the nodes was calculated using a bootstrap analysis of 1000 replicates. Finally, the tree was generated graphically in the Java application FigTree version 1.4.4.

Tolerance percentage to heavy metals

The percentage of tolerance to cadmium (Cd), copper (Cu) and silver (Ag) was estimated for the fungal strains. In the plate assay, fungal mycelium was inoculated by pitting on PDA medium, individually supplemented with Cd (8 mg/L), Cu (60 mg/L) or Ag (1 mg/L). PDA plates without HMs were considered as control. To observe fungal growth, the plates were incubated at 30 ℃ in the dark. Plates were photographed and fungal growth area was measured using digital vernier caliper. Eq. (1) was used to calculate the percent tolerance (%T) (Liaquat et al., 2020):

$$\%T = (Fm / F) \times 100 \quad (1)$$

where *Fm* (mm) and *F* (mm) represent the fungal growth area in the presence of metal and fungal growth area without metal exposure, respectively.

Confocal observation of hyphae

To analyze the stress caused by HMs on the hyphae of fungal strains. The fungi were grown on semi-solid MMA medium with Cd (8 mg/L). As a control, fungi were grown on MMA medium without Cd. For the observation of hyphae, the Olympus FluoView FV100 confocal microscope (Olympus, Japan) and the inverted agar block method described by Hickey et al. (2004) were used. For this, fungal hyphae were stained with 5.5 mmol/L FM4-64 (Molecular probes, Eugene, Oregon) and 0.1 g/L Calcofluor white (Thermo Fischer Scientific^{TM}), incubated for 10 min and examined under the confocal microscope. Samples stained with FM4-64 were observed at 514 nm excitation and 670 nm emission and samples stained with Calcofluor White were observed at 430 nm excitation and 470 nm emission.

Biosortion of Cd from media by fungal

Mycelial sample of fungal strains were inoculated in Minimal Medium Broth (MMB) composed of 3 g/L KNO_3, 62.5 mL/L Ustilage salts and 10 g/L glucose and supplemented with 8 mg/L Cd. MMB without Cd served as control. The pH of the media was adjusted to 5 ± 2 (Ozel, 2012) and incubated at 30 ± 1 ℃ in incubator with shaking at 150 rpm for 15 days. For Cd bioaccumulation determination, the fungal biomass was dried in convection oven at 70 ℃ for 48 h. 0.1 g of dried biomass was subjected to acid digestion for 48 h by adding 1 mL of perchloric acid ($HClO_4$) and 4 mL of HNO_3. Subsequently, the mixture was filtered using Whatman 42 filter paper. The final volume of the mixture was adjusted with deionized water to 25 mL (Zahoor et al., 2017). The determination of Cd in fungal biomass was carried out using atomic absorption spectrophotometry (AAS) using air-acetylene flame (Thermo Scientific ICE 3000) (Zahoor et al., 2017).

Phosphate solubilization

Pikovskaya's medium (PVK medium) with insoluble phosphate sources like tricalcium phosphate was tested to be able to resolve the phosphates by isolated endophytes (Vyas et al., 2007). After a 7-day incubation at 30 ℃, a clear zone around the fungal colony was measured. Phosphate solubilization percentage was calculated using Eq. (2).

$$Percentage = D_H / D_M \times 100 \quad (2)$$

where D_H is the diameter of the halo formed on the plate and D_M is the diameter of the medium on the plate.

Siderophore production

On chrome azurol S (CAS) plates, the two fungal strains were cultivated for 11 days at 30 ℃ to screen the siderophore formation (Andrews et al. 2016). Yellow halos around fungal colonies have been observed as good proof for the formation of siderophores. Three plate replicates of each strain were inoculated. Percentage of siderophore production was calculated according to Eq. (2).

Confrontation assay against pathogenic fungi

Confrontation assays were performed between *Talaromyces* isolates and species considered phytopathogenic: *Fusarium oxisporum* f. sp mori (Fom), *Fusarium oxysporum* (Fol4287), *Fusarium oxysporum* (Fo5176), *Fusarium solani* (Fs), *Fusarium nygamal* (Fn), *Fusarium languences* (Fl), *Botrytis cinerea* (Bc) and *Alternaria alternata* (Aa). *Talaromyces* isolates and plant pathogenic fungi were inoculated onto Petri dish rims containing PDA medium. Single axenic cultures of both *Talaromyces* isolates and phytopathogenic fungi were used as controls. All plates were incubated at 30 ℃ in darkness. The radial growth of colonies was measured and the percentage inhibition (%I) was calculated according to Eq. (3).

$$\% I = (A - B) / A \times 100 \quad (3)$$

where *A* is the growth diameter of phytopathogenic strains on control plates; *B* is the growth diameter of phytopathogenic strains on plates confronted with *Talaromyces* strains.

Chitinolytic activity

Colloidal chitin was prepared by dissolving 1 g of colloidal chitin in 10 mL of concentrated hydrochloric acid (HCl). The solution was allowed to stand for 6 h. 250 mL of cold 70 % ethanol was continuously added for 10 min, and the pH was adjusted to 7.0. The solution was centrifuged at 8,000 rpm for 30 min at 4 ℃. The sediment was washed with sterile deionized water and centrifuged at 3,000 rpm for 5 min at 4 ℃ (Agrawal and Kotasthane, 2012). One liter of basal medium (BM) was prepared consisting of 0.3 g $MgSO_4 \cdot 7H_2O$, 3.0 g $(NH_4)_2SO_4$, 2.0 g KH_2PO_4, 1.0 g citric acid monohydrate, 15 g agar, 200 µL Tween 80, 4.5 g colloidal chitin and 0.15 g bromocresol purple, pH was adjusted to 4.7 and sterilized at 121 ℃ for 15 min. The BM was poured into Petri dishes, once the medium solidified it was observed with a bright yellow coloration. Fungal sample was inoculated by pitting in the center of the medium. The cultures were incubated at 30 ℃ for 9 days. The test was positive by observing the formation of color zone, which should change from bright yellow to intense violet (Garcia-Espejo et al., 2016). To obtain the value of the chitinolytic activity index (CI), Eq. (4) was used.

$$CI = D_H / D_C \quad (4)$$

where D_H is the diameter of the halo formed on the plate and D_C is the diameter of the fungal colony (Parlido et al., 2023).

Effect of *Talaromyces* consortium on Cd accumulation by *T. latifolia*

Typha latifolia seedlings were inoculated by immersing their roots for 24 h in a suspension containing spores of fungi of the genus *Talaromyces* at a concentration of 1×10^6/mL of each fungus. Seedlings without fungal inoculation were immersed in sterile distilled water. Subsequently, seedlings (inoculated and non-inoculated) were transferred to a hydroponic system containing Hoagland's solution, which was either

contaminated or uncontaminated with Cd (8 mg/L). All seedlings were maintained at 28 ℃ with a 16 h light/8 h dark photoperiod. After 30 days, seedlings were harvested and sectioned into roots and shoots. Plant tissues were dried at 70 ℃ for 48 h, their dry weight was recorded and then digested with HNO_3 and H_2O_2, as mentioned previously (Carranza-Alvarez et al., 2008). The quantification of Cd concentration in the tissues was carried out by atomic absorption spectrophotometry with acetylene flame and air (Alonso-Castro et al., 2009). Finally, the translocation factor (TF) was calculated using Eq. (5) (Olguín and Sánchez-Galván, 2012).

$$TF = [Cd]_S / [Cd]_R \quad (5)$$

where $[Cd]_S$ (mg/kg) is the Cd concentration in the shoot and $[Cd]_R$ (mg/kg) is the Cd concentration in the root.

Chlorophyll determination

Total chlorophyll (Chl) was quantified according to the method described by Lichtenthaler (1987). For this purpose, 100 mg of fresh leaves were homogenized with 10 ml of acetone at 5 ℃ and in the dark. The supernatant was transferred to a cuvette and absorbance was measured in a UV-Vis spectrophotometer (Thermo Fischer Scientific, USA) at 644.8 nm, 661.6 nm. Chl was calculated according to Eq. (6).

$$Chl = (7.05 \times A_{661.6}) - (18.09 \times A_{644.8}) \quad (6)$$

where, $A_{661.6}$ is the absorbance value obtained at 661.6 nm and $A_{644.8}$ is the absorbance value obtained at 644.8 nm.

Statistical analysis

Statistical comparisons were performed using GraphPad Prism software (version 8.0.1) and were considered significant at a value $p < 0.05$. Normal distribution of the data was assessed with the Shapiro-Wilk test. Differences were tested by analysis of variance (ANOVA), followed by Tuckey's post hoc tests. All experiments were performed with three biological replicates.

Results

Heavy metal concentration in *T. latifolia* tissues

The concentrations of HMs found in the tissues of *T. latifolia*. Therefore, the root is the main tissue to bioaccumulate HMs, being the most suitable tissue to isolate EFs tolerant to HMs.

Identification of fungal strains

The four selected EF were identified using sequence data from ITS and β-tubulin gene regions to determine relationships within *Talaromyces*.

Macroscopically, *T. liani* showed rapid growth on PDA medium, forming raised colonies with a yellow corn color and slight orange hue.

Talaromyces trachyspermus on PDA medium, colonies were white, fast-growing, and raised, with hyaline mycelium and a cottony, granular texture.

Morphological characteristics of *Talaromyces austrocalifornicus* on PDA, YES and CYS media, colonies were white to cream-colored with a waxy central texture and cottony periphery, raised edges, and moderate growth.

The morphological characteristics of *Talaromyces* sp. on PDA and YES, the colonies were white with a reddish center.

Tolerance percentage to heavy metals

The percentage tolerance (%T) of the four *Talaromyces* exhibited high %T values for Ag. For Cu, *T. trachyspermus* presented the significantly higher %T, followed by *T. austrocalifornicus*. *T. liani* and *T.* sp. presented significantly lower. Regarding Cd, *T. liani* had the significantly highest %T, followed by *T.* sp. and *T. austrocalifornicus*. *T. trachyspermus* showed the significantly lowest %T.

Confocal observation of hyphae

Under control conditions, hyphae of all *Talaromyces* showed polarized growth. In contrast, hyphae exposed to Cd contamination showed aberrant, rhizoid-like growth, with increased hyphal branching, twisting, curvatures, and compact colonial structure.

Biosortion of Cd from media by fungi

T. liani was found to have significantly higher dry biomass on Cd-contaminated medium than on control medium. *T. trachyspermus* and *T*. sp. grown on Cd-contaminated medium, they had no significant difference compared to their controls. On the other hand, *T. austrocalifornicus* had a significant reduction in its dry biomass when grown in Cd-contaminated medium with respect to its control. Regarding Cd accumulation in fungal biomass, we found that *T. trachyspermus* had a significantly higher accumulation of Cd in its dry biomass.

Phosphate solubilization and siderophore production

T. liani and *T*. sp. reached the highest percentage of phosphate solubilization. *T. trachyspermus* reached 80 % solubilization and *T. austrocalifornicus* showed no capacity to solubilize phosphates. Regarding siderophore production, *T*. sp. showed the significantly higher percentage, followed by *T. liani* with and *T. trachyspermus*. *T. austrocalifornicus* showed no ability to produce siderophores.

Inhibition of phytopathogenic fungi

Botrytis cinerea (Bc) was found to be the phytopathogenic fungus most inhibited by the *Talaromyces* isolates. *T. austrocalifornicus* was the fungus that most reduced its growth, followed by *T*. sp. and *T. liani*. Although *T. trachyspermus* had a high percentage of inhibition, it was the one that least inhibited the growth of Bc.

The chitinolytic activity assay found that *T. liani* and *T*. sp. exhibited higher chitinolytic activity. *T. trachyspermus* showed the lowest CI.

Effect of *Talaromyces* consortium on Cd accumulation by *T. latifolia*

It was found that inoculated plants with *Talaromyces* spp. accumulated a higher concentration than plants without inoculation. The translocation factor (TF) was calculated and found to be significantly higher in inoculated plants than in plants without inoculation.

Discussion

The average concentrations of HMs found in the leaves and stems of *T. latifolia* were within the normal ranges reported by Kabata-Pendias and Mukherjee, 2007, Sabudak et al., 2007, and Hajar et al., 2014.

In the present study, endophytic fungi were isolated from the roots of *T. latifolia* because this was the organ in which the highest concentrations of HMs were found, some of which were at phytotoxic concentrations. The fungal isolates were identified morphologically and molecularly. The macroscopic and microscopic characteristics of each isolate were determined according to the taxonomic keys described by Yilmaz et al. (2014), Rico-Munoz et al. (2015), and Pangging et al. (2019). Molecular identification was based on a combined phylogeny of ITS and β-tubulin, accepted molecular markers for fungi, proposed by Yilmaz et al. (2014).

On the other hand, the fungi analyzed in this study showed high tolerance rates to silver (Ag), copper (Cu), and cadmium (Cd). Only *T. trachyspermus* showed very low tolerance to Cd. Interestingly, *T. trachyspermus* was the fungus that showed the greatest capacity for Cd biosorption in its biomass, compared to *T. liani*, *T. austrocalifornicus*, and *T*. sp., which also showed Cd biosorption capacity. Previous studies have reported the ability of fungi of the genus *Talaromyces* to tolerate and absorb HMs.

Exposure of *Talaromyces* to Cd-contaminated media showed that hyphal morphology was affected, with increased branching of hyphae and septa. This is consistent with the findings of Pervez et al. (2009), who reported that exposure of the ectomycorrhizal fungus Pleurotus ostreatus to Cd caused an increase in hyphal density

due to an increase in the number of branches and a decrease in the distance between branching points.

Our study also found that *T. liani*, *T. trachyspermus*, and *T*. sp. can solubilize phosphates from the medium. They also showed the ability to produce siderophores. These results are consistent with those reported by Sahu and Prakash (2021), who mention that *T. trachyspermus* can produce siderophores, specifically catecholate. In addition, they reported that *T. trachyspermus* can solubilize phosphates (550 ± 0.4 µg/ml).

We also evaluated the ability of *Talaromyces* to inhibit the growth of phytopathogenic strains. More than 70 % of plant diseases are caused by fungi or fungus-like pathogens that threaten food security (Tian et al., 2020).

Finally, the effect of *Talaromyces* fungi in consortium was evaluated. It was found that the consortium did not significantly increase the concentration of Cd in *T. latifolia* roots compared to non-inoculated plants.

Conclusions

In our study, we found that *Typha latifolia* plants, considered to be tolerant to heavy metals, contained toxic concentrations of As, Cd, Cu, and Pb in their roots. Endophytic fungal strains were isolated from the roots and characterized morphologically and molecularly, identifying them as *T. liani*, *T. trachyspermus*, *T. austrocalifornicus*, and *T*. sp. These strains were highly tolerant to Ag and Cu but showed moderate tolerance to Cd. The exception was *T. trachyspermus*, which showed very low tolerance to Cd. These strains also accumulated Cd in their biomass, with *T. trachyspermus* accumulating the highest concentration of Cd. However, toxic concentrations of Cd affected the growth of the fungi and the morphology of their hyphae. *T. liani*, *T. trachyspermus*, and *T*. sp. showed the ability to solubilize phosphates and produce siderophores, which means they can serve as biofertilizers. In addition, the isolates exhibited chitinolytic activity, which may have influenced their ability to inhibit the growth of phytopathogenic fungi. *T. autrocalifornicus* and *T*. sp. showed a higher percentage

of inhibition of phytopathogenic fungi. The *Talaromyces* consortium increased the Cd concentration in *T. latifolia* shoots and increased the translocation factor, as well as increasing the dry weight of *T. latifolia* plants. Based on the results presented in this study, we propose that *T. liani*, *T. trachyspermus*, *T. austrocalifornicus*, and *T*. sp. can be used in the bioremediation of HM-contaminated environments, as well as serving as phytostimulants (biofertilizers and biocontrol agents).

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CAPÍTULO III

In vitro evaluation of the tolerance of four *Talaromyces* strains to various abiotic stresses

Abstract

Fungi are organisms capable of tolerating a wide range of environmental stresses. Notably, endophytic fungi can enhance their host's tolerance to various stressors. Among them, members of the genus *Talaromyces* exhibit diverse biological functions that are valuable in medicine, industry, food, and agriculture. In agricultural applications, these fungi have been proposed as sustainable alternatives to agrochemicals, thereby improving plant health and crop yields. The objective of this study was to evaluate the ability of endophytic *Talaromyces* species to grow under different abiotic stresses and to determine the optimal conditions for their biotechnological application. *Talaromyces trachyspermus*, *T. austrocalifornicus*, *T. liani*, and *T.* sp. were exposed to osmotic stress (using NaCl, sorbitol, and SDS), oxidative stress (H_2O_2), caffeine stress, and cell wall stress (rose bengal). Results indicated that *T. trachyspermus* and *T.* sp. can grow at temperatures up to 38 ℃. All species grew at pH 4 and pH 7, but at pH 10, only *T. trachyspermus*, *T. liani*, and *T.* sp. showed growth. These three fungi also tolerated osmotic stress, though they were more sensitive to ionic stress. In addition, they grew under oxidative (H_2O_2) and caffeine stress. Under cell wall stress induced by rose bengal, only *T. liani* and *T.* sp. maintained growth; *T. trachyspermus* was inhibited, and *T. austrocalifornicus* showed complete growth inhibition under all treatments. This study demonstrates the capacity of these fungi to tolerate and grow under diverse stress conditions, supporting their potential use as biotechnological alternatives to help plants withstand environmental challenges and improve development.

Keywords: *Talaromyces*, endophytic fungi, abiotic stress, tolerance, growth inhibition

Introduction

Anthropogenic interventions and climate change have generated and spread stressful abiotic conditions worldwide, including drought, salinity, high temperatures, and pollution (Nehra et al., 2024). These stresses degrade the environment and exacerbate food security problems by reducing crop yields, survival, and quality (Ballesteros et al., 2023). Furthermore, continued global warming and decreased precipitation are predicted by the end of the 21st century (Williams, 2017). Thus, it is essential to implement strategies that improve crop resilience to environmental stresses. One promising approach is the use of beneficial microorganisms, such as endophytic fungi (Ballesteros et al., 2023). Fungi are considered among the most tolerant organisms to extreme conditions, exhibiting remarkable versatility and significant ecological and morphological plasticity (Coleine et al., 2022). They have been shown to tolerate osmotic stress (Liu et al., 2021), high temperatures (Nam et al., 2021), and a range of pH levels (Thuy et al., 2025). As a result, fungi have been utilized to restore and repair damaged habitats (Akpasi et al., 2023). Additionally, endophytic fungi have been observed to confer tolerance to their host plants against various types of stress (Majhi et al., 2025). Therefore, the application of endophytic fungi could enhance the ability of plants to withstand multiple environmental stresses.

Talaromyces is one of the largest genera of endophytic fungi within the family *Trichocomaceae* (Houbraken et al., 2020). To date, 171 species have been accepted within this genus (Nguyen and Lee, 2023). Members of *Talaromyces* are characterized by their production of white, yellow, or red globose ascomata that contain ascospores (Manoch and Dethoup, 2011). *Talaromyces* spp. have been isolated from a wide range of environments, including indoor air, dust, clinical specimens, leaf litter, honey, pollen, soil, and plant tissues (Sun et al., 2022). Several *Talaromyces* species exhibit multiple biological functions (Lei et al., 2022). They can decompose plant residues (Goyari et al., 2015), act as causative agents of diseases in humans and animals (Lau et al., 2017), produce various pigments useful in cosmetics and food (Venkatachalam et al.,

2018), and generate molecules with pharmaceutical potential (Dewapriya et al., 2018). Additionally, *Talaromyces* spp. have been used to remove heavy metals from contaminated wastewater (Wang et al., 2019) and have been proposed as a sustainable alternative to agrochemicals for improving plant health and crop yields (Nicoletti et al., 2023; Abbas et al., 2025). According to Nicoletti et al. (2023), an increasing number of studies report the endophytic presence of *Talaromyces* fungi, which are considered a valuable tool for enhancing agricultural production. For example, *Talaromyces* flavus has been shown to promote plant growth by solubilizing phosphates in the medium (Naraghi et al., 2012). Moreover, *Talaromyces* spp. have been reported to synthesize bioactive metabolites with potent antifungal, antibacterial, and nematicidal properties, highlighting their potential as pest biocontrol agents (Abbas et al., 2025). In addition, Chandra et al. (2025) indicated that *Talaromyces* sp. strain ST1 may serve as a biostimulant for stress mitigation and crop nutrition (Abbas et al., 2025). The objective of the present study is to evaluate the capacity of endophytic fungi from the genus *Talaromyces* to tolerate abiotic stresses, contributing to know the optimal conditions for their biotechnological applications.

Materials and methods

Fungal strains

The fungi used in this study were obtained from the fungal strain collection of the Department of Microbiology at the Center for Scientific Research and Higher Education of Ensenada (CICESE), Baja California, Mexico. *Talaromyces trachyspermus*, *Talaromyces austrocalifornicus*, *Talaromyces liani*, and *Talaromyces* sp. were isolated from the roots of *Typha latifolia* plants and identified morphologically and molecularly by Ponce-Hernández et al. (under review).

Fungal growth assay at different temperature

A micropipette was used to inoculate 1×10^3 spores of each fungal strain onto Petri dishes containing Minimum Medium Agar (MMA). Plates were incubated at 28 ℃, 30

℃, 34 ℃, and 38 ℃ in the dark. Five days post-inoculation (dpi), the diameter (mm) of each fungal colony was measured.

Fungal growth assay at different pH

Petri dishes were prepared with MMA at pH 4.0, 7.0, and 10.0, adjusted with a sterilized solution of sodium hydroxide (1 M NaOH) or hydrochloric acid (1 N HCl). Using a micropipette, 1×10^3 spores of each fungal strain were inoculated onto the Petri dishes mentioned above. All cultures were incubated at 28 ℃ in darkness. After five dpi, the colony diameters were measured.

Fungal growth under osmotic stress

For osmotic stress treatments, semi-solid MMA medium was supplemented with sodium chloride (0.6 M NaCl) (Rodríguez-Pupo et al. 2021), sorbitol (1 M) (Górka-Nieć et al., 2010), or sodium dodecyl sulfate (0.003% SDS) (Ma et al., 2023). Petri dishes containing MMA only served as controls. A micropipette was used to deposit 1×10^3 spores of each fungal strain onto the respective media. Plates were incubated at 28 ℃ in the dark. Colony radii were measured at five dpi, and the growth rate index (GRI) was calculated according to Eq. (1).

$$GRI = GR_{treatment} / GR_{normal} \quad (1)$$

where, $GR_{treatment}$ represents the radius (mm) of colonies exposed to stress, and GR_{normal} represents the radius (mm) of control colonies.

Fungal growth under oxidative stress

To assess oxidative stress in *Talaromyces* spp. strains, 1×10^3 spores of each fungus were inoculated onto MMA medium supplemented with sterile hydrogen peroxide (H_2O_2, 1 mM) according to Laothanachareon et al. (2023). The H_2O_2 solution was filtered through 0.2 μm syringe filters before being added to the MMA. Plates containing unsupplemented MMA served as controls. Plates were incubated at 28 ℃ in the dark. Colony radius was measured at five dpi, and the GRI was calculated according to Eq. (1).

Fungal growth under stress induced by caffeine

A micropipette was used to inoculate 1 × 10^3 spores of each fungal strain onto MMA medium supplemented with 2 mM caffeine (Ye et al., 2025). Sterile caffeine solution was added to sterile MMA, mixed, poured into Petri dishes, and allowed to solidify. Plates containing caffeine-free MMA served as controls. Plates were incubated at 28 ℃ in the dark. The colonies radii were measured at five dpi, and the GRI was calculated according to Eq. (1).

Fungal growth under cell wall stress

To evaluate oxidative stress in *Talaromyces* spp. strains, 1 × 10^3 spores were inoculated onto sterile MMA medium supplemented with 120 μg/mL rose bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein, sodium salt; Fisher Scientific) (Cronin et al., 2014). Plates containing MMA without rose bengal served as controls. Plates were incubated at 28 ℃ in the dark. Colony radii were measured at five days post-inoculation (dpi), and the growth rate index (GRI) was calculated according to Eq. (1).

Statistical analysis

All growth and confrontation experiments were conducted in triplicate. Data normality was assessed using the Shapiro-Wilk test. Differences among groups were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Treatments with p-values < 0.05 were considered statistically significant. All statistical analyses and graphs were generated using GraphPad Prism software (version 8.0.1).

Results

Growth of *Talaromyces* spp. under different temperature

The ability of *Talaromyces* spp. to grow at different temperatures.

Growth of *Talaromyces* spp. under different pH levels

The ability of *Talaromyces* spp. to grow at different pH values was evaluated. *T. trachyspermus* and *T. austrocalifornicus* exhibited the largest colony diameters at pH

4. *T*. *liani* and *T*. sp. displayed significantly larger diameters at pH 7. At pH 10, these fungi exhibited significantly smaller colonies.

T. sp. exhibited the largest colony diameter across all pH values tested, while *T*. *austrocalifornicus* consistently showed the smallest diameter at every pH tested.

Growth of *Talaromyces* spp. under osmotic stress

Fungi exposed to 0.6 M NaCl exhibited a significantly reduced growth rate compared to the other treatments. All fungi exposed to 0.003% SDS also showed a growth index below 1. Fungi exposed to 1 M sorbitol exhibited limited growth.

Growth of *Talaromyces* spp. under oxidative stress

T. *trachyspermus* exhibited the highest growth index at 1 mM H_2O_2. *T*. sp. had an moderate growth, while *T*. *austrocalifornicus* was completely inhibited.

Growth inhibition of *Talaromyces* spp. under stress induced by caffeine

All fungal strains studied exhibited limited growth when exposed to 2 mM caffeine.

Growth of *Talaromyces* spp. under cell wall stress

Exposure of *Talaromyces* spp. to 120 µg/mL rose bengal significantly limited the growth of these fungi.

Discussion

In this study, *T*. *trachyspermus* and *T*. sp. exhibited tolerance to the highest temperature tested (38 ℃). Previous research has demonstrated that several *Talaromyces* species can withstand extreme heat.

All studied fungi grew under both acidic and alkaline conditions, except for *T*. *austrocalifornicus*, which did not grow at pH 10. These findings align with those of Thuy et al. (2025), who found that *Talaromyces* spp. strains could grow in highly acidic environments (pH 1).

Under osmotic stress, all studied fungi exhibited reduced growth, with NaCl having the most pronounced inhibitory effect. This observation is consistent with previous

findings for *Talaromyces minioluteus*, *Talaromyces purpureogenus*, and *Talaromyces sayulitensis*, which also tolerated osmotic stress at various salinity levels but showed decreased growth as salinity increased (Ramatsitsi et al., 2023).

For oxidative stress (H_2O_2), only *T*. *trachyspermus*, *T*. *liani*, and *T*. sp. displayed mycelial growth, resembling the response observed in *Talaromyces marneffei* (Pruksaphon et al., 2022).

Conclusion

This study demonstrated the ability of *Talaromyces* fungi to grow under a range of adverse conditions. Notably, *T*. *trachyspermus* and *T*. sp. tolerated temperatures up to 38 ℃. All tested fungi grew under acidic (pH 4) and neutral (pH 7) conditions, whereas only *T*. *trachyspermus*, *T*. *liani*, and *T*. sp. grew at an alkaline pH (pH 10). These three fungi also grew under osmotic stress, though they were more sensitive to ionic stress, and were able to tolerate H_2O_2 and caffeine stress. Under rose bengal-induced stress, only *T*. *liani* and *T*. sp. exhibited growth, whereas *T*. *trachyspermus* was inhibited, and *T*. *austrocalifornicus* showed complete growth inhibition under all stress treatments. Overall, these results highlight the versatility of the tested *Talaromyces* species in tolerating and growing under a variety of stressful conditions. This versatility suggests their potential as endophytes to enhance stress tolerance in host plants, thereby improving crop production.

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CAPÍTULO IV

The fungus *Neosartorya* (*Aspergillus*) *fischeri* improves the fitness, tolerance and absorption of heavy metals in *Typha latifolia*

Abstract

Heavy metals (HMs) contamination is a global issue caused by anthropogenic activities, leading to severe negative effects on the environment and human health. To address this problem, bioremediation strategies using plants and rhizosphere-associated microorganisms, such as fungi have been recently implemented. In this study, the endophytic fungus *Neosartorya fischeri* was isolated from the roots of *Typha latifolia* plants growing in heavy metal-contaminated sites. *N. fischeri* colonizes the epidermis and root cortex, demonstrating high tolerance to toxic concentrations of silver (Ag), copper (Cu), and cadmium (Cd). Notably, N. fischeri removes Cd from the medium, bio-absorbing the metal into its biomass, and enhancing the tolerance and bioaccumulation of metals in the plant roots. Moreover, *N. fischeri* produces siderophores, volatile compounds, and degrades phosphates, which improve plant fitness. This is evidenced by the higher concentrations of photosynthetic pigments in *T. latifolia* plants colonized with *N. fischeri*. Additionally, *N. fischeri* inhibits the growth of important phytopathogens, such as *Fusarium* spp. All these findings highlight the significant role of *N. fischeri* in enhancing the fitness and resilience of *T. latifolia* in hostile environments, demonstrating the potential of *N. fischeri*-*T. latifolia* interaction for the bioremediation of contaminated sites.

Keywords: *Neosartorya fischeri*, Endophytic fungi, Heavy metals contamination, Cadmium, *Typha latifolia*, Bioremediation

Introduction

Heavy metals (HMs) are natural metals with an atomic number (Z) greater than 20 and an elemental density greater than 5 g/cm^3 (Ali and Khan 2017). The toxic concentrations of HMs in the environment have increased over the years due to anthropogenic activities such as mining, smelting, industrial processes, and agrochemicals (Ali et al., 2019). HMs are non-biodegradable elements and become toxic for all living organisms when they exceed the maximum acceptable limits in the environment (Briffa et al., 2020). In humans, exposure to HMs can cause a range of diseases, including cancer, damage to the central nervous system, kidneys, and lungs, as well as genotoxic, cardiac, gastrointestinal, endocrine, skin, liver, reproductive, immunological, poisoning, teratogenic, bones, hemolytic and growth development (Balali-Mood et al., 2021). Cadmium (Cd) is considered a non-essential HM and is highly toxic at low concentrations to living organisms (Tchounwou et al., 2012; Soni et al., 2024). Studies indicate that exposure to Cd can promote the development of cancers in several organs and tissues, including the kidneys, lungs, pancreas, breast, prostate, and digestive tract (Balali-Mood et al., 2021).

Phytoremediation is a biotechnological technique that uses plants to restore sites contaminated with HMs (Tan et al., 2023). This strategy has gained popularity because it is low-cost, ecologically friendly, non-invasive, and effective in contaminant absorption (Nedjimi, 2021). Plants from the *Typha* genus are widely used in the remediation of contaminated environmental matrices due to their high capacity to tolerate, accumulate and transform HMs into their less toxic forms (Carranza-Álvarez et al., 2008; Chandra and Yadav, 2010; Taufikurahman et al., 2019; Putri and Moersidik, 2021; Lei et al., 2023; Ebrahimbabaie et al., 2023). Through evolution, these plants have established close relationships with rhizosphere microorganisms, including bacteria and fungi, which enhance their survival and phytoremediation capacity (Compant et al., 2010). Fungi promote plant growth and fitness, reduce phytotoxicity caused by HMs, and increase the uptake of HMs by roots allowing plants to tolerate stressful environments (Zahoor et al., 2017; Deng and Cao, 2017). Endophytic fungi

produce siderophores, antimicrobial compounds and hormones, improving nutrient acquisition such as phosphate solubilization, promoting plant growth, and enhancing the biotransformation and absorption of HMs (Deng et al., 2014; Khan et al., 2017; Ali et al., 2019; El-Mahdy eet al.,2021).

Neosartorya fischeri, previously known as *Aspergillus fischeri*, is an Ascomycota fungus found in the soil or rhizosphere hat colonizes the roots of plants as an endophytic fungus (Šimonovičová et al., 2019). For example, it has been isolated from sunflower plants subjected to extreme environmental conditions (Maj et al., 2023). *N. fischeri* has been identified as a heat-resistant microorganism, equipped with mechanisms for adapting to changing environmental conditions including oxygen levels, chemical compounds, and fungicides (Panek et al., 2016). Although this fungus has been studied little, there is evidence of its capacity to promote plant growth through enzyme secretion and the production of gibberellins (Hamayun et al., 2011). Additionally, *N. fischeri* exhibits antimicrobial activity against pathogenic bacteria (Maj et al., 2023), making it potentially useful in agriculture and medicine (Maj et al., 2023). Promising studies indicate its efficacy in bioremediation of HMs, for instance, Rani et al., (2013) demonstrated that *N. fischeri* remove 93 % of nickel [Ni (II)] from the culture medium. Similarly, Litera et al., (2011) found that *N. fischeri* removes 0.317 mg/g of arsenic [As (V)] from contaminated water samples. However, the interaction between N. fischeri and plants like *Typha latifolia* for the bioremediation of polluted environments remains unexplored.

Therefore, this work aimed to study the potential of *N. fischeri* to tolerate HMs and the capacity of the fungus to biosorb metals within its biomass. Also, to evaluate the effect of the fungus in promoting plant fitness and biosorption of Cd in *Typha latifolia*. Collectively, our findings highlight the collaborative relationship between beneficial fungi and plants in remediating environments contaminated with HMs, presenting a cost-effective strategy to mitigate the harmful effects of HMs on the environment and human health.

Materials and methods

Isolation and identification of *N. fischeri*

Fungal isolates were obtained from the roots of several healthy *T. latifolia* plants collected from various sites in a heavy metal contaminated area in San Luis Potosí, Mexico (22° 09’ 10’’ N and 101° 02’ 12’’ W). For isolation of endophytic fungi, the roots were rinsed with sterile distilled water, washed with 70% ethanol for 5 min, treated with 10% $NaClO_2$ for 5 min, and subsequently washed several times with sterile distilled water. The roots were dried on sterile paper tissues. Samples were then cut crosswise, and portions of around 1 cm^2 were placed from the front of the cut side onto Potato Dextrose Agar (PDA) medium. The seeding of the roots from the collected plants was performed in triplicate, and all plates were incubated in a culture room at 30°C. Once mycelial growth was observed it was transferred to new PDA plates until axenic cultures were obtained (Salazar-Cerezo et al., 2018). The tolerance of the fungal isolates was tested on culture media contaminated with HMs as described below, and those isolates exhibiting the best tolerance were selected. The fungal isolates with the best metal tolerance were then cultured on Czapek's Yeast Extract Agar (CYA), Yeast Extract Sucrose Agar (YES), Malt Extract Agar (MEA), Oatmeal Agar (OA), PDA and Minimal Medium Agar (MMA). The plates were incubated at 30 °C in darkness. Fungal colonies were photographed after 7 days of growth, and their microscopic characteristics were registered. Moreover, fungal genomic DNA from isolates showing the same macroscopic and microscopic characteristics was extracted using the DNeasy® Plant Mini Kit (Qiagen®) and DNA quality was evaluated using a NanoDrop (Thermo Fischer Scientific™). The internal transcribed spacer (ITS) region was amplified by polymerase chain reaction (PCR) using TaKaRa LA Taq® DNA polymerase (Thermo Fischer Scientific™) following the manufacturer's instructions. The ITS region was amplified using the primers ITS-5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS-4 (5′-TCCTCCGCTTATTGATATATGC-3′) as described by Bellemain et al., (2010). The PCR products were verified by electrophoresis on a 1% agarose gel, purified using the QIAquick® PCR & Gel Cleanup Kit (Qiagen®), and sequenced by Eton Bioscience Inc.

The sequences were analyzed by BLAST in the NCBI. A phylogenetic analysis was performed using the Multiple Sequence Comparison by Log- Expectation (MUSCLE) alignment option in Jalview software version 2.11.3.3. The aligned data sets were analyzed using the Maximum Likelihood-based inference of phylogenetic trees with Smart Model Selection (PhyML + SMS). The best model was selected based on the Akaike Information Criterion (AIC) and Nearest-Neighbour-Interchange (NNI) based on 1000 bootstrap interactions (Yilmaz et al., 2014; Maj et al., 2024). Finally, the phylogenetic tree was visualized with FigTree version 1.4.4 (Guerra Sierra et al., 2022).

Tolerance of *N. fischeri* to heavy metals

Isolated *N. fischeri* isolates were inoculated onto PDA medium contaminated with silver (Ag) at 1 mg/kg, copper (Cu) at 60 mg/kg, and cadmium (Cd) at 8 mg/kg. The metal concentrations used in these experiments were those found in the roots of *T. latifolia* plants where *N. fischeri* was isolated (data not shown). PDA plates without metal served as controls. The cultures were incubated at 30 °C for nine days. The diameter of fungal growth was measured and photographed. The percentage tolerance (%T) was calculated using the following equation (Liaquat et al., 2020):

%T = area of fungal growth in the presence of metal / area of fungal growth without exposure to metal, multiplied by 100.

Staining and microscopy

The morphology of *N. fischeri* hyphae in media with and without Cd (8 mg/kg) was analyzed using a confocal microscope using the inverted agar block method described by Hickey et al. (2004). Fungal hyphae were stained with 5.5 mM FM4-64 (Molecular probes, Eugene, OR) and 0.1 g/L Calcofluor white (Thermo Fischer Scientific™), incubated for 10 min, and examined on an Olympus FluoView FV100 (Olympus, Japan) confocal microscope. Samples stained with FM4-64 or Calcofluor white were analyzed at 514-nm excitation to 670-nm emission or 430-nm excitation to 470-nm emission respectively.

The colonization of *T. latifolia* roots by *N. fischeri* under Cd (8 mg/kg) or uncontaminated conditions was analyzed via confocal microscopy using the WGA-

Alexa Fluor and propidium iodide staining method described by Martínez-Soto et al. (2023). WGA-Alexa Fluor was detected at 488 nm excitation and 540 nm emission staining the fungal structures green, while propidium iodide was detected at 561 nm excitation and 580-660 nm emission staining the plant tissue red.

Bioaccumulation and removal of Cd by *N. fischeri*

To assess the Cd removal capacity of *N. fischeri*, 10^6 spores/mL were inoculated into liquid minimal medium (MM) contaminated or not with 8 $mg \cdot L^{-1}$ Cd. The cultures were incubated at 30 °C and shaking conditions at 150 rpm for 15 days. Fungal biomass was recovered by filtration and dried at 70 °C for 48 h. Dried biomass (0.1 g) underwent acid digestion with 1 mL of perchloric acid ($HClO_4$) and 4 mL of concentrated nitric acid (HNO_3) for 48 h. The mixture was filtered using Whatman 42 filter paper (Whatman®) and the final volume was adjusted to 25 mL with deionized water. To evaluate the Cd removal capacity of the medium, 10 mL of concentrated HNO_3 was added to 50 mL of the filtered medium. The volume of the mixture was then reduced to 40 mL by evaporation in a water bath. Following filtration with Whatman 42 filter paper, the mixture was brought to a final volume of 50 mL with deionized water (Zahoor et al. 2017). Determination of Cd in the fungal biomass and filtered medium was performed by triplicate using atomic absorption spectrophotometry (AAS) with air-acetylene flame (Thermo Scientific ICE 3000).

Phosphate solubilization

The ability of the fungus solubilize phosphates was tested on Pikovskaya's medium (PVK) with insoluble phosphate sources (Vyas et al. 2007). After a 7-day incubation at 30 °C the transparent zone around the fungal colony was measured.

Siderophore production

Fungal isolates were cultivated on chrome azurol S (CAS) media plates for 11 days at 30 °C (Andrews et al. 2016). Yellow halos around fungal colonies indicated the production of siderophores.

Effect of *N. fischeri* on the Cd accumulation by *T. latifolia*

Typha latifolia seedlings were inoculated by immersing their roots in $1x10^6$/mL spore suspension or sterile distilled water as mock inoculation for 24 h. After fungal inoculation, seedlings were transferred to a hydroponic system containing Hoagland solution, which was either contaminated (8 mg/L) or not with Cd. All seedlings were maintained at 28 °C with 16 h light/8 h dark photoperiod. After 15 days, seedlings were collected and sectioned into roots and shoots, in preparation for the analysis. The Cd concentration in the sectioned plant tissues and the hydroponic medium was analyzed. Mock-inoculated plants served as negative controls. Plants tissues were dried and digested with acid for Cd determination, as mentioned above (Carranza-Álvarez et al. 2008). Cadmium concentrations were analyzed using atomic absorption spectrophotometry with air acetylene flame (Alonso-Castro et al. 2009). Finally, the translocation factor (TF), bioaccumulation factor (BAF), and bioconcentration factor (BCF) were calculated using the following formulas (Olguín and Sánchez-Galván, 2012):

$TF = [Cd]_S / [Cd]_R$

$BAF = [Cd]_S / [Cd]_M$

$BCF = [Cd]_R / [Cd]_M$

Where $[Cd]_S$ is the Cd concentration in the shoot (mg/kg), $[Cd]_R$ is the Cd concentration in the root (mg/kg) and $[Cd]_M$ is the initial Cd concentration in the medium.

Determination of chlorophyll and carotenoids

Chlorophyll (Chl) and carotenoids were quantified according to the method described by Lichtenthaler (1987). Specifically, 100 mg of fresh leaves were homogenized with 10 mL of acetone at 5 °C and the dark. The supernatant was transferred to a cuvette and absorbance was measured in UV-Vis spectrophotometer at 644.8 nm, 661.6 nm, and 470 nm. Chl a, Chl b, and carotenoids were calculated according to the following equations.

$Chl_a = 11.24_{A661.6} – 2.04_{A644.8}$

$Chl_b = 20.13_{A644.8} – 4.19_{A661.6}$

Carotenoides = (1000_{A470} – 1.90 Chl_a – 63.14 Chl_b) / 214

Total Chl was determined by the sum of Chl_a + Chl_b.

Growth promotion of *T. latifolia* by *N. fischeri*

Disinfested *T. latifolia* seeds were placed on one side of split Petri dishes containing Murashige and Skoog (MS) medium, which was either contaminated (8 mg/kg) or not with Cd. After germination, 30 µl of 10^5 spores/mL suspension was added to the other side of the Petri dish containing PDA either contaminated or not with Cd. The plates were properly sealed and incubated at 28 °C under 16 h light/8 h dark photoperiod. Petri dishes containing seeds without Cd and without *N. fischeri* spores served as controls. Seedling length was measured 10 days after the *N. fischeri* spores were added.

Inhibition of phytopathogenic fungi

Confrontation assays between *N. fischeri* isolates and three *Fusarium* species: *Fusarium oxisporum*, *Fusarium foetens,* and *Fusarium solani* were performed. The *N. fischeri* isolates and the respective phytopathogenic fungi were inoculated on the borders of the Petri dish media plate. Axenic cultures of both *N. fischeri* and the phytopathogenic fungi served as controls. All plates were incubated at 28 °C in the dark. The radial growth of the colonies was measured, and the inhibition percentage (%I) was calculated according to the equations below.

%I = (A-B) / A x 100

where A = growth diameter of *Fusarium* sp. in the control plates, B = growth diameter of *Fusarium* sp. in the treated plates with *N. fischeri*.

Statistical analysis

All experiments were performed with the respective technical and three biological replicates. The results are presented are means ± SD. Data were analyzed using GraphPad Prism software (version 8.0.1). Significant differences ($p < 0.05$) among the mean values of different treatments were determined using Tukey's multiple

comparisons test on a statistical analysis system (SAS). Graphs were created using GraphPad Prism.

Results

Identification *of N. fischeri*

In all media cultures, the fungal colonies presented a velutinous aspect with granular structures.

The ITS region showed 99 % query coverage with its best hit in the pBLAST run, demosntrating a 100 % identity with a species of *Neosartorya (Aspergillus) fischeri*.

N. fischeri, a fungus with tolerance to toxic concentrations of heavy metals

N. fischeri showed a tolerance to Cd, Ag and Cu.

Aberrant morphology of *N. fischeri* hyphae growing on Cd contaminated medium

Under control conditions, *N. fischeri* hyphae showed polarized growth and hyphae exposed to Cd contamination exhibited an aberrant, rhizoid-like growth with increased hyphae branching and a compact colony structure.

Bioaccumulation and remotion of Cd by *N. fischeri*

The fungus *N. fischeri* could grow on minimal medium contaminated with Cd. However, after 15 days of incubation, the dry biomass of fungi grown on Cd-contaminated medium was significantly lower compared to the control. *N. fischeri* removed Cd present in the culture medium. In the control conditions, *N. fischeri* formed large mycelial aggregates that filled the flask, and smaller and dispersed mycelial in the Cd-contaminated medium.

Phosphate solubilization and Siderophore production by *N. fischeri*

N. fischeri to solubilize phosphates, the halo indicating the phosphates solubilization. Siderophores production by *N. fischeri* was confirmed by the formation of a yellow halo surrounding the fungal colony on CAS medium.

Colonization of *T. latifolia* roots by *N. fischeri*

Following inoculation of *T. latifolia* with *N. fischeri*, the fungus colonized the root surface, epidermis, and root cortex.

N. fischeri enhances the Cd accumulation in *T. latifolia*

Seedlings inoculated with *N. fischeri* under Cd stress conditions exhibited a significantly higher concentration of Cd in their roots compared to non-inoculated plants.

N. fischeri improves the *T. latifolia* fitness

Seedlings colonized with *N. fischeri* exhibited significant increases in pigments content, showing enhancements of 1.4-fold in chlorophyll a, 1.2-fold in chlorophyll b, 1.3-fold in total chlorophyll, and 1.4-fold in carotenoids when compared to the mock-colonized seedlings.

Growth promotion of *T. latifolia* by *N. fischeri*

N. fischeri produces volatiles under Cd stress that promote seedling development.

Inhibition of phytopathogenic *Fusarium* species by *N. fischeri*

N. fischeri inhibited the growth of all tested *Fusarium* strains, *F. foetens* and *F. oxysporum* exhibited a high percentage of inhibition. *F. solani* showed a lower inhibition percentage.

Discussion

In this study, an endophytic fungus from *T. latifolia* roots growing in environments highly contaminated with HMs was isolated. The isolate was morphological- and molecularly identified as *N. fischeri*. The macroscopic and microscopic features of this fungi isolate were determined according to the *N. fischeri* characteristics previously described by Samson et al. (2007) and Maj et al. (2023).

Despite the high metal tolerance of this fungus, direct contact with the Cd-contaminated medium caused alterations in the hyphae morphology of *N. fischeri*. It has been noted that the bioavailability of HMs in the medium affects the mycelial morphology, fungal cell membrane physiology, exoenzymatic activity, secondary

metabolites synthesis, and cell wall components, resulting in increased aerial hyphal formation and branching (Urik 2017; Ding et al., 2022).

For the first time, we report that *N. fischeri* colonized both the epidermis and cortex of *T. latifolia* roots. Additionally, we found that *N. fischeri* increased the Cd accumulation capacity of the *T. latifolia* roots while reducing Cd translocation to aerial plant tissues. Although it is known that *T. latifolia* accumulates a higher concentration of Cd in roots than shoots (Carranza-Alvarez et al., 2008; Alonso-Castro et al., 2009), our results demonstrate that *N. fischeri* restricts Cd mobilization and enhances the plant's tolerance to elevated Cd concentration. Moreover, our findings show that *N. fischeri* uptakes Cd, suggesting its crucial role in the *N. fischeri-T. latifolia* interaction to help the plant withstand higher concentrations of metals. Similar effects have been reported in other fungal-plant associations (Kuang et al., 2021; Priyashantha et al. 2023). We also demonstrated that *N. fischeri* produces siderophores which induce metal immobilization, potentially explaining the higher Cd concentrations found in *T. latifolia* roots colonized by *N. fischeri*. They also enhance the levels of photosynthetic pigments and reduce the production of reactive oxygen species (ROS), fostering better plant growth (Yadav et al., 2023). We also found that *N. fischeri* can solubilize phosphates from the medium, consistent with previous research indicating that *N. fischeri* enhances phosphorus availability from biochar composts (Wilujeng et al., 2020).

N. fischeri also increased the concentration of carotenoids and total chlorophyll, including chlorophyll *a* and *b* indicating improved fitness of *T. latifolia* (Sarkar et al., 2021).

The results demonstrated that *N. fischeri* effectively suppressed the growth of *Fusarium* species. It has been described that *N. fischeri* produces extracellular antifungal proteins known as *Neosartorya fischeri* antimicrobial proteins (NFAP), which have significant bio-fungicidal potential.

Conclusions

In this study, it was found that the endophytic fungus *N. fischeri* is associated with roots of *T. latifolia* under HMs contaminated environments. This research demonstrates that

N. fischeri not only tolerates high concentrations of toxic metals like silver (Ag), copper (Cu), and cadmium (Cd), but also plays an active role in supporting plant development under such challenging conditions. By producing siderophores and breaking down phosphates, the fungus enhances nutrient availability and promotes more robust plant growth, even in the presence of metal-induced stress.

The colonization of *T. latifolia* roots by *N. fischeri* is particularly significant, because the fungus inhabits the epidermis and cortex layers, playing a crucial role in the plant's defense mechanisms. In response to metal stress, *N. fischeri* produces volatile organic compounds that further stimulate plant growth and enhance overall fitness, particularly by increasing the levels of photosynthetic pigments essential for energy production in plants.

Furthermore, *N. fischeri* removes Cd from the medium and bio-absorb the metal in its fungal biomass, enhancing the capacity of *T. latifolia* for accumulation of Cd in the roots and reducing the Cd translocation to the aerial plant tissues. This interaction not only allows the plant to sequester the toxic metal effectively, reducing its systemic toxicity, but also provides protection against root phytopathogens by inhibiting the growth of harmful fungi.

Collectively, these findings highlight the *N. fischeri-T. latifolia* partnership as an effective strategy for bioremediation of heavy metal-contaminated environments. This relationship not only boosts the plant's ability to grow and survive in harsh conditions but also serves as a natural method for phytoremediation, where plants and their microbial partners are used to detoxify polluted soils and water bodies. Further investigation of the association between *N. fischeri* and *T. latifolia* in field conditions is crucial to evaluating their bioremediation potential. Promising results could be expected since both *T. latifolia* and *N. fischeri*, can adapt to adverse environmental conditions and possess bioremediation capacities. Moreover, the mutual benefits of stimulating plant growth while suppressing pathogenic fungi make the *N. fischeri-T. latifolia* symbiosis particularly valuable for ecological restoration, addressing both plant health and environmental decontamination. The results from this study offer a promising

alternative for improving environmental health and alleviating the adverse effects of HMs contamination on both ecosystems and human health.

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CAPÍTULO V

Aspergillus sydowii, a tolerant fungus to heavy metals and pesticides

Abstract

The increase in population has led to a corresponding rise in industrial and agricultural activities, resulting in the release of significant levels of pollutants into our environment, particularly heavy metals (HMs) and pesticides. These pollutants are persistent and can contaminate soil and water, eventually enter the food chain and cause serious environmental and health issues. Mycoremediation offers a promising solution by utilizing fungi to efficiently and cost-effectively remove these toxic pollutants from the environment. In this work, we successfully isolated and identified the fungus *Aspergillus sydowii* from a heavily contaminated site in central Mexico. We assessed its ability to resist HMs and pesticides, as well as its potential to produce siderophores and facilitate phosphate solubilization in chromium azurol S medium (CAS) and Pikovskaya medium (PVK), respectively. This confirms its role as a plant biostimulant. We cultivated *A. sydowii* on PDA medium contaminated with silver (Ag) at 1 mg L^{-1}, copper (Cu) at 60 mg L^{-1}, and cadmium (Cd) at 8 mg L^{-1}. Additionally, we evaluated its growth in PDA medium contaminated with glyphosate at 20 mM and the fungicide Captan at 500 mg L^{-1}. Our results indicated that *A. sydowii* grows in environments contaminated with Ag, Cu, Cd, and pesticides. Microscopic analysis revealed that fungal hyphae under Cd-induced stress exhibited increased branching as response to the stress condition. Overall, our findings demonstrate that *A. sydowii* not only has the potential to solubilize phosphates and produce siderophores, but it also stands out as an excellent candidate for bioremediation efforts in environments tainted with metals and pesticides, further underlining its applicability in sustainable agricultural practices.

Keywords: *Aspergillus sydowii*, Heavy metals, Pesticides, *Typha latifolia* · Siderophores, Phosphate degradation.

Introduction

The enormous global population growth has led to increase economic development and rapid growth of agriculture and industry to satisfy the population needs (Alengebawy et al., 2021). However, those activities have also resulted in the release of significant environmental pollutants (Dagdag et al., 2023), mainly heavy metals (HMs) and pesticides (Shetty et al., 2023). HMs are natural elements found in the earth's crust (Briffa et al., 2020), although their normal concentrations in the environment have been disrupted by excessive anthropogenic activities, including industrial processes, mining, domestic usages, agricultural practices, and the burning of fossil fuels (Kumar et al., 2022). HMs are persistent, non-biodegradable and toxic when reach concentrations exceeding normal levels. In response, the World Health Organization (WHO) has established permissible limits for HMs in water and soil to protect the environment and human health. Exposure to toxic concentrations of HMs is linked to various health issues, including damage to the kidneys, liver, heart, skin, reproductive and immune systems, genotoxicity, and several types of cancer (Mitra et al., 2022).

On the other hand, pesticides are substances naturally or chemically synthesized designed to control agricultural pests such as weeds, fungi, bacteria, and insects (Alengebawy et al., 2021). Agriculture consumes about 85 % of the world's total pesticide production (Rajan et al., 2023), and although pesticides were developed to eliminate pests, several studies highlight concerns regarding their negative impacts on the environment and human health (Kim et al., 2016). Extensive research has associated pesticide exposure with various diseases, including cancers, asthma, diabetes, leukemia, Parkinson's disease, cognitive effects, and DNA damage (Azandjeme et al., 2013; Mehrpour et al., 2014; Amr et al., 2015; Raanan et al., 2015; Moisan et al., 2015; Bailey et al., 2015; Lee et al., 2016). Regarding glyphosate, it has been shown that its degradation metabolite, aminomethylphosphonic acid, can persist in the soil for a long time. This residue enters the food chain and has been found in drinking water, plants, animals and humans (Aluffi et al., 2020). Captan, on the other

hand, can persist in the soil depending on environmental conditions, being more stable in soils with low pH and moisture content. It has been reported that in humans it promotes mutagenicity and carcinogenesis, in animals it causes poisoning and is usually very toxic to fish and aquatic life. It also causes infertility in soil, because it eliminates nitrogen-fixing microorganisms, solubilizers of phosphate and other nutrients (Mohamed and Mostafa, 2018). Glyphosate and Captan are among the most widely used pesticides in the world (Maggi et al., 2019). Therefore, seeking efficient strategies to mitigate the high concentrations of HMs and pesticides in the environment is crucial. Bioremediation methods, utilizing living organisms, microorganisms, or plants, have been identified as effective approaches for eliminating pollutants, including toxic organic compounds, polycyclic aromatic hydrocarbons, explosives, pesticides, landfill leachates, and HMs (Abdel-Shafy and Mansour, 2018; Wu et al., 2023). For example, mycoremediation is a low-cost technology with promising results (Oladipo et al., 2018). It has been reported that diverse fungi have the potential to degrade pesticides and adsorb HMs, for instance, *Rhizopus* have been identified as biosorbents for lead (Pb) and cadmium (Cd), and *Aspergillus* species can remove chromium (Cr) (VI) (Zhang et al., 2020; Xu et al., 2020; Fauriah et al., 2021; Sharma and Kumar, 2021). Also, several metal-tolerant filamentous fungi, such as *Rhizopus*, *Trichoderma*, *Aspergillus*, *Penicillium*, and *Fusarium*, have been isolated from soils contaminated with Cd, Cr, copper (Cu), nickel (Ni), and cobalt (Co) (Oladipo et al., 2018). Interestingly, the tolerance mechanisms employed by fungi include cell wall adsorption of HMs, secretion of organic acids, and the uptake and accumulation of HMs (Garg et al., 2017). Moreover, fungi from genera like *Actinomucor*, *Aspergillus*, *Trichoderma*, *Penicillium*, *Mucor*, and *Fusarium* have been noted for their ability to tolerate and/or degrade various pesticides (Spinelli et al., 2021; Baumann et al., 2022).

The objective of this work was to identify and characterize *Aspergillus sydowii* as a tolerant fungus to toxic concentrations of HMs and pesticides like glyphosate and captan, as well as assessing its capacity to produce siderophores and degrade

phosphates. Collectively, the findings from this research suggest *A. sydowii* could be utilized as an eco-friendly technology for cleaning up contaminated environments.

Materials and methods

Isolation and identification of endophytic fungal strain

The endophytic fungus *A. sydowii* (PQ376633.1) was isolated from soil from a site (22° 09' 10" North and 101° 02' 12" West) reported with high heavy metal contamination (Aguilera et al., 2019) located in San Luis Potosí, State of San Luis Potosí, Mexico.

A. sydowii was identified through macro and microscopic observations, and by molecular DNA barcoding. For macroscopic characteristics, *A. sydowii* was grown on different culture media including Minimal Medium Agar (MMA), Potato Dextrose Agar (PDA), Oatmeal Agar (OA), Czapek's Yeast Extract Agar (CYA), Sucrose Yeast Extract Agar (YES) and Malt Extract Agar (MEA) using petri dishes of 55 mm diameter. Plates were incubated at 28 ± 2 ℃ in the darkness conditions. After 7 days, the colonies were photographed. Microscopic characteristics were observed by confocal microscopy using the inverted agar block technique (see section 2.3). For molecular identification, fungal genomic DNA was extracted using the DNeasy® Plant Mini Kit and evaluated for quality using NanoDrop Lite (Thermo Scientific), followed by polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS) region using TaKaRa LA Taq® DNA polymerase. The ITS1-ITS2 region was amplified using the primer set ITS-5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS-4 (5′-TCCTCCGCTTATTGATATATGC-3′). PCR was performed in a thermal cycler using the following amplification steps: preheating for 2 min at 94℃ followed by 32 cycles of 30 s at 94 ℃, 30 s at 55 ℃ and 1 min at 72 ℃ with a final extension of 5 min at 72 ℃. The size of the amplified PCR products was verified by electrophoresis in a 1% agarose gel. PCR products were purified using the QIAquick® PCR & Gel Cleanup Kit method and sequenced. The best matches of the raw sequences were searched for by BLAST using ntBLAST in NCBI GenBank. Homologous fungal ITS sequences were retrieved from NCBI, and a phylogenetic tree was constructed using the Muscle alignment option

in Jalview software version 2.11.3.3. The aligned data sets were analyzed using maximum likelihood (ML). The best model for ML was selected based on the Akaike Information Criterion (AIC). subsequent heuristic search performed using the Nearest-Neighbour-Interchange (NNI) option. The support at the nodes was calculated using a bootstrap analysis of 1000 replicates. Finally, the tree was generated graphically in the Java application FigTree version 1.4.4.

Heavy metals tolerance of *Aspergillus sydowii*

To evaluate tolerance to HMs, fungal mycelium of *A. sydowii* was inoculated by pitting on PDA medium contaminated with silver [Ag (1 mg L^{-1})], copper [Cu (60 mg L^{-1})] and cadmium [Cd (8 mg L^{-1})]. Metal-free PDA plates were used as controls. The plates were then incubated at 28 ± 2 ℃ for nine days. The grown colonies were photographed, and the growth diameter was measured. The equation below was used to calculate the percentage tolerance (%T) (Liaquat et al., 2020):

Tolerance percentage (%T) = area of fungal growth in the presence of metal ÷ area of fungal growth without exposure to metal, multiplied by 100.

Confocal microscopy of *Aspergillus sydowii*

Observation of the fungal hypha growing on contaminated PDA media with Cd and not contaminated media as a control were performed. The inverted agar block method was used to visualize the morphology of the hyphae. To observe cell membranes, the fluorophore FM® 4-64 (N-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino phenyl hexatrienyl) pyridinium dibromide) (5 µM) was used. To observe cell walls and septa, 0.01% calcofluor white dye was used. FM4-64 fluorescence was detected at 560-660 nm using an excitation wavelength of 488 nm, calcofluor fluorescence was detected at 430-470 nm using an excitation wavelength of 405 nm. All samples were analyzed under an Olympus FluoViewTM FV1000 laser scanning confocal microscope (Olympus, Japan) with 60X objective (1.42 AN).

Glyphosate and captan tolerance by *Aspergillus sydowii*

To evaluate pesticide tolerance, fungal mycelium of *A. sydowii* was inoculated by pitting on PDA medium contaminated with 20 mM Glyphosate (FAENA®, Mexico) and 500 mg L^{-1} Captan (Metfaver® Agroesa, Mexico). Concentrations were chosen according to previously conducted studies. (Carranza et al., 2017; Mohamed and Mostafa, 2018). The solutions of both pesticides were sterilized by filtration using 0.2 µm Whatman filters from sterile syringes. PDA plates without pesticides were used as a control. The plates were incubated at 28 ± 2 ℃ for nine days. The grown colonies were photographed, and the growth diameter was measured daily. The equation below was used to calculate the percentage tolerance (%T) (Liaquat et al., 2020):

Tolerance percentage (%T) = area of fungal growth in the presence of pesticide ÷ area of fungal growth without exposure to pesticide, multiplied by 100.

Phosphate solubilization

Mycelium of *A. sydowii* was inoculated by pitting on plates with Pikovskaya medium (PVK) containing insoluble phosphate sources such as tricalcium phosphate (Vyas et al. 2007). Plates were incubated at 28 ± 2 ℃ in the darkness conditons for 9 days. The formation of a clear circle around the fungal colony indicated the phosphate solubilization capacity. A measurement of the solubilization circle was made daily.

Siderophore production

Fungal sample of *A. sydowii* was inoculated on plates with chromium azurol S (CAS) medium. Plates were incubated for nine days at 28 ± 2 ℃ to examine siderophore formation (Andrews et al., 2016). The formation of a yellow halo around fungal colonies was interpreted as positive evidence of siderophore production. A measurement of the formed halo was made daily.

Statistical analysis

Experiments were performed in three replicates independently. Values represent the mean ± standard deviation. Data were analyzed using GraphPad Prism (version 8.0.1). Significant differences ($p < 0.05$) between the mean values of the different treatments

were determined using Tukey's multiple comparison test in a statistical analysis system (SAS, Cary, NC, USA). Graphs were made using GraphPad Prism software.

Results

Identification of *Aspergillus sydowii*

A. sydowii exhibited colonies of mid-sized growth, characterized by an almost circular shape in all growth media. After 9 days on MMA, the colonies show a greenish-gray coloration in the center and white at the periphery, with a velutinous texture and dense sporulation.

The highest quality sequence of the ITS region showed 99% query coverage, demonstrating identity with the *Aspergillus sydowii* species.

Aspergillus sydowii shows tolerance to toxic concentrations of heavy metals

A. sydowii demonstrated the ability to grow on media contaminated with Ag, Cu and Cd at 9 days post-innoculation.

Morphology of *Aspergillus sydowii* hyphae on contaminated medium with Cd

In contrast to hyphae growing under control conditions, the hyphae under to Cd stress displayed aberrant and deformed tip shapes, wavy apical growth, tangling, and increased branching.

Aspergillus sydowii shows high capacity for siderophore production and phosphate solubilization.

A. sydowii formed a yellow halo around the fungal colonies. This indicates the diffusion of siderophores into the CAS. Additionally, *A. sydowii* produced a straw-yellow halo on PVK medium around the fungal colony, demonstrating the capacity of the fungus for solubilizing phosphate.

Aspergillus sydowii shows tolerance to Glyphosate and Captan

A. sydowii exhibited tolerance to captan and glyphosate. Nevertheless, its growth under these stress conditions was lower compared to the controls.

Discussion

Previous studies have demonstrated the potential of *Aspergillus* species to eliminate and tolerate HMs. Demonstrating that *Aspergillus* species are potential candidate mycoremediators for the cleanup of environments contaminated by HMs. For *A. sydowii* it has been shown that it can remove Cd from the medium contaminated.

In our work, the potential of *A. sydowii* to tolerate pesticides was evidenced by its growth in medium contaminated with glyphosate and captan. Alvarenga et al. 2014, reported the ability of *A. sydowii* to completely degrade methyl parathion (organophosphate insecticide) after 20 days. It was also reported that this fungus can degrade the pesticide chlorpyrifos (Alvarenga et al. 2015).

We also found that *A. sydowii* possesses the ability to solubilize phosphates. This is an important characteristic found in microorganisms used as plant growth promoters.

It was found that *A. sydowii* can produce siderophores. It is known that most species of the genus *Aspergillus* can produce diversity of hydroxamate-type siderophores (Machuca and Milagres 2003).

The exceptional characteristics demonstrated by *A. sydowii* against toxic concentrations of heavy metals and pesticides are evidence of its bioremediation potential.

Conclusions

The present study highlights the remarkable abilities of *A. sydowii*, a fungus isolated from soil contaminated with heavy metals, specifically its tolerance to high concentrations of toxic elements like Ag, Cu, and Cd. This fungus also demonstrated resilience against pesticides commonly used agriculturally such as Glyphosate and Captan. Notably, the study reveals that exposure to toxic metal concentrations significantly affects cell morphology of *A. sydowii*, leading to unusual growth patterns and increased branching of hyphae. Additionally, the findings indicate that *A. sydowii* has the capacity to solubilize phosphates and produce siderophores, which are essential for plant promoting growth. These properties suggest to *A. sydowii* as a

promising biotechnological fungus for the remediation of contaminated sites with heavy metals and pesticides, and with potential application in sustainable agriculture practices.

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CAPÍTULO VI

Discusión general

Los metales pesados (MP) son elementos presentes en el medio ambiente que son esenciales para la vida, pero que pueden volverse tóxicos cuando sus concentraciones superan los niveles naturales y se acumulan en los organismos vivos. Debido al aumento de las actividades antropogénicas, las concentraciones de metales pesados (MP) y metaloides en el medio ambiente aumentan constantemente a lo largo de los años. Esto ha creado un problema mundial, ya que los MP son tóxicos, no son biodegradables y pueden entrar en la cadena alimentaria, causando graves daños a la salud humana y a los ecosistemas.

El problema de contaminación por metales pesados se ha reflejado en México, donde al menos 25 de los 31 estados del país han encontrado concentraciones en el suelo, los sedimentos y el agua que superan los límites máximos permitidos establecidos por las normas mexicanas. Estos datos se evidencian y analizan en la revisión presentada en el capítulo I de este documento con el objetivo de ofrecer una visión general de la situación actual de la contaminación por metales pesados en México, de esta manera sensibilizar a las entidades académicas y gubernamentales para que apliquen estrategias para resolver o mitigar el problema. Es imperativo aplicar estrategias que sean eficientes y eficaces para eliminar los metales pesados, sin descuidar el hecho de que dichas estrategias deben ser económicamente viables y respetuosas con el medio ambiente. La fitorremediación es un método biológico que cumple con todas las características mencionadas. En este método, las plantas utilizan diversos mecanismos, como la fitoextracción, la fitoestabilización, la fitodegradación, la fitovolatilización y la rizofiltración, que pueden llevarse a cabo de forma individual o varias de manera simultánea por la misma planta. Durante décadas, las plantas del género *Typha* se han utilizado para remediar entornos contaminados con metales pesados. *Typha latifolia* se ha destacado por su capacidad para hiperacumular una amplia gama de metales pesados y eliminar contaminantes orgánicos del agua, el

suelo y los sedimentos, lo que la hace adecuada para el tratamiento de aguas residuales. Para hacer frente al estrés metálico y mejorar la fitorremediación, las plantas establecen diversas asociaciones simbióticas con microorganismos en la rizosfera. Un ejemplo de ello son los hongos endófitos, que promueven el crecimiento y la producción de biomasa para mejorar su capacidad de biorremediación. Además, los hongos endofíticos pueden eliminar contaminantes como los metales pesados mediante reacciones biológicas y químicas y contribuir a la movilización de los contaminantes presentes en la rizosfera y su posterior absorción por las plantas, las cuales, a su vez, liberan exudados y enzimas que promueven actividades bioquímicas y microbianas que favorecen la fitoextracción. Esta información se evidencia en el manuscrito contenido en el capítulo II de este trabajo.

En el capítulo II se demostró el potencial de *T. latifolia* para tolerar concentraciones tóxicas de metales pesados como As, Cd, Cu y Pb, encontrando altas concentraciones de estos metales en sus raíces sin mostrar ningún efecto tóxico. Se propuso el uso potencial de esta planta en la biorremediación y como bioindicador para entornos contaminados. A lo largo de los capítulos II, III, IV y V se demuestra el papel que desempeñan los hongos endófitos de *T. latifolia* en la mejora 1) del bienestar de las plantas en situaciones de estrés y 2) de la fitorremediación de metales pesados. En el capítulo II, se aislaron y caracterizaron hongos del género *Talaromyces* (*T. liani*, *T. trachyspermus, T. austrocalifornicus* y *T*. sp.) y en el capítulo subsecuente se evaluó el potencial de los HE *Talaromyces* spp. para tolerar diversos estreses de tipo abiótico, con el fin de establecer las condiciones óptimas para el desarrollo de estos hongos. En el capítulo IV, se caracterizó y evaluó el potencial del hongo *Neosartoya fischeri* para tolerar los metales pesados y promover el desarrollo de las plantas. En el capítulo V, se caracterizó y evaluó el hongo *Aspergillus sydowii*. En general, se observó que estos hongos mostraban una alta tolerancia al Ag y al Cu y una tolerancia moderada al Cd, aunque la exposición a niveles tóxicos de Cd afectaba negativamente al crecimiento fúngico y a la morfología de las hifas. Además, todos los hongos mostraron la capacidad de solubilizar fosfatos y producir sideróforos, lo que destaca su potencial

como biofertilizantes y hongos promotores del crecimiento vegetal. Todos los hongos del género *Talaromyces* mostraron actividad quitinolítica, lo que probablemente contribuyó a su fuerte inhibición de los hongos fitopatógenos. Del mismo modo, *N. fischeri* mostró la capacidad de inhibir los hongos fitopatógenos, y se observó una fuerte colonización en las raíces de *T. latifolia*. Además, estos hongos mejoraron la salud de las plantas y la acumulación de Cd en los tejidos vegetales, lo que demuestra que los hongos endofíticos mejoran la acumulación de HM. Por último, *A. sydowii* mostró la capacidad de tolerar pesticidas como el glifosato y el captan.

En conjunto, estos hallazgos resaltan la posible aplicación de *T. liani*, *T. trachyspermus*, *T. austrocalifornicus*, *T*. sp., *N. fischeri* y *A. sydowii* en la biorremediación de entornos contaminados con metales pesados y como fitestimulantes. Estos microorganismos pueden funcionar como biofertilizantes y agentes de control biológico, mejorando el crecimiento y la tolerancia de las plantas en especies con capacidad para remediar sitios contaminados. En general, los resultados obtenidos permitieron alcanzar con éxito los objetivos específicos y general de este proyecto.

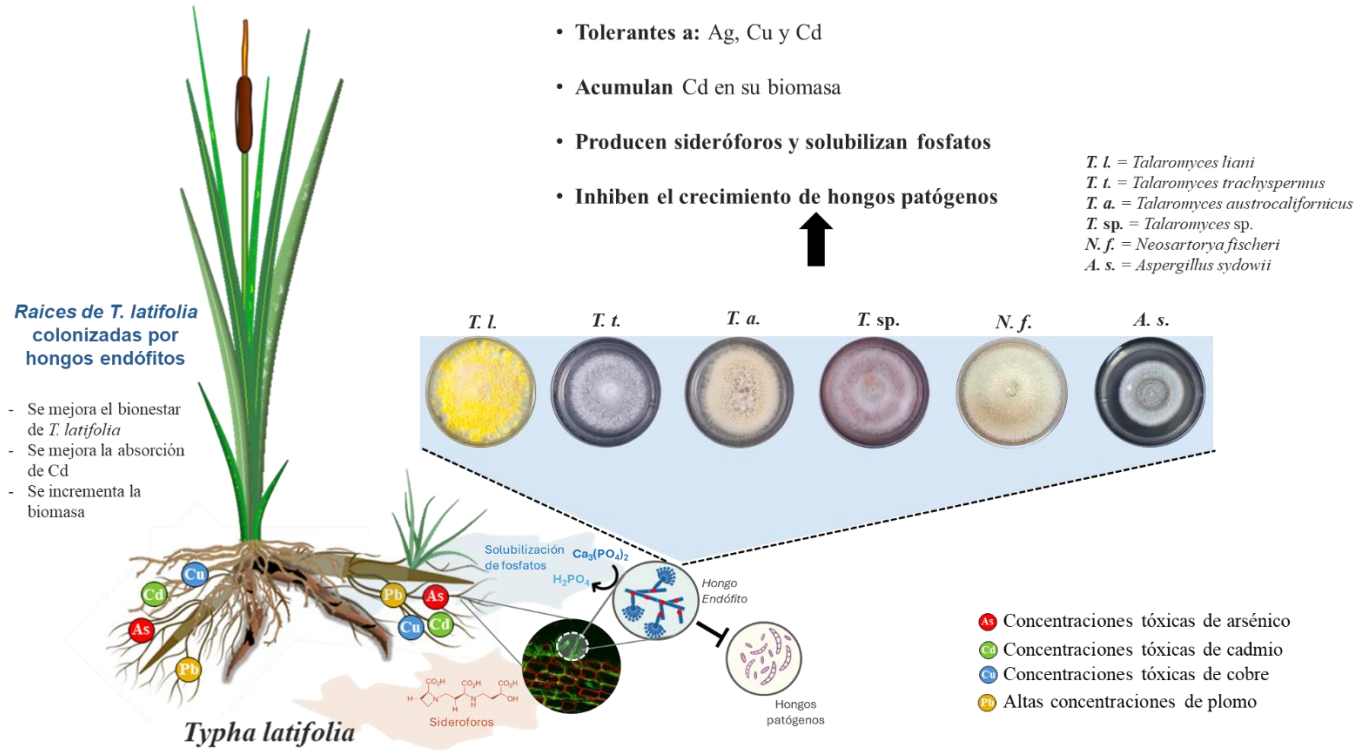
Conclusiones Generales

Los resultados de esta investigación conducen a cuatro conclusiones generales principales.

1. Existe un problema de contaminación ambiental por metales pesados, ya que en 25 estados del país al menos un metal pesado supera los límites permitidos por las normas mexicanas en el suelo, y en 26 estados al menos un metal pesado supera los límites permitidos en sedimentos y agua. La industria, aguas residuales urbanas, minería y agricultura han sido identificadas como las principales fuentes de contaminación.
2. *T. latifolia* es una especie vegetal adecuada para mitigar el problema de la contaminación, por su capacidad para bioacumular concentraciones tóxicas de metales pesados.

3. Los hongos endófitos aislados de las raíces de *T. latifolia* pueden tolerar y bioacumular concentraciones tóxicas de metales pesados. Sin embargo, la tasa de crecimiento y la morfología de las hifas se ven afectadas negativamente.
4. Los hongos endófitos aumentan la biomasa y el bienestar de las plantas de *T. latifolia* en condiciones de estrés por Cd. Esto se debe a su capacidad para producir sideróforos, degradar fosfatos, inhibir el crecimiento de hongos fitopatógenos y mejorar la capacidad de fitorremediación.

Resumen grafico



Perspectivas y recomendaciones

1. Realizar un análisis metabolómico a los hongos endófitos expuestos a medio contaminado con metales pesados, para conocer los compuestos que liberan los hongos endófitos frente a estrés causados por metales.
2. Realizar microscopía con fuente de luz sincrotrón a tejido vegetal y tejido fúngico expuesto a metales pesados, para conocer el sitio específico de la célula donde se confinan los metales.
3. Realizar ensayos de plantas de *T. latifolia* colonizadas por hongos endófitos expuestos a metales pesados utilizando suelo como sustrato para simular condiciones naturales.
4. Evaluar la capacidad de tolerancia y absorción de los hongos endófitos frente a otros metales pesados potencialmente tóxicos, tales como, plomo, arsénico y mercurio.
5. Evaluar la capacidad de tolerancia y absorción de los hongos endófitos frente a mezcla de metales pesados.
6. Evaluar de forma individual el efecto de los hongos *Talaromyces* spp. en la fitoextracción de metales pesados por *T. latifolia*.
7. Realizar ensayos de degradación de contaminantes emergentes, tales como fármacos o pesticidas, por hongos endófitos.
8. Evaluar el efecto para promover crecimiento de cada hongo endófito en plantas de interés agronómico.

ANEXO I

Artículos académicos y capítulos de libro generados

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REVIEW PAPER

Overview of the heavy metal contamination in Mexico: sources of the contamination and issues in human health

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Abstract This paper discusses information collected from original articles published between 1992 and 2022 regarding heavy metals (HMs) contamination in various environments across Mexico. The primary aim of this work was to identify the Mexican states where concentrations of HMs have been reported to exceed the maximum permissible limits for several types of soil, water, and sediment according to Mexican standards NOM-147-SEMARNAT/SSA1-2004, NOM-127-SSA1-2021, as well as international standards. The data collected indicates that 25 states in Mexico have reported at least one metal exceeding the maximum permissible limits in soil. Among these, Zacatecas, Nuevo Leon and Chihuahua had the highest number of HMs exceeding the standards. For sediment contamination, 26 states exceeded the permissible limits, with San Luis Potosí and Guerrero showing the highest number of HMs above the standards. Additionally, 26 states have reports of HMs exceeding the permissible limits in water, with Guanajuato and Guerrero having the highest number of HMs. Interestingly, the most frequent metals reported as soil contaminants are Cu, Fe, Pb and Zn; in sediment, they are Cd, Cr, Cu, Fe, Pb and Zn; and in water, they are Cd, Cr, Cu, Fe, Mn, Pb and Zn. The compiled information indicates that the primary anthropogenic sources of HMs release in Mexico include industrial activities, urban wastewater, mining, and agricultural practices. Furthermore, the data analyzed highlights several serious health risks associated with exposure to HMs, including cancer, central nervous system damage, DNA damage, and issues related to kidneys and lungs. This paper provides a comprehensive overview of HMs contamination in Mexico as well as the health challenges that arise from this contamination.

Keywords Heavy metals · Environmental contamination · Pollution · Human diseases · Mexico

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Introduction

Heavy metals (HMs) are elements found in the environment that are essential for life but can become toxic when their concentrations exceed natural

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Research Article

Fungus *Neosartorya* (*Aspergillus*) *fischeri* improves the fitness, tolerance and absorption of heavy metals in *Typha latifolia*

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Endophytic fungi
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Cadmium
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ABSTRACT

Heavy metal contamination is a global issue caused by anthropogenic activities leading to severe negative effects on the environment and human health. To address this problem, bioremediation strategies utilizing plants such as *Typha latifolia* and their symbiotic fungi have been adopted to remediate contaminated areas and mitigate the harmful effects of these pollutants. In this study, the endophytic fungus *Neosartorya fischeri* was isolated from the roots of *T. latifolia* plants growing in heavy metal-contaminated sites. *N. fischeri* colonized the epidermis and root cortex and showed high tolerance to toxic concentrations of silver (Ag) (1 mg/kg), copper (Cu) (60 mg/kg) and cadmium (Cd) (8 mg/kg). *N. fischeri* removed 8.7 % ± 0.5 % Cd from the medium, biosorbed 15.24 ± 0.2 mg/kg into its biomass, and enhanced the tolerance and bioaccumulation of Cd (184.18 ± 1.14 mg/kg) in plant roots. Moreover, *N. fischeri* produces siderophores, volatile compounds and solubilizes phosphates, which improve plant fitness. This was evidenced by a 28 % increase in photosynthetic pigments in *T. latifolia* plants colonized with *N. fischeri*. Additionally, *N. fischeri* inhibits the growth of important phytopathogens from the Fusarium genus. These findings highlight the important role of *N. fischeri* in enhancing the fitness and resilience of *T. latifolia* in hostile environments, demonstrating the potential of *N. fischeri*-*T. latifolia* association for the bioremediation of contaminated sites.

1. Introduction

Heavy metals (HMs) are natural metals with an atomic number (Z) greater than 20 and an elemental density greater than 5 g/cm^3 (Ali and Khan, 2017). The toxic concentrations of HMs in the environment have increased over the years due to anthropogenic activities such as mining, smelting, industrial processes, and agrochemicals (Ali et al., 2019). HMs are non-biodegradable elements and become toxic for all living organisms when they exceed the maximum acceptable limits in the environment (Briffa et al., 2020). In humans, exposure to HMs can cause a range of diseases, including cancer, damage to the central nervous system, kidneys, and lungs, as well as genotoxic, cardiac, gastrointestinal, endocrine, skin, liver, reproductive, immunological, poisoning, teratogenic, bones, hemolytic and in growth development (Balali-Mood et al., 2021). Cadmium (Cd) is considered a non-essential HM and is highly toxic at low concentrations to living organisms (Tchounwou et al., 2012; Soni et al., 2024). Studies indicate that exposure to Cd can promote the development of cancers in several organs and tissues, including the kidneys, lungs, pancreas, breast, prostate, and digestive tract (Balali-Mood et al., 2021).

Phytoremediation is a biotechnological technique that uses plants to restore sites contaminated with HMs (Tan et al., 2023). This strategy has gained popularity because it is low-cost, ecologically friendly, non-invasive, and effective in contaminant absorption (Nedjimi, 2021). Plants from the Typha genus are widely used in the remediation of contaminated environmental matrices due to their high capacity to tolerate, accumulate and transform HMs into their less toxic forms (Carranza-Álvarez et al., 2008; Chandra and Yadav, 2010; Taufikurahman et al., 2019; Putri and Moersidik, 2021; Lei et al., 2023; Ebrahimbabaie et al., 2023). Through evolution, these plants have established close relationships with rhizosphere microorganisms, including bacteria and fungi, which enhance their survival and phytoremediation capacity (Company et al., 2010). Fungi promote plant growth and fitness, reduce phytotoxicity caused by HMs, and increase the uptake of HMs by roots allowing plants to tolerate stressful environments (Zahoor et al., 2017; Deng and Cao, 2017). Endophytic fungi produce siderophores, antimi-

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Chapter 3

Endophytic Fungi and Bacteria

Enhancement of Heavy Metal Phytoextraction

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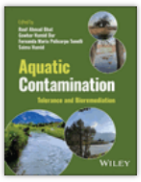

Aquatic Contamination: Tolerance and Bioremediation

Summary

Anthropogenic activities have increased the heavy metal (HMs) concentrations in the environment. Taking into consideration that HMs are not biodegradable, they can be accumulated in the soil, absorbed by plants and other living organisms, and later enter the food chains affecting human health. Phytoremediation is an alternative to handle this kind of contamination. Phytoremediation is a low-cost green technology using tolerant plants to remove contaminants from water or soil. Plants used in phytoremediation establish interactions with rhizosphere microorganisms to resist HMs toxicity and increase their phytoextraction capacities. These microorganisms include plant growth-promoting microorganisms, such as bacteria and endophytic fungi, which establish a mutualistic interaction with their hosts. Endophytic microorganisms play a key role in plant growth and development, e.g. they change the soil physicochemical characteristics, mineral and nutrient content, soil waste deposition, and soil water uptake. In addition, plant endophytic bacteria and fungi can remove and/or deactivate contaminants such as HMs through chemical-biological reactions. They contribute to removing contaminants at the rhizosphere which later can be absorbed by plants, which release exudates and enzymes that promote biochemical and microbial activities helping in phytoextraction. This chapter presents a thorough review of updated scientific documents about the interactions of plants with bacteria and/or fungi and their tolerance and absorption of HMs. Also, a deep review of their application in phytoremediation is included.

Keywords

phytoremediation phytoextraction fungi bacteria heavy metals

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Palabras clave: Hongos, metales pesados, contaminación, mecanismos de biorremediación.

Los hongos, microorganismos que ayudan en la descontaminación de ambientes contaminados con metales pesados

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Research article

Fungi of the *Talaromyces* genus associated to *Typha latifolia* roots, show capacity for bioremediation of heavy metals and enhancement of the plant fitness

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Bioremediation of heavy metals

ABSTRACT

The increase of heavy metals (HMs) in the environment has created a need for cost-effective and efficient remediation strategies. Among the most promising solutions is bioremediation, which utilizes living organisms to remove HMs from contaminated sites. This study aimed to assess the potential of endophytic fungi (EF) isolated from *Typha latifolia* roots to tolerate and accumulate HMs, promote plant growth, and inhibit phytopathogenic fungi. The isolated fungi were identified as *Talaromyces liani*, *Talaromyces trachyspermus*, *Talaromyces austrocalifornicus* and *Talaromyces* sp. Their tolerance to toxic concentrations of silver (Ag; 1 mg/kg), copper (Cu; 60 mg/kg), and cadmium (Cd; 8 mg/kg) ranged from 44.6 % to 98.0 %, except for *T. trachyspermus*, which showed only 5.5 % tolerance to Cd. In Cd-contaminated liquid medium, the fungi accumulated between 2.0 mg/g and 6.3 mg/g of metal in their biomass. Confocal microscopy revealed that exposure to toxic Cd concentrations caused aberrant hyphal growth. Interestingly, *T. liani*, *T. trachyspermus*, and *Talaromyces sp.* solubilized 80–100 % of the phosphate in the medium, and all fungi produced siderophores (24.7 %–62.7 %). Confrontation assays showed that *Talaromyces* spp. inhibited *Botrytis cinerea* and *Fusarium* spp., and all fungal isolates exhibited chitinolytic activity. Under an interaction assay of *Talaromyces* consortium and *T. latifolia*, the fungal consortium significantly increased the plant biomass and Cd accumulation in the shoots. These findings suggest that *Talaromyces* fungi exhibit strong potential for bioremediation of HMs-contaminated aquatic environments and may also act as effective biofertilizers and biocontrol agents against phytopathogenic fungi, thereby enhancing plant bioremediation capacity.

1. Introduction

Environmental concentrations of heavy metals (HMs) have steadily increased since 1950, making them a major category of globally distributed pollutants (Nriagu, 1996; Zhou et al., 2020; Gautam et al., 2016). This rise is primarily due to anthropogenic activities such as mining, industrial processes, urbanization, and excessive agrochemical use (Dagdag et al., 2023; Ponce-Hernández et al., 2025a). HMs are non-biodegradable, persist in the environment, and become toxic to organisms when concentrations exceed acceptable limits (Briffa et al., 2020). The most toxic HMs include cadmium (Cd), mercury (Hg), chromium (Cr), copper (Cu), arsenic (As), lead (Pb), nickel (Ni), zinc (Zn), and silver (Ag) (Mansouri et al., 2012; Afzaal et al., 2022; Padhye et al., 2023; Angon et al., 2024; Ponce-Hernández et al., 2025a). Specifically, Cd disrupts nitrogen and carbohydrate metabolism in plants, impairs photosynthesis, interferes with chlorophyll synthesis, and induces stomatal closure (Hocaoglu-Ozyigit and Genc, 2020). In humans, Cd exposure can cause cancer in various organs, hypercalcemia, nephrotoxicity, DNA damage, and lipid peroxidation (Tchounwou et al., 2012; Hocaoglu-Ozyigit and Genc, 2020; Chen et al., 2022). Meanwhile,

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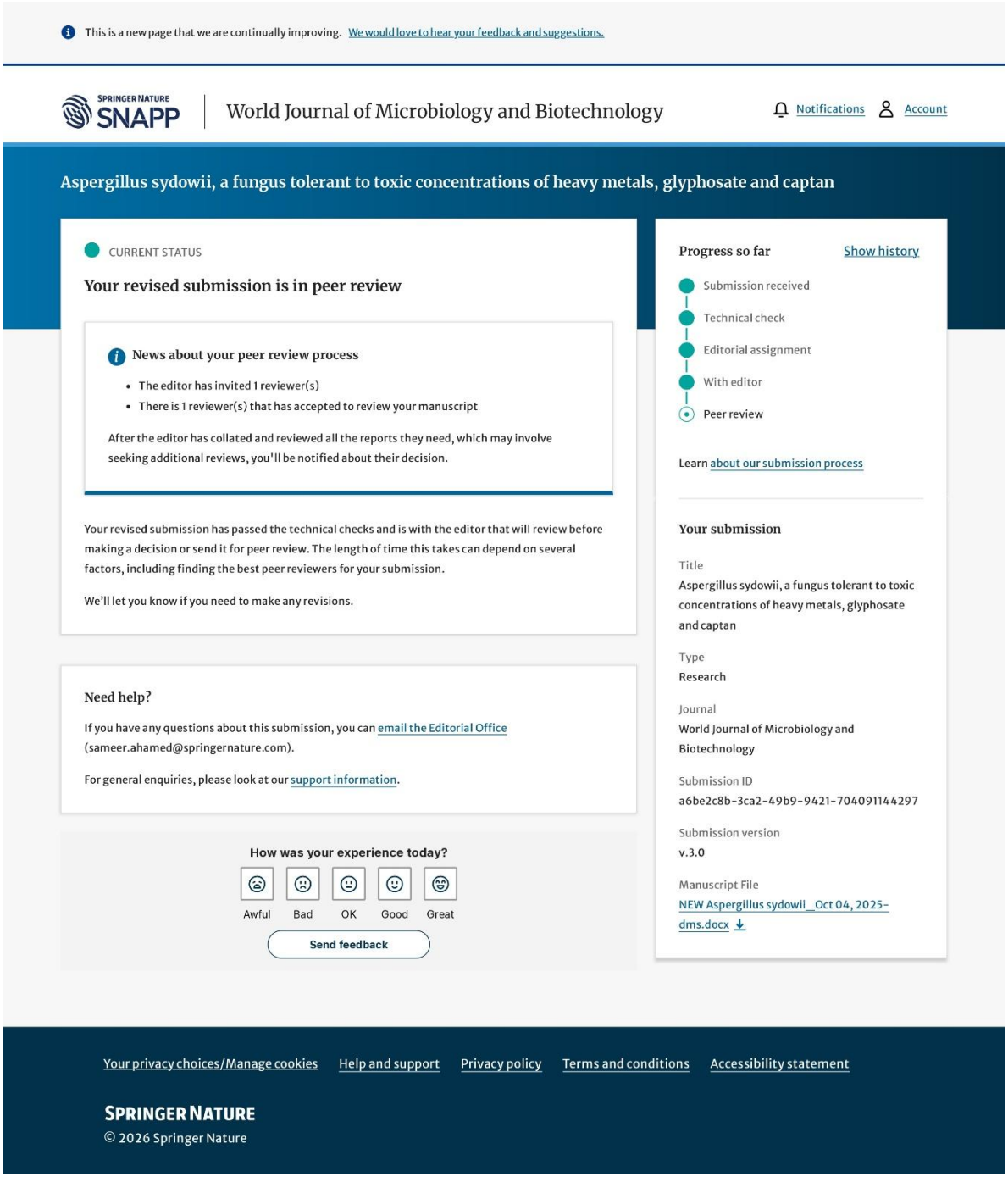
Nombre del artículo*: Aspergillus sydowii*, a fungus tolerant to toxic concentrations of heavy metals, glyphosate and captan



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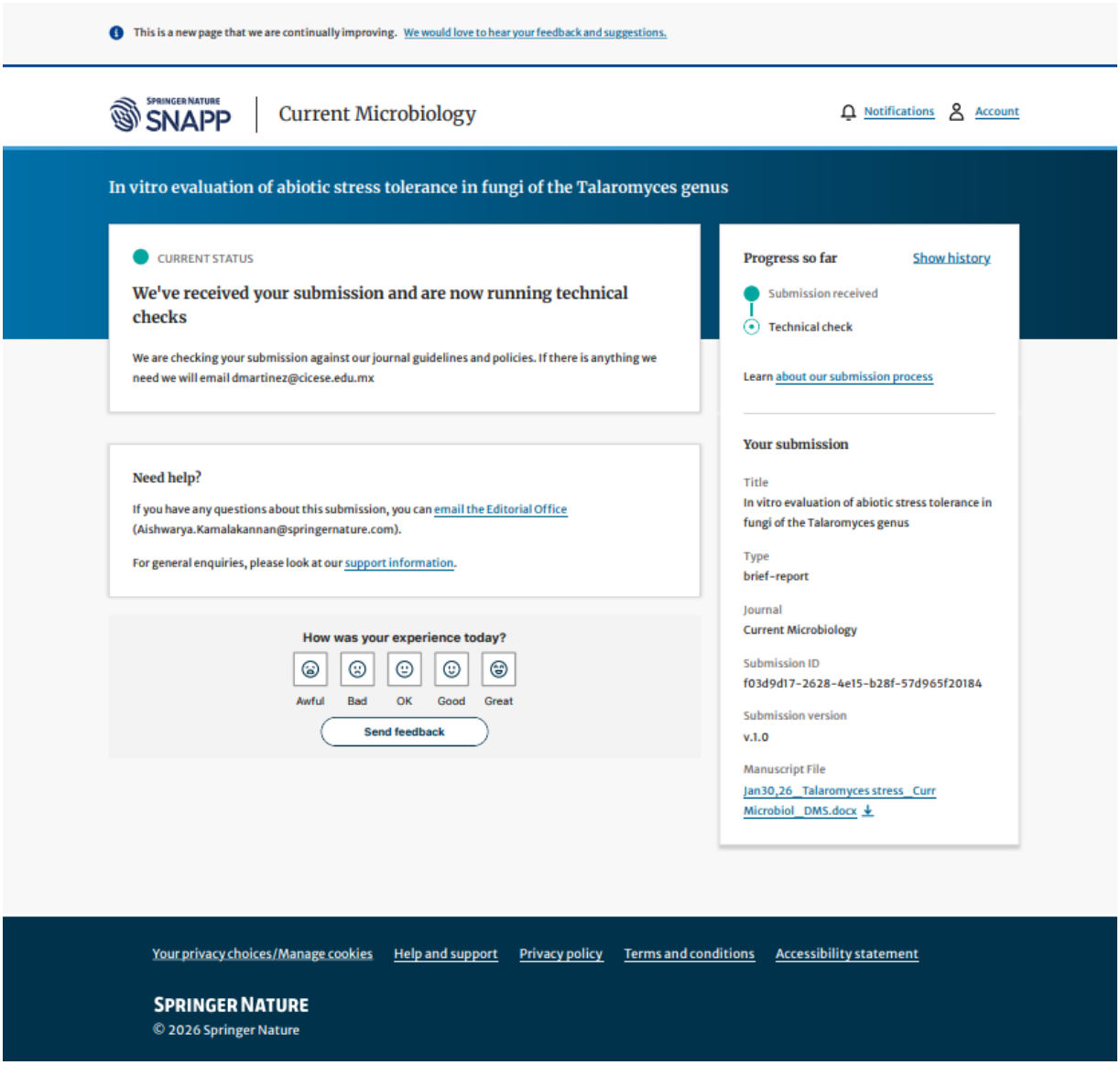

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Chapter 5
The Presence of Various Hazardous Pollutants in Agricultural Soils and Toxic Health Effects on Humans

Amauri Ponce-Hernández, Daniel Torres-Rico, Juan José Maldonado-Miranda, Candy Carranza-Álvarez, and Luis J. Castillo-Pérez

5.1 Introduction

The contamination of agricultural soils is an environmental problem of increasing global relevance (Toor et al., 2021). In a world where food security and ecological sustainability are main priorities, the degradation of agricultural soils due to the presence of hazardous contaminants poses significant challenges. These soils, which constitute the basis for the food production, fiber, fuel and even the survival of living beings, have been exposed for millennia to a diversity of toxic substances derived from multiple anthropogenic sources, mainly industrial, agricultural and urban (Zwolak et al., 2019; Fernandez-Marcos, 2022).

One of the main problems is the accumulation of heavy metals such as lead, cadmium, mercury and arsenic, which are introduced into the soil through intensive agricultural practices. In these practices the application of different types of agrochemicals, the irrigation with contaminated water and the atmospheric deposition are requires (Roychowdhury et al., 2002; Kubier et al., 2019; Mandal et al., 2020; Xing et al., 2020). Moreover, these metals, despite being present in trace concentrations, can be highly toxic and persist in the environment for decades, affecting the health of ecosystems and humans.

In addition to heavy metals, agricultural soils are also contaminated by residues of pesticides, herbicides and other agrochemicals used in modern agriculture to control crop pests and diseases (Meena et al., 2020; Lykogianni et al., 2021).

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Chapter 12
Cleaning the Environment: Using Fungi for Solving the Contamination by Heavy Metals and Toxic Agrochemicals

Fernanda García-Moreno, Rosario Razo-Belmán, Amauri Ponce-Hernández, and Domingo Martínez-Soto

12.1 Introduction

The increasing population and anthropogenic activities like manufacturing, mining, usage, and application of pesticides, fertilizers, and food additives have increased toxic pollutants to critical levels worldwide. Becoming a serious problem that has a negative impact on animal, plant as well as human health (Quintella et al., 2019). In recent decades, bioremediation has been employed as a new strategy to solve this problem based on the use of living organisms such as plants (called phytoremediation) and microorganisms such as algae, bacteria, and fungi (called microbial remediation), as well as their enzymes (Martínez-Soto et al., 2021; Ponce-Hernández et al., 2023). Recently, fungi have gained increasing attention for their ability to degrade, transform, and immobilize environmental contaminants by a process called mycoremediation (Bosco & Mollea, 2019; Ponce-Hernández et al., 2023). Mycoremediation is used to decontaminated the environment, because of the incredible versatility of fungi and their characteristics such as adaptability, morphology, physiology, and metabolism (García-Ortega et al., 2022). Furthermore, due to its

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