UNIVERSIDADE FEDERAL DE SÃO CARLOS

CENTRO DE CIÊNCIAS E TECNOLOGIA PARA A SUSTENTABILIDADE

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOTECNOLOGIA E MONITORAMENTO AMBIENTAL

GUILHERME ANDRADE NETO SCHMITZ BOEING

AVALIAÇÃO DO EFEITO DE MICROPLÁSTICOS DE TINTA SPRAY EM ÓRGÃOS INTERNOS DE OPERÁRIAS CAMPEIRAS DE *BOMBUS ATRATUS* (HYMENOPTERA: APIDAE, BOMBINI)

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Dissertação apresentada ao Programa de Pós-Graduação em Biotecnologia e Monitoramento Ambiental para obtenção do título de Mestre em Biotecnologia e Monitoramento Ambiental.

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“Science is a way of thinking much more than it is a body of knowledge."

- Carl Sagan

RESUMO

Estudos recentes demonstram os efeitos negativos das tintas spray no meio ambiente devido a sua disponibilização na forma de microplásticos (MPs) e a sua composição química. A fotodegradação dessas partículas pode aumentar a liberação de aditivos, incluindo os metais dos pigmentos. As abelhas neotropicais podem ser expostas a essas partículas contaminantes, que já foram encontradas no mel. Portanto, este estudo teve como objetivo avaliar os efeitos da fotodegradação em MPs de tintas spray expostos a diferentes intensidades de luz e tempos de exposição e também avaliar a potencial citotoxicidade desses MPs de tintas spray prístinas e fotodegradadas nas células colunares do intestino médio, túbulos de Malpighi e células do sistema hepatonefrocítico de operárias forrageiras de *Bombus atratus* expostas a 50 $mg.L^{-1}$ de MPs durante 96 horas. Os microplásticos prístinos foram obtidos através da pulverização de tinta e, em seguida, a forma e o tamanho foram caracterizados. A Fluorescência de raios-X (XRF) e a Espectroscopia de Infravermelho por Transformada de Fourier (FTIR) foram utilizadas para determinar a composição elementar e o tipo de polímero. Os MPs foram então expostos a diferentes intensidades de radiação UV-C e tempos de exposição. A liberação de elementos potencialmente tóxicos (EPTs) pelos MPs foi quantificada usando um espectrômetro de emissão atômica com plasma induzido por micro-ondas. Para avaliar a toxicidade dos MPs, realizamos análises histológicas e histoquímicas dos tecidos das abelhas. A análise por XRF revelou diversos elementos em ambas as tintas, incluindo cinco EPTs: Manganês, Cromo, Ferro, Cobre e Zinco. As partículas expostas à maior intensidade de UV-C mostraram mudanças significativas, como modificação da superfície, florescimento de aditivos e destaque de fragmentos da superfície resultando em *voids*, alterando seus valores de área e redondeza em relação aos prístinos. Além disso, exibiram um aumento na liberação de EPTs, especialmente Ferro, Manganês e Cobre, que excederam os limites seguros estabelecidos pelo Conselho Nacional do Meio Ambiente (CONAMA). A maior liberação de EPTs está relacionada com as alterações significativas observadas nos órgãos das abelhas expostas aos MPs fotodegradados, os quais apresentaram vacuolização celular, condensação nuclear e picnose. A liberação de EPTs das partículas fotodegradadas contribuiu para a desregulação da homeostase corporal das abelhas estudadas, potencialmente levando à redução da aptidão e alterações na alimentação e sobrevivência da espécie. Esses achados ressaltam a importância de entender a toxicidade de MPs em condições ambientais realistas, pois a composição do plástico e as condições de intemperismo podem influenciar a toxicidade das partículas.

Palavras-chave: Abelhas; Poliestireno; EPTs; e Poluição Plástica.

ABSTRACT

Recent studies have shown the negative environmental effects of spray paints due to microplastics (MPs) and their chemical composition. Photodegradation of these particles can increase the release of additives, including metals in the pigments. Neotropical bumblebees can be exposed to these contaminant particles, which have already been found in honey. Therefore, this study aimed to evaluate the effects of photodegradation on spray paints MPs exposed to different light intensities and exposure times, as well as assess the potential cytotoxicity of these pristine and photodegraded MPs on the columnar cells of the midgut, Malpighian tubules, and hepato-nephrocitic system cells of forager worker bees of *Bombus atratus* exposed to 50 $mg.L^{-1}$ of MPs for 96 hours. Pristine microplastics were obtained through paint spraying, then characterized by shape and size. Portable X-ray fluorescence (XRF) and Fourier Transform Infrared Spectroscopy (FTIR) were used to determine elemental composition and polymer type. The MPs were then exposed to different intensities of UV-C radiation and exposure times. The release of potentially toxic elements (PTEs) by the MPs was quantified using an atomic emission spectrometer with microwave plasma. To assess the toxicity of the MPs, we conducted histological and histochemical analyses of the bee tissues. The XRF analysis revealed several elements in both paints, including five PTEs: Manganese, Chromium, Iron, Copper, and Zinc. The particles exposed to the highest UV-C intensity showed significant changes, such as superficial modification, blooming of additives, and surface detachment resulting in voids, altering their area and roundness values in relation to the pristine. Additionally, they exhibited an increase in the release of PTEs, especially Iron, Manganese, and Copper, which exceeded the safe limits set by the National Environment Council of Brazil (CONAMA). The increased release of PTEs is associated with the significant changes observed in the organs of bees exposed to the photodegraded MPs, which showed cellular vacuolization, nuclear condensation, and pyknosis. The release of PTEs from the photodegraded particles contributes to the disruption of the body homeostasis of the studied bees, potentially leading to reduced fitness and alterations in feeding and survival of the species. These findings underscore the importance of understanding the toxicity of environmentally realistic MPs, as plastic composition and weathering conditions can influence particle toxicity.

Keywords: Bumblebees; Polystyrene; PTEs; and Plastic Pollution.

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LISTA DE ABREVIATURAS E SIGLAS

AO - Acridine Orange

APHA - American Public Health Association

CONAMA - Conselho Nacional do Meio Ambiente / National Environment Council of Brazil

Ctr – Controle

EPTs - Elementos Potencialmente Tóxicos

Exp - Exposed

FTIR - Espectroscopia de Infravermelho por Transformada de Fourier / Fourier Transform Infrared Spectroscopy

GB - Glossy Black spray paint

HE - Hematoxylin and Eosin

LOD - Limit of Detection

LOQ - Limit of Quantification

MP-AES - Microwave Plasma Atomic Emission Spectrometer / Espectrômetro de Emissão Atômica com Plasma Induzido por Micro-ondas

MPs - Microplasticos / Microplastics

MW - Matte White spray paint

OECD - Organization for Economic Cooperation and Development

oe - Oenocytes

pc - Pericardial Cells

pdGB MPs - Photodegraded Glossy Black Microplastics

pdMW MPs - Photodegraded Matte White Microplastics

pGB MPs - Pristine Glossy Black Microplastics

pMW MPs - Pristine Matte White Microplastics

PS - Polystyrene

PTEs - Potentially Toxic Elements

RH - Relative Humidity

SEM - Scanning Electron Microscope

SNH - Sistema Hepatonefrocítico

tr - Trophocytes

UV - Luz Ultravioleta / Ultraviolet Light

UV-C - Ultraviolet Light Type C

WHO - World Health Association

XRF - Fluorescência de Raios X / X-ray Fluorescence

1UVC24 - Sample exposed to 1 UV-C lamp for 24 hours

1UVC48 - Sample exposed to 1 UV-C lamp for 48 hours

3UVC24 - Sample exposed to 3 UV-C lamp for 24 hours

3UVC48 - Sample exposed to 3 UV-C lamp for 48 hours

SUMÁRIO

1. CAPÍTULO 1 – INTRODUÇÃO

Microplásticos (MPs), uma fonte diversificada e ubíqua de contaminação terrestre e aquática, representam uma ameaça ambiental significativa para os ecossistemas (Bank e Hansson, 2019; Brahney et al., 2020; Rillig e Lehmann, 2020; Bostan et al., 2023; Zhou et al., 2020; Roy et al., 2022). Com tamanhos variando de 1 μm a 5 mm e densidades que variam de 0,28 a 1,47 g cm^{-3}, essas partículas poliméricas podem ser encontradas em uma infinidade de ambientes e infiltram ecossistemas por meio de vários canais, incluindo instalações de tratamento de águas residuais, aterros, campos agrícolas, descargas industriais, escoamento doméstico, emendas de solo, cobertura morta, lodo, irrigação, inundação, deposição atmosférica e descarte de lixo (Hanvey et al., 2017; Bank et al., 2021; Rillig et al., 2017; Mai et al., 2018; Boots et al., 2019; Conley et al., 2019; Corradini et al., 2019; Gündoğdu et al., 2018; He et al., 2019; Li et al., 2018; Rochman 2018; Steinmetz et al., 2016).

MPs podem ser amplamente categorizados em tipos primários e secundários, onde os MPs primários são produzidos intencionalmente para aplicações específicas, como fibras têxteis artificiais e microesferas em cosméticos e produtos de cuidados pessoais, enquanto os MPs secundários resultam da degradação de materiais plásticos maiores por meio de intempéries, como erosão mecânica e exposição a raios ultravioleta (UV) (Cole et al., 2011; Järlskog et al., 2020; Fu e Wang, 2019; Rolsky et al., 2020; Hidalgo-Ruz et al., 2012; Duis and Coors, 2016). Ambos os tipos de MPs são poluentes pervasivos e persistentes, prontamente dispersos e integrados em sedimentos, solo e água, exibindo alta biodisponibilidade e tamanho microscópico, sendo encontrados em uma variedade de organismos em todos os níveis tróficos (Eerkes-Medrano et al., 2015; Prata et al., 2019; Wang et al., 2021; Zhou et al., 2020).

Tintas apresentam ligantes poliméricos como seus principais constituintes e são reconhecidas como uma fonte significativa de MPs, levantando preocupações sobre seu potencial risco ambiental (Gaylarde et al., 2021; Song et al., 2014; Turner, 2021). A avaliação e análise da contaminação, assim como o monitoramento de tintas como poluentes plásticos foi extensivamente explorada em contextos marinhos e de água doce (Ma et al., 2020; Ashrafy et al., 2022), e está recebendo um foco crescente em ambientes terrestres (Lima et al., 2014; Nizzetto et al., 2016; Imhof et al., 2016; Galafassi et al., 2019; Xu et al., 2022). A preocupação com os MPs derivados de tintas reside em sua composição química, que pode representar riscos ambientais. Notavelmente, tintas utilizadas em embarcações e equipamentos marinhos frequentemente contêm elementos potencialmente tóxicos (EPTs) como Cobre (Cu), Zinco (Zn)

e Chumbo (Pb), além de biocidas, utilizados para evitar organismos incrustantes (Gade et al., 2012; Soroldoni et al., 2017).

A liberação dessas substâncias no meio ambiente pode ocorrer por meio de processos naturais de abrasão, como mudanças de temperatura, chuva ácida e exposição à radiação ultravioleta (Sørensen et al., 2021; Costa et al., 2023). Em materiais poliméricos, a exposição à radiação ultravioleta pode induzir um processo de alteração química denominado fotodegradação (Gomaa et al., 2018; Yousif e Haddad, 2013). A interação entre polímero e luz ocorre principalmente por meio de grupos cromóforos, que absorvem a radiação e transferem sua energia para facilitar a clivagem homolítica, gerando consequentemente radicais livres (Curcio et al., 2018). Esse processo inicia um ciclo de auto-oxidação, causando alterações na superfície do material, que, à medida que a degradação avança, se estendem gradualmente para o interior do polímero (Blais et al., 1972; Curcio et al., 2018; de Freitas et al., 2022). A formação de fissuras é o principal fenômeno de fotodegradação em polímeros, associado à diminuição nas propriedades mecânicas, incluindo aumento da fragilidade (Rabello e White, 1997). A deterioração do material geralmente começa com o desenvolvimento de *microvoids*, progredindo para um processo de fibrilação que eventualmente leva à ruptura das fibrilas e formação de fissuras (Takahashi et al., 2010).

Com base nas dimensões ubíquas dos MPs, seu impacto em organismos marinhos está aumentando, especialmente em peixes (Avio et al., 2017), ostras (Sussarellu et al., 2016), mexilhões (Gündoğdu et al., 2020) e percas europeias (Lönnstedt e Eklöv, 2016). A exposição aos MPs em ambientes aquáticos confere efeitos tóxicos à biota, incluindo redução na aptidão, aumento do estresse oxidativo, disfunções imunológicas, bem como desequilíbrios na microbiota intestinal (Jin et al., 2018; Paul-Pont et al., 2016). Esses estudos predominantemente revelam uma redução na atividade de alimentação, diminuição na expectativa de vida e capacidade reprodutiva, juntamente com alterações comportamentais nos hospedeiros (Deng et al., 2021). A contaminação em ambientes terrestres é considerada potencialmente mais perigosa do que em ambientes aquáticos para os humanos, devido aos efeitos diretos nas cadeias alimentares que contem plantas, insetos e animais consumidos diretamente pela população (Toussaint et al., 2019).

Sendo cruciais para a manutenção e reprodução sexuada de espécies de plantas com flores, as abelhas desempenham um papel fundamental como principais insetos polinizadores (Kearns e Inouye, 1997). A polinização, um serviço ecossistêmico essencial, resulta em fertilização, produção de frutos e sementes, contribuindo significativamente para a estrutura e função dos ecossistemas naturais (Proctor et al., 2012). As abelhas não são apenas vitais para a

agricultura, mas também indispensáveis para a saúde geral e sustentabilidade de vários ecossistemas (Hung et al., 2018). Relatórios recentes estão apresentando os mesmos resultados que os mais antigos, destacando declínios substanciais nos serviços de polinização no noroeste da Europa, América do Norte e América do Sul, enfatizando a necessidade urgente de conservação das abelhas para enfrentar a alarmante diminuição de suas populações (Grixti et al., 2009; Carvell et al., 2006; Cameron et al., 2011; Pires et al., 2016; Wood et al., 2020).

O Brasil está localizado na periferia da distribuição do gênero *Bombus,* o que resulta na identificação de apenas seis espécies desse gênero no país. Entre elas, *Bombus morio* e *Bombus atratus* são particularmente abundantes nas regiões Sul e Sudeste (Moure e Sakagami, 1962). As abelhas do gênero *Bombus* desempenham um papel crucial na polinização de culturas agrícolas, contribuindo para o aumento da produtividade de diversas espécies. Seu maior porte permite que carreguem maiores quantidades de pólen e visitem várias flores antes de retornarem à colônia. Essas abelhas são extremamente rápidas, visitando o dobro de flores por minuto em comparação com a maioria das outras espécies, tornando-se polinizadores muito mais eficientes (Heinrich, 2000). Aproximadamente 95% de seu uso comercial ocorre em estufas de tomate, berinjela, pimentão, pepino, melão, abóbora, groselha vermelha e preta, framboesa e morango (Velthuis e Doorn, 2006). Em estufas de tomate, por exemplo, aumentos de rendimento podem superar 28%, além de resultar em frutos de qualidade superior (Sande, 1990; Fiume e Parisi, 1994). A urgência em entender as causas por trás do desaparecimento e declínio de várias espécies dentro desse gênero nas Américas e no Leste Europeu tem sido destacada por pesquisas recentes (Cameron et al., 2011; Grixti et al., 2009; Provase et al., 2021; Ceschi-Bertoli et al., 2020).

O principal fator que diferencia *Bombus* das abelhas melíferas e de outras espécies nativas é seu ciclo de vida distinto (Cameron et al., 2011). Mamangavas são especialmente suscetíveis a perturbações ambientais devido a esse ciclo de vida único. Se a rainha fundadora for exposta a substâncias tóxicas durante a fase solitária, o desenvolvimento potencial de uma colônia inteira originária de uma única rainha torna-se inatingível (Velthuis e van Doorn, 2006). Ao contrário de outras espécies sociais de abelhas, as colônias de *Bombus* não são perenes; elas são estabelecidas por um indivíduo solitário - a rainha fertilizada (Martins e Melo, 2009). Essa rainha, responsável por produzir todas as operárias, inicia a fase social da colônia (Martins e Melo, 2009; Cameron et al., 2011).

Existem duas maneiras de usar esses insetos como bioindicadores de estresse ambiental: por meio da taxa de mortalidade e análise de órgãos internos em configurações experimentais (ensaios toxicológicos) ou quantificando resíduos tóxicos presentes em ninhos, favos de mel ou

potes de pólen (Balestra et al., 1992). Portanto, a aplicação dos órgãos internos das abelhas em estudos ecotoxicológicos é de grande interesse, pois uma ampla variedade de compostos tóxicos pode se acumular em níveis moleculares, celulares e teciduais (Hyne e Maher, 2003). Como apresentado por Abdalla e Domingues (2015), um exemplo disso seria a avaliação das células do sistema hepatonefrocítico para uma compreensão sistêmica das alterações em outras células, como as células nutridoras dos folículos ovarianos no vitelário.

Estudos iniciais conduzidos por Liebezeit e Liebezeit (2015), demonstraram a presença de fibras plásticas, as quais variaram de 10 a 336 kg^{-1}, em 47 amostras de méis e em 22 espécies de flores visitadas por abelhas de interesse econômico. Os dados relativos às plantas com flores analisadas indicaram que uma grande proporção da carga de partículas encontradas pode originar-se de fontes externas, ou seja, essas partículas são trazidas para a colmeia pelas abelhas operárias durante a coleta de néctar (Liebezeit e Liebezeit, 2015). Adicionalmente, a presença de MPs foi confirmada em 12% das amostras de mel, cerveja, leite e bebidas coletadas no Equador, sendo principalmente compostos de polietileno, polipropileno e poliacrilamida (Diaz-Basantes et al., 2020). Abelhas podem obter essas partículas de poluição por meio da aderência aos pelos do corpo ou da ingestão de néctar, pólen e água contaminados com MPs, devido à sua ampla atividade de ferrageamento, e acabando por incorporar estes MPs no mel, cera e nas larvas da colmeia (Glenny et al., 2017; Alma et al., 2023).

O intestino é um órgão crucial em estudos ecotoxicológicos de insetos, pois contém um grande número de biomarcadores que podem ser analisados (Terra, 2001). É tipicamente composto pelo intestino anterior (estomodeu), intestino médio (ventrículo) e intestino posterior (proctodeu) (Cruz-Landim, 2009). O intestino médio desempenha um papel central na digestão e absorção de nutrientes e produtos químicos (Terra et al., 2019). O epitélio do intestino médio de insetos adultos é composto principalmente por células colunares ou digestivas e células regenerativas (Terra, 2001). As células colunares são responsáveis pela produção e secreção de enzimas digestivas, bem como pela absorção de substâncias digeridas e água (Terra et al., 1988). Em contrapartida, as células regenerativas, localizadas na base do epitélio, substituem as células colunares perdidas devido ao desgaste e ao envelhecimento através de um processo que envolve divisão e diferenciação (Cavalcante e Cruz-Landim, 1998).

Associado ao intestino, os túbulos de Malpighi das abelhas, assim como nos insetos em geral, são compostos por uma única camada de células piramidais ou cuboidais, com núcleos esféricos posicionados centralmente (Cruz-Landim, 1998). Esses túbulos exibem células especializadas ao longo de sua estrutura. Por exemplo, em *Drosophila melanogaster*, as células principais realizam o transporte de cátions, enquanto as células estreladas são responsáveis pelo

transporte de ânions cloreto para o lúmen (O'Donnell et al., 1996; Dow et al., 1998; Klowden, 2007; Beyenbach et al., 2010). Em *Rhodnius prolixus*, a porção distal dos túbulos de Malpighi transporta um filtrado para o lúmen, enquanto a porção proximal reabsorve parte desse filtrado de volta para a hemolinfa (Bradley, 1983). Em *Drosophila*, os túbulos têm origem embrionária tanto do ectoderma quanto do mesoderma, análogos aos rins dos mamíferos, pois têm origem embrionária de diferentes camadas germinativas e a função de filtração, conforme visto nos dados apresentados (Denholm et al., 2003; Jung et al., 2005).

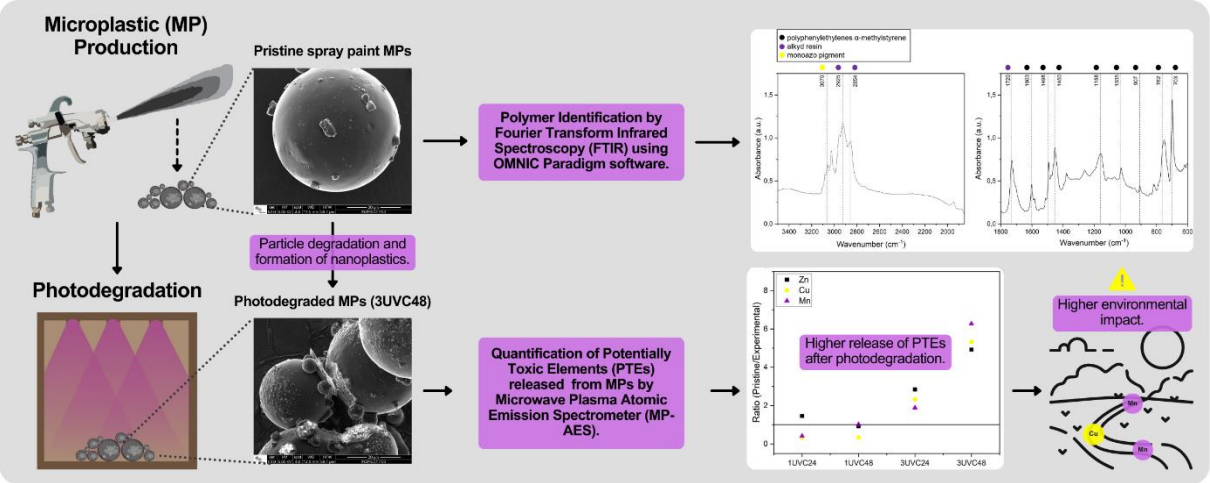
O estudo do sistema hepatonefrocítico (SHN) pode revelar respostas toxicocinéticas relevantes, já que suas células pericárdicas, enócitos e trofócitos participam do metabolismo intermediário e da biotransformação de compostos químicos e biomoléculas (Abdalla e Domingues, 2015). Além disso, os hemócitos do SHN são responsáveis pelas respostas imunológicas adaptativas e inatas em abelhas (Cruz-Landim, 2009), sendo assim, essas células podem indicar a potencial imunotoxicidade de diversos compostos.

Embora evidências crescentes tenham mostrado que os MPs contaminam um grande número de invertebrados, a maioria das pesquisas em abelhas vem utilizando MPs prístinos comerciais com formulações únicas e abelhas de um único grupo (*apis*) (Deng et al., 2021; Wang et al., 2022; Wang et al., 2021), o que pode não ser ambientalmente representativo. Desta forma, uma investigação sobre a influência e impacto de MPs de tinta spray prístinos e fotodegradados na fisiologia de abelhas do gênero *Bombus* seria algo inovador, utilizando um toxicante ambientalmente realístico e um organismo que nunca foi avaliado quanto a isso. Sob essa perspectiva, este estudo tem como objetivo entender os efeitos de dois tipos distintos de MPs de tinta spray nas células do intestino médio, túbulos de Malpighi e sistema hepato-nefrocítico, todos órgãos de alto interesse e impacto ecotoxicológico, de abelhas neotropicais da espécie *B. atratus*.

2. CAPÍTULO 2: ARTIGO 1

New insights on the photodegradation of spray paint MPs: increase in the release of potentially toxic elements

2.1. Graphical Abstract



2.2. Abstract

Recent studies have shown the negative environmental effects of spray paints due to microplastics (MPs) formation and their chemical composition. Photodegradation of these particles can increase the release of additives, including metals in the pigments. This study aimed to evaluate the effects of photodegradation on spray paint MPs exposed to different light intensities and exposure times. Pristine MPs were obtained through paint spraying, then characterized by shape and size. Portable X-ray fluorescence (XRF) and Fourier Transform Infrared Spectroscopy (FTIR) were used to determine elemental composition and polymer type. The MPs were then exposed to different UV-C radiation intensities and exposure times. The release of potentially toxic elements (PTEs) by the MPs was quantified using an atomic emission spectrometer with microwave plasma. XRF analysis revealed 12 elements in the paint, including five PTEs: Manganese, Chromium, Iron, Copper, and Zinc. The particles exposed to the highest UV-C intensity showed significant changes, such as superficial modification, blooming of additives, and surface detachment resulting in voids, altering their area and roundness values in relation to the pristine. Additionally, they exhibited increased release of metals, especially Manganese and Copper. Our findings expand knowledge about the photo-aging process of spray paint microplastics and reveal an increased release of potentially toxic elements. This study highlights the potential

environmental impact of spray paints as sources of various pollutants, not just plastic polymers.

Keywords: Polystyrene; Polymer; Plastic Pollution; and PTEs

2.3. Resumo

Estudos recentes mostraram os efeitos ambientais negativos das tintas spray devido à formação de microplásticos (MPs) e sua composição química. A fotodegradação dessas partículas pode aumentar a liberação de aditivos, incluindo metais nos pigmentos. Este estudo teve como objetivo avaliar os efeitos da fotodegradação em MPs de tintas spray expostos a diferentes intensidades de luz e tempos de exposição. MPs prístinos foram obtidos através da pulverização da tinta e, em seguida, caracterizados por forma e tamanho. A fluorescência de raios X portátil (XRF) e a Espectroscopia no Infravermelho por Transformada de Fourier (FTIR) foram usadas para determinar a composição elementar e o tipo de polímero. Os MPs foram então expostos a diferentes intensidades de radiação UV-C e tempos de exposição. A liberação de elementos potencialmente tóxicos (PTEs) pelos MPs foi quantificada usando um espectrômetro de emissão atômica com plasma de micro-ondas. A análise de XRF revelou 12 elementos na tinta, incluindo cinco PTEs: Manganês, Cromo, Ferro, Cobre e Zinco. As partículas expostas à maior intensidade de UV-C mostraram mudanças significativas, como modificação superficial, liberação de aditivos e desprendimento da superfície resultando em vazios, alterando seus valores de área e arredondamento em relação aos MPs prístinos. Além disso, exibiram um aumento na liberação de metais, especialmente Manganês e Cobre. Nossos achados expandem o conhecimento sobre o processo de fotoenvelhecimento dos microplásticos de tintas spray e revelam um aumento na liberação de elementos potencialmente tóxicos. Este estudo destaca o potencial impacto ambiental das tintas spray como fontes de vários poluentes, não apenas de polímeros plásticos.

Palavras-chave: Poliestireno; Polímero; Poluição Plástica; e EPTs

2.4. Introduction

Microplastics (MPs) represent diverse, ubiquitous sources of terrestrial and aquatic contamination (Bank and Hansson, 2019; Brahney et al., 2020; Rillig and Lehmann, 2020). They can be found in a myriad of environments, exhibiting different shapes and compositions, ranging in size from 1 μm to 5 mm, with densities varying from 0.28 to 1.47 g cm^{-3} (Hanvey et al. 2017; Bank et al. 2021). MPs can spread into the environment through various pathways,

such as wastewater treatment facilities, landfills, agricultural activities, industrial discharge, household runoff, soil amendments, mulching, sludge, irrigation, flooding, atmospheric deposition, and littering (Rillig et al., 2017; Mai et al., 2018; Boots et al., 2019; Conley et al. 2019; Corradini et al. 2019; Gündoğdu et al. 2018; He et al. 2019; Li et al. 2018; Rochman 2018; Steinmetz et al. 2016).

Featuring polymer binders as their main constituents, paints are recognized as a significant source of MPs (Gaylarde et al. 2021; Song et al. 2014; Turner 2021). The monitoring of paint as an MP source is extensively studied in marine and freshwater environments and it is gaining prominence in terrestrial settings as well (Lima et al., 2014; Nizzetto et al., 2016; Imhof et al., 2016; Galafassi et al., 2019; Xu et al., 2022). Paint MPs can pose environmental hazards due to their chemical composition; for instance, paints applied to ships and nautical equipment contain potentially toxic elements (PTEs) like Copper (Cu), Zinc (Zn), and Lead (Pb), alongside 'booster biocides' used to deter fouling organisms (Gade et al., 2012; Soroldoni et al., 2017).

Besides the plastic structure, paints and their primers may encompass corrosion inhibitors, extenders, and major and minor pigments such as Titanium (Ti), Barium metaborate ($Ba(BO_2)_2$), Chromium (Cr), Iron (Fe), and Tin (Sn) oxides (Singh and Turner, 2009). High emissions of any of these elements and components into the environment can pollute both biotic and abiotic systems (Cipro et al., 2017; de Souza Machado et al., 2018; Campanale et al., 2020). Therefore, more attention should be paid to the paint MPs (Xu et al., 2022).

The leakage of these substances into the environment can occur through natural processes such as temperature changes, acid rain, and exposure to ultraviolet radiation (Sørensen et al., 2021; Costa et al., 2023). This last one can induce chemical changes in polymers (Gomaa et al., 2018; Yousif and Haddad, 2013). The polymer-light interaction primarily takes place throughout the chromophore groups, which absorb light and transfer its energy to the system promoting homolytic scission and generating free radicals (Curcio et al., 2018). This process initiates an auto-oxidation cycle, causing alteration on the material surface, which, as the degradation proceeds, will gradually extend into the polymer’s bulk (Blais et al., 1972; Curcio et al., 2018; de Freitas et al., 2022).

Spray paints are extensively used in industrial, domestic, and graffiti applications, but their mass transferring efficiency ranges from 50 to 65%, causing the remaining material to be lost into the air during application, forming droplets, aerosols, and generating a range of particulates (Heitbrink et al.,1996; Poozesh et al, 2017). Thus, the environmental impact caused by pristine MPs originated from spray paint may be similar to that of secondary MPs formed from the degradation of larger plastic portions (Mølgaard et al., 2015; Chen et al., 2019).

Crack formation is a common phenomenon of polymer photodegradation, linked to the decline in mechanical properties, including increased brittleness (Rabello and White, 1997). Material deterioration usually begins with the development of microvoids, progressing into a fibrillation process that eventually leads to fibril rupture and cracks (Takahashi et al., 2010; de Freitas et al., 2022). The photodegradation process can occur in paint adhered to structures, as well as to the fragments lost to the environment during application (Christensen et al., 1999; Christensen et al., 2000; Groeneveld et al., 2023).

This study aims to investigate, for the first time, the effects of photodegradation on spray paint MPs exposed to different light intensities and exposure times to determine whether greater microparticle degradation leads to increased release of potentially toxic elements (PTEs) in aqueous environments. Given the existing gaps in scientific knowledge on this subject, this research seeks to elucidate the potential toxicity of photodegraded particles in aquatic ecosystems. The selected paint is environmentally relevant as it contains PTEs in its pigment composition.

2.5. Methodology

2.5.1. Determination of Elements in Pigments

Luxens® Glossy Black spray paint was applied to a paper surface, forming a 3 x 5 cm^2 area with uniform thickness. A portable X-ray fluorescence (XRF) system was used for analysis, featuring an Amptek® silver filament X-ray tube (30 kV and 10 μA) and an Amptek® Si-Drift detector. During measurements, the XRF system was positioned close to the sample without making contact, ensuring no damage occurred. Each measurement lasted 100 seconds and covered an area approximately 3 mm in diameter, identifying the primary elements in the paint composition.

2.5.2. Microplastic Production and Pristine Particles Characterization

The MPs were generated by spraying the graffiti can into the air in a clean, empty, closed room (12.02 m^2; 5.18 m x 2.32 m) until the paint was exhausted. This procedure ensured that the paint formed aerosol droplets, which were left to settle and dry for five days. The particles were then collected using wooden tools and horsehair brooms and subsequently sanitized. To standardize the pristine sample, the granulometric fraction of 250 to 53 μm was separated using mesh sieves. After production, the MPs were coated with carbon using a sputtering device (Leica EM ACE600) to a final nominal thickness of 16 nm and examined with a scanning electron microscope (Thermo Fisher Scientific Inspect F50). The resulting micrographs were

manually processed to estimate the area of the particles, and roundness was calculated using ImageJ® software and Equation 1.

Equation 1: $$4 \cdot \frac{[Area]}{\pi \cdot [Major\ Axis]^2} = \text{Roundness}$$

Where "Area" is the 2D visualized circumference of the sphere, manually calculated; "Major Axis" is the primary axis of the best-fitting ellipse; and "π" is approximately 3.14. The roundness and area parameters from the software were used, and a sole researcher conducted manual measurements to ensure quality control and prevent subjectivity in the sampling procedures. Particles observed in one micrograph per working group were analyzed and measured.

2.5.3. Polymer Identification

To remove the pigment from the spray paint and improve the accuracy of Fourier Transform Infrared Spectroscopy (FTIR) measurements of the polymer, 0.5 g of the pristine MP sample was dissolved in 10 mL of xylene (P.A.-A.C.S. 100%). This solution was then filtered through 0.45 μm acetate membranes using syringes to retain pigments and other particulates. The filtered solution was transferred to a Petri dish and left in a laminar flow hood for 48 hours to allow the xylene to evaporate, leaving only the polymer. The polymer residue was scraped from the dish and mixed with KBr (potassium bromide dried at 120ºC for 4 hours) to create pellets (200 mg of KBr and 2 mg of polymer). These pellets were analyzed by FTIR with 16 scans at a nominal resolution of 4.0 cm^{-1} (Thermo Scientific Nicolet Summit® iD1) using OMNIC Paradigm software. The online software Open Specy (Cowger et al., 2021) was utilized for polymer identification, with preprocessing enabled, a threshold signal-to-noise value set to 4, the signal threshold technique set to signal over noise, and the smoothing/derivative feature enabled.

2.5.4. Experimental Photodegradation

The pristine MPs were divided into four distinct exposure groups (Table 1). Each group consisted of a 3 g sample, which was dispersed into four Petri dishes (21.29 cm^2 each) to ensure the material formed a single layer and was evenly exposed (0.75 g of pristine MPs in each Petri dish). All groups were exposed in a photodegradation chamber, as described by Cacuro et al. (2018). The samples were positioned 0.2 m away from the 15 W UV-C fluorescent germicidal lamps (Osram brand, model TUV15W), which emit at a maximum wavelength of 254 nm, while maintaining a chamber temperature of 35ºC.

Table 1 - Presentation of the work groups and the treatments applied to each of them.

Sample Identification	Exposure Time	Number of UV-C Lamps	Incident Energy
1UVC24	24 hours	1 UV-C Lamp	6.1±0.1 μW.cm^{-2}
1UVC48	48 hours	1 UV-C Lamp	12.2±0.1 μW.cm^{-2}
3UVC24	24 hours	3 UV-C Lamps	18.2±0.1 μW.cm^{-2}
3UVC48	48 hours	3 UV-C Lamps	36.5±0.1 μW.cm^{-2}

The total incident energy was calculated using equation 2:

Equation 2: $$E = \frac{(P \cdot t)}{A}$$

Where "E" is the irradiated energy in joules (J), "P" is the lamp power in watts (W), "t" is the exposure time in seconds (s), and "A" is the area over which the energy is distributed (m²).

2.5.5. Photodegraded Microplastics Characterization

After exposure, the samples were collected, carbon coated (as described previously), and examined using a scanning electron microscope (Thermo Fisher Scientific Inspect F50). The shape and size of the particles in the captured micrographs were analyzed using the same ImageJ® software parameters applied to the pristine group. To ensure quality control and avoid subjectivity, the same researcher from the previous group conducted manual measurements of the particles in a single micrograph. The values obtained for each variable in each group were compared with those from the pristine MPs using the non-parametric Kruskal-Wallis test. This comparison aimed to understand the effects of different treatments on the exposed particles. The micrographs were also evaluated to identify patterns of photodegradation, such as the presence of cracks, formation of secondary MPs, and particle coalescence.

2.5.6. Release of Potentially Toxic Elements from Paint Microplastics in Aqueous Solution

2.5.6.1. Release

For determining the release of Potential Toxic Elements (PTEs) of both pristine and degraded paint MPs samples, 0.5 g from each of the five samples was added to 50 mL of ultrapure water. Two exposures were conducted for each group: one at a pH of 8 and another at a pH of 5. These dispersions were placed on an orbital shaking table (Tecnal model TE-1400) at 130 RPM for 24 hours. Following this period, the samples were filtered with 0.45 μm cellulose acetate membranes to remove plastic particles using a vacuum filtration system (Vix

model VPA 115), and the final solution's pH was measured using a portable pHmeter (Akso model AKLA56847).

2.5.6.2. Acid Digestion

To analyze the release of PTEs from polymers in aqueous solution, acid digestion was conducted in triplicate using the American Public Health Association's (APHA, 2000) method 3030E. In this procedure, 2.5 mL of concentrated nitric acid (HNO_3, 65% w/w) was added to the 50 mL dispersion solution and heated to 120 ºC on a hot plate. Post-digestion, the samples were diluted with ultrapure water in a 25 mL volumetric flask and subsequently transferred to Falcon tubes for the quantification of PTEs.

2.5.6.3. Quantification of PTEs released from MPs

The quantification of PTEs released from the MPs in aqueous solution was conducted using a microwave plasma atomic emission spectrometer (Agilent model 4200 MP-AES) (APHA, 2000). The emission lines, limits of detection (LOD), and quantification (LOQ) for the elements analyzed in each concentration range are presented in Table 2.

Table 2 - Emission lines, limits of detection (LOD) and quantification (LOQ) of the elements analyzed in the Agilent 4200 MP-AES.

Concentration range: 0, 0.01, 0.05, 0.1, 0.5, 1.0, 1.5 mg L^{-1}			
PTE	**Emission lines (nm)**	**LOD mg L^{-1}**	**LOQ mg L^{-1}**
Cr	425.433	0.0012	0.0040
Cu	324.754	0.0009	0.0031
Mn	403.076	0.0001	0.0003
Zn	213.857	0.0173	0.0577
Concentration range: 0, 0.1, 0.5, 1.0, 1.5, 2.5 mg L^{-1}			
PTE	**Emission lines (nm)**	**LOD mg L^{-1}**	**LOQ mg L^{-1}**
Fe	371.993	0.0034	0.0113

A synthesis of the whole applied methodology is didactically presented in Figure 1.

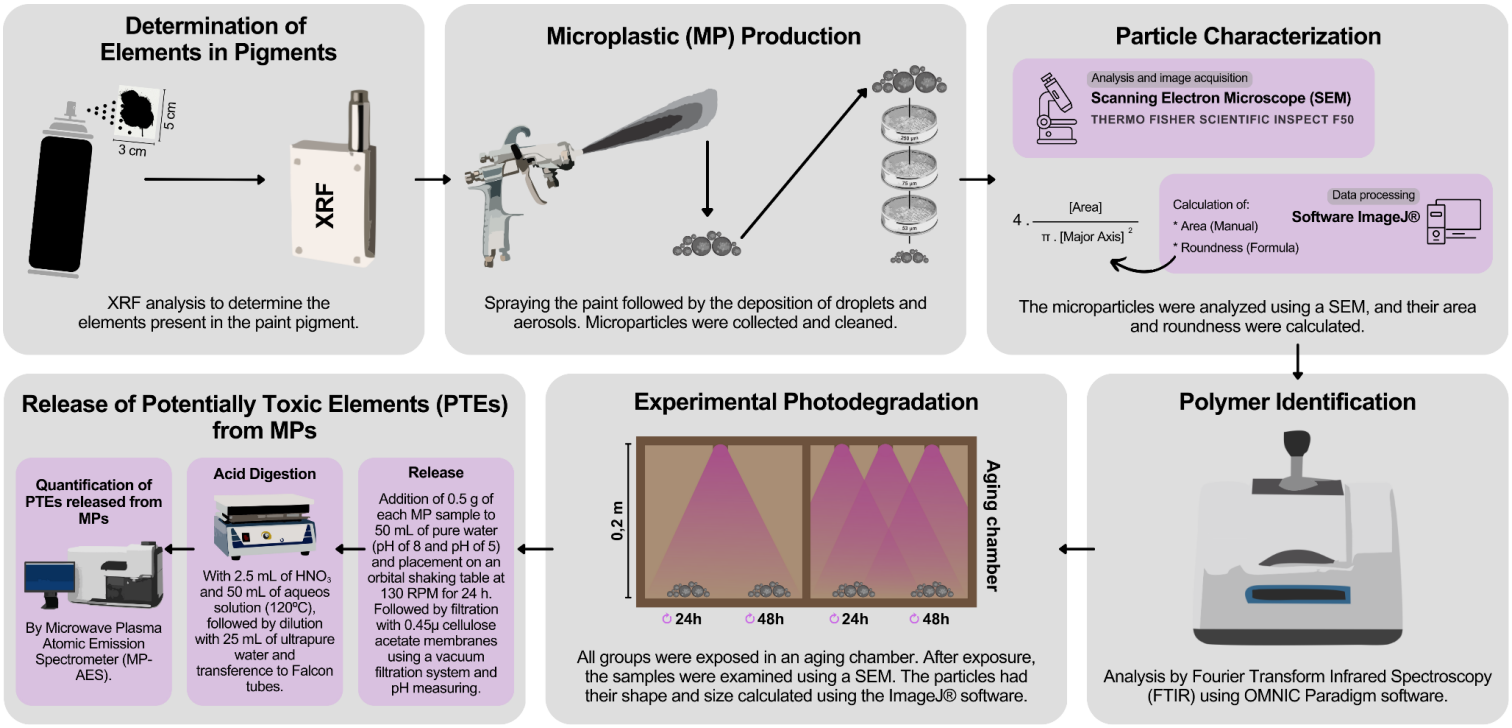


Figure 1 - Workflow and methodological overview applied in this paper.

2.6. Results and Discussion

The X-ray fluorescence (XRF) analysis showed the presence of twelve elements as different components of the spray paint. These elements include Silicon (Si), Sulfur (S), Chlorine (Cl), Potassium (K), Calcium (Ca), Titanium (Ti), Chromium (Cr), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn), and Strontium (Sr); their relative abundance in the paint sample is presented in Figure 2.

The variety of elements found is associated with the paint formulation; they may be part of antifouling agents, corrosion inhibitors, extenders, and primary/secondary pigments, which impart the color to the paint (Gaylarde et al., 2021). The presence of iron highlighted in Figure 2 is due to its usage in pigments like magnetite (Fe_3O_4), which has a dark color, or the blue color of Prussian blue ($Fe_4[Fe(CN)_6]_3$) (Cartechini et al., 2021).

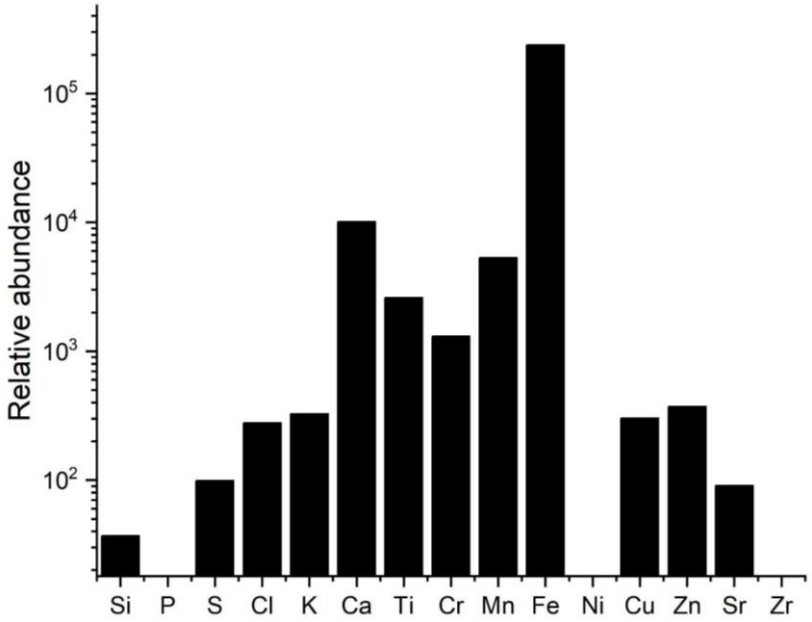


Figure 2 - Analysis of the relative abundance of elements present in the studied paint obtained by X-ray fluorescence spectrometry.

The assessment of the paint polymer using the Thermo Scientific Nicolet Summit® iD1 (FTIR) and the Open Specy virtual library (Cowger et al., 2021) revealed a high similarity (Pearson's coefficient of 81%) with polyphenylethylenes α-methylstyrene, a derivative of polystyrene (PS). The obtained similarity value may not have reached a higher rate due to the complex formulation of the paint, as it is a multicomponent material that may contain other polymers and additives, influencing the equipment readings, as depicted in Figure 3.

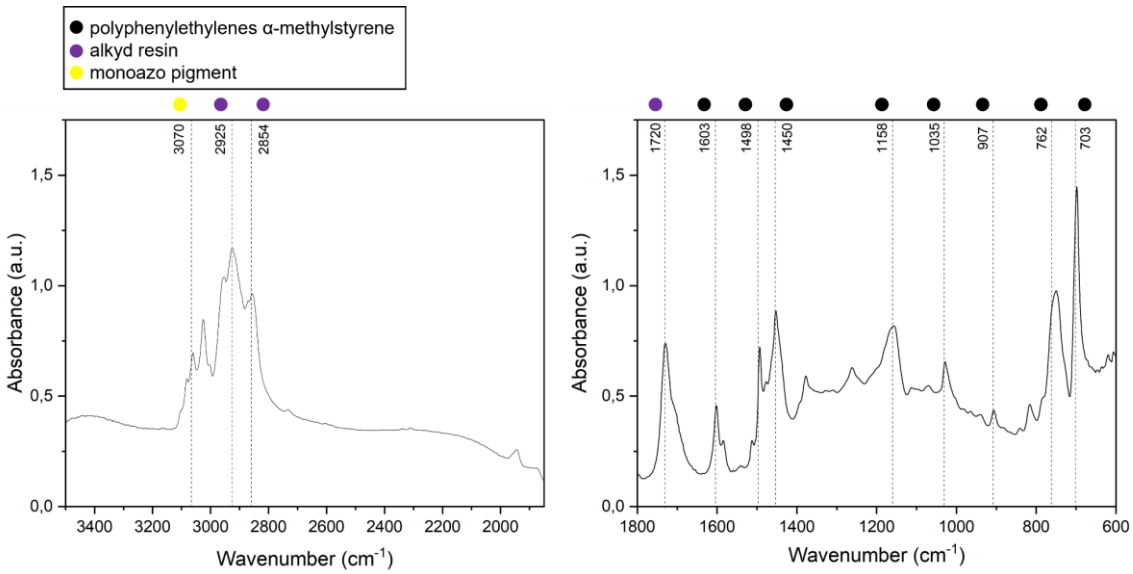


Figure 3 - FTIR analysis of the spray paint. The bands indicate the presence of polyphenylethylenes α-methylstyrene, alkyd resin, and monoazo pigment.

The FTIR bands indicate that, in addition to the presence of polyphenylethylenes α-methylstyrene, the paint also includes alkyd resin and monoazo pigment in its composition. Alkyd resin, like polyphenylethylenes α-methylstyrene, is a widely used binder for paint production and various plastic components (Cortea et al., 2020; Imhof et al., 2016).

The pristine MPs generated from the spray paint (Figure 4) exhibited an average area of 307.2 ± 349.4 μm^2 and a spherical shape, with a mean roundness value of 0.96 ± 0.03, approaching the value classified as completely circular (1.00) applied by the software. This spherical conformation of aerosol particles is generated from surface tension effects occurring during the spray application,imparting this characteristic shape to the microparticles (Alves, 2005).

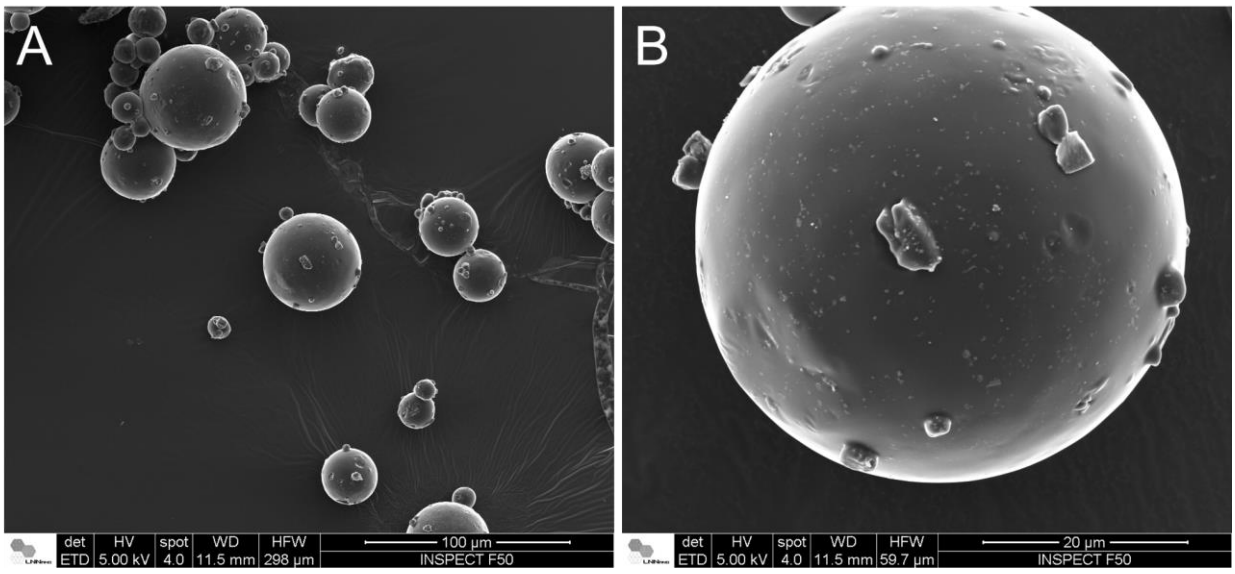


Figure 4 - Pristine spray paint MPs. Main characteristic is the spherical shape. Image captured using scanning electron microscopy. A) Particle agglomerate; B) Individual particle.

The pristine particles exposed to different intensities and exposure times of UV-C radiation showed alterations in the original values of area and roundness as presented in Table 3. To better compare the changes in the dimensional stability of the samples, all information was gathered into Figure 5.

Table 3: Area and roundness values of the sample groups exposed to different intensities and exposure times to UV-C. Their mean values were compared with those of the pristine samples, and the difference was presented.

Sample Identification	Average area	Comparison with pristine area	Average roundness	Comparison with pristine roundness
1UVC24	344.9 ± 385.8 μm^2	p > 0.9999	0.92 ± 0.09	p = 0.3191
1UVC48	144.8 ± 230.6 μm^2	p = 0.0086	0.78 ± 0.16	p < 0.0001
3UVC24	182.8 ± 199.8 μm^2	p > 0.9999	0.83 ± 0.16	p < 0.0001
3UVC48	168.6 ± 374.1 μm^2	p < 0.0001	0.73 ± 0.17	p < 0.0001

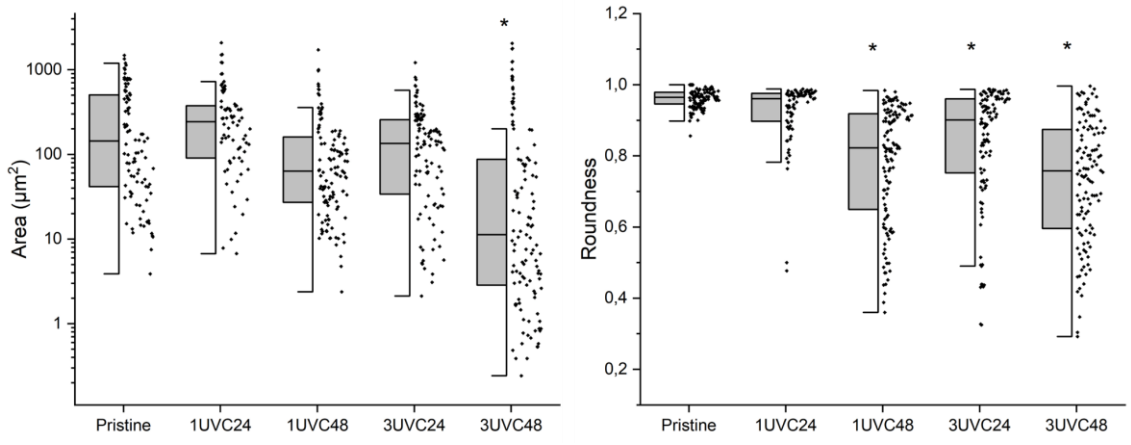


Figure 05: Comparison of the difference between the area and roundness of pristine and photodegraded particles. Black asterisk: significant difference from the pristine.

The degradation of the particles was gradual and followed the expected pattern, as the group exposed for a longer time and intensity (3UVC48) showed the most significant changes in the analyzed parameters. 1UVC24 appears to have reached the threshold value for roundness alteration in its particles, as an additional 24 hours was sufficient for 1UVC48 to exhibit this change. The reduction in the area of these particles may be related to the decrease in their roundness, as the degradation of larger particles, in addition to generating smaller secondary MPs, ends up altering their original shape. To better compare this group with the pristine one, we present these values in Figure 6. The prominent presence of nanoplastic particles in this group, with area sizes such as 0.388, 0.243, and 0.582 μm^2, formed from the degradation of larger particles, is an indication of the degradation of the polystyrene polymer that constitutes the particles.

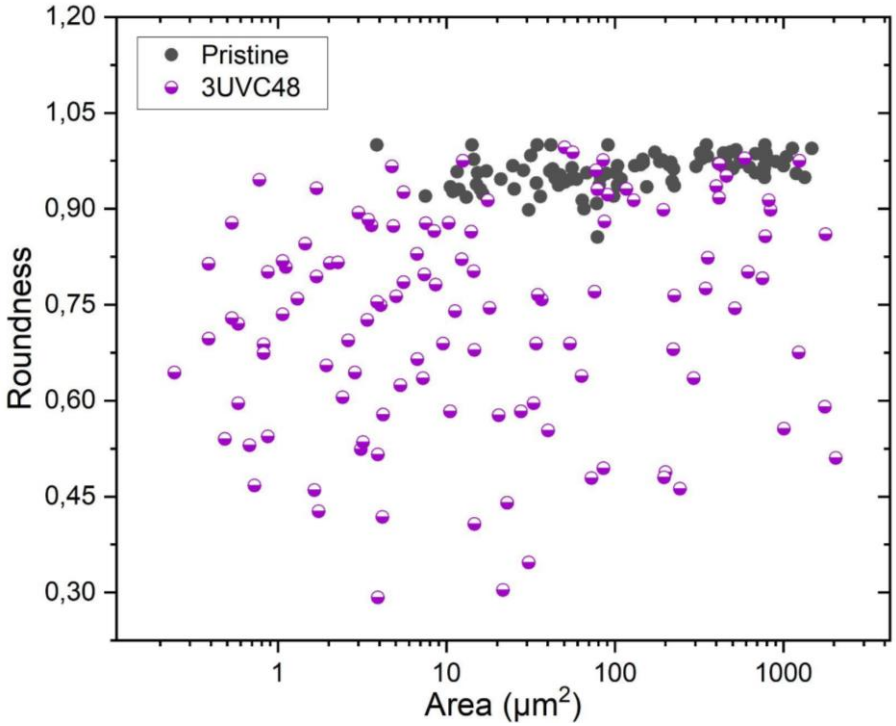


Figure 6 - Comparison between the values of area and roundness of pristine particles and 3UVC48, demonstrating a higher presence of smaller particles with a shape distinct from the original, likely caused by more extensive polymer degradation in this group.

The photodegradation of PS involves physical and chemical changes in its polymeric chains, potentially leading to breaks that generate free radicals and reduce its molecular weight, causing deterioration of the mechanical and structural properties of the polymer (Yousif and Haddad, 2013). The photodegraded samples showed superficial modification more often as a function of the higher incident energy (Figure 7B and 7D). One hypothesis is the blooming of additives as the degradation evolves (Nouman et al., 2017). Another effect of the degradation is the increase of the fragility (Waldman and Rillig, 2020), causing the detachment or breakage on the surface, as exemplified at the yellow arrows in Figures 7A, 7B and 7C, displaying the voids after the detachment, and in Figure 7D, a potential ongoing process of detachment. As it is observable by the measurement, the voids are at the order of magnitude of 5.6 to 4 micrometers. This degradation, if it occurs in the presence of oxygen, results in a color change (in translucent substrates) and gradual embrittlement of the particles (Schnabel, 1982) which also may explain the formation of cracks in these MPs (de Freitas et al., 2022).

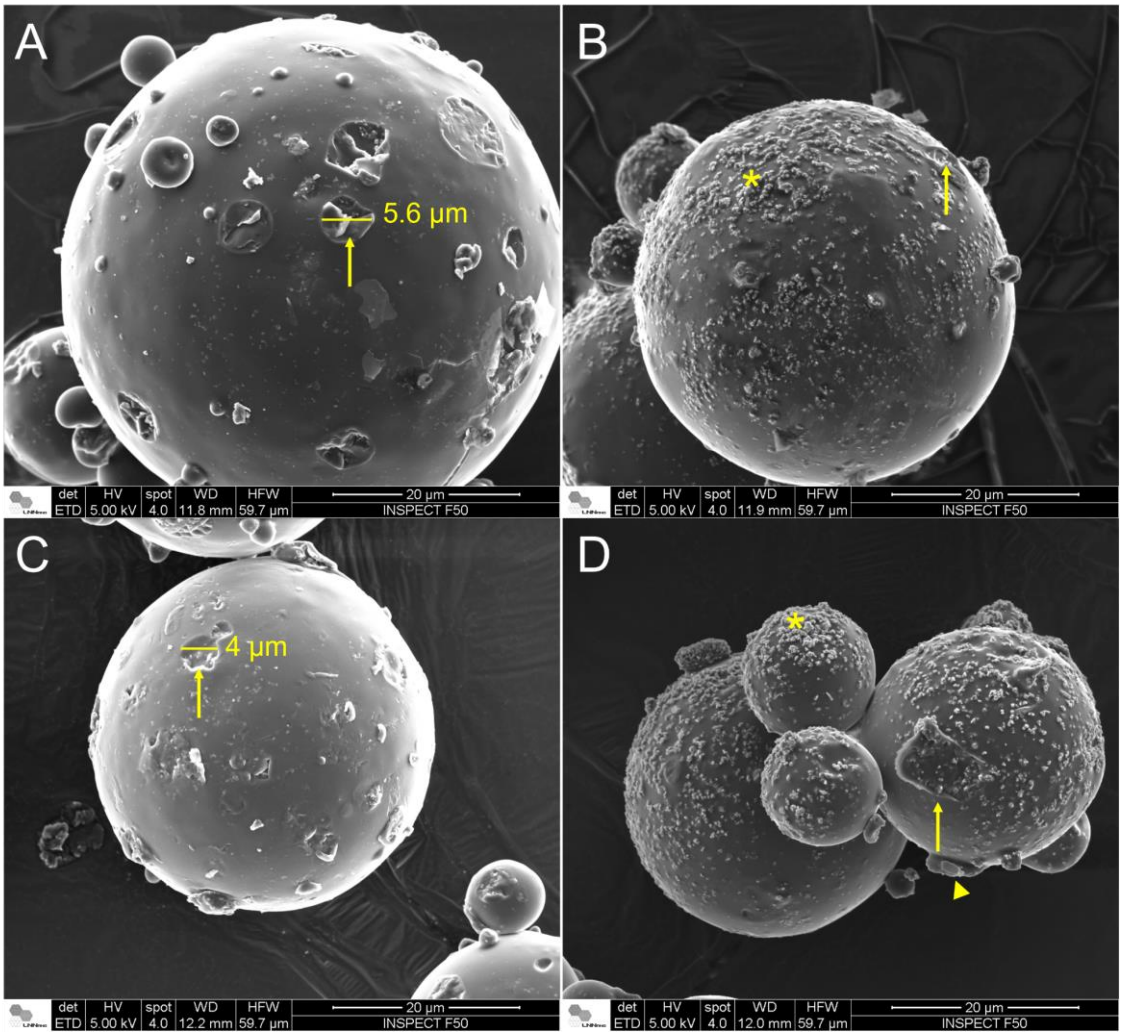


Figure 7 - Photodegraded MPs at different exposure times. A) 1UVC24; B) 1UVC48; C) 3UVC24; D) 3UVC48. Yellow arrow: Ongoing detachment of plastic portion; Yellow arrowhead: voids caused by the detachments of plastic portion; Yellow asterisk: blooming of additives.

This process of photodegradation on MPs can result in the release of internal substances from the particle, such as metals and other components of the paint (Gaylarde et al., 2021; Lassen et al., 2015), which might affect the environment in diverse ways, depending on the element or toxicant (Soroldoni et al., 2017; Soroldoni et al., 2018). Several studies evaluating the impacts of antifouling paint particles resulting from the degradation of vessel coatings have shown that component metals such as Pb, Cu, Cd, Cr and Zn are released into the environment due to weathering (Nakashima et al., 2016; Turner, 2010; Enders et al., 2019; Daehne et al., 2017; Takahashi et al., 2012). They also demonstrate that this release is higher in paint particles than in the hulls of fully painted ships, as particles have a larger surface area in contact with the environment, leading to their faster degradation (Gaylarde et al., 2021).

The release of components from the particles due to degradation can also be observed in Figure 8. The degraded MPs, especially from 3UVC24 and 3UVC48, showed an increase in metal release. Mn had the most prominent release among all; at pH 8, the release from 3UVC24 was 2.9 times higher than that of the pristine, while from 3UVC48, it was 7.9 times higher than the control. Both of these results exceed the values considered safe by the National Environment Council of Brazil (CONAMA, 2005) for freshwater, and the value for 3UVC48 also surpasses the limit stipulated by the World Health Organization (W.H.O., 2008) The values for this same metal at pH 5 are similar; the release from 3UVC24 was 1.8 times higher than that of the pristine group, and from 3UVC48, it was 6.2 times higher. Both values exceed the CONAMA safety threshold, and only the 3UVC48 exceeds the W.H.O. regulations. A Mann-Whitney test was applied to assess if there is a statistical difference in metal release at different pH levels, but it yielded a negative result ($p = 0.5476$), demonstrating that the pH difference, in this case, does not influence the release of these metals into the environment.

A

pH = 8.0	Zn	Cu	Mn	Cr	Fe
Pristine	0.083	0.006	0.068	<LOD	<LOD
1UVC24	<LOD	0.001	0.009	<LOD	<LOD
1UVC48	0.007	0.001	0.029	<LOD	<LOD
3UVC24	0.033	0.011	0.201	0.001	0.044
3UVC48	0.072	0.016	0.541	<LOD	0.177
CONAMA	0.18	0.009	0.1	0.005	0.3
W.H.O.	3	2	0.4	0.05	NA

B

pH = 5.0	Zn	Cu	Mn	Cr	Fe
Pristine	0.013	0.003	0.099	<LOD	<LOD
1UVC24	0.019	0.001	0.041	0.000	<LOD
1UVC48	0.012	0.001	0.101	<LOD	<LOD
3UVC24	0.037	0.007	0.187	0.003	0.053
3UVC48	0.064	0.016	0.621	<LOD	0.034
CONAMA	0.18	0.009	0.1	0.005	0.3
W.H.O.	3	2	0.4	0.05	NA

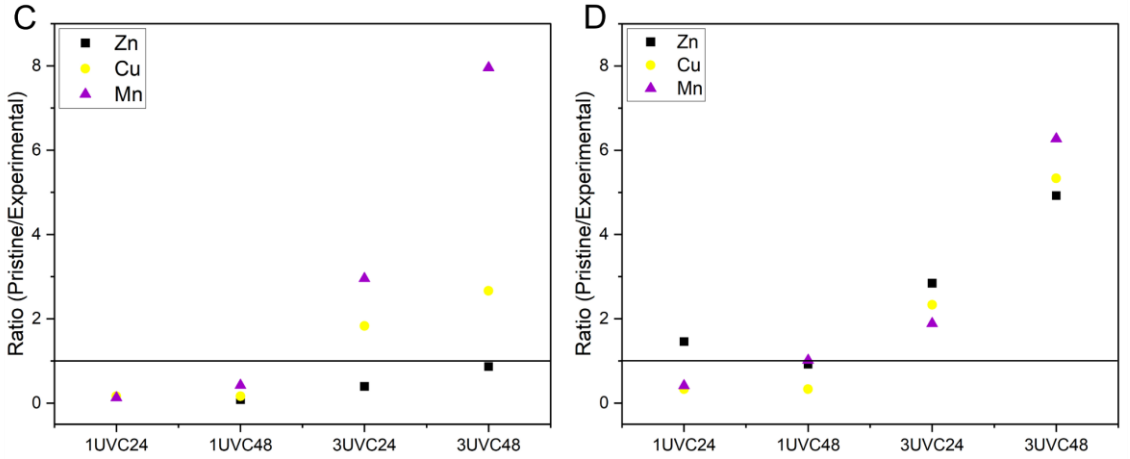


Figure 8 - Release values of the detected metals by the MP-AES of pristine particles and the four degraded levels during a 24-hour period at two pH values. The graph presents a ratio value between the pristine and the degraded groups of the three principal released metals. The graph C has a missing Zn value for 1UVC24, as it was <LOD (below the limit of detection). A) Release values of particles exposed to pH 8; B) Release values of particles exposed to pH 5; C) Ratio values of released metals between pristine and exposed groups at pH 8; D) Ratio values of released metals between pristine and exposed groups at pH 5.

Cu also showed an increase in its release in 3UVC24 in both treatments with different pH levels. At pH 8, its increase was 1.8 times, and its final value exceeds the safety threshold set by CONAMA, while at pH 5, its increase was 2.3 times compared to the pristine, but the value remains below environmental parameters. 3UVC48 in both treatments showed an increase in metal release, with the pH 8 treatment releasing 2.6 times more than the control, and the pH 5 treatment releasing 5.3 times more. Both final concentrations of the samples exceeded the value considered safe by CONAMA. Zn showed increases in its release only in 3UVC24 and 3UVC48, and the results of Cr and Fe release were inconclusive. The increased release of these elements by more degraded particles is expected due to the degradation process of polyphenylethylenes α-methylstyrene mentioned earlier.

An increase in soil Cu levels resulting from the degradation of plastic particles has the potential to disrupt the biotic environment (Daehne et al., 2017). Cu exhibits a strong affinity for soil organic matter and forms robust inner-sphere metal-organic ligand complexes through covalent-type bonds (Samarajeewa et al., 2021). These complexes are established with the organic constituents found in the soil, including decomposed plant and animal materials. The availability of binding sites for Cu is intricately linked to soil pH, with a lower pH leading to increased availability through deprotonation reactions (Senesi and Loffredo, 2018). This dynamic relationship between Cu and soil conditions can have profound implications, affecting various aspects of ecosystem dynamics and some groups, such as ruminants, insects and crop plants (Harris and Gitlin, 1996; Mastin and Rodgers, 2000; Gaetke, 2003).

Since the leaching values of Mn exceeded environmental limits, they may cause alterations to organisms. Symptoms of Mn toxicity and the threshold concentrations inducing toxicity exhibit considerable variability across plant species and even within different varieties of the same species (Fargašová et al., 1999). This variability may be attributed to distinct phytotoxic mechanisms of Mn that engage diverse biochemical pathways depending on the genotype of the plant (Ghosh et al., 2016; Singh et al., 2020). Mn's availability to plants relies on soil adsorption and the presence of root exudates for Mn chelation or reduction (Liu et al., 2020). Soils with heightened Mn sorption capacity tend to reduce the potential for Mn absorption by plants (Roy et al., 2021). Robust Mn tolerance is linked to constrained absorption, restricted translocation of excessive Mn to the aerial parts, or heightened tolerance to elevated Mn levels within the plant tissue (Mohanty et al., 2017).

An excess of Mn in the soil can cause alterations in the photosynthetic system of some plants, affecting their development and inhibiting the absorption of other nutrients (Ca, Mg, Fe,

and Zn); as well as the formation of brown necrotic leaf spots on the leaves, in addition to inhibiting the growth of shoots and roots, negatively impacting the physiological and biochemical processes of plants (Liu et al., 2020).

In relation to human health, chronic exposure to this metal pollutant can result in adverse effects and various symptoms related to neurotoxicity, including cognitive issues, Parkinson's disease, manganism, and dystonia (Das and Singh, 2011; Petitjean et al., 2021) The toxicity depends on several aspects, including the dose, route of exposure, species, and nutritional status of the individual (Bjerregaard et al., 2015). This element can be considered a systemic toxicant that can damage multiple organs in humans (Hudnell, 1999). Clinical neurotoxicity was reported in patients receiving long-term parenteral nutrition and in patients with chronic liver dysfunction or renal failure, as a result of their inability to eliminate and clear Mn from the blood (O'Neal and Zheng, 2015).

These cited alterations can occur more easily in environments where the particles analyzed in this study are present. MP pollution is already a global problem, and the polymers that compose them are just the beginning of the issue (Weinstein et al., 2019; Hale et al., 2020). The degradation of these particles will increase their toxicity to the environment (Cipro et al., 2017; de Souza Machado et al., 2018; Campanale et al., 2020). Additionally, their small size, wide distribution, and ability to adsorb other toxicants make MPs long-lasting and high-impact environmental pollutants and contaminants, with spray paint MPs being a prime example.

2.7. Conclusion

The photodegradation of particles under UV-C radiation followed an increasing pattern, in line with light intensity and exposure time. 3UVC24 and 3UVC48 exhibited the most significant alterations, such as the blooming of the additives, degradation of the particles edges and pieces detachment, thereby significantly altering their area and roundness values compared to the pristine group. These changes likely led to increased release of the metals Mn and Cu from these two samples, which can cause environmental impacts. The originality of the present research underscores the need for attention to spray paint photodegradation issues, and the development of new public policies and legislation is crucial, as understanding paints as a source of various pollutants, not just plastic polymers, is an emerging concern.

2.8. References

Alves, C. (2005). Aerossois atmosféricos: perspectiva histórica, fontes, processos químicos de formação e composição orgânica. Química Nova, 28(5), 859–870. doi: https://doi.org/10.1590/s0100-40422005000500025.

American Public Health Association – APHA, 2000. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, twenty ed., Washington, DC.

Bank, M.S., & Hansson, S.V. (2019). The Plastic Cycle: A Novel and Holistic Paradigm for the Anthropocene. Environmental Science & Technology, 53(13), pp. 7177–7179. doi: https://doi.org/10.1021/acs.est.9b02942.

Bank, M.S., Swarzenski, P.W., Duarte, C.M., Rillig, M.C., Koelmans, A.A., Metian, M., Wright, S., Provencher, J.F., Sanden, M., Jordaan, A., Wagner, M., Thiel, M., & Ok, Y.S. (2021). Global Plastic Pollution Observation System to Aid Policy. Environmental Science & Technology, 55(12), pp. 7770–7775. doi: https://doi.org/10.1021/acs.est.1c00818.

Bjerregaard, P., Andersen, C.B.I., & Andersen, O. (2015). Ecotoxicology of Metals—Sources, Transport, and Effects on the Ecosystem. Handbook on the Toxicology of Metals, 425–459. doi: https://doi.org/10.1016/b978-0-444-59453-2.00021-4.

Blais, P., Carlsson, D.J., & Wiles, D.M. (1972). Surface changes during polypropylene photo-oxidation: A study by infrared spectroscopy and electron microscopy. Journal of polymer science, 10(4), pp. 1077–1092. doi: https://doi.org/10.1002/pol.1972.150100412.

Boots, B., Russell, C.W., & Green, D.S. (2019). Effects of Microplastics in Soil Ecosystems: Above and Below Ground. Environmental Science & Technology, 53(19), pp. 11496–11506. doi: https://doi.org/10.1021/acs.est.9b03304.

Brahney, J., Hallerud, M., Heim, E., Hahnenberger, M., & Sukumaran, S. (2020). Plastic rain in protected areas of the United States. Science, 368(6496), pp. 1257–1260. doi: https://doi.org/10.1126/science.aaz5819.

Cacuro, T.A., Freitas, A.S.M., & Waldman, W.R. (2018). Demonstration of Polymer Photodegradation Using a Simple Apparatus. Journal of Chemical Education, 95(12), pp. 2222–2226. doi: https://doi.org/10.1021/acs.jchemed.7b00836.

Campanale, C., Massarelli, C., Savino, I., Locaputo, V., & Uricchio, V.F. (2020). A Detailed Review Study on Potential Effects of Microplastics and Additives of Concern on Human

Health. International Journal of Environmental Research and Public Health, 17(4), p. 1212. doi: https://doi.org/10.3390/ijerph17041212.

Cartechini, L., Miliani, C., Nodari, L., Rosi, F., & Tomasin, P. (2021). The chemistry of making color in art. Journal of Cultural Heritage, 50, 188–210. doi: https://doi.org/10.1016/j.culher.2021.05.002.

Chen, Y.-C., Lin, C.-H., Lung, S.-C.C., Chen, K.-F., Wang, W.-C.V., Chou, C.-T., & Lai, C.-H. (2019). Environmental concentration of spray paint particulate matters causes pulmonary dysfunction in human normal bronchial epithelial BEAS-2B cell. Process Safety and Environmental Protection, 126, pp. 250–258. doi: https://doi.org/10.1016/j.psep.2019.04.013.

Christensen, P.A., Dilks, A., Egerton, T.A., et al. (1999). Infrared spectroscopic evaluation of the photodegradation of paint Part I The UV degradation of acrylic films pigmented with titanium dioxide. Journal of Materials Science, 34, 5689–5700. doi: https://doi.org/10.1023/A:1004737630399.

Christensen, P.A., Dilks, A., Egerton, T.A., et al. (2000). Infrared spectroscopic evaluation of the photodegradation of paint Part II: The effect of UV intensity & wavelength on the degradation of acrylic films pigmented with titanium dioxide. Journal of Materials Science, 35, 5353–5358. doi: https://doi.org/10.1023/A:1004898913140.

Cipro, C.V.Z., Cherel, Y., Bocher, P., Caurant, F., Miramand, P., & Bustamante, P. (2017). Trace elements in invertebrates and fish from Kerguelen waters, southern Indian Ocean. Polar Biology, 41(1), pp. 175–191. doi: https://doi.org/10.1007/s00300-017-2180-6.

Conley, K., Clum, A., Deepe, J., Lane, H., & Beckingham, B. (2019). Wastewater treatment plants as a source of microplastics to an urban estuary: Removal efficiencies and loading per capita over one year. Water Research X, 3, p. 100030. doi: https://doi.org/10.1016/j.wroa.2019.100030.

Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E., & Geissen, V. (2019). Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. Science of The Total Environment, 671, pp. 411–420. doi: https://doi.org/10.1016/j.scitotenv.2019.03.368.

Cortea, I.M., Ratoiu, L., & Rădvan, R. (2020). Characterization of spray paints used in street art graffiti by a non-destructive multi-analytical approach. Color Research & Application, 46(1), 183–194. doi: https://doi.org/10.1002/col.22561.

Costa, J.P. da, Avellan, A., Mouneyrac, C., Duarte, A., & Rocha-Santos, T. (2023). Plastic additives and microplastics as emerging contaminants: Mechanisms and analytical assessment. TrAC Trends in Analytical Chemistry, 158, p. 116898. doi: https://doi.org/10.1016/j.trac.2022.116898.

Cowger, W., Steinmetz, Z., Gray, A., Munno, K., Lynch, J., Hapich, H., Primpke, S., De Frond, H., Rochman, C. and Herodotou, O. (2021). Microplastic Spectral Classification Needs an Open Source Community: Open Specy to the Rescue! *Analytical Chemistry*, 93(21), pp.7543–7548. doi:https://doi.org/10.1021/acs.analchem.1c00123.

Curcio, M.S., Canela, M.C., & Waldman, W.R. (2018). Selective surface modification of TiO2-coated polypropylene by photodegradation. European Polymer Journal, 101, pp. 177–182. doi: https://doi.org/10.1016/j.eurpolymj.2018.01.036.

Daehne, D., Fürle, C., Thomsen, A., Watermann, B., & Feibicke, M. (2017). Antifouling biocides in German marinas: Exposure assessment and calculation of national consumption and emission. Integrated Environmental Assessment and Management, 13(5), 892–905. doi: https://doi.org/10.1002/ieam.1896.

Das, A. and Singh, S. (2011). Occupational health assessment of chromite toxicity among Indian miners. Indian Journal of Occupational and Environmental Medicine, 15(1), 6. doi: https://doi.org/10.4103/0019-5278.82998.

de Freitas, A.d.S.M., Rodrigues, J.S., Botaro, V.R. et al. Formation of craze-like pattern in polypropylene UV-induced surface cracking. J Polym Res 29, 506 (2022). https://doi.org/10.1007/s10965-022-03357-z.

de Souza Machado, A.A., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., & Rillig, M.C. (2018). Impacts of Microplastics on the Soil Biophysical Environment. Environmental Science & Technology, 52(17), pp. 9656–9665. doi: https://doi.org/10.1021/acs.est.8b02212.

Enders, K., Käppler, A., Biniasch, O., Feldens, P., Stollberg, N., Lange, X., Fischer, D., Eichhorn, K.-J., Pollehne, F., Oberbeckmann, S., & Labrenz, M. (2019). Tracing microplastics in aquatic environments based on sediment analogies. Scientific Reports, 9(1). doi: https://doi.org/10.1038/s41598-019-50508-2.

Fargašová, A., Bumbálová, A., & Havránek, E. (1999). Ecotoxicological effects and uptake of metals (Cu+, Cu2+, Mn2+, Mo6+, Ni2+, V5+) in freshwater alga Scenedesmus quadricauda. Chemosphere, 38(5), 1165–1173. doi: https://doi.org/10.1016/s0045-6535(98)00346-4.

Gade, A.L., Heiaas, H., Lillicrap, A., & Hylland, K. (2012). Ecotoxicity of paint mixtures: Comparison between measured and calculated toxicity. Science of The Total Environment, 435-436, pp. 526–540. doi: https://doi.org/10.1016/j.scitotenv.2012.07.011.

Gaetke, L. (2003). Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology*, 189(1-2), pp.147–163. doi: https://doi.org/10.1016/s0300-483x(03)00159-8.

Galafassi, S., Nizzetto, L., & Volta, P. (2019). Plastic sources: A survey across scientific and grey literature for their inventory and relative contribution to microplastics pollution in natural environments, with an emphasis on surface water. Science of The Total Environment, 693, p. 133499. doi: https://doi.org/10.1016/j.scitotenv.2019.07.305.

Gaylarde, C.C., Neto, J.A.B., & da Fonseca, E.M. (2021). Paint fragments as polluting microplastics: A brief review. Marine Pollution Bulletin, 162, p. 111847. doi: https://doi.org/10.1016/j.marpolbul.2020.111847.

Ghosh, S., Mohanty, S., Akcil, A., Sukla, L.B., & Das, A.P. (2016). A greener approach for resource recycling: Manganese bioleaching. Chemosphere, 154, 628–639. doi: https://doi.org/10.1016/j.chemosphere.2016.04.028.

Gomaa, E. F., Abu-Serie, M.M., Hassan, M., & Elnouby, M. (2018). Hydroxyethyl cellulose hydrogel for wound dressing: Fabrication, characterization and in vitro evaluation. International Journal of Biological Macromolecules, 111, pp. 649–659. doi: https://doi.org/10.1016/j.ijbiomac.2018.01.040.

Groeneveld, I., Kanelli, M., Ariese, F., & van Bommel, M.R. (2023). Parameters that affect the photodegradation of dyes and pigments in solution and on substrate – An overview. Dyes and Pigments, 210, p. 110999. doi: https://doi.org/10.1016/j.dyepig.2022.110999.

Gündoğdu, S., Çevik, C., Güzel, E., & Kilercioğlu, S. (2018). Microplastics in municipal wastewater treatment plants in Turkey: a comparison of the influent and secondary effluent concentrations. Environmental Monitoring and Assessment, 190(11). doi: https://doi.org/10.1007/s10661-018-7010-y.

Hale, R.C., Seeley, M.E., La Guardia, M.J., Mai, L. and Zeng, E.Y. (2020). A Global Perspective on Microplastics. *Journal of Geophysical Research: Oceans*, [online] 125(1). doi: https://doi.org/10.1029/2018jc014719.

Hanvey, J.S., Lewis, P.J., Lavers, J.L., Crosbie, N.D., Pozo, K., & Clarke, B.O. (2017). A review of analytical techniques for quantifying microplastics in sediments. Analytical Methods, 9(9), pp. 1369–1383. doi: https://doi.org/10.1039/c6ay02707e.

Harris, Z.L. and Gitlin, J.D. (1996). Genetic and molecular basis for copper toxicity. *The American Journal of Clinical Nutrition*, 63(5), pp.836S841S. doi: https://doi.org/10.1093/ajcn/63.5.836.

He, P., Chen, L., Shao, L., Zhang, H., & Lü, F. (2019). Municipal solid waste (MSW) landfill: A source of microplastics? -Evidence of microplastics in landfill leachate. Water Research, 159, pp. 38–45. doi: https://doi.org/10.1016/j.watres.2019.04.060.

Heitbrink, W.A., Verb, R.H., Fischbach, T.J., & Wallace, M.E. (1996). A Comparison of Conventional and High Volume-Low Pressure Spray-Painting Guns. American Industrial Hygiene Association Journal, 57(3), pp. 304–310. doi: https://doi.org/10.1080/15428119691015043.

Hudnell, H.K. (1999). Effects from environmental Mn exposures: a review of the evidence from non-occupational exposure studies. Neurotoxicology, 20(2-3), 379–397. Available at: https://pubmed.ncbi.nlm.nih.gov/10385898/ [Accessed 14 Jan. 2024].

Imhof, H.K., Laforsch, C., Wiesheu, A.C., Schmid, J., Anger, P.M., Niessner, R., & Ivleva, N.P. (2016). Pigments and plastic in limnetic ecosystems: A qualitative and quantitative study on microparticles of different size classes. Water Research, 98, 64–74. doi: https://doi.org/10.1016/j.watres.2016.03.015.

Lassen, C., Hansen, S., Foss, Magnusson, K., Hartmann, N., Jensen, R., Nielsen, P., Gissel, T., Brinch, Lassen, C., Hansen, S., Magnusson, K., & Hartmann, N. (2015). Microplastics Occurrence, effects and sources of releases to the environment in Denmark. [Online] Available at: https://backend.orbit.dtu.dk/ws/files/118180844/Lassen_et_al._2015.pdf.

Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., & Zeng, E.Y. (2018). Microplastics in sewage sludge from the wastewater treatment plants in China. Water Research, 142, pp. 75–85. doi: https://doi.org/10.1016/j.watres.2018.05.034.

Lima, A.R.A., Costa, M.F., & Barletta, M. (2014). Distribution patterns of microplastics within the plankton of a tropical estuary. Environmental Research, 132, pp. 146–155. doi: https://doi.org/10.1016/j.envres.2014.03.031.

Liu, Y.; Xue, Y.; Xie, B.; Zhu, S.; Lu, X.; Liang, C.; Tian, J. (2020) Complex gene regulation between young and old soybean leaves in responses to manganese toxicity.Plant Physiology and Biochemistry, v. 155, p. 231-242. https://doi.org/10.1016/j.plaphy.2020.07.002.

Mai, L., Bao, L.-J., Wong, C.S., & Zeng, E.Y. (2018). Microplastics in the Terrestrial Environment. In Microplastic Contamination in Aquatic Environments (pp. 365–378). doi: https://doi.org/10.1016/b978-0-12-813747-5.00012-6.

Mastin, B.J. and Rodgers, J.H. (2000). Toxicity and Bioavailability of Copper Herbicides (Clearigate, Cutrine-Plus, and Copper Sulfate) to Freshwater Animals. *Archives of Environmental Contamination and Toxicology*, 39(4), pp.445–451. doi: https://doi.org/10.1007/s002440010126.

Mohanty, S., Ghosh, S., Nayak, S., & Das, A.P. (2017). Bioleaching of manganese by Aspergillus sp. isolated from mining deposits. Chemosphere, 172, 302–309. doi: https://doi.org/10.1016/j.chemosphere.2016.12.136.

Mølgaard, B., Viitanen, A.-K., Kangas, A., Huhtiniemi, M., Larsen, S.T., Vanhala, E., Hussein, T., Boor, B.E., Hämeri, K., & Koivisto, A.J. (2015). Exposure to airborne particles and volatile organic compounds from polyurethane molding, spray painting, lacquering, and gluing in a workshop. International Journal of Environmental Research and Public Health, 12(4), pp. 3756–3773. doi: https://doi.org/10.3390/ijerph120403756.

Nakashima, E., Isobe, A., Kako, S., Itai, T., Takahashi, S., & Guo, X. (2016). The potential of oceanic transport and onshore leaching of additive-derived lead by marine macro-plastic debris. Marine Pollution Bulletin, 107(1), 333–339. doi: https://doi.org/10.1016/j.marpolbul.2016.03.038.

National Environment Council of Brazil - CONAMA, Silva, M. (2005). Resolução CONAMA N° 357, de 17 de março de 2005, Brasília.

Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D., & Whitehead, P.G. (2016). A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. Environmental Science: Processes & Impacts, 18(8), pp. 1050–1059. doi: https://doi.org/10.1039/c6em00206d.

Nouman, M., Saunier, J., Jubeli, E. and Najet Yagoubi (2017). Additive blooming in polymer materials: Consequences in the pharmaceutical and medical field. Polymer Degradation and Stability, 143, pp.239–252. doi:https://doi.org/10.1016/j.polymdegradstab.2017.07.021.

O'Neal, S.L. and Zheng, W. (2015). Manganese Toxicity Upon Overexposure: a Decade in Review. Current Environmental Health Reports, 2(3), 315–328. doi: https://doi.org/10.1007/s40572-015-0056-x.

Petitjean, Q., Flavien Choulet, Anne-Véronique Walter-Simonnet, Anne-Lise Mariet, Laurent, H., Rosenthal, P., Annette de Vaufleury, & Gimbert, F. (2021). Origin, fate and ecotoxicity of manganese from legacy metallurgical wastes. Chemosphere, 277, 130337–130337. doi: https://doi.org/10.1016/j.chemosphere.2021.130337.

Poozesh, S., Akafuah, N., & Saito, K. (2017). Effects of automotive paint spray technology on the paint transfer efficiency – a review. Proceedings of the Institution of Mechanical Engineers, Part D: Journal of Automobile Engineering, 232(2), pp. 282–301. doi: https://doi.org/10.1177/0954407017695159.

Rabello, M.S., & White, J.R. (1997). Crystallization and melting behaviour of photodegraded polypropylene — I. Chemi-crystallization. Polymer, 38(26), pp. 6379–6387. doi: https://doi.org/10.1016/s0032-3861(97)00213-9.

Rillig, M.C., & Lehmann, A. (2020). Microplastic in terrestrial ecosystems. Science, 368(6498), pp. 1430–1431. doi: https://doi.org/10.1126/science.abb5979.

Rillig, M.C., Ingraffia, R., & de Souza Machado, A.A. (2017). Microplastic Incorporation into Soil in Agroecosystems. Frontiers in Plant Science, 8. doi: https://doi.org/10.3389/fpls.2017.01805.

Rochman, C.M. (2018). Microplastics research—from sink to source. Science, 360(6384), pp. 28–29. doi: https://doi.org/10.1126/science.aar7734.

Roy, P., Ashton, L., Wang, T., Corradini, M.G., Fraser, E.D.G., Thimmanagari, M., Tiessan, M., Bali, A., Saharan, K.M., Mohanty, A.K., & Misra, M. (2021). Evolution of drinking straws and their environmental, economic and societal implications. Journal of Cleaner Production, 316(128234), 128234. doi: https://doi.org/10.1016/j.jclepro.2021.128234.

Samarajeewa, A.D., Velicogna, J.R., Schwertfeger, D.M., Princz, J.I., Subasinghe, R.M., Scroggins, R.P., & Beaudette, L.A. (2021). Ecotoxicological effects of copper oxide

nanoparticles (nCuO) on the soil microbial community in a biosolids-amended soil. Science of The Total Environment, 763, 143037. doi: https://doi.org/10.1016/j.scitotenv.2020.143037.

Schnabel, W. (1982). Polymer Degradation. Principles and Practical Applications. [Online] Available at: https://cir.nii.ac.jp/crid/1573668923987157120 [Accessed 13 Jan. 2024].

Senesi, N., & Loffredo, E. (2018). Metal Ion Complexation by Soil Humic Substances. Soil Science Society of America book series, 563–617. doi: https://doi.org/10.2136/sssabookser8.c12.

Singh, N., & Turner, A. (2009). Trace metals in antifouling paint particles and their heterogeneous contamination of coastal sediments. Marine Pollution Bulletin, 58(4), pp. 559–564. doi: https://doi.org/10.1016/j.marpolbul.2008.11.014.

Singh, R. P.; Mishra, S.; Das, A. P. Synthetic microfibers: Pollution toxicity and remediation. Chemosphere, v. 257, p. 127199, 1 out. 2020. https://doi.org/10.1016/j.chemosphere.2020.127199.

Song, Y.K., Hong, S.H., Jang, M., Kang, J.-H., Kwon, O.Y., Han, G.M., & Shim, W.J. (2014). Large Accumulation of Micro-sized Synthetic Polymer Particles in the Sea Surface Microlayer. Environmental Science & Technology, 48(16), pp. 9014–9021. doi: https://doi.org/10.1021/es501757s.

Sørensen, L., Groven, A.S., Hovsbakken, I.A., Del Puerto, O., Krause, D.F., Sarno, A., & Booth, A.M. (2021). UV degradation of natural and synthetic microfibers causes fragmentation and release of polymer degradation products and chemical additives. Science of The Total Environment, 755(Part 2), 143170. ISSN 0048-9697. doi: https://doi.org/10.1016/j.scitotenv.2020.143170.

Soroldoni, S., Abreu, F., Castro, Í.B., Duarte, F.A., & Pinho, G.L.L. (2017). Are antifouling paint particles a continuous source of toxic chemicals to the marine environment? Journal of Hazardous Materials, 330, pp. 76–82. doi: https://doi.org/10.1016/j.jhazmat.2017.02.001.

Soroldoni, S., Martins, S.E., Castro, I.B. and Pinho, G.L.L. (2018). Potential ecotoxicity of metals leached from antifouling paint particles under different salinities. *Ecotoxicology and Environmental Safety*, 148, pp.447–452. doi: https://doi.org/10.1016/j.ecoenv.2017.10.060.

Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Tröger, J., Muñoz, K., Frör, O., & Schaumann, G.E. (2016). Plastic mulching in agriculture. Trading short-term

agronomic benefits for long-term soil degradation? Science of The Total Environment, 550, pp. 690–705. doi: https://doi.org/10.1016/j.scitotenv.2016.01.153.

Takahashi, C.K., Turner, A., Millward, G.E., & Glegg, G.A. (2012). Persistence and metallic composition of paint particles in sediments from a tidal inlet. Marine Pollution Bulletin, 64(1), 133–137. doi: https://doi.org/10.1016/j.marpolbul.2011.10.010.

Takahashi, J., Yamamoto, T., & Kazuyuki Shizawa (2010). Modeling and simulation for ductile fracture prediction of crystalline polymer based on craze behavior. International Journal of Mechanical Sciences, 52(2), pp. 266–276. doi: https://doi.org/10.1016/j.ijmecsci.2009.09.028.

Turner, A. (2010). Marine pollution from antifouling paint particles. Marine Pollution Bulletin, 60(2), 159–171. doi: https://doi.org/10.1016/j.marpolbul.2009.12.004.

Turner, A. (2021). Paint particles in the marine environment: An overlooked component of microplastics. Water Research X, 12, p. 100110. doi: https://doi.org/10.1016/j.wroa.2021.100110.

Waldman, W.R., & Rillig, M.C. (2020). Microplastic Research Should Embrace the Complexity of Secondary Particles. Environmental Science & Technology, 54(13), pp.7751–7753. doi:https://doi.org/10.1021/acs.est.0c02194.

Weinstein, J.N., Jr, H., Leads, R.R., Deloughry, E., Schandera, L., Liddy, K. and Scott, G.I. (2019). Microplastics: A Global Water Pollution Problem. pp.1–15. doi: https://doi.org/10.1002/9781119300762.wsts0203.

WHO (2008). Guidelines for Drinking Water Quality (3rd ed., pp. 5115). World Health Organization, Geneva, Switzerland.

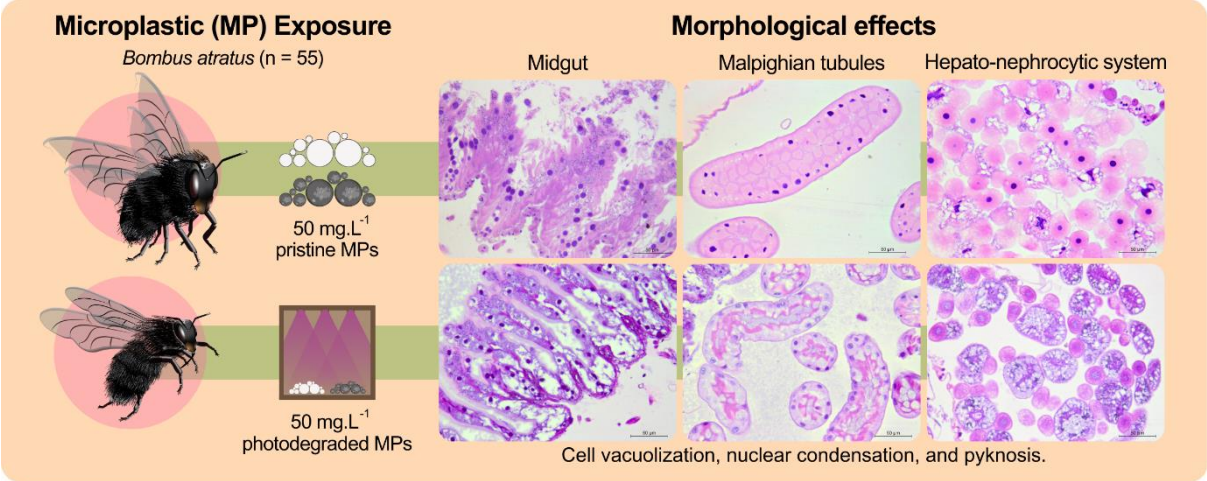
Xu, Y., Rillig, M.C., & Waldman, W.R. (2022). New separation protocol reveals spray painting as a neglected source of microplastics in soils. Environmental Chemistry Letters, 20(6), pp. 3363–3369. doi: https://doi.org/10.1007/s10311-022-01500-2.

Yousif, E., & Haddad, R. (2013). Photodegradation and photostabilization of polymers, especially polystyrene: review. SpringerPlus, 2(1). doi: https://doi.org/10.1186/2193-1801-2-398.

3. CAPÍTULO 3 – ARTIGO 2

Spray Paint Microplastics as Ecotoxicological Contaminants to Neotropical Bumblebees

3.1. Graphical Abstract



3.2. Abstract

Neotropical bees are declining due to anthropogenic actions. Individuals can be exposed to contaminants like microplastics (MPs), which have been found in honey. Thus, we evaluated the potential cytotoxicity of pristine and photodegraded spray paint MPs to the columnar cells of the midgut, Malpighian tubules, and hepato-nephrocitic system cells of *Bombus atratus* foraging workers exposed to 50 mg.L^{-1} MPs for 96 hours. To assess the toxicity of the MPs, we conducted histological and histochemical analysis of the tissues. The pristine MPs caused subtle changes in columnar and pericardial cells, while photodegraded MPs caused cell vacuolization, nuclear condensation, and pyknosis. These significant changes are related to the greater release of potentially toxic elements (PTEs), such as Copper, Manganese, and Iron, from the photodegraded MPs, which exceeded the safe limits set by the National Environment Council of Brazil (CONAMA). Photodegraded MPs also caused a decrease in body weight of the individuals. The release of PTEs from the photodegraded particles contributes to the disruption of body homeostasispotentially leading to reduced fitness and alterations in feeding and survival of the species. These findings underscore the importance of understanding the toxicity of environmentally realistic MPs, as plastic composition and weathering conditions can influence particle toxicity.

Keywords: Bumblebees; Ecotoxicity; Polystyrene; PTEs.

3.3. Resumo

As abelhas neotropicais estão em declínio devido às ações antropogênicas. Esses indivíduos podem ser expostos a contaminantes como microplásticos (MPs), que já foram encontrados no mel. Portanto, avaliamos a potencial citotoxicidade dos MPs de tintas spray prístinas e fotodegradadas nas células colunares do intestino médio, túbulos de Malpighi e células do sistema hepatonefrocítico de operárias forrageiras de *Bombus atratus* expostas a 50 $mg.L^{-1}$ de MPs durante 96 horas. Para avaliar a toxicidade dos MPs, realizamos análises histológicas e histoquímicas dos tecidos. Os MPs prístinos causaram mudanças sutis nas células colunares e pericárdicas, enquanto os MPs fotodegradados causaram vacuolização celular, condensação nuclear e picnose. Essas alterações significativas estão relacionadas à maior liberação de elementos potencialmente tóxicos (EPTs), como Cobre, Manganês e Ferro, dos MPs fotodegradados, que excederam os limites seguros estabelecidos pelo Conselho Nacional do Meio Ambiente (CONAMA). Os MPs fotodegradados também causaram diminuição do peso corporal dos indivíduos analisados. A liberação de EPTs das partículas fotodegradadas contribui para a desregulação da homeostase corporal das abelhas estudadas, potencialmente levando à redução da aptidão e alterações na alimentação e sobrevivência da espécie. Esses achados ressaltam a importância de entender a toxicidade de MPs em condições ambientais realistas, pois a composição do plástico e as condições de intemperismo podem influenciar a toxicidade das partículas.

Palavras-chaves: Mamangavas; Ecotoxicidade; Poliestireno; EPTs.

3.4. Introduction

Microplastics (MPs) pose as a significant environmental threat to ecosystems (Bostan et al., 2023; Zhou et al., 2020; Roy et al., 2022), carrying potential ecotoxicological risks and causing adverse effects on both aquatic and terrestrial organisms (Zhang, 2020; Wang et al., 2021). MPs are polymeric particles with a diameter smaller than 5 mm and can be broadly categorized into primary and secondary types (Cole et al., 2011; Järlskog et al., 2020). Primary MPs are intentionally produced for specific applications, such as artificial textile fibers and microspheres in cosmetics and personal care products (Fu and Wang, 2019; Rolsky et al., 2020). On the other hand, secondary MPs result from the degradation of larger plastic materials through weathering like mechanical erosion and exposure to UV rays (Hidalgo-Ruz et al., 2012; Duis and Coors, 2016). Both types of MPs are pervasive and persistent pollutants, readily dispersed and integrated into sediments, soil, and water (Eerkes-Medrano et al., 2015; Rillig et

al., 2017; Prata et al., 2019). Given their high bioavailability and microscopic size, MPs are found in a diverse array of organisms spanning all trophic levels (Zhou et al., 2020).

Painting sprays predominantly consist of polymer binders and emerge as a substantial source of MPs, raising concerns about their potential environmental risk (Gaylarde et al., 2021; Song and Li, 2014; Turner, 2021). The assessment of paint as a MP pollutant have been extensively explored in marine and freshwater contexts (Thompson, 2004; Tremblay, 2019; Ma et al., 2020; Ashrafy et al., 2022), with an increasing focus on terrestrial environments (Lima et al., 2014; Nizzetto et al., 2016; Imhof et al., 2016; Galafassi et al., 2019; Meyers and Trittin, 2020; Xu et al., 2022).

The ecotoxicological relevance of paint-derived MPs lies in their chemical composition, which can pose environmental hazards. Notably, paints used on ships and marine equipment often contain potentially toxic elements (PTEs) such as Copper (Cu), Zinc (Zn), and Lead (Pb), in addition to 'booster biocides' employed to deter fouling organisms (Gade et al., 2012; Soroldoni et al., 2017). The release of these substances into the environment can occur through natural processes such as temperature changes, acid rain, and exposure to ultraviolet radiation (Sørensen et al., 2021; Costa et al., 2023).

Based on the ubiquitous dimensions of MPs, their impact on marine organisms is increasing, notably for fish (Avio et al., 2017), oysters (Sussarellu et al., 2016), and mussels (Gündoğdu et al., 2020). MPs exposure to aquatic biota confers toxic effects, including decreased fitness, increased oxidative stress, immune dysfunctions, as well as disrupted gut microbiota (Jin et al., 2018; Paul-Pont et al., 2016). These studies mainly showed a reduction of feeding activity, shortened life span, and reproductive ability, as well as behavioral alterations in the host (Deng et al., 2021). The contamination in terrestrial environments is viewed as potentially more hazardous to humanity than in aquatic environments due to direct effects on food chains, plants, insects, and animals that are directly consumed by humans (Toussaint et al., 2019).

Critical for the maintenance and sexual reproduction of flowering plant species, bees play a pivotal role as the predominant pollinating terrestrial insects (Kearns and Inouye, 1997). Pollination, an essential ecosystem service, results in fertilization, fruit, and seed production, significantly contributing to the structure and function of natural ecosystems (Proctor et al., 1996). Bees are crucial for agriculture and also indispensable for the overall health and sustainability of various ecosystems (Hung et al., 2018). Recent reports are presenting the same results as older ones, highlighting substantial declines in pollination services in Northwestern Europe, North America, and South America, emphasizing the urgent need for bee conservation

to address the alarming decrease in their populations (Grixti et al., 2009; Carvell et al., 2006; Cameron et al., 2011; Pires et al., 2016; Wood et al., 2020; Zattara and Aizen, 2021).

The presence of MPs was confirmed in 12% of honey, beer, milk and refreshments samples collected in Ecuador, and it was mainly composed of polyethylene, polypropylene and polyacrylamide (Diaz-Basantes et al., 2020). Bees might obtain these particles through adhesion to body hair or ingestion of nectar, pollen, and water contaminated with MPs, due to their wide-range foraging activity (Glenny et al., 2017). Additionally, they may become susceptible to contamination by the elements present in the chemical composition of these MPs (Gade et al., 2012; Soroldoni et al., 2017).

Brazil is situated at the periphery of the distribution range for the bumblebee genus (Goulson, 2010). Consequently, only six species from this genus are identified in the country, with *Bombus morio* and *Bombus atratus* being particularly abundant in the South and Southeast regions (Moure and Sakagami, 1962). The urgency to understand the causes behind the disappearance and decline of numerous species within this genus in the Americas and Eastern Europe has been heightened by recent reports (Cameron et al., 2011; Grixti et al., 2009; Patel et al., 2020; Kevan, Rasmont and Martinet, 2024; IUCN, 2024).

There are two ways to use these insects as bioindicators of environmental stress: through mortality rate and internal organ analysis in experimental settings (toxicological assays) or by quantifying toxic residues present in nests, honeycombs, or pollen pots (Balestra et al., 1992; Martins, Gonçalves and Melo, 2013; Martínez-López et al., 2021). Therefore, the study of the internal organs of bees in ecotoxicological studies is of great interest, as a wide variety of toxic compounds can accumulate at molecular, cellular, and tissue levels (Hyne and Maher, 2003). As presented by Abdalla and Domingues (2015), an example of this would be the evaluation of cells of the hepato-nephrocytic system for a better understanding of the impact of chronicle and sublethal toxic concentrations on bees.

Unlike other social bee species, *Bombus* colonies are not perennial; they are established by a solitary fertilized queen (Velthuis and van Doorn, 2006; Goulson, 2010). The queen is responsible for producing all the workers that initiate the social phase of the colony (Martins and Melo, 2009; Cameron et al., 2011). Thus, these species are at risk, as the loss of a foundation queen could lead to the failure of a new colony to develop (Kevan, Rasmont and Martinet, 2024). The small population size makes even minor environmental changes significant for the species' survival (Zattara and Aizen, 2021).

Although increasing evidence has shown that MPs contaminate a large number of invertebrates, an investigation into how pristine and photodegraded spray paint MPs cause

mechanistic and physiological impacts on bumblebees has not yet been fully described. From this perspective, this study aims to understand the effects of two distinct types of spray paint MPs, in two different states of preservation, on the midgut, malpighian tubules and hepato-nephrocitic system cells of neotropical bees of the species *B. atratus* forager workers.

3.5. Methodology

3.5.1. Determination of Elements in Pigments

The paint was sprayed on a paper surface, creating an area of 3 x 5 cm² with a homogeneous thickness. A portable X-ray fluorescence (XRF) system, consisting of an Amptek® silver filament X-ray tube (30 kV and 10 μA) and an Amptek® Si-Drift detector, was used for the analysis. During measurements, the XRF system was positioned close to the object without touching it, causing no damage of any kind. Each measurement took 100 seconds and covered an area of approximately 3 mm in diameter, identifying the main elements present in the paint. This procedure was performed with both Luxens® spray paints: Matte White (MW) and Glossy Black (GB).

3.5.2. Polymer Identification

To remove the pigment from the spray paint and enhance the Fourier Transform Infrared Spectroscopy (FTIR) measurements of the polymer, 0.5 g of the pristine MP sample was dissolved in 10 mL of xylol (P.A.-A.C.S. 100%) and filtered through 0.45 μm acetate membranes using syringes to retain the pigments and other particulates. The filtered solution was placed in a Petri dish and kept in a laminar flow hood for 48 hours to facilitate xylol evaporation, leaving only the paint polymer. The polymer was subsequently removed from the dishes by scraping the surface, and then mixed to KBr (potassium bromide dried at 120ºC for 4 hours) for the production of pellets (200 mg of KBr and 2 mg of polymer), which were analyzed by FTIR with 16 scans and a nominal resolution of 4.0 cm^{-1} (Thermo Scientific Nicolet Summit® iD1) using the software OMNIC Paradigm. This procedure was performed with both studied paints.

3.5.3. Microplastic Production

The MPs were produced by spraying a graffiti can in the air, far from any surface, in a clean and empty closed room (12.02 m^2; 5.18 m x 2.32 m), until the paint ran out. This procedure ensured that most of the paint became aerosol droplets, which were left to deposition and dry for five days. Then, the particles were collected using wooden tools and horsehair

brooms, and sanitized. The granulometric fraction of 250 to 53 μm was separated in order to standardize the pristine sample. This procedure was performed with both studied paints and produced the pristine MPs used in this research: Matte White MPs (pMW) and pristine Glossy Black MPs (pGB).

3.5.4. Microplastic Photodegradation

The pristine MPs were divided into two exposure groups. Each group consisted of a 3 g sample, which was dispersed into four Petri dishes (21.29 cm² each) to ensure the material formed a single layer and was evenly exposed (0.75 g of pristine MPs in each Petri dish). Both groups were photodegraded in an aging chamber, as described by Cacuro et al. (2018). The samples were positioned 0.2 m away from the three 15 W UV-C fluorescent germicidal lamps (Osram brand, model TUV15W), which emit at a maximum wavelength of 254 nm, while maintaining a chamber temperature of 35ºC, for 48 hours. This procedure was performed with both studied paints and produced the photodegraded MPs used in this research: photodegraded Matte White MPs (pdMW) and photodegraded Glossy Black MPs (pdGB).

3.5.5. Release of Potentially Toxic Elements from Paint Microplastics in Aqueous Solution.

3.5.5.1. Release

For determining the release of Potentially Toxic Elements (PTEs) from both pristine and degraded MPs samples, 0.5 g from each of the four samples (pGB, pMW, pdGB, and pdMW) was added to 50 mL of ultrapure water (pH of 5). These dispersions were placed on an orbital shaking table (Tecnal model TE-1400) at 130 RPM for 24 hours. Following this period, the samples were filtered with 0.45 μm cellulose acetate membranes to remove plastic particles using a vacuum filtration system (Vix model VPA 115).

3.5.5.2. Acid Digestion

To analyze the release of PTEs from polymers in aqueous solution, acid digestion was conducted in triplicate using the American Public Health Association's (APHA, 2000) method 3030E. In this procedure, 2.5 mL of concentrated nitric acid (HNO_3, 65% w/w) was added to the 50 mL dispersion solution and heated to 120 ºC on a hot plate. Post-digestion, the samples were diluted with ultrapure water in a 25 mL volumetric flask and subsequently transferred to Falcon tubes for the quantification of PTEs.

3.5.5.3. Quantification of PTEs released from MPs

The quantification of PTEs released from the MPs in aqueous solution was conducted using a microwave plasma atomic emission spectrometer (Agilent model 4200 MP-AES) (APHA, 2000). The emission lines, limits of detection (LOD), and quantification (LOQ) for the elements analyzed in each concentration range are presented in Table 1.

Table 1.1 - Emission lines, limits of detection (LOD) and quantification (LOQ) of the elements analyzed in the Agilent 4200 MP-AES.

Concentration range: 0, 0.01, 0.05, 0.1, 0.5, 1.0, 1.5 mg L^{-1}			
PTE	**Emission lines (nm)**	**LOD mg L^{-1}**	**LOQ mg L^{-1}**
Cr	425.433	0.0012	0.0040
Cu	324.754	0.0009	0.0031
Mn	403.076	0.0001	0.0003
Zn	213.857	0.0173	0.0577
Concentration range: 0, 0.01, 0.05, 0.1, 0.5, 1.0 mg L^{-1}			
PTE	**Emission lines (nm)**	**LOD mg L^{-1}**	**LOQ mg L^{-1}**
Cu	324,754	0.0018	0.0060
Ni	352,454	0.0033	0.0110
Concentration range: 0, 0.1, 1, 5, 10 mg L^{-1}			
PTE	**Emission Lines (nm)**	**LOD mg L^{-1}**	**LOQ mg L^{-1}**
K	766,491	0.1832	0.6107
Concentration range: 0, 0.1, 0.5, 1.0, 1.5, 2.5 mg L^{-1}			
PTE	**Emission lines (nm)**	**LOD mg L^{-1}**	**LOQ mg L^{-1}**
Fe	371.993	0.0034	0.0113

3.5.6. Animal Collection and Exposure Procedures

Bombus atratus foraging workers were collected (n = 55) using an entomological net on flowers of the *Pleroma mutabile* species, in fragments of Atlantic Forest and Cerrado in the city of Sorocaba (São Paulo state, Brazil; 23º34'53.1"S 47º31'29.5"W), during summer. After capture, the individuals were transferred to Falcon tubes placed in a thermal box and transported to the laboratory, where they were weighted using an analytical balance (Beel Engineering® Mark 210A) and then placed individually into wooden entomological boxes (16 x 12 x 10 cm) as described by previous studies (Abdalla et al., 2018; Provase et al., 2021; Boeing et al., 2024). Each box had two feeders, one containing food (sucrose solution - 70%) and the other

containing filtered water or a MP contaminated solution (2 mL). The boxes were kept in an incubator maintained at 26°C, with a relative humidity (RH) of 70%, in the dark; the specimens were fed *ad libitum*, and the solutions were renewed everyday (Ceschi-Bertoli et al., 2020).

Despite the absence of data on the stability of *B. atratus* populations (IUCN, 2024), Cameron et al. (2011) reported significant declines in *Bombus* sp. populations in the Americas. Therefore, to prevent excessive collection of the organisms, the sample size was maintained within statistically suggested minimum values (Abdalla and Domingues, 2015; Domingues et al., 2016), with bees individually exposed (n = 11 individuals per experimental group), generating independent values for statistical analyses.

For the control group (n = 11), 2 mL of filtered water was offered. For the MPs exposures, a volume of 2 mL of the solutions was offered *ad libitum* to each of the experimental groups: the first group (hereafter "experimental group 1"; n = 11) was exposed to the concentration of 50 $mg.L^{-1}$ of pMW MPs; the second group ("experimental group 2"; n = 11) was exposed to the same concentration of the pGB MPs. The third group ("experimental group 3"; n = 11) was exposed to the concentration of 50 $mg.L^{-1}$ of pdMW MPs; and the fourth group ("experimental group 4"; n = 11) was exposed to the same concentration of the pdGB MPs. The concentration of 50 ppm was chosen based on the values that showed changes in *Apis mellifera* in the study conducted by Deng et al. (2021).

After 96 hours of exposure, all specimens were weighted again and cryo-anesthetized; the midgut, Malpighian tubules, fat body and pericardial cells surrounding the dorsal vessel were dissected under a stereomicroscope (Zeiss Stemi DV4). Dissection was carried out on a Petri dish with the organs immersed in distilled water at 4ºC, following Organization for Economic Cooperation and Development (OECD) guidelines (2017a, 2017b) for chemical testing on *Bombus terrestris*. For preservation, the organs were kept in fixative solution at 4ºC (Abdalla et al., 2018). The difference between the initial and final weights of the organisms was calculated and statistically analyzed.

3.5.7. Routine Morphological Analysis

The studied organs were removed entirely and fixed with 4% paraformaldehyde at 4ºC. Afterward, they were dehydrated in an increasing series of alcohol solutions: 15%, 30%, 50%, 70%, 80%, 85%, 90%, 95%, and 100% for 60 minutes each at 4ºC, with the last concentration repeated twice (Abdalla and Domingues, 2015; Domingues et al., 2016). The organs were immersed in a xylol + alcohol solution (1:1) for 60 minutes and then cleared in pure xylol for 48 hours. After this period, the organs were embedded in JB-4 historesin (Polysciences, Leica

Biosystems Nussloch GmbH, Heidelberg, Germany). The resin material was sectioned using a microtome with a thickness setting of 2 μm (Leica® - RM 2255 microtome). The slides containing the histological sections were subjected to the hematoxylin and eosin (H.E.) staining technique and sealed with ERV-Mount. Finally, the slides were analyzed under a Leica® Microscope (model DM1000) using Leica Application Suite V3.8 (LAS V3.8) software (Provase et al., 2021). The nuclei of mature columnar cells (n = 1100), and the cellular areas of pericardial cells (n = 1100) and oenocytes (n = 110) underwent a morphometric evaluation process using ImageJ® software. The obtained values from each group were then compared using the Kruskal-Wallis statistical test to determine if there were differences among the groups.

3.5.8. Histochemistry - Acridine Orange

The previously dehydrated midguts were embedded in histological paraffin (Histopar®, Brazil) through three 30-minute baths at 80ºC. After this process, the material was sectioned into 7 μm-thick slices using a microtome (Leica® - RM 2255 microtome). The sections were placed on polylysine-coated slides and stored at 4ºC until use. The slides underwent deparaffinization through a series of pure xylol baths for 30 minutes; xylol + 100% alcohol (1:1) for 10 minutes; and 100% alcohol for 15 minutes. Then, the slides were rehydrated in a descending series of alcohols (95%, 70%, and 50%) for 15 minutes each. After this process, the slides were submerged in an infiltration solution (0.1% Triton, 99.9% PBS) for 15 minutes, washed three times with distilled water, submerged in citrate buffer (pH 3.0) for 10 minutes, and washed three times again with distilled water. Finally, the slides were stained with Acridine Orange (AO) for 30 minutes in a covered rack (M920 - Stain Tray®, CA), washed three times with distilled water, and dehydrated in an ascending series of acetone (50%, 70%, and pure acetone) for 1 minute each. Slides were analyzed under a Leica® Microscope (model - DM4000) and Leica Application Suite V3.8 (LAS V3.8) software.

A synthesis of the whole applied methodology is didactically presented in Figure 1.

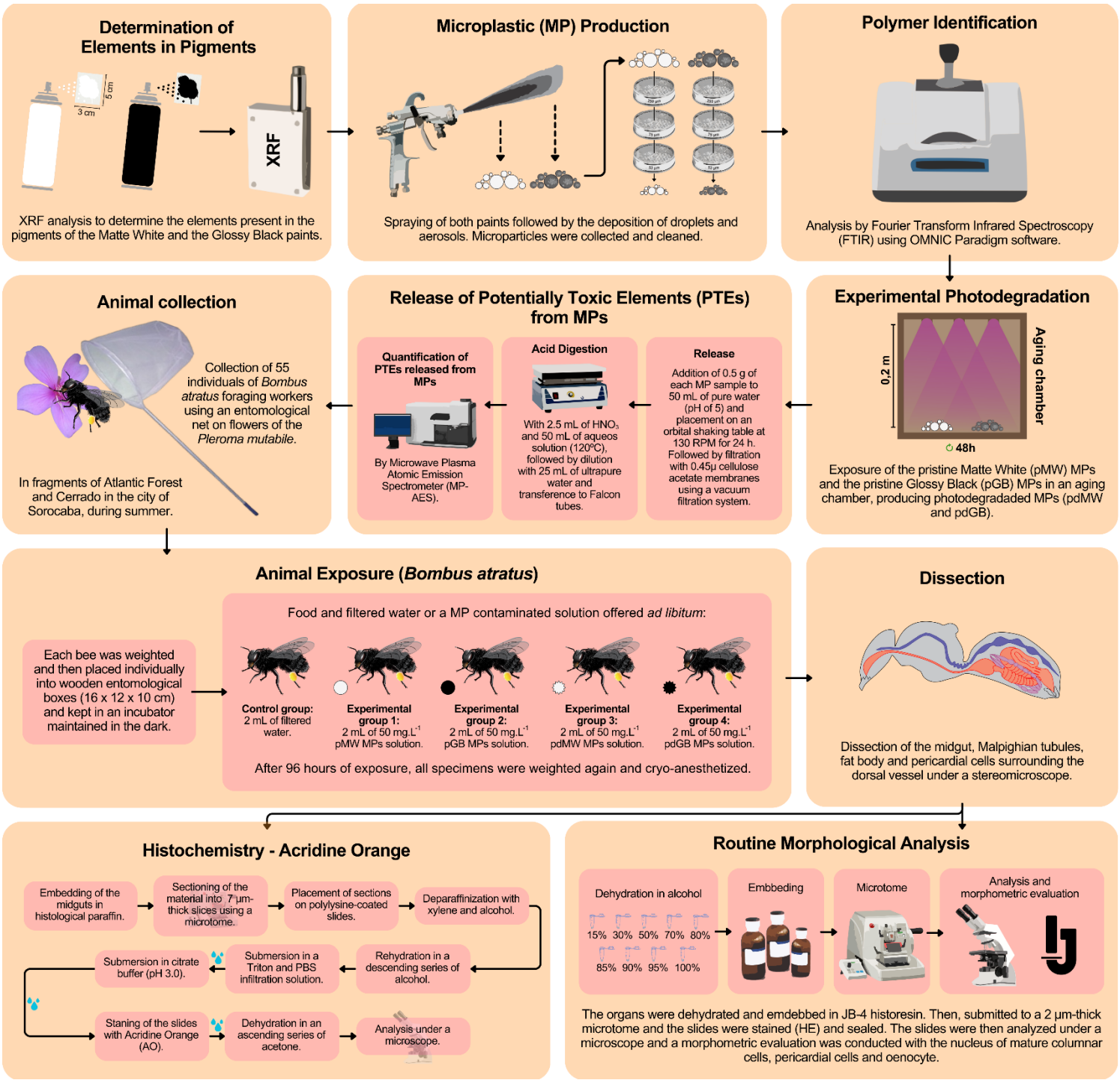


Figure 01: Workflow and methodological overview applied in this paper.

3.6. Results and Discussion

The XRF analysis revealed the presence of 10 chemical elements constituting different components of the MW spray paint, which are: Silicon (Si), Chlorine (Cl), Potassium (K), Calcium (Ca), Titanium (Ti), Iron (Fe), Nickel (Ni), Copper (Cu), Strontium (Sr), and

Zirconium (Zr). The GB spray paint showed 12 elements: Silicon (Si), Sulfur (S), Chlorine (Cl), Potassium (K), Calcium (Ca), Titanium (Ti), Chromium (Cr), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn), and Strontium (Sr). The relative abundance of these elements in the paint samples are presented in Figure 2.

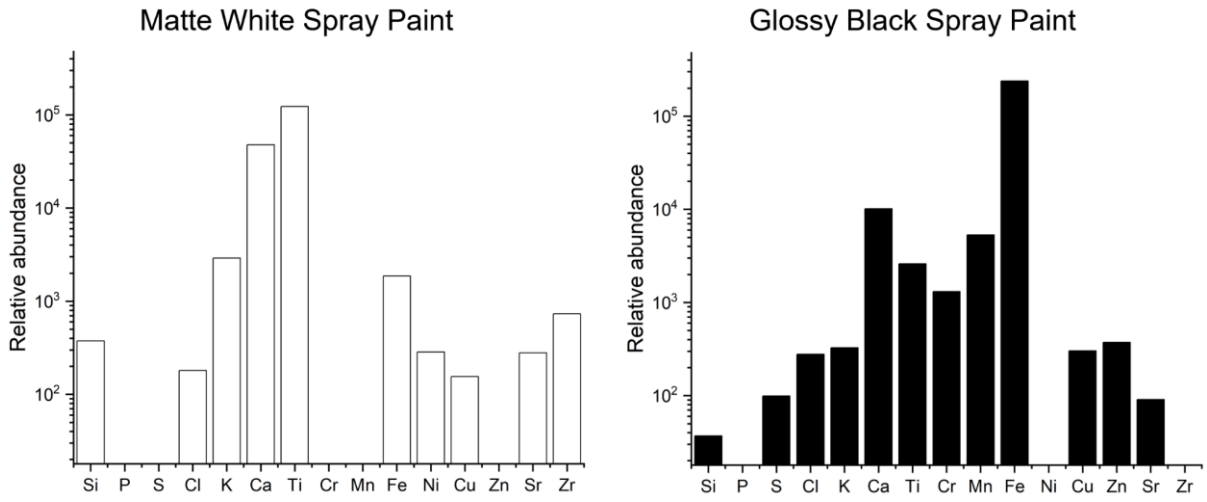


Figure 2 - Relative abundance of elements in each of the analyzed paints using X-ray fluorescence spectrometry.

The diversity of elements identified is closely tied to the paint's composition (Cortea et al., 2020). These elements present various roles within the formulation, including functioning as antifouling agents, corrosion inhibitors, extenders, and primary or secondary pigments that provide the paint with its distinctive coloration (Gaylarde et al., 2021). This array of elements underscores the complexity and versatility inherent in the paint formulation process (Cartechini et al., 2021). It can be observed that some of the elements fall under the classification of PTEs, such as Fe, Cr, Cu, Mn, Zn, and Ni (Nieder and Benbi, 2023). These elements, when present in the environment at concentrations above those considered environmentally safe by environmental agencies such as the World Health Organization (W.H.O.) and the National Environment Council of Brazil (CONAMA), can cause environmental changes in individuals and populations of various species (Li et al., 2021; Xu et al., 2023).

The assessment of the MW paint polymer using the FTIR technique revealed a high similarity with polyphenylethylenes α-methylstyrene, a derivative of polystyrene (PS). Components such as alkyd resin and styrene were also identified in the polymer bulk of the paint. In the GB paint, a high presence of polyphenylethylenes α-methylstyrene was identified, as well as alkyd resin and monoazo pigment. The obtained analysis is depicted in Figure 3.

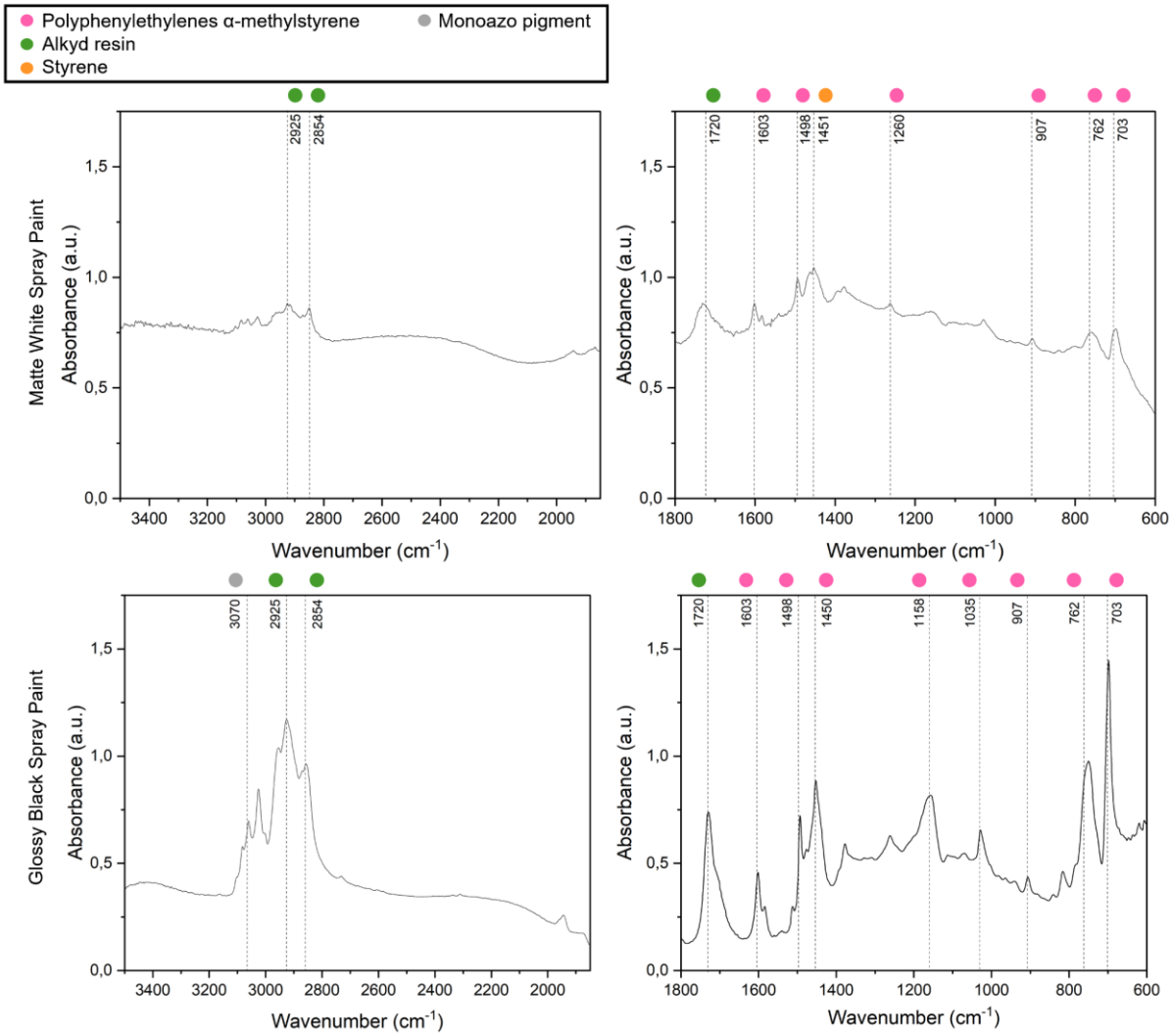


Figure 3 - FTIR analysis of the MW and GB paints. The bands indicate the presence of polyphenylethylenes α-methylstyrene, alkyd resin, styrene, and monoazo pigment.

This polymer type analysis was necessary because both paints lack this information on their labels, hindering a comprehensive understanding of their potential toxicity. The use of PS as a foundational polymer for spray paint production is commonplace; recent initiatives have repurposed degraded or discarded PS as a polymer source for creating waterproof paints, highlighting its versatility and broad applicability in various scenarios (Bellon et al., 2019). The use of alkyd resin is also common, as it is a widely used binder for paint production (Cortea et al., 2020). Styrene is one of the most important monomers in the production of polymers and copolymers, given its wide range of derivatives; it can be used in the production of everything from synthetic rubbers to paints (Miller, Newhook and Poole, 1994).

Both PS and styrene have significant environmental relevance, as they are well known pollutants (Kwon et al., 2015). The first records of environmental pollution from PS date back

to 1970, and this issue has been actively monitored by the academic community, as this polymer contains hazardous chemical components (Carpenter and Smith, 1972; Rochman et al., 2013; Bandow et al., 2017). Styrene can be found in the air, soil, and water due to accidental releases during production processes and effluents, or through the improper disposal of products containing this monomer (Arora and Manila, 2021). It is an active component of photochemical smog and thus contributes to air pollution (Wang, 2019).

The production of MPs from the spray paints was successful, resulting in spherical plastic particles with approximately 30 μm diameter. Additionally, the experimental photodegradation led to changes in the shape and integrity of the MPs, as superficial modification, detachment or breakage on the particles surface, and blooming of the additives (Nouman et al., 2017), as illustrated in Figure 04. Such structural alterations caused by photodegradation are well-documented phenomena (Waldman and Rillig, 2020). These changes occur due to the interaction between the polymer and light, primarily through the chromophore groups that absorb light and transfer its energy to the system, leading to homolytic scission and the generation of free radicals (Curcio et al., 2018). This process initiates an auto-oxidation cycle, causing surface alterations that, as degradation progresses, gradually extend into the polymer's bulk (Blais et al., 1972; Curcio et al., 2018; de Freitas et al., 2022).

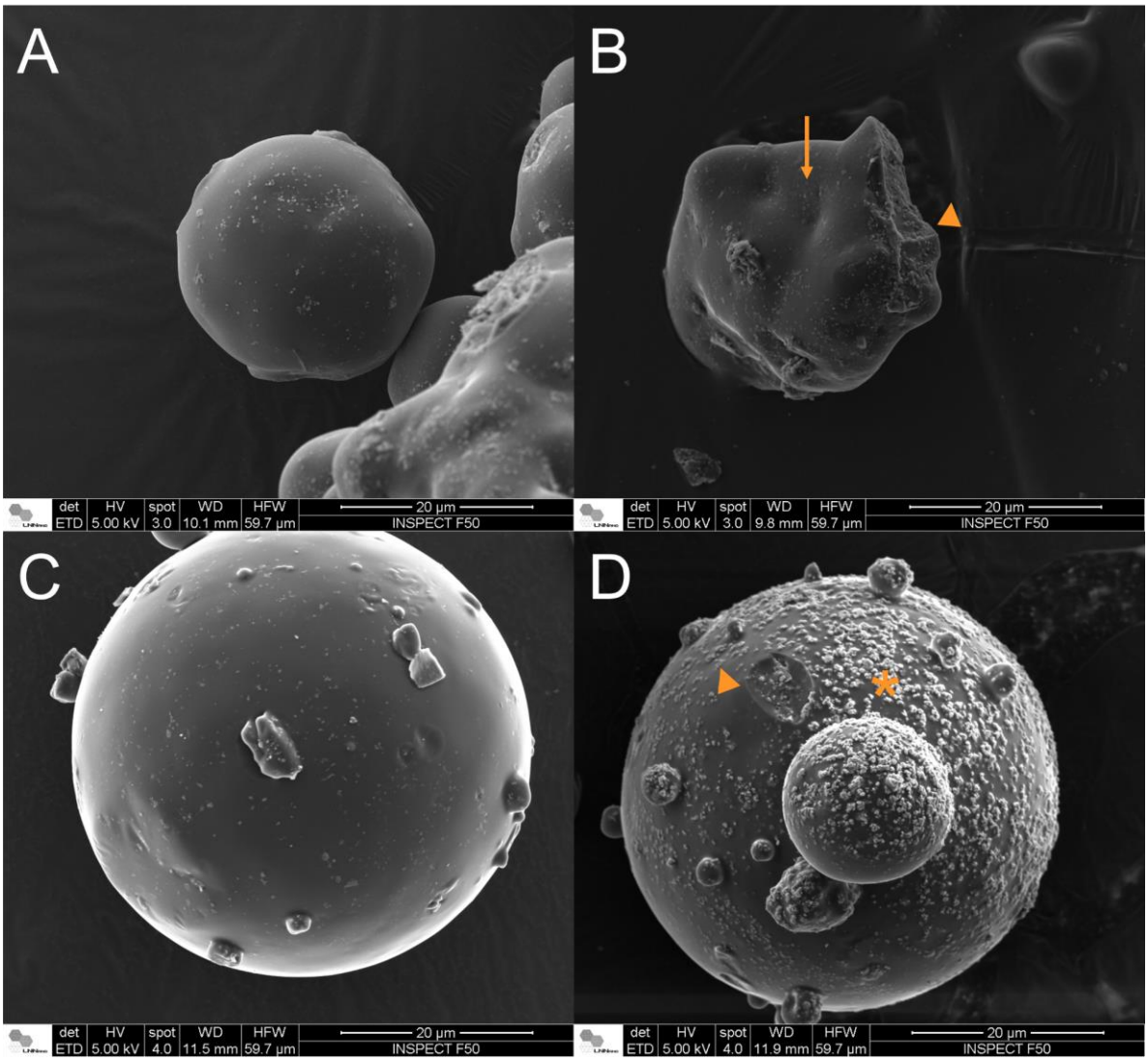


Figure 04: SEM micrographs of the MW and GB spray paint MPs. A) Pristine MW MPs; B) Photodegraded MW MPs; C) Pristine GB MPs; D) Photodegraded GB MPs. Orange arrow: superficial modification; orange arrowhead: detachment or breakage on the particle's surface; and orange asterisk: blooming of the additives.

These alterations caused by photodegradation resulted in a greater release of PTEs by the photodegraded MPs. As shown in Figure 5, the pdMW MPs exhibited increased Cu and Fe release compared to the pMW MPs. The Cu release from the photodegraded particles exceeded the environmentally permitted values by the National Environment Council of Brazil for fresh water (CONAMA, 2005), and the Fe release nearly reached the limit. For the pdGB particles, there was an increase in the release of Zn, Cu, Mn, and Fe, with the Cu value surpassing the CONAMA limit and the Mn value exceeding both CONAMA and World Health Organization (WHO, 2008) standards. It should also be noted that there was a high release of K from both pMW and pdMW; although K is not classified as a PTE, its high environmental releasing or

consumption can lead to alterations in the biota (Shabala and Pottosin, 2014; Cremades et al., 2016)

Matte White	Cu	Ni	K	Fe
Pristine	0.009	<LOD	9.664	<LOD
Photodegraded	0.282	<LOD	82.225	0.299
CONAMA	0.009	0.025	NA	0.3
W.H.O.	2.0	0.07	NA	NA

Glossy Black	Zn	Cu	Mn	Cr	Fe
Pristine	0.013	0.003	0.099	<LOD	<LOD
Photodegraded	0.064	0.016	0.621	<LOD	0.034
CONAMA	0.18	0.009	0.1	0.005	0.3
W.H.O.	NA	2.0	0.08	0.05	NA

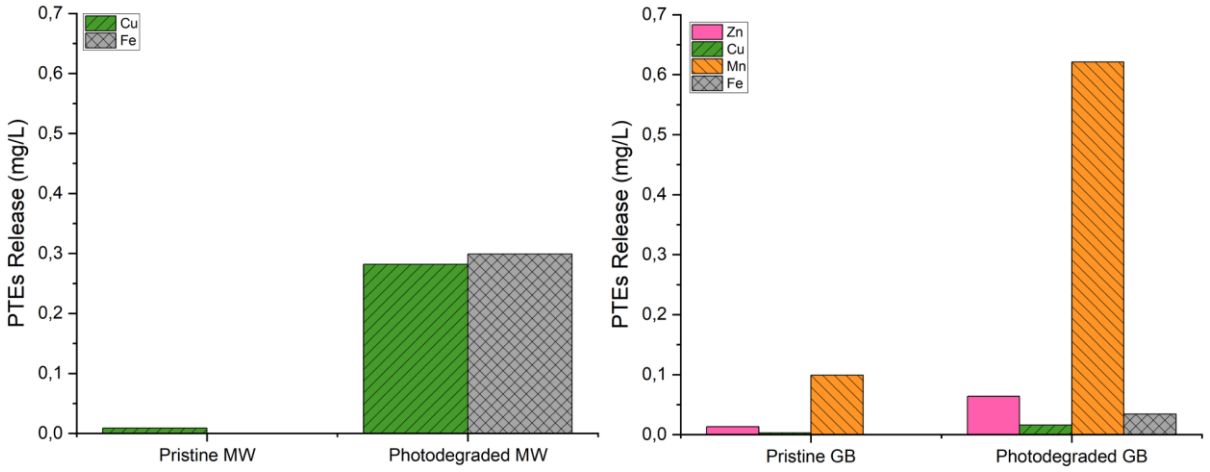


Figure 05: Comparison of the released PTE values (mg/L) from pristine and photodegraded MPs of the studied Matte White and Glossy Black spray paints.

The increase in the release of these metals, as previously mentioned, resulted in changes observable in the histological studies conducted. Morphometrics of the nuclei of columnar cells revealed a significant decrease in the nuclear areas. The average nuclear size in the control group was 65.43 ± 8.17 μm², while in experimental group 01, it was 61.92 ± 5.26 μm², and in experimental group 02, it was 58.77 ± 6.68 μm², with both differing significantly from each other and from the control group ($p < 0.0001$). The difference was even more pronounced in experimental groups 03 and 04, which presented average nuclear sizes of 29.58 ± 3.08 μm² and 21.96 ± 2.57 μm², respectively. These two latter groups also differed significantly from the control ($p < 0.0001$), from the groups exposed to pristine MPs ($p < 0.0001$), and from each other ($p < 0.0001$). The data on nuclear size distribution can be seen in Figure 06.

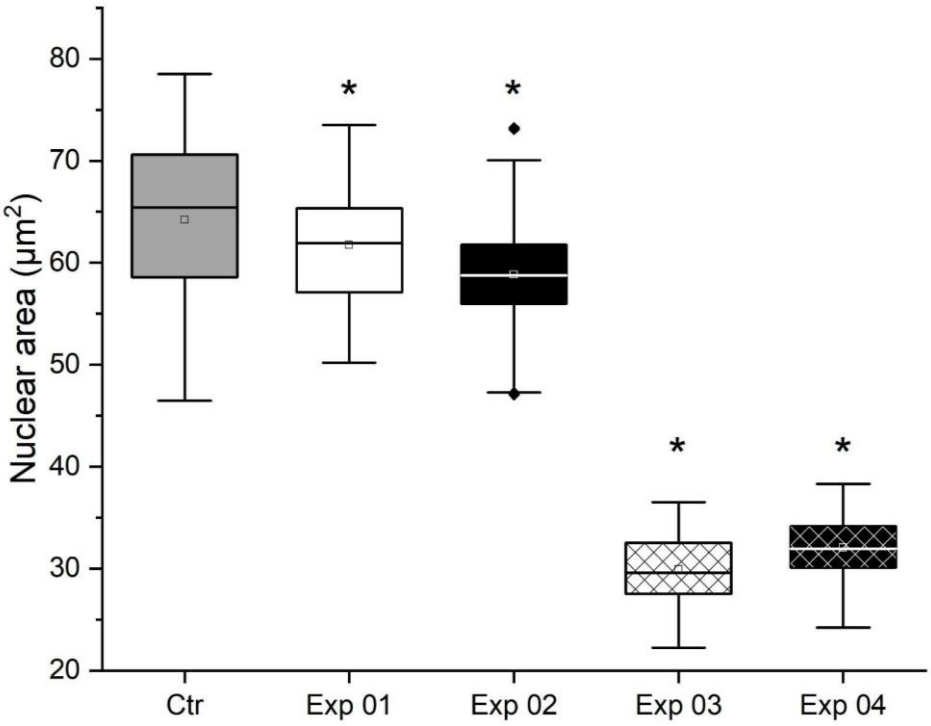


Figure 06: Comparison of the average nuclear areas of columnar cells in forager workers of *B. atratus*. Ctr: control group; Exp 01: individuals exposed to pMW MPs; Exp 02: individuals exposed to pGB MPs; Exp 03: individuals exposed to pdMW MPs; and Exp 04: individuals exposed to pdGB MPs. Black asterisk: significant difference from the control group.

The intestine is a crucial organ in ecotoxicological studies of insects, as it contains a large number of biomarkers that can be analyzed (Terra, 2001). It is typically composed of the foregut (stomodeum), midgut (ventriculus), and hindgut (proctodeum) (Cruz-Landim, 2009). The midgut plays a central role in the digestion and absorption of nutrients and chemicals (Terra et al., 2019). In most insects, food within this region is surrounded by an acellular semipermeable layer known as the peritrophic membrane, which plays several functions, such as protecting the midgut epithelium from mechanical damage by food, acting as a physical barrier against microbial infestation, and preventing the excretion of enzymes through the endo-ecto peritrophic circulation of digestive enzymes (Serrão et al., 2008; Cavalcante and Cruz-Landim, 1998; Malaspina and Silva-Zacarin, 2006).

The epithelium of the adult insect midgut primarily consists of columnar or digestive cells and regenerative cells (Terra, 2001). Columnar cells are responsible for the production and secretion of digestive enzymes, as well as the absorption of digested substances and water (Terra et al., 1988). Conversely, regenerative cells, located at the base of the epithelium, replace the lost columnar cells due to wear and aging through a process involving division and differentiation (Cavalcante and Cruz-Landim, 1998). As demonstrated by the previous

statistical analysis and visible in Figure 7, the nuclei of the columnar cells in the exposed groups showed a decrease in area, along with an attenuation of the presence of spherocrystals, which was more subtle in individuals exposed to pristine MPs and more pronounced in those exposed to photodegraded MPs, where there was a complete disappearance of spherocrystals.

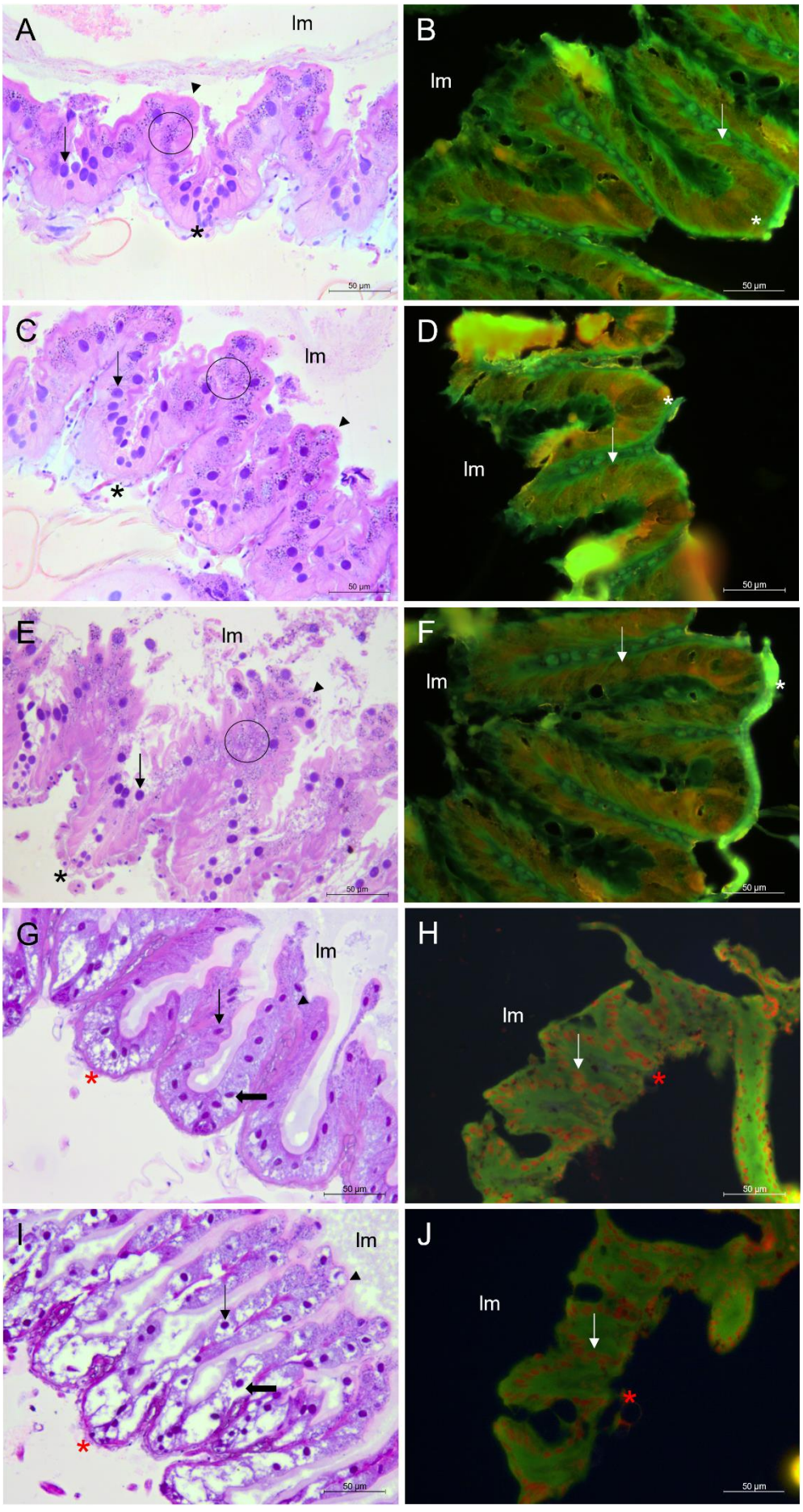

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Figure 07: Histological images of cross-sections of the midgut of forager worker bees of the species *B. atratus*. A and B) Control group; C and D) Experimental group 01 – pMW MPs; E and F) Experimental group 02 – pGB MPs; G and H) Experimental group 03 – pdMW MPs; I and J) Experimental group 04 – pdGB MPs. Thin black and white arrows: columnar cells; Black arrowheads: peritrophic membrane; Black circles: spherocrystals; lm: lumen; Black and white asterisks: cellular nest region (regenerative cells); Red asterisks: degradation of the cellular nest region; Thick black arrows: cellular vacuolization of columnar cells.

These spherocrystals may play a role in cellular osmoregulation and the storage/neutralization of potentially toxic metabolites for subsequent excretion (Serrão, da Cruz-Landim, 1996). Therefore, the decrease in their release and presence in the midgut epithelium indicates a disruption in tissue homeostasis (Serrão et al., 2008). The reduction in the number of spherocrystals, which could reflect a compromised cellular metabolism and excretion of toxic metabolic products, has already been observed in *A. mellifera* exposed to the fungicide pyraclostrobin (Domingues et al., 2020).

Changes like these can arise from the interaction of PS with these cells, as seen in red tilapia *Oreochromis niloticus* (Ding et al., 2018), freshwater mussel *Dreissena polymorpha* (Magni et al., 2018) and freshwater amphipods *Gammarus fossarum* (Kokalj et al., 2018). The midgut plays a vital role in bees, serving functions in digestion, nutrient absorption, detoxification, and the immune system (Wang et al., 2021). It is the primary tissue directly impacted by toxicants, like MPs contamination. Several studies have illustrated the adverse effects of MPs exposure in different organisms, such as inducing intestinal inflammation in zebrafish (Jin et al., 2018), causing intestinal barrier dysfunction in mice (Jin et al., 2019), and resulting in intestinal damage in *Caenorhabditis elegans* (Lei et al., 2018). A previous laboratory investigation using *Daphnia magna* as a model organism also revealed that MPs can enter their digestive system in concentration and time dependent patterns (Aljaibachi et al., 2020).

These types of interactions can lead to increased nuclear activity in these cells or even modifications in histones and, consequently, cellular chromatin, resulting in a decrease in their areas, a commonly found effect in animals under the influence of toxicants (Hamon et al., 2007; Chen et al., 2014; Wu et al., 2023). Additionally, Wang et al. (2021) observed that exposure to microplastics affects various stress-related genes in the midguts of bees. For example, lower and moderate doses of PS-MPs induced the expression of *Cat*, an antioxidant enzyme, but this inductive effect was not observed in the highest dose group of the study. At all levels of PS-MPs exposure studied by Wang et al. (2021), there were no changes in *Sod* expression, indicating that bees may not have sufficient endogenous enzymatic antioxidant systems to

metabolize reactive oxygen species, allowing the observed nuclear alterations (Borchi et al., 2010).

Another problem with MPs, besides the inherent toxicity of some of their polymers, is their ability to act as carriers of other toxicants such as heavy metals, additives, and other components used in their production (Bostan et al., 2023; Costa et al., 2023). Additionally, they can act as a source of adsorption for environmental contaminants, allowing other components present in the environment to attach to them, further increasing their overall impact on natural systems (Brennecke et al., 2016).

The groups exposed to degraded MPs (experimental groups 3 and 4) showed additional alterations beyond the decrease in nuclear area, such as cellular vacuolization and degradation of the regenerative region of the tissue, known as the cellular nest, which houses the regenerative cells. These changes can be observed with both H.E. and AO staining, where the nuclei of the cellular nest appear red, indicating nuclear damage and imminent tissue death (Vermes and Haanen, 1994). This increased cytotoxicity can be attributed to the photodegradation of the particles, as seen with the pdMW and pdGB paints, which tends to increase the release of its PTEs.

In the case of the pdMW particles, the higher release of Cu, above the values permitted by CONAMA, may be linked to the greater damage present in the tissue, with the metal acting synergistically with the PS, as seen in other studies (Wah Chu and Chow, 2002; Wu et al., 2016; Singh et al., 2017; Dortmans et al., 2022; Hawkins et al., 2021). Additionally, Brennecke et al. (2016) demonstrated that antifouling paints in particular are one of the main sources of PTEs for the marine environment, containing a copper-based biocidal pigment, which are applied to ship hulls and several other fixed structures. Although Cu is an essential element for many biological processes and plays an important role in growth, development, and cellular function (Puig and Thiele, 2002), the release of Cu into the environment at high levels can interfere with several biochemical processes, inhibiting enzymes and inducing oxidative stress (Mir et al., 2021).

The same seems to be happening with the individuals exposed to pdGB particles, as high concentrations of Mn, despite being essential, are often toxic and detrimental to the fitness of several aquatic and terrestrial organisms, such as the honeybee *A. mellifera* (Søvik et al., 2015), fruit fly *D. melanogaster* (Ternes et al., 2014), crab *Potamonautes warren* (Steenkamp et al. 1994), and zebrafish *Danio rerio* (Hernández et al. 2015). This toxicity impacts embryonic development, feeding behaviors, reproduction, immunity, and overall survivability. Furthermore, studies show that Mn induces neurotoxic effects, as this element can disrupt the

regulation of *γ-aminobutyric acid* (GABA) and *glutamatergic transmission*, compromising the cellular antioxidant defense mechanism, and disturbing neurotransmitter balance (Chen et al., 2019).

The same pattern of cytotoxicity can be observed in the Malpighian tubules in Figure 8; where the structure of experimental groups 01 and 02 resembles the control, with experimental group 01 even presenting spherocrystals in the tubules. Meanwhile, experimental groups 03 and 04 exhibit vacuolized pyramidal cells with decentralized nuclei positioned in the cell's peripheral region, indicating morphological changes in the tissue. The increase in the organ lumen area in these two groups also indicates greater activation in the secretion and filtration function, demonstrating its role in combating PTEs in the organism.

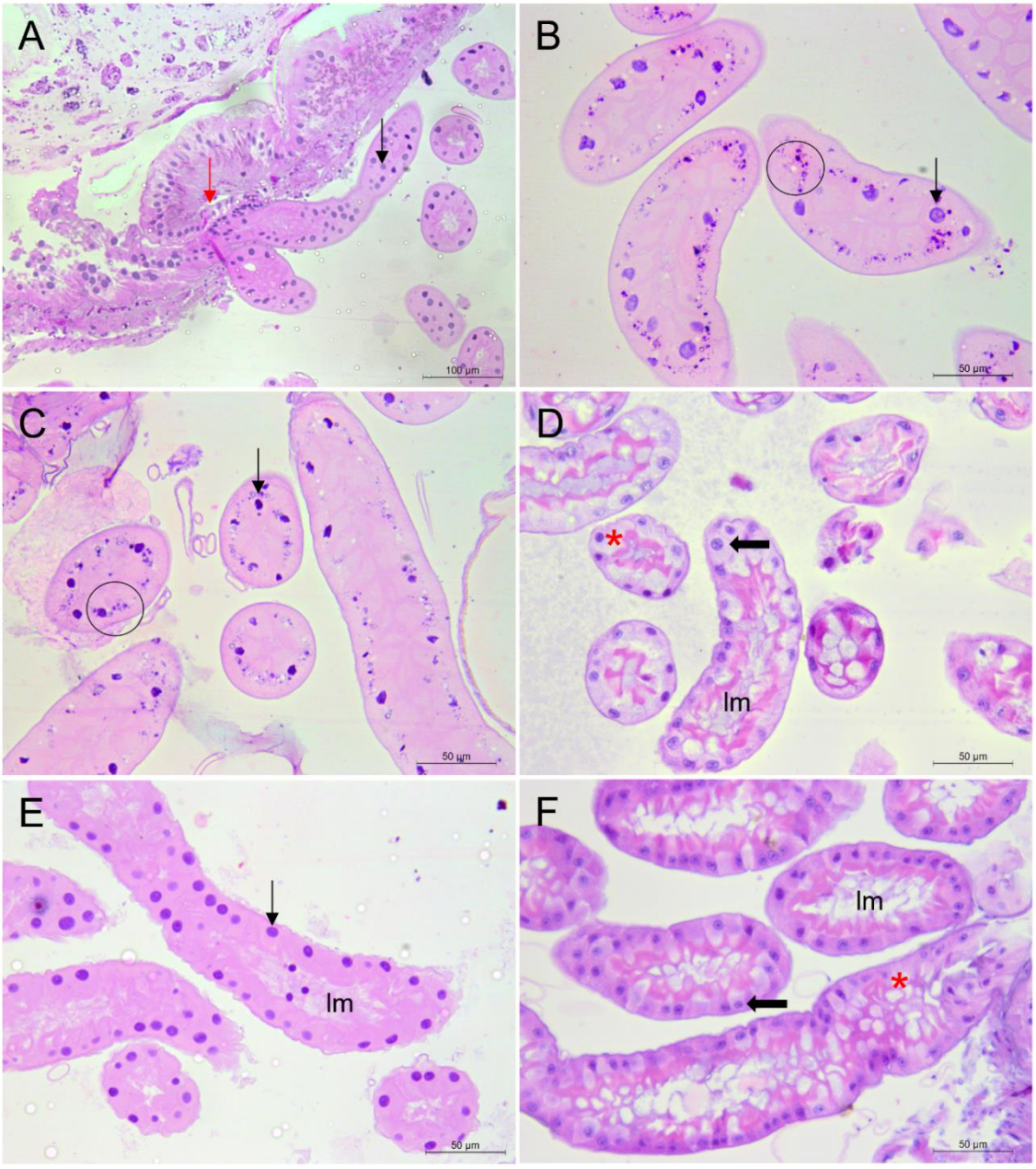

Figure 08: Histological images of transverse and horizontal sections of the Malpighian tubules of forager worker bees of the species *B. atratus*. A and B) Control group; C) Experimental group 01 – pMW MPs; D) Experimental group 03 – pd MW MPs; E) Experimental group 02 – pGB MPs; F) Experimental group 04 – pd GB MPs. Thin red arrow: incision region of the Malpighian tubule in the midgut; Thin black arrow: pyramidal or cuboidal cells; Black circle: spherocrystals; lm: lumen; Red asterisk: cellular vacuolization; and Thick black arrow: decentralized and apoptotic nuclei.

The Malpighian tubules of bees, like in insects in general, are composed of a single layer of pyramidal or cuboidal cells, with spherical nuclei positioned centrally (Cruz-Landim, 1998). These tubules exhibit specialized cells along their structure. For example, in *Drosophila melanogaster*, principal cells perform cation transport, while stellate cells are responsible for

chloride anion transport into the lumen (O'Donnell et al., 1996; Dow et al., 1998; Klowden, 2007; Beyenbach et al., 2010). In *Rhodnius prolixus*, the distal portion of the Malpighian tubules transports a filtrate into the lumen, while the proximal portion absorbs part of this filtrate back into the hemolymph (Bradley, 1983). In *Drosophila*, the tubules have embryonic origin from both ectoderm and mesoderm, analogous to the kidneys of mammals, as they have embryonic origin from different germ layers and the function of filtration, as seen in the presented data (Denholm et al., 2003; Jung et al., 2005).

Regarding the hepato-nephrocytic system cells, morphometric analysis of the pericardial cells indicated a significant difference in cellular area for all exposed groups compared to the control ($p < 0.0001$). The control group had an average cellular area of 303.6 ± 103.1 μm², while experimental group 01 had an average size of 400.2 ± 81.74 μm², and experimental group 02 had 399.4 ± 81.74 μm². Unlike previous analyses, the pericardial cells of experimental groups 03 and 04 followed the same alterations seen in those exposed to pristine microplastics, presenting an average area of 400.9 ± 83.42 μm² and 402.0 ± 78.10 μm², respectively. These values are presented in Figure 9.

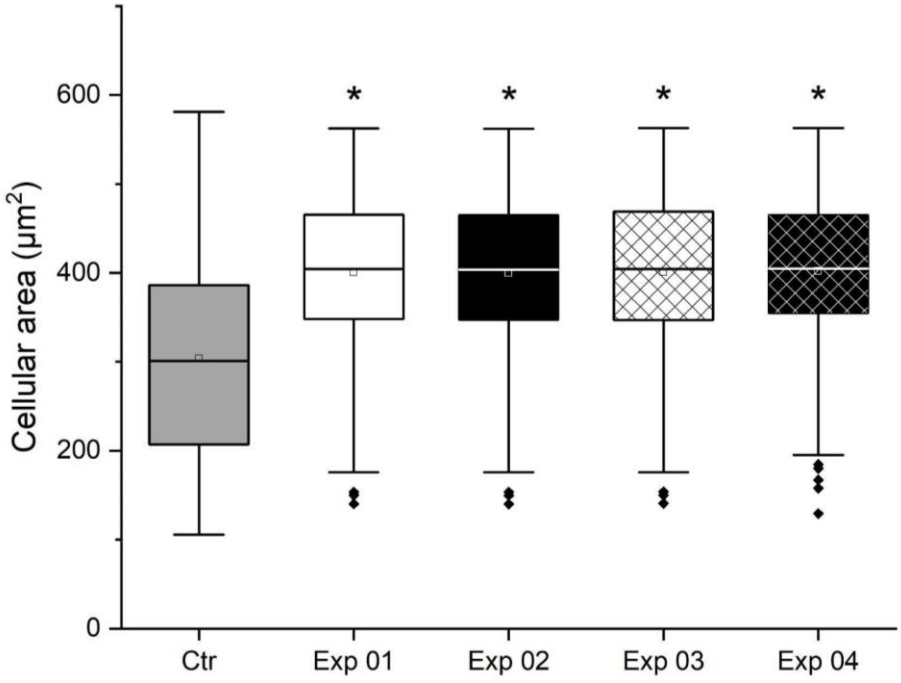

Figure 09: Comparison of the average cellular area of pericardial cells in forager workers of *B. atratus*. Ctr: control group; Exp 01: individuals exposed to pMW MPs; Exp 02: individuals exposed to pGB MPs; Exp 03: individuals exposed to pdMW MPs; and Exp 04: individuals exposed to pdGB MPs. Black asterisk: significant difference from the control group.

Oenocytes exhibited morphological alterations similar to those observed in the Malpighian tubules, with experimental groups 03 and 04 showing significant changes in their cellular areas, differing significantly from all other groups ($p < 0.0001$) but not differing from each other ($p > 0.9999$). Experimental groups 01 and 02 did not differ from each other or from the control group ($p > 0.9999$). The average cellular area in oenocytes for the control group was 1396 ± 224 μm², while experimental groups 01 and 02 had average areas of 1405 ± 217.3 μm² and 1387 ± 221.6 μm², respectively. Experimental groups 03 and 04 presented values of 2082 ± 267.4 μm² and 2118 ± 186.7 μm², respectively. These values are shown in Figure 10.

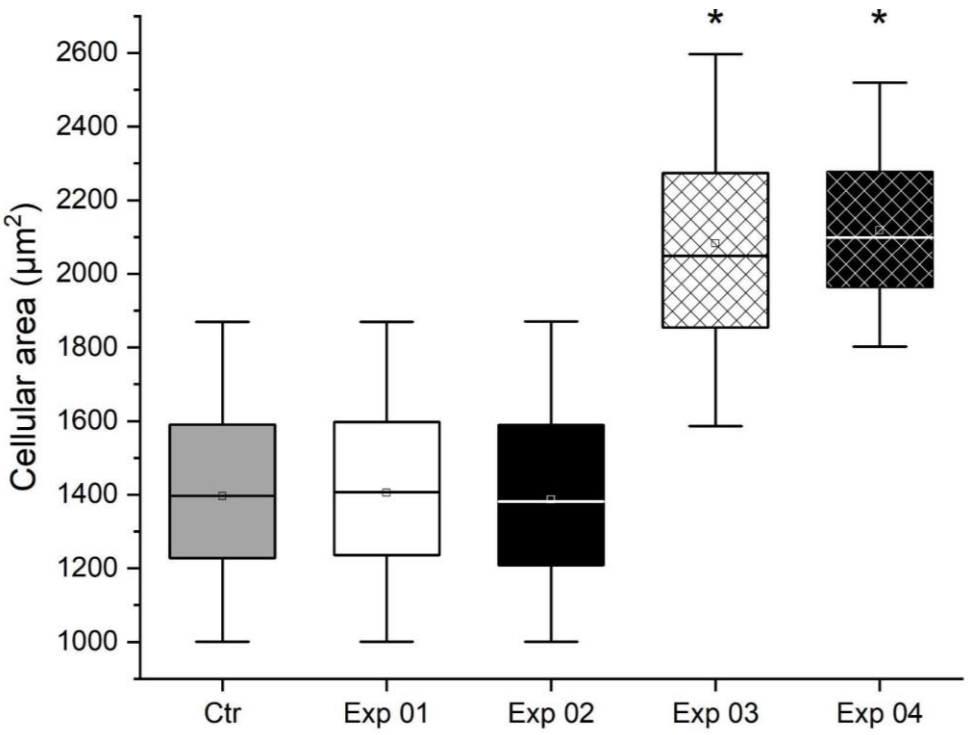

Figure 10: Comparison of the average cellular area of oenocytes in forager workers of *B. atratus*. Ctr: control group; Exp 01: individuals exposed to pMW MPs; Exp 02: individuals exposed to pGB MPs; Exp 03: individuals exposed to pdMW MPs; and Exp 04: individuals exposed to pdGB MPs. Black asterisk: significant difference from the control group.

Pericardial cells exposed to microplastics exhibited vacuolization in their cellular structure, justifying the increase in their cellular areas and indicating the toxicity of both pristine and photodegraded MPs, as both caused similar alterations in the areas of the studied cells. Pericardial cells from the control group (Figure 11 A) demonstrate the healthy state of the cells in the dorsal vessel, which play a crucial role in the circulatory system, ensuring the transport of nutrients, hormones, and other essential substances to various organs of *Bombus* (Poiani and Cruz-Landim, 2007; Das et al., 2007). Additionally, these cells are involved in immune

responses, capable of secreting antimicrobial peptides and other molecules related to the immune system (Cruz-Landim, 2009) that can act against pathogens or contaminants, such as PTEs.

Conversely, oenocytes in experimental groups 01 and 02 did not show alterations compared to the control, unlike those in experimental groups 03 and 04, which exhibited pyknotic nuclear states and swelling in cell size, indicating increased toxicity of the photodegraded MPs. The contribution of oenocytes to the detoxification process in individuals may have contributed to the greater alterations observed in experimental groups 03 and 04, as the photodegradation of the paints resulted in the release of PTEs present in their composition. Another cell type that showed alterations were trophocytes, where in experimental groups 01 and 02 they exhibited chromatin condensation, and in experimental groups 03 and 04, total cellular degradation and advanced pyknotic profile. Being the primary cells in the detoxification process and the first to be activated in the presence of contaminants (Abdalla and Domingues, 2015). These alterations are consistent with those observed in columnar cells, where cells exposed to degraded MPs showed the most significant changes for the reasons mentioned above. The micrographs containing the listed alterations are presented in Figure 11.

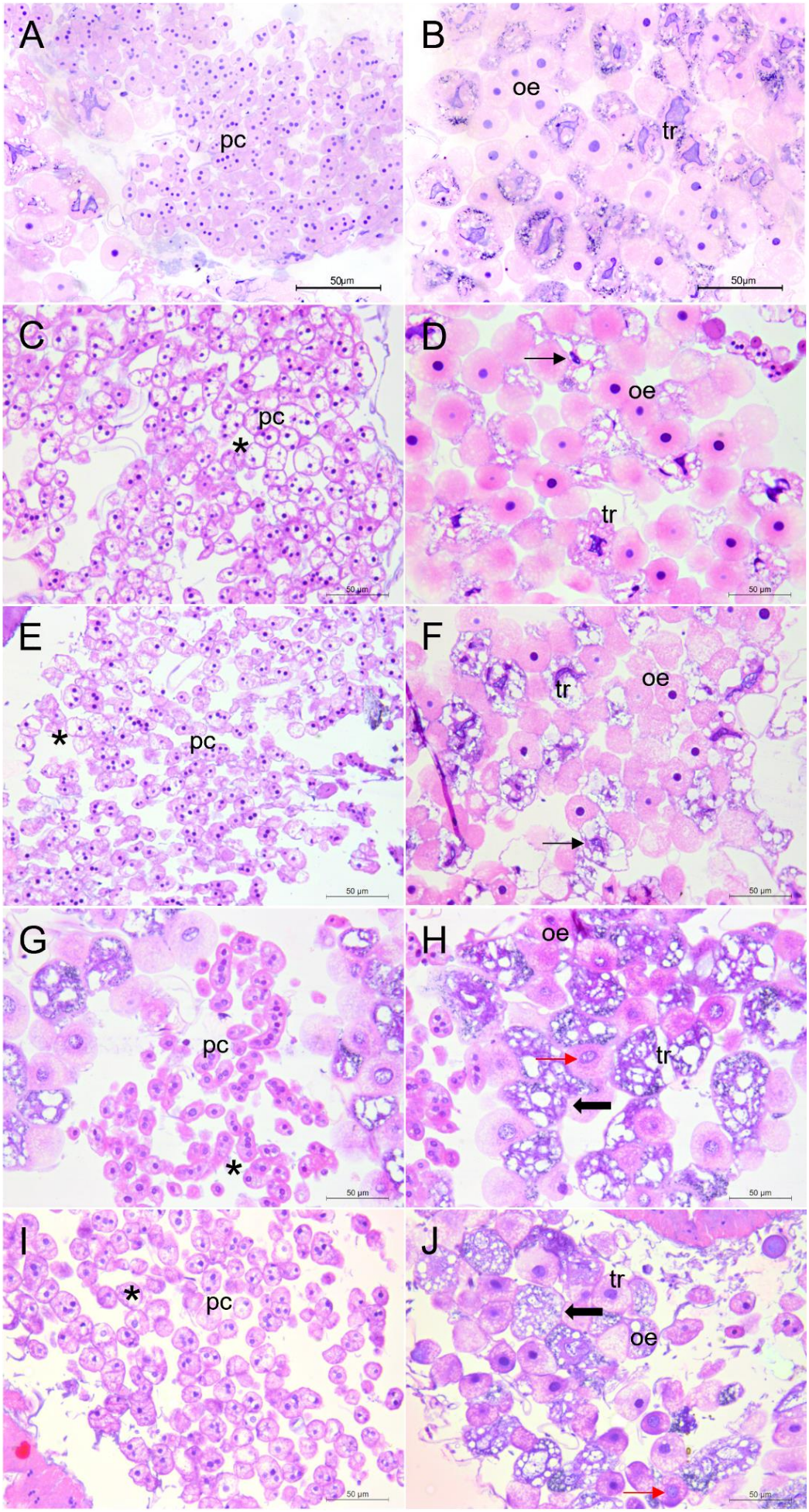
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Figure 11: Histological sections of transverse cuts of the Dorsal Vessel and associated fat body of worker bees from the species *B. atratus*. A and B) Pericardial cells, oenocytes, and trophocytes of control individuals; C and D) Pericardial cells, oenocytes, and trophocytes of individuals exposed to pMW MPs; E and F) Pericardial cells, oenocytes, and trophocytes of individuals exposed to pGB MPs; G and H) Pericardial cells, oenocytes, and trophocytes of individuals exposed to pdMW MPs; and I and J) Pericardial cells, oenocytes, and trophocytes of individuals exposed to pdGB MPs. pc: Pericardial Cell; oe: Oenocyte; tr: Trophocyte; Black asterisk: cellular vacuolization; Thin black arrow: nuclear condensation in Trophocytes; Thin red arrow: Oenocyte nucleus with pycnotic profile; and Thick black arrow: Trophocyte with pycnotic profile.

All these cellular alterations also reflected in the final body weight of the exposed individuals; as shown in Figure 12, individuals exposed to pdMW and pdGB MPs experienced a decrease in body mass after 96 hours of exposure, with both groups differing significantly ($p < 0.001$) from the control. Individuals exposed to pMW and pGB MPs showed no significant difference compared to the control ($p = 0.2367$ and 0.1082, respectively). These data demonstrate that the release of PTEs from the photodegraded particles contributes to the disruption of the body homeostasis of the studied bees, potentially leading to reduced fitness and alterations in feeding and survival of the species.

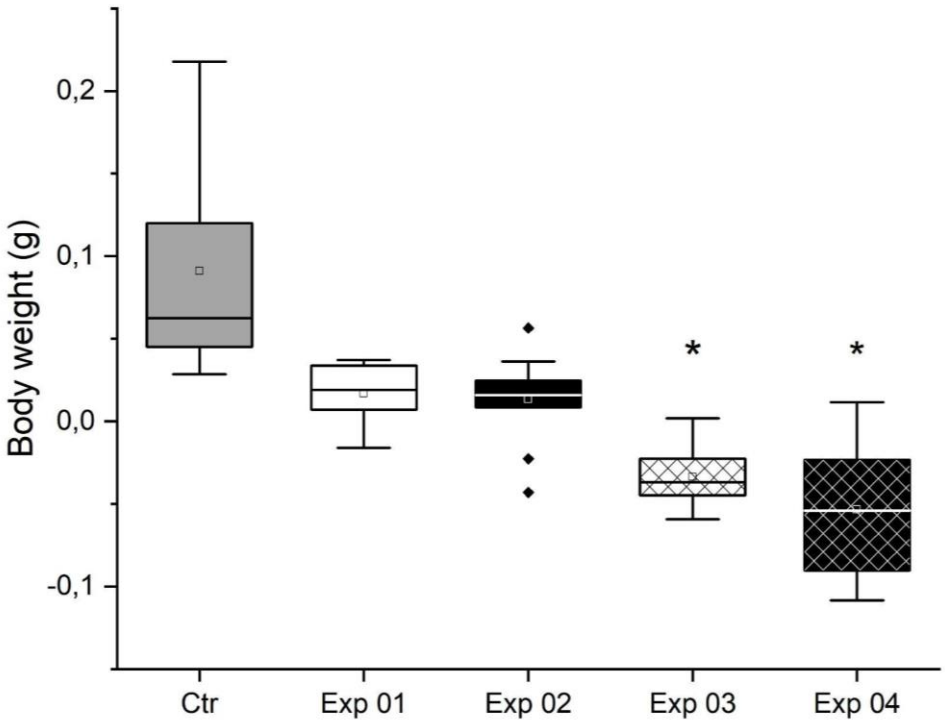

Figure 12: Graphical representation of the average difference between the final and initial weight of the studied organisms. Ctr: control group; Exp 01: individuals exposed to pMW MPs; Exp 02: individuals exposed to pGB MPs; Exp 03: individuals exposed to pdMW MPs; and Exp 04: individuals exposed to pdGB MPs. Black asterisk: significant difference from the control group.

The academic community is just beginning to understand the effects of MPs on terrestrial systems; studies indicate that plastic exposure affects organisms in different ways; in bees, these effects range from mortality, reproductive alterations, behavioral changes, and impacts on microbiota and intestinal structure (Balzani et al., 2022; Buteler et al., 2022; Wang et al., 2022). Balzani et al. (2022) demonstrated that acute and chronic ingestion of polyethylene affected the mortality of *A. mellifera*; this effect was observed at the highest concentration (50 mg L^{-1}), but not at lower concentrations, and feeding behavior showed dependency on the concentration offered. Specifically, the ingestion of low concentrations of polyethylene led to increased food intake, while ingestion of high concentrations impaired the bees' ability to respond to sucrose (Balzani et al., 2022). Thus, our results demonstrated that tissue degradation in exposed groups 3 and 4 may have influenced the organisms' nutrient absorption ability, leading to their weight loss, aligning with the results obtained by Naggar et al., 2023 in honeybees.

3.7. Conclusion

Pristine MPs caused subtle changes in columnar and pericardial cells, while photodegradation of MPs from spray paints led to greater release of PTEs, resulting in vacuolization, nuclear condensation, and pyknosis. Additionally, individuals exposed to pdMW and pdGB MPs showed a reduction in body weight. These findings underscore the importance of understanding the toxicity of environmentally realistic MPs, as plastic composition and weathering conditions can influence particle toxicity.

3.8. References

Abdalla, F. C., Domingues, C. E. C., Balsamo, P. J. (2018). Effect of Cadmium on Worker Ovary Morphology of *Bombus morio* (Hymenoptera: Bombini). Journal of Agricultural and Biological Sciences, 13, 28-37. doi: https://doi.org/10.3844/ajabssp.2018.28.37.

Abdalla, F.C., & Domingues, C. E. (2015). Hepato-Nephrocitic System: A Novel Model of Biomarkers for Analysis of the Ecology of Stress in Environmental Biomonitoring. PLOS ONE, 10(7), e0132349. doi: https://doi.org/10.1371/journal.pone.0132349.

Aljaibachi, R., Laird, W.B., Stevens, F., & Callaghan, A. (2020). Impacts of polystyrene microplastics on *Daphnia magna*: A laboratory and a mesocosm study. Science of The Total Environment, 705, 135800. doi: https://doi.org/10.1016/j.scitotenv.2019.135800.

American Public Health Association – APHA, 2000. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, twenty ed., Washington, DC.

ARORA, R.; MANILA. Styrene: Risk assessment, environmental, and health hazard. Hazardous Gases, p. 363–374, 2021.

Ashrafy, A., Liza, A.A., Islam, M.N., Billah, M.M., Arafat, S.T., Rahman, M.M., & Rahman, Sk.M. (2022). Microplastics Pollution: A Brief Review of Its Source and Abundance in Different Aquatic Ecosystems. Journal of Hazardous Materials Advances, 9, 100215. doi: https://doi.org/10.1016/j.hazadv.2022.100215.

Avio, C.G., Gorbi, S., & Regoli, F. (2017). Plastics and microplastics in the oceans: From emerging pollutants to emerged threat. Marine Environmental Research, 128, 2-11. doi: https://doi.org/10.1016/j.marenvres.2016.05.012.

Balestra, V., Celli, G., & Porrini, C. (1992). Bees, honey, larvae and pollen in biomonitoring of atmospheric pollution. Aerobiologia, 8(1), 122-126. doi: https://doi.org/10.1007/bf02291339.

Balzani, P., Galeotti, G., Scheggi, S., Masoni, A., Santini, G., & Baracchi, D. (2022). Acute and chronic ingestion of polyethylene (PE) microplastics has mild effects on honeybee health and cognition. Environmental Pollution, 305, 119318. doi: https://doi.org/10.1016/j.envpol.2022.119318.

Bandow, N., Will, V., Wachtendorf, V., & Simon, F.-G. (2017). Contaminant release from aged microplastic. Environmental Chemistry, 14(6), 394. doi: https://doi.org/10.1071/en17064.

Bellon, D., Zamudio, W.H., Tiria, L.C., Durán, S.M., Useche, I.E., & Peña, J. (2019). Effect of expanded polystyrene waste in the creation of waterproofing paint. Journal of Physics: Conference Series, 1386(1), 012075. doi: https://doi.org/10.1088/1742-6596/1386/1/012075.

Beyenbach, K.W., Skaer, H. and Dow, J.A.T. (2010). The Developmental, Molecular, and Transport Biology of Malpighian Tubules. Annual Review of Entomology, 55(1), pp.351–374. doi: https://doi.org/10.1146/annurev-ento-112408-085512.

Blais, P., Carlsson, D.J., & Wiles, D.M. (1972). Surface changes during polypropylene photo-oxidation: A study by infrared spectroscopy and electron microscopy. Journal of polymer science, 10(4), pp. 1077–1092. doi: https://doi.org/10.1002/pol.1972.150100412.

Boeing, G. A. N. S., Provase, M., Tsukada, E., Salla, R.F., Abdalla, F. C., (2024). Genotoxic effect of two environmentally safe doses of cadmium on the Hepato-nephrocytic system of *Bombus atratus* forager workers. *Environmental toxicology and pharmacology*, pp.104450–104450. doi:https://doi.org/10.1016/j.etap.2024.104450.

Borchi, E., Bargelli, V., Stillitano, F., Giordano, C., Sebastiani, M., Nassi, P.A., d'Amati, G., Cerbai, E., & Nediani, C. (2010). Enhanced ROS production by NADPH oxidase is correlated to changes in antioxidant enzyme activity in human heart failure. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1802(3), 331-338. doi: https://doi.org/10.1016/j.bbadis.2009.10.014.

Bostan, N., Ilyas, N., Akhtar, N., Mehmood, S., Saman, R.U., Shatid, A.A., Alfaifi, M.Y., Eldin, S., & Pandiaraj, S. (2023). Toxicity assessment of microplastic (MPs): a threat to the ecosystem. Environmental Research, 234, 116523. doi: https://doi.org/10.1016/j.envres.2023.116523.

Bradley, T.J. (1983). Functional design of microvilli in the Malpighian tubules of the insect *Rhodnius prolixus*. Journal of Cell Science, 60(1), pp.117–135. doi: https://doi.org/10.1242/jcs.60.1.117.

Brennecke, D., Duarte, B., Paiva, F., Caçador, I., & Canning-Clode, J. (2016). Microplastics as vector for heavy metal contamination from the marine environment. Estuarine, Coastal and Shelf Science, 178, 189-195. doi: https://doi.org/10.1016/j.ecss.2015.12.003.

Buteler, M., Alma, A.M., Stadler, T., Gingold, A.C., Manattini, M.C., & Lozada, M. (2022). Acute toxicity of microplastic fibers to honeybees and effects on foraging behavior. Science of The Total Environment, 822, 153320. doi: https://doi.org/10.1016/j.scitotenv.2022.153320.

Cacuro, T.A., Freitas, A.S.M. and Waldman, W.R. (2018). Demonstration of Polymer Photodegradation Using a Simple Apparatus. *Journal of Chemical Education*, 95(12), pp.2222–2226. doi:https://doi.org/10.1021/acs.jchemed.7b00836.

Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F., & Griswold, T.L. (2011). Patterns of widespread decline in North American bumble bees. Proceedings of the National Academy of Sciences, 108(2), 662-667. doi: https://doi.org/10.1073/pnas.1014743108.

Carpenter, E.J. and Smith, K.L. (1972). Plastics on the Sargasso Sea Surface. *Science*, 175(4027), pp.1240–1241. doi: https://doi.org/10.1126/science.175.4027.1240.

Cartechini, L., Miliani, C., Nodari, L., Rosi, F., & Tomasin, P. (2021). The chemistry of making color in art. Journal of Cultural Heritage, 50, 188–210. doi: https://doi.org/10.1016/j.culher.2021.05.002.

Carvell, C., Meek, W.R., Pywell, R.F., Goulson, D., & Nowakowski, M. (2006). Comparing the efficacy of agri-environment schemes to enhance bumble bee abundance and diversity on arable field margins. Journal of Applied Ecology, 44(1), 29-40. doi: https://doi.org/10.1111/j.1365-2664.2006.01249.x

Cavalcante, V.M., & da Cruz-Landim, C. (1999). Types of cells present in the midgut of the insects: A review. doi: https://api.semanticscholar.org/CorpusID:86106856.

Ceschi-Bertolli, L., Vidal, F. A. P., Balsamo, P. J., & Abdalla, F. C. (2020). Comet assay protocol for Bombus atratus fat body and pericardial cells (Hymenoptera, Bombini) at a safe concentration of mercury. Chemosphere, 261. doi: https://doi.org/10.1016/j.chemosphere.2020.127752.

Chen, O., He, Y., Sun, Y., Yang, L., Shi, W., & Li, S. (2019). Effects of Sub-Acute Manganese Exposure on Thyroid Hormone and Glutamine (Gln)/Glutamate (Glu)-γ- Aminobutyric Acid (GABA) Cycle in Serum of Rats. International Journal of Environmental Research and Public Health, 16(12), 2157. doi: https://doi.org/10.3390/ijerph16122157.

Chen, R., Kang, R., Fan, X.-G., & Tang, D. (2014). Release and activity of histone in diseases. Cell Death & Disease, 5(8), e1370. doi: https://doi.org/10.1038/cddis.2014.337.

Cole, M., Lindeque, P., Halsband, C., & Galloway, T.S. (2011). Microplastics as contaminants in the marine environment: A review. Marine Pollution Bulletin, 62(12), 2588-2597. doi: https://doi.org/10.1016/j.marpolbul.2011.09.025.

Cortea, I.M., Ratoiu, L., & Rădvan, R. (2020). Characterization of spray paints used in street art graffiti by a non-destructive multi-analytical approach. Color Research & Application, 46(1), 183-194. doi: https://doi.org/10.1002/col.22561.

Costa, J.P. da, Avellan, A., Mouneyrac, C., Duarte, A., & Rocha-Santos, T. (2023). Plastic additives and microplastics as emerging contaminants: Mechanisms and analytical assessment. TrAC Trends in Analytical Chemistry, 158, p. 116898. doi: https://doi.org/10.1016/j.trac.2022.116898.

Cremades, A., del Rio-Garcia, J., Lambertos, A., López-Garcia, C. and Peñafiel, R. (2016). Tissue-specific regulation of potassium homeostasis by high doses of cationic amino acids. *SpringerPlus*, 5(1). doi:https://doi.org/10.1186/s40064-016-2224-3.

Cruz-Landim, C. (1998). Especializações das células dos túbulos de Malpighi em uma abelha sem ferrão, *Melipona quadrifasciata anthidioides* Lep. (Hymenoptera, Apidae). Acta Microscópica 7, 26-33.

Cruz-Landim, C. (2009). Abelhas: Morfologia e Função de Sistemas. Editora Unesp, Sao Paulo, Brasil. 407 pp.

Curcio, M.S., Canela, M.C., & Waldman, W.R. (2018). Selective surface modification of TiO2-coated polypropylene by photodegradation. European Polymer Journal, 101, pp. 177–182. doi: https://doi.org/10.1016/j.eurpolymj.2018.01.036.

Das, D., Aradhya, R., Ashoka, D. and Inamdar, M. (2007). Post-embryonic pericardial cells of Drosophila are required for overcoming toxic stress but not for cardiac function or adult development. Cell and Tissue Research, 331(2), pp.565–570. doi: https://doi.org/10.1007/s00441-007-0518-z.

de Freitas, A.d.S.M., Rodrigues, J.S., Botaro, V.R. et al. Formation of craze-like pattern in polypropylene UV-induced surface cracking. J Polym Res 29, 506 (2022). https://doi.org/10.1007/s10965-022-03357-z.

Deng, Y., Jiang, X., Zhao, H., Yang, S., Gao, J., Wu, Y., Diao, Q. and Hou, C. (2021). Microplastic Polystyrene Ingestion Promotes the Susceptibility of Honeybee to Viral Infection. Environmental Science & Technology, 55(17), pp.11680–11692. doi: https://doi.org/10.1021/acs.est.1c01619.

Denholm, B., Sudarsan, V., Pasalodos-Sanchez, S., Artero, R., Lawrence, P., Maddrell, S., Baylies, M. and Skaer, H. (2003). Dual Origin of the Renal Tubules in *Drosophila*: mesodermal cells integrate and polarize to establish secretory function. Current Biology, 13(12), pp.1052–1057. doi: https://doi.org/10.1016/s0960-9822(03)00375-0.

Diaz-Basantes, M.F., Conesa, J.A. and Fullana, A. (2020). Microplastics in Honey, Beer, Milk and Refreshments in Ecuador as Emerging Contaminants. Sustainability, 12(14), p.5514. doi: https://doi.org/10.3390/su12145514.

Ding, J., Zhang, S., Razanajatovo, R. M., Zou, H., & Zhu, W. (2018). Accumulation, tissue distribution, and biochemical effects of polystyrene microplastics in the freshwater fish red tilapia (*Oreochromis niloticus*). Environmental Pollution, 238, 1-9. doi: https://doi.org/10.1016/j.envpol.2018.02.001.

Domingues, C.E.C., Balsamo, P.J., Oliveira, J.A., & Abdalla, F.C. (2016). Effects of Pb in the morphology of the worker's ovaries of Bombus morio (Hymenoptera: Apidae) in laboratory conditions. Neotropical Entomology, 45, 53-59. doi: https://doi.org/10.1007/s13744-015-0334-6.

Domingues, C.E.C., Inoue, L.V.B., Silva-Zacarin, E.C.M. and Malaspina, O. (2020). Fungicide pyraclostrobin affects midgut morphophysiology and reduces survival of Brazilian native stingless bee Melipona scutellaris. *Ecotoxicology and Environmental Safety*, 206, p.111395. doi: https://doi.org/10.1016/j.ecoenv.2020.111395.

Dortmans, J., Gralher, S., Ludwig, A., Kuckuk, R., Docter, D., & Rösch, P. (2022). Evaluation of the microplastic-associated bacterial biofilm formation and its interactions with bumble bees. Microorganisms, 10(4), 740. doi: https://doi.org/10.3390/microorganisms10040740.

Dow, J.A.T., Davies, S.A. and Sözen, M.A. (1998). Fluid secretion by the *Drosophila* Malpighian tubule. American Zoologist, 38(3), pp.450–460. doi: https://doi.org/10.1093/icb/38.3.450.

Duis, K. and Coors, A. (2016). Microplastics in the Aquatic and Terrestrial environment: Sources (with a Specific Focus on Personal Care products), Fate and Effects. Environmental Sciences Europe, 28(1). doi: https://doi.org/10.1186/s12302-015-0069-y.

Eerkes-Medrano, D., Thompson, R.C. and Aldridge, D.C. (2015). Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Research, 75, pp.63–82. doi: https://doi.org/10.1016/j.watres.2015.02.012.

Eirik Søvik, Perry, C., LaMora, A., Barron, A.B. and Yehuda Ben-Shahar (2015). Negative impact of manganese on honeybee foraging. Biology Letters, 11(3), pp.20140989–20140989. doi: https://doi.org/10.1098/rsbl.2014.0989.

Fu, Z. and Wang, J. (2019). Current practices and future perspectives of microplastic pollution in freshwater ecosystems in China. Science of The Total Environment, 691. doi: https://doi.org/10.1016/j.scitotenv.2019.07.167.

Gade, A.L., Heiaas, H., Lillicrap, A., & Hylland, K. (2012). Ecotoxicity of paint mixtures: Comparison between measured and calculated toxicity. Science of The Total Environment, 435-436, pp. 526–540. doi: https://doi.org/10.1016/j.scitotenv.2012.07.011.

Galafassi, S., Nizzetto, L., & Volta, P. (2019). Plastic sources: A survey across scientific and grey literature for their inventory and relative contribution to microplastics pollution in natural environments, with an emphasis on surface water. Science of The Total Environment, 693, p. 133499. doi: https://doi.org/10.1016/j.scitotenv.2019.07.305.

Gaylarde, C.C., Neto, J.A.B., & da Fonseca, E.M. (2021). Paint fragments as polluting microplastics: A brief review. Marine Pollution Bulletin, 162, p. 111847. doi: https://doi.org/10.1016/j.marpolbul.2020.111847.

Glenny, W., Cavigli, I., Daughenbaugh, K.F., Radford, R., Kegley, S.E. and Flenniken, M.L. (2017). Honey bee (Apis mellifera) colony health and pathogen composition in migratory beekeeping operations involved in California almond pollination. PLOS ONE, 12(8), p.e0182814. doi: https://doi.org/10.1371/journal.pone.0182814.

Goulson, D. (2010). *Bumblebees : behaviour, ecology, and conservation*. Oxford England ; New York: Oxford University Press.

Grixti, J.C., Wong, L.T., Cameron, S.A. and Favret, C. (2009). Decline of bumble bees (Bombus) in the North American Midwest. Biological Conservation, 142(1), pp.75–84. doi: https://doi.org/10.1016/j.biocon.2008.09.027.

Gündoğdu, S., Çevik, C. and Ataş, N.T. (2020). Stuffed with microplastics: Microplastic occurrence in traditional stuffed mussels sold in the Turkish market. Food Bioscience, 37, p.100715. doi: https://doi.org/10.1016/j.fbio.2020.100715.

Hamon, M.A., Batsché, E., Régnault, B., Tham, T.N., Seveau, S., Muchardt, C. and Cossart, P. (2007). Histone modifications induced by a family of bacterial toxins. Proceedings of the National Academy of Sciences of the United States of America, 104(33), pp.13467–13472. doi: https://doi.org/10.1073/pnas.0702729104.

Hawkins, D.S., Kavanagh, K., & Popowich, J.J. (2021). Microplastics and trace metals: A review of concentration, bioaccumulation, and effects on humans and aquatic ecosystems. Environmental Chemistry, 18(5), 298. doi: https://doi.org/10.1071/en20147.

Hernández, R.B., Nishita, M.I., Espósito, B.P., Scholz, S. and Michalke, B. (2015). The role of chemical speciation, chemical fractionation and calcium disruption in manganese-induced developmental toxicity in zebrafish (*Danio rerio*) embryos. Journal of Trace Elements in Medicine and Biology, 32, pp.209–217. doi: https://doi.org/10.1016/j.jtemb.2015.07.004.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C. and Thiel, M. (2012). Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. Environmental Science & Technology, 46(6), pp.3060–3075. doi: https://doi.org/10.1021/es2031505.

Hung, K.-L.J., Kingston, J.M., Albrecht, M., Holway, D.A. and Kohn, J.R. (2018). The worldwide importance of honey bees as pollinators in natural habitats. Proceedings of the Royal Society B: Biological Sciences, [online] 285(1870), p.20172140. doi: https://doi.org/10.1098/rspb.2017.2140.

Hyne, R.V. and Maher, W.A. (2003). Invertebrate biomarkers: links to toxicosis that predict population decline. *Ecotoxicology and Environmental Safety*, 54(3), pp.366–374. doi: https://doi.org/10.1016/s0147-6513(02)00119-7.

Imhof, H.K., Laforsch, C., Wiesheu, A.C., Schmid, J., Anger, P.M., Niessner, R., & Ivleva, N.P. (2016). Pigments and plastic in limnetic ecosystems: A qualitative and quantitative study on microparticles of different size classes. Water Research, 98, 64–74. doi: https://doi.org/10.1016/j.watres.2016.03.015.

IUCN. 2023. The IUCN Red List of Threatened Species. Version 2023-1. https://www.iucnredlist.org. Accessed on [12/06/2024].

Järlskog, I., Strömvall, A.-M., Magnusson, K., Gustafsson, M., Polukarova, M., Galfi, H., Aronsson, M. and Andersson-Sköld, Y. (2020). Occurrence of tire and bitumen wear microplastics on urban streets and in sweepsand and washwater. Science of The Total Environment, 729, p.138950. doi: https://doi.org/10.1016/j.scitotenv.2020.138950.

Jin, Y., Lu, L., Tu, W., Luo, T. and Fu, Z. (2019). Impacts of polystyrene microplastic on the gut barrier, microbiota and metabolism of mice. *Science of The Total Environment*, 649, pp.308–317. doi: https://doi.org/10.1016/j.scitotenv.2018.08.353.

Jin, Y., Xia, J., Pan, Z., Yang, J., Wang, W. and Fu, Z. (2018). Polystyrene microplastics induce microbiota dysbiosis and inflammation in the gut of adult zebrafish. Environmental Pollution (Barking, Essex: 1987), [online] 235, pp.322–329. doi: https://doi.org/10.1016/j.envpol.2017.12.088.

Jung, A.C., Denholm, B., Skaer, H. and Affolter, M. (2005). Renal Tubule Development in *Drosophila*: A Closer Look at the Cellular Level. Journal of the American Society of Nephrology, 16(2), pp.322–328. doi: https://doi.org/10.1681/asn.2004090729.

Kearns, C.A. and Inouye, D.W. (1997). Pollinators, Flowering Plants, and Conservation Biology. BioScience, 47(5), pp.297–307. doi: https://doi.org/10.2307/1313191.

Kevan, P.G., Rasmont, P. and Martinet, B. (2024). Thermodynamics, thermal performance and climate change: temperature regimes for bumblebee (Bombus spp.) colonies as examples of superorganisms. *Frontiers in bee science*, 2. doi: https://doi.org/10.3389/frbee.2024.1351616.

Klowden, M.J. (2013). Physiological systems in insects. Editorial: London: Academic Press Is An Imprint Of Elsevier.

Kokalj, A.J., Horvat, P., Skalar, T., & Kržan, A. (2018). Screening the effects of microplastics on freshwater amphipods (Gammarus fossarum): Searching for the causes of reduced feeding rate. Environmental Toxicology and Chemistry, 37(10), 2689-2697. doi: https://doi.org/10.1002/etc.4261.

Kwon, B.G., Koizumi, K., Chung, S.-Y., Kodera, Y., Kim, J.-O. and Saido, K. (2015). Global styrene oligomers monitoring as new chemical contamination from polystyrene plastic marine pollution. *Journal of Hazardous Materials*, 300, pp.359–367. doi: https://doi.org/10.1016/j.jhazmat.2015.07.039.

Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K.M. and He, D. (2018). Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. Science of The Total Environment, 619-620, pp.1–8. doi: https://doi.org/10.1016/j.scitotenv.2017.11.103.

Li, L., Zhang, B., Jiang, B., Zhao, Y., Qian, G. and Hu, X. (2021). Potentially toxic elements in weathered waste-rocks of Fushun western opencast mine: distribution, source identification, and contamination assessment. *Environmental geochemistry and health*, 44(6), pp.1813–1826. doi:https://doi.org/10.1007/s10653-021-01161-0.

Lima, A.R.A., Costa, M.F., & Barletta, M. (2014). Distribution patterns of microplastics within the plankton of a tropical estuary. Environmental Research, 132, pp. 146–155. doi: https://doi.org/10.1016/j.envres.2014.03.031.

Ma, H., Pu, S., Liu, S., Bai, Y., Mandal, S., and Xing, B. (2020). Microplastics in aquatic environments: Toxicity to trigger ecological consequences. Environmental Pollution, 261, p. 114089. doi: https://doi.org/10.1016/j.envpol.2020.114089.

Magni, S., Gagne, F., Andre, C., Della Torre, C., Auclair, J., Hanana, H., & Binelli, A. (2018). Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in a freshwater mussel (Dreissena polymorpha). Environmental Pollution, 234, 113-122. doi: https://doi.org/10.1016/j.envpol.2017.11.075.

Malaspina, O. and Silva-Zacarin, E.C.M. da (2006). Cell Markers For Ecotoxicological Studies In Target Organs Of Bees. *Braz. J. morphol. Sci.*, [online] 23(3-4), pp.303–309. Available at: http://www.jms.periodikos.com.br/article/587cb4627f8c9d0d058b463d.

Martínez-López, O., Koch, J.B., Martínez-Morales, M.A., Navarrete-Gutiérrez, D., Enríquez, E. and Vandame, R. (2021). Reduction in the potential distribution of bumble bees (Apidae: Bombus) in Mesoamerica under different climate change scenarios: Conservation implications. *Global Change Biology*, 27(9), pp.1772–1787. doi:https://doi.org/10.1111/gcb.15559.

Martins, A.C. and Melo, G.A.R. (2009). Has the bumblebee Bombus bellicosus gone extinct in the northern portion of its distribution range in Brazil? *Journal of Insect Conservation*, 14(2), pp.207–210. doi:https://doi.org/10.1007/s10841-009-9237-y.

Martins, A.C., Gonçalves, R.B. and Melo, G.A.R. (2013). Changes in wild bee fauna of a grassland in Brazil reveal negative effects associated with growing urbanization during the last 40 years. *Zoologia (Curitiba)*, 30(2), pp.157–176. doi:https://doi.org/10.1590/s1984-46702013000200006.

Meyers, K., & Trittin, M. (2020). Microplastics in agricultural soils: A review of the impacts on soil properties, plant health, and agricultural productivity. Environmental Science: Processes & Impacts, 22(1), 88-100. doi: https://doi.org/10.1039/c9em00357a.

Miller, R.R., Newhook, R. and Poole, A. (1994). Styrene Production, Use, and Human Exposure. *Critical Reviews in Toxicology*, 24(sup1), pp.S1–S10. doi: https://doi.org/10.3109/10408449409020137.

Mir, A.R., Pichtel, J. and Hayat, S. (2021). Copper: uptake, toxicity and tolerance in plants and management of Cu-contaminated soil. BioMetals, 34(4), pp.737–759. doi: https://doi.org/10.1007/s10534-021-00306-z.

Moure, J.S; Sakagami, S.F. As mamangabas sociais do Brasil (Bombus Latr.) (Hym. Apoidea). Stud. Entomol. Rio de Janeiro, v. 5, n. 1-4, p. 65-194, 1962.

Naggar, Y.A., Sayes, C.M., Collom, C., Ayorinde, T., Qi, S., El-Seedi, H.R., Paxton, R.J. and Wang, K. (2023). Chronic Exposure to Polystyrene Microplastic Fragments Has No Effect on Honey Bee Survival, but Reduces Feeding Rate and Body Weight. Toxics, [online] 11(2), p.100. doi: https://doi.org/10.3390/toxics11020100.

National Environment Council of Brazil - CONAMA, Silva, M. (2005). Resolução CONAMA N° 357, de 17 de março de 2005, Brasília.

Nieder, R. and Benbi, D.K. (2023). Potentially toxic elements in the environment – a review of sources, sinks, pathways and mitigation measures. *Reviews on environmental health*, 0(0). doi:https://doi.org/10.1515/reveh-2022-0161.

Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D., & Whitehead, P.G. (2016). A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. Environmental Science: Processes & Impacts, 18(8), pp. 1050–1059. doi: https://doi.org/10.1039/c6em00206d.

Nouman, M., Saunier, J., Jubeli, E. and Najet Yagoubi (2017). Additive blooming in polymer materials: Consequences in the pharmaceutical and medical field. *Polymer Degradation and Stability*, 143, pp.239–252. doi: https://doi.org/10.1016/j.polymdegradstab.2017.07.021.

O'Donnell, M.J., Dow, J.A., Huesmann, G.R., Tublitz, N.J. and Maddrell, S.H. (1996). Separate control of anion and cation transport in Malpighian tubules of *Drosophila melanogaster*.

Journal of Experimental Biology, 199(5), pp.1163–1175. doi: https://doi.org/10.1242/jeb.199.5.1163.

OECD (2017a). Guidelines for the Testing of Chemicals, Section 2, Effects on Biotic Systems. Bumblebee, Acute Contact Toxicity Test n.246.

OECD (2017b), Test No. 247: Bumblebee, Acute Oral Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.

Patel, V., Pauli, N., Biggs, E., Barbour, L. and Boruff, B. (2020). Why bees are critical for achieving sustainable development. *Ambio*, [online] 50(1). doi: https://doi.org/10.1007/s13280-020-01333-9.

Paul-Pont, I., Lacroix, C., González Fernández, C., Hégaret, H., Lambert, C., Le Goïc, N., Frère, L., Cassone, A.-L., Sussarellu, R., Fabioux, C., Guyomarch, J., Albentosa, M., Huvet, A. and Soudant, P. (2016). Exposure of marine mussels Mytilus spp. to polystyrene microplastics: Toxicity and influence on fluoranthene bioaccumulation. Environmental Pollution, 216, pp.724–737. doi: https://doi.org/10.1016/j.envpol.2016.06.039.

Pires, C.S.S., Pereira, F.M., Lopes, M.T. do R., Nocelli, R.C.F., Malaspina, O., Pettis, J.S., and Teixeira, É.W. (2016). Enfraquecimento e perda de colônias de abelhas no Brasil: há casos de CCD? Pesquisa Agropecuária Brasileira, [online] 51(5), pp. 422–442. doi: https://doi.org/10.1590/s0100-204x2016000500003.

Poiani, S.B. and da Cruz-Landim, C. (2007). Histology and ultrastructure of pericardial cells of Scaptotrigona postica Latreille (Hymenoptera, Apidae) in workers and queens of different ages. *Micron*, 38(7), pp.766–770. doi: https://doi.org/10.1016/j.micron.2006.11.008.

Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., & Rocha-Santos, T. (2019). Environmental exposure to microplastics: An overview on the factors affecting microplastic toxicity to animal and human health. Environmental Science and Technology, 53(13), 7747-7760. doi: https://doi.org/10.1021/acs.est.9b02600.

Proctor, M., Peter Frederick Yeo, Yeo, P., Lack, A.J. and Lack, A. (1996). *The Natural History of Pollination*. Timber Press (OR).

Provase, M., Salla, R. F., Requião de Lima, C., Abdalla, F. C. (2021). Effects of mercury at field estimated concentration in brain of Bombus atratus (Hymenoptera: Bombini). Chemosphere, 276, 130198. doi: https://doi.org/10.1016/j.chemosphere.2021.130198.

Puig, S. and Thiele, D.J. (2002). Molecular mechanisms of copper uptake and distribution. Current Opinion in Chemical Biology, 6(2), pp.171–180. doi: https://doi.org/10.1016/s1367-5931(02)00298-3.

Rillig, M.C., Ziersch, L., & Hempel, S. (2017). Microplastic transport in soil by earthworms. Scientific Reports, 7, 1362. doi: https://doi.org/10.1038/s41598-017-01594-7.

Rochman, C.M., Browne, M.A., Halpern, B.S., Hentschel, B.T., Hoh, E., Karapanagioti, H.K., Rios-Mendoza, L.M., Takada, H., Teh, S. and Thompson, R.C. (2013). Classify plastic waste as hazardous. *Nature*, 494(7436), pp.169–171. doi: https://doi.org/10.1038/494169a.

Rolsky, C., Kelkar, V., Driver, E. and Halden, R.U. (2020). Municipal sewage sludge as a source of microplastics in the environment. Current Opinion in Environmental Science & Health, 14, pp.16–22. doi: https://doi.org/10.1016/j.coesh.2019.12.001.

Roy, P., Ashton, L., Wang, T., Corradini, M.G., Fraser, E.D.G., Thimmanagari, M., Tiessan, M., Bali, A., Saharan, K.M., Mohanty, A.K., & Misra, M. (2021). Evolution of drinking straws and their environmental, economic and societal implications. Journal of Cleaner Production, 316(128234), 128234. doi: https://doi.org/10.1016/j.jclepro.2021.128234.

Serrão, J. E., & DA CRUZ-LANDIM, C. (1996). Ultrastructure and Histochemistry of the Mineral Concretions in the Midgut of Bees (Hymenoptera: Apidae) 1. *Netherlands Journal of Zoology*, *47*(1), 21-29. https://doi.org/10.1163/156854297X00210

Serrão, J.E., Ronnau, M., Neves, C.A., Campos, L.A.O. and Zanuncio, J.C. (2008). Ultrastructure of Anterior Midgut Region of Corbiculate Bees (Hymenoptera: Apidae). *Annals of the Entomological Society of America*, 101(5), pp.915–921. doi:https://doi.org/10.1603/0013-8746(2008)101[915:uoamro]2.0.co;2.

Shabala, S. and Pottosin, I. (2014). Regulation of potassium transport in plants under hostile conditions: implications for abiotic and biotic stress tolerance. *Physiologia Plantarum*, 151(3), pp.257–279. doi: https://doi.org/10.1111/ppl.12165.

Singh, N., Gupta, V.K., Kumar, A. and Sharma, B. (2017). Synergistic Effects of Heavy Metals and Pesticides in Living Systems. *Frontiers in Chemistry*, 5. doi: https://doi.org/10.3389/fchem.2017.00070.

Song, Q. and Li, J. (2014). Environmental effects of heavy metals derived from the e-waste recycling activities in China: A systematic review. *Waste Management*, 34(12), pp.2587–2594. doi: https://doi.org/10.1016/j.wasman.2014.08.012.

Sørensen, L., Groven, A.S., Hovsbakken, I.A., Del Puerto, O., Krause, D.F., Sarno, A., & Booth, A.M. (2021). UV degradation of natural and synthetic microfibers causes fragmentation and release of polymer degradation products and chemical additives. Science of The Total Environment, 755(Part 2), 143170. ISSN 0048-9697. doi: https://doi.org/10.1016/j.scitotenv.2020.143170.

Soroldoni, S., Abreu, F., Castro, Í.B., Duarte, F.A., & Pinho, G.L.L. (2017). Are antifouling paint particles a continuous source of toxic chemicals to the marine environment? Journal of Hazardous Materials, 330, pp. 76–82. doi: https://doi.org/10.1016/j.jhazmat.2017.02.001.

Steenkamp, V.E., du Preez, H.H., Schoonbee, H.J. and van Eeden, P.H. (1994). Bioaccumulation of manganese in selected tissues of the freshwater crab, Potamonautes warreni (Calman), from industrial and mine-polluted freshwater ecosystems. *Hydrobiologia*, 288(3), pp.137–150. doi: https://doi.org/10.1007/bf00006237.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P. and Huvet, A. (2016). Oyster reproduction is affected by exposure to polystyrene microplastics. Proceedings of the National Academy of Sciences, 113(9), pp.2430–2435. doi: https://doi.org/10.1073/pnas.1519019113.

Ternes, A.P.L., Zemolin, A.P., da Cruz, L.C., da Silva, G.F., Saidelles, A.P.F., de Paula, M.T., Wagner, C., Golombieski, R.M., Flores, É.M. de M., Picoloto, R.S., Pereira, A.B., Franco, J.L. and Posser, T. (2014). Drosophila melanogaster - an embryonic model for studying behavioral and biochemical effects of manganese exposure. EXCLI Journal, [online] 13, pp.1239–1253. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/pmc4464430/

Terra, W.R. (2001). The origin and functions of the insect peritrophic membrane and peritrophic gel. *Archives of Insect Biochemistry and Physiology*, 47(2), pp.47–61. doi: https://doi.org/10.1002/arch.1036.

Terra, W.R., Espinoza-Fuentes, F.P., Ribeiro, A.F. and Ferreira, C. (1988). The larval midgut of the housefly (Musca domestica): Ultrastructure, fluid fluxes and ion secretion in relation to the organization of digestion. *Journal of Insect Physiology*, [online] 34(6), pp.463–472. doi:https://doi.org/10.1016/0022-1910(88)90187-4.

Terra, W.R., Ignacio Granja Barroso, Dias, R.O. and Ferreira, C. (2019). Molecular physiology of insect midgut. *Advances in Insect Physiology*, pp.117–163. doi:https://doi.org/10.1016/bs.aiip.2019.01.004.

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., & Russell, A.E. (2004). Lost at sea: Where is all the plastic? Science, 304(5672), 838. doi: https://doi.org/10.1126/science.1094559.

Toussaint, B., Raffael, B., Angers-Loustau, A., Gilliland, D., Kestens, V., Petrillo, M., Rio-Echevarria, I.M. and Van den Eede, G. (2019). Review of micro- and nanoplastic contamination in the food chain. Food Additives & Contaminants: Part A, 36(5), pp.639–673. doi: https://doi.org/10.1080/19440049.2019.1583381.

Tremblay, L.A., Kraft, S., Maxwell, P., Statham, P., & Fath, B.D. (2019). Microplastics in water: Sampling, characterization, and analysis. Microchemical Journal, 149, 104066. doi: https://doi.org/10.1016/j.microc.2019.104066.

Turner, A. (2021). Paint particles in the marine environment: An overlooked component of microplastics. Water Research X, 12, p. 100110. doi: https://doi.org/10.1016/j.wroa.2021.100110.

Velthuis, H.H.W., and van Doorn, A. (2006). A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. Apidologie, [online] 37(4), pp. 421–451. doi: https://doi.org/10.1051/apido:2006019.

Vermes, I. and Haanen, C. (1994). Apoptosis and Programmed Cell Death in Health and Disease. *Advances in Clinical Chemistry*, pp.177–246. doi: https://doi.org/10.1016/s0065-2423(08)60336-4.

Wah Chu, K. and Chow, K.L. (2002). Synergistic toxicity of multiple heavy metals is revealed by a biological assay using a nematode and its transgenic derivative. *Aquatic Toxicology*, 61(1-2), pp.53–64. doi: https://doi.org/10.1016/s0166-445x(02)00017-6.

Waldman, W.R. and Rillig, M.C. (2020). Microplastic Research Should Embrace the Complexity of Secondary Particles. *Environmental Science & Technology*, 54(13), pp.7751–7753. doi: https://doi.org/10.1021/acs.est.0c02194.

Wang, K., Li, J., Zhao, L., Mu, X., Wang, C., Wang, M., Xue, X., Qi, S. and Wu, L. (2021). Gut microbiota protects honey bees (Apis mellifera L.) against polystyrene microplastics exposure risks. Journal of Hazardous Materials, 402, p.123828. doi: https://doi.org/10.1016/j.jhazmat.2020.123828.

Wang, K., Zhu, L., Rao, L., Zhao, L., Wang, Y., Wu, X., Zheng, H. and Liao, X. (2022). Nano- and micro-polystyrene plastics disturb gut microbiota and intestinal immune system in honeybee. *Science of The Total Environment*, [online] 842, p.156819. doi: https://doi.org/10.1016/j.scitotenv.2022.156819.

Wang, W., Fang, J.-L., Pan, X., Hua, M., Jiang, J., Ni, L. and Jiang, J. (2019). Thermal research on the uncontrolled behavior of styrene bulk polymerization. *Journal of Loss Prevention in the Process Industries*, 57, pp.239–244. doi: https://doi.org/10.1016/j.jlp.2018.11.020.

WHO (2008). Guidelines for Drinking Water Quality (3rd ed., pp. 5115). World Health Organization, Geneva, Switzerland.

Wood, T.J., Michez, D., Paxton, R.J., Drossart, M., Neumann, P., Gérard, M., Vanderplanck, M., Barraud, A., Martinet, B., Leclercq, N. and Vereecken, N.J. (2020). Managed honey bees as a radar for wild bee decline? *Apidologie*, 51(6), pp.1100–1116. doi:https://doi.org/10.1007/s13592-020-00788-9.

Wu, H., Liu, Q., Yang, N. and Xu, S. (2023). Polystyrene-microplastics and DEHP co-exposure induced DNA damage, cell cycle arrest and necroptosis of ovarian granulosa cells in mice by promoting ROS production. Science of The Total Environment, 871, pp.161962–161962. doi: https://doi.org/10.1016/j.scitotenv.2023.161962.

Wu, X., Cobbina, S.J., Mao, G., Xu, H., Zhang, Z. and Yang, L. (2016). A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. *Environmental*

Science and Pollution Research, [online] 23(9), pp.8244–8259. doi:https://doi.org/10.1007/s11356-016-6333-x.

Xu, Y., Rillig, M.C., & Waldman, W.R. (2022). New separation protocol reveals spray painting as a neglected source of microplastics in soils. Environmental Chemistry Letters, 20(6), pp. 3363–3369. doi: https://doi.org/10.1007/s10311-022-01500-2.

Xu, Z., Zou, G., Chu, H., Shen, Z., Zhang, Y., Abbas, M., Albogami, B.Z., Zou, G. and Abdelhafez, A.A. (2023). Biochar applications for treating potentially toxic elements (PTEs) contaminated soils and water: a review. *Frontiers in Bioengineering and Biotechnology*, 11. doi:https://doi.org/10.3389/fbioe.2023.1258483.

Zattara, E. and Aizen, M. (2021). Worldwide occurrence records suggest a global decline in bee species richness. *One Earth*, [online] 4(1), pp.114–123. doi:https://doi.org/10.1016/j.oneear.2020.12.005.

Zhang, H. (2020). Transport of microplastics in coastal sea waters: A review. Environmental Pollution, 250, 1099-1111. doi: https://doi.org/10.1016/j.envpol.2019.04.070.

Zhou, Y., Wang, J., Zou, M., Jia, Z., Zhou, S. and Li, Y. (2020). Microplastics in soils: A review of methods, occurrence, fate, transport, ecological and environmental risks. *Science of The Total Environment*, 748, p.141368. doi:https://doi.org/10.1016/j.scitotenv.2020.141368.

4. CONCLUSÕES

A fotodegradação de MPs de tinta spray ocasionou na maior liberação de EPTs presentes em sua composição, seguindo um padrão de quanto maior a intensidade de UV-C e o tempo de exposição, maior a liberação dos toxicantes. A exposição de abelhas da espécie *B. atratus* aos MPs prístinos de ambas as tintas apresentou resultados morfológicos sutis, com alterações na área nuclear e celular das células colunares e pericárdicas, respectivamente. Enquanto isso, a exposição de indivíduos a MPs fotodegradados levou a vacuolizações celulares, condensações nucleares e o aparecimento de núcleos em estado picnótico nos tecidos dos órgãos analisados. Adicionalmente, os indivíduos expostos aos MPs fotodegradados apresentaram uma diminuição do peso corporal total. Esses achados ressaltam a importância de compreender a toxicidade de MPs ambientalmente realistas, pois a composição plástica e as condições de intemperismo podem influenciar a toxicidade das partículas. Este estudo é o primeiro do Brasil a apresentar resultados dessa natureza e ressalta a necessidade da criação e atualização de políticas públicas em relação a poluição plástica e o seu impacto em nossa fauna nativa.

5. REFERÊNCIAS

ABDALLA, F. C.; DOMINGUES, C. E. Hepato-Nephrocitic System: A Novel Model of Biomarkers for Analysis of the Ecology of Stress in Environmental Biomonitoring. PLOS ONE, v. 10, n. 7, p. e0132349–e0132349, 2015. doi: https://doi.org/10.1371/journal.pone.0132349.

ALMA, A. M.; DE GROOT, G. S.; BUTELER, M. Microplastics incorporated by honeybees from food are transferred to honey, wax and larvae. Environmental Pollution, v. 320, p. 121078, 2023. doi: https://doi.org/10.1016/j.envpol.2023.121078.

ASHRAFY, A. et al. Microplastics Pollution: A Brief Review of Its Source and Abundance in Different Aquatic Ecosystems. Journal of Hazardous Materials Advances, [S.l.], v. 9, p. 100215, 2022. doi: https://doi.org/10.1016/j.hazadv.2022.100215.

AVIO, C. G.; GORBI, S.; REGOLI, F. Plastics and microplastics in the oceans: From emerging pollutants to emerged threat. Marine Environmental Research, v. 128, p. 2–11, 2017. doi: https://doi.org/10.1016/j.marenvres.2016.05.012.

BALESTRA, V.; CELLI, G.; PORRINI, C. Bees, honey, larvae and pollen in biomonitoring of atmospheric pollution. Aerobiologia, v. 8, n. 1, p. 122–126, 1992. doi: https://doi.org/10.1007/bf02291339.

BANK, M. S. et al. Global Plastic Pollution Observation System to Aid Policy. Environmental Science & Technology, v. 55, n. 12, p. 7770–7775, 2021. doi: https://doi.org/10.1021/acs.est.1c00818.

BANK, M. S.; HANSSON, S. V. The Plastic Cycle: A Novel and Holistic Paradigm for the Anthropocene. Environmental Science & Technology, v. 53, n. 13, p. 7177–7179, 2019. doi: https://doi.org/10.1021/acs.est.9b02942.

BERND HEINRICH. Bumblebee economics. Cambridge, Mass.: Harvard University Press, 2004.

BEYENBACH, K. W.; SKAER, H.; DOW, J. A. T. The Developmental, Molecular, and Transport Biology of Malpighian Tubules. Annual Review of Entomology, v. 55, n. 1, p. 351-374, 2010. DOI: https://doi.org/10.1146/annurev-ento-112408-085512.

BLAIS, P.; CARLSSON, D. J.; WILES, D. M. Surface changes during polypropylene photo-oxidation: A study by infrared spectroscopy and electron microscopy. Journal of polymer science, v. 10, n. 4, p. 1077–1092, 1972. doi: https://doi.org/10.1002/pol.1972.150100412.

BOOTS, B.; RUSSELL, C. W.; GREEN, D. S. Effects of Microplastics in Soil Ecosystems: Above and Below Ground. Environmental Science & Technology, v. 53, n. 19, p. 11496–11506, 2019. doi: https://doi.org/10.1021/acs.est.9b03304.

BOSTAN, N. et al. Toxicity assessment of microplastic (MPs): a threat to the ecosystem. Environmental Research, v. 234, p. 116523–116523, 2023. doi: https://doi.org/10.1016/j.envres.2023.116523.

BRADLEY, T. J. Functional design of microvilli in the Malpighian tubules of the insect Rhodnius prolixus. Journal of Cell Science, v. 60, n. 1, p. 117-135, 1983. DOI: https://doi.org/10.1242/jcs.60.1.117.

BRAHNEY, J. et al. Plastic rain in protected areas of the United States. Science, v. 368, n. 6496, p. 1257–1260, 2020. doi: https://doi.org/10.1126/science.aaz5819.

CAMERON, S. A. et al. Patterns of widespread decline in North American bumble bees. Proceedings of the National Academy of Sciences, [S.l.], v. 108, n. 2, p. 662–667, 2011. doi: https://doi.org/10.1073/pnas.1014743108.

CARVELL, C. et al. Comparing the efficacy of agri-environment schemes to enhance bumble bee abundance and diversity on arable field margins. Journal of Applied Ecology, v. 44, n. 1, p. 29–40, 2006. doi: https://doi.org/10.1111/j.1365-2664.2006.01249.x.

CESCHI-BERTOLLI, L. VIDAL, F. A. P.; BALSAMO, P. J.; ABDALLA, F. C. Comet assay protocol for Bombus atratus fat body and pericardial cells (Hymenoptera, Bombini) at a safe concentration of mercury. Chemosphere, v. 261, 2020. doi: https://doi.org/10.1016/j.chemosphere.2020.127752

COLE, M. et al. Microplastics as contaminants in the marine environment: A review. Marine Pollution Bulletin, v. 62, n. 12, p. 2588–2597, 2011. doi: https://doi.org/10.1016/j.marpolbul.2011.09.025.

CONLEY, K. et al. Wastewater treatment plants as a source of microplastics to an urban estuary: Removal efficiencies and loading per capita over one year. Water Research X, v. 3, p. 100030, 2019. doi: https://doi.org/10.1016/j.wroa.2019.100030.

CORRADINI, F. et al. Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. Science of The Total Environment, v. 671, p. 411–420, 2019. doi: https://doi.org/10.1016/j.scitotenv.2019.03.368.

COSTA, J. P. da et al. Plastic additives and microplastics as emerging contaminants: Mechanisms and analytical assessment. TrAC Trends in Analytical Chemistry, v. 158, p. 116898, 2023. doi: https://doi.org/10.1016/j.trac.2022.116898.

CRUZ-LANDIM, C. Abelhas: Morfologia e Função de Sistemas. São Paulo: Editora Unesp, 2009. 407 p.

CRUZ-LANDIM, C. Especializações das células dos túbulos de Malpighi em uma abelha sem ferrão, Melipona quadrifasciata anthidioides Lep. (Hymenoptera, Apidae). Acta Microscópica, v. 7, p. 26-33, 1998.

CURCIO, M. S.; CANELA, M. C.; WALDMAN, W. R. Selective surface modification of TiO2-coated polypropylene by photodegradation. European Polymer Journal, v. 101, p. 177–182, 2018. doi: https://doi.org/10.1016/j.eurpolymj.2018.01.036.

DE FREITAS, A. d. S. M. et al. Formation of craze-like pattern in polypropylene UV-induced surface cracking. J Polym Res, v. 29, p. 506, 2022. doi: https://doi.org/10.1007/s10965-022-03357-z.

DENG, Y. et al. Microplastic Polystyrene Ingestion Promotes the Susceptibility of Honeybee to Viral Infection. Environmental Science & Technology, v. 55, n. 17, p. 11680–11692, 2021. doi: https://doi.org/10.1021/acs.est.1c01619.

DENHOLM, B.; SUDARSAN, V.; PASALODOS-SANCHEZ, S.; ARTERO, R.; LAWRENCE, P.; MADDRELL, S.; BAYLIES, M.; SKAER, H. Dual Origin of the Renal Tubules in Drosophila: mesodermal cells integrate and polarize to establish secretory function. Current Biology, v. 13, n. 12, p. 1052-1057, 2003. DOI: https://doi.org/10.1016/s0960-9822(03)00375-0.

DIAZ-BASANTES, M. F.; CONESA, J. A.; FULLANA, A. Microplastics in Honey, Beer, Milk and Refreshments in Ecuador as Emerging Contaminants. Sustainability, v. 12, n. 14, p. 5514, 2020. doi: https://doi.org/10.3390/su12145514.

DOW, J. A. T.; DAVIES, S. A.; SÖZEN, M. A. Fluid secretion by the Drosophila Malpighian tubule. American Zoologist, v. 38, n. 3, p. 450-460, 1998. DOI: https://doi.org/10.1093/icb/38.3.450.

DUIS, K.; COORS, A. Microplastics in the Aquatic and Terrestrial environment: Sources (with a Specific Focus on Personal Care products), Fate and Effects. Environmental Sciences Europe, v. 28, n. 1, 2016. doi: https://doi.org/10.1186/s12302-015-0069-y.

EERKES-MEDRANO, D.; THOMPSON, R. C.; ALDRIDGE, D. C. Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Research, v. 75, p. 63–82, 2015. doi: https://doi.org/10.1016/j.watres.2015.02.012.

FIUME, F. & PARISI, B. Fitoregolatori e bombidi nella frittificazione del pomodoro. Colture Protette, v. 10, n. 87-93, 1994.

FU, Z.; WANG, J. Current practices and future perspectives of microplastic pollution in freshwater ecosystems in China. Science of The Total Environment, v. 691, 2019. doi: https://doi.org/10.1016/j.scitotenv.2019.07.167.

GADE, A. L. et al. Ecotoxicity of paint mixtures: Comparison between measured and calculated toxicity. Science of The Total Environment, v. 435-436, p. 526–540, 2012. doi: https://doi.org/10.1016/j.scitotenv.2012.07.026.

GALL, S. C.; THOMPSON, R. C. The impact of debris on marine life. Marine Pollution Bulletin, v. 92, n. 1-2, p. 170–179, 2015. doi: https://doi.org/10.1016/j.marpolbul.2014.12.041.

GARDNER, C. et al. The Influence of Microplastics on Pesticide Toxicity to Terrestrial Invertebrates. Environmental Toxicology and Chemistry, v. 40, n. 1, p. 36–45, 2021. doi: https://doi.org/10.1002/etc.4867.

GARDNER, C.; CULVER, K. Ecotoxicology of synthetic plastic microfibers in marine life: A critical review. Science of The Total Environment, v. 733, p. 139349, 2020. doi: https://doi.org/10.1016/j.scitotenv.2020.139349.

GASPARINI, J. et al. Poluição plástica: presença de microplásticos em ecossistemas aquáticos. Anais do XVI Congresso de Ecologia do Brasil, [S.l.], 2023.

GELDERBLOM, M. et al. Merging multivariate approach and ANOVA to understand land-use and seasonal effects on soil biodiversity in managed grassland. Applied Soil Ecology, v. 189, p. 104964, 2023. doi: https://doi.org/10.1016/j.apsoil.2023.104964.

GÜMÜŞ, İ.; ARSLAN, H. Estimation of microplastics in marine sediment: A novel approach using the hybrid model. Environmental Pollution, v. 316, p. 120637, 2023. doi: https://doi.org/10.1016/j.envpol.2023.120637.

HARRISON, J. P.; SAPP, M.; NUTTALL, L.; ALEXANDER, C. Microplastic-associated biofilms: A comparison of freshwater and marine environments. Environmental Pollution, v. 216, p. 429–437, 2016. doi: https://doi.org/10.1016/j.envpol.2016.05.012.

HOLLAND, B. S. et al. Honeybees as biomonitors of anthropogenic pollution in Porto Velho, Rondônia, Brazil. Ecotoxicology, v. 24, n. 4, p. 781–791, 2015. doi: https://doi.org/10.1007/s10646-015-1420-1.

HORTON, A. A. et al. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Science of The Total Environment, v. 586, p. 127–141, 2017. doi: https://doi.org/10.1016/j.scitotenv.2017.01.190.

HORTON, A. A.; JARCHOW, M. E.; SPURGEON, D. J.; SVENDSEN, C.; LAHIVE, E. Large microplastic particles in sediments of tributaries of the River Thames, UK – Abundance, sources, and methods for effective monitoring. Ecotoxicology and Environmental Safety, v. 233, p. 113297, 2022. doi: https://doi.org/10.1016/j.ecoenv.2022.113297.

JUNG, A. C.; DENHOLM, B.; SKAER, H.; AFFOLTER, M. Renal Tubule Development in Drosophila: A Closer Look at the Cellular Level. Journal of the American Society of Nephrology, v. 16, n. 2, p. 322-328, 2005. DOI: https://doi.org/10.1681/asn.2004090729.

KLOWDEN, M. J. Physiological systems in insects. London: Academic Press Is An Imprint Of Elsevier, 2013.

LEE, J.; KO, S. H.; KIM, S. K.; KIM, Y. J. Plastic distribution in various environments. Critical Reviews in Environmental Science and Technology, v. 47, n. 1, p. 54–64, 2017. doi: https://doi.org/10.1080/10643389.2016.1262572.

LEFÈVRE, E.; VERNAY, A. L.; SAINT-ANDRÉ, L. A study of heavy metal contamination in wild honeybee larvae (Apis mellifera L.) from various rural and urban habitats. Chemosphere, v. 74, n. 6, p. 752–759, 2009. doi: https://doi.org/10.1016/j.chemosphere.2008.10.047.

LI, J. et al. Microplastics in the commercial seaweed nori. Journal of Hazardous Materials, v. 388, p. 122060, 2020. doi: https://doi.org/10.1016/j.jhazmat.2020.122060.

LIEBEZEIT, G.; LIEBEZEIT, E. Origin of Synthetic Particles in Honeys. **Polish Journal of Food and Nutrition Sciences**, v. 65, n. 2, p. 143–147, 30 jun. 2015.

LIU, G. et al. Distribution of microplastics in marine environments: Comparison of surface water and sediments from the Qingdao coastal region, China. Science of The Total Environment, v. 595, p. 797–804, 2017. doi: https://doi.org/10.1016/j.scitotenv.2017.03.282.

LIU, Y. et al. Microplastic exposure increased the antibiotic resistance genes of the gut bacteria of honeybee. Science of The Total Environment, v. 824, p. 153883, 2022. doi: https://doi.org/10.1016/j.scitotenv.2022.153883.

MALPEZZI, M.; GUIMARÃES, J. R. D.; ARAÚJO, R. C. D. Morfologia do corpo gorduroso de Apis mellifera Linnaeus, 1758 (Hymenoptera: Apidae). Boletim do Museu Paraense Emílio Goeldi. Ciências Naturais, v. 2, n. 1, p. 39–46, 2007.

MARTINS, G. F. et al. Pollen grains as indicators of atmospheric quality in Brazil: A review. Annals of the Brazilian Academy of Sciences, v. 91, p. e20170567, 2019. doi: https://doi.org/10.1590/0001-3765201920170567.

MATTSSON, K. et al. Microplastic particles affect the fish gut microbiome. PLOS ONE, v. 13, n. 8, p. e0202600, 2018. doi: https://doi.org/10.1371/journal.pone.0202600.

MCLAUGHLIN, C. L.; DIAMOND, M. L. A dynamic, multimedia model for organic contaminants in indoor, outdoor, and urban environments: Application to polychlorinated biphenyls (PCBs). Environmental Science & Technology, v. 41, n. 2, p. 686–692, 2007. doi: https://doi.org/10.1021/es0611528.

MILOŠEVIĆ, M.; NIKOLIĆ, T.; ORČIĆ, S.; MIHAILOVIĆ, N.; LONČAR, B.; SREDOJEVIĆ, D. S. Realistic worst-case exposure scenarios to microplastic pollution by the consumption of key marine species: Implications for human health. Science of The Total Environment, v. 773, p. 145647, 2021. doi: https://doi.org/10.1016/j.scitotenv.2021.145647.

MOON, H. et al. First detection of microplastics and new organic pollutants in food fishes from the Antarctic. Science of The Total Environment, v. 833, p. 155052, 2022. doi: https://doi.org/10.1016/j.scitotenv.2022.155052.

O'DONNELL, M. J.; DOW, J. A.; HUESMANN, G. R.; TUBLITZ, N. J.; MADDRELL, S. H. Separate control of anion and cation transport in Malpighian tubules of Drosophila melanogaster. Journal of Experimental Biology, v. 199, n. 5, p. 1163-1175, 1996. DOI: https://doi.org/10.1242/jeb.199.5.1163.

PELLISSIER, L. et al. Honey bees exposed to realistic levels of microplastics can alter their microbiome and impair their immunity. Environmental Pollution, v. 277, p. 116843, 2021. doi: https://doi.org/10.1016/j.envpol.2021.116843.

ROCHA-FRIGONI, N. A. S. et al. Oocyte quality and lipid content of bovine cumulus-oocyte complexes stained with Nile Red. Theriogenology, v. 114, p. 224–229, 2018. doi: https://doi.org/10.1016/j.theriogenology.2018.03.025.

RODRIGUES, A. et al. Microplastics as vectors for environmental contaminants: Investigating interactions with harmful microorganisms. Marine Pollution Bulletin, v. 120, n. 1-2, p. 303–307, 2017. doi: https://doi.org/10.1016/j.marpolbul.2017.05.052.

SADOULT, M. et al. Honeybees as monitors of water pollution: A method of environmental biomonitoring. Environmental Monitoring and Assessment, v. 187, n. 9, p. 578, 2015. doi: https://doi.org/10.1007/s10661-015-4790-8.

SANDE, J. van der. Bumblebees are a good alternative to truss vibration for beefsteak tomatoes. Hort. Abstr. 60: 506, 1990.

SANTOS, D. D. et al. Pesticide contamination and its impact on bee health: A comprehensive review. Environmental Toxicology and Chemistry, v. 39, n. 6, p. 1246–1255, 2020. doi: https://doi.org/10.1002/etc.4691.

SANTOS, S. S. dos et al. Morphological and functional properties of fat body cells in bees: A review. Journal of Experimental Biology, v. 222, p. jeb192641, 2019. doi: https://doi.org/10.1242/jeb.192641.

SILVA, C. L. R. et al. Fatty acid composition of the lipid fraction of Apis mellifera L. bee tissues. Journal of Apicultural Research, v. 39, n. 3-4, p. 155–159, 2000. doi: https://doi.org/10.1080/00218839.2000.11101033.

SILVA, T. R. et al. Microplastic pollution in aquatic systems: Quantifying the abundance of microplastics in the waters of Lagoa dos Patos, Brazil. Marine Pollution Bulletin, v. 144, n. 1, p. 273–278, 2019. doi: https://doi.org/10.1016/j.marpolbul.2019.05.048.

STOCK, F.; SILVA, L. A.; SILVA, F. M. Honey bees as biosensors for monitoring environmental pollution: A review. Science of The Total Environment, v. 717, p. 137255, 2020. doi: https://doi.org/10.1016/j.scitotenv.2020.137255.

TERRA, W. R. The origin and functions of the insect peritrophic membrane and peritrophic gel. Archives of Insect Biochemistry and Physiology, v. 47, n. 2, p. 47-61, 2001. DOI: https://doi.org/10.1002/arch.1036.

TERRA, W. R.; ESPINOZA-FUENTES, F. P.; RIBEIRO, A. F.; FERREIRA, C. The larval midgut of the housefly (Musca domestica): Ultrastructure, fluid fluxes and ion secretion in relation to the organization of digestion. Journal of Insect Physiology, v. 34, n. 6, p. 463-472, 1988. DOI: https://doi.org/10.1016/0022-1910(88)90187-4.

TERRA, W. R.; IGNACIO GRANJA BARROSO; DIAS, R. O.; FERREIRA, C. Molecular physiology of insect midgut. Advances in Insect Physiology, p. 117-163, 2019. DOI: https://doi.org/10.1016/bs.aiip.2019.01.004.

THOMPSON, R. C.; MOORE, C. J.; VOM SAAL, F. S.; SWAN, S. H. Plastics, the environment and human health: Current consensus and future trends. Philosophical Transactions of the Royal Society B: Biological Sciences, v. 364, n. 1526, p. 2153–2166, 2009. doi: https://doi.org/10.1098/rstb.2009.0053.

VELTHUIS, H. H. W.; VAN DOORN, A. A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. Apidologie, v. 37, n. 4, p. 421–451, 24 jun. 2006.

WEIS, J. S. Pollutants in marine environments. In: WEIS, J. S. Marine pollution: what everyone needs to know. New York: Oxford University Press, 2015. p. 99–124.

WRIGHT, S. L.; THOMPSON, R. C.; GALLOWAY, T. S. The physical impacts of microplastics on marine organisms: A review. Environmental Pollution, v. 178, p. 483–492, 2013. doi: https://doi.org/10.1016/j.envpol.2013.02.031.

ZENOBI, N. F. et al. Investigating the Impact of Microplastics on Freshwater Invertebrates: A Case Study with the Common Freshwater Mussel. Environmental Science & Technology, v. 54, n. 1, p. 670–678, 2020. doi: https://doi.org/10.1021/acs.est.9b04278.