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Miscanthus as a Productive Biofuel Crop for Phytoremediation

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Table of Contents

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There are many locations where soil quality improvements would be beneficial because of contamination, erosion, flooding, or past human activities. Miscanthus, a C-4 grass related to sugarcane, grows well in mildly contaminated soil and on sites where soil quality is poor, particularly with respect to nitrogen. Because of its high biomass yield, it is of interest as an energy crop, and as a plant to use for simultaneous crop production and phytoremediation. Here we review recent literature on using miscanthus for combined biomass production and phytoremediation of contaminated and marginal lands. We analyze both advantages and disadvantages for production of this crop along with phytoremediation of sites contaminated with metals and petroleum hydrocarbon. Reports of laboratory and field investigations, which use *Miscanthus spp*. for stabilizing and removing metals are considered. The potential for growing miscanthus commercially at contaminated and marginal sites in the regions of Central and Eastern Europe as well as the United States appears to be good because large quantities of biomass can be produced and effective phyto-stabilization can be achieved with very slow metal removal over time. In addition, soil quality is improved in many cases.

Keywords heavy metals, miscanthus, petroleum compounds, Phytoremediation, second generation biofuels

I. INTRODUCTION

There are many reviews of miscanthus as a bioenergy crop, estimating productivity, adaptability and relative merit compared to other crops (c.f. Clifton-Brown *et al*., 2001; Fowler *et al*., 2003; Lewandowski *et al*., 2003; Fischer *et al*., 2005; DEFRA, 2007; Fischer *et al*., 2007; Eisentraut, 2010; Heaton *et al*., 2010; Zub and Brancourt-Hulmel, 2010; Brosse *et al*., 2012; Kim *et al*., 2012). Our focus is not mainly on production in good, agricultural soils but on lands in need of remediation. This review examines the production of miscanthus as a useful crop on lands with contaminants and on marginal disturbed lands where improvements in soil quality are needed. Both crop production and soil quality improvement are desirable and studies that relate to this focus are included.

A. Phytoremediation Principles

Phytoremediation, as first defined by Cunningham and Berti (1993), is now an established set of technologies for remediation and is considered an effective alternative to physical or chemical processes (Salt *et al*., 1995; 1998; USEPA, 2000; Mulligan *et al*., 2001; Pivetz, 2001; Suresh and Ravishankar, 2004; Arthur *et al*., 2005). In this approach plants are used for the *in situ* treatment of soils or waters polluted with different inorganic chemicals including heavy metals (Nanda Kumar *et al*., 1995; Salt *et al*., 1995; Dahmani-Mullera *et al*., 2000; Hemen, 2011), many organic substances (Davis *et al*., 2002; McCutcheon and Schnoor, 2003) including persistent organic pollutants (Lunney *et al*., 2004; Whitfield and Zeeb, 2010) and radioactive elements (Dushenkov *et al*., 1999; Dushenkov, 2003; Grytsyuk and Arapis, 2005; Abdel-Sabour, 2007; Cerne *et al*., 2011; Noskova *et al*., 2010).

Phytoremediation is considered an environmentally friendly and potentially economical approach, well suited for large areas, which have relatively low levels of contamination (Suresh and Ravishankar, 2004; Vangronsveld *et al*., 2009; Kulakow and Pidlisnyuk, 2010). Having a safe, marketable product of the phytoremediation process can greatly enhance the benefits in the overall economic balance. By use of a perennial energy crop that shows low accumulation of contaminants, one both manages the contamination, and receives a cash return on the use of the land. Forest products may be comparably effective and may show a net economic gain, but the return of most timber crops is low on an annualized basis.

B. Phytoremediation Processes

Five fundamental processes can be identified when plants are used for remediation of contaminated sites (Salt *et al*., 1998; USEPA 2000; Pivetz, 2001; Nixon, 2002; Vassilev *et al*., 2004; Yang *et al*., 2005):

1. *Phyto-immobilization:* plants prevent transport of dissolved contaminants in the soil.
2. *Phyto-stabilization:* plants mechanically stabilize polluted soils, and prevent bulk erosion and airborne transport to other environments.
3. *Phyto-extraction:* plants extract metallic and organic compounds from soil to plant tissue.
4. *Phyto-volatilization:* plants volatilize contaminants in soil or water to air.
5. *Phyto-degradation*: plants mineralize or assimilate contaminants in soil or water.

For most inorganic contaminants and crops, the first two phytomethods are the major processes that prove useful. Phyto-extraction of some elements can effectively be done by some species, but few such species are agricultural crops. The highly effective “hyperaccumulators” have been identified growing on soils with high metal content. For instance, amongst horticultural or agronomic crops only *Brassica juncea* has shown some promise, while a number of small members of the brassicaceae (*Thlaspi*, *Arabidopsis*, *Alyssum*) are hyperaccumulators of zinc and cadmium (Verbruggen *et al*., 2009). Phyto-volatilization of elements such as selenium and mercury is possible under certain conditions or with transgenic crops that are not yet on the market (often for regulatory reasons). Phyto-extraction plus phyto-degradation is a common fate of organic compounds (Davis *et al*., 1998; 2002). Related to this last process, an active microbial community in the rhizosphere may be the main driver in degradation of many compounds (Davis *et al*., 1998; Harvey *et al*., 2002). This process, if characterized, may be called rhizoremediation. Both grasses and trees are effective in supporting a microbial community capable of degradation of organic contaminants (Davis *et al*., 1998; Cook and Hesterberg, 2013). Warm season (C-4) and cool season (C-3) grasses may differ greatly in their ability to stimulate rhizodegradation and to take up and metabolize particular contaminants (Lin *et al*., 2008). For instance, the C-4 grass commonly called switchgrass (*P. virgatum* L.) much more effectively stimulated detoxification of atrazine compared to four other C-3 grasses. Miscanthus, another C-4 grass, has not been evaluated.

C. Phytoremediation Potential

The main advantage of phytoremediation is that it treats contaminated sites without excavation (Marmiroli and McCutcheon, 2003; Vanek *et al*., 2010; Zhu *et al*., 2010). However, in situ treatment requires more time, and may result in less uniform treatment than engineering solutions because of variable soil characteristics, climate and other in-field conditions (Davis *et al*., 1998; 2002; Liu *et al*., 2003).

Trees, crops and wild plants have been successfully used for phytoremediation of metals (Baker and Brooks, 1989; Pulford and Watson, 2003; Hammer *et al*., 2003), specifically through selective metal uptake and sequestration (Vassilev *et al*., 2004; Yang *et al*., 2005), and for organic contaminants including hydrocarbons, explosives, halogenated solvents and dyestuffs (Davis *et al*., 2002; Harvey *et al*., 2002; McCutcheon and Schnoor, 2003; Cook and Hesterberg, 2013). Some laboratory successes have been obtained with genetically engineered plants or associated bacteria (James and Strand, 2009; Van Aiken *et al*., 2010), but thus far, most examples in field settings are using unaltered and sometimes arbitrarily selected plants. For instance, native cottonwood may be equally effective as a hybrid poplar, and the hybrid is used merely because there is a readily accessible commercial source, though the disease resistance of the native tree is superior (personal observation, and personal communication, C. Barden).

Many of the processes of phytoremediation are common to many genera of plants, and in many instances only convenience or climatic adaptation is important. For instance, phytovolatilization of solvents seems to happen through any species of tree tested. So the choice of tree is determined by price, availability and adaptation to a particular climate. The main criteria in selecting plant species for phytoremediation of a particular site are the following: tolerance to contaminant substances known to exist at the site; extent of accumulation, translocation and potential uptake; climatic adaptation to e.g. heat, cold, wind; tolerance to water-logging and/or extreme drought conditions; availability and affordability; habitat preference, e.g. terrestrial, aquatic, semi-aquatic; tolerance to high or low pH and/or high salinity; growth rate and biomass partitioning and yield; and economics including potential for marketable product (Dickinson *et al*., 2009; Hemen, 2011).

The potential to use miscanthus and other biofuel crops for phytoremediation has been identified already (Xie *et al*., 2008; Masarovicova *et al*., 2009). There are several advantages when using biomass fuel crops such as miscanthus for phytoremediation, but also some disadvantages (Chaney *et al*., 1997; Nixon, 2002; Vassiliev *et al*., 2004; Masarovicova *et al*., 2009; Miller and Gage, 2011) (Table 1).

II. SECOND GENERATION BIOFUEL CROPS

The first generation biofuels, which largely used food crops to produce biofuel, met with concern because they directly displace food crops and negatively affect food security (Eisentraut, 2010). Second generation biofuel crops, generally non-food crops, or crop by-products, are preferable because they are not in direct conflict with food crops and may not directly affect food prices (Lewandowski *et al*., 2000; Fowler *et al*., 2003; Rosillo-Calle *et al*., 2006). Second generation biofuels are produced from cellulose, hemicellulose or lignin, i.e. biomass that can be derived from natural ecosystems like forests, grassland or aquatic ecosystems, or by cultivation of bioenergy crops (Corma et al., 2007; Pu et al., 2008; Naik et al., 2010). In addition, any kind of lignocellulosic waste like straw or sawdust could be used (Rosillo-Calle *et al*., 2006; Clifton-Brown *et al*., 2007).

As a result of resource availability, scientific and technological advances, and favorable policy, the concept of "biorefinery," the biomass-based parallel of the traditional petroleum refinery, has emerged (Naik *et al*., 2010; Daoutidis *et al*., 2013). It is envisaged that a biorefinery will draw a complex feedstock of biomass and convert it, in a series of processing steps, into valuable products. More than 200 processing facilities of varying complexity have been created across the U.S. to currently produce corn-ethanol, biodiesel, and cellulosic biofuels (Biorefinery locations, 2012).

Crops for second generation biofuel production (dedicated energy crops) can be divided into the following two main categories: short rotation trees, e.g. *Salix*, *Populus*, *Eucalyptu*s, and *Robinia* (black locust) species, and grasses (annual and perennial). Biofuel perennial grasses belong to family Poaceae and are represented by miscanthus (*M. sinensis*, *M. sacchariflorus*, *M. hybrida x giganteus*); switchgrass (*Panicum virgatum* L.); reed canary grass (*Phalaris arundinacea* L.); common reed (*Phragmites australis*); Johnson grass (*Sorghum halepense* Cav.); sugarcane (*Saccharum spp*.) (Hoogwijk *et al*., 2003). Both categories of perennial crops are cultivated in plantations with typical harvest periods of three to seven years for woody plants and annually for grasses (Eisentraut, 2010). In Taiwan, miscanthus may be harvested twice each year (Chou, 2009).

TABLE 1
Advantages and disadvantages of using biofuels energy crop Miscanthus x giganteus for phytoremediation (Fernando A. L. *et al*., 2004; Fischer G. *et al*., 2005; Heaton E.A. *et al*., 2010; Masarovicova E. *et al*., 2010; USDA, 2011; Brosse N. *et al*., 2012)

Advantages	Disadvantages
Perennial crop, does not have to be replanted every year	Uncertainty in transformation to energy products
High productivity and production of large quantities of biomass compared to other energy crops (20–35 t/ha)	Is large, tall, dense growing perennial grass with few wildlife friendly uses
Possibility to clean the site which may be transformed for future use	As a hybrid the seeds are not viable but the plant may be invasive through rhizome spread
Economic return can be obtained from the land with employment and accelerated market penetration of biomass fuels	May produce an extremely small number of viable seeds but a few viable seeds may be enough to cause invasive spread
Potential for income generation through carbon credits through CO_2 sequestration	Yields are influenced by water availability. Productivity under low-rainfall conditions may be poor
Reduction of soil erosion due to rainfall	Dedicated energy crops can result in displacement of other crops, which may lead to significant changes in land use
Reduction of windblown dust	
Does not require as much N as forage sorghum and sweet sorghum	
Grows at lower spring temperature and stops growing later in the season than other warm season grasses	
Crop uses water efficiently	
The harvested crop is relatively dry and drying costs are low	

A. Second Generation Biofuels in Phytoremediation

Recently, studies have been conducted worldwide using different energy crops for phytoremediation purpose (Meers *et al*., 2007; Van Ginneken *et al*., 2007; Meers *et al*., 2010; Nyesvyetov, 2010). Potential synergy exists between cultivation of second generation biofuel crops and phytoremediation of contaminated and marginal lands (Vassiliev *et al*., 2004; Lord *et al*., 2008; Witters *et al*., 2012; Pidlisnyuk V., 2012). First, using marginal land has the benefit of providing a partial solution to the problem of limited agricultural land (Cai *et al*., 2011; Gopalokrishnan *et al*., 2011; Qin *et al*., 2011). Second, reusing derelict industrial sites provides an economic advantage, since the major capital cost of land is avoided compared to using productive agricultural land. Poorly vegetated contaminated sites are frequent sources of diffuse pollution damaging the quality of natural resources; reusing these sites for bioenergy crop production could be a beneficial solution (Gallagher, 2008; Kechavarzi and Lord, 2009). As discussed by Witters *et al*. (2012), the carbon abatement value of phytoremediation adds to its benefits relative to conventional clean-up approaches.

The majority of literature on phytoremediation of marginal lands is about using fast-growing woody plant species (Riddell-Black *et al*., 1996; Punshon *et al*., 1996; Punshon and Dickinson, 1997). Much less attention has been devoted to using non-woody perennial crops (Techer *et al*., 2012a;b;c) and only a few sources have reported using second generation biofuel perennial crops for phytoremediation or restoration of marginal land especially in Eastern and Central Europe (Prasad, 2006; Rakhmetov, 2007; Barbu *et al*., 2009; Barbu, 2010; Hromadko *et al*., 2010; Los *et al*., 2011; Kocon and Matyka, 2012). Some information is available for EU countries (e.g. Riddell-Black, 1998; Pourrut, 2011), but there is very little information on the use of miscanthus for phytoremediation in the U.S. Below, we review both laboratory and field studies of miscanthus in phytoremediation.

B. Miscanthus Composition, Value, and Processing

Miscanthus contains about 22% lignin, 36% α-cellulose, and 24% hemicellulose (Kim *et al*., 2012). While miscanthus may significantly contribute to the future energy supply, cost of processing cellulosic biofuels is still a major barrier to wide commercial production in comparison with other fuels (Carriquiry *et al*., 2011; Vassilev *et al*., 2004; Katzer, 2011). Technologies are not yet fully developed for separation of hemicelluloses and lignin from cellulose, conversion of cellulose to fermentable sugar, and utilization of the hemicelluloses and lignin fractions for production of value-added products. The annual harvestable energy production of miscanthus is favorable at > 17 MJ/kg dry matter (Collura *et al*., 2006) and $> 10{,}000$ kg.ha^{-1} yield (total 170,000 MJ/ha/year). Some detailed combustion research with miscanthus has been reported (Dahl and Obernberger, 2004; Collura *et al*., 2006; Dorge *et al*., 2011). Biomass yields over 20,000 kg.ha^{-1} are reported, but typical late winter harvest gives a lower practical yield. However, the late harvest has an advantage of allowing the leaching of nutrient ions back to the soil and also deposits organic litter on the soil surface. Together, these lower the demand for fertilizer, and improve soil quality and heavy metal sequestration.

With hydrothermal pretreatment followed by enzymatic hydrolysis, a sugar yield of 55.1 g of glucan plus xylan per 100 g of dry *M. x giganteus* has been reported (Zhang *et al.*, 2012). An alternative strategy of thermochemical conversion of biomass has witnessed tremendous progress recently (Naik *et al.*, 2010; Daoutidis *et al.*, 2013). For instance, a variety of chemical processes, spanning gas phase free-radical pyrolysis to homogeneous and heterogeneous acid, metal, and base catalysis have been developed. Catalytic pyrolysis and reforming of lignocellulose, dehydration of fructose to produce 5-hydroxymethylfurfural (HMF), isomerization of sugars, dehydration and hydrogenolysis, Diels-Alder addition to oxygenates, and hydroxyalkylation are but a few examples of these rich and rapidly growing research activities (Heaton *et al.*, 2003; Corma *et al.*, 2007; Resasco and Crossley, 2009; Serrano-Ruiz *et al.*, 2010; Dapsens *et al.*, 2012). Pyrolysis research with miscanthus has resulted in gas phase and liquid phase products including oxygenated liquids which can serve as feedstocks for petrochemical conversions (Yorgun and Simsek, 2008; Melligan *et al.*, 2011). For instance, phenol derivatives may be processed further to obtain valuable products such as resorcinol and phenolic based adhesives (Melligan *et al.*, 2011). These studies bring miscanthus into line with agricultural crops including sorghum, maize and wheat which are being similarly processed as chemical feedstocks. Progress with fast pyrolysis for biomass conversion was recently reviewed by Dickerson and Soria (2012).

C. Miscanthus Productivity as a Biofuel Crop

Among perennial grasses to be used for production of biofuel *M. x giganteus* and *P. virgatum* both have C-4 photosynthesis and are considered among the most promising crops (Ercoli *et al.*, 1999; Clifton-Brown *et al.*, 2001; Long and Beale, 2001; Lewandowski *et al.*, 2003; DEFRA, 2007; Heaton *et al.*, 2010; Los *et al.*, 2011; Zub and Brancourt-Hulmel, 2010; Brosse *et al.*, 2012). The hybrid *M. x giganteus* is a large, sterile triploid perennial grass derived from a cross between *M. sinensis* and *M. sacchariflorus,* native to southern and eastern Asia respectively. The hybrid is adaptable to areas not experiencing deep freezing of the soil and neither excessively wet or dry. Genetic efforts to improve miscanthus have been reviewed (Chou, 2009). There are 20 different species of miscanthus and under some conditions, higher production may be obtained with species other than those in common use (Liu *et al.* 2013). In Hunan Province, People's Republic of China, *M. lutarioriparius* yields twice as much biomass as *M. sinensis*. As illustrated below, most work on biomass production or phytoremediation has been done with the sterile *M. x giganteus*. Fertile species are invasive in some settings and some have been designated as invasive species (USDA 2013a; b). Miscanthus species are normally early colonizers, for instance on newly forming volcanic soils in Japan (Stewart *et al.*, 2009).

It has been predicted that as a biofuel crop *M. x giganteus* may supply up to 12% of the European Union's energy need by 2050 (Fruhwirth and Liebhard, 2004). Its total above-ground biomass yield in European conditions may reach 20 to 35 $t.ha^{-1}.yr^{-1}$ (van der Werf *et al.*, 1993; Hotz, 1996; Himken *et al.*, 1997; Venendaal *et al.*, 1997). Similar total production for miscanthus - 24–35 $t.ha^{-1}.yr^{-1}$, is reported for the U.S. (Lewandowski, 2003; USDA, 2011). In addition, miscanthus can grow well in contaminated soils and is adaptable in a relatively cool European or warm climate such as southern Missouri, U.S.A. where around 5000 hectares are established (The Doe Run Company project, 2012).

Compared with other biofuel crops, miscanthus grass produces more mass and hence more energy per hectare (Fischer *et al.*, 2007; Heaton *et al.*, 2010). This difference is partly attributable to higher leaf photosynthetic rates at low temperatures as confirmed in two complete growing seasons in the field (Dohleman *et al.*, 2009). Thus, although miscanthus is a C-4 grass expected to be efficient in water use under high temperatures and light intensity, it is adapted to continue operating in a C-4 mode even at relatively low temperature, compared to switchgrass. Another important factor compared to annual maize is the longer seasonal duration of active photosynthesis, because miscanthus grows from rhizomes, not seeds, and establishes a closed canopy earlier in the season. It also senesces later in autumn because there is no need to ripen harvestable grain, unlike maize that is selected to senesce and translocate nutrients to the ripening grain prior to frost. The advantages and disadvantages of growing *M. x giganteus* as a productive energy crop for phytoremediation are illustrated in Table 1.

D. Miscanthus Production on Marginal Land

When attempting to produce miscanthus on marginal, or contaminated soils, soil amendments may be essential; they are certainly beneficial. Kilpatrick (2012) reported in detail on benefits of biosolids from wastewater treatment for increasing miscanthus productivity on marginal (low pH and low nutrients) land in southern Ohio, U.S.A. A shorter summary of the work is provided by Islam *et al.* (2012). Organic by-products and distillery effluents have been applied to miscanthus plots in Ireland to investigate the potential to dispose the effluents while improving soil quality (Galbally *et al.*, 2012). Irrigation with such effluents benefits the crop both by the water supplied in climates where water might be limiting, and by the nutrients present in the effluent stream. However, on the particular soil being studied, at the highest application rate of 15 tons P $.ha^{-1}.yr^{-1}$, phosphate release to ground water exceeded by two-fold the allowable limits for Ireland (35 $ug.L^{-1}$). This is not surprising because the P demand of miscanthus is low, only 1–2 $kg.t^{-1}$ dry matter if harvested in winter, implying a maximum off-take of 20-40 $kg.ha^{-1}.yr^{-1}$ for typical maximum yields of 20 $t.ha^{-1}.yr^{-1}$.

The potassium needs of miscanthus may be 10 times those of P, with the K content of dry matter varying with the date of harvest and the climate. With winter rain a significant fraction of the K is leached, returning to the soil (Brosse *et al.*, 2012). This level of K off-take is appreciably higher (∼3 times) than that of coppiced willow, an alternative bioenergy crop for some regions (Potash Development Association, 2009). However the P requirement of miscanthus is ∼2 times lower than that of coppiced willow. Christian *et al.* (2008) recommend addition of 7 $kg.ha^{-1}.yr^{-1}$ P_2O_5 and 100 $kg.ha^{-1}.yr^{-1}$ K_2O in typical production conditions for miscanthus in southern England (e.g. at Rothamsted research area). Economic production of crops requires inclusion of nutrient input costs, which become non-trivial in long-term production.

Nitrogen is another commonly limiting nutrient for high yields of most crops. Depending on soil type, and perhaps the microbial ecosystem, the N demand and fertilizer responsiveness of miscanthus has been reported to vary widely (Wang *et al.*, 2012; Christian *et al.*, 2008). One important factor as discussed by Heaton *et al.* (2009) is the return of N from foliage to roots during senescence in winter. There is some circumstantial evidence for N fixation in miscanthus, including the presence of potentially N2 -fixing species, as reported by Davis *et al.* (2010). However, there is no definitive evidence for fixation in field conditions. One needs to show specific incorporation of ^{15}N label (Boddey *et al.*, 1991), or reduction of deuteroacetylene (Lin-Vien *et al.*, 1989).

Christian *et al.* (2008) observed in the U.K. that with continuous cropping over 14 years there was no decline in production on soil with no added N. Similarly, Dohleman *et al.* (2012) have obtained yields of 38 tons/ha in Illinois, USA with no added N. However, this latter result was found on fertile agricultural soils that may have large reserves of slowly mineralizable N in organic matter (∼1–2% by weight in the top 0.2 m, or 20–50 thousand $kg.ha^{-1}$). The N content of miscanthus harvested in winter is below 2 $g.kg^{-1}$ so that only about 40 $kg.ha^{-1}.yr^{-1}$ would be needed on soils with a typical yield of 20 $t.ha^{-1}$. Atmospheric deposition of N already exceeds 10 $kg.ha^{-1}.yr^{-1}$ in many parts of Europe and the United States (Bobbink *et al.*, 2010) and averages 7 $kg.ha^{-1}.yr^{-1}$ even on undisturbed prairie in eastern Kansas, USA (CASTNET, 2013). The net off-take of N would be a very small fraction of the total deposited plus mineralizable N in good soils.

Miller (2010) analyzed the nitrogen and land use intensity for production of bioenergy for a wide range of potential crops. On a land area basis, for crops other than algae, sugarcane was highly favored with sugar beet doing equally well or better. However, sugar beet requires about 4 times more N input per unit bioenergy compared to sugarcane. Willow and miscanthus rank behind oil palm on energy per area, but much ahead of it in terms of N input. If miscanthus can in fact fix nitrogen, that may place it ahead of everything except sugarcane, for which there is also evidence of N fixation under suitable conditions (Boddey *et al.*, 1991). In areas other than tropics where sugarcane and oil palm grow, miscanthus is favored overall.

E. Miscanthus and Carbon Sequestration

Miscanthus production on mineral-rich, organic matter deficient marginal lands will result in increases of soil C over time. The magnitude of increase depends on total production and time of harvest because a significant fraction of foliage drops slowly during autumn and winter. There is significant carbon sequestration for marginal lands (Clifton-Brown *et al.*, 2007). Those authors monitored a miscanthus (*M. x giganteus*) field that had been established for 15 years in Ireland and by using C isotope dilution, found an average of 0.6 $tons.ha^{-1}.yr^{-1}$ carbon sequestration.

Stewart *et al.* (2009) reported values in the range of 2 $t.ha^{-1}.yr^{-1}$ soil C accumulation for *M. sinensis*, native to Japan. Information for net soil C gain in other environments, and for the hybrid *M. x giganteus,* is limited. Borzecka-Walker *et al.* (2008) compared miscanthus and willow for a location in Poland and calculated that about 0.6 $t.ha^{-1}.yr^{-1}$ of C was sequestered by miscanthus, compared to about 0.3 $t.ha^{-1}.yr^{-1}$ for willow. Zimmermann *et al.* (2012) estimated C sequestration amounts that varied widely in southeast Ireland, depending on the patchiness of the miscanthus stands in established fields. They used stable isotope ratios to derive values ranging from ∼0.2 up to > 1 $t.ha^{-1}.yr^{-1}$.

Long-term study fields of miscanthus in Germany were analyzed by Schneckenberger and Kuzyanov (2007). They estimated that in a loamy soil with the crop grown for 9 years there was stable incorporation of about 0.23 $t.ha^{-1}.yr^{-1}$ soil organic C in the top 1 m of soil, while after 12 years in a sandy soil the annual accumulation was about half as much. These values are comparable to what is observed for permanent C-3 grasslands in the same climatic conditions.

F. Miscanthus in Phytoremediation Applications

The water use efficiency of miscanthus is high, because it is a C-4 type grass. When available sunlight is high and temperatures are adequate, miscanthus produces large crops of standing biomass, hence using significant amounts of water, typically 1 $m.yr^{-1}$ or more. Thus it may be useful for phyto-immobilization and phyto-extraction applications (Clifton-Brown *et al.*, 2001; Van Loocke *et al.*, 2012; Zhuang *et al.*, 2013). A typical application would be as a cover crop on a closed landfill, or down gradient of a landfill to capture leachate. Gopalokrishnan *et al.* (2012) investigated the use of a buffer strip of miscanthus to produce a crop while improving water quality by capturing nitrate in ground water draining from fertilized agricultural fields. Estimated nitrate decreases were >60%, but would depend on the starting nitrate value and the vigor of the crop. Miscanthus would likely be also effective in capturing atrazine run-off (c.f. Lin *et al.*, 2008)

One key aspect of research is management of the phytoremediation process, such as the biofuel crop has commercial value while also stabilizing or removing the heavy metals or other contaminants at a useful rate. Schmidt (2003) has reviewed the processes that alter metal uptake into plants, including the use of

TABLE 2
The results of heavy metal determinations in agricultural soils of Slovakia (mg/kg) (Kobza, 2005)*

Heavy metals	Total content***			Content in 2 mol/l HNO3****			Content in 0.05 mol/l EDTA		
	Geometric mean x_G	min	max	Geometric mean x_G	min	max	Geometric mean x_G	min	Max
Cd	0.285	0.050	9.05	0.169	0.010	6.85	0.088	0.010	3.60
Pb	24.9	9.5	1050	14.2	3.70	649	3.56	0.160	268
Cr	72.7	10.5	170	2.09	0.100	43.1	0.162	0.010	2.90
Ni	12.8	0.3	57.5	3.22	0.200	19.1	1.04	0.110	8.60
Cu	22.3	5.0	156	7.55	1.00	171	3.27	0.300	80.5
Zn	64.3	11.0	1070	12.3	2.05	565	2.35	0.050	126
Hg	0.075	0.009	6.69	–	–	–	–	–	–

Note. *Altogether 429 sites were detected, among them 314 agricultural sites and 112 forestland sites**.
** Soil samples were collected from the surface layer (depth 0–0.1m) and treated.
*** Total content was estimated after treatment of soil samples by mixture of acids ($HCl+HNO_3+HF$).
**** EPA standard.

both organic and inorganic agents to form water-soluble metal complexes. Plants differ in their uptake of metals, so plant selection is also important (Peng *et al*., 2006). Plants are selected for the commercial value of the product and their ability to take up metals at the desired rate, which depends on the acceptable metal concentration for processing the harvested crop. For combustion, safe management of ash or volatile elements such as mercury or selenium, is an important consideration.

Miscanthus generally takes up only small amounts of the toxic heavy metals. However, it should be noted that not all researchers are in agreement with the assumption that these levels of uptake are safe (Pogrzeba *et al*., 2010). Their central argument is that *M. sacchariflorus* contains significantly higher amounts of toxic heavy metals including Zn (10 x), Cd (2 x) and Pb (20 x) compared to coal on the basis of metal amount per energy yield. Uptake into harvested plant parts was relatively low on a basis of $mg.kg^{-1}$. For instance in a soil containing Pb = 8.73, Cd = 4.07, Zn = 385 $mg.kg^{-1}$ the values for biomass were Pb = < 3, Cd = 0.3 and Zn = 25 $mg.kg^{-1}$. The energy density of miscanthus is much lower than coal, so that the metal amount per unit energy is still relatively high.

The impact of miscanthus cultivation on soil metal availability was assessed by Iqbal *et al*. (2013) at a site in France. A field near a large lead smelter for over a century, until 2003, had been converted from agricultural production with annual crops to miscanthus three years previously. Heavy metals of concern included Cd, Cu, Pb and Zn. Selective equilibrium extraction with EDTA, and rate of extraction, were used as proxy measures of potential bioavailability. Different particle size classes were compared. Both Cu and Pb extractability were decreased under miscanthus, compared to annual crops, which included wheat, broad beans and sugar beets. For Cd and Zn no clear conclusions could be drawn for this relatively short time period of perennial vs annual cropping. Organic carbon levels were higher in some soil fractions in the miscanthus cropped area, but increased net carbon accumulation (if any) was not reported.

G. Extent of Degraded Lands Available

In the U.K., over 66,000 ha of brownfields land may be potentially available for cropping with miscanthus (Hartley *et al.* 2009). Nearly twice that area is indicated as present in Germany, but less than half as much in France, while Poland and Romania may have well over 500,000 ha each (Oliver *et al*., 2005). Definitions of what constitutes a brownfield, and the extent of contamination varies widely.

Central and Eastern Europe are characterized by an intensively developed mining industry, so that many regions are polluted by heavy metals from industrial processing, including metalliferous (mining, smelting industry, and paint factories) (Banasova, 2004; Krizani *et al*., 2009; Buchek *et al*., 2011), agricultural operations (fertilizers and pesticides), and municipal activities (sludge and landfills) (Kalembasa and Malinowska, 2009a; Kulakow and Pidlisnyuk, 2010). There are some former Ag, Cu and Au mining sites in Slovakia polluted by heavy metals (Andras *et al*., 2008). Some data, which illustrate the magnitude of pollution of land in Slovakia and Ukraine, are presented in Tables 2 and 3, respectively. The data for Slovakia represent a nationwide survey done prior to ~2003 (Kobza, 2005). Digestion to determine metal content used three standard methods. The total metals content is indicative of the maximum potential for metal availability. The USEPA method is a standard one used to determined metal content of soils in areas where contamination is a specific concern, such as in the Tri-State mining district of SE Kansas, NE Oklahoma and SW Missouri. The less vigorous use of EDTA extraction is often used in an attempt to determine bioavailable metal content (John and Leventhal, 1995). It is evident that levels of potentially bioavailable Pb and Cd are rather high at some sites in Slovakia, although overall the geometric mean is in a quite acceptable range.

TABLE 3
Contamination of soil by heavy metals in selected places in Ukraine (Ministry of Ecology and Natural Resources of Ukraine, 2012)

		Maximum analyzed concentration, mg/kg of dry soil					
Place*	year	Cd	Mn	Cu	Ni	Pb	Zn
Kyiv	2007	0.8	1200.0	275.0	34.0	188.8	1104.0
	2008	0.1	750.0	44.0	25.5	137.6	264.5
	2009	0.3	1050.0	44.0	51.0	179.2	276.0
	2010	1.0	1050.0	88.0	59.5	227.2	368.0
	2011	0.5	1350.0	148.5	25.5	32.0	115.0
Kostyantunivka, Donetsk oblast	2007	10.5	1500.0	346.5	34.0	528.0	644.0
	2008	43.5	4500.0	214.5	51.0	947.2	1702.0
	2009	25.5	4950.0	286.0	42.5	1424.0	2104.5
	2010	10.5	4950.0	44.0	42.5	544.0	2323.0
	2011	11.5	8100.0	126.5	68.0	848.0	2633.5
Mariupol, Donetsk oblast	2007	1.8	2700.0	374.0	34.0	377.6	644.0
	2008	2.8	3600.0	319.0	93.5	320.0	655.5
	2009	2.8	3300.0	165.0	85.0	611.2	264.5
	2010	4.3	11550.0	82.5	59.5	918.4	931.5
	2011	1.5	5550.0	242.0	93.5	313.6	954.5

Note. Spellings are Ukrainian in accordance with http://www.mapofukraine.net/travel_info/list-of-ukrainian-cities-and-towns.html; oblast is a geographic region.

One of the monitoring reports in Poland (Oleszek *et al.*, 2003) shows an increase of Zn and Pb concentration in the soil and agricultural products over time. In addition, the regions of Central and Eastern Europe have many former military sites now classified as marginal lands, which have to be remediated for future use (Ministry of the Environment of Czech Republic, 2009; Klein, 2012).

A similar contamination is observed at the Tri-State mining district, which contained a major source of lead and zinc ores in the U.S. in the 19–20th centuries. Ranges in concentrations now found in streambed sediments are 0.6 to 460 (median 13) $mg.kg^{-1}$ for Cd, 22 to 7400 (median 180) $mg.kg^{-1}$ for Pb, and 100 to 45,000 (median 1800) $mg.kg^{-1}$ for Zn (Pope, 2005). Comparable levels are found in nearby fields, while mine tailings and overburden piles cover thousands of hectares. On these lands, organic matter is a limiting factor for the establishment of crops to stabilize and remediate the soils (Ganga Hettiarachchi, personal communication).

On a worldwide basis it has been estimated that 300–700 million ha of marginal and contaminated land are available for miscanthus production and in need of improvement (Cai *et al.*, 2011). Price *et al.* (2004) used maps of modeled climatic conditions and experimentally measured yields of miscanthus at many locations to predict the extent to which climate limits yield for locations in the U.K. There was reasonable agreement between predicted yields and rainfall data across the different regions. Land use classification by GIS has been described by Lovett *et al.* (2009), specifically for the U.K. This is a realistic extension of the work of Price *et al.* (2004). The types of crops or forests grown now, the quality of the land for agriculture, the predicted yield of miscanthus, and the socioeconomic factors including settlement patterns were all considered by Lovett *et al.* (2009). Such use of GIS can indicate whether a large enough crop can be grown in a particular region to economically support a second generation biofuel system.

Worldwide there must be places for several thousand such biofuel processing facilities, but for many regions the crop may not find a high value market. In other regions the density of planting sites within a reasonable radius is too small to support a large processing facility. As a specific instance, production in Ontario, Canada would not be an economically viable program if only marginal lands were used. It is suggested however, that use of a relatively small fraction of higher quality lands would make the production of considerable biomass a feasible proposition (Kludze *et al.*, 2013)

H. Ecological Impacts of Miscanthus as a New Large-Scale Crop

Second generation biofuel production from perennial grasses is likely to rise in importance in Eastern and Central European countries (Kahle *et al.*, 2001; Lyubun and Tychinin, 2007; Stasiak, 2007; Majtkowski *et al.*, 2009) including Ukraine and Slovakia (Fischer, 2005; Masarovicova *et al.*, 2009; Rakhmetov, 2007; Stefanovska *et al.*, 2011a; Pidlisnyuk, 2012), as well as in the United States (USDA, 2011). On highly productive land in the United States, some studies have been conducted to determine the overall impacts of the crop on nutrient cycles, including C and N. (Behnke *et al*., 2012).

In Slovakia, research on growing miscanthus and switchgrass for biofuel production has just recently started (Gubisova *et al*., 2011; Masarovicova *et al*., 2009). In Ukraine, miscanthus and switchgrass have been growing for commercial evaluation since 2005 at several experimental stations (Zinchenko *et al*., 2006; Rakhmetov, 2007; Gumentik, 2010; Stefanovska *et al*., 2011a). A survey of the most appropriate areas for planting miscanthus in Ukraine showed that many are in the eastern and southeastern parts of the country, which has the best potential for productive harvest (Geletukha *et al*., 2011), and this part of the country is the most polluted (Table 3).

Recent research has identified potential environmental concerns related to large-scale development of miscanthus in a food grass-crop dominated agricultural landscape in this region (Fernando *et al*., 2010). First, plants like switchgrass and miscanthus have not been grown in massive monoculture to produce biomass, so there are no current guidelines for their production (Thomson and Hoffmann, 2011). Second, if these crops are to be grown on marginally arable land, they may be affected by herbivore pests and plant diseases at a rate that exceeds what would be expected if the plants were not stressed in this manner. Research carried out in the United States indicates a growing number of insect pests causing damage on miscanthus, but identity of pests or their effects on biomass are uncertain (Prasifka *et al*., 2009; Spencer and Raghu, 2009; Prasifka *et al*., 2012). Populations of invertebrates are present in larger numbers in miscanthus fields compared to wheat fields (Hedde *et al*., 2013).

Our original survey of insect herbivore populations and natural enemies on miscanthus and switchgrass started in Ukraine in 2009. Preliminary results indicate that different life stages of insects from six orders were present on miscanthus during the growing season: Hemiptera, Homoptera, Diptera, Coleoptera, Lepidoptera and Thysanoptera (Stefanovska *et al*., 2011). No significant plant injury by insects was observed with the exception of the Hessian fly, *Mayetiola destructor* (Diptera: Cecidomyiidae), which was observed inside the stems of miscanthus. Hessian fly along with other species from the families Chrolopidae and Anthomyiidae are destructive pests of several cereal crops so that there is a potential risk to not only reduce yield of miscanthus, but also damage adjacent food crops.

Introduction of second generation perennial biofuel crops is promising both in terms of energy production and phytoremediation of contaminated/marginal lands, but caution is needed. For successful introduction and use of perennial biofuel grasses it is necessary to understand and critique the current state of development, and to develop effective strategies for using perennial grasses as phytoremediation agents while avoiding negative effects on other crops, populations or the environment (Emmerson *et al*., 2011; Bellamy *et al*., 2008). In the U.S. and EU most work has focused on the sterile hybrid *M. x giganteus* because of concern for potential invasiveness of other species.

III. LABORATORY RESEARCH USING MISCANTHUS FOR PHYTOREMEDIATION

There have been very few large scale field studies of metal phytoremediation with perennial grasses, other than some phytostabilization research such as that performed in the Tri-State mining district (e.g. in Galena, KS), although those studies were not performed with biofuel grasses. Various grasses have been used in remediation of petroleum hydrocarbons, but again not perennial biofuel crops (Van Epps, 2006; Cook and Hesterberg, 2013). The available field studies with miscanthus for metal phytoremediation are reviewed in section 4. The main lab-scale phytoremediation research using miscanthus has examined uptake of metals and metalloids. There is also some limited amount of work on growth in presence of and degradation of petroleum hydrocarbons. Results are summarized below.

A. Metals and Miscanthus

Miscanthus spp. grown on metalliferous soils in a pot study under greenhouse conditions took up Cu, As and Zn with little difference in uptake of metals from polluted and unpolluted soils (Wilkins, 1997). This study demonstrated that miscanthus was able to grow and survive on highly polluted soils. Using inorganic fertilizer and/or lime greatly improved the yield of miscanthus in contaminated soils with very little influence on the uptake of metals. The author concluded that there was little cause for concern with growing miscanthus as a biofuel crop on mining polluted soil.

The use of miscanthus as an accumulator of heavy metals was studied in pots (Fernando *et al*., 1996; Fernando and Oliveira, 2004). Results indicated that higher heavy metal concentrations in the soil negatively affected plant growth and productivity. Miscanthus was able to accumulate and remove heavy metals (Cd, Cr, Cu, Ni, Pb and Zn) from the soil into the below ground hypogeal part of the plant, but there was no significant accumulation of heavy metals in aerial parts. Tolerance to Ni, Pb, Zn and Cr near their Portuguese sludge permissible content limits was greater than that for Cd, Cu, Hg. A related experiment was done in the field with different levels (0-200 $t.ha^{-1}$, 25% solids) of domestic sewage sludge (Fernando and Oliveira, 2005). Measured sludge metal levels in units of $mg.\ kg^{-1}$ on a dry matter basis were: Cd = 0. 7, Cr = 55, Cu = 134, Hg = 5, Ni = 25, Pb = 94, Zn = 940. Productivity, plant height and stem number increased with increasing levels of sewage sludge (up to 100 $t.ha^{-1}$), but levels of heavy metals (Cd, Cr, Cu, Ni, Pb and Zn) in the aerial part of miscanthus did not significantly increase with increasing metal concentrations in the soil. The ash, nitrogen and phosphorus content in the aerial part of the plants did increase with increasing levels of contaminants (Fernando *et al*., 2004). The belowground material did accumulate higher concentrations of heavy metals with increasing levels of contaminants (Nixon, 2002.)

Ezaki *et al*. (2008) reported that *M. sinensis* showed tolerance to aluminum (up to 900 uM), chromium, and zinc. Several tolerance mechanisms have been identified, including suppression of oxidative damage by increased levels of superoxide dismutase and catalase, exclusion of Al from the root tip, perhaps by exudation of chelators, and transport of Al from root to shoot, where it is presumably sequestered. This was a screening test done with seeds germinated in a dilute calcium chloride solution and then exposed to the Al stressor for 14 days. The effect of long-term exposure to Al was not studied.

The impact of low micromolar concentrations of cadmium on growth and antioxidant enzymes activities in *M. sinensis* under hydroponic conditions was investigated by Scebba *et al*. (2006). Levels up to 6.6 uM ($\sim$0.74 mg.L^{-1}) of Cd were tested. Dry matter accumulation was affected with few visible signs, except that roots became shorter and thicker and root systems became more dense and compact after treatment with the highest Cd concentration.

Arduini *et al*. (2003; 2004; 2006a) investigated the possibility of using miscanthus for Cd uptake and explored different conditions of the process. Cadmium as Cd $(NO_3)_2$ was tested on plants grown in sand culture. Results confirmed that miscanthus is not able to effectively adapt to available Cd concentrations above 2.5 mg.L^{-1} and is sensitive to Cd concentrations higher than 0.5 mg.L^{-1} ($\sim$ 4 uM). At 0.25 and 0.5 mg.L^{-1} Cd levels there was some stimulation of plant growth in the first month of culture, but all plants markedly decreased growth between one and three months, indicating a limiting factor other than Cd.

Response of *M. sinensis* Var Giganteus (probably synonymous with *M. hybrida x giganteus*) during long-term application of chromium in hydroponic conditions was investigated by the same research group (Arduini *et al*., 2006b;c). Plants were exposed to Cr for 36 days, at the stage just before heading. Plants were grown from 20 g rhizome pieces in pots of sand and transplanted to a hydroponic nutrient film system with added nutrients optimized for corn and sorghum. The Cr was supplied beginning at day 81 of the nutrient film culture (with a plant weight of about 60 g). Plant biomass gain was reduced with all tested concentrations of $Cr^{(III)}$, as the nitrate salt, at levels of 50–200 mg.L^{-1}. Most of chromium taken up by the plant was retained by the hypogeal part, but growth of all parts was affected, with about 25% decrease of biomass accumulated at 50 mg.L^{-1} and >50% decrease at 100 mg.L^{-1}. Chromium concentrations reached 6000 mg.kg^{-1} in the hypogeal portion of the plant. This would not be part of the field-grown harvest, being underground.

Following up on the work of Arduini *et al*., Sharmin *et al*. (2012) developed a proteomic analysis of how Cr, provided as potassium dichromate (K_2CrO_4), affects protein expression in roots of *Miscanthus sinensis*. Plants were grown from seeds for 3 weeks and then maintained in 1/2 strength Hoagland's solution hydroponically for 1 week. Exposure to Cr lasted an additional 3 days. At levels of Cr below 250 uM (13 mg.L^{-1}), there was little effect on root growth or plant dry weight increase. There was a notable shortening of both roots and shoots compared to controls at or above 500 uM Cr, with a 50% decrease at 1 mM Cr (52 mg.L^{-1}). Given that the plants were 4 weeks old at the time and had been in 1/2 strength Hoagland's solution for a week, following 3 weeks after germination in a potting mixture, this is a very striking effect. It may represent complete inhibition of new growth immediately following exposure to the Cr source. No data was provided on the appearance or weight of plants immediately prior to the Cr exposure. At the highest level of Cr exposure, root tissue accumulated 1300 mg.kg^{-1} Cr. It is important to note that the Cr was supplied as Cr^{VI} in this study, although no measurements are provided to indicate whether it remained in that form while in aerated 1/2 strength Hoagland's solution. Three dozen proteins showed significantly altered (increased or decreased) levels in electrophoretic analyses of root extracts. Major responding protein classes included stress response, defense, nitrogen metabolism, energy metabolism, ion transport, metal detoxification, cell division. There may be a threshold for Cr toxicity, and Cr^{VI} may be more toxic than Cr^{III}.

The influence of sewage sludge fertilization applied at different rates to *M. sacchariflorus* was compared with plant treatment by mineral fertilizers for uptake of different metals (Pb, Cd, Cr, Co, Cu, Zn, and Ni) into stems and leaves during two years of observation (Kalembasa and Malinovska, 2009a;b;c). Higher concentrations of heavy metals (except Zn) were found in leaves than in stems of the grass. Cadmium was not detected in *M. sacchariflorus* biomass in the first year whereas large amounts of the metal were recorded in the second year, 6–9 mg.kg^{-1}. The variation of doses of sewage sludge did not influence the accumulation levels of metals in either year.

In the third year of pot greenhouse experiment, Kalembasa and Malinowska (2009b) compared the impact of fertilization with sewage sludge vs mineral fertilization on *M. sacchariflorus* biomass production in the presence of Fe, Mn, Mo, B, Ba, Sr, As, Sn, Li and Ti. Yield of plants was evaluated on the basis of two harvests, in June and December. Significant impact of sewage sludge fertilization on the plant yield was found. Results showed that biomass harvested in December contained higher metal concentrations compared with those harvested in June. All metal uptake was higher for *M. sacchariflorus* fertilized by sewage sludge than those fertilized by mineral fertilization. Management of brownfields land under different settings was considered by Hartley *et al*. (2009) for three sites in the U.K. One site had been a depository for alkaline coal flyash, another had canal sediments and a third was a former landfill. All had moderately high As (78, 59 and 72 mg.kg^{-1}). The sediments had comparatively high Cd (36 mg.kg^{-1}), Cu (508 mg.kg^{-1}) and Ni (44 mg.kg^{-1}). One 10 cm rhizome was planted in each pot of $\sim$5 kg soil, unmodified, or supplemented with green waste compost (30% v/v), or biochar (20% v/v). Triplicates were established and maintained in a greenhouse for 8 mo., watered with tap water. While the As level in roots reached $\sim$ 8 mg.kg^{-1} for the 1st soil, root levels were much lower in the other two, and levels in above-ground biomass never exceeded 1 mg.kg^{-1} in any treatment. Total biomass production was enhanced in each soil by addition of green waste compost and unaffected by addition of biochar, but all yields were 1/3 to 1/2 that of a peat-based standard soil. The low transfer ratio of < 0.01, comparing above-ground biomass to soil, indicates that miscanthus may be suitable for production of biomass on such contaminated soils in the U.K.

Two recent publications (Ollivier *et al*., 2012; Wanat *et al*.,2013) describe the tolerance of miscanthus to the heavy metal lead, and the metalloid arsenic in soils that were naturally developed from long-term contamination in settling ponds at a former gold mine that had been abandoned 45 years. Three levels of As, along with Pb were tested under the headings of severe (S), moderate (M), and low (L) (Ollivier *et al*., 2012). The soil samples for this pot study were collected and pooled from sections to 20 cm depth. The contaminants were well aged in situ. Although the soil S contained 83,000 $mg.kg^{-1}$ As and $\sim$ 1500 $mg.kg^{-1}$ Pb at a pH of 3.4, there was no significant decrease in miscanthus growth compared to soil M with 9300 $mg.kg^{-1}$ As and 200 $mg.kg^{-1}$ Pb at pH 3.5 or soil L with 1700 $mg.kg^{-1}$ As and 300 $mg.kg^{-1}$ Pb at pH 5.6. The extractable As level with 10 mM $CaCl_2$ was below 2 $mg.kg^{-1}$ for soil S, 6 $mg.kg^{-1}$ for M and 10 $mg.kg^{-1}$ for L at the end of the three month experiment. Extractable Pb levels were about 65, 1.6 and 0 $mg.kg^{-1}$ for those same soils. Leaf and stem tissues contained moderate levels of As and Pb not obviously correlated to either total or $CaCl_2$ extractable soil levels. We can calculate that the combined above-ground biomass contained (all as $mg.kg^{-1}$) about 10 As and 35 Pb in soil S, 40 As and 20 Pb in soil M and 4 As and 0.8 Pb in soil L. The accumulation ratio of toxic elements in plant aboveground biomass (based on leaf vs root concentrations) did not exceed 0.13 for Pb or 0.013 for As, indicating a very low translocation even if the soils are highly contaminated (Wanat *et al*.,2013). It was concluded that *M. x giganteus* showed a good potential for phytostabilization of metal-contaminated soil and its biomass can be used as energy source. Further study is obviously needed to determine whether the ash from biofuel produced with these levels of soil contamination is toxic. It is important to note that when the two soils S and L were compared with a standard "soil compost" the above-ground biomass at final harvest after 12 weeks was only 1/10 the below-ground portion for the two soils S and L, while it was $\sim$1:1 for the compost. So the impoverished soils appear strongly inhibitory of above-ground growth (Wanat *et al*., 2013). Unfortunately the (estimated) input dry mass of rhizome was not reported, only the final harvest weight. So the actual above- or below-ground net biomass accumulation during the three month study cannot be calculated.

B. Organic Contaminants and Miscanthus

There have been very few reports of research in which miscanthus has been investigated in soils with petroleum contaminants, either aged *in situ* or added for the study. Petroleum biodegradation is often affected by plant roots, root exudates, and microbes that can benefit in multiple ways from the root zone environment as they transform contaminants. Techer *et al.* (2011; 2012a;b; c) have reported on laboratory studies with *M. x giganteus* root exudates and whole plants with the petroleum contaminants pyrene and phenanthrene. A microcosm study (Techer, 2012b) showed that biodegradation was enhanced when miscanthus plants were present.

Investigations of root exudates from *M. x giganteus* showed that biodegradation was stimulated by root exudates for contaminant systems with (i) pyrene, (ii) pyrene + phenanthrene, (iii) pyrene + salicylate, and (iv) pyrene + diesel fuel (Techer *et al*., 2011). A second study with root exudates and a mixture of pyrene and phenanthrene showed that degradation of these contaminants was enhanced when root exudates were present (Techer *et al*., 2012c). Quercetin was identified as a root exudate that stimulated biodegradation. These studies depended on use of a commercial standard soil with organic contaminants added just before the study. Freshly added contaminants are typically more bioavailable than those in aged soils (Maliszewska-Kordybach, 2005).

In soils containing longterm polycyclic aromatic hydrocarbons (PAH) contamination, there was very little effect of plants (Techer *et al*., 2012a;b). Greenhouse studies were conducted with five replicates each of treatment (planted and unplanted) in two soils from an industrial site with PAHs and metals (Techer *et al*., 2012a). The initial concentrations of PAHs were 26 $mg.kg^{-1}$ and 324 $mg.kg^{-1}$, in soils designated M and H. The soil designated M also had high heavy metals with Cr (range 48–140), Pb (range 530–10,000) and Zn (range 880–7700) while soil H had lower heavy metals Cr (range 58–67), Pb (range 220–290) and Zn (range 740–830). The *M. x giganteus* was grown in these two soils and in an uncontaminated synthetic reference soil. Plant growth was about twice as great (total dry matter accumulation) in the synthetic soil (Techer *et al*., 2012a). Some PAHs were decreased in planted or unplanted soil during the treatment period. In no instance was the planted condition significantly better than the unplanted.

We conclude that the number of laboratory studies for in-depth investigation of phytoremediation using miscanthus is rather small and more fundamental research is needed, especially with hydrocarbon contaminants. There is a need to investigate the impact of bioavailable metal concentrations in the soil and their effect on miscanthus yield, growth and uptake kinetics, as well as to observe the impact of general soil conditions and their modification on the effectiveness of phytoremediation. It is very important that studies use "aged" metals, or naturally occurring minerals, not just freshly introduced contamination. It is well documented that the bioavailability of metals differs greatly, decreasing after prolonged contact with soils in most cases (McLaughlin, 2001).

IV. FIELD EXPERIMENTS USING MISCANTHUS FOR PHYTOREMEDIATION

The possible use of second generation biofuel perennial grasses for phytoremediation of metal contaminated sites has been investigated and in some cases real positive effects have been achieved. Here we present the cases reported using various miscanthus species.

M. floridulus (Labill) showed good results for cleaning nonferrous mining sites (Sun *et al*., 2006) in Hunan Province, PRC. The soil was contaminated with high concentrations of Pb (>760 $mg.kg^{-1}$), Cd (>4 $mg.kg^{-1}$), Zn (>370 $mg.kg^{-1}$) and Cu (>95 $mg.kg^{-1}$) due to Pb-Zn mine tailings and mine toxic water pollution. The concentrations of heavy metals in stems and leaves were higher than in roots. Results indicated that *M. floridulus* (along with other species tested) had a potential for (slow) remediation of mining contaminated sites. This species needs relatively warm growing conditions with an optimum of 30 C day/25 C night temperature, and abundant water (Kao *et al*., 1998). It produces fertile seeds and is naturalized in some parts of the U.S. such as Arkansas and Missouri, indicating a risk for unwanted distribution if used for energy crops (USDA, 2013a;b).

Indigenous plants of *M. floridulus* with several other species were examined on a uranium mill tailings repository in South China (Li *et al*., 2011). The research site covers approximately 1.70 km^2 and contains almost 2×10^8 tons of uranium mill tailings. The metal concentration found in *M. floridulus* aboveground biomass was <1/30 of the tailings level for elements including Pb, U, Ba, Sr (but nearer 1/2 for Ni in stalks). A halophyte, *Paspalum paspaloides,* accumulated metal to concentrations nearer 1/4 the tailings contaminant level, whereas another grass, *P. orbiculare* was intermediate in its accumulation. *Phragmites australis* (reeds) stalks, and several species of sedges (Cyperaceae) had Pb and U levels approaching half that of the mine tailings.

Interesting results were reported in Ukraine while using *M. x giganteus* for phytoremediation of radioactively polluted land after Chernobyl fallout (Zinchenko *et al*., 2006), where levels of Cs-137 in the biomass were 0.10–0.22 ($Bq.kg^{-1}$) per (Bq $x10^3$ $.m^{-2}$). With a yield of 1 $kg.m^{-2}$ biomass it would take a very long time to remove a significant fraction of the total contamination.

A recent paper describes results of a pilot scale field study on yields of *M. x giganteus* in soil contaminated with Pb and Zn during three years of cultivation in Poland (Kocon and Matyka, 2012). Another possible perennial energy crop for Poland, *Sida hermaphrodita* (Virginia mallow) was compared to miscanthus. For ∼ 60 years the C-3 dicot sida was developed as cattle forage in Poland and more recently it has been tested as an energy crop. The two soils used for the study were artificially contaminated with two metals, Pb and Zn, over 20 years prior to this work. Thus, the metals were well aged in situ. The initial added concentrations (all in $mg.kg^{-1}$) were: Pb- 700 for loam and 600 for sand; Zn- 1100 for loam and 900 for sand. Analytical values by the time of this experiment were somewhat lower, 408–626 for Pb and 635–1002 for Zn. Rhizome or root sections of miscanthus and sida were planted in spring 2008 at a density of two plants per plot (1 m^2). Plots were fertilized each year with N, P & K.

TABLE 4
Yields of aerial part of *Miscanthus giganteus* and *Sida hermaphrodita* for soil contaminated by Zn and Pb (in g/plot) Kocon and Matyka, 2012)

Plant	Soil	pH	2008	2009	2010
Miscanthus	Loam	5.7	194	1216	1518
giganteus		6.3	375	1390	2014
	Sand	5.2	379	2067	3084
		6.1	546	2087	3454
Sida	Loam	5.7	49	255	854
hermaphrodita		6.3	130	429	1199
	Sand	5.2	248	720	1171
		6.1	499	1531	2128

Note. Plot size was 1m × 1m; each plot was filled with loamy or sandy soil, being at two different pH levels, more than 20 years ago from the experimental time (2008–2010); two plants were set per one plot; the soil in each plot was artificially contaminated by metals; loam was contaminated by 700 mg/kg of soil by Pb and 1100 mg/kg of soil by Zn; sand was contaminated by 600 mg/kg of soil by Pb and 900 mg/kg of soil by Zn; and yield was determined for biomass dried several days at 60°C.

Yield of aerial biomass of miscanthus and sida varied with the degree of contamination, pH and soil type (Table 4). Yields increased each year for each crop, but miscanthus (C-4) always outyielded sida (C-3) and reached ∼30 $t.ha^{-1}$ in sandy soil in the 3rd year. No direct comparison to uncontaminated soils is reported and only two pH levels were examined.

Both Zn and Pb were accumulated to higher tissue concentrations by sida than by miscanthus, which confirmed earlier laboratory research on phytoremediation (Kuboi *et al*., 1986). However, total Zn removal from soil was greater for miscanthus than for sida (∼200 $mg.m^{-2}$ $.yr^{-1}$ vs <100) while for Pb in later years it was about 1 $mg.m^{-2}.yr^{-1}$. The lower pH in sand was an exception with miscanthus removing over 8 $mg.m^{-2}$ Pb in the 3rd year. Tissue levels were very low in most cases, perhaps too low for reliable measurement. These are all negligible fractions of the total soil burden of metal, which is in the range of $g.m^{-2}$ within the root zone.

The French Institute National de la Recherche Agronomique (INRA) carried out trials with *M. x giganteus* and confirmed that it tolerates high levels of heavy metals in the soil, while only accumulating low levels of cadmium in its leaves as it grows (Cadoux *et al*., 2008). Given this profile, miscanthus has been used to clean up ancient industrial sites in the Parisian suburbs. Miscanthus was the only grass biofuel crop being tested, together with several species of wheat and fast-growing energy trees such as hybrid poplar. The stated objective of the phytoremediation effort is "to create a new system of sustainable agricultural activities on polluted sites, aimed at generating nonfood products such as fuels and biomaterials for industry."

As described in a recent presentation (Pourrut, 2011), an extensive phytostabilization effort is being conducted by the PHYTENER consortium in the city of Lille, France. Two soils were examined near a large closed (from 2003) metal smelter, with average soil Pb levels of 200 to 500 $mg.kg^{-1}$. Three cultivars of *M. x giganteus* were planted at different densities on 5 × 10 m plots with added mycorrhizal fungi in some treatments. There was different partitioning into leaves vs stems for each of three metals examined, Cd, Pb and Zn. Whole plant Cd levels were about 1 $mg.kg^{-1}$, those for Pb were 15 $mg.kg^{-1}$ and for Zn 45–65 $mg.kg^{-1}$. Leaf levels were always higher than those for stems. Oddly, for Pb, reported levels were nearly two-fold higher for leaves or stems, than for whole plants, which are composed of stems plus leaves, suggesting analytical difficulties. Median soil Cd values in the region are 33 $mg.kg^{-1}$ and Zn levels >5000 $mg.kg^{-1}$. Another grass *Arrhenantherum eliatus* was found to attain biomass metal levels equal to the EDTA extractable Cd (∼ 30 $mg.kg^{-1}$) and Zn (1000 $mg.kg^{-1}$) levels (Deram *et al.*, 2006). Therefore miscanthus appears favorable for its low accumulation, if biofuel use rather than phytoaccumulation is the desired end.

Miscanthus species along with other crops of willow (Salix), eucalyptus and the grass *Phalaris* were screened for remediation of contaminated and/or degraded soils across four countries: Germany, Spain, Sweden and Austria within the European Union BIORENEW project (Riddell-Black, 1998). Altogether over 20 varieties of miscanthus were studied for metal uptake characteristics, at four marginal metal-contaminated sites for which encouraging data were received. The types studied included 5 varieties planted in Wales which has a different climate than the four EU countries. A rapid screening test that reflects the long-term response of fuel crops to metalliferous growing media was proposed, and a computer-based decision support tool was developed for remediation planning and implementation. The research addressed demands for sustainable environmental technologies and provided a closed loop system for contaminated land remediation, reuse of the recovered metals and recycling of the principal by-product of the technology, namely biomass ash, as a fertilizer and liming agent.

Rumanian researchers (Barbu *et al.*, 2009; Iordache *et al.*, 2010; Barbu, 2010) investigated the possibilities to use *M. x giganteus* for cleaning up soils contaminated with Pb and Cd at the region of Copsa Mica, Rumania. Average concentrations of metals in the soil at the depth of 20 cm were >680 $mg.kg^{-1}$ for Pb and >13 $mg.kg^{-1}$ for Cd (Iordache *et al.*, 2010). The testing field had an area of about 5000 m^2 and was located in Copsa Mica town, one km from the pollution source. Miscanthus rhizomes were planted in spring in rows (1 m between rows, 1 m between plants at a depth of 10–12 cm). After one year plants were cropped and analyzed for concentration of heavy metals in leaves, stems and rhizomes. The amount of metals accumulated by *M. x giganteus* from the contaminated soil was rather low, in particular small concentrations were detected in leaves and stems. The average uptake amount was about 35–55 $g.ha^{-1}.yr^{-1}$ of heavy metals, which permitted authors to conclude that *M. x giganteus* showed good adaptation properties and could be cultivated in acidic soil polluted with Pb and Cd.

Miscanthus has been assessed for ability to grow on land polluted with heavy metals as a result of tin mining (Visser *et al.*, 2001). The growth and metal uptake by miscanthus from soils and mine waste polluted with Cu, Zn, and As were studied over a two year period in West Cornwall, U.K. The metal content in aboveground biomass was just slightly lower when miscanthus was grown on unpolluted soil and compared with data from the polluted one. Therefore, miscanthus grown on mine waste did not show greatly enhanced metal uptake.

Miscanthus was demonstrated to grow well on soils amended with sewage sludge, despite high concentrations of phytotoxic metals, and also in Pb-contaminated soils (Kerr *et al.*, 1998). However, as in the above-described case, metal uptake by miscanthus was rather small. Researchers concluded that this biofuel grass could not be considered as a means of significant phytoextraction in the selected contaminated places. In soil contaminated with artificial spoils, miscanthus did show significant concentration of Zn at 500 $mg.kg^{-1}$ in the plant. For some other metals, the available concentrations proved too phytotoxic for the plants to survive (Kerr *et al.*, 1998).

Techer *et al.* (2012a) planted two areas of 16 m^2 with *M. x giganteus* at the industrial site where soils H and M had been collected for the laboratory work described earlier (see discussion of studies above). Growth was reported in both soils. For the initial planting, growth in the M soil resulted in a greater stem height, while in a second season results were similar. The plants produced only 2–3 shoots per square meter in these establishment years. This is the only reported field study of soils with significant organic contamination.

Only one study has addressed macroscopic organism biodiversity in miscanthus fields with contaminated soils. Invertebrate communities in fields of *M. x giganteus* that were three years old at one location in soils affected by metal smelter atmospheric dust (AD) and at a second location watered with urban wastewater (UW) were investigated by Hedde *et al.* (2013). Both soils had elevated levels of Zn (129–590 $mg.kg^{-1}$) and Pb (62–376 $mg.kg^{-1}$). The density of soil-dwelling macroinvertebrates was 3 to 7-fold greater in miscanthus fields compared to wheat wheat fields. Taxonomic diversity was significantly higher in the miscanthus fields. This investigation concluded that both density and diversity of soil-dwelling invertebrates were larger in the miscanthis plantations. This effect did not hold for highly mobile invertebrates.

The positive impacts on soil quality of miscanthus crops in terms of increases in soil organic matter, microbial populations, and soil-dwelling invertebrate populations have economic value because the land becomes more productive and it can be used for a greater variety of crops. Lands that have low concentrations of soil organic carbon will experience significant increases in organic carbon by growing miscanthus as a commercial crop

(Anderson *et al*., 2009). Successful phytoremediation includes more than just removal of specific contaminants. Increase of biodiversity, carbon sequestration, and longterm stabilization of disturbed or erodible soils is an important further benefit of the use of properly selected plants.

V. CONCLUSIONS

The perennial grass miscanthus (primarily defined by results with *M. x giganteus*) has good potential for growing at contaminated and also marginal sites, which are not polluted to a high concentration. Some research reports metal uptake by different plant parts, but concentrations of the contaminants within plants differ with location and depend on the nature of contaminant substances, soil conditions, time of exposure and level of growth. The level of contaminant substances taken up by aerial biomass growth is small and biomass can be used for energy production. In some cases reported biomass growth was higher in the presence of contamination.

Miscanthus has potential to stabilize and possibly remove metal contaminants slowly over time while being grown for its energy value. The water use and surface stabilization help prevent metal transport away from the site due to wind, soil erosion, and water movement. Soil quality, organic matter concentration, and organism diversity are enhanced by growing miscanthus in contaminated and marginal soils.

The effective practical use of perennial grasses for phytoremediation of contaminated sites in Central and Eastern Europe, as well as in central US depends on simultaneously producing biofuel perennial crops. The roles of soil properties and agricultural conditions in those regions on crop growth and phytoremediation effectiveness will be subjects of additional research and outreach.

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REFERENCES

Abdel-Sabour, M. F. 2007. Remediation and bioremediation of uranium contaminated soils. *Electr. J. Environ., Agric. Food Chem.* **6**: 2009–2023.

Anderson, K. J., Davis, S. C., Masters, M. D., and Delucia, E. H. 2009. Changes in soil organic carbon under biofuel crops. *GCB Bioenergy* **1**: 75–96.

Andras, P., Lichý, A., Rusková, J., and Matúšková, L. 2008. Heavy metal contamination of the landscape at the Lubietova deposit (Slovakia). *World Acad. Sci., Engineer. Technol.* **44**: 96–100.

Arduini, I., Masoni, A., Ercoli, L., and Mariotti, M. 2003. Growth and cadmium uptake of Miscanthus sinensis as affected by cadmium. *Agricultura Mediterranea* **133**: 169–178.

Arduini, I., Masoni, A., Mariotti, M., and Ercoli, L. 2004. Low cadmium application increases miscanthus growth and cadmium translocation. *Environ. Exper. Bot.* **52**: 89–100.

Arduini, I., Ercoli, L., Mariotti, M.,and Masoni, A. 2006a. Response of miscanthus to toxic cadmium applications during the period of maximum growth. *Environ. Exper. Bot.* **55**: 29–40.

Arduini, I., Ercoli, L., and Masoni, A. 2006b. Response of Miscanthus sinensis to long term application of low Cr levels in hydroponics. *Agrochimica* **12**: 12–18.

Arduini, I., Masoni, A., and Ercoli, L. 2006c. Effects of high chromium applications on miscanthus during the period of maximum growth, *Environ. Exper. Bot.* **58**: 234–243.

Arthur, E. L., Rice, T. A., Anderson, T. A., Baladi, S. M., Henderson, K. L. D, and Coats, J. R. 2005. Phytoremediation: an overview. *Crit. Rev. Plant Sci.* **24**: 109–122.

Baker, A.J.M. and Brooks, R. R. 1989. Terrestrial higher plants which hyperaccumulate metallic elements- a review or their distribution, ecology and phytochemistry. *Biorecovery* **1**: 81–126.

Banasova, V. 2004. Unique vegetation of the old mining sites. *Chraneme uzemia Slovenska* **62**: 42–43.

Barbu, C.-H., Pavel, B.-P., Sand, C., and Pop, M.-R. 2009. *Miscanthus sinensis x giganteus' behavior on soils polluted with heavy metals, Metal Elements in Environment, Medicine and Biology*, Tome 9, pp. 21–24, Cluj University Press, (Proc. of 9th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca, 2009, October 16–17, Cluj-Napoca, Romania), available at web: http://www.rutsolmeg.ro/raport02.pdf.

Barbu, C.-H. 2010. *Miscanthus sinensis x giganteus* cultivated on soils polluted with heavy metals – a valuable replacement for coal. **In:** International Conference on Green Remediation, Conference Summary Papers, University of Massachusetts Amherst, pp. 2–5.

Behnke, G. D., Davis, M. B., and Voight, N. B. 2012. Greenhouse gas emissions, nitrate leaching, and biomass yields from production of *Miscanthus x giganteus* in Illinois, U.S.A. *Bioenergy Res.* **1**: 801–813.

Bellamy, P. E., Croxton, P. J., Heard, M. S., Hinsley, S. A., Hulmes, L., Hulmes, S., Nuttall, P., Pywell, R. F., and Rothery, P. 2008. The impact of growing miscanthus on farmland bird populations. *Biomass and Bioenergy* **33**: 191–199.

Biorefinery locations, 2012. Available at: http://www.ethanolrfa.org/biorefinery-locations

Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., and 12 others. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol. Appl.* **1**: 30–59.

Boddey, R. M., Urquiaga, S., Reis, V., and Dobereiner, J. 1991. Biological nitrogen fixation associated with sugarcane. *Plant and Soil* **137**: 111–117.

Borzecka-Walker, M., Faber, A., and Borek, R. 2008. Evaluation of carbon sequestration in energetic crops (Miscanthus and coppice willow). *Int. Agrophysics* **22**: 185–190.

Breslavetz, A. I. and Yurchenko, A. I. 2009. Technohenno contaminated soil and ways for improvement, available at: http://www.nbuv.gov.ua/portal/natural/Ponp/2009/2009-Articles/UkrNDI-EP_ 2009_17.pdf (in Ukrainian).

Brosse, N., Dufour, A., Meng, X., Sun, X., and Ragauskas, A. 2012. Miscanthus: a fast-growing crop for biofuels and chemicals production. *Biofuels, Bioprod. Bioref.* **6**: 580–598.

Buchek I. Y, Svjatenko A. I., and Pidlisnyuk V. V. 2011. Analysis of the conditions of waters mining contamination at the open-cast iron-ore mines, *Scientific Bulletin of Kremenchuk National University*, **67**(2): 110–113 (in Ukrainian).

Cadoux S., Vanderdriessche V., Machet J. M., Mary B., Beakdouin N., Lemaire G., and Gosse G. 2008. Potential yield and main limiting factors of *Miscanthus giganteus* in France, identification of the needs for further research. **In:** 16th European Biomass Conference and Exhibition, Valencia, Spain.

Cai, X., Zheng, X., and Wang, D. 2011. Land availability for biofuel production. *Environ. Sci. Technol.* **45**: 334–339.

Carriquiry, M. A., Du, X., and Timilsina, G. R. 2011. Second generation biofuels: economics and policies. *Energy Policy* **39**: 4222–4234.

CASTNET USEPA 2013 www.epa.gov/castnet/javaweb/site_pages/KN2184.html

Cerne, M., Smodis, B., and Strok, M. 2011. Uptake of radionuclides by a common reed (*Phragmites australis* (Cav.) Trin. Ex Steud.) grown in the vicinity of the former uranium mine at Zirovsky vrh, *Nucl. Engin. Design* **24**: 1282–1286.

Chaney, F. L., Malik, M., Yin, M. L., Brown, S. L., Brewer, E., Angle, J. S., and Baker, A.J.M. 1997. Phytoremediation of soils metals. *Curr. Opin. Biotechnol.* **8**: 279–284.

Chou, C.H. 2009. Miscanthus plants used as an alternative biofuel material: The basic studies on ecology and molecular evolution, *Renew. Energy* **34**: 1908–1912.

Christian, D. G., Riche, A. B., and Yates, N. E. 2008. Growth, yield and mineral content of *Miscanthus x giganteus* grown as a biofuel for 14 successive harvests. *Industrial Corps and Products* **28**: 320–327.

Clifton-Brown, J., Joern, B., and Jones, M. 2007. Carbon mitigation by the energy crop, miscanthus, *Global Change Biology* **13**: 2296–2307.

Clifton-Brown, J. C., Long S. P., and Jorgensen U. 2001. Miscanthus productivity. **In:** *Miscanthus for Energy and Fiber*, pp. 46–67. Jones, M. B. and Wash, M., Eds. James & James, London.

Collura, S., Azambre, B., Finqueneisel, G., Zimny, T., Weber, J.W. 2006. *Miscanthus x giganteus* straw and pellets as sustainable fuels. Combustion and emission tests. *Environ. Chem. Lett.* **4**: 75–78.

Cook, R. L. and Hesterberg, D. 2013. Comparison of trees and grasses for rhizoremediation of petroleum hydrocarbons. *Int. J. Phytorem.* **15**: 844–860.

Corma, A., Iborra, S., and Velty, A. 2007. Chemical routes for the transformation of biomass into chemicals, *Chem. Rev.* **107**: 4044–4098.

Cunningham, S. D. and Berti, W. R. 1993. Remediation of contaminated soils with green plants: an overview. *In Vitro Cell. Develop. Biol.* **29P**: 207–212.

Dahl, J. and Obernberger, I. 2004. Evaluation of the combustion characteristics of four perennial energy crops (*Arundo donax, Cynara cardunculus, Miscanthus x Giganteus,* and *Panicum virgatum*), *Proceedings 2nd World Conference on Biomass for Energy, Industry, and Climate Protection*, Rome Italy, May, 2004.

Dahmani-Mullera, H., van Oort, F., Gelieb, B., and Balabanea, M. 2000. Strategies of heavy metal uptake by three plant species growing near a metal smelter. *Environ. Poll.* **109**: 231–238.

Daoutidis, P., Marvin, W. A., Rangarajan, S., and Torres, A. I. 2013. Engineering biomass conversion processes: A systems perspective. *AIChE J.* **59**: 3–18.

Dapsens, P. Y., Mondelli, C., and Prez-Ramrez, J. 2012. Biobased chemicals from conception toward industrial reality: Lessons learned and to be learned. *ACS Catal.* **2**: 1487–1499.

Davis, L. C., Castro, S., Zhang, Q., and Erickson, L. E. 2002. Benefits of vegetation for soils with organic contaminants. *Crit. Rev. Plant Sci.* **21**: 457–491.

Davis, S. C., Parton, W. J., Dohleman, F. G., Smith, C. M., Del Grosso, S. J., Kent. A. D., and DeLucia, E. H. 2010. Comparative biogeochemical cycles of bioenergy crops reveal nitrogen-fixation and low greenhouse gas emissions in *Miscanthus x giganteus* agro-ecosystem. *Ecosystems* **13**: 144–156.

Davis, L. C., Banks, M. K., Schwab, A. P., Narayanan, M., Erickson, L. E., and Tracy, J. C. 1998. Plant-based bioremediation. **In:** *Bioremediation: Principles and Practice Vol 2 Biodegradation Technology Developments*, pp. 183–219. Sikdar, S. K. and Irvine, R. L., Eds. Technomic Publ. Co., Lancaster, PA.

Davis, S. C., Parton, W. J., Del Grosso, S. J., Keough, C., Merx, E., Adler, P. R., and DeLucia, E. H. 2012. Impact of second-generation biofuel agriculture on greenhouse-gas emissions in corn-growing regions of the U.S. frontiers. *Ecol. Environ.* **10**: 69–74.

DEFRA. 2007. Planting and growing miscanthus, Best practices guideline, available at: www.naturalengland.org.uk

Deram, A., Denayer, F.-O., Petit, D., and Van Haluwyn, C. 2006. Seasonal variation of cadmium and zinc in *Arrhenantherum elatius*, a perennial grass species from highly contaminated soils. *Environ. Poll.* **140**: 62–70.

Dickerson, T. and Soria, J. 2013. Catalytic fast pyrolysis: a review. *Energies* **6**: 514–518.

Dickinson, N. M., Baker, A.J.M., Doronila, A., Laidlaw, S., and Reeves, R. D. 2009. Phytoremediation of inorganics: realism and synergies. *Int. J. Phytorem.* **11**: 97–114.

Dohleman, F. G., Heaton, E. A., Arundale, R. A., and Long, S. P. 2012. Seasonal dynamics of above- and below-ground biomass and nitrogen partitioning in *Miscanthus x giganteus* and Panicum virgatum across three growing seasons. *GCB Bioenergy* **4**: 534–544.

Dohleman, F. G., Heaton, E. A., Leakey, A., and Long, S. P. 2009. Does greater leaf-level photosynthesis explain the larger solar energy conversion efficiency of miscanthus relative to switchgrass? *Plant Cell Environ.* **32**: 1525–1537.

Dorge, S., Jeguirim, M., and Trouve, G. 2011. Thermal degradation of Miscanthus pellets: kinetics and aerosol characterization. *Waste and Biomass Valorization* **2**: 149–155.

Dushenkov, S., Mikheev, A., Prokhnevsky, A., Ruchko, M., and Sorochinsky, B. 1999. Phytoremediation of radiocesium-contaminated soil in the vicinity of Chernobyl, Ukraine. *Environ. Sci. Technol.* **33**: 469–475.

Dushenkov, S. 2003. Trends in phytoremediation of radionuclides. *Plant & Soil* **249**: 167–175.

Eisentraut, A. 2010. Sustainable production of second-generation biofuels; Potential and perspectives in major economies and developing countries. *Review.* Information paper published by International Energy Agency, 221 pp.

Emmerson, E., Bourke, D., Dauber, J., O'Rourke, E., Stanley, D., Thompson, R., Whelan, P., and Stout, J. 2011. The food versus fuel debate – what effect will replacing traditional crops with *Miscanthus x giganteus* have on farmland biodiversity? TEAGASC Biodiversity Conference, May 2011. www.teagasc.ie/publications/2011/996/Biodiversity_Conf_Proceedings.pdf

Ercoli, L., Mariotti, M., Masoni, A., and Bonari, E. 1999. Effect of irrigation and nitrogen fertilization on biomass yield and efficiency of energy use in crop production of miscanthus. *Field Crops Res.* **63**: 3–11.

Ezaki, B., Nagao, E., Yamamoto, Y., Nakashima, S., and Enomoto, T. 2008. Wild plants*, Andropogon virginicus* L. and *Miscanthus sinensis* Anders, are tolerant to multiple stresses including aluminum, heavy metals, and oxidative stresses. *Plant Cell Rep.* **27**: 951–961.

Fernando, A., Duarte, P., and Oliveira, J. F. S. 1996. Bioremoval of heavy metals from soil by *Miscanthus sinensis x giganteus*. *Biomass for Energy and the Environment*, Proceedings of the 9th European Bioenergy Conference, pp. 531–536.

Fernando, A. L., Godovikova, V., and Oliveira, J.F.S. 2004. *Miscanthus x giganteus*: Contribution to a sustainable agriculture of a future/present- oriented biomaterial. *Advanced Materials Forum II*, Proceeding of the II International Materials Symposium, April 14–17, 2003. Materials Science Forum, Martins R., Fortunato E., Ferrera, I., Eds. pp. 437–441.

Fernando, A. L. and Oliveira, J. S. 2004. Effects on growth, productivity, and biomass quality of *Miscanthus x giganteus* of soils contaminated with heavy metals. *2nd World Congress on Biomass for Energy, Industry, and Climate Protection*, Rome, Italy, pp. 387–390.

Fernando, A. L. and Oliveira, J.F.S. 2005. Phytoremediation by *Miscanthus x giganteus* of soils contaminated with heavy metals. *REWAS'04-Global Symposium on Recycling, Waste Treatment and Clean Technology*, pp. 2419–2427.

Fernando, A.L., Duarte, M.P., Almeida, J., Boleo, S., Mendes, B. 2010. Environmental impact assessment of energy crops cultivation in Europe, *Biofuels, Bioproducts and Biorefining*, **4**: 594–604.

Fischer, G., Prieler, S., and van Velthuizen, H. 2005. Biomass potentials of miscanthus, willow and poplar: results and policy implications for Eastern Europe, Northern and Central Asia, *Biomass Bioenergy*. **28**: 119–132.

Fischer, G., Hizsnyik, E., Prieler, S., and van Velthuizen, H. 2007. Assessment of biomass potentials for bio-fuel feedstock production in Europe: Methodology and results, available at: www.refuel.eu/uploads/media/Refuel-D6-Jul2007-final6.pdf.

Fowler, P. A., McLauchlin, A. R., and Hall, L. M. 2003. The Potential Industrial Uses of Forage Grasses Including Miscanthus, Report published by BioComposites Centre, University of Wales, UK, 40 pages; available at: http://www.bc.bangor.ac.uk

Fruhwirth, P. and Liebhard, P. 2004. *Miscanthus sinensis Giganteus*. Produktion, Inhaltsstoffe und Verwertung, **In:** *Miscanthus sinensis Giganteus. Chinaachilf als nachwachsender Rohstoff, Landliches Forbildingsinstitut und Landwirtschaftskammer Osterreich*, pp. 34–38.

Galbally, F., Fagan, C., Ryan, D., Finnan, J., Grant, J., and McDonnell, K. 2012. Biosolids and distillery effluent amendment to Irish *Miscanthus x giganteus* plantations: Impacts on groundwater and soil. *J. Environ. Qual.* **41**: 114–123.

Gallagher, E. 2008. *The Gallagher Review of the Indirect Effects of Biofuels Production*, Renewable Fuels Agency, St. Leonards-on-Sea, East Sussex, UK.

Geletukha, G. G., Zelezna, T. A., Zovmirm, M. M., and Matveev- Drozdova, O. I. 2011. Evaluation of biomass energy potential in Ukraine. Part 2. Energy crops, liquid fuels, biogas. *Promislova teplotechnica* **33**(1): 57–64 (in Ukrainian).

Gopalokrishnan, G., Negri, M. C., and Snyder, S. W. 2011. A novel framework to classify marginal land for sustainable biomass feedstock production. *J. Environ. Qual.* **40**: 1593–1600.

Gopalokrishnan, G., Negri, M. C., and Salas, W. 2012. Modeling biogeochemical impacts of bioenergy buffers with perennial grasses for a row-crop field in Illinois. *GCB Bioenergy* **4**: 729–750.

Grytsyuk, N. and Arapis, G. 2005. Phytotechnologies in the rehabilitation of radioactively polluted Areas, *COST Action 859 - 1st Scientific Workshop and Management Committee Meeting*, Italy, pp. 152–154.

Gubisova, M., Porvaz, P., Gubis, J., Zofajova, A., Mihalik, D., and Kraic, J. 2011. Growing technology and biotechnology of miscanthus in Slovakia. Proceeding of Pannonian Plant Biotechnology Workshops. The bioenergy question: reality or wishful thinking, May 16–18, 2011, Vienna, Austria, available at: ttp://www.pannonbiotech.hu/_user/browser/File/PPBA_Tulln_2011_Book.pdf.

Gumentik, Ya. M., 2010. Perspective of growing perennial grasses for biofuel production. *Sugar Beet* **4**: 21–22 (in Ukrainian).

Hammer, D., Kayser, A., and Keller, C. 2003. Phytoextraction of Cd and Zn with *Salix viminalis* in field trials. *Soil Use and Management* **19**: 187–192.

Hartley, W., Dickinson, N. M., Riby, P., and Lepp, N. W. 2009. Arsenic mobility in brownfield soils amended with green waste compost or biochar and planted with *Miscanthus*. *Environmental Pollution* **157**(10): 2654–2662.

Harvey, P. J., Campanella, B. F., Castro, P.M.L., Harms, H., Lichtfouse, E., Schaffner, A. R., Smrcek, S., and Werck-Reichhart, D. 2002. Phytoremediation of polycyclic aromatic hydrocarbons, anilines and phenols. *Environ. Sci. Pollution Res.* **9**: 29–47.

Heaton, E. A., Clifton-Brown, J., Voigt, T., Jones, M. B., and Long, S. P. 2003. Miscanthus for Renewable Energy Generation: European Union Experience and Projections for Illinois. *Mitigation and Adaptation Strategies for Global Change* **8**.

Heaton, E. A., Dohleman, F. G., and Long, S. P. 2009. Seasonal nitrogen dynamics of *Miscanthus x giganteus* and *Panicum virgatum*. *GCB Bioenergy* **1**: 297–307.

Heaton, E. A., Dohleman, F. G., Miguez, A. F., Juvik, J. A., Lozovaya, V., Widholm, J., Zabotina, O. A., McIsaac, G. F., Davis, M. B., Voigt, T. B., Boersma, N. N., and Long, S .P. 2010. Miscanthus. A promising biomass crop. *Adv. Botanical Res.* **56**: 76–137.

Hedde, M., van Oort, F., Boudon, E., Abonnel, F., and Lamy, I., 2013. Responses of soil macroinvertebrate communities to miscanthus cropping in different trace metal contaminated soils. *Biomass and Bioenergy*, http://dx.doi.org/10.1016/J.biombioe.2013.01.016.

Hemen, P. 2011. Metal hyperaccumulation in plants: A review focusing on phytoremediation technology. *J. Environ. Sci. Technol.* **4**: 118–138.

Himken, N., Lammel, J., Neukirchen, D., Czypionka-Krause, U., and Olfs, H.-W. 1997. Cultivation of miscanthus under West European conditions: Seasonal changes in dry matter production, nutrient uptake and remobilization. *Plant & Soil* **189**: 117–126.

Hoogwijk, M.M., Faaij, A., van den Broek, R., Berndes, G., Gielen, D., and Turkenburg, W. 2003. Exploration of the ranges of the global potential of biomass for energy. *Biomass and Bioenergy* **25**: 119–133.

Hotz, A. 1996. Screening of different miscanthus cultivars in respect of their productivity and usability as a raw material for energy and industry. **In:** Proceeding of the 9th European Bioenergy Conf., 24–27 June 1996, Chartier, P., Ferrero, G. L., Henius, U. M. and Wiinblad, M., Eds. Copenhagen, Denmark. Elsevier Science, Oxford, UK, pp. 2294.

Hromadko, L., Vranova, V., Techer, D., Laval-Gilly, R., Rejsek, K., Formanek, P.,and Falla, J. 2010. Composition of root exudates of *Miscanthus x giganteus* Greef et Deu., *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* **58**(1): 71–76.

Iordache, V., Mihail, D., Neagoe, A., Sandu, R., Bodescu, F.,and Marinoiu, L. 2010. Decision support for the management of agrosystems contaminated with metals: research program and preliminary results. **In:** *Modern Technologies and Biotechnologies for Environmental Protection*, pp. 83–93. Barbu, C. H. and Sand, C., Eds. "Lucian Blaga" University of Sibiu.

Iqbal, M., Bermond, A., and Lamy, I. 2013. Impact of miscanthus cultivation on trace metal availability in contaminated agricultural soils: complementary insights from kinetic extraction and physical fractionation. *Chemosphere* **91**: 287–294.

Islam, K. R., Kilpatrick, L. A., Reeder, R. C., Raut, T., Copple, A., and Michel, F. C. 2012. Growing miscanthus for biofuels on marginal land amended with sewage sludge and flue gas desulfurization (FGD) gypsum. http://sungrant.tennessee.edu/NR/rdonlyres/DOA5701F-E268-43A9-B312-DACBDBA937B0/3680/38Islam_Khandakar.pdf

James, C. A. and Strand, S. E. 2009. Phytoremediation of small organic contaminants using transgenic plants. *Curr. Opin. Biotechnol.* **20**: 337–241.

John, D. A. and Leventhal, J. S. 1995. Bioavailability of metals. **In:** *Preliminary Compilation of Descriptive Geoenvironmental Mineral Deposit Models*, pp. 10–18. DuBray, E., Ed. United States Geological Survey, Denver, CO. Available at pubs.usgs.gov/of/1995/ofr-95–0831/CHAP2.pdf

Kahle, P., Beuch, S., Boelcke, D., Leinweber, P., Schulten, H.-R. 2001. Cropping of miscanthus in Central Europe: biomass production and influence on nutrients and soil organic matter, *European J. Agron.* **15**: 171–184.

Kalembasa, D. and Malinowska, E. 2009a. Influence of sewage sludge fertilization on heavy metal content in biomass of *Miscanthus sacchariflorus* during field experiment. *Environ. Protect. Engin.* **35**: 149–155.

Kalembasa, D. and Malinowska, E. 2009b. The yield and content of trace elements in biomass of *Miscanthus sacchariflorus* (Maxim.) Hack. and in soil in the third year of a pot experiment. *J. Elementology* **14**(4): 685–691 (in Polish).

Kalembasa, D. and Malinowska, E. 2009c. Influence of after effect of waste activated sludge used in soil of pot experiment on heavy metals content in *Miscanthus sacchariflorus*. *Acta Agrophysica* **13**: 377–384.

Kao, W-Y., Tsai, T-T., and Chen, W-H. 1998. A comparative study of *Miscanthus floridulus* (Labill) Warb. and *M. transmorrisonensis* Hayata: Photosynthetic gas exchange, leaf characteristics and growth in controlled environments. *Ann. Bot.* **81**: 295–299.

Katzer, J. R. 2011. Coal and coal/biomass-based power generation. **In:** *Global Climate Change- The Technology Challenges*, pp. 51–89. Princiotta, F. T., Ed. Springer, New York, NY, USA.

Kechavarzi, C. and Lord, R. A. 2009. Expanding the potential biomass crop production: reusing brownfield sites and biodegradable wastes, available at: http://www.bay-publishing.com/article - 335.

Kerr, J., Pulford, I., Duncan, H., and Wheeler, C. 1998. Phytoremediation of heavy metal contaminated sites by potential fiber crops. **In:** Proceedings of the 6th International FZK/TNO Conference Contaminated Soil '98, pp. 1119–1120. London: Telford.

Kilpatrick, L. A. 2012. Sustainable growth of miscanthus on marginal lands amended with flue gas desulfurization gypsum and sewage biosolids. Paper No. 12-1337624, ASABE; www.asabe.org 37 pp.

Kim, S. J., Kim, M. Y., Jeong, S. J., Jang, M. S., and Chung, I. M. 2012. Analysis of the biomass content of various miscanthus genotypes for biofuel production in Korea. *Industrial Crops and Products* **38**: 46–49.

Klein, M. 2012. *Russian military capabilities, great power ambitious and reality*, SWP Research paper, Berlin, 34 pages, available at: http://www.swp-berlin.org/fileadmin/contents/products/research_papers/2009_RP12_kle_ks.pdf.

Kludze, H., Deen, B., Weersink, A., van Acker, R., Janovick, K., and DeLaporte, A. 2013. Impact of land classification on potential warm season grass biomass production in Ontario, Canada. *Can. J. Plant Sci*. **93**: 249–260.

Kobza, J. 2005. Soil and plant pollution by potentially toxic elements in Slovakia. *Plant Soil Environ*. **51**: 243–248.

Kocon, A., Matyka, M. 2012. Phytoextractive potential of *Miscanthus giganteus* and *Sida hermaphrodita* growing under moderate pollution of soil with Zn and Pb. *J. Food Agric. Environ*. **10**: 1253–1256.

Krizani, I., Andras, P., and Šlesarova, A. 2009. Percolation modeling of the dump and settling pit sediments at the Banska Štiavnica ore-field (Western Carpathians, Slovakia). *Carpathian J. Earth Environ. Sci*. **4**(1): 109–126.

Kuboi, T., Noguchi, A., and Yasaki, A. 1986. Family-dependent cadmium accumulation characteristics in higher plants. *Plant & Soil* **92**: 405–415.

Kulakow, P. and Pidlisnyuk, V. Eds., 2010. Application of Phytotechnologies for Cleanup of Industrial, Agricultural and Wastewater Contamination, Proceedings of the NATO Advanced Research Workshop, Kamenetz-Podilsky, Ukraine, 2007. Springer, 198 pages.

Lewandowski, I., Clifton-Brown, J., Scurlock, J., and Huisman, W. 2000. Miscanthus: European experience with a novel energy crop. *Biomass & Bioenergy* **19**: 210–227, available at: www.sciencedirect.com/science/article/pii/S0961953400000325.

Lewandowski, I., Scurlock, J.M.O., Lindvall, E., and Christou, M. 2003. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass and Bioenergy* **25**: 335–361.

Li, G.-Y., Hu, N., Ding, D.X., Zheng, J.-F., Liu, Y.-L., Wang, Y.-D., and Nie, X.-Q. 2011. Screening of plant species for phytoremediation of uranium, thorium, barium, nickel, strontium and lead contaminated soils from a uranium mill tailing repository in South China. *Bull. Environ. Contam. Toxicol*. **86**: 646–652.

Lin, C. H., Lerch, R. N., Garrett, H. E., and George, M. F. 2008. Bioremediation of atrazine contaminated soil by forage grasses: transformation, uptake and detoxification. *J. Environ. Qual*. **37**: 196–206.

Lin-Vien, D., Fateley, W. G., and Davis, L. C. 1989. Estimation of nitrogenase activity in the presence of ethylene by use of deuterated acetylene as a substrate. *Appl. Environ. Microbiol*. **55**: 354–359.

Liu, S. L., Luo, Y. M., Ding, K. Q., and Cao, Z. H. 2003. Phytoremediation of organic pollutants in soils. *Soils* **35**: 187–192.

Liu, C., Xiao, L., Jiang, J., Wang, W., Gu, F., Song, D., Yi, Z., Jin, Y., and Li, L. 2013. Biomass properties from different Miscanthus species. *Food and Energy Security* DOI:10.1002/fes3.19

Long, S. P. and Beale, C. V. 2001. Resource capture by miscanthus. **In:** *Miscanthus for Energy and Fibre*, pp. 10–20. Jones, M. B. and Walsh, M., Eds. James & James, London.

Lonsdale, W. M. and FitzGibbon, F. 2011. The known unknowns-managing the invasion risk from biofuels. *Curr. Opin. Environ. Sustain*. **3**: 31–35.

Lord, R. A., Atkinson, J., Lane, A. N., Scurlock, J.M.O., and Street, G. 2008. Biomass, remediation, regeneration (BioReGen Life project): Reusing brownfield sites for renewable energy crops, Proceedings of the 10th International UFZ/TNO conference on Soil-Water Systems, Milan, June 3–6, 2008.

Los, L. V., Zinchenko, V. O., and Zajvoronovskyi, V. P. 2011. Production of biofuel crops as effective way for solving energetic and ecological problems: case of *Miscanthus giganteus*, *Release of Zytomir national agroecological university. N2* **29**(1): 46–57 (in Ukrainian).

Lovett, A. A., Sunnenberg, G. M., Richter, G. M., Dailey, A. G., Riche, A. B., and Karp, A. 2009. Land use implications of increased biomass production identified by GIS-based suitability and yield mapping for miscanthus in England. *Bioenergy Res*. **2**: 17–28.

Lunney, I. A., Zeeb, B. A., and Reimer, K. J. 2004. Uptake of weathered DDT in vascular plants: potential for phytoremediation. *Environ. Sci. Tech*. **38**: 6147–6154.

Lyubun, Y.V. and Tychinin, D. N. 2007. Phytoremediation in Russia. **In:** *Methods in Biotechnology, vol.23: Phytoremediation: Methods and Reviews*, pp. 423–434. Willey, N., Ed. Humana Press Inc., Totowa, NJ, available at: Springer Protocols, http://link.springer.com/content/pdf/10.1007%2F978–1–59745–098–0_29.

Majtkowski, W., Mayjtkowska, G., Pilat, J., and Mikolajczak, J. 2009. Grass species from C-4 carbon fixation group: Polish experiment with a novel energy and forage purposes crops, *J. Central European Agric*. **10**: 211–216.

Maliszewska-Kordybach, B. 2005. Dissipation of polycyclic aromatic hydrocarbons in freshly contaminated soils- the effect of soil physico-chemical properties and aging. *Water, Air Soil Pollution*. **168**: 113–128.

Marmiroli, N. and McCutcheon, S. C. 2003. Making phytoremediation a successful technology, In *Phytoremediation: Transformation and Control of Contaminants*, pp. 85–119. McCutcheon, S.C. and Schnoor, J. L., Eds., Wiley-Interscience, Hoboken, NJ.

Masarovicova, E., Kralova, K., and Pesko, M. 2009. Energetic plants- cost and benefit. *Ecol. Chem. Engin*. **16**: 263–276.

McCutcheon, S. C. and Schnoor, J. L. Eds. 2003. *Phytoremediation: Transformation and Control of Contaminants*. Wiley Interscience, Hoboken, NJ.

McLaughlin, M. J. 2001. Aging of metals in soils changes bioavailability. *ICME Fact Sheet on Risk Assessment 4*. http://www.icmm.com/document/55

Melligan, F., Auccaise, R., Novotny, E. H., Leahy, J. J., Hayes, M.H.B.,and Kwapinski, W. 2011. Pressurized pyrolysis of miscanthus using a fix bed reactor. *Bioresource Technol*. **102**: 3466–3470.

Meers, E., Vandecasteele, B., Ruttens, A., Vangronsveld, J., and Tack, F. 2007. Potential of five willow species (*Salix* spp.) for phytoextraction of heavy metals. *Environ. Exp. Bot*. **60**: 57–68.

Meers, E., Van Slychen, S., Adriaensen, K., Ruttens, A., Vangronsveld, J., Du Laing, G., Witters, N., and Tack, F.M.C. 2010. The use of bioenergy crops (*Zea mays*) for "phytoattenuation" of heavy metals on moderately contaminated soils: a field experiment. *Chemosphere* **78**: 35–41.

Miller, A. C. and Gage, C. L. 2011. Potential adverse environmental impact of greenhouse gas mitigation strategies. **In:** *Global Climate Change—the technology challenges*, pp. 377–415. Princiotta, F. T., Ed. Springer, New York, NY, USA.

Miller, S. A. 2010. Minimizing land use and nitrogen intensity of bioenergy. *Environ. Sci. Technol*. **44**: 3932–3939.

Ministry of the Environment of Czech Republic, 2009. Removal of contaminated sites caused by the former Soviet Army, available at: http://www.mzp.cz/C1257458002F0DC7/cz/pasportizace_lokalit/$FILE/OES-mapa_priority_lokality_SA-20090825.pdf (in Czech).

Ministry of Ecology and Natural Resources of Ukraine, 2012. Enriched five-years report regarding land desertification and erosion, available at: www.menr.gov.ua/Zvit5rokOpus2013.doc (in Ukrainian).

Mulligan, C. N., Yong, R. N., and Gibbs, B. F. 2001. Remediation technologies for metal contaminated soils and groundwater: An evaluation. *Eng. Geol*. **60**(1–4): 193–207.

Naik, S. N., Goud, V. V., Rout, P. K., and Dalai, A. K. 2010. Production of first and second generation biofuels: a comprehensive review. *Renew. Sustain. Energy Rev*. **14**: 578–597.

Nanda Kumar, P. B. A., Dushenkov, V., Motto, H., and Raskin, I. 1995. Phytoextraction: the use of plants to remove heavy metals from soils. *Environ. Sci. Technol*. **29**: 232–1238.

Nixon, P. 2002. Bioremediation of contaminated sites. **In:** *Bioenergy Crops and Bioremediation—a Review*. Contract Report by ADAS for the Department for Food, Environment and Rural Affairs, pp. 73–80. Britt, C. and Garstang, J., Eds. available at: http://www.ienica.net/usefulreports/adasbioenergy.pdf.

Noskova, L. M., Shuktomova, I. I., and Simakova, Y. S. 2010. Simulation of biological uptake of uranium and radium in an industrially polluted area. *Russian J. Ecol*. **41**(5): 405–411 (in Russian).

Nyesvyetov, O. 2010. Phytoremediation: outlook for integrated environmental and energy issues. *Energy Saving and Alternative Sources of Energy* **7**(26): 184–191.

Oleszek, W., Terelak, H., Maliszewska-Kordybach, B., and Kukula, S. 2003. Soil, food and agro product contamination monitoring in Poland. *Polish J. Environ. Studies* **12**(30): 261–268.

Oliver, L., Ferber, U., Grimski, D., Millar, K., and Nathanail, P. 2005. The scale and nature of European brownfields, 8 pp., available at www.cabernet.uk.org/resourcefs/417.pdf

Ollivier, J., Wanat, N., Austruy, A., Hitme, A., Joussein, E., Welzl, G., Munch, J. C., and Schloter, M. 2012. Abundance and diversity of ammonia-oxidizing prokaryotes in the root-rhizosphere complex of *Miscanthus x giganteus* grown in heavy metal-contaminated soils. *Microb. Ecol.* **64**: 1038–1046.

Peng, K., Li, X., Luo, C., and Shen, Z. 2006. Vegetation composition and heavy metal uptake by wild plants at three contaminated sites in Xiangxi area, China. *J. Environ. Sci. Health: Part A.* **41**: 65–76.

Pidlisnyuk, V. 2012. Expanding the potential of second generation biofuels crops by using for phytoremediation of sites contaminated by heavy metals: laboratory stage. *Scientific Bulletin of Kremenchuk National University* **74**(4): 104–108 (in Ukrainian).

Pivetz, B. E. 2001. Phytoremediation of contaminated soil and ground water at hazardous waste sites., Ground Water Issue, USEPA, February, 2001, 35 pages.

Pogrzeba, M., Kryzyzak, J., Sas-Nowosielska, A., Majtkowski, W., Malkowski, E., and Kita, A. 2010. A heavy metals environmental threat resulting from combustion of biofuels of plant origin. In *Environmental heavy metal pollution and effects on child mental development: risk assessment and prevention strategies*, NATO Science for Peace and Security Series C: Environmental Security, Simenov, L. I., Kochubovski, M. V., and Simenova, B.G., Eds., pp 213–225.

Pope, L. M. 2005. Scientific investigation report 2005-5251 U.S. Department of the Interior USGS, available at: http://pubs.usgs.gov/sir/2005/5251/pdf/SIR20055251.pdf.

Potash Development Association. 2009. Potash offtakes by miscanthus and SRC willow bio-energy crops. Available at www.pda.org.uk/news/nf68.php

Pourrut, B. and PHYTENER Consortium. 2011. The PHYTENER project: Development of phytostabilization combined with energy crop production on agricultural soils highly contaminated by metals, available at: presentations.copernicus.org/EGU2011–9395_ presentation.pdf.

Prasad, M.N.V. 2006."Stabilization, remediation, and integrated management of metal-contaminated ecosystems by grasses (Poaceae). **In:** *Trace Elements in the Environment*, pp. 405–424. Prasad, M.N.V., Sajwan, K.S., and Naidu, R., Eds. Taylor and Francis, Boca Raton, Florida.

Prasifka, J. R., Bradshaw, J. D., Meagher, R. L., Nagoshi, R., Steffey, K. L., and Gray, M. E. 2009. Development and feeding of fall armyworm on *Miscanthus x giganteus* and switchgrass. *J. Econ. Entomol.* **102**: 2154–2159.

Prasifka, J. R., Bradshaw, J. D., and Gray, M. E. 2012. Potential biomass reductions to *Miscanthus* × *giganteus* by stem-boring caterpillars. *Environ. Entomol.* **41**: 865–871.

Price, L., Bullard, M., Lyons, H., Anthony, S., and Nixon, P. 2004. Identifying the yield potential of *M. x giganteus*, an assessment of the spatial and temporal variability of *M. x giganteus* biomass productivity across England and Wales. *Biomass Bioenergy* **26**: 3–13.

Pu, Y., Zhang, D., Singh, P. M., and Ragauskas, A. J. 2008. The new forestry biofuels sector, *Biofuels Bioprod. Bioref.* **2**: 58–73.

Pulford, I. D. and Watson, C. 2003. Phytoremediation of heavy metal-contaminated land by trees: a review. *Environ. Int.* **29**: 529–540.

Punshon, T. and Dickinson, N. M. 1997. Mobilization of heavy metals using short rotation coppice. *Aspects of Applied Biology, 49. Biomass and Energy Crops*, pp. 285–292.

Punshon, T., Dickinson, N. M., and Lepp, N. W. 1996. The potential of *Salix* clones for bioremediating metal polluted soil. **In:** *Heavy Metals and Trees*,.pp. 93–104. Glimmerveen, I., Ed. Institute of Chartered Foresters, Edinburgh.

Qin, Z., Zhuang, Q., Zhu, X., Cai, X., and Zhang, X. 2011. Carbon consequences and agricultural implications of growing biofuel crops on marginal agricultural lands in China. *Environ. Sci. Technol.* **45**: 10765–10772.

Rakhmetov, D. B. 2007. Role of new cultures in bioenergy, *Scientific Release of National Agricultural University* **116**: 13–20 (in Ukrainian).

Resasco, D. E. and Crossley, S. 2009. Molecular engineering approach in the selection of catalytic strategies for upgrading of biofuels, *AIChE J.* **55**: 1082–1089.

Riddell-Black, D. 1998. The BIORENEW Phytoremediation project, available at: http://www.contaminatedland.co.uk/ask-guru/thred-33.htm.

Riddell-Black, D. M., Rowlands, C., and Snelson, A. 1996. The take up of heavy metals by wood fuel crops - implications for emission and economics, *Biomass for Energy and the Environment*. Proceeding of the 9th European Bioenergy Conference, **3**, pp. 1754–1759.

Robinson, B. H., Banuelos, G., Conesa, H. M., Evangelon, W. H., and Schulin, R. 2009. The phytomanagement of trace elements in soil. *Crit. Rev. Pl. Sci.* **28**: 240–266.

Rosillo-Calle, F., de Groot, P., Hemstock, S. L., and Woods, J., Eds. 2006. *The Biomass Assessment Handbook: Bioenergy for a Sustainable Environment*. Earthscan Publications Ltd., London.

Salt, D. E., Blayblock, M., Nanda Kumar, N. P. B. A., Dushenkov, V., Ensley, B. D., Chet, I., and Raskin, I. 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnology* **13**: 468–474.

Salt, D. E., Smith, R. D., and Raskin, I. 1998. Phytoremediation. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **49**: 643–668.

Scebba, F., Arduini, I., Ercoli, L., and Sebastiani, L. 2006. Cadmium effects on growth and antioxidant enzymes activities in *Miscanthus sinensis*, *Biologia Plantarum* **50**: 688–692.

Schmidt, U. 2003. Enhancing phytoextraction: The effect of chemical soil manipulation on mobility, plant accumulation and leaching of heavy metals. *J. Environ. Qual.* **32**: 1939–1954.

Schneckenberger, K. and Kuzyanov, Y. 2007. Carbon sequestration under miscanthus in sandy and loamy soils estimated by natural abundance ^{13}C abundance. *J. Plant Nutr. Soil Sci.* **170**: 538–542.

Schwarz, H. 1993. *Miscanthus sinensis* 'Giganteus' production on several sites in Austria. *Biomass Bioenergy* **5**: 413–419.

Serrano-Ruiz, J. C., West, R. M., and Dumasic, J. A. 2010. Catalytic conversion of renewable biomass resources to fuels and chemicals. *Ann. Rev. Chem. Biomol. Eng.* **1**: 79–100.

Sharmin, S. A., Alam, I., Kim, K.-H., Kim, Y.-G., Kim, P.-j., Bahk, J. D., and Lee, B. H. 2012 Chromium induced physiological and proteomic alterations in roots of *Miscanthus sinensis*. *Plant Sci.* **187**: 113–126.

Spenser, J. L. and Raghu, S. 2009. Refuge or reservoir? The potential impacts of the biofuel crop *Miscanthus* x *giganteus* on a major pest of maize. *PLoS ONE* **4**(12): e8336. DOI:10.1371/journal.pone.0008336, available at http://www.plosone.org/article/info:doi%2F10.1371%2Fjournal.pone.0008336

Stasiak, M. 2007. Miscanthus genotypes as a source of biomass and their selected utilizable feature. Proceeding of the 18th EUARPIA Genetic Resources Section Meeting, 23–26 May, 2007, Piestany, Slovak Republic, p. 106.

Stefanovska, T., Lewis, E., and Pidlisnyuk, V. 2011a. Evaluation of potential risk for agricultural landscapes from second generation biofuel production in Ukraine: the role of pests. *Aspects of Applied Biology*, **109**, *Agricultural Ecology Research: Its Role in Delivering Sustainable Farm Systems*, pp. 165–169.

Stefanovska, T., Likar, Ya., Rakhmetov, D., and Pidlisnyuk, V. 2011b. Herbivorous pests of *Miscanthus x giganteus*. *Quarantine and Plant Protection* **5**: 6–8 (in Ukrainian).

Stewart, J. R., Toma, T., Fernandez, F. G., Nishiwaki, A., Yamada, T., and Bellero, G. 2009. The ecology and agronomy of *Miscanthus sinensis*, a species important to bioenergy crop development, in its native range in Japan: A review. *GCB Bioenergy* **1**: 126–153.

Sun, J., Tie, B.-Q., Qin, P.-F., Isao, A., and Luo, R. 2006. Investigation of contaminated soil and plants by heavy metals in Pb-Zn mining area. *J. Plant Res. Environ.* **15**: 63–67.

Suresh, B. and Ravishankar, G. A. 2004. Phytoremediation: a novel and promising approach for environmental clean-up. *Crit. Rev. Biotechnol.* **24**: 97–124.

Techer, D., Laval-Gilly, P., Henry, S., Bennasroune, A., Formanek, P., Martinez-Chois, C., D'Innocenzo, M., Muanda, F., Dicko, A., Rejsek, K., and Falla,

J. 2011. Contribution of *Miscanthus x giganteus* root exudates to the biostimulation of PAH degradation: An in vitro study, *Sci. Total Environ.* **409**: 4489–4495.

Techer, D., Martinez-Chois, C., Laval-Gilly, P., Henry, S., Bennarroune, A., D'Innocenzo, M., and Falla, J. 2012a. Assessment of *Miscanthus x giganteus* for rhizoremediation of long term PAH contaminated soils. *Appl. Soil Ecol.* **62**: 42–49.

Techer, D., Laval-Gilly, P., Henry, S., Bennasroune, A., Martinez-Chois, C., D'Innocenzo, M., and Falla, J. 2012b. Prospects of *Miscanthus x giganteus* for PAH phytoremediation: A microcosm study. *Industrial Crops and Products* **36**: 276–281.

Techer, D., D'Innocenzo, M., Laval-Gilly, P., Henry, S., Bennasroune, A., Martinez-Chois, C., and Falla, J. 2012c. Assessment of *Miscanthus x giganteus* secondary root metabolites for the biostimulation of PAH-utilizing soil bacteria. *Appl. Soil Ecol.* **62**: 142–146.

The Doe Run Company project, 2012, available at: http://news.yahoo.com/doe-run-plants-biofuel-crop-test-program-restore-070401697.html.

Thomson, L. J. and Hoffmann, A. A. 2011. Pest management challenges for biofuels production. *Curr. Opin. Environ. Sustain.* **3**: 95–99.

USEPA. 2000. Introduction to phytoremediation, available at nepis.epa.gov/Exe/ZyNET.exe.

USDA. 2011. United States Department of Agriculture, Natural Resources Conservation Service, Plant Materials Program. Planting and managing giant miscanthus as a biomass energy crop, Technical Note no. 4, 33 pp.

USDA. 2013a. United States Department of Agriculture, Natural Resources Conservation Service. Invasive species fact sheet, Pacific Islands area. *Miscanthus floridulus*.

USDA. 2013b. United States Department of Agriculture, Plant profiles *Miscanthus floridulus*.

Van Aiken, B., Correa, P., and Schnoor, J. L. 2010. Phytoremediation of polychlorinated biphenyls: new trends and promises. *Environ. Sci. Technol.* **44**: 2767–2776.

Van der Werf, H. M. G., Meijer, W. J. M., Mathijssen, E. W. J. M., and Darwinkel, A., 1993. Potential dry matter production of *Miscanthus sinensis* in the Netherlands. *Industrial Crops Products* **1**: 203–210.

Van Epps, A. 2006. Phytoremediation of petroleum hydrocarbons. clu-in.org/download/studentpapers/A_Van_Epps-Final.pdf

Van Ginneken, L., Meers, E., Guisson, R., Rutterns, A., Elst, K., Tack, F. M. G., Vangronsveld, J., Diels, L., and Dejongle, W. 2007. Phytoremediation of heavy metal-contaminated soils combined with bioenergy production. *J. Environ. Eng. Landscape Manage.* **15**: 2227–2237.

Vanek, T., Podlipna, R., and Soudek, P. 2010. General factors influencing application of phytotechnology techniques. **In:** *Application of Phytotechnologies for Cleanup of Industrial, Agricultural and Wastewater Contamination*, pp. 1–13. Kulakow, P. and Pidlisnyuk, V. Eds. Proceedings of the NATO Advanced Research Workshop, Kamenetz-Podilsky, Ukraine, 2007. Springer, New York, NY, USA.

Vangronsveld, J., Herzig, R., Weyens, N., Boulet, J., Adriaensen, K., Ruttens, A., Thewys, T., Vassilev, A., Meers, E., Nehnevajova, E., van der Lelie, D., and Mench, M. 2009. Phytoremediation of contaminated soils and groundwater: lessons from the fields. *Environ. Sci. Pollut. Res.* **16**: 765–794.

Van Loocke, A., Twine, T. E., Zeri, M., and Bernacchi, C. J. 2012. A regional comparison of water use efficiency for miscanthus, switchgrass, and maize. *Agricult. Forest Meteorol.* **164**: 82–95.

Vassilev, A., Schwitzguebel, J.-P., Thewys, T., van der Lelie, D., and Vangronsveld, J. 2004. The use of plants for remediation of metal-contaminated soils. *Scientific World J.* **4**: 9–34.

Venendaal, R., Jorgensen, U., and Foster, C. A. 1997. European energy crops: a synthesis. *Biomass Bioenergy* **13**: 147–185.

Verbruggen, N., Hermans, C., and Henk, S. 2009. Molecular mechanisms of metal hyperaccumulation in plants. *New Phytologist* **181**: 759–776.

Visser, P., Pignatelli, V., Jørgensen, U., and Santos Oliveira, J. F. 2001. Utilisation of miscanthus. **In:** *Miscanthus for Energy and Fibre*, p. 150. Jones, M. B. and Walsh, M., Eds. James and James, London.

Wanat, N., Austruy, A., Joussein, E., Soubrand M., Hitmi, A., Gautier-Moussard, C., Lenain, J.-F., Vernay, P., Munch, J. C., and Pichon, M. 2013. Potentials of *Miscanthus x giganteus* grown on highly contaminated technisols. *J. Geochem. Exploration* **126**: 78–84.

Wang, D., Maughan, M.W., Sun, J., Feng, X., Miguez, F., Lee, D., and Dietze, M. C. 2012. Impact of nitrogen allocation on growth and photosynthesis of miscanthus (*Miscanthus x giganteus*). *GCB Bioenergy* **4**: 688–697.

Whitfield, M. A. and Zeeb, B. A. 2010. A review of recent research developments into the potential for phytoextraction of persistent organic pollutants (POPs) from weathered, contaminated soil. **In:** *Application of Phytotechnologies for Cleanup of Industrial, Agricultural and Wastewater Contamination*, pp. 35–59. Kulakow, P., Pidlisnyuk, V., Eds., Proceedings of the NATO Advanced Research Workshop, Kamenetz-Podilsky, Ukraine. Springer, New York, NY, USA.

Wilkins, C. 1997. The uptake of copper, arsenic and zinc by miscanthus-environmental implications for use as an energy crop, *Aspects of Applied Biology*, **49**. *Biomass and Energy Crops*, 335–340.

Witters, N., Mendelsohn, R. O., Van Slycken, S., Weyers, N., Schreurs, E., Meers, E., Tack, F., Carleer, R., and Vangronsveld, J. 2012. Phytoremediation, a sustainable remediation technology? Conclusions from a case study. I: Energy production and carbon dioxide abatement. *Biomass and Bioenergy* **39**: 454–469.

Xie, X. M., Zhou, F., Zhao, H. Y., and Lu, X. L. 2008. A summary of ecological and energy producing effects of perennial energy grasses. *Acta Ecologica Sinica* **28**: 2329–2342.

Yang, X. E., Jin, X. F., Feng, Y., and Islam, E. 2005. Molecular mechanisms and genetic basis of heavy metals tolerance/hyperaccumulation in plants. *J. Integr. Plant Biol.* **47**: 1025–1035.

Yorgun, S. and Simsek, Y. E. 2008. Catalytic pyrolysis of *Miscanthus x giganteus* over activated alumina. *Bioresource Tech.* **99**: 8095–8100.

Zhang, T., Wyman, C. E., Jakob, K., and Yang, B. 2012. Rapid selection and identification of miscanthus genotypes with enhanced glucan and xylan yields from hydrothermal pretreatment followed by enzymatic hydrolysis. *Biotechnology for Biofuels* **5**: 56–69.

Zhu, K., Chen, H., and Nan, Z. 2010. Phytoremediation of loess soil contaminated by organic compounds. **In:** *Application of Phytotechnologies for Cleanup of Industrial, Agricultural and Wastewater Contamination*, pp. 159–176. Kulakow, P. and Pidlisnyuk V., Eds. Springer, New York, NY, USA.

Zhuang, Q., Zhangcai, Q., and Chen, M. 2013. Biofuel land and water: maize, switchgrass or miscanthus? *Environ. Res. Lett.* **8**: 015020.

Zimmermann, J., Daubert, J., and Jones, M. B. 2012. Soil carbon sequestration under *Miscanthus x giganteus*. A large scale survey. **In:** Conference Proceedings SIMBIOSYS June 28 2012, *Integrating Biodiversity Research, Policy, and Practice. Sectorial Impacts on Biodiversity and Ecosystem Services*; www.tcd.ie/research/simbiosys/images/zimmermann.pdf

Zinchenko, V., Kusaylo V., and Zinchenko O. 2006. Biohelioenergetics is our energetic future. *Proposition* **8**: 130–132 (in Ukrainian).

Zub H. W., and Brancourt-Hulmel M. 2010. Agronomic and physiological performances of different species of miscanthus, a major energy crop. A review. *Agron. Sustain. Develop.* **30**(2): 201–214.