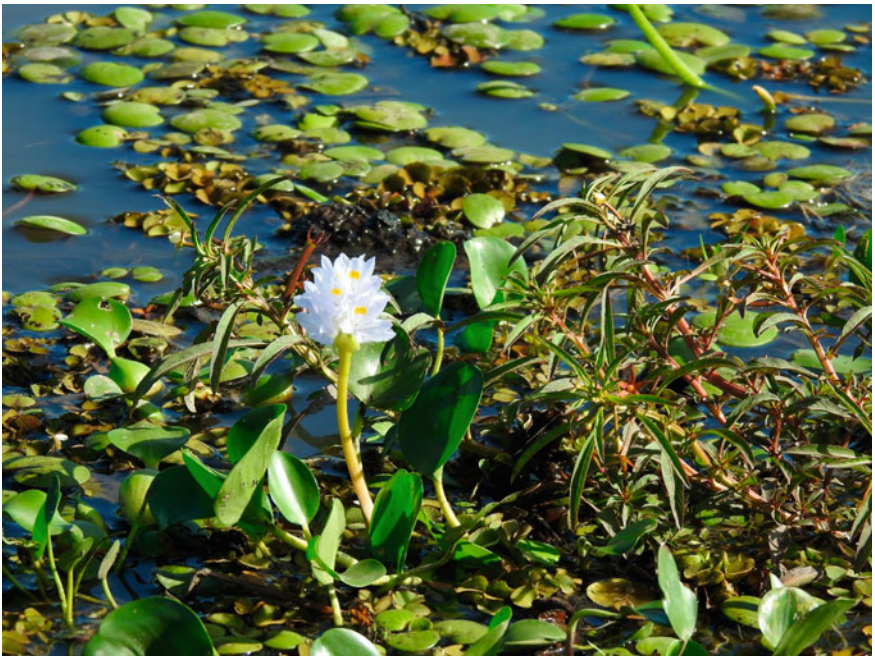

Chapter 1
The Ecotoxicology of Aquatic Macrophytes: An Overview

Mirta L. Menone, Braedon W. Humeniuk, and Chris D. Metcalfe

Abstract Aquatic macrophytes are a morphologically and physiologically diverse group of vascular plants that are distributed all over the world in a variety of aquatic habitats. They provide a range of ecological services, as well as habitat for aquatic vertebrates and invertebrates, and are important primary producers that support both herbivores and detritivores. Aquatic macrophytes are exposed to a range of contaminants of both geogenic and anthropogenic origin. In order to protect aquatic ecosystems from the impacts of these contaminants, toxicity studies with species of aquatic macrophytes should be essential components of ecological risk assessments. This chapter provides an overview of the challenges and the opportunities for ecotoxicology studies using aquatic macrophytes and provides an introduction to the more detailed reviews and reports in subsequent chapters of the book.

1.1 Introduction

Aquatic macrophytes constitute an assemblage of taxonomically diverse macroscopic plants that are characterized by a life cycle that takes place completely or partially in the aquatic environment. Macrophytes have evolved mechanisms that allow them to adapt to environmental heterogeneity (e.g., changing water levels) and to inhabit various types of aquatic habitats, including lakes, rivers, streams, wetlands, swamps, seasonally flooded areas, as well as brackish and marine environments (Lesiv et al. 2020). Vascular plants represent the largest group among macrophytes, including aquatic ferns (*Azolla* spp., *Salvinia* spp.) but mostly Angiosperms;

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both monocots and dicots (Rejmánková 2011). These vascular aquatic macrophytes (hereafter referred to as aquatic macrophytes) are represented by 33 orders and 88 families, with about 2,614 species distributed worldwide. Overall, the diversity is highest in the Neotropics (984 species), intermediate in the Indomalayan, Nearctic and Afrotropics (664, 644 and 614 species, respectively), lower in the Palearctic and Australasia (497 and 439 species, respectively), and in the Oceanian (108 species), while only a very few vascular macrophyte species have been found in the Antarctica bioregion (Chambers et al. 2008).

The most common classification for aquatic macrophytes is by their growth form or the basis of attachment to the substratum, which includes four groups: (1) emergent macrophytes that are rooted in sediments or soils that are periodically inundated, but with aerial leaves; (2) floating leaved macrophytes rooted to the bottom substrate in streams and lakes with leaves that float on the surface of the water; (3) free-floating macrophytes that typically float on or under the water surface but are not attached to the bottom; and (4) submerged macrophytes that grow completely submerged under the water, with roots attached to, or closely associated with the substrate (Wetzel 1975; Chambers et al. 2008; Srivastava et al. 2008; Hanson 2013). Examples of the types of macrophytes are illustrated in Fig. 1.1.

This introductory chapter describes the importance of aquatic macrophytes for the functioning of aquatic ecosystems and for the well-being of humans, and also provides an overview of approaches for using aquatic macrophytes in ecotoxicology studies and for risk assessments. In subsequent chapters in this book, experts in the field of the ecotoxicology of aquatic macrophytes provide in-depth descriptions of the use of these plants for assessing the impacts of environmental pollution through biomonitoring and biomarkers, evaluating recoveries from contamination and for conducting risk assessments, as well as the potential for using macrophytes for bioremediation.

1.2 The Importance of Macrophytes in Aquatic Ecosystems

Aquatic macrophytes are primary producers at the base of both herbivorous and detritivorous food chains. They also provide physical structure to aquatic ecosystems, increase habitat complexity and heterogeneity, affect oxygen and nutrient concentrations, provide refuge from predation and release dissolved organic carbon which can be used by microbial complexes in periphyton or plankton (Bakker et al. 2016). Thus, aquatic macrophytes play an important role in the structure and the functioning of aquatic ecosystems. Photosynthesis driving primary production by macrophytes provides energy flow to the food webs of a range of aquatic ecosystems. In addition to the role of carbon derived from microalgae to higher trophic levels, there is evidence that carbon from the detritus generated by macrophytes may be an important carbon source for invertebrates and fish. In addition to providing organic matter for detritivores, macrophytes also provide food resources to aquatic and terrestrial herbivores (Thomaz 2021).

Plant biodiversity is also the foundation of food security for humans and in some cases, the basis for identifying new medicines. Aquatic macrophyte communities

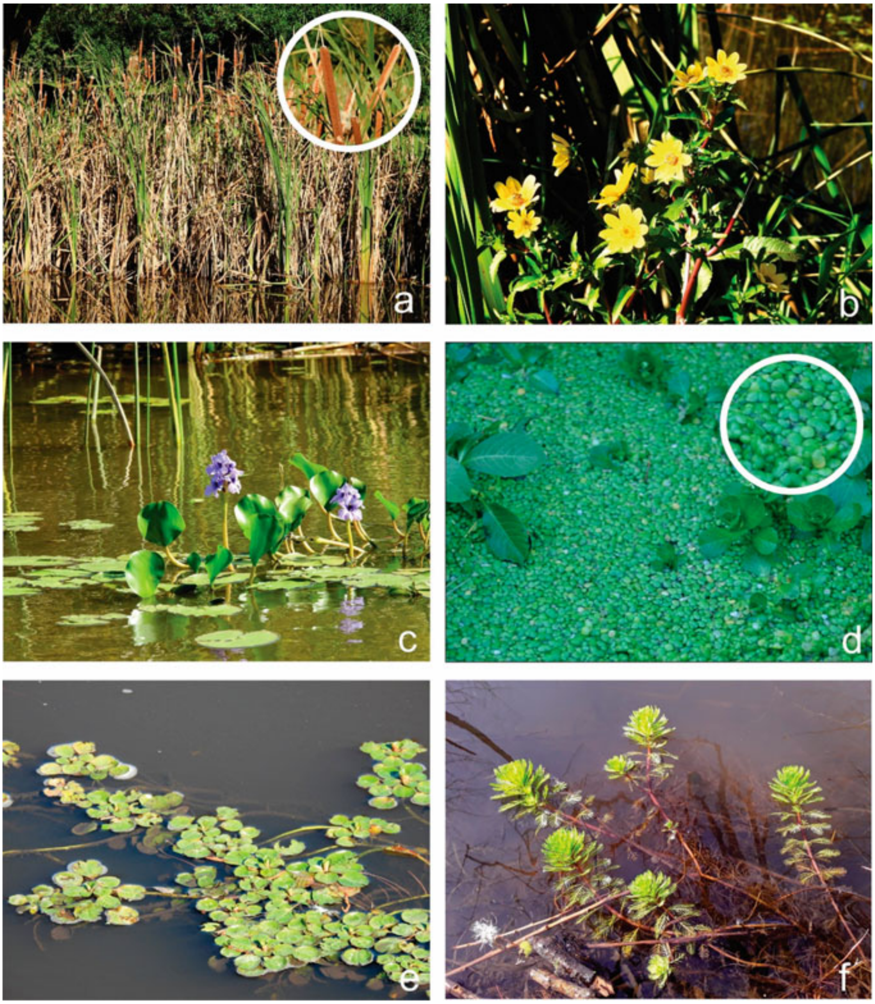

Fig. 1.1 Selected freshwater macrophytes that have been used in ecotoxicology studies: (a) cattail (*Typha* spp.), (b) beggartick (*Bidens* spp.), (c) water hyacinth (*Eichhornia* spp.), (d) duckweed (*Lemna* spp.), (e) primrose-willow (*Ludwigia* spp.), (f) water milfoil (*Myriophyllum* spp.). Photographs a, b, c and e were provided by Silvina Bachmann, photograph d by Nicolás Chiaradía and photograph f by Débora Pérez

offer multiple other benefits to humankind in terms of ecosystem functions, as well as resilience to climate change and other perturbations (Ebert and Engels 2020). Thomaz (2021) recognized these benefits within the paradigm of "ecosystem services" and identified more than 26 types of ecosystem services provided by aquatic macrophytes. These services were classified into supporting (e.g., photosynthesis and production of oxygen), provisioning (e.g., food and fiber provided by plant biomass), regulating (e.g., water purification through retention of nutrients and pollutants) and cultural (e.g., local knowledge systems of communities which depend on ecosystems with macrophytes for survival).

1.3 Ecotoxicology Studies with Aquatic Macrophytes

Ecological stressors such as climate change, eutrophication, acidification or introduced species have been recognized as drivers of reduced macrophyte diversity in aquatic ecosystems (Chambers et al. 2008). In addition, natural ecosystems are subject to contamination by a number of elements of geogenic or anthropogenic origin, as well as xenobiotics. Anthropogenic activities such as discharges of industrial and municipal wastewater, and wastes originating from households, industry and agriculture are the main sources of the contaminants transported into aquatic ecosystems (Piwowarska and Kiedrzynska 2022). Aquatic macrophytes have been used as bioindicators of water quality in lentic and lotic systems in studies that focus on changes in plant communities (Thiebaut and Muller 1999; Ceschin et al. 2010), as well as studies of effects at the organismal level (Menone et al. 2000; Bonanno et al. 2017; Pérez et al. 2017). Despite the crucial role of macrophytic plants in aquatic ecosystems, these organisms have been underemployed for evaluating the impacts of anthropogenic activities, if compared to the number of comparable studies conducted with animals. Even so, the majority of ecotoxicology studies with aquatic macrophytes have focused on a narrow range of plant species, including *Lemna* spp., *Myriophyllum* spp. and *Hydrilla* spp. (Ceschin et al. 2021). These and other macrophytes species that have been used in ecotoxicology studies conducted in the laboratory and in the field are listed in Table 1.1.

Ecological risk assessments typically involve two main experimental or predictive approaches, as illustrated in Fig. 1.2. "Exposure Assessments" consist of measurements of the concentration of a toxicant of interest in a relevant environmental matrix (e.g., water, sediment, soil, air) or alternatively, calculations to predict what the concentration is expected to be. These data are used to determine a Predicted Exposure Concentration (PEC). "Effects Assessments" consist of measurements of the acute or chronic toxicity of the toxicant of interest to a range of organisms and these data are used to determine a Predicted No Effect Concentration (PNEC). The "Risk Characterization" step involves comparing the PEC to the PNEC to determine if exposure concentrations are likely to exceed the thresholds for toxicity (Fig. 1.2). Risk Management steps may be needed if there is a clear risk of impacts to aquatic or terrestrial species. For Effects Assessments that focus on threats to aquatic ecosystems where there are macrophytes (e.g., wetlands), there is no specific species or taxonomic group that is consistently more sensitive to the toxic effects of contaminants, including the standard duckweed (*Lemna* spp.) test organisms (Fairchild et al. 1998; Arts et al. 2008; Giddings et al. 2013). This highlights the need to incorporate toxicity studies with a suite of macrophytic test species into risk assessments (Lemly et al. 1999; Hanson and Arts 2007; Repetto 2013).

Duckweed species offer several advantages as a model test organism, as they have a wide geographic range (i.e., environmentally relevant), are exceptionally easy to

Table 1.1 Aquatic macrophytes that have been used in ecotoxicological studies, classified as emergent, floating leaved, free floating and submerged

Emergent	Free floating	Floating leaved	Submerged
Bidens laevis (FW)	*Eichhornia crassipes* (FW)	*Ludwigia peploides* (FW)	*Elodea canadensis* (FW) *E. nutalli*
Bruguiera gymnorrhiza (MG) *Kandelia candel* (MG) *Rhizophora mucronate* (MG)	*Ceratophyllum demersum* (FW)	*Potamogeton natans* (FW)	*Hydrilla verticillate* (FW)
Glyceria maxima (FW)	*Lemna minor* (FW) *L. gibba*		*Myriophyllum aquatium* (FW) *M. alterniflorum*, *M. quitense*, *M. spicatum*
Oryza sativa (FW)	*Spirodela polyrhiza* (FW)		*Posidonia oceánica* (M)
Phragmites australis (FW)			*Vallisneria neotropicalis* (FW) *V. natans*
Spartina densiflora (SM) *S. alterniflora*			*Zostera marina* (M)
Typha latifolia (FW) *T. domingensis*			

FW: freshwater (lakes, streams, wetlands); SM: saltmarsh; MG: mangrove; M: marine

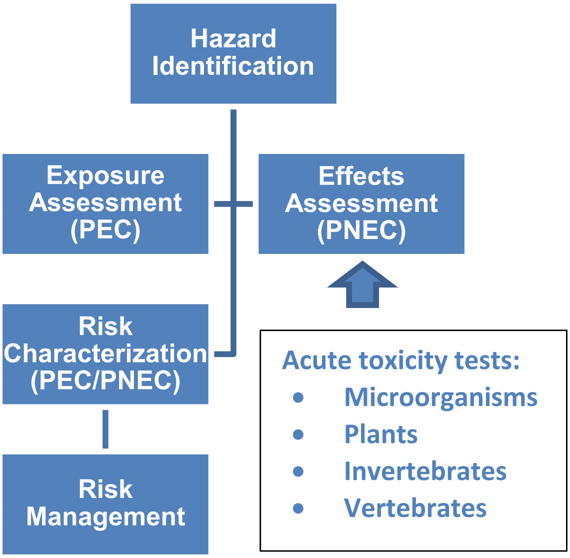

Fig. 1.2 The elements of an ecological risk assessment. PEC = Predicted Exposure Concentration; PNEC = Predicted No Effect Concentration; PEC/PNEC = Hazard Quotient

culture, bioassays are relatively inexpensive and simple to conduct, and it is possible to measure toxicity over a relatively short period of time (Rand et al. 1995; Brain and Solomon 2007; Hanson 2013). Duckweed species have been used to evaluate the cytotoxic and mutagenic effects of several classes of contaminants, including pesticides, pharmaceuticals, polycyclic aromatic hydrocarbons, metals, metalloids, organometal compounds and radionuclides (Mkandawire et al., 2013). However, duckweed species lack stems, true leaves and a sediment-interacting root system, and therefore, concerns have been raised about the suitability of *Lemna* spp. as a surrogate for all macrophytes, especially when testing compounds with herbicidal activities or assessing the risks to wetland ecosystems (Maltby et al. 2009; Arts et al. 2010; Hanson 2013). There are also limitations when evaluating responses under controlled field assessments (e.g., microcosm or mesocosm studies), as these systems are not typically eutrophic. This can mean that growth responses of duckweed are reduced under conditions of nutrient deficiency, especially in comparison to rooted submerged and emergent macrophytes, which are able to access nutrients available from both the sediments and the water column (Maltby et al. 2009; Hanson 2013). Additionally, effects of stressors that may impair light availability within the water column (e.g., turbidity) are not effectively captured by duckweed, as they typically float at the surface with ample access to light (Brain et al. 2005).

Because of the limited predictive capabilities of duckweed for evaluating the effects of sediment-bound contaminants, there are also standardized test methods for the rooted, submerged eudicot, *Myriophyllum* spp. Toxicity tests with *M. sibiricum* or *M. spicatum* have been applied when assessing the risks from exposure to herbicides that partition into sediments or for studies of eudicot targeted herbicides such as chlorophenoxy compounds (Arts et al. 2010; OECD 2014). Although there have been numerous other macrophytes used in toxicity tests (Table 1.1), the widespread adoption of additional test species for risk assessments has been limited, due in part to the lack of standardization and validation of testing procedures (Hanson 2013). However, there is ample evidence that macrophytes should be an essential component of effects assessments for a range of aquatic ecosystems (Hanson and Arts 2007; Arts et al. 2010; Giddings et al. 2013; Hanson 2013).

Because of the diversity of growth forms or the basis of attachment to substrata, macrophytes can be exposed to contaminants through several pathways, such as in sediments, in the water column, or through aerial exposure (Vonk and Kraak 2020). It is imperative for risk assessments to address the different pathways of exposure that apply to a particular ecosystem or to a specific toxicant of interest. Single-species toxicity testing introduces high levels of uncertainty for an effects assessment, especially when used as a sole line of evidence rather than in a weight-of-evidence approach (Maltby et al. 2009; Taylor and Scroggins 2013). To reduce uncertainty when characterizing the risk to non-target organisms, studies with macrophytes with different morphologies and exposure pathways must be included in the standard regulatory risk assessment process. In Chap. 5, wild rice (*Zizania* spp.) is presented as a candidate species for assessing risks to wetland ecosystems, as this rooted and emergent plant can be exposed to contaminants in sediment, water and air. Chapter 4 provides a global perspective on the use of macrophytes for risk assessments.

As was pointed out decades ago, simply determining the contaminant loads of organisms does not necessarily provide information on the toxicological significance of the body burden, or on the many factors which can influence the accumulation of contaminants. An alternative and potentially more useful approach is to evaluate indexes of sublethal stress, or "biomarkers" (Padinha et al. 2000). There are several studies in the literature on biomonitoring with macrophytes that include data on stress biomarkers, which are mostly biochemical responses (Lytle and Lytle 1998; Nimptsch et al. 2005; Turull et al. 2017; Bertrand et al. 2019). Chapter 3 in this book, provides a review of studies of bioaccumulation and biomarker responses with an emergent freshwater macrophyte, *Potamogeton pusillus,* and with mangrove species exposed in the laboratory and in the field to metals and metalloids. In this book, Chap. 2 provides a review of physiological, biochemical and genotoxicity biomarkers that have been measured in aquatic macrophytes in response to exposures to different classes of contaminants, including metals and metalloids, current use pesticides and emerging contaminants such as pharmaceuticals and personal care products (PPCPs) and per- and polyfluoroalkyl substances (PFASs). In addition, Chap. 6 includes a discussion of the potential for recovery by aquatic macrophytes from the effects of exposure to herbicides.

Due to the detrimental effects of toxic elements and xenobiotics on living organisms, there is a pressing need to develop strategies for eliminating or mitigating exposures to the contaminants that are discharged into the aquatic environment (Piwowarska and Kiedrzynska, 2021). On this subject, Chap. 7 describes best practices using drainage ditches vegetated with macrophytes as a management strategy to reduce the levels of contaminants (primarily pesticides and nutrients) entering surface waters in runoff from agricultural lands. Similarly, Chap. 8 describes "Green Liver" systems applied at laboratory and field scales, as low-impact, low-energy and low-cost systems for the remediation of pollutants in water.

1.4 Conclusions

Overall, this book provides a valuable addition to the literature on the use of macrophytes to assess the impacts of contaminants in aquatic ecosystems, and also, the potential for using macrophyte communities to reduce pollutant loading to the environment. Clearly, there is a need to develop standardized methods for toxicity testing using alternative test species, in addition to the standard operating procedures that have been developed with *Lemna* spp. and *Myriophyllum* spp. Continued work is needed to identify stress responses that can be used as biomarkers of exposure to toxicants, including employing -omics approaches. Finally, communities of macrophytes offer promise as "Nature-based Solutions" for mitigating the effects of substances that enter the aquatic environment from geogenic and anthropogenic sources.

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