El Colegio de la Frontera Sur

Interacción entre plaguicidas y la diversidad funcional de macroinvertebrados edáficos en sitios agrícolas de la región de los Chenes, Campeche, México

TESIS

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Con orientación en Gestión de ecosistemas y territorios

Por

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Las personas abajo firmantes, miembros del jurado examinador de: Elida Lucero Sánchez del Cid hacemos constar que hemos revisado y aprobado la tesis titulada:
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DEDICATORIA

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INDICE

RESUMEN

En la península de Yucatán, Campeche, México, los pesticidas se usan en cantidades excesivas para el cultivo de soja (*Glycine max*) y maíz (*Zea mays*). En esta investigación, se evaluó el efecto de los plaguicidas organoclorados y del producto de degradación del herbicida glifosato (AMPA) en la diversidad y composición de los rasgos funcionales de los macroinvertebrados edáficos. Se muestreó en tres sistemas: a) cultivo de *Glycine max* y b) cultivo de *Zea mays* y c) en la vegetación natural circundante. Se realizaron cuatro repeticiones por cada sistema con un total 12 unidades experimentales. En cada unidad se realizaron cinco monolitos de acuerdo al método TSBF (Anderson and Ingram, 1993) para colectar a los macroinvertebrados del suelo y caracterizarlo. Para determinar las concentraciones de plaguicidas en el suelo, se realizó una muestra compuesta de los cinco monolitos por cada unidad experimental (n: 12) y para el AMPA se realizaron muestras por cada monolito (n: 60). En el sistema tecnificado de *Glycine max* se encontró el contenido más alto de AMPA (0.297±0.27 mg kg-1). Los PHPs presentaron la concentración más alta en el cultivo de *Zea mays* con el compuesto cis clordano alfa (1.17±0.77 µg-kg-1). El porcentaje de arcilla y el contenido de AMPA en el suelo tuvieron una correlación fuertemente significativa (rho=0.90; *p*: 0.002). El sitio con mayor densidad de macroinvertebrados fue el cultivo de *Zea mays* (37.89±56.29 ind m^2). En sitios con mayor concentración de AMPA se encontraron especies con menor tamaño, una dureza del exoesqueleto alta y con un tipo de alimentación omnívora. Los resultados muestran que el AMPA tiene un efecto en la homogenización de la diversidad taxonómica y funcional de los macroinvertebrados edáficos. Los PHPs no mostraron una relación fuerte con la diversidad de rasgos funcionales ni con la riqueza taxonómica de los macroinvertebrados.

Palabras claves: ácido aminometilfosfónico (AMPA) derivado del Glifosato, plaguicidas históricos persistentes, macroinvertebrados edáficos, rasgos funcionales.

CAPÍTULO I

INTRODUCCIÓN

La intensificación agrícola ha reducido la biodiversidad del suelo en terrenos cultivados (Pelosi et al. 2013) y se considera como una de las causas principales de la pérdida de biodiversidad (Gámez-Virués et al. 2015). En comunidades de invertebrados del suelo se han reportado efectos negativos en la biodiversidad por labranza, el uso de plaguicidas, la simplificación de la rotación de cultivos y la gestión de uso de la tierra (Forrest et al. 2015; Gámez-Virués et al. 2015; Spake et al. 2016; Ding et al. 2017). En particular, los macroinvertebrados edáficos conocidos como bioindicadores de la calidad del suelo (Velasquez et al. 2007a; Huerta et al. 2009), se han visto afectados por los plaguicidas (Garcia-Perez et al. 2014; Pelosi et al. 2014; Fontana et al. 2016; Araya et al. 2017). Sin embargo, aunque se sabe acerca de la importancia de poner a prueba el efecto de los plaguicidas en los invertebrados del suelo, los estudios principales se llevan a cabo en el laboratorio y no en campo (Huerta et al., preparación) (Jansch et al. 2006).

Una mayor demanda en la producción agrícola ha dado lugar a un aumento aproximado del 30 al 40% en el uso de plaguicidas en la agricultura en los últimos 50 años (Yang 2016). Por ello, se prevé que esta tendencia aumentará en las próximas décadas (Liu et al. 2015) y en particular en los herbicidas a base de glifosato que se han convertido en los más aplicados en todo el mundo, especialmente en cultivos modificados genéticamente (Brookes and Barfoot 2015; Yang 2016). Por ejemplo, en los Estados Unidos, desde 1974 se han aplicado más de 1.600 millones de kilogramos de glifosato lo que representa el 19% del uso mundial estimado (8,6 billones de kilogramos) (Benbrook 2016). A nivel mundial, el uso de glifosato ha aumentado casi 15 veces desde que se introdujeron los cultivos resistentes al glifosato en 1996. En los Estados Unidos de 1974 a 2014 se ha aplicado dos tercios del volumen total del glifosato. La proporción correspondiente a nivel mundial es del 72% (United States Department of Agriculture 2014).

En México el uso de plaguicidas durante el 2014 se estimó en 98 814 toneladas de insecticidas (33%), herbicidas (27%), fungicidas y bactericidas (40%) (FAOSTAT 2017). El AMPA (ácido aminometilfosfónico) se considera como el principal producto de degradación del glifosato y se ha encontrado frecuentemente en el suelo, sedimentos y cuerpos de agua (Borggaard and Louise 2008). El tiempo de vida media del glifosato oscila entre 1.7 y 197 días en el suelo (Degenhardt et al. 2011; Yin et al. 2015). El AMPA tiene un tiempo de vida medio más largo que oscila entre 9 y 240 días en el suelo. Los estudios demuestran que la variación en los tiempos de vida media de glifosato y el AMPA dependen de las propiedades del suelo (como pH, porcentaje de materia orgánica y textura) y las condiciones climáticas (Al-Rajab and Hakami 2014). Además, los suelos agrícolas son las principales fuentes de plaguicidas orgánicos persistentes (COPs) ya que actúan como un depósito definitivo de residuos actuales y pasados (Weaver et al. 2012; Pozo 2017). En consecuencia, los productos químicos tóxicos, especialmente los COPs en el suelo se pueden acumular en las plantas o en la fauna del suelo. Lo que trae como resultado un riesgo para los ecosistemas terrestres (Walker 2014). Los plaguicidas organoclorados, como una clase típica de COPs siguen siendo ubicuos en el ambiente actual (Cai et al. 2008) debido a su larga historia de uso y su difícil degradación (Sultana et al. 2014) .

Los plaguicidas afectan a todos los niveles de organización biológica, desde individuos hasta ecosistemas (Walker et al. 2012). La toxicidad de una sustancia química depende del tiempo de exposición, la concentración, dosis, características de los factores ambientales y de la mezcla de los diferentes tipos de contaminantes (Fent 2004). Se puede desarrollar resistencia por parte de los organismos del suelo hacía los diferentes plaguicidas. Sin embargo, esto es más evidente en las especies con una amplia distribución geográfica las cuales toleran más la presencia de los plaguicidas que las especies nativas o locales

(Vandewalle et al. 2010). De igual manera, la susceptibilidad del organismo puede variar entre regiones (Fent 2004).

En los últimos años se ha dado más énfasis en el estudio del efecto de plaguicidas sobre las lombrices de tierra. En especial sobre *Eisenia foetida,* la cual es una lombriz epigea, nativa de ambientes templados. Sin embargo, el papel de las lombrices de tierra en los sistemas intensivos de producción agrícola está limitado por una perturbación excesiva del suelo (Evans et al. 2011). Los otros grupos taxonómicos de macroinvertebrados del suelo han recibido poca atención.

Las lombrices tropicales que se caracterizan por ser en su mayoría endogeas/geófagas están propensas a una mayor toxicidad potencial a los contaminantes (Fragoso and Lavelle 1992). Esto se debe a que por su metabolismo tienen una tasa de ingestión de sustrato mayor (Buch et al. 2013). Por ejemplo, una población de la especie de la categoría geófaga *Pontoscolex corethrurus* (Müller, 1857) puede ingerir hasta 400 toneladas de suelo/ha/año en un pastizal de Laguna Verde, México (Lavelle et al. 1983).

A pesar de que numerosos estudios de ecotoxicidad se han llevado a cabo en los últimos años con el uso de las lombrices de tierra, éstos se centraron principalmente en los plaguicidas individuales (Spurgeon et al. 2003).

Por lo tanto, los resultados de los estudios de una sola especie no reflejan situaciones de campo donde se utilizan múltiples plaguicidas o mezclas. Por lo que puede subestimar el riesgo ecológico que realmente se presenta en el ambiente (Zhou et al. 2011). Tomar una sola especie como indicadora de exposición no siempre representará lo que está ocurriendo al conjunto de organismos (van Gestel van Dis, W.A. 1988).

Jansch et al. (2006) probaron la sensibilidad de lombrices y colémbolos a concentraciones peligrosas al 5 % de diferentes tipos de plaguicidas. El 95% de todos los plaguicidas tuvieron datos de toxicidad, al menos para tres especies. Los artrópodos y oligoquetos presentaron diferencias pronunciadas en su sensibilidad a la mayoría de estos plaguicidas. *Eisenia foetida* fue la especie menos sensible a los insecticidas basada en la mortalidad aguda, mientras que una especie de Collembola, *Folsomia candida* fue una de las especies más sensibles para una amplia gama de tóxicos (Biocida, fungicida, herbicida e insecticida).

Zhou et al. (2011) evaluaron la toxicidad de un piretroide (cipermetrina) y un insecticida organofosforado (clorpirifos) individualmente y en combinación. Se realizaron pruebas sobre diferentes respuestas (agudas, crónicas y de comportamiento) en las lombrices de tierra de las especies *Eisenia foetida y andrei* en la evaluación del riesgo ecológico de estos plaguicidas. Se encontró que la toxicidad de la mezcla de cipermetrina y clorpirifos fue significativamente mayor que cualquiera de estos plaguicidas individualmente, especialmente en las respuestas crónicas de la lombriz de tierra.

Alshawish et al. (2004) probaron el efecto de la cipermetrina, clorpirifos y dicofol, mancozeb sobre su toxicidad crónica en *Aporrectodea caliginosa* en laboratorio. Encontraron que el clorpirifos era el plaguicida más tóxico, lo que puede producir impactos significativos en la fecundidad de la lombriz a 50 mg / kg de suelo seco, mientras que la cipermetrina en la misma dosis era la menos tóxica. Sin embargo, produjo una reducción del 20% en la viabilidad del capullo y no se encontraron efectos en el desarrollo de las crías.

En un estudio en el centro de Veracruz, México, se evaluaron las diferencias en los procesos del suelo inducidos por lombrices en sitios donde se aplicó el herbicida glifosato. Se encontró una mayor tasa de mineralización de carbono neto y un cambio en las propiedades físico-químicas del suelo (pH, contenido de arcilla, limo y contenido de calcio en el suelo), en parcelas donde se aplicaba más glifosato y la ausencia de *Amynthas corticis.* La cual se vio afectada por el herbicida al ser una especie de lombriz anécica (ya que ingiere también hojarasca). También se vio un efecto negativo en la biomasa, densidad y número de especies de lombrices en parcelas donde se aplicaba glifosato (García-Pérez et al. 2014).

Los invertebrados del suelo juegan un papel importante en la dinámica de los ecosistemas, ya que están involucrados en el funcionamiento del suelo (Pelosi et al. 2014).

La diversidad funcional (DF) se define como el valor, rango y abundancia de los rasgos funcionales en una comunidad o ecosistema (Córdova-Tapia and Zambrano 2015). La selección de rasgos depende de la función a estudiar en la comunidad y su cuantificación es, generalmente, un valor único por especie en la comunidad, representado por la media o la mediana. La abundancia de valores de los rasgos está asociada a la abundancia de especies en la comunidad (Córdova-Tapia and Zambrano 2015). El primer paso para evaluar la diversidad funcional de una comunidad consiste en identificar los rasgos funcionales que se tomarán en cuenta para el estudio. Los rasgos funcionales son rasgos biológicos que influyen en el desempeño de los organismos y que pueden estar relacionados con los procesos ecosistémicos (flujo de materia y energía), la estabilidad de los ecosistemas (resistencia y resiliencia), las interacciones biológicas (intra e inter específicas) y/o la modificación del hábitat (Hooper et al. 2005). La descripción funcional basada en rasgos eco-morfológicos toma en cuenta la relación entre la forma, el desempeño y la ecología de los organismos (Córdova-Tapia and Zambrano 2015).

La DF de una comunidad se puede medir mediante varios enfoques (Bernhardt-Röermann et al., 2008), que incluyen *a priori* (i) la diversidad de grupos funcionales a priori, (ii) la diversidad de grupos funcionales determinados, e (iii) índices basados en rasgos funcionales. Los enfoques basados en rasgos se usan actualmente en diferentes campos de la ecología, por ejemplo en ecología de invertebrados de arroyos y de plantas (Ilunga et al. 2015; Wood et al. 2015). Han sido menos estudiados en ecología del suelo, aunque los perfiles de rasgos funcionales se han enfatizado como una forma consistente de revelar las respuestas de las lombrices de tierra a las perturbaciones ambientales (Hedde et al. 2013; Fontana et al. 2016).

Actualmente se usan parámetros biológicos para evaluar los efectos ecotoxicológicos que se miden actualmente por los biomarcadores, bioindicadores o una combinación de ambos (van Gestel et al. 2009; Capowiez et al. 2010). Estos indicadores dependen de diferentes factores como la textura del suelo o el microclima que aparecen como factores de confusión y dificulta la calibración de estas herramientas a gran escala (Hobbelen et al. 2006). Para superar estos inconvenientes los conceptos de rasgos funcionales surgen como una forma prometedora para comprender procesos que impulsan las respuestas del organismo a las perturbaciones ambientales. Por ejemplo la teoría de hábitat templet (Southwood 1977) provee el marco que relación las características de la taxocenosis con la variabilidad espacio-temporal del hábitat. Esta teoría supone al hábitat como molde dentro del cual la evolución forja las características morfológicas y las estrategias de vida, y filtra aquellos rasgos que faciliten la supervivencia (Petchey et al. 2007; Pey et al. 2014).

La intensificación del uso del suelo (uso de maquinaria pesada, monocultivos, gran uso de insumos externos etc) puede reducir en gran medida la riqueza de especies y el funcionamiento de los ecosistemas (Flynn et al. 2009). Es decir, propiedades funcionales y servicios que prestan al ecosistema o agroecosistema y que apoyan al bienestar humano. Por ejemplo, el almacenamiento de carbono, mantenimiento de la estructura del suelo, resistencia a invasión de plagas y ciclaje de nutrimentos (Morerira 2012). Sin embargo, la riqueza de especies determina estas funciones a través de la diversidad y valores de rasgos funcionales de las especies presentes (Flynn et al. 2009).

La homogeneización biótica causada por la intensificación de la agricultura da como resultado comunidades monoespecificas, con valores de rasgos más estrechos lo que puede limitar las funciones proporcionadas por la comunidad y su capacidad para responder a las perturbaciones rasgos (Sandra and Cabido 2001). Es decir, puede contar con rasgos similares pero estos tendrán menor variación (rango/atributos) que los del sistema natural y por lo tanto una menor diversidad funcional (Gámez-Virués et al. 2015).

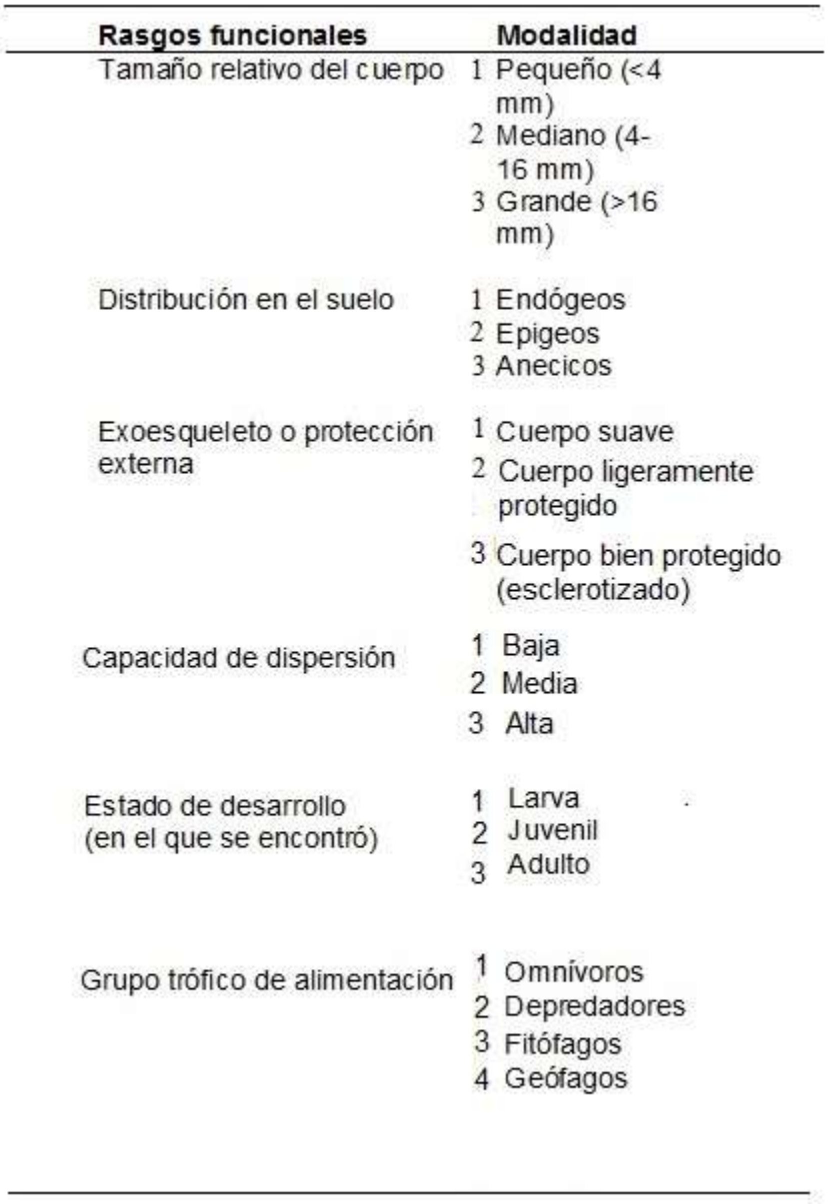
En algunos estudios se han encontrado diferentes patrones de rasgos perdidos a través del gradiente de intensificación agrícola. El uso de la diversidad funcional para evaluar las consecuencias del cambio de uso del suelo revela que la diversidad de rasgos cambia en gran medida. Sin embargo, este enfoque se aplica poco sobre la ecología del suelo (Gámez-Virués et al. 2015).

El perfil rasgo funcional ha sido señalado por varios autores como un parámetro importante para revelar las respuestas a la contaminación por parte de invertebrados (Ribera et al. 2001; Skalski et al. 2010). Sin embargo, el desarrollo del enfoque de rasgos funcionales se ha visto obstaculizado por diversos factores (Skalski et al. 2010). Uno de los factores limitantes más importantes puede ser la ausencia de una base de datos unificada, compartida, que contenga múltiple información de un gran número de taxas (Hedde et al. 2012).

Los diferentes índices de diversidad tradicionales (Índice de Shannon-Wiener, Simpson, Margalef) ignoran la diferencia en la composición de especies, basándose más en la abundancia relativa (Botta-Dukát 2005). Los estudios que abordan de forma conjunta la riqueza de especies, riqueza funcional y composición funcional sugieren que la importancia de la composición y riqueza funcional tienden a ser más grandes que la riqueza de especies para influir en las funciones del ecosistema (Petchey and Gaston 2002; Naeem and Wright 2003; Laliberté and Legendre 2010).

En el estado de Campeche, el progreso de la agricultura mecanizada, la ganadería y la urbanización han dado como resultado una tasa anual de deforestación de -0.74% de 1976 a 2005, superior al promedio nacional de -0.43% para el período 2000-2010 (Ellis et al. 2017). En el municipio de Hopelchén, Campeche, México, en años recientes se ha tenido un incremento en las hectáreas dedicadas a la agricultura bajo mecanizado (Aguilar y Rendón von Osten 2016). En el 2013 más de 38 mil hectáreas de cobertura forestal en el estado de Campeche desaparecieron. Dentro de los principales cultivos sembrados en la región se encuentra la soya (*Glycine max*) transgénica, lo que ha generado un aumento en la aplicación del herbicida glifosato ya que la cepa RR-SOJA (Roundup Ready) o soja 40-3-2 es resistente al glifosato (Huacuja 2016). Hopelchén, es donde se ubica la mayor parte de las colonias menonitas que producen ese grano, cuyos integrantes cultivaron en 2013 alrededor del 80% de las 6.851 hectáreas de soya cosechadas en el municipio (Huacuja 2016). Entre dichas colonias, destacan Las Flores y La Nueva Trinidad, que producen la mayoría de la soya de Campeche (Huacuja 2016). Sin embargo, su uso no sólo se limita a este tipo de cultivo, sino también al maíz (*Zea mays*), sandia (*Citrullus lanatus*), calabaza (*Cucurbita argyrosperma*) entre otros. Dentro de estos cultivos existe también un alto uso de otros plaguicidas dentro de la gama de organofosforados, piretroides, carbamatos y algunos organoclorados que aún están permitidos (Arellano-aguilar and Rendón von Osten 2016). A pesar de que hay un uso importante de los plaguicidas en la península de Yucatán hay poca información documentada acerca de la cantidad de residuos de plaguicidas que se encuentra en el suelo y del efecto de estos sobre los macroinvertebrados edáficos (Huerta et al., en preparación). La mayoría de los estudios anteriores sobre las relaciones rasgos funcionales y variables ambientales se han centrado en macroinvertebrados bentónicos en ambientes acuáticos (Hein et al. 2005) y muy pocos estudios en los macroinvertebrados edáficos y menos en relación con plaguicidas (Hedde et al. 2013) . Por lo que en el presente trabajo se seleccionaron seis rasgos funcionales de macroinvertebrados del suelo dentro de los que se consideran características morfológicas, ecológicas y de comportamiento, además de complementar con la densidad, riqueza taxonómica e índices de diversidad. Con el fin de analizar y evaluar la respuesta de los macroinvertebrados del suelo a los plaguicidas.

Tabla1. Descripción de los rasgos seleccionados y sus modalidades



Rasgos funcionales	Modalidad
Tamaño relativo del cuerpo	1 Pequeño (<4 mm) 2 Mediano (4-16 mm) 3 Grande (>16 mm)
Distribución en el suelo	1 Endógeos 2 Epigeos 3 Anecicos
Exoesqueleto o protección externa	1 Cuerpo suave 2 Cuerpo ligeramente protegido 3 Cuerpo bien protegido (esclerotizado)
Capacidad de dispersión	1 Baja 2 Media 3 Alta
Estado de desarrollo (en el que se encontró)	1 Larva 2 Juvenil 3 Adulto
Grupo trófico de alimentación	1 Omnívoros 2 Depredadores 3 Fitófagos 4 Geófagos

OBJETIVOS

Objetivo general

Evaluar y caracterizar la respuesta de los rasgos funcionales y la diversidad de macroinvertebrados edáficos a la presencia o usencia de residuos de plaguicidas encontrados en los cultivos de maíz (*Zea mays*), soya (*Glycine max)* y en la vegetación natural circundante en sitios agrícolas de la región de los Chenes.

Objetivos específicos

- Determinar las concentraciones de residuos de plaguicidas organoclorados y del producto de degradación del glifosato (AMPA) que se encuentren presentes en los suelos agrícolas.
- Identificar y clasificar a los macroinvertebrados en morfoespecies de acuerdo a su grupo taxonómico.
- Determinar si los plaguicidas afectan a los macroinvertebrados edáficos a nivel individuo, población y comunidad de acuerdo a: riqueza taxonómica, rasgos funcionales, densidad y su composición biológica encontrados en los diferentes sistemas.

HIPOTESIS

Hipótesis de investigación

1. Teniendo en cuenta el efecto de los residuos de plaguicidas encontrados en el suelo como una variable de estrés ambiental, los rasgos funcionales, la diversidad, riqueza taxonómica y la densidad de macroinvertebrados del suelo disminuyen en los sitios con una mayor concentración de plaguicidas en el suelo.

2. Existen diferencias entre los sistemas agrícolas (*G. max y Z. mays*) y la vegetación nativa circundante a estos sitios en términos de: i: diversidad taxonómica y funcional y ii: composición de rasgos funcionales.

CAPITULO 2

ARTÍCULO SOMETIDO EN LA REVISTA SCIENCE OF THE TOTAL ENVIRONMENT: SOIL MACROINVERTEBRATES FUNCTIONAL TRAITS UNDER THE INFLUENCE OF AMINOMETHYLPHOSPHONIC ACID (AMPA) AND HISTORICALLY PESTICIDES AT CAMPECHE, MEXICO

Soil macroinvertebrates functional traits under the influence of aminomethylphosphonic acid (AMPA) and historically pesticides at Campeche, Mexico.

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Abstract

At the Yucatan peninsula, Campeche, Mexico, pesticides are used in excessive quantities for the cultivation of soybean (*Glycine max*) and maize (*Zea mays*). In this research, the effect of historically persistent pesticides organochlorines and the metabolite AMPA (product of the degradation of glyphosate herbicides) on the diversity and composition of functional traits of edaphic macroinvertebrates were evaluated. Samples were taken in three systems: a) technified cultivation of *Glycine max*, b) semi-technified cultivation of *Zea mays* and c) in the surrounding natural vegetation. Four repetitions per system were done, with a total of twelve experimental units.

In each experimental unit, five monoliths were extracted according to the TSBF method (Anderson and Ingram, 1993) for collecting the soil macroinvertebrates and to characterize the soil. Additionally, a monolith of 10 x 10 cm was extracted to collect macroinvertebrates and to determine if they contained residues of pesticides. To determine the concentrations of pesticides in the soil, a composed sample of the five monoliths for each experimental unit (n: 12) was processed and for the glyphosate and AMPA, samples for each monolith (n: 60) were processed. The highest amount of AMPA (0.297±0.27 mg kg-1) was found in Glycine max. The historically persistent pesticides cis-Chlordane alpha was high found in *Zea mays* (1.17±0.77 μg-kg-1). The highest concentration of the p,p´-DDD (0.0068 μg kg-^{1}) in macroinvertebrates was found in *G. max*. The percentage of clay and the AMPA content in soil had a strong correlation (rho=0.90; p: 0.002). The site with the highest density of macroinvertebrates was the semi-technified system of *Z. mays* (37.89±56.29 ind m^{2}). In places with high AMPA concentration, were found morphospecies with smaller size, with a hard exoskeleton and an omnivorous type of diet.

Key words: aminomethylphosphonic acid (AMPA), historically persistent pesticides, soil macroinvertebrates, functional traits.

Introduction

The loss of biodiversity can affect the viability of the ecosystems by diminishing the capacity of the communities to respond to disturbances and environmental changes (Gámez-Virués et al. 2015). Agricultural intensification has reduced the soil's biodiversity of cultivated land (Pelosi et al. 2013) and is considered as one of the mayor causes of the loss of biodiversity. Practices like the simplification of the crop's rotation, farming with heavy machinery and the use of external inputs like fertilizers and pesticides have provoked a serious degradation of the soil (Brunet et al. 2009). A higher demand in production originated an increase of approximately 30 to 40% of the use of pesticides in agriculture (Yang 2016). It is foreseen that this tendency will increase over the next decades (Liu et al. 2015).

The use of pesticides in Mexico in the year 2014 is estimated at 98 814 tons of insecticides (33%), herbicides (27%), fungicides and bactericides (40%) (FAOSTAT 2017). The glyphosate-based herbicides have become the most widely applied herbicide in the world, especially on genetically modified crops (Brookes and Barfoot 2015; Yang 2016). The adverse effects of pesticides on the environment and human health depend of multiple factors like the pesticide's toxicity, the dose and application practices, the pesticide's purpose in the soil, its half-life and degradation products (Fan et al. 2015; Yang 2016). AMPA (aminomethylphosphonic acid) is considered to be the main degradation product of Glyphosate and is frequently found in soil, sediments and water bodies (Borggaard and Louise 2008). The half-life of Glyphosate ranges between 1.7 and 197 days in soil (Degenhardt et al. 2011; Yin et al. 2015). AMPA has a longer half-life that oscillates between 9 and 240 days in soil. Research shows half-life variations of Glyphosate and AMPA strongly depend on the soil's properties (like pH, % of organic matter and texture) and climatic conditions (Al-Rajab and Hakami 2014). In addition, agricultural grounds are the principal sources of persistent organic pollutants (POPs) because they act like a permanent deposit of actual and past waste (Weaver et al. 2012; Pozo 2017). Therefore, toxic chemical products, especially those persistent organic pollutants in the soil, can accumulate in plants or wildlife in the soil, resulting in a risk for the earth's ecosystems. Organochlorine pesticides, a typical kind of POPs, are still present in the actual environment (Cai et al. 2008) because of its long usage history and its difficult degradation (Sultana et al. 2014) .

In particular, edaphic macroinvertebrates, known as bioindicators, fulfill important functions in several ecological soil processes (Velasquez et al. 2007b; Huerta and van der Wal 2012). However, there's very little information about the field-scale effect of pesticides on invertebrates (Jansch et al. 2006). The functional traits and the functional biodiversity of macroinvertebrates have a close relationship with environmental variables (Ding et al. 2017). In the last two decades, the functional traits have been demonstrated to help understand how species respond to different environmental disturbances like the change of use of land (Forrest et al. 2015) and the introduction of pesticides (Petchey et al. 2007; Lamanna et al. 2014). The reaction of the functional traits to the environmental variables is predictive and stable, even throughout the biogeographic regions, since the environmental restrictions or stress factors play a dominant role in the configuration of the composition of local community features (Statzner and Beché 2010). A homogenization of the functional traits diversity through agricultural intensification has been reported in edaphic macroinvertebrates (Pey et al. 2014). This acts as an ecological filter that can eliminate entire communities in the biotic homogenization process. A change in the communities with shared traits can restrict the reaction to environmental disturbances of species (Gámez-Virués et al. 2015).

In the State of Campeche, mechanized agricultural progress, cattle raising and urbanization have resulted in an annual deforestation rate of 0.74% from 1976 to 2005, superior to the national average of 0.43% during the period 2000-2010 (Esparza and Martinez 2011). In the municipality of

Hopelchén, Campeche, Mexico, there has been an increase in hectares used for mechanized agriculture in recent years (Arellano-aguilar and Rendón von Osten 2016). In 2013, more than 38 thousand hectares of forest disappeared in the State of Campeche. The main crops cultivated in the region are transgenic soybeans (*Glycine max*), which has generated an increase in the application of glyphosate pesticides, since the RR-SOJA strain (Roundup Ready) or soy 40-3-2 is resistant to glyphosate (Huacuja 2016). Hopelchén is the place where the biggest part of the Mennonite colonies that produce this crop is located and whose members cultivated around 80% of the 6.851 hectares of soya harvested in the municipality in 2013. Between these colonies, Las Flores and La Nueva Trinidad stand out for being the biggest soy producers of Campeche (Huacuja 2016). However, their cultivation is not only limited to these types of crops and they also produce corn (*Zea mays*), watermelon (*Citrullus lanatus*), gourd (*Cucurbita argyrosperma*) and other crops. There's a major usage of pesticides on these crops, which range from de organophosphates, pyrethroids and carbamates to some organochlorines that are still allowed (Arellano-aguilar and Rendón von Osten 2016). Despite the important use of pesticides in the Yucatán peninsula, there's very little documented information about the quantity of pesticide residues in the soil and the effect of those on macroinvertebrates (Huerta et al., in preparation). Most previous research about the relation between functional traits and environmental variables has focused on benthic macroinvertebrates in aquatic environments (Hein et al. 2005), very little research concentrated on soil macroinvertebrates and even less have investigated the relation with pesticides (Hedde et al. 2013) .

The main objective of this research was to evaluate and describe the reaction of functional traits and the diversity of soil macroinvertebrates to the crops *Zea mays* (*Z. mays*), *Glycine max* (*G. max)* and the natural surrounding vegetation in a Mennonite community and cultivation areas in Chenco, Hopelchen considering 90% of the total volume of soy produced in Campeche comes from the Mennonites who have a high purchasing power and the remaining 10% are locals (SAGARPA 2015). Considering the effect of the found pesticide residues in the soil as a variable, we hypothesis that the functional traits, diversity and density of the soil macroinvertebrate population would decrease in sites with a higher pesticide concentration in the soil.

Materials and Methods

Studied area

The investigation took place in the municipality of Hopelchén, on the granted lands of Chenco (communal lands) and the Mennonite countryside “Las Flores” (communal lands of Chenco and private properties). This last one is located 15 km from the Chenco-Cano Cruz highway. Both sites are located within the physiographic plain of the Yucatán peninsula, in the region named Sub Provincia Carso and Lomeríos de Campeche (INEGI 2009). The climate is Aw (tropical savanna climate or tropical humid and dry climate) as described in the Köppen-Geiger system (INEGI 2014). Precipitation ranges from 972 to 1,223 mm, with an average of 1,128 mm. The type of soil found at the research sites is luvisol (INEGI 2014). Its natural vegetation is spiny and arboreal semi-deciduous medium Tropical Rain Forest (INEGI 2014).

Sampling design

Samples of soil and macroinvertebrates were taken in three different systems: a) technified cultivation of *Glycine max* (Mennonite community ´Las Flores’), b) semi-technified cultivation of *Zea mays* (granted lands of Chenco) and c) in the surrounding natural vegetation. The difference between technified cultivation and semi-technified cultivation is based on the use of heavy machinery to complete the different cultivation practices. Plowing, sowing, harvesting and the use of external inputs is done with machinery in technified cultivation; while in semi-intensive cultivation only while plowing heavy machinery is used. Four plots for each setting were chosen, 12 in total, and in each of them five monoliths were extracted (TSBF, Anderson and Ingram, 1993) each at 100 m from another. In total 20 monoliths per system were obtained, 60 in total.

Macroinvertebrates sampling

The macroinvertebrates were collected by hand- sorting according to the Tropical Soil Biology and Fertility Institute’s method (TSBF), which is widely used in tropical environments (Anderson and Ingram 1993). Monoliths of 25 x 25x 30 cm were collected, at regular intervals of 100 m.

Soil Samples extraction

A compound soil sample per plot was taken to analyze and detect the concentration of the pesticide’s residues. For glyphosate and its degradation product AMPA, the 60 monoliths of each setting were analyzed, as well as for the description of the texture, organic material and pH. These last three parameters are considered because they maintain a strong relationship with the adsorption of pesticides in the soil (Zhou et al. 2004; Yu and Zhou 2005). The soil samples for the analyzes were taken from the monoliths described in the previous section.

Pesticides determination

The detection analysis of organochlorines took place in the persistent organic pollutants laboratory (POPs) of the EPOMEX Institute from the University of Campeche. All solvents used in laboratory procedures were 98% high purity grade (HPLC). Silica gel, alumina, florisil and sodium sulfate were purified following the protocols of NMX-AA-071-1981 (1981). The glass material was washed with Extran®, dried in the oven for 4 h at 200 C and rinsed with acetone and hexane. A CEM MARS Xpress Microwave Accelerated Reaction System (CEM Corporation, Matthews, NC, USA) was used. In this study, portions of 5 g soil were weighed into 55 mL perfluoroalkoxy (PFA) polymer extraction vessels equipped with Teflon-sealed lip-tight caps and polyetheretherketone (PEEK)-liners. Microwave power was 1200W (100%). The extraction solvent was 25mL *n*-hexane and acetone (1:1, v/v). The extraction was performed in temperature-controlled mode. The extraction temperature was 110 ◦C and programmed as follows: ramp to 110 ◦C for 10 min, holding at 110 ◦C for 10 min. After the extraction completed, soil and solvent were separated by filtration and the solvent was decanted into a pear-shaped flask. The sample went through the clean-up procedure. The extracts were transferred to 100-mL pear-shaped flasks and evaporated to nearly dryness under reduced pressure in a 35◦C water bath using a rotary evaporator (R-201, Shanghai Shenshen). An additional 10 mL *n*-hexane was added to the concentrated extracts and evaporated to a small volume ‘about 1 mL). The concentrated extracts and two 2-mL portions of *n*-hexane from rinsing the sample flask were transferred to top of a chromatography column (30cm×10mm i.d.) filled with 10 g silica gel (100–200 mesh) to separate the PAHs, DDTs and HCHs fraction from other interfering matters. The silica gel was wet-loaded as slurry in n-hexane and capped with a thin layer of absorbent cotton to prevent the gel from spilling, and approximately 2 cm length of anhydrous sodium sulfate was added in the top. The column was sequentially eluted with 30 mL of n-hexane and 35 mL of DCM to produce fractions enriched in aliphatic hydrocarbons and PAHs, DDTs, HCHs at flow rate of ∼2mL min−1. The elute of the PAHs, DDTs and HCHs fractions was concentrated to 1 mL according to the extracts, transferred into a Kuderna-Danish concentrator and rinsed three times with *n*-hexane. The final volume was adjusted to 1mL under a gentle stream of N_2, and an appropriate volume (50 µL) of 2,4,5,6-tetrachloro-*m*-xylene, 2-Fluoro-1,1¨-biphenyl and pterphenyl- d_{14} (J&K chemical Ltd. USA) was spiked to sample solution as internal standards. Then the sample was transferred to 1.5 mL vial for gas chromatography analysis.

To identify and quantify POPs a mix of standards was used: a, band c-HCH, heptachlor, aldrin, heptachlor epoxide, endosulfan I, dieldrin, p, p DDE, endrin, endosulfan II, endrin aldehyde, p, p DDD, endosulfan sulfate and p, p DDT (SUPELCO47426-U CLP Organochlorine Pesticide Mix).

The pesticides in soil and macroinvertebrates were quantified using a gas chromatograph Varian 3800 equipped with an electron capture detector Ni ^{63} and a capillary column HT8 (60 m x 0.25 mm, film thickness of 25 μm) (SGE Analytical Science, EE.UU.).

The glyphosate analysis was carried out in the Soil and Land Management Degradation Department of the University of Wageningen, Netherlands. The concentrations of glyphosate and AMPA in soil were determined by the mobile phases of liquid chromatography-tandem mass spectrometry (LC-MS / MS) using an XBridge ™ Shield RP C18 column (particle size of 3,5 \ mu m, 150 mm of length, 2,1 mm of diameter.) (Waters, Netherlands). The mobile phases consisted of NH4Ac 5 mM in Millipore water (dissolvent A, pH = 9) and a solution of Me OH: H_2O (Millipore) 9: 1 (dissolvent B, pH = 9). The pH adjusted itself at approximately 9, using NH3 at 25%. The gradient times of LC for the separation were: isocratic from 0 to 1 min (100% A: 0% B); from 1 to 6 min, a linear increase of B from 0 to 100%; isocratic from 6 to 8 min (0% A: 100% B); from 8 to 9 min, a linear decrease of B from 100% to 0%; and isocratic from 9 to 14 min (100% A: 0% B). The initial conditions reestablished in 1 min, for a total operating time of 15 min. The temperature of the column was 35,0◦C and the flow rate 0,4 ml min -1. The optimizations of the ionization and fragmentation conditions for the analytes were obtained through the infusion of individual FMOC analyte solutions. Optimal responses were obtained by electrospray ionization in negative ion mode, using the following source parameters: 3.5 kV capillary voltage, 20 V cone voltage, 120 ° C source temperature, 400 ° C desolvation temperature and gas flows of 160 -200 L h -1 (cone) and 580-600 L h -1 (desolvation).

Determination of soil physicochemical parameters

The percentage of organic carbon (CO) was determined by the method proposed by Gaudette et al. (1974), which consists of the titration of the excess of potassium dichromate with ferrous sulfate. Subsequently, the CO % was multiplied by 1,724 to determine the percentage of organic matter (OM). The soil texture analysis was performed by densimetry: soil dispersion by agitation with sodium hexametaphosphate and measurement of the distribution of particles according to their size with a hydrometer (NORM-021 2002).

The pH was determined according to the electrometric method (NORM-021 2002), which is based on determining the activity of the ion H by the use of an electrode whose membrane is sensitive to H. In the case of soils, the pH is measured potentiometrically in the supernatant suspension of a 1:2 soil-to-water ratio.

Functional traits selection

Five groups of functional traits were selected: body size, exoskeleton or external protection, dispersal capacity, development stage, trophic group and vertical distribution which were then divided into 22 quantitative modalities. A discrete score was defined for each category within each group of traits starting with the numbers 1, 2, 3, … consecutively (Ding et al. 2017). Each morphospecies was categorized with a score within each functional trait. The selection of traits was based on literature and published articles that defined agricultural intensification and pesticides as environmental stressors (Barbaro and Van Halder 2009; Flynn et al. 2009; De Lange et al. 2013; Céline Pelosi et al. 2014; Gagic et al. 2015; Gámez-Virués et al. 2015; Ding et al. 2017). The trait 'body size' was defined as a relative size by calculating the difference between the average body size of the population for each taxonomic group. Considering size as a relative measure within each order reduces the influence of taxonomic differences on the community's response to the presence of pesticides. The 'development stage' trait was considered as the state in which the individual was found to be collected, either larva, juvenile or adult, and was defined considering the life cycle of the taxonomic group to which it belongs and its morphological characteristics.

Statistical analysis

For the functional traits, the average of a trait in a taxonomic group was taken, considering the relative abundance of the morphospecies of that taxonomic group, this according to Vandewalle et al. (2010) and Ding et al. (2017).

A Kolmogorov-Smirnov test was performed to know if the data followed a normal distribution and a Levene test was done to know if homogeneity of variances applied. An analysis of variance (ANOVA) was performed when the data were normal and when this was not the case, a Kruskal

Wallis test or a Mann Whitney test was performed for cases where the number of observations was different. This was done to determine if there are significant differences among the management areas related to the number of morphospecies, density and diversity of the macroinvertebrates, organic matter content, pH, soil texture type and pesticides (organochlorines and AMPA). A Spearman correlation analysis was performed for non-parametric data (since they did not have a normal distribution) with all variables, from macroinvertebrates and soil to pesticides, in order to find possible correlations with the studied variables. Finally, a canonical correspondence analysis was carried out between the three sites to be able to differentiate the composition of functional traits and the independent variables per site. Statistical analyses were carried out with the programs Statistica 7 and R (R Core Time 2017) with the stats and vegan packages (Oksanen et al. 2017).

The frequency of occurrence (Fo) of pesticides was determined by the total number of samples (12) collected for each compound:

$$Fo = x^{*}\ 100/12$$

Results

Soil macroinvertebrates

731 individuals were found throughout the study, with a total of 65 morphospecies (Table 2). The *Z. mays* semi-technified system was the site with the highest number of taxa (25) and the *G. max* technified setting was the site with the lowest number of taxa (4), but that was not significant. In the two crop settings, the ants > termites > beetles predominated in presence and abundance. 12 ant morphospecies of four subfamilies were found, of which two species were exclusively found in the natural vegetation, two in the cultivation of *Z.mays* and two in the cultivation of *G. max*. For its part, in the technified system of *G. max* species of ants were reported as invasive and indicators of disturbance (*Solenopsis geminata, Solenopsis sp2, Pheidole protensa and dorymyrmex sp1*). In the cultivation of *Z. mays*, the semi-technified cultivation and the natural surrounding vegetation, the primitive subfamily *Ectatomminae* with the species *Ectatomma tuberculatum* was found, which is considered an important group for the biological control of plagues. In the *Z. mays* and *G. max* systems, species of *coleopteran*, which are considered to be predators of soybean crop pests such as *Calleida decora* and *pseudoophonus rufipes*, were encountered.

The site with the highest density of macroinvertebrates was the *Z. mays* cultivation (37.89±56.29 ind m²) followed by the natural vegetation site (15.08±4.63 ind m^2). However, no significant differences were found between any of the three systems (Table 3).

Pesticides

Eighteen pesticide compounds were found throughout the study (in soil and macroinvertebrates), 16 in the *G.max* cultivation, 14 in the *Z. mays* and 8 in the natural surrounding vegetation. The most abundant ones were: p,p´ DDE (n: 14) > methoxychlor (n: 6) > Dieldrin (n: 6). No significant differences were found in the concentration of the compounds (methoxychlor and DDE) in soil content and macroinvertebrates between systems (Table 4).

Of the macroinvertebrates that were collected, only samples of the *G. max* and *Z. mays* were obtained. In all of these, pesticide residues were detected. However, the cultivation of *G. max* was the one with the highest concentrations compared to the *Z.mays* one. The compound p, p'-DDD, that was only found in macroinvertebrates, represented the highest concentration (0.0068 µg kg^{-1}). AMPA was found in soil at the three sites where samples were taken; *G. max* (0.29±0.27 mg kg^{-1}) > *Z. mays* (0.039± 0.002 mg kg^{-1}) > natural vegetation (0.007± 0.003 mg kg^{-1}), being significantly higher in *G. max* and *Z. mays* than in the natural vegetation (*F*= 4.072; *p*= 0.049*)* (Table 4). Regarding the frequency of occurrence, p,p´-DDE was 100 % present, followed by methoxychlor (50%) and aldrin, dieldrin, endosulfan sulfate, epoxide heptachlor and delta HCH (33.3% , Table 3).

Functional traits of macroinvertebrates in the gradient of agricultural technification

The number of functional traits found per system differ significantly. They are distributed as follows; natural vegetation (n: 20.5 ± 0.57**a**) > *Z. mays* (n: 18. 25±3.59**a**) > *G. max* (n: 13.75±3.77**b**) (Table 1). In terms of functional trait modalities, significant differences were found between systems; in the modalities: “bland bodies, slightly sclerotic, endogenous, low dispersion (sessile) and average (walkers), phytophagous and geophagous" (Fig. 1).

In the three modalities of the size trait, modality 1 (small < 4mm) predominated in 50% of the *G. max* taxa, while in the natural vegetation and *Z. mays* the average size (4-16 mm) was the dominant modality of that trait. The size ‘large’ was the one that showed the lowest proportion in all three sites. In *Z. mays,* 58 % of the taxa were endogeic, while in *G. max* and in the natural vegetation the modality represented 40 %. In *G. max* and the natural vegetation, the modality of epigeic was the dominant modality of this trait. On the other hand, the anecic modality was the one that displayed in smaller proportions in the sites. The ‘external protection’ trait predominated the modalities of ‘well protected’ and ‘slightly protected body’ in the natural vegetation (70%) and *Z. mays* (65%). In *G. max,* 'bland bodies' were represented in 50% and the other half was distributed in 'well protected' and 'slightly protected' (Fig. 1). Of the ‘developmental stage’ trait, the ‘adult’ modality predominated with 80% in *G. max*, 60% in *Z. mays* and 55 % in the natural vegetation. On the other hand, the ‘dispersal capacity’ trait with the ‘low dispersion’ modality presented a 62% increase in the soybean taxa, while the *Z. mays* had a higher percentage of taxa that present medium and high dispersal capacity. The ‘trophic group’ trait with the geophagus and phytophagous modalities were the least representative in all three sites, while the omnivorous and predatory modalities were represented in greater proportions.

Soil properties

Regarding the soil texture, although all the sites where samples were taken had the same type of soil (Luvisoles), significant differences were found (p: 0.0001) in the percentage of clay and sand in the soil between the surrounding natural vegetation and the two cultivation sites (Table 3). The distribution of the percentage of clay was found as follows; the cultivation of *G. max* > *Z. mays* > natural vegetation, and the percentage of sand was inverted; natural vegetation > *Z. mays* > *G. max* (Table 3). There were no significant differences in pH, but the organic matter content (OM) was significantly higher (*p*: 0.02) in the natural vegetation (3.85 ± 0.93) than in the *Z. mays* (2.52 ± 0.22) (Table 3).

Relation between functional traits and pesticides

After a multiple correlation analysis, a positive and significant correlation was found between the percentages of clay and AMPA (rho= 0.90; *p*=0.002). Both AMPA and clay had a strongly negative correlation with some biological variables of macroinvertebrates and functional traits. The number of traits and taxonomic richness had a negative correlation with AMPA (rho = -0.60) and clay (rho= -0.80). The endogeic macroinvertabrates were negatively correlated only with clay (rho = -0.65), the epigeic and anecics with clay and AMPA (Table 5).

Methoxychlor found in the soil was positively correlated with the functional trait of omnivorous feeding (Table 5). The pH had a negative relation with the diversity index of Shannon Wiener. The percentage of sand presented a positive correlation with the diversity of macroinvertebrates and with the number of functional traits, as with most of the modalities of all functional traits (Table 4). The analysis of the canonical correspondence gave us a variance of 65% of the composition of the macroinvertebrates' functional traits explained by the management sites, where can be observed that AMPA and clay are found in the *G. max* cultivation and are negatively correlated with the diversity and number of functional traits (Fi). Metoxichloro in the soil and a high pH were detected in the *Z. mays* cultivation. The natural vegetation was positively correlated with the high diversity of functional traits and with the percentage of sand (Tabla.5). Although the DDE is included for the canonical analysis, this did not have a strong correlation with any of the variables.

Discussion

Macroinvertebrates'functional traits and their relation with pesticides

Many studies have investigated the reactions of functional traits in benthic macroinvertebrates and little was known about the relationship of environmental factors and functional traits of soil macroinvertebrates. They also focused on studying this relationship with a target species such as spiders (Lambeets et al. 2009), beetles (Ribera, Dolédec, Downie, and Foster 2001) and earthworms (Pelosi et al. 2013) separately. However, none of these studies focused on the effect of pesticides on the set of macroinvertebrates taxa that exist in agricultural soils at the field scale. This study is the first to characterize the effect of the main glyphosate degradation product (AMPA) and historically persistent (organic) pesticides in macroinvertebrates, using the functional traits as a variable response. One of the main results was that if the concentrations of pesticides in the soil increased, a decrease in the number of functional traits and macroinvertebrates' diversity was found, which confirms part of our hypothesis that the number of functional traits would tend to decrease in locations where the concentrations of AMPA and historically persistent pesticides in the soil were higher. Although the statistical analyzes performed not all pesticides considered because there was a large amount of data undetected culture *G. max* was where the highest number of persistent organic compounds was found and with the highest concentration (1.79 mg cis chlordane). Residues of different types of persistent organic pesticides (10) were also found in the bodies of all the macroinvertebrates that were taken for the sample. This information is considered only informative and complementary since there was no specific methodology for the selection of organisms.

On the other hand, our results demonstrate that pesticides and especially AMPA have an effect on the composition and abundance of functional traits of soil macroinvertebrates. This shows that functional traits can better characterize the composition of macroinvertebrates than diversity and density indexes; since these did not present significant differences between systems. These results have been consistent in different studies where both diversity indexes and functional features in natural and agricultural areas were measured (Lock et al. 2001; Pižl et al. 2009; Hedde et al. 2012).

Different studies have shown that species small in size are those that predominate in disturbed environments (Gámez-Virués et al. 2015; Ding et al. 2017). This was reflected in our study, since in the cultivation of *G. max* this modality predominated. This may be due to the fact that small species in some taxa have a greater dispersal capacity. Ishitani et al. (2003) and Barbaro and Van Halder (2009) found that small beetles predominate in disturbed habitats because they have a greater dispersal capacity than large beetles. High dispersal capacity is considered to be an adaptation to disturbed habitats and may prevent the number of species from decreasing (Ribera, Dolédec, Downie, and Foster 2001; Barbaro and Van Halder 2009). However, in this study; although a small size predominated in sites with high concentrations of pesticides (*G. max*) it did not have a high dispersal capacity. This may be due to the difference between the taxa and their biological conditions and the ecological characteristics of each group (Ding et al. 2017).

In our study, we found that the functional feeding trait had a constant response regarding the content of methoxychlor (*Z. mays*) and AMPA (*G.max*). This is similar to reported in (Ding et al. 2017) who found that the trait "functional feeding" were significantly related to management and land use variables. The omnivorous modality was the one that predominated (40%) in sites with higher concentrations of AMPA *(G. max*) and the phytophagous species decreased when they were in direct contact with AMPA. The macroinvertebrates with a geophagous diet were the ones that were found to a lesser extent since they are the most affected in the presence of contaminants by being more in contact with these as found by Hedde et al. (2012). An important group for the cultivation of crops and affected by AMPA were the predators. In a study about the impacts of pesticides on beneficial species reported that 80% of a population of predatory beetles for plant pests died when exposed to glyphosate (Hassan et al. 1988).

Anecics in general in all three systems were represented to a lesser extent. This is because in Mexico there are not many organisms that fall into this ecological category (Fragoso and Lavelle 1992) due to the soil’s properties (ie. percentage of organic matter). The endogeic soil invertebrates presented a smaller proportion in the cultivation of *G. max* (40%) than in the *Z. mays* (8%). On the other hand, the natural vegetation also presented a low proportion (38%). However, this may be due to the fact that in the natural vegetation the three ecological categories are equitably distributed. This is similar what found by (Hedde et al. 2012) who showed a decrease in the proportion of endogeic from 49% (unpolluted) to 29% (low polluted) and less than 6% in the most contaminated plots. However, in the *G. max* only a small number of species were present and these are of very small size and have an omnivorous diet, so the exposure would not be directly as in geophagous organisms.

The external protection of the macroinvertabrates varied between locations and the bland and moderately protected bodies were negatively correlated with the AMPA concentrations. Statzner and Beché (2010) found that the exoskeleton of beetles varied in the presence of organic pollutants. This may be due to what was mentioned by (Hedde et al. 2012) who found a relationship between soft body macroinvertebrate with a geophages in the most contaminated sites and suggests that they respond to exposure by contact and ingestion.

Finally, one important result was that there was no negative correlation of DDT, DDE and methoxychlor on the functional traits of macroinvertebrates like with the AMPA. Although the effect of this organic compound on the functional features of macroinvertebrates has not been studied, the fact that they have not correlated does not imply that it is not causing or has caused an effect of another type that was not considered in this work (at the enzymatic, genetic and others).

Since in the body of macroinvertebrates if residues of different types of POPs were found, it is important to consider a study that encompasses other variables that can better explain the relationship between these pesticides (COPs) and soil macroinvertebrates. Although there were no significant differences in density, in general the three sites, including the natural vegetation, have a low density of macroinvertebrates (natural vegetation: 15.08, corn: 37.89 and *G.max*: 8 ind m^2). In a previous study done in Calakmul, Campeche, in fallow lands, an average density of 179 ind m^2 of soil macroinvertebrates was found and in the tropical rain forest of that area 432 ind m^2 (Sánchez del Cid et al. 2015). Since in this study pesticide residues were found in the surrounding natural vegetation, it is suggested that they have an effect on the abundance, richness and functional composition of soil macroinvertebrates.

Pesticides

Our results coincide with those found by Cantu-Soto et al.(2011) in agricultural soils of the Sonora Valley, where b-endosulfan, methoxychlor and DDE were detected in most of the samples (n: 4, 6 and 12 respectively). However, the concentrations in our study were higher with respect to the three compounds, for example our average DDE level was higher (0.42 µg / kg) than theirs (0.011 µg / kg), also methoxychlor levels in our study was higher (0.90 µg / kg) than reported by them (0.004 µg / kg). Comparing to HCH and DDT concentrations in soil from other regions of agricultural areas, the average HCH level (0.63 µg / kg) in the present study was y lower than that of India (0.85 µg / kg) (Mishra et al. 2012) but superior to the one of Guangdong, China (0.062 µg / kg) (Yu et al. 2013). The average level (0.42 µg / kg) of DDE in the present study was also much lower than in India (0.90 µg / kg) (Mishra et al. 2012), but higher than in the United States (0.21 µg / kg) (Bidleman and Leone 2004) and Poland (0.11 µg / kg) (Falandysz et al. 2001). Soil samples from the last two sites were collected at the such sites as near the mine machines factory close to a transformer station and close to a coal mine.

Our results are similar to those of Sultana et al. (2014), who found a higher level of DDE in 86% of their samples (n: 16). The DDT level in our study exceeded the available Chinese and Dutch soil quality standards of 0.01 µg / kg 0.05 µg / kg, respectively (Netherlands Target Values for Soil Remediation 2000 and Chinese Environmental Quality Standard for Soils (GB15618-1995). In our study no relationship was found between the measured soil properties and POPs. This compares with what was found in Zhang et al. (2013) and Sultana et al. (2014). There is very little information about the effect of DDT and almost none of its degradation products on specific soil macroinvertebrates, in which it has been shown that pesticides have a negative effect on the reproduction of earthworms and their density and biomass (Pelosi et al. 2013). In insects there has been seen an negative effect on their fertility, prolificness and longevity (Araya et al. 2017) which may be causing a low density and taxonomic richness in our cultivation sites and in the surrounding natural vegetation.

Soil properties

The results showed that the three locations studied were different in terms of some soil properties and that this plays an important role in the effect of pesticides on soil macroinvertebrates. The clay content was strongly correlated with the AMPA concentrations, which is evident since the glyphosate is more easily adsorbed in clay soils,

which predominate in the Yucatan peninsula (Zhou et al. 2004). However, strong adsorption may oppose degradation and may give rise to relatively long persistence (Barja and Afonso 2005).

Conclusions

Our results confirm that pesticides, especially the main degradation product of glyphosate herbicides (AMPA), have an adverse effect on the composition and richness of soil microinvertebrates' functional traits. Soil macroinvertebrates from the natural surrounding vegetation are also affected by the pesticides. The functional traits showed a clear at the three locations: the loss of diversity and the macroinvertebrates' taxonomic abundance

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FIGURES AND TABLES

Table 2. Taxonomic classification of soil macroinvertebrates**.**

Plyllum	Subphyllum/ Class	Order	Family	Sub family	Gender	Morpho specie
Annelida	Oligochaete	Lumbricina	Acanthodrilidae		Lavellodrilus	1
					Dichogaster	1
Arthropod	Insect	Hymenoptera	Formicidae	Dolichoderinae	Dorymirmex	2
				Myrmicinae	Solenopsis	2
				Myrmicinae	Pheidole	2
				Dorylinae	Neivamyrmex	1
				Ponerinae	Cryptopone	1
				Ponerinae	NI	1
				Ponerinae	Hypoponera	1
				Ectatomminae	Ectatomma	2
		Coleóptera	Elateridae	Agrypninae	Conoderus	1
			Carabidae	NI	NI	1
				Lagriinae	NI	1
				-	Calleida	1
					Pseudoophonus	1
			Chrisomelidae	NI	NI	1
			Curculionidae	NI	NI	1
			Tenebrionidae	Pimelinae	NI	1
			Staphylinidae	Paederinae	NI	1
				NI	NI	1
		Isóptera	Rhinotermitidae	NI		1
			Termitidae	Nasutitermitinae		1
				NI		1
		Diplura	Japygidae			2
			Campodeidae			1
		Hemyptera	Reduvidae			1
		Blattodea	Blattidae			2
		Dermáptera	Forficulidae			1
		Collembola				1
	Chelicerata	Araneae	Aranaidae			4
	Myriapoda	Chilopodos	Geophilimorpha	Ballophilidae	Ityphilus	3
		Diplopodos	Polidésmida			3
			Spirobolida			2

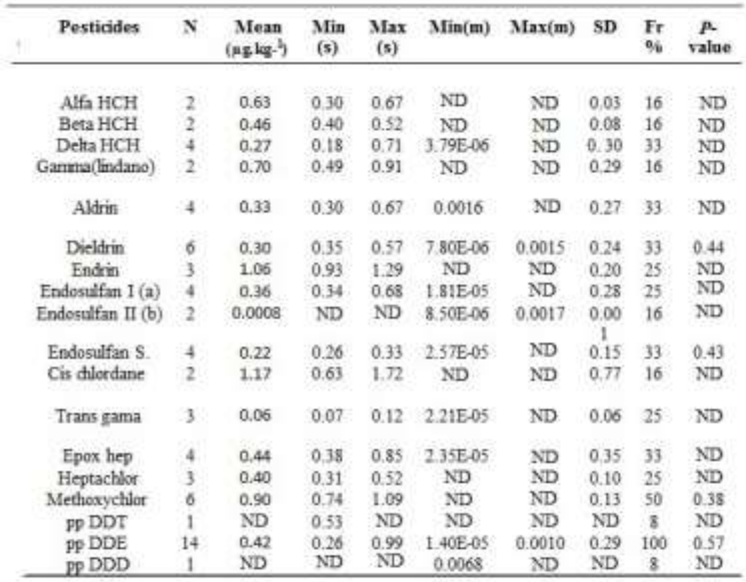
Table 3. Soil macroinvertebrates characteristics and soil properties along the studied sites. NV: natural vegetation. Different letters indicate significant differences among the treatments ($p<0.05$).

Biological and environmental variables	**NV** Mean± SD	***Z.mays*** Mean± SD	***G.max*** Mean± SD	***P <0.05***
Density of macroinvertebrates (Ind m²)	15.08±4.63	37.89±56.29	8±6.786	0.30
Richness	15±3.55	11.75±9.21	5.5±2.38	0.11
Shannon Wiener diversity	1.95±0.90	1.09±0.27	1.42±0.08	0.13
Evenness	0.54±0.26	0.40±0.27	0.82±0.23	0.11
No functional traits	20.5±0.57 **a**	18.25±3.59**a**	13.75±3.77**b**	**0.02***
Soil characterization				
Clay (%)	35.51±5.96**b**	52.98±4.31**a**	60.81±5.06**a**	**0.0002***
Sand (%)	47.07±7.74**a**	28.36±3.98**b**	20.25±3.57**b**	**0.0001***
Silt (%)	17.40±2.25	18.63±1.18	18.92±2.78	0.59
OM (%)	3.85±0.93	2.52±0.22	2.79±0.32	**0.02***
pH	7.43±0.31	7.58±0.35	7.05±0.68	0.32
AMPA (mg kg−1)	0.007±0.003**b**	0.039±0.002**a**	0.297±0.27**a**	**0.049***

Means among the rows with different letters are significantly different ($p < 0.05$)

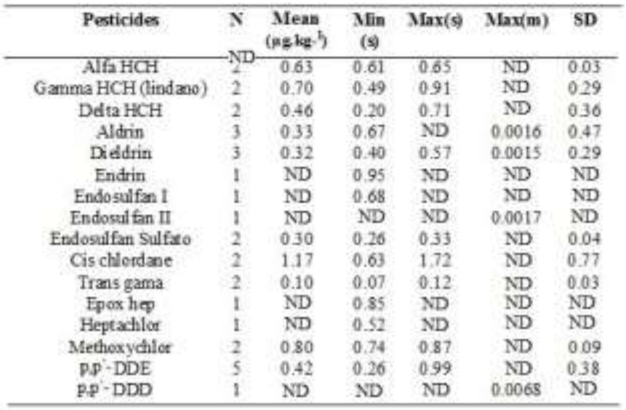
Table 4. Pesticides concentration in the 'Ejido Chenco' and in the Mennonite community 'Las Flores' Campeche, Mexico. Mean, Max and minimum contents. In soil (s) and soil macroinvertebrates (m); (a) In the technified soybean crop; (b) semi-technified maize crop; (c) natural vegetation that surrounds the crops; and (d) in soil macroinvertebrates.

a



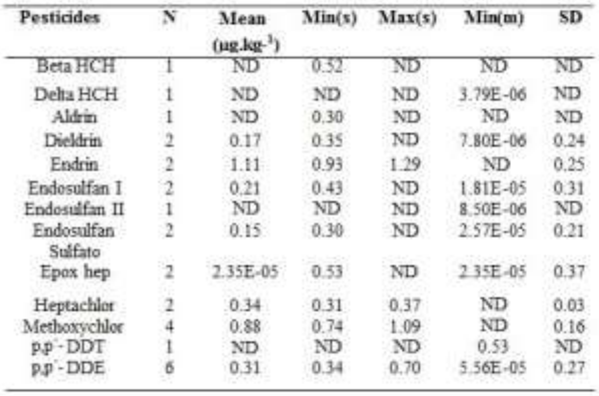
Pesticides	N	Mean (µg.kg-^{1})	Min (s)	Max (s)	Min(m)	Max(m)	SD	Fr %	p-value
Alfa HCH	2	0.63	0.30	0.67	ND	ND	0.03	16	ND
Beta HCH	2	0.46	0.40	0.52	ND	ND	0.08	16	ND
Delta HCH	4	0.27	0.18	0.71	3.79E-06	ND	0. 30	33	ND
Gamma(lindano)	2	0.70	0.49	0.91	ND	ND	0.29	16	ND
Aldrin	4	0.33	0.30	0.67	0.0016	ND	0.27	33	ND
Dieldrin	6	0.30	0.35	0.57	7.80E-06	0.0015	0.24	33	0.44
Endrin	3	1.06	0.93	1.29	ND	ND	0.20	25	ND
Endosulfan I (a)	4	0.36	0.34	0.68	1.81E-05	ND	0.28	25	ND
Endosulfan II (b)	2	0.0008	ND	ND	8.50E-06	0.0017	0.00 1	16	ND
Endosulfan S.	4	0.22	0.26	0.33	2.57E-05	ND	0.15	33	0.43
Cis chlordane	2	1.17	0.63	1.72	ND	ND	0.77	16	ND
Trans gama	3	0.06	0.07	0.12	2.21E-05	ND	0.06	25	ND
Epox hep	4	0.44	0.38	0.85	2.35E-05	ND	0.35	33	ND
Heptachlor	3	0.40	0.31	0.52	ND	ND	0.10	25	ND
Methoxychlor	6	0.90	0.74	1.09	ND	ND	0.13	50	0.38
pp DDT	1	ND	0.53	ND	ND	ND	ND	8	ND
pp DDE	14	0.42	0.26	0.99	1.40E-05	0.0010	0.29	100	0.57
pp DDD	1	ND	ND	ND	0.0068	ND	ND	8	ND

b



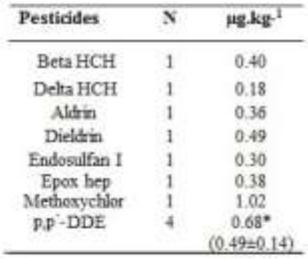
Pesticides	N	Mean (µg.kg-^{1})	Min (s)	Max(s)	Max(m)	SD
Alfa HCH	ND 2	0.63	0.61	0.65	ND	0.03
Gamma HCH (lindano)	2	0.70	0.49	0.91	ND	0.29
Delta HCH	2	0.46	0.20	0.71	ND	0.36
Aldrin	3	0.33	0.67	ND	0.0016	0.47
Dieldrin	3	0.32	0.40	0.57	0.0015	0.29
Endrin	1	ND	0.95	ND	ND	ND
Endosulfan I	1	ND	0.68	ND	ND	ND
Endosulfan II	1	ND	ND	ND	0.0017	ND
Endosulfan Sulfato	2	0.30	0.26	0.33	ND	0.04
Cis chlordane	2	1.17	0.63	1.72	ND	0.77
Trans gama	2	0.10	0.07	0.12	ND	0.03
Epox hep	1	ND	0.85	ND	ND	ND
Heptachlor	1	ND	0.52	ND	ND	ND
Methoxychlor	2	0.80	0.74	0.87	ND	0.09
p.p´-DDE	5	0.42	0.26	0.99	ND	0.38
p.p´-DDD	1	ND	ND	ND	0.0068	ND

c



Pesticides	N	Mean (µg.kg-^{1})	Min(s)	Max(s)	Min(m)	SD
Beta HCH	1	ND	0.52	ND	ND	ND
Delta HCH	1	ND	ND	ND	3.79E-06	ND
Aldrin	1	ND	0.30	ND	ND	ND
Dieldrin	2	0.17	0.35	ND	7.80E-06	0.24
Endrin	2	1.11	0.93	1.29	ND	0.25
Endosulfan I	2	0.21	0.43	ND	1.81E-05	0.31
Endosulfan II	1	ND	ND	ND	8.50E-06	ND
Endosulfan Sulfato	2	0.15	0.30	ND	2.57E-05	0.21
Epox hep	2	2.35E-05	0.53	ND	2.35E-05	0.37
Heptachlor	2	0.34	0.31	0.37	ND	0.03
Methoxychlor	4	0.88	0.74	1.09	ND	0.16
p.p´-DDT	1	ND	ND	ND	0.53	ND
p.p´-DDE	6	0.31	0.34	0.70	5.56E-05	0.27

d



Pesticides	N	µg.kg-^{1}
Beta HCH	1	0.40
Delta HCH	1	0.18
Aldrin	1	0.36
Dieldrin	1	0.49
Endosulfan I	1	0.30
Epox hep	1	0.38
Methoxychlor	1	1.02
p.p´-DDE	4	0.68* (0.49±0.14)

Table 5. Spearman correlation between soil macroinvertebrates characteristics and soil properties and pesticides* significant at p<0.05.

	AMPA	**Methoxychlor**	**Sand**	**Clay**	**pH**
AMPA		-0.038	-0.87*		-0.38
Sand	-087*	0.10		-0.96*	0.24
Clay	0.90*	-0.11			-0.22
No functional	-0.61*	0.17	0.79*	-0.81*	0.26
Richness	-0.49	0.12	0.73*	-0.71*	0.18
Shannon Wiener diversity	-0.05	-0.32	0.03	-0.07	-0.58*
Medium (4-16 mm)	-0.60*	0.25	0.79*	-0.75*	0.32
Epigeic	-0.47	0.11	0.67*	-0.68*	0.13
Endogeic	-0.61*	0.10	0.75*	-0.69*	0.20
Anecic	-0.65*	-0.04	0.76*	-0.80*	0.26
Soft- body	-0.61*	-0.15	0.77*	-0.74*	0.15
Lightly sclerotize	-0.62*	-0.24	0.75*	-0.82*	0.20
Low dispersal capacity	-0.68*	0.11	0.76*	-0.77*	0.15
Medium dispersal capacity	-0.53	0.25	0.75*	-0.73*	0.12
Juvenile	-0.35	0.28	0.54	-0.61*	0.32
Adult	-0.73*	0.07	0.80*	-0.87*	0.17
Predators	-0.59*	0.13	0.72*	-0.78*	0.06
phytophagous	-0.61*	0.15	0.80*	-0.78*	0.68*
Omnivorous	0.39	0.62*	-0.11	0.18	-0.37

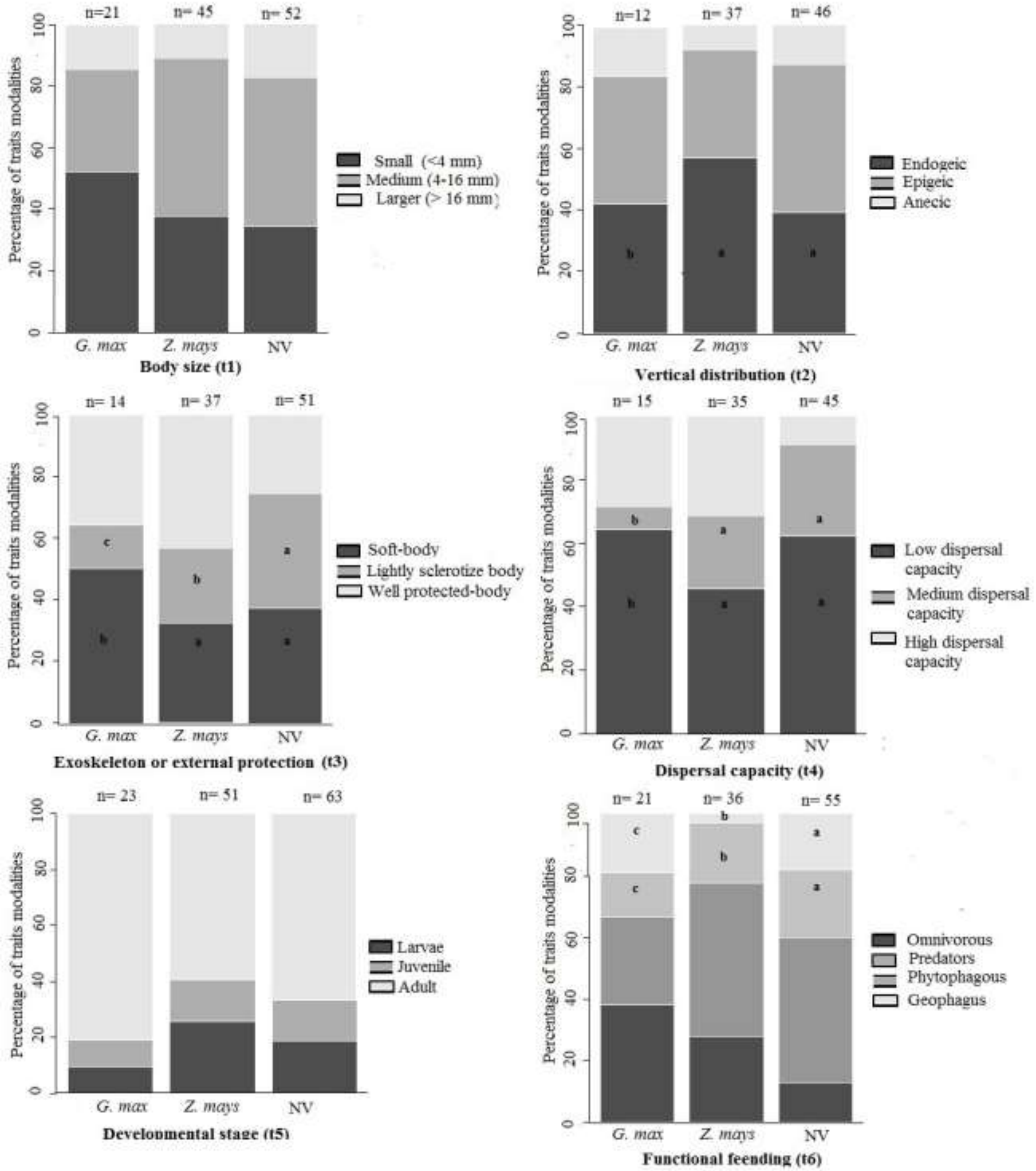


Fig. 1. The structure of 19 modalities of six traits at technified (*G.max*), semi – technified system (*Z. mays*) and in the vegetation natural. Each bar represents the percentage of each trait modality in each system. The letters represent the significant differences of each modality per system.

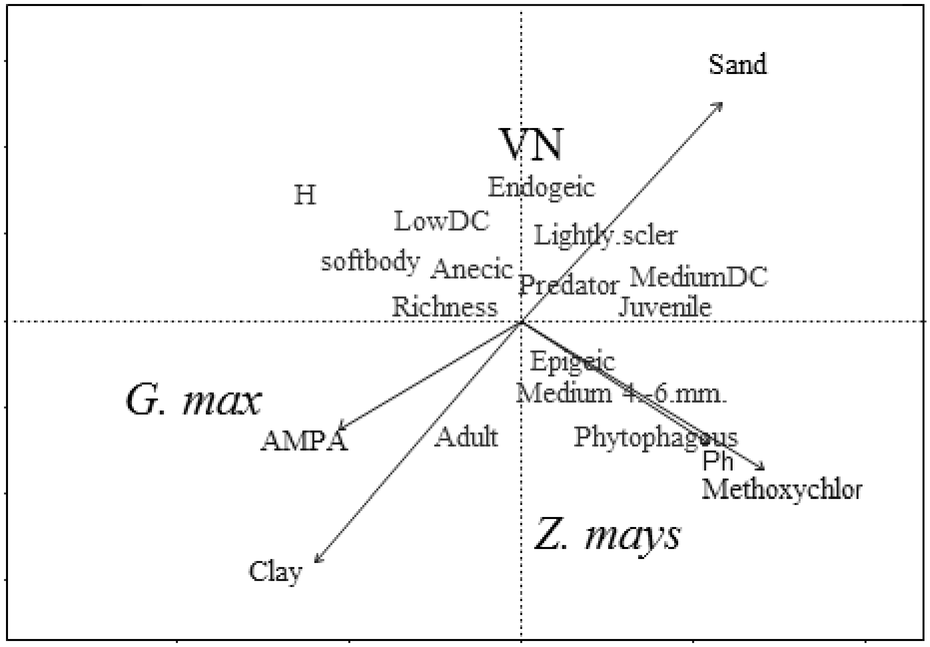


Fig.2 Canonical analysis of the three systems and the functional traits that had a significant correlation in the analysis of coefficient of correlation of spearman. NV: natural vegetation

CAPÍTULO 3

Conclusiones generales

La diversidad, riqueza taxonómica y densidad de macroinvertebrados no difirió significativamente entre sistemas. Sin embargo, si hubo diferencias en cuanto a la composición y riqueza de rasgos funcionales. En el cultivo de *G. max* se encontró el menor número de rasgos funcionales y el mayor contenido del AMPA. Esto se puede deber a que en el cultivo de *G. max* la comunidad de macroinvertebrados presenta especies con los mismos tipos de rasgos; lo que se puede considerar como una homogenización funcional. El enfoque de rasgos funcionales en múltiples taxas de macroinvertebrados podría ser una opción para revelar los efectos de la intensificación agrícola y de lo que ésta conlleva como en este caso lo fueron los plaguicidas.

Los grupos más representativos en los tres sistemas fueron las hormigas, termitas y escarabajos. Lo cual se puede deber a su menor tamaño y a que la exposición a los contaminantes por contacto en el caso de hormigas y escarabajos es menor debido a su exoesqueleto endurecido. Un factor importante también fue su tipo de alimentación (omnívora en su mayoría) y el que muchas de las especies de hormigas encontradas se consideran plagas, lo que les da un rango mayor de supervivencia en sitios con disturbio. Sin embargo, en los dos campos de cultivo se encontraron especies de insectos cómo hormigas (*Ectatomma tuberculatum)* y escarabajos (*Calleida decora, Pseudoophonus rufipes*), las cuales están catalogadas como control biológico de diferentes plagas incluso de la misma *G. max* (ej. *Velvetbean caterpillar y Anticarsia gemmatalis)*. Un gran problema es que el uso indiscriminado de plaguicidas en los agroecosistemas acaba no sólo con la especie o grupo objetivo, sino también con la fauna nativa la cual juega un papel importante como control natural de plagas.

Los parches de vegetación natural circundante a los campos agrícolas sirven como fuente de especies para los sitios agrícolas. Sin embargo, los resultados demuestran que el suelo de la vegetación natural también tiene residuos de plaguicidas. Esto se puede deber a la cercanía con las parcelas (500m) y a la forma de aplicar los plaguicidas, más en el campo de cultivo de *G.max* donde muchas veces se fumiga con avioneta lo que genera un arrastre por viento a todas las zonas aledañas. Sólo el segundo producto de degradación de DDT (DDE) se encontró en todas las parcelas de la vegetación natural. Sin embargo, aunque se encontraron sólo en una de las cuatro parcelas, las concentraciones del metoxicloro, dieldrin y beta HCH fueron parecidas a las que se detectaron en los campos de cultivo. El AMPA también se detectó en todas las parcelas de la vegetación natural, aunque fue significativamente el lugar con menor concentración. Esto pone en manifiesto la importancia de encontrar alternativas viables para implementar en estos tipos de cultivo que pudieran disminuir de forma paulatina la dependencia del uso excesivo de los plaguicidas.

Se encontraron 18 tipos de plaguicidas organoclorados, se detectaron en los tres sitios y en los macroinvertebrados muestreados en el cultivo de *G.max y Z. mays*. Sólo se midieron variables ecológicas de los macroinvertebrados por lo que podría ser necesario en investigaciones posteriores utilizar biomarcadores enzimáticos para caracterizar de forma más completa el escenario y determinar a nivel bioquímico cómo estos contaminantes afectan a los organismos expuestos.

Los rasgos funcionales de los macroinvertebrados que mostraron patrones claros y predecibles ante la concentración de plaguicidas, en específico al AMPA fueron: la protección externa del cuerpo o exoesqueleto. Es decir, especies o grupo taxonómico que presentan un exoesqueleto endurecido se encontraron en los sitios con mayores concentraciones de plaguicidas. Al igual que capacidad de dispersión y el grupo de alimentación funcional. Por su parte, el rasgo de tamaño, aunque se ha considerado en otros trabajos como preponderante en la respuesta de las especies al estrés ambiental en este estudio la variación se vio marcada dentro de cada sistema y no entre sistemas. Es decir, hubo modalidades que predominaron dentro de los sitios como el tamaño pequeño en el cultivo de *G.max.* Estos resultados sugieren que las especies que colonizan estos sitios tienen una composición de rasgos de acuerdo a las condiciones del ambiente que les permite facilitar su supervivencia.

En el presente trabajo se encontró que el contenido del AMPA y el porcentaje de contenido de arcilla en el suelo varió entre sistemas y tuvieron una correlación negativa significativa con las variables biológicas que se midieron de los macroinvertebrados. Sin embargo, la correlación negativa fue más alta y significativa con la arcilla que con el propio AMPA. Esto se puede deber a que a las partículas de acilla se le adhieren no sólo a este compuesto sino también a otros tipos de contaminantes que son muy usados en estos sitios cómo organofosforados, piretroides, carbamatos y neonicotinoides que pudieran estar teniendo un efecto en los macroinvertebrados y que no fueron analizados en el estudio.

Por último, cabe destacar que este trabajo de tesis es el primero en caracterizar el efecto del principal producto de degradación de glifosato (AMPA) y plaguicidas históricos (orgánicos) persistentes en los macroinvertebrados usando los rasgos funcionales como variable respuesta.

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ANEXOS

Run			Resultados			Elemental Ratios
Run	Run #	Peso	Carbón	Hidrogeno	Nitrógeno	C/N
P1M2FSA	1	9.141	8.14	2.32	0.99	9.587
P3M1FA	2	8.371	2.14	1.3	0.22	11.342
P1M4FSA	3	9.794	5.73	1.81	0.54	12.373
P4M5CHA	4	8.338	3.17	1.61	0.24	15.401
P4M2CHA	5	7.975	3	1.53	0.26	13.454
P3M3SCHA	6	8.002	3.51	1.45	0.29	14.113
P1M3FSA	7	8.382	5.29	1.86	0.44	14.019
P3M1SCHA	8	8.88	4.21	1.68	0.39	12.587
P3M1SCHB	9	8.553	4.05	1.7	0.33	14.31
P3M1SCHC	10	8.775	4.09	1.69	0.41	11.632
P4M3CHA	11	7.657	2.96	1.43	0.23	15.006
P3M2SCHA	12	8.541	7.29	1.92	0.64	13.281
P4M2FA	13	9.137	2.45	1.46	0.18	15.871
P1M2FA	14	8.899	2.89	1.43	0.22	15.317
P1M5FA	15	8.985	2.5	1.46	0.12	24.292
P1M1FA	16	8.151	2.83	1.43	0.2	16.499
P2M5FA	17	8.221	2.59	1.45	0.18	16.777
P3M4SCHA	18	8.238	3.78	1.67	0.33	13.356
P3M4SCHB	19	9.881	3.7	1.72	0.33	13.073
P3M4SCHC	20	7.998	3.77	1.7	0.36	12.211
P4M3FA	21	9.77	1.32	1.55	0.02	76.956
P3M1CHA	22	8.642	2.74	1.43	0.18	17.749
P2M5FSA	23	8.335	3.85	1.51	0.34	13.203
P2M2CHA	24	7.304	2.71	1.44	0.24	13.166
P3M3FA	25	8.625	1.86	1.54	0.12	18.073
P1M3FA	26	7.969	2.36	1.33	0.15	18.345
P2M4CHA	27	9.992	2.83	1.5	0.21	15.713
P3M5SCHA	1	9.35	5.34	1.95	0.5	12.453
P3M5SCHB	2	7.683	5.26	1.78	0.39	15.726
P3M5SCHC	3	7.461	5.23	1.98	0.55	11.088

P3M4FA	4	9.899	1.86	1.65	0.12	18.073
P3M2FA	5	8.885	2.23	1.68	0.18	14.445
P2M2FSA	6	9.575	5.52	1.79	0.5	12.873
P3M4CHA	7	7.255	2.44	1.4	0.2	14.225
P4M4CHA	8	9.991	3.21	1.53	0.24	15.595
P4M4CHSA	9	7.56	4.37	1.62	0.32	15.923
P4M5SFA	10	8.8	1.69	1.63	0.02	98.527
P4M1FA	11	7.793	6.37	1.3	0.42	17.684
P4M1FB	12	9.724	6.88	1.38	0.34	23.594
P4M1FC	13	9.698	6.9	1.34	0.4	20.114
P1M4FA	14	7.408	2.19	1.29	0.18	14.186
P1M1FSA	15	8.842	7.14	1.86	0.62	13.428
P2M2FA	16	7.999	2.9	1.5	0.5	6.763
P4M3CHSA	1	9.337	4.61	1.82	0.48	11.198
P4M3CHSB	2	9.343	4.58	1.79	0.42	12.715
P4M3CHSC	3	8.829	4.61	1.76	0.44	12.217
P1M3CHA	4	8.871	3	1.56	0.31	11.284
P1M2CHA	5	8.033	3.35	1.51	0.35	11.16
P4M1CHSA	6	8.043	5.72	1.78	0.58	11.499
P1M5CHA	7	8.253	2.51	1.46	0.27	10.839
P4M5CHSA	8	7.333	5.92	1.84	0.63	10.957
P1M4CHA	9	7.963	2.44	1.42	0.27	10.537
P3M5CHA	10	7.45	2.7	1.41	0.2	15.741
P4M2CHSA	11	7.684	9.39	2.07	0.87	12.585
P3M5FA	12	7.912	1.91	1.54	0.22	10.123
P3M5FB	13	8.557	1.89	1.55	0.27	8.162
P3M5FC	14	8.162	1.92	1.54	0.21	10.661
P4M1CHA	15	9.242	2.98	1.57	0.21	16.546
P2M3FA	16	8.485	3.15	1.55	0.34	10.803
P3M3CHA	17	7.915	2.11	1.37	0.3	8.201
P1M5FSA	18	8.962	3.05	1.33	0.34	10.46
P3M2CHA	19	8.575	2.19	1.43	0.25	10.214
P2M1CHA	20	9.215	2.85	1.53	0.28	11.868
P2M4FSA	21	9.8	5.18	1.77	0.49	12.326
P2M3CHA	22	8.025	3.01	1.61	0.32	10.968
P2M3CHB	23	7.675	2.95	1.58	0.32	10.749
P2M3CHC	24	8.883	2.95	1.59	0.31	11.096
P1M1CHA	25	7.278	3.14	1.54	0.34	10.768
P2M3FSA	26	7.338	5.28	1.8	0.5	12.313
P2M5CHA	1	7.544	3.35	1.81	0.43	9.084
P2M4FA	2	8.933	2.99	1.83	0.3	11.621
P2M1FSA	3	7.822	10.18	2.72	1.05	11.305
P4M4FA	4	7.71	2.04	2.06	0.21	11.327