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Quantification of pesticide residues in soil of *Cynomys mexicanus* habitat in San Luis Potosi and Zacatecas, Mexico

Plaguicidas detectados en suelo de colonias de *Cynomys mexicanus* en San Luis Potosí y Zacatecas, México

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ABSTRACT

The Mexican prairie dog (*Cynomys mexicanus*) is an endemic rodent of the grasslands of northern Mexico, considered a keystone species for the maintenance of its ecosystem. The main problem it faces is the increase in the agricultural frontier, which means that it is constantly exposed to chemical products used in nearby farming areas, such as pesticides. The objective of this study was to detect and quantify pesticide residues in soil samples of *C. mexicanus* colonies from the states of Zacatecas and San Luis Potosí. High Performance Liquid Chromatography (HPLC) analyzed five soil samples. Eight pesticides were detected in 100 % of the samples analyzed, of which glyphosate and methamidophos had higher concentrations, up to 5.5 mg/g and 4.8 mg/g respectively. The results show that high concentrations of the mentioned pesticides are found in the soil compared to those detected in other studies and higher than the DL_{50} established for both. Therefore, soil is an important source of exposure and means of entry into the organism of *C. mexicanus*.
Keywords: soil, pesticides, *Cynomys mexicanus*, high efficiency liquid chromatography, glyphosate

RESUMEN

El perrito de la pradera (*Cynomys mexicanus)* es un roedor endémico de los pastizales del Norte de México, considerada una especie clave para el mantenimiento de su ecosistema. La principal problemática a la que enfrenta es el aumento de la frontera agrícola, por lo que se encuentra en constante exposición a productos químicos utilizados en zonas de cultivo cercanas, como los plaguicidas. El objetivo de este estudio fue detectar y cuantificar residuos de plaguicidas en muestras de suelo de colonias de *C. mexicanus* de los estados de Zacatecas y San Luis Potosí. Se analizaron un total de 5 muestras de suelo y se analizaron por medio de la técnica de Cromatografía Líquida de Alta Eficiencia (HPLC). Se detectaron ocho plaguicidas presentes en el 100% de las muestras analizadas, de los cuales el glifosato y el metamidofos tuvieron concentraciones mayores, de hasta 5.5 mg/g y 4.8 mg/g respectivamente. Los resultados muestran que en el suelo se encuentran altas concentraciones de los plaguicidas mencionados comparadas con las detectadas en otros estudios y mayores a la DL_{50} establecida para ambos. Por lo que el suelo es una fuente de exposición importante y medio para la entrada hacia el organismo de *C. mexicanus.*
Palabras clave: suelo, plaguicidas, *Cynomys mexicanus*, cromatografía líquida de alta eficiencia, glifosato.

INTRODUCTION

Pesticide consumption in Mexico has increased dramatically, between 2000 and 2016 it increased between 57-65 %, by 2017 it is estimated that more than 106 000 tons of pesticides were produced, with fungicides being the most produced, followed by insecticides and finally herbicides (Moo-Muñoz *et al*., 2020). The increase in demand for these products has led to their massive production, reflecting the excessive use by producers, causing contamination of nearby ecosystems (Blanco *et al*., 2020). Pesticides according to their chemical composition maintain certain properties such as persistence, bioaccumulation and biomagnification, toxicity and volatility. Because of this they can be transported to sites other than where they are applied and can remain present in soil, water bodies, can bioaccumulate in plants and animal tissues and can biomagnify through the food chain (Cantú *et al*., 2019; Flores *et al*., 2018; García *et al.*, 2018).
By having the ability to accumulate in the organisms of animals, pesticides can cause damage to their functions such as thermoregulation, behavior (foraging), learning and reproductive capacity, mainly in birds and mammals (Chi-Coyoc *et al*., 2016). It can also suppress the immune system, cause malformations in fetuses, because endocrine disruption and can lead to death in case of acute poisoning (Blanco *et al*., 2020). On the other hand, it can have an impact on population dynamics, since the alteration of the aforementioned processes can reduce survival rates, thus reducing wildlife populations (Badii *et al*., 2006). There is evidence of the presence of pesticides in animals; DDT residues have been found in tissues of whales, dolphins and sea lions. Dieldrin has been detected in turtle eggs and crocodile blood, DDE, ∑HCH and ∑endosulfan have been detected in bird feathers and eggs (García *et al*., 2018). Organochlorines such as DDT have been detected in some small mammals such as mice (Chi-Coyoc *et al*., 2016).

One of the animals in direct contact with pesticides is the Mexican prairie dog (*Cynomys mexicanus*), a diurnal rodent characteristic of grasslands, and endemic to northern Mexico and endangered according to NOM059-SEMARNAT-2010 (SEMARNAT, 2010).
The Mexican prairie dog is considered a keystone species for the maintenance of its ecosystem as it provides several benefits to its habitat. Through the construction of their burrows, they increase water infiltration capacity, oxygenation and the incorporation of organic matter, maintaining the vegetation height, which is favorable for other grassland species such as birds. Currently populations are threatened due to the increase of the agricultural frontier; the area of distribution has decreased drastically by up to 73 % compared to its historical distribution (SEMARNAT, 2018). The distribution of this species is restricted to an area where Coahuila, Nuevo León, San Luis Potosí states and Zacatecas converge. San Luis and Zacatecas states have the lowest number of colonies; until 2008, 12 active colonies were reported in San Luis Potosí and in Zacatecas, it was considered extinct, but it was reintroduced and there is currently one active colony

(Carrera, 2008; Medellín & Bárcenas, 2021). As the colonies are often found close to cultivation areas, it is of great importance to detect whether pesticides are present in the soil where their burrows are located, as well as to determine the concentration of the substance, in order to analyze whether it can be considered a factor in the decline of the populations.

MATERIAL AND METHODS

Sampling was carried out in May 2022, according to the location of the colonies in the states of Zacatecas and San Luis Potosí (Carrera, 2008). Two sampling points were determined in Zacatecas state in the Tanque Nuevo ejido, taking as a reference the reports of PROFUAUNA A.C for the location, as in this state the species was already considered extinct, sampling was carried out in a recently reintroduced colony. For San Luis Potosí, three colonies located within the municipality of Vanegas were sampled, two in the locality of El Gallo and one in the locality of Vanegas (Table 1). Because most colonies in San Luis Potosí are located within private ranches, it was not possible to access all colonies; a total of 12 colonies have been reported in the state (Carrera, 2008; Medellín & Bárcenas, 2021).

Table 1. UTM coordinates of soil sampling sites in colonies of Mexican prairie dogs in Zacatecas and San Luis de la Plata states

State	Sample	X	Y
Zacatecas	M1 Tanque Nuevo	290829.25	2698473.02
Zacatecas	M2 Tanque Nuevo	290985.00	2696484
San Luis Potosí	M1 Gallo1	306681.8	2679409.4
San Luis Potosí	M2 Gallo 2	306715.1	2677716.5
San Luis Potosí	M3 Vanegas	304362.04	2650102.45

Soil samples were collected according to the methodology established by the Mexican Official Standard NOM-AA-105-1988 for the analysis of pesticides in soil. For this purpose, sub-samples of approximately 100 g. of soil were taken within the colonies, using a zigzag pattern in an attempt to cover as much area as possible (DOF, 1988).

Samples were taken from the burrow mounds and from the loose soil inside the burrows, taking care not to destroy the mounds, which serve as a watchtower for the individuals, in order to avoid affecting the habitat.

Eight pesticides (chlorpyrifos, methamidophos, glyphosate, endosulfan, imidacloprid, deltamethrin and methomyl), comprising organophosphorus, organochlorine, carbamate and pyrethroid compounds, were chosen for identification. These pesticides are regularly used on maize, tomato and potato crops, crops present in the study areas (Flores *et al*., 2018).

For the extraction of pesticides, the Soxhlet technique was used (Maldonado, 2021), using 15 g soil and 180 ml of hexane as solvent, allowing 5 cycles for each extraction. The final product was evaporated and was reconstituted with distilled water for subsequent analysis.
For identification and quantification, a calibration curve was made for each pesticide and six standard dilutions were prepared with concentrations ranging from 0.001 mg/mL to 0.5 mg/mL in distilled water. These dilutions were injected into the HPLC and the area values were plotted to determine the equation of the line and determine the coefficient of determination. With these values, the quantification of the pesticides detected in the samples was performed.
For the detection and quantification of pesticides, the High Performance Liquid Chromatography (HPLC) technique was used using an Agillent 1100 Series UV-Vis detector, with an Agillent Varían Pursuit column of 5 mm diameter, 150 mm length and 4.6 mm particle diameter at a temperature of 25.5 °C. Acetonitrile (A) and HPLC-grade water (B) were used at a flow rate maintained at 0.5 mL/min at an injection volume of 20 µL. Detection was performed at a wavelength of 234 nm.

RESULTS AND DISCUSSION

Pesticide residues were detected in all samples analyzed from Zacatecas and San Luis Potosí states. For Zacatecas, glyphosate was detected in higher concentrations compared to the other pesticides detected, with values of 5.3 mg/g and 5.4 mg/g. For the state of San Luis Potosi, glyphosate was also detected in higher concentrations, with values between 4.9- 5.5 mg/g, these ranges were similar to those detected in the state of Zacatecas (Table 2 and 3). Glyphosate concentrations have already been detected in colonies in Coahuila and Nuevo León states, where glyphosate levels were found to be higher than in this study, ranging from 4.2 to 13.5 mg/g (Cano-García *et al.*, 2022). Much lower herbicide levels of up to 0.026 mg/g were detected in agricultural soils in Venezuela (Rojas-Fernández *et al*., 2019). The presence of glyphosate in higher concentration in all samples analysed may be due to the frequent use of this product for weed control. In Mexico, two out of every three-production units use glyphosate for weed control (Hernández *et al*., 2021). In addition, the chemical properties of glyphosate give it the ability to persist in the environment, as it is chemically stable and volatile, which facilitates its dispersion in the environment (Flores *et al*., 2018).

The insecticide methamidophos also presented high concentrations compared to the other samples, with values of 4.5 and 4.7 mg/g for Zacatecas (Table 2). These results coincide with similar ranges for the state of San Luis Potosí, where concentrations from 4.3 to 4.8 mg/g were detected (Table 3). Methamidophos is an insecticide belonging to the organophosphate group. This pesticide has a high residual power and is mainly used for the control of mites in maize, potato, tomato and chilli crops, which are present in the study area (Iannacone *et al.*, 2007). This insecticide is classified as Highly Hazardous (class Ib) according to the World Health Organization (OMS, 2020). In the states of Coahuila and Nuevo León, lower concentrations of methamidophos than those obtained in this study have been detected in potato and apple crop soils, ranging from 0.018 mg/g to 1.16 mg/g (Maldonado *et al*., 2021). However, in the states of Coahuila and Nuevo León in prairie dog colonies, methamidophos was found in higher concentrations ranging from 3.2 to 11.6 mg/g (Cano-García *et al.*, 2022).

The pesticides chlorpyrifos, malathion, endosulfan, imidacloprid, deltamethrin and methomyl were detected at lower concentrations. Chlorpyrifos was detected at 0.2 mg/g and malathion at 0.3 and 0.4 mg/g (Table 2 and 3), compared to those detected in the states of Coahuila and Nuevo León in potato and apple soil. Here higher concentrations of chlorpyrifos were found, ranging from 0.33 mg/ to 3.8 mg/g and malathion from 0.07 to 1.01 mg/g (Maldonado *et al.,* 2021).

Table 2. Concentrations (mg/g) of pesticides detected in soil samples obtained from prairie dog colonies in the state of Zacatecas and San Luis Potosi

Sample	Chlorpyrifos	Methamidophos	Malathion	Glyphosate	Endosulfan	Imidacloprid	Deltamethrin	Methomyl
M1 ZAC	0.2	**4.5**	0.4	**5.3**	0.1	0.06	0.02	0.07
M2 ZAC	0.2	**4.7**	0.4	**5.4**	0.1	0.06	0.02	0.08

The highest concentrations are highlighted in bold

Table 3. Concentrations (mg/g) of pesticides detected in soil samples obtained from prairie dog colonies in the state of San Luis Potosi

Muestra	Chlorpyrifos	Methamidophos	Malathion	Glyphosate	Endosulfan	Imidacloprid	Deltamethrin	Metomilo
M1 SLP	0.2	**4.8**	0.4	**5.5**	0.1	0.06	0.02	0.08
M2 SLP	0.2	**4.3**	0.3	**4.9**	0.09	0.06	0.01	0.07
M3 SLP	0.2	**4.6**	0.4	**5.4**	0.1	0.06	0.02	0.07

The highest concentrations are highlighted in bold

Pesticides applied in crop fields are dispersed in the environment; they can be present in different elements such as soil in this case. This causes organisms such as the prairie dog and all those found in the habitat to be directly exposed to these chemicals, causing damage to both individuals and populations. The United States Environmental Protection Agency (EPA) as a class II toxicity pesticide, with class I being the most toxic, considers Glyphosate (Salazar & Aldana, 2011). In Mexico, glyphosate is one of the pesticides considered highly hazardous with the highest number of authorised registrations in COFEPRIS (Bejarano, 2017). It has a toxicity (oral in rats) of LD_{50} greater than 5000 mg kg-1 and an Acceptable Daily Intake (ADI) of 0.3 mg kg^{-1} (Salazar & Aldana, 2011). If we compare the highest concentration of glyphosate detected in this study, which was 5.5 mg/g present in soil, it is higher than the LD_{50} and ADI. Studies in mice treated daily with doses of 250 or 500 mg/kg at a concentration of 50 g/l (50 mg/mL) glyphosate, in subchronic treatments of six weeks and chronic treatments of 12 weeks, caused alterations in recognition and retention memory. Chronic exposure had an effect on working memory, and a decrease in acetylcholinesterase (AChE) enzyme activity in the brains of mice was demonstrated (Bali *et al.*, 2019).

Although the action of glyphosate in plants is focused on the inhibition of the enzyme 5-enolpyruvyl shikimate 3-phosphate synthetase which mammals do not have (Hernández *et al*., 2021), glyphosate is considered an anticholinergic pesticide, as it prevents the hydrolysis of acetylcholine (ACh), a neurotransmitter, a process carried out by AChE. This causes ACh to accumulate in nerve endings, leading to interference in thermoregulatory functions; behavior of individuals such as foraging time, learning ability, food and water consumption; it can cause weight loss; developmental problems and low reproductive success in mammals (Chi-Coyoc *et al.*, 2016; Dallegrave *et al*., 2007; García, 2015; Salazar & Aldana, 2011). The presence of glyphosate in the soils of *C. mexicanus* colonies, while not demonstrating its presence in their bodies, does make it clear that pesticides applied on nearby crops are transported to their colonies, leaving them chronically exposed. The residues can remain for a long time; in the case of glyphosate, it has been found that up to 21 months after its application it can be present in the soil (Simonsen *et al*., 2008).

There are few studies carried out on wild mammals where pesticide residues are determined, there are few studies that actually demonstrate that, most of the damage is demonstrated in *in vitro* tests with rats. In contrast, several studies have been carried out on bees and birds where it has been shown that pesticides affect populations by reducing their populations and causing damage to their reproduction (Botías & Sánchez-Bayo, 2018; Martin-Culma & Arenas-Suárez, 2018; Cobos *et al.*, 2011).

Organochlorine pesticides are very stable, persistent in the environment and have a high capacity for bioaccumulation as they are fat-soluble (Sierra-Cortés *et al*., 2019). Endosulfan is an organochlorine and is considered a persistent organic pollutant (POP), its lipophilic properties allow it to accumulate in the fatty tissues of animals (Sharma *et al*., 2012). This compound functions as a GABA receptor antagonist in the Central Nervous System (CNS) of mammals, it has been shown that exposures in pregnant rats between 12 and 22 days with doses of 3 mg/kg, causes weight loss in the mother and in the offspring decreases sperm production (Richardson *et al*., 2019; Silva & Gammon, 2009).

In brown bears and grizzly wolves in Croatia, organochlorine residues of hexachlorobenzene (HCB), hexachlorocyclohexane isomers (a-, b- and c-HCH), 1,1-dichloro-2,2-di(4-chlorophenyl) ethane (DDE), 1,1-dichloro-2,2-di(4-chlorophenyl) ethane (DDD) and 1,1,1-trichloro-2,2-di(4-chlorophenyl) ethane (DDT). Concentrations ranged from 0.45 to 4.09 ng/g in bears and from 1.18 to 5.67 ng/g in wolves, these concentrations were considered low and not posing a danger to the studied species, contrary to the concentration of the organochlorine insecticide endosulfan detected in the analyzed soil samples of 0.1 mg/g which are much higher (Romanić *et al*., 2015). It has been found that pesticides can be transported to sites other than where they are applied, reaching even higher places such as mountains, bioaccumulating in small mammals that are present. Endosulfan has been detected in high mountain regions in Brazil at concentrations ranging from 0.6 to 144 ng/g, equivalent to 0.000144 mg/g, which is well below the concentrations detected in this study of 0.1 mg/g (Capella *et al.*, 2023). In the Gulf of Mexico area in the state of Campeche, different species of wild mice that have been exposed to pesticides due to their proximity to agricultural areas were analysed. Endosulfan was detected from 4.62 to 2 899.5 ng/g suggesting that concentrations can cause long-term damage such as oxidative stress, damage to cells of different organs, can influence reproduction by causing alterations in spermatozoa and can even be teratogenic and even carcinogenic (Chi-Coyoc *et al*., 2016).

One of the few studies that has documented physical damage in wildlife was conducted in Uganda, where populations of chimpanzees and baboons were studied that have certain deformities such as cleft lip, nasal deformities ranging from sinking to absent nostrils and varying degrees of skin damage. The study focused on the analysis of samples of maize seeds and fresh stalks that were consumed by individual chimpanzees and baboons. The results showed that maize seeds and stalks contained concentrations of chlorpyrifos higher than the Maximum Residue Limit (MRL) at 0.372 mg/kg; imidacloprid was also detected in seeds at elevated concentrations of up to 460 mg/kg. These concentrations are higher than what was detected in prairie dog soil samples, although no studies have been conducted directly on individuals, it shows that there is exposure to pesticides through food (Krief *et al.*, 2017). Imidacloprid is a pyrethroid insecticide that is classified as moderately hazardous by the WHO with an LD_{50} of 450 mg/kg (OMS, 2020). In this study up to 60 mg/kg was detected so long-term exposure of prairie dogs could cause problems as it has been shown that doses lower than the LD_{50} of imidacloprid (31 mg/kg) can affect thyroid homeostasis and reproduction (Pandey & Mohanty, 2015). Chlorpyrifos is also considered moderately hazardous with an LD_{50} of 135 mg/kg (OMS, 2020) and the results of this study exceeded these ranges as 200 mg/kg were detected.

CONCLUSIONS

Eight pesticides were detected in 100 % of the samples collected in Zacatecas and San Luis Potosí states. The herbicide glyphosate and the insecticide methamidophos showed higher concentrations than the other pesticides. The presence of glyphosate in the soil may cause adverse effects on the organism of *C. mexicanus*, as the compounds can be absorbed through the skin. Although these results do not demonstrate the presence of residues in the body of the prairie dog, it is clear that they are present in its habitat, and chronic exposure may be causing damage to populations. These results provide important information and reference to an important factor that may be influencing the decline in prairie dog populations, such as pesticide application activity in agricultural areas. They provide a guideline for Integrated Pest Management actions to reduce the impact on wildlife in ecosystems.

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Errata Erratum

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