**Lancaster Environment Centre**
**Faculty of Science and Technology**
**Lancaster University**

# Investigating the Efficiency and Toxicity of Biochar Adsorption of Aqueous Pesticides and Metals on *Daphnia* spp.

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BSc. (Hons), MSc.

Submitted for the degree of Doctor of Philosophy

November 2024.

# Declaration

This is to certify that:

i. This thesis comprises only my original work towards a PhD except where otherwise indicated.

ii. Appropriate acknowledgement has been made for all materials used; as in-text citations and compiled in the reference list.

iii. This project has been conducted in compliance with the University's Research Ethics Policy and has not been submitted for a higher degree elsewhere.

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November 2024.

# Abstract

# Investigating the Efficiency and Toxicity of Biochar Adsorption of Aqueous Pesticides and Metals on *Daphnia* spp.

The increase in the frequency and extent of aquatic pollution by pesticides and metals remains a serious worldwide problem, largely due to anthropogenic industrial activities since the latter half of the nineteenth century. Pesticides and heavy metals, which are frequently reported to exceed regulatory limits, have direct and indirect impacts on the aquatic ecosystem, pose environmental and health risks to humans and animals, and are more prevalent in developing countries than in their developed counterparts. Biochar, a well-studied and established carbonaceous soil amendment, has recently been investigated as a viable adsorbent for water pollutants.

This thesis evaluates the aqueous adsorption of Isoproturon (IPU) by biochar derived from agricultural wastes: rice husk (RH), hardwood (HW), and coconut shells (CS), compared with commercial activated carbon. Secondly, the acute toxicity of untreated and carbon-amended (0.001 -10 mg $L^{-1}$) IPU, cypermethrin (CP), and the heavy metals arsenic (As), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) on *D. magna* and *D. pulex* over 72- 96 h without feeding, decreased in the order: Ni > Cd ≈ Cu ≈ As > Zn > Pb. . Thirdly, the acute toxic effects of biochar treatment of CP, IPU, As, and Cd on the percentage and cumulative $CO_2$ production of *D. pulex* were studied. Integrating $LD_{50}$, $EC_{50}$, and condition index analyses in the evaluation of Daphnia ecotoxicity suggests that the toxicity of all tested pesticides and metals followed the decreasing order Cypermethrin > Cadmium ≈ Copper > Zinc ≈ Nickel > Lead > Arsenic > Isoproturon. This ranking reflects both acute and chronic toxicity patterns observed in the experiments.

The overall findings reveal that the adsorption capacities for IPU were in the decreasing order: PAC (97%) > RH biochar (91%) > HW biochar (68%) > GAC (67%) > CS biochar (33%). RH-biochar adsorption of IPU was higher than that of GAC. The respiration of *D. pulex* in treated and untreated pesticide and metal solutions was significantly affected by the bioavailability of the pollutants, their mode of action on the organism, and the organism's ability to acclimatize to these effects. Furthermore, although biochar amendment of the solutions reduced their toxicity, it also reduced the oxygen levels. This suggests that biochar treatment of water in closed systems could lead to synergistic toxicity between hypoxia and the compounds present.

Finally, CP was approximately 100-fold more toxic than IPU, and D. pulex was about 10-fold more sensitive to both pesticides than *D. magna*, which showed higher resistance to the toxic effects of all pollutants tested except As, Cd, and Zn. RH-biochar is an effective and promising adsorbent for removing pesticides and heavy metals from aquatic systems and could be an environmentally friendly, cost-effective, and efficient technology, but further research is needed to determine production parameters that suit the pollutant types it is intended to treat.

*Keywords*: Adsorption, Biochar, Pesticides, Metals, Toxicity, *Daphnia*

# Dedication

To God, thank you for making this dream a reality.

To my precious and ever-loving husband and children – you all own this!

NDDC
Niger Delta Development Commission
*Determined to Make A Difference*

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# Glossary of Terms

% - Percentage

ANOVA – Analysis of Variance

ANOVA- Analysis of Variance

As- Arsenic

Cd- Cadmium

CP- Cypermethrin

CS- Coconut Shell

Cu- Copper

DDT - Dichlorodiphenyltrichloroethane

d-i – deionized water

DMSO- Dimethyl Sulfoxide

dpm - disintegrations per minute

EA - Environment Protection Agency

EDCs- Endocrine Disrupting Compounds

g – gram

GAC- Granular Activated Carbon

h - hours

HH Combo – High Hardness Combination Medium

HW- Hardwood

IPU – Isoproturon

$LC_{50}$ - Lethal Concentration 50

MCLs - Maximum Contaminant Limits

MeOH- Methanol

mg – milligram

Ni- Nickel

OCPs - Organochlorine pesticides

PAC- Powdered Activated Carbon

Pb- Lead

POPs -Persistent Organic Pollutants

RH- Rice Husk

RM – Repeated Measures

rpm - rotations per minute

SDG- Sustainable Development Goals

spp.- species

UK- United Kingdom

UN- United Nations

USEPA - United States Environmental Protection Agency

VOC- Volatile Organic Compounds

Zn – Zinc

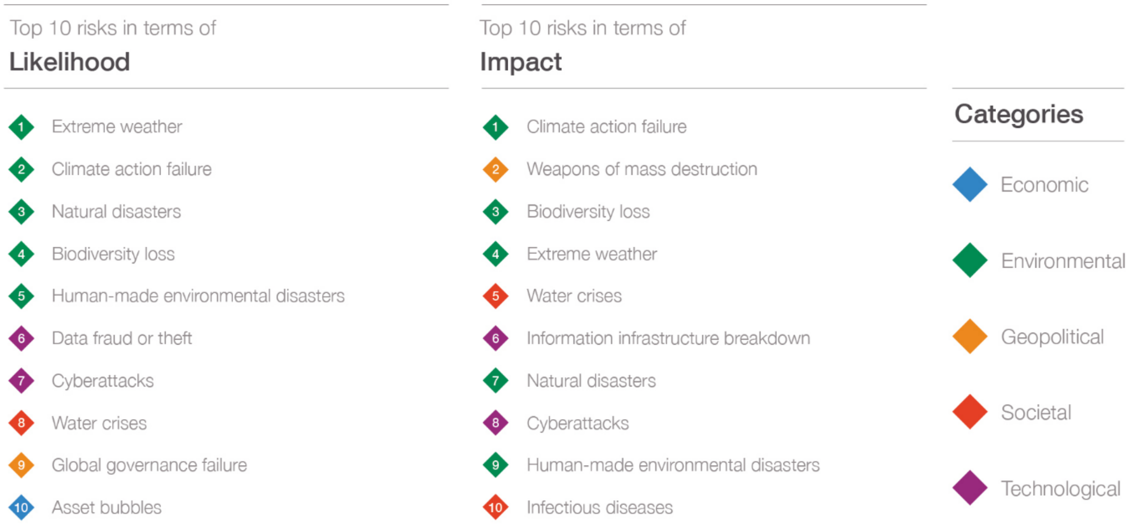
# Chapter One

# General Introduction and Literature Review

## 1.1. Water Pollution

Anthropogenic pollutants cause significant environmental harm, leading to soil, air, and water pollution, and affecting developed and developing countries (Ukaogo *et al.*, 2020; National Water Quality Report to Congress, 2009). Water pollution, one of the three significant forms of pollution, is a critical issue due to global water scarcity and projections of clean water scarcity by 2025 (Edo *et al.*, 2024; UN World Water Development Report, 2018). In 2021, the world's population in water-stressed countries was estimated at over 2 billion and is forecasted to increase in some regions due to climate change and population growth (World Health Organization, 2023).

Recent scientific findings have highlighted an increasing trend in human water use and a decreasing per-capita availability of freshwater for domestic, agricultural, and industrial use (Ogou *et al.*, 2022; Huang *et al.*, 2021). According to Dangui and Jai (2022), water demand is projected to increase by 55% by 2050. Consequently, around 40% of the world's population is expected to live in regions facing severe water stress. Factors contributing to this situation include population growth, rising living standards, and climate change. (Dangui & Jia, 2022).



| | Top 10 risks in terms of **Likelihood** | | Top 10 risks in terms of **Impact** |
|---|---|---|---|
| 🟩 1 | Extreme weather | 🟩 1 | Climate action failure |
| 🟩 2 | Climate action failure | 🟧 2 | Weapons of mass destruction |
| 🟩 3 | Natural disasters | 🟩 3 | Biodiversity loss |
| 🟩 4 | Biodiversity loss | 🟩 4 | Extreme weather |
| 🟩 5 | Human-made environmental disasters | 🟥 5 | Water crises |
| 🟪 6 | Data fraud or theft | 🟪 6 | Information infrastructure breakdown |
| 🟪 7 | Cyberattacks | 🟩 7 | Natural disasters |
| 🟥 8 | Water crises | 🟪 8 | Cyberattacks |
| 🟧 9 | Global governance failure | 🟩 9 | Human-made environmental disasters |
| 🟦 10 | Asset bubbles | 🟥 10 | Infectious diseases |

**Categories**

| | |
|---|---|
| 🟦 | Economic |
| 🟩 | Environmental |
| 🟧 | Geopolitical |
| 🟥 | Societal |
| 🟪 | Technological |

Figure 1: Excerpt from the Global Risks Report 2020, World Economic Forum.

The Global Risk Report has placed water crises in its top five global risks for several years based on their impact on society. In the 2020 report, water comes after weapons of mass destruction and extreme weather events and ahead of natural disasters. Consequently, for the next decade, the water crisis remains the most significant risk of concern (Dos Santos *et al.*, 2017; World Economic Forum, 2020), and environmental issues have remained top priorities over the years (Figure 1).

Water pollution by organic and inorganic chemicals has been a concern for several decades and remains a significant environmental issue affecting terrestrial and aquatic ecosystems. Aquatic pollution occurs when harmful chemicals, pathogens, and debris enter water bodies, lower water quality and posing a significant threat to human health and the ecosystem. As of 2011, water pollution by pesticides, fertilizers, and metals, among numerous pollutants, had intensified over three decades, owing to industrialization and conventional agricultural activities (Mohan *et al.*, 2011; Geiger et al., 2010; Gibbs et al., 2009; Dutcher, 2007). These contaminants affect human health, contaminate food sources, and create non-target effects on wildlife, functional agrobiodiversity, and ecosystems (Ogwu & Izah, 2023; Ukaogo *et al.*, 2020).

## 1.2. Pollution in Aquatic Systems

According to Goel (2006), water pollutants are physical, chemical, or biological substances that can cause aesthetic effects such as discoloration on aquatic organisms or on people who consume water. They are broadly classified as organic and inorganic.

### 1.2.1. Sources of Aquatic Pollutants

Water pollution, while a significant issue, is largely preventable and can originate from natural processes, industrial activities, agricultural practices, and urban and municipal waste. In general, atmospheric deposition of organic and inorganic pollutants, leachate from waste dumps or landfills, point source effluent discharges, agricultural runoffs from farms, urban runoffs from paved roads, untreated or partially treated sewage and solid waste dumping (Edokpayi *et al.*, 2017; Kabir *et al.*, 2019; Fayiga *et al.*, 2017; Patidar, 2015; Hill, 1997). Identifying pollution sources is critical for implementing effective preventive measures.  Figure 2 general sources of water pollution.

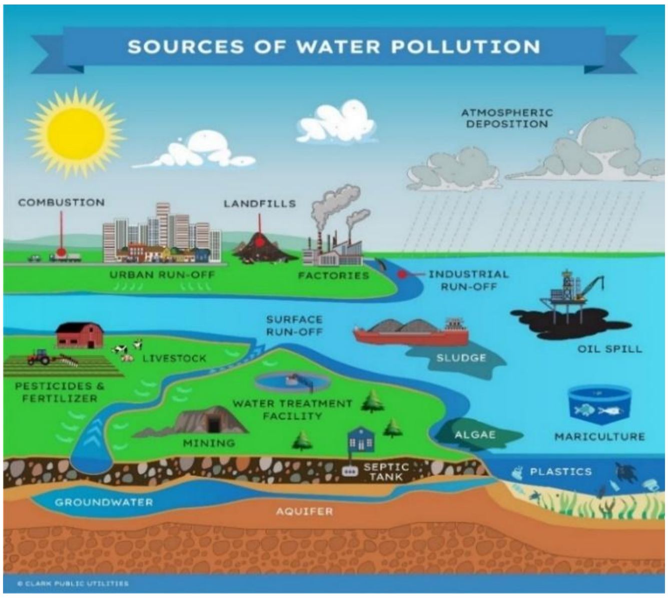


Figure 2: General Sources of Water Pollution (Source: Powerzone, 2024)

Emissions and releases to surface water, groundwater, and soil also occur through a range of processes, including deliberate discharge, spillage (for example, from storage, during transport, or processing and usage), leakage, and runoff (for example, agricultural chemicals), all of which are important in terms of aqueous pollutants. Many industries aim to keep contamination levels within accepted limits by setting legal limits for stream discharges.

Natural sources such as decayed organic matter and algae blooms can also contribute to the presence of organic pollutants in water bodies. While these sources may not have the same impact as human activities, they can still degrade water quality and harm aquatic ecosystems. Industries also release harmful substances, such as heavy metals and toxic chemicals, directly into water bodies or indirectly through air and land pollution (Patidar, 2015). Discharging these industrial effluents without proper treatment threatens water quality (US Environmental Protection Agency, 2017).

Table 1 below summarises water pollutants from agricultural activities, including crops, livestock, and aquaculture. Agricultural activities often contribute to water pollution through the excessive use of fertilizers and pesticides (Mateo-Sagasta *et al.,* 2018; Maghar *et al.,* 2018). Herbicides are usually released into the environment through agricultural practices, warehouse spillages, and domestic and industrial wastewater effluents, which pose a significant concern for environmental health (Li *et. al.,* 2017; Minh *et al.,* 2016; Pavlaki *et al.,* 2014; Alvez-Pereira *et al.,* 2012; Lapworth *et al.,* 2012; Lessard & Frost, 2012; Nitschke *et. al.,* 1999).

Table 1: Agricultural Sources of Water Pollutants and their indicators or examples (Source: Mateo-Sagasta et. al., 2018)

| **Pollutant Category** | **Indicators/ examples** |
|---|---|
| Nutrient | Nitrogen and phosphorus are present in organic fertilizers, animal droppings found in water as nitrate, ammonia, or phosphate |
| Pesticides | Herbicides, insecticides, fungicides and bactericides |
| Salts | Sodium ions, chloride, potassium, magnesium, sulfate, calcium, and bicarbonate. These are measured in water either directly as total dissolved solids or indirectly as electric conductivity |
| Sediments | Measured in water as total suspended solids or turbidity |
| Organic matter | Chemical or biochemical oxygen-demanding substances (e.g. organic materials such as plant matter and livestock droppings), which use up dissolved oxygen in water for degradation |
| Pathogens | Bacteria and pathogen indicators e.g. *E. coli*, total coliforms, faecal coliforms, and *enterococci* |
| Metals | Selenium, lead, copper, mercury, arsenic and manganese |
| Emerging contaminants | Drug residues, hormones, and feed additives |

Spray drift, volatilization, drainage, and leaching after boom spraying are the main pathways of herbicide dispersion (Lessard & Frost, 2012; Le *et al.*, 2010). Such chemicals can be washed into groundwater, rivers, and lakes, causing nutrient enrichment, eutrophication, and harmful algal blooms. The occurrence of these algal blooms results in hypoxia, an oxygen-depleted condition of the aquatic environment causing deadly effects on living organisms (Fletcher *et al.*, 2021; Maghar *et al.*, 2018; Smith *et al.*, 1999). Figure 3 illustrates the origins and pathways of agricultural water pollutants.

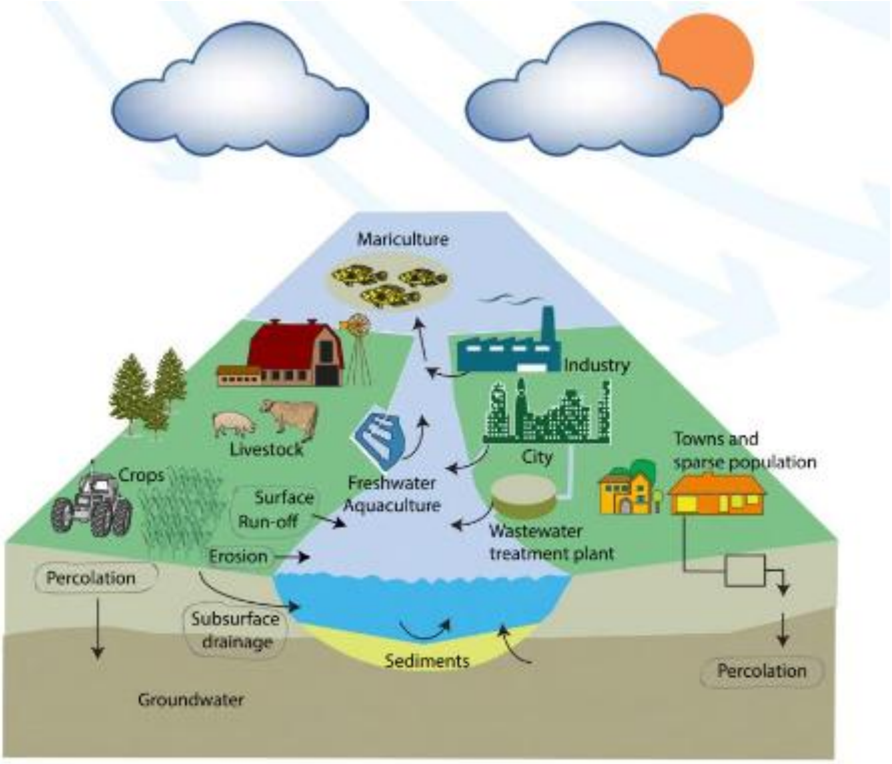


Figure 3: Sources and fates of Agricultural water pollutants (Source: Mateo-Sagasta et. al., 2018)

Wind and rain can wash pesticides from agricultural fields into nearby water bodies, making them toxic and unsuitable for various applications (Ajia, 2017) and altering the water's physical, chemical, or biological features. Herbicides are frequently used in agriculture, allowing these chemicals to reach the soil through various farming activities. These application modes include solid or granular deposition on crops, direct treatment of plants (foliar or root application), interception by plant structures (leaves), pesticide dust application, liquid spraying on soil, agrochemical industrial production processes, discharges from agrochemical facilities, effluents from production and animal treatment tanks, accidental chemical spills in storage or transport, and incorporation of herbicide-contaminated soil amendments (compost or manure) (Meena *et al.*, 2020; Ortiz-Hernandez *et al.*, 2013; Chauhan *et al.*, 2012; Akatar *et al.*, 2009). Other potential sources of environmental contamination with herbicides are weak storage and disposal practices of obsolete or prohibited chemicals, especially in low-income nations (Ortiz-Hernandez *et al.*, 2013; Dasgupta *et al.*, 2010).

Flow from urban surfaces such as roads, parking lots, and lawns contributes significantly to organic water pollutants (Edokpayi *et al.*, 2017). Pollutants include oil and grease, heavy metals, synthetic chemicals, pet waste, and litter. Urban stormwater runoff carries contaminants into rivers, lakes, and coastal waters, adversely affecting water quality and aquatic ecosystem health (Letchinger, 2000).

Sewage contains pathogens that can cause waterborne diseases such as cholera, typhoid, and dysentery. This presents a significant risk, especially in developing nations where sewer systems are often inefficient (Agbasi *et al.*, 2023).

Improper waste disposal significantly contributes to water pollution. Plastics, metals, and other debris accumulate in water bodies, harming aquatic life and degrading water quality. Plastics are particularly problematic because they persist in the environment and can break down into toxic microplastics that may enter the food chain and pose risks to human health. The disposal of solid waste into water bodies is a common practice in densely populated areas of developing countries with inadequate sanitation infrastructure (United Nations Environment Programme, 2018; Sharma *et al.*, 2016). Additionally, landfill sites can be sources of diverse pollutants that may affect distant areas, such as groundwater, through various environmental media (Wong *et al.*, 2012; Schmid *et al.*, 2011).

Moreover, climate change and melting ice caps have revealed glaciers as a secondary source of persistent organic pollutants (POPs). When glaciers melt, these long-accumulated pollutants are released into the environment (Schmid *et al.*, 2011). Furthermore, complex mixtures of EDCs from industrial chemicals, agrochemicals, and pharmaceuticals pose challenges in determining their origin in aquatic environments. Expectations of COVID-19 pandemic response will further introduce pharmaceuticals to these environments (Horn *et al.*, 2020). They might disrupt the regulation and expression of critical endocrine-related genes in fish species. Because EDCs generally contain hormone-active chemicals, they can have synergistic, additive, or even cumulative effects on aquatic organisms, potentially harming them (Jacobsen *et al.*, 2012; Ma *et al.*, 2019; Papalou *et al.*, 2019; Archer & Van Wyk, 2015).

The main categories of water pollutants are organic and inorganic. Chemical pollutants constitute the majority of water pollutants found in solution or suspension. These include prominent organic pollutants, such as pesticides, petroleum products, and by-products produced after the application of disinfectants (also identified as persistent organic pollutants (POPs)), as well as volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), specific emerging contaminants (EDCs), and natural organic matter (Chen *et al.*, 2015; Ifelebuegu, 2013; Wong *et al.*, 2012; Liu & Amy, 1993).

Inorganic pollutants include heavy metals and metalloids such as arsenic, cadmium, nickel, lead, and zinc, as well as micro- and macronutrients. Figure 4 shows these sources.

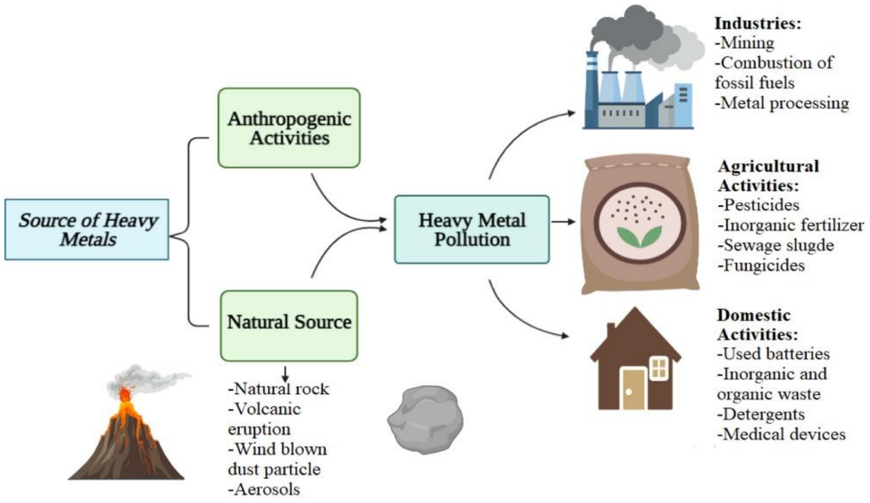


Figure 4: Sources of Metal Water Pollution (Adapted from Zamora-Ledezma et. al., 2021)

Metal pollutants that reach a water body may have their origins in both natural and human-made ecosystems: from processes of weathering of rocks and soils, respiratory deposition of toxic particulates, proper effluents of domestic (untreated) and industrial origin (such as those from paper, paint, and textile industries) (Park *et al.*, 2015; Valentín *et al.*, 2013).

## 1.3. Organic Pollutants in Aquatic Systems

Organic pollutants are harmful chemicals that can enter our water bodies from various sources, including industry, agriculture, pharmaceuticals, and urban areas. Understanding these sources is important because they contribute to the different types of organic contaminants we encounter. This knowledge is critical for effectively managing water quality. One group of organic water pollutants is the Persistent Organic Pollutants (POPs), such as Petroleum and related products, Nitroaromatic compounds, and Polycyclic Aromatic Hydrocarbons (PAHs), which are commonly found in soil due to their physicochemical properties and exist in low concentrations in water (Krithiga *et al.*, 2022).

A second group of organic water pollutants is emerging contaminants, called Pharmaceutical and personal care products (PPCPs), i.e., pharmaceuticals, disinfectant by-products, flame retardants, surfactants, per- and polyfluorinated compounds, benzotriazoles, and benzothiazoles, as well as transformation products and metabolites (Ifelebuegu, 2013; Valentín *et al.,* 2013; Fischer *et al.,* 2012). A typical example is Alkylphenol polyethoxylates and their metabolites. The widely used ethoxylate derivatives include nonylphenol and octylphenol, which are active ingredients in pharmaceuticals, disinfectants, surfactants, per- and polyfluorinated compounds, benzotriazoles, benzothiazoles, organometallic compounds, flame retardants, detergents, pesticides, wetting agents, emulsifiers, lubricants, and many cosmetic products. Other emerging chemical sources include pentabromo-diphenylether, bisphenol A, and di(2-ethylhexyl) phthalate (Ifelebuegu, 2013; Xu et al., 2013; Fischer et al., 2012; Wong *et al.,* 2012).

A third group, called priority organic water pollutants are grouped as Volatile Organic Compounds (VOC), base neutral extractable (like anthracene and benzo[a]pyrene), acid extractable (like pentachlorophenol and phenol), and pesticides, commonly found in groundwater (Wong *et al.,* 2012; Chen *et al.,* 2015; Ahmad *et al.,*2014; Xu *et al.,* 2012; Teixidó *et al.,* 2011; Beesley *et al.,*2010; Zheng *et al.,* 2010; Qiu *et al.,* 2009). Examples of VOCs commonly found in groundwater are trichloroethylene, carbon tetrachloride, benzene, and tetrachloroethylene, detected in groundwater supplies in California and later in other locations in the USA in the 1970s.

### 1.3.1. Pesticides

Pesticides are organic or inorganic substances that reduce populations of insect pests, weeds, plant pathogens, and other potentially damaging organisms to suitable levels (Ikpesu & Arlyo, 2013). They are commonly used to prevent, destroy, repel, or mitigate any pest ranging from insects (insecticides), rodents (rodenticides), and weeds (herbicides) to microorganisms (algicides, fungicides, or bactericides) and nematicides

(Yadav*et. al.,* 2015; Ikpesu & Arlyo, 2013; Wong *et al.,* 2012). Among the most effective are the chlorinated hydrocarbons (Ikpesu & Arlyo, 2013).

Pesticides boost crop yields, cut post-harvest losses, and enhance food security. They also help control insect-borne diseases such as malaria and typhus, thereby improving overall health and life expectancy. Pesticides have been instrumental in preventing crop losses caused by weeds, insects, and diseases, ensuring a stable food supply for growing populations.

## 1.3.2. Classification of Pesticides

Pesticides are typically categorized by the active ingredient's functional class and the type of organism they target, such as organophosphate insecticides, thiocarbamate herbicides, and triazine herbicides. Most commercial pesticide formulations are complex mixtures of active and other ingredients. To increase the cost-effectiveness and efficacy of these commercial pesticides, these 'other ingredients' may include solvents, mineral clays, stickers, and wetting agents. The information about these other ingredients is not publicly available and is considered proprietary business information.

### *1.3.2.1. Classification of Pesticides by Purpose or Target Organisms*

The United States Environmental Protection Agency (USEPA) classifies these agrochemicals by target organism (Singh *et al.*, 2020; USEPA, 2012). Pesticides are either classified by their purpose or target organisms. For example, pesticides that kill rodents are called rodenticides, pesticides that kill weeds or unwanted plants are called herbicides, and those that kill insects are called insecticides (Rasooly *et al.*, 2022). Table 2 below shows the different classes of pesticides by target organism and further highlights the purpose of each class.

Table 2: Pesticides Classifications based on their purpose (Adapted from Rasooly et al., 2022 and USEPA, 2012)

| Type of Pesticide | Purpose/ Target Pests |
|---|---|
| **Acaricides** | Kill members of the arachnid subclass such as ticks, mites that live on plants or livestock. |
| **Algaecides** | Kill and prevent the growth of algae e.g. in swimming pools |
| **Antimicrobials** | Kill microorganisms to prevent disease |
| **Attractants** | Attract specific pests using natural insect chemicals called pheromones, to confuse the mating behaviour of insects |
| **Avicides** | Control pest birds |
| **Bactericides** | Control bacteria |
| **Biopesticides** | Naturally occurring substances with pesticidal properties |
| **Fumigants** | Produce vapours or gases to control air- or soil-borne insects and diseases. Targets termites and bed bugs. |
| **Fungicides** | Destroy fungi that infect plants, animals or people |
| **Herbicides** | Kill weeds or undesired plants competing with farm produce |
| **Insect Growth regulators (IGRs)** | Accelerate or retard the growth of insect growth |
| **Insecticides** | Control or eliminate insects |
| **Larvicides** | Target insect larvae |
| **Molluscicides** | Kill snails and slugs |
| **Nematicides** | Kill nematodes (microscopic worm-like organisms that live in soil and cause damage to food crops. |
| **Ovicides** | Control insect eggs |
| **Piscicides** | Control pest fish |
| **Plant Growth Regulators (PGRs)** | Accelerate or retard the growth rate of a plant. Substances excluding fertilizers or other plant nutrients that alter the expected growth, flowering, or reproduction rate of plants. |
| **Predacides** | Control vertebrate pests |
| **Repellents** | Repel pests such as mosquitoes, flies, ticks and flea |
| **Rodenticides** | Kill mice, rats and other rodents |

#### *1.3.2.2. Classification of Pesticides by Mode of Action*

Mieldazys *et al.* (2016) proposed a more technical method for classifying pesticides by mode of action. They identified six classes of pesticides:

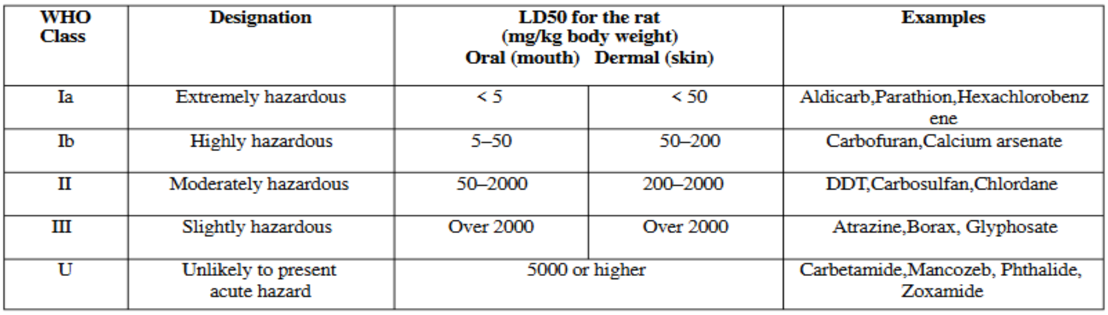
- **Direct-contact pesticides** are applied directly to the site of need. They come in direct contact with the pest (usually outside the plant). Treating all parts of the plant, including the underside of the leaves, is essential when applying these pesticides.

- **Systemic Pesticides** are usually absorbed by the plant and act only after the pest consumes a plant part (usually living inside the leaf, stem, or root).
- **Broad-spectrum pesticides** are non-selective and used for various pests. Due to their indiscriminate nature, these pesticides can also harm beneficial organisms.
- **Curative pesticides** are applied to prevent further damage caused by pests. They are used after pest damage has begun and are meant to treat or lessen the effects of pests.
- **Preventive Pesticides** stop pests from causing damage before they begin.
- **Selective Pesticides** act against specific pests.

### *1.3.2.3. Classification of Pesticides by their Toxicity*

Another category of pesticides is classified by their toxicity, which is mainly determined by their chemical composition. The toxicity of pesticides to humans can be acute, sub-chronic, or chronic. (Damalas & Eleftherohorinos, 2011). Acute toxicity can occur almost immediately and is caused by the absorption of a single dose (within seconds or minutes) or by repeated exposure over a short period, typically within 24 hours. This type of toxicity mainly occurs through inhalation or dermal contact. Symptoms may include skin irritation, sensitization, and neurotoxicity (Damalas & Eleftherohorinos, 2011). Acute toxicity is measured using the lethal concentration (LD/ $LC_{50}$) determined by test animals and the dose at which half of the tested animals die during the study (Table 3). This procedure ensures compliance with safety standards in pesticide classification (Damalas & Eleftherohorinos, 2011).

Table 3: World Health Organization (WHO) 2020 Pesticides Classification by Acute Toxicity (Source: Rasooly *et al.*, 2022)



| WHO Class | Designation | LD50 for the rat (mg/kg body weight) Oral (mouth) | Dermal (skin) | Examples |
|---|---|---|---|---|
| Ia | Extremely hazardous | < 5 | < 50 | Aldicarb,Parathion,Hexachlorobenzene |
| Ib | Highly hazardous | 5–50 | 50–200 | Carbofuran,Calcium arsenate |
| II | Moderately hazardous | 50–2000 | 200–2000 | DDT,Carbosulfan,Chlordane |
| III | Slightly hazardous | Over 2000 | Over 2000 | Atrazine,Borax, Glyphosate |
| U | Unlikely to present acute hazard | 5000 or higher | | Carbetamide,Mancozeb, Phthalide, Zoxamide |

### 1.3.2.4. *Classification of Pesticides by Chemical Structures/ Functional Groups*

Pesticides are grouped by chemical structures, functional groups, or families because the similarity in their chemical structures causes them to exhibit similar physicochemical characteristics. Figure 5 below shows the chemical classification of pesticides with a few examples.

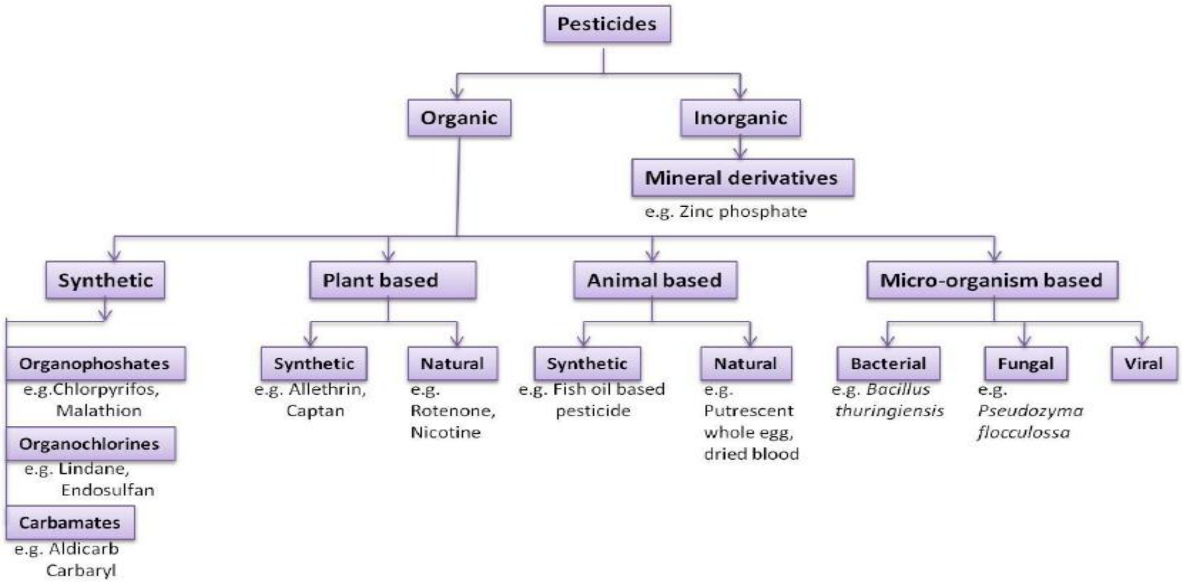


Figure 5: Chemical Classification of Pesticides (European and Mediterranean Plant Protection Organization, EMPPO 2015)

According to the European and Mediterranean Plant Protection Organization (2015), the major groups of pesticides are organochlorines (banned in most countries due to their high toxicity), organophosphates, carbamates, and pyrethroids. Pesticides' active ingredients (base compounds) are mixed with other compounds, which can be solids, liquids, or gases, to improve their effectiveness, safety, handling, and storage, such as solvents, mineral clays, stickers, wetting agents, or other adjuvant materials. This mixture is called 'pesticide formulation' (BCMA, 2015).

## 1.3.3. Fate and Behaviour of Pesticides in the Environment

The fate of environmental pollutants refers to what happens to contaminants after they are released into the environment. Figure 6 illustrates that, after their release, pollutants may be transported through multiple processes and pathways, often moving from one medium to another and undergoing a wide range of transformations. Some contaminants may break down naturally over the years through chemical reactions or microbial degradation; others may undergo sorption and transport in the environment. The fate and behaviour of these substances, along with their physicochemical properties—such as form, stability, water solubility, and the octanol/water partitioning coefficient—are influenced by various external environmental factors. These factors include temperature, pH, and the presence of other ions and compounds at any given time (Valentín *et al.*, 2013; Fischer *et al.*, 2012; Wong *et al.*, 2012). Together, these elements determine how these contaminants behave, their metabolites, preferred environmental compartments, and the potential means of human exposure. (Williams & Kookana, 2018; Valentín *et al.,* 2013; Shetty *et al., 2023*; Barcelo, 1991).

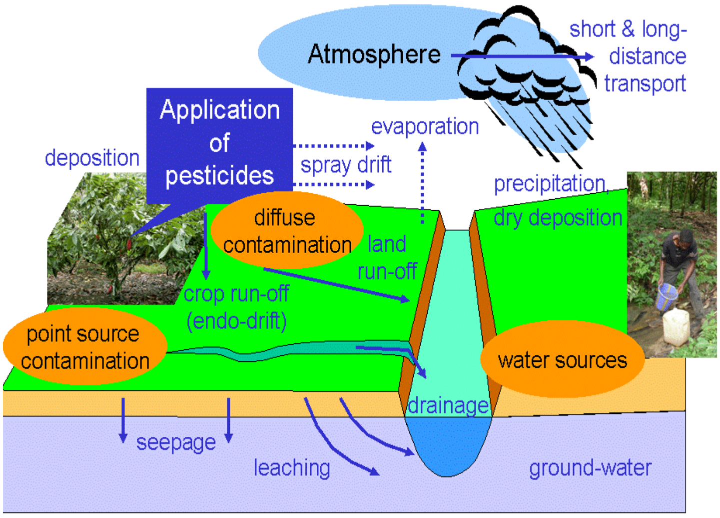


Figure 6: Fate of Pesticides in the Environment (Source: Agriculturenigeria.com)

Pesticides travel through different environmental pathways, as shown in Figure 6. Some of the processes that determine the fate of herbicides include off-site transport processes like leaching, run-off, atmospheric transport, and volatilization (Hill, 2020; Gish *et al.,* 2011) and on-site processes including plant uptake and release, photodegradation, and degradation (Ortiz-Hernandez *et al.,* 2013; Chauhan *et al.,* 2012; Mudhoo & Garg, 2011), dissolution, adsorption, bioaccumulation and biomagnification (Ding *et. al.,* 2017; Wong *et al.,* 2012).

- **Dissolution, Dilution, and Dispersion**

The dissolution of pesticides in water bodies is a key pathway for their transformation in the aquatic environment. The dissolution rate depends on environmental factors like water temperature, pH, and the pesticide's solubility (Xiao *et al.*, 2022). As pesticides dissolve, their concentration decreases through dilution, influenced by the water volume and chemical properties (Sarraute, Husson, and Gomes, 2019). Pollutants may accumulate in specific locations or organisms, leading to higher concentrations. Generally, pollutant concentrations diminish with distance due to increased opportunities for dilution, decomposition, or settling. This is why many epidemiological studies consider distance as a measure of exposure.

Pollution dispersion and concentration can also vary based on meteorological conditions. Greater mixing leads to more consistent dispersion of pollutants in surface and groundwater, creating uniform contamination patterns regionally. In developed countries, water quality is relatively uniform following treatment and distribution, but local variations can occur. In developing countries, shallow wells can exhibit varying water quality due to nearby pollution sources like latrines or agricultural activities.

- **Leaching and Mobility**

The leaching and mobility of pesticides in soil and the risk of groundwater contamination are among the most significant challenges associated with their use. Factors like the chemical nature of the herbicide, the herbicide concentration, the soil type, structure/texture, and environmental factors like heavy rainfall determine contamination (Singh *et al.,* 2015; Sorenson *et al.,* 2003). The physicochemical properties of pesticides, like solubility, stability, and reactivity, influence their fate in the environment. For instance, water-soluble pesticides are more prone to leaching into groundwater, while volatile pesticides can undergo atmospheric transport over long distances.

- **Run-off and Transport**

Pesticides can travel within water bodies through currents, leading to dispersion to other areas. Agricultural and urban run-off can carry pesticides from land to water bodies, contaminating surface water. Pesticides move and persist in water based on their solubility, volatility, and sediment interaction. Some can travel long distances through surface runoff and water flow, risking contaminating downstream ecosystems.

- **Volatilization**

Some pesticides can evaporate from water into the atmosphere, with the rate influenced by the pesticide's volatility, water temperature, and air movement.

The evaporation of pesticides results in their spread and possible re-deposition in different environmental compartments. Additionally, volatilization and runoff affect how pesticides are found in groundwater. Because of this, it's essential to understand how these chemicals interact with surface waters to evaluate where they end up and how they break down (Hintze, Glauser, and Hunkeler, 2020).

- **Adsorption and Desorption**

Pesticides can adsorb to soil particles, slowing their movement and increasing persistence. This process is influenced by soil properties, such as organic matter content and clay mineral type, which affect pesticide mobility and bioavailability.

Compounds with high adsorption will stay on soil top layers, while others, with lower adsorption can leach to groundwater and, therefore, affect human and environmental health. (Meftaul *et al.*, 2020). Pesticides may be retained by sediment, organic matter and plant tissues through strong bond formations (Esclapez *et al.*, 2017), thereby decreasing their transportation and bioavailability in the environment (Bian *et al.*, 2022; Zhang *et al.*, 2020).

The retention and release mechanisms are affected by factors like pH, temperature, and organic matter (Iwuozor *et al.*, 2023; Mojiri *et al.*, 2020; Cosgrove *et al.*, 2019). High desorption rates can increase pollutant bioavailability, threatening aquatic life (Chavoshani *et al.*, 2020) and affecting pesticide removal methods (Mojiri *et al.*, 2020).

- **Degradation**

The persistence of a pesticide, or how long it remains active in the environment, dramatically affects its fate and behaviour. Highly persistent pesticides can accumulate in soil and water, leading to long-term contamination. Degradation occurs through biotic processes, like microbial metabolism, and abiotic processes, such as hydrolysis and photolysis. These processes depend on the pesticide's chemical structure and on prevalent environmental factors such as temperature, pH, and the presence of other chemicals (Lougkovois et al., 2024).

Pesticides can degrade in aquatic environments through chemical reactions, biological processes, or exposure to sunlight. Microorganisms can break down certain pesticides into less toxic substances, while some are prone to hydrolysis, which simplifies their chemical structure.

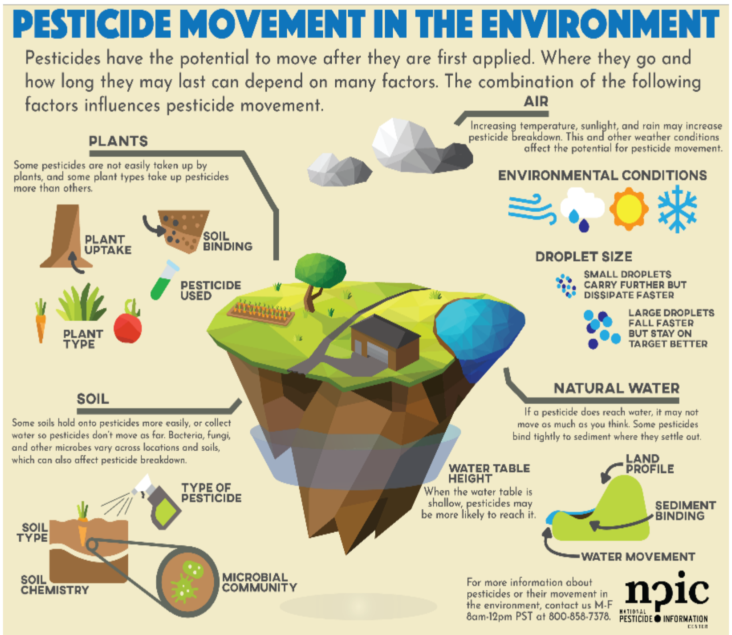
- **Bioaccumulation and Biomagnification**

POPs possess a range of physicochemical properties, which impact their resistance to degradation (recalcitrance), toxicity, accumulation in living organisms (bioaccumulation), increase in concentration along the food chain (biomagnification), transboundary dispersion and the ability to travel long distances through different environmental compartments (Ding *et al.,* 2017; Wong *et al.,* 2012; Schmid *et al.,* 2011). Bioaccumulation is a significant consequence of the accumulation of non-degraded pesticides in the tissues of aquatic organisms. When predators consume these organisms, the pesticide concentration can increase through biomagnification, leading to higher levels of pesticides in higher trophic levels of the food chain. Pesticides can be absorbed by plants and animals, leading to bioaccumulation in food webs.

The environmental or aquatic fate of pesticides is complex and multifaceted. Pollution patterns are more complex than simple distance-based models, and they vary by pollutant and environmental media because of the transport behaviours involved. For example, a microcosm study on atrazine showed that 0.33% was volatilized, 0.25% was mineralized, and 6.89% ended up in leachate (Mudhoo & Garg, 2011). Another study suggested that Isoproturon and its byproduct, chlorotoluron, may persist in soil and leach for up to 3 years after field application (Sorenson *et al.,* 2003). Overall, the fate and behaviour of pesticides in the environment are complex processes influenced by many factors. By studying these processes, researchers can better understand and predict how pesticides behave in the environment and the environmental risks associated with pesticide use and develop sustainable practices to minimize their impact.

### 1.3.4. Factors That Determine the Fate of Pollutants in Water

Understanding the fate of pesticides in water is necessary as pesticides may raise environmental and health concerns after application. Once applied, pesticides undergo various processes that determine their behaviour in the environment. To safeguard our ecosystem, it is crucial to understand the intricate interactions between pesticides and environmental factors. This knowledge is essential for predicting how pesticides behave and developing effective management strategies (see Figure 7).



PESTICIDE MOVEMENT IN THE ENVIRONMENT

Pesticides have the potential to move after they are first applied. Where they go and how long they may last can depend on many factors. The combination of the following factors influences pesticide movement.

Some pesticides are not easily taken up by plants, and some plant types take up pesticides more than others.

Increasing temperature, sunlight, and rain may increase pesticide breakdown. This and other weather conditions affect the potential for pesticide movement.

Some soils hold onto pesticides more easily, or collect water so pesticides don't move as far. Bacteria, fungi, and other microbes vary across locations and soils, which can also affect pesticide breakdown.

If a pesticide does reach water, it may not move as much as you think. Some pesticides bind tightly to sediment where they settle out.

When the water table is shallow, pesticides may be more likely to reach it.

For more information about pesticides or their movement in the environment, contact us M-F 8am-12pm PST at 800-858-7378.

Figure 7: Factors that Control the Pathways of Pesticide Movement in the Environment (Source: National Pesticide Information Centre)

Studies demonstrate that the physicochemical properties of substances significantly affect the fate of organic contaminants in water. The physicochemical properties of organics, including hydrophobicity (Omoni *et al.*, 2020), water solubility, pH, absorptivity, volatility, ionic property, structure, molecular weight (Ogbonna & Semple, 2013), reactivity, polarity, form of the compound, its stability, octanol/water partitioning coefficient, the presence or interference from other ions and compounds, the temperature and pH of the environmental compartment, climate conditions and the presence of organisms, play a pivotal role in determining the fate of pesticides in water and the environment at large (Valentín *et al.*, 2013; Fischer *et al.*, 2012; Wong *et al.*, 2012).

- **pH**

pH is a crucial factor in the environmental behaviour of pesticides, affecting their degradation and persistence. Acidic conditions typically enhance pesticide breakdown, while alkaline conditions stabilize them (Lewis *et al.*, 2016). Fluctuations in pH can also influence the solubility and toxicity of organic pollutants, with low pH increasing the solubility of certain compounds and increasing their potential harm to aquatic life (Priyadarshanee, Mahto & Das, 2022). Moreover, pH is essential for microbial degradation. Specific microbial communities that effectively break down persistent contaminants thrive within specific pH ranges (Singh *et al.*, 2021). Additionally, pH changes can alter how pollutants adsorb to various substrates, impacting their retention and distribution in aquatic environments (Yu *et al.*, 2019).

pH fluctuations from human activities can trigger toxicity in aquatic systems by enhancing the mobilization of metals in aeolian aquifer sediments. The interaction between contaminants and sediments is significantly affected by pH (Zhai *et al.*, 2021). This influence on the solubility and toxicity of pesticides underscores the need to analyse and regulate pH in these ecosystems. Understanding these interactions is crucial for assessing ecological risk and developing strategies to mitigate chemical pollutants (Gheorghe *et al.*, 2017; Riaz *et al.*, 2021).

- **Temperature**

Temperature also significantly affects pesticide degradation. High temperatures can increase the metabolic activity of soil microorganisms, thereby improving the biodegradation of pesticides (Tudi et *al.*, 2021). Consequently, the hottest climates can reduce the effectiveness of pesticides, even though they potentially increase the risk of environmental eluviation and outflow. On the contrary, the coldest temperatures can inhibit microbial activity, resulting in prolonged pesticide stability and greater toxicity over time (Kah et *al.*, 2016). For instance, elevated temperatures can accelerate pesticide degradation, while acidic soils can enhance their leaching into groundwater.

- **Presence of other ions, compounds, and organisms**

The release and use of anthropogenic chemicals from daily and agricultural processes often result in water pollution in the environment as mixtures. Other chemicals can influence the efficacy and behaviour of these pollutants in the environment (El-Nahlal & Hamdona, 2017). Some effects may be synergistic, antagonistic, or competitive, as observed between Cd and Zn. Understanding these interactions and their ecological impacts is paramount in protecting aquatic ecosystems.

- **Volatility**

The form of a pesticide controls its fate in the environment. For example, a pesticide's gaseous or liquid formulation can be more susceptible to volatilization or dilution into water bodies than its solid formulation. However, a powdered formulation can also be more susceptible to wind transport than pellets or heavier solid formulations.

- **Water Solubility**

Solubility plays a vital role in the movement of pesticides within the environment. Highly soluble pesticides tend to leach into groundwater and surface water, raising significant ecological concerns. Conversely, pesticides with low solubility are more likely to adsorb to soil particles, thereby reducing their mobility and causing soil contamination (Khorram *et al.*, 2016). The ionic properties of a pesticide affect its water solubility, with ionic herbicides being more soluble than non-ionic ones. Adsorption increases with higher hydrophobicity. Improved water solubility enhances bioavailability and soil adsorption, reducing soil retention, persistence, and dissipation time. The high molecular weight of organic compounds reduces their water solubility, depending on ionic strength. According to Omoni (2020), the hydrophobicity of an organic compound can slow down its degradation through natural attenuation and biological or chemical processes (Trelluet *et. al.*, 2016). Ogabonnaya & Semple (2013) further suggest that the persistence and loss of HOCs are controlled by hydrophobicity, polarity, aqueous solubility, and molecular structure or size.

According to Fragoeiro (2005), a pesticide can reach groundwater if its water solubility exceeds approximately 30 mg $L^{-1}$, its absorptivity (Koc = partition coefficient between soil organic carbon and water) is less than 300-500 cm3 g-1, its soil half-life is longer than about 2-3 weeks, its hydrolysis half-life is longer than approximately six months, and its photolysis half-life is longer than three days. (Okeke, 2002; Barceló, 1991).

- **Octanol/Water Partitioning**

This factor affects only organic pollutants. The octanol/water partition coefficient (Kow) of a chemical is the ratio of the concentration of the chemical in the octanol phase to its concentration in the aqueous phase of a two-phase octanol/water system, usually measured at room temperature (USGS, 2015; Yamamoto, 2014). Thus, the mathematical representation of Kow in Equation 1 as:

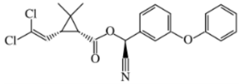
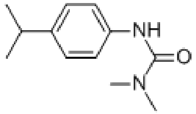
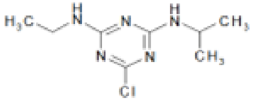
Equation 1: Calculation for Kow

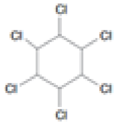
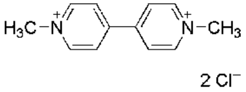
$$Kow = Concentration\ in\ octanol\ phase\ /\ Concentration\ in\ aqueous\ phase$$

Kow estimates the tendency of a chemical to partition itself between an organic phase (for example, fish, soil) and an aqueous phase.

In recent years, Kow has gained recognition as a key parameter in studies of the biological, toxicological, and environmental fate of organic chemicals. There have been confirmations on its relationship with water solubility, soil/sediment adsorption coefficients, and bioconcentration factors for aquatic and human life (USGS, 2015; Yamamoto, 2014; Mackay *et. al.,* 1997). This has contributed to its increased use in estimating these other properties and its consideration as an essential property in studies of emerging or problematic chemicals. Chemicals with low Kow values (less than 10, for instance) may tend to have high water solubilities (relatively hydrophilic), small soil/sediment adsorption coefficients, and small bioconcentration factors for aquatic life. On the other hand, chemicals with high Kow values (e.g., greater than 104) are highly hydrophobic (Yamamoto, 2014).

Table 3: Summary of the Physicochemical Properties of Some Pesticides

| **Compound, Chem. Formula, Mol. Wt.** | **Chemical Structure** | **Physical State** | **Log Kow** | **Water Solubility (mg L$^{-1}$)** | **Environmental Ranges** | **Half-life** | **References** |
|---|---|---|---|---|---|---|---|
| Cypermethrin<br>$C_{22}H_{19}O_3NCl_2$<br>416.3 g moL$^{-1}$ |  | Yellowish brown, odourless viscous semi-solid at ambient temperature | 3.98-6.2 | 72 at 20 °C | Food: 0.05 to 2 mg kg$^{-1}$ | Air: 6-20 d<br>Soil: 4-12 d<br>Water: 2 wks | Jones (1995) |
| Isoproturon<br>$C_{12}H_{18}N_2O$<br>206.29 g moL$^{-1}$ |  | Polar herbicide: Colourless, odourless transparent crystals | 2.2-2.5 | 55 - 70.2 at 20-25 °C<br>65 at 22 ºC<br>72 at 20 °C | S/Water: 0.1-0.125 µg L$^{-1}$<br>G/Water: 0.05 -0.1 µg L$^{-1}$<br>Food: 0.01 to 0.1 mg kg$^{-1}$ | Water: 15-30 d at pH 7.<br>Soil: 40 days at 20 °C | Leoci & Ruberti (2020)<br>PubChem (2016)<br>Yin *et. al.* (2008)<br>WHO (2003)<br>DoE (1989)<br>Kulshrestha & Muckerjee (1986) |
| Atrazine<br>$C_8H_{14}ClN_5$<br>215.69 g moL$^{-1}$ |  | Polar triazine herbicide: Odourless, Colourless powder or crystals | 2.05 - 2.8 | 30 - 33 | S/Water:0.45–700 µg L$^{-1}$<br>Soil: 0.031-0.119 mg kg$^{-1}$ | | Ikpesu&Arlyo (2013)<br>ATSDR (2003)<br>Pubchem (2016)<br>(Mackay *et. al.,* 1997)<br>Xing *et. al.* (1996) |

| **Compound,<br>Chem. Formula,<br>Mol. Wt.** | **Chemical Structure** | **Physical State** | **Log Kow** | **Water Solubility (mg L$^{-1}$)** | **Environmental Ranges** | **Half-life** | **References** |
|---|---|---|---|---|---|---|---|
| Lindane<br>$C_6H_6Cl_6$<br>290.8 g moL$^{-1}$ |  | Insecticide: colourless to white crystalline powder/solid with slight musty or aromatic odour. | 3.3- 3.8 | 2-10 | S/Water: 0.13-1.2 µg L$^{-1}$<br>Soil: 2.1 – 15.4 mg g$^{-1}$ |  | USDHHS (2015);<br>Fu *et. al.* (2003);<br>Nantel (2001);<br>Bentum *et. al.* (2006) |
| Paraquat<br>$C_{12}H_{14}N_2Cl_2$<br>257.16 g moL$^{-1}$ |  | Polar herbicide: Yellowish-white solid, usually dark blue-green in solution. | 2.44 | 700,000 | S/Water: 1.45-2800 µg L$^{-1}$<br>Soil: 0.2 –15 mg g$^{-1}$ |  | Watts (2011);<br>Ashton&Leahy (2000);<br>INCHEM (2000) |

### 1.3.5. Isoproturon

Isoproturon (IPU), a phenyl urea herbicide widely used in agricultural systems, is a colourless, odourless, transparent crystalline substance with the IUPAC name, 3-(4-isopropyl phenyl)-1,1-dimethylurea or 3-p-cumenyl- 1,1-dimethylurea (WHO, 2003). It's melting point ranges between 155–156°C and exhibits a vapour pressure of 0.003×10-3 Pa at 20°C. At this temperature, its solubility is as follows: methanol is 75 g $L^{-1}$ (in dichloromethane), 63 g $L^{-1}$ (in acetone), 38 g $l^{-l}$ (in benzene), 5 g $L^{-1}$ (in xylene), 4 g $L^{-1}$ (in n-hexane) (Leoci & Ruberti, 2020). During combustion, IPU emits toxic fumes containing nitrogen oxides (NOx) and carbon oxides (CO and CO2). It is highly stable in the presence of acids, alkalis, and light, but can decompose under alkaline conditions and at elevated temperatures (Loccufier *et al.*, 2020; Leoci & Ruberti, 2020; Fragoeiro, 2005). In soil, it degrades under different factors (hydrolysis, photodegradation, biodegradation, etc.), i.e., hydrolysis of phenyl urea and demethylation of urea nitrogen. On soils, its half-life is around 40 days at an average ambient temperature of 20 °C (Leoci & Ruberti, 2020). Table 4 presents some physicochemical properties of IPU.

IPU is used to cultivate wheat, sugar cane, citrus fruits, cotton, asparagus, and other cereal crops (Leoci & Ruberti, 2020; Liu, 2010), in various regions, creating a significant path for aquatic contamination (Mohammed *et. al.*, 2024). Its effectiveness in controlling the germination of a wide range of broadleaf and longleaf weeds has led to its continued use in modern agriculture despite its persistence in soil and water (Leoci & Ruberti, 2020) and the resulting environmental and health impacts (Javaid et *al.*, 2020). Studies show that although IPU effectively improves crop yields, its residual effects can affect non-target plant species and soil health (Kaur et al., 2019; Garg et *al.*, 2022).

#### *1.3.5.1. Environmental Fate of Isoproturon*

Like most pesticides, IPU is known for its mobility in soil. It undergoes various degradation processes that influence its fate in soil. The main degradation pathways include microbial degradation and chemical transformation, which help reduce IPU concentrations in soils (Hussain *et al.*, 2017). IPU degradation begins with demethylation, yielding “monodemethyl Isoproturon” (MDIPU) and “didemethyl Isoproturon” (DDIPU) (Leoci & Ruberti, 2020; Renyi et al., 2017). Additionally, the phenyl urea side chain hydrolyzes, yielding 4-IA, the aniline metabolite of IPU (Hussain *et al.*, 2009). Studies indicate that the nature of agricultural management practices significantly impacts the persistence of herbicides in ecosystems, with certain conditions facilitating faster degradation (Carpio *et al.*, 2021). Generally, IPU has moderate persistence, with field studies reporting detectable residues for several weeks, depending on soil conditions and microbial activity (Papadopoulou *et al.*, 2016; Degrendele *et al.*, 2022). This persistence raises concerns about the quality of the soils and water because IPU can contaminate water bodies with leachate and runoff, potentially compromising aquatic ecosystems (Sondhia, 2019; Pietrzak *et al.*, 2020).

It is persistent in water and hydrolyses slowly, with a half-life of approximately 30 days (WHO, 2003; DoE, 1989), depending on these environmental conditions. They mostly range from a few days to several weeks (Singh *et al.*, 2023). The aquatic degradation processes of IPU involve both microbial activities and photolysis. Hussain *et al.* (2017) show that fioriture through microbial strains can efficiently degrade the IPU in agricultural land, suggesting a potential for similar processes in aquatic environments. Recent studies, such as those by Locufier et al. (2020), show that IPU degradation kinetics vary significantly with environmental conditions, underscoring its persistence as a potential threat to water sources. In addition, IPU transformations can produce harmful by-products, as noted by Wang et al. (2021), which could harm aquatic ecosystem health.

#### *1.3.5.2. Mode of Isoproturon Action and Toxicity*

IPU can exert severe toxic effects on ecosystems, influencing microbial activity, soil composition, and environmental conditions. It can persist in the environment for varying durations and lead to bioaccumulation in aquatic organisms (Barbieri *et al.*, 2021), including algae and invertebrates, and ultimately affects the entire food chain (Kazeem & Fischer, 2020; Shehu *et al.*, 2022). Its presence significantly impacts aquatic biodiversity and non-target organisms. Additionally, IPU disrupts soil and microbial health and function (Storck *et al.*, 2018), affecting the nutritional cycle and ecosystem resilience (Narayanan *et al.*, 2022). The primary IPU metabolite, 4-IA, mentioned, appears more toxic than IPU (Leoci & Ruberti, 2020; Papadopoulou *et al.*, 2016; Tixier *et al.*, 2002).

The biochemical mechanisms underlying IPU's herbicidal effectiveness primarily involve inhibition of photosynthesis and growth in target plant species, specifically by interrupting the electron transport chain in photosystem II (Sing et *al.*, 2024). This inhibition blocks electron flow, preventing the conversion of electrical energy into chemical energy, which is essential for plant growth and survival (Marlatt & Martyniuk, 2017).IPU acts by interfering with the photosystem II (PSII) complex, generating reactive oxygen species (ROS) within plant cells, which can induce oxidative stress in susceptible species and lead to chlorosis and plant death (Lushchak *et al.*, 2018). Singh *et al.* (2024) report that while IPU effectively controls populations of specific weeds, molecular interactions can also lead to non-intentional toxicity in other plant species and beneficial organisms.

In addition, the interactions between IPU and various environmental factors have complex ecological implications. Qiu *et al.* (2024) examined the bioaccumulation of IPU in the green algae *Chlamydomonas reinhardtii*, revealing synergistic toxicity when combined with metals such as cadmium. These interactions illustrate the potential for toxicity to accumulate in the bodies of those exposed to agricultural outflow, which contains multiple contaminants. Environmental toxicity evaluations reveal that Isoproturon poses risks to aquatic plants, such as *Lemna minor*, affecting their growth and physiology (Varga *et al.*, 2019). In addition, the potential impacts on human health, including endocrine disruption and carcinogenicity, demand careful consideration given their generalized use (Leoci & Ruberti, 2020; Qiu *et al.*, 2024).

### 1.3.6. Cypermethrin

Cypermethrin (CP) is a synthetic pyrethroid insecticide available in several formulations, including emulsifiable concentrate and wettable powder. Its chemical name is (±)-B-Cyano-(3-phenoxy phenyl) methyl (±)-cis/trans-3-(2,2-dichlorovinyl)-2,2 dimethyl cyclopropane carboxylate (Jones, 1995). CP occurs as a mixture of cis- and trans-isomers. The cis-trans ratio of technical-grade cypermethrin is 1:1 (Kidd & James, 1991). The cis isomers are more active than the trans isomers by a factor of two (Takahashi et al., 1985). It possesses high insecticidal activity, low avian and mammalian toxicity, and adequate stability in air and light (Kaufman et al., 1981; USDA, 1995). Table 3 displays some physicochemical properties of cypermethrin.

CP is effective against many lepidopterous pests that target cotton, fruits, and vegetables. It is also employed for crack, crevice, and spot treatments to manage insect infestations in various settings, including retail stores, warehouses, industrial facilities, residential buildings, greenhouses, laboratories, and on ships, railcars, buses, trucks, and aircraft. Additionally, it may be utilized in non-food areas of schools, nursing homes, hospitals, restaurants, hotels, and food processing establishments, serving as a barrier treatment insect repellent for horses.

#### *1.3.6.1. Environmental Fate of Cypermethrin*

CP is more stable in sunlight compared to natural pyrethrins. While photodegradation significantly affects it on leaves and surface waters, its impact on soil is minimal. Under aerobic conditions, these metabolites may break down into the very polar primary metabolites, which move readily through the soil. Microbes play an essential role in breaking down cypermethrin. CP degrades more slowly under anaerobic and waterlogged conditions (Walker & Keith, 1992). The anaerobic half-life was reported at 50 days. It is also stable for photolysis with a half-life of >100 days. In a sterile solution in sunlight, cypermethrin photodegrades slowly with water (EA, 2019).

In water bodies, CP undergoes a series of reactions that determine its fate and impact on aquatic ecosystems. It can undergo hydrolysis, oxidation, and photolysis reactions. Hydrolysis is the reaction of cypermethrin with water molecules, leading to the breakdown of the compound into smaller molecules. This process can create various metabolites, some of which may be more toxic than the original compound. Oxidation occurs when cypermethrin interacts with atmospheric oxygen, producing breakdown products. Photolysis happens when cypermethrin is exposed to sunlight, leading to the formation of different byproducts. CP and its degradation products can persist in the aquatic environment for extended periods, leading to potential bioaccumulation in aquatic organisms. This can harm aquatic ecosystems, including toxicity to fish and other marine organisms. CP and its breakdown products in water sources can also harm human health by contaminating drinking water supplies (EA, 2019; EHC, 1992).

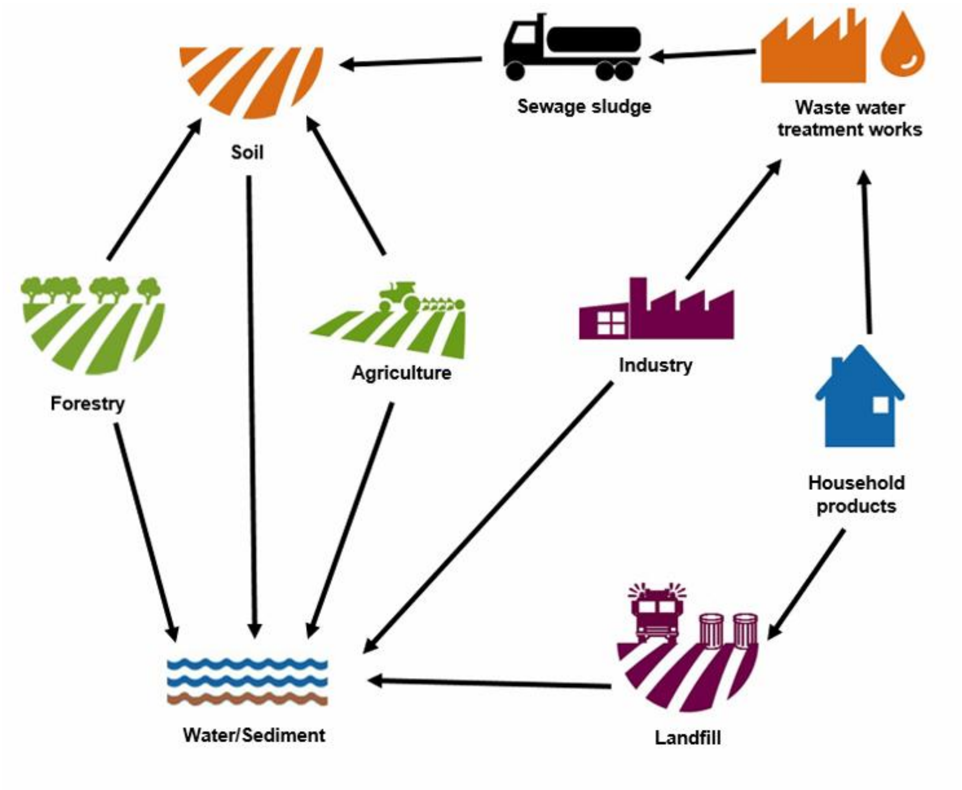


Figure 8: Industrial Sources and Pathway of Cypermethrin Transformation (Source: Environment Agency, 2019).

Cypermethrin has limited mobility in surface waters, remaining mainly in the surface film when used in agricultural applications. It does not penetrate deep into sediments. In natural water bodies, it degrades quickly, with an average half-life of about two weeks, due to photochemical and biological factors. Spray drift from treated fields does not create lasting residues in adjacent waters. Fish absorb cypermethrin rapidly, and in rainbow trout, the residue half-life is about eight days. Given the low expected concentrations in water and their rapid decline, detectable residues in fish are unlikely. Field studies show that at recommended application rates, cypermethrin and its degradation products remain at minimal levels in soil and surface waters, indicating potential environmental implications of its use (EA, 2019; EHC, 1992*)*.

### *1.3.6.2. Mode of Cypermethrin Action and Toxicity*

Cypermethrin is highly toxic to fish and other aquatic life. In both vertebrates and invertebrates, it primarily targets the nervous system. This compound acts as both a stomach poison and a contact insecticide (Jin & Webster, 1998). In the peripheral nervous system of frogs, it triggers repetitive activity and generates nerve impulse sequences by altering the ion permeability of nerve membranes (Vijverberg & van den Bercken, 1990;

Siegfried, 1993). These prolonged sequences can generate thousands of repetitive nerve impulses in the sensory organs. This repetitive activity results from pyrethroid-induced damage to voltage-dependent sodium channels, which causes them to remain open significantly longer than usual (Vijverberg & van den Bercken, 1990). Studies show that cypermethrin inhibits ATPases that are crucial for the movement of ions against their concentration gradient via active transport. This inhibition is particularly vital for fish and aquatic insects, as ATPases supply the energy required for active transport and play a significant role in oxygen exchange. The inhibition of ATPase and disruption of active transport potentially impair ion movement and the organism's ability to maintain ionic balance, thereby affecting respiratory surfaces and indicating that cypermethrin poses a greater toxicity risk to aquatic organisms (Siegfried, 1993).

Research indicates that the primary action site of cypermethrin (and pyrethroid insecticides in general) within the vertebrate nervous system is the sodium channel located in the nerve membrane. This modulation of sodium channels in nerve cells increases neuronal excitability and considerable neurotoxicity (Kaisarevic *et al.*, 2019; Ali, 2021). The α-cyano pyrethroids, including cypermethrin, induce a prolonged increase in sodium permeability of the nerve membrane during excitation, leading to extended sequences of repetitive impulses in sensory organs and a frequency-dependent reduction of nerve impulses in nerve fibres. Since the mechanisms that generate and conduct nerve impulses are consistent throughout the nervous system, pyrethroids exhibit similar effects across different central nervous system regions. Additionally, research suggests that the facial skin sensations experienced by individuals who handle cypermethrin stem from the repetitive firing of sensory nerve terminals in the skin, potentially serving as an early warning indicator of exposure.

The acute oral toxicity of CP is considered moderate, with significantly varying LC50 values among different animal species, depending on the mode of exposure and the isomeric ratios. The toxic responses across species are largely comparable. Symptoms of poisoning include salivation, tremors, heightened startle reflex, sinuous writhing of the entire body (choreoathetosis), and clonic seizures (Jones, 1995).

## 1.4. Inorganic Water Pollutants

The history of notable inorganic pollutants in water spans several decades, and the evolution of environmental monitoring priorities is a testament to the progress in this field. It has advanced from faecal contamination in the 1950s to nutrients (Nitrogen and Phosphorus) in the 1970s and finally, heavy metals since the 1970s and 1980s due to rapid industrialization (Wong *et al.*, 2012). During the 1970s and 1980s, some heavy metals were considered highly toxic; hence, testing was essential to safeguard water quality and human health. Rapid industrial development during the period led to the discharge of highly toxic heavy metals, such as arsenic (As, a metalloid), cadmium (Cd), lead (Pb), and mercury (Hg), into aquatic systems via various industrial effluents and wastes. Inorganic water pollutants are commonly categorized into two main groups: metals and nutrients.

Nitrogen (N) and phosphorus (P) are essential nutrients found in fertilizers used in agriculture. When these fertilizers are applied, especially in excessive amounts, they can wash into lakes and rivers, leading to a phenomenon known as eutrophication. This process is characterized by the rapid growth of algae, which can significantly reduce oxygen levels in the water. Furthermore, excessive algal growth can produce harmful toxins that harm aquatic life and human health. The decline in water quality can ripple effect on fisheries and local communities that rely on these water resources for their livelihoods. The repercussions include water eutrophication, toxicity, groundwater contamination, air pollution, soil degradation, and irreversible ecosystem changes (Chen *et al.*, 2017; Mateo-Sagasta *et al.*, 2018).

Heavy metals are elements with high atomic weight and densities greater than 5 g $cm^{-3}$. They can be toxic to living organisms, even at low concentrations. Common heavy metals include lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), and zinc (Zn). While certain heavy metals, such as copper (Cu) and iron (Fe), are essential in trace amounts for biological functions, excessive levels can disrupt cellular processes, accumulate in ecosystems, and pose serious health risks. Their persistence in the environment makes them a significant concern in studies related to pollution and toxicology (Duffus, 2002). Heavy metals are introduced into the ecosystem from several sources and accumulate in the three major environmental compartments: air, water, and land. In the air, they exist as particulate matter (PMs) emitted from chimneys and industrial flare stacks. They exist in aqueous forms in water and are introduced through effluent discharges from wastewater treatment plants and other industrial activities. (Bailey *et al.*, 1999; Pagnanelli, 2001; Shreshtha, 2004; Khan, Ibrahim & Subramaniam, 2004). Heavy metals on land are often associated with several sources, including military bases, war zones, metal manufacturing and finishing plants, mine tailings, areas with high metal waste disposal, the use of leaded gasoline and paints, the application of land fertilizers, animal manure, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, and the spillage of petrochemicals, among others (Ahmad *et al.*, 2014; Wuana & Okieimen, 2011; Zhang *et al.*, 2010; Khan *et al.*, 2008; Chen *et al.*, 2005; Shrestha, 2005; Zahir *et al.*, 2005; Pagnanelli *et al.*, 2001; Bailey*et al.*, 1999).

Heavy metals cause harm to biological and environmental systems in low and high quantities due to their "bio-accumulation tendencies and recalcitrant nature" as opposed to organic contaminants, which biodegrade in the presence of microbes over time (Sud *et al.*, 2008; Khan *et al.*, 2004; Bateman, 1999; Osmond *et al.*, 1995). They are recalcitrant as they do not degrade by physical or biological processes but persist in the environment and are transported into different environmental spheres depending on their forms (Biard & Cann, 2005). Manganese (Mn), chromium (Cr), iron (Fe), cobalt (Co), and nickel (Ni) serve as essential nutrients in biological systems and are catalysts in industrial processes (Alloway, 2004; Holden, 2005).

### 1.4.1. Fate and Behaviour of Metals in Water

Metals are vital players in many biological pathways and are critical micronutrients. In high concentrations, however, they can be toxic and pose significant environmental and human health risks. As a result, knowledge of the behaviours and interactions of metals in the environment is essential for managing their potential consequences effectively. Also, since metals are inorganic, they are carbon-free. Their interaction with other environmental compounds is primarily chemical alteration. They go through different processes, including adsorption, precipitation, dissolution, and transformation. Such processes can influence metal mobility and bioavailability in the environment. Transport mechanisms, including sedimentation, adsorption, and bioaccumulation, affect their distribution and persistence in such systems (Wu *et al.*, 2016). Some of the most important reactions that metals experience are:

• **Sedimentation**: the ionic properties of heavy metals that affect their bioavailability and toxicity are dependent on the interactions between heavy metals and water and aquatic sediments (Kolarova & Napiorkowski, 2021). Miranda *et al.* (2022) emphasized the significance of sediment and metal ion characteristics on the adsorption-desorption behaviour of heavy metals, noting that sediment characteristics can amplify or attenuate the ecological impacts of contaminants. Moreover, when heavy metals compete with one another, they frequently produce synergistic effects, making environmental risk assessment more complex (Lead *et al.*, 2018; Areal *et al.*, 2020).

• **Oxidation**: Metals burn with oxygen to give metal oxides. Oxidation can also occur at a slower rate without the combustion process (Hill, 2020). Iron, for instance, may oxidize in the presence of oxygen, producing various iron oxides which may precipitate in the water, compromising its quality (Miranda *et al.*, 2022) • Dissolution: metals solubility is mainly pH-dependent, thus changing the chemical forms of these pollutants alters their bioavailability, reactivity and therefore their afters toxicity for water organisms (quo *et al.*, 2016). Metals in water can be found in both dissolved and particulate forms, including as ions, inorganic and organic complexes. Surprisingly, the toxicity and mobility of such metal species are strongly influenced by pH, redox conditions, total organic carbon content, and metal speciation (Luoma & Rainbow, 2008). Examples include changes in pH, affecting metal solubility, resulting in precipitation of metals such as copper and lead, and thus reducing their bioavailability (Batley *et al.*, 2013).

Metals may also interact with the natural organic matter (NOM) in water bodies. In addition, this transient interaction usually leads to metal–organic complexes forming that may increase or decrease the mobility and toxicity of metals. For example, copper typically binds strongly to NOM, forming stable complexes that can reduce its free ion concentration and limit toxicity to aquatic organisms (Xue & Sigg, 1993). Furthermore, redox conditions in surface water can modulate the oxidation state of metals, determining their form and reactivity. For instance, under reducing conditions, mercury is transformed into methylmercury, a potent, bioaccumulating toxin in aquatic food webs (Morel *et al.*, 1998).

Biological processes, such as uptake, influence the fate and cycling of metals in aquatic systems, and bioturbation is mediated by benthic fauna. Metal bioavailability depends on the organisms, and they can absorb metals from water or their diet, leading to bioaccumulation and possibly biomagnification in the food chain (Rainbow, 2002).

Interactions with sediments are equally crucial as metals bind to particles and are deposited into sediments, where they can be remobilized with changes in environmental conditions (Förstner & Wittmann, 2012). Generally, metals in water undergo complex behaviours and fates that require a multidisciplinary approach to be fully understood. Thus, monitoring and modelling activities are critical to predicting metal behaviour and reducing their effects on aquatic ecosystems (Chapman *et al.*, 2013).

### 1.4.2. Factors that determine the fate of metals in water

Various environmental, chemical, and biological factors significantly influence the behaviour of metals in aquatic systems, impacting both ecosystems and human health. Understanding these dynamics is essential for managing and restoring polluted waters. Key factors affecting metal behaviour include pH, temperature, redox potential, dissolved oxygen, and the presence of other substances (Fayiga *et al.*, 2017). These elements influence metal solubility, transport, and toxicity. Additionally, physicochemical properties such as size, shape, atomic number, and valence state play a role. In aquatic environments, factors such as water chemistry—including temperature, pH, and salinity—affect the solubility and bioavailability of metals. Salinity, in particular, influences metal distribution in estuarine systems, driving biogeochemical transformations (Derman *et al.*, 2021). Figure 9 summarizes the key factors that shape the behaviour of metals in water, discussed below.

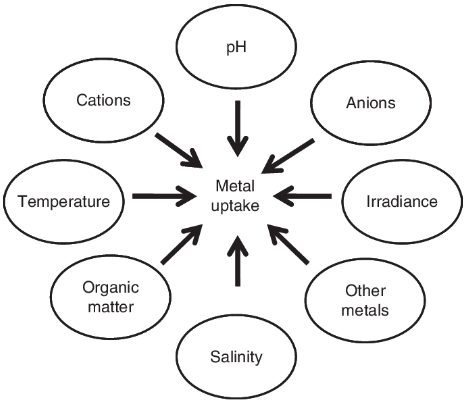


Figure 9: Factors that Control Metal Behaviour and Uptake in Water (Source: Olguín & Sánchez-Galván, 2011).

- **pH**

pH is a key environmental factor that affects the behaviour of heavy metals in aquatic ecosystems. It determines the ionic forms of metals, influencing their toxicity and bioavailability. Acidic conditions increase metal solubility, posing greater risks to aquatic organisms (Gautam *et al.*, 2016). The acidity or alkalinity of water significantly affects the solubility and toxicity of metals such as lead, cadmium, and mercury (Ali *et al.*, 2019). A lower pH enhances metal solubility, increasing their mobility and bioavailability (Gheorghe *et al.,* 2017). This can lead to bioaccumulation in the food web, threatening biodiversity and ecosystem health (Alengebawy *et al.*, 2021). pH levels are crucial for understanding how pollutants behave and their potential toxicity. Different pH levels alter the chemical forms of metals, thereby affecting their toxicity (Mojiri *et al.*, 2020). Metals may exist as ions, complexes, or free precipitates, each having different impacts on aquatic life. Higher pH can result in the formation of less toxic metal complexes, reducing the immediate effects on organisms (Gheorghe *et al.,* 2017). Additionally, factors like dissolved organic matter and sediment characteristics influence how pesticides and heavy metals interact in aquatic environments (Sumudumbi & Jayawarda, 2021). Lower pH often increases the solubility of organic matter, thereby enhancing interactions between contaminants and potentially leading to greater combined toxicity (Abuqamar *et al.,* 2024). In conclusion, pH significantly impacts the solubility, bioavailability, and toxicity of metals in aquatic ecosystems. Changes in pH can have serious implications for water bodies and ecosystem health. Understanding these dynamics is essential for assessing ecological risks and developing strategies to manage chemical pollutants effectively (Gheorghe *et al.*, 2017; Riaz *et al.,* 2021).

- **Temperature**

Temperature also controls the fate and behaviour of metals in the aquatic environment. For example, an increase in temperature can improve metal mobility, influencing their absorption by aquatic organisms (Paxa *et. al.*, 2022).

- **Presence of other ions, compounds, and organisms**

The release and use of anthropogenic chemicals from daily and agricultural processes often result in water pollution in the environment as mixtures. Other chemicals can influence the efficacy and behaviour of these pollutants in the environment (El-Nahlal & Hamdona, 2017). Some effects may be synergistic, antagonistic, or competitive, as observed between Cd and Zn. Understanding these interactions and their ecological impacts is paramount in protecting aquatic ecosystems.

Chemical interactions, particularly those involving organic and inorganic ligands, also play a fundamental role in modelling the fate of metals. The formation of metal-ligand complexes can seize metals, reduce their bioavailability or improve their transport within water systems (Jeong *et. al.*, 2023). For example, the transformation and bioavailability of metal oxide nanoparticles are worrying, as they can behave differently than their bulk counterparties, leading to unexpected ecological effects (Amde *et. al.*, 2017; Turran *et. al.*, 2019). Furthermore, the continuous use of nanoparticles complicates metal interactions, as their behaviour is influenced by the surrounding chemical environment (Joo & Zhao, 2017).

Biological factors, including the behaviour and physiology of aquatic organisms, further influence metal dynamics. Many microorganisms, such as bacteria and microalgae, play crucial roles in the biotransformation of toxic metals, influencing their mobility and toxicity (Duan et al., 2020; Kunhikrishnan et *al.*, 2017). For example, some bacterial strains can mobilize arsenic, altering its form and availability, posing risks to aquatic life and human health through the food network (Barral-Fraga et *al.*, 2020). The concept of biomagnification highlights how metals can accumulate to trophic levels, potentially leading to higher concentrations in predators, including humans (Saidon *et. al.*, 2024).

- **Salinity**

Salinity is vital in determining the distribution of metals in estuarine systems, where salinity gradients influence biogeochemical transformations (Derman *et al.*, 2021). Variable salinity affects the solubility, bioavailability, and toxicity of metals, impacting both ecosystems and human activities. Salinity affects the adsorption and desorption of heavy metals in aquatic systems, with higher salinity often increasing metal solubility (Miranda *et al.*, 2022). This process enhances bioavailability, especially for metal-based engineering nanoparticles (Peng et al., 2017), and also affects their toxicity (Lead *et al.*, 2018). Additionally, factors such as pH can further influence metal adsorption by microplastics in different salinity conditions (Ahechti *et al.*, 2022). Recent studies suggest that the changes brought about by salinity in metal behaviour are particularly significant in semi-arid coastal aquifers (Gantayat & Elumalai, 2024). Therefore, understanding these interactions is crucial for assessing ecological risks and developing mitigation strategies.

### 1.4.3. Metals of Interest and their Applications

Metals are a significant group of toxic water contaminants (Khan et *al.,* 2008). The metals employed in this study include Arsenic (As), Cadmium (Cd), Copper (Cu), Nickel (Ni), Lead (Pb), and Zinc (Zn), commonly found in paper, paint, and textile industries (Park *et. al.,* 2015; Valentín *et. al.,* 2013). Generally, metals as toxicants exhibit several modes of action, including neurotoxicity and blocking respiration and biosynthesis (Sanchez-Bayo, 2011). The sources, applications, and properties of the metals of interest in this study are summarized in Table 4 below.

Table 4: Physicochemical properties, Occurrences, Sources, Application and Health Effects of Metals of Interest.

| **Metal / Valencies** | **WHO MCL ($\mu g\ L^{-1}$)** | **Sources and Applications** | **Health Effects** | **References** |
|---|---|---|---|---|
| As (III, V) | 10 | Coal burning, smelting, agriculture and mining, erosion of sedimentary rocks, waste disposal plants, compost, landfills, industrial wastewater, fly ash, sewage sludge, pesticides, manufacturing of electronics, special alloys, manufacture of certain ceramics and glasses, wood preservatives. | Short-term effects include vomiting, numbness, diarrhoea, muscle cramping, skin disorders, and skin and eye burns on contact. Long-term effects are skin and visceral cancers, hyperkeratosis, change in pigmentation, bladder cancer and lung cancer. infant mortality and health damage of child, vascular diseases. Damage to the stomach, kidney, liver, heart, lungs, and nervous system, circulatory system issues, and the risk of getting cancer. | Zamora-Ledezma *et. al.* 2021; Public Health England, 2019; Quansah *et al.*, 2015. |
| Cd (II) | 3 | Electroplating and Metallurgical industries, Petroleum products, Insecticides and Synthetic chemicals | Kidney damage, renal disorder, lung cancers, “Itai-Itai” disease, and damage to respiratory systems, liver, and reproductive organs, induce tissue injury and apoptosis of tubular cells, sensory alteration, mutation, osteoporosis, hypochromic bone degeneration, anaemia and osteomalacia | Zamora-Ledezma *et. al.* 2021; Kumar *et al.*, 2021; WHO, 2017; Cooper & Davies, 2015 |
| Cu (I, II) | 200 | Mining industries, metallurgy chemical manufacturing, steel industries, | Liver damage, Wilson disease, insomnia, Hair loss, anaemia, liver and kidney damage, headache, hypotension, melena, hematemesis, coma, jaundice, gastrointestinal | Zamora-Ledezma *et. al.* 2021; Kumar *et al.*, 2021; |

| Metal / Valencies | WHO MCL (μg L⁻¹) | Sources and Applications | Health Effects | References |
|---|---|---|---|---|
| | | electroplating industries, fertilizers, pesticides | distress, vomiting, nausea, respiratory problems, and abdominal pain. | |
| Ni (II) | 70 | Metal alloys, Battery plants, Electroplating industries, Pulp and paper mills, fertilizers and petroleum refineries | Dermatitis, nausea, chronic asthma, coughing, cancers, Dry cough, chest pain, breathing problem, diarrhoea, skin eruption, pulmonary fibrosis, gastrointestinal ache, renal oedema | Zamora-Ledezma *et. al.* 2021; Cooper & Davies, 2015. |
| Pb (II, IV) | 10 | Metal purifying, Pesticides, Vehicular emissions, Fertilizers Coal, petrol | Kidney disease, foetal brain damage, circulatory and nervous system damage, mental retardation, and cancer in the human body | Zamora-Ledezma *et. al.* 2021; Cooper & Davies, 2015. |
| Zn (II) | 300 | Metal alloys pigments, electroplating, industrial waste mining, coal combustion | Depression, lethargy, neurological impairment, increased thirst, Pain, skin inflammation, fever, vomiting, anaemia | Zamora-Ledezma *et. al.* 2021 |

#### *1.4.3.1. Arsenic (As)*

Arsenic is a naturally occurring metalloid found in the earth's crust, air, and water, and is recognized as a carcinogen that contaminates drinking water globally. Research shows that arsenic exposure increases the risk of malignancies (Karagas *et al.*, 2012). While typically present at low levels in air and water (Rasmussen & Andersen, 2003; Uthus, 2003), arsenic is toxic to human cells, causing mutagenesis, carcinogenesis, and teratogenesis (Liu & Lu, 2010). It leads to chromosomal abnormalities and promotes uncontrolled cell growth through increased cellular tyrosine phosphorylation (Hossain *et al.*, 2000; Flora, 2011).

Arsenic disrupts ATP synthesis by competing with phosphorus in phosphorylation pathways, and some organo-arsenic compounds have neurotoxic effects (Sanchez-Bayo, 2011). Major anthropogenic sources of arsenic pollution stem from fossil fuel energy generation and metal smelting (Ravenscroft *et al.*, 2011). Biochar has been shown to adsorb arsenic similarly to chromium (Deng *et al.*, 2020). Remediation methods for arsenic include activated carbon, immobilization, coagulation, filtration, phytoremediation, and precipitation (Kumar *et al.*, 2021; Mahimairaja *et al.*, 2005).

#### *1.4.3.2. Cadmium (Cd)*

Cadmium is a toxic, soft, silver-white metal that occurs naturally in low levels in rocks and soil. It is primarily used in nickel-cadmium batteries, electroplating, alloys, pigments, and plastic stabilizers. Environmental sources include the smelting of metals like zinc, fossil fuel combustion, solid waste, and certain fertilizers (Davis *et al.*, 2001). With an atomic number of 48 and an atomic weight of 112.4, cadmium mainly exists as a divalent ion, similar to zinc (Wuana & Okieimen, 2011). This similarity contributes to its toxicity, as it often replaces zinc in biological processes (Deitsch, 2009). Other exposure sources include soft drinks, water softeners, pesticides, fungicides, and cigarette smoke (Manahan, 2003).

Cadmium is highly carcinogenic and can lead to serious health issues such as kidney failure, bone disorders, and various cancers (Santiago, 2015; Adams, 2014). Its elimination half-life is 10 to 30 years, and it accumulates in the kidneys, with urinary excretion serving as a biomarker (Su *et al.*, 2011). Prolonged exposure is linked to tubular dysfunction and bone diseases (Nair *et al.*, 2013; Verougstraete *et al.*, 2003). The toxicity largely arises from cadmium's ability to substitute zinc in biological reactions (Sanchez-Bayo, 2011; Golovine *et al.*, 2010). Remediation techniques for cadmium in wastewater include biosurfactants, chemical precipitation, flotation, micellar-enhanced ultrafiltration (MEUF), and adsorption (Kumar *et al.*, 2021; Renu and Singh, 2017).

#### *1.4.3.3. Copper (Cu)*

Copper is a transition metal with an atomic number of 29 and an atomic weight of 63.5. It has a density of 8.96 g cm$^{-3}$, a melting point of 1083°C, and a boiling point of 2595°C. Sources of copper in water include runoff from urban areas (Adams, 2014). While copper is an essential micronutrient for both plants and animals, its effective range is narrow (Santiago, 2015). In humans, copper aids haemoglobin production in the blood, while in plants, it supports seed production, disease resistance, and water regulation. Importantly, copper does not biomagnify or bioaccumulate in organisms.

In soil, copper complexes with organic materials and is rarely found in solution. Health issues related to copper exposure include anaemia and liver damage (Wuana & Okieimen, 2011), and it may affect brain function, leading to mental health issues (Santiago, 2015; Adams, 2014). Copper ions from salts like copper sulphate can inhibit the germination of algae and fungi, disrupt sodium regulation in fish, and cause liver damage in mammals (Sanchez-Bayo, 2011). Remediation techniques for copper ions include membrane filtration, electrodialysis, electrocoagulation, and ion exchange (Kumar *et al.*, 2021; Arbabi & Golshani, 2016).

#### *1.4.3.4. Nickel (Ni)*

Nickel is a transition metal prized for its unique properties, including corrosion resistance, high tensile strength, and good thermal and electrical conductivity (Buxton *et al.*, 2019). It primarily occurs in minerals like pentlandite and garnierite and is found in ultramafic rocks (Kumar *et al.*, 2021). Nickel is widely used in metallurgy, electronics, and renewable energy, playing a crucial role in stainless steel production and serving as a catalyst (Shahzad *et al.*, 2018). While it has beneficial roles in human health, evidenced by essential Ni-containing enzymes in plants and microorganisms, there are also significant health and environmental concerns. Nickel compounds can induce cancer and lead to respiratory issues and dermatitis (Vinken *et al.*, 2010; Fasinu & Orisakwe, 2013). Its accumulation in ecosystems threatens aquatic life, contaminates agricultural land, affects plant growth, and enters the food chain, posing further health risks (El-Kady & Abdel-Wahhab, 2018; Okereafor *et al.*, 2020; Sanchez-Bayo, 2011).

#### *1.4.3.5. Lead (Pb)*

The term "lead" comes from the Latin word "plumbum," meaning "pipe," due to its historical use in water pipes. Lead is the heaviest non-radioactive element, with an atomic number of 82, an atomic mass of 207.2, and a density of 11.4 g cm$^{-3}$. It commonly occurs in nature as divalent ions, oxides, hydroxides, and metal oxyanion complexes (Wuana & Okieimen, 2011). Lead is found in chocolate, toothpaste, batteries, and old paints (Wuana & Okieimen, 2011; Deitsch, 2009). Exposure routes include inhalation and ingestion, leading to accumulation in organs such as the brain and kidneys, and chronic exposure can result in reduced IQ and difficulties in adapting to new situations (Santiago, 2015; Adams, 2014).

A large percentage of extracted lead comes from the mineral galena, which contains lead sulfide. Key consumers are the automobile industry and manufacturers of batteries and additives. In soil, lead concentrations range from 2 to 200 mg/kg and can reach higher levels in drinking water and urban air (Nriagu *et al.*, 2008). Lead is toxic and affects various body systems, inhibiting haemoglobin synthesis and interfering with calcium ions (Sanchez-Bayo, 2011).

Studies indicate that inorganic lead compounds increase cancer risk through mechanisms such as DNA damage and oxidative stress. Lead can disrupt zinc's role in enzyme functions, affecting gene expression and tumor growth in laboratory animals (Witkiewicz-Kucharczyk & Bal, 2005). Lead adsorption by biochar is promoted by high temperatures, with precipitation being a key mechanism (Shen *et al.*, 2019). Standard mechanisms for lead and cadmium adsorption by biochar include cation exchange and electrostatic attraction (Deng *et al.*, 2020).

#### *1.4.3.6. Zinc (Zn)*

Zinc is a transition metal with an atomic number of 30 and an atomic mass of 65.4. It has a density of 7.14 g $cm^{-3}$, a melting point of 419.5°C, and a boiling point of 906°C. As an essential trace element for human health, zinc is found naturally in drinking water and soil, occurring at about 70 mg $kg^{-1}$ in crustal rocks. Human activities like mining, coal burning, waste incineration, and steel production release additional zinc into the environment.

Zinc deficiency can lead to congenital disabilities, while excess zinc can raise water acidity and pose health risks. It also negatively impacts microorganisms and earthworms, inhibiting the breakdown of organic matter (Wuana & Okieimen, 2011). Zinc is vital for synthesizing DNA, RNA, and proteins and is necessary for the activity of zinc-containing enzymes. It can reduce the carcinogenic effects of cadmium (Cd) in animal studies (Saïd *et al.*, 2010), indicating their antagonistic relationship. Zinc competes with copper for uptake in biomolecules, and its free ion is a corrosive acid with biocidal activity (Sanchez-Bayo, 2011). Research shows that biochar is more effective than activated carbon in adsorbing zinc (II) from aqueous solutions (Deng *et al.*, 2020).

## 1.5. Consequences of Water Pollution

Research highlights the benefits of pesticides for food production and public health. However, their large-scale and unregulated use has caused severe socio-economic, environmental, and health issues. Different species absorb and accumulate water pollutants, such as pesticides and metals, in different ways. Depending on the pollutant's chemical composition and dose, and on the energy cost of uptake versus detoxification, it can be transformed and excreted without risk of organismal toxicity. Conversely, a pollutant may be resistant to chemical or biotransformation, resulting in accumulation in the nervous system, liver, and kidneys and toxicity (Zeitoun & Mehana, 2014; Gabriel et al., 2006).

These harmful substances can affect aquatic organisms, disrupt ecosystem processes, and thereby reduce biodiversity. Additionally, some of these chemicals can bioaccumulate through the food chain and pose a major health threat to humans who consume contaminated fish or shellfish. Industrialization, urbanization, and population growth have also increased pressure on water resources, leading to higher levels of pollution

(Thompson et al., 2019). This challenge must be addressed urgently, as water pollution can harm aquatic organisms, contaminate water bodies, and pose major environmental and human health risks.

Groundwater aquifers are the main source of drinking water in developing countries. Groundwater is vulnerable to pesticide pollution because pesticide mobility in soil is governed by physicochemical properties, such as water solubility, weather, season, soil type, and the concentration applied (Singh et al., 2015). Studies on pesticides in India indicate that all freshwater bodies, from rivers and lakes to estuaries, are polluted by pesticides, as only 10% of wastewater is treated; the balance is released untreated into the water system. These effluents seep into groundwater, rivers, and other water sources and are transmitted to families through several pathways, including aquatic foods (Yadav et al., 2015). Significantly high levels of surface water bodies were reported to be contaminated by largely organochlorine pesticides (OCPs), chlorpyrifos, endosulfan (both alpha- and beta-), dichlorvos, and parathion-methyl, while a lower level of groundwater of endosulfan and hexachlorobenzene (HCB) was also found (Yadav et. al., 2015; Lari et. al., 2014).

### 1.5.1. Pesticide Resistance

One effect of using pesticides is the emergence of pesticide-resistant pests. Many pests can develop resistance over time and become resistant to specific pesticides, making them ineffective. This creates a spiral of increased pesticide use as farmers turn to more potent, and consequently more toxic, formulations to counteract resistant pests, thereby maintaining health and environmental risks.

### 1.5.2. The socio-economic Impacts

Lack of access to potable water is exacerbated by water source contamination, leading to increased water-related conflicts, especially in developing countries (UN Water, 2020). In particular, the presence of polluted water bodies can impair the visual and recreational appeal of an area to visitors, further impacting tourism, which is highly identified with water quality (European Commission, 2019). Economic impact encompasses reduced marine resources, costly procedures for producing potable water, and wastewater decontamination. The resulting food shortage intensifies socio-economic inequity and can fuel socio-political volatility as communities compete for limited resources. But the loss of productivity attributable to water pollution must also be considered, along with direct health effects. It reduces the labour force's capacity to contribute effectively to economic growth. Those suffering from waterborne illnesses are often unable to work, worsening labour shortages in other sectors. This productivity loss constrains household income but also has larger consequences for the national economy.

The socio-economic impact of water pollution is huge. Individual health, eco-system, and economic development are all affected by pollution at the national level. Countries struggling with water quality problems grapple with multiple cost factors, ranging from lower agricultural production to hospitalisation costs from water-borne diseases, as well as lost economic opportunities across clean-water-dependent sectors like tourism and fisheries. his complex situation illustrates how access to clean water, economic growth, and public health are interconnected, with poverty serving as both a driver and a result of water pollution.

### 1.5.3. Environmental Impacts of Water Pollutants

The environmental consequences of water pollution with pesticides and metals are multifaceted and far-reaching. Firstly, Water pollutants such as heavy metals, pesticides, and oil spills contaminate water sources, leading to waterborne diseases, disruption of aquatic ecosystems, and biodiversity loss. Soil pollutants, including pesticides, industrial chemicals, and heavy metals, can affect crop yields, food safety, and soil fertility, harm plant and animal life, and seep into groundwater resources. The environmental consequences of water pollution include eutrophication of surface waters, including freshwater lakes, streams, estuaries, and coastal seas; loss of biodiversity; changes in species composition in terrestrial and aquatic ecosystems; groundwater pollution; and the acidification of sensitive freshwaters (Tilman et *al.,* 2001). These consequences of water pollution have led to stringent effluent and water quality laws worldwide.

Pesticide use has caused significant harm to the environment, particularly in aquatic ecosystems. Runoff from agricultural fields containing pesticides contributes to water pollution, contaminating lakes, rivers, and groundwater. The accumulation of pesticides in aquatic environments harms fish, amphibians, and other aquatic organisms, disrupting ecosystems and reducing biodiversity. Furthermore, pesticides can persist in soil and sediment for extended periods, causing long-term damage to terrestrial ecosystems. For instance, pesticide metabolites contaminate water bodies, rendering them unsuitable for their intended use and causing aquatic toxicity and water pollution, among other consequences (Ajia, 2017). The environmental consequences of pesticides are numerous, including ecological harm, loss of pollinators, and soil and water pollution.

- **Water Pollution**

Pesticides enter aquatic ecosystems through surface runoff, leaching, and spray drift. These substances pollute water bodies, endangering aquatic organisms, entire food webs, and human health. Moreover, pesticide runoff can contaminate drinking water sources and groundwater. Contamination of groundwater is particularly concerning, as it poses a direct threat to drinking water supplies. Pesticides can persist in groundwater for extended periods and may harm human health with prolonged exposure. Additionally, agricultural fields treated with pesticides can experience runoff, contaminating surface waters. This process inevitably pollutes freshwater bodies and aquatic ecosystems (Safa, 2014).

- **Eutrophication**

This eutrophication and habitat destruction would cause unprecedented ecosystem simplification, loss of ecosystem services, and species extinction. Significant scientific advances and regulatory, technological, and policy changes are needed to control the environmental impacts of agricultural expansion (Tilman *et. al.,* 2001).

- **Ecosystem Disruption**

Pesticides that target specific pests usually have unintended consequences on non-target organisms. This imbalance disrupts ecosystem dynamics, reducing biodiversity, altering predator-prey interactions, and declining ecosystem resilience. The consequences of pesticide use on the ecosystem are detrimental, as these pollutants can persist for long periods and have severe ecological impacts on aquatic organisms (Thompson *et. al.,* 2019; UN-Water, 2020). The toxicity to non-target organisms, including fish, amphibians, and birds, can be significant, disrupting ecosystem balance. One of the primary ecological effects of water pollution is the degradation and loss of freshwater habitats (Vörösmarty *et al., 2010),* which lead to a decline in biodiversity, as many species depend on these habitats for their survival *(Vörösmarty et al.,* 2010). The contamination of water bodies can disrupt the ecological balance, resulting in declining fish stocks and other commercially valuable species (Vörösmarty *et. al.,* 2010). This disruption affects the livelihoods of communities dependent on fishing and disrupts the food chain, leading to cascading ecological impacts (Vörösmarty *et. al.,* 2010).

Ecological effects include reduced fish populations, transformed microbial communities, decreased self-purifying capacity of natural waters, polluted river sediments, and increased secondary pollution (Shaumyan, 2007). Furthermore, the adverse effects of water pollution on ecosystems cannot be overstated. Polluted water bodies adversely affect aquatic life, degrading biodiversity and essential habitats. Ecosystems critical to food security and livelihoods are under siege from nutrient loading and chemical contaminants, disrupting the ecological balance. In rural communities that depend on fishing and agriculture, the decline of healthy water sources translates into reduced fish catches and lower agricultural productivity.

Aquatic ecosystems are highly vulnerable to the toxic effects of pesticides. Pesticides can accumulate in the tissues of aquatic organisms, causing physiological disorders, reproductive abnormalities, and even mortality. Fish, amphibians, and invertebrates are often the most affected, as they are directly exposed to contaminated water. Moreover, pesticides can disrupt the balance of aquatic ecosystems by selectively reducing populations of certain species, leading to cascading ecological effects throughout the food chain (De Marval, Pelissero, & Jaffrezic, 2018). Figure 10 summarizes the ecological impacts of herbicides and insecticides in the freshwater ecosystem.

For example, photosynthesis is an important ecosystem process that influences water quality, and its inhibition by herbicides results in lower daytime dissolved oxygen (DO) concentrations and lower pH. Furthermore, if a herbicide causes acute mortality of macrophytes, the decomposition of dead macrophytes may further reduce pH and DO concentrations. This deterioration of water quality can then have detrimental impacts on sensitive invertebrate species, representing indirect effects resulting from species-habitat relationships. A reduction in Dissolved Oxygen (DO) can greatly reduce cladoceran populations (Schafer *et al.*, 2011).

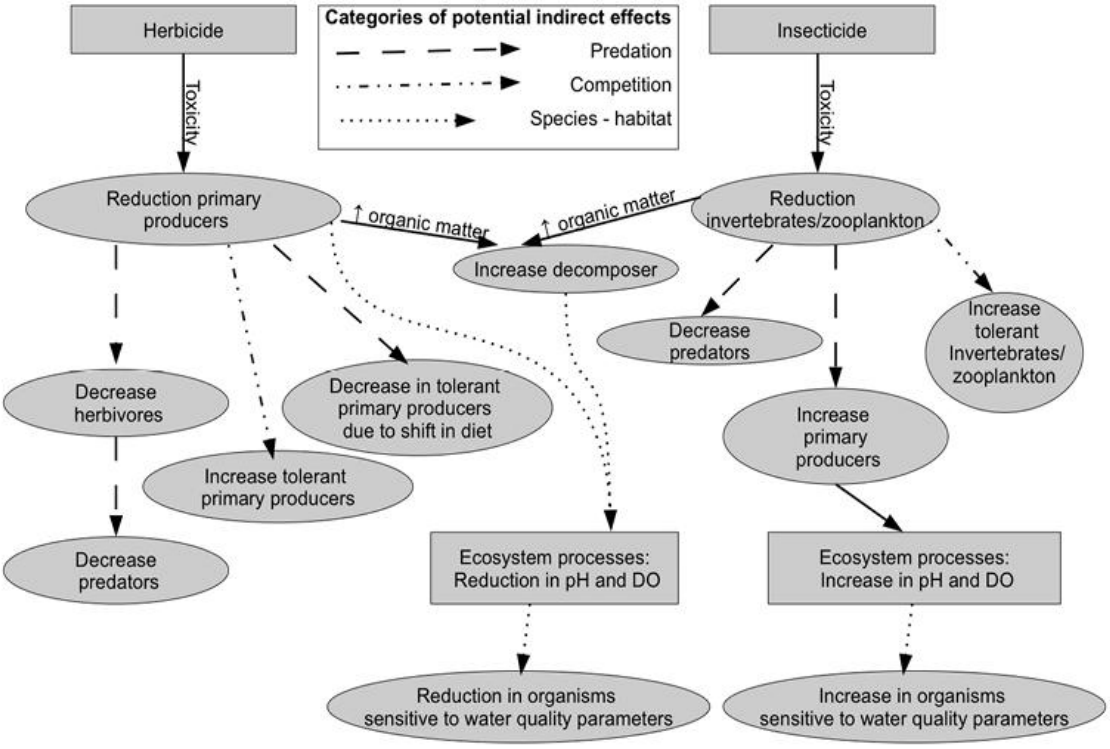


Figure 10: Schematic representation of direct (solid line) and indirect (dashed and dotted lines) potential effects of pesticides in freshwater ecosystems (Source: Schafer *et al.*, 2011).

- **Loss of Pollinators**

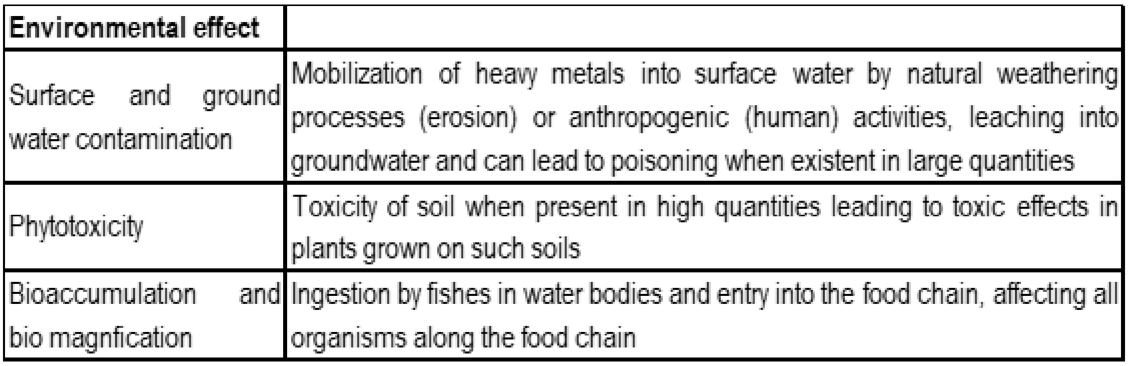
The indiscriminate use of pesticides affects pollinators, such as bees, butterflies, and birds, which are vital for the reproduction of many plant species. The decline in pollinator populations jeopardizes global food security and disrupts critical ecosystem services.

- **Soil Contamination**

Pesticides can accumulate in soil, persist for years, and negatively affect soil health. This contamination can hinder beneficial microbial activity, reduce nutrient availability, and ultimately impede sustainable agriculture.

The persistence and mobility of pesticides in the environment can lead to various adverse effects. Groundwater contamination by pesticides is of particular concern, as it poses a direct risk to drinking water sources. Pesticides can persist in groundwater for extended periods, potentially affecting human health through chronic exposure. Moreover, surface water contamination can result from runoff from treated fields or accidental spills, thereby contaminating aquatic ecosystems. The impact on non-target organisms, including fish, amphibians, and birds, can be significant, disrupting ecosystem balance. Apart from their direct effects on the environment, pesticides can also indirectly impact human health through contamination of food sources. Certain factors influence crop residue levels, including pesticide persistence, plant metabolism, and the interval between pesticide application and harvest. Heavy metals have environmental impacts on water sources, plant toxicity, and bioaccumulation along the food chain. Table 5 below summarizes some of these impacts.

Table 5: Environmental effects of Heavy metal toxicity (Source: Ambrose, 2013).



| **Environmental effect** | |
|---|---|
| Surface and ground water contamination | Mobilization of heavy metals into surface water by natural weathering processes (erosion) or anthropogenic (human) activities, leaching into groundwater and can lead to poisoning when existent in large quantities |
| Phytotoxicity | Toxicity of soil when present in high quantities leading to toxic effects in plants grown on such soils |
| Bioaccumulation and bio magnfication | Ingestion by fishes in water bodies and entry into the food chain, affecting all organisms along the food chain |

## 1.5.4. Health Effects of Water Pollution

Contaminated water sources are a potential route of pesticide exposure, especially in rural areas where groundwater is the primary source of drinking water. Consuming water contaminated with pesticides can lead to acute or chronic health effects, ranging from nausea and skin irritation to more severe conditions such as cancer and reproductive disorders (Karthikeyan & Muthukumar, 2017).

In developing and underdeveloped countries with ineffective implementation of environmental policies, there has been a proliferation of cancer, lung, and skin diseases from contamination of water sources by toxic contaminants (Shaumyan, 2007). Moreover, pesticides can also enter the food chain, as they are absorbed by plants and subsequently consumed by animals and humans. This indirect pesticide exposure further magnifies the potential health risks highlighted below. The persistence and mobility of pesticides in the environment can lead to various adverse effects. Waterborne diseases such as cholera, typhoid, and hepatitis can spread through consuming polluted water (Yarkwan, 2023; Sarker *et. al.*, 2021; World Health Organization, 2020). Exposure to toxic water pollutants can have severe and potentially life-threatening impacts, ranging from skin irritation to cancers, developmental issues, and endocrine disruption (World Health Organization, 2020). This emphasizes the seriousness of the situation. The economic ramifications of water pollution are significant, including healthcare costs that strain communities' limited resources. Diseases caused by waterborne contaminants increase healthcare expenses, diverting funds from education, infrastructure, and development initiatives (WHO, 2021).

#### *1.5.4.1. Health Impacts of Pesticide Use*

- **Pesticide Residues in Food**

Pesticides are crucial in modern agriculture because they help control pests and increase crop yields. However, the use of pesticides comes with numerous health concerns. Certain factors may influence crop residue levels, such as the pesticide's persistence, plant metabolism, and the time interval between pesticide application and harvest. Stringent food safety regulations ensure that pesticide residues in food do not exceed acceptable limits, protecting consumers from potential health risks. The presence of pesticide residues in food is a significant concern. Studies have identified residues on fruits, vegetables, and grains, highlighting the need to regulate pesticide use and promote sustainable and organic farming practices.

Pesticides pose significant health problems due to their 'stability, long-range transport properties and bioaccumulation tendencies in living tissues'. Bioaccumulation is the process by which substances, such as pesticides, accumulate in the adipose tissues of humans and animal organisms, often reaching higher concentrations as they move up the food chain (Ahmad *et. al.,* 2014; Xu *et. al.,* 2013). Water pollutants are absorbed or accumulated in species differently. In some cases, pollutants may undergo chemical or biotransformation and excretion without the risk of toxicity to an organism, depending on the chemical composition and dose of the pollutant. However, a pollutant may resist chemical or biotransformation in other cases, leading to accumulation and toxicity in nerves, the liver, and kidneys (Zeitoun & Mehana, 2014; Gabriel *et. al.,* 2006). Pesticides ingested by animals can bioaccumulate and biomagnify throughout the food chain, posing risks to higher trophic levels, including humans. This phenomenon underlines the interconnectedness between environmental contamination and human health.

- **Acute and Chronic Human Health Impacts**

Understanding the potential impact of pesticides on humans is crucial. Pesticides can impact humans through inhalation, ingestion, and skin absorption. These agrochemicals are biologically active and very toxic to wildlife and humans. Research posits that the health effects of pesticide exposure can range from mild skin irritation and neuro-behavioural disorders to cancers and ultimately death, depending on the route of exposure and the concentration (Ikpesu & Arlyo, 2013; Valentín et. al., 2013; Ahmad et. al., 2010; Metcalf, 1997). Pesticides pose a significant threat to aquatic species, as they can be lethal even at low concentrations (Ikpesu & Arlyo, 2013; Adeniyi et al., 2008; Adeyemi et *al.,* 2008). Prolonged exposure to these chemicals has been associated with a range of harmful health effects, including an elevated risk of cancer, reproductive disorders, neurotoxicity, and disruption of endocrine functions.

Research posits that in addition to the risks they pose to humans and other non-target organisms, they also exert toxic/and/or lethal effects on many other arboreal, aquatic, and terrestrial organisms (de Souza *et. al.,* 2016; Aktar *et. al.,* 2009). Previous studies have linked herbicides to the destruction of foliage and inhibition of photosynthesis in plants, oxidative stress in non-mammalian sp., chromosomal aberrations in fish, a decline of soil microbial populations and diversity, mutation, abnormal behaviour, increased mortality, reduced reproduction, and growth rates among other deleterious effects (de Souza *et. al.,* 2016; Aktar *et. al.,* 2009). Their health effect on humans can range from mild skin irritation to genetic defects, endocrine disruption, cancer, and death. Human health impacts from pesticide use extend beyond ecosystems and pose significant risks to human health. Farmers and agricultural workers are particularly vulnerable due to prolonged exposure during pesticide application and handling.

Pesticides can cause acute and chronic health effects on those exposed, such as farmworkers, residents of agricultural areas, and consumers of food treated with pesticides. These effects may include respiratory issues, neurological disorders, reproductive disorders, and even cancer. Prolonged exposure to pesticides has been linked to various health issues, including organ damage, neurodevelopmental disorders, increased risk of cancer, reproductive disorders, and endocrine disruption. Several studies have demonstrated a correlation between pesticide exposure and increased risk of Parkinson's disease, leukaemia, and reproductive disorders. Furthermore, occupational exposure makes farmers and agricultural workers who handle pesticides directly more susceptible to these adverse health effects. Farmers and agricultural workers are particularly vulnerable due to prolonged exposure during pesticide application and handling.

For example, India was one of the first countries to experience epidemic pesticide poisoning caused by leakage at a pesticide plant in Bhopal. A well-documented case illustrating the connection between pesticide exposure and health impacts is the 1984 tragedy in Bhopal, India. A toxic gas leak from a pesticide plant resulted in the exposure of over 500,000 people to methyl isocyanate gas. Thousands died within days, and many more suffered long-term health consequences. More than 2,500 people and about 335,000 people were victimized (Shaumyan, 2007). This catastrophe highlighted the urgent need for stricter regulations and improved safety measures to prevent such incidents.

Table 6: Summarized Health Effects and Maximum Contaminant Limits of Pesticides of Interest

| **Pesticide** | **MCL (ug $L^{-1}$)** | **Plant and Human Health Effects** | **References** |
|---|---|---|---|
| CP | 10 | In humans, exposure can cause symptoms such as headaches, nausea, and in serious cases, respiratory distress. Animals, in particular aquatic species, are notably affected, because cypermethrin induces toxicities in fish, with potential results unfavourable for health. Among mammals, studies have highlighted its reproductive toxicity and its impact on myocardial tissue | Cooper & Davies, 2015; Ullah *et al.*, 2018;<br>Farag *et al.*, 2021;<br>Ghazouani *et al.*, 2020; Sharma *et al.*, 2014. |
| IPU | 10 | The results are yellowing, chlorosis, lightening effects, and finally the drying of floras, Genotoxic (chromosomal aberrations in bone marrow cells, for example, chromatid gaps, chromatid breaks, acentric fragments, chromatid exchanges, ring chromosomes, and metacentric chromosomes) for mammals (Swiss albino mice)<br>Reproductive problems, infertility, and fetal malformations in humans. Growth effects potentially associated with thyroid disruption. | Leoci & Ruberti, 2020; Cooper & Davies, 2015; Behera & Bhunya, 1990;<br>Caux *et. al.*, 1998; Mittler, 2002; Hassan & Nemat-Alla, 2005;<br>Yin *et. al.*, 2008;<br>Hoshiya *et. al.*, 1993; Peres *et. al.*, 1996). |

#### *1.5.4.2. Health Impacts of Metals Use*

Heavy metal toxicity in water depends on valency and form (Akgun, 2005). Most toxic trace metals, such as $Pb^{2+}$, $Cu^{2+}$, $Ni^{2+}$, $Cd^{2+}$, $Co^{2+}$, and $Zn^{2+}$, occur in solution as cations and become increasingly insoluble as pH increases. Oxyanion-forming elements such as Chromium, Arsenic, Uranium, and Selenium are commonly found as trace contaminants in groundwater because they can persist at the usual near-neutral pH of groundwater (Dzombak & Morel, 1990).

Heavy metals in industrial wastewater, such as mine effluents, are often found alongside other organic and inorganic contaminants. Pollution of drinking or agricultural water sources by heavy metals can lead to severe public health epidemics. A few historical cases are the Minamata Bay mercury incident of the 1950s, with which over 500 fatalities due to ingestion of mercury-contaminated fish (Zahir *et. al.,* 2005; Tsuda *et. al.,* 2009), and the arsenic contamination of groundwater sources in Bangladesh since the 1970s (Mehovic & Blum, 2004; Uddin & Huda, 2011; Thakur & Semil, 2013). Some other countries with arsenic toxicity challenges include the USA, Taiwan, Mexico, Mongolia, Argentina, India, and Chile (Uddin & Huda, 2011; Thakur & Semil, 2013). Other historical incidents were the Iraqi epidemic from 1971 to 1972 caused by the consumption of bread prepared from grains sprayed with alkylmercury fungicides (Tsuda *et. al.,* 2009), the "itai-itai" disease in Japan resulting from long-term consumption of rice grown in cadmium-contaminated areas, the congenital disabilities caused by lead toxicity in Fallujah, Iraq (The Huffington Post 2013, 2013; Wuana & Okieimen, 2011), and the lead poisoning of children in Zamfara state, Nigeria (Terradaily, 2010).

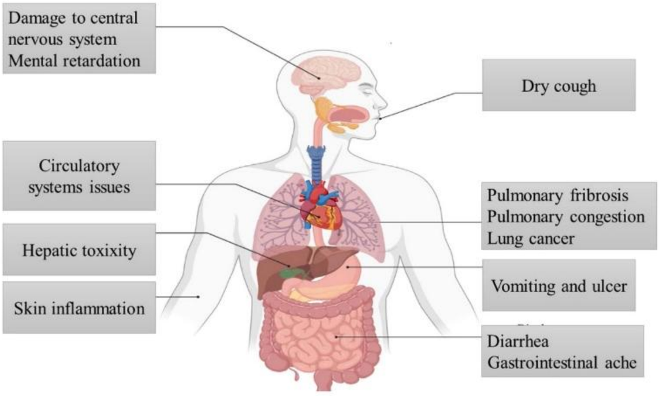


Figure 11: Health Effects of Metal Water Pollution (Adapted from Zamora-Ledezma et. al., 2021)

The impact of water pollution with arsenic, cadmium, copper, nickel, lead, and zinc in developed and developing countries is significant, with harmful effects on aquatic ecosystems, wildlife, and human populations. Heavy metals accumulate in the food chain, threatening the health of aquatic organisms and humans who consume contaminated water or fish. Studies have shown that metal pollution can harm wildlife, including fish, birds, and mammals, as well as plants and soil quality. These impacts can also affect human health, with exposure to high levels of metal pollutants linked to respiratory problems, neurological disorders, respiratory diseases, and cancer, as illustrated in Figure 11.

The ecological impacts of heavy metals include toxicity to aquatic organisms, which can disrupt food webs and ecosystem functions (Souza Machado *et al.*, 2016; Gautam *et al.*, 2016). In addition, these contaminants can persist for long periods, as many heavy metals are recalcitrant and accumulate in aquatic environments (Zhou *et al.*, 2023).

#### *1.5.4.3. Ecotoxicity of Aquatic Pollutants*

Heavy metals are considered among the most toxic water contaminants (Khan et *al.,* 2008). Cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg) and nickel (Ni) are the commonly encountered heavy metals known to cause health problems (WHO, 2004; WHO, 2006; ESCO global 2009).

Cypermethrin and its metabolites have acute effects on aquatic organisms, particularly *Daphnia* spp. Studies have shown that exposure to cypermethrin can harm *Daphnia* survival, growth, mobility, respiration, and reproduction. Furthermore, cypermethrin metabolites can accumulate in the environment and cause long-term effects on aquatic ecosystems. The acute immobilization test aims to determine the concentration that causes immobilization in 50% of neonates at 24 and 48 h. As a result, EC50 at 48 h for immobilization is estimated (Tkaczyk *et. al.,* 2021; OECD, 2004). Cypermethrin is very toxic for honeybees, fish, and aquatic invertebrates. The presence of suspended solids decreases toxicity by at least a factor of 2 because of the adsorption of cypermethrin to the solids. Under normal agricultural conditions (during which drifts may reach adjacent ditches or streams), the only effects seen in surface-breathing or dwelling insects were hyperactivity or immobilization.

In recent years, there has been a growing concern about the effects of Isoproturon and its metabolites on the respiration of *Daphnia* sp. in water. Various studies have shown that exposure to Isoproturon can result in reduced oxygen consumption and altered respiratory patterns in *Daphnia*, leading to potential respiratory distress and even death. The findings raise concerns about the potential ecological consequences of the widespread use of Isoproturon in agricultural practices. Among the challenges in studying the effects of Isoproturon on *Daphnia* sp. is the complex interplay between the herbicide and other environmental factors. For example, studies have shown that certain co-contaminants can exacerbate the toxicity of Isoproturon, underscoring the need to consider the broader environmental context when assessing the risks associated with pesticide use.

## 1.6. The Use of Bioindicators for Water Quality Testing and Monitoring

Bioindicators are crucial for evaluating environmental quality, as they use specific organisms to reflect ecological health. In addition, studies have shown that the physiological state of bioindicators can reveal long-term discrepancies in environmental quality (Kuznetsova & Kholodkevich, 2015), thus providing an estimated timeline of past environmental conditions. According to Holt & Miller (2010), a good bioindicator should have the following properties:

- Provide a measurable response in proportion to the degree of contamination
- Abundant and common
- Have a well-understood ecology and life history
- Be economically or commercially important, i.e., public interest in or awareness of the species.

Water bioindicators play a crucial role in studies aimed at understanding the ecological effects of aquatic pollution. They can also be used to assess the health of streams and rivers. Consequently, bioindicators, through various detection methods, enhance our understanding of ecosystem health and the impact of human activities on aquatic environments (Parmar *et. al.*, 2016; Ovaskainen *et. al.*, 2019; Stagencheva & Sheath, 2016; Kumari & Paul, 2020). Some aquatic bioindicators include:

- **Aquatic plants,** such as waterlily, lotus, mosquito fern, floating heart, water milfoil, mare's tail, water lettuce, water hyacinth, and duckweed, have been identified as an essential class of bioindicators for water quality determination.
- **Marine microorganisms**: A reduced abundance of these organisms can indicate poor water quality. Cyanobacteria have become bioindicators because of their rapid responses to pollutants (Mateo *et. al.*, 2015).
- **Coral pigments**: Darkening of these pigments can indicate poor water quality.
- **Periphyton:** a microbial community that grows on submerged surfaces in aquatic ecosystems and is made up of many different types of organisms, including algae, bacteria, fungi, protozoa, and invertebrates
- **Macroinvertebrates** are standard indicators in river biomonitoring. They lack a backbone, are visible without a microscope, and are found in and around water bodies at some point in their lives. Macroinvertebrates are effective bioindicators due to their sensitivity to changes in aquatic ecosystems (Castillo-Figueroa *et. al.*, 2018). In many lotic environments, the macroinvertebrate community consists of several hundred species from numerous phyla (e.g., Morse *et. al.* 1980, Benke *et. al.* 1984, Roy *et. al.* 2003, Hose *et. al.* 2004), including arthropods (insects, *Daphnia* spp., mites, scuds, and crayfish), molluscs (snails, limpets, mussels, and clams), annelids (segmented worms and leeches), nematodes (roundworms), and turbellarians (flatworms). Benthic (meaning “bottom-dwelling”) macroinvertebrates are small aquatic animals and the aquatic larval stages of insects. They include dragonfly and stonefly larvae, snails, worms, and beetles.

For example, mayfly and stonefly larvae are sensitive to low oxygen levels and can indicate clean water. Other species, such as *Baetis* and *Paraleptophlebia*, can increase abundance after anthropogenic disturbances. Furthermore, the presence and diversity of plankton have been identified as significant indicators, highlighting their potential for monitoring water quality (Chandel et *al.*, 2024). Macroinvertebrate communities are particularly effective in agricultural environments, where they respond to land-use changes (Selvianita et *al.*, 2024; Shimba & Jonah, 2016). Plankton populations reflect changes in water chemistry and serve as indicators of nutrient pollution (Chandel *et. al.*, 2024). Mussels can accumulate contaminants such as hydrocarbons, metals, and other pollutants in their tissues. *Daphnia* spp. are well-studied bioindicators.

- Fish (Okwuosa *et. al.*, 2019) and macroinvertebrates play an essential role in evaluating water quality because they react to pollution and degradation of habitat (López-lópez and Sedeño-Díaz, 2015).

These toxicological assessments, with their ability to provide a holistic vision of ecosystem health, offer a comprehensive approach to environmental quality evaluation. They integrate interactions and complex responses to environmental stressors, providing a more complete picture than chemical analyses (Ovaskinen et *al.*, 2019). While chemical methods are informative, they often fall short of capturing biotic responses to contamination (Hassan *et. al.*, 2019). Bioindicators present a holistic approach to understanding and managing water quality.

### 1.6.1. *Daphnia* Species

*Daphnia* spp., or water fleas, are tiny crustaceans in diverse freshwater habitats. Their sensitivity to chemicals, ease of cultivation, crucial role in aquatic food webs, and the availability of their genetic sequence have led to their extensive use in ecotoxicological and biological research since the early 20th century (Siciliano *et al.*, 2015; Ebert, 2005). Planktonic organisms from the Cladoceran family, particularly *D. magna and D. pulex,* are commonly used in bioassays to monitor water quality. These species are effective bio-indicators in acute toxicity assessments, as they exhibit strong reactions to common environmental toxins (Siciliano *et al.*, 2015). *Daphnia* are essential bioindicators in various aquatic environments, such as acidic swamps, freshwater lakes, and ponds. They play a crucial role in ecotoxicological research due to their high sensitivity to environmental stressors. The two most prevalent species are *D. pulex,* which is smaller and more common, and *D. magna*, which is larger and more widespread (Figure 12).

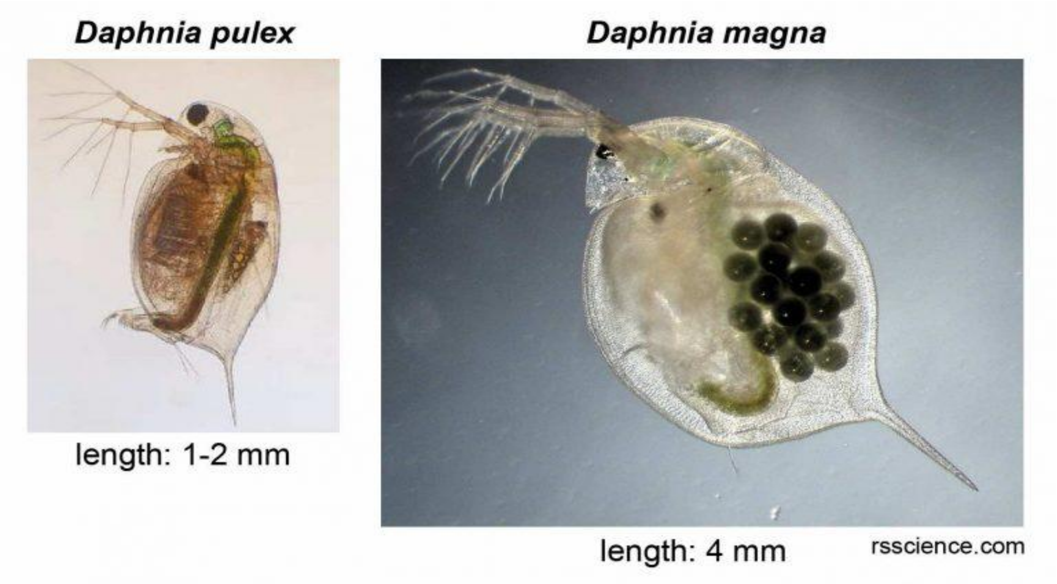


Figure 12: Two common Daphnia spp. Left: *D. pulex* with a body length of around 1-2 mm. Right: female *D. magna* with a clutch of eggs (Source: rsscience.com)

*D. magna* is particularly noted as a model organism for evaluating the toxicity of pharmaceuticals (Farkas *et al.*, 2022; Tkaczyk *et al.*, 2021), perfluorinated alkyl substances (Seyoum *et al.*, 2020), and cadmium (Cd). These assessments follow OECD standardized protocols for acute immobilization, reproduction, and lethality. Adult female *D. magna* can be distinguished from similar species, including *D. pulex,* by the absence of a comb on the abdominal claw and two distinct combs on the abdomen. Males, smaller than females, can be identified by their larger first antennae, a distinguishing characteristic.

#### *1.6.1.1. Daphnia Physiology and Metabolism*

The life cycle of *Daphnia* may be characterized as a sexual or asexual mode of reproduction. In favourable conditions, *Daphnia* undergoes cyclic parthenogenesis (Tkaczyk *et. al.,* 2021; Ebert, 2005; Tatarazako & Oda, 2007). *Daphnia* feed on small particles suspended in the water through a filtering mechanism. This mechanism uses flattened leaf-like legs (phyllopods) that create a water current. As water flows from the front to the back of the organism, Daphnia collects particles with special setae, transfers them into a food groove, and then into the mandible, where the food is mechanically processed (Ebert, 2005; Tatarazako & Oda, 2007). They typically consume bacteria, algae, cyanobacteria, protozoans, and other small particles ranging from 1 μ m to 50 μ m in size. However, larger particles up to 70 µm can sometimes be found in the gut of larger organisms.

*Daphnia* are also capable of absorbing ions through chloride-absorbing glands. Their primary food source is usually planktonic algae, mainly green algae, such as *Scenedesmus, Chlamydomonas,* and *Chlorella* spp. When the food concentration is below a certain level (known as the incipient limiting level), the rate at which *Daphnia* take up food from the water (feeding rate) is directly proportional to the food concentration, and the filtering rate (amount of water filtered per unit time) is at its maximum. However, when the food concentration is above this level, the feeding rate remains constant, while the filtering rate decreases as the food concentration in the water increases.

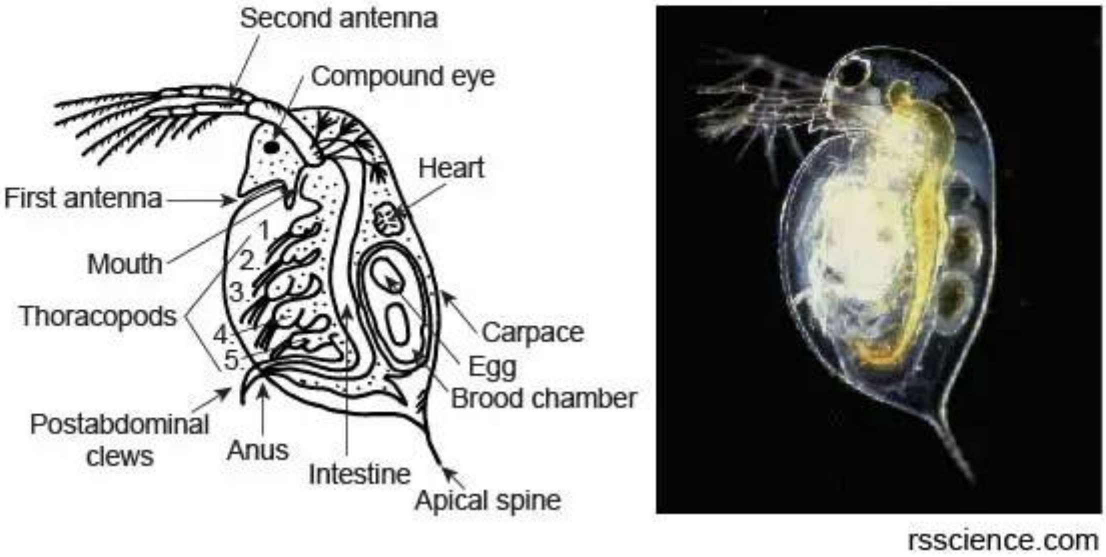


Figure 13: Anatomy of Daphnia (Source: Tapdiqova, 2018)

Daphnia typically adopt a colour that matches the predominant food in their diet. *Daphnia* that feed on green algae appear transparent with a slight green or yellowish tint, while those that feed on bacteria are usually white or salmon pink. Well-fed organisms are more intensely pigmented than starved individuals (Ebert, 2005). *Daphnia* play a vital role as primary consumers in the freshwater aquatic food chain because they serve as food for predatory invertebrates and fish. Moreover, daphnids consume algae; thus, they improve water quality (Dietrich *et. al.,* 2010; Tatarazako & Oda, 2007).

Daphnia have an open circulatory system; their heart is dorsally and anterior to the brood chamber. At 20 °C, the heart beats about 200 times per minute, but this rate slows at lower temperatures. In response to environmental changes, such as oxygen concentration and temperature, haemoglobin concentration can vary by about 20-fold. When loaded with oxygen, haemoglobin appears red and reddish to the otherwise transparent Daphnia. In general, low oxygen levels affect the entire Daphnia population, resulting in reddish colouration in all individuals. Daphnia's nervous system is characterized by the cerebral ganglion located near the gut and the eye. Juvenile and adult *Daphnia* possess a large compound eye, while embryos display two brownish eye spots that fuse during the later stages of development. The compound eye helps the organism orient while swimming. A smaller structure, known as the naupliar eye, lies between the mouth and the compound eye, above the cerebral ganglion (Ebert, 2005).

According to Tkaczyk *et al. (2021),* the transparent carapace allows assessment of different physiological endpoints, including heart activity: heart rate (Bownik et al., 2019a; Villegas-Navarro et al., 2003), and parameters of feeding activity: filtration rate and ingestion rate (Nkoom et al., 2019). Also, other physiological parameters, based on Daphnia limb or organ movements, such as eye activity, post-abdominal claw activity, thoracic limb activity, mandible movement, or whole-organism movements, are used. Moreover, others proposed swimming behaviour parameters, such as swimming activity, swimming time, swimming speed, behavioural strength, and hopping frequency, as highly sensitive endpoints (Bownik, 2017; Jeong et *al.,* 2018) (Figure 14). Recently, Bownik (2019) described in detail different physiological endpoints that could be used in tests to assess the toxicity potential of various compounds (Bownik, 2019).

#### *1.6.1.2. Respiration in Daphnia species*

Respiratory gas transfer occurs via the Daphnia carapace's inner walls and the epipodites located within the filtering chamber (Tkaczyk et al., 2021; Pirow et *al.,* 1999). Oxygen consumption is measured using a polygraph system that consists of a chamber with an oxygen electrode. Daphnia are placed in a chamber where the tested pharmaceutical oxygen concentration is measured during exposure over a defined period (Bownik, 2019). Respiration rate was measured in studies of such pharmaceuticals as antibiotics (procaine penicillin) (Bownik *et. al.,* 2019a) and beta-blockers (propranolol and metoprolol) (Dzialowski *et. al.,* 2006).

### *1.6.1.3. Factors that Affect Daphnia Physiology, Metabolism, and Respiration.*

The physiology, metabolism, and respiration of *Daphnia* spp. can be influenced by environmental factors such as temperature, pH, salinity, and feeding, as well as other biological factors. Temperature has a profound impact on mitochondrial metabolism and respiratory functions. Research shows that *Daphnia* significantly adjusts these parameters during acclimation to different thermal environments (Hoffschröer et *al.*, 2024).

Salinity is another crucial factor that interacts with temperature and hypoxia, collectively influencing *Daphnia* metabolism. Research has shown that exposure to elevated salinity and temperature can induce metabolic changes that influence growth rates and overall fitness (Garreta-Lara et *al.*, 2018). In addition, the implications of hypoxia (low dissolved oxygen) on *Daphnia* physiology are noteworthy, as low oxygen conditions may invoke specific metabolic phenotypes. Such adaptations reflect a propensity to change their metabolic pathways in response to low oxygen availability, which prevails in many freshwater ecosystems (Lee *et. al.*, 2022).

Predation pressures also significantly sculpted Daphnia's physiological traits. Increasing $PCO_2$ levels in aquatic environments can impair predator-induced *Daphnia* defences, affect their metabolism, and increase their vulnerability to predation (Weiss et *al.*, 2018). This highlights the interaction between ecological threats and organism adaptations, emphasizing the importance of understanding these dynamics in evolving ecosystems.

Food quality and availability, including seasonal variations, further impact *Daphnia*'s physiological metrics. Nutritional stoichiometry, determined by the quality of available food resources, plays a vital role in biomass growth and production (Pater *et. al.*, 2018). The interactive effects of these factors demonstrate how Daphnia can modulate their physiological responses to optimize energy use for growth, leading to vital changes in body stoichiometry and biochemistry. Furthermore, Daphnia's ability to adapt to food conditions involves metabolic adjustments that enable optimal management of energy reserves (KlimmPen et *al.*, 2021). This adaptability is a testament to the species' evolutionary tactics for exploiting resources under stress. In chemical exposure contexts, such as pharmaceutical products, *Daphnia*'s physiological endpoints (Figure 14) reflect significant changes in metabolism and growth, highlighting their usefulness in ecological risk assessments (Tkaczyk *et. al.*, 2021). It's equally important to understand how human activities, such as chemical pollution, affect Daphnia's physiology. For instance, research by Boyd *et. al.* (2021) showed that exposure to organic ultraviolet filters negatively impacts both behavioural patterns and essential physiological processes. This highlights our responsibility to manage our environmental impact.

In addition to external stimuli, biological factors such as genetic predisposition influence Daphnia's breathing rates, but research suggests no age-related changes in respiratory performance, suggesting a consistent metabolic capacity across different life stages (Anderson et *al.*, 2022). This implies that Daphnia can maintain metabolic efficiency in moving environments, which is critical for population resilience at any age. However, the OECD recommendations are for the use of neonates in ecotoxicological testing.

In summary, Daphnia's metabolic and respiratory responses are shaped by complex interactions among environmental and biological factors, demonstrating a remarkable ability to adapt. The synthesis of research findings emphasizes Daphnia's relevance in ecological studies and its role as an indicator species for assessing ecosystem quality and health, particularly given ongoing global changes (Niinemets et *al.*, 2017). This underscores the importance of ongoing research into its physiological ecology, as it can provide insights into the broader implications of environmental change for freshwater biodiversity.

#### *1.6.1.4. Acute Toxicity Endpoints Studied with Daphnia*

Most toxicity tests with *Daphnia* are based on the commonly used OECD guidelines for the Acute Immobilisation Test (OECD, 2004). The major physiological endpoints frequently assessed are displayed in Figure 14. However, this study will focus on the swimming behaviour (weakness), immobilization, and mortality of *Daphnia* spp. studied.

Swimming behaviour in *Daphnia* is a critical aspect of their survival, allowing them to avoid predators and locate food. It has been well established that exposure to several pesticides can significantly alter *Daphnia* swimming patterns. For example, zinc administration resulted in standard and biochemical toxicological effects in Daphnia *magna*, including deteriorated locomotion (Sousa & Nunes, 2020). These changes can compromise not only individual aptitude but also the dynamics of the population within aquatic ecosystems since altered behaviour can affect feeding, predatory-pressure interactions, and reproductive success.

The influence of heavy metals on *Daphnia*'s swimming behaviour is similar. Research has shown that cadmium, copper, and lead exposure exacerbate swimming impairments, with contrasting effects across species of *Daphnia* (Araujo *et al.*, 2019; Bownik, 2017). The mechanisms by which heavy metals or metal nanoparticles (Gaiser *et al.*, 2011) decrease *Daphnia* swimming speed have not been elucidated. However, it could be hypothesized that oxidative stress, known to be induced by these substances in other animal species, may also be responsible for the toxic effects on crustacean behaviour (Fan et al., 2012; Manke et al., 2013; Cano *et al.*, 2017). The interruption of normal swimming activities can increase vulnerability to predation, affecting the community's structure and biodiversity within the affected habitat. Consequently, alteration in Daphnia swimming behaviour should be considered an early warning sign of ecological degradation.

Breathing rates in Daphnia also serve as a vital physiological endpoint to assess the impact of pollutants. Pesticides and heavy metals often reduce oxygen intake and disrupt metabolic processes. Studies have shown that the introduction of sulfamethoxazole altered Daphnia's locomotion behaviour and hindered lipid metabolism, resulting in a double interruption of breathing and swimming efficiency (Zhang *et al.*, 2023). Such physiological impediments can spread through trophic levels, affecting the health of fish and other aquatic organisms that depend on *Daphnia* as a food source.

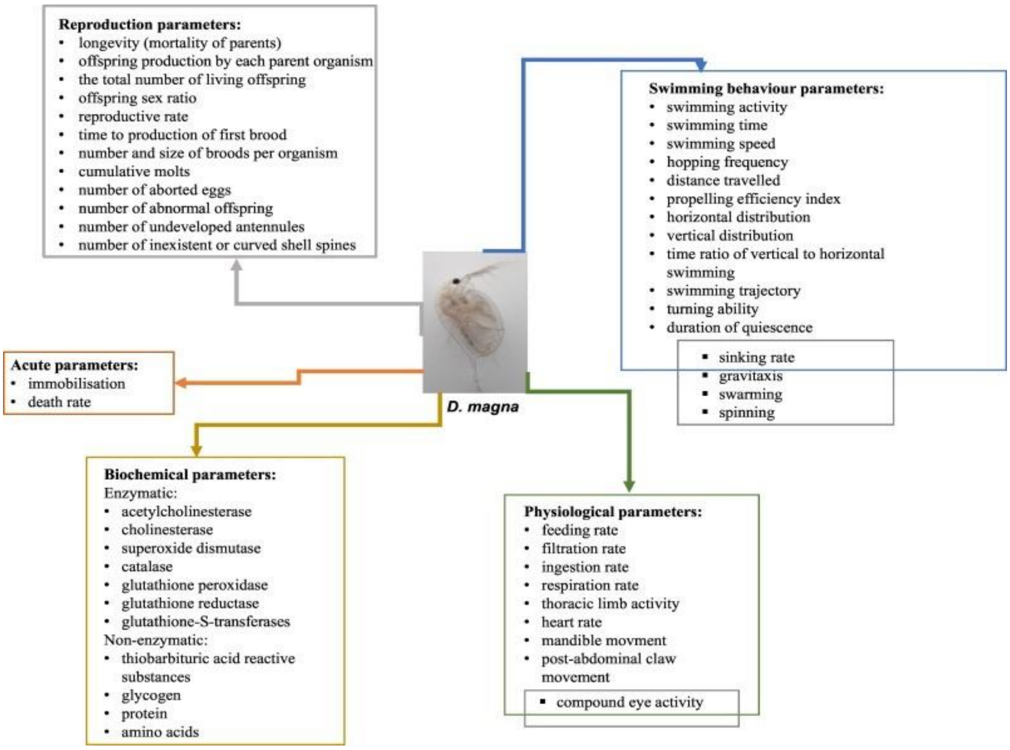


Figure 14: Parameters Commonly Used in the Assessment of Daphnia Toxicity (Source: Tkaczyk et. al., 2021)

In addition, immobilization is a critical endpoint in toxicological evaluations of Daphnia, with numerous studies demonstrating significant correlations between pollutant concentration and immobilization rates. *Daphnia* exposed to high pesticide concentrations exhibited a marked increase in immobilization, indicating acute toxicity (Silva *et al.*, 2021). The ingestion of contaminated food or water can cause paralysis, rendering organisms unable to feed or reproduce and further decreasing their populations. The ecological ramifications of high immobilization rates can lead to cascading effects, alter food webs, and disrupt nutrient cycles in aquatic ecosystems (Amatey & Baawain, 2019).

Mortality is the most direct indicator of toxicological impact. Several studies involving *Daphnia* have demonstrated the lethality of pesticides and metal solutions. For example, evaluations of acute herbicide toxicity revealed a direct relationship between exposure levels and mortality rates in *Daphnia* spp. (Lebrun *et al.*, 2023). The disappearance of Daphnia due to pollution can have long-term implications for aquatic ecology, as these organisms are a fundamental component of the food web, serving as primary consumers and prey to higher trophic levels.

Beyond individual organisms, the impact of such toxic substances raises significant concerns regarding ecological balance and biodiversity. Introducing pollutants can select for resistant species, leading to changes in community composition and the potential domain of taxa more tolerant to pollution, displacing sensitive species such as *Daphnia* (Giannouli *et al.*, 2023). This change can decrease general biodiversity, making ecosystems less resistant to disturbances and altering functional dynamics. In addition, the implications of pesticide and metal pollution extend beyond immediate aquatic environments since these substances can bioaccumulate and enter terrestrial food systems through runoff and sedimentation (Abuqamar *et al.*, 2024). The long-term exposure of several organisms to these pollutants can compromise ecological integrity and human health throughout the food chain. Therefore, it is imperative to recognize and mitigate the risks raised by such environmental risks.

Research on ecological remediation and restoration potential has highlighted the need for sustainable management practices that limit the introduction of hazardous substances in aquatic environments. Phytoremediation techniques can be integrated with ecotoxicological evaluations to improve the recovery of ecosystems exposed to pesticides and heavy metals (Alkimin *et al.*, 2020). Using biological indicators, such as *Daphnia*, to assess the effectiveness of these remediation strategies can provide valuable information for restoring ecological balance. Some of the toxic effects of pesticides and metals are displayed in Table 7.

Table 7: Review of Toxic Effects of Metals and Pesticides of Interest on Bioindicator (Adapted from Ahmed 2023; Bownik 2017 & Kim et. al., 2015)

| ***Organism*** | **Chemical Exposure** | **Effect** | **Reference** |
|---|---|---|---|
| ***D. magna*** | Cadmium at 6, 20, and 37 µg $L^{-1}$ for 24 h | Reduced survival and somatic growth | Connon et. al., 2008 |
| ***D. magna*** | Cadmium chloride at 71 µg $L^{-1}$ for 24 h | Increased mortality, reduced survival, depleted glutathione level, and induced oxidative stress | Taylor et. al., 2010 |
| ***C.*** *dubia* ***and D. carinata*** | Copper, lead, zinc (at 1.3, 1.1, 13.0 mg $L^{-1}$, respectively) for 48 h or 7 days | Increased mortality and reduced reproduction ability | Cooper et. al., 2009 |
| ***D. magna*** | Cadmium at 6, 20, and 37 µg $L^{-1}$ for 24 h | Reduced survival and somatic growth | Connon et. al., 2008 |
| ***D. magna*** | Cadmium chloride at 71 µg $L^{-1}$ for 24 h | Increased mortality, reduced survival, depleted glutathione level, and induced oxidative stress | Taylor et. al., 2010 |
| ***D. magna*** | Copper (Cu) | Inhibition of growth, impaired reproduction, and altered behaviour. | Ahmed, 2023 |
| ***D. magna*** | Lead (Pb) | Growth inhibition, altered behaviour, and impaired reproduction. | Ahmed, 2023 |
| ***D. magna*** | Zinc (Zn) | Impaired reproduction and altered behaviour. | Ahmed, 2023 |
| ***D. magna*** | Cadmium (Cd) | Growth inhibition, altered behaviour, and impaired reproduction. | Ahmed, 2023 |
| ***D. magna*** | Copper | Decrease in swimming time and speed | Untersteiner *et al.*, 2003 |
| ***D. magna*** | Binary mixtures of glyphosate and copper [Cu (II)] | Decreased swimming speed, acceleration, and distance moved. Increased inactive time | Hansen and Roslev, 2016 |
| ***D. magna*** | Cypermethrin | Decreased swimming speed | Christensen *et al.*, 2005 |
| **C. silvestrii** | Herbicide (used in sugarcane) | 80% mortality at 3.5 µg $L^{-1}$ (Lower respiration rates in herbicide exposure) | Silva *et al.* (2021) |

| ***Organism*** | **Chemical Exposure** | **Effect** | **Reference** |
|---|---|---|---|
| ***D. magna*** | Metals (Ni, Zn, Cu, Cd) | Increased mortality with mixed metals (Reduced feeding and respiration rates) | Lari *et al.* (2017) |
| ***D. magna*** | Pesticide (methomyl) | 75% at 0.5 mg $L^{-1}$ (Decrease in lipid metabolism and respiration) | Suarez *et al.* (2023) |
| ***D. magna*** | Cu oxide nanoparticles | 65% mortality at 0.2 mg $L^{-1}$ (Impacts on electron transport and mitochondrial respiration) | Santos-Rasera *et al.* (2019) |
| ***D. magna*** | Heavy metals, PAHs | High mortality across metal levels (Diminished respiration under high pollutant loads) | Terra & Gonçalves (2013) |
| ***D. magna*** | Imidacloprid, thiacloprid (pesticides) | Variable mortality (up to 70% Accumulated respiration impacts with continuous exposure) | Pavlaki (2009) |
| ***Daphnia* spp.** | Heavy metals (Cu, Ag) | 100% mortality in extreme concentrations (Accumulation of metals affects respiration) | Buikema & Herricks (1978) |
| **Various *Daphnia* spp.** | Organophosphates, acrolein | Severe mortality (not quantified) (Bioaccumulation led to respiratory distress) | Stanley & Preetha (2016) |

In conclusion, the impact of pesticides and metal solutions on the swimming behaviour of Daphnia, breathing rates, immobilization, and mortality reveals significant ecological implications and environmental risks. As *Daphnia* species serve as vital indicators for aquatic health, understanding and monitoring their responses to pollutants is essential for conserving aquatic ecosystems. Future research must continue to elucidate the complex interactions between pollutants and aquatic organisms while advocating sustainable practices that protect and restore our bodies of water. By prioritizing these efforts, we can safeguard biodiversity and maintain the ecological functions that support the health of our planet's aquatic systems.

## 1.7. Adsorption

Adsorption among other conventional and sustainable methods of pollutant removal is a remediation technique used to remove pesticides and metals from environmental media. Adsorption techniques are increasingly recognized for their effectiveness in eliminating pesticides and heavy metals from contaminated water sources. Various materials, such as carbon-activated frames and metal-organic frameworks, have demonstrated higher adsorption capacities through mechanisms involving Van der Waals forces and ionic interactions (Mojiri *et al.*, 2020; Bonilla-Petriolet *et al.*, 2017). Recent criticism highlights the comparative efficiency of these techniques in various contexts (Cosgrove *et al.*, 2019; Jatoi *et al.*, 2021). In addition, the kinetics and isotherm models governing the adsorption processes have been widely studied (Alrefaee *et al.*, 2023; Rajabi *et al.*, 2023). These studies pave the way for innovative solutions to eliminate pollutants (Marican & Durán-Lara, 2018; Saleh *et al.*, 2020; Mondol & Jhung, 2021; Garba *et al.*, 2021).

Adsorption is a widely used technique for treating water and wastewater. Activated carbon and other modified sorbents are commonly used to decontaminate water of organic contaminants. The reactions involved in the use of modified sorbents are complex. Reactions include ion exchange, complexation, charge-transfer reactions, physical adsorption of ion clouds, aqua- and hydroxo-complexes, and interactions between metal ions and n-electrons of aromatic structures (Biniak *et. al.,* 1997). Adsorption is popularly achieved using carbonaceous material such as activated carbon, chemically or physically modified or unmodified Biochar, and other waste materials. This treatment method is operationally expensive if the carbonaceous material, e.g. activated carbon, is purchased. These costs can be evaluated in terms of financial and environmental costs.

Adsorption is another necessary fate process that affects the mobility and bioavailability of pesticides. Pesticides can adsorb onto soil particles, reducing their movement in soil and increasing their persistence. Soil properties, such as organic matter content and clay mineral composition, greatly influence pesticide adsorption. Highly adsorbed pesticides tend to stay in the soil surface layer, while those with low adsorption capacity can leach into groundwater, posing risks to human health and the ecosystem. Adsorption is the accumulation of a substance (the adsorbate) on the surface of a solid (the adsorbent) (Chowdhury & Saha, 2012). The process involves the accumulation of substances to the numerous binding sites of highly carbonaceous compounds at the interface between two phases, which could be liquid and liquid, gas and liquid, gas and solid or liquid and solid (Grassi *et. al.,* 2012).

Adsorption processes, usually based on activated carbon, are widely used sorbent for water and wastewater purification from contaminants such as colour, odour, and organic and inorganic pollutants. Other commercially available sorbents are metal hydrides, synthetic resins (Choong *et. al.,* 2007), alumina, silica gel, and zeolites (Akgun, 2005). Adsorption can be applied in batch and column continuous flow industrial operations. Studies on the treatment of metal-laden wastewater reveal that adsorption is highly effective (Lesmana et *al.,* 2009). Adsorption technology is widely used as an end-of-pipe water treatment option, with activated carbon the most commonly commercially available material due to its advantages**.** The adsorption technology applies in simple and reliable passive sampling or extraction in environmental (air, water, soil, and biota) analysis (Namieśnik *et. al.,* 2005).

In recent years, adsorption with alternative materials such as plant residues and agricultural waste has been a fast, easy, effective, and cheap wastewater treatment method applicable to organic and inorganic pollutants (Park *et. al.,* 2015; Demirbas,2008). Water treatment is achieved through adsorption using activated carbon and Biochar produced from natural waste materials. Although there are numerous studies on the adsorption of contaminants from water with Biochar, activated carbon, and other modified sorbents, there is a need for the study of the mobility and stabilization of contaminants in multi-element contaminated or polluted water (Ahmad *et. al.,* 2014; Valentin *et. al.,* 2013) and regeneration techniques of these cheap materials (Mohan *et. al.,* 2014). Numerous batch adsorption studies have been conducted on metals and organic spot tests using Biochar from the same feedstock, followed by adsorption of the same organic contaminants, which is not popular. This stresses the need for disability to easily compare these crop carbonaceous materials**.**

### *1.7.1. Factors that affect Adsorption*

The factors that affect the adsorption efficiency of an adsorbent include the surface area, temperature, interfering substances (Grassi *et. al.,* 2012), proximate composition of adsorbent, adsorbent dose, adsorbent particle size, exposure time of adsorbate to adsorbent, agitation, solution pH, and temperature (Saikaew & Kaewsarn, 2009). The physicochemical properties of organics, including hydrophobicity (Omoni *et. al.,* 2020), water solubility, pH, absorptivity, volatility, ionic property, structure, molecular weight (Ogbonna & Semple, 2013), reactivity, and polarity, affect the sorption, retention, mobility, and dissipation in the environment (Cortez *et. al.,* 2019; Fingler *et. al.,* 2004; Pizzigallo *et. al.,* 2001; Sarmah *et. al.,* 1998; Watt *et. al.,* 2010).

Metal biosorption, according to Demirbas (2008), is a complex process affected by factors such as the speciation or metal-ion complex, pH, surface area, surface composition or oxygen functionality, and the particle size of adsorbent, which control the extent of adsorption (Dias *et. al.,* 2007; Plazinski, 2013) (Biniak *et. al.,* 1997). Saeed, Iqbal, and Akhar (2005) posited that competition among metals and other contaminants for binding sites on an adsorbent in multi-metal systems is influenced by the initial concentrations, nature, and quantity of adsorbent. In summary, the physicochemical properties of the adsorbent and adsorbate affect the rate and capacity of adsorption, while the solubility of the adsorbate influences the adsorption equilibrium (Grassi *et. al.,* 2012).

### *1.7.2. Alternative Adsorbents*

Among the most promising methods is the use of sustainable materials, from macro- to nanoscale cellulose, which have shown efficiency in adsorbing pesticides from contaminated water (Rana et *al.*, 2021).

- **Waste streams**

Waste streams have been considered cost-effective alternatives for sorbents in the decontamination of water and soil. These include natural materials or certain waste products from municipal, industrial, or agricultural operations that are available in large quantities (Bhatnagar & Silanpaa, 2010). Contribution of agricultural wastes, food and fruit wastes (20-50%) and other domestic wastes to the waste volume generated in the UK, tough shells pose a serious disposal challenge because of their contribution to the volume of waste incinerated or sent to landfills (Kosseva & Webb, 2013).

The availability of these wastes has made them suitable for research in recent years. The possibility of using waste without expensive pretreatment and its abundance are advantages for using waste as sorbents (Kurniawan et al., 2006; El-Sheikh et al., 2013). The cost of using agro waste is justified because it poses a significant disposal challenge that can be addressed by converting it into a valuable adsorbent for heavy metal removal (Dias et *al.,* 2007; Kosseva & Webb, 2013). Recent studies reveal that these wastes can be converted to activated carbon and Biochar, which are both useful for water and soil decontamination (Verla *et. al.,* 2004; Inbaraj & Sulochana, 2006; Dias *et. al.,* 2007; Dermirbas, 2008; Milenkovic *et. al.,* 2009; Bhatnagar & Sillanpaa, 2010).

- **Agricultural wastes**

Agricultural wastes (agro waste) are used for several purposes, including biofuels, enzymes, chemicals, animal feeds, and composites (Iqbal *et. al.,* 2013). Researchers have confirmed that agro-waste containing cellulose has had high adsorption capacity for various organic and inorganic pollutants in the last few decades. The proximate constituents of agricultural waste materials include hemicellulose, lignin, lipids, proteins, simple sugars, water, hydrocarbons, and starch.

The advantages of their application in adsorption include low cost, large-scale availability, renewable nature, and environmental friendliness (Bhatnagar & Sillanpaa, 2008; Demirbas, 2008). The drive for environmental preservation through waste reduction and recycling has led researchers to incorporate locally available, reusable components into environmental management (Babel & Kurniawan, 2003; Dias et *al.,* 2007; Bhatnagar & Silanpaa, 2010). Agricultural waste has been used as a precursor for producing carbonaceous materials and is considered suitable due to its low ash content and reasonable hardness. Lower carbonisation temperatures make their production process more cost-effective compared to commercial AC production, which uses coal and intricate shells and requires high temperatures (Cuhadar, 2005; Dias *et. al.,* 2007; Das, Vimala, and Karthika, 2008; Bhatnagar & Silanpaa, 2010). Pine-needle-derived biochar has been successfully used for the sorption of naphthalene, nitrobenzene, and m-dinitrobenzene from water (Chen *et. al.,* 2008). Sheng & Yang (2003) showed that soil amended with rice- or wheat-straw-derived biochar enhanced the sorption of duiron and atrazine. Table 9 displays the proximate composition of rice husk, hardwood, and coconut shells.

Table 8: The proximate constitution of rice husk, hardwood, and coconut shells.

| **Material** | **Cellulose** | **Hemicellulose** | **Lignin** | **Ash** | **Extractives** | **References** |
|---|---|---|---|---|---|---|
| **Rice Husk** | 28–36 | 16–22 | 20–25 | 15–20 | 1–2 | N.S. Ahmed *et al.* (2016), D.K. Mishra *et al.* (2020) |
| **Hardwood** | 40–50 | 15–30 | 20–30 | 1–3 | 2–5 | A.P. Kollmann *et al.* (1951), H. Gunning *et al.* (2017) |
| **Coconut Shell** | 26–36 | 20–30 | 27–33 | 0.5–3 | 2–6 | Prauchner *et al.* (2011),<br>T. Tsamba *et al.* (2016) |

- **Biosorbents**

Biosorbents, particularly those derived from agricultural waste, have attracted considerable attention for their effectiveness in removing pesticides and heavy metals from contaminated water sources. Numerous studies have shown that chemically modified agricultural waste has significant biosorption capacity for heavy metals (Syeda *et al.*, 2022). The fungal biomass of species such as Aspergillus Niger has also been used effectively in this context (Cabrera-Barjas *et al.*, 2020).

The main biosorption mechanisms include ion exchange, surface complexation, and adsorption, which improve elimination efficiency (Tiwari *et al.*, 2023). This approach, characterized by environmental respect, minimizes hazardous waste generation and promotes resource recovery (Saini *et al.*, 2020; Karić *et al.*, 2022). In addition, spent biosorbents exhibit multi-point adsorption capacities and effectively remove glyphosate herbicides (Ramrakhiani *et al.*, 2019).

Functionalized soy waste biomass indicates promising results in achieving a high elimination rate of heavy metals (Bulgariu & Bulgariu, 2018). Agricultural by-products are effective biosorbents for organic pollutants (Aguiar *et al.*, 2019). The critical review of Elgarahy *et al.* (2021) also establishes biosorption as a vital green technology for wastewater sanitation. This aligns with results on the application of biosorption techniques for removing heavy metals from wastewater (Kanamarlapudi et al., 2018), highlighting the environmental benefits of biosorbent use across multiple facets.

- **Nanomaterials**

The use of nanomaterials also presents exciting opportunities for sustainable remediation due to their high surface and reactivity (Mukhopadhyay *et. al.*, 2022). The integration of these technologies into existing water treatment systems is feasible, as evidenced by their compatibility with conventional processes (Kumar *et. al.*, 2022). Ultimately, evaluating the environmental impact of these techniques is essential to ensure that they improve, rather than compromise, ecological integrity (Boudh & Singh, 2019; Cosgrove et *al.*, 2019).

### *1.7.3. Carbonaceous Compounds used for adsorption*

There are several types of black carbon, which include activated charcoal and biochar. Activated carbon (AC) possesses high removal efficiency for a wide range of contaminants (organic and inorganic) and is not associated with undesirable by-products, making it easy to design and operate. Its main disadvantage is its high cost of adsorbent production (Gupta *et. al.* 2009). The industrial applications of AC are mainly purification and separation procedures. Examples of these processes include solvent recovery, heavy metal removal, solution decolourization, water treatment, air purification (deodorization), and decolourization of sweeteners (Cuhadar, 2005). AC is efficient at adsorbing numerous contaminants; its major limitation is the high cost of preparation or purchase, due to energy, gas, and chemical requirements, storage, and transportation of bulk quantities. This has led to numerous studies on low-cost options for its preparation or alternative adsorbents with similar adsorption efficiencies (Gupta *et. al.* 2009).

### 1.7.4. Biochar and Its Applications

Biochar is a carbonaceous co-product of organic biomass pyrolysis at temperatures between 200 - 1000 ° C in the presence of limited oxygen (Zhelezova *et. al.,* 2017). It can be produced at low temperatures of below 500 °C or higher temperatures (burning with little or no oxygen) (Foereid, 2015; Bierderman & Harpole, 2013). It can be produced from biomass of different types, ranging from agricultural wastes to biosolids (Zama *et. al.,* 2018). Historically, the use of biochar in agriculture dates to ancient civilizations over 2,000 years ago when the Amazonians used it to create highly fertile soils known as *terra preta* (Portuguese for "black earth"). These soils contained large amounts of charcoal, bone, and organic waste, which created rich, long-lasting soil fertility that remains evident today (Kumar *et al.*, 2021).

Its intended use was for soil amendment because it sequesters applied carbon and reduces atmospheric carbon emissions (Foereid, 2015; Bierderman & Harpole, 2013; Lehmann & Joseph, 2009; Lehmann & Rondon, 2006), but its application bestowed several other positive effects on soil structure, quality, and fertility that have accompanying unintended positive and negative consequences. Researchers posit that biochar has agricultural benefits, such as nutrient trapping and reduced leaching, resulting in efficient use of soil nutrients, increased nutrient availability, increased water-holding capacity, microbial stimulation for enhanced biodegradation of toxic organic residues, and possible mineralization of some organics. improved crop yield, reduced crop disease incidents, and reduced bioavailability of metals in soil and ecosystem function (Foereid, 2015; Bierderman & Harpole, 2013).

Biochar studies also reveal their ability to diffuse gas, retain water (Quin *et. al.,* 2014), retain soil micro and macronutrients (Ayaz *et. al.,* 2021), improve OC and DOM (Yavari *et. al.,* 2015; Lehmann & Rondon, 2006), and plant protection from pests (Graber *et. al.,* 2010). Omoni (2020) further suggests that biochar stimulates accessory biodegradation enzymes in soil, including β-glucosidase, phosphatase, aryl alcohol oxidase, glyoxal oxidase, and dehydrogenase (Oleszczuk *et. al.,* 2014). Biochar improves aeration, porosity, moisture (Hossain *et al.,*2020; Pathy et al., 2020; Razzaghi et al., 20202; Lehmann et al., 2011; Jha et al., 2010), and the bioavailability of organic and mineral nitrogen (Singh *et al.,* 2010). The improved soil properties endowed by biochar addition aid in the biodegradation and dissipation of herbicides in soils.

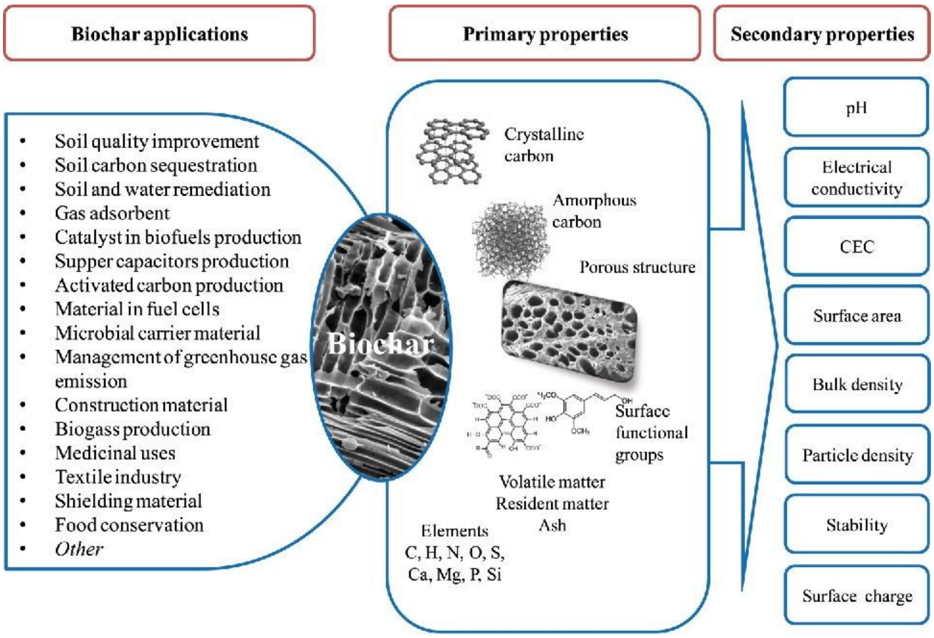


Figure 15: Primary and Secondary Properties of Biochar and its Potential Applications (from Igalavithana et. al., 2018)

Unlike PAHs, the binding of certain herbicides to soil and its amendments has been reported to improve their biodegradation (Mendes *et al., 2018;* Jablonowski *et al., 2013;* Lashermes *et al.,* 2010). Consequently, it increases herbicide persistence, degradation half-life, and dissipation time, and decreases mineralisation, photodegradation, bioavailability, and herbicidal action. Biochar has emerged as a new material capable of modifying solubility and, consequently, the behaviour of pesticides in soil environments (Khorram *et. al.*, 2016).

Biochar is stable, eco-friendly, cost-effective, and easy to handle (Tang *et. al.,* 2013). Reports on biochar addition to soil suggest that it enhances the physical, chemical, and biological properties of soil (Yavari *et. al.,* 20015). Numerous reviews and studies have reported the advantages of biochar use as a potent soil amendment (Shaaban et al., 2018;

Khorram et al., 2016; Xie et al., 2016; Lu et al., 2012; Kookana et *al.,* 2011). Due to its basic pH, biochar has been reported to act as a soil conditioner through its liming effect (Zhelezova et al., 2017; Yavari et *al.,* 2015).

Biochar has gained significant attention in recent years for its potential to adsorb pesticides and metals in water systems. Figure 16 summarizes the mechanisms involved. This has sparked research interest in the application of biochar in water and wastewater management (Foereid, 2015).

Historically, the use of biochar for adsorbing pesticides in water systems dates back to ancient civilizations, where charcoal was used to filter water for drinking. Studies have shown that biochar can effectively adsorb metals such as lead, cadmium, and copper, reducing their concentrations in water and decreasing their toxicity to aquatic organisms. Smith *et. al.* (2010) conducted pioneering studies demonstrating biochar's high affinity for organic contaminants, including Isoproturon. Subsequent research by Wang *et. al.* (2015) further confirmed the efficacy of biochar in removing Isoproturon from water, highlighting its potential for practical applications in water treatment technologies. Other indirect advantages of biochar use are reducing global warming and producing bioenergy (Igalavithana *et. al.* 2018).

#### *1.7.4.1. Physicochemical properties of biochar*

Biochar properties that control or impact its ability to adsorb contaminants include its Mass and Atomic Ratio, pH, zeta potential (Tadros, 2015), streaming potential, sedimentation potential, pore volume (Biniak *et. al.,* 1997), micropore volume (Adinata *et. al.,* 2007), surface functional groups, high surface, porosity, and stable organic carbon content, plays a crucial role in its adsorption capacity, facilitating the retention of nutrients and the binding of contaminants (Tomczyk *et al.*, 2020; He and al., 2018).

The functional groups on the surface of carbonaceous compounds affect their adsorption capacity and the types of ions adsorbed, as illustrated in Figure 16. Oxygen functional groups are generally known to increase adsorption in activated carbon, while Nitrogen groups increase the sorption of anions (Biniak *et. al.,* 1997). The major mechanisms involved in metal removal from aqueous solutions using biochar (Figure 16) are physisorption, surface complexation with functional groups, precipitation, ion exchange, electrostatic interaction, precipitation, and reduction and oxidation for some redox-sensitive elements (Manikandan *et al.*, 2023; Deng *et al.*, 2020). Biochar with a high surface area and pore volume exhibits a higher affinity for metal ions because they can be physically entrapped within the pores on its surface, as shown in Figure 16.

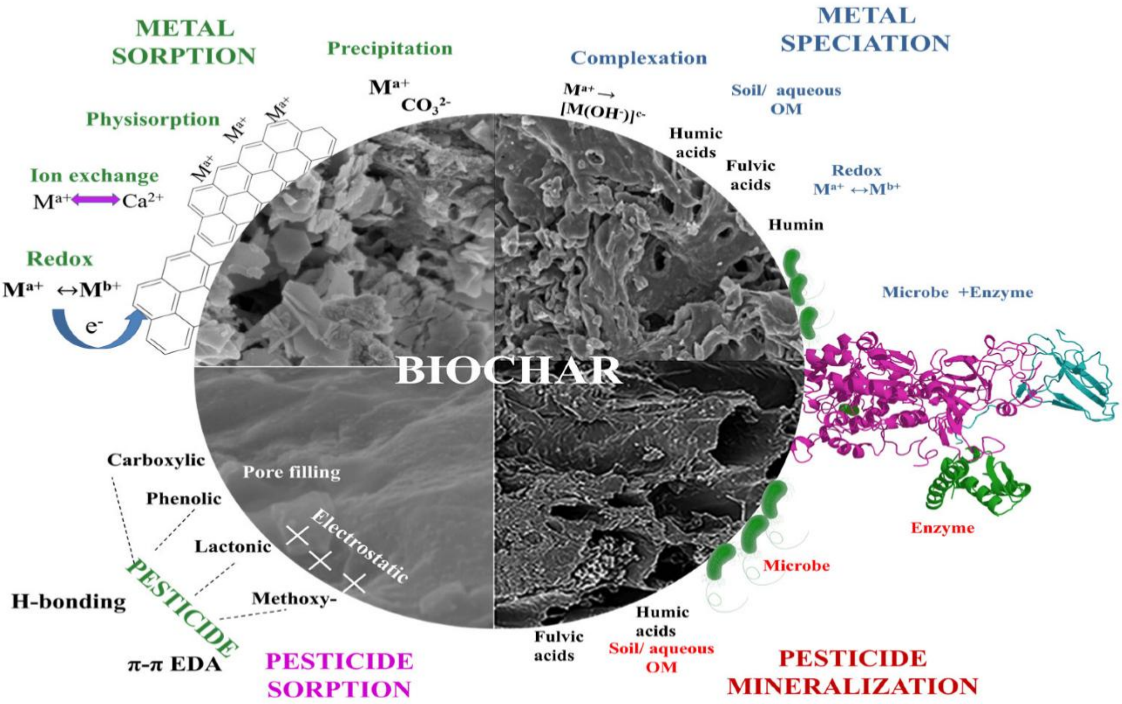


Figure 16: Species of Ions Identified on Biochar Surface responsible for Pesticide and Metals Adsorption (Source: Manikandan *et al.*, 2023).

The negatively charged surfaces of biochar can adsorb positively charged metal ions via electrostatic attraction (Manikandan *et al.*, 2023). Some physicochemical properties of the biochar used in this study, as reported by Muhammad (2016), are adapted and presented in Table 9.

Table 9: Reported Physicochemical Properties of Biochar Used in this Study (adapted from Muhammad 2016).

| **Parameter** | **RH** | **CS** | **HW** |
|---|---|---|---|
| **% C** | 38.64 | 72.95 | 71.38 |
| **% N** | 0.53 | 0.53 | 0.45 |
| **C/N ratio** | 72.59 | 139.71 | 158.68 |
| **CEC (meq 100g$^{-1}$)** | 43.28 | 31.17 | 34.36 |
| **Inorganic P** | 1.75 | 0.39 | 0.41 |
| **pH** | 8.46 | 8.33 | 9.05 |

* %C= percentage carbon, %N = percentage nitrogen, CEC = Cation Exchange Capacity, Inorganic P= Inorganic phosphorus, RH= rice husk derived biochar, CS= coconut shell derived biochar, HW= hardwood derived biochar.

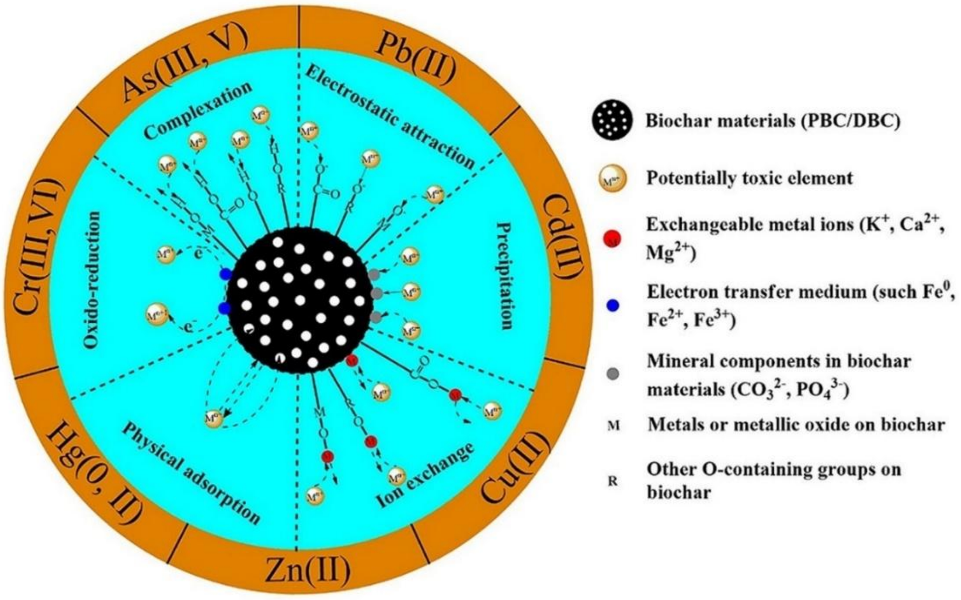


Figure 17: Reported Mechanisms of Metal Adsorption by Biochar (Source: Deng *et al.*, 2020)

Pesticides can be absorbed by biochar through two main mechanisms, depending on the strength of the interaction between the pesticide (sorbate) and the biochar (sorbent).

**Physisorption**: involves weak, reversible interactions such as hydrogen bonding and Van der Waals forces. It is used in gas storage and temporary adsorption processes, such as air purification (Lee *et al.*, 2021).

**Chemisorption**: relies on strong and irreversible interactions, often through covalent bonding or complex formation. It is important for applications that require long-term connections, such as catalysis and chemical sensor modification (Singh *et al.*, 2020).

Both physisorption and chemisorption are distinct processes that serve different roles in adsorption applications and can operate independently or together, depending on the specific needs. While physisorption is beneficial for processes requiring rapid, reversible adsorption with low energy expenditure, chemisorption is more suitable for applications requiring strong, permanent adsorption due to the formation of chemical bonds (Punniyakotti *et al.*, 2024).

Table 10: Summary of Differences Between Physisorption and Chemisorption

| **Aspect** | **Physisorption** | **Chemisorption** |
|---|---|---|
| **Nature of interaction** | Van der Waals forces | Chemical bond formation |
| **Energy range** | 5–40 kJ/mol (Smith & Brown, 2019) | 40–400 kJ/mol (Lee *et al.*, 2021) |
| **Temperature dependence** | Favoured at low temperatures | Favoured at higher temperatures |
| **Adsorption type** | Multilayer possible | Monolayer only |
| **Reversibility** | Reversible | Often irreversible |
| **Rate** | Rapid | Slower due to bond formation |

### *1.7.4.2. Biochar Production*

Common methods for biochar production include slow pyrolysis, intermediate pyrolysis, fast pyrolysis, and gasification (Ogbonnaya & Semple, 2013). Slow pyrolysis is more commonly used for biochar production as it gives higher yields. Reviews of biochar production suggest that biochar produced at lower temperatures may be useful for the reduction of herbicide leaching (Li *et. al.,* 2013) and, on the other hand, biochar produced at higher temperatures is more suitable for the sorption of herbicides and other organic chemicals (Igalavithana *et. al.* 2018; Ogbonnaya & Semple, 2013). Figure 18 summarizes the most common biochar production methods and the average percentage yields of the different end products.

In biochar production, feedstock and pyrolysis conditions are the two main factors that control the characteristics and stability of the resulting biochar yield, fixed carbon content, porosity, surface area, ash content, aromaticity, nutrients, and other physiochemical parameters (Ding *et. al.,* 2017; Foereid, 2015; Biederman & Harpole, 2013; Kookana *et. al.,* 2011). In addition, biochar can be treated with acids, bases, steam and other ions to increase its adsorptive capacity before or after production (Igalavithana *et. al.* 2018). The impact of biochar preparation feedstock and temperature on its adsorbent capacity for organics and inorganics is significant in determining the effectiveness of biochar as a remediation tool.

According to Yavari *et al.* (2015), the heating rate, gas pressure, retention time, and pyrolysis temperature are sequentially relevant pyrolysis variables that determine the characteristics of the produced biochar, as shown in Figure 18.

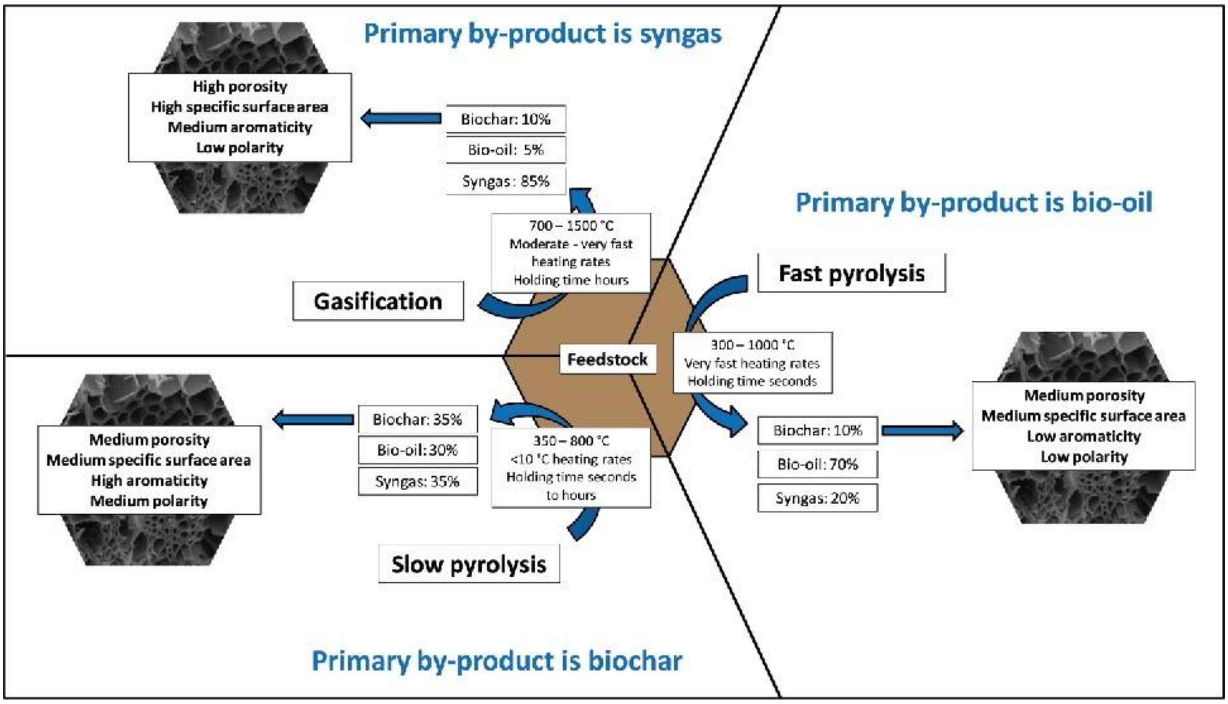


Figure 18: Schematic Representation of Biochar Production Conditions and How They Control the Biochar End Product (adapted from Igalavithana et. al., 2018).

### *1.7.4.3. Biochar Feedstock and Its Impacts on Adsorption*

Biochar can be produced from many types of feedstocks, under various production conditions, and used in diverse environmental conditions and compartments; these numerous factors affect the adsorption behaviour of biochar, thus their suitability for applications (Foereid, 2015; Biederman & Harpole, 2013).

Yavari *et. al.* (2015) posit that the nature of biochar is additionally determined by the quantity of lignocellulosic compounds, mineral content, particle size, and structure of the initial biomass. The type and proximate composition of biochar feedstock are responsible for its chemical composition, the number of macropores and nutrient content, and its pyrolysis conditions, such as time, pressure, and temperature, influence its carbon, hydrogen (C/H) content, pH, CEC, surface structure, and morphology (Zhelezova *et. al.* 2017, Ahmad *et. al.,* 2014; Gaskin *et. al.*, 2008).

Biochar from plant materials has notably demonstrated commendable adsorption for organic and inorganic (metals and nutrients) pollutants from solution (Foereid, 2015), (Cao *et. al.,* 2009). Recently, biochar has been widely studied, and reports confirm that it can be produced from a wide range of feedstock (Mayakaduwa *et. al.* 2016). Some examples of feedstock used in biochar production in recent past include wood (Cederlund *et. al.* 2017; Dechene *et. al.* 2014; Cabrera *et. al.* 2011), rice straw (Dan *et. al.* 2021), wheat straw (Cweilag-Pisasecka *et. al.* 2018), manure (Zhelezova *et. al.* 2017), nut shells (Brair *et. al.,* 2016; Cabrera *et. al.,* 2014), cassava waste (Deng *et. al.* 2017; Luo *et. al.* 2016), sewage sludge (Ding *et. al.* 2019), poultry litter sludge (Martin *et. al.* 2012), wastewater biosolids (Oh *et. al.* 2013), cow bones (Mendes *et. al.* 2018), corn cobs (Hao *et. al.* 2013), tea stalks (Jiao *et. al.* 2019), pineapple stubble, palm oil fibre and coffee hull (Chin-Pampillo *et. al.,* 2021).

Biochar prepared from dairy manure was used for the adsorption of atrazine and lead, and the results obtained showed that it adsorbed both compounds up to six times better than Activated carbon, which was used as a control. Atrazine was partitioned into its organic phase, whilst sorption with AC was surface sorption. Pb sorption was successful, up to 680 mmol $kg^{-1}$. In solutions where both compounds were sorbed by biochar, there was little competition between them for binding sites, in contrast to the fierce competition observed with AC (Cao et *al.,* 2009).

The type of biochar feedstock used plays a crucial role in determining its effectiveness in removing pesticides from water. The choice of biochar feedstock is critical in determining its effectiveness in adsorbing pesticides from water. Studies have shown that biochar produced from different feedstocks, such as wood, agricultural residues, municipal solid waste, and manure, can exhibit varying adsorption capacities due to differences in their chemical composition and surface characteristics.

The impact of research on the effects of biochar feedstock on pesticide adsorption from water has been significant, with numerous studies demonstrating biochar's effectiveness as a pesticide adsorbent. For example, a study published in the Journal of Agricultural and Food Chemistry found that biochar derived from bamboo can effectively adsorb the fungicide chlorothalonil from water, reducing its concentration by up to 90%. For example, biochar derived from high-lignin feedstocks, such as woody biomass, has been shown to exhibit high adsorption capacities for certain pesticides due to its porous structure and surface chemistry. On the other hand, biochar produced from low-lignin feedstocks, such as rice husks, may have lower sorption capacities but could be more cost-effective and readily available.

Research has shown that the type of feedstock used to produce biochar can greatly influence its adsorption properties. Different types of biochar, such as those produced from woody biomass, agricultural residues, or manure, have different surface properties and chemical compositions that affect their adsorption capacity. For example, biochar produced from coconut shells has been found to have a high surface area and porosity, making it an effective adsorbent for metals such as lead and cadmium. According to Korram et. al. (2022), rice husk biochar accounts for 20% of rice weight. It contains 50% cellulose, 25–30% lignin, 15–20% silica, and 10–15% moisture (Singh, 2018). The biochar yield from rice husk is approximately 35% of its feedstock material (Shackley *et al.*, 2011; Shackley *et al.*, 2012). Table 11 illustrates the proximate composition of biochars of interest from previous research.

Table 11 highlights how pyrolysis alters the properties of these agricultural residues. Significant reductions in volatile matter and increases in fixed carbon make biochar more thermally stable.

Table 11: Comparison of the proximate composition (Volatile Matter (VM), Fixed Carbon (FC), Ash, and Moisture Content (MC)) of rice husk, hardwood, and coconut shell feedstocks against their respective biochars from previous studies

| Feedstock | Feedstock Composition (VM/FC/Ash/MC) | Biochar Composition (VM/FC/Ash/MC) | Reference |
|---|---|---|---|
| **Rice husk at 450°C** | 60.2 / 15.4 / 12.5 / 7.1 | 18.5 / 75.4 / 6.1 / 3.2 | Cabrera *et al.* (2011) |
| **Hardwood** | 72.3 / 15.0 / 8.0 / 5.4 | 20.0 / 74.3 / 5.7 / 2.9 | Devens *et al.* (2018) |
| **Mixed wood chips at 550°C** | No data | 10.8/ 85.3/ 3.9/ 2.1 | Suliman *et al.* (2016) |
| **Coconut shell at 700°C** | 68.2 / 12.1 / 7.6 / 6.5 | 12.9 / 82.5 / 4.6 / 1.8 | Vikraman *et al.* (2024),<br>Vijayaraghavan (2019) |
|  | 75.996/14.757/0.047/9.200 |  | Kyu *et al.* (2020) |

#### 1.7.4.4. *Biochar Production Temperature and Its Impacts on Adsorption*

The effects of biochar preparation temperature on pesticide adsorption from water are significant for addressing water pollution in environmental science and agriculture. The preparation temperature of biochar has been found to influence its adsorption capacity, with higher temperatures generally resulting in greater adsorption efficiency, which can be optimized to target specific contaminants in water. High-temperature pyrolysis can yield biochar with greater stability and adsorption capacity, whereas low-temperature pyrolysis may produce biochar with higher nutrient content but lower adsorption capacity.

Several studies have examined the impact of high biochar preparation temperature on pesticide adsorption from water. For example, a study by Zhang *et al.* (2016) found that biochar produced at temperatures above 500°C exhibited higher adsorption capacities for pesticides than biochar produced at lower temperatures. The increase in adsorption was attributed to the greater surface area and pore volume of high-temperature biochar, which provides more sites for pesticide molecules to bind. Some studies have shown that biochar produced at elevated temperatures exhibits higher surface area and pore volume, thereby increasing its metal adsorption capacity. For example, a study by Zhang *et al.* (2016) found that biochar produced at 700°C had a higher copper adsorption capacity than biochar produced at lower temperatures. Similarly, Fu *et. al.* (2019) reported that biochar produced at 800°C had a higher lead adsorption capacity than biochar produced at lower temperatures.

On the other hand, some researchers have raised concerns about the use of high-temperature biochar for water treatment. A study by Liu *et. al.* (2018) suggested that biochar produced at temperatures above 700°C may have reduced adsorption capacities for certain pesticides due to the formation of aromatic structures that hinder pesticide binding. These findings highlight the complexity of the relationship between biochar preparation temperature and pesticide adsorption, as well as the need for further research to optimize biochar properties for water purification. Some studies also suggest that high biochar preparation temperature may not always favour metal adsorption from water. For example, Ahmed *et al. (*2018) found that biochar produced at 400°C had a higher cadmium adsorption capacity than biochar produced at higher temperatures. This discrepancy in results highlights the complexity of the factors that influence metal adsorption on biochar and underscores the need for further research in this area.

In conclusion, the question of whether high biochar preparation remains a topic of ongoing research and debate. While high-temperature biochar may offer advantages, including increased surface area and pore volume, it also has potential drawbacks, including changes in biochar structure that could affect pesticide adsorption. Future developments in the field are likely to focus on optimizing biochar properties through temperature control and other methods to enhance its effectiveness for removing pesticides and other contaminants from water. By gaining a better understanding of the factors that influence biochar's adsorption capacity, researchers can develop more efficient and sustainable water treatment solutions.

## 1.8. Research Problem

The primary concern related to water pollution is its impact on environmental degradation. This research examines the effectiveness of carbonaceous materials in adsorbing pesticides from water. With the increasing levels of water pollution and its serious consequences, the urgency of this research has heightened. Generally, evidence of environmental deterioration is more conspicuous in developing and underdeveloped countries in Asia, Latin America, and Africa due to resource scarcity or mismanagement and the absence of government structures for environmental management, monitoring, and policy implementation (Pure Earth Blacksmith Institute, 2016). Although present in developed countries such as England, environmental pollution is continually monitored and reduced to the barest minimum, as far as reasonably practicable. This study aims to evaluate lessons learned by developed countries regarding pollution and the methods of pollution control that are transferable to the Nigerian landscape.

The environmental consequences of pesticide usage are water pollution through the eutrophication of surface waters, particularly freshwater lakes, streams, estuaries, and coastal seas, loss of biodiversity diversity, changes in species composition in terrestrial and aquatic ecosystems, groundwater pollution with nitrate and nitrite and resulting tropospheric smoke and ozone and acidification of soils, and sensitive freshwaters (Tilman *et al.,* 2001).

Furthermore, clean water scarcity is exacerbated by water shortages, poor surface water quality, flooding, and climate change. Many countries are also depleting their groundwater reserves to meet the demands of agriculture and food production; further, electricity generation has also increased, all of which has driven research into water treatment and reuse. Based on these occurrences, water projections infer long-term global economic stress for food production by 2025 and severe water stress by 2050 if mitigation measures are not implemented (Clapper, 2013; WHO, 2013).

Several researchers argue that the world's natural resources, including water, are on the verge of exhaustion (Shaumyan, 2007; McMichael *et al.*, 2003; Shetty *et al.*, 2023; Raven, 2002). This looming potential for water scarcity highlights the importance of long-term planning and the development of sustainable solutions and has contributed to the ongoing reduction of Maximum Contaminant Levels (MCLs) for pesticides, metals, and other potentially toxic compounds (Oluyemi *et al.,* 2010). These issues reiterate the need for agricultural and industrial wastewater treatment and reuse. Water management strategies to improve water quality have continually advanced in developed countries such as England, where significant changes have occurred over the past six decades (Osbeck *et al.,* 2013). On the other hand, it is a new area of research in developing African countries (Adeniyi, 2004; Fakayode, 2005).

The second issue of interest is the resulting health impacts of water pollution. The health impacts of water pollutants can be acute (short-term) and chronic (long-term), and can arise from exposure via different pathways (Hill, 2020; Gish *et al.,* 2011; Damalas & Eleftherohorinos, 2011**).** Most human exposure occurs through inhalation, ingestion, and skin absorption. According to Jorgenson (2009), water pollutants such as pesticides and metals, ubiquitous in the environment, can travel along the food chain, bioaccumulate, and bio magnify in the fatty tissue of mammals, including humans. In humans, these effects range from headaches and mild skin irritations to congenital disabilities, spontaneous abortion, neuro-behavioural disorders, severe health conditions like cancers, and death in some cases (Ahmad *et al.,* 2010; Cadbury, 2000; McGinn, 2000). In women, elevated levels of some toxic chemicals are passed to infants and young children during pregnancy or through breastfeeding (Barakat, 2010; Burns *et al.,* 2003; Xu *et al.,* 2013; Czub & McLachlan, 2004; Tilman *et al.,* 2001).

These health effects are also evident and often similar in aquatic and animal life, sometimes including dermatitis, organ (kidney, liver) damage, circulatory and nervous system impairment, lethargy, depression, foetal brain damage, cancer, and death. This toxicity warrants a more in-depth study to mitigate its impacts (Kaur & Singh, 2021; Kansal et *al.*, 2023).

Furthermore, the health effects of metals are more organ-targeted, depending on the type and concentration of the metal. These health effects are noticeable in aquatic life, animals, and humans far from the points of release or many years after release (Khan *et al.,*2008). The evidence discussed earlier confirms that humankind and the ecosystem at large are affected by water pollutants, further emphasizing the importance of wastewater treatment before discharge.

The third remarkable issue of water pollution is the sustainability and costs of the methods employed in wastewater treatment. Usually, the extent and type of treatment required for polluted water are determined by factors such as the nature of the pollutants (organic or inorganic), their concentration, and the planned end use, among others. The nature of water pollutants often determines their oxidation state or dissolution potential, as well as the range of concentrations frequently found in water.

The removal of organic and inorganic pollutants from the environment can be achieved over time using physical, chemical, or biological methods; technologies such as chemical precipitation, ion exchange, membrane filtration, electrodialysis, photocatalysis, and adsorption have been explored for this purpose. The disadvantage of most of these treatment methods is cost related. The high cost of water treatment has shifted the focus towards developing cheaper, more effective water treatment methods, pollutant removal technologies, and the potential recycling of applicable waste materials (Kurniawan et al., 2006; El-Sheikh et al., 2013; Dias *et al.,* 2007; Kosseva & Webb, 2013). As a result of the high cost of environmental remediation of organics, research focus has shifted in recent decades towards improving contaminant removal technologies that incorporate the recycling of waste materials. (Park *et al.,* 2015 ; Singh *et al.,* 2015 ; Ahmad *et al.,* 2014 ; Mohan *et al.,* 2014 ; Yakout *et al.,* 2013).

Adsorption is achieved using carbonaceous materials such as activated carbon, chemically or physically modified biochar, and agricultural waste, among others, used in recent studies. This treatment method is operationally expensive if the carbonaceous material (e.g., activated carbon) is purchased. These costs can be evaluated from both financial and environmental perspectives.

In recent years, adsorption with alternative materials such as plant residues and agricultural waste has been a fast, easy, effective, and cost-effective wastewater treatment method for organic and inorganic pollutants (Park *et al.,* 2015; Demirbas, 2008). Water treatment is widely achieved through adsorption using activated carbon and biochar derived from natural materials.

Although numerous studies have examined the adsorption of contaminants from water using biochar, activated carbon, and other modified sorbents, there is a need to study the mobility and stabilization of contaminants in polluted water (Ahmad *et al.,* 2014; Valentin *et al.,* 2013). Biochar is a carbonaceous coproduct of pyrolysis of organic material, widely used and established as a soil amendment with numerous benefits (Foereid, 2015; Bierderman & Harpole, 2013). Recent research has sparked interest in the application of biochar in water and wastewater management (Foereid, 2015), as it is a new area of research (Inyang & Dickenson, 2015; Tan *et al.,* 2015; Yavari *et al.,* 2015; Ahmad *et al.,* 2014; Ahmad *et al.,* 2012).

The fourth topic of interest concerns the challenges of applying biochar. Although biochar has numerous benefits and successful applications as a soil and water amendment, it introduces metal and organic contaminants from its feedstock biomass or toxic co-products when applied. Some of these contaminants include furans, dioxins, PAHs (Wu *et al.*, 2017), PCBs, and metals; Al *et al.* (Zama *et al.,* 2018; Hilber *et al.*, 2017), with the potential to accumulate and transport contaminants during or after its application (Yavari *et al.,* 2015; Kookona *et al.*, 2011).

Although a review of over 50 biochars by Hale *et al.* (2012) reports that the levels of these contaminants do not pose significant risks to soil and its properties, there is little information on their risk to water. Furthermore, these compounds are potentially toxic to living organisms upon exposure. Thus, the mechanisms underlying their production and interactions during biochar production should be further studied. These metals and compounds may compete for binding sites on biochar surfaces. They may synergize or antagonize the efficacy of biochar bioremediation.

Research on biochar applications for adsorbing organics in surface water, wastewater, and groundwater is limited, even though its utilization in soil has been extensively studied and established (Inyang & Dickenson, 2015; Tan *et al.,*2015; Yavari *et al.,* 2015; Ahmad *et al.,* 2014; Ahmad *et al.,* 2012). Additionally, the short- and long-term effects of biochar use on water bioindicators, and aquatic organisms (natural flora and fauna) have not been widely studied (Singh *et al.,* 2015; Ahmad *et al.,* 2014; Valentín *et al.,* 2013).

Although there are numerous studies on the adsorption of contaminants with biochar, activated carbon, and other modified adsorbents, there is a need for the study of mobility and stabilization of contaminants in polluted water (Ahmad *et al.,* 2014; Valentín *et al.,* 2013) and regeneration techniques of these materials (Mohan *et al.*, 2014). For example, rice husk biochar efficiently removes aqueous glyphosate and aminomethylphosphonic acid (Karram *et al.*, 2022; Garba et. al., 2019).

Numerous biochar adsorption studies have been conducted on organics, but few studies on biochar reproducibility allow easy comparison with other carbonaceous materials (Mohan *et al.*, 2014). While biochar shows excellent potential for water treatment, some challenges remain. One of the main concerns is the long-term environmental stability of biochar. Biochar can degrade over time, releasing adsorbed contaminants into water. The impact of biochar adsorption on *Daphnia* spp. can be positive and negative. On the positive side, biochar has been shown to effectively adsorb pesticides, reducing their concentration in water and potentially mitigating harmful effects on aquatic organisms. This could benefit *Daphnia* spp. by enhancing water quality and decreasing their exposure to toxic chemicals. However, there are also concerns about the potential adverse effects of biochar on *Daphnia* spp., including changes in water chemistry, alterations in ecological interactions, and impacts on respiratory function, as well as the long-term effects of biochar on aquatic ecosystems.

Additionally, studies on the effects of water treatment with carbonaceous materials on natural flora and fauna are limited (Singh et al., 2015; Ahmad et al., 2014; Valentín et al., 2013) and not well understood (Lima *et al.*, 2022). Consequently, there is insufficient study on the possible toxic effects of biochar remediation of contaminated water on aquatic organisms. This study aims to employ *Daphnia* spp., a renowned bioindicator.

### 1.8.1. Research Aim and Objectives

#### *Aim*

This thesis aims to evaluate the suitability and impacts of five carbonaceous materials on the adsorption of the pesticides cypermethrin (CP) and Isoproturon (IPU), metals arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) from water, and the possible acute toxic effects of biochar-remediated water on *Daphnia* spp.

#### *Research Objectives*

1. To evaluate and compare the adsorption of C-14 IPU (herbicide) from water using rice husk, hardwood, and coconut shell biochar, granular (GAC), and powdered activated carbon.
2. To investigate and compare the possible acute toxic effects of GAC and RH-biochar treatments with untreated pesticide-and-metal solutions (CP, IPU, As, Cd, Cu, Ni, Pb, and Zn) on the physiology of *Daphnia* spp.

3. To compare the effects of GAC and RH-biochar treatments with untreated pesticide-and-metal solutions (CP, IPU, As, Cd) on the percentage and cumulative $CO_2$ production (respiration) of Daphnia *pulex* in water.
4. To proffer evidence-based solutions for challenges associated with biochar use in water treatment and recommendations for future research in water treatment.

### 1.8.2. Thesis Structure/ Summary of Chapters

This chapter introduced the rationale for the study, the main areas of focus, and the literature review. Chapter Two details the materials and methods employed in this study, and Chapter Three presents the adsorption of Isoproturon with Carbonaceous Materials. Chapters four and five evaluate the acute toxicity of Biochar-treated and untreated pesticides and metals on neonate Daphnia spp. respectively. Chapter six investigates the effects of Biochar treatment of pesticide and metal solutions on the respiration of *D. pulex*. Chapter 7 summarises the research results, discusses their implications, presents evidence-based conclusions, and offers recommendations for future research.

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# Chapter Two

# Materials and Methods

# 2. Materials and Methods

This chapter gives a brief overview of the methods employed in the studies reported in this thesis including, the sources of all the materials used, the preparation of laboratory-based aqueous herbicide and metal solutions for laboratory analyses, the standard operating procedures used for the preservation of Daphnia spp. and bioassays preparation, the methods for the measurement of organic and inorganic samples in solution, quality control and assurance of all experiments conducted, the principles and equations for data calculation and the statistical methods of data analysis.

## 2.1 Materials

Approximately 99% pure analytical grade Cypermethrin (CP) and Isoproturon (IPU) were purchased from Sigma-Aldrich, UK, and Chem Service, USA, respectively. The C-14 analogues of CP and IPU were obtained from Supelco in the UK. Dichloromethane (DCM) was obtained from Ratbourn Chemicals, UK. Methanol and dimethyl sulfoxide (DMSO) were sourced from Fisher Scientific, UK. Combusto-cones were obtained from Perkin Elmer, UK. Goldstar multipurpose liquid scintillation cocktail, carbon trap solutions for $CO_2$ trapping, and carbon counting solutions used in the liquid scintillation counter were procured from Meridian, UK. All metal salts (As, Cd, Cu, Ni, Pb, Zn) and chemicals used for the preparation of High Hardness (HH) Combo media and Bold's Basal Media (BBM) were purchased from Thermofisher Scientific, UK.

The bioindicators (*Daphnia* spp.) used for this study were obtained from several sources; Daphnia *magna* was obtained from the University of Birmingham Daphnia facility and Aquarea Live Fish Foods, while *Daphnia pulex* and *Chlorella vulgaris* (*Daphnia* food) were purchased from Blades Biological Ltd., UK. The procedures used for *Daphnia* culture maintenance followed those outlined in the Birmingham University Daphnia Facility Standard Operating Procedures (SOPs) attached as Appendix I.

The biochar used for all experiments in this thesis was sourced from Malaysia and the UK. Rice husk (RH) and coconut shell (CS)- derived biochar were purchased from Malaysian farmers, and hardwood (HW)- derived biochar was purchased and prepared within the UK. The granular activated carbon (GAC) and powdered activated carbon (PAC), used as controls, were purchased from Sigma Aldrich, UK.

## 2.2 Methods

### 2.2.1 Biochar Preparation

#### *2.2.1.1 Rice Husk and Coconut Shell-derived Biochar Preparation*

The rice husk and coconut shell biochars used in this study were produced in tropical climates. Both biochars contain high amounts of carbon and nutrients and are produced differently. The rice husk (RH) biochar was produced by fast pyrolysis in a rotary husk furnace (RHF) in Tanjung Karang, Selangor, Malaysia. This production method resulted in a higher biochar yield at (900 °C to 1000 °C) within a few minutes. Coconut shell (CS) biochar was produced by slow pyrolysis in drums by farmers for 6-8 h at a temperature of up to 400 °C.

#### *2.2.1.2 Hardwood Biochar Preparation*

The hardwood biochar used in this study was purchased from Bodfari Environmental, St. Asaph, UK. This biochar has a very high carbon content, and the production method was slow pyrolysis for 24 h in a ring kiln at 400 ° C.

#### *2.2.1.3 Physicochemical Characterization of Biochar*

##### Preparation of Biochar for Physicochemical Analysis

All biochar was crushed and sieved using a 50 - 150 µm mesh. The biochar samples' known weights were dried at 30°C, placed in a vacuum oven overnight, and then transferred to a quartz U-tube for weighing before analysis.

### 2.2.2. Preparation of Solutions

#### *2.2.2.1 Preparation of Stock Solutions*

Due to their low solubility in water, stock solutions of herbicides used in this research were prepared by dissolving known weights of their salts in organic solvents (methanol for adsorption and DMSO for toxicity experiments), then made up to the volumetric mark with dilution water (Guillén-Jiménez *et al.*, 2012). Analytical grade cypermethrin (CP) and Isoproturon (IPU) were used for pesticide studies. On the other hand, the metal stock solutions were prepared by dissolving known weights of their metal salts in deionized water, then made up to the volumetric mark. All pesticide stock solutions were frozen in properly labelled amber bottles at -20 °C (Zhang *et al.,* 2011).

#### *2.2.2.2 Preparation of Pesticide and Metal Test Solutions*

Each test metal solution was prepared by diluting known volumes of stock solutions in deionized water to the required test concentrations. Stock herbicide test solutions were prepared and diluted with solvents (MeOH or DMSO) to working concentrations of 0.5-10000 µg $L^{-1}$ and stored at 4 °C for weekly use.  A critical factor in this study was the difference in the solubilities of the pesticides of interest in the solvent. This led to different DMSO volumes used to prepare the two pesticides, CP and IPU. The CP and IPU solutions used for preliminary tests contained 0.02 % and 0.04% DMSO, respectively. During the pesticide experiment, three controls were used: HH Combo Medium (dilution water), 0.02% and 0.04% DMSO solutions as CP and IPU solvent controls, respectively.

The solvent control solutions used for pesticide studies were prepared by diluting the highest-volume solvent used to dissolve each pesticide with deionized water. All test solutions were prepared in quintuples. The reagents used to prepare media for the growth and maintenance of the test organisms are specified in the OECD 2004 manual. Finally, the test solutions used for toxicity and respiration experiments were prepared following the methods described above, except that the stock solutions were diluted with HH Combo medium (used for *Daphnia* spp. culture maintenance) to working concentrations.

### 2.2.3 Test Organisms

*Daphnia* species were used as test organisms in all bioassay experiments. Several species were considered for this study, but preliminary tests and supplier availability led to the selection of *D. magna* and *D. pulex*. The procedures used to maintain the Daphnia culture followed those outlined in the Standard Operating Procedure (SOP) used in the University of Birmingham Daphnia facility. Figure 19 shows the culture setup for *Daphnia and C. vulgaris* and the preparation of HH Combo Medium.

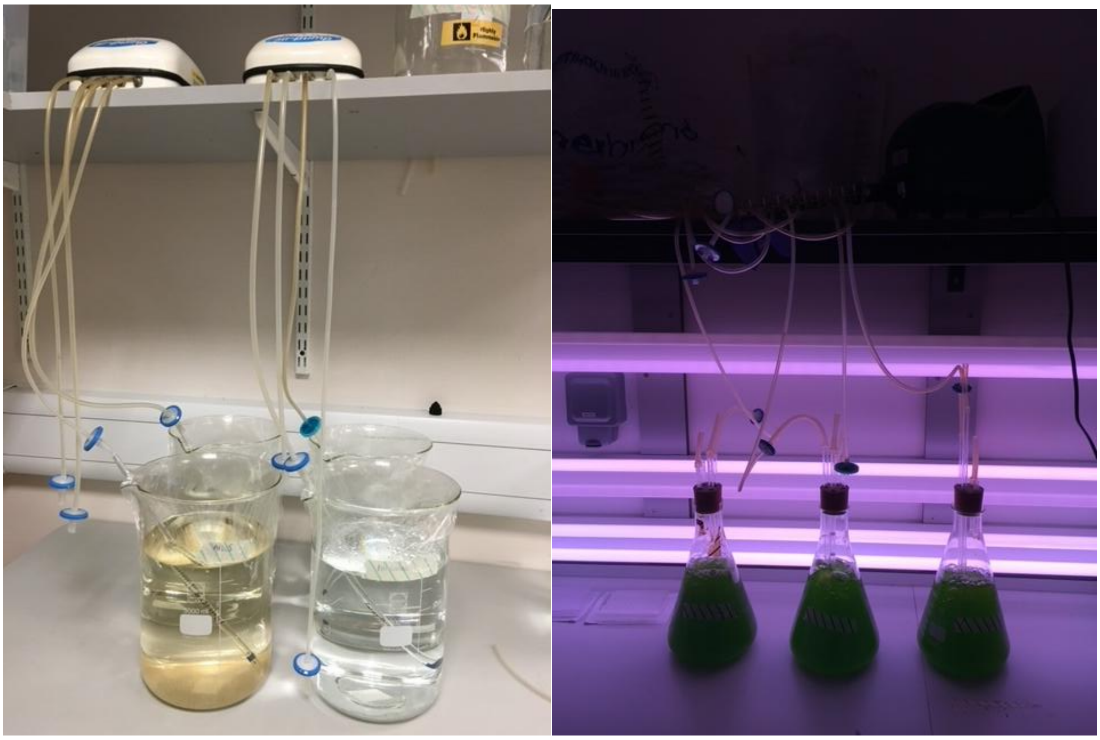

Figure 19: Setup for *Daphnia* Culture using HH Combo Medium (Left), and Light and Temperature Controlled Chamber for *C. vulgaris* (Daphnia food) Culture (right)

## 2.3. Experimental Procedures

### 2.3.1. Batch Biochar Adsorption of Pesticides in Solution.

Approximately 0.1g of RH biochar was weighed into 50 ml reaction vials using a Mettler Toledo PB 5001-S analytical weighing balance, then 20 ml of IPU solution was introduced into the centrifuge tubes, which were sealed with parafilm and placed on an orbital shaker at 100 rpm for 0 - 72 h. At each time point, the samples were filtered using Whatman No. 1 (110 Ø) filter papers into 35 ml amber vials, pipettes were used to transfer 5 ml of the filtrate into 20 ml scintillation vials, and 15 ml of Goldstar scintillation cocktail was added to the samples (Struthers *et al.*, 1998).

Samples were gently swirled to homogenize and then kept in the dark for up to 12 h before scintillation analysis using a Tri-carb 2300TR Liquid Scintillation Counter (Xing *et al.*, 1996; Lu *et al.*, 1977). The RH biochar residue on the filter papers was allowed to dry in a fume hood overnight, and then known weights of the residues were stored in Combusto-cones (306/307) purchased from Perkin Elmer, Netherlands. The residues were analysed immediately using an Isotech (Model 307) Sample oxidiser (Figure 20). All samples that were not oxidized immediately were wrapped with aluminium foil and frozen at 4 °C in a freezer pending combustion in the sample oxidizer. The resulting samples were gently swirled to homogenize, stored in the dark for 24 h, and analysed using a Tri-Carb 2300 TR Liquid Scintillation Counter.

Figure 20: The Isotech (Model 307) Sample Oxidizer

All experiments were conducted in triplicate, and destructive sampling was employed. A Mettler Toledo pH meter was used to measure pH throughout the experiment. All experiments were conducted in a cool, dark, constant-temperature room at 20 ± 3 °C to prevent photodegradation of the solutions. Sterile MQ-water was used throughout the experiment to prevent microbial activity. The principle used in the quantification of all pesticide samples was radiolabelling. This method is faster and easier to use, and details of its comparison with other methods are presented in Appendix I.

### 2.3.2 Batch Biochar Adsorption of Metals in Solution.

Known weights of biochar and black carbon (approximately 0.1g) were placed in glass vials, and 20 ml of known concentrations (0.0001-10 mg $L^{-1}$) of metal solutions were added and shaken at 120 rotations per minute (rpm) on platform shakers for 48 hours at 20 °C.

### 2.3.3 Bioassays for the Determination of Pesticide and Metal Toxicity to *Daphnia* spp.

30ml of organic solutions with known contaminants was measured into 50ml glass beakers. Then, ten *D. magna* neonates (<24 h) harvested 6 h before the experiment will be introduced into each of these beakers before incubation at 20 ± 1 °C in 16:8 photo and dark periods (Li *et al.*, 2018, Liu *et al.*, 2017). The neonates were monitored at 10 time points over 72 h, during which the mobile, moribund, and immobile daphnids were counted in each glass beaker (Kim *et al.,* 2007) and recorded. All experiments were conducted in triplicate; experiments with control solutions of medium only and solvent only will also undergo observation. All the organisms used in this experiment were not fed during the test period. The No Observable Effects Concentration (NOEC) and Lethal Concentration on 50% of daphnids ($LC_{50}$) were calculated and recorded for each chemical.

#### *2.3.3.1 Acute Immobilization Test in Untreated Pesticide and Metal Solutions*

This procedure was similar to the bioassay described in Section 2.3.2. For untreated test solutions, unfed *Daphnia* spp. was exposed to different pesticide solutions of known concentrations over 48 h. The organisms were closely monitored at specific time points for immobilization in accordance with the OECD 2004 acute immobilization procedure.

#### *2.3.3.2 Procedure for Toxicity Tests on all Biochar-Treated Solutions*

Known amounts of biochar were measured into 50 ml glass beakers. 30ml of pesticide test solutions of known concentrations were prepared by diluting stock solutions with deionised. water. The prepared mixtures were agitated on an orbital shaker for a known duration (depending on the type of biochar and based on previous adsorption and optimization experiments). After adsorption, the solutions were filtered, and the supernatants were used as test solutions. Then, the test solutions were introduced to glass beakers and ten *D. magna* neonates (<24 hr) that were harvested a maximum of 6 h before experiments, were introduced into each of these beakers before incubation at 20 ± 1 °C in 16:8 photo and dark periods (Li *et al.*, 2018; Liu *et al.*, 2017). The neonates were monitored at 10 time points over 72 h, and mobile, moribund, and immobile daphnids were counted in each glass beaker (Kim *et al.,* 2007). All experiments were conducted in triplicate, and control solutions of medium-only and solvent-only bioassays were also observed during the 96-h test period.

### 2.3.4 Bioassays to Determine the Effect of Biochar Treatment of Pesticide and Metal Solutions on *Daphnia* Respiration

After preliminary respiration tests using varying numbers of daphnids, results were analysed, and a minimum of 50 daphnids produced detectable respiration ($CO_2$). 50 daphnids were harvested and placed in air-tight 250 ml flasks connected to a Micro-Oxymax $CO_2$ trapping machine (Figure 21), 100-120 ml of known concentrations of pesticides and metal solutions were introduced into the flasks and then sealed. The experiments were run for 24- 96 h, and the results were copied from the machine and analysed.

Figure 21: Respiration Experiment Setup using Micro Oxymax $CO_2$ Trapping machine

## 2.4 Quality Assurance and Control

All Daphnia tests were designed and assessed in accordance with the OECD 202 Acute Immobilisation Test guideline, with any modifications clearly referenced. Toxicity experiments were validated in accordance with the OECD 202 (2004) criteria. The pH of all experiments was measured before and after each experiment, or daily if the experiment lasted more than 24 hours (Cao & Harris, 2010; Cao *et al.,* 2009; Chen *et al.,* 2009). Biochar and solution pH were measured prior to use (Sopeña *et al.*, 2012**).**

Physicochemical properties of test solutions—including dissolved oxygen (DO), conductivity, pH, and temperature—were measured at the start, during destructive sampling, and at the end of each experimental batch (Li *et al.*, 2018). Agitation speed and light conditions were kept as constant as possible and recorded periodically. All experiments were performed in a cool, dark room to minimize photodegradation of organic chemicals.

A preliminary mass-balance experiment using various vial types identified the most suitable containers. Amber glass vials were used for pesticide adsorption and toxicity tests, while acid-cleaned clear glass vials were used for metal-related experiments.

Blanks were employed as experimental controls and factored into all result calculations to ensure quality standards. Controls included sample blanks (no biochar), matrix controls (test water only), solvent-only blanks, and biochar-only blanks (biochar in water without analyte). Procedural blanks were used throughout all sample handling steps.All laboratory experiments were conducted with 3–5 replicates, with results expressed as means ± standard error (SE). Adsorption and respirometry experiments were done in triplicate, while toxicity tests were performed in quintuplets.

Calibration and standard solutions for pesticides and metals were freshly prepared or sourced from validated stocks, and their preparation and storage were thoroughly documented. Chain of custody and unique sample identification were maintained throughout all experiments. Destructive sampling was used due to the long sampling periods. Batch experiments were rejected if Daphnia mortality exceeded 10% in control solutions.

### 2.4.3 Preliminary and Range-finding Experiments

Preliminary adsorption experiments were conducted with 0.1 g of each raw sorbent in 50 ml single-metal solutions to select four metals for mixed-metal solutions and three sorbents for further adsorption studies. These preliminary tests also established the concentration ranges for definitive toxicity tests. Acute tests were observed for up to 72 hours without feeding. Immobility was defined as the inability of Daphnia to swim for 15 seconds after gentle agitation (OECD, 2004). Weakness was classified as the ability to swim, with or without erratic movement, after swirling the vessel. Mortality was defined as complete death, with dead daphnids counted at the vessel bottom using magnifying lenses.

The control water for toxicity tests was the same culture medium used for Daphnia rearing. Daphnids were not fed during acute exposure, but a consistent pre-test feeding regime was maintained. All exposures involved solutions of dissolved-phase pesticides and metals.

## 2.5 Data Analysis

### 2.5.1 General Data Acceptance Criteria and Quality Control Limits

Accepted Daphnia immobilization in control solutions was ≤10% for all test batches. Matrix spike recovery for all C-14 solutions ranged from 70–130% (RSD ≤20%). Duplicate relative percent difference (RPD) was typically ≤20–30%, depending on analyte concentration. Method blanks were less than 10% of the lowest sample concentration.

Photos of the experimental setup are included in this study. Instrument logs, calibration results, and all raw data were archived and remain accessible for audits or reanalysis until the final submission.

Assuming no losses, the percentage adsorption of pesticides and metals was calculated using the formulae below:

Equation 1: Percentage Pesticide/ Metal Adsorbed

$$\%\ \text{Adsorption}\ (C) = (C_a * 100)/C_0$$

Where:
$C_a$ = *Concentration Adsorbed (mg/l)*
$C_0$ = *Initial Metal Concentration (mg/l)*

Equation 2: Mass per mass Adsorption Capacity of Adsorbent

$$\text{Adsorption Capacity (mg/g)} = (C_a * V)/M$$

Where:
$C_a$ = *Concentration Adsorbed (mg/l)*
$V$ = *Volume of sample treated (l)*

*C= Final Metal Concentration (mg/l)*

***Adsorption Capacity (mg/g) = (Ca * V)/M***

Where: *Ca = Concentration Adsorbed (mg/l)*

LC/EC$_{50}$ toxicity values were calculated using probit or logit analysis, with goodness-of-fit metrics applied where appropriate. All data were reported as means ± standard error for three or five replicates. Statistical analysis was conducted using SigmaStat, with significance set at the 95% confidence level. Shapiro-Wilk normality tests, exploratory data analysis, and ANOVA (including Friedman repeated-measures, Wilcoxon signed-rank, Bonferroni, Brown-Forsythe, Chi-square, and Tukey post-hoc tests) were performed as applicable to assess statistical significance. Plots were generated with Sigma Stat. Raw statistical data were archived, and significant plots are included in the appendices. Limitations and challenges encountered are discussed in a later section.

# Chapter Three

# Adsorption of Isoproturon with Carbonaceous Materials.

## 3.1 Introduction

Isoproturon (IPU) is a phenylurea herbicide widely used in global agricultural practices. It is characterized as a colourless, odourless, transparent crystalline compound, formally designated by the IUPAC as 3-(4-isopropylphenyl)-1,1-dimethylurea or 3-p-cumenyL$^{-1}$,1-dimethylurea (WHO, 2003). Herbicides constitute a major subclass of pesticides, employed to suppress or eradicate unwanted vegetation that could adversely affect crop development and agricultural productivity (Mehdizadeh et al., 2021; USEPA, 2012; Norman et al., 1950). While the informal utilization of herbicidal agents has historical precedence spanning over two centuries, the advent of synthetic herbicides occurred in the mid-twentieth century (Mesnage et al., 2021).

Herbicides account for about 30-50% of the pesticides currently used worldwide, with rapid increases in their use (Asad et al., 2017; de Souza et al., 2016). Herbicide application in agriculture has led to a marked increase in agricultural yield, a decrease in vector-borne diseases, and significant satisfaction of the ever-rising need to meet food production demands (Pillegi et. al., 2020; Mieldazys et al., 2016; Yavari et al., 2015; Ortiz-Hernandez et al., 2013; Gupta, 2011; Mudho & Garg, 2011; Aktar et al., 2009; Abate & Masini, 2005; Norman et. al., 1950).

However, their unintended severe negative impacts on the environment and health resulting from their biological activity and toxicity to wildlife and humans cannot be overemphasized (Pillegi et. al., 2020; Mieldazys et. al., 2016; Yavari et. al., 2015; Ortiz-Hernandez et. al., 2013; Gupta, 2011; Mudho & Garg, 2011; Aktar et. al., 2009; Abate & Masini, 2005; Norman et. al., 1950).

Herbicides have been reported to produce acute and chronic health effects in humans, ranging from mild skin irritation and cancers to death in extreme cases. In addition to the risks they pose to humans and other non-target organisms, they also exert toxic and/or lethal effects on many other arboreal, aquatic, and terrestrial organisms (de Souza *et al.,* 2016; Aktar *et al.,* 2009). Previous studies have linked herbicides to the destruction of foliage and inhibition of photosynthesis in plants, oxidative stress in non-mammalian sp., chromosomal aberrations in fish, decline of soil microbial populations and diversity, mutation, abnormal behaviour, increased mortality, reduced reproduction and growth rates, among other deleterious effects (de Souza *et al.,* 2016; Aktar *et al.*, 2009). These effects have stressed the importance of their removal from environmental compartments. One notable method of pesticide remediation is adsorption. Adsorption employs the use of adsorbents, which include modified or unmodified commercially available activated charcoal, biochar, nanotubes, and agricultural wastes, among other sorbents.

Biochar is a co-product of biomass pyrolysis at temperatures between 200 - 1000 °C in the presence of limited oxygen (Zhelezova *et al*., 2017). It can be produced from various biomass sources, including agricultural wastes and biosolids (Zama *et al*., 2018). Biochar is stable, eco-friendly, cost-effective, and easy to handle (Tang *et. al.,* 2013). Reports on biochar addition to soil suggest that it enhances the physical, chemical, and biological properties of soil (Yavari *et. al.,* 20015). Several reviews and studies have reported the advantages of biochar use as a potent soil amendment (Shaaban et *al.,* 2018; Khorram et *al.,* 2016; Xie et *al.,* 2016; Lu et *al.,* 2012; Kookana et *al.,* 2011). Due to its neutral pH, biochar has been reported to act as a soil conditioner through its liming effect (Zhelezova et *al.,* 2017; Yavari et *al.,* 2015).

Biochar has gained significant attention in recent years for its potential to adsorb pesticides and metals in water systems. Figure 16 summarizes the mechanisms involved. This has sparked research interest in the applications of biochar in water and wastewater management (Foereid, 2015). Historically, the use of biochar for adsorbing pesticides in water systems dates back to ancient civilizations, where charcoal was used to filter water for drinking. Studies have shown that biochar can effectively adsorb metals such as lead, cadmium, and copper, reducing their concentrations in water and decreasing their toxicity to aquatic organisms.

Smith *et. al.* (2010) conducted pioneering studies demonstrating biochar's high affinity for organic contaminants, including Isoproturon. Subsequent research by Wang *et. al.* (2015) further confirmed the efficacy of biochar in removing Isoproturon from water, highlighting its potential for practical applications in water treatment technologies. Other indirect advantages of biochar use include reducing global warming and producing bioenergy (Igalavithana et *al.,* 2018).

This chapter aims to evaluate and compare the adsorption capacities of five unmodified (virgin) carbonaceous materials: rice husk, hardwood, and coconut shell biochar; granular activated carbon (GAC); and powdered activated carbon (PAC) for aqueous C-14 IPU. It further aims to investigate the optimal adsorption time and capacity of these five adsorbents.

## 3.2 Materials and Methods

Five carbonaceous materials (rice husk, hardwood, and coconut shell biochar, granular (GAC), and powdered activated carbon (PAC)) were employed in the adsorption of three to seven logarithmic concentrations of C-14 IPU. Preliminary Experiments involving the destructive sampling of batch adsorption experiments comparing the adsorption capacity of all carbonaceous materials for C-14 Isoproturon (IPU) in solution were conducted as described in **Section 2.3.1**. Batch experiment one tested RH biochar only, pilot experiment two compared RH, CS, and HW biochar with PAC and GAC.

Pilot experiments were conducted using RH to adsorb C-14 from IPU solutions at three concentrations: 0.10, 1.00, and 10.00 mg $L^{-1}$. Preliminary experiments were conducted using different glass vials, including transparent and amber glass, as well as glass centrifuge tubes. The average amount of chemical stuck on the walls of all three tested vial types was negligible, <1% of activity in the spiked solutions used for the experiment.

In summary, the results indicated that adsorption was similar, and amber glass vials were used in subsequent experiments to prevent photodegradation of the chemical of interest. Secondly, filtration of the supernatants and separation of the biochar pellets from the solution after batch adsorption were also compared. The amount of activity left on the filter paper after filtration of biochar pellets was also confirmed to be <1%.

All the results obtained from this experiment were triplicated and expressed as mean values with standard deviation. For this experiment, the target radioactivity in each 20ml of IPU solution was 1000 disintegrations per minute (dpm), which equals 50 dpm $mL^{-1}$.

## 3.3 Results and Discussion

### *3.3.1. Physicochemical Properties of Biochar and Pesticide Solutions*

Biochar derived from three types of agricultural waste—rice husk (RH), hardwood (HW), and coconut shells (CS)—was used to assess the adsorption capabilities of five concentrations of Isoproturon, compared to commercially available powdered and granular activated carbon (PAC and GAC). Table 9 (pg.78) indicates that the pH values of the RH, HW, and CS biochars are 8.46, 9.05, and 8.33, respectively. These values are consistent with findings from previous studies, which reported a pH of 8.01 and 9.26 for RH biochar (Xu *et al.*, 2013; Kanagasuppurathinam *et al.*, 2024). Additionally, Kanagasuppurathinam *et al.* (2024) reported a pH of 9.60 for CS biochar and approximately 10 for wood-derived biochar. Further research suggests that the alkalinity of biochar produced from agricultural feedstock, as shown in Table 1 (pg. 5), may be influenced by the release of alkali salts during pyrolysis (Tran *et al.*, 2016; Kim *et al.*, 2013; Ahmad *et al.*, 2012).

### *3.3.2 Adsorption of IPU Solutions with Rice Husk Biochar*

All initial radioactivity values were factored into the calculations because they differed and were far lower than the target values expected during spiking. The initial pH of the Milli-Q (MQ) water used for this experiment was **6.50 ± 0.01.** Table 12 displays the actual initial radioactivity in each IPU concentration level.

Table 12: C-14 Activity in each prepared IPU solution

| IPU Concentration (mg $L^{-1}$) | Activity (dpm $mL^{-1}$) |
|---|---|
| Blank (MQ water) | 7.07 ± 7.13 |
| 0.00 | 19.16 ± 0.64 |
| 0.10 | 12.69 ± 0.75 |
| 1.00 | 14.45 ± 2.23 |
| 10.00 | 20.55 ± 0.72 |

The background activity and pH of the biochar and water in all reactions throughout the experiment are shown in Table 13.

Table 13: Background C-14 activity of biochar + water at each reaction time point.

| *Reaction Time (h)* | *Activity (dpm mL$^{-1}$)* | *pH* | *Corresponding temperature ($^{o}$ C)* |
|---|---|---|---|
| *0* | 29.85 | 8.32 | 21.00 |
| *12* | 11.09 | 8.30 | 22.00 |
| *24* | 51.27 | 8.65 | 21.00 |
| *36* | 28.13 | 8.60 | 22.00 |
| *48* | 29.13 | 8.30 | 22.10 |

Table 13 confirms that RHBC is alkaline, as it increased the water's pH from 6.50 to above 8. The results obtained from the experiment and the graphical representation in Figure 22, which show the percentage adsorption of IPU at different concentrations and time points, respectively, further suggest that IPU was increasingly adsorbed across all time points shown: T0 to T48.

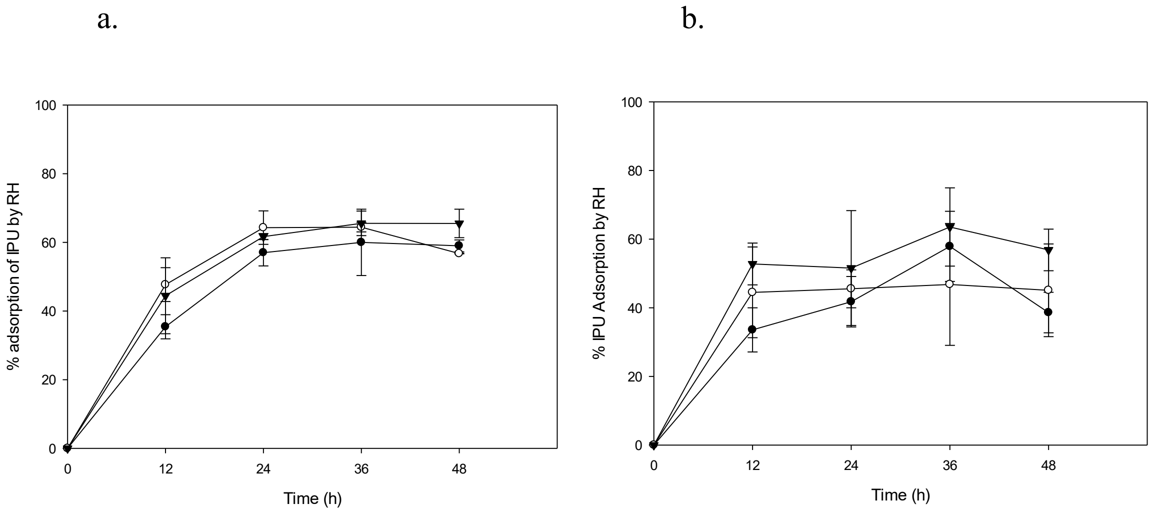

Figure 22: Percentage Rice Husk (RH) Biochar Adsorption of 0.01 mg L$^{-1}$ (●), 1 mg L$^{-1}$ (○), and 10 mg L$^{-1}$ (▼) Isoproturon from water over 48 h.

For uniformity, only data for 0-48 h is displayed in the figures. However, data for 0-72h were statistically analysed and discussed below. Figure 22a suggests that biochar is a good adsorbent for IPU, with adsorption exceeding 60% at 48 h and 90% at 72 h. However, the adsorption rate decreased between 36 and 48 h at 0.1 and 1 mg L$^{-1}$, whereas it remained steady at 10 mg L$^{-1}$, increasing around 72 h into the reaction. There was no statistically significant difference ($P = 0.717$). Power of the performed test with alpha = 0.050.

However, for Figure 22b, a comparison of the mean adsorption of IPU by Rice husk biochar at all time points against T0 at (p=0.05) confirmed that there was a statistically significant difference between the mean adsorption of IPU by RH biochar at the different time points (all p values were less than 0.05). This further suggests that reaction time is an important factor in the adsorption of IPU from water. However, further comparison of the mean IPU adsorption by rice husk biochar indicates statistically significant differences between T12 and T24, and between T48 and T72 (p-values are less than 0.05). However, there were no statistically significant differences in IPU adsorption rates at any time point beyond 72 h.

For Figure 22b, the data obtained for all time points except 0 h were normally distributed at the 95% confidence level. At 72 h, there was maximum adsorption of IPU by RH biochar (approx. 97%). The adsorption data obtained from this experiment were statistically analysed using repeated measures one-way ANOVA. The data were normally distributed. Data distribution passed the Shapiro-Wilk normality test (P = 0.057) and the Brown-Forsythe Equal Variance Test (EVT) (P = 0.423). The differences in mean values among the IPU concentrations (treatment groups) are not large enough to rule out random sampling variability; there was no statistically significant difference (P = 0.076). The power of the performed test (0.373) was below the desired power of 0.800.

Based on the pilot experiment, it was concluded that at 21-23 °C and pH about 8, RH biochar could adsorb IPU from water with an efficiency of approximately 68%, with adsorption highest at 36 h. However, additional optimization tests were important for determining the optimal temperature, pH, adsorbent dosage, and reaction time to improve the adsorbent's performance in removing IPU from water.

### *3.3.3 48 h Adsorption of Isoproturon Solutions with Carbonaceous Materials*

Pilot experiments were conducted using three biochars: rice husk (RH), coconut shell (CS), and hardwood (HW) biochar, and two commercially purchased activated carbons – granular activated carbon (GAC) and powdered activated Carbon (PAC) for the adsorption of 0.1, 1, and 10 mg $L^{-1}$ C-14 IPU solutions. The method is detailed in **Section 2.3.1.** The initial C-14 activity in each IPU concentration level is presented in Table 14.

Table 14: Background C-14 activity in each prepared IPU solution. All values are expressed as the mean of triplicates and standard error.

| IPU Concentration (mg $L^{-1}$) | Activity (dpm $mL^{-1}$) |
|---|---|
| Blank (MQ water) | 41.23 ± 1.33 |
| 0.10 | 31.20 ± 3.34 |
| 1.00 | 31.93 ± 2.98 |
| 10.00 | 30.68 ± 3.01 |

The pH of MQ water used for this experiment was 7.91 ± 0.34 at about 21.6 ° C. The pH of RH biochar ranged from 8.30 to 8.58 at 21 to 23 °C. The pH readings for the prepared IPU solutions before and after biochar addition at 0 h and 72 h are illustrated in Table 15.

Table 15: pH of IPU solution before use and when exposed to biochar at 0 h and 72 h. All values are expressed as the mean of quintuplets and standard error.

| Biochar Type | IPU solution before use | IPU solution + BC 0 h | IPU solution + BC 72 h |
|---|---|---|---|
| RH | 7.18 ± 0.20 | 8.72 ± 0.02 | 8.00 ± 0.04 |
| CS | 7.18 ± 0.20 | 6.96 ± 0.09 | 7.02 ± 0.16 |
| HW | 6.70 ± 0.25 | 8.96 ± 0.04 | 7.58 ± 0.02 |
| PAC | 6.79 ± 0.15 | 3.29 ± 0.01 | 6.90 ± 0.02 |
| GAC | 7.18 ± 0.20 | 6.58 ± 0.08 | 6.70 ± 0.10 |

The results in Table 15 suggest that the pH of IPU in solution is almost neutral. It further suggests that the RH and HW are alkaline in nature as they increased the pH of IPU solution at 0 h. CS and GAC slightly reduced the pH of IPU solution at 0 h but increased it after 72 h. On the other hand, PAC acidified the solution at 0 h and increased the pH to almost neutral after 72 h.

Tables 15 indicates that both CS biochar and GAC slightly reduced the pH of the IPU solutions at 0 h, decreasing it from 7.18 to 6.96 and 6.58, respectively. In contrast, PAC acidified the solutions from a pH of 6.79 to 3.79 at 0 h. This can be explained by the release of protons (from surface functional groups) into the solution during agitation, or by the interaction of IPU with PAC's acidic binding sites, which release more protons and thus acidify the solution. However, without pH adjustments, the pH of all pesticide solutions returned to nearly neutral at 72 h for all biochars used in this study. This change can be attributed to the continuous agitation of the vials and to interactions between the adsorbate and adsorbent, which aligns with findings reported by Taha et al. (2014), who noted that 15 pesticides were efficiently adsorbed from water using rice straw biochar at pH 7.

Furthermore, previous studies support this study's findings, reporting that adding biochar to all test solutions reduced dissolved oxygen (DO) levels, ranging from 6.60 to 9.20 before the 72 h acute toxicity tests and from 3.01 to 7.63 afterward. These values align with values obtained in this study (Table 15).

In conclusion, adding biochar to water can release intrinsic contaminants from the biochar itself or alter the chemistry of the solution into which it is introduced. This modification can ultimately affect physicochemical properties, including solution pH and the types and rates of reactions that occur.

Adsorption of C-14 IPU was conducted with different biochar types, rice husk, hardwood, coconut shell, and granular activated carbon. Results of IPU adsorption with RH, HW, and CS biochar and GAC over 48 h are displayed in Figure 23.

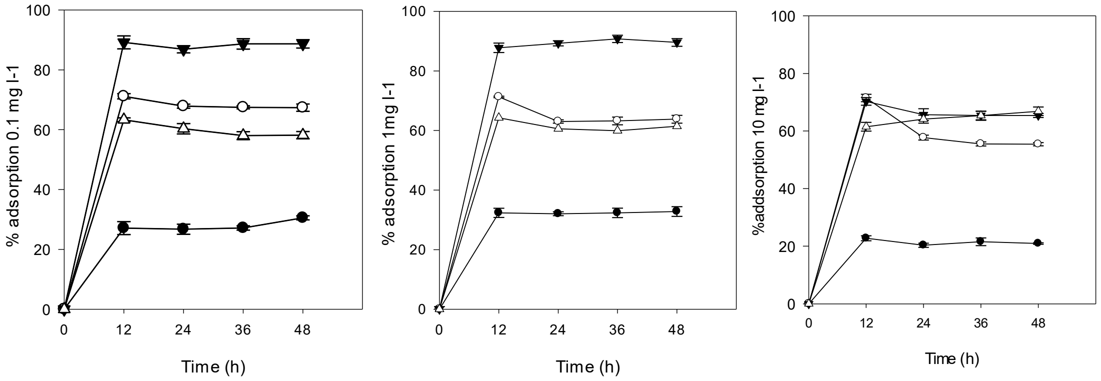

Figure 23: Adsorption of 0.1mg $L^{-1}$, 1 mg $L^{-1}$, and 10 mg $L^{-1}$ C-14 IPU by granular activated carbon (Δ•), coconut shell (●), hardwood (○), and Rice Husk (▼•) biochars over 48 h.

The results in Figure 23 suggest that the adsorption efficiency of C-14 IPU at 0.1 mg $L^{-1}$, 1 mg $L^{-1}$, and 10mg $L^{-1}$ by the different biochars of interest occurred in the order RH > HW > GAC > CS. The results also advocate that the maximum adsorption of IPU by these biochars occurred within 24 h.

The adsorption trend in this experiment is similar to the first. Adsorption equilibrates within about 24 h for most reactions, and the maximum adsorption efficiency by RH was 60.8%. The major difference between the results obtained in this batch and those of the first experiment is the incorporation of moisture content into the sample oxidizer (SOx) calculations and the reduction of human error.

Figure 23 indicates that different sorbents follow distinct kinetic mechanisms. GAC exhibits rapid uptake and a clear equilibrium plateau, consistent with pseudo-second-order kinetics, where adsorption is controlled by site availability and adsorption capacity, with minimal diffusion limitation. CS displays similar behaviour but with a slower approach to equilibrium, suggesting pseudo-second-order kinetics with a significant intraparticle diffusion contribution, reflected by a multi-stage adsorption process.

HW shows weaker initial uptake and prolonged adsorption, indicating that early-stage adsorption may follow pseudo-first-order kinetics, while later stages are increasingly governed by intraparticle diffusion. RH demonstrates the fastest uptake and highest adsorption capacity, with adsorption predominantly controlled by intraparticle diffusion, likely due to its high ash content and poorly developed pore structure. Overall, the results highlight a transition from capacity-controlled adsorption in GAC and coconut shell biochar to diffusion-limited adsorption in hardwood and rice husk biochars.

The adsorption data obtained from this experiment were statistically analysed using repeated measures one-way ANOVA. The data were not normally distributed. Data distribution failed the Shapiro-Wilk normality test ($P < 0.050$), so a Friedman Repeated Measures Analysis of Variance on Ranks was conducted. Chi-square was 59.103 with 11 degrees of freedom. ($P = < 0.001$). There was a statistically significant difference ($P < 0.001$), and to isolate the group (s) that differ from the others, a multiple-comparison procedure, the Tukey Test, was conducted. Figure 24 suggests that significant differences were observed between the percentages of adsorption of RH and CS at all levels, and between HW and CS at 10 mg $L^{-1}$. However, there were no significant differences between HW and CS adsorption at 0.1 mg $L^{-1}$, and between HW and CS adsorption at 0.1 mg $L^{-1}$ and 10 mg $L^{-1,}$ respectively$^{.}$ Furthermore, the relationships between all other combinations were neither conclusive nor significant.

At increasing Isoproturon molarity (from ~$10^{-7}$ to $10^{-5}$ M), all materials show higher qe (mmol/g), but the rate at which qe increases with molarity and the apparent onset of saturation differ among CS, GAC, HW, and RH. This reflects differences in their molar adsorption capacity and affinity towards Isoproturon.

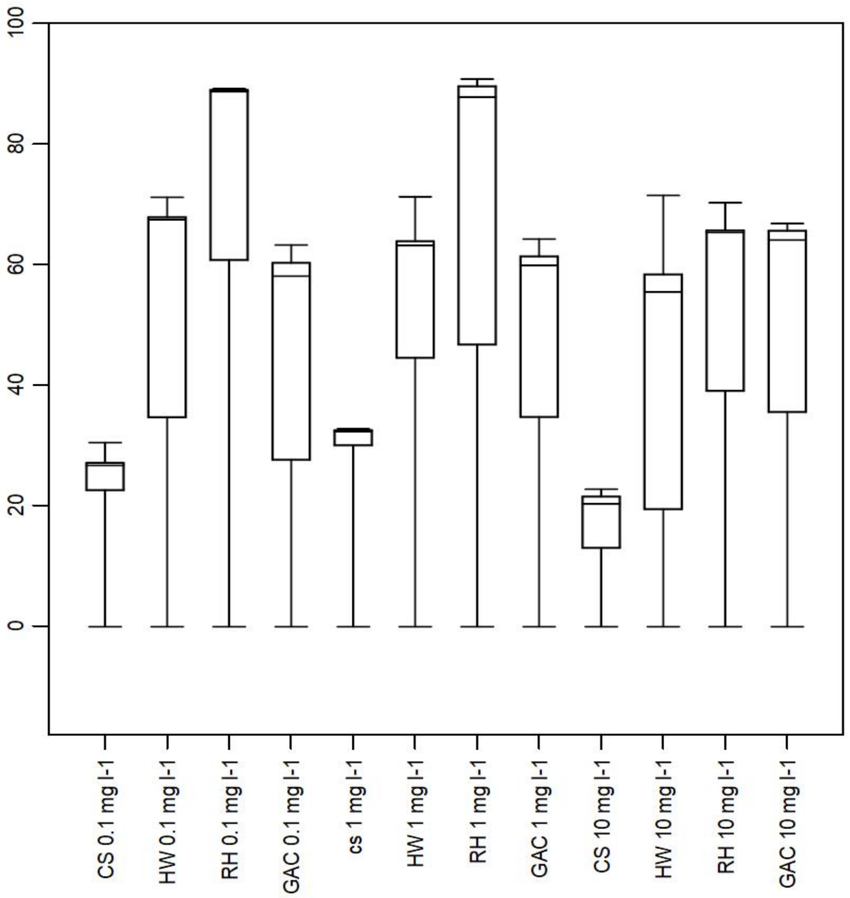

Figure 24: Box Plot Showing the Percentage Adsorption of All biochars Tested and their Inter-relationships.

### *3.3.4. 9 h Adsorption of Isoproturon Solutions with Five Carbonaceous Materials*

The results obtained from this experiment were similar to previous results in Section 3.2.2 above. All experiments using different char types for IPU adsorption from aqueous solutions suggest that IPU adsorption with biochar is rapid, but desorption back into solution is also possible. Furthermore, results suggest that Rice husk and Hardwood biochar perform best among the five types of chars tested, and their adsorption capacities can be comparable to, or even exceed, those of commercial Granular Activated Carbon.

Further studies using biochar for the adsorption of in discharges from waste treatment plants could yield interesting findings for water treatment.

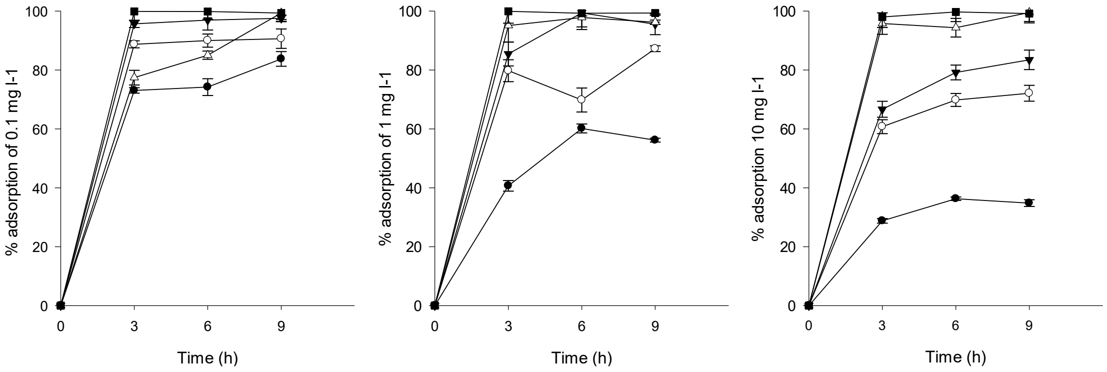

Figure 25: Adsorption of 0.1mg $L^{-1}$, 1 mg $L^{-1}$, and 10 mg $L^{-1}$ C-14 IPU by Granular Activated Carbon (Δ), powdered activated carbon (■), coconut shell (●), hardwood (○), and Rice Husk (▼) biochars over 9 h.

Figure 25 shows the percentage concentration of IPU in water over 0-9 h after adsorption with RH, HW, and CS biochars, GAC, and PAC. The results for PAC adsorption were significantly different from those obtained with other carbonaceous compounds. The PAC treatments required a faster method of sorbent-sorbate separation after treatment because the solutions took more than half an hour to pass through the filter papers used for filtration, and this was suspected to impact the results due to the extra contact time of the adsorbent and adsorbate. The PAC treatments were therefore excluded after this batch of experiments.

Figure 25 highlights clear differences in early-stage adsorption kinetics exhibited by all carbonaceous materials used. Adsorption is rapid for all sorbents during the initial contact period, indicating dominance of surface adsorption and external mass transfer. PAC exhibits the fastest uptake and highest short-term removal efficiency due to its smaller particle size and greater accessible surface area, followed by GAC.

Among the biochars, RH showed the greatest adsorption, while HW and, particularly, CS demonstrated slower uptake and lower removal efficiencies, reflecting increasing diffusion limitations and less developed pore structures. Increasing initial IPU concentration results in higher absolute adsorption but reduced relative removal, especially for biochar, suggesting rapid saturation of available sites under short contact times. Overall, the figure demonstrates that activated carbons, especially PAC, are more effective for rapid IPU removal, whereas biochar requires longer contact times to achieve comparable performance.

Data obtained in this experiment were analysed using repeated measures of one-way ANOVA. All the data was normally distributed. There were no statistically significant differences in the adsorption data for CS, HW, RH, GAC, and PAC. However, a comparison of all biochar adsorption values across all biochar groups identified a significant difference in PAC and CS sorption at all IPU concentrations. The p-values obtained are displayed in Table 16.

Table 16: p-values for all adsorption data for the 9 h study.

| Test | CS | HW | RH | GAC | PAC | All Biochar |
|---|---|---|---|---|---|---|
| Normality (Shapiro-Wilk): | $P < 0.713$ | $P = 0.747$ | $P = 0.317$ | $P = 0.078$ | $P = 0.238$ | $P < 0.050$ failed |
| Equal Variance (Brown-Forsythe): | $P = 0.575$ | $P = 0.823$ | $P = 0.554$ | $P = 0.512$ | $P = 0.790$ | |

### 3.3.4. Adsorption Percentage of Biochar

Previous research suggests that biochar is efficient in the adsorption of phenyl urea herbicides like Isoproturon, linuron, diuron, chloroturon, and monuron from water, with pH, temperature, and biochar feedstock being the most significant controlling factors (Kanagasuppurathinam *et al.*, 2024; Alsherbeny *et al.*, 2022; Taha *et al.*, 2014; Tran *et al.*, 2016; Kim *et al.*, 2013; Ahmad *et al.*, 2012). Mendes *et al.* (2018) found that biochar can reduce the aqueous presence of herbicides by 73-83%, and Patil *et al.* (2022) reported that biochar could adsorb up to 90.6% of phenyl urea herbicides from aqueous solutions.

These studies collectively highlight biochar's effectiveness in adsorbing phenylurea herbicides, with removal rates ranging from 73% to over 90%, depending on biochar type, application method, and environmental conditions. All preliminary adsorption tests suggest that RH biochar increasingly adsorbed 0.1, 1, and 10 mg $L^{-1}$ of aqueous IPU (approximately 91%) at a temperature of 21 - 23 °C and pH of approximately 8 over 72 h. The adsorption equilibrium for all adsorption tests was observed to peak around 24 h, which informed the following two batch adsorption experiments, each lasting 9h and 12 h.

Adsorption experiments comparing all biochar and activated carbon of interest for 0.001, 0.01, 0.1, 1, and 10 mg $L^{-1}$ C-14 IPU in solution over 12 and 9 h, respectively showed decreasing adsorption capacity for both pesticides in the order PAC (97%) > RH biochar (91%) > HW biochar (68%) > GAC (67%) > CS biochar (33%). Considering the acidic pH of the PAC used in this study, previous adsorption reports suggest that acidic conditions favour increased adsorption of pesticides from water and might have contributed to PAC's performance in adsorbing C-14 IPU from water.

Similarly, Alsherbeny *et al.* (2022) reported that the removal percentage of atrazine increased from % to 74% as the pH decreased from 9 to 3. This report contrasts with the report by Taha et al. (2014) and this study, but additional factors, such as the prolonged sorbate-sorbent contact time during PAC filtration from the C-14 IPU solutions, might have contributed to the higher adsorption efficiency exhibited by PAC due to its high surface area. However, increased pesticide concentration and adsorption time generally have an asymptotic effect on adsorption capacity (Manikandan *et al.*, 2023).

RH biochar exhibited the highest percentage of C-14 IPU adsorption in solution, significantly different ($p \leq 005$) from CS biochar at all concentrations and HW biochar at only 10 mg $L^{-1}$. This can be explained by the position that the proximate constitution, pesticide concentration, contact time, and pyrolysis temperature significantly control the type of adsorption and the adsorption behaviour of biochar for pesticides in water (Kanagasuppurathinam *et al.*, 2024; Kameyama *et al.*, 2016; Cabrera *et al.*, 2011).

Table 10 (pg. 80) shows that the RH biochar used in this study was produced at a high temperature of 900-1000 °C, and biochar produced at high temperatures is renowned for its increased surface area, pore volume, and stability for better pesticide binding, as reported by Zhang *et al.* (2016) for biochar produced at temperatures above 500°C. Additionally, RH biochar was produced using fast pyrolysis. These factors explain the high adsorption percentage of RH biochar. Furthermore, the adsorption performance of

HW and CS biochar was not very high or similar, despite their similarity in proximate properties, as shown in Tables 9 and 10 (pages 78 & 80). The slow pyrolysis preparation method might have been responsible for the lower percentage adsorption of IPU from water compared to RH-biochar.

## 3.4. Conclusions

The results of this study suggest that IPU adsorption biochar is also rapid, and desorption back into solution is also possible. Ruzickova *et al.* (2021) posit that some biochars cause fluctuations in pesticide adsorption-desorption rates, inadvertently re-releasing bound pesticides back into the water, which may increase toxicity levels. This can be explained by the different types of forces responsible for the adsorption of pesticide molecules, including hydrophobic effects, π–π electron donor–acceptor interactions, pore filling, electrostatic interactions, ionic bonding, and H-bonding (Manikandan *et al.*, 2023).

Physisorption, which involves weak van der Waals forces, is rapid and may lead to the desorption of contaminants back into solution (Smith & Brown, 2019), whereas chemisorption involves slower formation of stronger bonds that may be irreversible, depending on the prevailing conditions (Deng *et al.*, 2018). Although pollutants coexist in real life, single-pollutant adsorption with biochar helps determine optimal conditions for adsorbing specific water pollutants, thereby optimizing adsorption systems for these pollutants (Lima *et al.*, 2022). Acute toxicity studies provide information for pesticide- and metal-polluted aquatic systems.

Finally, the rapid uptake of C-14 Isoproturon by PAC demonstrates its suitability for short-residence-time applications, such as rapid mixing or emergency treatment, while GAC remains effective for fixed-bed and filtration systems with moderate contact times. In contrast, biochar exhibits slower adsorption kinetics, particularly for hardwood and rice husk, thereby limiting its effectiveness in time-constrained systems. RH performs best among the biochars but still requires longer contact times to achieve comparable removal. Overall, this study indicates that activated carbons are best suited for rapid treatment processes, whereas biochars are more appropriate for long-contact or low-flow systems, where sustainability and cost considerations may outweigh kinetic limitations.

# Chapter Four

## Acute Toxicity of Biochar-Treated and Untreated Aqueous Pesticide Solutions on Neonate *Daphnia* spp.

## 4.1 Introduction

Pesticides can be toxic to aquatic organisms, including marine life, algae, fish, invertebrates, and aquatic plants. The increasing presence of pesticide residues in aquatic environments poses a significant risk to non-target organisms, particularly sensitive freshwater invertebrates and humans (Carvalho, 2017; Stehle & Schulz, 2015). Exposure to pesticides can result in acute or chronic toxicity, affecting their reproduction, growth, and survival. Their toxicity depends on the specific pesticide, its concentration, exposure duration, and the sensitivity of the exposed organisms. The health effect of a commercial pesticide product may be a consequence of the active ingredient, the other ingredients in the formulation, or both. Unfortunately, toxicological testing is usually restricted to the active ingredient.

The importance of ecotoxicity studies of pesticides cannot be overemphasized, given the serious health impacts of aquatic pollutants at low and high levels. One of the recently studied health effects of pesticides is endocrine disruption (ED). This is common in aquatic organisms exposed to these chemicals. Agrochemicals are an essential class of Endocrine disrupting Compounds (EDCs), with various studies noting the ED effects associated with certain insecticides (Horak, 2021; De Angelis *et. al.,* 2009), herbicides (Harada *et. al.,* 2016), fungicides (Kackar *et. al.,* 1997), algaecides (Hiromori *et. al.,* 2016), nematicides, acaricides (Yang *et. al.,* 2019), fumigants (Goldman *et. al.,* 2007), fertilizers (Poulsen *et. al.,* 2018), and plant growth regulators (Xiagedeer *et. al.,* 2020). Therefore, when these agrochemicals reach non-target aquatic environments, they can elicit ED effects on wildlife species through different modes of action. They can influence the regulation and expression of essential genes involved in normal hormone function in fish species (Truter et *al.*, 2016).

Environmentally relevant concentrations of herbicides have also caused oestrogenic and anti-androgenic effects in tilapia species elsewhere, including Nile tilapia (O. niloticus), native to northern Africa (De Almeida et *al.,* 2018). Other ED effects have also been reported in South African wildlife. Bornman *et. al.* (2010) investigated the formation of calcifications inside the seminiferous tubules, known as testicular microlithiasis. Different environmental mixtures of agrochemicals can (Jacobsen et *al.,* 2012; Ma et *al.,* 2019; Papalou et *al.,* 2019) but do not always elicit synergistic, additive, or combined effects (Archer & Van Wyk, 2015).

While adsorption-based treatment technologies, such as activated carbon and biochar, have demonstrated effectiveness in reducing pesticide concentrations in water (Ahmad *et al.*, 2014; Mohan *et al.*, 2014), there remains a critical need to assess the capacity of these treatments to concurrently reduce biological toxicity, rather than chemical concentration alone (Escher & Fenner, 2011). This distinction is essential, as partial removal, transformation products, or desorption effects may still result in severe short- or longer-term adverse ecological impacts (Boxall *et al.*, 2004; Fenner *et al.*, 2013).

This chapter investigates the acute toxicity of biochar-treated and untreated aqueous pesticide solutions using neonate *Daphnia* spp. as a model organism. *Daphnia* species are widely used in ecotoxicological assessments due to their high sensitivity to contaminants, rapid life cycles, and ecological relevance in freshwater food webs (OECD, 2004; Lampert, 2011). By comparing immobilisation and mortality responses between treated and untreated solutions, this chapter evaluates the extent to which biochar adsorption translates into meaningful reductions in acute toxicity (Escher *et al.*, 2014).

The findings provide an important link between physicochemical removal processes and the reduction of ecological risk, supporting a more holistic assessment of biochar-based treatment strategies for pesticide-contaminated water systems (Giesy *et al.*, 2010; van der Oost *et al.*, 2003). This study investigated the acute toxicity of five concentrations of the pesticides Cypermethrin (CP) and Isopoturon (IPU) to two *Daphnia* spp. (*D. magna* and *D. pulex*) over 48 h to determine the range for the test solutions that will be used in the upcoming experiments (Li *et al.,* 2016).

## 4.2. Materials and Methods

The medium used to maintain the *D. magna* and *D. pulex* cultures (HH Combo Medium) was used as dilution water for all test solutions and as the “water only” control in the experiment. The method employed in this experiment is detailed in **Section 2.3.3.**

## 4.3 Results and Discussion

### 4.3.1 Acute Toxicity of Untreated Pesticide Solutions to *Daphnia pulex* in Methanol

Pilot experiments were conducted using *D. pulex* neonates and three to five concentrations of CP and IPU. All ± standard error of the mean. Methanol was used as the solvent, and each pesticide concentration required a different volume of solvent due to differences in solubility. Pesticide concentration required a different volume of solvent due to differences in solubility. CP contained 0.04% MeOH, and IPU contained 2% MeOH. Solvent controls were prepared for each pesticide type. Table 17 displays the dissolved oxygen (DO) readings before and after the treatment.

Table 17: Average Dissolved Oxygen (DO) mg $L^{-1}$ for CP and IPU test solutions using MeOH as solvent. "n.d." represents "no data".

| **Concentration** | **Pesticide** | **0 h** | **72 h** |
|---|---|---|---|
| MeOH control | CP | 7.90 | 6.24 |
| | IPU | 7.40 | 6.13 |
| 0.001 mg $L^{-1}$ | CP | 7.50 | 5.30 |
| | IPU | n.d. | n.d. |
| 0.010 mg $L^{-1}$ | CP | 9.00 | 7.63 |
| | IPU | n.d. | n.d. |
| 0.100 mg $L^{-1}$ | CP | 9.10 | 6.03 |
| | IPU | 7.50 | 6.17 |
| 1.000 mg $L^{-1}$ | CP | 6.60 | 5.43 |
| | IPU | 9.10 | 5.90 |
| 10.000 mg $L^{-1}$ | CP | 9.20 | 5.37 |
| | IPU | 8.60 | 3.01 |

The DO data recorded in Table 18 were normally distributed ($P = 0.063$), and paired t-tests were conducted. The dissolved oxygen values at the start of the experiment differed significantly from those at the end ($P < 0.001$).

### 4.3.2. Mortality *of D. pulex in Pesticides Prepared with MeOH*

Figure 26 below illustrates the percentage mortality rate of *D. pulex* in pesticide solutions used during the pilot toxicity experiment, employing MeOH as the solvent.

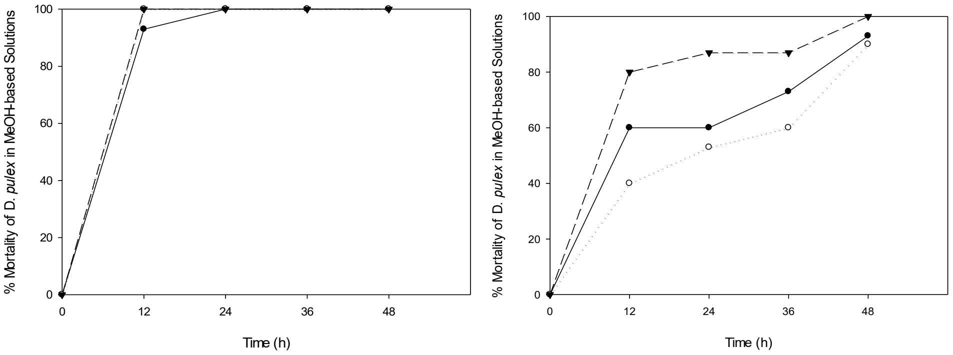

Figure 26: Percentage mortality of *D. pulex* in MeOH control and pesticide solutions. MeOH solvent control (●), 0.1 mg L$^{-1}$ (○), 1 mg L$^{-1}$ (▼), and 10 mg L$^{-1}$ (Δ) pesticide solutions, error bars are the standard error of the mean, n=3.

For toxicity tests using MeOH as the solvent, the statistical comparison between the toxicity of the toxicity of MeOH quantities used for CP and IPU controls failed the Shapiro-Wilk normality test ($P < 0.050$). A Wilcoxon signed-rank test was then carried out, and the results indicate a significant difference ($P = 0.008$). Analysis of the toxicity of different concentrations of CP to *D. pulex* passed the Shapiro-Wilk normality test ($P = 0.222$) and the Brown-Forsythe Equal Variance Test ($P = 0.669$).

Bonferroni t-test confirms a statistically significant difference in toxicity between the control solution and all concentrations of CP to *D. pulex* ($P < 0.001$). On the other hand, the Bonferroni t-test suggests that although the toxicity of IPU solutions showed a statistically significant difference between the control and 0.1 and 10 mg L$^{-1}$, there was no significant difference between the toxicity of the control solution and the toxicity of the 1 mg L$^{-1}$ IPU solution ($P = <0.001$). The values of the Shapiro-Wilk normality test ($P = 0.725$) and the Brown-Forsythe Equal Variance Test ($P = 0.196$) were passed by this data set.

One-way repeated-measures ANOVA was used to test the overall toxic effects of CP and IPU solutions on *D. pulex* (Figure 27). The data set passed the Shapiro-Wilk Normality Test ($P = 0.572$) and the Brown-Forsythe equal variance test ($P = 0.113$). However, the Bonferroni t-test suggests that at $P = <0.001$, there were significant differences between the CP and IPU control solutions, IPU control, and all concentrations of CP, 1 mg L$^{-1}$ IPU vs 0.1 and 10 mg L$^{-1}$ CP. Figure 27 displays the interactions between all test solutions.

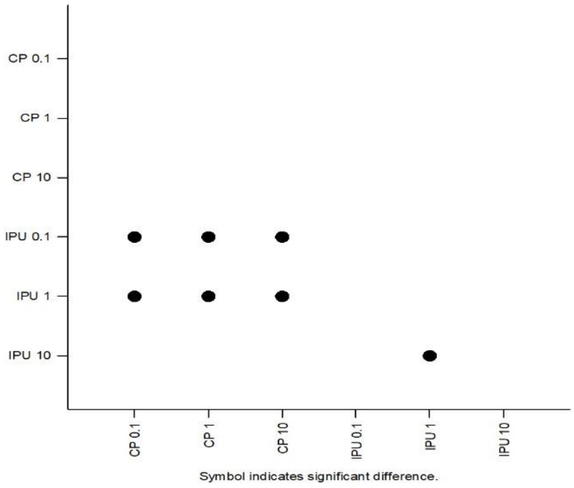

Figure 27: Multiple comparison plot for the toxicity of all MeOH-based pesticide solutions to *D. pulex*. The dots represent relationships with significant differences.

The toxicity of pesticides to *D. pulex* was reinvestigated using DMSO as the dilution solvent, and the percentage survival of daphnids in MeOH and DMSO is shown in Figure 28.

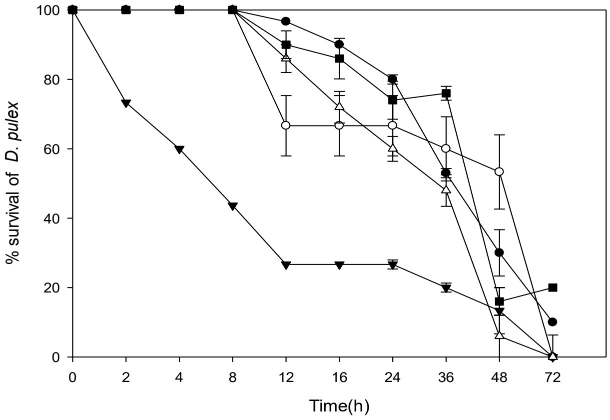

Figure 28: Comparison of *D. pulex* survival in HH Combo Medium, MeOH, and DMSO controls over 72h. MeOH solvent control (●), 0.1 mg $L^{-1}$ (○), 1 mg $L^{-1}$ (▼), and 10 mg $L^{-1}$ (Δ) pesticide solutions, error bars are the standard error of the mean, n=3.

The results obtained suggest that DMSO is less toxic to *D. pulex* than MeOH. Data obtained from this experiment were analysed statistically using the one-way repeated measures ANOVA. The data set comparing HH combo medium control with MeOH controls passed the Shapiro-Wilk normality test ($P = 0.256$) and the Brown-Forsythe equal-variance test ($P = 0.400$). The Bonferroni t-test indicates that, at the 95% confidence level, there is a significant difference ($P < 0.050$).

The data set comparing HH combo medium control with DMSO controls failed the Shapiro-Wilk normality test ($P < 0.050$), and the Friedman Repeated Measures Analysis of Variance on Rank test suggested a significant difference ($P = 0.009$). Tukey's test was conducted, and the result suggests that, at the 95% confidence level, there were no significant differences ($P < 0.050$). The box plot for this comparison is displayed in Figure 29. The data set comparing all the controls used in this experiment passed the Shapiro-Wilk normality test ($P = 0.590$) and the Brown-Forsythe equal variance test ($P = 0.232$). The Bonferroni t-test suggests that at 95% confidence level, there were significant differences ($P < 0.050$). The significant differences are shown in the multiple comparison plots (Figure 29).

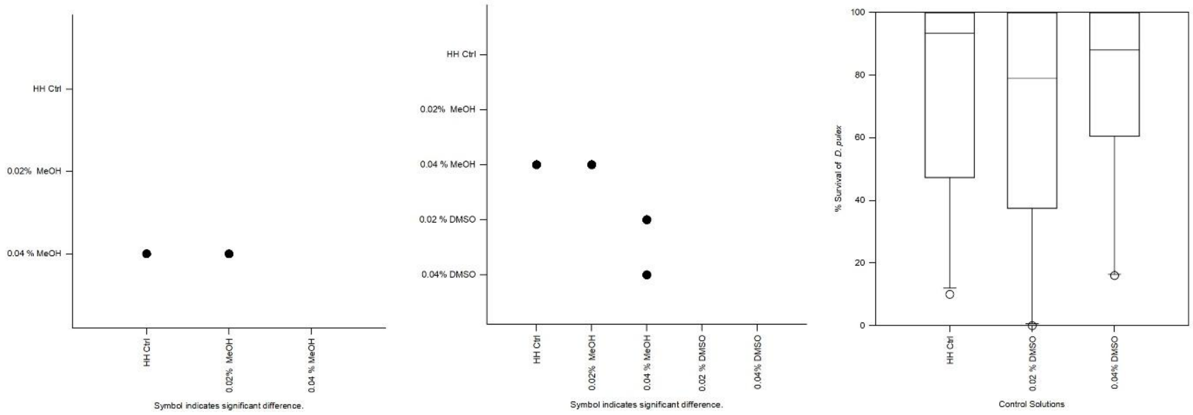

Figure 29: Multiple Comparison Plots (left and middle), and box plots (right) illustrating the significant differences and relationships between the control types used in toxicity tests on *D. pulex*. Black dots on comparison plots represent significant differences.

A comparison of methanol and dimethyl sulfoxide (DMSO) as dilution solvents for the pesticides cypermethrin (CP) and Isoproturon (IPU) indicated that the mortality percentage of *Daphnia* in pesticide-DMSO solutions was significantly lower ($p \leq 0.05$) than in pesticide-methanol solutions. This aligns with the reports by Marcin & Alaksander (2023), indicating no mortality of Daphnia in test solutions at a 1% DMSO dilution. However, Christou *et al.* (2020) reported minor behavioural changes without mortality at low DMSO concentrations, and Dave et al. (1981) reported significant toxicity to *D. magna* at various methanol concentrations. Both solvents generally aid in delivering hydrophobic substances like pesticides in water; however, methanol tends to increase the toxicity of these substances to *Daphnia*, while DMSO generally has a milder, less toxic effect unless used at higher concentrations of above 0.05 - 1% v/v (Hafner *et al.*, 2015), which was avoided in this study.

### 4.3.3. Acute Toxicity of Untreated Pesticide Solutions to *Daphnia* spp. in Dimethyl Sulfoxide (DMSO)

This study investigated the acute toxicity of Cypermethrin and Isoproturon to *Daphnia magna* over 72 h. The method employed is the same as in 3.2.1 above, except that the solvent MeOH is replaced with DMSO. Different concentrations of DMSO in the two groups of test solutions. The CP and IPU stock solutions contained 0.02% and 0.04% DMSO, respectively. During the experiment, three controls were used: water-only (HH Combo), 0.02% solvent, and 0.04% solvent. All test solutions were prepared in quintuples. Table 18 presents the physicochemical readings before and after the toxicity experiments conducted.

Table 18: Average Dissolved Oxygen (DO), pH, and temperature values for CP and IPU test solutions using DMSO as solvent. Values were recorded in triplicate at the beginning and end of the experiment.

| | | DO (mg $L^{-1}$) | | pH | | Temp. ($^{o}$ C) | |
|---|---|---|---|---|---|---|---|
| Concentration | Pesticide | 0 h | 72 h | 0 h | 72 h | 0 h | 72 h |
| Water control | CP | 8.90 | 7.33 | 7.61 | 7.89 | 20.20 | 22.20 |
| | IPU | 8.90 | 7.50 | 7.84 | 7.72 | 20.20 | 22.10 |
| DMSO control | CP | 8.20 | 7.67 | 7.38 | 7.95 | 20.90 | 22.80 |
| | IPU | 8.40 | 6.83 | 7.44 | 7.81 | 21.10 | 23.30 |
| 0.001 mg $L^{-1}$ | CP | 8.40 | 7.77 | 7.77 | 7.96 | 20.40 | 22.00 |
| | IPU | 8.50 | 8.30 | 7.73 | 7.93 | 20.50 | 22.20 |
| 0.010 mg $L^{-1}$ | CP | 8.30 | 7.17 | 7.73 | 7.96 | 20.30 | 22.50 |
| | IPU | 8.30 | 8.20 | 7.74 | 7.93 | 20.20 | 22.30 |
| 0.100 mg $L^{-1}$ | CP | 8.20 | 8.10 | 7.74 | 7.92 | 20.10 | 22.60 |
| | IPU | 8.30 | 8.20 | 7.70 | 7.90 | 20.30 | 22.80 |
| 1.000 mg $L^{-1}$ | CP | 8.20 | 7.93 | 7.75 | 7.94 | 20.40 | 22.70 |
| | IPU | 8.35 | 8.20 | 7.73 | 7.75 | 20.70 | 23.00 |
| 10.000 mg $L^{-1}$ | CP | 8.10 | 7.20 | 7.74 | 7.95 | 20.40 | 22.10 |
| | IPU | 8.45 | 8.30 | 7.77 | 7.86 | 20.70 | 22.00 |

The DO values obtained during this toxicity experiment with DMSO as solvent failed the normality test ($P < 0.050$). The Wilcoxon Signed Rank t-test was conducted, and there was a significant difference in DO values between the beginning and end of the toxicity experiment ($P < 0.001$). The pH values for this experiment were statistically analysed using a paired t-test. The data passed the normality test ($P = 0.116$), and the differences in pH at the beginning and end of this experiment were statistically significant ($p < 0.001$). The temperature values were compared using a paired t-test, and the data were normally distributed ($P = 0.721$). The temperature changes from the beginning to the end of this experiment were also significantly different ($p <0.001$).

### *4.3.4 Mortality of Daphnia spp. in Pesticide Solutions prepared with DMSO*

The mortality of Daphnia spp. (*pulex* and *magna*) in five concentrations of CP and IPU were recorded and expressed as percentages over 72h. Figure 30 suggests that the mortality rate observed across all controls was directly proportional to the amount of solvent in the control solution. *D. magna* displayed over 50% mortality after 60 h of exposure to control solutions, and *D. pulex* displayed a similar percentage of mortality after 36 h without feeding. In both scenarios, *D. pulex* showed higher mortality rates of up to 100% overall, whilst mortality for *D. magna* in controls peaked at 66%.

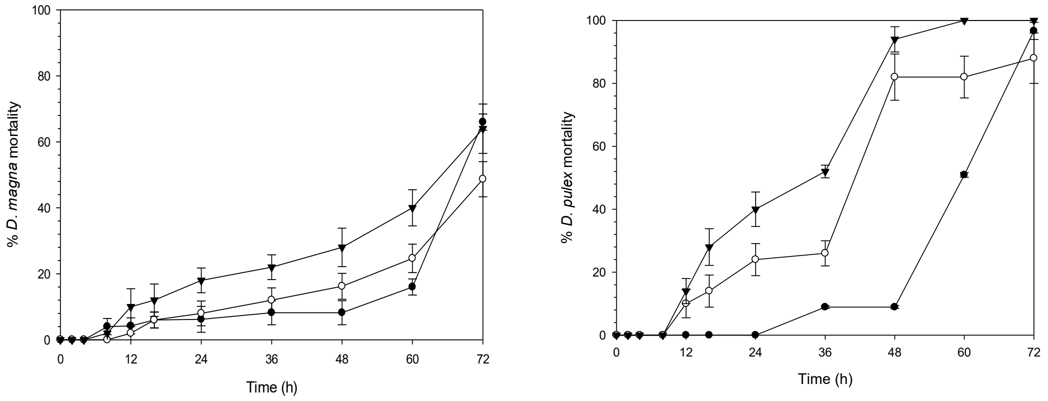

Figure 30: Percentage Mortality Rates of *D. magna* (left) vs. *D. pulex* (right), in HH combo medium (●), 0.02% DMSO control (○), and 0.04% DMSO control (▼) over 72 h. All values are averages of five samples ± standard error of the mean.

At the 95% confidence level, Figure 30 shows that 0.04% DMSO produced a higher mortality than the other negative controls. *D. pulex* and *D. magna* displayed a steep increase in percentage mortality after 36 h and 60 h. In conclusion, the mortality in all control solutions was less than 10% within 24 h, therefore satisfying the requirements of OECD 2004. Furthermore, there was a steep decrease in *D. pulex* activity in water and solvent controls after 48 h and 36 h, respectively. On the other hand, *D. magna* showed a steep decrease in activity after 60 h across all control groups.

Figure 31 and Table 19 detail the LD50 curves and values of the controls (culture media (HH Combo and solvent controls) on *D. magna* and *D. pulex*. Table 19 further suggests that *D. pulex* is more sensitive to DMSO controls, whereas *D. magna* exhibits delayed mortality progression across all control solutions.

Table 19: Comparative LD50 Values for *D. pulex* vs *D. magna* in all Control Solutions

| **Control** | **LD$_{50}$ *D. magna* (h)** | **LD$_{50}$ *D. pulex* (h)** |
|---|---|---|
| *HH Combo* | 68.47 | 62.88 |
| *0.02% DMSO* | 74.17 | 34.90 |
| *0.04% DMSO* | 64.37 | 26.98 |

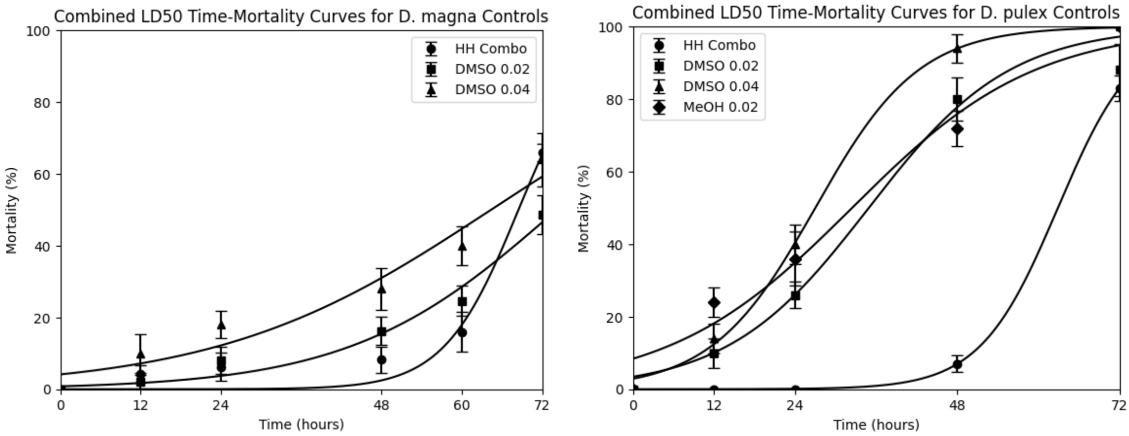

Figure 31: Comparison of the LD50 Plots of all the Control Solutions Used in this Study on *D. pulex and D. magna.*

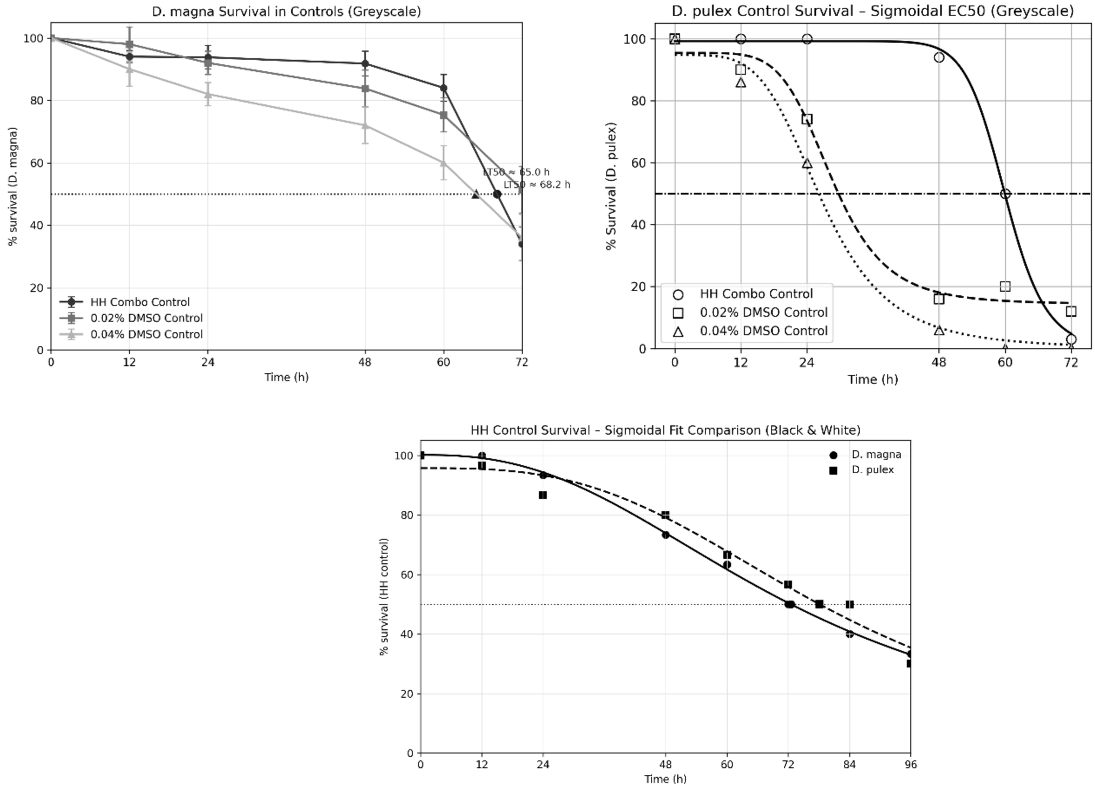

Figure 32: Compares the LD50 and EC50 Comparison Plots of all the Control Solutions Used in this Study on *D. pulex and D. magna*.

Figure 32 displays the mortality of *D. pulex* in pesticide solutions prepared with DMSO. This suggests that DMSO is more appropriate, as the percentage mortality observed in the controls and in the pesticide solutions is reduced. The figure further shows that EC50 was achieved in *D. magna* at approximately 69 h, 65 h, and 74 h in HH Combo, 0.02 % DMSO, and 0.04 % DMSO, respectively. On the other hand, EC50 was achieved at 59 h, 36 h, and 27 h in the HH Combo Control, 0.02 % DMSO control, and 0.04 % DMSO control, respectively.

In summary, the solvent DMSO clearly accelerates mortality in *D. pulex* relative to the HH combo control. The 0.04% DMSO treatment is the most toxic, reaching 50% survival fastest. HH Combo control shows much slower mortality, confirming the solvent effect. Furthermore, *D. pulex* declines faster than *D. magna* in the solvent controls. 0.04% DMSO is the most toxic solvent condition for both species. *D. pulex* is slightly more tolerant than *D. magna* under HH control conditions, and HH Combo control maintains the highest survival, confirming baseline conditions.

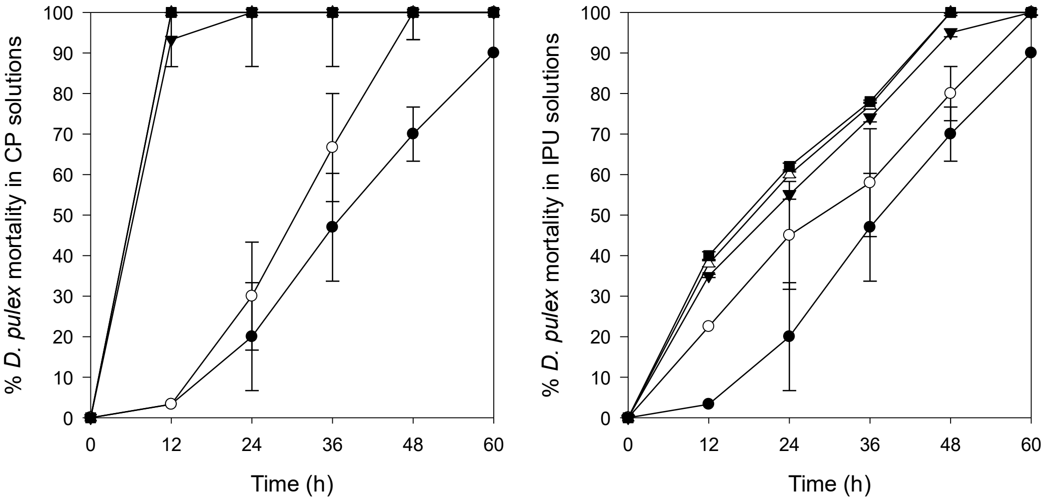

Figure 33: Percentage mortality of *D. pulex* in MeOH control (●), and pesticide solutions; 0.1 mg $L^{-1}$ (○), 1 mg $L^{-1}$ (▼), and 10 mg $L^{-1}$ (Δ).

Figure 33 illustrates that the percentage mortality of *D. magna* in 0.1, 1, and 10 mg $L^{-1}$ CP peaked around 36 h of exposure. In addition to the steep increase in mortality displayed in 0.01 mg $L^{-1}$ CP after 48 h, *D. magna* showed a gradual death response to the toxicity of 0.001, 0.0001, and 0.00001 mg $L^{-1}$ CP solutions. On the other hand, *D. magna* exhibited a similar mortality trend across all IPU solutions, with a steep increase after 60 h and a peak of 72% at 72 h.

The percentage mortality produced by all concentrations of CP in both Daphnia spp. showed a statistical difference ($p \leq 0.05$) in comparison with the control solutions except *D. magna* in 1 and 10 mg $L^{-1}$ IPU solutions within 72 h. Furthermore, the percentage mortality produced by CP and IPU in both daphnia species at all concentrations was significantly different except at 0.001 mg $L^{-1}$, where no significant difference was observed in both daphnia species ($p \leq 0.05$). In addition, observation data for both daphnia species at all pesticide concentrations showed a significantly higher percentage mortality of *D. pulex* in 1 and 10 mg $L^{-1}$ CP solutions ($p \leq 0.05$).

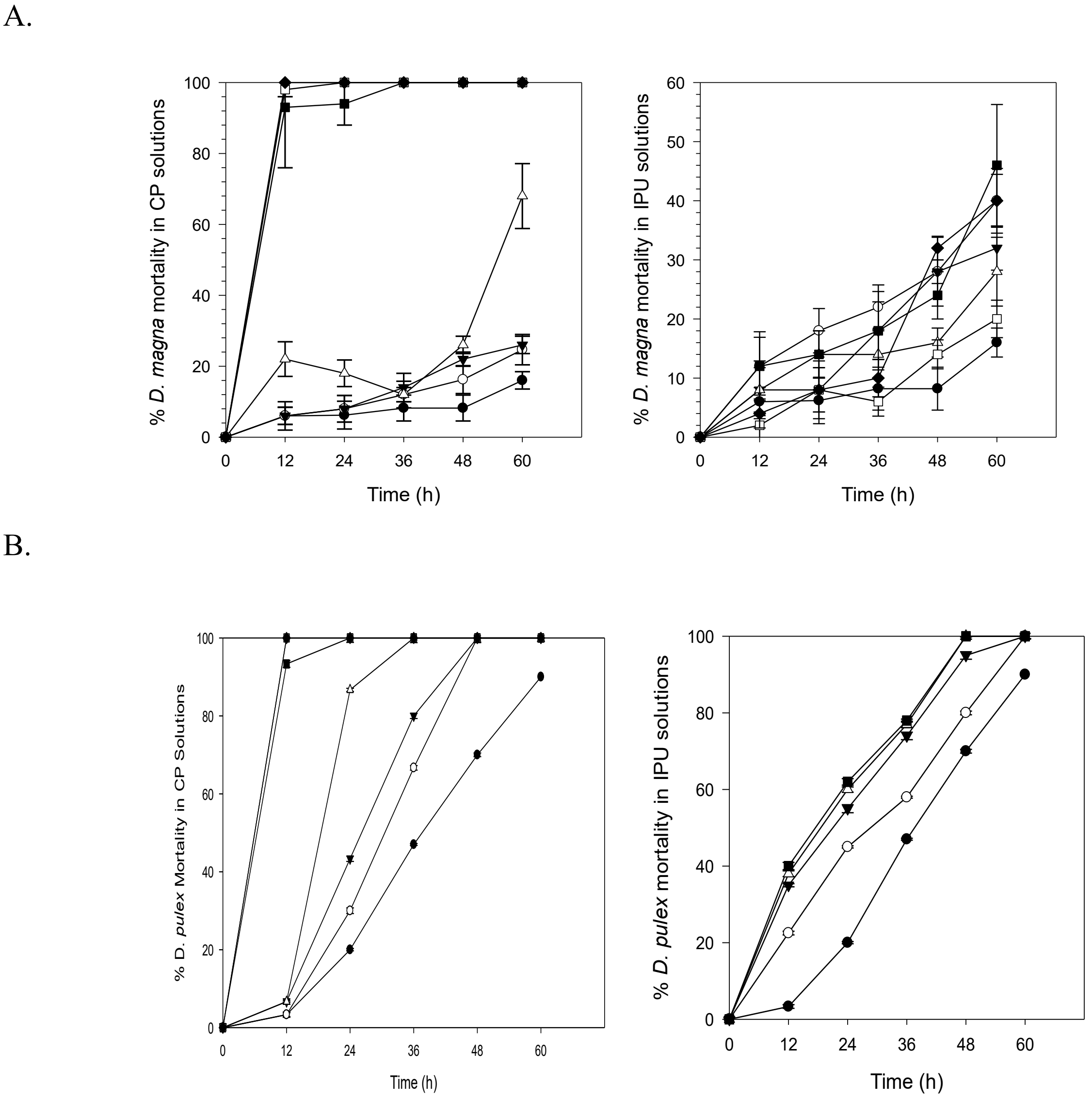

Figure 34: Percentage mortality of *D. magna* (A) and *D. pulex* (B) in HH Combo control (●), DMSO controls (○), and pesticide solutions: 0.001 mg $L^{-1}$ (▼), 0.01 mg $L^{-1}$ (Δ), 0.1 mg $L^{-1}$ (■), 1 mg $L^{-1}$ (□), 10 mg $L^{-1}$ (♦) pesticide solutions; CP (left) and IPU (right). All values are averages of five samples ± standard error of the mean.

*D. magna* showed similar activity trends in the highest and lowest three CP concentrations. However, there was a steep decrease in activity after 48 h of exposure to 0.01 mg $L^{-1}$ CP. Activity rates of *D. magna* in IPU solutions followed a similar trend. A steep decrease in *D. pulex* activity in water and solvent controls was observed after 48 h and 36 h, respectively. On the other hand, *D. magna* showed a steep decrease in activity after 60 h across all control groups.

### 4.3.5 *Effective Concentrations of Cypermethrin and Isoproturon*

The results for the toxic responses of both *Daphnia* spp. were recorded as percentage activity during toxic exposure (i.e., combination of viable and moribund daphnids). Other factors, such as the percentage of viable, moribund, and mortal daphnids, were measured and recorded at each time point over 72 h. The percentage response (combination of dead and moribund daphnids), the "No Observable Effects Concentration" (NOEC), Effective Concentration (EC50), and Lethal Dose (LD50) of all pesticide solutions were determined. Finally, a complete physical and statistical analysis of all results and plots was obtained, interpreted, and briefly summarized. Figure 33 displays the log-dose and linear regression plots of *D. magna* and *D. pulex* in pesticide solutions.

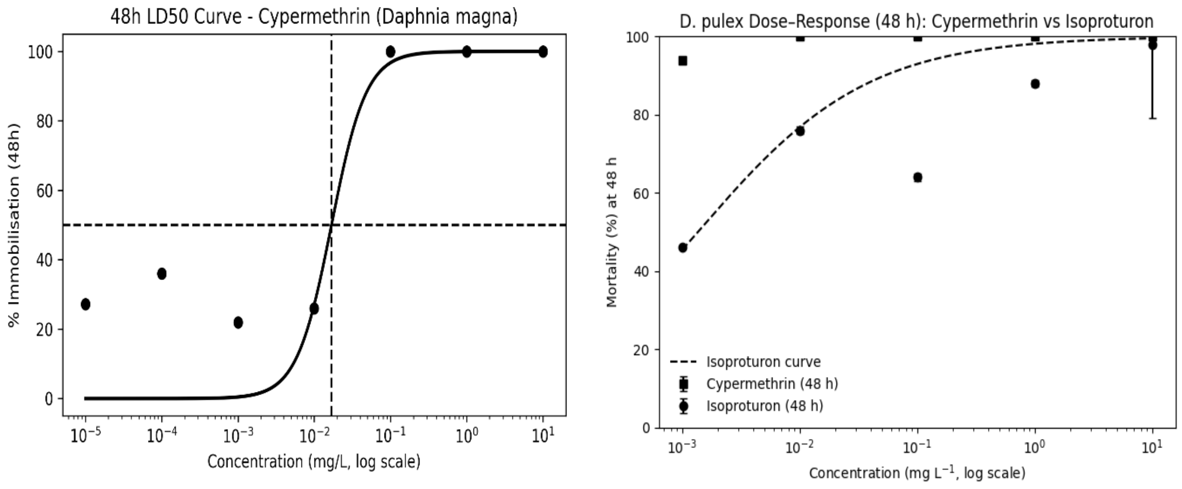

Figure 35: 48-h Log Dose-Response Curve for Daphnia *spp.* in untreated Cypermethrin (CP) and Isoproturon (IPU) Solutions. All percent mortality values are means of quintuple observations at each time point.

Figure 35 displays the relationships between the pesticide doses and the percentage mortality of daphnids. The 48 h LD50 of *D. magna* in CP is approximately 0.165 mg $L^{-1}$, which is 16.85 µg $L^{-1}$. Isoproturon had no acute effect within the concentration range tested, so the results could not be fitted to an LD50 curve. This means that the LD50 of Isoproturon was >10mg $L^{-1}$. On the other hand, the 48-h LD50 for *D. pulex* in CP was <0.001 mg $L^{-1}$ (boundary statement; 50% mortality could not be observed in the plot (right) because the lowest test concentration already produced

94% mortality). However, the $LD_{50}$ of *D. pulex* in IPU was approximately 0.00136 mg $L^{-1}$, obtained by log-linear interpolation between 0.001 and 0.01 mg $L^{-1}$.

Table 20 illustrates the NOEC, EC50, LC50, 100% response, and mortality exhibited by both *Daphnia* spp. in both pesticide test solutions over 72 h without feeding. According to OECD (2004), the recommended EC50 calculation is based on 48 h of exposure, with a 24 h determination optional. However, the result of this experiment shows that at 48 h, all daphnids are immobilized.

Table 20: Controls tested Showing *Daphnia* spp., the duration (h) that produced total response and mortality, and the No Observable Effects Concentration (NOEC) over 72 h.

| | | *Time (h)* | | |
|---|---|---|---|---|
| *Control* | *Daphnia* spp. | NOEC | 100% response | 100% mortality |
| *HH Combo Medium* | *Magna* | 24 | n/a | n/a |
| | *Pulex* | 8 | n/a | n/a |
| *0.02% DMSO* | *Magna* | 8 | n/a | n/a |
| | *Pulex* | 8 | 72 | n/a |
| *0.04% DMSO* | *Magna* | 4 | n/a | n/a |
| | *Pulex* | 4 | 48 | 60 |

* n/a *signifies that the mortality or response was less than 100%, where applicable*

Table 20 suggests that there was no tested concentration of CP and IPU at which daphnid behaviour, with respect to mortality and weakness, was the same as in the control solutions. The average concentration/ timeframe for NOEC exhibited by Daphnia spp. in all CP solutions was 0.001 mg $L^{-1}$ at 2 h, but the duration was 4 h in 0.001 and 1 mg $L^{-1}$ IPU *for D. pulex and D. magna,* respectively. It further confirms that *Daphnia* spp. survived in the controls beyond 72 h without feeding, mortality was not up to 100% except for *D. pulex* in both DMSO control solutions. In addition, the percentage mortality of *Daphnia* spp. Across all control solutions, there was no significant difference, except in the 0.02% DMSO control.

The mortality rates caused by both DMSO controls across the two Daphnia species differed statistically ($p \leq 0.05$). The EC50 for all controls was reached at the same time for both species, except for the 0.04% DMSO control, where *D. pulex* reached EC50 earlier, within 24 hours. The LC50 was achieved sooner in *D. pulex* than in *D. magna*, especially in 0.04% DMSO solutions, as expected. *D. magna* reached LC50 after 72 hours, which explains the 'n/a' value. Only *D. pulex* achieved 100% mortality in the 0.04% DMSO solution, unlike in other control solutions and in *D.*

*magna* across all controls. *D. magna* did not reach 100% mortality in any control solution, while *D. pulex* did in 0.02% and 0.04% DMSO after 72 and 48 hours, respectively. The 48-hour EC50 of CP was consistent with values reported by Kim et al. (2007) and Lakota et al. (1989). Overall, these toxicity results suggest that Cypermethrin is more toxic to *D. pulex* than IPU.

### *4.3.5 Survival of Daphnids in Pesticide Solutions*

As established earlier, the survival of daphnids was recorded at all time points in all experiments. However, survival included both viable and moribund daphnids. Figure 36 shows the percentage survival of daphnids across all pesticide concentrations over 72 h.

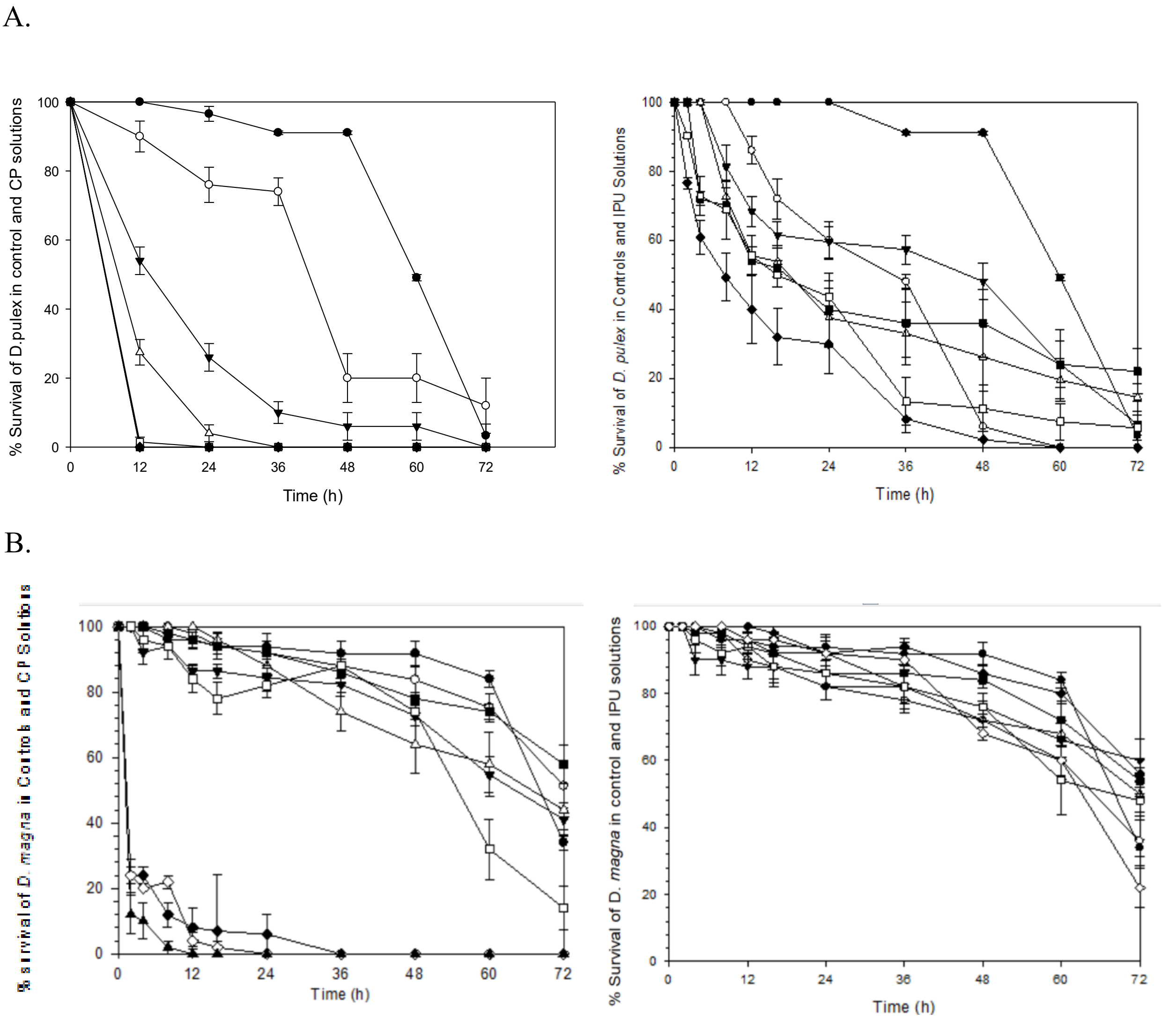

Figure 36: Percentage Survival of *D. pulex* (A) and *D. magna* (B) in Water and Solvent Controls, and Five concentrations: 0.001 mg $L^{-1}$ (●), 0.01 mg $L^{-1}$ (○), 1 mg $L^{-1}$ (▼), (Δ), 0.1 mg $L^{-1,}$ and 10 mg $L^{-1}$ (■) of CP and IPU pesticide solutions over 60 h. n = 3 ± standard error of the mean.

The activity of *D. pulex* in all CP solutions had reached a steady state, but activity in all IPU solutions seemed to be in a kinetic stage at 72 h. Figure 34 suggests that after 36 h, the average mortality of daphnids exceeded 20%, whilst the average activity rate fell below 80% after 48 h. Statistical analysis of data on the survival of *D. magna* across all control and all CP solutions showed that the data were normally distributed.

However, the data for *D magna* survival in all IPU solutions, all controls, and pesticide solutions failed the normality test. The statistical difference in the percentage survival of *D. magna* and *D. pulex* across all control and pesticide solutions was analysed; only their behaviour in water and 0.02% DMSO differed significantly. Figure 37 displays box plots of *D. magna* survival across all pesticide solutions.

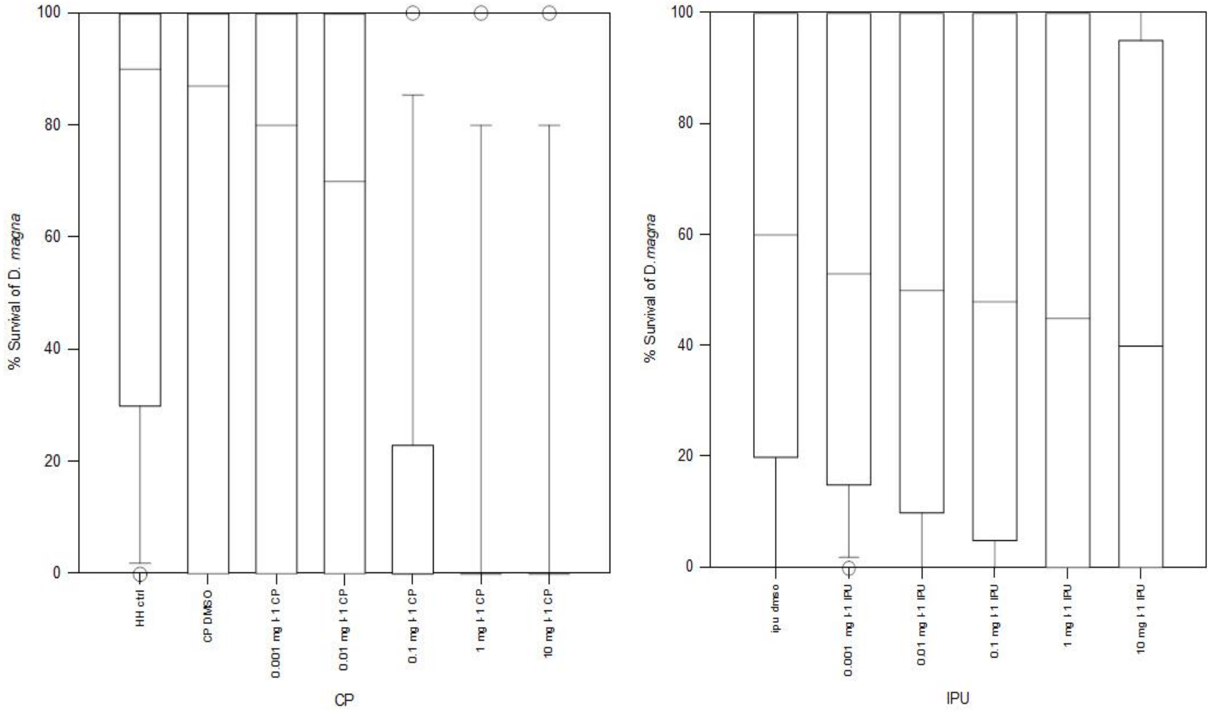

Figure 37: Box plots of the survival (%) of *D. magna* in CP (left) and IPU (right) solutions over 72 h.

It is noteworthy that, at the 95% confidence level, the survival percentages of both Daphnia spp. Across all control solutions, there were no significant differences. The percentage survival of *D. pulex* in all CP solutions failed the normality test ($p < 0.050$) and showed significant differences only between the toxicity of the highest three concentrations (0.1, 1, and 10 mg $L^{-1}$) and the dilution water (HH Combo media) when analysed with ANOVA on ranks. However, in IPU solutions, the survival percentage data of *D. pulex* passed the normality test ($p = 0.245$), failed the Brown-Forsythe equal variance test ($p < 0.050$) and only showed significant differences in the comparison of the toxicity of the highest (10 mg $L^{-1}$) and the lowest concentration (0.001 mg $L^{-1}$) and the solvent (DMSO) control.

On the other hand, the percentage survival of *D. magna* in all CP solutions passed the normality test ($p < 0.050$). Further ANOVA on ranks revealed that only the highest two concentrations (1 and 10 mg $L^{-1}$) showed significantly different toxicity compared with all other concentrations of CP and the control solution. However, in IPU solutions, the survival percentage data of *D. magna* failed the normality test ($p = 0.245$), failed the Brown-Forsythe equal variance test ($p < 0.050$), and only showed significant differences in the comparison of the toxicity of the highest (10 mg $L^{-1}$) against every other concentration of IPU and all control solutions.

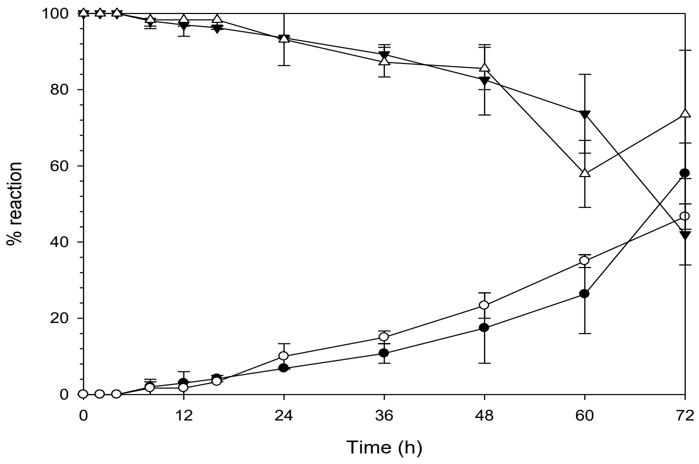

Figure 38: Average mortality and activity rates of *Daphnia* spp. Observed for 72h in three different Toxicity Experiments. Where activity accounts for viable and moribund daphnid neonates, and all values are averages of five samples ± standard error of the mean.

### 4.2.2.4 *Acute Display of Weakness by Daphnids in Pesticide Solutions*

This behaviour in daphnids was observed during all bioassay experiments and recorded at each time point. Notably, the moribund factor is a subset of the percentage survival of daphnids, accounting for both viable and moribund organisms. Most graphs displaying the percentage of moribund daphnids in this experiment followed a parabolic shape, as seen in Figure 39.

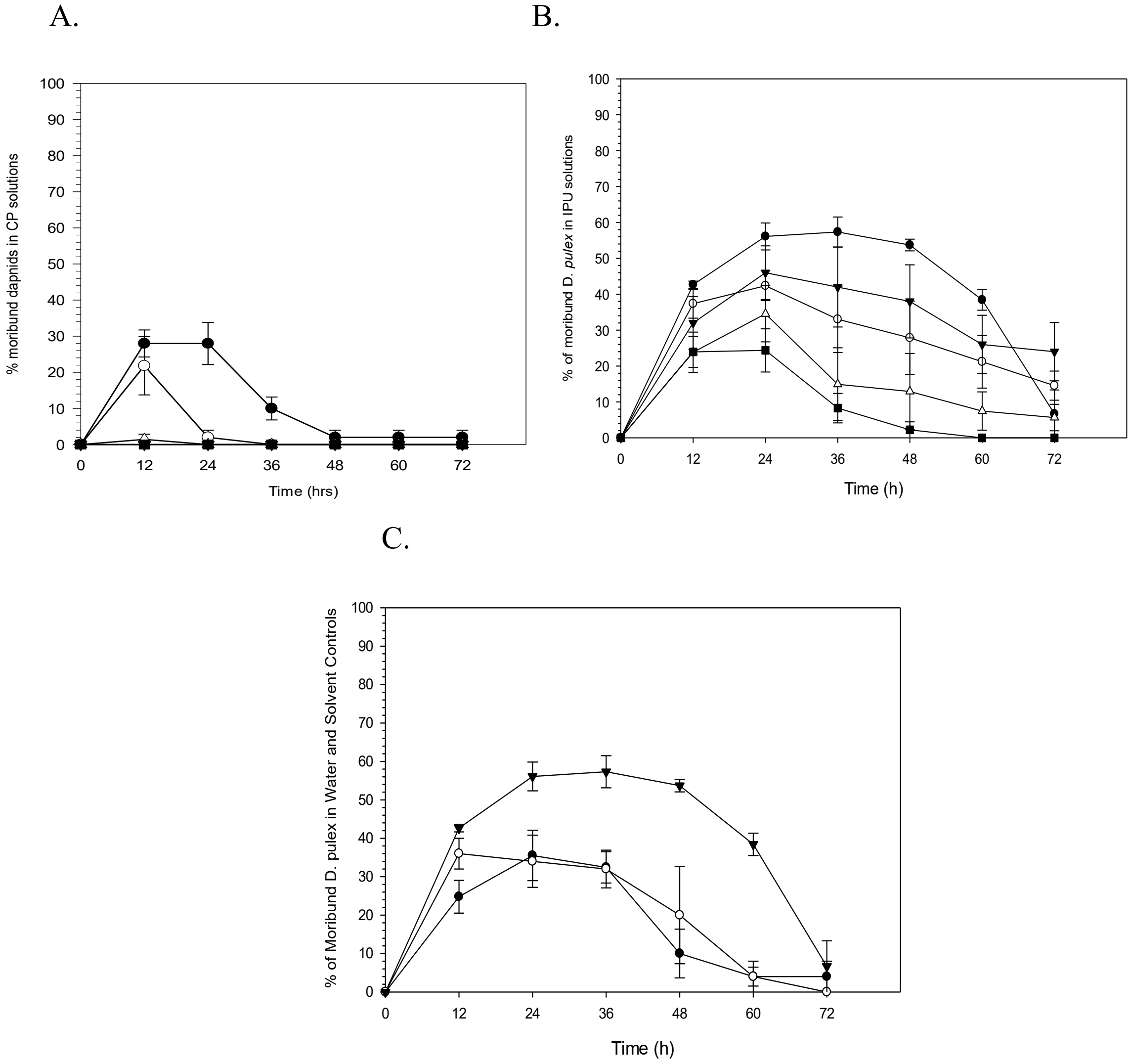

Figure 39: The percentage of moribund *D. pulex* in five concentrations of cypermethrin (A), Isoproturon (B), and all control solutions (C); water, 0.02%, and 0.04% DMSO Controls over 72 h. HH Combo control (●), DMSO controls (○), and pesticide solutions: 0.001 mg $L^{-1}$ (▼), 0.01 mg $L^{-1}$ (Δ), 0.1 mg $L^{-1}$ (■), 1 mg $L^{-1}$ (□), 10 mg $L^{-1}$ (♦) pesticide solutions; CP (left) and IPU (right). All values are means of quintuple observations at each time point. Error bars are standard errors of the mean.

At 95% confidence level, assuming

a) H0: that the mean mortality and responses of *D. magna* and *D. pulex* neonates to pesticide solutions are equal, and that,

b) Ha: that the mean mortality and responses of *D. magna* and *D. pulex* neonates to pesticide solutions are unequal

Paired t-tests were conducted in Sigmaplot on the mean results obtained from the acute toxicity experiments. Paired t-tests were used to examine the effects of species type and concentration on the percentage mortality and activity rates of Daphnia in pesticide solutions. T-tests comparing the mean percentage mortality of both *Daphnia* spp in HH Combo medium, 0.02% DMSO, and 0.04% DMSO control solutions. The results showed that, at $p \leq 0.05$, all mean percentage mortalities were significantly different only between the DMSO control solutions and between the HH Combo medium and the highest solvent concentration (0.04%).

The mean percentage mortality of both *Daphnia* spp. at each pesticide concentration at all observed time points, and the results showed that at $p \leq 0.05$, all mean percentage mortalities were significantly different for both species except at 0.001 mg $L^{-1}$. Also, the mean percentage mortality and activity of *Daphnia* spp. in both pesticide solutions at each concentration for both species showed no significant differences at $p \leq 0.05$, except for *D. pulex* mortality in 1 and 10 mg $L^{-1}$ CP solutions.

The mean percentage mortality of both daphnia species in HH Combo medium and solvent controls at the different concentrations of pesticide solutions and all timepoints. The results obtained suggest that at 0.001 mg $L^{-1}$, all mortality rates in pesticide solutions were significantly different from the mortality rates in water and solvent controls, except the mortality rates of *D. magna* vs. solvent and water controls, the mortality rates of *D. magna* in IPU *vs.* HH Combo medium, and the mortality rates of *D. pulex* in IPU compared with mortality in solvent control.

At 0.01 mg $L^{-1}$, all *Daphnia* spp. mortality rates in pesticide solutions were significantly different from those in water and solvent controls, except for *D. pulex* in IPU, which was compared with the solvent control. At 0.1 mg $L^{-1}$, all *Daphnia* spp. mortality rates in pesticide solutions were significantly different from those in water and solvent controls, except for *D. pulex* in the IPU vs. solvent control. At 1 mg $L^{-1}$, all the *Daphnia* spp. mortality rates in pesticide solutions were significantly different from those in water and solvent controls, except for *D. magna* in IPU, where its mortality was significantly lower than in the HH combo medium. At 10 mg $L^{-1}$, all *Daphnia* spp. mortality rates in pesticide solutions were significantly different from those in water and solvent controls, except for *D. magna* in IPU, where mortality was similar to that in water and solvent controls.

The results above suggest that the pesticide concentrations that produced significantly different activities are 0.001 mg $L^{-1}$, 0.01 mg $L^{-1}$, 1 mg $L^{-1,}$ and 10 mg $L^{-1}$. Additionally, during the comparison of mortality and total responses, both data trends became similar for pesticide concentrations from 1 to 10 mg $L^{-1}$ for CP and IPU, because their toxic effects on Daphnia spp.

were greater and more lethal. Overall, this study suggests that *D. magna* exhibited immediate response to the toxicity of pesticides, but were more resistant and produced lower mortality compared to *D. pulex*, which showed resistance to the toxicity of pesticides at the initial hours but rapidly died off later in the experiment

The exposure of Daphnia *magna* and Daphnia *pulex* to HH combo medium control (dilution water), solvent controls for CP and IPU (0.02% and 0.04% DMSO) respectively, five concentrations each of CP and IPU (0.001, 0.01, 0.1, 1 and 10 mg $L^{-1}$ ) over 72 h without feeding suggest that there was no concentration of pesticides solutions tested, that produced no observable effect on the daphnids (no NOEC), this correlates with reports of 0.01 µg $L^{-1}$ NOEC by Yordanova (1997). Even the control solutions and the lowest pesticide concentration tested (0.001 mg $L^{-1}$) produced average toxic responses in *D. pulex* and *D. magna* at 2 and 4 h, respectively.

This is because the concentrations studied ranged from 100 times below to 100 times above the World Health Organization (WHO) maximum permissible limits for these pesticides in water, mimicking a spill scenario or extreme pollution in a developing country with poor monitoring infrastructure. The average NOEC for *D. magna* and *D. pulex* across all control and pesticide solutions is 8 h and 2 h, respectively. The average NOEC time points suggest that the absence of food might have contributed to the daphnids' low tolerance to the controls' toxicity and, in turn, to their toxicity in the pesticide solutions.

Both species of *Daphnia* survived in the controls beyond 72 h without feeding; the percentage mortality did not exceed 100% except for *D. pulex* in both DMSO control solutions. The highest solvent control concentration (0.04% DMSO) produced significantly higher percentage mortality in both *Daphnia* species ($p \leq 0.05$), as expected, and the percentage mortality and response of all daphnids in all pesticide concentrations were significantly different from their mortality and response in their corresponding control solutions ($p \leq 0.05$).

This can be attributed to the inherent mild toxicity of DMSO and its synergistic combined toxicity with the pesticides in solution. *D. magna* achieved LC50 later than 72 h and did not show 100% toxic response in any of the control solutions, while *D. pulex* exhibited 100% response to 0.02% and 0.04% DMSO after 72 h and 48 h, respectively. The percentage mortality displayed in all controls was directly proportional to the quantity of solvent in the control solution. At 95% confidence level, 0.04% DMSO produced a higher mortality than other negative controls.

Furthermore, *D. magna* displayed over 50% mortality after 60 h of exposure to control solutions, and *D. pulex* displayed a similar percentage of mortality. This is lower than the reports by Mungi et al., which indicate that 1 µg $L^{-1}$ CP produced 100% mortality immediately after application.

The 24 and 48 h LC50 of CP to *D. magna* were approximately 40 µg $L^{-1}$ and 30 µg $L^{-1}$, respectively. Previous studies with *D. magna* reported lower 24 h LC50 values of 1 and 2 µg $L^{-1}$ by Lutnicka *et al.* (2014) and Yordanova et al. (2009), respectively. Similarly, lower 48 h LC50 values ranging between 0.2 - 0.7 µg $L^{-1}$ have been reported (Houssou *et al.*, 2017; Kim *et al.*, 2008; Mokry & Hoagland, 1990; Lakota *et al.*, 1989). However, a higher 48 h LC50 of 150 µg $L^{-1}$, comparable to the results of this study, was reported for CP on *D. magna* by the pesticide manufacturer Wefco-Africa (2024).

On the other hand, the 24 h and 48 h LC50 for CP on *D. pulex* were 0.675 µg $L^{-1}$ and 0.525 µg $L^{-1}$, respectively. There is limited research data to confirm its similarity or difference from previous research. On the other hand, for IPU, the 24 and 48 h LC50 on *D. pulex* were 4 µg $L^{-1}$ and 0.85 µg $L^{-1}$, respectively, and *D. magna* had no LC50 or EC50 values because it did not exhibit up to 50% mortality in any IPU solution over 72 h. These results are comparable to reports of 80% mortality of *Ceriodaphnia silvestrii* at 3.5 µg $L^{-1}$ and 100% mortality across all pesticide solutions containing Vinasse by Silva *et al.* (2021).

Additionally, the percentage mortality produced by CP and IPU in both *Daphnia* spp. at all concentrations were significantly different except at 0.001 mg $L^{-1}$ ($p \leq 0.05$). In addition, both *Daphnia* species showed a considerably higher mortality rate for *D. pulex* in CP solutions ($p \leq 0.05$), possibly due to CP toxicity. *D. magna* showed similar activity trends across the highest and lowest three CP concentrations. However, there was a steep decrease in activity after 48 h of exposure to 0.01 mg $l^{-1}$ CP. The activity percentage of *D. magna* in IPU solutions followed a similar trend. The moribund organisms were a subset of the surviving daphnids, which accounted for (viable + moribund organisms). It is noteworthy that all plots showing the percentage of moribund daphnids in pesticide solutions were parabolic. This can be explained by the increasing death of the daphnids at later timepoints.

Furthermore, the percentage mortality and total response of both Daphnia species to the highest two concentrations (1 and 10 mg $L^{-1}$) of both pesticides tested were not significantly different, likely due to the pesticides' very high toxicity, resulting in lethality. Overall, this study suggests that *D.*

*magna* exhibited immediate response (were moribund) to the toxicity of pesticides but was able to acclimatize over time and exhibited lower mortality compared to *D. pulex*, which showed resistance to the toxicity of pesticides (low response) at the initial hours but rapidly died off later in the experiment. In conclusion, the 0.04% DMSO control solution was more toxic to *Daphnia* than expected, because although DMSO has milder toxic effects on Daphnia, the stress it induced was directly proportional to the amount of DMSO used (Haap *et al.*, 2008). *D. magna* is more robust and has shown the ability to withstand the toxic effects of water pollutants over a longer period than *D. pulex* in all tested pesticide concentrations and control solutions.

This study suggests that CP is more toxic to *Daphnia* than IPU despite the higher solvent concentration in IPU. Previous research suggests that the different modes of action of both pesticides could be responsible for this observation. CP is classified as a highly neurotoxic insecticide that binds to sodium channels in the nervous systems of aquatic insects, leading to nerve overstimulation, paralysis, and death in extreme cases (Munoz et al., 2010). *Daphnia* are highly vulnerable due to their permeable exoskeleton (Chandler *et al.*, 2004). On the other hand, IPU is a herbicide that inhibits photosynthesis by binding to the D1 protein in Photosystem II, thereby increasing the accumulation of reactive oxygen species (ROS) and further intensifying cellular damage (El Amrani *et al.*, 2012). Studies by Cedergreen & Rasmussen (2017) and Schafer *et al.* (2011) further suggest that macroinvertebrates are among the most sensitive organisms to insecticides, whereas herbicides target primary producers, such as macrophytes, that perform photosynthesis (Sanchez-Bayo, 2011). This explains the high toxicity of CP compared to IPU.

### 4.3.3 Acute Toxicity of Biochar-Treated Aqueous Pesticide Solutions on *D. pulex.*

Due to the scarcity of *D. magna*, all studies involving the pre-treatment of pesticide and metal solutions with rice husk (RH) biochar and granular activated carbon (GAC) were continued with only *D. pulex*. Figure 38 illustrates that the mortality of *D. pulex* exposure to all CP solutions peaked after about 36 h, and the mortality trends of water and solvent control exposures were similar. On the other hand, the mortality trend of *D. pulex* in all IPU solutions followed a similar path and was lower than CP mortality overall.

Repeated-measures ANOVA was used to analyse these data. At a 95% confidence level, all percentage mortality data for *D. pulex* in untreated and GAC-treated CP solutions were normally distributed and passed the equal variance test. However, there were significant differences in the mortality of *D. pulex* between the untreated 0.1, 1, and 10 mg $L^{-1}$ CP solutions and the 0.001, 0.01, and 10 mg $L^{-1}$ CP solutions treated with GAC.

A multiple comparison test was employed to confirm this. At the 95% confidence level, there were no significant differences in the mortality percentage of *D. pulex* between untreated and GAC-treated IPU solutions. Furthermore, among all solutions used in this study, only the 1 and 10 mg $L^{-1}$ CP solutions showed significant differences relative to all control solutions, as confirmed by the Tukey test.

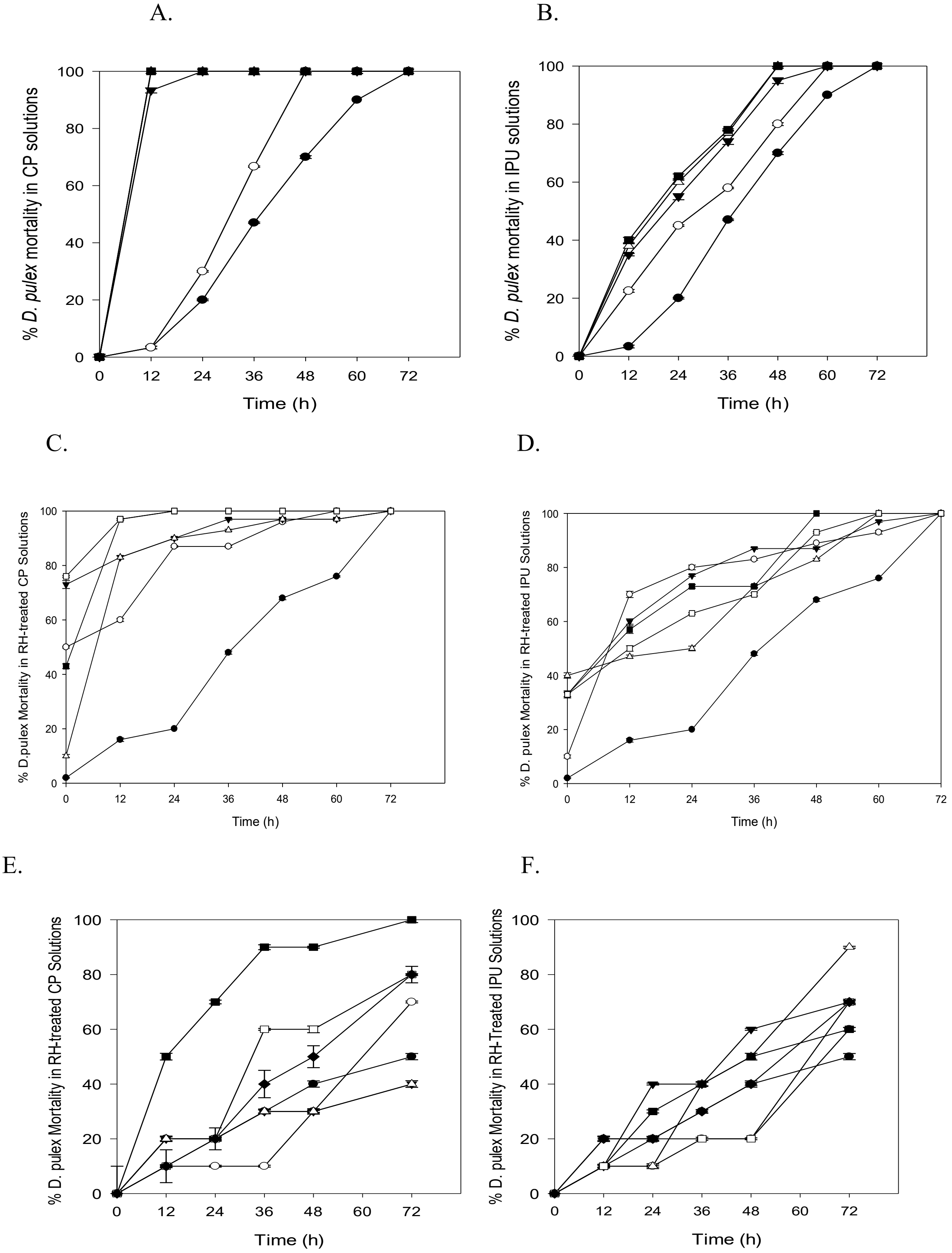

Figure 40: Percentage Mortality of *D. pulex* in different concentrations of (A and B) untreated, (C and D) RH-treated, (E and F) GAC-treated CP (left) and IPU (right) solutions, respectively, over 60 -72 h. HH Combo control (●), DMSO controls (○), and pesticide solutions: 0.001 mg $L^{-1}$ (▼), 0.01 mg $L^{-1}$ (Δ), 0.1 mg $L^{-1}$ (■), 1 mg $L^{-1}$ (□), 10 mg $L^{-1}$ (♦) pesticide solutions; CP (left) and IPU (right). All values are averages of five samples ± standard error of the mean.

At a 95% confidence level, all percentage mortality data for *D. pulex* in untreated and GAC-treated CP solutions followed a similar trend. However, there were significant differences in the percentage mortality of *D. pulex* between the untreated 0.1, 1, and 10 mg $L^{-1}$ CP solutions and the 0.001, 0.01, and 10 mg $L^{-1}$ CP solutions treated with GAC. There were no significant differences in the percentage mortality of *D. pulex* between untreated and GAC-treated IPU solutions, but a marked reduction in mortality in the GAC-treated solutions. Furthermore, when comparing all solutions used in this study at the 95% confidence level, only the 1 and 10 mg $L^{-1}$ CP solutions showed significantly higher percentage mortality than all control solutions, as confirmed by the Tukey test. This, as previously mentioned, can be attributed to the higher toxicity of CP than of IPU and to the higher CP concentration.

This study recorded a high mortality percentage of *D. pulex* in RH-treated pesticide solutions, especially in CP. Research suggests that adding biochar to soil or water alters its chemistry. In the case of water, biochar addition can lead to the release of metals and other intrinsic pollutants, increased turbidity, pH changes, and reduced dissolved oxygen (Lima *et al.*, 2022; Shanmuganathan *et al.*, 2023). Sanchez-Bayo (2011) further posited that the addition of herbicides and the decomposition of dead aquatic plants lower dissolved oxygen (DO) concentrations and pH, which stress Daphnia and lead to physiological and metabolic changes. This agrees with and can explain the findings of this study. The health indices study further consolidates the earlier finding that *D. pulex* is more severely affected by pesticides than *D. magna*, especially by CP. Lima *et al.* (2022) reported similar findings in their review of the sensitivity of different bioindicators to Atrazine, that *D. pulex* is more sensitive (the second most sensitive bioindicator) than *D. magna*. Furthermore, CP generally impacts the health condition of *Daphnia* spp. negatively than IPU. This further confirms the earlier results presented in this study and is significant in the early detection of environmental stress (Scilliano *et al.*, 2015) and insights into the behavioural and physiological adaptations of *Daphnia* in polluted aquatic ecosystems (Stinson *et al.*, 2023).

### 4.3.4 Health Index of *Daphnia* spp. in Untreated and Treated Pesticide Solutions

This section briefly illustrates the health indices of *D. magna* and *D. pulex* in untreated pesticide solutions, then compares the health indices of *D. pulex* in RH- and GAC-treated pesticide solutions.

Figure 41 briefly summarizes the health condition of *Daphnia* spp. across five pesticide concentrations at each time point over 72 h.

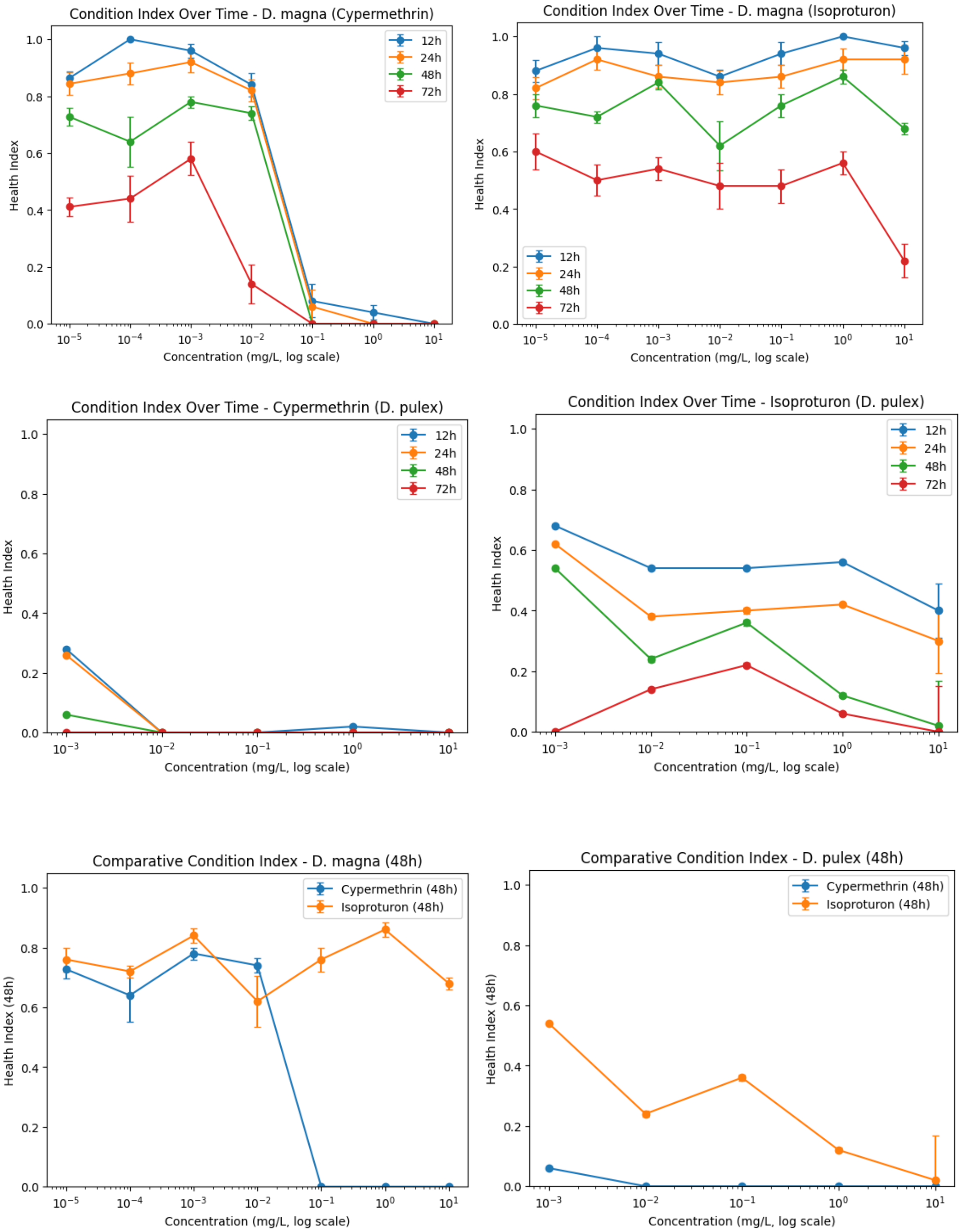

Figure 41: Health Indices of *D. magna* and *D. pulex* in CP and IPU pesticide solutions: 0.001 mg L⁻¹, 0.01 mg L⁻¹, 0.1 mg L⁻¹, 1 mg L⁻¹, 10 mg L⁻¹ solutions over 72 h

The plots above suggest that *D. pulex* is more severely impacted by pesticides than *D. magna,* especially CP. Furthermore, CP generally impacts the health condition of Daphnia spp. negatively than IPU. This further confirms the earlier results presented in this study.

A careful examination of the pesticide ecotoxicity result plots indicates that CP was generally more toxic than IPU. None of the metal concentrations tested produced an observable effect on the daphnids (no NOEC), including the control media. This is because even the control media used yielded total responses of 20% and 30% in D. magna and D. pulex, respectively, at 24 h. Both species produced 40% responses to control media at 48 hrs of exposure. On the other hand, mortality results for *D. magna* were approximately 7% and 27% at 24 h and 48 h, respectively; for *D. pulex*, 13% and 20% at 24 h and 48 h, respectively.

Furthermore, during the comparison of mortality and total responses, both data trends became similar for pesticide concentrations from 1 to 10 mg $L^{-1}$ because their toxic effects on *Daphnia* spp. were greater and more. The health indices of both *Daphnia* spp. were analysed using a repeated-measures ANOVA, and at the 95% confidence level, D. magna showed a significant difference in health when exposed to 0.001 mg $L^{-1}$ CP compared with all other CP concentrations studied. Across all IPU solutions, there were no significant differences in health. A statistical comparison of all health indices data for *D. magna* in both pesticide solutions still showed significant differences at only 0.001 mg $L^{-1}$ CP.

In conclusion, *D. magna* is more robust and has shown the ability to withstand the effects of toxicity over a longer period than *D. pulex* in all tested pesticide concentrations and solutions.

## 4.4 Conclusions

This chapter demonstrated that acute pesticide toxicity in aqueous systems is strongly influenced not only by the active ingredients of the pesticides, but also by formulation and experimental factors such as solvent choice, exposure duration, and species sensitivity. Across the preliminary and main bioassays, cypermethrin consistently produced greater adverse effects than Isoproturon, reflected in higher mortality, faster onset of immobilisation, and poorer health index outcomes, particularly for *Daphnia pulex*. In contrast, Isoproturon exhibited comparatively limited acute toxicity over the concentration range tested and, in some cases, did not reach LC50/EC50 thresholds for *D. magna* during the exposure period, reinforcing the pesticide-specific and mode-of-action-dependent nature of the observed responses.

A key methodological finding was the importance of solvent selection for reliable ecotoxicity assessment. Methanol increased apparent toxicity relative to DMSO, and even low concentrations of DMSO contributed measurable stress that scaled with solvent concentration. Control performance confirmed that solvent effects can confound interpretation if not tightly managed, and the decline in activity and survival over time also indicated that prolonged exposure without feeding likely reduced tolerance and shortened NOEC time points. These outcomes highlight the need for careful control design, strict adherence to validity criteria, and cautious interpretation of "no-effect" thresholds when multiple stressors (solvent, starvation, and chemical exposure) coexist.

The chapter also provided an essential link between chemical removal and biological risk reduction by assessing treated versus untreated pesticide solutions. Granular activated carbon treatment produced clearer reductions in acute toxicity than rice husk biochar, while biochar-treated solutions sometimes showed elevated mortality, plausibly reflecting changes to water chemistry (e.g., turbidity, dissolved oxygen, or release of intrinsic contaminants) that can offset adsorption benefits. Together, these results demonstrate that treatment efficacy cannot be evaluated solely by concentration reduction; it must be validated using organism-level endpoints to confirm real reductions in ecological hazard.

Overall, the findings establish *D. pulex* as the more sensitive and discriminating indicator species in this study and confirm cypermethrin as the dominant acute risk driver relative to Isoproturon under the tested conditions. The evidence presented here supports the chapter's central premise: adsorption-based remediation approaches must be assessed using integrated physicochemical and ecotoxicological lines of evidence to ensure that observed removal translates into meaningful protection of freshwater biota.

# Chapter Five

## Acute Toxicity of Biochar-Treated and Untreated Aqueous Metal Solutions on *Daphnia* spp.

## 5.1 Introduction

Dissolved trace metals and metalloids remain persistent stressors in freshwaters because they are not degradable and can exert toxicity at low concentrations, particularly in their bioavailable (often dissolved/ionic) forms. Among the priority contaminants frequently associated with mining, metallurgical effluents, urban runoff, and industrial discharges are arsenic (As) and the metals cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn). These elements can impair aquatic invertebrates through multiple mechanisms, including disruption of ion regulation and membrane transport, oxidative stress, and interference with enzyme function and energy metabolism. In cladocerans such as *Daphnia*, acute exposures commonly translate into immobilisation and mortality, making these organisms sensitive indicators of bioavailable metal hazards (Traudt *et al.*, 2017; Cui *et al.*, 2018).

*Daphnia* species are widely used as model organisms in aquatic ecotoxicology, owing to their critical ecological role as primary consumers that link autotrophic producers to higher trophic levels, their rapid life cycles, and their reproducible, quantifiable responses to diverse classes of environmental contaminants. While interspecific, ontogenetic, and experimental variables can modulate sensitivity, comparative toxicological investigations consistently demonstrate the broad responsiveness of Daphnia spp. to a spectrum of heavy metals, underscoring their value for acute hazard screening across various contaminant types and exposure matrices (Traudt et al., 2017; Cui et al., 2018).

Moreover, emerging evidence indicates that contaminant mixtures frequently display non-additive toxicity; for instance, co-exposure to Cu, Zn, and As may produce synergistic or concentration-dependent interactive effects, highlighting the necessity for mechanistic toxicity assessments under environmentally relevant, multi-chemical scenarios (Limouni *et al.*, 2026). Even for individual substances, acute toxicity thresholds can vary substantially among Daphnia species. For example, the acute lethality of arsenic in Daphnia *pulex* has been characterised by 48-hour LC50 values in the milligram per litre range under controlled laboratory conditions, demonstrating that arsenic can induce measurable acute toxic effects contingent on both exposure chemistry and organismal physiology (Schultz *et al.*, 2024).

In parallel with the imperative of hazard characterisation, there has been considerable scholarly and applied interest in the development of treatment technologies to attenuate dissolved metal concentrations and, by extension, mitigate ecological risks. Among these, biochar—a porous, carbonaceous material derived from the thermochemical conversion of lignocellulosic biomass—has attracted substantial attention as a cost-effective and potentially circular-economy adsorbent for aquatic remediation. Systematic reviews of biochar applications in water treatment have documented rapid expansion of this research domain, demonstrating that biochar can effectively sequester cationic metals such as Pb(II), Cd(II), and Cu(II) under optimised conditions, while simultaneously identifying unresolved challenges related to sorbent regeneration, end-of-life management, and operational performance in chemically complex matrices (Phiri *et al.*, 2024; Tadesse *et al.*, 2025).

The mechanistic basis for heavy metal sequestration by biochar is multifaceted, encompassing both physical adsorption (notably pore filling) and a suite of chemical interactions at the biochar–solution interface. Dominant processes elucidated in the literature include complexation with oxygenated functional groups, ion exchange, electrostatic attraction, cation–π interactions, and (co-)precipitation with mineral constituents present in the ash fraction. The relative prominence of these mechanisms is determined by variables such as biochar feedstock type, pyrolysis or activation parameters, system pH, and the presence of competing ions (Mei *et al.*, 2025).

Nevertheless, reliance on metrics such as 'removal efficiency' is insufficient for robust environmental decision-making, as biochar-mediated treatment can induce complex alterations in exposure chemistry that modulate biological responses beyond total dissolved metal reduction alone. For instance, adsorption processes may shift metal speciation toward less bioavailable forms; concurrently, treatment may alter parameters such as pH and dissolved organic carbon, while particulate matter or residual contaminants inherent to certain biochars can introduce novel or independent stressors. As a result, there is a growing consensus on the need to integrate physicochemical performance metrics with organism-level bioassays to verify that remediation interventions yield tangible reductions in ecotoxicity within relevant biota (Tadesse *et al.*, 2025).

Empirical studies have substantiated that the ecotoxicological profile of biochar materials is highly contingent on feedstock composition and production methodology, with biochar employed as an adsorbent capable of influencing Daphnia responses via particle-mediated effects or leachable chemical constituents (Coelho et al., 2025). Furthermore, investigations evaluating activated biochar in water treatment scenarios underscore the critical need to directly assess Daphnia endpoints in both sorbent-only and sorbent–contaminant exposure regimes, given that toxicological outcomes can diverge markedly between 'pristine' and contaminant-laden matrices (Shirani *et al*., 2024).

Against this backdrop, acute ecotoxicological investigations that systematically compare biochar-treated and untreated solutions containing As, Cd, Cu, Ni, Pb, and Zn offer a rigorous framework for evaluating whether adsorption-driven modifications in exposure chemistry are sufficient to attenuate or reduce short-term biological effects in *Daphnia* spp. Acute immobilisation assays serve as a particularly informative first-tier assessment tool, demonstrating high sensitivity to bioavailable metal fractions, responsiveness to changes in solution chemistry, and the capacity to detect unanticipated outcomes in which treatment by-products or altered metal speciation negate expected reductions in ecological risk.
The integration of detailed chemical characterisation with organismal endpoints in Daphnia therefore provides a critical link between conventional treatment efficacy metrics and the ultimate objective of safeguarding ecological integrity in metal-contaminated aquatic environments (Phiri *et al*., 2024; Mei *et al*., 2025; Shirani *et al*., 2024).

This study aims to investigate the acute ecotoxicity of untreated and biochar-treated aqueous solutions of Arsenic (As), Cadmium (Cd), Copper (Cu), Nickel (Ni), Lead (Pb), and Zinc (Zn) to *Daphnia* spp. (*D. magna* and *D. pulex)*

## 5.2 Materials and Methods

All stock metal solutions were prepared by dissolving their salts in distilled water. During the experiment, triplet controls and quintuple test solutions were used. The same method described in **Section 2.3.3** was employed.

## 5.3 Results and Discussion

### 5.3.1 Effective Concentration Studies of Metal Solutions

The daphnids were observed for the toxic effects of metal solutions; moribund organisms (showing weakness), dead organisms (mortality), and healthy organisms (showing viability) were recorded at each time point for both daphnid species.  Next, the results for the activity rates (i.e., combination of viable and moribund organisms) are presented. Finally, the percentage responses (combination of dead and moribund organisms), the “No Observable Effects Concentration” (NOEC), Effective Concentration (EC50), and Lethal Dose (LD50) of all metal solutions were determined, and the statistical analysis and comparison of results for the different metals and species of *Daphnia* are briefly summarized.

Table 21: Solutions tested Showing *Daphnia* spp., the graphically determined EC50 and LD50, and the corresponding response time. Values expressed in mg $L^{-1}$

| | | **EC50 (mg $L^{-1}$)** | | **LD50 (mg $L^{-1}$)** | |
|---|---|---|---|---|---|
| Metals | Species | 48 h | 96 h | 48 h | 96 h |
| As | *Magna* | $5.94 \times 10^{-5}$ | $5.34 \times 10^{-4}$ | 0.594 | < 0.001 |
| | *Pulex* | $9.02 \times 10^{-3}$ | $6.72 \times 10^{-4}$ | 0.155 | < 0.001 |
| Cd | *Magna* | 0.0167 | < 0.001 | 0.0646 | < 0.001 |
| | *Pulex* | 0.0134 | < 0.001 | 0.504 | 0.000227 |
| Cu | *Magna* | < 0.001 | < 0.001 | 0.0104 | < 0.001 |
| | *Pulex* | 0.00766 | < 0.001 | 0.0149 | < 0.001 |
| Ni | *Magna* | $1.98 \times 10^{-8}$ | $8.13 \times 10^{-10}$ | 0.018 | 0.0000286 |
| | *Pulex* | 0.00447 | $1.80 \times 10^{-8}$ | 0.0447 | 0.0000091 |
| Pb | *Magna* | 0.106 | < 0.001 | ≈ 3.94 | 0.0024 |
| | *Pulex* | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Zn | *Magna* | 0.00108 | $2.9 \times 10^{-5}$ | 0.0091 | 0.00104 |
| | *Pulex* | 0.00081 | $5.8 \times 10^{-8}$ | 0.134 | < 0.001 |

** n.d. represents results with less than 50% mortality overall.*

Table 22 shows the concentrations of test solutions that produced total response and mortality. Furthermore, in comparing percentage mortality and total responses, both data trends became similar for metal concentrations from 0.1 to 10 mg $L^{-1}$ because metal toxicity was higher and the effects were more lethal for most metals. Additionally, Table 23 displays that both *Daphnia* spp. did not exhibit 100% response or mortality throughout the 96 h of observation.

No metal solution concentration tested produced an observable effect on the daphnids (no NOEC), including the control media. This is because even the control media used produced total responses of 20% and 30% responses from *D. magna* and *D. pulex* at 24 h, respectively. Both species produced 40% responses to control media at 48 h of exposure. On the other hand, mortality results for *D. magna* were approximately 7% and 27% at 24 h and 48 h, respectively; for *D. pulex*, 13% and 20% at 24 h and 48 h, respectively. The LOEC for all metal solutions was 0.001 mg $L^{-1}$ or lower, as at 24 h the lowest metal concentrations used in the experiment produced almost 50% toxic responses for all metals except copper. The NOEC for most metal solutions was observed between 2 h and 4 h of observation within 0.001-1 mg $L^{-1}$

Figure 42 shows the LD50 plots for *D. pulex* and *D. magna* for all metal solutions. *D. pulex* is more sensitive to arsenic than *D. magna* at 48 h, and by 96 h, mortality exceeds 50% at the lowest concentration, so the true LD$_{50}$ lies below the lowest tested concentration (0.001 mg $L^{-1}$). *D. magna* is more sensitive to Cd than *D. pulex* at 48 h.

At 96 h, toxicity increases dramatically, with LD$_{50}$ approaching or below the lowest tested concentration. Both species of Daphnia are highly sensitive to copper. By 96 h, mortality is already >50% at the lowest concentration tested, meaning the true LD$_{50}$ lies below 0.001 mg $L^{-1}$. *D. magna* is more sensitive to Lead at 48 h, but by 96 h, *D. pulex* becomes slightly more sensitive.

Lead toxicity increases strongly with exposure time, with LD$_{50}$ dropping by ~3 orders of magnitude from 48 h to 96 h. *D. pulex* is far more sensitive to Pb than *D. magna*. Mortality in *D. pulex* exceeds 50% even at the lowest concentration, so the true LD$_{50}$ is below 0.001 mg/L. *D. magna* shows delayed toxicity, with LD$_{50}$ dropping from ~3.9 mg $L^{-1}$at 48 h to 0.0024 *mg* $L^{-1}$at 96 h. *D. magna* is more sensitive to Zn at 48 h. By 96 h, toxicity increases, and *D. pulex* becomes extremely sensitive, with LD$_{50}$ falling below the lowest tested concentration.

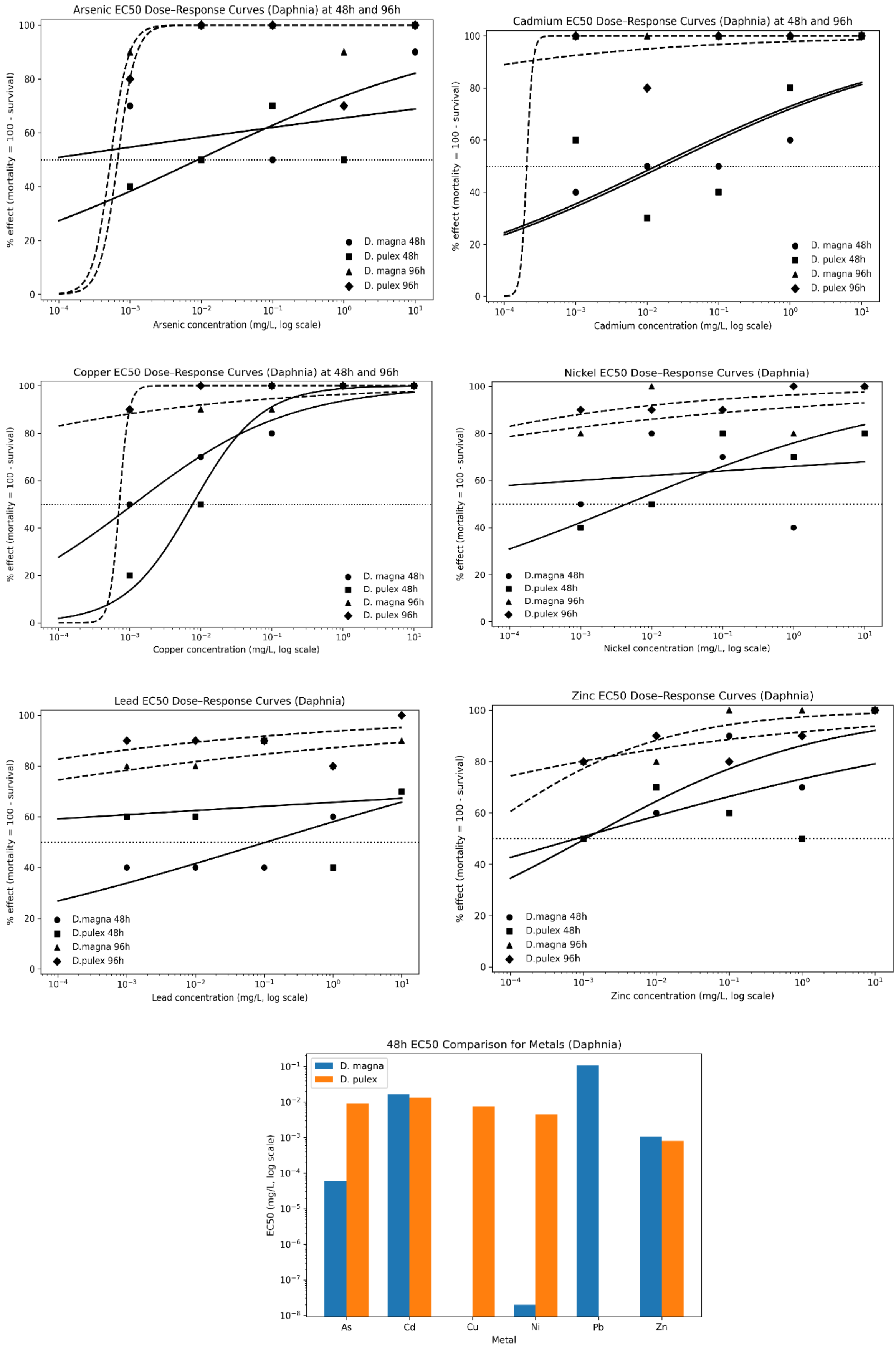

Figure 42: EC50 Plots and Comparison Chart Showing Metals Toxicity to *D. pulex* and *D. magna*

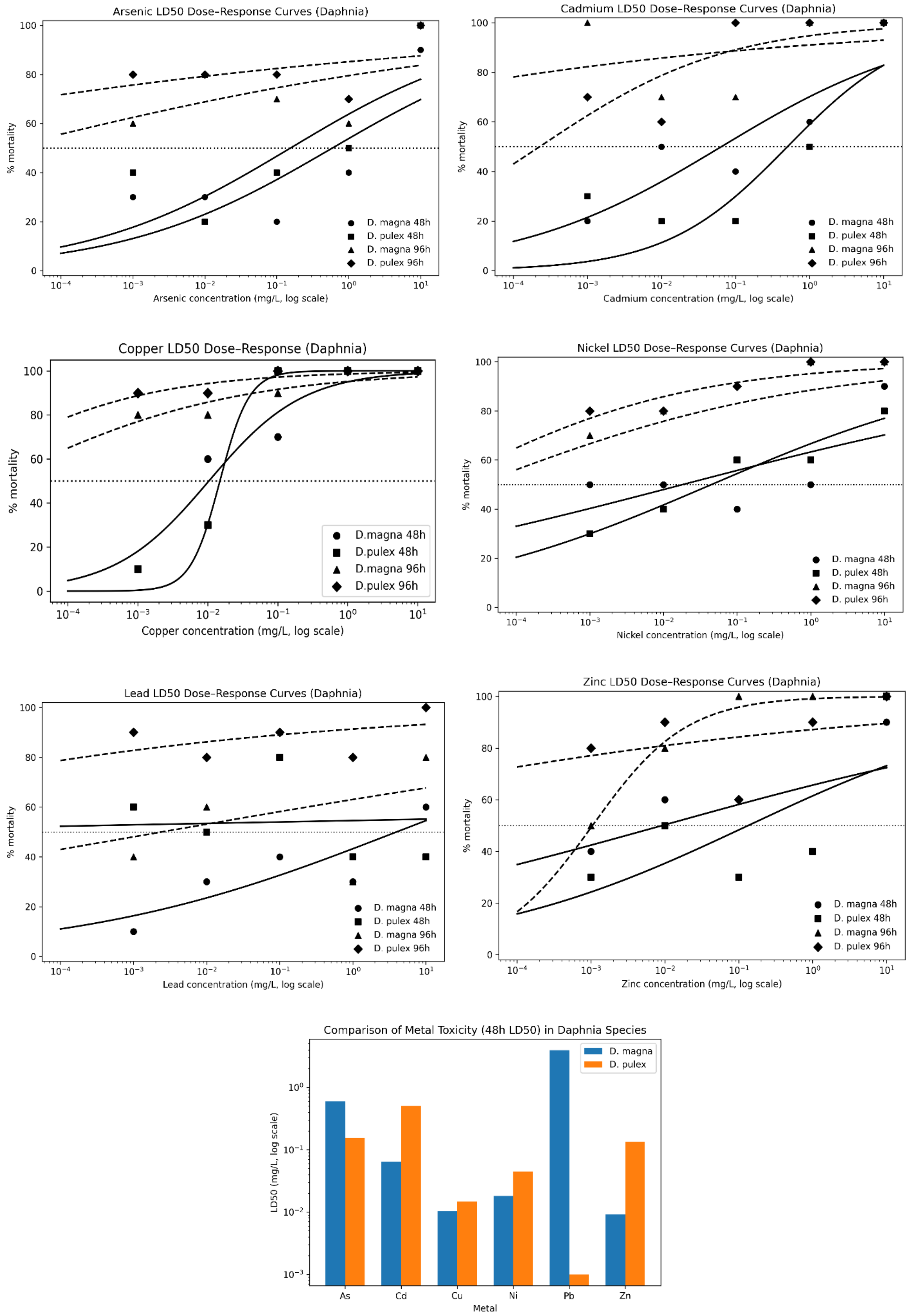

Figure 43: LD50 Plots and Comparison Chart Showing Metals to *D. pulex* and *D. magna*

Figure 43 further suggests that at 96h, Cd EC50 data show ≥80–100% mortality at all tested concentrations, meaning the 50% effect point is below your lowest test concentration (0.001 mg $L^{-1}$). Copper is highly toxic to Daphnia, especially as exposure time increases. 96 h exposure causes near complete mortality even at the lowest concentration, indicating very high chronic toxicity.

For several Ni treatments, mortality is already >50% at the lowest tested concentration (0.001 mg $L^{-1}$), indicating the true $EC_{50}$ is below that concentration. The logistic model, therefore, extrapolates values that appear extremely small. Lead toxicity increases strongly with exposure time. By 96 h, mortality exceeds 50% even at the lowest concentration, so the true $EC_{50}$ lies below 0.001 mg $L^{-1}$ for both species. At 96 h, mortality is already ≥80–100% at the lowest concentrations, meaning the true $EC_{50}$ is likely below the lowest tested concentration (0.001 mg $L^{-1}$).
Overall, based on the EC50 data, the results indicate that the metal toxicity ranks as Ni ≈ As > Zn > Cu > Cd > Pb, with Ni and As being the most toxic.

### 5.3.2 Mortality of *D. magna* and *D. pulex* in Untreated Metal Solutions over 72 h.

The figures below show the mortality rates of *D. magna* and *D. pulex* in water control solutions over 72 h, followed by the mortality of both *Daphnia* spp. in five concentrations of all tested. Figure 45 below displays the percentage mortality and responses of *D. magna* and *D. pulex* to HH Combo media (water control) without feeding over 96 h. Figures 45 and 46 compare the percentage mortality of *D. magna* and *D. pulex* at five exponential concentrations of six metal solutions (As, Cd, Cu, Ni, Pb, and Zn) over 72 h.

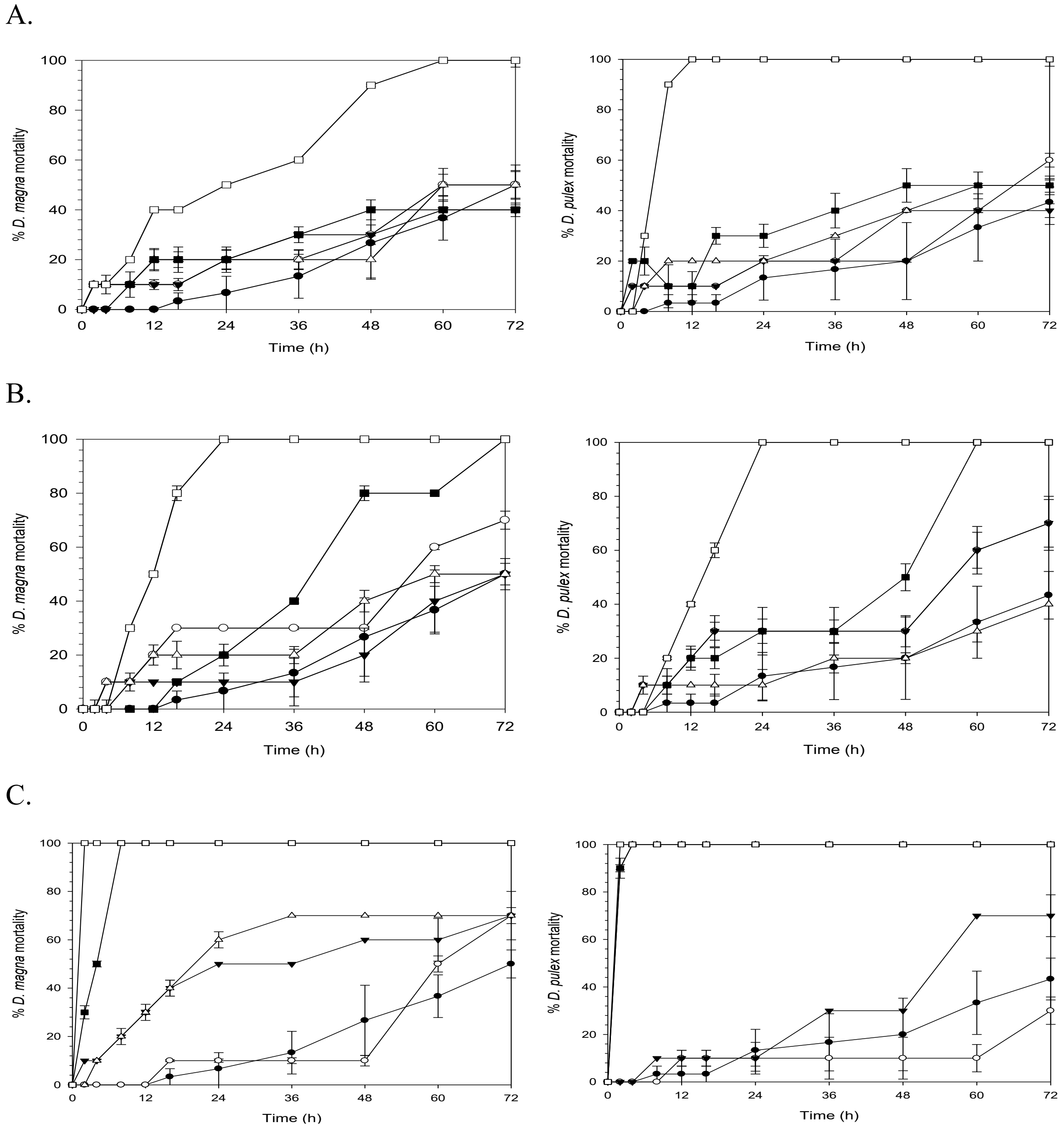

Figure 44: Percentage mortality of *D. magna* vs. *D. pulex* in HH Combo control (●), 0.001 mg $L^{-1}$ (○), 0.01 mg $L^{-1}$ (▼), 0.1 mg $L^{-1}$ (Δ), 1 mg $L^{-1}$ (■), and 10 mg $L^{-1}$ (□) Arsenic (A), Cadmium (B), and Copper (C) Solutions for 72 h. All values are averages of five samples ± standard error of the mean.

Figure 44 shows that the mortality rates of *D. magna* in As solutions reached a steady state before 72 h, with the highest values at 10 mg $L^{-1}$ As. Mortality in all other As solutions seemed to reach a steady state between 48-60 h of *D. magna* exposure. On the other hand, the mortality of *D. pulex* to arsenic solutions peaked at 10 mg $L^{-1}$ As after 12 h, and all other As concentrations produced similar mortality trends that reached a steady state before 72 h, except at the lowest concentration (0.001 mg $L^{-1}$). It further illustrates that *D. magna* showed higher mortality even at low Cadmium concentrations than *D. pulex.* The figure suggests that the three highest copper concentrations produced the highest mortality in both Daphnia spp., which were in steady state by 72h of exposure. *D. magna* and *D. pulex* mortality for 1 and 10 mg L$^{-1}$ peaked after 8 h and 4 h, respectively. However, for both species, mortality at the lowest three cadmium concentrations did not appear to have reached a steady state by 72 h. Percentage mortality followed expected trends in *D. magna,* but *D. pulex* displayed lower mortality in 0.001 mg L$^{-1}$ cadmium than in the water control.

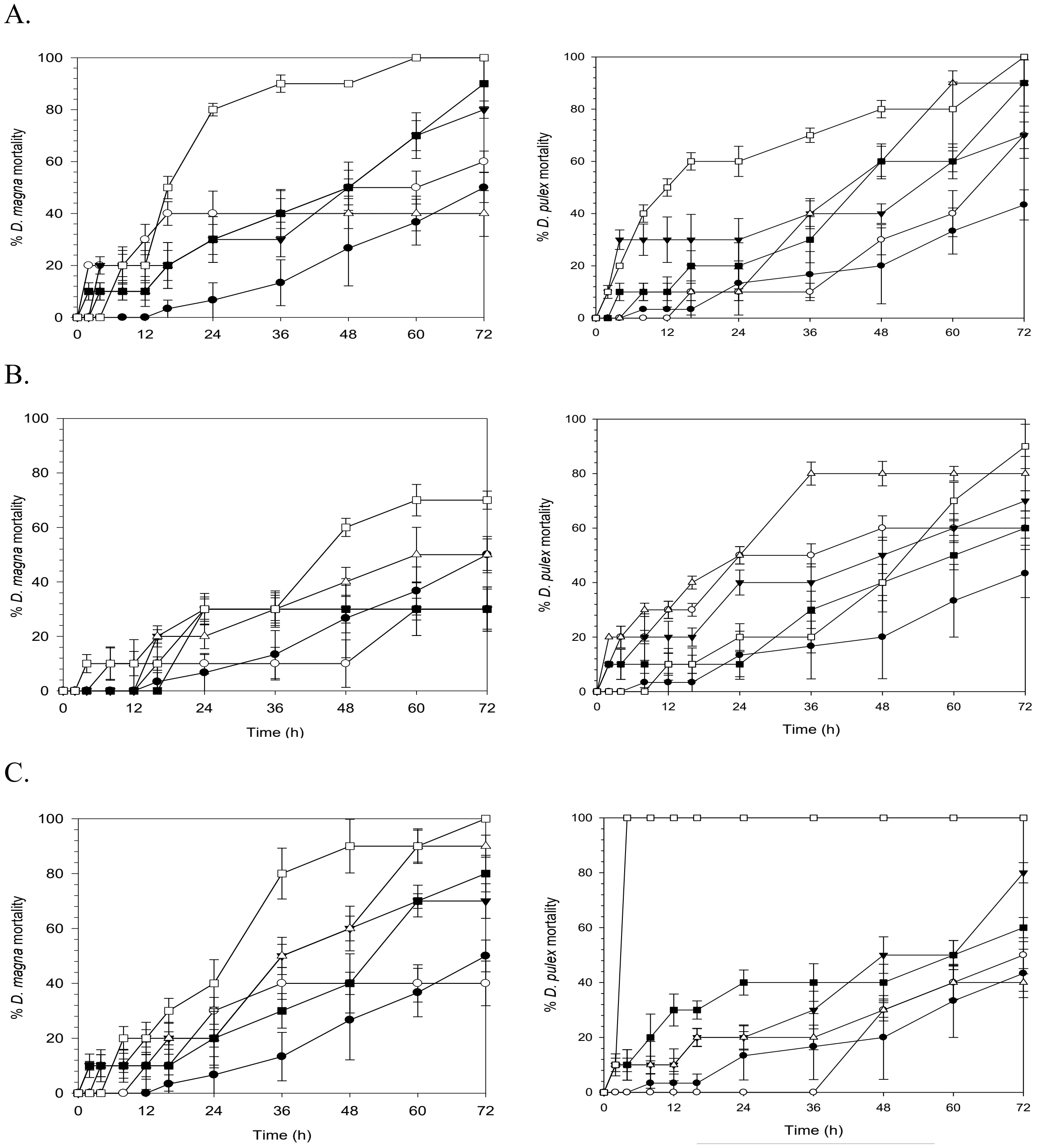

Figure 45: Percentage mortality of *D. magna* vs. *D. pulex* in HH Combo control (●), 0.001 mg L$^{-1}$ (○), 0.01 mg L$^{-1}$ (▼), 0.1 mg L$^{-1}$ (Δ), 1 mg L$^{-1}$ (■), and 10 mg L$^{-1}$ (□) Nickel, Lead, and Zinc Solutions for 72 h. Where all values are averages of five samples ± standard error.

Figure 45 suggests that, overall, *D. magna* exhibited higher mortality in response to Nickel than *D. pulex.* Both plots illustrate that both *Daphnia* species were at their initial steady state or later stages of kinetic state reactions by 72 h of exposure. Furthermore, as expected, the highest concentrations produced the highest mortality in both species.

In addition, it illustrates that the mortality rate of *D. magna* at 0.1 mg $L^{-1}$ Pb was higher than at 1 mg $L^{-1}$ Pb, and that mortality at 1 mg $L^{-1}$ Pb was lower than in the HH combo medium. On the other hand, mortality of *D. pulex* was highest in 0.1 mg $L^{-1}$ Pb, and mortality in 0.001 mg $L^{-1}$ Pb was the second highest. It shows the large difference in mortality rates of *D. pulex* across different Zinc solutions. Mortality in 10 mg $L^{-1}$ Zn produced 100% mortality in about 4 h, but the lower concentrations produced gradual mortality rates with similar trends. *D. magna* mortality trend was generally similar and higher than the mortality displayed by *D. pulex.*

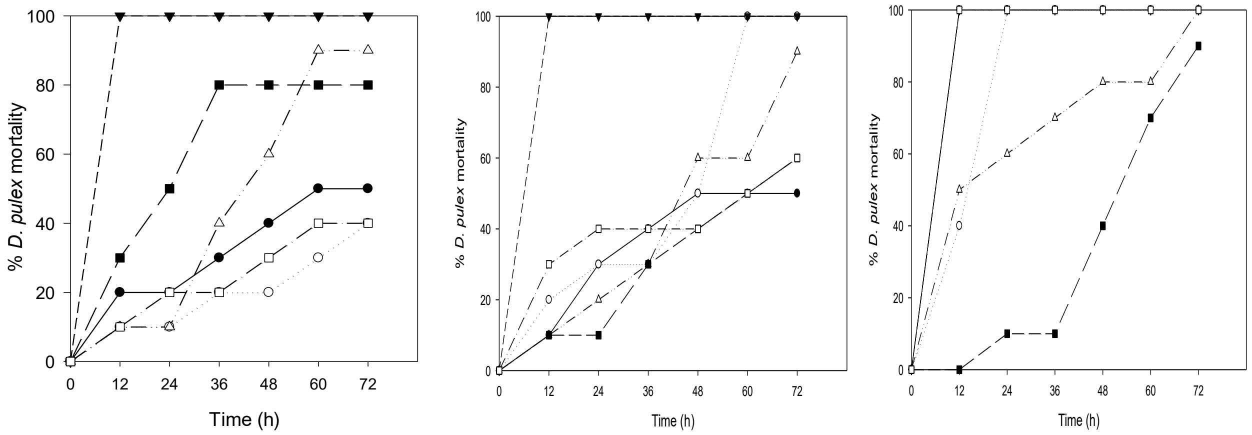

Figure 46: Mortality of *D. pulex* in 0.1, 1, and 10 mg $L^{-1}$ As (●), Cd (○), Cu (▼), Ni (Δ), Pb (■), and Zn (□) Solutions. All values are averages of three samples ± standard error of the mean.

Figure 46 displays the mortality rates of *D. pulex* in metal solutions by concentration, indicating the metal with the highest toxicity to *Daphnia* among the metals studied. It suggests that the toxicity of the metal solutions was in the decreasing order Cu > Cd > Ni > As > Zn > Pb. Furthermore, during the comparison of mortality and total responses, both data trends became similar for metal concentrations from 0.1 to 10 mg $L^{-1}$ because metal toxicity was higher and the effects were more lethal for most metals.

Unlike *D. magna*, the *pulex* showed higher and more rapid responses to the toxicity of the metal solutions at 10 mg $L^{-1}$. Three of the metals of interest, copper, zinc, and arsenic, produced 100%

mortality within 4-8 h of exposure, while the other three metals (nickel, cadmium, and lead) did the same after 60 h of exposure, with lead being the lowest. Nickel and lead produced the lowest total responses at this concentration.

At 95% confidence level, assuming:

a) H0: that the mean mortality and responses of *D. magna* and *D. pulex* daphnids to metal solutions are equal, and that,

b) Ha: that the mean mortality and responses of *D. magna* and *D. pulex* daphnids to metal solutions are unequal

Paired and unpaired t-tests were conducted on the average results obtained. The unpaired t-tests highlighted the differences between the responses of both *Daphnia* spp. to control medium and test solutions at different time points, the differences between the responses to a particular metal at different time points, and the differences between the type of responses at the different time points.

Paired t-tests were employed to investigate the possible influence of exposure times; for example, at 24 and 48 h of daphnia exposure and 0 to 24 or 48 h of exposure. The t-tests further described the effects of response type (mortality and total response, including weakness). The time points and metal concentrations evaluated with this test were those that showed significant differences in the unpaired t-tests. Comparison of the percentage mortality and response of both Daphnia species to each metal solution at all observed time points showed that, at $p \leq 0.05$, all mean percentage mortalities were not significantly different across arsenic, cadmium, and nickel test solutions. However, there were significant differences between the percentage mortality and responses displayed by both species to 0.1 mg $L^{-1}$ copper solutions, the percentage mortality exhibited by both species in response to 0.001 mg $L^{-1}$ and 0.1 mg $L^{-1}$ lead solutions, and the percentage mortality exhibited by both species in 10 mg $L^{-1}$ zinc solutions.

The percentage 24 h and 48 h mortality and total response of both *Daphnia* spp. to different metal solutions at each concentration. The results showed that, at $p \leq 0.05$, all means were not significantly different across test solutions. The percentage mortality and total responses of both daphnia species to different concentrations of all the test metal solutions at 24 h and 48 h. The results observed at the 24-h time point highlighted significant differences in the percentage mortality of *D. magna* between the 0.01 mg $L^{-1}$ metal solutions at $p \leq 0.05$. On the other hand, the percentage mortality of *D. pulex* was significant only at 0.01 mg $L^{-1}$ metal concentrations. At the 48-h time point, *D. magna* produced significant total responses at 0.01 mg $L^{-1}$ while *D. pulex* showed no significantly different responses at any metal concentration.

In summary, the results above suggest that the metal concentrations that produced significantly different total responses are 0.001 mg $L^{-1}$, 0.01 mg $L^{-1}$, 0.1 mg $L^{-1,}$ and 10 mg $L^{-1}$. Toxicity results for these metal concentrations were further evaluated at 24 h and 48 h time points using the paired t-test.

Paired t-tests were used to compare the differences in toxic responses exhibited by both daphnia species at the beginning and end of the experiment, in the control solutions, the lowest metal concentrations studied, and at the significantly different metal concentrations deduced from the unpaired t-tests. At a 95% confidence level, the control results were statistically compared to the results at different time points.

At 0 and 96 h, the percentage mortality and percentage total responses were statistically significant. This suggests that the exposure time influences the responses of both *Daphnia* spp. At 0 and 12 h, the percentage mortality of D. magna differed significantly among As, Ni, and Zn. Also, *D. pulex* showed statistical differences in percentage mortality for Cd, Cu, Ni, and Pb. However, all percentage total response data analysed for both species were statistically different at 95% confidence level. At 0 and 24 h, the percentage mortality of D. magna differed significantly for As, Cu, Ni, Pb, and Zn, and for *D. pulex* in Cu, Ni, and Pb.

However, all percentage total response data analysed for both species were statistically different at the 95% confidence level. At 0 and 48 h, all results obtained at both time points were significantly different at 95% confidence level for percentage mortality and total response of both *Daphnia* spp. in all metal solutions. At 24 and 48 h, the percentage mortality of D. magna differed significantly among Cd, Ni, and Zn, whereas D. pulex showed significant mortality only in Ni and Pb. On the other hand, the total response percentage of *D. magna* was statistically significant for Cd, Pb, and Zn, whereas *D. pulex* showed significant differences in toxicity for Pb and Zn at the 95% confidence level.

At 24 h and 48 h, the specific responses of both daphnia species at each concentration per metal type were analysed using paired t-tests at 95% confidence level. *D. magna* only showed statistically significant mortality in cadmium, nickel, and zinc, but its percentage total responses were significant to cadmium, lead, and zinc. On the other hand, *D. pulex* showed statistically significant mortality only for nickel and lead, whereas its total responses were significant for Pb and Zn solutions.

## Acute Toxicity of Biochar-Treated and Untreated Aqueous Metal Solutions on *Daphnia* spp.

Neonates of *Daphnia magna* and *Daphnia pulex* were exposed to HH combo medium control (dilution water), five concentrations each of arsenic ($As^{3+}$), cadmium ($Cd^{2+}$), copper ($Cu^{2+}$), nickel ($Ni^{+}$), lead ($Pb^{2+}$), and zinc ($Zn^{2+}$) (0.001, 0.01, 0.1, 1 and 10 mg $L^{-1}$) over 72 - 96 h without feeding. The pH and DO values observed in this study align with those reported by Murano et al. (2007), ranging from 6.4 to 8 and from 6.6 to 8.3, respectively.

### 5.4.1 Acute Toxicity of Untreated Metal Solutions to *Daphnia* spp.

The exposure of *D. magna* and *D. pulex* to HH combo medium control (dilution water), five concentrations each of CP and IPU (0.001, 0.01, 0.1, 1 and 10 mg $L^{-1}$ ) over 72 h without feeding suggest that there was no concentration of metal solutions tested, that produced no observable effect on the daphnids (no NOEC) and both *Daphnia* spp. did not exhibit 100% response or mortality throughout the 96 h of observation. However, the NOEC for most metal solutions was observed between 2 and 4 h for 0.001-1 mg $L^{-1}$. The LOEC for all metal solutions was 0.001 mg $L^{-1}$ or lower, even the lowest metal concentrations used in the experiment produced approximately 50% toxic responses for all metals except copper, with higher values. Conversely, Teodorovic *et al.*, (2009) reported a LOEC of 0.61 mg $L^{-1}$ for cadmium, 1.14 mg $L^{-1}$ for Zn, and 62.56 mg $L^{-1}$ for Pb. The disparity between the values in this study and the reported values might have been caused by differences in the concentration ranges and physicochemical properties of the test solutions.

For arsenic toxicity on *D. magna*, Fikirdesici *et al.* (2012) reported a 24 h LC50 of 509 µg $L^{-1}$ and Lima et al (1984) reported a 96-h $EC_{50}$ estimate of 1,500 µg $L^{-1}$. The value calculated in this study is 10,000 µg $L^{-1}$ for D. magna and 4,000 µg $L^{-1}$ for *D. pulex,* respectively. For cadmium toxicity on *D.magna*, reports for 24h LC50 ranged from 44 - 220 µg $L^{-1}$ (Fikirdesici *et al.*, 2012; Teodorovic *et al.*, 2009), which is almost 90 – 180 times lower than reports from this study with LC50 of 4,000 µg $L^{-1}$ for both *Daphnia* spp. A 48 h LC50 of 39 - 610 µg $L^{-1}$ was reported by Nelson *et al.* (1984), Aguilar ‐ Alberola & Mesquita ‐ Joanes (2012), Ferreira et al (2008), and Teodorovic et al. (2009).

However, Kim *et al.* (2017) calculated a 48 h EC50 of 21 µg L$^{-1}$ comparable to the value in this study (10 µg L$^{-1}$).

For copper toxicity on *D. magna*, the 24 h LC50 was 60 µg L$^{-1}$, which is comparable to 160 µg L$^{-1}$ reported by Santos-Rasera et al. (2019), although Deshpande (2016) reported a 48h LC$_{50}$ for Cu of 2.43 µg L$^{-1}$. The 24 h EC50 of Ni for *D. magna* reported by Khangarot and Ray (1989) was 4.89 mg L$^{-1}$, and the 48 h LC50 for Ni reported by Deshpande (2016) was 995 µg L$^{-1}$. These values are comparable to 3.5 mg L$^{-1}$ and 1000 µg L$^{-1}$ calculated in this study and fall within the LC50 range (0.63-5 mg L$^{-1}$) reported by Mano & Shinohara (2020). Cu is one of the most toxic metals on *D. magna* (Khangarot & Ray, 1989)

For Pb toxicity on *D. magna*, the 24 h LC50 was 162.88 mg L$^{-1}$ (Aguilar-Alberola & Mesquita-Joanes, 2012). This study calculated a 48 h LC50 value of 500 µg L$^{-1}$, which is comparable to 694.6 and 160 µg L$^{-1}$ reported by Santos-Rasera et al. (2019), although Deshpande (2016) reported a much lower 48h LC$_{50}$ value for Cu of 2.43 µg L$^{-1}$. For Zn toxicity to *D. magna*, the 24 h EC50 reported by Khangarot and Ray (1989) was 1 mg l$^{-1}$, and the 48 h EC50 of 0.22 mg L$^{-1}$ was reported by Gebara et al. (2021). These reports align with the values in this study: 0.1 and 0.001 mg L$^{-1}$ for the 24- and 48-h EC50, respectively. The 48 h LC50 of 3.379 mg l$^{-1}$ reported by Lee et al. (2021), and the 24 and 48 h LC50s of 180 and 41 mg L$^{-1}$, respectively, reported by Teodorovic et al. (2009), align closely with the 48 h LC50 of 0.05 mg L$^{-1}$ calculated for this study.

There are insufficient studies and consequently data on the sensitivity of *D. pulex* to metals and pesticides, resulting in limited comparability with the results obtained in this study. The effective concentration data from this study suggest that *D. pulex* was more sensitive than *D. magna* to all the metals studied except As and Zn. The large disparity between the results of this study and those of previous reports on D. magna could be due to the absence of biochar or physicochemical property modification in this study, compared with other reports on *D. magna*. Variations in acute metal toxicity may be attributed to differences in hardness, pH, DOC, and other physicochemical properties of the test waters (Mano & Shinohara, 2020), given that some studies were conducted in field (real-life) settings.

The results obtained indicate that the toxicity of the metal solutions was in the decreasing order Cu > Cd > Ni > As > Zn > Pb. This is comparable with the toxicity trends observed by Teodorovic *et al.* (2009) and Cui et al. (2018). Unlike *D. magna*, *D. pulex* showed higher and more rapid toxicity responses to the metal solutions at 10 mg $l^{-1}$. Data from this study suggests that *D. magna* exhibited rapid responses to Ni and slow and low-toxic responses to zinc compared to all other test solutions at this concentration. This is similar to reports by Teodorovic *et al.* (2009) that the sensitivity of *D. magna* to metals is in the decreasing order Cd > Zn > Pb. Cadmium produced the highest and immediate toxic responses overall, while Pb had the lowest overall toxic effects up to 60 h. Mortality percentage was observed in the decreasing order Zn > Cu > Pb > As/Cd > Ni after 96 h of exposure to 0.1 mg $L^{-1}$ metal solutions. The highest total responses were produced by Zinc, followed by Pb at this concentration. Mortality was observed in the decreasing order Cu > Cd > Ni > Pb > As > Zn after 96 hrs of exposure to 0.1 mg $L^{-1}$ metal solutions. The lowest total responses were produced by cadmium and zinc. Mortality was observed in the decreasing order Cu > Cd > Ni > Zn > As > Pb after 96 h of exposure to 1 mg $l^{-1}$ metal solutions. The highest total responses were observed for copper, with 100% mortality after 8 h, while lead showed the lowest mortality percentage at 16 h at this concentration.

The toxicity of metals to *D. magna* produced rapid, similar responses and mortality percentages at 1 mg $L^{-1}$. Copper produced the highest mortality of 100% within less than 2 h of exposure, followed by lead, with mortality peaking at 48 h. All test solutions showed similar response trends as mortality trends, except Pb, which produced the lowest total responses at 1 mg $L^{-1}$.
D. magna showed higher sensitivity to Cd solutions than D. pulex, which even showed lower mortality percentage in 0.001 mg $l^{-1}$ Cd than in the control. *D. magna* showed a higher tolerance for up to 1 mg $L^{-1}$ Pb solution than the control. *D. pulex*, on the other hand, showed the highest percentage mortality in 0.1 mg $L^{-1}$ Pb and the second highest in 0.001 mg $L^{-1}$ Pb, but seemed to adapt at higher Pb concentrations, and was very sensitive to high doses of Zn (10 mg $L^{-1}$), with 100% mortality in 4h.

According to Khangarot and Ray (1989), metal toxicity has been linked to the physicochemical properties of metals or metal ions. This, in summary, means that metals with higher reactivity often exhibit higher toxicity. In an ideal environmental setting, this could explain the toxicity trends observed in this study. However, the observed toxicity trend does not completely follow the decreasing reactivity trend (Zn > Cd > Ni > Pb > Cu > As) of these metals, possibly due to other interfering physicochemical factors. The result of this study, therefore, disagrees with the position.

### 5.3.3 Mortality of *D. pulex* in Biochar-Treated Metal Solutions over 72 h.

Figures 47 and 48 show the mortality rates of *D. pulex* neonates at different concentrations of metal solutions. Figures 48 and 49 display the mortality rates of *D. pulex* neonates in different metal solution concentrations under different conditions over 72 h. Figure 48 compares the effect of RH biochar and GAC treatment of the different concentrations of As, Cd, and Cu, but Figure 44 compares the effect of RH biochar treatment and “no treatment” on the mortality rates of *D. pulex* neonates in different concentrations of Ni, Pb, and Zn solutions over 72 h.

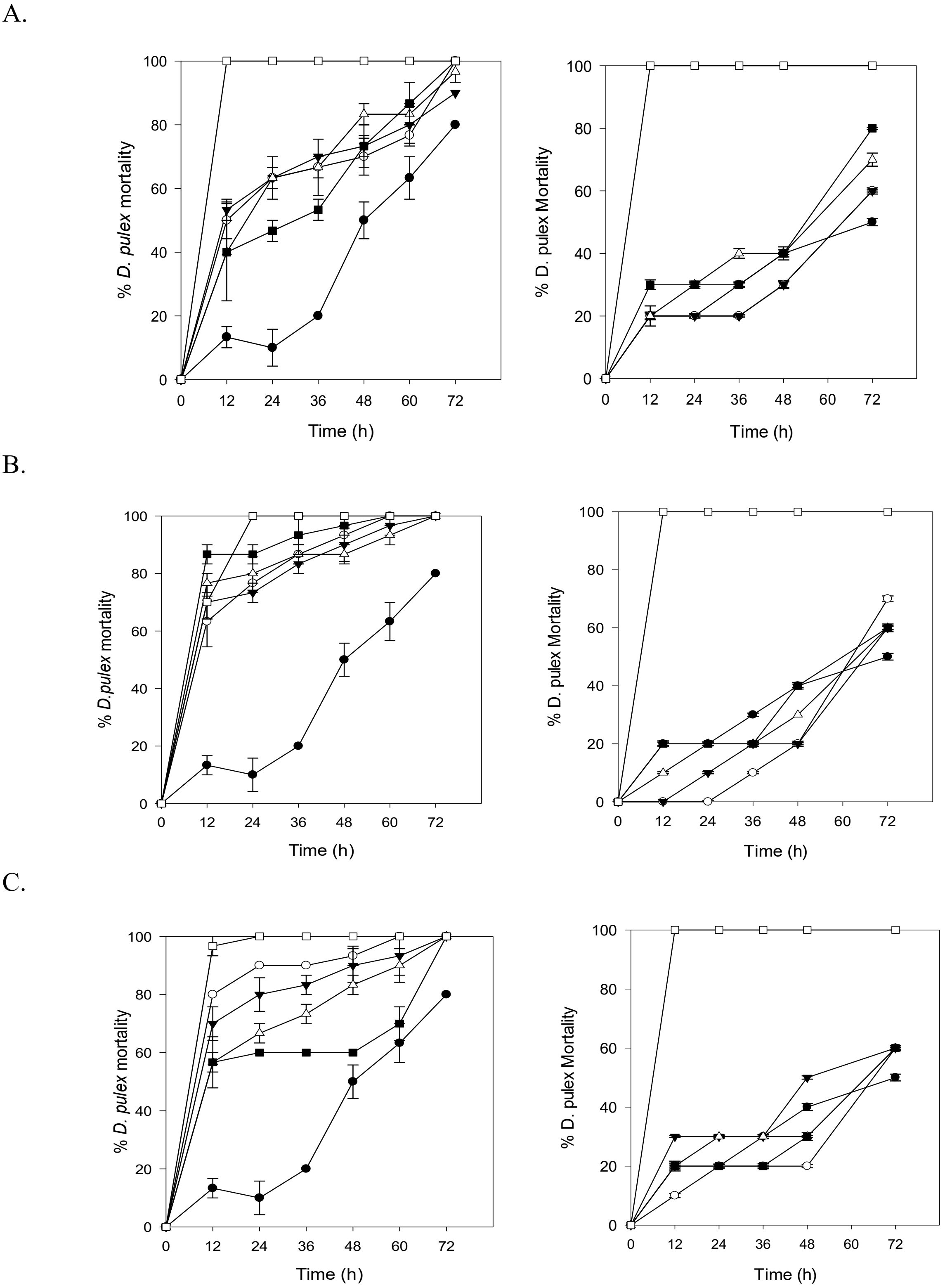

Figure 47: Percentage mortality of *D. pulex* in rice husk (RH) and granular activated carbon (GAC)-treated solutions of arsenic (A), cadmium (B), and Copper (C) solutions over 72 h. HH Combo control (●), 0.001 mg L$^{-1}$ (○), 0.01 mg L$^{-1}$ (▼•), 0.1 mg L$^{-1}$ (Δ•), 1 mg L$^{-1}$ (■), and 10 mg L$^{-1}$ (□). All left plots for RH and all right plots for GAC, (n = 3) ± standard error of the mean.

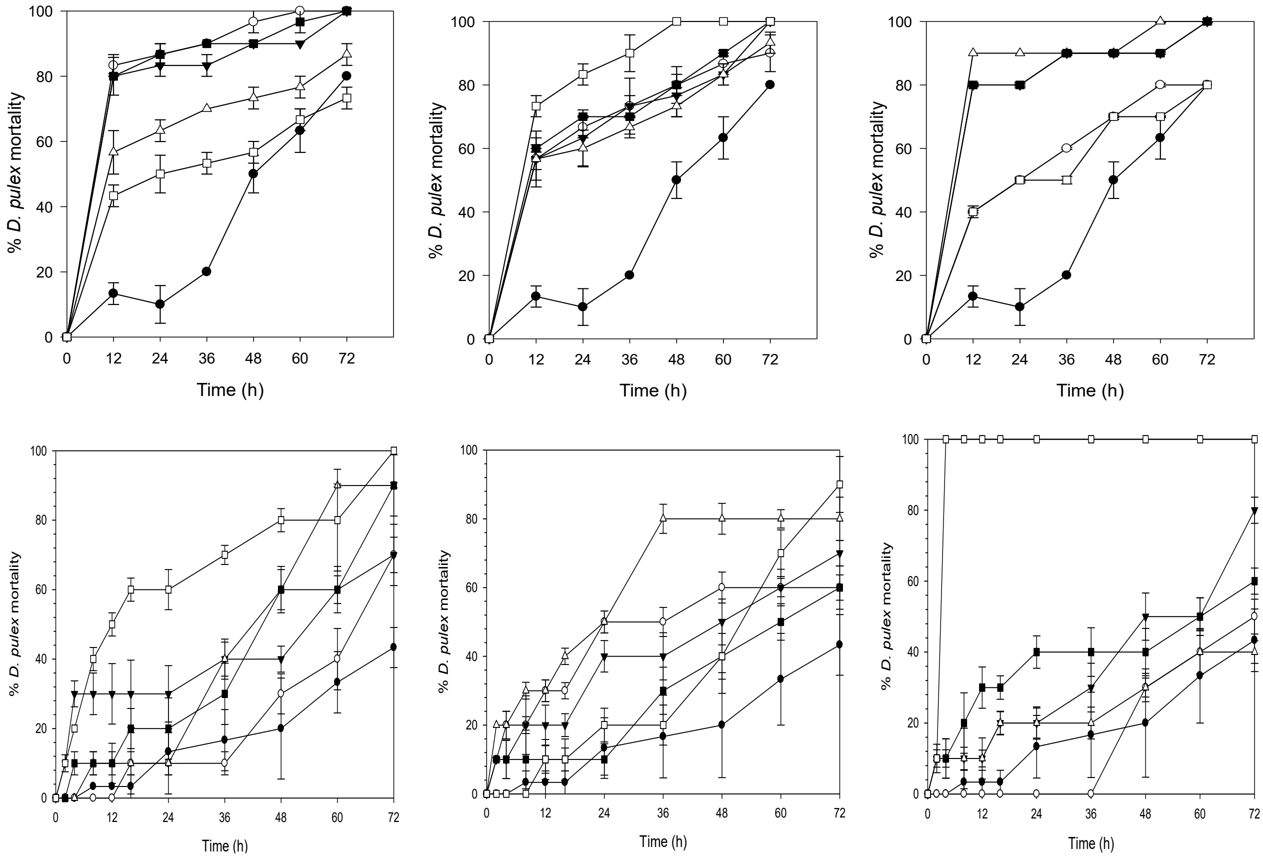

Figure 48: Percentage mortality of *D. pulex* in untreated and rice husk (RH)-treated metal solutions of nickel (left), lead (middle), and zinc (right) solutions over 72 h. HH Combo control (●), 0.001 mg L$^{-1}$, (○), 0.01 mg L$^{-1}$ (▼), 0.1 mg L$^{-1}$ (Δ), 1 mg L$^{-1}$ (■), and 10 mg L$^{-1}$ (□). All top plots for RH biochar and all bottom plots for untreated solutions, (n = 3) ± standard error of the mean.

## Acute Toxicity of Biochar-Treated Aqueous Metal Solutions of As, Cd, and Cu on *Daphnia pulex.*

On average, treatment with metal solutions and RH biochar was more effective at reducing their toxic effects on *D. pulex* than GAC treatment. This is reflected in the acute toxicity results and the health index plots. However, the lowest two concentrations of these metals seemed to have lower toxic effects on the Daphnids than their treatment with carbonaceous compounds. This could be explained by reduced oxygen levels during treatment with carbonaceous materials. According to Ferreira *et al.* (2008), hypoxia impairs physiological processes such as respiration, circulation, and metabolism in Daphnia spp. it further induces haemoglobin synthesis

### 5.3.4 Health Index of *Daphnia* spp. in Untreated and Treated Metal Solutions

This section briefly illustrates the health indices of *D. magna* vs *D. pulex* in untreated metal solutions (Figures 49 and 50), then compares the health indices of *D. pulex* in RH- and GAC-treated metal solutions at all studied timepoints over 96 h in Figure 51.

The condition indices (CIs) generated for *Daphnia magna* and *Daphnia pulex* in metal solutions reveal clear concentration- and time-dependent declines in the health of these organisms across all tested metals. In general, higher concentrations produced faster and steeper reductions in survival, indicating acute toxic effects, whereas lower concentrations caused gradual declines, consistent with chronic toxicity. These results demonstrate the sensitivity of cladocerans to dissolved metals and highlight their importance as bioindicator species in freshwater ecotoxicology.

The condition index plots for arsenic (Figure 49) showed moderate declines in health indices over time, particularly at higher concentrations. However, survival remained relatively high at lower concentrations during early exposure periods, suggesting that arsenic exerts primarily chronic rather than acute toxicity in these organisms. Arsenic toxicity in aquatic organisms is typically associated with interference with cellular respiration and enzyme function, particularly by inhibiting ATP production pathways. Previous studies have shown that arsenic disrupts energy metabolism and oxidative phosphorylation in aquatic invertebrates, leading to reduced physiological performance before mortality. The relatively gradual decline in CI observed in this study aligns with earlier findings that arsenic toxicity often manifests as delayed mortality following prolonged exposure rather than immediate lethal effects.

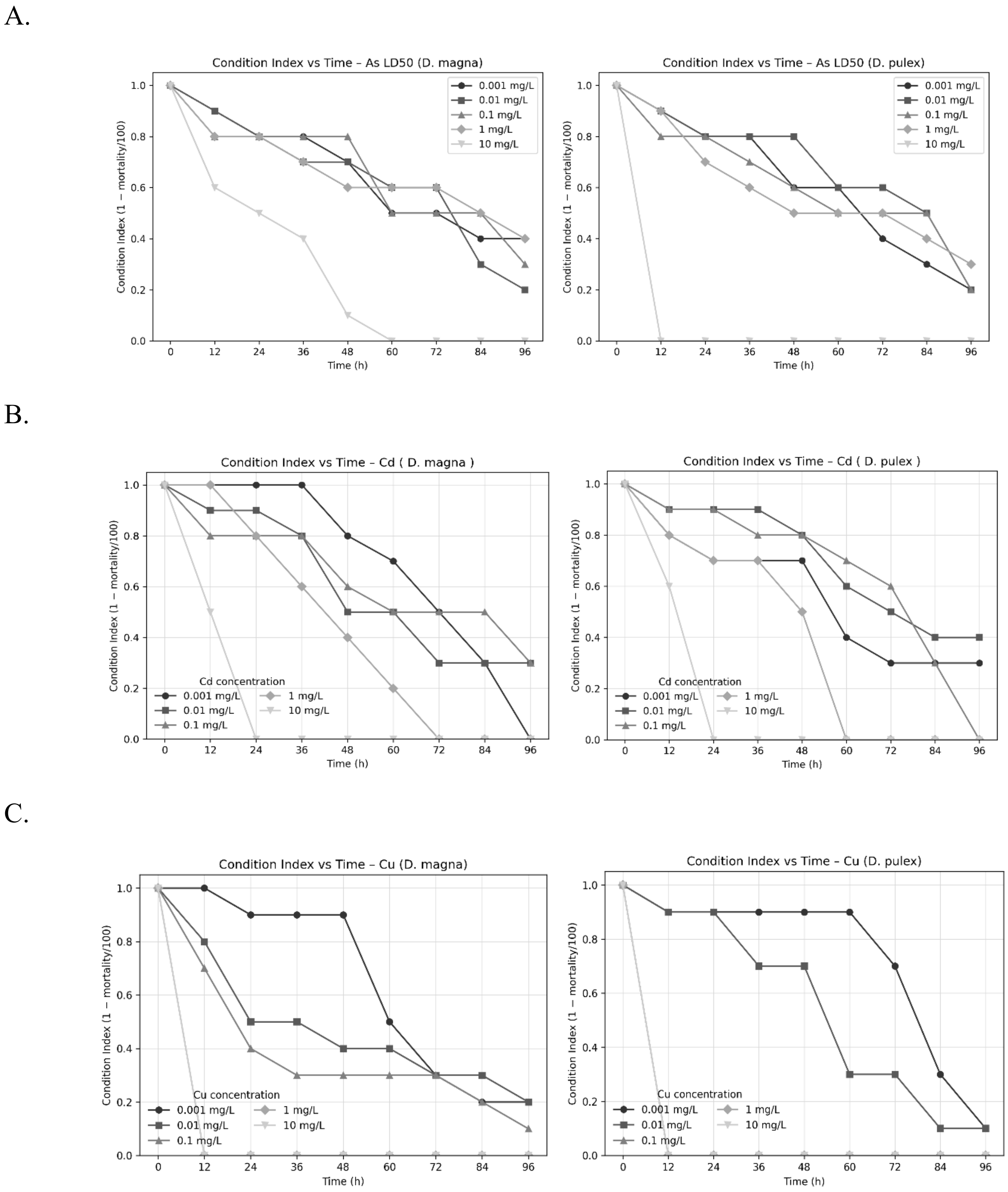

Figure 49: The health index of *Daphnia* spp. in five metal concentrations over 72 h. *D. magna* (left) and *D. pulex* (right) in untreated metal solutions of arsenic (A), cadmium (B), and copper (C). HH Combo control (●), 0.001 mg L⁻¹, (○), 0.01 mg L⁻¹ (▼•), 0.1 mg L⁻¹ (∆•), 1 mg L⁻¹ (■), and 10 mg L⁻¹ (□). (n = 3) ± standard error of the mean.

Cadmium exposure of Daphnids resulted in rapid declines in health indices, particularly after 48–60 hours of exposure. At higher concentrations, survival dropped sharply, indicating strong acute toxicity. Cadmium is known to disrupt calcium homeostasis and ion regulation, particularly in aquatic crustaceans, where calcium uptake is critical for physiological function. It also interferes with membrane permeability and enzyme systems involved in metabolism. The rapid reduction in CI observed in this study supports previous research demonstrating that cadmium is highly toxic to freshwater zooplankton, even at relatively low concentrations.

Copper exposure (figure 49) of both Daphnia species produced the steepest declines in condition indices among all tested metals. Survival decreased rapidly even at moderate concentrations, indicating that copper is one of the most toxic metals for both *Daphnia* species in this study. Copper toxicity is often associated with oxidative stress, damage to respiratory enzymes, and disruption of sodium and calcium transport across gill membranes. Additionally, in aquatic crustaceans, copper also interferes with osmoregulation and metabolic processes, leading to rapid physiological collapse at elevated concentrations. The pronounced reduction in CI observed in this study is consistent with numerous ecotoxicological studies identifying copper as one of the most toxic trace metals to freshwater invertebrates.

Figure 50 compares the health indices of *D. magna* and *D. pulex* in five concentrations of untreated metal solutions of Ni, Pb, and Zn over 96 h. Nickel exposure (Figure 50) produced moderate declines in condition indices, with toxicity becoming more pronounced at higher concentrations and later time points. Nickel toxicity mechanisms include disruption of enzyme activity, interference with cellular metabolism, and induction of oxidative stress. Compared to copper and cadmium, nickel tends to produce less immediate mortality, instead causing progressive physiological impairment. This pattern was evident in the CI plots, where survival declined steadily rather than abruptly.

Lead exposure (Figure 50) resulted in gradual reductions in CI, indicating a primarily chronic toxic response. Mortality increased with both exposure time and concentration, but the decline in health indices was less severe than observed for copper or cadmium. Lead toxicity in aquatic organisms is often associated with neurological impairment, inhibition of enzymatic processes, and interference with ion transport systems. These mechanisms may lead to longer-term physiological stress rather than immediate lethality, which explains the slower decline in CI observed in the plots.

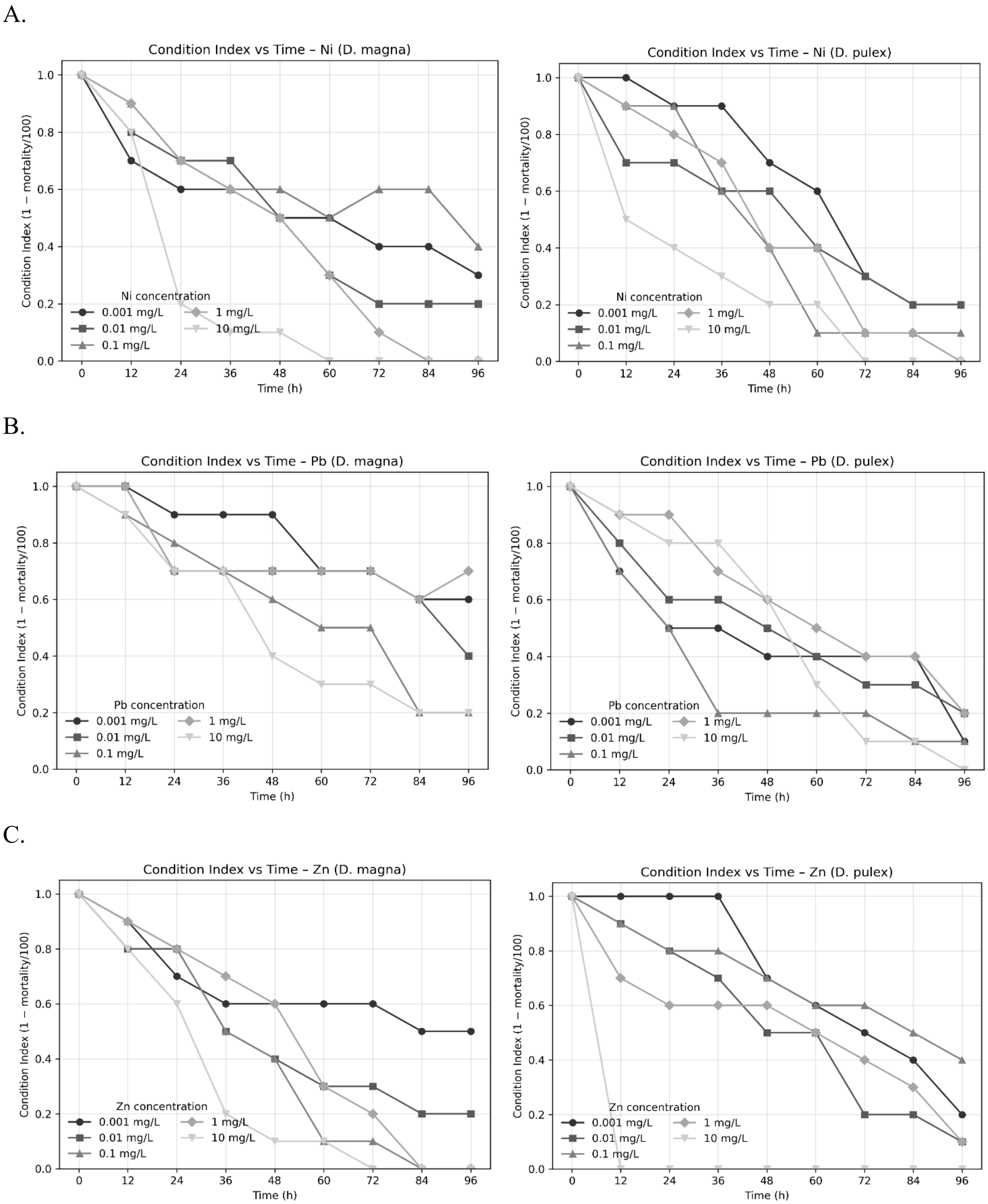

Figure 50: The health index of *Daphnia* spp. in five metal concentrations over 72 h. *D. magna* (left) and *D. pulex* (right) in untreated metal solutions of nickel (A), lead (B), and zinc (C). HH Combo control (●), 0.001 mg $L^{-1}$, (○), 0.01 mg $L^{-1}$ (▼), 0.1 mg $L^{-1}$ (∆), 1 mg $L^{-1}$ (■), and 10 mg $L^{-1}$ (□). (n = 3) ± standard error of the mean.

Figure 50 further shows that Zinc exhibited moderate toxicity, with CI values declining substantially at higher concentrations after prolonged exposure. However, survival remained relatively stable at low concentrations during early exposure periods. Unlike many of the other metals tested, zinc is an essential trace element required for normal biological function. As a result, organisms may tolerate low concentrations before toxicity occurs. However, elevated concentrations disrupt enzyme activity and metabolic processes, thereby reducing survival. The observed CI patterns reflect zinc's dual role as both an essential nutrient and a potential toxicant at high concentrations.

The temporal Patterns of Toxicity revealed by the CI plots fall into three general phases. The first was early exposure (0–24 h), which was characterised by minimal mortality for most metals, except at the highest concentrations. Organisms likely experience initial physiological stress yet survive. Secondly, the intermediate exposure (48–60 h) showed substantial declines in CI occurred for highly toxic metals such as copper and cadmium. This period likely represents the onset of acute toxicity. Finally, the late exposure (72–96 h) suggests that daphnid survival decreased dramatically at higher concentrations, particularly for metals with strong toxic effects. This phase reflects cumulative physiological damage leading to mortality. These patterns emphasize the importance of exposure duration in toxicity assessments, as some metals exhibit delayed toxic responses.

Figure 51 compares the health indices of *D. pulex* in untreated, RH-treated, and GAC-treated metal solutions of As, Cd, and Cu at five concentrations over 72 h. The health index (HI) plots illustrate the temporal physiological response of *Daphnia* exposed to arsenic (As), cadmium (Cd), and copper (Cu) across five concentrations under three treatment conditions: untreated metal solutions, rice husk biochar (RH) treated solutions, and granular activated carbon (GAC) treated solutions. The health index ranges from 0 to 1 and represents the proportion of surviving organisms relative to the initial population, thereby serving as a quantitative indicator of organismal stress and toxicity over time. Across all treatments, the HI values decrease over time, reflecting progressive physiological impairment and mortality under metal exposure. However, the magnitude and rate of decline vary substantially depending on both the metal type and the presence of sorbent-based pre-treatment.

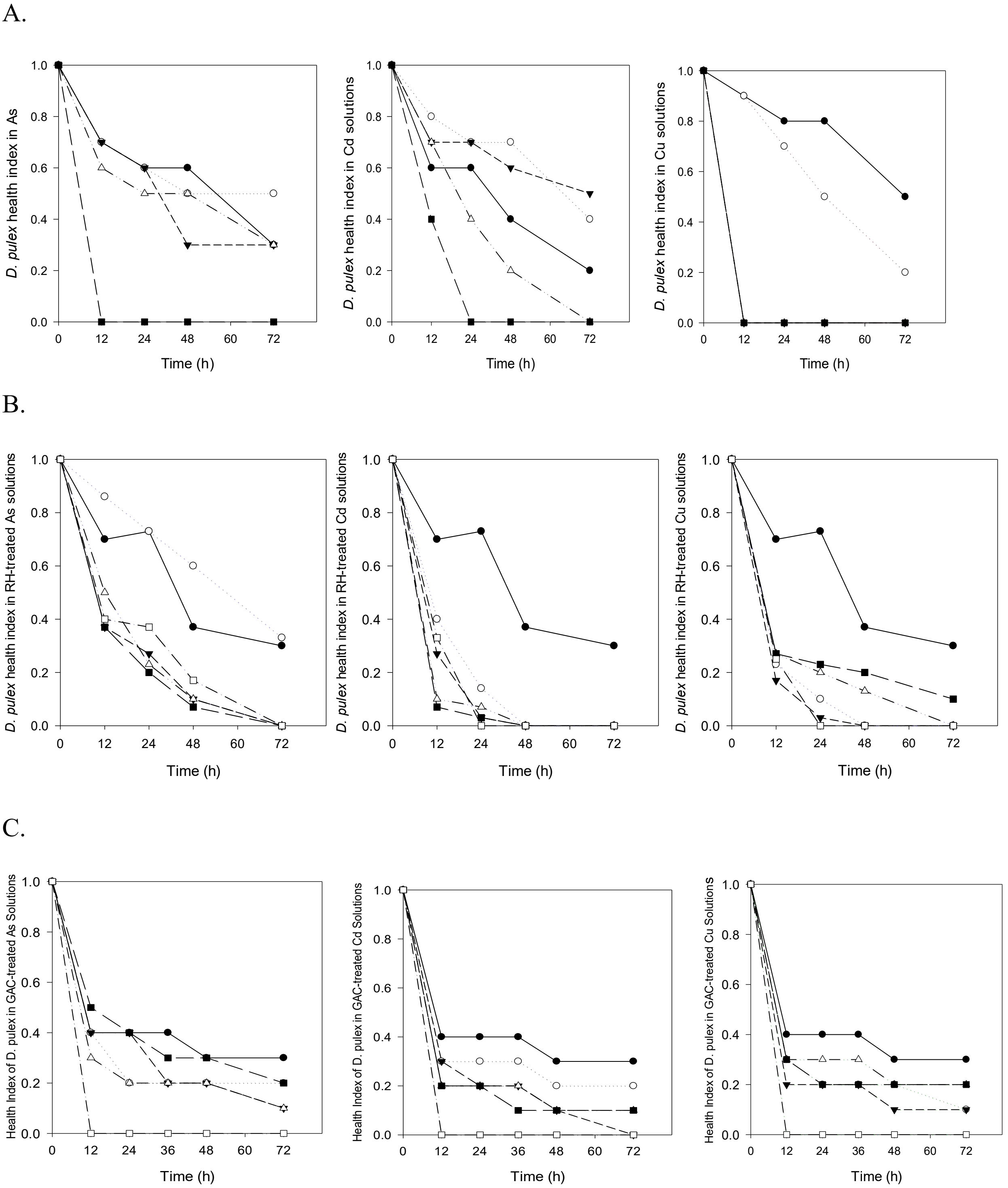

Figure 51: The health index of *Daphnia pulex* in five metal concentrations of untreated metal solutions (A), rice husk (RH)-treated (B), and GAC-treated (C), metal solutions of arsenic, cadmium, and copper. HH Combo control (●), 0.001 mg $L^{-1}$, (○), 0.01 mg $L^{-1}$ (▼), 0.1 mg $L^{-1}$ (∆), 1 mg $L^{-1}$ (■), and 10 mg $L^{-1}$ (□). (n = 3) ± standard error of the mean.

In Figure 51, the untreated solutions show a clear concentration-dependent decline in health index for all three metals. At the lowest concentrations (0.001 and 0.01 mg $L^{-1}$), health index values remain relatively high during the early exposure period but gradually decline by 72 h, indicating progressive physiological stress and mortality. This trend is particularly evident for arsenic and cadmium, where HI values decrease from approximately 0.7 at 12 h to values between 0.3 and 0.5 by 72 h. At higher concentrations (1 and 10 mg $L^{-1}$), the toxic effects are markedly more severe, with health index values declining rapidly to zero within 24–48 h. Copper appears to exhibit the most acute toxicity in the untreated solutions, with HI values approaching zero rapidly at moderate concentrations, suggesting strong physiological disruption even at relatively low exposure levels. The untreated datasets demonstrate a typical heavy metal toxicity profile, where increasing concentration leads to faster and more severe physiological decline. The sharp reductions in HI values at higher concentrations reflect rapid mortality and severe metabolic impairment, consistent with known mechanisms of metal toxicity, including oxidative stress, disruption of ion regulation, and interference with enzymatic systems.

Furthermore, the RH-treated solutions show markedly different behaviour. In contrast to the untreated systems, health index values decline more rapidly at early time points for several treatments. This pattern suggests that the RH treatment did not consistently reduce bioavailable metal concentrations in the exposure system and may have introduced additional environmental stress, possibly due to dissolved oxygen depletion. For arsenic exposures, the HI values decline sharply across all concentrations, reaching zero by 72 h even at the lowest concentrations. This suggests that either the RH treatment did not effectively remove arsenic from the solution or that residual metal concentrations remained sufficiently high to induce physiological stress.

Cadmium responses under RH treatment are even more pronounced. Health index values decline rapidly to near zero by 48 h across all concentrations, indicating severe toxicity and limited protective effect of the RH biochar. Similarly, copper exposures under RH treatment show rapid decreases in HI values, with several concentrations reaching zero before the end of the experiment. These results suggest that the adsorption capacity of rice husk biochar for these metals may be limited under the experimental conditions, potentially due to insufficient sorption capacity, saturation of adsorption sites, or metal desorption processes during exposure. These results further suggest that RH treatment in this system may have altered water chemistry, thereby increasing organismal stress. Potential explanations include the release of dissolved organic compounds from the biochar, changes in pH or ionic composition, or incomplete adsorption of the metal species.

Overall, the RH-treated results indicate that although biochar materials are often reported to reduce metal bioavailability, the effectiveness of such treatment can vary depending on the specific metal, biochar properties, and experimental conditions. In this case, the RH biochar did not consistently improve organismal health outcomes.

In contrast, the GAC-treated solutions exhibited a different response pattern compared with RH treatment. Although HI values still decline over time, the rate of decline is generally slower than in the RH-treated systems and, in some cases, comparable to or slightly better than the untreated exposures. For arsenic treatments, HI values stabilise between approximately 0.1 and 0.3 after the initial decline, suggesting that partial adsorption of arsenic may have reduced its effective bioavailability. Cadmium responses under GAC treatment also demonstrate partial mitigation, showing a similar trend, with moderate HI values maintained throughout much of the exposure period except at the highest concentrations. This indicates that GAC treatment mitigated metal toxicity, likely due to the strong adsorption capacity of activated carbon for dissolved metal ions and their associated complexes. This pattern suggests that GAC may reduce the effective bioavailable fraction of cadmium, slowing the progression of physiological stress. Copper responses under GAC treatment remain severe at higher concentrations, but lower concentrations show improved survival compared with untreated systems. This suggests that GAC adsorption may reduce copper bioavailability to some extent, although the metal’s strong toxicity still leads to substantial physiological effects.

Comparison between the three metals reveals important differences in their toxicological behaviour; clear differences emerge in both the magnitude and timing of toxicity effects. Untreated solutions show the expected concentration-dependent toxicity patterns, with rapid HI decline at higher metal concentrations. Arsenic exposures generally produce smoother declines in HI over time, suggesting progressive physiological impairment. Cadmium exposures, by contrast, display sharper reductions in HI at intermediate time points, consistent with cadmium’s well-known ability to disrupt enzymatic systems and induce oxidative stress rapidly. These mechanistic differences likely contribute to the variations in survival patterns observed across treatments. Among the three metals, copper appears to exhibit the most rapid toxicity, followed by cadmium, while arsenic shows slightly more gradual effects in untreated systems. This hierarchy of toxicity is consistent with previous studies demonstrating strong copper toxicity in freshwater invertebrates due to its disruption of ion transport mechanisms across epithelial membranes.

When comparing the two sorbent materials, Rice husk biochar treatment does not appear to provide consistent protection and, in several cases, results in health index trajectories that are similar to or worse than those of untreated systems. In contrast, GAC treatment demonstrates some capacity to moderate the toxic effects of certain metals, particularly at lower concentrations. Activated carbon has a highly developed pore structure and a very large surface area, enabling effective adsorption of metal ions and organic complexes. Rice husk biochar, while also porous, may have lower adsorption capacity or different surface chemistry depending on its production conditions. If the biochar was insufficiently activated or released soluble compounds, this could partially explain the lower health indices observed in the RH-treated systems.

Overall, the health index analysis highlights the strong influence of both metal concentration and sorbent treatment on organism survival. It suggests that metal exposure causes progressive physiological decline in *Daphnia*, with toxicity strongly influenced by concentration and exposure duration. Untreated exposures clearly demonstrate the expected dose-dependent toxicity of arsenic, cadmium, and copper, while the sorbent-treated systems illustrate how remediation materials can alter biological outcomes; their effectiveness depends on the sorbent's physicochemical properties. In this dataset, neither treatment completely eliminates toxic effects at higher concentrations. GAC treatment appears more effective at moderating toxicity, whereas rice husk biochar shows limited or inconsistent mitigation effects. These findings emphasise the importance of evaluating sorbent performance under realistic biological exposure conditions, as the ecological effectiveness of remediation materials depends not only on their adsorption capacity but also on their interactions with aquatic organisms and surrounding water chemistry. Even when adsorption processes remove a portion of dissolved metals, residual concentrations may still be sufficient to cause substantial physiological stress in sensitive aquatic organisms.

Across most metal exposures, *Daphnia pulex* appeared slightly more sensitive than Daphnia magna, as indicated by faster declines in condition indices and earlier onset of mortality at comparable concentrations. The differences in sensitivity between these species may be attributed to several biological factors, including differences in body size and metabolic rate, variation in metal uptake and accumulation, and species-specific detoxification mechanisms. Previous studies have reported similar differences in metal sensitivity between *Daphnia* species, supporting the use of multiple test organisms when assessing ecological risk. Overall, the metal toxicity results suggest that *D. pulex* may be more hesitant to respond, but once responses set in, it is more fragile than *D. magna* except in cases where extreme toxicity was species-specific.

## 5.4 Conclusions

In summary, *D. magna* is widely studied and has sufficient toxicity data for most metals, but there is little or no toxicity data on metals in *D. pulex* to compare with this study's results. *D. magna* seemed more sensitive to the toxic effects of the aqueous metals than *D. pulex* in the first few hours of exposure. However, it seemed to adapt to the toxicity and outlast *D. pulex* in the metal solution over the 72-96 h of the study without feeding. The LD50 and EC50 values for arsenic and its compounds vary but indicate high toxicity to *Daphnia* spp. However, the type of arsenic compound, exposure duration, and species significantly influence toxicity levels. The results of this study indicate that *D. magna* is more robust and can withstand metal toxicity for a longer period than *D. pulex* across all tested metal solutions. However, the results suggest that, compared to *D. magna*, *D. pulex* may be more hesitant to display responses, but once they set in, exhibit rapid progression.

Mano & Shinohara (2020) studied the acute toxicity of dissolved Ni on *D. magna* and suggested that the acute toxic effects of dissolved nickel on the mobility and survival of *D. magna* can vary among river waters with different water quality parameters, which affect nickel bioavailability. This applies to most aquatic pollutants, as water quality parameters influence their bioavailability. When considering the toxicity of metals to aquatic organisms, it is important to consider the bioavailable background metal concentrations present in culture media in the evaluation of the toxicity test results, especially when the toxicity data are used for water quality guideline derivation and/or ecological risk assessment for metals (Bossuyt & Janssen, 2003). This could explain the high sensitivity of *Daphnia* to some metals, namely Zn, Cu, and Ni, which were already added to their growth and maintenance media and subsequently used as the dilution water for this study.

In summary, the metal toxicity ranking (48 h) for *D. magna* followed the order Zn ≈ Cu > Ni > Cd > As >>> Pb, and for *D. pulex* it was Pb > Cu > Ni > Zn > As > Cd. Toxicity increases strongly with exposure time. Most metals show $LD_{50}$ values decreasing by several orders of magnitude between 48 h and 96 h. Nickel shows the highest chronic toxicity and produced the lowest $LD_{50}$ values at 96 h, indicating strong delayed toxicity. Copper and Zinc show strong acute toxicity. These metals already caused high mortality at 48 h, especially in *D. magna*. Lead shows species-specific toxicity; it was very toxic to *D. pulex* and less toxic to *D. magna* initially. *D. pulex* generally shows higher sensitivity to most metal toxicants, as $LD_{50}$ values are lower for *D. pulex* than for *D. magna*.

The observed declines in health indices suggest that metal contamination in freshwater ecosystems could significantly affect zooplankton populations, which play a crucial role in aquatic food webs. Because *Daphnia* species serve as key grazers of phytoplankton and as prey for fish and other organisms, reductions in their populations could have cascading ecological consequences. The strong toxicity observed for copper and cadmium indicates that these metals may pose particularly significant risks to freshwater ecosystems.

The condition index analysis demonstrated that exposure to dissolved metals resulted in significant declines in organism health and survival in both *Daphnia magna* and *Daphnia pulex*. Toxicity increased with both concentration and exposure time, with copper and cadmium producing the most pronounced effects. Arsenic and lead exhibited more gradual toxicity consistent with chronic exposure effects. Across the metals tested, toxicity patterns suggested the following decreasing order: Cu > Cd > Zn ≈ > Ni > Pb > As.

Copper and cadmium exhibited the most pronounced effects on organism health, while arsenic and lead produced more gradual declines in survival over time. Overall, *Daphnia pulex* showed slightly greater sensitivity to metal exposure than *Daphnia magna*, highlighting the importance of species-specific responses in ecotoxicological assessments.

# Chapter Six

# Respiration of *D. pulex* in Biochar-Treated and Untreated Aqueous Pesticide and Metal Solutions.

## 6.1 Introduction

Freshwater ecosystems are increasingly exposed to complex mixtures of contaminants arising from agricultural, industrial, and urban activities. Among the most prevalent pollutants are pesticides and trace metals, both of which can exert acute and sub-lethal effects on aquatic invertebrates. Herbicides such as Isoproturon and pyrethroid insecticides such as cypermethrin are widely detected in surface waters due to agricultural runoff and diffuse pollution (Stehle & Schulz, 2015; Silva et al., 2019). Similarly, dissolved metals including arsenic (As), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) persist in aquatic systems and are recognised for their toxicity, bioaccumulation potential, and ecological risk (Ali et al., 2019; Tchounwou et al., 2018). Although regulatory frameworks aim to limit environmental concentrations, episodic contamination events can result in short-term exposures that can induce physiological stress in aquatic organisms.

*Daphnia* spp. is widely used as a sentinel organism in aquatic ecotoxicology due to its ecological relevance, sensitivity to contaminants, and established role in regulatory testing (OECD, 2023). While traditional acute toxicity assessments focus on immobilisation or mortality endpoints, there is increasing recognition that sub-lethal physiological indicators, such as respiration rate, provide more sensitive and mechanistic insights into contaminant-induced stress (Barata et al., 2016; Jemec Kokalj et al., 2018). Respiration reflects metabolic activity and energy expenditure, and alterations in oxygen consumption or carbon dioxide production may indicate disruption of cellular respiration, ion regulation, or neurophysiological function prior to overt toxicity.

Pesticides and metals differ in their primary modes of toxic action, but both may interfere with metabolic processes. Pyrethroids such as cypermethrin primarily affect sodium channel function in nerve membranes, leading to neurotoxicity and increased metabolic demand (Bradberry et al., 2017). Isoproturon, a phenylurea herbicide, has been associated with oxidative stress and altered enzymatic activity in aquatic organisms (Silva et al., 2019). Trace metals can disrupt mitochondrial function, induce oxidative stress, and impair respiratory enzyme systems (Ali et al., 2019). Measurement of respiration, therefore, provides a functional endpoint that can detect early metabolic disturbances resulting from exposure.

Biochar has emerged as a promising remediation material for reducing contaminant bioavailability in aquatic environments. Produced by the pyrolysis of biomass, biochar has a high surface area and functional groups that can adsorb both organic contaminants and metal ions (Tan et al., 2020).

Recent studies demonstrate that biochar can significantly reduce the mobility and toxicity of pesticides and metals in aqueous systems through adsorption and complexation mechanisms (Lyu et al., 2018; Ahmad et al., 2021). However, chemical reduction in contaminant concentration does not necessarily equate to reduced biological stress. Direct assessment of physiological endpoints following biochar treatment is therefore necessary to evaluate the effectiveness of remediation.

Automated respirometry systems such as the Micro-Oxymax carbon dioxide monitoring system enable continuous measurement of $CO_2$ production as an indicator of metabolic rate. Such systems provide high-resolution, non-invasive monitoring of organismal respiration and are increasingly applied in aquatic ecotoxicology to quantify sub-lethal effects (Reid et al., 2019). By measuring carbon dioxide output, metabolic perturbations that precede immobilisation or mortality can be detected.

By comparing respiration rates across treatments, this study aims to determine whether biochar treatment reduces contaminant-induced metabolic stress and to evaluate respiration as a sensitive endpoint for assessing remediation performance. The findings contribute to improved understanding of sub-lethal ecotoxicological responses and the biological effectiveness of biochar-based water treatment strategies.

## 6.2 Materials and Methods

This chapter investigates the acute respiratory responses of *Daphnia* spp. exposed to biochar-treated and untreated aqueous solutions of Isoproturon, cypermethrin, and selected trace metals (As, Cd, Cu, Ni, Pb, Zn) using Micro-Oxymax $CO_2$ monitoring.

## 6.3 Results and Discussion

### *6.3.1 Respiration Studies of D. pulex in Pesticide Solutions*

This study began with a pilot experiment to determine the range of percentage and cumulative $CO_2$ production by different numbers of neonates and adult *D. pulex*. The results obtained after 24 h are displayed in Figure 52 A. Figure 52B displays the respiration trend for the same experiment over 60 h, and Figure 53 shows the respiration of fifty adult daphnids in different concentrations of DMSO solvents over 24 h.

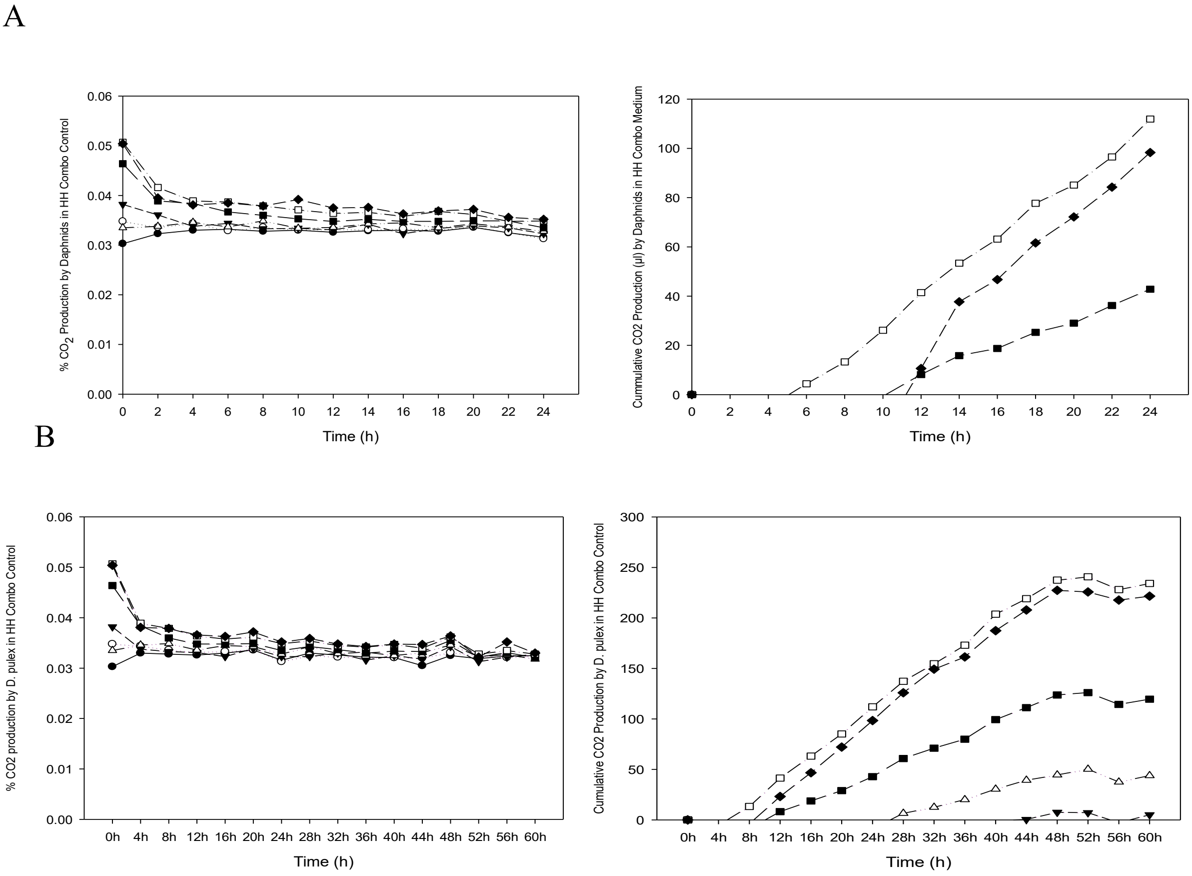

Figure 52: Respirometry results comparing the percentage and cumulative $CO_2$ production of five neonates (●), ten neonates (○), ten adults (▼), fifteen adults (Δ), twenty adults (■), thirty adults (□), and forty adults (♦) *D. pulex* in HH combo media (control solutions) over 24 and 60 h.

Figure 52A displays an observation over 24 h, and Figure 52B shows the measurements over 60 h to ensure that the respiration rates, percentages, and cumulative CO2 production were suitable for further experiments. However, the 24 h time scale was suitable for the reporting of respiration data.

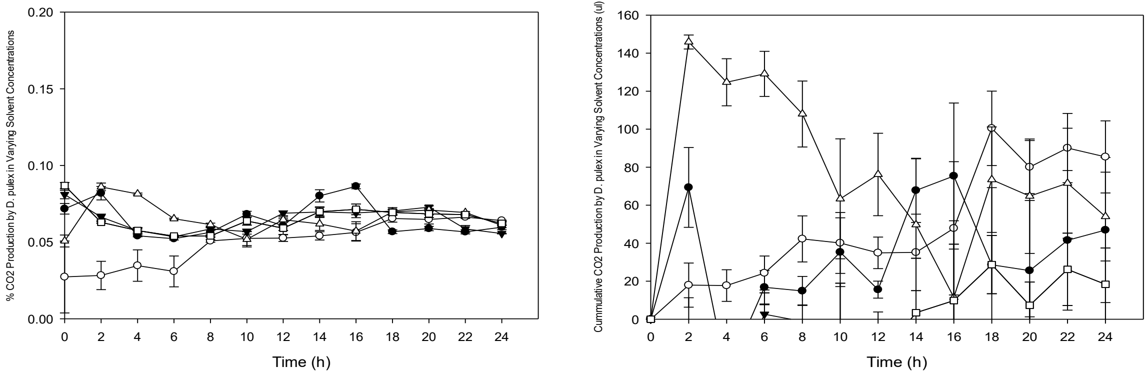

Figure 53: The percentage and cumulative $CO_2$ production (µl) of fifty adult *D. pulex* in different concentrations; 0.025% (●), 0.04% (○), 0.05% (▼), 2% (Δ), 5% (■), and 10% (□), of DMSO solvents in HH combo media (control solutions) over 24 h.

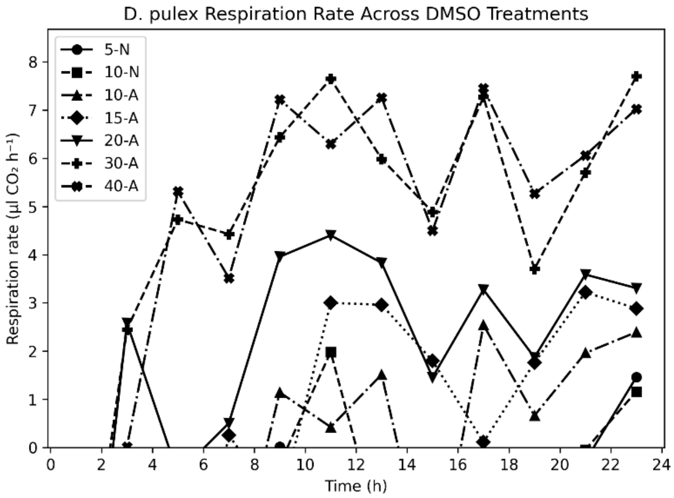

Figure 54: *D. pulex* Hourly Respiration Rates for Varying Populations in Closed HH Combo Control Systems. Where N= neonates and A= adults.

The respiration rate (µl $CO_2$ $h^{-1}$) in the closed-system pilot experiment using different Daphnia populations exhibited different patterns (Figure 54). Population systems with 5 and 10 neonates showed that the respiration rates stabilized quickly after early chamber equilibration. The rates approach ~0 µl $CO_2$ $h^{-1}$ by ~10–12 h, indicating metabolic steady state. Moderate daphnid numbers, i.e., 10 and 15 adults, exhibited an initially suppressed respiration with gradual recovery after ~12–16 h, suggesting physiological compensation, whilst high solvent daphnid numbers led to rapid transition to positive respiration rates after ~8–10 h, with the highest stimulation in the highest two daphnia population groups, indicating stress-induced metabolic stimulation.

In summary, the cumulative $CO_2$ production by 5 and 10 neonates was not detectable until around 60 h of observation. The cumulative $CO_2$ production by 10 and 15 daphnids was similarly below the detection limits of the micro-oxymax machine during the first 60 h of observation. However, the $CO_2$ produced by 20, 30, and 40 adult daphnids was measurable by the machine and followed similar trends: the percentage CO2 production was high at the beginning and decreased gradually, and the cumulative CO2 production increased gradually, peaking at 48 h. Finally, all cumulative $CO_2$ production values peaked at 48 h and became constant afterward.

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### *6.3.2 Respiration of D. pulex in Treated and Untreated Pesticide Solutions*

This study investigated the possible effects of biochar treatment of cypermethrin and Isoproturon on the respiration of adult *D. pulex*. Figures 51 and 52 show the percentage and cumulative $CO_2$ production of *D. pulex* in treated and rice husk biochar-treated solutions of Isoproturon and cypermethrin over 24 h, respectively.

The percentage of $CO_2$ production of daphnids in CP solutions ranged from 0.04 to 0.063% in CP and 0.051 to 0.097%. CO2 production in IPU solutions was higher than in CP solutions, and the highest percentage was observed at 2 h with 10 mg $L^{-1}$ of both pesticides. Overall, the highest percentage $CO_2$ production was observed within the first 2 h of *D. pulex* exposure in all untreated pesticide solutions. However, treated solutions followed the same trend but showed a slight increase in percentage $CO_2$ production between 22 and 24 h of *D. pulex* exposure to RH-biochar-treated pesticide solutions.

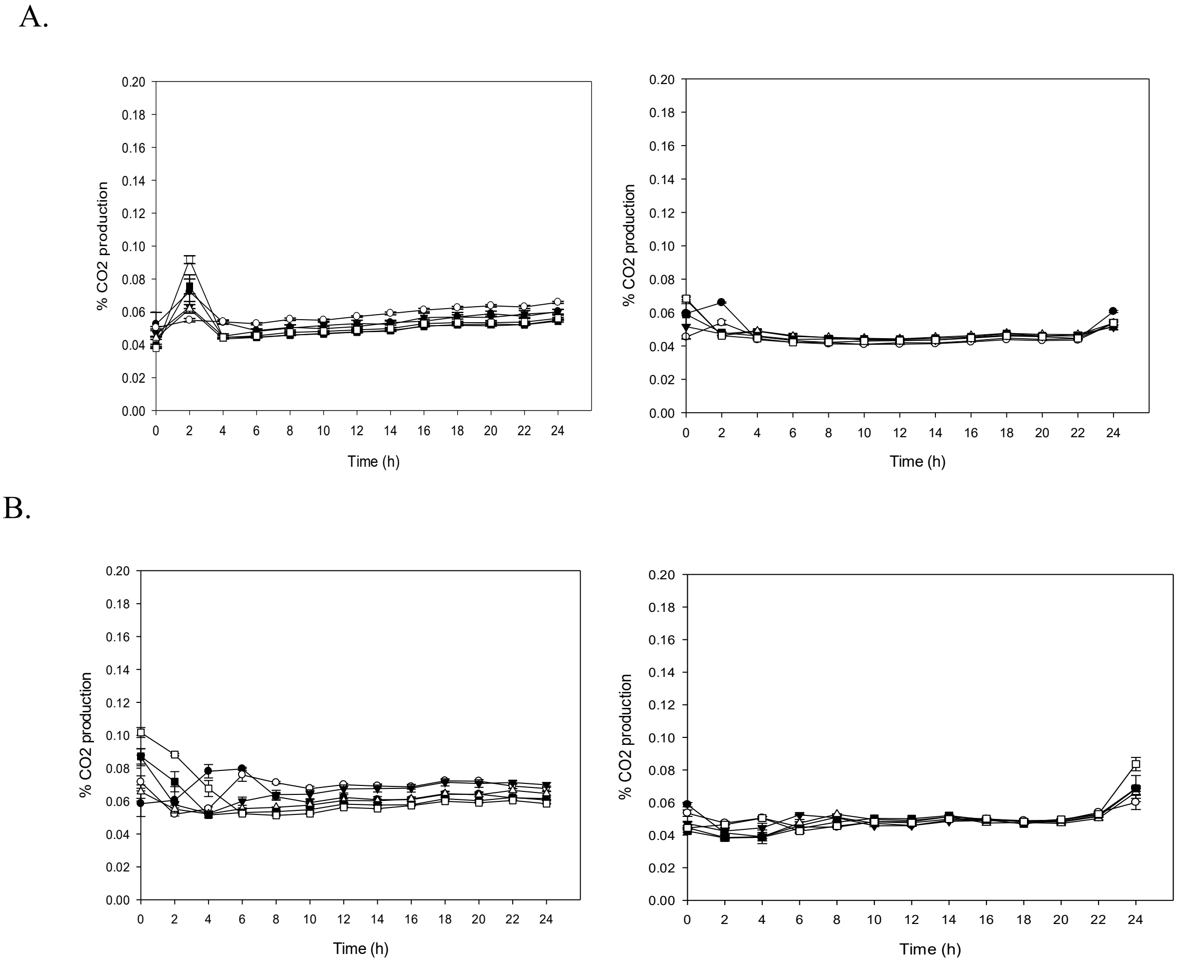

Figure 55: The percentage $CO_2$ production of fifty adult *D. pulex* in HH combo media control (●), DMSO control (○), and different concentrations; 0.001 mg $L^{-1}$ (▼), 0.01 mg $L^{-1}$ (Δ), 0.1 mg $L^{-1}$ (■), 1 mg $L^{-1}$ (□), and 10 mg $L^{-1}$ (♦) of untreated (left) and rice husk biochar-treated (right) cypermethrin (A) and Isoproturon (B) solutions over 24 h. Values are mean ± standard error, expressed in mg $L^{-1}$, (n = 3).

Overall, the percentage $CO_2$ production of *D. pulex* seemed to be slightly higher in untreated pesticide solutions than in RH-treated pesticide solutions as seen in Figure 55. Still, the cumulative CO2 production *D. pulex* in CP seemed to be improved by RH-biochar treatment (from approximately 60 to 100 µl). In comparison, the cumulative $CO_2$ production *D. pulex* in IPU solutions were lowered from approximately 120 to 30 µl by RH-biochar treatment.

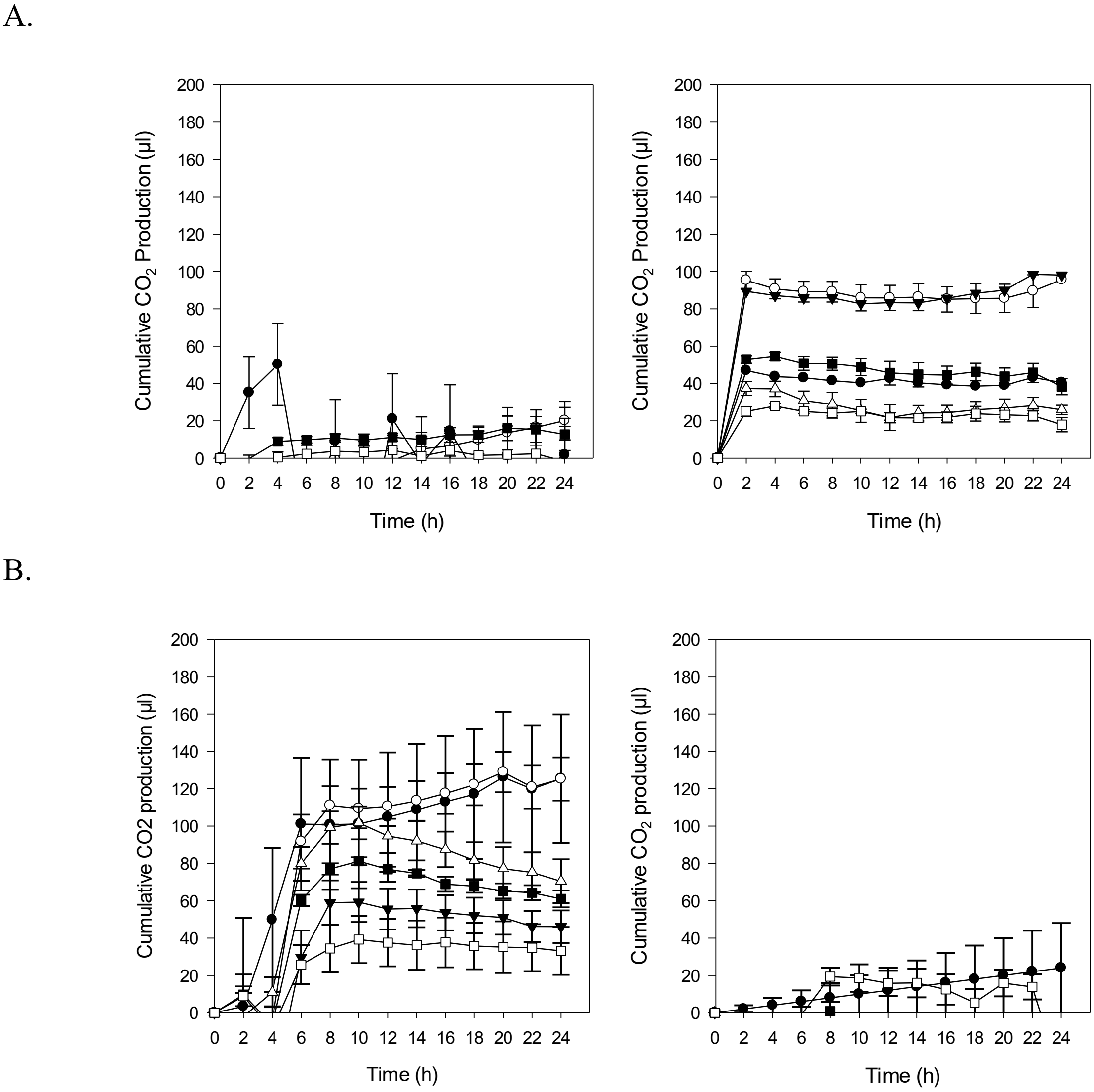

Figure 56: The cumulative $CO_2$ production of fifty adult *D. pulex* in HH combo media control (●), DMSO control (○), and different concentrations; 0.001 mg $L^{-1}$ (▼), 0.01 mg $L^{-1}$ (Δ), 0.1 mg $L^{-1}$ (■), 1 mg $L^{-1}$ (□), and 10 mg $L^{-1}$ (♦) of untreated (left) and rice husk biochar-treated (right) cypermethrin (A) and Isoproturon (B) solutions over 24 h.

Data obtained from Figure 56, at 0, 4,12 and 24 h was analysed using ANOVA and at 95% confidence level, the percentage $CO_2$ production of *D. pulex* in untreated solutions were only significant for 0.001 mg $L^{-1}$ CP at 4 and 12 h, 0.1 mg $L^{-1}$ at 4 and 24 h, 0.001 mg $L^{-1}$ at 4 h, 1 mg

$L^{-1}$ at 4 and 24 h, 10 mg $L^{-1}$ at 4h. In summary, the percentage $CO_2$ production of *D. pulex* differed significantly across most pesticide concentrations, especially at 0.001 mg $L^{-1}$ at 4h. The treatment of CP solutions with RH-biochar seemed to improve *D. pulex* respiration in these solutions (from approximately 60 to 100 µl), and the opposite was the case in IPU solutions, as the highest cumulative value of $CO_2$ dropped from approximately 120 to 30 µl.

As expected, the percentage and cumulative $CO_2$ production in IPU solutions were higher than in CP solutions, and the highest percentage $CO_2$ production was observed within the first 2 h of *D. pulex* exposure in all untreated pesticide solutions. This might be explained by the higher toxicity CP exhibits to Daphnia spp. compared to IPU. However, the highest percentage $CO_2$ production was observed at 2 h in 10 mg $l^{-1}$ of both pesticides. This can be explained by a stress response triggered by the high toxicity of the pesticides, as the daphnids might have increased cellular activity and metabolism to cope with the toxic stress. Another explanation is that daphnids may attempt to metabolise the pesticides, causing a temporary spike in their respiration.

Furthermore, treated solutions followed the same trend but showed a slight increase in percentage $CO_2$ production between 22-24 h of *D. magna* exposure to RH-biochar-treated pesticide solutions. This might have resulted from the acclimatization of these organisms to the toxic effects over the long exposure time of almost 24 h or the effects of hypoxia and oxygen demand; the organisms might have needed to increase their respiration activities to obtain enough oxygen in a rapidly oxygen-depleting environment (a closed system for almost 24 h). On the other hand, the observed improvement in the cumulative CO2 production of *D. pulex* in RH-biochar-treated CP solutions and the reduction in RH-treated IPU solutions might be explained by the different modes of toxicity of both pesticides. CP would have a more paralytic effect on *Daphnia,* thereby reducing respiration, while IPU would elicit metabolic stress, leading to an increase in respiration rates as Daphnia attempts to cope with the induced stress.

### *6.3.3. Respiration of D. pulex in Treated and Untreated Metal Solutions*

This study investigated the behaviour of adult *D. pulex* in rice husk biochar-treated arsenic and cadmium solution, as displayed in Figure 57 and 58. Data obtained from this experiment were analysed using ANOVA and Tukey's post hoc tests to determine significant differences at the concentration and chemical levels. The results obtained are interpreted below.

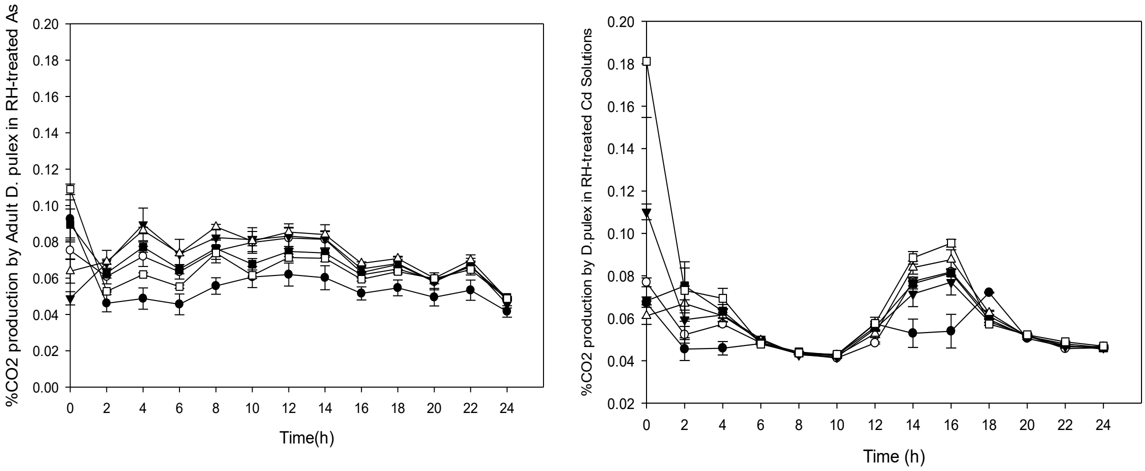

Figure 57: The percentage $CO_2$ production of fifty adult *D. pulex* in HH combo media control (●) and different concentrations; 0.001 mg $L^{-1}$ (○), 0.01 mg $L^{-1}$ (▼), 0.1 mg $L^{-1}$ (Δ),1 mg $L^{-1}$ (■), and 10 mg $L^{-1}$ (□) of rice husk biochar-treated arsenic (left) and cadmium (right) solutions over 24 h. Values are mean ± standard error, expressed in mg $L^{-1}$, (n = 3).

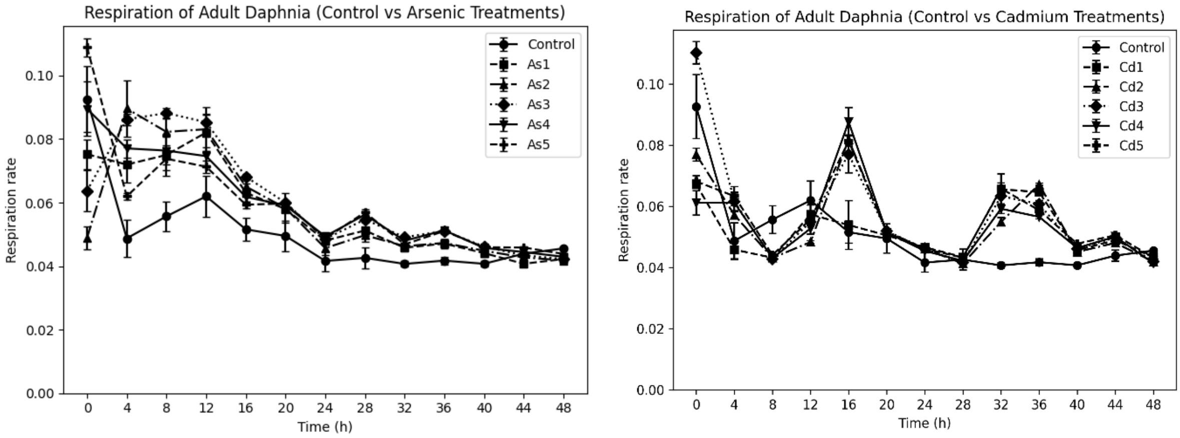

Figure 58: Respiration Rates of Adult D. pulex in Biochar-Treated Arsenic vs Cadmium Solutions over 48 h

The highest and lowest percentage $CO_2$ production (0.109) at 0h and (0.001) at 12h were in 10 mg $L^{-1}$ arsenic, and the highest and lowest percentage of $CO_2$ production was 0.181 for 10 mg $L^{-1}$ Cd at 0 h, and 0.036 for the HH combo control at 2 h after rice husk biochar treatment.

At 0 h, the percentage of $CO_2$ production in 10 mg $L^{-1}$ Cd was significantly higher ($p < 0.05$) compared to all other concentrations of pesticide, metal, and control solutions, respectively. There were no observable significant differences ($p > 0.05$) in the percentage of $CO_2$ production for the other concentrations, and they were not significantly different ($p > 0.05$) from the control solutions. At 4 h, the percentage of $CO_2$ produced under different concentrations did not differ significantly from that in the control. Also, a significantly similar percentage of $CO_2$ production was observed across all tested pesticide and metal concentrations.

At 8 h, there was a significantly higher percentage of $CO_2$ production ($p < 0.05$) observed in all Cd concentrations compared to the control, except 0.001 mg $L^{-1}$ Cd, which showed statistically similar $CO_2$ production with the control. However, there was no significant difference ($p > 0.05$) in the $CO_2$ production observed for all the Cd concentrations at this time interval. At 12 h, there was a significantly higher percentage of $CO_2$ production ($p < 0.05$) observed in 0.01 mg $L^{-1}$ Cd compared to the control and other concentrations, except for 0.1 mg $L^{-1}$ Cd, which showed a statistically similar percentage $CO_2$ production rate. No significant difference ($p > 0.05$) in $CO_2$ production was observed for the Cd concentrations (0.001, 0.1, 1, & 10) and control at this time interval

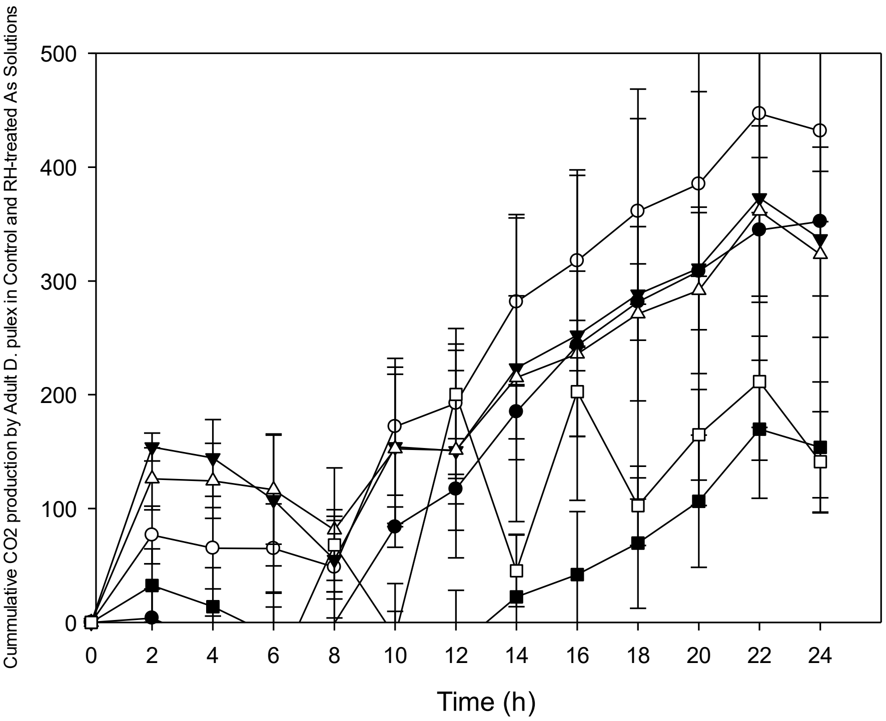

Figure 59: The cummulative $CO_2$ production of fifty adult *D. pulex* in HH combo media control (●) and different concentrations; 0.001 mg $L^{-1}$ (○), 0.01 mg $L^{-1}$ (▼), 0.1 mg $L^{-1}$ (Δ),1 mg $L^{-1}$ (■), and 10 mg $L^{-1}$ (□) of rice husk biochar-treated arsenic solutions over 24 h. Values are mean ± standard error, expressed in mg $L^{-1}$, (n = 3).

Figure 59 shows that, on average, *D. pulex* produced a higher percentage of cumulative $CO_2$ across all metal solutions studied, with approximately 500 µl, compared to pesticide solutions, which produced around 200-250 µl. Additionally, there is no graphical comparison of cumulative $CO_2$ production between arsenic and cadmium, as all recorded cadmium values, although high and within the range observed for arsenic, were negative. Overall, the highest and lowest cumulative $CO_2$ production of adult *D. pulex* in rice husk biochar-treated arsenic solution was observed in 0.01 mg $L^{-1}$ at 24 h and 10 mg $L^{-1}$ at 14 h, respectively. The highest and lowest control values were 127.7 µl at 24 h and 0 µl at 12 h, respectively.

The respiration of *D. pulex* in cadmium solutions was lower than in arsenic solutions, as there was no detectable cumulative $CO_2$ production at all concentrations of cadmium. This correlates with earlier toxicity findings in this research that cadmium is more toxic to *Daphnia* spp. than arsenic. Furthermore, cadmium elicited high $CO_2$ production in 10 mg $L^{-1}$ solutions within the first 2 h of exposure. This can be explained by the induction of toxic stress in *Daphnia* by cadmium. On average, *D. pulex* produced a higher percentage and cumulative CO2 in all metal solutions studied (approximately 500 µl cumulative) than in the pesticide solutions (approximately 200-250 µl cumulative). This buttresses the suggestion that *Daphnia* spp. is more sensitive to CP and IPU than the metals studied.

Additionally, acute respiration studies of *Daphnia* spp. in polluted and biochar pre-treated polluted water are critical for understanding the sublethal effects (Pérez *et al.*, 2020; Liang *et al.*, 2019) of pesticides and metals on the physiological health and disrupted metabolic functions (Hannas *et al.*, 2021; Strayer & Alexander, 2017) of these sensitive aquatic organisms. It also provides insights into the ecological impact and trophic dynamics of aquatic pollution in the ecosystem (Xu *et al.*, 2018; Schulz & Dabrowski, 2001).

The health and survival of *Daphnia* populations influence species at higher trophic levels, including fish and amphibians (Bodar *et al.*, 2017). High mortality rates in *Daphnia* can signal potential population declines across the food web, indicating ecosystem instability (Arrighetti & Ramírez, 2020). Additionally, *Daphnia* may become more prone to predation under stress from polluted water, thereby shifting the ecological balance in the aquatic ecosystem. Respiration studies can serve as an early warning system for water quality (Ahmed & Faggio, 2019; Santos *et al.*, 2019) and can help predict *Daphnia* adaptation and resistance to aquatic pollutants. Although respiration studies of *Daphnia* spp. It is critical for pollution monitoring and prediction models.

This study emphasises that it is not a standalone method for this purpose, as a high percentage and cumulative respiration are not signs of good health but could be signals of toxic stress.

Respiration measurements provide a sub-lethal toxicity indicator, often detecting stress earlier than mortality endpoints. Thus, the respiration-based CI and EC50 provide mechanistic insight, showing that metabolic impairment precedes organism death. Respiration rate is widely used as a sensitive physiological indicator of stress in aquatic invertebrates because it reflects the metabolic energy required to maintain cellular homeostasis under environmental disturbance. In the present study, respiration responses of adult *Daphnia* were measured over a 48-hour exposure period following treatment with arsenic (As) and cadmium (Cd). The plots generated for both metals illustrate temporal changes in respiration rate across multiple treatment levels relative to the control. These results provide insight into the sublethal physiological effects of metal exposure and the metabolic strategies employed by *Daphnia* to cope with toxic stress.

Because the population size was fixed at 50 organisms, the cumulative $CO_2$ curve represents the integrated metabolic output of the entire population. The RH-treated control shows a progressive increase in cumulative $CO_2$, reaching ~380 units by 48 h, indicating the absence of severe physiological stress, sustained metabolic activity, and stable respiration in the closed system. The average population respiration rate can be approximated as the highest value divided by the total population size: 381/50, which equals approximately 7.6 µl. Thus, the control population maintained a relatively constant metabolic throughput.

The commonly known mechanism of action for arsenic includes the inhibition of oxidative phosphorylation, interference with ATP production, and interference with mitochondrial electron transport. Because respiration represents the whole population's metabolism, these results indicate that arsenic affects energy allocation in the studied Daphnia population, that metabolic stress occurs before mortality endpoints, and that the respiration metrics provide early indicators of toxicity. This aligns with the ecotoxicology literature, in which respiration changes precede declines in survival and are consistent with toxicological hormesis.

Analysis of the cumulative Daphnia respiration data suggests a classic hormetic pattern: the control population exhibits more stable metabolic activity; lower arsenic concentrations stimulate respiration (hypermetabolism), as well as stress-induced elevation of respiration and/or detoxification energy demand, which are common sub-lethal toxic responses in aquatic invertebrates. The moderate concentrations produce metabolic compensation through increased respiration, whereas the high concentrations strongly suppress respiration through mitochondrial impairment or inhibition, preventing energy production and consequent reduced respiration capacity, indicating progressive physiological stress.

The results further indicate that arsenic causes progressive metabolic disruption in *Daphnia pulex*. The sequence of toxic effects appears to follow the sequence of physiological stress - increased metabolic demand - respiratory compensation - metabolic inhibition - eventual mortality. Thus, respiration-based metrics are sensitive indicators of early toxic stress in aquatic crustaceans. Across both arsenic and cadmium treatments, respiration rates followed a broadly similar temporal structure, comprising an initial disturbance phase, a mid-exposure metabolic peak, and a subsequent stabilisation phase. However, the magnitude and dynamics of these responses differed between the two metals, reflecting their distinct modes of toxic action.

At the start of the arsenic exposure, respiration rates were elevated in several treatment groups relative to the control, indicating an immediate physiological response to the toxicant. The highest arsenic treatments displayed the greatest metabolic elevation, suggesting that exposure triggered a rapid increase in metabolic demand. This early increase in respiration likely reflects the activation of detoxification pathways and stress response mechanisms, including increased ATP production, enhanced ion regulation, and the initiation of cellular repair processes. Arsenic is known to interfere with phosphate metabolism and disrupt mitochondrial oxidative phosphorylation, thereby reducing metabolic efficiency. Consequently, organisms must increase respiratory activity to compensate for impaired energy production.

During the intermediate exposure period, particularly between approximately 8 and 16 hours, respiration rates increased further in several arsenic treatments, producing a distinct metabolic peak around the midpoint of the experiment. This phase likely represents the period of greatest physiological stress, during which organisms mobilise energy reserves to support detoxification and maintain internal equilibrium. Such transient increases in metabolic rate are characteristic of sublethal toxicological responses in aquatic invertebrates, where organisms initially attempt to compensate for toxicant-induced disruption before reaching a new metabolic equilibrium.

Following this peak, respiration rates gradually declined and converged toward control levels. By approximately 24 h and continuing through to the end of the 48-h exposure period, respiration rates stabilised across treatments at values similar to those observed in the control group. This stabilisation suggests that *Daphnia* partially acclimated to arsenic exposure, restoring metabolic balance after the initial disturbance. Although arsenic clearly induced measurable physiological stress, the convergence of respiration rates suggests that the organisms retained sufficient physiological capacity to compensate for the toxicant over the course of the experiment.

In contrast to the arsenic response, cadmium exposure produced a more pronounced acute metabolic disturbance. The cadmium treatments exhibited elevated respiration rates at the start of the experiment, with the highest treatment exceeding those observed in the control group. Cadmium is a highly reactive heavy metal that exerts toxicity through several mechanisms, including protein binding, enzyme inhibition, and oxidative stress induction. These effects can rapidly impair cellular function, increasing metabolic demand as organisms attempt to counteract toxic damage.

The cadmium respiration profile was characterised by stronger oscillations during the early and intermediate exposure phases. A pronounced metabolic peak occurred at approximately 16 hours in several cadmium treatments. This peak was more distinct than that observed for arsenic, indicating a more intense physiological response. The increased respiration during this period likely reflects elevated energy expenditure associated with detoxification processes, including the synthesis of metallothioneins and other metal-binding proteins that help sequester cadmium within the organism. The production of these protective molecules requires considerable metabolic energy and therefore contributes to the observed increase in respiration.

After this peak period, respiration rates in the cadmium treatments declined and gradually approached the control baseline. From approximately 24 hours onward, respiration values across treatments stabilised within a relatively narrow range, suggesting that metabolic equilibrium had been re-established. Nevertheless, some cadmium treatments maintained slightly elevated respiration rates compared with the control, indicating that residual physiological stress may have persisted even after the acute response phase had subsided.

Comparison of the arsenic and cadmium respiration profiles reveals important differences in Daphnia's physiological responses to these metals. While both toxicants produced measurable metabolic disturbances, cadmium exposure resulted in a stronger and more variable respiration response, particularly during the early stages of exposure. This observation is consistent with the known biochemical behaviour of cadmium, which interacts strongly with proteins and enzymes and can disrupt multiple metabolic pathways simultaneously. Arsenic, by contrast, produced a more gradual metabolic response and a smoother return to baseline respiration levels.

The results also demonstrate that respiration rate is an effective sublethal indicator of metal-induced stress in freshwater invertebrates. Changes in metabolic respiration occurred well before any potential mortality effects would be expected, highlighting the sensitivity of this endpoint for detecting early physiological disruption. Such sublethal metabolic responses are ecologically significant because they can influence energy allocation within the organism. Increased metabolic expenditure associated with detoxification and stress responses may reduce the energy available for growth, reproduction, and other essential biological processes.

## 6.4 Conclusions

Regulatory agencies often require mortality studies on *Daphnia* to set permissible levels of pollutants, ensuring that these levels remain within ranges that prevent harmful effects on aquatic life (Barmentlo *et al.*, 2015; O'Brien *et al.*, 2017). As such, percentage mortality data are integral to developing water quality guidelines. Furthermore, the different performances of the three biochars studied, compared with GAC, provide insight into the need for research into the engineering of biochars to suit water amendment needs, depending on pollutant type.

*D. magna* is more robust and has shown the ability to withstand the effects of toxicity over a longer period than *D. pulex* in all water and solvent controls, tested pesticides, and metal concentrations, and control solutions without feeding. It is noteworthy that although RH biochar had the highest percentage adsorption of pesticides from solution than GAC and HW biochar, its use for the pre-treatment of pesticide and metal solutions seemed to elicit higher toxicity to *D. pulex*, except in CP solutions. Several factors might be responsible for this occurrence, including the possibility of RH biochar binding most of the oxygen species in the solutions and inducing stress due to low oxygen in the daphnids, the possibility of release of intrinsic pollutants with synergistic toxic effects on the daphnids, or the desorption of adsorbed pollutants back into solution.

The respiration analyses show that CP, IPU, arsenic, and cadmium induce measurable metabolic stress in adult *Daphnia*. However, cadmium exposure produced a more pronounced and variable metabolic response, while arsenic exposure resulted in a more gradual disturbance followed by relatively rapid stabilisation. These findings reinforce the value of respiration measurements as sensitive indicators of sublethal toxic effects and highlight the importance of metabolic endpoints for understanding the physiological consequences of heavy metal contamination in freshwater ecosystems. Furthermore, the responses elicited by *Daphnia*, in biochar pre-treated pesticide and metal solutions, would be expected responses to reduced toxicity and consequent metabolic disturbance.

Lower dissolved toxicant concentrations would decrease the physiological stress associated with detoxification processes, thereby reducing the metabolic energy required for ion regulation, oxidative stress mitigation, and repair of metal-induced cellular damage. Consequently, respiration rates in biochar-treated systems would likely remain closer to control levels and exhibit reduced temporal fluctuations compared with untreated aqueous toxicant exposures.
Notably, biochar also altered metal speciation and pesticide structures in solution, promoting the formation of less bioavailable complexes or precipitates. By reducing the concentration of free metal ions—the fraction most readily taken up by aquatic organisms—biochar treatment effectively lowers the toxicological pressure experienced by exposed organisms.

From an ecological perspective, sustained metabolic stress in *Daphnia* populations could have broader consequences for freshwater ecosystems. As a key component of aquatic food webs, *Daphnia* plays an important role in regulating phytoplankton populations and transferring energy to higher trophic levels. If heavy metal exposure leads to prolonged metabolic stress, reductions in reproductive output or survival could ultimately affect population stability and ecosystem functioning. The use of biochar as a pre-treatment or remediation material could therefore mitigate sublethal metabolic stress in aquatic invertebrates such as *Daphnia*. Reduced metabolic disruption would help maintain normal physiological functioning, preserving energy allocation for growth and reproduction and ultimately supporting population stability. These findings highlight the potential role of biochar-based remediation strategies in reducing the ecological impacts of heavy metal contamination in freshwater systems.

Another important outcome of this study is the demonstration of the importance of temporal resolution when assessing sublethal toxicological responses. The respiration peaks observed between approximately 12 and 16 h would not have been detected if measurements had been limited to only the beginning and end of the experiment. Continuous or high-frequency monitoring, therefore, provides a more accurate representation of physiological stress dynamics and allows identification of critical periods during which organisms experience the greatest metabolic challenge.

Overall, the data obtained from this study would be relevant in the tracking of acute aquatic pollution timelines, estimating the lethal and sublethal effects of biochar or activated carbon pre-treatment on the entire aquatic ecosystem, understanding the mechanisms of stress adaptation utilized by *Daphnia* spp. in polluted aquatic environments, and the development of guidance for worldwide aquatic pollution regulation for proactive safeguarding biodiversity and humans.

# Chapter Seven

# Conclusions and Future Work

## 7.1 Conclusions

The effects of aqueous pesticides and metals on non-target organisms cannot be overemphasized. This study investigated the adsorption percentage of different biochars produced by varying methods and feedstocks for the removal of aqueous C-14 IPU. The aqueous concentrations of pesticides and metals studied ranged from 100 times below to 100 times above the acceptable limits, mimicking severe spillage or pollution scenarios. The percentage adsorption of unmodified carbonaceous materials studied followed the trend: PAC > RH > HW > GAC > CS, with RH biochar adsorbing more than 90% of IPU from solution. Also, the alkaline nature of biochar increased the pH of all pesticide solutions when introduced, and the adsorption performance of RH and HW biochar is comparable to that of commercial GAC for water pollutants.

The toxicological effects of cypermethrin (CP) and Isoproturon (IPU) on *Daphnia* species (*D. magna* and *D. pulex*) were systematically investigated. At a 95% confidence level, the toxicity of both pesticides was significantly affected by concentration and exposure duration, contradicting some previous reports. All tested pesticide and metal solutions exhibited noticeable effects on Daphnids tested across all tested durations (72–96 h). i.e., No NOEC. The diluent, dimethyl sulfoxide (DMSO), exhibited toxicity proportional to its concentration in control solutions.

CP is a synthetic pyrethroid insecticide that targets voltage-gated sodium channels in nerve membranes, causing prolonged neuronal excitation, paralysis, and eventual mortality. It produced the most rapid declines in condition indices and survival, indicating strong acute toxicity to Daphnia, as demonstrated by its $LD_{50}$ and $EC_{50}$ analyses, particularly at longer exposure times. The steep response curves observed in the $EC_{50}$ plots are consistent with this neurotoxic mode of action, which often produces rapid immobilisation even at low concentrations. Condition index plots further showed that even relatively low concentrations produced progressive physiological deterioration over time, indicating that sub-lethal exposure still imposes substantial metabolic stress.

IPU is a phenylurea herbicide that inhibits photosystem II in plants but may indirectly affect aquatic invertebrates through interference with metabolic pathways and energy regulation. It exhibited lower acute toxicity than CP but still produced clear concentration-dependent effects on survival and organism health. The IPU $EC_{50}$ curves exhibited more gradual sigmoidal transitions, suggesting slower toxicodynamic responses than those of CP.

Condition index plots revealed progressive declines in organism health over extended exposure periods, indicating that IPU acts primarily through chronic toxicity mechanisms rather than rapid acute toxicity. These findings are consistent with previous ecotoxicological studies demonstrating that herbicides typically produce sub-lethal physiological stress rather than immediate lethal effects in zooplankton.

Among the tested metals, copper (Cu) was among the most toxic, producing rapid reductions in condition index and steep $EC_{50}$ responses. It's known mechanism of action impairs metabolic processes and osmoregulatory function, resulting in rapid mortality. The steep decline in the condition index observed for copper confirms its high acute toxicity to freshwater zooplankton. Cadmium (Cd) exhibited similarly strong toxicity, particularly after 48–72 h of exposure. The $LD_{50}$ and $EC_{50}$ analyses revealed pronounced mortality responses, while condition index curves showed rapid deterioration of organism health with increasing concentration. Cadmium, therefore, represents a high-risk contaminant in freshwater systems, particularly under conditions of chronic exposure.

Zinc demonstrated moderate toxicity, with condition indices declining gradually at lower concentrations but sharply at higher concentrations. Although zinc is an essential trace element, elevated concentrations disrupt enzyme activity and metabolic pathways, ultimately leading to mortality. The $EC_{50}$ plots indicated delayed toxicity relative to copper and cadmium, consistent with zinc's dual role as both a nutrient and a toxicant. Nickel exhibited moderate but progressive toxicity, characterised by gradual declines in condition indices and less steep $EC_{50}$ curves than those of copper and cadmium. Nickel toxicity is often associated with oxidative stress and interference with enzymatic processes, which may explain the slower onset of mortality observed in the experimental data.

Lead produced relatively slow declines in survival, suggesting predominantly chronic toxicity rather than acute lethal effects. The $LD_{50}$ and condition index analyses suggest that lead exposure causes gradual physiological impairment rather than rapid mortality. Arsenic exhibited the lowest acute toxicity among the tested metals, with condition index plots showing relatively slow declines in organism health. Arsenic toxicity typically arises from interference with ATP production and energy metabolism, resulting in delayed physiological stress. The gradual reduction in CI observed in the experiments supports the interpretation that arsenic acts primarily as a chronic toxicant under the tested conditions.

Across most treatments, Daphnia pulex generally exhibited slightly greater sensitivity than Daphnia magna. This pattern was evident in lower $EC_{50}$ values, faster declines in condition indices, and earlier onset of mortality. CP demonstrated approximately 100-fold greater toxicity than IPU in Daphnia, with *D. pulex* being about ten times more sensitive than *D. magna* to both pesticides. Additionally, *D. magna* showed greater resistance to IPU compared to CP and did not exhibit complete mortality at any IPU concentration within 72 h of exposure without feeding. Compared with *Daphnia magna, Daphnia pulex* showed slightly greater sensitivity to cypermethrin, suggesting species-specific differences in pesticide uptake, detoxification capacity, or neurological susceptibility. Species differences may arise from variation in metabolic rates, body size, and surface area-to-volume ratio, metal uptake, and detoxification mechanisms. These findings highlight the importance of using multiple test species in aquatic toxicity assessments.

Pre-treating pesticide solutions with biochar and activated carbon decreased *D. pulex* mortality rates. Despite RH biochar exhibiting the highest IPU adsorption, granular activated carbon (GAC) was more effective in reducing solution toxicity to *D. pulex*, particularly for CP. The investigated metals—arsenic (As), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn)—are prevalent in industrial effluents. Their toxicity to Daphnia species decreased in the order: Ni > Cd ≈ Cu ≈ As > Zn > Pb. *D. pulex* was generally more sensitive than *D. magna*, except in response to As and Zn, and Cu was the most toxic metal for both species.

From an integrated toxicity perspective, the combined $LD_{50}$, $EC_{50}$, and condition index analyses demonstrate clear concentration-dependent and time-dependent toxicity for both pesticides and metals in *Daphnia magna* and *Daphnia pulex*. Across treatments, organism health declined progressively with exposure time and contaminant concentration, reflecting cumulative physiological stress that led to immobilisation and mortality. The integrated results indicate that pesticides (cypermethrin and Isoproturon) generally exhibited stronger acute toxicity than most metals, whereas metals displayed variable chronic toxicity depending on their physicochemical properties and biological interactions. Across all tested substances, toxicity generally increased with exposure duration, confirming the importance of temporal dynamics in aquatic toxicology experiments.

Pre-treatment of metal solutions with RH biochar and GAC significantly mitigated metal toxicity but induced hypoxia in the organisms. Acute toxicity and declining oxygen levels triggered increased respiration during initial (0–4 h) and final (22–24 h) observation periods. These findings highlight that respiration studies, while informative, should be integrated with additional endpoints to fully characterize organismal toxic responses. Respiration rates in *D. pulex* were modulated by pollutant bioavailability, mechanism of action, and the species' acclimatization capacity.

The health index of *Daphnia* in all pesticide and metal test solutions provided a clearer illustration of the toxic responses of these organisms to these pollutants and corresponds with the percentage mortality and survival of the organisms over time. This study suggests that CP and IPU were more toxic to *Daphnia* than all the metals studied, with Cu the most toxic. Suffice it to say that *D. magna* are tougher than *D. pulex* in their adaptation to toxic pesticide and metal solution exposures - *D. magna* showed an immediate slow response to toxic pesticides and metals but acclimatized and showed the ability to withstand the effects of toxicity over a longer period than *D. pulex,* which exhibited delayed responses and quicker mortality in all tested pesticide and metal concentrations. They are suitable for experiments without feeding for more than 24 h and can thrive in the absence of sufficient food for up to 72-96 h.

The effective and lethal concentrations of CP, IPU, and the heavy metals studied are relevant in determining the predictive value of these pollutants and identifying toxic exposure thresholds (Elphick *et al.*, 2011; Arrighetti & Ramírez, 2020). *Daphnia* serves as a reliable metric for ongoing environmental monitoring programs. Researchers can track changes in pollution levels and detect emerging contaminants by periodically assessing mortality rates in water samples from different sites. Consistent mortality data over time aids in identifying pollution trends and can prompt timely responses to pollution events (O'Brien *et al.*, 2017). This would contribute to the development of robust environmental risk assessments, monitoring, and protection matrices. In summary, *D. magna* is more robust and has shown the ability to withstand the effects of toxicity over a longer period than *D. pulex* in all water and solvent controls, tested pesticides, and metal concentrations, and control solutions without feeding.

Integrating $LD_{50}$, $EC_{50}$, and condition index analyses in the evaluation of Daphnia ecotoxicity suggests that the toxicity of all tested pesticides and metals followed the decreasing order Cypermethrin > Cadmium ≈ Copper > Zinc ≈ Nickel > Lead > Arsenic > Isoproturon. This ranking reflects both acute and chronic toxicity patterns observed in the experiments. The observed toxicity responses demonstrate that both pesticides and metals can significantly affect zooplankton survival and physiological health, potentially disrupting aquatic ecosystems. Because *Daphnia* species play a central role in freshwater food webs as grazers and prey organisms, contaminant-induced mortality could lead to cascading ecological effects, including altered phytoplankton dynamics and reduced energy transfer to higher trophic levels. The integrated toxicity analysis indicates that cypermethrin is the most hazardous compound tested, producing rapid and severe toxic responses in both *Daphnia* species. Among the metals, cadmium and copper were the most toxic, while arsenic and lead produced slower chronic effects. Condition index analysis proved particularly valuable for revealing sub-lethal physiological stress preceding mortality, complementing traditional $LD_{50}$ and $EC_{50}$ endpoints. Overall, the results demonstrate the importance of combining multiple toxicity metrics to obtain a comprehensive understanding of contaminant effects in aquatic organisms.

Finally, biochar is a promising adsorbent for soil and water amendment. In soil, it degrades and adsorbs pollutants; in water, it adsorbs them. It requires controlled production processes and environments to achieve optimal results, as it behaves differently across varying environmental conditions and media. Further research into the optimal production and environmental parameters for different individual and multi-element pollutants, and a robust study of their potential toxic effects on bioindicators, are essential for the successful use of biochar for bioremediation. This implies a need for guidelines on the recommended maximum levels of contaminants that can be introduced to the environment through biochar applications (Singh et *al.,* 2014).

Notably, this study confirms that biochar can exhibit different functions in water remediation, depending on the dominant production conditions, the physicochemical properties of the water or soil, the chemical compounds present, and the pedoclimatic conditions. It is therefore pertinent to consider the intended use, the pros and cons of biochar, and the production conditions to make an informed decision on its application for water_remediation. Finally, the inconsistencies in reports on biochar applications in bioremediation and other potential future uses in industry underscore the importance of biochar engineering to suit its intended purposes (Zhelezova et *al.*, 2017).

## 7.2 Limitations and Future Research

### 7.2.1. Limitations

The limitations of this research include not studying the physicochemical properties of all the carbonaceous materials before and after pesticide adsorption as planned. To overcome this challenge, data from previous research were employed. Secondly, the chemistry underlying adsorption and studies using different biochars and activated carbons are not described using adsorption models. Due to the number of compounds studied, many in-depth experiments, such as sorption-desorption and the effects of varying physicochemical parameters, could not be completed. Finally, although studying single compounds in solution provides critical insight into their toxicity to aquatic biota and the consequences for the food chain, the results may not be applicable to real-world situations in which pollutants occur in mixtures. Future research to address these limitations is suggested below.

### 7.2.2 Future Research

Rice husk showed the highest adsorption performance for all single pesticide and metal concentrations treated. Given the coexistence of water pollutants, further field studies using water from polluted rivers and from waste treatment plant discharges, and laboratory studies on multi- and mixed-pesticide and metal solutions using this biochar could yield interesting findings for water treatment. Furthermore, longer-term studies of biochar as a water amendment are key to better understanding its behaviour over extended periods in aquatic environments, as its behaviour in soil has been widely studied and established (Vijayaraghavan, 2019). Further studies of the adsorption behaviour of different biochars, in correlation with their production conditions and characterization, are encouraged to better understand this complex relationship.

Mortality studies can also reveal the effects of multiple pollutants combined, a common occurrence in natural water bodies. Interactions between pollutants can sometimes lead to synergistic effects, in which the combined toxicity exceeds the sum of the individual toxicities. Studying mortality rates under different pollutant combinations provides critical insights into these effects, helping to assess real-world risk scenarios (Pane *et al.*, 2004; Bodar *et al.*, 2017). Additionally, the investigation of environmental factors, such as pH and temperature, in combination with co-contaminants, should be studied using RH biochar.

Toxicity studies employing *D. magna* are well established; however, studies on *D. pulex* are few and require more attention, given their seemingly higher (10-fold) sensitivity compared to *D. magna*. Other sub-lethal and chronic effects of toxicity should be studied in *D. pulex* to better understand their physiological and metabolic responses to aquatic pollutants. Pulse (short-term) and long-term exposure studies of the physiological and metabolic responses to pesticides would determine whether *Daphnia* populations develop adaptive responses to chronic pollutant exposure

(Cedergreen & Rasmussen, 2017). Observing respiration over time helps researchers determine whether *Daphnia* can develop resistance to specific contaminants or whether cumulative exposure leads to persistent declines in health (Martins *et al.*, 2020; Santos *et al.*, 2019). Respiration studies of *D. pulex* over longer periods with feeding should be studied. Additionally, further studies should examine these species' sensitivity to a range of aquatic contaminants.

More research is needed to develop stable biochar materials for safe use in water treatment. Regarding perspectives on the topic, opinions on the use of biochar for pesticide adsorption from water are mixed. Proponents argue that biochar offers a sustainable, cost-effective solution for removing pesticides from water, thereby protecting human health and the environment. They highlight its potential to reduce pesticide contamination in water bodies and improve water quality for various uses. However, critics raise concerns about the long-term stability and effectiveness of biochar in removing pesticides, as well as potential risks associated with the release of adsorbed pesticides back into the environment and the subsequent effects on the metabolism and survival of soil flora and fauna, and of aquatic organisms such as *Daphnia* spp.

Finally, there have been research suggestions that focus on enzyme stimulation rather than microbes for degradation. Further research in this direction, in addition to studies of the interactions between herbicides and target and non-target organisms, would better inform policymakers on herbicide and ecotoxicological risk assessments. In conclusion, in-depth studies on potential methods for standardizing biochar production are required before large-scale field applications of biochar for water remediation. Additionally, it is essential to fully understand the potential adverse consequences that accompany the numerous benefits of biochar use in bioremediation, both in the short and long term.

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# Appendix I: All Procedures used for Daphnia Culturing

**UNIVERSITY OF BIRMINGHAM**

**SCHOOL OF BIOSCIENCES, *DAPHNIA* FACILITY**

**STANDARD OPERATING PROCEDURE**

**SOP No.1 VERSION #1**

## PREPARATION OF HIGH-HARDNESS COMBO (MODIFIED) MEDIUM

**SOP No. 1 VERS #1**

The purpose of this Standard Operating Procedure (SOP) is to describe the preparation of High Hardness COMBO (modified) medium.

### Preparation of aqueous stock solutions

All stock solutions are to be prepared in volumetric glassware and using deionised water ($dH_2O$). The quality of the $dH_2O$ must be 15MΩ.

| No# | Compound | Chemical Formula | Concentration (g/L) | Notes |
|---|---|---|---|---|
| 1 | Calcium chloride dihydrate | $CaCl_2.2H_2O$ | 110.28 | |
| 2 | Magnesium sulphate heptahydrate | $MgSO_4.7H_2O$ | 113.5 | ≡ 55.45 $MgSO_4$ |
| 3 | Potassium phosphate dibasic | $K_2HPO_4$ | 1.742 | |
| 4 | Sodium nitrate | $NaNO_3$ | 17.0 | |
| 5 | Sodium metasilicate nonahydrate | $Na_2SiO_3.9H_2O$ | 13.267 | ≥98% purity |
| 6 | Boric acid | $H_3BO3$ | 24.0 | Requires heating |
| 7 | Potassium chloride | KCl | 5.96 | |
| 8 | Sodium bicarbonate | $NaHCO_3$ | 63.0 | |
| 9 | Sodium selenite | Na $SeO_3$ | 40 µg/l | |

**Preparation of Animate stock solutions**

All stock solutions are to be prepared in volumetric glassware and using deionised water ($dH_2O$). The quality of the $dH_2O$ must be 15MΩ.

| No# | Compound | Chemical Formula | Concentration (g/L) | Notes |
|---|---|---|---|---|
| 10 | Lithium chloride | LiCl | 31 | Store at -20°C |
| 11 | Rubidium chloride | RbCl | 7.0 | Store at -20°C |
| 12 | Strontium chloride hexahydrate | $SrCl_2.6H_2O$ | 15 | Store at -20°C |
| 13 | Sodium bromide | NaBr | 1.6 | Store at -20°C |
| 14 | Potassium iodide | KI | 0.33 | Store at -20°C |

All stock solutions are to be dispensed into 1.5 ml aliquots in Eppendorf tubes and stored at -20°C.

To prepare the Animate stock solution add 1 ml of each of stock solutions 10 to 14 to approximately 500 ml of $dH_2O$ and the volume adjusted to 1 litre. The Animate stock solution is stored at approximately +5°C prior to use.

## Preparation of Vitamin stock solution (VIM)

All stock solutions are to be prepared using deionised water ($dH_2O$). The quality of the $dH_2O$ must be 15MΩ.

| No.# | Compound | | |
|---|---|---|---|
| 15 | Biotin (*d*-biotin) | 10 mg into 96 ml of $dH_2O$ | Aliquot to 1.5 ml and store at -20°C |
| 16 | $B_{12}$ (cyanocobalamin) | 10 mg into 89 ml of $dH_2O$ | Aliquot to 1.5 ml and cover with foil, keep sterile and store at -20°C |

To prepare the vitamin solution, remove one aliquot of Biotin and $B_{12}$ from the freezer at - 20°C and allow to defrost. To a 50 ml volumetric flask, add 30 ml of $dH_2O$, 0.5 ml biotin, 0.5 ml $B_{12}$ and 10 mg of Thiamine HCl. Adjust the volume to 50 ml with $dH_2O$ then transfer the whole volume to a Duran bottle covered with foil to shield from light and store at approximately +5°C in a fridge for up to a maximum of 1 week.

Optionally the stock solution can be prepared using autoclaved $dH_2O$ but do not autoclave the VIM after preparation.

## Preparation of High Hardness COMBO (modified) medium.

To approximately 3.5 litres of $dH_2O$ add 4 ml of stock solutions 1 to 7, 8 ml of stock solution 8 and 200 µl of stock solution 9 and adjust the volume to 4 litres.

Aerate the medium for approximately 24 hours then adjust the pH to 7.6 – 7.8 prior to the addition of 4 ml of Animate and 2 ml of VIM stock solutions.

Alternative volumes of medium can be prepared with the appropriate adjustment to the volumes of stock solutions used.

**Approved by:** Ian Sewell

**Signature:** Ian Sewell

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## UNIVERSITY OF BIRMINGHAM

## SCHOOL OF BIOSCIENCES, *DAPHNIA* FACILITY

## STANDARD OPERATING PROCEDURE

## SOP No.3 VERSION #1

# PREPARATION OF BOLD’S BASAL MEDIUM (BBM)

## SOP No.31 VERS #1

The purpose of this Standard Operating Procedure (SOP) is to describe the preparation of Bold's Basal Medium (BBM).

## Preparation of aqueous stock solutions

All stock solutions are to be prepared in volumetric glassware and using deionised water ($dH_2O$). The quality of the $dH_2O$ must be 15MΩ.

| No# | Compound | Chemical Formula | Concentration (g/L) | Volume added to final 5L volume |
|---|---|---|---|---|
| 1* | Potassium di-hydrogen orthophosphate | $KH_2PO_4$ | 17.50 | 50 |
| 2* | di-potassium hydrogen orthophosphate | $K_2HPO_4$ | 7.50 | 50 |
| 3* | Magnesium sulphate heptahydrate | $MgSO_4.7H_2O$ | 7.50 | 50 |
| 4* | Sodium nitrate | $NaNO_3$ | 25.00 | 50 |
| 5* | Calcium chloride dihydrate | $CaCl_2.2H_2O$ | 2.50 | 50 |
| 6* | Sodium chloride | NaCl | 2.50 | 50 |
| $7^+$ | EDTA tetrasodium salt<br>+Potassium hydroxide | EDTA-$Na_4$<br>KOH | 50.00<br>31.00 | 5 |
| $_8$+ | Ferrous sulphate heptahydrate<br>+ conc. Sulphuric acid | $FeSO_4.7H_2O$<br>$H_2SO_4$ | 4.98<br>10 ml/L | 5 |
| $9^+$ | Boric acid | $H_3BO_3$ | 11.42 | 5 |
| $10^+$ | Zinc sulphate heptahydrate | $ZnSO_4.7H_2O$ | 14.12 | 0.5 |
| $11^+$ | Manganese chloride tetrahydrate | $MnCl_2.4H_2O$ | 2.32 | 0.5 |
| $12^+$ | Copper sulphate pentahydrate | $CuSO_4.5H_2O$ | 2.25 | 0.5 |
| $13^+$ | Cobaltous nitrate hexahydrate | $Co(NO_3)_2.6H_2O$ | 0.80 | 0.5 |
| $14^+$ | Sodium molybdate dihydrate | $Na_2MoO_4.2H_2O$ | 1.92 | 0.5 |

*Volume prepared 1 litre

- Volume prepared 100 ml

## Preparation of Bold's Basal Medium (BBM).

To approximately 3.5 litres of $dH_2O$ add 50 ml of stock solutions 1 to 6, 5 ml of stock solutions 7 to 9 and 0.5 ml of stock solutions 10 to 14 and adjust the volume to 5 litres. In order to avoid precipitation of the chemicals, each stock solution should be added separately and the flask shaken to mix, prior to addition of the next stock solution. Stocks do not need autoclaving as this will be performed on the final medium prepared.

Adjust the pH to 6.6 (6.4 – 6.8 acceptable range) with HCl (1M or 0.1M) or 1M NaOH.

Alternative volumes of medium can be prepared with the appropriate adjustment to the volumes of stock solutions used.

The medium is then dispensed to 2L conical flasks prior to autoclaving.

The usage of this medium can be high. Therefore if less than 100 ml of stock solutions 1 – 6 and less than 10 ml of stock solutions 7 – 9 remains after preparation then fresh stock solutions should be made for subsequent users.

**Approved by:** Ian Sewell

**Signed:** Ian Sewell

UNIVERSITY OF BIRMINGHAM
SCHOOL OF BIOSCIENCES, DAPHNIA FACILITY

STANDARD OPERATING PROCEDURE

SOP No.5 VERSION #2

# ALGAL CULTURE

The purpose of this Standard Operating Procedure (SOP) is to describe the culture of algae in the facility.

Culture of Algae

The algal species cultured will be Chlorella vulgaris. Other species may be cultured for specific experimental purposes.

The culture of algae will generally be performed once weekly with preparation and harvesting on Mondays. Alternatively, if there is greater demand for algae then preparation and harvesting can be done on Monday and Thursday mornings. For holiday periods the regime will be altered accordingly. The number of flasks prepared will be dependent on demand in the laboratory.

Harvesting algae – Make up new cultures by inoculating freshly autoclaved BBM medium in conical flasks from two or more good existing cultures by adding an aliquot of algae to each flask. Decant the remaining algal culture into clean beakers and cover with cling-film to prevent aerial contamination. The aliquot volume added to the fresh medium will normally be 20 – 25 mL for 7 days incubation. Volumes for alternative incubation periods will be 100 – 110 mL on Monday, for a 3-day incubation period and 75 – 80 mL on Thursday, for a four day incubation period. Other volumes may be used for other incubation periods.

Aerate the culture flasks vigorously using fresh autoclaved aeration tubes.

After use place the used conical flasks in a sink and add ≈1 teaspoon of sodium bicarbonate. Fill with hot water then place used aeration tubes in the flasks. Leave to soak for > 1 hour.

Clean conical flasks and aeration tubes and leave to dry.

Centrifugation of Algae

Centrifuge the algae in 500 ml centrifuge tubes using a Sorval Centrifuge (located in room S203 off S204) at 3500 rpm for 15 mins using the rotor SLA3000, min temp +4oC and max temp +9oC. The rotor can be found in the corner cupboard in room S204.

After centrifugation, decant off the supernatant and re-suspend the concentrated algae in the minimum amount of modified standard Combo then decant into a clean beaker. In order to ensure the maximum amount of algae is recovered rinse the centrifuge tubes with minimum amount of standard Combo so as not to over dilute, then add this to the collection beaker.

After centrifugation measure the optical density (OD) using a spectrophotometer. Measure the absorbance of a 1 in 10 dilution of the algal concentrate at 440 nm using 1 cm cuvettes. Dilute with standard Combo until the absorbance is approx. 0.80.

After adjustment to the correct OD, decant the algae into 1L Duran bottles and store in the fridge at +4oC.

Clean the centrifuge tubes and leave to dry.

Preparation of Fresh Algal Medium

Prepare 10 litres, either in 10 L or 2 x 5 L volumes of BBM medium (SOP No.3) in 10 L aspirators or 5 L Duran bottles and dispense (approx. 1650 mL) to an appropriate number of conical flasks and autoclave. The volume prepared will vary dependent on the demand for experimental procedures. Prepare above on Monday morning and autoclave ready for Thursday and repeat preparation on Thursday morning (pick up from Central Services Friday) ready for Monday morning.

Approved by: Ian Sewell

Signed:

# UNIVERSITY OF BIRMINGHAM
# SCHOOL OF BIOSCIENCES, *DAPHNIA* FACILITY

## STANDARD OPERATING PROCEDURE

## SOP No.7 VERSION #1

## *DAPHNIA* CULTURE

The purpose of this Standard Operating Procedure (SOP) is to describe the culture of *Daphnia* clones held in the facility.

## **<u>Culture of *Daphnia* held at 10°C</u>**

The stock cultures of *Daphnia* sp. clones are held in Room NB8 at a temperature of 10°C. The cultures will be maintained at this temperature unless otherwise advised.

Two replicate cultures of each clone will be maintained in bottled mineral water or borehole water in glass jars of nominal volume 200 - 250 ml with loosely fitted lids. Replicates 1 and 2 of each clone will be stored on separate shelves. Each individual vessel will be labelled with the species, clone and replicate number to ensure unique identification of each vessel.

The cultures will have the culture medium changed once every four weeks. The time between medium changes can be shortened or extended for specific purposes such as during holiday periods. However the feeding regime should be adjusted accordingly to prevent build-up of uneaten algae and bacterial growth. Clean culture vessels will be filled with approximately 50 – 75% of volume with fresh medium and daphnids from the old cultures poured over into the fresh medium. If insufficient numbers of daphnids are poured over then they can be transferred by glass pipette. Ephyppia should not be transferred to the new cultures.

Each culture will be fed once per week. The amount of concentrated algae fed to each culture vessel will typically be as follows

| | |
|---|---|
| *Daphnia magna* | 400 - 500 µl |
| *Daphnia pulex, pulicaria, longicephala* | 300 - 400 µl |

The volume of concentrated algae fed to the cultures can be varied depending on the number, age and life stage of the daphnids. The amount fed should be such that the culture medium has a very faint green tinge at the end of the feeding period.

New cultures will be established using neonates from second or third broods. If one replicate culture is lost then the replicate can be re-established using neonates or adult daphnids from the other replicate. This action will be recorded in the culture record book held in the laboratory.

Cultures known as the zoo will have the culture medium changed once every four weeks and fed twice per week on Monday and Friday. 250 ml vessels will be fed approx. 6 drops of concentrated algae from a plastic Pasteur pipette and smaller (~100 ml) vessels 2 drops

## Culture of *Daphnia* held at 20°C

The stock cultures of *Daphnia* sp. clones held in Room NB9 will be maintained at a temperature of 20°C. The cultures will be maintained at this temperature unless otherwise advised.

Stock cultures of each clone will be maintained in an appropriate medium in glass jars of nominal volume between 200 mL and 5 litres with loosely fitted lids or clingfilm. The size of culture vessel will depend on the number of daphniids and experimental requirements for neonates. Each individual vessel will be labelled with the species, clone and replicate number to ensure unique identification of each vessel.

Depending on experimental requirements the cultures will have the culture medium changed once, twice (e.g. Monday and Thursday) or three times (e.g. Monday, Wednesday, Friday) per week. The time between medium changes can be shortened or extended for specific purposes such as during holiday periods. However the feeding regime should be adjusted accordingly to prevent build-up of uneaten algae and bacterial growth. Clean culture vessels will be filled with fresh medium and daphniids from the old cultures poured or pipetted over into the fresh medium. Ephyppia should not be transferred to the new cultures.

Each culture will be fed during the water change period. The volume of concentrated algae fed to the cultures can be varied depending on the number, age and life stage of the daphnids but generally the amount fed will be 2 mL of concentrated algae per 1 litre of medium with a loading rate of 20 daphnids/L. Alternatively the amount fed should be such that the culture medium has a very faint green tinge at the end of the feeding period. This amount can be determined from observation of the cultures and how long it takes for the daphnids to clear the algae.

An alternative feeding regime would be 20 neonates per 1 litre of medium and fed 1.0 ml of algae on days 1-2, 1.5 ml algae on days 3-7 and 2.0 ml on day 8+. Algal rations are doubled on Friday to ensure sufficient food over a weekend. New cultures will be established using neonates from second or third broods.

**Approved by**: Ian Sewell

**Signed:**

# Appendix II: Conventional and Sustainable Remediation Methods for Aquatic Pollutants

## Remediation of Polluted Aquatic Environments

Organic (pesticide) and inorganic (metal) pollutants can be removed from the environment by physical, chemical, or biological methods. Removing pesticides from water and wastewater is a major environmental challenge worldwide, as significant amounts of pesticides and their metabolites have been found in surface and groundwater bodies (Ahmad *et. al.,* 2010).

Organics removal from water usually involves adsorption or degradation of the compounds. Fenton oxidation ($H_2O_2/Fe^{2+}$) and Fenton-like oxidation ($H_2O_2/Fe^{3+}$) are widely employed for the oxidative degradation of EDCs and other organics from wastewater (Xu *et. al.,* 2004; Gotvajn & Zagorc-Koncan, 2005; Lucas & Peres, 2009; Mendez-Arriaga *et. al.,* 2010).

Conventional water treatment methods effectively ensure safe drinking water and protect environmental health. Techniques like coagulation, sedimentation, membrane filtration, and disinfection (Zinicovsaia, 2016; Crini & Lichtfouse, 2018) are commonly used to purify water and wastewater contaminated with pesticides and heavy metals (Demessie *et al.*, 2015; Mohan and Singh, 2002; Khan *et al.*, 2004; Das *et al.*; Saikaew & Kaewsarn, 2009; Zein *et al.*, 2010). Many of these methods use a multi-step approach. For instance, coagulation and sedimentation (Gul Zaman *et al.*, 2021) may be followed by filtration and disinfection with chlorine or ultraviolet light to remove pathogens before distribution (Collignarelli *et al.*, 2017). A key benefit of conventional methods is their reliability. They are cost-effective compared to advanced technologies (Adeleye *et al.*, 2016) and have consistently provided safe water over decades (Saravanan *et al.*, 2021). Established practices like chlorination are widely used in municipal water systems (Hasan & Muhammad, 2020).

Conventional water treatment methods face criticism, especially regarding their environmental impact. The use of chemical coagulants and disinfectants can produce harmful by-products like trihalomethanes, which may pose carcinogenic risks (Jiménez *et al.*, 2018; Miklos *et al.*, 2018). Additionally, these chemicals can create health risks for workers and increase the likelihood of accidental spillages (Hanafi & Sapawe, 2020). These treatment systems are also energy-intensive, contributing to their carbon footprint and highlighting the need for more sustainable practices (Rout *et al.*, 2021). Furthermore, conventional methods are often ineffective against emerging pollutants such as pharmaceuticals and microplastics (Nasiri *et al.*, 2017; Eniola *et al.*, 2022), raising concerns amid rising pollution levels.

To combat these issues, advanced treatment methods like membrane technologies and advanced oxidation processes have been developed. However, they come with higher costs and complex procedures, particularly at low metal concentrations (Obotey Ezugbe & Rathilal, 2020; Zajda & Aleksander-Kwaterczak, 2019; Grassi *et al.*, 2012; Saeed *et al.*, 2005). This highlights the need for safer alternatives that maintain efficiency while reducing risks. High water treatment costs—financial and environmental—have led to a focus on developing cheaper, effective methods and pollutant removal technologies. Research now emphasizes sustainable approaches, including waste recycling (Park *et al.*, 2015; Singh *et al.*, 2015; Ahmad *et al.*, 2014; Mohan *et al.*, 2014; Yakout *et al.*, 2013). Innovations in wastewater treatment, such as biological treatments and membrane filtration, aim not just to clean water but also to enable its reuse for agricultural and industrial needs.

Green remediation techniques (Singh *et al.*, 2017) offer integrated solutions for pesticide pollution while promoting ecosystem health. Sustainable adsorbents (Ponnuchamy *et al.*, 2021) and bioremediation methods (Hussain *et al.*, 2021) are effective approaches for degrading organic pollutants. In conclusion, while conventional water treatment methods are reliable, their environmental impact and limitations in addressing emerging contaminants necessitate re-evaluation. Balancing public safety, environmental sustainability, and technological innovation is crucial for the future of water treatment (Zeng *et al.*, 2023; Crini *et al.*, 2019). This focus will significantly shape future water management, particularly in regions facing water stress, such as many African countries

(OECD, 2022). The following section discusses sustainable water remediation techniques.

### 1.7.1. Phytoremediation

*Phytoremediation* is a sustainable technology that uses plant species to remove pollutants from water bodies, particularly pesticides and heavy metals (Bawa-Allah, 2023). It involves using plants to absorb and detoxify heavy metals (Abdel-Shafy et al., 2018). Research indicates that it is an ecologically sustainable method (Bhat *et. al.*, 2022; Haq *et. al.*, 2020), promoting ecological balance and sustainability (Jeevanantham *et al.*, 2019; Kanwar *et al.*, 2020).

The effectiveness of this approach lies in its mechanisms, such as the absorption, accumulation, and transformation of contaminants (Nedjimi, 2021; Saxena *et al.*, 2020), and it is effective for several metals, but may be limited by plant growth conditions and the time required for remediation (Zhang *et. al.*, 2023). Aquatic macrophytes, such as Typha Latifolia and Eichhornia Crassipes, play crucial roles in mitigating toxic water pollution. These plants have demonstrated the ability to absorb heavy metals and effectively degrade pesticide residues (Anand *et al.*, 2019; Bhalla *et al.*, 2022). In addition, the integration of phytoremediation technologies, particularly using specific plant species, is essential for environmental remediation efforts (Takkar *et al.*, 2022; Sumiahadi & Acar, 2018).

### 1.7.2. Biodegradation and biosorption

Complementing phytoremediation, microbial technologies have become another viable alternative (Zehra *et. al.*, 2020). Bioremediation techniques have emerged as effective strategies to eliminate pesticides and heavy metals from contaminated water sources. Several methods, including microbial remediation, use bacteria, fungi, and algae to degrade pollutants (Hussain *et al.*, 2021).

The factors that influence the effectiveness of bioremediation include environmental conditions and microbial tension selection (Zhang *et al.*, 2020; Ahmad *et. al.*, 2020). Challenges such as pollutant toxicity and variable degradation rates (Tarfeen *et al.*, 2022) should be addressed. Successful applications include a microalgae consortium that effectively eliminates pesticides and heavy metals from wastewater (Abdel-Razek *et al.*, 2019). In addition, actinobacteria show promise in this field, highlighting the need for more research to optimise bioremediation techniques (Alvarez *et al.*, 2017; Karimi *et al.*, 2022).

Furthermore, using biochar in combination with microorganisms has proven valuable in detoxifying contaminated environments (Manikandan *et al.*, 2023). Recent studies indicate substantial progress in addressing pesticide waste through bioremediation, which offers a sustainable approach to environmental management (Giri *et al.*, 2021). Additionally, microbial agents are crucial in mitigating heavy metals (Medfu Tarekegn *et al.*, 2020). Therefore, synthesising various biological strategies offers a promising direction for environmental cleaning efforts (Singhal *et al.*, 2021).

In general, integrating different bioremediation techniques is essential to improve the effectiveness of eliminating water pollutants (al., 2020; Singh et *al.*, 2022). In addition, the application of nanotechnology can improve the efficiency of heavy metal elimination, making it a promising research field (Ekrami et al., 2022; Tatarchuk et *al.*, 2019). Despite the effectiveness of these techniques, their widespread implementation depends on economic viability and public acceptance, highlighting the need for integrated evaluations of environmental impacts (Gaur et al., 2021; Azubuike et al., 2020; Azam et *al.*, 2021).