Gaurav Sablok *Editor*

Plant Metallomics and Functional Omics

A System-Wide Perspective

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Preface

The origin of life required a stimulating and binding element, and metal served the purpose by integrating into the backbone soup of life. The integration of metal dates back to the origin of life—starting from the basic building blocks in the form of its integration with heme and leading to the origin and diversification of the human era. However, this integration was seen across all the diverse forms of plants, thus allowing them to sustain and adapt to the changing environment and providing a sustainable source of food and energy. With the rapidly advancing sequencing technologies, indispensable efforts have been leveraged to understand the connecting link between the metal abundance and the genetic gain and loss from a plant adaptation perspective. Several approaches such as next generation genome sequencing, transcriptome sequencing, laser-associated transcriptome sequencing, localization imaging techniques, and posttranscriptional and translational modifications have been widely used to establish the connecting link between the metal and the associated plant growth in a metal-contaminated environment. A significant proportion of the crop genetic research is focused on establishing and finding the elusive blocks of knowledgeable connecting links between the physiological significance of metal integration and relative associated toxicity of the transient flow of the metal from the roots to the shoots as well as abaxial and adaxial surface of plant leaf, thus affecting the plant biomass. This is relatively important to establish several lines of the genetic research to advance the understanding of the metal translocation and the involvement of the metal in several physiological responses. We believe that the biological implication of this underpinning phenomenon will not only broaden the scope of crop domestication but will also allow for the breeding of the sustainable production of breeding lines to meet the demand of functional metal-resistant crops in the event of the metal-contaminated soils. *Plant Metallomics and Functional Omics* is a bridging volume, which brings together the collective knowledge on understanding the biological mechanism behind the metal tolerance from several dimensions such as expression-based approaches, high-throughput imaging techniques, mutant-based screening scans, posttranscriptional events, small RNAs, and relative roles of metals in crop biomass production. The present volume, by bringing several aspects together of metal tolerance and functional omics, will allow for the deeper understanding of the metal tolerance and might allow to address the following question: How do we plan to feed ever-increasing human food demand in 2050?

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Sydney, NSW, Australia Gaurav Sablok

Contents

Chapter 1
Energy Crop at Heavy Metal-Contaminated Arable Land as an Alternative for Food and Feed Production: Biomass Quantity and Quality

Marta Pogrzeba, Jacek Krzyżak, Szymon Rusinowski, Jon Paul McCalmont, and Elaine Jensen

1.1 Introduction

Unsustainable development of heavy industry in the second half of the twentieth century, including processing of metal ores and the overexploitation of natural resources, has seriously impacted the quality of large areas of agricultural land. Heavy metal contamination (HMC) particularly has resulted in a significant proportion of arable land now being unsuitable for food or feed production (Tóth et al. 2016). This heavy metal contamination of soil is one of the most pressing concerns in the debate about food security and food safety in Europe. The large number of contaminated sites in the European Union, plus total land area affected by other kinds of pollution (Van Liedekerke et al. 2014), underlines the extent of the problem in the continent. Estimates suggest that remediation of these areas could cost €17.3bn annually (CEC 2006). Apart from soil contamination, which may lead to the degradation of water quality and a series of negative impacts on the environment, the propagation of heavy metals throughout the food chain has serious consequences for human health (Järup 2003; Mulligan et al. 2001; Rattan et al. 2005; Tóth et al. 2016).

Industrial and post-industrial areas are frequently a source of contaminants which can affect surrounding arable lands. In regions associated with Zn, Fe, Cu and Pb mining and smelting, many 'hot-spots' have developed, which are associated with trace element (TE)-contaminated soils, and as a result, plants grown in these areas are often contaminated with TE by root uptake and/or foliar exposure

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(Alloway 2013; Dudka et al. 1995; Nicholson et al. 2003). In light of this, food crop production should be restricted or forbidden altogether in such areas, particularly root crops such as carrot, parsnip and potato (Liu et al. 2013; Roba et al. 2016).

Biomass production from non-food and dedicated energy crop plants could be an alternative use for such contaminated arable land, particularly where soils are improved with site appropriate agro-techniques such as fertilisation, tillage practices or irrigation management (Kidd et al. 2015). Extensive literature already exists investigating the potential of energy crop cultivation in TE-contaminated soils (e.g. Meers et al. 2010; Van Ginneken et al. 2007; Zhang et al. 2015).

Remediation of contaminated soils has become a long term but pivotal challenge; beyond its scientific and technical aspects, it is key to addressing a range of social issues (rehabilitation of former industrial sites in eco-districts, restoration of ecosystem services, improved economic viability of land-based industries and the provision of biomass feedstock to accelerate the growth of the new bio-economy) (Alkorta et al. 2010). Recognising the importance of management options for sustainable and safe use of heavy metal-contaminated (HMC) soils, investigations have looked at combining the production of energy crops on contaminated areas with phytoremediation of the soil. Whereas HMC soils are unsuitable for food production, dedicated energy crops can allow a sustainable commercial exploitation of these soils by establishing biomass feedstock production systems. In addition, the cultivation of crops offers opportunities for soil stabilisation and phyto-management of contaminated soils (Ollivier et al. 2012). Nowadays, biomass production is focused on second-generation, low-input perennial energy crops, for example *Panicum virgatum, Spartina pectinata, Miscanthus* spp. (Dohleman et al. 2012; Guo et al. 2015; Clifton-Brown et al. 2017). Such plants have much lower input requirements and produce more energy and less greenhouse gas emissions per hectare than first-generation annual food crop species (e.g. *Zea mays*) which have been used previously (Schrama et al. 2016). There are a number of typical energy crop species available commercially which have also been tested with success for their phytoremediation effects on HMC arable land. However, further research is very much needed under exposure to a range of heavy metals to demonstrate their robustness for large-scale applications. To date, the main energy crop species utilised in EU countries have been different clones of willow (*Salix* spp.) and poplar (*Populus* spp.) (El Kasmioui and Ceulemans 2012), Miscanthus (*Miscanthus* × *giganteus)* (Smeets et al. 2009; Michalska et al. 2012), switchgrass (*Panicum virgatum*) (Howaniec and Smoliński 2011; Michalska et al. 2012) and Virginia mallow (*Sida hermaphrodita*) (Borkowska and Molas 2012). While all these species are usually grown on uncontaminated sites, several have also been tested for phytoremediation of HMC soils: willow (Witters et al. 2009; Mleczek et al. 2010), switchgrass (Chen et al. 2012), Miscanthus (Ollivier et al. 2012; Pogrzeba et al. 2017a) and Virginia mallow (Pogrzeba et al. 2018a; Antonkiewicz et al. 2017).

1.2 Second-Generation Energy Crops Grown on Heavy Metal-Contaminated Soil

In this review, six emerging second-generation energy crop species (*Miscanthus* × *giganteus*, *Sida hermaphrodita, Spartina pectinata, Panicum virgatum, Phalaris arundinacea* and *Arundo donax* spp.) were taken into consideration in terms of general characteristics, biomass elemental composition and the potential disposal of contaminated biomass with associated energy generation.

1.2.1 Miscanthus × giganteus (Family: Poaceae)

Miscanthus × *giganteus* is a perennial rhizomatous grass with the C4 photosynthetic pathway (Lewandowski et al. 2000); it is an allotriploid, naturally occurring hybrid of *Miscanthus sinensis* and *Miscanthus sacchariflorus.* As a consequence of its triploidy, *M.* × *giganteus* is sterile and cannot produce viable seeds and is, therefore, established clonally through rhizome propagation (Linde-Laursen 1993; Naidu et al. 2003), although progress is being made on the commercialisation of novel seed-based hybrids (Clifton-Brown et al. 2017; Krzyżak et al. 2017). The genus *Miscanthus* has its origins in the tropics and subtropics, but different species are found throughout a wide climatic range in East Asia. *M.* × *giganteus* was first cultivated in Europe in the 1930s where it was introduced from Japan. However, agricultural establishment of *M.* × *giganteus,* especially in the temperate climates of Europe and North America, can be challenging with relatively high establishment costs, narrow genetic base and low hardiness in the first winter following establishment (Clifton-Brown et al. 2017). However, extensive field trials across Europe, and a rapidly growing commercial market, have shown that *M.* × *giganteus* biomass can be an economically viable biomass crop with a range of end uses, for example used as a solid fuel, in construction materials such as pressed particle board and as a source of cellulose (Lewandowski et al. 2000, 2016).

Miscanthus is harvested annually in late winter or spring of the following year. At this time, mineral nutrient content has been reduced by remobilisation to rhizomes and natural weathering. A low mineral content at harvest is desirable in biomass intended for thermal conversion because it minimises the impact on combustion efficiency and lowers stack emissions (Christian et al. 2008). The economic lifetime of the crop is estimated at 10–15 harvesting years (J. Clifton-Brown personal communication) from a single cultivation, during which time biomass production undergoes two distinct phases: a yield-building phase, where yields of *M.* × *giganteus* increase each year for 2–5 years, depending on climate and plant densities, and a plateau phase where the mature yield is maintained and relatively stable. Because of its C4 photosynthetic pathway and perennial rhizome, *Miscanthus* displays a good combination of radiation-, water- and nitrogen-use efficiencies for biomass production (Zub and Brancourt-Hulmel 2010).

1.2.2 *Arundo donax Spp. (Family: Poaceae)*

Giant reed (*Arundo donax* spp.) is a robust perennial grass native to the 'Old World' from the Iberian Peninsula of Europe to south Asia, including North Africa and the Arabian Peninsula (Goolsby and Moran 2009; Mariani et al. 2010). Despite being a C3 species, rates of photosynthesis and productivity are similar to those of a C4 (Nackley and Kim 2015), and it has a large amount of energy production per unit of dry weight (Mariani et al. 2010; Tho et al. 2017). *A. donax* spp. can grow on a wide range of soil types, from loose sands and gravelly soils to heavy clays and river sediments. It is also able to tolerate a wide range of soil salinity (Nackley and Kim 2015). It can be used as an ornament as well as for fibre uses, to produce cellulose pulp and paper (Cosentino et al. 2014). *A. donax* spp. is characterised by easy vegetative propagation, high water and nitrogen efficiencies, relatively high yields and a fast growth rate of around 10 cm per day (Barbosa et al. 2015; Cosentino et al. 2014). It has deep, dense, extensive root systems and spreads rapidly by rhizomes, thereby helping to reduce the risk of soil erosion (Cosentino et al. 2014). Further, it is resistant to wind, water and biological erosion and can be cultivated on contaminated soils (Barbosa et al. 2015). It is a promising energy crop of the Mediterranean areas and is regarded as one of the top potential biofuel crops (Mariani et al. 2010; Nackley and Kim 2015; Tho et al. 2017). According to Barbosa et al. (2015), *A. donax* also prevents the leaching of heavy metal and reduces groundwater contamination.

1.2.3 *Panicum virgatum (Family: Poaceae)*

Panicum virgatum is a native, cross-pollinated, perennial warm-season grass with a C4 photosynthetic pathway originating from North America (Hultquist et al. 1996). *P. virgatum* is a high-yielding and low-input bioenergy feedstock which reaches a height of 1–2 m and, rarely, 3 m. It can be grown on light or moderately heavy saline or alkaline soils with full, mature yields being reached 3 years after planting. It is emphasised that *P. virgatum* can be used as a productive species in the reclamation and stabilisation of contaminated sites as well as for the bioaccumulation of heavy metals and energy production (Pogrzeba et al. 2017b). *P. virgatum*, like *Miscanthus*, has the ability to collect and store large amounts of carbon in below-ground biomass and to produce large quantities of above-ground harvestable biomass with minimal agricultural inputs (Dohleman et al. 2012). *P. virgatum* can also be used as a cellulosic biomass feedstock for bio-refineries and bio-fuel production (Sokhansanj et al. 2009).

1.2.4 *Phalaris arundinacea (Family: Poaceae)*

Phalaris arundinacea L. (reed canary grass, RCG) is a coarse, vigorous, rhizomatous perennial grass distributed throughout Europe and in temperate regions of North America and Asia (Christian et al. 2006). It has been an important cultivated forage grass in northern temperate regions of the world for nearly two centuries (Galatowitsch et al. 1999) The grass is tall (60–200 cm) and leafy, but its forage value is limited to the young succulent shoot stage; older stems are less palatable to livestock. In natural conditions, it is most commonly found growing along water margins, but when established, it has drought resistance. Early trials showed that it is tolerant of a range of soil textures from silty loam to heavy clay. Because RCG has a wide geographic adaptation, genetic variation is present that can be used to select genotypes for specific environments. Adaptability and high yield led to RCG being evaluated as a potential bioenergy crop especially for the UK (Christian et al. 2006; Jensen et al. 2018). Number of *Phalaris* shoots is highest during the second season, from then the shoot count remains fairly constant throughout the crop lifetime (Vymazal and Krőpfelová 2005). Productive lifespan of this plant ranges between 5 and 10 years (Smith 2008).

1.2.5 Spartina pectinata (Family: Poaceae)

Spartina pectinata is a C4, rhizomatous, perennial, warm-season grass originating from North America (Guo et al. 2015; Kim et al. 2012; Rofkar and Dwyer 2011) and is characterised by a very wide range of occurrence, from New Foundland and Quebec (Canada) to Arkansas, Texas and New Mexico (USA) (Guo et al. 2015). The harvestable biomass of *S. pectinata* consists of leaves and stems, reaching a height of about 1–3 m. The plant is predominantly found in lower, poorly drained soils along roadsides, ditches, streams, marshes, wet meadows and potholes where soils are overly saturated (Kim et al. 2012; Prasifka et al. 2012; Guo et al. 2015). *S. pectinata* can reproduce both by seeds and by rhizomes (Prasifka et al. 2012). According to Guo et al. (2015), the species is well adapted to various abiotic stresses, including cold, water saturation and saline soils. It can grow in humid environments, tolerates acidified areas and is resilient in changing environmental conditions (Kim et al. 2012); as a result, *S. pectinata* is able to produce biomass even on degraded lands (Prasifka et al. 2012). It has been shown to be a useful energy crop (Kowalczyk-Jusko et al. 2011) which can be helpful in the reclamation of soils contaminated with heavy metals (Korzeniowska and Stanislawska-Glubiak 2015; Pogrzeba et al. 2018b).

1.2.6 Sida hermaphrodita (Family: Malvaceae)

Virginia mallow (*Sida hermaphrodita*) originates from the southeastern regions of North America. The plant was brought to Europe in the first half of the twentieth century, initially to Ukraine and then into Poland (Kasprzyk et al. 2013). *S. hermaphrodita* is characterised by a deep root system, rapid growth and an ability to quickly adapt to different climatic and soil conditions, though it is sensitive to drought pressure as well as pests and disease (Šiaudinis et al. 2015). Despite this sensitivity, ease of establishment and rapid growth potential made it a valuable raw material used in power generation, biogas production and as a source of fibre and feed (Kasprzyk et al. 2014). It has been used in textiles, food, medicines and the pulp and paper industries. From an environmental point of view, according to Nabel et al. (2014), the extensive root system of *S. hermaphrodita* also offers the benefit of sequestering large amounts of carbon in this below-ground biomass while, because of the slow rate of seed germination and the low competitiveness of cuttings, it is not expected to be an invasive species (Nabel et al. 2016). It can be grown on the slopes of eroded areas, land which is excluded from agricultural use and on chemically degraded areas, also on dumps and landfills (Kasprzyk et al. 2014). The species has a high potential of phytoextraction of HMs (Ni, Cu, Zn and Cd) in comparison to other species used as energy crops (Borkowska and Molas 2012; Antonkiewicz et al. 2017; Pogrzeba et al. 2018a).

1.3 Biomass Yield and Elemental Composition of Second-Generation Energy Crops

In terms of harvestable feedstock characterisation, it is the above-ground biomass that is the most important consideration. Understanding the yield and elemental composition of the biomass produced is essential with regard to processing, energy generation and the post-processing of residues to fall within the remit of a circular economy (Ghisellini et al. 2016; Pogrzeba et al. 2018a). In addition, the elemental composition of biomass is key to assessing the uptake and accumulation of HM when determining plant selection for phytoextraction or phytostabilisation (Nsanganwimana et al. 2014) and for informing utilisation pathways. For example residual material from metal excluding crops rich in nutrients might be successfully used as a soil conditioner after processing (e.g. ash, digestate, biochar) or, on the contrary, contaminated material from accumulators could be problematic due to the re-introduction, and concentration, of contaminants back to the environment if applied back to the fields (Pogrzeba et al. 2018a). Perhaps the best opportunity would be offered by plants which stabilise contaminants in the soil rather than extract them to above-ground parts.

1.3.1 Biomass Yield on Heavy Metal-Contaminated Sites

Results summarising biomass yields across several studies for the selected energy crop species cultivated on HM-contaminated and HM-uncontaminated soil are presented in Table 1.1. It was found that yields are generally lower for plants cultivated on HM-contaminated sites, though the magnitudes of the impacts of HMC varied between the species. The smallest differences between contaminated and uncontaminated soils were found for *S. hermaphrodita*, *P. arundinacea* and *S. pectinata*

Table 1.1 Biomass yield of described species cultivated on HM-contaminated and HM-uncontaminated soil

Species	Experiment duration	Plant density	Heavy metals	Yield	References
Miscanthus sp.	3 years	N/A	Yes	16–37 Mg ha^{-1}	Kocoń and Jurga (2017)
	3 years	2 plants m^{-2}	No	28.7 Mg ha^{-1}	Angelini et al. (2009)
Arundo donax	1 year	2.7 plants m^{-2}	Yes	12 Mg ha^{-1}	Fiorentino et al. (2013)
	1 year	2 plants m^{-2}	No	29 Mg ha^{-1}	Angelini et al. (2009)
Panicum virgatum	3 years	2.7 plants m^{-2}	Yes	4–4.5 Mg ha^{-1}	Rusinowski et al. (2019)
	3 years	1 g seeds m^{-2}	No	15.4 Mg ha^{-1}	Vamvuka et al. (2010)
Phalaris arundinacea	3 years	1.5 plant m^{-2}	Yes	5.5 Mg ha^{-1}	Lord (2015)
	3 years	N/A	No	5.5–7.5 Mg ha^{-1}	Jasinskas et al. (2008)
Spartina pectinata	2 years	3 plants m^{-2}	Yes	11 Mg ha^{-1}	Pogrzeba et al. (2018b)
	4 years	1 g seeds m^{-2}	No	11.7 Mg ha^{-1}	Boe et al. (2009)
Sida hermaphrodita	3 years	N/A	Yes	6–23 Mg ha^{-1}	Kocoń and Jurga (2017)
	2 years	2.7 plants m^{-2}	No	23.3 Mg ha^{-1}	Jablonowski et al. (2017)

(Kocoń and Jurga 2017; Lord 2015; Pogrzeba et al. 2018b), while the greatest differences were seen in *A. donax*, *M.* × *giganteus* and *P. virgatum* (Kocoń and Jurga 2017, Fiorentino et al. 2013, Rusinowski et al. 2019). However, despite this overall impression, there are other crucial factors which can drive biomass yield aside from the presence of HM, particularly planting density, nutrient status and climatic conditions. Kocoń and Jurga (2017) cultivated plants in well-prepared microplots (1 m × 1 m × 1 m), they did not specify their planting density, but it might be assumed, due to the small plot size, that this exceeded 3 plants m^{-2}, double that of the commercial norm of around 1.6 plants m^{-2}. Issues such as this make review comparisons across studies difficult as similar biomass yields reported from contaminated sites compared to uncontaminated sites in different studies for *S. hermaphrodita* and *M.* × *giganteus* could possibly be explained by higher planting densities. The influence of climatic conditions on yields could be explained in the *P. virgatum* example where significantly lower yields were produced in a cooler Poland climate on HMC soil (Rusinowski et al. 2019) compared to those produced in a warmer Greek climate on uncontaminated soil (Vamvuka et al. 2010). Among the selected plant species, the highest yielding crops on HM-contaminated sites seem to be *M.* × *giganteus* (Kocoń and Jurga 2017) and *S. hermaphrodita* (Kocoń and Jurga 2017), while on uncontaminated sites *A. donax* (Angelini et al. 2009) and *M.* × *giganteus* (Angelini et al. 2009). The lowest yields were found for *P. virgatum* (Rusinowski et al. 2019) and *P. arundinacea* (Lord 2015) when cultivated on contaminated sites, while on uncontaminated site, it was *P. arundinacea* (Jasinskas et al. 2008). From this review, it would appear that *P. arundinacea* produces the lowest yields across the species regardless of soil heavy metal status.

1.3.2 Primary Macronutrients

The concentrations of primary macronutrients in this set of energy crop species have been investigated predominantly in terms of their use efficiency when grown in uncontaminated soils (Dierking et al. 2017; Rancane et al. 2017; Ameen et al. 2018); there is a scarcity of articles reporting accumulation and utilisation of these in biomass cultivated in HM-contaminated soils (Table 1.2). All of the described plant species accumulate, in their above-ground biomass, about 0.5–10 g kg^{-1} DM of N (Table 1.2). The results of P concentration in harvested plant biomass (in the range of 0.5–0.7 g kg^{-1} DM) show less variation between plant species when compared to the nitrogen contents, while the range of concentration of K is similar to that obtained for N at 0.5–7 g kg^{-1} DM. Differences within species between experiments are not only driven by different nutrient status in the soils (1.2–2.5 g kg^{-1}, 0.1–1 g kg^{-1} and 0.6–2.1 g kg^{-1} for N, P and K, respectively) but also between crop age, growing conditions and harvest timing. Pogrzeba et al. (2018a) and Rusinowski et al. (2018) presented results from the same *S. hermaphrodita* plantation after the first and third growing seasons; elemental analyses performed on plant biomass samples collected in March (brown harvest) revealed significantly lower nutrient values than for samples collected in October (green harvest) indicating advanced overwinter relocation of macronutrients. Thus, it is difficult to assess, based on reviewed reports, which plants accumulate more nitrogen, phosphorus and potassium, as it is an effect of many variables. More work is needed to assess the level of accumulation of these elements in energy crops cultivated on HM-contaminated soils.

1.3.3 Heavy Metals

Among the range of common heavy metal contaminants (Pb, Cd, Zn, As, Cu), the greatest attention found in the reviewed reports was for Pb and Zn; in contrast, the least investigated HM was As (Table 1.2). Only a few investigations focused on this element, among which only one was performed in field conditions (for *P. arundinacea* where As levels exceeded 7 mg kg^{-1} (Lord 2015). Among the studies we reviewed, the highest concentration of Pb in plant biomass was found for *P. virgatum* (Pogrzeba et al. 2017b; Aderholt et al. 2017; Gleeson 2007) and the lowest for S. *hermaphrodita* (Kocoń and Jurga 2017; Antonkiewicz et al. 2006; Pogrzeba et al. 2018a; Rusinowski et al. 2018). For *P. virgatum,* the highest value of Pb concentration among reports was 210 mg kg^{-1} DM (Gleeson 2007), while the highest value for *S. hermaphrodita* was 6.4 mg kg^{-1} (Antonkiewicz et al. 2006), though other studies on this species (Kocoń and Jurga 2017; Pogrzeba et al. 2018a; Rusinowski et al. 2018) have shown results below 1 mg kg^{-1} DM. Concentrations of Zn in plant biomass samples taken from mature plantations suggested that the range for *M.* × *giganteus*, *P. arundinacea* and *S. hermaphrodita* is between 50 and

Table 1.2 Soil characteristics and biomass composition of described species

Species	pH	Experiment type	Exposure duration	Soil characteristics								Biomass composition								References
				N[a]	P[a]	K[a]	Pb	Cd	Zn	As	Cu	N	P	K	Pb	Cd	Zn	As	Cu	
				(g kg^{-1})			(mg kg^{-1})					(g kg^{-1})			(mg kg^{-1})					
Miscanthus sp.	7.5	Field	5–6 years	2.5	0.10[b]	0.6[b]	486.2	8.8	511.8			1	0.7	6	0.05	0.4	35			Nsanganwimana et al. (2016)
	8.2	Field	8 years			17.4	2200	15.4	1700		870			0.8	0.94	0.41	107		8.24	Laval-Gilly et al. (2017)
	7.0	Field	2 years	1.4	0.18[b]	0.2[b]	411.5	17.3	1994			16.5	1.18	7	75	5.08	85			Pogrzeba et al. (2017a)
	6.1	Pot	3 months		0.07[b]		325			1727					0.6			3.6		Wanat et al. (2013)
Arundo donax	7.7	Field	1 year	1.8[c]			86.9	3.4	114.6		62.9	10				4.5	8		10	Fiorentino et al. (2013)
	7.7	Pot ASC	2 years	0.3	0.70	2.1	464		457						4.5		92			Barbosa et al. (2015)
	3.8	Pot	2 years	0.3			2161		1534	22,661	411.6				37		50	2.5	20	Castaldi et al. (2018)
		Pot	1 year						900			5	0.6				175			Barbosa et al. (2013)
Panicum virgatum	6.5	Field	2 years	1.5	0.80	1.0	514.7	17.9	1659			6.5	0.7	7.2	54.2	1.1	397			Pogrzeba et al. (2017b)
	6.5	Pot	1 month				108		237				0.8		3		28			Aderholt et al. (2017)
		Pot	3 months		0.60	1.4	36,105	35.7	2557	393	6658				210					Gleeson (2007)

(continued)

Table 1.2 (continued)

Species	pH	Experiment type	Exposure duration	Soil characteristics								Biomass composition								References
				N[a]	P[a]	K[a]	Pb	Cd	Zn	As	Cu	N	P	K	Pb	Cd	Zn	As	Cu	
				(g kg^{-1})			(mg kg^{-1})					(g kg^{-1})			(mg kg^{-1})					
Phalaris arundinacea	5.5	Microplots ACS	2 years		0.09[b]	0.1[b]			705		224						987		5.3	Korzeniowska and Stanislawska-Glubiak (2017)
	6.6–7.9	Field					14.3–44.8	0.06–2.11	112–194		18.8–37.2				1.3–1.6	BDL-0.03	27.6–123		1.24–5.61	Polechońska and Klink (2014)
		Field	3–5 years	2.4	0.30	1.4	23–498	0.1–0.9	57–636	7–47	23–277	0.6–5.4			0.5–9.2	0.1	12–62	0.5	1.7–11	Lord (2015)
	7.9	Pot	5 months	0.5	0.02[b]		46.6	0.9	112		29.4				0.9	0.1	43.6		8.2	Kacprzak et al. (2014)
Spartina pectinata	5.5	Microplots ACS	2 years		0.09[b]	0.2[b]			652		419						571		7.3	Korzeniowska and Stanislawska-Glubiak (2015)
	6.6	Field	2 years	1.2	0.66	0.9	372.5	14	1329			0.44	0.4	5.2	41.3	0.3	103.2			Pogrzeba et al. (2018b)
Sida hermaphrodita	6.2	Field	3 years		0.10[b]	0.1[b]	769.3	3.6	1215		82.5				0.5	0.4	26		1.9	Kocoń and Jurga (2017)
	6.0	Pot ACS	5 years				240.0	40	400		160				6.4	6.5	60		4.1	Antonkiewicz et al. (2006)
	6.5	Field	1 year	1.8	1.02	0.9	635.6	25.7	2360			8.4	0.2	0.5	BDL	13.9	2000			Pogrzeba et al. (2018a)
	6.5	Field	3 years	1.8	1.02	0.9	635.6	25.7	2360			7.4	0.9	9.28	BDL	2.5	163.3			Rusinowski et al. (2018)

ASC artificially contaminated soil, *BDL* below detection limit
[a]Total concentration
[b]Available concentration
[c]Organic nitrogen concentration

100 mg kg^{-1} DM (Nsanganwimana et al. 2016; Laval-Gilly et al. 2017; Lord 2015; Antonkiewicz et al. 2006). While higher values than this have been seen, they tend to come from samples taken from immature plantations (Pogrzeba et al. 2018a) or from plants cultivated in artificially contaminated soils (Korzeniowska and Stanislawska-Glubiak 2015). Similar results can be seen in Cd concentrations in plant biomass; long-term experiments show that Cd levels are between 0.1 and 0.5 mg kg^{-1} with higher values seen in the immature plants though there is one notable exception (Antonkiewicz et al. 2006). In this case, results could be driven by a relatively high concentration of Cd in the growing medium (40 mg kg^{-1}). For Cu accumulation in above-ground plant biomass, concentrations range between 2 and 10 mg kg^{-1} DM though data are not available for *P. virgatum* for this particular element.

Even where HM concentrations in the soils do not exceed toxicity thresholds prescribed by Kabata-Pendias (2010), there may still be problems presented by the level of bio-availability of even low-level concentrations of heavy metals. Where these contaminants are particularly mobile, they may still contaminate food beyond safety thresholds; this is a particular problem where there may be a legacy from excessive application of plant protection products containing HM as active substances (Huang et al. 2007). Sarwar et al. (2017) reviewed a wide range of factors affecting this bioavailability of metals in soil, including soil organic matter, pH, competitive ions concentration, root exudates and plant species and age. Pogrzeba et al. (2018a) reported that heavy metal concentration in *S. hermaphrodita* biomass depended primarily on the bioavailability rather than absolute concentration of metals in the soil. Calculated bioaccumulation factors (BCF) were higher for plants cultivated on heavy metal-contaminated arable land, 0.21–0.55 for cadmium and 0.23–0.86 for zinc, depending on treatment, while on a sewage sludge dewatering site (high organic matter content), those values did not exceed 0.1.

Manipulations to manage the levels of mobility have been studied with some success; as an example, applications of 'red mud' (a waste product of alumina production) have been shown to enhance phytostabilisation and reduce the bioavailability of heavy metals. Pavel et al. (2014) showed that the application of red mud to soils caused a significant decrease in the labile fraction of heavy metals and their corresponding uptake by *Miscanthus* plants tissues, especially in the harvestable stems, with a corresponding increase in yield. These findings show that the application of red mud to soils can contribute to increased biomass production, reduced metal concentrations in plant tissues and also, potentially, a lower risk of metal leaching to subsoil layers or groundwater.

There is little doubt that understanding all these factors contributing to the level of crop uptake of HM contaminants and subsequent impacts on feedstock quality and processing options is a significant challenge for anyone assessing the economic and practical viability of crop production and utilisation at particular sites.

1.4 Biomass Conversion Technologies

Renewable sources of energy could be an alternative that can replace fossil fuels. The production of biofuels from lignocellulosic feedstocks can be achieved through two very different processing routes (Sims et al. 2010):

- Thermo-chemical—(also known as biomass-to-liquids, BTL), where pyrolysis/gasification technologies produce a synthesis gas (CO + H2) from which a wide range of long carbon chain biofuels, such as synthetic diesel, aviation fuel or ethanol, can be reformed, based on the Fischer–Tropsch conversion (Sims et al. 2010).
- Biochemical—in which enzymes and other micro-organisms are used to convert cellulose and hemicellulose components of the feedstocks to sugars prior to their fermentation to produce ethanol; or under anaerobic digestion methane where a biogas is produced (Appels et al. 2011).

Table 1.3 gives a list of studies looking at particular conversion technologies (e.g. heat, electricity, biogas, syngas and bioethanol) related to crop species. It should be noted, however, that these studies address the processing of uncontaminated biomass, and there is a scarcity of literature on conversion of contaminated material with more studies being much needed.

Table 1.3 Possibilities of described species biomass conversion methods

Conversion route	Species	References
Combustion and co-combustion	*Miscanthus* sp., *Spartina pectinata, Sida hermaphrodita, Arundo donax, Phalaris arundinacea*	Iqbal et al. (2017), Baxter et al. (2014), Kiesel et al. (2017), Kowalczyk-Juśko (2017), Corno et al. (2014), Jayaraman and Gökalp (2015) and Čížková et al. (2015)
Gasification	*Miscanthus* sp., *Spartina pectinata, Sida hermaphrodita, Panicum virgatum, Phalaris arundinacea*	Werle et al. (2017), Jayaraman and Gökalp (2015) and Čížková et al. (2015)
Pyrolysis	*Arundo donax, Miscanthus* sp., *Panicum virgatum, Phalaris arundinacea*	Saikia et al. (2015), Liu et al. (2017), Jayaraman and Gökalp (2015), Orts and McMahan (2016) and Čížková et al. (2015)
Anaerobic digestion	*Miscanthus* sp., *Arundo donax, Sida hermaphrodita, Phalaris arundinacea*	Kiesel et al. (2017), Corno et al. (2014), Zieliński et al. (2017), Pokój et al. (2015) and Čížková et al. (2015)
Bioethanol production	*Panicum virgatum, Arundo donax, Miscanthus* sp., *Phalaris arundinacea*	Elia et al. (2016), Corno et al. (2014), Boakye-Boaten et al. (2016), Orts and McMahan (2016) and Čížková et al. (2015)

1.4.1 Thermochemical Conversion

Among renewables, biomass is unique in that it can be directly converted to high value end products (bioenergy and biofuel) in any form (solid, liquid, or gas) using thermochemical conversion technology (Patel et al. 2016). The thermochemical conversion of biomass to produce useful end products from the initial feedstock can occur through any of the following conversion pathways: pyrolysis, gasification and/or combustion (Sims et al. 2010). Pyrolysis is a process of heating biomass without oxygen, which decomposes feedstocks into bio-oil, bio-gas and biochar (Bridgwater and Peacocke 2000; Kung and Zhang 2015), all of which can be used for electricity generation. However, if biochar is not used to generate electricity in the pyrolysis plant but applied to the cropland as a soil amendment, net negative carbon dioxide (CO_2) emissions across the energy production process may be achievable (Kung and Zhang 2015). In gasification, all different types of biomass can be converted into a syngas, composed mainly of hydrogen, carbon monoxide, carbon dioxide and methane. From this syngas, a very wide range of energy or energy carriers—heat, power, biofuels, hydrogen, biomethane—as well as chemicals, can be provided (Heidenreich and Foscolo 2015). Simple combustion is the most mature technology for biomass utilisation (Carroll et al. 2015) and, in general, is defined as the rapid chemical combination of a substance with oxygen, resulting in the production of heat and light. The combustion quality of biomass is determined by: (a) composites that affect the heating value of the biomass, for example ash, moisture and lignin; (b) composites that lead to harmful emissions, for example nitrogen, sulphur, chloride and heavy metals; (c) composites that have an impact on ash fouling, slagging and corrosion, for example chloride, potassium, phosphorus, magnesium, silicon, calcium and sodium (Iqbal and Lewandowski 2016). There are few reports which consider the use of energy crop biomass cultivated on HM-contaminated soils as the energy carrier for thermochemical conversion. Liu et al. (2017) reported that *A. donax* used for phytoremediation purposes could be successfully used to produce biochar with stabilised heavy metals through pyrolysis as a method for contaminated biomass disposal. On the other hand, Werle et al. (2017) showed a potential use of *M.* × *giganteus, S. hermaphrodita, P. virgatum* and *S. pectinata*-contaminated biomass in energy generation via gasification, which is suggested in the literature as a safe method for HMC biomass conversion due to the capacity to control the fate of the heavy metals during the process (Pinto et al. 2008; Nzihou and Stanmore 2013). In addition, Pogrzeba et al. (2018a) showed a potential use of ashes after *S. hermaphrodita* gasification process as a soil amendment, where the permissible level of HM is not exceeded.

1.4.2 Biochemical Conversion

Anaerobic digestion is a microbial conversion method that occurs in an aqueous environment, meaning that biomass sources containing high water levels (even above 60%) can be processed without any pre-treatment (Appels et al. 2011).

Energy yield from the biogas (methane) derived from biomass via anaerobic digestion has proved to be competitive in energy yield when compared to simply burning to produce steam for electricity or for ethanol production (Parawira et al. 2008). When supplied by perennial, low-input energy crops, such as the species reviewed here, biogas production can be a key sustainable technology for energy production from agrarian biomass with high-energy yields per hectare being possible with current technologies and agronomy (Table 1.3).

Biochemical conversion of biomass includes three main processes: the physicochemical pre-treatment of the biomass, the enzymatic hydrolysis of the carbohydrates to a fermentable sugar stream by cellulases and finally the fermentation of the sugars by suitable microorganisms to the target molecules (Sawatdeenarunat et al. 2015). There are no reports referring to biochemical conversion of our selected energy crops cultivated on HM-contaminated sites; however, there are studies reporting the effect of HM on those conversion processes. Mudhoo and Kumar (2013) reviewed that HM could have stimulatory, inhibitory or even toxic effect on the anaerobic digestion process; however, these effects depended on the metal species and its concentration in the biomass feedstock. On the other hand, Xie et al. (2014) performed research on bioethanol production from sugarcane cultivated on HM-contaminated soil in which authors concluded that even high levels of HM presence in sugarcane juice did not affect the fermentation process and the resulting ethanol production when appropriate yeast species were used.

1.5 Concluding Remarks

Cultivation of energy crop species on HM-contaminated soil can offer an economically viable alternative for food and feed crop production when considering health risks and social, environmental and economic aspects. As presented in the review, all described species could be effectively cultivated on HM-contaminated soils; however, more research is needed in field experiments on HM-contaminated sites, particularly for *A. donax*, *P. virgatum* and *S. pectinata*, across a wide range of agroecological and climatic conditions.

Despite the fact that, in mature plantations, our described species did not accumulate HM at the levels which could result in significant toxicity symptoms, yield reductions are likely when compared to plantations established on uncontaminated sites.

Without doubt, there is a still a significant research gap in knowledge around the conversion of contaminated biomass and management of subsequent residues. There is a need for more research particularly around biomass composition and feedstock quality in terms of HM accumulation. Long-term investigations need to focus on elements such us Cd, Cu and As, of which the first two were crucial components in field applied plant protection products in the past and have now resulted in arable land contamination for these elements and a significant problem in soils today.

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Chapter 2
Systems Biology of Metal Tolerance in Plants: A Case Study on the Effects of Cd Exposure on Two Model Plants

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2.1 Scientific Background

Plant growth and biomass production are affected by environmental stresses of natural and anthropogenic origin, significantly restricting their full valorisation potential for economic and societal use. Especially, environmental pollution with metals, notably cadmium (Cd), is of great concern. Cadmium enters the plant through metal transporters, which are embedded in the plasma membrane of root cells, thereby competing with the uptake of essential nutrients and altering the nutrient balance (Fig. 2.1, unpublished data).

Following its uptake, Cd gets distributed throughout the plant where it provokes parallel and/or consecutive events that cause Cd-toxicity symptoms either as a direct or indirect consequence of increasing Cd concentrations. On a physiological level, Cd reduces a.o. plant growth, causes leaf chlorosis, disturbs the water balance and disrupts photosynthesis (Sanità Di Toppi and Gabbrielli 1999; Perfus-Barbeoch et al. 2002). On a cellular level, it interferes with the redox status and stimulates the production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), inducing oxidative stress (Cuypers et al. 2010, 2011). Free oxygen radicals cause

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