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Bioremediation of Trifluralin via macroplastics in freshwater ecosystems compared to soil ecosystems

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BIOREMEDIATION OF TRIFLURALIN VIA MACROPLASTICS IN FRESHWATER ECOSYSTEMS COMPARED TO SOIL ECOSYSTEMS

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Yr wyf drwy hyn yn datgan mai canlyniad fy ymchwil fy hun yw'r thesis hwn, ac eithrio lle nodir yn wahanol. Caiff ffynonellau eraill eu cydnabod gan droednodiadau yn rhoi cyfeiriadau eglur. Nid yw sylwedd y gwaith hwn wedi cael ei dderbyn o'r blaen ar gyfer unrhyw radd, ac nid yw'n cael ei gyflwyno ar yr un pryd mewn ymgeisiaeth am unrhyw radd oni bai ei fod, fel y cytunwyd gan y Brifysgol, am gymwysterau deuol cymeradwy.

ABSTRACT

Bioremediation is the process of environmental detoxification of toxic contaminants. Plastic is capable of the sorption of toxic chemicals and microplastics are capable of adsorbing to the surfaces of macrophytes within the water column. There is a lack of mitigation studies focusing on microplastics and sorbed contaminants within a freshwater context. Here, the aim was to quantify whether there is potential for bioremediation technologies to be implemented using *Lemna minor* and the plastic mulch films LDPE and PLA-PBAT to degrade the herbicide trifluralin. ^{14}C-labelled trifluralin solutions of varying concentrations (1, 10 and 100%) were created and applied to LDPE and PLA-PBAT plastic mulch films to calculate sorption potential. Bioremediation of ^{14}C-trifluralin at these concentrations were tested via LDPE and PLA-PBAT within soil and within freshwater (100% concentration only) using *Lemna minor* and river sediment. Measurements were undertaken using 1 M NaOH traps, capturing $^{14}CO_2$ and a Wallac 1404 liquid scintillation counter to calculate the amount of $^{14}CO_2$ collected. A key result of this study was that *L. minor*, freshwater, river sediment and trifluralin via PLA-PBAT have equal bioremediation potential compared to bioremediation via PLA-PBAT within soil. Interestingly, PLA-PBAT was the most functional film for potential bioremediation applications due to its higher adsorption of trifluralin, however, LDPE was promising also. Additionally, a 1% trifluralin concentration sorbed best for both plastic film types. PLA-PBAT and *L. minor* within a water matrix have equal bioremediation potential comparable to PLA-PBAT within soil supporting their possible application for environmental mitigation technologies within a freshwater context.

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CHAPTER 1: INTRODUCTION

1.1. PLASTICS AND PESTICIDES IN THE WORLD

Plastics, their additives and pesticides are toxic to environments globally. The Stockholm Convention on Persistent Organic Pollutants (POPs) (2001) aimed to eliminate or restrict the production and usage of POPs, which included plastic additives and pesticides. The Stockholm Convention was formally annexed to EU legislation in EC Regulation No 850/2004. Plastics and pesticides are commonly detected in effluents, surface water and tap water, regardless of treatment in urban wastewater treatment plants (Sousa *et al.* 2018).

1.2. PLASTICS

Globally, the dominant plastic types consist of: thermoplastic types of polypropylene (PP) (21%), low and linear low-density polyethylene (LDPE and LLDPE) (18%), polyvinyl chloride (PVC) (17%), and high-density polyethylene (HDPE) (15%) (Hahladakis *et al.* 2018). Other high demand plastics include: polystyrene (PS) and expandable PS (8%) polyethylene terephthalate (PET) (7% excluding PET fibre) and the thermosetting plastic polyurethane (Hahladakis *et al.* 2018). Fragmentation of plastic via weathering by sunlight, rain, wind and ocean waves (Bhattacharya *et al.* 2010) results in the creation of secondary microplastics. Primary microplastics are classed as being <1 mm in diameter (Kalčíková. 2020). Unfortunately, plastic waste is exported internationally by highly economically developed nations to less economically developed countries. In 2014/2015 46% wt. of plastics collected for recycling in Europe were ultimately exported abroad, with 90% wt. ending up in China (Hahladakis *et al.* 2018).

A study conducted by Song *et al.* (2015) focused on the need for a suitable microplastic identification method to be created. Prior to 2015, and this study, no comparison of microplastic identification methods had been made (Song *et al.* 2015). To date, the analysis of microplastics within environmental samples lacks standardised protocols (Mateos-Cardenas *et al.* 2019; Song *et al.* 2015) for variables such as size range, solid state, shape, colour,

origin, and chemical composition (Mateos-Cardenas *et al.* 2019). Non-plastics that resemble plastics and plastics that resemble non-plastics can make it difficult to correctly identify microplastics (Song *et al.* 2015).

The Identification of microplastics using three methods was investigated by Song *et al.* (2015): Naked eye and /or microscope (1); microscope (and the naked eye), and by a spectroscopic method using a Fourier transform infrared spectroscope (FT-IR) (2); spectroscopic method using an FT-IR or a Raman spectroscope (3). Microplastics were compared according to type (fragment, fibre, sheet and expanded polystyrene (EPS)) and size.

It was concluded that microplastics of >1 mm are suitable for microscope identification (method 1). Method 2, using a spectroscopic method was more accurate than the microscope, small microplastics (<1 mm) were detected by FT-IR, including those <50 µm. FT-IR advantageously can distinguish the polymer type and can provide information that helps to identify the origin of plastic and any further behaviours that may occur in the environment, however a downside to FT-IR is that it is expensive and time consuming. Raman spectroscopy can detect microplastics down to 1–2 µm in size. It was recommended by Song *et al.* (2015) to use the spectroscopic method to identify small microplastics (<1 mm) and if few samples are being assessed use a mixture of FT-IR or Raman methods. If many samples are being assessed, then it is advisable to use a combination of both microscope and spectroscopic methods. A screening analysis using FT-IR or Raman should be conducted if using a combination of methods, to identify sample groups, according to matrix, season, and location.

Plastic debris is continuously observed in the open ocean, the benthos of the deep sea, shorelines and within organisms, posing health risks. Some sources of marine plastic waste derive from: littering in coastal areas, plastics blown from open dumpsites, leached sewage effluents and spillage during transport. Within the open ocean, plastic waste can be transported by oceanic gyres or accumulate in the centre of gyres (Hahladakis *et al.* 2018).

The bioavailability of plastic in the aquatic environment increases with a decrease in size (Song *et al.* 2015), ultimately resulting in ingestion of macroplastic, microplastic and nanoplastics in organisms. Plastic can be mistaken as food by biota such as birds and fish (Teuten *et al.* 2007). Ingestion can negatively affect oxygen input, growth, development, feeding and behavioural patterns and can cause death (Kalčíková. 2020).

1.2.1. Pesticides

Pesticides such as insecticides and herbicides are toxic contaminants. These agrochemicals can increase the likelihood of cancer, genetic mutations (increases hybridisation rate and decreases genetic diversity), affects development, physiology, behaviour, reproduction, and causes diseases affecting the liver and the central nervous system (de Oliveira *et al.* 2020; Sousa *et al.* 2018). The EU lists many pesticides as priority substances (PSs) to be monitored from discharges (EU Directive EC/76/464). Some (PSs) are classified as potentially carcinogenic to humans (Kapsi *et al.* 2019). 91 pesticides are listed as a confirmed or possible endocrine disruptors by the UK Environment Agency, The German Environment Agency, The European Union Community Strategy for Endocrine Disruptors, the Oslo and Paris Commission and the World Wildlife Fund (Kapsi *et al.* 2019).

1.3. PLASTICS AND PESTICIDES IN FRESHWATER COMPARTMENTS

The accumulation of POPs in freshwater compartments is paramount. The distribution of micropollutants within the freshwater aquatic environment can vary due to inputs depending on seasonality such as weather conditions, temperature, and consumption trends as well as water flow and biodegradation and photodegradation rates (Sousa *et al.* 2018). Between 1970 and 2014 there was an 83% decline in freshwater organism populations, significantly higher compared to declines in terrestrial and marine systems (Reid *et al.* 2019). Coastal and estuarine waters are the most vulnerable environments to discharges of micropollutants (Sousa *et al.* 2018).

Studies on the effects of microplastics in freshwater ecosystems are increasingly fewer compared to those in a marine context, particularly in the case of aquatic plants. There are less studies supporting the mitigation of microplastics compared to that of pharmaceuticals and personal-care products in freshwater ecosystems (Reid *et al.* 2019). Most plastic is less dense than water and floats at the surface microlayer in freshwater ecosystems and microplastics are mistaken for food by animals such as birds and fish (Teuten *et al.* 2007). Micropollutants also change seasonally, with pesticides being at greater concentrations during the growing season in freshwater compartments due to runoff, however micropollutants can be released from other freshwater compartments at any time.

Kapsi *et al.* (2019) assessed the environmental risk of pesticide residues in the Louros River to aquatic organisms such as algae, zooplankton and fish. Risk quotients were calculated. Algae had low risk ($0.01<RQ<0.1$) except for acetochlor, that had medium risk ($0.1<RQ<1$). Zooplankton had low risk except for pirimiphos-methyl, endosulfan-a and azinphos-ethyl. Fish had a medium risk from endosulfan-a, other compounds showed no risk.

1.3.1. Legislation

EU legislation for freshwater water quality includes The EU Water Framework Directive (2000/60/EC), that was established to identify priority substances (PSs), that pose a significant risk to the aquatic environment and to set EU Environmental Quality Standards that measured the concentration of a pollutant in water, sediment or biota which should not be exceeded (Sousa *et al*. 2018). The Decision 2455/2001/EC in 2001 established the first list of 33 PSs and the first list of EU Environmental Quality Standards for the 33 priority substances (PSs) (Sousa *et al*. 2018). A third of the list of these chemicals consisted of pesticides (Kapsi *et al.* 2019). Plastic additives and pesticides are included as PSs such as di-(2-ethylhexyl) phthalate (DEHP), hexabromocyclododecanes, trifluralin and atrazine (Sousa *et al*. 2018).

The EU REACH Regulation (2007) protects water quality regulation, concerning the registration, evaluation, authorisation and the restriction of chemicals (Kapsi *et al.* 2019).

The EU Directive 2008/105/EC amended the EU Water Framework Directive (2000/60/EC) by adding eight other certain pollutants (Kapsi *et al.* 2019). Directive 2013/39/EU recommended monitoring 45 PSs and proposed a Watch List of substances for EU monitoring, published in Decision 2015/495/EU (Sousa *et al.* 2018). The Watch List consisted of 17 organic compounds that were classed as Contaminants of Emerging Concern (CEC) (Sousa *et al.* 2018). Environmental Quality Standards are not present for CECs, rather, two indicators are used based on maximum concentrations found (Sousa *et al.* 2018). The literature survey from Sousa *et al.* (2018) concerning monitoring programs of organic pollutants published between 2012-2017 shows that the area of research involving monitoring of PSs and CECs in surface and ground waters has been increasing. Publications related to the pesticides listed in Decision 2015/495/EU Watch List was increasing prior to 2018 (Sousa *et al.* 2018).

1.4. PLASTIC CHEMISTRY AND ADDITIVES

1.4.1. Plastic chemistry

Plastics are xenobiotic contaminants that consist of monomers that form via polymerisation (Fred-Ahmadu *et al.* 2020). A polymers molecular chain arrangement can be defined as linear, cross-linked, network or branched (Fred-Ahmadu *et al.* 2020). These polymers contain plastic additives by addition or condensation reactions (Lithner *et al.* 2011). However, many of these additives are not chemically bound to plastic, rather, by reactive organic additives (Hahladakis *et al.* 2018).

1.4.2. Plastic additives

Plastic additives are chemical compounds used in all plastic products to enhance properties and prolong their life (Hahladakis *et al.* 2018; Turner & Filella 2021) or remain as residues or contaminants derived from the manufacturing process (Turner & Filella 2021). Common additives include: plasticisers (improves flexibility and reduces melt flow), colourants, flame retardants (prevents ignition) and fillers and mineral reinforcements (increases surface

hardness and bulk). Furthermore, fibre properties improves mechanical strength, heat resistance avoids oxidation, UV resistance avoids oxidation under sunlight and anti-static and conductive properties enhances electrical conductivity and prevents electrostatic discharge (Fred-Ahmadu *et al.* 2020; Hahladakis *et al.* 2018; Turner & Filella. 2021).

1.4.3. Plasticisers

Plasticisers are used for improving flexibility and stretchability (Wagner & Schlummer 2020; Hahladakis *et al.* 2018). Flexibility is present due to the increased distance between polymer chains due to the addition of plasticisers, resulting in dipole – dipole interactions weakening, allowing chains to slide against each other, as illustrated in figure 1.3.3. (a). Dipolar charges are equal and opposite electrical charges (Wagner & Schlummer 2020).

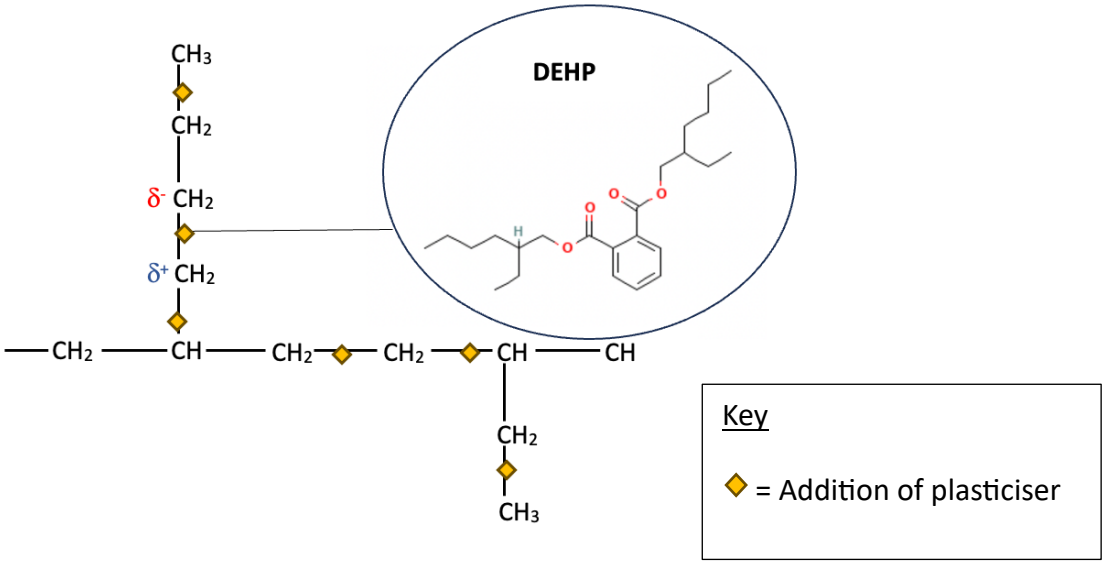
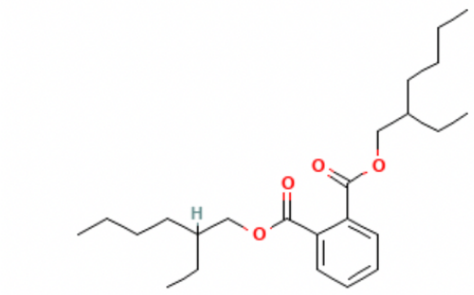


Fig. 1.4.3. (a) Addition of the phthalate di-(2-ethylhexyl) phthalate (DEHP) to the polymer LDPE (low density polyethylene) increasing flexibility via the weakening of dipole – dipole interactions.

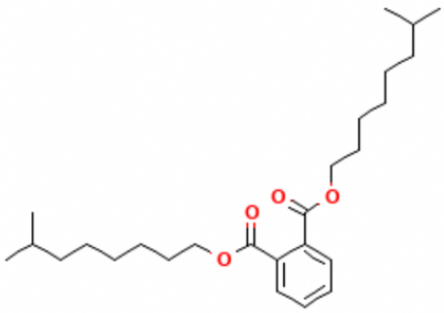
Phthalates accounted for > 70% global plasticiser production in 2012. This is due to their low cost of achieving the broadest range of processing and performance requirements (Bamai & Yu 2020). These additives are phenolic acid esters (Fred-Ahmadu *et al*. 2020) and are particularly applied to PVC (polyvinyl chloride) (Bamai & Yu 2020; Fred-Ahmadu *et al.*

2020). Phthalate esters are classified into low or high molecular weight phthalates depending on their carbon chain length (Bamai & Yu 2020), as illustrated in figure 1.3.3. (b). High molecular weight phthalates include: di-(2-ethylhexyl) phthalate (DEHP), di-isononyl phthalate (DiNP), di-iso-decyl phthalate (DiDP), and di-n-octyl phthalate (DNOP). Examples of low molecular weight phthalates include: di-iso-butyl phthalate (DiBP), di-n-butyl phthalate (DnBP), dimethyl phthalate (DMP), diethyl phthalate (DEP). Four phthalates DEHP, BBP (Benzyl butyl phthalate), DBP (Dibutyl phthalate), DiBP are on the candidate list of SVHCs in Annex XIV of the EU REACH Regulation (Wagner & Schlummer 2020).

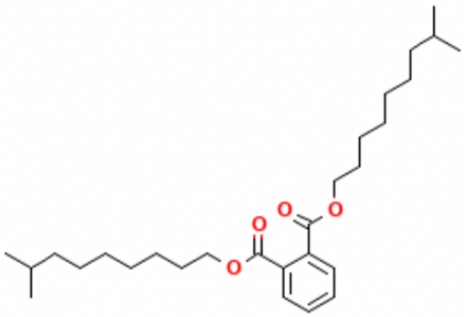
High molecular weighted phthalates



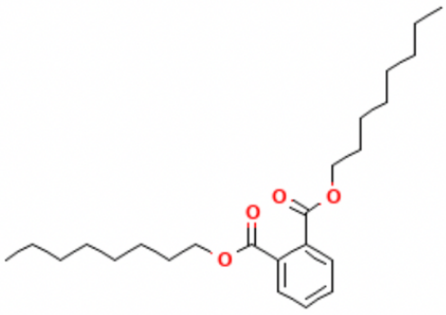
Di – (2-ethylhexyl) phthalate (DEHP)
$C_{24}H_{38}O_4$



Di - isononyl phthalate (DiNP)
$C_{26}H_{42}O_4$



Di-iso-decyl phthalate (DiDP)
$C_{28}H_{46}O_4$



Di-n-octyl phthalate (DNOP)
$C_{24}H_{38}O_4$

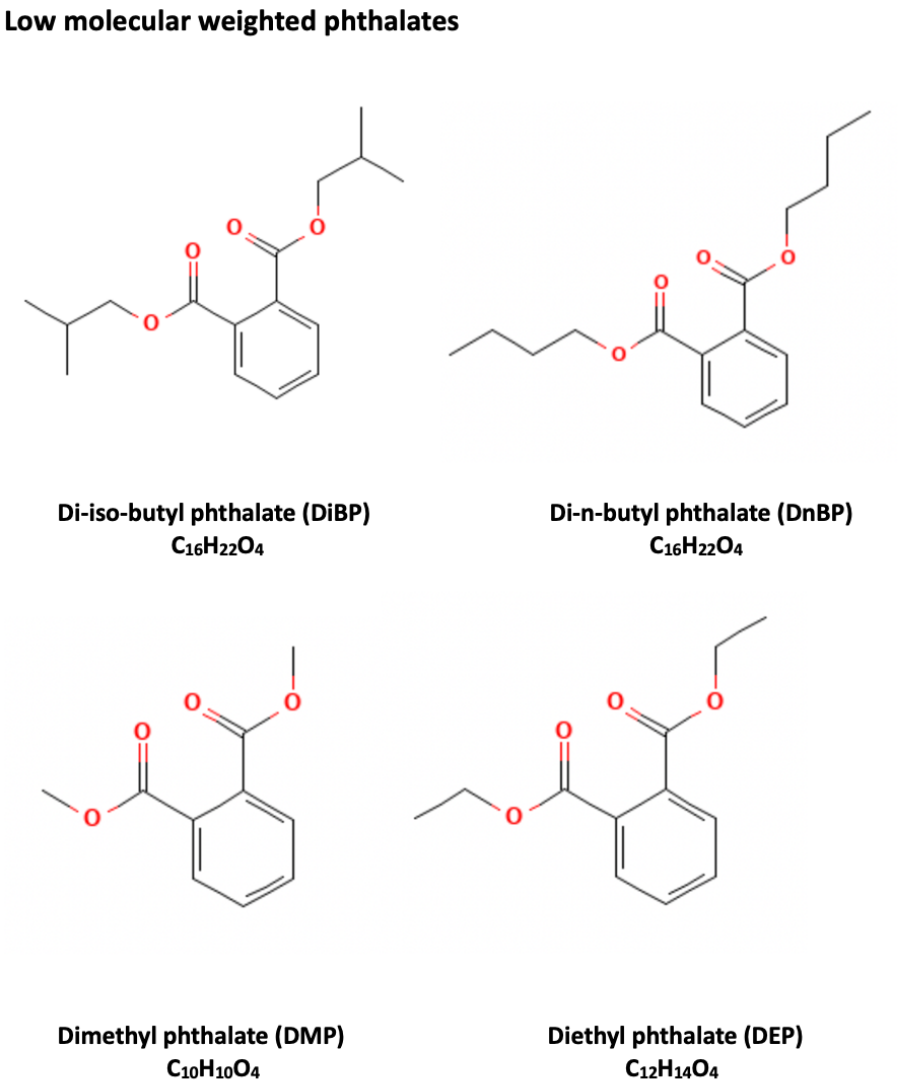


Fig. 1.4.3. (b) Examples of phthalate ester structures grouped by high and low molecular weight.

1.4.4. Environmental toxicity

Additives can contaminate the environment via leaching, abrasion, and degradation, ultimately entering the food chain (Wagner & Schlummer 2020) through soil, air, water and food (Hahladakis *et al.* 2018). Migration from food packaging can cause human exposure and leaching can derive from recycling and recovery processes and from the products produced from recyclates. Organisms with longer gut retention times may be more affected by the

leaching of additives such as fish (Hahladakis *et al.* 2018). EU legislation, Directive 2013/39/EU and Decision 2015/495/EU, list some phthalates, as priority substances (PSs) in the area of water policy, due to their high frequency of detection in the environment, persistence and bioaccumulation ability (Sousa *et al.* 2018). DEHP is classified as one of the most severe environmental contaminants (Sousa *et al.* 2018). DnBP, DEHP and BBP cause endocrine disruption, they are prohibited in toys and childcare items. Regulation of these chemicals in high fat food content materials occurs in the EU, USA, Canada and Japan due to migration (Bamai & Yu 2020; Fred-Ahmadu *et al.* 2020). Experimental studies have shown phthalates to be toxic in terms of renal, reproductive, cardio and neurotoxicity in organisms (Fred-Ahmadu *et al.* 2020). Flame retardants such as brominated diphenyl ethers are classified as PSs, that accumulate in the aquatic environment, and have been found in shellfish, birds and marine mammals (Sousa *et al.* 2018).

1.4.5. End of life options and legacy additives

In the past, many additives used in plastics were based on compounds of toxic metals and metalloids such as arsenic, cadmium, chromium (VI) and lead. There have been restrictions on these due to concerns over human and environmental health, and organic compounds or non-metal-based alternatives have been used instead, however they are still present in the environment due to their pervasiveness and are cause for concern (Turner & Filella 2021). These plastic additives are referred to as ‘legacy additives’ and some are classified as substances of very high concern (SVHC) or POPs and improper disposal, recycling and reuse of legacy additives results in constant circulation of these toxic additives within the environment (Wagner & Schlummer 2020). End of life options for legacy additives include incineration (energy recovery), landfill and recycling. Export of plastic waste occurs between countries, generally from high economically developed countries to low, legally and illegally, it is illegal to export legacy additives. This is not an end-of-life option, however legacy additives are illegally transported to low-income nations through insufficient controls and thus enter the environment far from the point of original use (Wagner & Schlummer 2020).

1.5. TRIFLURALIN BIOCHEMISTRY

1.5.1. Overview

An example of a toxic organohalogen pesticide is trifluralin ($C_{13}H_{16}F_3U_3O_4$), a herbicide in the chemical group dinitroaniline and is a yellow-orange crystalline solid that can be available as an emulsifiable concentrate (Fernandes *et al.* 2013; PubChem 2023). It is a substituted aniline with nitro groups at positions 2 and 6 or 3 and 5 of the benzene ring and a trifluoromethyl group at position 4 (Fernandes *et al.* 2013; PubChem 2023) as shown in figure 1. Trifluralin is a C-nitro compound and a member of the trifluoromethyl benzenes (PubChem 2023). Substituents are added to the benzene ring and the amine group of dinitroanilines (Coleman *et al.* 2020) to improve functionality, enhancing trifluralin's use as a herbicide. In the case of trifluralin, nitro groups were added to the benzene ring. Trifluralin is a largely insoluble compound in water, 0.22 mg/L at 20 °C (Coleman *et al.* 2020) and notably volatile (vapour pressure 6.7 mPa at 20 °C (Coleman *et al.* 2020), 1.1 10^{-4} mm Hg pressure vapour at 25 °C (Fernandes *et al.* 2013)) as well as an organohalogen (PubChem 2023; Tubić *et al*. 2021). The agrochemical is a selective pre-emergence herbicide (Coleman *et al.* 2020; PubChem 2023), selective herbicide's only target certain plant species (Fernandes *et al.* 2013). Moreover, trifluralin has been used in agriculture since 1963 (Coleman *et al.* 2020; Fernandes *et al.* 2013) and is primarily used in the production of tomato, alfalfa, cotton, and soybean crops (Coleman *et al.* 2020; de Oliveira *et al.* 2020). The herbicides mode of action is the inhibition of microtubule assembly in cell mitosis (Anthony and Hussey 1999; Callahan *et al.* 1996; Coleman *et al.* 2020; Fernandes *et al.* 2013; de Oliveira *et al.* 2020).

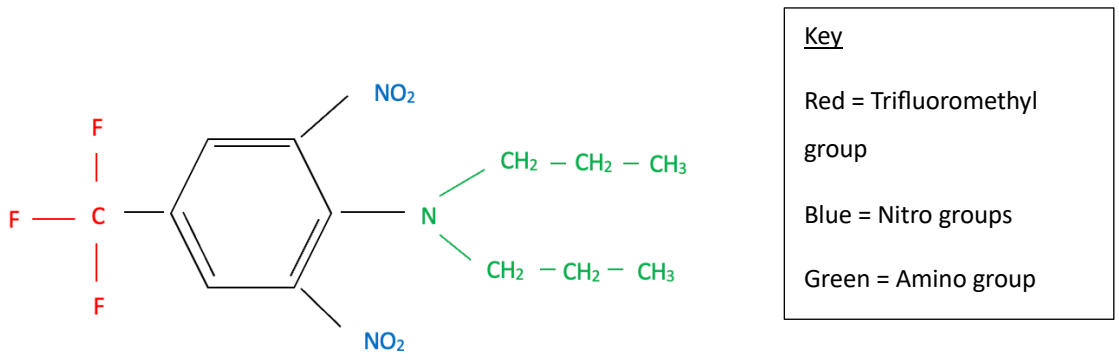


Fig. 1.5.1. Trifluralin ($C_{13}H_{16}F_3U_3O_4$) chemical structure.

1.5.2. Legislation

Within the European Union (EU), trifluralin is a priority substance (PS) as it is an organohalogen (Tubić *et al*. 2021). Organohalogens have high toxicity at low concentrations, persist in the environment and bioaccumulate in organisms (Tubić *et al*. 2021) and causes long-term environmental pollution (120-240 days to degrade (Fernandes *et al.* 2013)). EU Directive 2013/39/EU and the Watch List of Decision 2015/495/EU requires PSs and contaminants of emerging concern (CECs) to be monitored in surface water (Sousa *et al.* 2018; Tubić *et al*. 2021). Trifluralin has been banned in the EU since 2008 due to concerns over its toxicity (Coleman et al. 2020). The American Environmental Protection Agency (EPA) classes trifluralin as a possible human carcinogen (Fernandes *et al.* 2013; PubChem. 2023).

1.5.3. Persistence of trifluralin in the environment

Organohalogens, including trifluralin decompose extremely slowly (residues of trifluralin have been observed in soil for more than 1 year in multiple studies (Coleman *et al*. 2020)), and can induce high toxicity at low concentrations. Further, its long-term persistence and transport mean that it causes long-term pollution (PubChem 2023). Trifluralin has been observed in soil and water months after application (de Oliveira *et al.* 2020). The agrochemical is lipophilic and prone to bioaccumulation (de Oliveira *et al.* 2020) and high affinity to soil results in leaching into surface water (Fernandes *et al.* 2013). However, the toxicity mechanisms of trifluralin are not completely described (de Oliveira *et al.* 2020).

A study by Laabs *et al*. (2002) in Brazil, found significant amounts of trifluralin in rainwater (0.31 μg/L) (de Oliveira *et al.* 2020). Kapsi *et al.* (2019) quantified pesticides in the Louros River (Greece). One of the most common pesticides observed was trifluralin: minimum concentration 0.082 μg/L and maximum concentration 0.084 μg/L. Sousa *et al*. (2018) focused on reviewing the occurrence of priority substances and contaminants of emerging concern (EU guidelines) in freshwaters worldwide. Trifluralin was one of the priority

substances observed at the highest concentrations in freshwater, at least once at a concentration higher than 0.5 μg/L (Tubič *et al*. 2021).

1.5.4. Effect of trifluralin on biota

The EPA has determined that dogs exposed to chronic trifluralin levels within their diet ultimately resulted in weight loss and negative effects on the blood and liver (PubChem 2023). Furthermore, offspring of rodents exposed to gavage of trifluralin has skeletal abnormalities and depressed foetal weight (PubChem 2023). In a study by Weichenthal *et al*. (2010), colon cancer in farmers was found to have been derived from trifluralin exposure (de Oliveira *et al.* 2020). Trifluralin has also been shown to disrupt the endocrine system in humans (Coleman *et al*. 2020; Orton *et al.* 2009).

A study by de Oliveira *et al*. (2020) investigated whether trifluralin and tebuthiuron (concentrations ranging from 1-100 μM) affect isolated rat liver mitochondria. Trifluralin induced mitochondrial swelling in this research. Mitochondrial permeability transition (MPT) causes the organelle to swell and can result in the breach of the outer membrane and release of apoptogenic factors. Trifluralin was also shown to cause cell death. The agrochemical induced the opening of the permeability transition pore (PTP) and altered ATP levels which are both related to cell death. The herbicide alters mitochondrial respiration particularly at the highest concentration; however it was not shown to induce oxidative stress. This research used concentrations that were high compared to those generally found in the field, however bioaccumulation and biomagnification could result in higher concentrations (de Oliveira *et al*. 2020).

Aquatic organisms are susceptible to trifluralin toxicity, notably fish: lethal dose for fish can be as low as 50 μg/L (Coleman *et al*. 2020; de Oliveira *et al.* 2020). A study by Poleksić and Karan (1999) tested the effects of acute and subacute toxicity of trifluralin on carp. A decrease in relative growth rate in relation to an increase in trifluralin concentration was observed. The kidneys of the carp had degenerative and vascular changes. Chrondocytes in

the gill filament cartilage had degenerated in the study at the highest concentration of trifluralin.

1.5.5. Mechanism to which trifluralin inhibits plants

The mode of action of trifluralin is to inhibit microtubule polymerisation (Fernandes *et al.* 2013). This results in physical misconfiguration and loss of function, the mitotic spindle does not form, inhibition can cause misalignment and chromosome separation during mitosis and the spindle apparatus does not form (Senseman 2007). Microtubule inhibition impacts cellular function: microtubules are involved in multiple cellular processes such as cellular structure maintenance, chromosome migration, cell wall formation, intracellular formation and cellulose microfibril orientation (Fernandes *et al.* 2013). Tubulin has been shown to be the primary subcellular target for dinitroaniline action (Anthony and Hussey 1999).

1.5.6. Biotic and abiotic degradation of trifluralin

Microbial biodegradation occurs via cometabolic reactions. Catabolic reactions rarely occur in trifluralin biodegradation as its structure represents a poor substrate for naturally occurring enzymes, due to the presence of a trifluoromethyl group and low aqueous solubility (Coleman *et al*. 2020). Aniline: 2, 6 dinitroaniline, a by-product of trifluralin degradation, is toxic to the kidneys and liver (Fernandes *et al.* 2013). Photodecomposition and chemical reduction are the major abiotic degradation pathways of trifluralin (Coleman *et al*. 2020).

1.6. PLASTIC SORBING AND DESORBING CHEMICALS

Plastic expresses sorption and desorption behaviour of toxic chemicals within a water matrix. Such chemicals can include pesticides leached from agricultural land such as trifluralin. Microplastics are more likely to absorb and desorb toxic chemicals, as they have a large surface area to volume ratio (Song *et al.* 2015). Compared to macroplastics, microplastics will be more abundant in the environment and therefore will be the majority in ‘clean-up’

strategies. Plastics can also leach their additives as well as sorb them. The leaching of these additives is dependent on pore size, the size of the additive molecule and the physio-chemical properties of the surrounding media (Fred-Ahmadu *et al.* 2020; Teuten *et al.* 2009). Sorption is also contaminant specific (Fred-Ahmadu *et al.* 2020). Biofilm formation on microplastics via biofouling (biomass accumulation e.g. via microbes and phytoplankton) decreases the hydrophobicity of microplastics, increasing sorption potential for hydrophilic contaminants (Rai *et al.* 2021; Fred-Ahmadu *et al.* 2020). Within the literature it is stated that organic pollutants (e.g. polycyclic aromatic hydrocarbons or organochlorines such as DDT), polychlorinated biphenyls, antibiotics, herbicides, pesticides, and trace metals can absorb to plastic surfaces (Leifheit *et al*. 2021). For example trifluralin is hydrophobic and binds to glass and plastic (Anthony & Hussey 1999).

1.6.1. Polymer type

The sorption potential of microplastics to toxic chemicals differs depending on polymer type (Song *et al.* 2015). Surface charge, surface area, molecular chain formation and the acid-base character influences sorption (Fred-Ahmadu *et al.* 2020).

1.6.2. Crystallinity

Crystallinity phase affects the sorption potential of microplastics to toxic chemicals (Fred-Ahmadu *et al.* 2020; Tubić *et al*. 2021). A polymer will be designated as one of three phases depending on the alignment of their molecular chains: crystalline, semi-crystalline and amorphous. The extent of crystallinity of the polymer is given as a mass fraction or volume fraction. The range is from a few percentage points to around 90% crystallinity. Crystallisation in polymers is signified by straight chains with regularly spaced side groups, whereas amorphous polymers do not form a rigid arrangement within the polymer chain. Semi-crystalline and amorphous phases are suitable for sorption. The crystalline area within a polymer is not suitable for sorption.

1.6.3. pH

Liu *et al.* (2019) found no significant effect of pH on the sorption of phthalate esters to polyethylene, polystyrene and polyvinyl chloride (PVC) due to high electrostatic repulsion. However, as pH increased hydrophobic interactions reduced between particles (Fred-Ahmadu *et al.* 2020; Liu *et al.* 2019). pH increase may cause the dissociation of hydrophobic neutral sorbate molecules into hydrophilic negatively charged species, resulting in reduced hydrophobic interaction (Liu *et al.* 2019).

1.6.4. Salinity and ionic strength

Salinity is not thought to greatly affect the sorption process by microplastics (Fred-Ahmadu *et al.* 2020). Freshwater bodies naturally have dissolved salts within them, contributing to their ionic strength. The overall ion concentration in a solution is equal to the ionic strength. Absorption of contaminants is affected by the competition for binding sites on plastic between ions in solution and contaminants (Fred-Ahmadu *et al.* 2020).

1.6.5. Age/weathering

Pristine or virgin plastics are polymers that have not undergone physiochemical changes to their surface from thermal, mechanical, biological, radiative, or oxidative pressures (Fred-Ahmadu *et al.* 2020). Furthermore, biofilm formation around microplastics is aided by the ageing of microplastic (Rai *et al.* 2021). A study by Tubić *et al.* (2021) deduced that polyethylene derived from two personal care products showed greater adsorption of chlorobenzenes and trifluralin compared to virgin polyethylene, demonstrating that physiochemical changes can increase binding sites for potential sorbed contaminants.

1.6.6. Chemical properties of contaminant

Multiple factors affect plastic sorption of contaminants that differ depending on a compound's physiochemical properties and environment and cannot be generalised (Tubić *et al*. 2021). The hydrophobicity of a compound will equate to the chemicals sorption affinity to plastic. A compound that has high hydrophobicity will adsorb greater to plastic (Wu *et al*. 2016).

A study by Tubić *et al*. (2021) found trifluralin to adsorb the most on polyethylene, in comparison to five chlorobenzenes (333 μg/g compared to 1,2,3-TeCB trichlorobenzene at 227 μg/g). Polyethylene has a strong sorption affinity for trifluralin, due to its hydrophobicity derived from a higher number of chlorine atoms (Tubić *et al*. 2021).

1.7. PHYTOREMEDIATION

Bioremediation is the process of environmental detoxification of toxic contaminants via microbes such as bacteria and fungi, using biological organisms, processes, or products (Ansari *et al.* 2015) and can assist with clean-up strategies of contaminants such as trifluralin. Phytoremediation is within the scope of bioremediation. Phytoremediation consists of the removal of contaminants from soil, water, and air via high tolerance plants that are hyperaccumulators. There are various types of phytoremediation: phytoextraction, phytodegradation, phytovolatilisation, rhizofiltration and phytostabilisation (Ansari *et al.* 2015). However, it is important to note that not all these processes are relevant to aquatic plants such as duckweed; phytoextraction requires soil to occur (Ansari *et al.* 2015). Phytoextraction involves hyperaccumulators storing contaminants in aboveground tissues. Phytodegradation involves plants absorbing and storing toxicants, at which point contaminants are biochemically degraded or converted into non-toxic by-products. Phytovolatilisation involves contaminants vaporising from foliage. Rhizofiltration is the precipitation and concentration of toxic pollutants via roots grown in aerated water (Ansari *et al.* 2015). The rhizosphere is the root zone that consists of the root surface and soil/water zone around the root that allows biotransformation of organics (Cunningham *et al.*1997).

Phytostabilisation allows contaminants to bind to a plants' roots and leaves and are demobilised and stabilised, reducing bioavailability (Kalčíková 2020). Micropollutants taken up by the roots can be converted by the plant and redeposited into the rhizosphere in a different chemical form (Cunningham *et al.*1997).

1.7.1. Microbial phytoremediation

Microbial consortia can assist with phytoremediation, by having characteristics that allow the degradation of organic contaminants. Co-metabolism can occur in phytoremediation, where enzymes or cofactors that are produced by microbes to degrade their metabolic substrate, degrade target contaminants (Cunningham *et al.*1997). Plants can influence their microbial consortia by providing substrates, holding sway on the spatial arrangement of microbial species, allowing the assemblage of biofilms on root surfaces and by promoting growth-linked degradation (Crowley *et al.*1997; Cunningham *et al.*1997). However, bioaugmentation (addition of microbial cultures to enhance microbial consortia for a particular function i.e. degrading particular contaminants) may also enhance phytoremediation (Speight 2016).

The rhizosphere consists of a diverse spectra of microorganisms with dense populations, therefore genetic exchange and gene arrangements are diverse (Crowley *et al.* 1997). A high turnover of microbes occurs within the rhizosphere and changes in the types and quantities of substrates that are available to microbial life occur due to protozoa and nematodes grazing bacteria (Crowley *et al.* 1997). The root zone is oligotrophic (Crowley *et al.* 1997), and therefore highly competitive. The microbial communities vary along the length of the root (Cunningham *et al.* 1997) and high microbial activity is focused on the root tips and the site of lateral root emergence (due to temporary carbon from root exudates or root cell lysates) (Crowley *et al.* 1997). Within phytoremediation, all exudates from biotransformation of contaminants are deposited from the plant roots via rhizodeposition (Crowley *et al.* 1997). The rhizosphere is known to have microbial consortia that are able to degrade pesticides (Cunningham *et al.* 1997), however the rhizosphere may not be able to degrade xenobiotics, depending on whether the degrader population is present, or if the microbial species is non-competitive in the rhizosphere (Crowley *et al.* 1997).

Plants are living organisms; therefore they have reduced capacity regarding contaminant removal compared to traditional methods. They have specific oxygen, nutrient, water, pH limits that must be maintained (Cunningham *et al.* 1997). Furthermore, time frame, depth and concentration of contaminant limitations are also present (Cunningham *et al.* 1997). The lifespan of a plant will be much shorter compared to engineered technology. A plant will be limited by its biology as to where it can grow in the water column in terms of depth, the concentration of a contaminant will have to be within a plants' tolerance.

There is a reduced risk of secondary contamination within phytoextraction as one will be eliminating or minimising the need to move contaminated soils. The majority of sites will be able to be treated in situ. Furthermore phytoextraction is less expensive compared to traditional methods such as excavation or combustion (Cunningham *et al.* 1997).

1.7.2. Duckweed

Duckweed, such as the common duckweed *Lemna minor* that are native to Britain (Botanical Society of the British Isles. 2014), are hyperaccumulators and highly tolerant to environmental toxicity (Ali *et al.* 2016; Crowley *et al.* 1997; Lewis 1995) including microplastics. Microplastics do not significantly affect the growth rate of duckweeds (Kalčíková. 2020). In the study by Kalčíková *et al.* (2017), which aimed to determine whether polyethylene microbeads from cosmetic products significantly affected the duckweed species *Lemna minor*, it was found that leaf growth rate and content of photosynthetic pigments chlorophyll *a* and *b* were unaffected. In the study by Mateos – Cárdenas *et al.* (2019), *Lemna minor* was subjected to absorbance of up to seven polyethylene microplastics per mm^2. Over seven-day exposure experiments, photosynthetic efficiency and plant growth was unaffected by microplastics. Dovidat *et al.* (2020) exposed the duckweed species *Spirodela polyrhiza* to nano and microplastics and found that plant growth and chlorophyll production were unaffected. Furthermore, external attachment (adsorption) of nanoplastics were observed on *Spirodela polyrhiza* roots.

Throughout the literature, species of duckweed have been tested for tolerance to herbicides and plastic as well as testing whether plastic binds to macrophytes. Phytoremediation has been used at a mass scale for metal 'clean-up' via terrestrial soils. Duckweed, however, has not been used to phytoremediate on a mass scale in freshwater ecosystems. There is potential for species of duckweed to be used as a 'clean-up' strategy to remove microplastics with absorbed contaminants such as herbicides via phytoremediation from freshwater ecosystems. The species consists of free-floating mats, present in shaded or open areas of freshwater (Strzałek & Kufel 2021) that are gently free-flowing or stagnant. The fronds consist of a leaf-like structure, with a root attached (Ghanem *et al.* 2019). Duckweed grows rapidly and reproduces asexually (Strzalek & Kufel 2021; Ghanem *et al.* 2019). Plant biomass can double in two or three days under optimal conditions (Vymazal 2008). Species within the family *Lemnaceae* are commonly known as duckweeds. The five genera include: *Landoltia, Spirodela, Lemna, Wolffia, Wolffiella,* with thirty-eight species in total (Ali *et al.* 2016). *Lemnaceae* distribution is extensive from temperate to tropical climates and varied freshwater habitats such as lakes, streams, and effluents (Lewis 1995).

Aquatic plants such as duckweeds have been used in the role of phytotoxicity testing in pesticide toxicity tests (Lewis 1995) and are therefore highly tolerant to such chemicals. According to Fernandes *et al.* (2013), studies on plant resistance to dinitroanilines show that plants that express tolerance to herbicides have a mutation that causes a change in their genetic code. A study by Rice *et al.* (1997) undertook an experiment to test the hypothesis that herbicide-tolerant aquatic plants can phytoremediate herbicide-contaminated waters. Herbicides used were ^{14}C-labelled metolachlor (MET) and atrazine (ATR). Aquatic plants used were *Ceratophyllum demersum* (coontail, hornwort), *Elodea canadensis* (American elodea, Canadian pondweed), or *Lemna minor.* These aquatic plants significantly reduced the concentration of MET in the contaminated water treatment after a sixteen-day incubation period. Overall, 1.44% (*C. demersum*), 4.06% (*E. canadensis*), and 22.7% (*L. minor*) of ^{14}C-labelled MET remained in the water treatments. Comparably, 61% of the MET was present in water treatments that did not have aquatic plants. Pond water was used, which accounts for some MET bioremediation occurring in treatments without aquatic plants. In terms of atrazine, *C. demersum* (41.3%) and *E. canadensis* (63.2%) significantly reduced the concentration of ATR in the contaminated water treatment after a sixteen-day incubation period. *Lemna minor* was not as successful and was similar in ATR concentrations (84.9%) to

treatments that did not have aquatic plants (85%). Overall *Lemna minor* was more successful at phytoremediation of MET than ATR.

It is important to note that duckweed will not be entirely tolerant to microplastics or herbicides. For example in the study previously mentioned conducted by Kalčíková *et al.* (2017) in-lab conditions, microplastic had negative effects on the root length of *Lemna minor* due to mechanical blocking as well as root cell viability due to the sharpness of microbeads tested. However, smoother surfaces of microbeads were not tested, but it can be suggested that it is most likely that the smoother the surface, the less impact on roots.

1.8. CURRENT KNOWLEDGE

1.8.1. Possible mechanisms of how microplastics adsorb to the surfaces of macrophytes

Throughout the literature microplastic and macrophyte interactions within freshwater ecosystems are scarce, however previous research has established that microplastics adsorb to the surfaces of macrophytes. Bhattacharya *et al*. (2010) focused on a model cellulose film and freshwater algae, Gutow *et al*. (2016) and Schaffelke (1999) focused on marine macroalgae, Goss *et al*. (2018) focused on marine seagrass and Dovidat *et al.* (2020) focused on duckweed.

Bhattacharya *et al*. (2010) observed hydrophobic interactions between algae and polystyrene beads. Surface charge effected sorption, photo-oxidation functionalises plastic surfaces, resulting in different charged polarities. Differing surface charged plastics adsorb to macrophytes due to intermolecular hydrogen bonds. Bhattacharya *et al*. (2010) found positively charged polystyrene beads to adsorb slightly more than negatively charged beads in cellulose, this derived from electrostatic repulsion caused by the carboxyl and sulfate groups in cellulose.

Gutow *et al*. (2016) and Schaffelke (1999) showed how surface morphology affects adsorption of microplastics. In macroalgae, the complex thalli structure were shown to retain more microplastics. The roughness of the thalli provides multiple binding sites for microplastics. Furthermore, the roughness of the cellulose structure had the same outcome in the work by Bhattacharya *et al*. (2010).

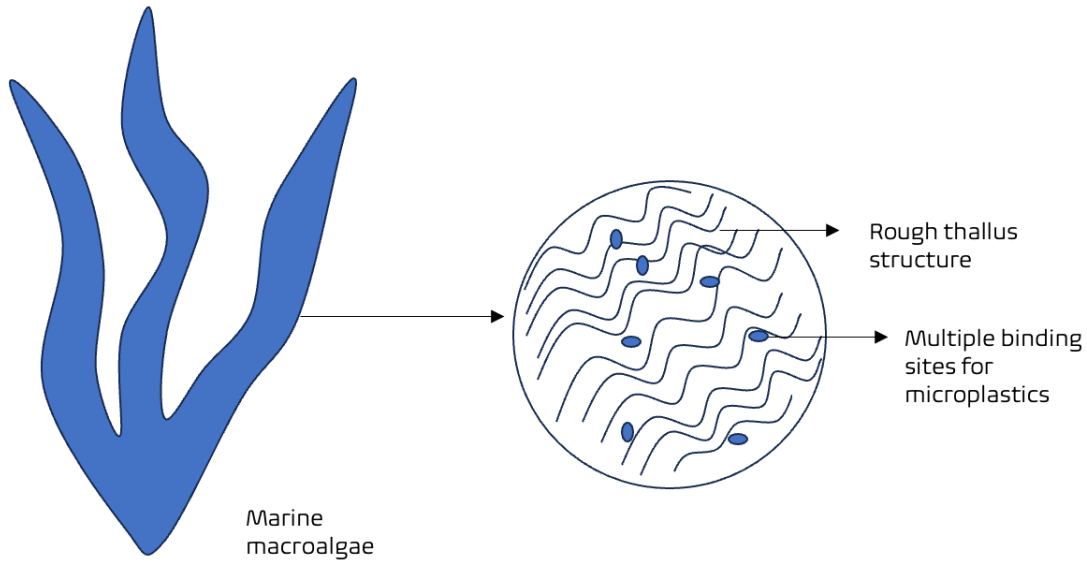


Fig. 1.8.1. (a). A possible mechanism of how microplastics adsorb to the surfaces of macrophytes presented by Gutow *et al.* (2016) and Schaffelke (1999). Thalli rough structure equates to multiple binding sites for microplastics.

Periphyton biofilms on plant surfaces increases the retention of microplastics via surface stickiness and epibiotic algae that assists the periphyton layer in binding microplastics to submerged plants (Goss *et al*. 2018; Rai *et al.* 2021). Further understanding is needed of the mechanisms to which microplastics adsorb to macrophytes, i.e. to understand whether microplastics can be carried away with other particulate matter or stay adsorbed to the plants' surface (Kalčíková 2020).

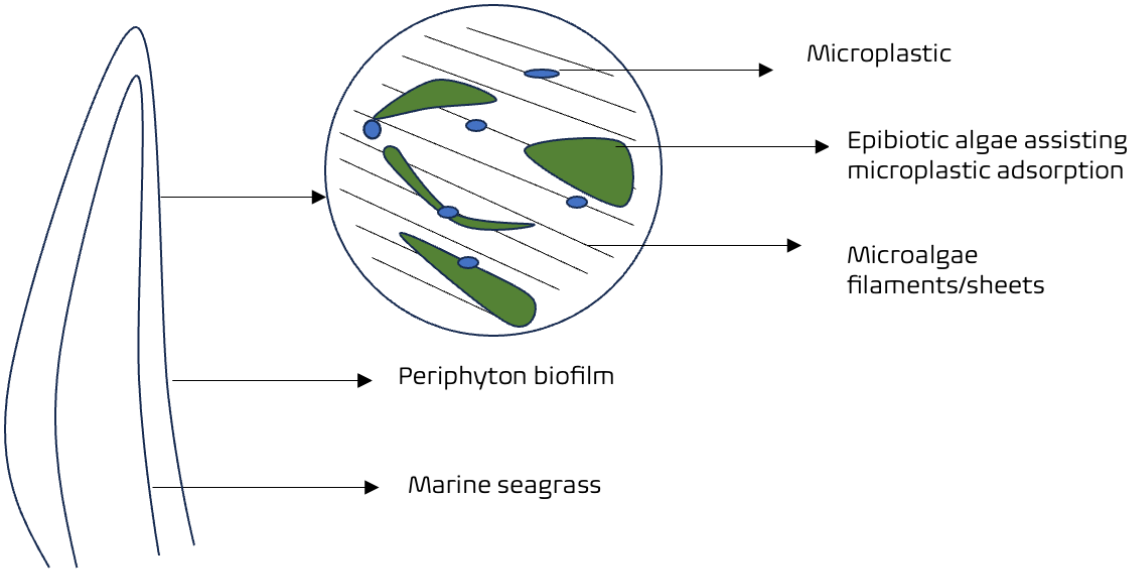


Fig. 1.8.1. (b). A possible mechanism of how microplastics adsorb to the surfaces of macrophytes presented by Goss *et al.* (2018). Periphyton biofilms on plant surfaces increases the retention of microplastics via surface stickiness and epibiotic algae that assists the periphyton layer in binding microplastics to submerged plants.

1.8.2. Biofilms

A point of interest are biofilms that form on microplastic. Biofilms on microplastics consist of microbial communities that form epiplastic biofilms located within a plastisphere, as depicted in figure 1.7.2. The plastisphere is the niche surrounding microplastics that harbour microbial consortia. Epiplastic biofilms are microbial communities localised on plastispheres. These consortia can vary depending on habitat such as freshwater columns and sediment, as well as competition between microbes (Rai *et al.* 2021). Biofouling on plastic surfaces leads to an increase in density and therefore submersion, transporting microplastics further into the water column and sediment (Kalčíková 2020; Rai *et al.* 2021). Biofilms have potential for bioremediation of microplastics and their absorbed contaminants. Shiu *et al.* (2020) found that 25-nm polystyrene nano plastic formed a microgel upon interacting with dissolved organic matter in freshwater ecosystems. This increased the transition of dissolved organic matter to particulate organic matter and thus increased biodegradation.

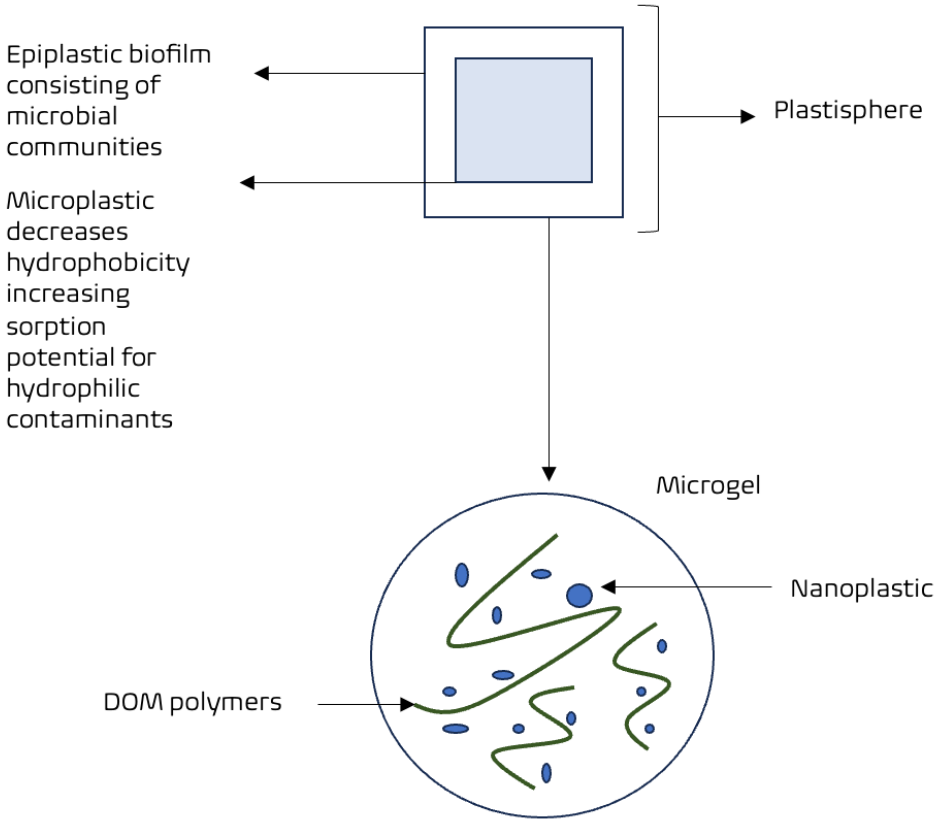


Fig. 1.8.2. Epiplastic biofilm formation on microplastics via biofouling within the plastisphere.

1.8.3. Knowledge gaps and research potential

Within the literature, knowledge gaps in this area of research are prevalent. The microbial degradation pathways of plastic types in the freshwater environment is understudied. There is research potential for microbial species that aid in plastic degradation to be studied by adding these microbes to a site for bioremediation or for research focusing on genetically engineered microbes/macrophytes to enhance bioremediation/ phytoremediation. The fate of contaminants in freshwater ecosystems are not thoroughly understood and the fate of chemical products produced by microbial bioremediation of said contaminants has not been researched. Furthermore, due to the lack of knowledge of how microplastics interact in freshwater ecosystems there is a lack of knowledge of how plastic additives behave. There is research potential for the bioremediation of microbes on epiplastic biofilms surrounding microplastics to bioremediate plastic with sorbed contaminants such as pesticides as well as

testing if there is a significant difference between duckweed, freshwater and freshwater sediment in terms of microbial bioremediation efficiency.

Species of duckweed could adsorb and phytoremediate microplastics near dams, where high concentrations of microplastics accumulate. The majority of microplastics float up to the surface microlayer of still waters (e.g. lakes and water reservoirs) where interaction with duckweed can occur (Kalčíková 2020). Hydrophobic compounds can be at a concentration 500 times greater at the surface microlayer compared to the water column (Teuten *et al.* 2007). Plant biomass with adsorbed microplastics could be removed multiple times reducing microplastics and their absorbed contaminants within freshwaters (Kalčíková 2020).

1.9. RESEARCH OBJECTIVES

The aim of this research is to quantify whether there is potential for bioremediation technologies to be implemented using *Lemna minor* and the plastic mulch films LDPE and PLA-PBAT. The films have the potential to sorb trifluralin. The research also aims to compare bioremediation of trifluralin between freshwater and soil ecosystems.

The following chapters aim to assess:

1. The capacity for the plastic mulch films LDPE and PLA-PBAT to sorb trifluralin, potentially aiding bioremediation in freshwater or soil ecosystems.

2. To determine the tolerance of *Lemna minor* under exposure of trifluralin and the plastic additive DEHP. This was undertaken to ensure that *L.minor* is a suitable aquatic plant to be used in potential phytoremediation clean-up strategies in freshwaters.

3. The rate of mineralisation of trifluralin within soil via sorption to LDPE and PLA-PBAT and trifluralin directly applied to the soil. Additionally, quantification of any differences between bioremediation output between plastic films and soil and a later experiment focusing on bioremediation in a freshwater context was assessed.

4. The rate of bioremediation of trifluralin via the plastic film PLA-PBAT and microbes derived from the duckweed species *Lemna minor,* freshwater and freshwater sediment. This was to quantify bioremediation and phytoremediation efficiency of trifluralin in freshwater ecosystems.

CHAPTER 2: METHODOLOGY

2.1. TRIFLURALIN SORPTION TO LDPE AND PLA-PBAT PLASTIC MULCH FILMS

2.1.1. Overview

Previous research has indicated that plastics have the potential to sorb chemicals dissolved within the water matrix. To critically evaluate this, the ability of two contrasting plastic mulch films were tested, namely LDPE and PLA-PBAT, to sorb the herbicide trifluralin. LDPE is a conventional film (30 μm thick) derived from GroMax Industries Ltd, Hadleigh, UK. PLA-PBAT is a biodegradable film (15:85 *w/w*, 15 μm thick), also derived from GroMax Industries Ltd. The agricultural mulch films were tested to determine how well the toxic herbicide trifluralin sorbs to the films to determine their capacity for removing trifluralin from the water column.

2.1.2. Preparation of ^{14}C-labelled trifluralin solutions

The experiments used a commercial formulation of trifluralin (43% trifluralin concentration, other ingredients 57%) Weed Stopper II, manufactured for Montere, Lawn and Garden Products Inc. To make up the ^{14}C-labelled trifluralin solutions, the end of a 20 μl pipette tip was placed in 500 μl of trifluralin for 1 hour in a 20 ml glass vial. This initial step was designed to fill the surface sorption sites in the plastic pipette tip in order for later aliquots to be more accurate. Using this pipette tip, 9.4 μl of trifluralin was added to 1000 μl of distilled water in a new 20 ml glass vial; this represented the 100% stock solution. Trifluralin is hydrophobic and binds to glass and plastic (Anthony & Hussey. 1999). Glass vials were used due to trifluralin's tendency to adsorb greater to plastic than glass (Sharom & Solomon. 1981). 100 μl was taken of the 100% stock solution and added to 900 μl of distilled water in a new glass vial, this was the 10% stock solution. 100 μl was taken of the 10% stock solution and added to 900 μl of distilled water in a new glass vial, this was the 1% stock solution. The ^{14}C-trifluralin was

removed from the freezer and left to reach room temperature (1 h). For a batch of 45 samples 37 kBq (44,000 DPM per sample) of ^{14}C-trifluralin bought from American Radiolabelled Chemicals Ind, St Louis, MO, USA was added to each 500 µl stock solutions. Solutions were mixed carefully.

2.1.3. Trifluralin sorption to LDPE and PLA-PBAT

Treatments consisted of 24 individual 1.5 cm^2 macroplastic squares of LDPE (*n*=12) and PLA-PBAT (*n*=12). Each of the trifluralin concentrations (1, 10, 100%) was replicated 4 times. Each 1.5 cm^2 plastic square was placed flat inside individual 20 ml glass vials and 10 µl trifluralin was placed in the centre of the plastic film. The glass vials were kept closed, preventing evaporation of the pesticide. Samples were then left for 24 h or 14 d in the dark at room temperature. After incubation, 10 ml of distilled water was added to the vials and the samples shaken at 200 rev/min for 30 min. To measure the amount of ^{14}C-trifluralin desorbed, 1 ml of the water was removed and added to 4 ml of HiSafe3 scintillation fluid and mixed with a Genie vortexer. The LDPE and PLA-PBAT were then removed from the water wash solution and placed in new vials containing 10 ml of methanol and shaken (200 rev/min, 30 min). 1 ml of the methanol wash solution was then removed and mixed with scintillation fluid. A Wallac 1404 liquid scintillation counter with automated quench correction was the used to calculate the amount of ^{14}C-trifluralin in both water and methanol washes as a percentage of the total added.

LDPE and PLA-PBAT were removed from the methanol wash solution and dried. A Hidex Biological Oxidiser (Hidex Inc., Finland) was used to quantify the amount of trifluralin retained within the plastic matrix. 1ml of the oxidiser fluid (Oxisolve C400) was removed and as above, added to 4 ml of Hisafe3 scintillation fluid and mixed with a Genie vortexer. Subsequently, a Wallac 1404 liquid scintillation counter was used to calculate the amount of trifluralin retained within the plastic matrix.

2.1.4. Statistical considerations

All data was tested for normality using the Shapiro-Wilk test. Differences between the methanol and water washes over 24 hours and 14 days were calculated using Paired Samples t-Tests and Wilcoxon Signed-Rank Tests, the latter due to non-parametric data. Sorption variability of LDPE and PLA-PBAT was quantified via t-Tests. Sorption over 24 hours and 14 days were compared between plastic film type and trifluralin concentration. t-Tests were used to determine differences in sorption between films as well as between trifluralin concentrations. Mann-Whitney U Tests were undertaken due to non-parametric data to determine the quantity of retention of trifluralin within the plastic matrix of LDPE and PLA-PBAT after 24 hours and 14 days.

2.2. TRIFLURALIN AND DEHP TOLERANCE IN *LEMNA MINOR*

2.2.1. Overview

Within the literature, it is stated that *Lemna minor* is highly tolerant to herbicides (Lewis 1995; Fernandes *et al.* 2013; Rice *et al.* 1997) and specifically to trifluralin by Knežević *et al.* (2016) and Fairchild *et al.* (1997). The tolerance of DEHP was also quantified, to determine whether *Lemna_minor* is a suitable aquatic plant to be used in potential phytoremediation clean-up strategies in freshwaters. Plant biomass can double in two or three days under optimal conditions (Vymazal 2008). Growth rate indicates plant health, thus showing whether frond doubling time has been negatively affected by trifluralin.

2.2.2. Culturing *Lemna minor*

Duckweed (*L. minor*) was cultured over 7 days in hydroponic culture using Long Ashton Nutrient Solution containing sulphur (Hewitt. 1966). As trifluralin was found to strongly adsorb to plastic surface, 100 ml glass beakers containing 50 ml of nutrient medium were used to culture the duckweed (Khvatkov 2019). Cling-film was placed over the top to reduce

evaporation. Based on Ghanem *et al.* (2019), cultivation was undertaken in a plant growth cabinet with continuous light conditions (24 h), light intensity of 280 µmol m^{-2} s^{-1} and a temperature of 26°C. Duckweed plants were derived from a commercial aquaria supplier.

2.2.3. *Lemna minor* tolerance to DEHP and trifluralin via PLA-PBAT and water

The trifluralin stock solutions were made as described in section 2.1.2. 10 µl of DEHP was pipetted onto PLA-PBAT (12 × 1.5cm^2 squares) and left for 24 h. 10 µl of various trifluralin concentrations were then added to PLA-PBAT + DEHP 1.5 cm^2 squares of plastic (1% x 4 replicates, 10% x 4, 100% x 4 = 12) and left for 24 h. The duckweed was rinsed with distilled water to remove the snails and algae.

The first treatment was one 1.5 cm^2 square of PLA-PBAT + DEHP + Trifluralin (1% x 4 replicates, 10% x 4, 100% x 4) added to each 100 ml glass beaker with 50 ml of water and 15 fronds of *L. minor*. The second treatment involved 10 µl of various trifluralin strengths (1% x 4 replicates, 10% x 4, 100% x 4) pipetted into the water of 100 ml glass beakers containing 50 ml nutrient medium and 15 fronds of *Lemna minor* (12 replicates). 15 duckweed fronds and 50 ml of nutrient medium in each of four glass beakers were prepared as the control. Treatments were left in the growth cabinet for 6 days. Frond number was counted at the end of the experiment to draw comparisons and relative growth rate was calculated using Magahud and Dalumpines (2021) equation.

2.2.4. Statistical considerations

All data was tested for normality using the Kolmogorov-Smirnov test, and data was parametric. One-Way ANOVA tests were undertaken to establish whether there was a difference of *Lemna minor* tolerance to trifluralin between water and plastic film inputs. To determine differences between *Lemna minor* under natural conditions and *Lemna minor* under toxic conditions via trifluralin input by plastic or within water One-Way ANOVA tests were used once again.

2.3. TRIFLURALIN MINERALISATION VIA LDPE, PLA-PBAT AND SOIL

2.3.1. Overview

The testing of the mineralisation of trifluralin via the two plastic film types LDPE and PLA-PBAT and soil was undertaken to compare bioremediation output between plastic films and soil and a later experiment focusing on bioremediation in a freshwater context (section 2.4). The method was derived from the paper by Viljoen *et al.* (2023). The method derived from this paper was used due to its similarity to the objectives of this research, ^{14}C-labelled plasticiser was added to soil to determine mineralisation, whereas this research aimed to quantify mineralisation of ^{14}C-labelled trifluralin.

2.3.2. Trifluralin mineralisation via LDPE and PLA-PBAT and soil

Soil derived from Henfaes Agriculture research Station (53◦14′ N, 4◦ 01′ W) was used for these experiments. Sample 2 of soil was used for trifluralin mineralisation via LDPE and PLA-PBAT within soil (section 3.3.2.) and sample 3 was used for trifluralin mineralisation in soil (section 3.3.1.). Soil sample characteristics are presented below in table 2.3.2. Miranda *et al.* (2001) was used to quantify available nitrate and Murphy and Riley (1962) was used to determine available phosphorus.

Measurement	Sample 2	Sample 3
Soil moisture (%)	19.35	17.93
pH	5.9	5.8
Soil Organic Matter (SOM - %)	5.92	5.59
Soil Organic Carbon (SOC - %)	2.25	2.12
Available Phosphorus (Conc. mg P kg^{-1} DW)	28.86	23.19
Available Nitrogen (Conc. mg NO_3 kg^{-1} DW)	24.65	23.51

Table 2.3.2. Soil characteristics of soil samples used in experiments in section 3.3.

The soil was passed through a sieve to (< 5 mm). The trifluralin stock solutions were made as described in section 2.1.2. A 10 µl drop of ^{14}C-trifluralin was added to LDPE and PLA-PBAT and treatments consisted of 1.5 cm^2 of LDPE and PLA-PBAT as in section 2.1, with 1% trifluralin concentration x 4 replicates and 10% x4, 100% x 4, with a total of 12 replicates per plastic type (24). Field-moist soil (5 g) was placed in 24 sterile polypropylene tubes. LDPE and PLA-PBAT with 10 µl of ^{14}C-trifluralin were placed in the appropriately labelled polypropylene tubes. Another 5 g of field-moist soil was added to the tubes, burying the plastic films within soil.

The soil treatment consisted of: 1% trifluralin x 4 replicates, 10% x 4, 100% x 4 (12). In the soil treatment ^{14}C-trifluralin was added directly to the surface of 10 g of soil. 10 µl of the starting solutions were added to a further 0.49 ml of water and then added to the soil. This was to allow the solution to cover the same area as the plastic. 1 M NaOH traps were then suspended above the soil surface to catch any respired $^{14}CO_2$. The experiment for the treatments LDPE and PLA-PBAT lasted 4 weeks and the experiment for the soil treatment lasted 6 weeks. Using the method described in 2.1.3. the amount of $^{14}CO_2$ from LDPE, PLA-PBAT and soil treatments were measured using a scintillation counter.

A limitation of this experiment was that initially treatments were to be done at the same time using the same trifluralin solutions, however, a mistake was made during the trifluralin stock solution making, resulting in not enough radioactivity being added to the trifluralin stock solutions for the soil. Therefore the soil treatment needed to be repeated. Trap change frequency was daily for the LDPE and PLA-PBAT treatments. However, the soil treatment trap change frequency was changed to twice a week due to such low $^{14}CO_2$ results daily from the LDPE and PLA-PBAT treatments.

2.3.3. Statistical considerations

All data was tested for normality using the Kolmogorov-Smirnov test. Differences between trifluralin concentrations concerning mineralisation rates where trifluralin was applied directly to the soil, were measured via a Kruskal-Wallis test due to non-parametric data and in order to compare three treatments. Testing mineralisation rates between differing plastic films (LDPE and PLA-PBAT) was measured via a t-Test for parametric data and a Mann-Whitney U test for non-parametric data. Differences between mineralisation rates depending on trifluralin concentration (via plastic films) was measured via Kruskal-Wallis tests due to non-parametric data.

One-Way ANOVA tests for parametric data and a Kruskal-Wallis test for non-parametric data was undertaken to compare mineralisation rates of the same trifluralin concentration with differing inputs (via LDPE, PLA-PBAT, soil).

2.4. BIOREMEDIATION OF ^{14}C-TRIFLURALIN VIA PLA-PBAT AND MICROBES DERIVED FROM *LEMNA MINOR*, FRESHWATER, AND FRESHWATER SEDIMENT

2.4.1. Overview

This experiment was undertaken to quantify the biochemical bioremediation of trifluralin via the plastic film PLA-PBAT and microbes derived from the duckweed species *Lemna minor*, freshwater and freshwater sediment. This is to quantify bioremediation and phytoremediation efficiency of trifluralin in freshwater ecosystems.

2.4.2. Adsorption of ^{14}C-trifluralin to PLA-PBAT

A 100% strength trifluralin stock solution was made as described in section 2.1.2. The 100% solution was used as it had already been determined that *L. minor* was tolerant to 100% strength trifluralin (section 2.2). 10 µl of ^{14}C-labelled trifluralin was adsorbed to PLA-PBAT for 24 h and a wash was then performed as described in section 2.1.3.

2.4.3. Preparing sodium hydroxide trap treatments

L. minor was cultured prior to the start of this experiment as described in section 2.2.2. 50 ml beakers were used to culture duckweed in this experiment. Spherical 10 ×10 cm transparent containers were used to house the 50 ml beakers containing each treatment and a 1 M NaOH trap placed inside to collect $^{14}CO_2$. The experiment consisted of 3 treatments, each with 4 replicates and is represented in figure 2.4.3.:

Treatment 1 - (^{14}C-trifluralin + PLA-PBAT) + (*L. minor* + Sediment + Freshwater);

Treatment 2 - (^{14}C-trifluralin) + (*L. minor* + Sediment + Freshwater);

Treatment 3 - (^{14}C-trifluralin) + (*L. minor* + Freshwater).

The freshwater and freshwater sediment was derived from the Afon Gamlan, near to the Rhaeadr Ddu waterfalls in southern Eryri National Park (52° 48' N, 3° 53' W). 10 g of sediment was used per replicate to ensure an even layer at the bottom of the 50 ml beaker. 30 ml of freshwater was used for treatments 1 and 2 as sediment filled space within the beakers, 40 ml was used in treatment 3. 10 ml of Long Ashton Nutrient Solution with sulphur (Hewitt. 1966) was used in all treatments. 15 *L. minor* fronds were planned to be used per replicate however a change to this was made as described in section 3.4.4. Treatment 1 had ^{14}C-trifluralin adsorbed to the biodegradable film PLA-PBAT, treatments 2 and 3 had 10 µl of ^{14}C - trifluralin pipetted into the freshwater/nutrient medium.

The containers were placed in a growth cabinet to maintain the duckweed and the NaOH traps were changed twice a week for experiment 1, however experiment 2 trap change was daily except for weekends (see section 3.4.4.). Experiment 1 had a time scale of 25 days and experiment 2 had a duration of 14 days. Using the method described in 2.1.3. the amount of $^{14}CO_2$ from treatments were measured via a scintillation counter.

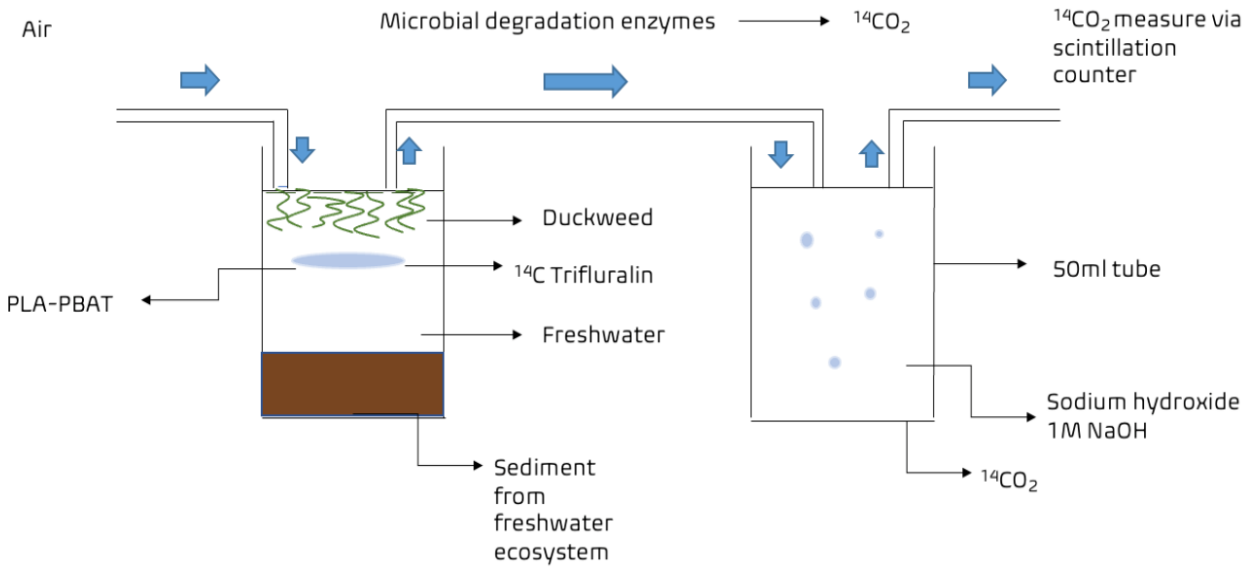


Fig. 2.4.3. A sodium hydroxide trap measuring bioremediation via $^{14}CO_2$ from freshwater sediment, duckweed, and freshwater. $^{14}CO_2$ is derived from microbial degradation.

2.4.4. Statistical considerations

All data was tested for normality using the Kolmogorov-Smirnov test. Tests undertaken to quantify differences between experiment 1 treatments, were a One-Way ANOVA and a t-Test. Differences between experiment 2 treatments were determined via a One-Way ANOVA. t-Tests were undertaken to compare experiment 1 and experiment 2 treatments as well as a Mann-Whitney U Test due to non-parametric data. Statistical tests undertaken to compare trifluralin mineralisation via treatment PLA-PBAT 100% in section 3.3. and experiment 1 and 2 of this experiment were a t-Test and a Mann-Whitney U Test due to non-parametric data.

CHAPTER 3: RESULTS

3.1. TRIFLURALIN SORPTION TO LDPE AND PLA-PBAT PLASTIC MULCH FILMS

3.1.1. Sorption of trifluralin to LDPE and PLA-PBAT over 24 hours

Figure 3.1.1. shows that the amount of trifluralin recovered in the methanol wash (ca. 50-90% of the total recovered) was much higher than that recovered by a water wash (ca. 5-10%) across both plastic types. This is indicative of strong sorption of trifluralin to the surface of both plastic films. Further, higher amounts of trifluralin was adsorbed by the bio-plastic film PLA-PBAT compared to the conventional film LDPE (based on the amount recovered in the water washes). The sorption response was also concentration-dependent with the 10% and 100% trifluralin concentrations having greater recovery from the PLA-PBAT film ($p < 0.001$) compared to the 1% trifluralin concentration ($p = 0.017$). The PLA-PBAT biofilm associated water washes and methanol washes were significantly different from each other with regards to the 10 and 100% trifluralin concentrations ($p < 0.001$), but this response was not significant at the 1% concentration ($p = 0.068$). Overall, the data indicates that 10% and 100% trifluralin concentrations adsorb less strongly to the film PLA-PBAT than the 1% treatment. With regards to the LDPE film associated water washes and methanol washes, treatments 10% and 100% concentrations were not significantly different ($p = 0.068$ and $p = 0.257$, respectively), while those at a 1% concentration showed a significant difference ($p < 0.001$). This indicated that the 1% trifluralin concentration adsorbed more strongly and to a greater extent to the LDPE film than the 10% and 100% treatments.

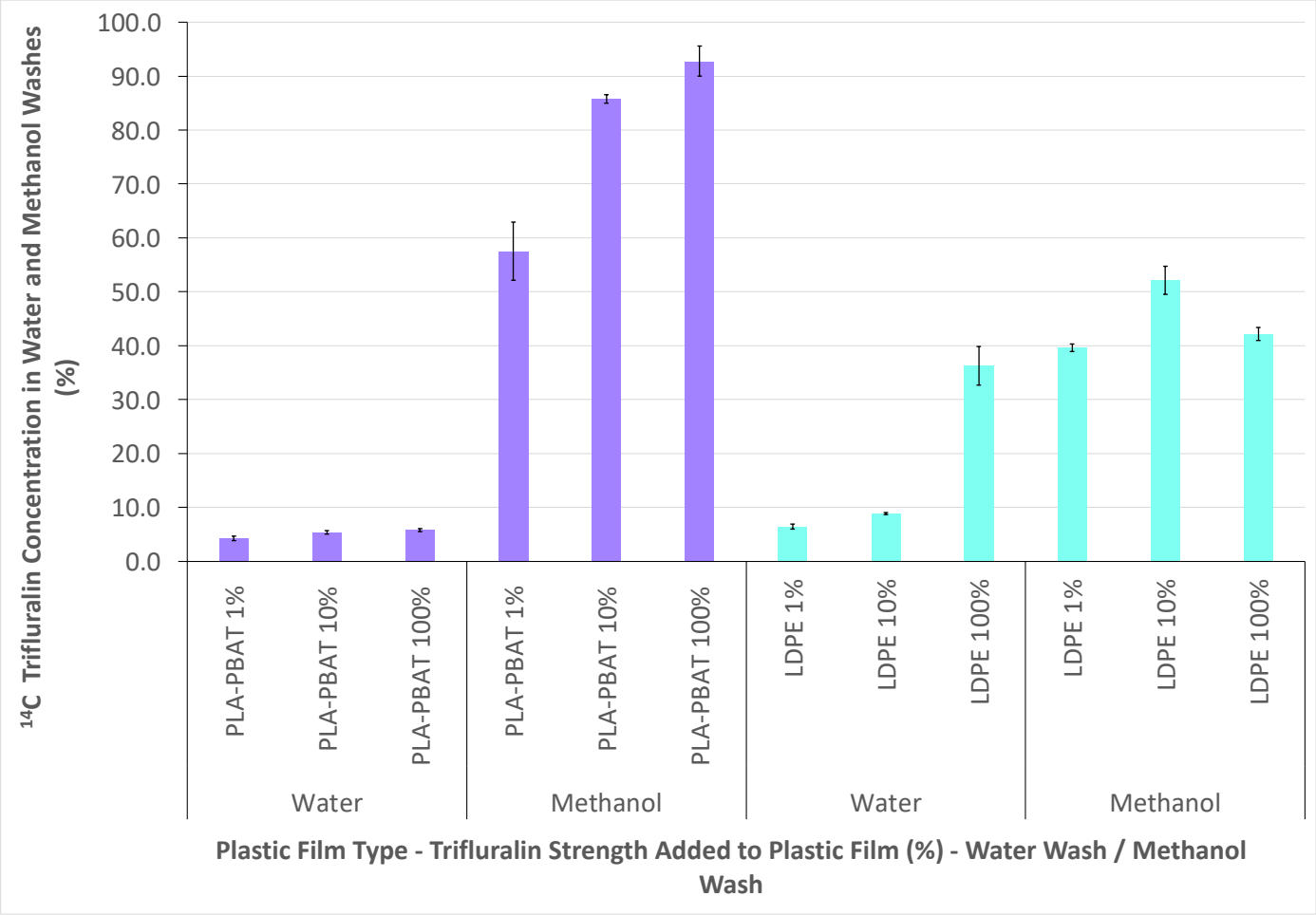


Fig. 3.1.1. Amount of trifluralin recovered in either a water wash or methanol wash after contact of the ^{14}C-trifluralin solution to LDPE or PLA-PBAT based plastic mulch films over 24 h. Values represent means ± SEM (n = 4).

3.1.2. Sorption of trifluralin to LDPE and PLA-PBAT over 14 days

Similar amounts of trifluralin was adsorbed by PLA-PBAT compared to LDPE with over 80% bound to the surface of the plastic (Fig. 3.1.2.). All three trifluralin concentrations, 1%, 10% and 100% adsorbed strongly to PLA-PBAT (p <0.001). The PLA-PBAT biofilm associated water washes and methanol washes were significantly different from each other with regards to treatments PLA-PBAT 1% (p = 0.002) and PLA-PBAT 10% (p <0.001), but not so with regards to treatment PLA-PBAT 100% treatment (p = 0.068). Overall, this indicates that 1% and 10% trifluralin concentrations are sorbed to a greater extent to the PLA-PBAT film compared to the 100% treatment. The LDPE conventional film associated

water and methanol washes were significantly different from each other in the 10% and 100% LDPE treatments ($p = 0.007$ and $p = 0.001$, respectively), however, no significant difference was seen in the 1% treatment ($p = 0.110$). The results indicate that trifluralin in the 10% and 100% treatments adsorb more greatly to the film LDPE then the 1% treatment.

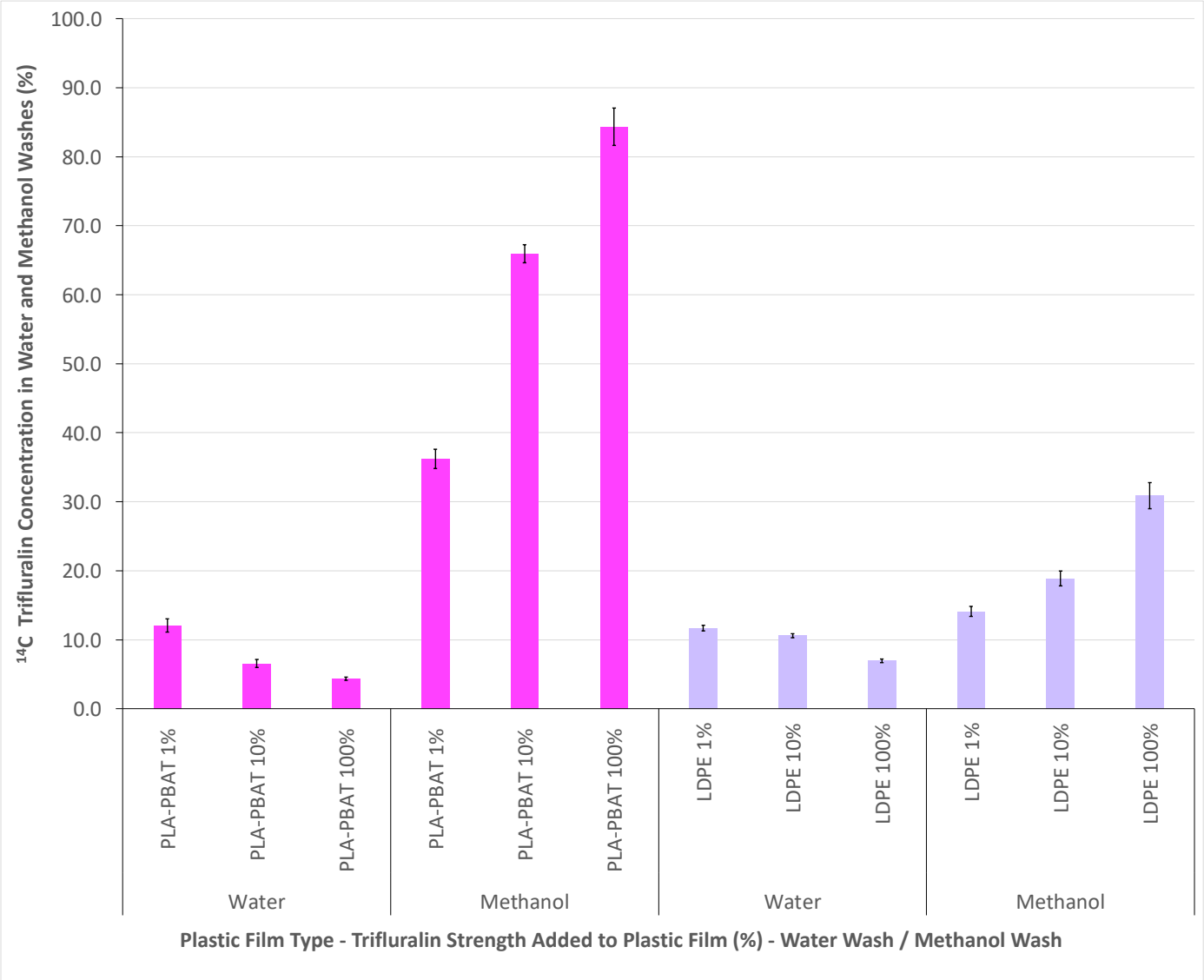


Fig. 3.1.2. Amount of trifluralin recovered in either a water wash or methanol wash after contact of the ^{14}C-trifluralin solution to LDPE or PLA-PBAT based plastic mulch films over 14 days. Values represent means ± SEM ($n = 4$).

3.1.3. Comparison of sorption of trifluralin to LDPE and PLA-PBAT over 24 hours and 14 days

All treatments showed significant differences in sorption response between trifluralin concentrations dependent on time except for PLA-PBAT 100% ($p = 0.071$). A trend here was that the 14 days results had a lower sorption response than the 24 hours results, which was not expected. LDPE 1% was significantly different between 24 hours and 14 days ($p < 0.001$), with sorption of 40% over 24 hours and >10% over 14 days. LDPE 10% was significantly different ($p < 0.001$), with sorption of >50% over 24 hours and <20% over 14 days. LDPE 100% was significantly different ($p = 0.002$), with sorption of >40% over 24 hours and 30% over 14 days. PLA-PBAT 1% was significantly different ($p = 0.009$), with sorption of <60% over 24 hours and >30% over 14 days. PLA-PBAT 10% treatment was significantly different ($p < 0.001$), with sorption of >80% over 24 hours and >60% over 14 days. PLA-PBAT 100% was significantly different ($p = 0.071$), with sorption of >90% over 24 hours and >80% over 14 days.

PLA-PBAT and LDPE films both had significant differences in sorption response between 24 hours and 14 days, however LDPE had a stronger response ($p < 0.001$) compared to PLA-PBAT ($p = 0.046$). The 14 days results had a lower sorption response than the 24 hours results, which was not expected. This may be due to error or evaporation of pesticide regardless of the sample being sealed. Over 80% sorption of trifluralin bound to the surface of the PLA-PBAT film after 14 days (Figure 3.1.2.) whereas it was over 90% after 24 hours (Figure 3.1.1.). 30% sorption of trifluralin bound to the surface of the LDPE film after 14 days (Figure 3.1.2.) whereas it was over 50% after 24 hours (Figure 3.1.1.).

3.1.4. Trifluralin retained in LDPE and PLA-PBAT plastic films

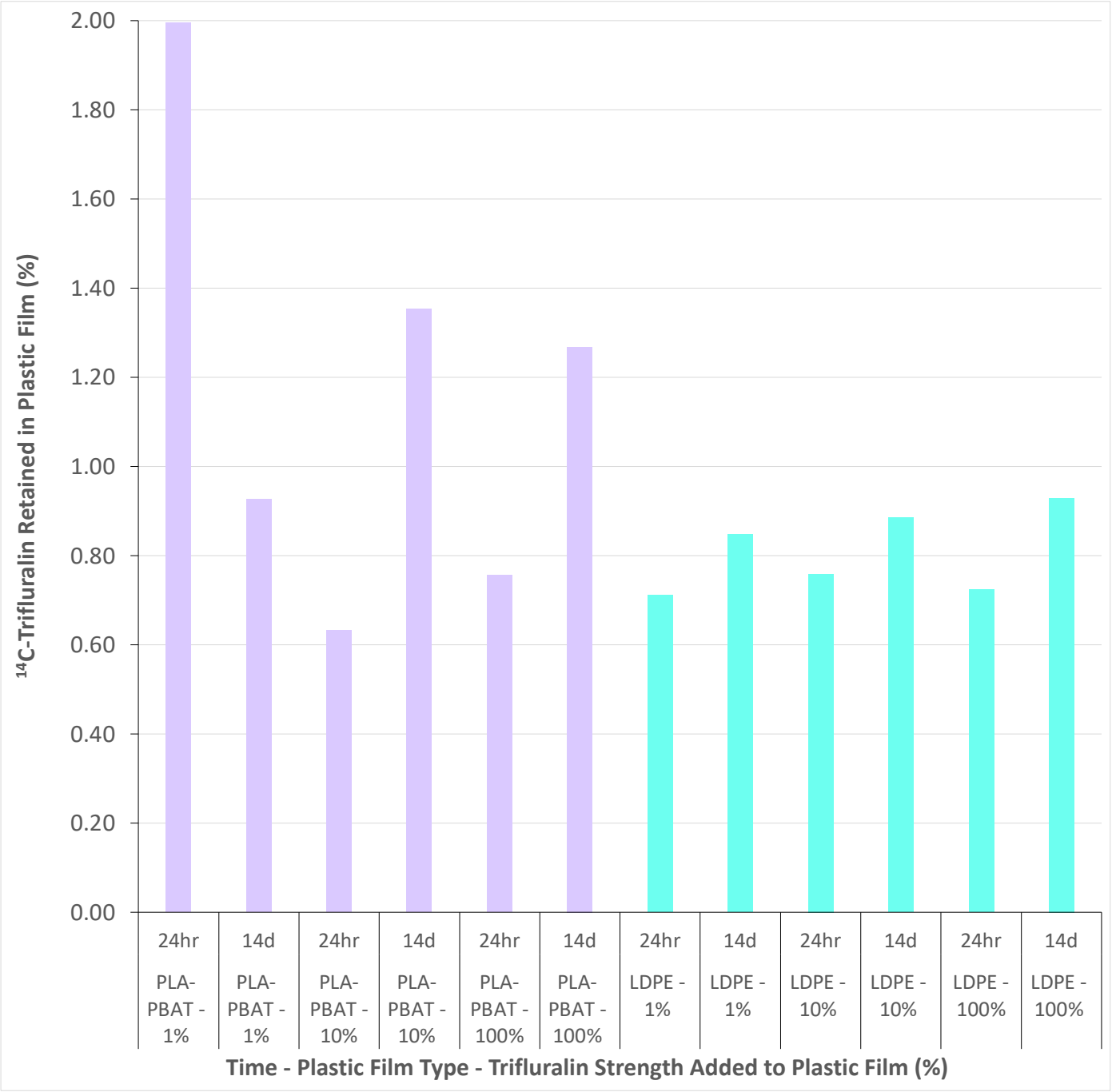


Fig. 3.1.4. Trifluralin retention within the plastic matrix of films LDPE and PLA-PBAT over 24 hours and 14 days.

This data was quantified via the oxidisation of plastic films. Values are negligible, trifluralin retained within the plastic matrix is minute. Looking at the trends in data, it could be assumed that PLA-PBAT samples retained more trifluralin than the LDPE samples. However, differences between films were insignificant; over 24 hours (p = 0.658) and over 14 days (p

= 0.077). This data reflects the fact that trifluralin strongly adsorbed to the films as described in sections 3.1.1. and 3.1.2.

3.2. TRIFLURALIN AND DEHP TOLERANCE IN *LEMNA MINOR*

Overall, the results suggested that *Lemna minor* was shown to be tolerant to both trifluralin and DEHP exposure.

RGR	PLA-PBAT 1% + DEHP	PLA-PBAT 10% + DEHP	PLA_ PBAT 100% + DEHP	Control	Water 1% + DEHP	Water 10% + DEHP	Water 100% + DEHP
Sample 1	2.95	3.10	2.68	2.68	3.01	2.95	2.68
Sample 2	2.92	2.84	3.01	3.26	2.84	3.16	2.77
Sample 3	3.01	3.10	3.42	2.84	2.84	2.73	3.16
Sample 4	2.92	3.05	3.33	2.81	2.77	2.73	2.88
Mean	2.95	3.02	3.11	2.90	2.87	2.89	2.87
SEM	0.02	0.06	0.17	0.13	0.05	0.10	0.10

Table. 3.2. Relative growth rate (RGR) of *Lemna minor* exposed to trifluralin concentrations at 1, 10 and 100% and 10 µl of DEHP over 6 days.

The relative growth rate (RGR) of *L. minor* over 6 days is presented in table 3.2. The equation used to quantify RGR of *L. minor* was derived from Magahud and Dalumpines (2021). Unlike Magahud and Dalumpines (2021), this work did not consider fronds that protruded from the mother frond, only fronds that had seperated from the mother frond. The RGR equation is presented below.

$$RGR = \frac{[ln(Nti)] - [ln(Nto)]}{ti - to}$$

ln is natural logarithm; *Nti* is frond number at day number; *Nto* is frond number at day 0; *ti-to* period between *Nti* and *Nto* expressed in days.

As described by Vymazal (2008), frond number can double in two or three days under optimum conditions. In table 1, all samples have more than doubled over 6 days. However, this does not consider the fronds that protruded from the mother frond and not separated, which the equation considers. It could be assumed that if these were considered frond count may have quadrupled.

L. minor was shown to not differ in frond count between exposure to various trifluralin concentrations (1, 10 and 100%) along with 10 µl of DEHP adsorbed to the surface of PLA-PBAT and various trifluralin concentrations in water ($p = 0.866$, $p = 0.216$ and $p = 0.143$, respectively). Further, both the PLA-PBAT + DEHP samples ($p = 0.484$) and water samples ($p = 0.988$) were not significantly different from the control treatment in terms of frond number, indicating tolerance by *L. minor* to both trifluralin at differing concentrations and DEHP. One limitation of this study is that the DEHP added to each PLA-PBAT sample may have not completely adsorbed/absorbed to the surface of the plastic as this was not quantified.

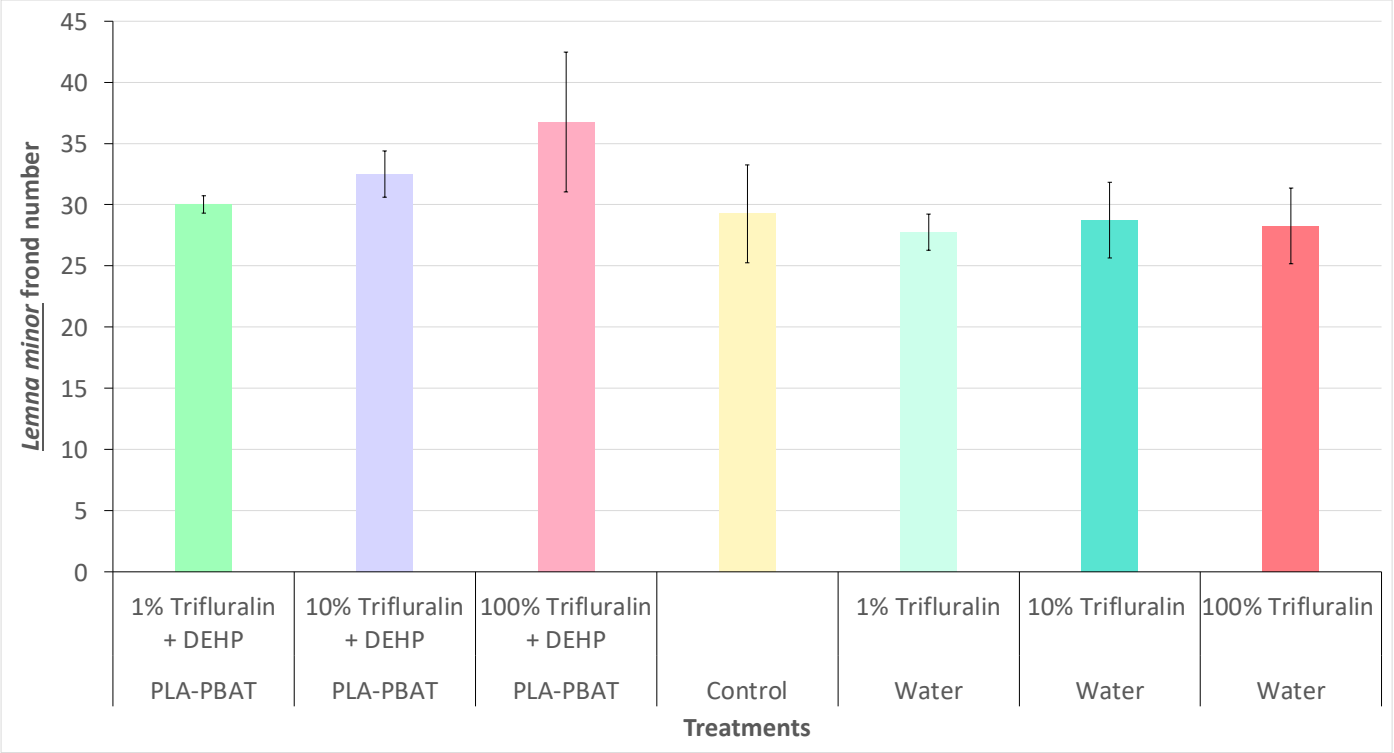


Fig. 3.2. Response of *Lemna minor* (frond number) to trifluralin and DEHP exposure on the surface of PLA-PBAT plastic mulch film suspended in the water column. Values represent means ± SEM (n = 4).

3.3. TRIFLURALIN MINERALISATION VIA LDPE, PLA-PBAT AND SOIL

3.3.1. Trifluralin mineralisation in soil

Mineralisation rates of trifluralin between treatments were non-significant ($p = 0.416$).

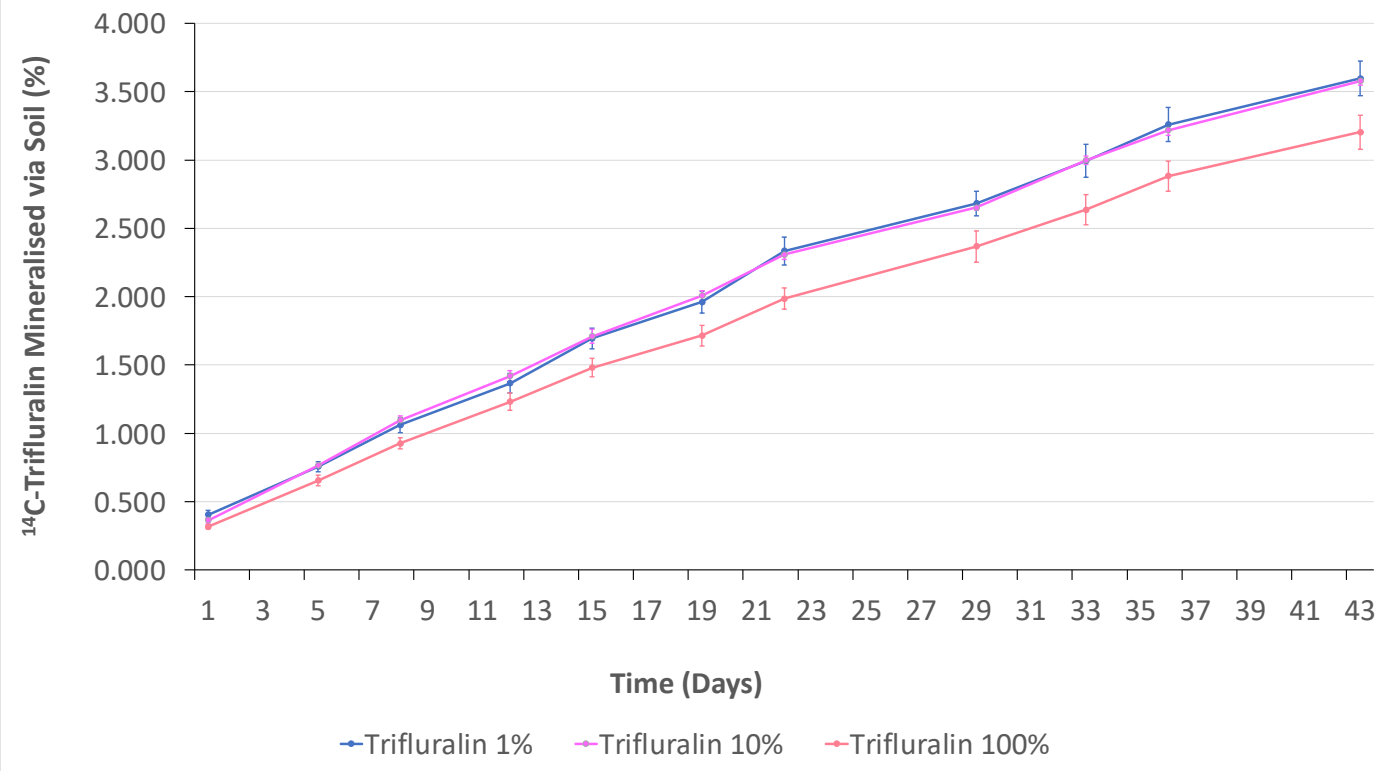


Fig. 3.3.1. Mineralisation of trifluralin via direct application of trifluralin to soil. Trifluralin concentrations 1, 10 and 100%. Values represent means ± SEM ($n = 4$).

3.3.2. Trifluralin mineralisation via LDPE and PLA-PBAT within soil

Treatments LDPE 1% and PLA-PBAT 1% had a much higher mineralisation rate of trifluralin compared to the 10% and 100% treatments ($p < 0.001$) indicating that a lower concentration of trifluralin was mineralised at a faster rate. A similar trend is seen here to the data showing mineralisation of trifluralin via soil, the 1% treatment had the highest percentage of trifluralin mineralisation, with 10% then 100% treatments following. However, differences between plastic mulch films were minor. Films with 1% trifluralin adsorbed to the surface had no

significant difference depending on film type (p = 0.935) nor did the 10% treatment (p = 0.352) and the 100% treatment (p = 0.554). Treatments LDPE 1% and PLA-PBAT 1% had greater mineralisation compared to treatment trifluralin 1% (In Soil) p (< 0.001), indicating that plastic films aided in higher mineralisation of trifluralin with a lower concentration of herbicide (1%). In treatments with 10% and 100% trifluralin concentrations however, mineralisation between trifluralin adsorbed onto plastic films and trifluralin added directly to the soil was negligible; 10% treatments (p = 0.248) and 100% treatments (p = 0.208).

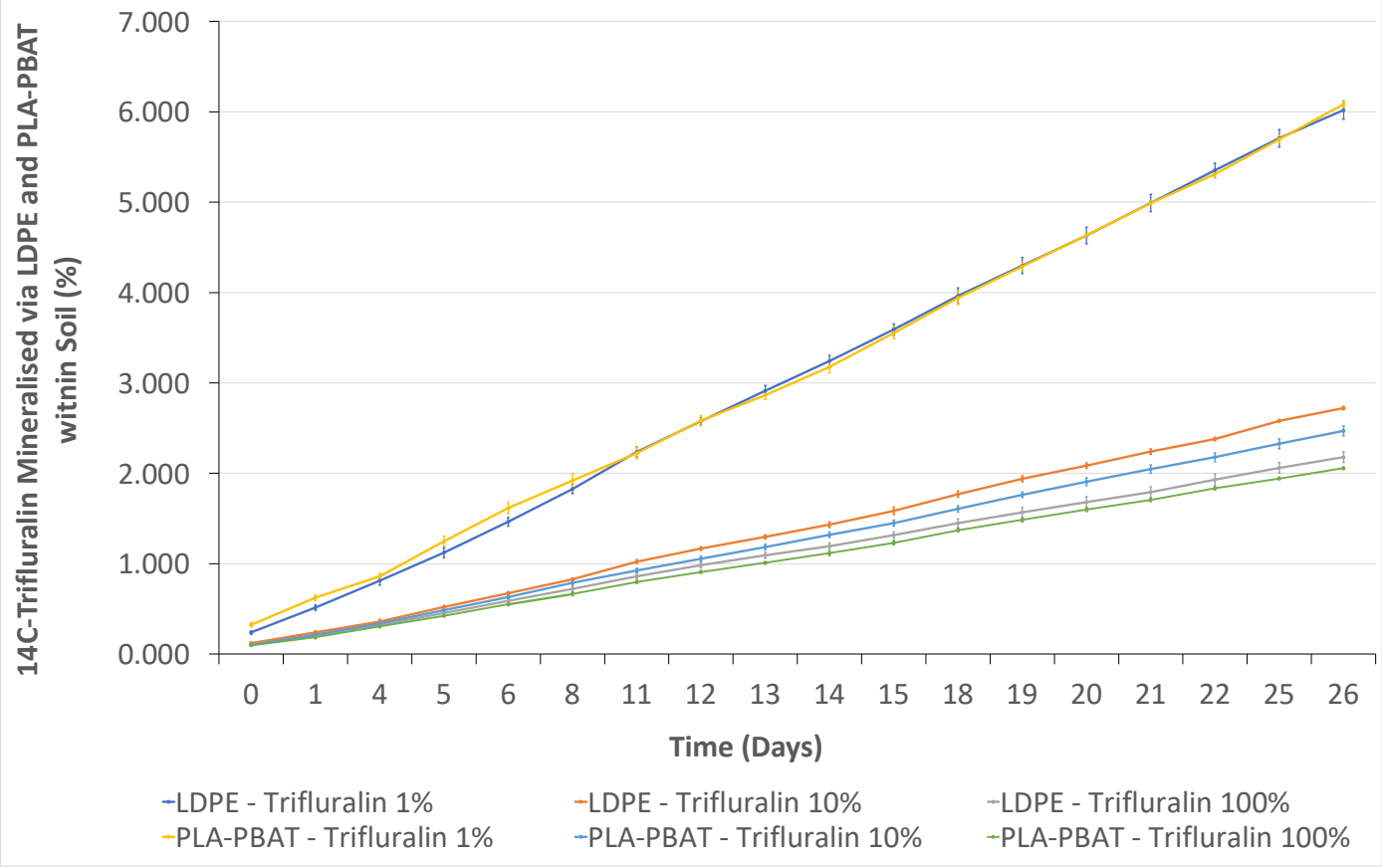


Fig. 3.3.2. Mineralisation of trifluralin via application to plastic mulch films LDPE and PLA-PBAT. Trifluralin concentrations 1, 10 and 100%. Values represent means ± SEM (n = 4). LDPE 100% treatment (n = 3).

3.3.3. Limitations

Initially it would have been better to test the mineralisation rates via plastic mulch films and directly to soil using the same trifluralin stock solutions. However, an error was made when adding radioactive ^{14}C activity (not enough activity was added). This meant that the data was null and the soil aspect of the experiment had to be repeated. Therefore mineralisation rates will not be completely comparable as activity added was slightly different. Also, treatment LDPE trifluralin 100% in figure 3.3.2. had three replicates rather than four due to an error whilst preparing samples. Furthermore, the third replicate of LDPE trifluralin 100% in figure 3.3.2. had too much soil added to the sample.

3.4. BIOREMEDIATION OF ^{14}C-TRIFLURALIN VIA PLA-PBAT AND MICROBES DERIVED FROM *LEMNA MINOR*, FRESHWATER, AND FRESHWATER SEDIMENT

3.4.1. Bioremediation within experiment 1 and experiment 2

Treatment 1 of experiment 1 was significantly different from treatments 2 and 3 with treatment 1 having < 1.5% degradation over 25 days ($p < 0.001$). Treatments 2 and 3 were not significantly different from each other ($p = 0.627$) and had > 1.5% degradation over 25 days. Experiment 2 treatments were not significantly different from each other ($p = 0.778$) with degradation rates > 1.5% over 14 days. Treatment 1 between experiments 1 and 2 were not significantly different from each other ($p = 0.866$). Whereas treatments 2 and 3 were significantly different between experiments ($p = 0.001$) and ($p = 0.002$) respectively. A trend here is that experiment 2 had higher degradation rates of trifluralin, potentially due to an increase in microbial communities.

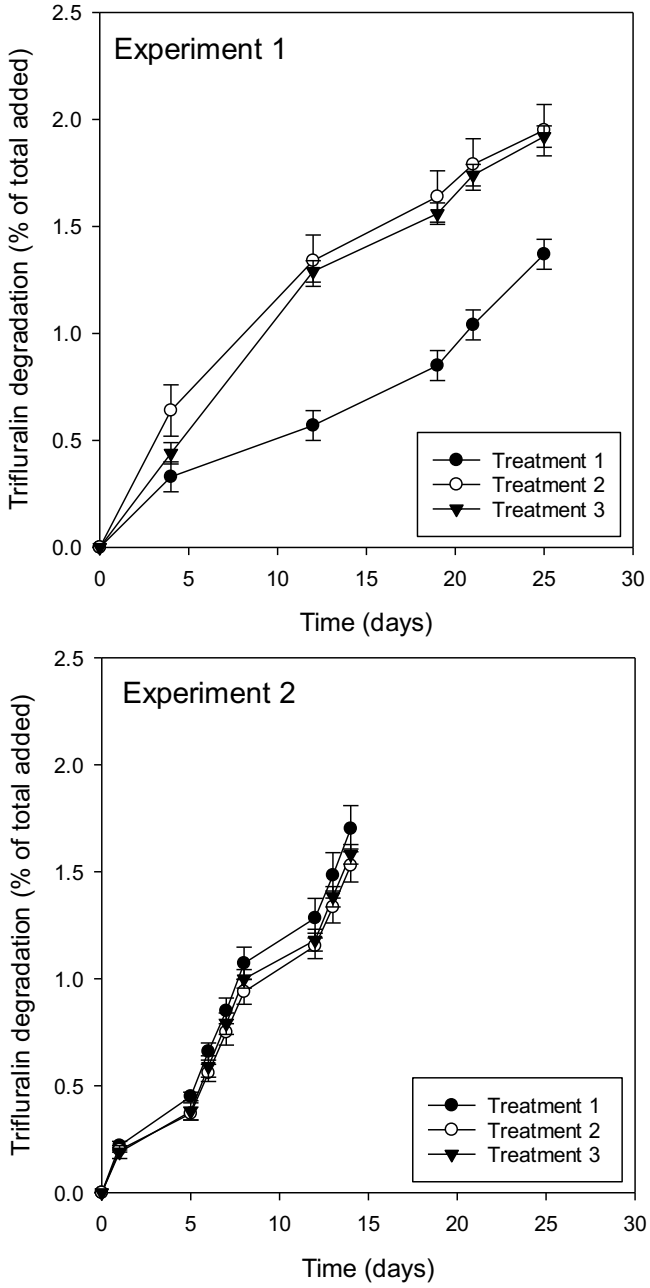


Fig. 3.4.1. Bioremediation and phytoremediation of trifluralin via application to PLA-PBAT plastic film or directly to freshwater. Trifluralin concentration 100%. Experiment 1 involved phytoremediation by microbes via duckweed that had perished whereas Experiment 2 involved phytoremediation by microbes via duckweed that was alive. Treatment 1: (Trifluralin + PLA-PBAT) + (Duckweed + River Sediment + Freshwater). Treatment 2: (Trifluralin) + (Duckweed + River Sediment + Freshwater). Treatment 3: (Trifluralin) + (Duckweed + Freshwater). Values represent means ± SEM (n = 4).

3.4.2. Comparison of bioremediation between experiments within sections 3.3. and 3.4.

A comparison was made between trifluralin degradation via the PLA-PBAT film with 100% trifluralin concentration within soil (section 3.3.) and within a freshwater context (section 3.4.) Treatment 1: (Trifluralin + PLA-PBAT) + (Duckweed + River Sediment + Freshwater) with 100% trifluralin concentration via PLA-PBAT.

The conclusion can be drawn that *L. minor*, freshwater, river sediment and trifluralin via PLA-PBAT can equally bioremediate compared to degradation via PLA-PBAT within soil (section 3.3). There were no differences between trifluralin degradation via the PLA-PBAT film with 100% trifluralin concentration within soil (section 3.3) and experiment 1 treatment 1 ($p = 0.215$) nor when comparing with experiment 2 ($p = 0.776$).

3.4.3. Limitations

A limitation of this study is the error that was made during experiment 1 which resulted in the need to repeat the experiment (i.e. experiment 2). Sodium hydroxide traps were not removed during the day from the containers containing the plants preventing photosynthesis. Although phytoremediation would still have occurred due to the microbes present, this experiment needed to be repeated to determine if there was a difference in degradation perhaps due to a difference in microbial communities.

Experiment 2 initially had ≤ 30 fronds (a small spoonful) added per treatment in the second experiment. More fronds were added compared to the experiment stated in section 3.2. to reduce damage, as fronds were delivered matted together. Additionally, the fronds were very small. These fronds were delivered without roots. This resulted in having to add new duckweed on day 10 of the experiment. ≤ 15 of larger *L. minor* was added (less was added due to the increase in size). Thus, the same strain of *L. minor* was not used throughout experiment 2, limiting results.

CHAPTER 4: DISCUSSION AND CONCLUSIONS

4.1. MACROPLASTIC FILM SORPTION OF TRIFLURALIN

Trifluralin sorption to LDPE and PLA-PBAT plastic mulch films was quantified over 24 hours. PLA-PBAT adsorbed over 90% of trifluralin across all concentrations tested, exhibiting a stronger sorption response than LDPE. LDPE adsorbed over 50% of trifluralin. For both plastic films, sorption was highest in the 1% trifluralin treatment compared to the 10% and 100% treatments. Specifically, sorption to LDPE at 1% trifluralin was significantly higher than its 10% and 100% counterparts. Overall, the 1% concentration elicited the strongest sorption to the plastic films among the conditions evaluated. This may have derived from a decrease in sorption sites on the plastic mulch films as concentration of trifluralin increased, resulting in the filling of adsorption sites (Wang *et al*. 2012). Weathering of these plastic film types over time would increase the number of adsorption sites available, deriving from the formation of functional groups on the polymer surface containing oxygen or surface oxidisation (Fred-Ahmadu *et al.* 2020).

Over 14 days, PLA-PBAT maintained a strong trifluralin sorption response, with over 80% recovered, similar to the 24-hour results. However, sorption to LDPE dropped from 50% at 24 hours to 30% at 14 days. The sorption responses differed between films and timepoints. For PLA-PBAT, the 1% and 10% trifluralin concentrations exhibited greater sorption than the 100% treatment. In contrast, the 10% and 100% concentrations sorbed more strongly to LDPE compared to the 1% concentration at 14 days.

PLA-PBAT adsorbed higher trifluralin amounts versus LDPE over 24 hours and 14 days, indicating superior potential for absorbing organopollutants and also potentially for providing sorbent surfaces for remediating contamination in ecosystems. This may have been due to the lower crystallinity of PLA-PBAT compared to LDPE, presented in figure 4.1. PLA has a crystallinity of 15% (Zhang *et al*. 2012), PBAT of < 10% (Sousa *et al*. 2019), and LDPE of 30% - 50% (Yuan & Xu 2023). Lower crystallinity equates to greater sorption, as the crystalline area within a polymer is not suitable for sorption (Fred-Ahmadu *et al.* 2020).

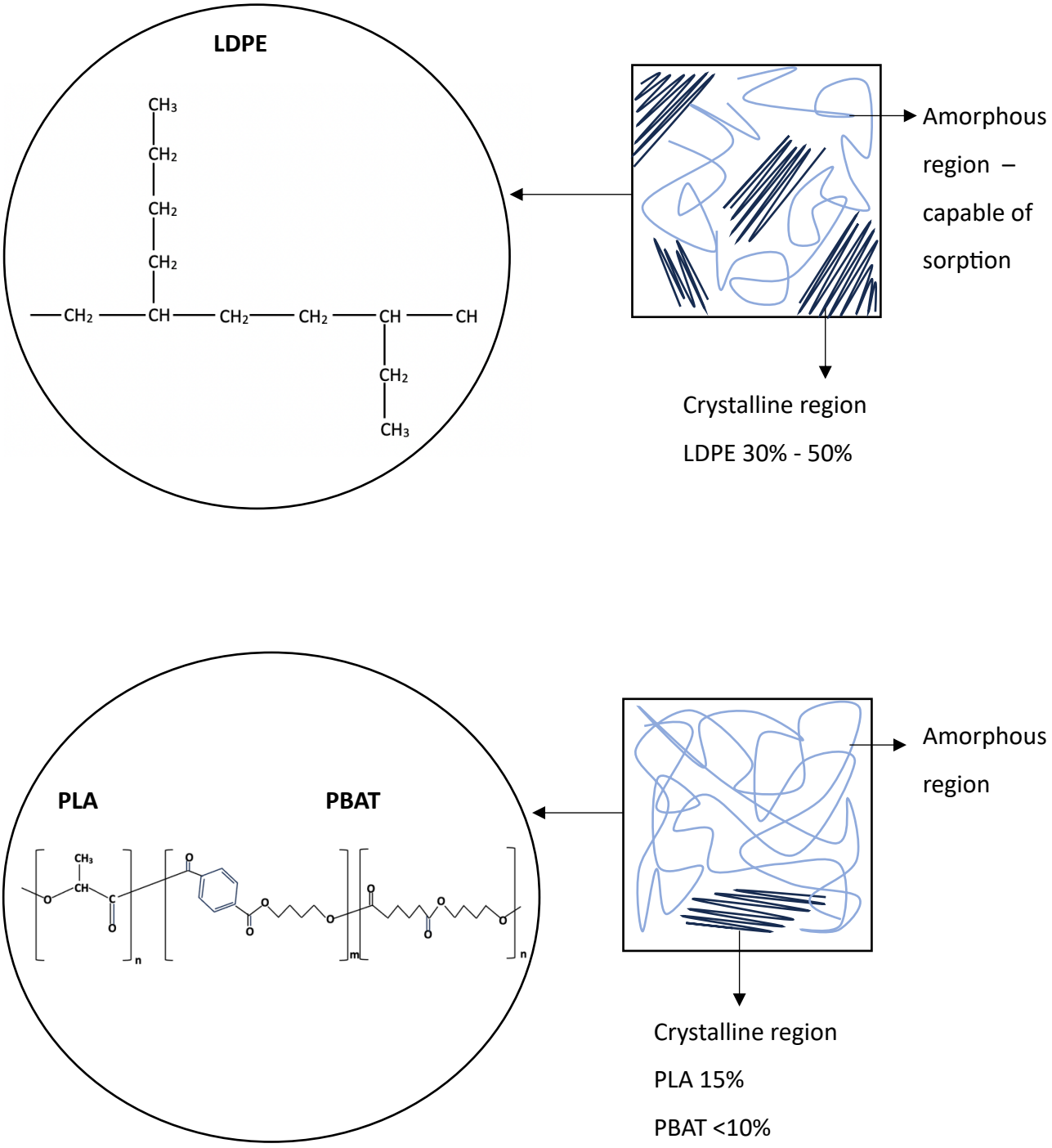


Fig. 4.1. Comparison of crystalline and amorphous regions of LDPE and PLA-PBAT.

Still, the 30-50% sorption to LDPE reveals promise for bioremediation approaches. Contrary to assumptions, sorption decreased from 24 hours to 14 days, potentially attributable to procedural errors or evaporation losses during extended pesticide exposure. The limited trifluralin retained within the plastic matrix, coupled with the efficacy of water washes in recovering sorbed trifluralin, verifies the strong binding to film surfaces rather than penetration into the internal polymer matrix. Overall, both PLA-PBAT and LDPE successfully adsorb trifluralin in freshwater conditions, supporting their possible application for environmental mitigation technologies.

Due to their higher surface area to volume ratio compared to the macroplastics (1.5 cm^2) tested here, microplastics may exhibit even greater sorption capacity (Song *et al.* 2015). Moreover, environmental prevalence of microplastics exceeds that of macroplastics, enhancing the feasibility of implementing bioremediation strategies based on these ubiquitous small plastic particles as bioavailability of plastic increases with a decrease in size (Song *et al.* 2015).

4.2. *LEMNA MINOR* TOLERANCE TO XENOBIOTICS

Results from section 3.2 suggested that *L. minor* is tolerant to trifluralin and the plasticiser DEHP. *L. minor* frond count did not differ between exposure to differing trifluralin concentrations (1,10 and 100%) and 10 µl DEHP to all treatments except for the control. Frond numbers also doubled over the six-day period, without considering fronds protruding from the mother frond that had not seperated. Thus, it could be assumed that frond count may have quadrupled if these additional fronds had been considered.

L. minor is known to be tolerant to xenobiotics (Ali *et al.* 2016; Crowley *et al.* 1997; Lewis. 1995) and the results presented here are in accordance with this. Tolerance specifically to trifluralin has been shown by Knežević *et al.* (2016) and Fairchild *et al.* (1997). Whether *L. minor* is tolerant to the transformation products of trifluralin, however, remains unknown. Further tests over longer time periods are still needed. Golab *et al*. (1979) identified 28 transformation products from the mineralisation of trifluralin in an anaerobic field soil in a three-year study.

L. minor was shown to be tolerant (section 3.2) to the plasticiser DEHP. *W. arrhiza*, another duckweed species was studied by Kotowska *et al.* (2018) to determine the effectiveness of the species to degrade the eight most frequently detected phthalates in freshwaters, including DEHP. After 24 hours, a 59% concentration reduction occurred for the phthalate DEHP. This was much greater compared to the ≤ 1.64% reduction of trifluralin over 14 days via *L. minor* within experiment 2 of the bioremediation experiment presented in section 3.4. Consequently, duckweed species should also be suitable for phytoremediation strategies designed to remove phthalates from freshwater.

4.3. TRIFLURALIN BIOREMEDIATION VIA MICROBES

4.3.1. Soil microbes that mineralise trifluralin

It is unknown what microbes were present in the bioremediation experiment presented in section 3.3, however, there are some known trifluralin degraders within soil that may have been present in the soil used in this experiment. Bellinaso *et al.* (2003) identified trifluralin degrading bacteria; *Klebsiella* sp., *Herbaspirillium* sp., *Bacillus* sp., and *Pseudomonas* sp. The research aimed to identify trifluralin-resistant bacteria from a soil where trifluralin had been in used for four decades and to quantify their mineralisation ability. *Klebsiella* sp. reduced the amount of trifluralin tested (50 mg 1^{-1}) after 30 days by 24.6%, *Herbaspirillium* sp. by 16.4%, and two strains of *Bacillus* sp. by 16%. *Pseudomonas* sp., *Bacillus* sp., and *Klebsiella* sp. have been cited as degraders of xenobiotics (Bellinaso *et al.* 2003; Sato. 1992; Spanggord *et al*. 1991). Furthermore, other bacterial genera have been cited as trifluralin degraders; *Moraxella* sp. degraded trifluralin by 95% after 28 days (Sato. 1992) and *Brevundimonas diminuta* is a known trifluralin metaboliser (Bellinaso *et al*. 2003; Hamdi and Tewfik. 1969).

Pseudomonas sp. have shown to bioremediate trifluralin effectively in soil. *Pseudomonas* sp. degraded 95% trifluralin over 21 days in a study conducted by Hamdi and Tewik (1969). *Pseudomonas* sp. originated from soil of a cotton field that had been treated with trifluralin. Koksoy and Uraz (2023) found in a laboratory study quantifying the most effective medium for trifluralin biodegradation that *Pseudomonas aeruginosa* in the presence of activated carbon biodegraded trifluralin at a rate of 99.3% and performed best over 72 hours. However, Bellinaso *et al.* (2003) found three *Pseudomonas* sp. that reduced levels by < 5%. This demonstrates how

there can be variability within genera of the ability to degrade trifluralin and that not all species of a certain genus can degrade trifluralin as effectively.

Golab *et al*. (1979) identified a transformation product of trifluralin; α, α,α trifluorotoluene-3,4,5-triamine, that could not be recovered from field soil and was the major compound in soil residues, whereas the other 27 transformation products identified could be recovered from the soil. Metabolism of α, α,α trifluorotoluene-3,4,5-triamine will depend on soil microbial communities.

Bioremediation of trifluralin via LDPE and PLA-PBAT mineralised at the highest rate with a concentration of 1%. This was also the case with the 1% treatment of trifluralin directly applied to the soil. Comparing these treatments, treatments LDPE 1% and PLA-PBAT 1% had significantly higher mineralisation compared to Trifluralin 1% (in soil), therefore aiding bioremediation. The 1% trifluralin concentration treatment may have performed the best due to lower toxicity levels of trifluralin and/ or its transformation products, or perhaps due to a greater amount of trifluralin available to be mineralised at the 1% concentration due to stronger sorption to LDPE and PLA-PBAT.

The higher the K_{OC} value (organic carbon partition coefficient) of an organic chemical such as a pesticide, the stronger it will bind to the soil (Antonious. 2012). Trifluralin has a high K_{OC} value (8000 ml g^{-1}) therefore the herbicide binds extremely well to soil particles (Antonious 2012). The soil organic matter (SOM) content of the soils used for experiments in section 3.3 were generally high (5.9% for the soil sample used for experiment in section 3.3.2., and 5.6% for the soil used in the experiment in section 3.3.1). Humified substances are the major component of soil organic matter (Antonious. 2012). According to Tavares and Rezende (1998) the carboxylic and phenolic groups of humic substances were the key sites of adsorption and mineralisation of trifluralin within soil. Due to the high K_{OC} and high SOM values, trifluralin bound strongly to the soil used in the experiments in section 3.3, thus facilitating mineralisation. However, mineralisation rates were not completely comparable due to the need to repeat the soil aspect of the experiment, therefore differences in soil organic matter between experiments are null.

4.3.2. Trifluralin degradation in freshwater ecosystems

It is unknown what microbes were present in the bioremediation experiment presented in section 3.4, however, there are some known trifluralin degraders within freshwaters that may have been present in this experiment. Furthermore, there are fewer microbes known to specifically biodegrade trifluralin in freshwater ecosystems compared to soil. Zablotowicz *et al*. (2001) identified the bacteria *Pseudomonas fluorescens* and *Pseudomonas putida* from the Mississippi Delta oxbow lakes under microaerophilic conditions that were capable of metabolising trifluralin (used in agricultural practices in the local area). Cultures were isolated from three lakes. Of these, 53 of 54 isolated bacteria degraded trifluralin via nitro reduction.

The repeat of the bioremediation experiment (experiment 2) due to the lack of photosynthesis, presented in section 3.4 had a higher degradation rate of trifluralin, compared to experiment 1. This may have been due to an increase in microbial communities along the rhizosphere (Crowley *et al.* 1997; Cunningham *et al.* 1997). *L. minor*, freshwater, river sediment and trifluralin via PLA-PBAT (100% trifluralin concentration - section 3.4.) can equally bioremediate compared to degradation via PLA-PBAT within soil PBAT (100% trifluralin concentration - section 3.3.). Therefore showing equal potential for bioremediation strategies concerning the film PLA-PBAT using *L. minor* when compared to soil. Epiplastic biofilms and biofilms located on *L. minor* may have aided bioremediation (Kalčíková 2020; Rai *et al.* 2021; Shiu *et al.* 2020).

Bioremediation via plastic mulch films presented in sections 3.3. and 3.4. was low. A maximum of 3.2% was mineralised via the treatment LDPE 1% over 14 days in soil as shown in figure 3.3.2. A maximum of 1.64% was degraded in Treatment 3 of Experiment 2 over 14 days in freshwater as shown in figure 3.4. Previously identified microbiota that degrades trifluralin at a high rate if present may have been present at lower populations, outcompeted by other microbial communities, or may have been non-competitive in these specific niches.

4.4. CONCLUSIONS

The key findings of this study were that PLA-PBAT was the most useful film for bioremediation/ phytoremediation strategies to remove trifluralin from soil and freshwaters as it adsorbed the highest amount of trifluralin. However, LDPE is a good candidate also. A conclusion can be drawn that *L. minor*, freshwater, river sediment and trifluralin via PLA-PBAT have equal bioremediation potential compared to degradation via PLA-PBAT within soil. Regarding bioremediation of trifluralin within soil, trifluralin via LDPE and PLA-PBAT and treatments (In Soil) were mineralised at the highest rate at a concentration of 1%. Comparing these treatments, treatments LDPE 1% and PLA-PBAT 1% had significantly higher mineralisation compared to Trifluralin 1% (In Soil), therefore the plastic mulch films aided bioremediation. Further investigation is needed to quantify the specific microbes that metabolise trifluralin on *L. minor* and other freshwater macrophytes as well as specific microbes that metabolise other pesticides within freshwaters as this is a large gap within the literature. *L. minor* has shown to be tolerant to trifluralin and will therefore most likely be tolerant to its transformation products. However, this has not been tested over a large expanse of time (experiments undertaken had a range of 6-14 days), so further investigation is needed to determine whether *L. minor* can be tolerant to trifluralin and its transformation products over a longer period of time. Additionally, sorption of trifluralin to LDPE and PLA-PBAT could be quantified using microplastics of the film rather than macroplastics to evaluate if greater surface area to volume ratio increases trifluralin binding. The plastic mulch films could also be tested to assess whether biofilm accumulation differs depending on whether the films are weathered or pristine ultimately affecting sorption of pesticide. Further, trifluralin removal efficiencies could be piloted by pumping contaminated water through columns with *L.minor* and plastic mulch fragments.

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