El Colegio de la Frontera Sur

Exposición del Zopilote de cabeza negra (*Coragyps atratus*) a microplásticos y contaminantes en vertederos a cielo abierto y rellenos sanitarios de Campeche, México.

Tesis
presentada como requisito parcial para optar al grado de
Doctora en Ciencias en Ecología y Desarrollo Sustentable
Con orientación en Conservación de Biodiversidad
Por

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Anexo 13

PORTADILLA DE TESIS DE DOCTORADO

El Colegio de la Frontera Sur

ECOSUR

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Las personas abajo firmantes, miembros del jurado examinador de:

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para obtener el grado de **Doctor (a) en Ciencias en Ecología y Desarrollo Sustentable**

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Exposición del Zopilote de cabeza negra (*Coragyps atratus*) a microplásticos y contaminantes en vertederos a cielo abierto y rellenos sanitarios de Campeche, México.

RESUMEN

El manejo inadecuado de la basura en el estado de Campeche, México, al igual que en el resto del país, representa una problemática que no es exclusiva de las zonas urbanas, en donde se concentra la mayor parte de la población, sino también comienza a ser frecuente en las comunidades rurales, que carecen de sistemas de recolección y sitios destinados para el depósito, manejo y control de los residuos.

En el estado de Campeche, la basura está concentrada en vertederos a cielo abierto o bien en rellenos sanitarios donde la disposición final de los residuos, tales como los plásticos, se acumulan. Por efectos mecánicos, los macroplásticos (>25 mm) se van fragmentado con el tiempo, dando lugar a meso (<25 mm y >5 mm) y microplásticos (<5 mm), en los cuales se encuentran substancias químicas toxicas (ftalatos) y otras se pueden adherir sustancias químicas tóxicas, convirtiéndose en un foco de riesgo para los seres vivos expuestos a estas partículas. En particular, los vertederos a cielo abierto y los rellenos sanitarios son focos de atracción para aves y mamíferos carroñeros como los zopilotes de cabeza negra (*Coragyps atratus*) que buscan restos animales entre los desperdicios humanos y eso conlleva a la exposición a través de la ingesta accidental de plástico y otros contaminantes. Por ello, el objetivo del presente trabajo es estudiar la exposición de los zopilotes a microplásticos (MPs), y contaminantes tóxicos asociados a estas partículas como hidrocarburos aromáticos policíclicos (HAPs), plaguicidas organoclorados (POCs) y metales, estudiando las egagrópilas y plumas del zopilote de cabeza negra en el estado de Campeche.

Palabras claves: Aves carroñeras, egagrópilas, microplásticos, plumas, POCs, HAPs, metales, rellenos sanitarios, vertederos a cielo abierto.

CAPÍTULO I

1.Introducción

Desde 1999 se ha demostrado un descenso vertiginoso de las poblaciones de especies carroñeras en el sur de Asia, África y Europa (Prakash et al., 2003). La situación en América es parcialmente conocida para las dos especies de cóndores (*Vultur gryphus* y *Gymnogyps californianus*) que están globalmente amenazados y en peligro de extinción, respectivamente (SEMARNAT, 2010; Méndez et al., 2019) Recientemente se ha demostrado que *C. atratus* presenta una disminución de 30% o más de su población desde los Estados Unidos, México y varios países en Centro América (Fink et al., 2022).

En México habitan cinco especies de la familia Cathartidae: el zopilote de cabeza negra (*Coragyps atratus*), el aura común (*Cathartes aura*), el aura sabanera (*Cathartes burrovianus*), el zopilote rey (*Sarcoramphus papa*) y el Condor de California (*Gymnogyps californianus*) (Iñigo, 1987). Para este estudio la especie *Coragyps atratus* fue seleccionada en vista de su amplia distribución en el país y su estrecha relación con los basureros, además tienen un servicio ambiental como saneadores ambientales al consumir carroña y eliminar posibles infecciones bacterianas y virales en los desperdicios de cadáveres por lo que se encuentran protegidos por el tratado de aves migratorias de 1918 (Federal Register, 2013) y del cual México es signatario.

Los zopilotes de cabeza negra y los zopilotes de cabeza roja se alimentan de animales muertos, y por ello se adaptan fácilmente a vivir en centros urbanos y en contacto con personas (sinantropía), a diferencia los zopilotes sabaneros que prefieren áreas costeras donde se alimentan de peces y pequeños reptiles o el zopilote rey (*Sarcoramphus papa*) y el cóndor (*Gymnogyps californianus*) que prefieren ambientes más naturales (Del Hoyo, 1994). Por ello, los vertederos a cielo abierto o rellenos sanitarios constituyen a menudo puntos atractivos para distintas especies de aves carroñeras. En particular el zopilote negro (*Coragyps atratus*), se encuentra en mayor número de individuos en comparación con otras especies y usan a estos basureros como fuentes alternativas o principales de alimentación (Pomeroy, 1975).

El 87% de los basureros urbanos en México son a cielo abierto y 13% son rellenos sanitarios. Se estima que el 79% de los desechos se acumula en basureros o en el medio natural, alrededor del 12% es incinerado y un 9% es reciclado (UNEP, 2019). Los plásticos desechados en el ambiente sufren transformaciones por factores físicos, químicos y biológicos, fragmentándolos y dando lugar a los microplásticos (MPs) los cuales tienen dimensiones menores a 5 mm.

Los plásticos ya fragmentados pueden ser ingeridos por los zopilotes al consumirlos de forma accidental o intencional. Estos materiales antrópicos pueden ser recipientes de plástico rotos, bolsas de papel, ligas, biberones y poliestireno (Iñigo-Elías 1987; Kelly et al., 2007). Los zopilotes tienen una fisiología compleja, al igual que muchas de las aves rapaces diurnas (zopilotes, cóndores, gavilanes, águilas y gavilanes) y nocturnas (búhos y lechuzas), dado que tienen estómagos pequeños que no pueden digerir estructuras duras como pelos, plumas y huesos, regurgitándolos en forma de bolas, denominadas “egagrópilas o pellets” (Iñigo-Elías, 1987).

En egagrópilas de zopilote de cabeza negra (*C. atratus*) en Chiapas, México, Íñigo (1987) encontró productos sintéticos, principalmente bolsas de plástico que representaron el 39.1% del total de las muestras examinadas. Estos plásticos tienen substancias químicas toxicas como esteres de ftalatos (Provencher et al., 2020; Wang et al., 2021; Allen et al., 2022; Badrya et al., 2022) y también se pueden adsorber diferentes sustancias como los plaguicidas organoclorados (POCs) e hidrocarburos aromáticos policíclicos (HAPs) y metales pesados del ambiente (UNEP/GPA, 2006; Syberg, 2015). Los plaguicidas son sustancias o mezclas de sustancias que se destinan a controlar plagas, incluidos los vectores de enfermedades humanas, de fauna silvestre y doméstica, así como las especies no deseadas que causan perjuicio o interfieren con la producción agropecuaria, forestal o urbana (USEPA, 2010). Los HAPs son generados por la combustión de combustibles fósiles y materia orgánica tales como las quemas agrícolas (Acampora et al., 2018) y, en el caso de los metales pesados, pueden ser emitidos por varias actividades antropogénicas como la industrialización, procesos de fundición, tráfico vehicular, minería, escurrimientos agrícolas, actividad petrolera, entre otros (Naccari et al., 2009). Una de las principales propiedades de los contaminantes antes mencionados es que tienen una alta dispersión ambiental, estos compuestos pueden ser transportados vía atmosférica a largas distancias desde su lugar de origen, aplicación o uso (Wania y MacKay, 1996).

Las aves están altamente expuestas a la contaminación atmosférica, la lluvia, las caídas, el contacto con el suelo, el polvo y el agua, que pueden representar fuentes de contaminación externa (Jasper et al., 2004; Cardiel et al., 2011). Sin embargo, los PAHs, metales and POCs pueden ser ingeridos por las aves durante la alimentación, el acicalamiento y la respiración (Rutkowska et al., 2018). Estos contaminantes se distribuyen a través del torrente sanguíneo uniéndose a las moléculas de proteína en la pluma durante su crecimiento (Dauwe et al., 2003; Rutkowska et al., 2018).

El uso de las plumas como método no invasivo nos permiten evaluar los niveles de contaminantes en las aves sin tener que sacrificarlas (Jaspers et al., 2011). Varios estudios han reportado efectos en aves por la exposición a POCs, desde intoxicación hasta alteración del sistema inmunológico, desarrollo de cáncer, problemas reproductivos, efectos neurológicos, genotóxicos y en último caso la muerte (Cooper, 2002), por su parte, los HAPs son considerados compuestos mutagénicos, carcinogénicos y teratogénicos (Singh and Gupta, 2016).

Los zopilotes tienen un papel fundamental en los ecosistemas y en la salud publica pues ayudan a disminuir los focos de infección provenientes de animales muertos. Sin embargo, la exposición a contaminantes puede poner en riesgo la salud de estas aves. En el continente americano, existen diversas indicaciones de que varias poblaciones de esta especie han sido exterminadas y otras continúan en declinación (Fink et al., 2022). Íñigo (1999), explicó que las poblaciones en México de esta especie están desapareciendo localmente de muchas regiones del país. Debido a lo anterior, el objetivo del estudio es evaluar la exposición del zopilote de cabeza negra (*Coragyps atratus*) a microplásticos POCs, HAPs y metales procedentes de vertederos a cielo abierto y rellenos sanitarios del estado de Campeche.

1.1. Rellenos sanitarios y vertederos a cielo abierto

En México, los vertederos a cielo abierto son terrenos en donde se depositan y acumulan los residuos sólidos municipales sin ningún control técnico sanitario y operativo, así como la ausencia de obras de infraestructura para minimizar los impactos negativos al ambiente. Uno de los problemas asociados a la presencia de basureros a cielo abierto, es que la mayoría de estos sitios se convierten en puntos clave para el depósito ilegal de residuos peligrosos, lo cual provoca que en estos sitios sean un gran problema de contaminación ambiental y de riesgo a la salud humana (SEDESOL, 1996).

Por el contrario, un relleno sanitario es un sitio donde se compacta la basura y se cubre con tierra, tienen un sistema de recolección de lixiviados y un sistema de recolección de gases, sin embargo, en México no todos los rellenos sanitarios presentan todos estos elementos (Greenpeace, 2010).

Durante el año 2012, en México se generaron 42.1 millones de toneladas de residuos sólidos urbanos (RSU) anuales, lo que equivale a un promedio de 1.2 kg diarios per cápita, un 15% más que en el año 2000. En los últimos años la generación de estos residuos se ha incrementado en más del 90% pasando de 21.9 millones de toneladas en 1992 a 47,156,600 de toneladas en el 2017 (INEGI, 2017). a 42.1 millones de toneladas en el 2012. Por otro parte, el estado de Campeche es uno de los estados con menor generación de basura con un volumen de 271 mil toneladas. La generación de RSU dependerá de la densidad de personas que viven o pasan por la localidad, ya que hay una relación entre las grandes ciudades y una mayor producción de residuos sólidos en comparación con las ciudades pequeñas (SEMARNAT, 2012).

Tanto en los rellenos sanitarios como en los vertederos a cielo abierto es posible la existencia de sustancias peligrosas en los residuos urbanos como los materiales usados comúnmente en el hogar y o industriales que pueden contener sustancias químicas peligrosos. A continuación, se resumen algunos de ellos (ATSDR, 2001):

i. Los plásticos usados normalmente pueden contener: cloruro de vinilo, polietileno, formaldehído y ácido ftálico o esteres ftálicos.
ii. También pueden encontrarse metales pesados en los desechos urbanos, los productos electrónicos como televisiones, radios, vidrios, cerámicas, plásticos, objetos de bronce y los aceites usados pueden contener plomo. Las baterías de níquel-cadmio, los plásticos, el lavavajillas, el lavarropas, los pigmentos, las cerámicas, los aceites usados y el caucho contienen cadmio. Las lámparas fluorescentes, los restos de pinturas, los termómetros, los pigmentos de tintas y los plásticos pueden contener mercurio.

1.2. Plásticos y microplásticos (MPs)

Los plásticos son polímeros de alto peso molecular compuestos de moléculas orgánicas, sintetizados a partir de derivados del petróleo y otras sustancias naturales (Segura et al., 2007). Los plásticos entran al ambiente a través de los procesos de fabricación, lixiviación, evaporación, difusión en los basureros y sitios de disposición de residuos (Derraik, 2002; Wang et al., 2022).

Los plásticos desechados en el ambiente se degradan lentamente en meso y microplásticos a través de factores físicos, químicos y biológicos; sin embargo, muchos de estos polímeros plásticos son altamente resistentes y pocos pueden reciclarse, por lo tanto, la acumulación de la basura plástica ha ido aumentando en el ambiente. Los microplásticos son pequeñas partículas de plástico ubicuas de menos de cinco milímetros de tamaño y se originan en dos fuentes: los primarios que se fabrican intencionalmente para aplicaciones industriales o domésticas particulares, como exfoliantes faciales, pastas dentales y “pellets” de resina utilizados en la industria del plástico (microplásticos primarios); y los que se forman a partir de la descomposición de artículos de plástico más grandes bajo radiación ultravioleta o abrasión mecánica (microplásticos secundarios) (GESAMP, 2015). Estos microplásticos presentan un nuevo conjunto de problemas ambientales, debido a dos razones principales: (i) son lo suficientemente pequeños para ser absorbidos por la biota y, por lo tanto, pueden acumularse en la cadena alimentaria; y (ii) pueden absorber contaminantes en sus superficies (Browne et al., 2011).

1.2.1. Microplásticos como vehículos de transportes de contaminantes

Una característica muy importante de los MPs es que al material del cual están constituidos los polímeros pueden adsorber o se le adhieren contaminantes persistentes, bioacumulables y tóxicos como los plaguicidas organoclorados (POCs), hidrocarburos aromáticos policíclicos (HAPs) y metales que se encuentran en el ambiente, entre otras muchas substancias tóxicas, de tal manera que los MPs actúan como vehículos de transporte. Además, liberan o desprenden contaminantes originados durante su fabricación, como los ftalatos o aditivos plásticos que forman parte de este (Bogusz y Oleszczuk, 2016; Wang et al., 2022).

Los MPs pueden actuar como vectores para el transporte de compuestos químicos como:

i. Compuestos directamente relacionados a la fabricación de plásticos para proporcionarles ciertas propiedades (plastificantes), como los ftalatos que los hacen más maleables, el Bisfenol A, los retardantes de llama, los antimicrobianos y aquellos que evitan los daños oxidativos (nonilfenoles).
ii. Metales pesados y contaminantes orgánicos hidrófobos (COHs) que se adsorben en ellos, como cobre, zinc, plomo, bifenilos policlorados (PCBs), hidrocarburos aromáticos policíclicos (PAHs) (Rochman, 2015).

Estudios científicos demuestran que contaminantes tales como los ftalatos o aditivos plásticos, que son sustancias químicas que se añaden al plástico para darle flexibilidad, se pueden liberar de los microplásticos y ocasionar efectos adversos en los organismos, incluyendo las aves (Provencher et al., 2020; Wang et al., 2021; Allen et al. 2022; Badyra et al., 2022). Por ejemplo, el Di (2-ethyl hexyl) phthalate (DEHP) es un plastificante que se utiliza en envases de alimentos, dispositivos médicos, productos domésticos y plástico industrial, etc. (Wang et al., 2012). Li et al., (2018), demostraron que la exposición de la codorniz (*Coturnix japonica*) al DEHP es perjudicial para sus riñones.

Algunos polímeros como el polietileno, polipropileno, nylon y policloruro de vinilo pueden acumular contaminantes orgánicos persistentes en su estructura (UNEP/GPA 2006; Syberg, 2015).

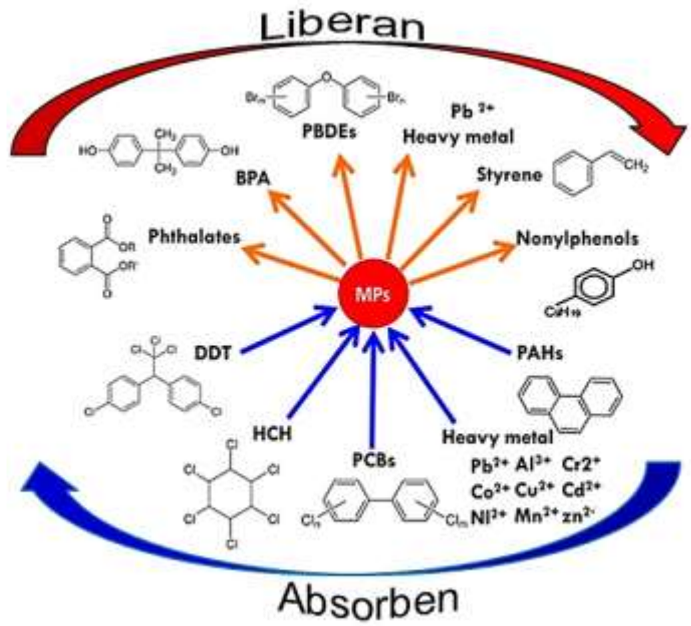

Figura 1. Incorporación de contaminantes químicos por microplásticos.

1.3. Plaguicidas organoclorados (POCs)

Los POCs son sustancias o mezclas de sustancias que se destinan a controlar plagas, incluidos los vectores de enfermedades humanas y de animales (USEPA, 2010); su duración en el transcurso del tiempo indica que toleran los cambios en la naturaleza y por ende sufren transformación, la cual a veces los vuelve más persistentes a la degradación química y bioquímica (Ramírez y Lacasaña, 2001), esta es una de sus propiedades más importantes en el ambiente, biótico y abiótico, pues son muy estables químicamente y solo en condiciones ambientales extremas, se degradan con lentitud. Así mismo, son insolubles al agua, pero solubles en disolventes orgánicos.

Una de las formas de medir la persistencia de un compuesto es mediante la vida media en años de este, por ejemplo, que el DDT tiene una vida media de 10 años, el toxafeno de 11, el endrín de 10, el clordano de 8 y el lindano de 2 años (Calva y del Rocío, 1998). La gran mayoría de los POCs pasan por un proceso de biotransformación cuando se encuentran en el ambiente o dentro del ser vivo, dando origen a otras sustancias más toxicas, más estables y/o persistentes que el original. Tal es el caso del dicloro difenil tricloroetano (DDT) que puede ser transformado por acción de las enzimas microsomales del hígado de animales a dicloro difenil dicloro etano (DDD) y convertido lentamente a dicloro difenil dicloro etileno (DDE) (ATSDR, 2002). El principal efecto adverso de los POCs en las aves se da en la reproducción (Vorkamp et al., 2009). El DDE, principal metabolito del DDT inhibe la disponibilidad del carbonato de calcio durante la formación del cascarón de los huevos de aves y reptiles haciéndolos delgados y frágiles (Vos et al., 2000).

1.4. Hidrocarburos aromáticos policíclicos (HAPs)

Los HAPs compuestos que pueden tener de 2 a 6 anillos aromáticos fusionados y son generados principalmente por incendios forestales y/o por motores de combustión. Existen 16 HAPs que han sido identificados como contaminantes prioritarios por la Agencia de Protección del Medio Ambiente de los Estados Unidos (US-EPA) (EPA, 1984), ya que muchos de los HAPs poseen propiedades mutagénicas, carcinogénicas y teratogénicas, además de ser persistentes, bioacumulables, tóxicos y susceptibles a ser transportados a grandes distancias por vía atmosférica (Genualdi et al., 2009).

Los incendios emiten una gran cantidad de hidrocarburos aromáticos policíclicos. Así, la principal fuente de HAPs es la combustión incompleta de materiales orgánicos (Kim et al., 2003). Esto ocurre principalmente en incendios de vegetación, actividades volcánicas y procesos diagenéticos. Otros incendios se producen por el sistema de roza, tumba y quema (RTQ) que es una práctica ancestral de muchos campesinos en México, como en la Península de Yucatán (Nigh and Diemont, 2013), en donde gran parte de la, población rural se dedica a actividades agrícolas extensivas donde se practica el método RTQ con el fin de preparar las áreas cultivables. Es muy probable que, durante cada temporada de quemas, y de incendios forestales, se emita al ambiente una gran cantidad de HAPs de los cuales se desconocen los impactos ecológicos que pudieran tener. Por ejemplo, en el 2019, en la Península de Yucatán se tuvieron un total de 96 incendios con 33 mil hectáreas quemadas (CONAFOR, 2019).

Se sabe que los HAPs afectan a las aves durante la producción de huevos causando teratogénesis, causando cambios en el tamaño y grosor del cascaron. Además, la exposición a estos hidrocarburos también puede producir cáncer en aves adultas (Stubblefield et al., 1995).

1.5. Metales

Entre los contaminantes ambientales se encuentran los metales, estos son elementos que están presentes naturalmente en el ambiente a través del ciclo geológico. El aumento de la urbanización y la industrialización introduce grandes cantidades de metales a los ecosistemas, impactando de forma negativa en su estabilidad a nivel ecológico (Pan et al., 2008). Los iones de metales son altamente estables, asimismo en bajas concentraciones pueden biomagnificarse en la red trófica, convirtiéndose en un peligro creciente para el ser humano y la vida silvestre (Parra et al., 2014).

Al igual que otros organismos, las aves se ven afectadas por la acumulación de metales pesados, ya que éstos pueden suprimir su sistema inmune, las enzimas que depositan el calcio en el cascarón, incrementar el comportamiento agresivo territorial en paseriformes, afectar negativamente el sistema endocrino y causar disfunciones reproductivas como, por ejemplo, una menor tasa de crecimiento en polluelos de gorrión (*Passer montanus*) (Pan et al., 2008). El Hg causa reducciones en la fecundidad (Brasso y Cristol 2008), la reproducción aviar es sensible por toxicidad a este metal. Otro ejemplo, es la bioacumulación de Hg en el ave canora conocida como saltapared de Carolina (*Thryothorus ludovicianus*) con concentraciones de 0.7 ppm en sangre (Jackson et al., 2011).

1.6. Exposición de las aves a plásticos y contaminantes

La ingestión de plásticos por las aves puede ser 1) Indirecta (accidental) cuando rompen las bolsas de plástico para llegar a los desechos domésticos o cuando consumen una presa o carroña que tiene microplásticos o fragmentos de plásticos en sus tejidos y 2) directa (intencional) cuando los plásticos se asemejan a la forma o textura de un pedazo de tejido lo que lleva a los zopilotes a ingerir estos; por ejemplo, pueden confundir las bandas de goma con la carne por su textura y color (Íñigo, 1987), también pueden confundir los fragmentos de plásticos con huesos, por la necesidad de calcio (Ferro, 2000). Grandes pedazos de plásticos pueden provocar la sofocación y obstrucción en esófago, buche y estómago de las aves, en particular los zopilotes y aves marinas, presentan alteraciones estomacales y afectación de otras funciones como la reproducción (Wang et al., 2021).

Se sabe que las aves carroñeras se alimentan de basura humana en áreas pobladas (Íñigo, 1987). Por ejemplo, el cóndor de California (*Gymnogyps californianus*) se ve afectado por la ingestión de desechos antropogénicos ya que se han encontrado vidrio, metal y plástico en nidos, con una reducción en la supervivencia de los polluelos causada por la desnutrición (Mee et al., 2007).

Una vez que los plásticos entran por el pico, estos pasan al través del esófago y son retenidos temporalmente en la molleja, después continúan al estómago. En el proventrículo, primera sección del estómago en los zopilotes es pequeño y con paredes poco musculosas, por lo que no pueden digerir estructuras duras como pelos, plumas y huesos y, por ello, son regurgitados en forma de bolas, denominadas “egagrópilas o pellets” (Figura 2; Errington, 1930). El contenido de las egagrópilas depende de la dieta de las aves, y puede contener huesos, piel, pelos, exoesqueletos, materia vegetal, plumas, uñas, dientes, picos, quitinas de insectos, espinas de peces, etc. (Íñigo, 1987).

Figura 2. Consumo de plásticos por las aves carroñeras como el zopilote negro (*Coragyps atratus*) en los rellenos sanitarios y/o vertederos a cielo abierto de Campeche, México.

Las condiciones de acidez estomacal en los zopilotes producen efectos diferenciales de degradación sobre el alimento consumido (Carvalho et al., 2003). Por lo tanto, se digieren más fácilmente los tejidos, huesos y queratinas (como pelos, plumas), a diferencia de los plásticos y otros materiales sintéticos (Iñigo, 1987).

Los POCs, HAPs y metales al estar ampliamente distribuidos en el ambiente contaminan diferentes compartimentos ambientales (Agua, aire, suelo y biota). Debido a lo anterior los zopilotes pueden estar expuestos a contaminantes a través de los alimentos que ingieren de manera accidental. Así, cuando un contaminante es ingerido, los compuestos pasan del tracto digestivo a la sangre y se distribuye dentro del organismo a los diferentes órganos y tejidos (Wang et al., 2021).

Además, se ha demostrado que muchos contaminantes llegan a las plumas de las aves a través del torrente sanguíneo durante su periodo de crecimiento y se enlazan a la molécula de queratina durante el periodo de muda (Dauwe et al., 2005). Cuando las plumas ya están maduras, las conexiones vasculares se atrofian y las concentraciones de los contaminantes dentro de la pluma permanecen estables (Figura 3; Burger and Gochfeld, 2000). Por lo tanto, las plumas pueden contener información sobre las concentraciones circulantes en la sangre en el momento de su crecimiento (Espín, 2010). A pesar de que el uso de las plumas es una técnica no destructiva, hay pocos estudios de metales en plumas de aves carroñeras.

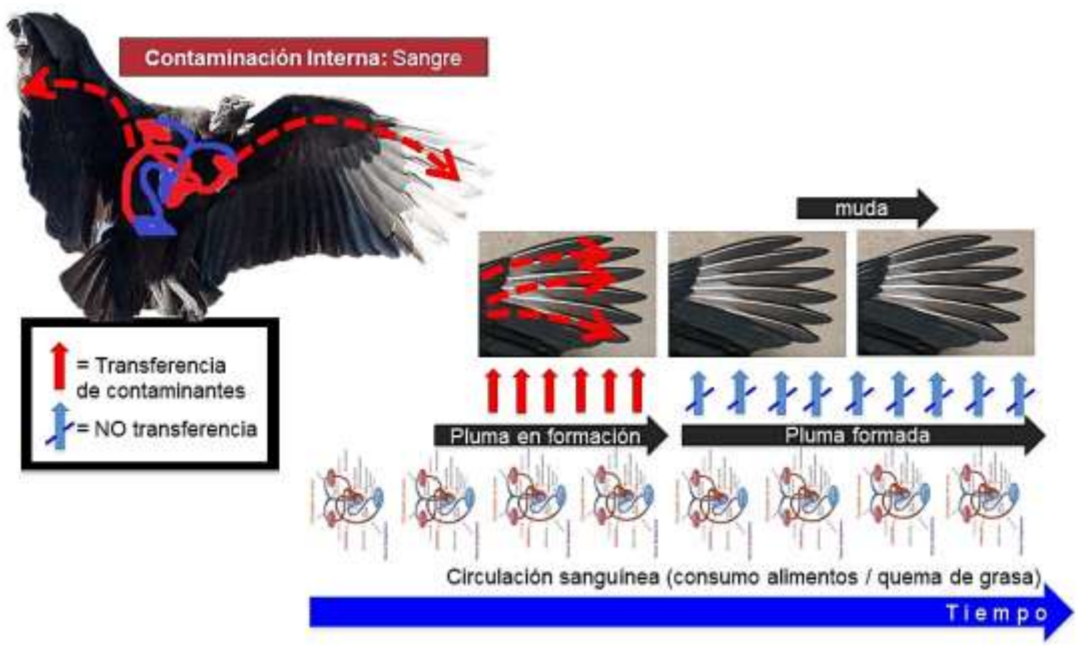

Figura 3. Esquema de la transferencia de contaminantes en las plumas de las aves.

Por ejemplo, López Berenguer et al. (2021) determinaron metales en plumas de Cathartes aura al sur de Atacama (Chile), cuyas concentraciones medias fueron de 0.68 ± 0.84 $\mu g \cdot g^{-1}$ para Cd, 1.97 ± 2.01 $\mu g \cdot g^{-1}$ para Pb, y 59.11 ± 80.69 $\mu g \cdot g^{-1}$ para Cu. En otro estudio realizado por Durmus (2018) reportó Hg con concentraciones de 0.45 $\mu g \cdot g^{-1}$ en plumas de *Gypaetus barbatus* de la región este de Turquía, donde la contaminación puede deberse a actividades agrícolas y urbanas en el sitio.

1.7. El zopilote de cabeza negra (*Coragyps atratus*)

Coragyps atratus es una especie monotípica del género Coragyps que posee una longitud total entre 58 y 68 cm, carece de dimorfismo sexual aparente, tienen un plumaje negro, con la cabeza y el cuello desnudos y de aspecto rugoso, pico negruzco, iris pardo y cola corta redondeada (Londoño et al., 2006). El zopilote de cabeza negra es el ave rapaz diurna más abundante en el continente americano, incluido México. Posee una masa corporal entre 1 y 2 kg y sus alas abiertas llegan a medir hasta 1.2 m (Iñigo, 1999).

1.7.1. Distribución

El zopilote *Coragyps atratus* conocido en México como zopilote o chombo de cabeza negra, se distribuye desde el centro de Estados Unidos de América (entre ambas costas) hasta la Patagonia (fig. 4). En México existen tanto poblaciones residentes como algunas migratorias en todos los estados del país y en algunas islas del Golfo de California, de la costa del Pacífico y del mar Caribe (Iñigo, 1999).

Figura 4. Distribución global del zopilote negro (*Coragyps atratus*) en el Neotrópico.

1.8. Justificación del estudio

Las aves ocupan un amplio rango en las cadenas tróficas, están extensamente distribuidas, presentan un periodo de vida largo y son sensibles a cambios atmosféricos o ambientales, lo que las convierte en buenos bioindicadores de exposición temporal y espacial a contaminantes, ideales para valorar la salud ambiental. Las aves pueden reflejar los impactos de contaminantes en todo el ecosistema o en un área amplia.

La importancia de este trabajo es conocer si los zopilotes de cabeza negra del estado de Campeche están expuestos a microplásticos y por lo tanto a diversos contaminantes adheridos a estos polímeros plásticos. Así también, evaluar las concentraciones de metales en rellenos sanitarios y de los vertederos a cielo abierto que incorporan estas aves carroñeras en sus plumas, debido a que estos contaminantes podrían representar un riesgo no solamente para estas aves sino también a la salud humana con relación a los sitios donde se vierten estos desechos sólidos urbanos.

La especie *C. atratus* fue seleccionada para este estudio en vista de su amplia distribución en el estado y su estrecha relación con los basureros. El zopilote de cabeza negra y otras especies de zopilotes del nuevo mundo son sensibles a los efectos que ocasionan estos contaminantes como el adelgazamiento del cascaron ocasionado por el DDT, entre otros. En México, al igual que en muchas partes del mundo, los basureros y los rellenos sanitarios son áreas de forrajeo del zopilote de cabeza negra (*C. atratus*) en donde es probable que estas aves estén expuestas no solo a los microplásticos sino a otros compuestos tóxicos, debido a que hurgan en la basura en busca de alimento.

Asimismo, los microplásticos (MPs) son un grave problema ambiental ya que a estas partículas plásticas pueden adherir y liberar de su estructura contaminantes orgánicos persistentes y, debido a las dimensiones de estos plásticos y a su ubicuidad ambiental se encuentran fácilmente disponibles para ser ingeridos por los zopilotes. Una vez ingeridos, los MPs pueden inducir daño mecánico, efectos subletales y diversas respuestas celulares, exacerbados por la posible liberación de contaminantes adsorbidos. Sin embargo, aunque se desconocen los efectos fisiológicos relacionados con la ingestión de los MPs en la especie *C. atratus*, esta ave está expuesta a estos compuestos tóxicos.

Aunque el zopilote regurgita los microplásticos a través de las egagrópilas, están expuestos a diversos contaminantes intrínsecos de los mismos plásticos como los compuestos ftalatos, así como por metales de forma externa a través del contacto físico y de forma interna por el consumo de alimentos contaminados de los vertederos a cielo abierto y rellenos sanitarios. Los metales ingeridos por los zopilotes a través de los alimentos cruzan al torrente sanguíneo al ser digeridos y llegan a las plumas durante su período de crecimiento. Sin embargo, cuando las plumas ya están maduras las conexiones vasculares se atrofian y las concentraciones de los metales pueden permanecer estables. Por lo tanto, las plumas dan información sobre las concentraciones de estos compuestos tóxicos circulantes en la sangre durante su desarrollo.

1.9. Hipótesis

(H1) Debido al incremento de los RSU en la ciudad de San Francisco de Campeche, así como la compactación de la basura en el relleno sanitario, es posible que los zopilotes que se alimentan en este sitio presenten un aumento de microplásticos y contaminantes adheridos a estos polímeros entre los años 2019 y 2020.

Predicción: Habrá mayores cantidades de microplásticos y mayores concentraciones de contaminantes adheridos a los polímeros plásticos en las egagrópilas del 2019 a diferencia del 2020.

(H2) En los vertederos a cielo abierto se quema la basura cuando sobre pasa el volumen del sitio, en comparación al relleno sanitario donde solamente se compacta la basura y se cubre con tierra. Por lo tanto, se espera que los zopilotes procedentes de localidades con vertederos a cielo abierto tengan mayor concentración de metales.

Predicción: En localidades con vertederos a cielo abierto habrá mayores concentraciones de metales en comparación con los zopilotes del relleno sanitario.

2. Objetivos

2.1. Objetivo general

Evaluar la exposición del Zopilote de cabeza negra (*Coragyps atratus*) a microplásticos y contaminantes en vertederos a cielo abierto y rellenos sanitarios de Campeche, México.

2.2. Objetivos particulares

i. Cuantificar y comparar entre años la cantidad de microplásticos presentes en las egagrópilas de zopilotes procedentes de un relleno sanitario y las concentraciones de HAPs, metales y POCs adheridos a estos polímeros plásticos.

ii. Comparar las concentraciones de metales de las plumas de zopilotes procedentes de vertederos y un relleno sanitario de localidades urbanas, semiurbanas y rurales.

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Capítulo II

Artículo 1. Heavy metals, organochlorine pesticides and polycyclic aromatic hydrocarbons in microplastics found in regurgitated pellets of Black vulture from Campeche, Mexico.

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Organochlorine pesticides, polycyclic aromatic hydrocarbons, metals and metalloids in microplastics found in regurgitated pellets of black vulture from Campeche, Mexico

Heavy metals, organochlorine pesticides and polycyclic aromatic hydrocarbons in microplastics found in regurgitated pellets of Black vulture from Campeche, Mexico.

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Abstract

Plastics are produced by the millions of tons worldwide each year, with their final deposition in landfills (LFs). Plastics deposited in LFs can fragment over time, giving rise to mesoplastics and later to microplastics (MPs), in which toxic chemicals such as heavy metals, organochlorine pesticides, and polycyclic aromatic hydrocarbons can adhere. MPs can be vectors for the exposure to pollutants of black vultures (*Coragyps atratus*) due to feeding in LFs, resulting in accidental ingestion of MPs. It is also possible that MPs can adsorb pollutants from vultures during the digestion process. These birds are exposed to contaminants from the LF. In LFs, they burn most of the stored plastics and generate intense emissions of chemical substances linked to particles and toxic and greenhouse gases (Kumar et al., 2015). Therefore, the aim of this study was to estimate the risk of black vulture exposure to MPs, heavy metals (HMs), organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs). Fifty-eight black vulture pellets were collected at the

Campeche LF during 2019 (n = 24) and 2020 (n = 34). The MPs were extracted using a sieving method, making a distinction between the sizes of each particle. The pellets, on average, had an MP load per pellet of 6.7 ± 5.8 MPs/total pellets. The greatest abundance of MPs was detected in 2019, with 225 particles in total. The concentrations of Cd, Pb, Cu, Cr, Hg, As, and Al were detected in the MPs, with the greatest average concentration of 35.59 ± 32.39 $\mu g \cdot g^{-1}$ (2019) and 15.82 ± 17.47 $\mu g \cdot g^{-1}$ (2020) for Al. From the organochlorine compounds detected, ∑dienes were found in all the MPs of 2019 with average concentrations of 1.03 ± 1.11 $ng \cdot g^{-1}$. In 2020, ∑endosulfans were present in all MPs at 0.97 ± 1.47 $ng \cdot g^{-1}$. Among the PAHs, 15 of the 16 compounds indicated as priorities by the US EPA were quantified. The compound with the greatest total concentration for both years was acenaphthylene (3 rings), with 10.51 ± 7.88 $ng \cdot g^{-1}$ (2019) and 10.61 ± 18 $ng \cdot g^{-1}$ (2020). More research is needed regarding the origin of the contaminants detected in the MPs extracted from the pellets, since the contaminants may come from the environment or possibly from the digestion processes in the stomach of avian raptors and scavengers.

Graphical abstract

Key words: Heavy metals, landfill microplastics, organochlorine pesticides, polycyclic aromatic hydrocarbons, scavenger birds.

1. Introduction

Plastics have been used in our daily lives since 1950. Since then, plastic production has been increasing, reaching 360 million tons in 2018, 40% of which is single-use (PlasticEurope, 2018). A person generates approximately 1 kg of garbage per day, 20% being plastic (Huerta-Lwanga et al., 2017). Worldwide, these plastics are deposited in landfills (LFs) (Nizzetto et al., 2016). Many of these MPs are derived from the fragmentation of macroplastics. MPs are tiny plastic particles less than 5 mm in size and have two origins. Primary MPs are those manufactured for industrial or household applications, such as facial scrubs, toothpaste, and resin plastic nurdles used in the industry. Secondary MPs are those formed from the breakdown of larger plastics after they are degraded in the environment (Cole et al., 2011).

MPs can be released to the terrestrial environment through uncontrolled landfills and other human activities, resulting in the contamination of terrestrial systems, especially the soil (Nizzetto et al., 2016). Due to their small size, MPs can be easily ingested by organisms with different trophic levels and accumulate along trophic networks (Huerta-Lwanga et al., 2016, 2017).

LFs are attractive sites for different scavenger species, particularly for the black vulture (*Coragyps atratus*), which is more abundant than other species and uses these dumps as an alternative or primary source of food (Pomeroy, 1975). All vultures accidentally ingest synthetic materials such as plastics that they cannot digest and regurgitate them in the form of balls, called “pellets” (Iñigo, 1987). Pellets are a fundamental element for analyzing the type of diet that vultures consume (Blanco, 2012).

Ingestion of MPs by avian scavengers can be indirect (accidental) when they break plastic bags to obtain household waste or consume prey or carrion with MPs in their tissues. Direct ingestion (intentionally) occurs when plastics resemble the shape or texture of a piece of tissue, which leads to vultures ingesting these materials. For example, they confuse automobile tires with pieces of meat due to their texture and color (Iñigo, 1987).

Due to the dimensions of these plastics and their environmental ubiquity, they are readily available to be ingested by vultures. If they are not regurgitated, MPs can induce mechanical damage to the stomach or intestine (Iñigo, 1987), including obstruction of the gastrointestinal tract, particularly if they are offered to young individuals (Walters et al. 2010).

There are few studies of the plastics in pellets from carrion birds. In one study, Iñigo (1987) found synthetic products (39.1%), mainly plastic from bags, in the pellets of black vultures (*C. atratus*) from Chiapas, Mexico. Augé (2017) analyzed *C. aura* pellets from the Malvinas Islands, of which 58% of the pellets contained plastics. Ballejo et al. (2020) studied pellets from three vulture species (*Vultur gryphus*, *Coragyps atratus*, and *Cathartes aura*) and determined that 17.4% of the pellets studied contained synthetic residues. Zhao et al. (2016) found MPs present in the digestive tract of 94% of dead land birds with various feeding behaviors in China.

Worldwide, there are few studies on the transfer of MPs in terrestrial systems. In Mexico, there is only one study of MPs in terrestrial organisms; Huerta-Lwanga et al. (2017) determined the bioaccumulation of MPs in earthworms to be 14.8 ± 28.8 MPs and that in chicken feces to be 129.8 ± 82.3.

MPs have a large surface area, and therefore, these particles can absorb and transfer toxic substances (Salvador et al., 2017), such as heavy metals (HMs) (Alam et al., 2018), polycyclic aromatic hydrocarbons (PAHs), and organochlorine pesticides (OCPs) (Brennecke et al., 2016). These compounds are considered persistent, bioaccumulative, and toxic. Most of them are characterized by low solubility. They tend to breakdown outside of water in environmental media with similar hydrophobic properties, such as sediments, organic matter, and polymeric materials like plastics (Rochman, 2015). These toxic substances are found in the leachate from LFs so that MPs can act as vectors for environmental pollutants. Thus, given their ability to adsorb chemical pollutants, MPs represent a growing environmental concern.

Several studies have been carried out worldwide in which pollutants in MPs have been identified in other matrices, among which MPs have been examined in sediments and soils. For example, Camacho et al. (2018) analyzed pollutants in MPs on two beaches in the Canary Islands (Spain). The researchers found concentrations of polycyclic aromatic hydrocarbons (PAHs) ranging from 52.1–17,023.6 $ng \cdot g^{-1}$ and 35.1–8,725.8 $ng \cdot g^{-1}$, while organochlorine pesticides (OCPs) varied from 0.4–13,488.7 and 0.4–3,778.8 $ng \cdot g^{-1}$. Shi et al. (2020) analyzed PAHs and OCPs in MPs collected from Guangdong's eastern beaches. The concentrations of ΣPAHs and

ΣOCPs ranged from 11.2–7,710 ng·g^{-1} and from 2.2–1,970 ng·g^{-1}, respectively. Zhou et al. (2019) determined the heavy metal concentrations in MPs from soils of central China. The mean contents of Cd, Cr, Pb, Ag, Cu, Sb, Hg, Fe, and Mn in MPs were 0.6, 14.2, 13.1, 0.2, 13.7, 0.5, 0.1, 3367.4, and 14.5 μg·g^{-1}, respectively.

Vultures are the birds that are most affected by various human activities. This guild of birds is suffering significant population declines, leading to the extinction of several species (Buechley and Şekercioğlu, 2016). Approximately 70% of vulture species are threatened globally (IUCN, 2019).

The aims of the study were to evaluate the exposure of black vultures (*C. atratus*) to MPs and to determine the concentrations of heavy metals, polycyclic aromatic hydrocarbons and organochlorine pesticides in the MPs found in pellets from a landfill in Campeche, Mexico. This is the first study of contaminants from MPs in the pellets of avian raptors in Mexico.

2. Materials and methods

2.1 Study area

The study area is the Campeche city landfill, which occupies approximately 12.5 ha. Eight hectares have been used as a controlled dump since 1985. The LF has a life expectancy of 25 years, and wastes are deposited daily. There are two types of waste, which are classified as urban solid waste (USW) and special handling waste (SHW). The latter is in this category according to NOM-083SEMARNAT-2003 because more than 100 tons of this waste are received daily. Currently, between USW and SHW, an average of 350 tons is received daily, of which approximately 190 tons corresponds to USW (De Vecchi-Galindo et al., 2015).

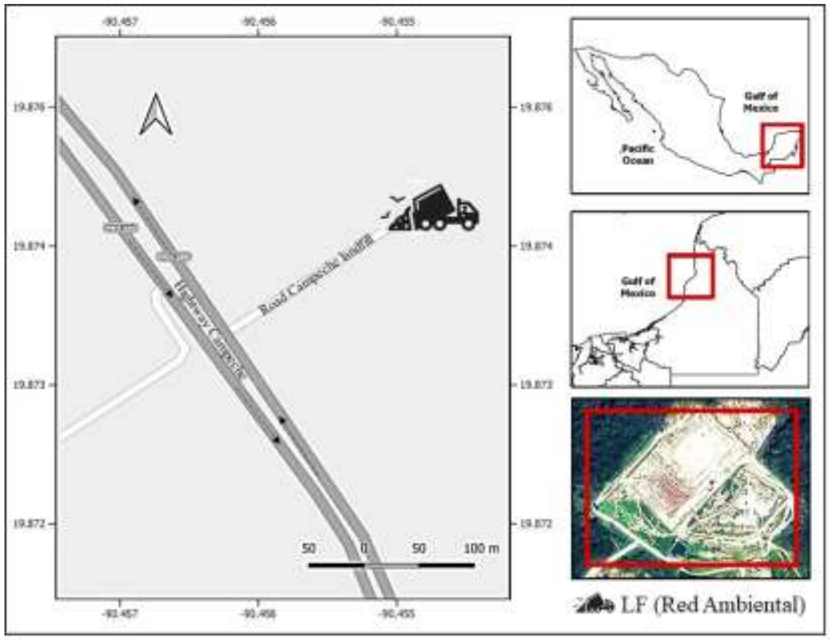

Fig. 1. Sampling site in the landfill (Red Ambiental) of Campeche, Mexico.

2.2 Sample collection

In 2019, the LF was monitored to find the spots where black vultures (*Coragyps atratus*) were perching (Figure 1). Once the perch was located, the area under the roost was visited two days in a row each year (in accordance with the permission of the Red Ambiental company to enter the city landfill). Soil was examined during the search for fresh pellets. To limit resampling of the same group of vultures in the same day, researchers only collected approximately 10 to 15 random pellets in two polygons of different areas according to the perches of the vultures. The first polygon was 38 m2, and the second was approximately 79 m2. Each pellet was placed in a No. 2 paper 10 x 24.5 cm bag labeled with the perch’s name and the date. In 2020, the pellets were collected in the same perch. In total, 58 vulture pellets were collected in 2019 (n = 24) and 2020 (n = 34).

Pellets were then taken to the laboratory, and each pellet's length and width were measured with a Vernier and frozen to prevent organic matter from degrading.

2.3. Microplastics identification

The pellets were thawed in an oven at 45 °C for 4 to 6 h (depending on each sample's humidity). Later, they were weighed using an analytical balance (VELAB Ve-204) with a precision of 0.1 mg. Each pellet was placed on a tray and dissected with forceps following the protocol of Augé, 2017. The proposed protocol consisted of 1) measuring the length and width of the pellets and 2) crumbling manually with tweezers. Subsequently, the organic materials were separated from the macro-, meso-, and MPs.

For identifying MPs, the modified method proposed by Hidalgo-Ruz et al. (2012) was followed. The MP selection criteria were their color, appearance, and shape. The content of the pellets was passed through a set of sieves with varying mesh sizes (5, 2.36, 1, 0.5, and 0.25 mm). The contents were handled with fine tweezers and a dissection needle to separate the particles. The MPs found (plastic nurdles, plastic fragments, synthetic fibers, and films) were transferred to vials, each with a respective label (key and site). The material contained in each sample and each fraction was identified with a stereoscopic magnifying glass (8–32 magnification) and later with a LEICA DM2500 microscope.

2.3.1. Fourier transform Infrared (FTIR) analysis

All All objects recognized as MPs were identified using the technique described by Matsuguma et al. (2017) in a Nicolet iS5 FTIR spectrophotometer

(Thermo Fisher Scientific) to analyze their composition. OMNIC software was used to identify MP compounds for FTIR spectroscopy and libraries.

FTIR is a fingerprinting technique based on spectra with specific bands and characteristics that is used to identify compounds present in MPs (Hummel, 2002). In this study, the spectral assignment characteristics were applied to selected samples with a greater than 80% match.

The FTIR analysis details were as follows: spectrum resolution, 4 cm^{-1}; 3–5 scans per sample; automatic atmosphere adjustment on; baseline correction, advanced ATR correction. For polymer identification, HR Hummel polymer and additives, HR rubber composites, and Sprouse polymer from ATR libraries were used. The ATR plate was wiped with alcohol before each reading. At the end of a batch of readings, the surfaces were sterilized.

2.3.2. Instrumental conditions and quality control of microplastics

Sources of contamination for MPs were minimized using the procedures of Hidalgo-Ruz et al. (2012). For example, to avoid possible contamination of the sample with synthetic fibers in the air, the process of opening the pellets was done quickly, and the contents were placed in glass vials and covered immediately to avoid possible contamination by airborne fibers. Latex gloves, glass utensils, and cotton lab coats were used at all times.

2.4. Trace metals analysis

Selected trace metals (Cd, Pb, Cu, Cr, Al and Hg) and metalloids (As) were determined in the MPs of vulture pellets using differential pulse anodic stripping voltammetry (DPASV), a modified version of the procedure proposed by Maciel et al. (2019). The weight of the representative mass of MPs per pellet for the determination of metals and metalloids presented a range of 0.012–1.305 g (2019) and 0.011–0.103 g (2020). Digestion was carried out in a MARS 5 microwave digestion system (CEM) and then transferred to a Teflon container with 10 ml of an acid mixture containing 9 ml of 65% HNO_3 and 1 ml of 37% HCl. Mercury and rotary disk electrodes were used for metal and metalloid determination (HMDE, RDE); Ag/AgCl/3 M KCl electrode was used as the reference, and a platinum electrode was used as the auxiliary electrode (Metrohm, Switzerland); metal and metalloid concentrations are given as $\mu g \cdot g^{-1}$ of dry weight.

The blank tests were prepared following the same procedure but without MP samples to determine background contamination. For the control test, three types of plastic were used: soft drink cap (polyethylene), hose (tygon), and fishing line (polyamides) as standard reference material. The glassware was washed with tap water, rinsed with deionized water, soaked in detergent for 24 h, and washed again with deionized water. They were then placed in an acid bath with 25% HNO_3 for 24 h and rinsed with deionized water.

To determine the concentration of metals and metalloids that the MPs were absorbing in the landfill, a test was carried out in which the plastics that were most frequently found in the MPs of the vulture pellets were analyzed. The polymer type of the MPs was identified, and new plastic material was obtained as a reference material to determine the basal concentrations of metals and metalloids that plastics contain in the “normal” state. For this, metals were analyzed in eight replicates of each reference polymer used, 5 with a standard and 3 without a standard.

Subsequently, to infer how much metals and metalloids had been absorbed by the MPs, the concentration of metals and metalloids present in the clean or reference plastic was subtracted from them. The preparation of the MP control was carried out with the modified methodology of Wang et al. (2020).

Adsorption experiments were carried out in five-fold excess to determine the potential of MPs to adsorb metals and metalloid ions. MPs (0.10 g) were used (clean food grade plastics). The initial concentration of the added solution for cadmium (Cd), lead (Pb), copper (Cu), chromium (Cr), aluminum (Al), arsenic (As), and mercury (Hg) was 2.5 mg/L in a background of 0.1 mol/L HNO_3. They were left to rest for 24 hours to see their ability to adsorb metals. The MP pools were placed in Teflon bottles (PTEE) containing 10 mL of solution with the initial concentration of the metal ion standards. Next, they were subjected to the same analytical procedure to evaluate the precision of the method to establish the recovery percentage. The metallic results of each polymer without a pattern were subtracted from the plastics of the same material that were found in the pellets to have the actual adsorption value at the landfill. The detection limits (LODs) of each metal and metalloid were Cd 0.05, Pb 0.03, Cu 0.05, Cr 0.05, Hg 0.02, As 0.02 and Al 0.05 $ng \cdot g^{-1}$.

2.5. Organochlorine pesticide (OCPs) and Polycyclic aromatic hydrocarbon (PAHs) analysis

The analysis of OCPs and PAHs in the MPs extracted from pellets was adapted from the method described by Tan et al. (2019). The weight of the representative mass of microplastics per pellet for the determination of OCPs and PAHs presented a range of 0.010–1.258 g (2019) and 0.011–0.109 g (2020). The dry mass microplastics from each pellet were transferred to 12-ml capped test tubes previously rinsed with acetone and hexane (J.T. Baker HPLC grade). Subsequently, ultrasonic extraction was carried out with 5 ml of hexane at 150 W for 10 min. After ultrasonic extraction, the extract was transferred to a 25-ml round-bottom flask. This ultrasonic extraction was repeated three times.

To optimize the clean-up and fraction steps in a chromatography column, the column was packed with silica gel (3 g) and sodium sulfate (1 g), previously conditioned with 5 mL of n-hexane. Elution procedures were established to obtain the clean-up and fractions of the analytes. The procedures were as follows:

Five milliliters of n-hexane, 5 mL of hexane/dichloromethane mixture (1:1, v/v), and 5 mL of dichloromethane were added. All the fractions were evaporated to dryness and resuspended in 0.5 mL of hexane for analysis by gas chromatography.

A Thermo Scientific TRACETM 1300 Series GC coupled with a TSQTM 8000 Evo triple quadrupole MS and TriPlusTM RSH liquid autosampler was employed for GC-MS/MS (Thermo Fisher Scientific, Austin, TX) analysis. Thermo Scientific TraceFinderTM 4.0 software was used to set up the data acquisition and data processing methods. The analysis was performed after injection of 1 µL of the sample in splitless mode. Chromatographic separation was carried out using a Trace™ TR-PCB 8 MS column, 50 m x 0.25 mm x 0.25 µm. The GC oven temperature was programmed as follows: initial temperature of 70 °C, hold at 70 °C for 2 min, increase the temperature to 300 °C at a rate of 20 °C/min, and hold at 300

°C for 8 min. The injection port temperature was set at 150 °C, and helium was used as the carrier gas at a flow rate of 1.0 mL/min. For the MS measurements, the ion source and transference line temperatures were 300 and 280 °C, respectively; argon was used as the collision gas; and the electron impact voltage was 70 eV. The GC–MS/MS program used ran under selective reaction monitoring (SRM) operating mode, detecting three transitions of the 20 pesticides (α, β, χ, δ-HCH; heptachlor, heptachlor epoxide; aldrin, dieldrin, endrin, endrin aldehyde, endrin ketone; endosulfan I, endosulfan II, endosulfan sulfate; chlordane cis (I) and tans (II); p,p′-DDE, p,p′-DDD, and p,p′-DDT, metoxcichloro) and 16 PAHs (naphthalene, acenaphthylene, acenaphthene, phenantrene, fluorene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, dibenz[a,h]anthracene and indenol[1,2,3-cd]pyrene). The range of the limit of detection (LOD) for each chemical group was HCHs 0.002 – 0.004 $\mu g \cdot mL^{-1}$, dienes 0.004 to 0.010 $\mu g \cdot mL^{-1}$, endosulfans 0.005 to 0.006 $\mu g \cdot mL^{-1}$, DDTs 0.010 to 0.031 $\mu g \cdot mL^{-1}$, chlordanes 0.004 $\mu g \cdot mL^{-1}$, heptachlors 0.004 to 0.006 $\mu g \cdot mL^{-1}$, mirex 0.006 $\mu g \cdot mL^{-1}$, and PAHs 0.006 to 0.018 $\mu g \cdot mL^{-1}$.

A quality control sample was tested for OCPs, PAHs, and a reagent blank sample for each batch of samples. Quality control samples were spiked with each of the standards to monitor the efficiency of extraction and analysis. The PAHs were identified by comparing their retention times with those of the aromatic standards of Supelco 48743 according to the priority PAHs of the EPA 610 method, and the recovery of PAHs was > 86%. The OCPs were analyzed using a mixture of standards of SUPELCO 47426-U CLP Organochlorine Pesticide Mix, and the recovery percentages for the OCPs were > 89% (Vallarino and Rendón von Osten, 2017). GC–MS/MS conditions scheduled with ion monitoring indicating retention time (min) by detected compound are shown in Supporting Information S1.

2.6. Source of the PAHs contamination

Combustion processes and petroleum products are the two main sources of anthropogenic PAHs found in the environment (Yunker et al., 1996). To distinguish pirogenic and petrogenic PAH sources from MPs in vulture pellets, we used three indices described by Khabouchi et al. (2020) in which a phenanthrene/anthracene ratio less than 10 reflected a pyrolytic origin, while values greater than 10 indicated a petrogenic origin. A fluoranthene/pyrene ratio lower than 1 indicated the pyrolytic origin of PAHs, and a ratio higher than 1 indicated that the source was petrogenic. The relationship between PAHs of low molecular weight (LMW) and high molecular weight (HMW) lower than 1 indicates a pyrogenic origin, but a value greater than 1 suggests a petrogenic source.

2.7. Statistical analysis

All data were evaluated to determine the normality of the residual distributions (Shapiro-Wilk and Kolmogorov-Smirnov test (KS test) $P > 0.05$). The mean data of MPs and the concentrations of pollutants between years followed a nonnormal distribution. Therefore, the Kruskal-Wallis-H test was used to determine the difference between the mean MPs per pellet and was used to analyze the differences between the following factors: size of the MPs between mesh, shape (fiber, fragment, nurdles), and the concentrations of PAHs, OCPs, metals and metalloids by year. All statistical analyses were carried out using Statistica® V.71 software (StatSoft, Inc, 2005).

3. Results and discussion

In total, 58 vulture pellets were analyzed in 2019 (24 pellets) and 2020 (34 pellets). In 2019, all the vulture pellets presented organic material (feathers, hairs, tissue, vegetation) with a total weight of 53.70 g (67.94%). The synthetic products had a total weight for microplastics of 3.86 g (2.44%), mesoplastics of 20.79 g (26.29%), and macroplastics of 2.64 g (3.33%).

Likewise, the samples in 2020 presented organic material with a total weight of 44.58 g (83.74%). Synthetic products had a total weight for microplastics of 1.62 g (1.53%), mesoplastics of 2.60 g (4.86%) and macroplastics of 5.44 g (10.17%).

MP numbers ($KS = 0.235$; $p < 0.01$) and MP weights ($KS = 0.386$; $p < 0.01$) were not normally distributed between years.

3.1 Abundance and weight of microplastics by mesh size

Seventy-seven percent of the pellets presented MPs, with a total of 389 microplastic particles from 58 vulture pellets with an average of 6.70 ± 9.28 MPs per pellet. In 2019, there were 225 MPs/total pellets (57.84%) with an average of 9.37 ± 12.69 MPs per pellet, and by 2020, there were 164 MPs/total pellets (42.15%) with an average of 6.83 ± 5.05 MPs per pellet. The highest amounts of MPs were found in the 5-mm mesh in 2019, with a total of 153 MPs and an average of 6.37 ± 11.40 per pellet. In 2020, a total of 87 MPs presented with an average of 3.62 ± 4.39 per pellet, as shown in Figure 2A.

The total weight of the MP mass in 2019 was 3.869 g with a total average of 0.184 ± 0.547 g. IN comparison, in 2020, the total mass weight was 1.622 g with a total average of 0.067 ± 0.653 g, approximately one-third of the weight of 2019. According to the sizes, the 5-mm mesh had the highest weight of MPs for both years, with 2.842 g (2019) and 0.869 g (2020) (Figure 2B). Details of the total MPs were classified by size and shape (fibers, fragments, films, and plastic nurdles).

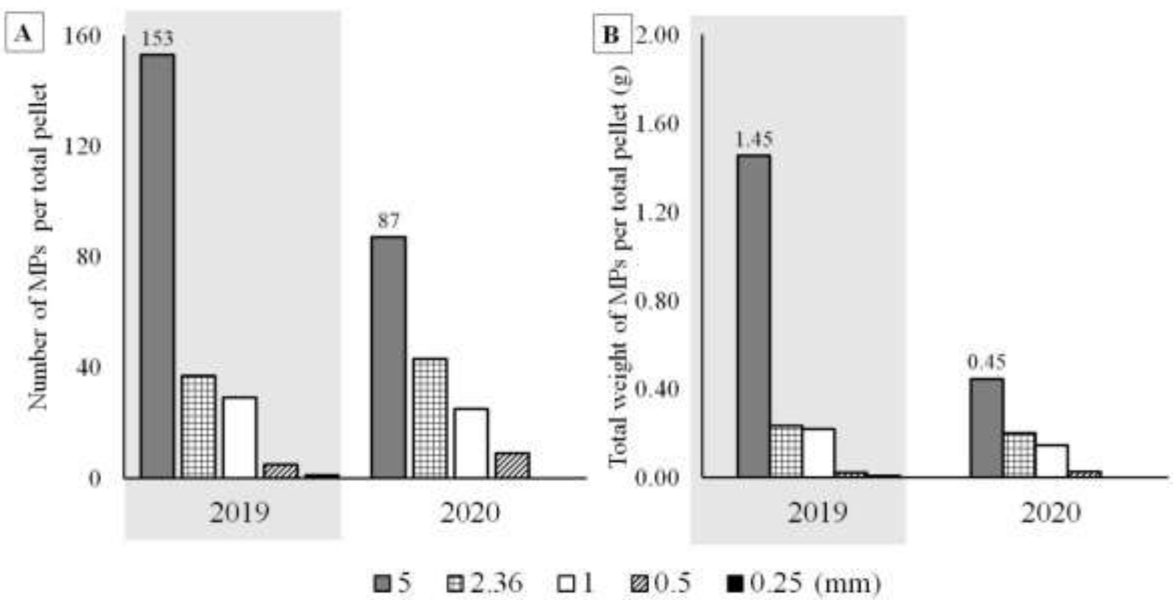

Fig. 2. Microplastic in Black vulture´s pellets per sampling year: A) MP counts by size and B) MPs weight by size. Different letters indicate significant differences among the treatments.

Vultures ingested different percentages of anthropogenic waste in both years, with 32.06% (2019) and 16.56% (2020) in proportion to the total weights of pellets per year. In 2019, they ingested approximately twice as much plastic in weight, as evidenced by the recovery of MPs in their pellets. They feed in the city’s LFs because the food supply is continuous since there is always organic waste that arrives every day. The ingestion of plastics in the diet of *C. atratus* may be due to the accidental consumption of this material when breaking bags to feed on organic waste. They can eliminate tiny pieces of food since their beak is proportionally longer and thinner than that of any other species of the Cathartidae family (Sazima, 2007).

In landfills, scavengers find large amounts of organic waste along with different synthetic materials, especially plastics, while foraging (Ballejo et al., 2021). MPs may adhere to organic debris and are easily ingested by vultures. It is also likely that macroplastics are ingested and in turn breakdown into MPs during corrosive digestion in the stomach due to the extreme acidity of gastric acid, which has pH values of approximately 1 (Houston and Cooper, 1975). Therefore, the MPs ingested by birds are good indicators of spatiotemporal fluctuations and differences in the abundance of plastic waste (van Franeker and Law, 2015).

3.2 Shape and color classification of microplastics from pellets

In prey and scavenger birds, the time that food spends in the crop predigestion is short, passing very quickly to the gizzard, where all indigestible elements, such as integument, hair, nails, feathers, culms, and bones, accumulate in a bolus called the pellet, which is expelled by the stimulation of the acidity of their stomach (Muñoz-Pedreros and Rau, 2004).

Figure 3 shows the MPs found in the pellets of black vultures (*C. atratus*) that perch in the LF. Large plastics were also found, such as hair nets, pieces of bags, and synthetic textiles, as shown in the figure in Supporting Information S2.

The highest number of fibers was found in 2019, with 142 (63.11%) MPs/total pellets, and the lowest amounts were found in 2020, with 47 (28.65%) MPs/total pellets. The fragments were presented in greater quantity in 2020, with 106 (64.63%) MPs/total pellets, compared to 2019, with 62 (27.55%) MPs/total pellets. However, in 2019, only films and plastic nurdles were found with 19 (8.44%) and 2 (0.88%) MPs/total pellets (Figure 4A).

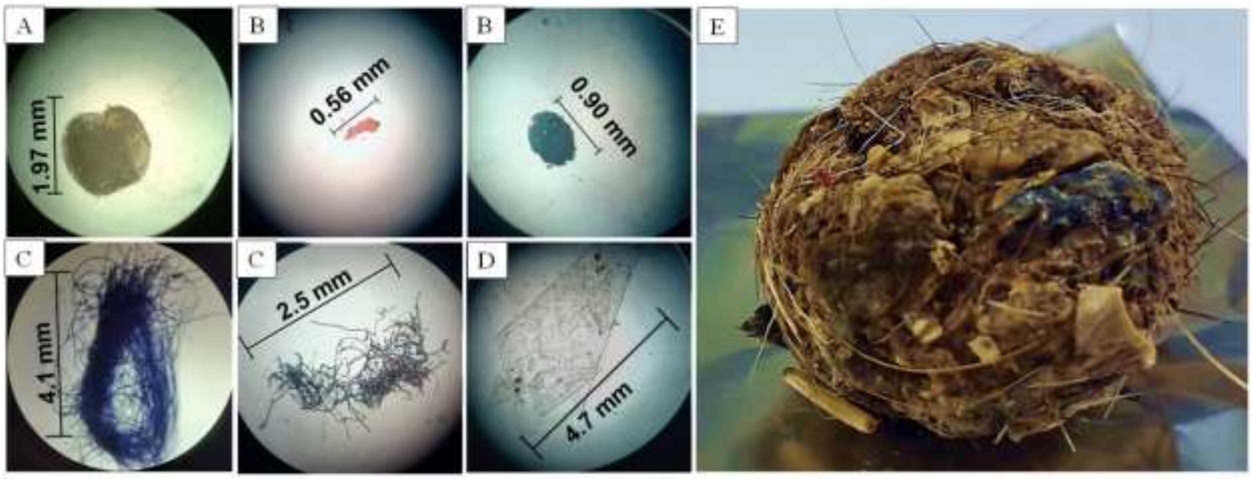

Fig. 3. Microplastics extracted from *C. atratus* pellets from Campeche: (A) plastics nurdles, (B) plastics fragments, (C) synthetic fibers, (D) films, (E) pellet´s Overview.

The fibers of MPs showed significant differences between years (KW-H $_{(1,58)}$ = 3.67, $p < 0.05$) but the fragments (KW-H $_{(1,58)}$ = 0.20, $p < 0.65$), films (KW-H $_{(1,58)}$ = 2.01, $p < 0.15$) and plastic nurdles (KW-H $_{(1,58)}$ = 1.41, $p < 0.23$) did not.

The black vulture (*C. atratus*) is a diurnal bird of prey species that feeds using sight to find carcasses more successfully in open areas than in closed areas such as forests (Houston, 1988). They unintentionally consume some plastics because they mistake them for carrion or bones due to their similar color (Iñigo, 1987).

MP color is essential, as some animal species are visual predators and can selectively hunt prey based on color (e.g., Boerger et al., 2010; Chen et al., 2020). Colors can be associated with the original polymeric components or added to the polymers during the manufacturing process. Over time, these colors can fade or change due to environmental weathering (Chen et al., 2020).

The MPs extracted from the *C. atratus* pellets presented nine colors. The most abundant pellets for 2019 were black, white, and yellow, with 35, 61, and 75 MPs/total pellets, respectively. Similarly, in 2020, the most abundant colors were black and white, with 55 and 80 MPs/total pellets, respectively, as shown in Figure 4B.

In 2019, yellow and white fibers were the most abundant, with 68 and 46 MPs/total pellets, respectively. In addition, 2019 contained the highest number of films, which were all transparent, with 19 MPs/total pellets and only two white nurdles. In 2020, black and white plastic fragments were the most abundant at 53 and 37 MPs/total pellets.

The low-calcium diet could explain the higher consumption of white MPs, with percentages of 27.22% (2019) and 33.53% (2020), and black MPs, with percentages of 15.55% (2019) and 48.78% (2020). Black vultures can confuse plastics for food when looking for small bones to easily digest (Ramsay and Houston, 1999).

One of the characteristics of avian scavengers' digestive system is the extreme gastric acidity, which has pH values of approximately 1 (Houston and Cooper, 1975). Therefore, the conditions of extreme acidity in the stomach facilitate the digestion of these volumes of carrion, producing corrosive effects in the foods they eat, such as bones (Andrews, 1990), and synthetic materials, such as plastics.

The MPs that these scavengers accidentally ingest are likely to turn black or yellowish in color due to digestive corrosion. As shown in Figure 4B, the percentage of yellowish plastics was 33.33%, and they were only present in 2019.

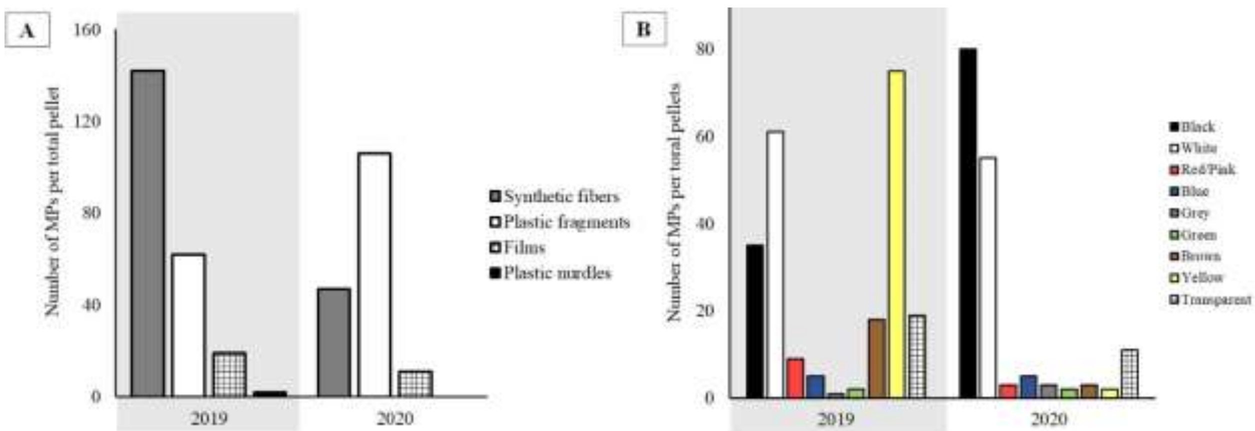

Fig. 4. Classification of microplastics by shape (A) and colors (B).

The first studies of contamination by MPs in Campeche have already been registered. Huerta-Lwanga et al. (2017) determined the bioaccumulation of MPs in earthworms to be 14.8 ± 28.8 items and that in chicken feces to be 129.8 ± 82.3 items. Other studies have been performed in aquatic organisms; Borges-Ramírez et al. (2020, 2019) found 316 MPs in six species of commercial consumption, on coasts with 1,392 items/m^2, and in urban canals with 756 items/m^2. In Mexico, there is no information on pollutants in MPs extracted from the pellets of birds of prey.

There is scarce information available worldwide on plastic ingestion by vultures. Nevertheless, some studies, such as that of Iñigo (1987), found synthetic products (39.1%), mainly plastic from bags in the pellets of black vultures (*C. atratus*) from Chiapas, Mexico. Mee et al. (2007) studied ten nests of the California Condor, where only one hatchling of 8 individuals survived. The dead chicks contained plastic garbage in their entrails (30–204.5 g). Additionally, another study by Augé (2017) analyzed *C. aura* pellets from the Falkland Islands, of which 58% of the pellets contained plastics.

3.4 Chemical composition of microplastics

MPs chemical composition can vary greatly depending on the material or materials from which they were manufactured. In this study, the spectral assignment characteristics were considered for selected samples with a coincidence greater than 80%, considering that the remaining percentage may be another type of plastic material. A total of 35 chemical compounds were identified in the pellets' MPs (Figure 5), as shown in Supporting Information S4.

The chemical characterization of the MPs in 2019 was composed of the following: polyethylene (97.2–99.5%) and oxidized polyethylene (96.1–98.7%) in the fibers; polyethylene (93.6–99.3%) in the fragments; polyethylene (94.4–98.2%) in the films; and fluoroelastomer (98.7%) in the plastic nurdles. Of the materials found in 2020, the fibers were composed of polyamides (89.5–99.3%), fragments of polyethylene (96.7–98.7%), and films of polyethylene (87–98.2%).

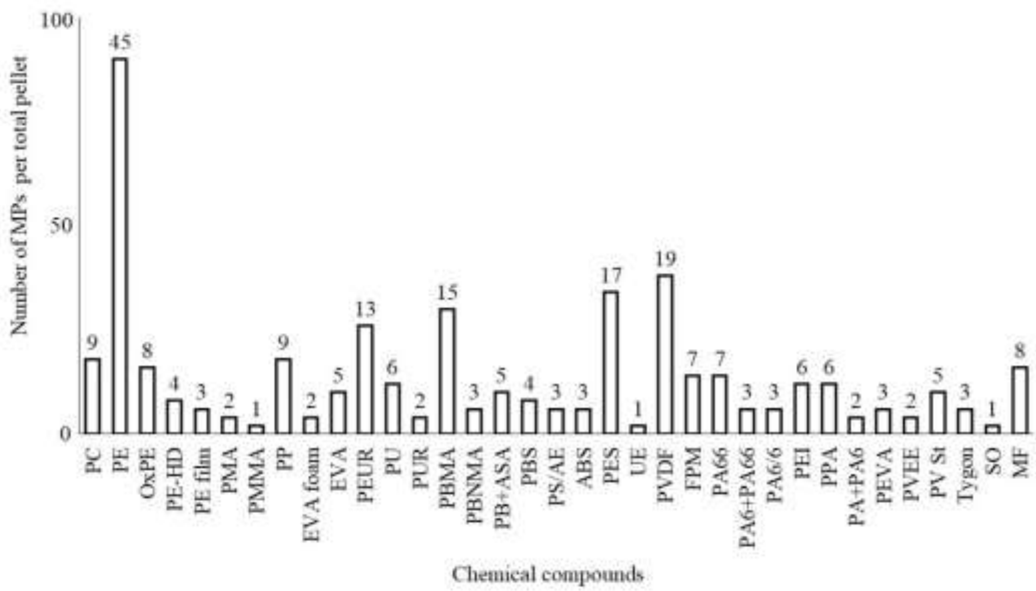

Fig. 5. Percentage of the frequency of chemical compounds in microplastics found in *C. atratus* pellets per year. The numbers inside the bars refer to the pellets´ frequency. PC:Polycarbonate, PE: Polyethylene, OxPE: Oxidised polyethylene, PE-HD: Polyethylene marlex catalyst, PE film: Polyethylene film, PMA: Polymethyl acrylate, PMMA: Polymethyl methacrylate, PP:Polypropylene, EVA foam: Ethylene vinyl acetate foam, EVA: Ethylene vinyl acetate, PUR: Plyurethane rubber, PU: Polyurethane, PEUR: Polyether urethane, PBMA: polybuthyl methacrylate, PBNMA: Polybenzyl methacrylate, PB + ASA: Polybutadiene acrylonitrile styrene, PBS:Polybutadiene styrene, PS/AE: Polystyrene acrylate ester, ABS:Polystyrene acrylonitrile butadiene, PES: Polyester, UE:Mixture urethane and polyester, PVDF: Polyvinylidene fluoride, FPM: Fluoroelastomer, PA66: Polyamide 66, PA6+PA66: Polyamide 6+polyamide 66, PA 6/6: Nylon 6/6, PEI: Polyetherimide, PPA: Polyphthalamide, PA+PA6: Nylon+poyamide 6, PEVA: Polyvinyl acetate ethylene, PVEE: Polyvinyl ethyl ether, PV St: Polyvinyl stereate, SO: Silicone oil, MF: Melamine resin.

Polyethylene and polyamide (89–99%) were the most abundant polymers in the vulture pellets. Only 7 compounds were found in 2020 (polymethyl acrylate, polymethyl methacrylate, polybutadiene styrene, mixture of polyester and urethane, nylon mixture and polyamide 6, polyvinyl acetate ethylene and polyvinyl ethyl ether), and 3 compounds were found in 2019 (polyethylene film, ethylene vinyl acetate polymer, and silicone oil).

3.5 Heavy metals (HMs) and aluminum in microplastics

Out of the 35 polymers identified in the pellets of *C. atratus*, three polymers (polyethylene, tygon, and polyamides) were used as reference materials to measure the metal and metalloid concentrations normally present in these materials. The three polymers selected were present in 30% of the MPs found in the pellets. Table 1 shows the recovery percentage and the detection limits of each of the metal and metalloid standards added to the replicas of these plastic polymers.

Table 1. Metals recovery rate from plastics particles as reference material (% recovery and RSD of 3 replicates samples)

Metals	Source material / polymer composition					
	Soda bottle lid / Polyethylene		Hose / Tygon		Fishing line / Polyamides	
	Recovery rate (%)	RSD	Recovery rate (%)	RSD	Recovery rate (%)	RSD
Cd	90	2.9	89	3.7	92	7.5
Pb	91	3.9	90	1.5	93	3.7
Cu	84	7.8	91	7.7	93	6.4
Cr	94	9.3	82	2	86	2.4
Hg	90	3.8	83	2	88	2.3
As	82	3.2	90	2.8	87	5.3
Al	91	2.9	89	5.2	89	7.9

The range values of metals and metalloids in the MPs of the *C. atratus* pellets for 2019 and 2020 presented the following order: Cd (0.0003 and 0.0006 $\mu g \cdot g^{-1}$), Cu (0.005 and 0.016 $\mu g \cdot g^{-1}$), Cr (0.12 and 0.16 $\mu g \cdot g^{-1}$), Pb (0.15 and 0.25 $\mu g \cdot g^{-1}$), Hg (0.40 and 0.61 $\mu g \cdot g^{-1}$), As (1.26 and 1.65 $\mu g \cdot g^{-1}$) and Al (35.60 and 15.82 $\mu g \cdot g^{-1}$).

The only metal and metalloid with high concentrations in both years were Al and As. This may be because MPs can absorb these pollutants in two ways: 1) when they are disposed of in the LF and 2) during digestion in the stomach of vultures.

Heavy metal leaching from plastic waste occurs in response to heat, light, and pH variation (Alam et al., 2018a; Cheng et al., 2010). Therefore, it is possible that MPs release or absorb heavy metals from the landfill because large amounts of aluminum cans, electronic waste, and batteries, among others, are thrown away.

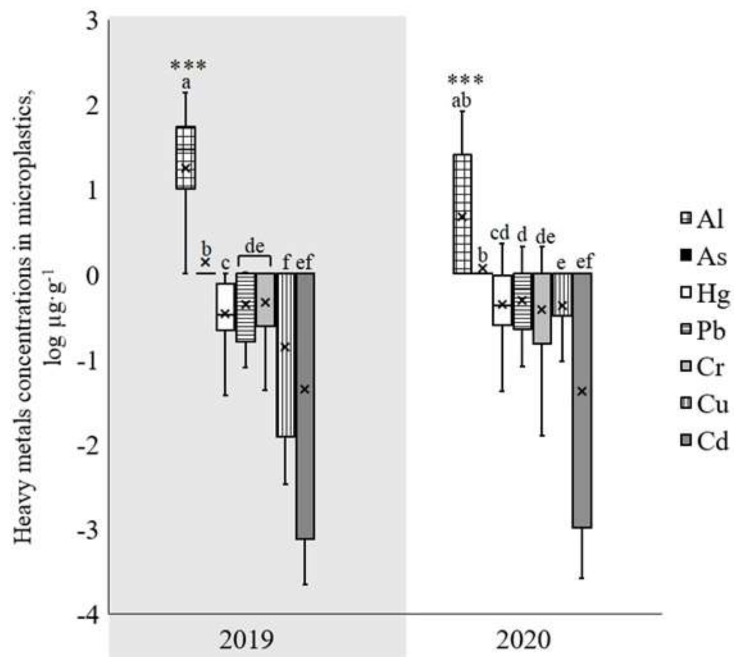

Fig. 6. Metal concentrations in microplastics per year (logarithmic values of concentrations in µg·g^{-1}). Each box plot indicates the median value (▬), the quartile values (x), and the lines go from the 10th to the 90th percentile. Statistical differences according to the Kruskal–Wallis test *** ($p \leq 0.001$). Different letters indicate significant differences between treatments (a,b,c).

The concentration of aluminum was much higher than the concentration of the other metals and showed significant differences between 2019 and 2020 (KW-H $_{(1,45)}$ = 6.86, p < 0.008). The concentrations of Cd (KW-H $_{(1,45)}$ = 0.18, p < 0.67), Pb (KW-H $_{(1,45)}$ = 0.65, p < 0.41), Cu (KW-H $_{(1,45)}$ = 0.57, p < 0.44), As (KW-H $_{(1,45)}$ = 2.19, p < 0.13), Cr (KW-H $_{(1,45)}$ = 0.16, p < 0.68) and Hg (KW-H $_{(1,45)}$ = 0.65, p < 0.41) did not present significant differences between years.

Maršić-Lučić et al. (2018) determined the levels of traces of metals in MPs in beach sediments in the Adriatic Sea of Croatia and showed concentrations of Cd: 1.3–5.44, Cr: 0.03–0.74, Cu: 0.08–0.61, and Pb: 0.04–0.85 $\mu g \cdot g^{-1}$. In another study, Dobaradaran et al. (2018) analyzed metals in MPs from coastline sediment along the Persian Gulf, and the mean metal concentrations in MPs were Al: 115, Fe: 531, Mn: 32.2, Cd: 0.035, Cr: 0.915, Ni: 2.03, Pb: 4.59, and Cu: 3.61 $\mu g \cdot g^{-1}$.

On the other hand, Kiyataka et al. (2014) determined the levels of HMs in high-density polyethylene yogurt containers. The results showed that the highest levels of As and Pb were 0.87 and 462.3 $mg \cdot kg^{-1}$, respectively.

Nnorom and Osibanjo (2009) analyzed HMs in mobile phone plastics (of different models and brands), where the mean for each metal was Pb: 58.3 ± 50.4 $mg \cdot kg^{-1}$, Cd: 69.9 ± 145 $mg \cdot kg^{-1}$, Ni: 432 ± 1905 $mg \cdot kg^{-1}$ and Ag: 403 ± 1888 $mg \cdot kg^{-1}$.

MPs can absorb heavy metals on their surface. Additionally, as the particles degenerate during ultraviolet (UV) light degradation, their ability to adsorb metals can change. Bandow et al. (2017) found that after 2000 h of UV irradiation, MPs increased the amount of Cu and Zn adsorption. Therefore, landfills can be the source of general environmental contamination by metals and metalloids. *Coragyps atratus* can serve as interesting biological indicators of contamination by metals and other anthropogenic pollutants. There are studies that show that birds are affected by the accumulation of metals, as these metals can suppress their immune systems. The enzymes that deposit calcium in the shell are adversely affected by traces of metals.

Metals also damage the endocrine system and cause reproductive dysfunction (Pan et al., 2008).

3.6 Levels of organochlorine pesticides (OCPs)

Organochlorine pesticides were grouped by chemical family for better interpretation. Thus, ΣDDTs comprise the sum of p,p′-DDE + p,p′-DDD + p,p′-DDT. Likewise, Σendosulfans is made up of the sum of endosulfan I + II + endosulfan sulfate. Aldrin + dieldrin + endrin aldehyde + endrin ketone are within Σdienes, and in the case of ΣHCHs, the α + β + χ + δ isomers of hexachlorocyclohexane (HCH) are grouped, since at least one of its congeners was found in each sample.

Figure 7 combines all the mean concentrations obtained in this study; ΣDDTs predominated in the 45 samples from both years with mean concentrations of 0.020 ± 0.027 ng·g^{-1} (2019) and 0.011 ± 0.010 ng·g^{-1} (2020). Another of the substances found was Σdienes, which were present in the 45 samples (45/45) and had the highest mean concentration in 2019, with 1.03 ± 1.14 ng·g^{-1}, as opposed to 2020, with a concentration of 0.78 ± 0.74 ng·g^{-1}.

In the case of endosulfan, it has only been banned for two years in Mexico; due to its persistence and possible recent use, it was found in all samples (45/45) with concentrations of 0.11 ± 0.10 ng·g^{-1} (2019) and 0.97 ± 1.50 ng·g^{-1} (2020). ΣHCHs were recorded in 43 samples (43/45), presenting concentrations of 0.008 ± 0.009 ng·g^{-1} (2019) and 0.005 ± 0.008 ng·g^{-1} (2020).

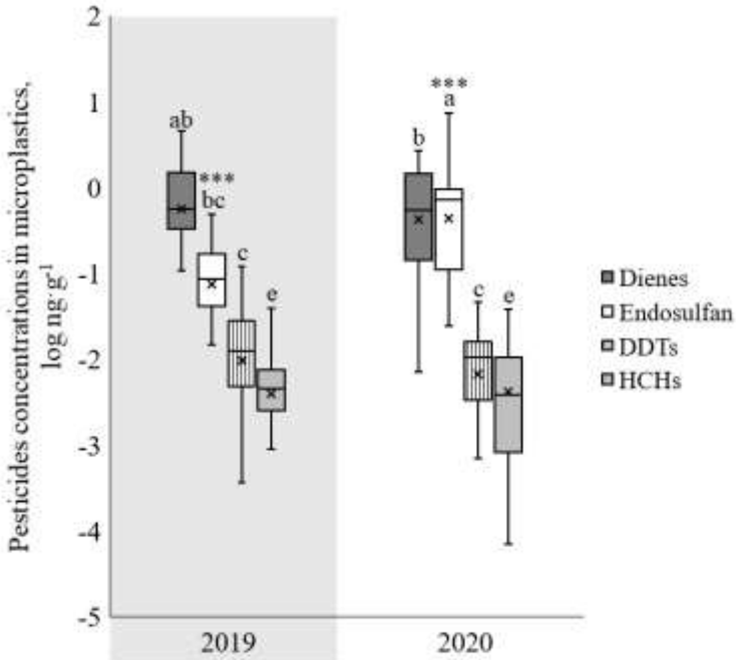

Fig. 7. Organochlorine pesticides' concentration in microplastics by year (logarithmic values of concentrations in ng·g^{-1}). Each box plot indicates the median value (▬), the quartile values (x), and the lines go from the 10th to the 90th percentile. Statistical differences according to the Kruskal–Wallis test *** ($p \leq 0.001$). Different letters indicate significant differences between treatments (a,b,c).

The concentrations of the four families of pollutants showed significant differences between years: ∑DDTs (KW-H $_{(1,45)}$ = 30.31, p < 0.001), ∑dienes (KW-H $_{(1,45)}$ = 16.03, p < 0.001), ∑endosulfans (KW-H $_{(1,45)}$ = 32.60, p < 0.001) and HCHs with (KW-H $_{(1,45)}$ = 8.74, p < 0.003).

The mean concentrations of OCPs in the MPs in this study varied in both years. For ∑DDTs and ∑HCHs, in 2019, they presented concentrations of 0.020 and 0.008 ng·g^{-1}, and the concentrations determined in 2020 were 0.011 and 0.005 ng·g^{-1}, respectively. ∑Dienes and ∑endosulfans were the two families with high concentrations, containing 1.03 and 0.11 ng·g^{-1} in 2019 and 0.78 and 0.97 ng·g^{-1} in 2020. The city of Campeche is the largest generator of municipal solid waste (MSW) in the state. These concentrations were generally lower than the values reported elsewhere. For example, Capriotti et al. (2021) reported concentrations of ∑HCHs in MPs from inshore areas of 99.17 $ng·g^{-1}$ and ∑DDTs in MPs from offshore areas of 77.79 $ng·g^{-1}$ among the surface waters of the central Adriatic Sea (Italy). Another study by Shi et al. (2020) estimated the ∑DDTs and ∑HCHs concentrations in sedimentary MPs from eastern Guangdong at 1180 and 782 $ng·g^{-1}$, respectively. They suggest that contamination by OCPs is likely due to agricultural, aquaculture, and fishing activities in the study area.

MPs from the coastal areas of central Chile presented concentrations of ∑DDTs of 12.61 $ng·g^{-1}$ (Pozo et al., 2020). In Chile, DDTs have been banned since 1984 by SAG (SAG, 1980). The authors suggest that the concentrations of DDTs they found are those that are already in the environment (Pozo et al., 2014). Other studies by Lo et al. (2018) examined OCPs in sedimentary MPs from Hong Kong and determined that the ∑DDT concentrations ranged from 1.96–626 $ng·g^{-1}$ and ∑HCHs ranged from 5.02–63.5 $ng·g^{-1}$. The authors suggest that these OCP concentrations were higher, probably due to aquaculture activities in the South China Sea.

There are no studies on the determination of OCPs in MPs extracted from pellets of avian raptors. However, there are studies of these contaminants in birds. For example, Albert et al. (1989) determined that DDE residue levels ranged from 3.4 – 20 ppm in the shells of *C. atratus* eggs from Chiapas, Mexico, and that the shells were 5.2% thinner than the average estimated before DDT use.

Bakir et al. (2012) found that DDT had a higher affinity for MPs from polyethylene and polyvinyl chloride, indicating that MPs could enrich the contamination of organochlorine pesticides in the environment. The importance of analyzing contaminants in MPs is that they can absorb and transport toxic substances such as OCPs in organisms that accidentally or intentionally consume them. The vultures are exposed to MP consumption and their contaminants since they feed in the LF.

Adverse effects of OCPs in birds include impaired reproductive behavior and impaired metabolic liver activities (Giesy et al., 2003; Mineau and Whiteside, 2013). One of the well-known sublethal effects caused by DDT metabolites, particularly p,p'-DDE, is the thinning of the eggshell, leading to reproduction failure (Blus, 2011; Ratcliffe, 1967).

3.7 Levels of polycyclic aromatic hydrocarbon (PAHs)

The presence of 15 (15/16) hydrocarbons was observed in the MPs extracted from pellets. Nine of these presented significant differences between years: acenaphthene ($K\text{-}W_{(1,45)} = 6.49$, $p < 0.01$), anthracene ($K\text{-}W_{(1,45)} = 19.30$, $p < 0.001$), benzo{b}fluoranthene ($K\text{-}W_{(1,45)} = 10.14$, $p <0.001$), benzo {a} anthracene ($K\text{-}W_{(1,45)} = 4.97$, $p < 0.02$), benzo{a}pyrene ($K\text{-}W_{(1,45)} = 6.03$, $p < 0.01$), chrysene ($K\text{-}W_{(1,45)} = 6.49$, $p < 0.01$), dibenzo{a, h}anthracene ($K\text{-}W_{(1,45)} = 11.03$, $p < 0.001$), indenol{123cd}pyrene ($K\text{-}W_{(1,45)} = 12.11$, $p < 0.001$), and naphthalene ($K\text{-}W_{(1,45)} = 5.52$, $p < 0.01$).

According to the number of rings, the compound with the highest average concentration for both years was acenaphthylene (3 rings), with 10.51 ± 7.88 $ng \cdot g^{-1}$

(2019) and 10.61 ± 18 ng·g^{-1} (2020), followed by fluoranthene (4 rings), with 6.07 ± 5.22 ng·g^{-1} (2019) and 8.21 ± 10.38 ng·g^{-1} (2020), and finally fluorene (3 rings), with 5.97 ± 5.34 ng·g^{-1} (2019) and 5.32 ± 10.49 ng·g^{-1} (2020), as shown in Figure 8B. As shown in Figure 8A, the highest average concentrations occurred for compounds with 3 and 5 rings for both years, with 24.03 and 13.36 ng·g^{-1} for 2019 and 20.20 and 21.91 ng·g^{-1} for 2020.

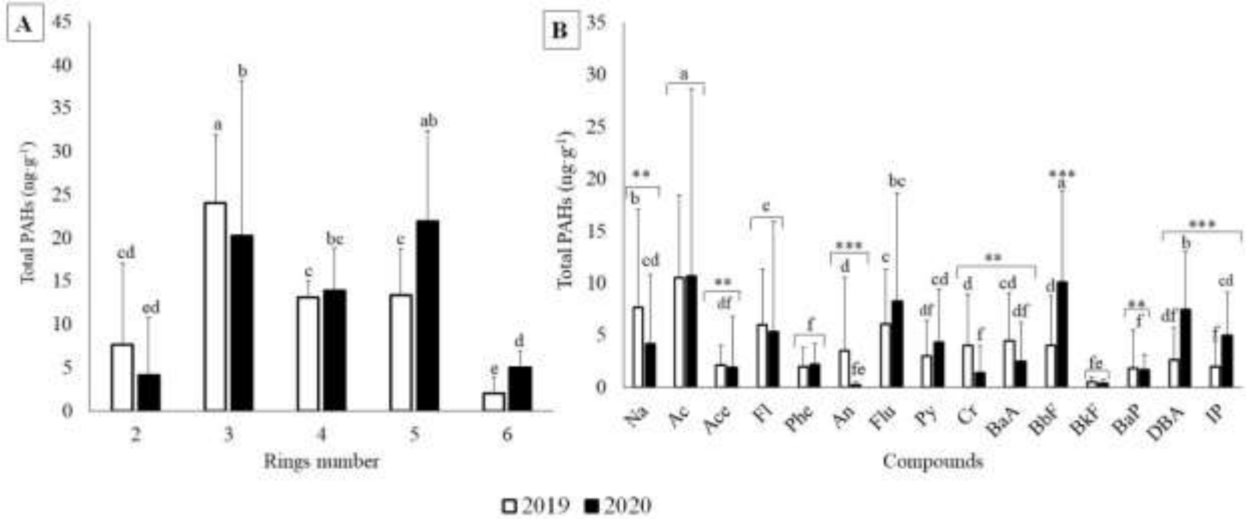

Fig. 8. Polycyclic aromatic hydrocarbons in microplastics by A) ring number and B) total compound. Statistical differences according to the Kruskal–Wallis test ** ($p \leq 0.01$), *** ($p \leq 0.001$). Significant differences between treatments are indicated by different letters (a,b,c).

The Phe/Ant index (2014) showed that the origin of PAHs is pyrogenic, with 35 (35/45) samples having values <10. Similarly, the Flu/Py ratio gave us values of less than 1 for 35 (35/45) samples. The LMW/HMW rate showed that the sources of PAHs were pyrogenic with 32 (32/45) samples with values <1, in which the main source of pyrogenic PAHs in Campeche is probably the result of slash-and-burn agriculture, an ancestral practice of many farmers in Mexico (Ortiz-Álvarez et al., 2003). PAHs released into the environment can be transported long distances by atmospheric pathways (Primbs et al., 2008; Genualdi et al., 2009; Xue and Warshawsky, 2004). Therefore, PAHs determined in MPs in vulture granules may have been absorbed through atmospheric deposition in the city landfill.

The concentrations of ∑PAHs in the MPs found in this study ranged from 0–31.25 $ng \cdot g^{-1}$ in 2019 and from 0–71.35 $ng \cdot g^{-1}$ in 2020. These concentrations were generally lower than the values reported elsewhere.

Mai et al. (2018) carried out PAH measurements in MPs collected in the Bohai and Huanghai Seas, China, and indicated that the concentrations of the sum of 16 PAHs were in the range of 3,400 to 119,000 $ng \cdot g^{-1}$. The authors suggested a mixed origin of PAHs absorbed from the MPs in the Bohai and Huanghai seas, with 70% coming from a diesel source and 30% from a gasoline source. Camacho et al. (2019) determined that PAHs in MPs ranged from 35.1–17,023.6 $ng \cdot g^{-1}$ in marine plastic debris from Canary Island beaches (Spain), suggesting contamination of pyrolytic origin rather than petrogenic origin. Another study by Capriotti et al. (2021) reported that the highest concentrations of ∑PAHs were found in coastal MPs: 408 $ng \cdot g^{-1}$ in surface waters of the central Adriatic Sea (Italy), with a dominance of low ring PAHs, suggesting that petroleum-derived pollutants are probably the primary sources of MP contamination.

There are no studies on the determination of PAHs in MPs extracted from vulture pellets. However, studies indicate that PAHs can cause a broad spectrum of health effects in birds, from altering molecular and physiological processes to modifying liver and immune function, increasing physical deformities, and reducing reproductive success and growth (Albers, 2006; Paruk et al., 2016). The PAH concentrations found in MPs in black vulture granules in this study indicate significant exposure to PAHs absorbed by MPs from LFs and accidental ingestion of plastics by these scavengers.

4. Conclusions

The number of MPs found in the pellets in the two years was 225 ± 13.04 (2019) and 164 ± 5.05 (2020). The pellets, on average, had an MP load per pellet of 6.7 ± 5.8 MPs/total pellets.

A total of 35 chemical compounds were identified in the MPs. Polyethylene and polyamide (89–99%) were the most abundant polymers in the vulture pellets. Our results show that MP ingestion by vultures is both accidental and intentional because this species selects its food through sight and not by smell, similar to the other Cathartids. The majority of MPs found in the pellets were colored black and white, similar to carrion and bones.

Only one of the seven metals analyzed (Cd, Pb, Cu, Cr, Hg, Al, and As), namely, Al, was detected in all the microplastic samples. The amounts of Al (35.59 and 15.82 ng·g-1) increased from 7 to 17 times in the MPs based on the polymers used as reference material. However, there was a decrease of half of the Al between the two years, probably due to metal leaching through aging and fragmentation of plastics to MPs in the city landfill.

Four families of OCPs (ΣDDTs, Σdienes, Σendosulfans, and ΣHCHs) were present in the MPs, with 100% in 2019 and 97% in 2020. The highest average concentrations were Σdienes at 1.03 ± 1.14 $ng\cdot g^{-1}$ (2019) and Σendosulfans at 0.97

± 1.50 $ng \cdot g^{-1}$ (2020). The concentrations of OCPs in the MPs of this study were low compared to those of other studies. However, OCPs in the MPs indicate adsorption, acting as vectors for the transport of contaminants in the pellets of *C. atratus*.

Of the PAHs considered carcinogens by the IARC (2016), benzoa{a}pyrene was found in both years at 37.80 $ng \cdot g^{-1}$ (2019) and 40.22 $ng \cdot g^{-1}$. The concentrations of PAHs by their number of rings indicate that they have a pyrogenic and petrogenic origin. This may be a result of the burning seasons for agricultural activities and of the adsorption of oils derived from petroleum found in the garbage. Many of these PAHs dispersed in the environment reach city landfills and are adsorbed by MPs.

The index of Phe/An, Flou/Py and LMW/HMW suggested that the PAHs found in the MPs of vulture pellets probably originated from the incomplete combustion of biomass during agricultural activities and forest fires and their transportation by atmospheric sources to the sanitary landfill, which indicates the absorption of these hydrocarbons by MPs.

Chemical contamination from MPs is a global concern, given MPs' potential to enter living systems through plastic waste. Vultures regurgitate MPs and their pollutants through their pellets, and in this way, we have evidence that they are exposed to these toxic compounds from the moment of ingestion. More research is required to assess the exposure of vultures to MPs and contaminants. Vultures perform an important ecosystemic service by removing decomposing organic material from the environment, reducing the production of pathogens when consuming carrion and reducing the risks of human diseases. Scavengers such as vultures are exposed to various pollutants; therefore, it is necessary to study the possible effects of pollutants and MPs in vultures. More research is needed on the origin of the contaminants detected in the MPs extracted from the pellets, since the contaminants may come from the environment or possibly from the digestion processes in the stomach of avian raptors and scavengers.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Capítulo III

Artículo 2. Black vulture (*Coragyps atratus*) feathers as bioindicators of exposure to metal contamination in urban, semi-urban, and rural areas from Campeche, Mexico.

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Co-Authors: Merle Borges Ramirez; Griselda Escalona Segura; Esperanza Huerta Lwanga; Eduardo Iñigo Elias; Marina Rodriguez Yah
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Black vulture (*Coragyps atratus*) feathers as bioindicators of exposure to metal contamination in urban, semi-urban, and rural areas from Campeche, Mexico.

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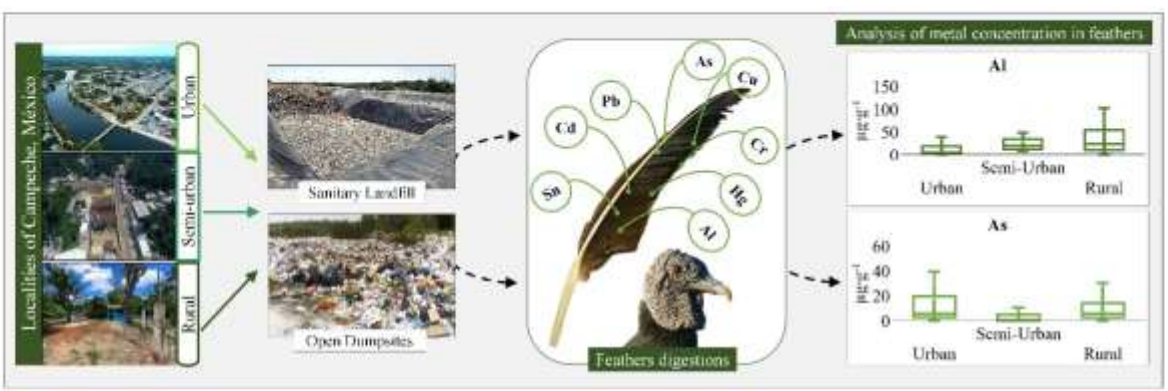
Abstract

Population growth and humanity dependency of “single-use plastic products (SUPs)’ has led to the generation of large amounts of garbage around the world. This urban and rural garbage ends in sanitary landfills (SL) and open dumpsites (OD). Both landfills are one of the sources of soil, water, and air pollution due to the release of toxic compounds such as metals. However, in the ODs, contamination by metals is greater since in these sites is a common practice to burn the garbage openly when it exceeds the volume of the site. In both tropical and temperate environments scavenging birds such as the Black Vulture (*Coragyps atratus*) that use these sites as a feeding area are probably exposed to metals and other “persistent bioaccumulative toxic substances (PBTs)" released in OD and SL. The objective of this study is to evaluate the presence of toxic metals, which places have more or less contamination, as well as the distribution of metals in molting feathers of *C. atratus* from OD and SL from urban, semi-urban and rural locations in Campeche, Mexico. A total of 125 Black Vulture primary and secondary wing feathers were collected from the OD and SL. Metals were determined by voltammetry, through acid digestion. The highest levels of metals occurred in landfills in urban, semi-urban, and rural locations. The metals with the highest concentration were Al with an average of 35.67 ± 33.51 $\mu g \cdot g^{-1}$ (Rural) and As with 16.20 ± 30.06 $\mu g \cdot g^{-1}$ (Urban). Black vulture from dumpsites are good bioindicators of what human consume in urban, semi-urban and rural environments, however the conservation of vultures is of great importance, since these scavenger birds perform ecosystem services by feeding on decomposing organic material.

Graphical abstract



Key words: Metals, sanitary landfill, open dumpsite, scavenger birds.

1. Introduction

The mismanagement of municipal solid waste (MSW) has led to the generation of open-air dumps. The state of Campeche generates around 930 tons of garbage daily among all its municipalities, equivalent to 27,900 tons per month (López-Méndez, 2019). Only the state capital, San Francisco de Campeche (SFC), has one sanitary landfill (SL), where the MSW is deposited (Borges-Ramírez et al., 2021), and in the other localities of the state, there are open dumpsites (OD) (INEGI, 2015).

Solid waste is heterogeneous and includes electronic products, plastics, paint residues, used batteries, old clothes, syringes, paper, and organic materials, which are the origin of the high levels of metals in the soil of open dumpsites and sanitary landfills (Praveena and Rao, 2016). The release of metals occurs through the incineration of MSW, which is one of the most used practices in OD to reduce the volume of garbage by 90%, producing the release of metals that are concentrated in the ashes (Cogut, 2016; Abanades et al., 2002). During garbage combustion some low boiling point metals undergo volatilization processes such as mercury and cadmium, other metals such as copper are trapped almost entirely in the bottom ash. Metals from bottom ash accumulate in the solid material left over from burning garbage, contaminating the soil. Metals from fly ash are dispersed by the wind and can travel long distances entering food chains (Adama et al., 2016). Lead and arsenic are distributed almost 1/3 in flying ash and 2/3 in bottom ash (Belevi and Moench, 2000). The amount and type of metals emitted vary significantly depending on the content of the waste being burned; for example, electronic waste, which is made of different metals, releases large amounts of these pollutants when burned (Mavropoulos, 2015).

SL and OD harbor large numbers of scavenger birds such as vultures, crows, and gulls, among others (Karimian, et al., 2021). Birds can be exposed to multiple pollutants such as organochlorides and metals both externally through physical contact and internally through consumption of contaminated food scraps (Borges-Ramírez et al., 2021; Roux and Marra, 2007).

However, other sources of metal input in OD and SL are charcoal kilns and cement manufacturing (Iqbal et al., 2016; Kumar et al., 2008). These charcoal kilns release metals during wood combustion (Orłowska et al., 2014). Similarly, cement production plants also release metals, spreading them through industrial dust (Hua et al., 2016). Metals in cement plants are present in complex particulate forms, oxides, salts, or gaseous forms (Rühling and Tyler, 2001). Therefore, individuals of *C. atratus* that use landfills have a high probability of accumulating metals in their feathers.

It has been documented since the 1960s that some metals can kill or harm birds; for example, they are related to a reduction of bird body weight, mortality of embryos in the egg, reduced growth rate, damage to their immune system, and other physiological systems such as the endocrine or reproductive. (Vallverdú-Coll et al., 2019). Metals bioaccumulate and biomagnified in organisms through tissues such as feathers in the case of birds (Markowski et al., 2013; Ganz et al., 2018; Jaspers et al., 2019). Feathers, during their growth, can incorporate large amounts of metals into protein molecules because they are directly connected to the bloodstream

(Abbasi et al., 2015). Therefore, large feathers (for example, primary or secondary wing feathers, as well as tail feathers) are considered good indicators of metal contamination (Carneiro et al., 2015; Ganz et al., 2018; Jaspers et al., 2019).

Around the world, bird feathers have been used in many biomonitoring studies because birds have different trophic levels, and the use of molted feathers is considered a non-invasive test. Molting is the process of shedding some or all the feathers and replacing them with new feathers. Adult birds continue molting in yearly cycles, which vary across species. Some bird species shed their flight feathers simultaneously once a year in late summer after breeding (Legagneux et al., 2013). Black Vultures replace their flight feathers in a staggered manner, taking approximately two years to complete all their molted feathers (Graves et al., 2020).

There are few studies of metals in scavenger bird feathers; for example, Kushwaha (2016) analyzed *Gyps indicus* feathers from the Bundelkhand region (India) determining Cd, Pb and Cu with a range of 0.1 – 0.4 $\mu g \cdot g^{-1}$, 0.47 – 6.4 $\mu g \cdot g^{-1}$ and 2.9 – 8.11 $\mu g \cdot g^{-1}$. Another study by Durmus (2018) reported Hg with concentrations of 0.45 $\mu g \cdot g^{-1}$ in *Gypaetus barbatus* feathers from the eastern region of Turkey, where Hg contamination may be due to agricultural and urban activities on the site.

Vultures play a crucial role in the functioning and stability of ecosystems; between 60 and 95% of carcasses are located and consumed by these birds (Ogada et al., 2012). The reduction of scavenger bird species has ecological, social, and economic implications related to regulatory and cultural ecosystem services (Wilbur and Jackson, 1983; Houston, 1988; Whelan et al., 2015). However, most vulture species have experienced drastic global declines because of multiple causes including metal contamination (Wilbur and Jackson, 1983; Kelly and Johnson, 2011; Krüger and Amar, 2018). In the American continent, there are various indications that various populations of this species have been exterminated and others continue to decline (Fink et al., 2022). Íñigo (1999) explained that the populations of this species in Mexico are disappearing locally from many regions of the country.

There are five species of vultures currently exist in Mexico: The Black Vulture (*Coragyps atratus*), Turkey Vulture (*Cathartes aura*), Lesser Yellow-headed Vulture (*Cathartes burrovianus*), King Vulture (*Sarcoramphus papa*) and the California Condor (*Gymnogyps californianus*) (Iñigo, 1987). The *C. atratus* is the most abundant bird of prey on the American continent, and it is distributed from the center of the United States of America to Patagonia. In Mexico, this carrion bird is very common. However, they have been disappearing locally in many regions of the country (Iñigo-Elías, 1987; Buckley, et al. 2022).

Vultures are exposed to metals because they feed in sanitary landfills and open dumpsites (Iñigo-Elías, 1987; Ballejo et al., 2021), in addition to the fact that scavenger birds occupy high trophic levels and are long-lived (Ogada et al., 2012). Therefore, this study aims to evaluate which type of metal is the most abundant in urban, semi-urban and rural localities, and to determine if Black Vultures are more exposed to metal contamination in OD or SL.

2. Materials and methods

2.1. Study area

The state of Campeche is in southeastern Mexico (Fig. 1). It covers an area of approximately 60,000 km^2 with a population of around 928,363 inhabitants (INEGI, 2020). Campeche has the largest protected area (22,787.65 km^2) Calakmul in the Yucatan Peninsula, which represents 40% of the state's area (Rada et al., 2015).

There are three types of population settlement in the state: rural (<2,500 inhabitants), semi-urban (≥2,500 and <15,000) and urban (≥15,000) (INEE, 2016).

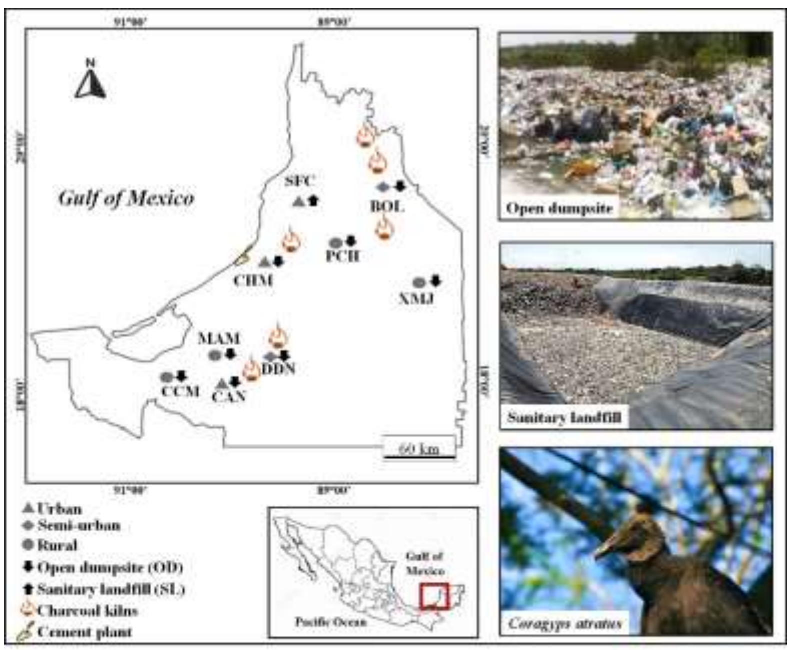


Fig. 1. Sample sites of open dumpsites and a sanitary landfill from localities in urban, semi-urban, and rural areas of Campeche, Mexico.

2.2. Sampling Method

Samples of flight feathers of *C. atratus* were collected in nine localities in the state of Campeche (Fig. 1, Table 1). For each site, between 8 to 15 feathers were collected, to limit resampling from the same group of vultures on the same day, only feathers were collected at random. Each feather was placed in a 10 × 24.5cm No. 2 paper bag labeled with the site´s name and the date. Prior to analysis, samples were washed in triton and deionized water to minimize adherent external contamination and then dried at 45°C for 24 hours.

Table 1. Feather collection localities in the state of Campeche, Mexico according to the type of area (urban, semi-urban, and rural).

Localities	Key	Habitants	Area	Type of Garbage Site
San Francisco de Campeche	SFC	294,077	Urban	Sanitary Landfill (SL)
Candelaria	CAN	43,879	Urban	Open dumpsites (OD)
Champotón	CHM	30,881	Urban	
Bolonchén	BOL	3,975	Semi-urban	
División del Norte	DDN	3,600	Semi-urban	
Pich	PCH	2,055	Rural	
Mamantel	MAM	1,417	Rural	
Xmejía	XMJ	1,123	Rural	
Conquista Campesina	CCM	887	Rural	

2.3. Trace metals analysis

Selected trace metals (Cd, Pb, Cu, Cr, Al, As, Hg, and Sn) were determined in *C. atratus* feathers using differential pulse anodic stripping voltammetry (DPASV) VA Computrace 797 Metrohm (Switzerland), a modified version of the procedure proposed by Tajik and Beitollahi (2020). The representative weight mass of feathers for the determination of metals and metalloids was 0.3 g. Digestion was carried out in a MARS 5 microwave digestion system (CEM), transferring the samples to a Teflon container with 3 mL of an acid mixture of 9 mL of 65% HNO_3 and 1 mL of 37% HCl. For the determination of metals, mercury electrodes and rotating discs (HMDE, RDE) were used; an Ag/AgCl (3M KCl) electrode was used as a reference and a platinum electrode (Metrohm, Switzerland) as auxiliary; metal concentrations are given as μg·g^{-1} of dry weight.

2.4. Statistical analysis

The Shapiro-Wilk and Kolmogórov-Smirnov tests (KS test) did not show a normal distribution in the data ($p < 0.05$). The differences in the concentrations of metals in the feathers were determined with the Kruskal-Wallis-H test for multiple comparisons between localities and areas (urban, semi-urban and rural). All statistical analyses were performed using Statistica V.7.1 software (StatSof, 2005).

3. Results and discussion

A total of 125 *C. atratus* feathers from a sanitary landfill and eight from open dumpsites from localities were analyzed in the state of Campeche, Mexico. The eight metals analyzed are detected in all the feathers, which indicates that the Black Vulture of OD and SL are exposed to metal contamination. Only Cr, Cd, and As are considered carcinogenic contaminants for humans (IARC, 2011). However, the toxic concentration of metals can cause teratogenic, mutagenic, and carcinogenic effects in biological organisms, including birds (Hashmi et al., 2013; Albayrak and Karadeniz Pekgöz, 2021).

The localities with the highest concentrations of metals in the feathers of *C. atratus* were CHM with Cu: 0.35, Cr: 21.86 and As: 30.23 μg·g^{-1}; BOL with Cd: 0.18 μg·g^{-1}; DDN with Al: 46.08, Sn: 9.33 and Pb: 0.44 μg·g^{-1}; PCH with Hg: 0.29 μg·g^{-1}; MAM with Al: 58.76, Cu: 0.15 and Pb: 0.76 μg·g^{-1}; XMJ with 23.86 μg·g^{-1}; CCM with Sn: 9.98, Cd: 0.11 and As: 28.51 μg·g^{-1}. The average values of each metal of the three areas are shown in Table 2.

The data on metal concentrations in feathers did not present a normal distribution between the locations (Kolmogorov-Smirnov KS test): Al (KS = 0.12; $p < 0.01$), As (KS = 0.32; $p < 0.01$), Cr (KS = 0.31; $p < 0.01$), Sn (KS = 0.27; $p < 0.01$), Cd (KS = 0.26; $p < 0.01$), Pb (KS = 0.18; $p < 0.01$), Cu (KS = 0.32; $p < 0.01$), and Hg (KS = 0.23; $p < 0.01$). The concentrations of each metal presented significant differences by location, Al ($KW\text{-}H_{(8,125)} = 49.11$, $p < 0.001$), As ($KW\text{-}H_{(8,125)} = 39.43$, $p < 0.001$), Cr ($KW\text{-}H_{(8,125)} = 49.12$, $p < 0.001$), Sn ($KW\text{-}H_{(8,125)} = 50.45$, $p < 0.001$), Cd ($KW\text{-}H_{(8,125)} = 46.41$, $p < 0.001$), Pb ($KW\text{-}H_{(8,125)} = 44.73$, $p < 0.001$), Cu ($KW\text{-}H_{(8,125)} = 34.56$, $p < 0.001$), Hg ($KW\text{-}H_{(8,125)} = 37.73$, $p < 0.001$).

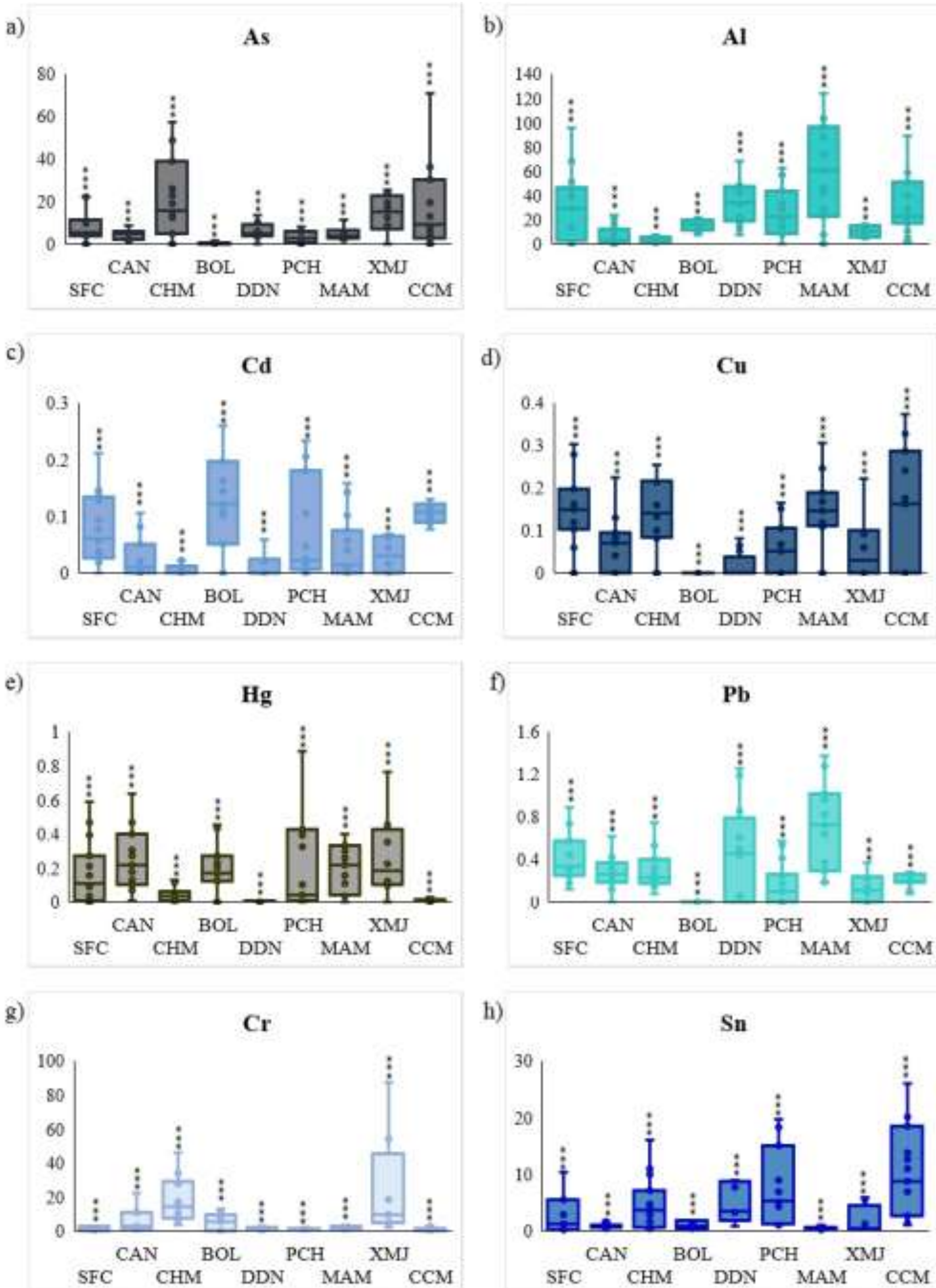


Fig. 2. Metal concentrations ($\mu g \cdot g^{-1}$) in the feathers of *C. atratus* from nine locations in the state of Campeche, Mexico.

The metals that showed significant differences between areas (urban, semi-urban and rural) were Al (KW-H $_{(2,125)}$ = 23.15, p<0.001), As (KW-H $_{(2,125)}$ = 17.54, p<0.001), Cr (KW-H $_{(2,125)}$ = 7.09, p< 0.01), Pb (KW-H $_{(2,125)}$ = 10.99, p< 0.001), Cd

(KW-H $_{(2,125)}$ = 5.86, p< 0.001) and Cu (KW- H $_{(2,125)}$ = 24.33, p< 0.001). The Sn (KW-H $_{(2,125)}$ = 1.77, p < 0.41) and Hg (KW-H $_{(2,125)}$ = 3.70, p < 0.15) did not present significant differences between areas.

Table 2. Average metal concentration (μg·g^{-1}) in *C. atratus* feathers from localities in urban, semi-urban and rural areas of the state of Campeche, Mexico.

		Metal concentration in feathers/μg·g^{-1} dry weight							
Areas	**Sites**	**Al**	**Sn**	**Hg**	**Cu**	**Pb**	**Cd**	**Cr**	**As**
Urban	SFC	31.286	4.714	0.178	0.153	0.412	0.078	1.871	11.855
	CAN	6.837	0.923	0.244	0.117	0.336	0.026	6.745	6.513
	CHM	3.835	4.561	0.044	0.358	0.300	0.006	21.861	30.236
Semi-urban	BOL	17.098	1.518	0.186	0.129	0.070	0.183	5.080	0.249
	DDN	46.081	9.333	0.006	0.017	0.449	0.016	1.240	5.944
Rural	PCH	26.412	7.313	0.299	0.083	0.167	0.095	1.801	5.396
	MAM	58.763	0.739	0.245	0.159	0.769	0.044	4.549	5.734
	XMJ	10.292	1.756	0.267	0.059	0.131	0.032	23.864	14.212
	CCM	35.405	9.988	0.024	0.140	0.217	0.112	1.114	28.515

The feathers of *C. atratus* from OD in the three areas presented higher concentrations of metals than those that feed in the SL (Fig. 2). The difference between the metal values per site could be related to the characteristics of the food present in the dumps and to the management practices of burning the garbage at OD sites.

In emergent nations, garbage at OD is often burned to reduce the volume of waste in these sites because it exceeds the capacity of the place (Lemieux et al., 2004). Unlike the LF, where only the garbage is spread, compacted, and covered (Oakley and Jiménez, 2012).

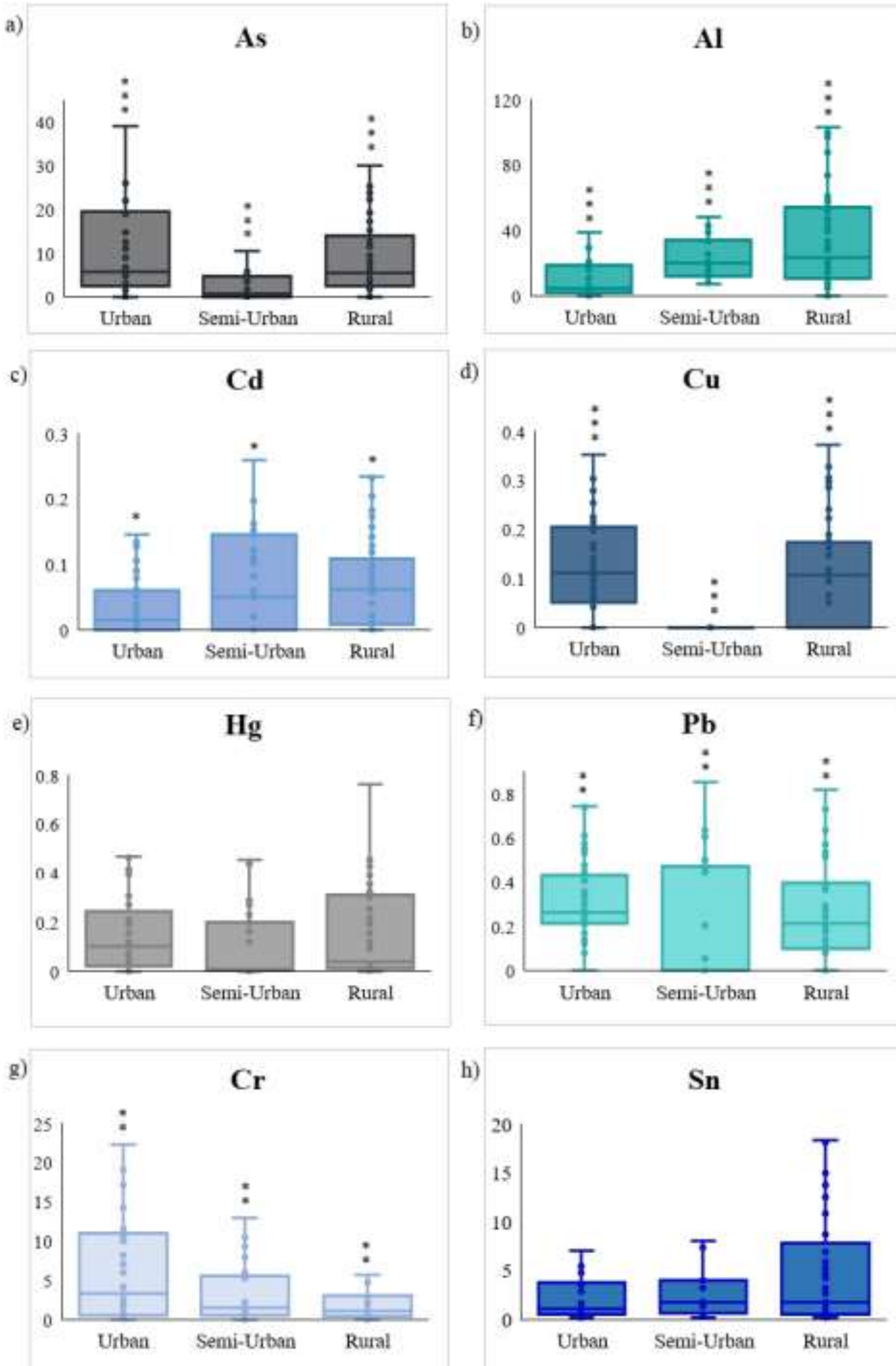


Fig. 3. Metal concentrations (µg·g^{-1}) in the feathers of *C. atratus* from urban, semi-urban and rural areas of the state of Campeche.

3.1. Metals concentrations in feathers

3.1.1. Arsenic

The highest mean concentrations of arsenic (As) in this study were found in the feathers of *C. atratus* from the OD of the urban locality of Champotón (CHM) with 30.24 ± 46.61 $\mu g \cdot g^{-1}$ and in the rural locality of Conquista Campesina (CCM) with 28.52 ± 53.39 $\mu g \cdot g^{-1}$ (Fig. 2a). Significant differences were found in the As concentrations of feathers from urban, semi-urban and rural areas (Fig. 3a).

Some studies of birds exposed to As, such as the exposure of Zebra finch chicks to concentrations of 36 to 72 $\mu g \cdot g^{-1}$ of As, caused their death (Albert et al., 2008). Another study carried out by Sánchez-Virosta et al. (2018) exposed Great tits (*Parus major*) chicks for 14 days to sodium arsenite (1 $\mu g \cdot g^{-1} \cdot d$), determining the distribution of As in feathers: 13.6 ± 5.88 $\mu g \cdot g^{-1}$, liver: 4.19 ± 5.92 $\mu g \cdot g^{-1}$, and bones: 3.37 ± 3.85 $\mu g \cdot g^{-1}$. According to the study by Sánchez-Virosta et al. (2018), the concentration of As found in the feathers of *C. atratus* of this study indicates that the daily intake of As in vultures in the open dumpsites of CHM is 2.2 $\mu g \cdot g^{-1}$, and in the OD of the CCM locality was approximately 2 $\mu g \cdot g^{-1}$.

Compared to scavenger birds from other nations, As concentrations in this study were higher, e.g. Yamac et al. (2019) reported As in the Eusarian Black Vulture (*Aegypius monachus*) feathers from the Sündiken and Türkmenbaba mountains in the Iberian Peninsula, the mean As value was 0.554 $\mu g \cdot g^{-1}$. The concentrations of this metal in *C. atratus* from our study were 50 to 55 times higher than in *A. monachus*, possibly due to the release of metals with the burning of wastes in the OD (Lemieux et al., 2004; Cogut, 2016). In addition, the Monserrat cement industry, and the charcoal kiln of the municipality of Champotón are located 36.1 km and 21.7 km from the OD of CHM, as well as the charcoal kiln of the Miguel Alemán ejido is 34.5 km from the OD of CCM. Therefore, it is possible that the metallic residues released by charcoal kiln and the cement industry reach DOs through the air (DOF, 2016; Vázquez-Lule, 2009; Nyde, 2005). In addition, arsenic contamination in the mountainous areas of Turkey is less because it is an uninhabited area.

3.1.2. Aluminum

In this study, the highest mean concentrations of aluminum (Al) were found in the feathers of *C. atratus* from the open dumpsites of the rural locality of Mamantel (MAM) with 58.76 ± 40.96 $\mu g \cdot g^{-1}$ and in the semi-urban locality of División del Norte (DDN) with 46.08 ± 51.15 $\mu g \cdot g^{-1}$ (Fig. 2b). Significant differences were found in Al concentrations in feathers from urban, semi-urban and rural areas (Fig.3b).

Furthermore, Al concentrations in this study were generally lower than values reported elsewhere. There are few studies of Al in feathers of scavenger birds; for example, Cardiel et al. (2011) determined Al in *Gyps fulvus* feathers with concentrations of 1.144 $\mu g \cdot g^{-1}$. Aluminum contamination in *G. fulvus* was 19 times higher than in *C. atratus* feathers from the MAM site and 24 times higher than in NDD. The authors suggest that the high contamination of *G. fulvus* in Spain is due to the distribution of these pollutants through the air, especially around mining operations.

Adout et al. (2007) determined Al in the Rock Pigeon (*Columba livia*) feathers in an industrial area of the city of Beer-Sheva, Israel (urban area) and in a rural area near Kibbutz Urim and Kibbutz Zeelim, the mean concentrations were 134 and 139

$\mu g \cdot g^{-1}$. The aluminum concentration in *C. livia* feathers was 2 to 3 times higher than the concentrations in *C. atratus* feathers from MMA and DDN from Campeche. Metal contamination in *C. livia* may be due to the use of fuels in the industrial zone, as well as the accumulation of toxic waste in the city of Beer-sheva, which has a human population of 204,707. Compared with *C. atratus* from the MAM (rural) and DDN (semi-urban) localities that have primary activities in a lower proportion than Beer-sheva. Therefore, the generation of garbage and the release of metals is also related to the activities of the sites.

Although our study sites are open dumpsites, Al concentrations were lower in *C. atratus* feathers. This contamination may be due to the type and volume of garbage burned at both sites, and to indirect contamination and to a lesser extent by flying particles of the charcoal kiln of the Miguel Alemán common, which is 32.9 km away, and in DDN by the Pakal-che charcoal kiln located 8.8 km from our sampling point (Nyde, 2005; Reyes-Gómez and Vázquez-Lule, 2009).

3.1.3. Cadmium

In the feathers of *C. atratus* from the semi-urban locality of Bolonchén de Rejón (BOL) and the rural locality of Conquista Campesina (CCM) the highest mean concentrations of cadmium (Cd) were found with 0.18 ± 0.21 and 0.11 ± 0.04 $\mu g \cdot g^{-1}$ (Fig. 2c). Significant differences were found in Cd concentrations in feathers from urban, semi-urban and rural areas (Fig. 3c).

Some studies of birds exposed to Cd, such as the exposure of duck chicks (*Aix sponsa*) for 13 weeks to Cd (100 $\mu g \cdot g^{-1}$) to determine the distribution of Cd in feathers: 29.56 $\mu g \cdot g^{-1}$, liver: 208.88 $\mu g \cdot g^{-1}$, and kidney: 132.17 $\mu g \cdot g^{-1}$ (Mayac et al., 1981). According to the study by Mayac et al. (1981), we can infer that it is possible that the concentration of Cd by in the diet of *C. atratus* by ingestion through the consumption of food in the open dumpsite of BOL is a concentration of 0.6 $\mu g \cdot g^{-1}$ exposed during half a day and in the open dumpsite of the CCM locality there is a concentration of 0.3 $\mu g \cdot g^{-1}$ exposed for approximately eight hours.

However, the Cd levels in this study were lower than the values reported in other scavenger birds. For example, López-Berenguer et al. (2021) found Cd in *Cathartes aura* feathers from three cities in southern Atacama: Caldera (Atacama Coast), Chile, with a human population of 16,150; in Copiapó (Interior Atacama) with 175,162 and in the Coquimbo Region with 216,623. This presented concentrations of 0.68, 0.55 and 0.63 $\mu g \cdot g^{-1}$, Cd in the three urban sites of Atacama were 3 to 4 times higher than in BOL and CCM.

These results may be since the economy of the Atacama is based mainly on mining and its large population compared to the localities of BOL (semi-urban) and CCM (rural). Even though in the BOL locality there may be indirect metal contamination through the Nohalal and Montebello charcoal kilns, which are located approximately 17.7 and 21.6 km from the open dumpsites, and in the CCM locality, the charcoal kilns of the Miguel Aleman ejido located 34.5 km (Pat-Fernández et al., 2011; Nyde, 2005). Lower Cd concentrations were found in *C. atratus* feathers from our study.

Another study by Abbasi et al. (2015) in the Egyptian Vulture (*Neophron percnopterus*) feathers presented mean concentrations of 1.42 $\mu g \cdot g^{-1}$. Most of the samples were collected in the province of Punjab, Pakistan, an urban area with more than 100 million inhabitants and the center of industrial and agricultural activities. Cd concentrations in *N. percnopterus* feathers were 7 to 8 times higher than those found in our study in *C. atratus* from BOL and 12 times higher than in CCM. The authors suggest that metal contamination in Punjab province is not only due to runoff from industrial activities but also due to its large population, unlike BOL (3,975 inhabitants) and CCM (887 inhabitants) where their economic activities are low as well as their population.

3.1.4. Copper

The highest concentrations of copper (Cu) in *C. atratus* feathers occurred in the urban locality of Champotón (CHM) with 0.35 ± 0.63 $\mu g \cdot g^{-1}$ and the rural locality of Mamantel (MAM) with 0.15 ± 0.11 $\mu g \cdot g^{-1}$ (Fig. 2d). Significant differences were found in Cu concentrations in feathers from urban, semi-urban and rural areas (Fig. 3d).

Of the works that exist on Cu in scavenger bird feathers, there is, for example, the study carried out by Nighat et al. (2013) reporting this metal with concentrations of 6.28 $\mu g \cdot g^{-1}$ in *Sarcogyps calvus* feathers from Punjab province (urban area). These Cu concentrations in *S. calvus* were 17 times higher than those reported in our study with *C. atratus*. This may be due to the heavy pollution in Punjab, Pakistan (e.g., sewage, urban waste, pesticides, automobiles, industrial effluents, and local leather tanning). In addition, the generation of urban waste in Punjab is much higher as it has a population of approximately 100 million inhabitants, unlike CHM and MAM, which have a small population. Therefore, metal contamination will not only depend on the activities on the site but also on the generation of garbage. In addition, the release of metals will also depend on the type and volume of waste from the site (Cogut, 2016).

Black Vultures, like other scavenger birds, perch near open dumpsites because these sites represent a potential food source for them (Novaes and Cintra, 2013). At these sites is where we think birds are exposed to metal contamination.

3.1.5. Mercury

In this study, the highest mean levels of mercury (Hg) in feathers from the open dumpsites of the rural localities of Pich (PCH) with 0.29 ± 0.42 and Xmejia (XMJ) with 0.26 ± 0.24 $\mu g \cdot g^{-1}$ (Fig. 2e). Significant differences were found in Hg concentrations in feathers from urban, semi-urban and rural areas (Fig. 3e).

Some studies of birds exposed to Hg, such as the exposure of Common Loon (*Gavia imer*) chicks for 15 weeks to methylmercury chloride (1.2 $\mu g \cdot g^{-1}$), to determine the distribution of this metal in feathers: 59.6 $\mu g \cdot g^{-1}$, brain: 3.1 $\mu g \cdot g^{-1}$, kidney: 8.3 $\mu g \cdot g^{-1}$, liver: 14.8 $\mu g \cdot g^{-1}$, and carcass: 4 $\mu g \cdot g^{-1}$ (Kenow et al., 2007). According to the study carried out by Kenow et al. (2007), it is possible that the input concentration of Hg in *C. atratus* in our study came through food consumption in the open dumpsites of PCH and XMJ is 0.005 $\mu g \cdot g^{-1}$ exposed for half a day. Because toxic compounds, such as metals, are released during garbage burning, it is possible that *C. atratus*, which use OD as a feeding area (Noreen and Sultan, 2021), may ingest Hg by consuming the carrion of these sites.

Of the existing investigations of Hg in scavenger birds, there is the study carried out by Di Marzio et al. (2018), who reported concentrations of Hg in feathers of *C. atratus* from two urban sites, El Valle and Bariloche de la Patagonia (Argentina) with mean values of 1.02 and 0.22 $\mu g \cdot g^{-1}$. The authors suggest that Hg contamination in *C. atratus* from Patagonia is due to residues from a chlor-alkali plant that closed in 1995. However, Hg values from the valley are 3 to 4 times higher than reported in PCH and XMJ. This may be because the valley includes the cities of Cipolletti and Allen, with populations of 87,343 and 22,859 inhabitants and 40,000 hectares dedicated to agriculture: in addition to a gold and silver mining area. Unlike Bariloche, which presents concentrations like those of PCH and XMJ, these sites are rural locations with less activity.

Another study conducted by Días dos Santos et al. (2021) determined Hg in feathers of *C. atratus* from Lavras in the western Amazon, Brazil, with values of 9.93 $\mu g \cdot g^{-1}$. The authors suggest that Hg concentrations are magnified in the food web because it bioaccumulates in scavenger birds consuming dead animals or contaminated waste. However, the Hg values in the feathers of *C. atratus* from Amazons were 33 to 37 times higher than the values we found in our sites PCH and XMJ. This may be due to the mining activities of Lavras, and the generation of garbage due to the size of its population with 94,228 inhabitants, much larger than the populations of PCH and XMJ in Campeche with 2,055 and 1,123 inhabitants.

Although in the localities of PCH and XMJ there may be indirect contamination in the open dumpsites by metallic particles released from the Hopelchén coal mines (32.8 km from PCH) and the Xmabén coal mines (8.4 km from XMJ) (Mejía, 2007; Nyde, 2005). The highest concentrations occurred in Lavras; this may be due to direct contamination from mining at this site.

3.1.6. Lead

In our study, the highest mean concentrations of lead (Pb) in feathers from the open dumpsites of the rural locality of Mamantel (MAM) with 0.76 ± 0.54 $\mu g \cdot g^{-1}$ and in the semi-urban locality of División del Norte (DDN) with 0.44 ± 0.43 $\mu g \cdot g^{-1}$ (Fig. 2f). Significant differences were found in Pb concentrations in feathers from urban, semi-urban and rural areas (Fig. 3f).

Some studies of birds exposed to Pb, such as the exposure of Herring Gull (*Larus argentatus*) chicks for 45 days to lead (200 $\mu g \cdot g^{-1}$) to determine the distribution of this metal in feathers: 9.20 $\mu g \cdot g^{-1}$, muscle: 0.16 $\mu g \cdot g^{-1}$, salt gland: 0.27 $\mu g \cdot g^{-1}$, brain: 1.61 $\mu g \cdot g^{-1}$, kidney: 41.07 $\mu g \cdot g^{-1}$, liver: 21.50 $\mu g \cdot g^{-1}$, and bone: 130.55 $\mu g \cdot g^{-1}$ (Burger and Gochfeld, 1990). According to the study carried out by Burger and Gochfeld (1990), it is possible that the input concentration of Pb in *C. atratus* in our study came from the consumption of food in the open dumps of the MAM and DDN localities with a concentration of 16.52 and 9.56 $\mu g \cdot g^{-1}$ of exposure for approximately 4 days.

The Pb values found by our research was higher compared to scavenger birds from other places, for example, Abbasi et al. (2015) reported Pb in *Neophron percnopterus* feathers from the Punjab province, Pakistan (urban area with more than 100 million inhabitants) with mean concentrations of 4.01 µg·g-1. Pb values in *C. atratus* feathers from MAM and DDN in Campeche were 5 to 9 times lower than those found in *N. pernocterus* from Punjab. Lead contamination in Punjab may be due to runoff from industrial and agricultural activities in the area. Unlike MAM and DDN, which have a small population as well as their agricultural and livestock activities.

Although in the localities of MAM and DDN they have the indirect entry of metals through the charcoal kilns of the Miguel Alemán ejido (32.9 km from MAM), and the Pakal-che charcoal kilns (8.8 km from DDN) (Nyde, 2005; Reyes-Gómez and Vázquez-Lule, 2009). Both localities have low agricultural and livestock activities. Therefore, metal contamination will depend on the generation of garbage from the sites, as well as the type and volume of garbage and leachates generated (Kanmani and Gandhimathi, 2013).

3.1.7. Chrome

In the feathers of *C. atratus* from the rural locality of Xmejía (XMJ) and the urban locality of Champotón (CHM) the highest mean concentrations of chromium (Cr) were found with 23.86 ± 30.46 and 21.86 ± 22.10 $\mu g \cdot g^{-1}$ (Fig. 2g). Significant differences were found in Cr concentrations in feathers from urban, semi-urban and rural areas (Fig. 3g).

Experiments with Cr have been carried out, for example, a study by Koivula and Eeva (2010) added Cr to the diet of black ducks (*Anas rubripes*). Cr affected the growth and survival rates of these birds and their young. Another study by El-Kholy et al. (2017) exposed Japanese quail chicks for 6 weeks to chromium (0.5 $\mu g \cdot g^{-1}$) determining the distribution of this metal in feather: 0.0042 $\mu g \cdot g^{-1}$, carcass: 0.075 $\mu g \cdot g^{-1}$, liver: 0.0019 $\mu g \cdot g^{-1}$, and gizzard: 0.0016 $\mu g \cdot g^{-1}$. According to the study by El-Kholy et al. (2017) it is possible that the input concentration of Cr in *C. atratus* is due to food consumption in the open dumpsites of the XMJ and CHM localities with concentrations of 2,840 and 2,602 $\mu g \cdot g^{-1}$.

Of the existing investigations of Cr in scavenger birds, there is the study carried out by Yamac et al. (2019) who reported Cr in Eurasian black vulture (*Aegypius monachus*) feathers, the mean Cr concentrations were 9.03 $\mu g \cdot g^{-1}$. Compared to our study, Cr concentrations in *C. atratus* feathers were 2.4 to 2.6 times higher than in *A. monachus*, this could be since the mountainous area of Turkey is not populated and pollution by metals, probably by atmospheric deposition.

Unlike our XMJ and CHM sites in Campeche, which are populated areas where trash is commonly burned at open dumpsites, therefore metals such as Cr can be released faster and become available for *C. atratus* feeding on these sites. In addition, these sampling sites may have indirect contamination by flying metal particles from the Monserrat cement plant and the charcoal kilns of the municipality of Champotón, which are located approximately 36.1 km and 21.7 km from the locality of CHM. Similarly, the Xmabén charcoal kilns are located approximately 8.4 km from the locality of XMJ (DOF, 2016; Vázquez-Lule et al., 2009; Nyde, 2005). The cement and charcoal kiln production industries release metals, which are dispersed by air currents, so it is possible for these contaminants to reach the CHM and XMJ open dumpsites. Therefore, Cr concentrations in scavenger bird feathers vary depending on anthropogenic activities at the site.

3.1.8. Tin

The highest concentrations of Sn in our study were found in the OD at the rural locality of Conquista Campesina (CCM) with 9.98 ± 8.00 $\mu g \cdot g^{-1}$ and the semi-urban locality of División del Norte (DDN) with 9.33 ± 13.29 $\mu g \cdot g^{-1}$ (Fig. 2h).

Significant differences were found in Sn concentrations in feathers from urban, semi-urban and rural areas (Fig. 3h). Sn was the only metal that was present in all the feathers of the three areas.

There are no studies of Sn in scavenger bird feathers, but there are in other bird species, for example, Burger and Gochfeld (2000) reported Sn in feathers of different seabird species from the Midway Island in the Pacific Ocean.

Sn concentrations by species were as follows: *Diomedea immutabilis* (5.95 $\mu g \cdot g^{-1}$), *Pterodroma hypoleuca* (2.63 $\mu g \cdot g^{-1}$), *Puffinus nativitatis* (1.80 $\mu g \cdot g^{-1}$), *Phaethon rubricauda* (4.64 $\mu g \cdot g^{-1}$), *Puffinus pacificus* (1.16 $\mu g \cdot g^{-1}$), *Anous stolidus* (2.31 $\mu g \cdot g^{-1}$), *Sterna fuscata* (3.66 $\mu g \cdot g^{-1}$), *Sterna lunata* (2.89 $\mu g \cdot g^{-1}$) and *Gygis alba* (15.20 $\mu g \cdot g^{-1}$). The authors suggest that Sn contamination in Midway birds is due to residues of antifouling paints used on ships and the accumulation of hazardous waste emitted during World War II.

Compared to our study, Sn concentrations in *C. atratus* from the CCM and DDN localities were 1.5 times lower than those found in the Midway species *G. alba*. However, the other Midway bird species had concentrations 1.6 to 8.5 times lower than those of *C. atratus*, from Campeche. The authors suggest that Sn contamination on Midway Island is due to the type of diet of each bird species (differences in the proportion of specific prey or in the size of the prey).

In the case of contamination by Sn in the feathers of *C. atratus*, it will depend on the feeding site, which are open dumpsites, where they burn the garbage from time to time when the waste exceeds the volume of the site, therefore, *C.atratus* is exposed to the metals that are released from the burning of garbage, because this species uses the OD as its main source of food.

4. Conclusions

Black Vultures are important in their ecosystems by providing environmental services, cleaning, and removing dead animals, and helpful bioindicator species for ecosystem health because they are at the top of the food chain and may be exposed to contaminants through their diet. The feathers of *C. atratus* can be used as an indicator of metal contamination.

The highest concentrations of diverse metals found through this study in these scavenger birds occurred in the open dumpsites of the three areas (urban, semi-urban, and rural). This metal contamination may be due to the type of waste that reaches these sites and the burning of local garbage. Of the eight metals analyzed (Cd, Pb, Cu, Cr, Hg, Al, As, and Sn), Sn was the only metal present in all the feathers of the three areas.

In rural and semi-urban localities, the highest concentrations were of Al with 35.67 $\mu g \cdot g^{-1}$ and 29.97 $\mu g \cdot g^{-1}$ and in urban localities the highest levels were of As with 16.20 $\mu g \cdot g^{-1}$.

Metal contamination is a topic of interest since the discharge of metals in open dumpsites can affect not only the birds that feed on these sites but also humans, since during the burning of garbage in these sites, they release metals into the atmosphere, posing a long-term risk at the population level for both animals and humans. However, more research is required to document the exposure of Black

Vultures to metals from sanitary landfill and open dumpsites. The conservation of vultures is of great importance since these scavenger birds perform ecosystem services by feeding on decomposing organic material, reducing the production of pathogens in the environment.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Capítulo IV

Conclusiones generales

Con base en los resultados de este estudio y las hipótesis postuladas se concluye que:

a) A pesar del incremento de basura en el relleno sanitario de Campeche, los zopilotes de cabeza negra ingirieron más microplásticos en el año 2019 que en el año 2020. Estas variaciones en las cantidades de MPs por años, puede depender de la degradación del tipo de polímero plástico. Es decir, si es un plástico resistente y termoestable tardará años en fragmentarse, a diferencia de un plástico frágil que se romperá y/o fragmentará más rápido, formando meso y microplásticos. Estos resultados nos muestran que en el año 2019 la mayoría de estas partículas plásticas estaban constituidas por polietilenos o derivados de este polímero que tiene una degradación de aproximadamente 150 años, a diferencia de los plásticos encontrados en el año 2020 que estaban conformados en mayor proporción por polibutadieno-estireno y mezclas de poliéster con uretano que tardan aproximadamente en degradarse 500 años. De los contaminantes analizados en los MPs, el metal que se presentó con mayor concentración entre años fue el Al con 35.60 $\mu g \cdot g^{-1}$ (2019) and 15.82 $\mu g \cdot g^{-1}$ (2020). Se puede observar que en el año 2020 las concentraciones de Al fue la mitad de lo que se presentó en el año 2019, probablemente debido a la lixiviación de metales por envejecimiento y fragmentación de los MPs. Otro de los contaminantes analizados fueron los POCs, siendo los drines los que se encontraron con mayor concentración en el año 2019 con 1.03 ± 1.14 $ng \cdot g^{-1}$. Y en el año 2020 los endosulfanes fueron el compuesto con mayor concentración con 0.97 ± 1.50 $ng \cdot g^{-1}$. Los drines se prohibieron en México en el año de 1991, pero se emplearon tantas cantidades de este plaguicida que lo que se determinó en los MPs son residuos que quedaron de este contaminante en el ambiente. En el caso del Ensodulfan su prohibición en México fue en el año 2019. En cuanto a los HAPs el benzopireno que es uno de los compuestos considerados como carcinógeno por el Centro Internacional de Investigaciones sobre el Cáncer (IARC), se presentó en ambos años con concentraciones de 1.80 ± 3.68 $ng \cdot g^{-1}$ (2019) y 1.67 ± 1.45 $ng \cdot g^{-1}$ (2020). La liberación de los HAPs en el ambiente se produce durante las temporadas de quemas de actividades agrícolas y por incendios forestales, estos HAPs emitidos al ambiente pueden llegar a los rellenos sanitarios a través de las corrientes de aire. Ya que en el relleno sanitario de la ciudad de SFC no se quema la basura, solamente se compacta y se cubre con tierra. Sin embargo, la determinación de estos contaminantes en los MPs nos indica la adsorción que tienen estas partículas plásticas y se demuestra que sirven como transporte de contaminantes.

b) Las mayores concentraciones de metales en *C. atratus* se presentaron en los vertederos a cielo abierto de las localidades urbanas, semi-urbanas y rurales. Los valores de metales entre localidades podrían estar relacionada con los desechos de los basureros y con la quema de basura en los vertederos a cielo abierto, ya que comúnmente queman la basura en estos sitios cuando el volumen de desechos generados sobrepasa el lugar de depósito. De los ocho metales analizados (Cd, Pb, Cu, Cr, Hg, Al, As y Sn) el Sn fue el único metal que estuvo presente en todas las plumas de los sitios de muestreo. Sin embargo, los dos metales con las concentraciones más altas en las plumas fueron el aluminio con 35.67 $\mu g \cdot g^{-1}$ en las localidades rurales y 29.97 $\mu g \cdot g^{-1}$ en las localidades semiurbanas; y el As con concentraciones promedios de 16.20 $\mu g \cdot g^{-1}$ en las localidades urbanas. Con este estudio se tiene la evidencia de que los zopilotes están más expuestos a metales en los vertederos a cielo abierto que en el relleno sanitario. Sin embargo, los zopilotes no solamente están expuestos a los microplásticos sino a contaminantes como los metales que se liberan de la basura a través de lixiviados o por volatilización durante la quema de la basura.

Con este estudio se tiene la evidencia de que los zopilotes están expuestos a contaminantes de los vertederos y rellenos sanitarios. El zopilote de cabeza negra (*C. atratus*) es un buen organismo bioindicador. El uso de sus egagrópilas y sus plumas como métodos no invasivos para esta especie, nos permite conocer el tipo de contaminantes al que están expuestos no solamente ellos, sino los organismos que emplean estos sitios como fuente de alimentación.

Sin embargo, se requiere más investigación para evaluar la exposición de los zopilotes no solo a los MPs sino a sus contaminantes. Así también evaluar los posibles efectos que producen estos contaminantes en los zopilotes de cabeza negra, ya que estas aves carroñeras realizan un importante servicio ecosistémico al remover del medio ambiente materia orgánica en descomposición, reduciendo la producción de patógenos al consumir carroña y reduciendo los riesgos de enfermedades humanas.

Se necesita implementar acciones preventivas con normas y estrategias de manejo, para cumplir con la gestión integral de residuos sólidos y prevenir la contaminación ambiental que ocasiona la basura plástica. Y evitar que se sigan creando vertederos al aire libre que ponen en riesgo al ecosistema. Para ello es importante el compromiso social de las personas para disminuir el uso de plásticos en nuestra vida cotidiana.