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GIANT MISCANTHUS (*MISCANTUS* × *GIGANTEUS* GREEF ET DEU.) – A PROMISING PLANT FOR SOIL REMEDIATION: A MINI REVIEW

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Abstract

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Giant miscanthus (*Miscanthus* × *giganteus* Greef et Deu.) is a perennial rhizomatous grass with C4 type photosynthesis, which is distinctive by its resistance to cold temperatures when maintaining a sufficient photosynthesis rate. We revised potential of *Miscanhus* for use in soil bioremediation, especially from biological point of view. Translocation rate from roots to aerial part is low in general, but *Miscanthus* is able to grow even on highly contaminated soils without artificial fertilization. We also discussed the role of root exudates in pollutant immobilization, chelation and uptake. Commetabolism of polycyclic aromatic hydrocarbons with assistance of soil microbes shows promising results and significant reduction of tetracyclic PAHs in soil. *Miscanthus* is therefore suitable for immobilization of inorganic pollutants in soil and removal of organic pollutants, which makes it suitable to create buffer zones for surface waterway protection, stabilization of heavily contaminated substrates (e.g. reclaimed burrows of mining industry and sedimentation pools). According to low content of pollutants in aerial biomass the harvested plant material is deemed safe for further agricultural or industrial use.

Keywords: *Miscanthus* × *giganteus*, phytoremediation, phytoextraction, root exudates, contamination, soil, heavy metals

INTRODUCTION

Miscanthus × *giganteus* Greef et Deu. (giant Miscanthus, *M* × *g*) is a perennial rhizomatous grass species with C4 photosynthesis, originating from south-east Asia. This hybrid is probably naturally occurring cross-breed of *M.* × *sacchariflorus* (diploid) and *M. sinensis* (tetraploid) with triploid chromosome and unable to produce fertile seeds (Greef and Deuter, 1993; Linde-Laursen, 1993). Its artificial propagation is therefore performed by micropropagation of tissue cultures or by rhizome cuttings. Naturally the grass propagates by rhizome growth in soil. Giant miscanthus was discovered the first time in 1935 in Japan, where it was denominated *Miscanthus sinensis* "Giganteus" hort. (Greef and Deuter, 1993; Greef *et al.*, 1997).

Plant height in coherent stand can reach up to 4 meters; its life-span is about 20–25 years (Lewandowski, *et al.*, 2003). *M* × *g* belongs to group of *Miscanthus* cultivated in Europe and USA for its potential use as a biofuel source. It is also suitable for industrial use for production of paper pulp, composts, geotextiles, construction materials (e.g. pressed particle boards) or bio-composite manufacturing, etc. (Stander, 1989; Greef and Deuter, 1993; Huisman *et al.*, 1997; Lewandowski *et al.*, 2000; Kirwan *et al.*, 2007; Marín *et al.*, 2009).

BIOLOGICAL DISPOSITIONS FOR USE IN BIOREMEDIATION

Distinctive for *Miscanthus* among other C4 plants is its ability to photosynthesize even in low temperature conditions (even in just above 5 °C), which most likely significantly contributes to maximum capacity of absorbed solar radiation conversion (up to 2%) to harvested biomass (Swaminathan *et al.*, 2010). These values are specified by Strašil (2009), who demonstrates the plant physiological minimum between 8 and 12 °C. Lower temperature (8 °C) resulted in photosynthetic capacity reduction by 50%. Adaptation of this C4 phenotype to cold tolerance is in correlation with elevated accumulation of pyruvate-orthophosphate dikinase, the key enzyme of C4 photosynthesis (Swaminathan *et al.*, 2010). The C4 plants (contrary to C3 plants) contain larger portion of carbon 13C isotope and exude a specific mixture of chemical substances from roots; certain chemicals are demonstrating chemoatractive properties towards the microbial species (e. g. *Azospirillum lipoferum*), which chemoatractivity hasn't been reported in C3 species root exudates (Formánek and Ambus, 2004; Vranová *et al.*, 2013).

The above-mentioned characteristics of giant miscanthus suggest its suitability for growth even in colder northern areas, eventually at higher above-sea levels. The limit is just the lower plants tolerance to frost, especially in one-year old plants (LT50 = –4.2 °C rhizomes, –8.0 °C aerial part), but plants left on-site through winter are more cold-resistant. The acclimatization of rhizomes to colder temperatures before planting proved useful (Płażek *et al.*, 2011).

Miscanthus is undemanding on nutrient supply; it is able to grow on wide scale of soils from humus sandy substrates to deeper loamy soils rich in soil organic matter. As inappropriate for plant growth are considered sandy soils, which are quick to dry-out in longer drought periods, as well as cold clayey soils (Strašil, 2009). *M × g* is able to grow even on poor, contaminated substrates without necessity of further mineral nutrition application (Lewandovski *et al.*, 2003) and shows certain potential for remediation of soils contaminated with heavy metals (Arduini *et al.*, 2006; Vranová *et al.*, 2009).

Contamination of soil with organic and inorganic polluting substances is a serious problem in areas with intense agronomic production, mining and industrial enterprises. With increasing human demand for utilization of natural resources and used materials more and more harmful substances are entering and affecting the ecosystems. Their effect therefore encompasses both soil environment (e.g. soil enzymatic activity) and vitality of plants growing on contaminated substrates (growth inhibition and development anomalies), as well as health of consumers of these plants including humans (Arduini *et al.*, 2006). The danger of multitude of these substances lies in possibility of entering the food chains, bioaccumulation in organisms and subsequent harmful and toxic effects, which occur even in very small concentrations (Gratao *et al.*, 2005).

ORGANIC AND INORGANIC POLLUTANT BIOREMEDIATION

Heavy metals (HM, e.g. Cr, Cu, Ni, Zn, Cd, Hg, Pb) are listed among the most important pollutants. From organic substances we could mention polycyclic aromatic hydrocarbons (PAH), petrol hydrocarbons, polychlorinated biphenyls (PCB), various pesticides, dyes, explosives, medical products, detergents etc. (Soudek *et al.*, 2008). Organic pollutant remediation is based on its degradation by soil microorganisms with direct catabolism or cometabolism with assistance of water-soluble root exudates (Brimecombe *et al.*, 2007). Physico-chemical approach to soil decontamination is economically, technologically and also personally highly demanding, the phytoremediation-based approach is generally looked upon as a viable alternative (Bhargava *et al.*, 2007; Técher *et al.*, 2011).

Most significant advantage in comparison with other methods is its applicability *in situ*, without necessity to move and mix the substrate, which results into reduced operation, manipulation and maintenance costs and environmentally conscious approach. The greatest disadvantage of phytoremediations is the long period necessary to achieve considerable effect (in order of decades; Soudek *et al.*, 2008).

Main complication in phytoremediation of HM contaminated soils is the HM toxicity to plants. The plants have various strategies to overcome the adverse effect of excessive heavy metal concentration in soil, e.g. tolerance strategy (metabolism modification to prevent HM from interfering with metabolical processes, mostly adsorption, absorption and deposition in roots) or hyperaccumulation strategy (HM are deposited in specialized vacuoles or thick cell walls). To designate plant species a hyperaccumulator it must be able to absorb at least 100× larger quantity of trace element ions than other plant species growing on identical substrate.

From the phytoremediation point of view the use of hyperaccumulating species would be optimal, but a major hindrance is the low growth and low ability to produce biomass (Macková and Macek, 2005). This is also a reason why they are unsuitable for mechanized harvest. Here the plants with lower bioaccumulation factor seem more suitable, especially when demonstrating higher biomass growth and are able to be harvested for further use, e.g. as an energetic crop or raw material for further agricultural or industrial use.

Tolerance of *M × g* to inorganic pollutants was measured mainly in laboratory conditions (Arduini *et al.*, 2006a; Arduini *et al.*, 2006b), remediation of organic pollutants was tested both in laboratory and field conditions (Técher *et al.*, 2012). Miscanthus also seemspromising for wastewater and dump leachate treatment (Jones *et al.*, 2006). Técher *et al.* (2012) demonstrated that in PAH-contaminated and miscanthus-planted substrate reported reduction of tetracyclic PAH content and enhancement of soil properties due to organic C input. Fernando *et al.* (2004) studied biomass production and suitability of *Miscanthus* for phytoremediations under fertilization with domestic sludge at 0, 50, 100 and 200 $t.ha^{-1}$. Plants responded well to fertilization on every tested level compared to no addition of domestic sludge, but they also gradually showed decline in biomas production with increasing fertilization. This trend could be linked to greater metabolic cost of pollutant detoxification and countering their harmful effects.

However there are only few studies on this topic, which deserves further exploration.

Important mechanism regulating the pollutant uptake by plant is root exudation, which is defined as release of low-molecular-weight (LMW) and high-molecular-weight (HMW) organic substances into the rhizosphere, namely amino acids, LMW carboxylic acids, saccharides, simple phenolic compounds of flavonoid type, mucilages, root cell lysates and enzymes (Curl and Truelove, 1986; Rejšek *et al.*, 2012b; Vranová *et al.*, 2013). Plant root exudates contain numerous enzymes necessary for organic matter decomposition, e.g. acid phosphomonoesterase and proteases (Rejšek *et al.*, 2008; Rejšek *et al.*, 2012a; Rejšek *et al.*, 2012b). Root exudates are an important interaction medium between plant and soil environment. They act directly as a protective layer on roots preventing them from drought, ensure the plant nutrition when reabsorbed by roots (especially amino acids), help to increase the solubility and availability of soil nutrients for plants and simultaneously prevent the intake of toxic substances to roots (Marschner, 1995; Técher *et al.*, 2011). The indirect effect of root exudates encompasses mainly the nutrition of soil microbial communities and regulation of their abundance. Soil-borne bacteria are associated with organic matter decomposition and making nutrients available for plant uptake.

Root exudates could also play an important part in HM contaminated soils phytoremediation by assisting the chelation of pollutants and facilitating their transport to the plant. As an example it is possible to mention histidine, citric, malic, malonic acids and other substances (Lee *et al.*, 1977; Mench and Martin, 1991). LMW organic compounds (sugars, amino acids and organic acids) occur in root exudates in form of L- and D- enantiomers (Vranová *et al.*, 2012), where D- enantiomers are decomposed at a much slower rate in soil and are less efficient in heavy metal chelation (Pospíšilová *et al.*, 2011). Chiral separation of root exudates LMW compounds of *M × g* was not yet reported and remains a topic for further research. It is safe to assume that root exudates have an important role as a HM uptake mediator and regulatory mechanism, therefore the rate of plant-root exudation will correspond to substrate saturation with heavy metal ions up to borderline toxic HM concentration and metallic ion uptake (Mench and Martin, 1991; Barceló and Poschenrieder, 2002). Root exudates are also a feasible substrate for microbial metabolism and degradation of these compounds is regularly limited by presence of inorganic ions (Vranová *et al.*, 2009; Vranová *et al.*, 2011).

Quantitative and qualitative analysis of *M × g* root exudates was conducted by Técher *et al.* (2011), who also assessed the influence of target compounds on cometabolic degradation of PAHs by bacteria in substrate and biostimulating effect of some exuded compounds (quercetine, rutine etc.) on soil microflora. Qualitative composition of individual compound divisions (sugars, amino acids and organic acids) and ratio of their occurrence was examined by Formánek *et al.* (2009), who proved the dominance of aspartic acid, arginine, alanine and glutamic acid in *M × g* root exudates, when these amino acids were also confirmed as dominant in soil solution gathered from various types of ecosystems (Lojková *et al.*, 2006).

These compounds are supposed to significantly affect the nutrient uptake and selective uptake of ions into plant roots. The role of root exudates in promoting metabolism of decompositors for acceleration of pollutant removal, especially with regard to LMW compound enantiomers, represents a trend of contemporary research.

DISCUSSION

Miscanthus is able to maintain satisfactory growth rate even in severe conditions, e.g. on ash dumps (Técher *et al.*, 2012), or on industrially highly contaminated substrates (Wanat *et al.*, 2013). It is resistant to comparatively high concentrations of HM in soil, which in case influence the growth rate and biomass increase, but don't affect the rate of metabolic processes in plant (Arduini *et al.*, 2006a, b; Wanat *et al.*, 2013). Even in conditions of HM ion concentration exceeding the toxicity limit miscanthus maintains sufficient increase of aerial biomass (Arduini *et al.*, 2006a), in subtoxic concentration levels the HM could even promote growth and ion translocation, as demonstrated in experiments with cadmium by Arduini *et al.* (2004). Wanat *et al.* (2013) confirmed the adaptability of *M × g* to industrially highly contaminated soils (former settling basins after shut-down gold mining operation, contaminated with As, Pb and Sb), when plants created rather smaller amount of aerial biomass, but were able to grow on site with no added mineral nutrition. Fernando *et al.* (2004) tested phytoremediation cappacity of *M × g* to soils contaminated with heavy metals on soils treated with domestic sludge. They concluded that aerial part of plants shows differences in HM content among four levels of applied sludge quantities, however, these differences aren't statistically significant. Therefore we assume that present HM were witheld in the rhizosphere or in root part of the plant without further translocation into aerial biomass.

Also the low translocation factor of plant (HM ion transport from roots to aerial part) and its high oxidation resistance were proved in this experiment. The disadvantage of this plant for decontamination purpose is therefore the retention of absorbed HM in roots and rhizomes (up to 90% of total absorbed cadmium – Arduini *et al.*, 2006a), but miscanthus plants seem valuable for short-term phytostabilisation of contaminated landscapes. One way of possible decontamination is also harvesting the whole plant including the rhizomes and subsequent replanting the site with new planting material.

Miscanthus appears more suitable for planting on sites contaminated with organic pollutants, especially because of its root system, reaching up to 250 cm deep and showing the greatest density in depths about 30 cm (Neukirchen *et al.*, 2005). These parameters correlated with nature and quality of root exudates released into the soil give the preconditions for very effective retention and decomposition of namely PAHs with cooperation of soil microbes (Técher *et al.*, 2011; Técher *et al.*, 2012; Rejšek *et al.*, 2012a). This process could be modified by addition of synthetically prepared root exudates into the soils (Técher *et al.*, 2011).

Miscanthus also seems suitable to be planted on sites contaminated by both organic and inorganic pollutants, where organic pollutants will be degraded microbially and HM will be slowly taken up by plant roots.

CONCLUSION

In this mini review we conclude that *Miscanthus × giganteus* is viewed as promising alternative in bioremediation strategies. However, it is not the best option for quick removal of anorganic pollutants, especially heavy metals, from contaminated soils. Its potential lies mostly in stabilization of reclaimed burrows of mining industry, protection of surface waterways from contaminated leachate waters, and finally its suitability for root zone wastewater treatment. When considering employing *Miscanthus* in intensive bioremediation strategies, it is recommended to harvest the roots along with aerial biomass and replant fresh plants on the site. This is needed to be done in continuous strip harvest and replanting to minimize the mobilization of pollutants in barren soil. Recommended sites for *Miscanthus* planting lie in border teritories of recultivated areas, where it can also be harvested for technical purposes. Biological characteristics of *Miscanthus* confirm its suitability for its year-round presence on amended soils, where it prevents soil erosion and leaching of the pollutants. Plant cover prevents the aerial dispersion and runoff, provides cover for local wildlife and aesthetically complements the landscape.

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