PHYTO2ENERGY

Phytoremediation driven energy crops
production on heavy metal degraded areas as
local energy carrier

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1. Introduction

This document serves as a guidance on the use of 4 energy crop species tested under the Phyto2Energy project to implement the novel approach consisting of phytoremediation driven energy crops production on heavy metals contaminated soils. It is based on data from a four-year field experiment carried out in the Phyto2Energy project under the leadership of research fellows from the Institute for Ecology of Industrial Areas (IETU), Poland in cooperation with experts from VITA34 GmbH, Germany. One of the aims of this experiment was to investigate some pre-selected energy crop species from the viewpoint of producing satisfactory biomass yield both from a phytostabilization and /or phytoextraction process under real conditions. The four tested energy crop species include:

- Miscanthus *(Miscanthus* x *giganteus),*
- Virginia mallow *(Sida hermaphrodita),*
- Switchgrass *(Panicum virgatum),*
- Cordgrass *(Spartina pectinata).*

This document provides a practical knowledge to engineering companies dealing with soil remediation as well as engineering companies dealing with green energy production to support heavy metal contaminated site owners and land use planners on the selection of the most appropriate energy crops depending on the targeted heavy metal land management option, site conditions, climatic conditions.

General scheme of biomass production on HM contaminated areas is presented below (Fig1).

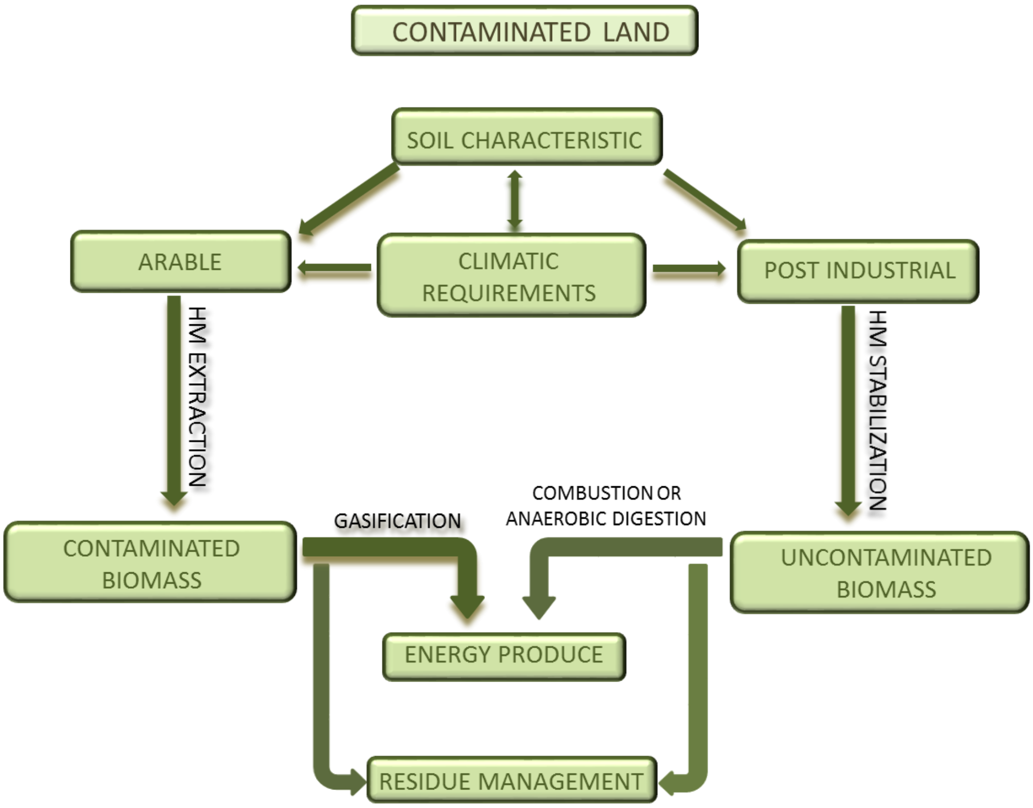


Fig. 1. A general overview of the phytoremediation driven energy crops production on heavy metal contaminated areas.

Under the Phyto2Energy project two targeted heavy metal land management options were considered:

a) Phytoextraction of heavy metals with energy crops to restore soil for agricultural food production.
b) Phytostabilization with energy crops for economic restoration of an abandoned heavy metal contaminated site by producing non-contaminated biomass for energy purposes.

These options are further discussed in the section 3 – Land management options.

2. Energy crop characteristics

Current, biomass production is focused on second generation, low input perennial bioenergy crops (e.g. *Panicum virgatum, Spartina pectinata, Miscanthus spp*.) [4, 5, 6]. Such plants have much lower input requirements, produce more energy and reduce greenhouse gas emission compared to the first generation annual food crop species [7].There is a number of typical energy crop species available on the market which have also been tested with success for phytoremediation effect on HMC arable land. They, however, need further tests for different heavy metals to prove their robustness for large scale applications. Until now species used so far in Poland as well as in other EU countries as energy crops are miscanthus [9, 10], switchgrass [10, 11] and virginia mallow [12]. All these species are normally grown on non-contaminated agricultural land. Among above listed plant species only switchgrass [15] and miscanthus [16] were also used for phytoremediation of heavy metal contaminated sites. Surprisingly, little has been investigated on the effects of combining the production of energy crops with phytoremediation using safe biomass utilization derived from HMC soils [17].

Characteristics of plants commonly used as a energy crops, including: physiological features, yield and possible uptake of heavy metals in aboveground biomass are presented below.

2.1. *Miscanthus* x *giganteus*

Figure 2. *Miscanthus* x *giganteus* growth on the heavy metal contaminated arable land (second growing season).

General characteristics

Miscanthus x *giganteus* is a perennial rhizomatous C4 grass species, contains the genomes of *Miscanthus sinensis* and *Miscanthus sacchariflorus* [19, 20]. It is the one of 23 species of *Miscanthus* that is a great of significance for energy purposes [39]. Because of its C4 photosynthetic pathway and perennial rhizome, Miscanthus displays quite good combination of radiation-, water- and nitrogen-use efficiencies for biomass production [24].

Origin

The genus *Miscanthus* has its origins in the tropics and subtropics, but different species are found throughout a wide climatic range in East Asia. *M.* x *giganteus* was first cultivated in Europe in the 1930s, when it was introduced from Japan.

Nutrient requirements

Miscanthus has a low nutrient requirements, but the addition of nitrogen, phosphorus and potassium may at times be necessary, especially on sandy soils. The solid fertilizer applied before planting should contain: nitrogen 70 kg ha^{-1}, phosphorus 30 kg ha^{-1} as P_2O_5 and potassium 45 kg ha^{-1} as K_2O [16, 21]. Plant development and biomass production per square meter depends also on soil type. On agricultural soils, even contaminated, miscanthus grows well without any toxic symptoms. On a former post-industrial site the plant growth could be affected by low water content and nutrient deficiency due to the ground structure.

Harvesting

Miscanthus is harvested annually when stems have low water content, which is normally in late winter or spring of the following year. At this time mineral nutrient content has been reduced by remobilization to rhizomes and natural weathering. A low mineral content at harvest is desirable in biomass intended for thermal conversion because it minimizes the impact on combustion efficiency and lowers stack emissions [22]. *Miscanthus sp.* can give the highest crop yield, caloric value and energy yield per hectare compared to other energy plant species, with crop yield between 12 and 30 tons of dry mass per hectare [23]. The lifetime of the crop is estimated between 20 and 25 years, *Miscanthus* biomass is produced during two phases: a yield-building phase, which in *M.* x *giganteus* lasts for two to five years, depending on climate and plant densities, and a plateau phase where the yield is maintained [24].

Breeding/Propagation

Cultivation of *M.* × *giganteus* especially in Europe and North America in temperate climates has a few disadvantages such as relatively high establishment costs, narrow genetic base and low hardiness in the first winter following establishment [6]. As a consequence of its triploidy, *M.* x *giganteus* is sterile and cannot form fertile seeds [19, 20]. The most popular methods propagation of *M.* x *giganteus* are in vitro tissue culture and rhizome cutting.

Ecological requirements

M. x *giganteus* has no special ecological requirements and can be grown through the Europe. Species is well adapted to different types of soil, prefers humid condition and higher temperatures. The photosynthesis efficiency and crop yield are depend on temperatures. The growing season ends with the first frost [39].

Heavy metals accumulation

The uptake of metals strongly depend on the level of bioavailable forms both on clean and polluted soils. *M.* x *giganteus* is capable of accumulating about 2 mg Pb kg^{-1}, 0.3 mg Cd kg^{-1} and 25 mg Zn kg^{-1}on clean soil while on loamy soil contaminated with heavy metals - up to 200 mg Pb kg^{-1}, 5 mg Cd kg^{-1} and 700 mg Zn kg^{-1} [25]. Furthermore, on the clean haplic luvisols *M.* x *giganteus* may accumulated from 2.2to 2.8mg kg^{-1} Cu, 0.5to 0.5mg kg^{-1} Ni and 12.6 31.7mg kg^{-1} Zn while on the contaminated haplic luvisols the plants may accumulated 3.7 to 11.4mg kg^{-1} Cu, 12.5 to 54.1mg kg^{-1} Ni and 264.0to 1086.0mg kg^{-1} Zn [26].

Measures to maximize phytostabilisation effect

M. × *giganteus* is a valuable plant that can be successfully grown on soils polluted with Zn, Cd and Pb. Aided phytostabilisation using red mud lead to a significant decrease in the labile (mobile fraction) concentration of heavy metals in soil and corresponding uptake by plant tissues, especially in stems. Also, addition red mud to the soil causes an increase of biomass production. All these findings show that *M.* × *giganteus* can be used for the production of renewable biomass on metal-contaminated soils and the application of red mud can contribute to increase the biomass production, to reduce metal concentrations in plant tissues and to a potentially reduced risk of metal leaching to subsoil layers or groundwater [23]. Moreover, the application of solid NPK fertilizer (once before planting) can diminish HM uptake. For biomass production it would be better to obtained low HM content in biomass which reduces the problem of HMs in further biomass processing [16].

Applications

M. x *giganteus* biomass can be used as solid fuel, in construction materials such as pressed particle-board, and as a source of cellulose [18].

2.2. *Sida hermaphrodita* (virginia mallow)

Figure 3. *Sida hermaphrodita* growth on the heavy metal contaminated arable land (second growing season).

General characteristics

Virginia mallow (*Sida hermaphrodita*) belongs to the *Malvaceae* family, thereby the reference to mallow in one of its common names [27]. It is characterized by a deep root system, rapid growth and high yield from 12 to 20 t ha^{-1} dry matter [28]. It has the ability to accumulate carbon in the root system [29].

Origin

Sida hermaphrodita originates from the Southeastern parts of North America. In the 20th century, the plant was brought to Europe, specifically to Ukraine, and then it was introduced in Poland [27].

Nutrient requirements

Before planting, depending on soil fertility status addition of solid fertilizer is needed: nitrogen 100 kg ha^{-1}, phosphorus 80 kg ha^{-1} as P_2O_5 and potassium 120 kg ha^{-1} as K_2O [27].

Harvesting

The most advantageous harvest time can take place from January through April; however before new stems regrow. Thus, it makes possible, without expensive biomass drying and storage, to proceed to granulation or combustion of biomass delivered directly from the field [43].

Breeding/Propagation

S. hermaphrodita produces seeds, but also it can reproduces vegetative via rhizomes spreading. The shoots emerge in April from a well-developed rhizome, buds being situated at the base of the stems of the previous year. Species is considered a susceptible for pests and diseases. It is estimated that the plantation life time is 20 years. The flowers appear from July until the first frost. *S. hermaphrodita* achieves more than 4 m height and more than 40 shoots per square meter during single growing season [45]. Because of the slow rate of seeds germination and the low competitiveness of cuttings, the virginia mallow is not considered to be an invasive species [30]. It can be grown on the slopes of eroded areas, land which is excluded from agricultural use, on chemically degraded areas, also on dumps and landfills [27].

Ecological requirements

Sida hermaphrodita is characterized by quick adaptation to different climatic and soil conditions [28], however is sensitive to water shortages as well as diseases or pests [27]. It doesn't have any special ecological requirements. It is well adapted to different types of soil, but in low-condition soil the yield will decrease. Also, the temperature have an influence on the amount of yields in growing season, the low temperature causes downturn of stems grown and seeds germination. Virginia mallow is well adapted to water deficiency and low-temperatures (up to −35 °C) in winter season. Its growth is more dependent to water deficiency than other nonwoody energy crops [43, 45]. Good plant growth without any toxic symptoms is expected on agricultural soils (even if the soil is contaminated) while on post-industrial sites, plant growth could be affected by low water content and nutrient deficiency.

Heavy metals accumulation

S. hermaphrodita has abilities to accumulate contaminants such as cadmium, nickel, lead and zinc. It has ability to accumulate more amount metals in the above-ground part of the plant than in the soil, showing good phytoextraction coefficient [44].

Measures to maximize phytoextraction effect

S. hermaphrodita has a high potential of phytoextraction of HMs (Ni, Cu, Zn, and Cd) in comparison to other species used as energy crops [12, 31]. Virginia mallow can be used in heavy metal phytoextraction. The heavy metal phytoextraction by *S. hermaphordita* depends on the bioavailable form of the metals in the soil, additionally fertilization can reduce HM accumulation in plants. Bioaccumulation factors are higher for plants cultivated on heavy metal contaminated arable land, 0.21-0.55 for cadmium and 0.23-0.86 for zinc, depending on treatment, while on sewage sludge dewatering site those values does not exceed 0.1 [21].

Applications

S.hermaphrodita is a valuable raw material used in power generation, biogas production and also used as a source of fibers and feed [27]. Also, virginia mallow can be used in textile, food, medicinal and pulp and paper industries. The calorific value of *S. hermaphrodita* vary between 17,000 – 18,500 kJ kg^{-1} [28].

2.3. *Panicum virgatum* (switchgrass)

Figure 4. *Panicum virgatum* growth on the heavy metal contaminated arable land (second growing season)

General characteristics

Panicum virgatum (switchgrass) is a native, cross-pollinated, perennial warm-season grass with a C4 photosynthetic pathway.[32]. *P.virgatum* reaches a height of 1-2, rarely 3 meters. Inflorescences reaches from 15-50 cm in length. Full yield is reached 3 years after planting. The yield is estimated at 7-9 Mg d.m. ha^{-1}, while the energy value is 15-17 GJ kg^{-1} [34].

Origin

It is originates from the North America (USA and Canada, primarily east of the Rocky Mountains and south of Hudson Bay) [32].

Nutrient requirements

Panicum virgatum (switchgrass) is a high-yielding and low-input bioenergy feedstocks which need before planting fertilizer addition which consist of nitrogen 80 kg ha^{-1}, phosphorus 50 kg ha^{-1} as P_2O_5 and potassium 75 kg ha^{-1} as K_2O. After 3–5 years, the yields are 10.4 ± 1.0 Mg ha^{-1} [33]. Adding phosphorus and potassium to seedbeds can cause favorable growth seeds. In contrast to nitrogen, which added to seedbeds over first phase of growth can causes undesirable weed growth [40].

Harvesting

It can be cut and harvested using traditional grass-harvesting machinery. The thin woody stems allow good dry-down in the winter. No records of harvesting methods of storage in the European context have been found [40].

Breeding/Propagation

Switchgrass is established from the seeds. To be able to breed switchgrass it requires seedbeds using traditional ploughing and insurance that the surface is free from weed. Sowing is normally carried out using small drills. The optimal temperature to sow is 10-15 °C and it should be carried out to the depth of 1 cm and to 400 plants per m2 [40].

Ecological requirements

P. virgatum can withstand low-temperatures without any problems [40]. It can be grown on any type of soil - light, moderately concise, saline or alkaline soil [34] and has resistance to acid soils but it has been recorded that most beneficial growth is on neutral soil [40]. Good plant growth is expected without any toxic symptoms on agricultural soils (even if the soil is contaminated) while on former post-industrial sites the plant growth could be affected by low water content and nutrient deficiency due to the ground structure.

Heavy metals accumulations

Switchgrass can be used to remove heavy metals as a cadmium, chromium, and zinc from soil using its ability to uptake metals on contaminant lands. In a sand culture the Switchgrass can accumulate Cd at a low soil pH. The maximum uptake of heavy metals as Cd, Cr, Zn using *P .virgatum* amounts to 40 and 34 µg pot^{-1}, 56 µg pot^{-1}, 358 and 254 µg pot^{-1}, respectively. One can adopt the optimal timing of harvest as plant Cd, Cr, and Zn approach 450 and 526 mg kg^{-1}, 266 mg kg^{-1}, and 3022 and 5000 mg kg^{-1}, respectively [46]. Heavy metals in soil can affect mineral macronutrients status in *P. virgatum*, especially Mg and K [34].

Measures to maximize phytoextraction effect

Switchgrass has a high biomass and can be successfully used in phytoremediation of contaminated soils. Addition EDTA (ethylenediaminetetraacetic acid) or natural acids can cause increase phytoextraction efficiency with the use of Switchgrass. It was considered that the optimal phytoextraction of Pb occurs with applications of 1.0 mmol EDTA kg−1 soil. The second alternative to maximize phytoextraction efficiency is application citric acid as an alternative to EDTA. The desired soil pH to maximize Pb-phytoextraction is 4.0-4.5. Both methods maximized phytoextraction can causes side effects. Also exogenous application of plant hormones as a gibberellic acid (GA3) or indole-3-acetic acid (IAA) has been used to improve phytoextraction [49]. Uptake of heavy metals by energy crop is determined by the metal bioavailability. Better remedial effect is reached during the winter sampling [47]. Moreover, Switchgrass has been successful in absorbing Cd from contaminated soil. The optimal phytoextraction conditions were found to be in a pH range from 4.1 to 5.9 and a Cd concentration range from 100 to 175 µM [48].

Applications

It is emphasized that the Switchgrass can be used as an alternative species in the reclamation and stabilization of contaminated sites as well as to bioaccumulation of heavy metals and for bioenergy production [34]. *P. virgatum*, like Miscanthus, has the ability to collect and store underground coal and to produce large quantities of biomass with minimal agricultural inputs [4]. As well as switchgrass can be used as cellulosic biomass feedstock for biorefineries and biofuel production [35]. It is also used as an ornamental plant [40].

2.4. *Spartina pectinata* (cordgrass)

Figure 5. *Spartina pectinata* growth on the heavy metal contaminated arable land (second growing season).

General characteristics

Spartina pectinata (cordgrass) is a C4, rhizomatous, perennial, and warm-season grass belongs to *Poaceae* family [5]. Occurs in salt marsh ecosystem [41]. It has high biomass production (5.0–9.7 Mg ha^{-1}) in eastern South Dakota, USA [5]. While, in eastern England achieves 12-14 Mg·ha^{-1} and in northern Germany 12.8 Mg·ha^{-1} [38]. The biomass of *S. pectinata* primarily consisting of leaves and stems reaches a height of about 1-3 m.

Origin

It is originates from the North America and is characterized by a very wide range of occurrence, from New Foundland and Quebec (Canada) to Arkansas, Texas and New Mexico (USA) [5].

Nutrient requirements

Typical solid fertilization before planting should contains: nitrogen 80 kg ha^{-1}, phosphorus 50 kg ha^{-1} as $P_2O_5$and potassium 75 kg ha^{-1} as K_2O.

Harversting

The location and season have influence a lot on amount of yield and moisture content, but long-term production depends on time of harvesting. The most appropriate time for harvesting is the time after stems have died in the winter. For harvesting dead stems standard machinery can be used [41].

Breeding/Propagation

Cordgrass reproduces both generative through seeds and vegetative by rhizomes [37]. Seeds vitality and germinate depends on temperature and humidity. In controlled storage increase of temperature and moisture causes decreased vitality. Although humid soil causes seeds to develop rapidly [42].

Ecological requirements

Spartina pectinata is predominantly found in lower, poorly drained soils along roadsides, ditches, streams, marshes, wet meadows, and potholes where soils are overly saturated [36, 37, 5]. It is well adapted to various abiotic stresses including cold, water saturation, and saline soils. It can grows in humid environment and tolerates acidified areas, and is resistant to changing environmental conditions [36]. Sometimes especially when grown on arable land or postindustrial site can produce higher biomass per square meter than *Miscanthus.* The cordgrass is able to growth on degraded lands [37].

S. pectinata is resistant to cold winter (-15 to -20 °C), different types of soil and high groundwater levels, but has not adapted to prolonged flooding. Soil moisture has an beneficial effect to accelerate seeds germination. The optimal conditions is the temperature of 20 °C at night and up to 30 °C during day time [42].

Heavy metals accumulations

On the Haplic Luvisols clean soil *Spartina pectinata* may accumulated from 3.5 to 4.3 mg kg^{-1} Cu, 0.4 to 0.9 mg kg^{-1} Ni and 20.0 to 35.6 mg kg^{-1} Zn. While grown on the contaminated haplic luvisols soil may accumulated 6.1 to 8.8 mg kg^{-1} Cu, 13.7 tom 39.4 mg kg^{-1} Ni and 911.0 to 1038.0 mg kg^{-1} Zn. Corgdrass is more tolerant to soil contamination with Cu, Ni, and Zn than *M.* × *giganteus* [26]

Measures to maximize phytoextraction effect

Phytoremediation potential of plants may change together with the growth of plants, and therefore, the duration of the experiment [26].

Applications

S. pectinata could be treated as energy crops and as fuel material [38] and can be used for the reclamation of soils contaminated with heavy metals [26].

3. Land management options

Depending on future land use, energy crops grown on heavy metal contaminated land can be applied for two purposes:

1. PHYTOEXTRACTION: uptake of contaminants to the aboveground biomass with a simultaneous production of biomass for energetic use. This option is mainly feasible on heavy metal contaminated agricultural land which due to that fact is excluded from food crops production.

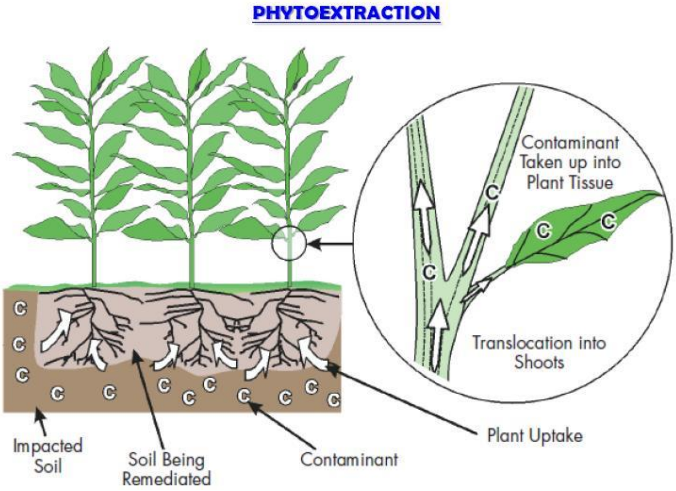


Figure 6. Scheme of phytoextraction
(source: https://knowhowtogmo.wordpress.com/2011/01/31/phytoextraction/)

2. PHYTOSTABILISATION: to stabilize contaminants into the root system and uptake heavy metals but in limited quantities. This option finds application in particular for postindustrial sites with elevated HM content in the ground which are idle and as such may pose some risk to the environment. Phytoextraction allows to reduce this risk and return the land into economic use by biomass production. Despite contamination, appropriate selection of energy crops enables production on non-contaminated biomass that may have multiple application including energy production.

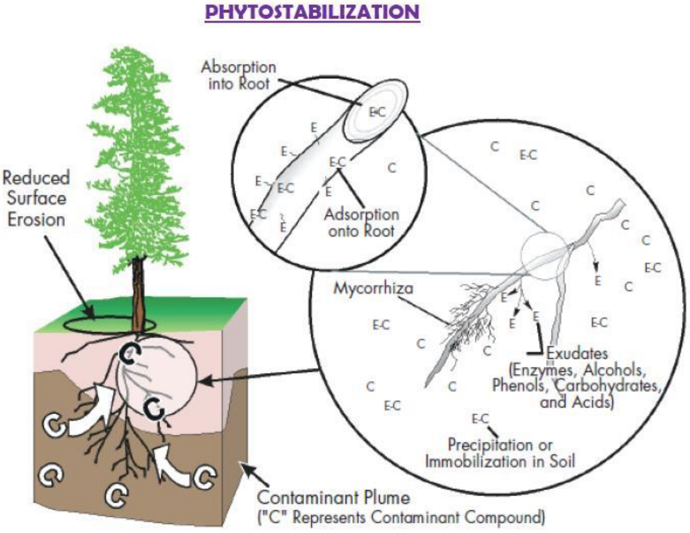


Figure 7. Scheme of phytostabilisation (source: https://denisesteinert81.wordpress.com/)

Comparison of phytoextraction and phytostabilisation approach are presented in Table 1.

Table 1. Comparison of phytoextraction and phytostabilisation approach

	Phytoextraction driven energy crops production	**Phytostablisation driven energy crops production**
Purpose	Remove contaminants from soil to restore the land for agricultural production (food or feed) by production of biomass	Produce biomass for energy purposes while preventing the spread of contamination , reduce environmental risk
Application	Agricultural lands with HM contamination	Industrial sites
Key advantages	Improvement of the soil quality , removal of contaminants, future possibilities for food or feed production, temporal change in land use with lack of income (energy crop instead of food production)	Land recovery for biomass production with calculated income for at least 15 years
Key disadvantages	Long term process depend on heavy metal levels and planned land use in the future	
Biomass	Contaminated biomass with limited application options , optimal conversion to energy by gasification	Production of non-contaminated biomass, optimal option of the conversion to energy by combustion or anaerobic digestion,

4. Criteria to use energy crops for phytoremediation approach

There are three main factors which determine the use of phytotechnology on heavy metals contaminated areas:

1. Level of soil contamination
2. Appropriate soil conditions for plant growth
3. Appropriate climatic conditions for plant development

To investigate these factors it is necessary to obtain the following data on the site specific conditions:

- ✓ site characteristics form the contaminants point of view,
- ✓ existing vegetation influence on the energy crop plantation (weeds etc.),
- ✓ hydrological and climate conditions.

Soil, climate parameters and plant requirements needed for energy crop cultivation are listed below.

4.1. Soil parameters

Soil parameters which should be analyzed to assess the possibility of energy crop plantation on heavy metal contaminates land are as follows:

- ✓ type of soil,
- ✓ pH,
- ✓ electrical conductivity,
- ✓ organic matter,

- ✓ total concentration of contaminants (for example Pb, Cd, Zn *etc.*),
- ✓ bioavailable concentration of contaminants (contaminants presence in soil solution),
- ✓ total and available macronutrients: N, P, K, Ca, Mg,

4.2. Plant growth requirements

Each plant need the optimal condition to growth.

What is important in plants growth:

1) **Fertilization** based on plant requirement, for proposed energy crops should be added before planting:
   i) NPK standard fertilization (ammonium sulphate and phosphorous - 4% N, 22% P_2O_5, 32% K_2O), applied directly to the soil before planting:
   - *Miscanthus* x *giganteus* - nitrogen 70 kg ha^{-1}, phosphorus 30 kg ha^{-1} as P_2O_5 and potassium 45 kg ha^{-1} as K_2O);
   - *Sida hermaphrodita* - nitrogen 100 kg ha^{-1}, phosphorus 80 kg ha^{-1} as P_2O_5 and potassium 120 kg ha^{-1} as K_2O);
   - *Panicum virgatum* - nitrogen 80 kg ha^{-1}, phosphorus 50 kg ha^{-1} as P_2O_5 and potassium 75 kg ha^{-1} as K_2O);
   - *Spartina pectinata* - nitrogen 80 kg ha^{-1}, phosphorus 50 kg ha^{-1} as P_2O_5 and potassium 75 kg ha^{-1} as K_2O);

   or during the plant growth:
   ii) Commercial liquid fertilizers applied directly on plant or soil surface

2) **Weed control** is an important factor, especially during the establishment and first two years of the crop. It is recommended that before planting the field should be completely cleared of all perennial weeds. It is very important to do it before planting of energy crop grasses because after we cannot use the herbicide against perennial weeds.

4.3. Site climatic requirements

All 4 energy crop species demonstrate high resistance against disadvantageous climate conditions. The plant has evolved in regions of the world that have large temperature fluctuations between summer and winter. Energy crops are able to growth in wide climates from drought to moderate.

Climatic parameters which should be controlled during plant growth:

- ✓ Temperature,
- ✓ Precipitation.

5. Recommendations for energy crops use for phytoremediation approach

Summary of the P2E project results concerning possible use of energy crop in phytoremediation of heavy metals contaminated sites is presented in the Table 2.

Table 2. Matrix of possible energy crop use in phytoremediation of HM contaminated land

Energy crop	Phytoextraction			Phytostabilisation		
	Heavy metal					
	Pb	Cd	Zn	Pb	Cd	Zn
Miscanthus x giganteus		🟢	🟢	🟢		
Sida hermaphrodita		🔵		🟢		🟢
Panicum virgatum				🔵	🟢	🔵
Spartina pectinata	🟢		🔵		🟢	

🔵 Medium potential for phytoremediation

🟢 High potential for phytoremediation

6. Implementation depending of future land use – case studies – Polish (Bytom) and German (Leipzig)

For implementation of the idea to use energy crops in phytoremediation two case studies were chosen:

1) For **the first site** - **Polish case study** (Bytom) which was an agricultural land affected by HMs soil contamination (especially zinc, cadmium and lead) resulting from a decommissioned lead and zinc smelter activity, **phytoextraction approach was implemented**. The contents of Pb, Cd and Zn in soil exceed the limits set by Polish law for arable lands. They exclude this area from food production. Soil texture at the site has been classified as silty loam. Phytoextraction of HM by energy crops can help to diminish bioavailability of contaminants in soil and give possibility to back to feed or food (mainly cereals) production in future. Contaminated energy crop biomass can be used as energy source but technology to obtain energy from biomass should be safe for environment (direct combustion is prohibited).
2) For **the second site**, located in Leipzig called **German case study** which was a former sewage sludge dewatering site that was operational from 1952 to 1990, **phytostabilisation approach was implemented**, which give the possibility to obtain less contaminated biomass. Following its closure, approximately 650,000 tons of sewage sludge remained in several basins. Because exceeded level of HMs was found, phytoremediation of the site using *Phragmites australis* (Cav.)Trin. ex Steud was carried out. Nevertheless, the sewage sludge still remained contaminated with lead, cadmium and zinc. Soil texture at the site has been classified as sandy loam. Phytostabilisation (due to the low bioavailability of contaminants) give possibility to demonstrate that such postindustrial site could be used for "safe" biomass production and can give some profits to the owner (selling the biomass for energy purposes) in future.

6.1. Energy crop plantation establishment (soil and plant requirements)

Experimental trials in Poland – arable land (Fig. 8a) and Germany – postindustrial land (Fig. 8b) were established in May/June 2014 and last for three growing seasons.

Before planting, composite soil samples were taken from each sites at 0-20 cm depths, to determine the parameters characterizing soil/ground (see the chapter 3.1 Soil requirements)

Rhizomes (*Miscanthus* x *giganteus*), roots seedlings (*Sida hermaphrodita, Spartina pectinata*) and seedlings (*Panicum virgatum*) were planted at 10 cm depths.

To see the difference how energy crops reacts on different type of fertilization (soild or liquid) and how it can change the biomass production, different options of ferritization were used:

- C – Control, no treatment;
- NPK- NPK standard fertilization (as ammonium sulphate and phosphorous - 4% N, 22% P_2O_5, 32% K_2O), applied directly to the soil before planting, calculate based on plant requirements (see chapter: 3.2 Plant requirements)
- INC - Commercial microbial inoculum Emfarma Plus® ProBiotics Poland was applied as an alternative for the standard chemical fertilization.

Figure 8. General view of energy crop trials established on: a) arable land contaminated with HM (Polish case study); b) postindustrial site (German case study)

6.2. Soil characteristics on case studies

Physio-chemical soil initial parameters were generally homogenous within the experimental sites. Differences were observed between the sites due to the historical uses and the source of the contaminations, resulting from smelting activity and sewage sludge deposition for Polish and German site, respectively (Table 3). Soil pH was neutral and did not differ significantly between the sites. The soil EC and OM content was about 6-fold higher at the Leipzig site when compared to the Bytom site. The Pb total soil content was slightly higher in the Bytom site (about 637 mg kg^{-1}) when compared to the Leipzig site (about 615 mg kg^{-1}). The total content of Cd and Zn was 36% and 63% higher at the Leipzig site, respectively. The bioavailability of Pb was below detection limits in both sites. Higher bioavailability of Cd was found at the Bytom site, while for the Leipzig site, it was below the detection limit (control plot). The bioavailability of Zn in the soil was almost 6.5-fold higher for the Bytom site.

Table 3**.** Heavy metals content and physico-chemical parameters of initial soil on two experimental test sites.

Property	CASE STUDY	
	POLISH (Bytom)	GERMAN (Leipzig)
pH (1 : 2.5 soil/KCl ratio)	6.79	6.37
Electrical conductivity (µS/cm)	127	797
Organic matter content (%)	6.2	32.95
Sand (1 – 0.05 mm), %	28	58
Silt (0.05 – 0.002 mm), %	56	19
Clay (< 0.002 mm), %	16	23
Total heavy metal concentration (extraction with aqua regia)		
Pb (mg kg^{-1})	635	612
Cd (mg kg^{-1})	25.7	34.20
Zn (mg kg^{-1})	2360	3880
$CaCl_2$ extractable metal fraction [a]		
Pb (mg kg^{-1})	BDL	BDL
Cd (mg kg^{-1})	1.87 (7.27) [b]	0.280 (0.81) [b]
Zn (mg kg^{-1})	110 (4.66) [b]	12.63 (0.32) [b]

Values represent mean of three replicate samples ± SE

[a] – extraction with 0.01 M $CaCl_2$

[b] – in parentheses percentages of total metal concentrations are presented

6.3. Biomass production on case studies

Based on the results of the obtained biomass per plot, biomass yield in t/ha was calculated for each plant and respective treatment variant. Figure 9 presents the calculated biomass yield after the third growing season. Significantly higher yield for *M*. x *giganteus*, *S. hermaphrodita* and *S. pectinata* was calculated for the Bytom site, while for *P. virgatum* obtained biomass yield was comparable for both of the sites. Among all of the tested species, the highest biomass yield was found for *M*. x *giganteus* (over 20 t ha^{-1} for inoculated plot) and *S. pectinata* (over 20 t ha^{-1} under standard NPK fertilization) cultivated at the Bytom site. The yield of *M. x giganteus*, *S. pectinata* cultivated at HM contaminated arable land in Poland was over twice time higher when compared to the former sewage sludge deposit site. In case of *S. hermaphrodita,* the obtained yield at Polish test site was 1.5-fold higher for inoculum treatment and over 2-fold higher for NPK fertilization. At control plots, the yield of these species was slightly higher at the German test site.

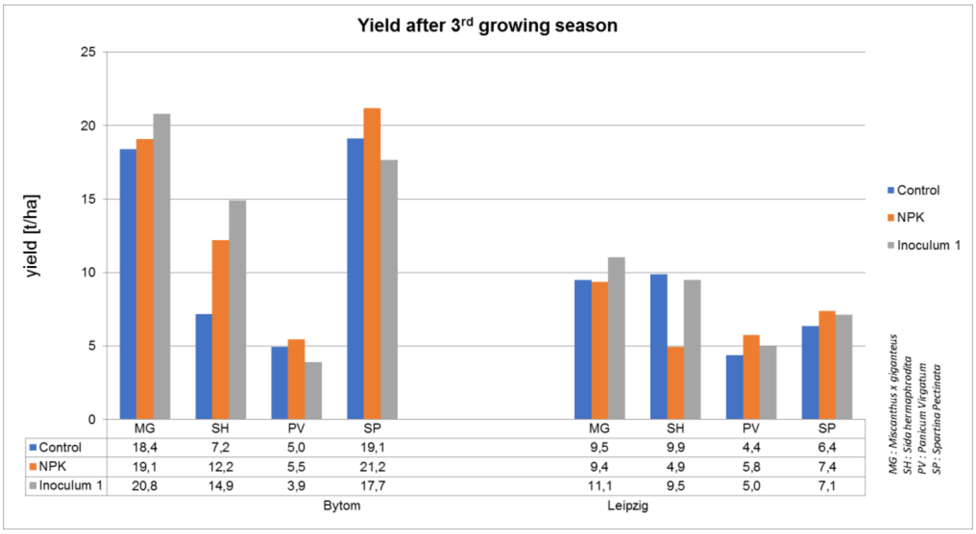
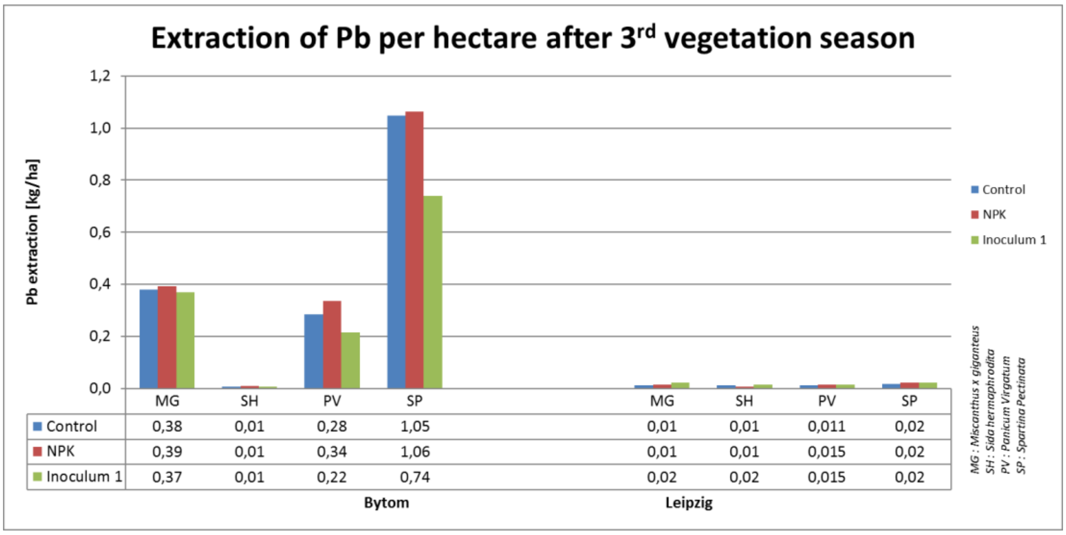


Figure 9. Comparison of the biomass yield after the 3rd growing season.

Summarizing – Inoculation enhanced biomass production for *Miscanthus* x *giganteus* from both sites and *Sida hermaphrodita* (virginia mallow) grown on arable land contaminated with HM in comparison to control and NPK fertilization (solid fertilization). But the cost of applying inoculum to the ground (once before planting and on each month spraying on soil surface) are higher in comparison to NPK fertilizer application (once before planting).

6.4. Remediation potential of energy crops

Based on the results of the heavy metal uptake by plants and the biomass yield after the third growing season, the extractions of heavy metals per hectare were calculated. Figure 10 illustrates the extraction of Pb kg ha^{-1}. The highest lead extraction was found for *Spartina pectinata*. Even if this species is not cumulating the highest amount of lead among all tested species, a high biomass yield caused that using this species, it is possible to extract up to 1 kg of lead per hectare per year. *Miscanthus* and *Panicum* are able to extract twice lower amount of lead, while *S. hermaphrodita* is able to extract only a very limited amount of this element. Because of a lower lead uptake and biomass yield, plants cultivated at the Leipzig site extracted a very limited amount of lead.



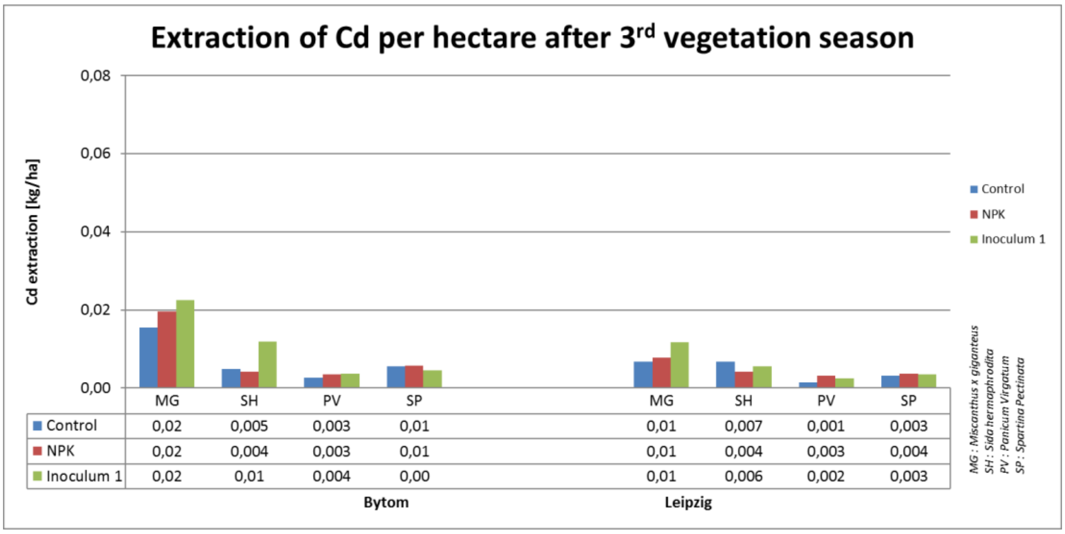
	Bytom				Leipzig			
	MG	SH	PV	SP	MG	SH	PV	SP
■ Control	0,38	0,01	0,28	1,05	0,01	0,01	0,011	0,02
■ NPK	0,39	0,01	0,34	1,06	0,01	0,01	0,015	0,02
■ Inoculum 1	0,37	0,01	0,22	0,74	0,02	0,02	0,015	0,02

Figure 10. Lead extraction by plants after third growing season.

The data shows that at the Pb extraction levels demonstrated by *Spartina pectinata* , cultivation of cordgrass on a heavy metal contaminated arable soil for the period of 15- 20 years may allow to diminish the concentration of Pb in soil to the levels that enable restoring feed or food crops production.

On the other hand a very low Pb extraction found in biomass of *Miscanthus* x *giganteus* and *Sida hermaphodita* (virginia mallow) from the postindustrial site suggest, that these species could be used for non-contaminated or slightly contaminated biomass yield production.

Figure 11 presents cadmium extraction per ha after 3rd growing season. The highest efficiency in cadmium extraction was found for *Miscanthus* cultivated at the Bytom site, especially under microbial inoculation. Also for *Sida hermaphrodita* it was found that the microbial inoculation stimulated the extraction of this element. The lowest cadmium extraction was found for *Panicum virgatum*. The main reason was due to the lowest biomass production among all the tested plant species. Plants cultivated at the Leipzig site were able to extract significantly lower amounts of Cd when compared to the Bytom site. It was determined both by low metal bioavailability in the soil and a low biomass yield.



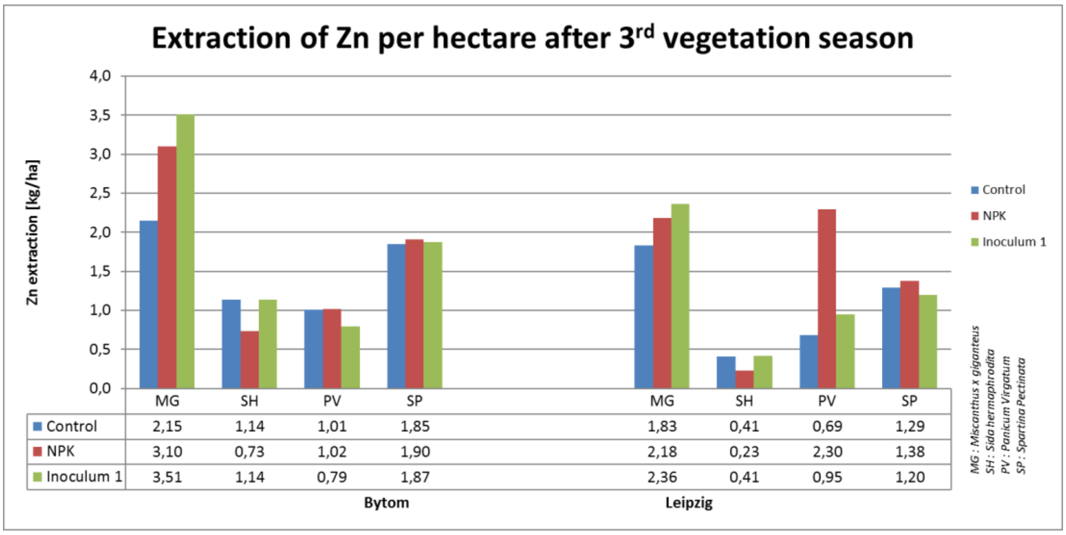
	Bytom MG	Bytom SH	Bytom PV	Bytom SP	Leipzig MG	Leipzig SH	Leipzig PV	Leipzig SP
Control	0,02	0,005	0,003	0,01	0,01	0,007	0,001	0,003
NPK	0,02	0,004	0,003	0,01	0,01	0,004	0,003	0,004
Inoculum 1	0,02	0,01	0,004	0,00	0,01	0,006	0,002	0,003

Figure 11. Cadmium extraction by plants after third growing season.

Summarizing - Cd extraction by *Miscanthus x giganteus* after 15 to 20 years of growth on arable land contaminated with heavy metals can diminish concentration of Cd in soil to the levels potentially enabling restoration of feed or food crops production.

On the other hand, low Cd extraction found in biomass of *Panicum virgatum* grown on arable and postindustrial land suggest that it is possible to produce "safe" (low metals content) biomass on such areas.

Figure 12 presents zinc extraction per ha after the 3rd growing season. The highest extraction of this element was found for *Miscanthus* cultivated at the Bytom site under the microbial inoculum fertilization. Taking into account the obtained biomass yield it is possible to extract over 3.5 kg of zinc each year. Almost twice lower (about 2kg/ha/year) extraction was found for *Miscanthus* cultivated at the Leipzig site, where the yield was the main determining factor. A similar trend was found for the Bytom site, where the highest extraction was at the plots with the microbial inoculum addition. Due to a high biomass yield *Spartina pectinata* was able to extract nearly 2kg of Zn/ha/year. The lowest Zn extraction was found for *Sida hermaphrodita* cultivated at the Leipzig site.



	Bytom MG	Bytom SH	Bytom PV	Bytom SP	Leipzig MG	Leipzig SH	Leipzig PV	Leipzig SP
Control	2,15	1,14	1,01	1,85	1,83	0,41	0,69	1,29
NPK	3,10	0,73	1,02	1,90	2,18	0,23	2,30	1,38
Inoculum 1	3,51	1,14	0,79	1,87	2,36	0,41	0,95	1,20

Figure 12. Zinc extraction by plants after the third growing season.

Zinc was the most extracted metal among all the tested HMs, because of its high concentration in soil and a relatively high bioavailability at both sites, what, multiplied by the yield, resulted in a high extraction.

Summarizing – at the level of up to 3 kg of Zn extraction per growing season, *Miscanthus* x *giganteus* provides as real opportunity to diminish the levels of contamination to the limits allowing for feed or food crops production after 20 years of cultivation on arable land contaminated with heavy metals.

On the other hand on postindustrial site, very low Zn extraction found in biomass of *Sida hermaphodita* (virginia mallow) suggest that it is possible to produce "safe" (low metals content) biomass.

7. Literature

[1]. Scarlat, N., Banja, M. Possible impact of 2020 bioenergy targets on European Union land use. A scenario-based assessment from national renewable energy action plans proposals. Renew Sust Energ Rev. 2013;18:595–606. doi:10.1016/j.rser.2012.10.040.

[2] Alkorta, I., Becerri, J.M. and Garbisu C. Recovery of Soil Health: The Ulti-mate Goal of Soil Remediation Processe., In: Trends in Bioremediation and Phy-toremediation, Płaza G. (Ed.), Research Signpost, 2010, India, 1-9.

[3] Ollivier, J., Wanat, N., Austruy, A., Hitmi, A., Joussein, E., Welzl, G., Munch, J.Ch. and Schloter, M. Abundance and diversity of ammonia- oxidizing prokaryotes in the root-rhizosphere complex of Miscanthus x giganteus grown in heavy metal-contaminated soils. Microbial Ecology, 2012, 64, 1038-1046.

[4] Dohleman, F.G., Heaton, E.A., Arundale, R.A., Long, S.P. Seasonal dynamics of above-and below-ground biomass and nitrogen partitioning in Miscanthus× giganteus and Panicum virgatum across three growing seasons. GCB Bioenergy 4, 2012, 534-544. doi:10.1111/j.1757-1707.2011.01153.x

[5] Guo, J., Thapa, S., Voigt, T., Rayburn, A. L., Boe, A., & Lee, D. K. Phenotypic and biomass yield variations in natural populations of prairie cordgrass (Spartina pectinata Link) in the USA. BioEnergy Research, 2015, 8(3), 1371-1383.

[6] Clifton-Brown J., Hastings A., Mos M., McCalmont J.P., Ashman Ch., Carroll D., Cerazy J., Chiang Y-Ch, Cosentino S., Cracroft-Eley W., Scurlock J., Donnison I.S., Glover Ch., Gołąb I., Greef J.M., Gwyn J., Harding G., Hayes Ch., Helios W., Hsu T-W, Huang L. S., Jeżowski S., Kim D-S, Kiesel A., Kotecki A.,Krzyzak J., Lewandowski I., Lim S.H., Liu J., Loosely M., Meyer H., Nelson W., Pogrzeba M., Robinson G., Robson P., Rogers Ch., Scalici G., Schuele H., Shafiei R., Shevchuk O., Schwarz K-U., Squance M., Swaller T., Thornton J., Truckses T., Botnari V., Vizir I., Wagner M., Warren R., Yamada T., Youell S., Xi Q., Zong J., Flavell R., 2017. Progress in upscaling Miscanthus biomass production for the European bioeconomy with seed based hybrids. GCB Bioenergy, 9(1), 6-17, DOI:10.1111/gcbb.12357

[7] Schrama, M., Vandecasteele, B., Carvalho, S., Muylle, H., Putten, W.H.,. Effects of first-and second-generation bioenergy crops on soil processes and legacy effects on a subsequent crop. GCB Bioenergy, 2016, 8, 136-147. doi:10.1111/gcbb.12236

[8] El Kasmioui, O. and Ceulemans R. Financial analysis of the cultivation of poplar and willow for bioenergy. Biomass and Bioenergy, 2012, 43, 52-64.

[9] Smeets, E.M.W., Lewandowski, I.M. and Faaij A.P.C. The economical and environ-mental performance of miscanthus and switchgrass production and supply chains in a European setting. Renewable and Sustainable Energy Reviews, 2009, 13, 1230-1245.

[10] Michalska, K., Miazek, K., Krzystek, L and Ledakowicz S. Influence of pretreatment with Fenton's reagent on biogas production and methane yield from lignocellulosic biomass. Bioresource Technology, 2012, 119, 72-78.

[11] Howaniec, N. and Smoliński A. Steam gasification of energy crops of high cultivation potential in Poland to hydrogen-rich gas. International Journal of Hydrogen Energy, 2011, 36, 2038-2043.

[12] Borkowska, H. and Molas R. Two extremely different crops, Salix and Sida, as sources of renewable bioenergy. Biomass and Bioenergy, 2012, 35, 234-240.

[13] Witters, N., Van Slycken, S., Ruttens, A., Adriaensen, K., Meers, E., Meiresonne, L., Tack, F.M.G., Thewys, T., Laes, E. and Vangronsveld J. Short-rotation coppice of willow for phytoremediation of a metal-contaminated agricultural area: a sustainability assessment. BioEnergy Research, 2009, 2, 144-152.

[14] Mleczek, M., Rutkowski, P., Rissmann, I., Kaczmarek, Z., Golinski, P., Szentner, K., Strażyńska, K. and Stachowiak A. Biomass productivity and phytoremediation potential of Salix alba and Salix viminalis. Biomass and Bioenergy, 2010, 34, 1410-1418.

[15] Chen, B., Lai, H., Juang, K. Model evaluation of plant metal content and biomass yield for the phytoextraction of heavy metals by switchgrass. Ecotoxicology and Environmental Safety, 2012, 80, 393-400.

[16] Pogrzeba M., Rusinowski S., Sitko K., Krzyżak J., Skalska A., Małkowski E., Ciszek D., Werle S., McCalmont J.P., Mos M., Kalaji H.M., 2017. Relationships between soil parameters and physiological status of Miscanthus x giganteus cultivated on soil contaminated with trace elements under NPK fertilisation vs. microbial inoculation. Environmental Pollution, 225,163-174.

[17]. Werle S, Bisorca D, Katelbach-Woźniak A, Pogrzeba M, Krzyżak J, Ratman-Kłosińska, I, Burnete D. Phytoremediation as an effective method to remove heavy metals from contaminated area–TG/FT-IR analysis results of the gasification of heavy metal contaminated energy crops. J Energy Inst. 2016. doi:10.1016/j.joei.2016.04.002.

[18] Lewandowski, I., Clifton-Brown, J. C., Scurlock, J. M. O., Huisman, W. (2000). Miscanthus: European experience with a novel energy crop. Biomass and Bioenergy, 19(4), 209-227.

[19] Linde-Laursen, I. (1993). Cytogenetic analysis of Miscanthus 'Giganteus', an interspecific hybrid. Hereditas, 119(3), 297-300.

[20] Naidu, S. L., Moose, S. P., Al-Shoaibi, A. K., Raines, C. A., & Long, S. P. (2003). Cold tolerance of C4 photosynthesis in Miscanthus× giganteus: adaptation in amounts and sequence of C4 photosynthetic enzymes. Plant Physiology, 132(3), 1688-1697.

[21] Pogrzeba M., Krzyżak J., Rusinowski S., Werle S., Hebner A., Milandru A., 2018. Case Study on Phytoremediation Driven Energy Crop Production Using Sida hermaphrodita. International Journal of Phytoremediation, (doi.org/10.1080/15226514.2017.1375897

[22] Christian, D. G., Riche, A. B., & Yates, N. E. (2008). Growth, yield and mineral content of Miscanthus× giganteus grown as a biofuel for 14 successive harvests. Industrial crops and products, 28(3), 320-327.

[23] Pavel, P. B., Puschenreiter, M., Wenzel, W. W., Diacu, E., & Barbu, C. H. (2014). Aided phytostabilization using Miscanthus sinensis× giganteus on heavy metal-contaminated soils. Science of the Total Environment, 479, 125-131.

[24] Zub, H. W., & Brancourt-Hulmel, M. (2010). Agronomic and physiological performances of different species of Miscanthus, a major energy crop. A review. Agronomy for Sustainable Development, 30(2), 201-214.

[25] Pogrzeba, M., Krzyżak, J., Sas-Nowosielska, A., Majtkowski, W., Małkowski, E., Kita, A. (2011). A heavy metal environmental threat resulting from combustion of biofuels of plant origin. Environmental Heavy Metal Pollution and Effects on Child Mental Development, 213-22.

[26] Korzeniowska, J., Stanislawska-Glubiak, E. (2015). Phytoremediation potential of *Miscanthus* × *giganteus* and *Spartina pectinata* in soil contaminated with heavy metals. Environmental Science and Pollution Research, 22(15), 11648-11657.

[27] Kasprzyk, A., Leszczuk, A., & Szczuka, E. (2014). Virginia Mallow (Sida hermaphrodita (L.) Rusby)-properties and application. Modern Phytomorphology, (6).

[28] Šiaudinis G, Jasinskas A, Šarauskis E, Steponavičius D, Karčauskienė D, Liaudanskienė I. The assessment of Virginia mallow (Sida hermaphrodita Rusby) and cup plant (Silphium perfoliatum L.) productivity, physico–mechanical properties and energy expenses. Energy. 2015;93:606–612. doi:10.1016/j.energy.2015.09.065.

[29] Nabel, M., Barbosa, D. B., Horsch, D., & Jablonowski, N. D. (2014). Energy crop (Sida hermaphrodita) fertilization using digestate under marginal soil conditions: A dose-response experiment. Energy procedia, 59, 127-133.

[30] Nabel, M., Temperton, V. M., Poorter, H., Lücke, A., & Jablonowski, N. D. (2016). Energizing marginal soils–The establishment of the energy crop Sida hermaphrodita as dependent on digestate fertilization, NPK, and legume intercropping. Biomass and Bioenergy, 87, 9-16.

[31] Antonkiewicz J, Kołodziej B, Bielińska EJ. Phytoextraction of heavy metals from municipal sewage sludge by *Rosa multiflora* and *Sida hermaphrodita*. International Journal of Phytoremediation. 2016:1549–7879. doi:10.1080/15226514.2016.1225283.

[32] Hultquist, S. J., Vogel, K. P., Lee, D. J., Arumuganathan, K., & Kaeppler, S. (1996). Chloroplast DNA and nuclear DNA content variations among cultivars of switchgrass, *Panicum virgatum* L. Crop Science, 36(4), 1049-1052.

[33] Arundale, R. A., Dohleman, F. G., Voigt, T. B., and Long, S. P. (2014). Nitrogen fertilization does significantly increase yields of stands of *Miscanthus* × *giganteus* and *Panicum virgatum* in multiyear trials in Illinois. BioEnergy Res. 7, 408–416. doi: 10.1007/s12155-013-9385-5

[34] Pogrzeba M., Rusinowski S., Krzyżak J., 2017 Macroelements and heavy metals content in *Panicum virgatum* cultivated on contaminated soil under different fertilization. International Journal Agriculture & Forestry, Vol. 63 Issue 1: 69-76, Podgorica, DOI: 10.17707/AgricultForest.63.1.08.

[35] Sokhansanj, S., Mani, S., Turhollow, A., Kumar, A., Bransby, D., Lynd, L., & Laser, M. (2009). Large scale production, harvest and logistics of switchgrass (*Panicum virgatum* L.)-current technology and envisioning a mature technology. Biofuels, Bioproducts and Biorefining, 3(2), 124-141.

[36] Kim, S., Rayburn, A. L., Parrish, A., & Lee, D. K. (2012). Cytogeographic distribution and genome size variation in prairie cordgrass (Spartina pectinata Bosc ex Link). Plant molecular biology reporter, 30(5), 1073-1079.Fraser, A., & Kindscher, K. (2001). Tree spade transplanting of *Spartina pectinata* (Link) and *Eleocharis macrostachya* (Britt.) in a prairie wetland restoration site. Aquatic Botany, 71(4), 297-304.

[37] Prasifka, J. R., Lee, D. K., Bradshaw, J. D., Parrish, A. S., & Gray, M. E. (2012). Seed reduction in prairie cordgrass, *Spartina pectinata* Link., by the floret-feeding caterpillar *Aethes spartinana* (Barnes and McDunnough). Bioenergy Research, 5(1), 189-196.

[38] Kowalczyk-Jusko, A., Kulig,R., & Laskowski, J. (2011). The influence of moisture content of selected energy crops on the briquetting process parameters. Teka Komisji Motoryzacji i Energetyki Rolnictwa, 11.

[39] Qingguo, Xi. Miscanthus (Miscanthus spp.) in: El Bassam, N. (2010). *Handbook of bioenergy crops: a complete reference to species, development and applications*. Routledge.

[40] Dudley, G.C., Elbersen H.W. Switchgrass (Panicum virgatum L.) in: El Bassam, N. (2010). *Handbook of bioenergy crops: a complete reference to species, development and applications*. Routledge

[41] Dudley, G.C. Cordgrass (Spartina spp.) in: El Bassam, N. (2010). *Handbook of bioenergy crops: a complete reference to species, development and applications*. Routledge

[42] Beringen, R., van Duinen, G. A., de Hoop, L., De Hullu, P. C., Matthews, J., Odé, B., ... & Leuven, R. S. E. W. (2017). Risk assessment of the alien prairie cordgrass (spartina pectinata). *Reports Environmental Science*, *526*, 1-62.

[43] http://bioenergycrops.com/virginia_fanpetals/

[44] Prelac, M., Bilandžija, N., & Zgorelec, Ž. (2016). The phytoremediation potential of heavy metals from soil using Poaceae energy crops: A review. *Journal of Central European Agriculture*, *17*(3).

[45] Nahm, M., & Morhart, C. Virginia mallow (Sida hermaphrodita (L.) Rusby) as perennial multipurpose crop: Biomass yields, energetic valorization, utilization potentials, and management perspectives. *GCB Bioenergy*.

[46] Chen, B. C., Lai, H. Y., & Juang, K. W. (2012). Model evaluation of plant metal content and biomass yield for the phytoextraction of heavy metals by switchgrass. *Ecotoxicology and environmental safety*, *80*, 393-400.

[47] Krzyżak, J. (2016, November). The Use of Energy Crop Species for Phytoremediation of Heavy Metal Contaminated Sites. In *Proceedings of the 18th International Conference on Heavy Metals in the Environment*.
[48] Wang, Q., Gu, M., Ma, X., Zhang, H., Wang, Y., Cui, J., ... & Gui, J. (2015). Model optimization of cadmium and accumulation in switchgrass (Panicum virgatum L.): potential use for ecological phytoremediation in Cd-contaminated soils. *Environmental Science and Pollution Research*, *22*(21), 16758-16771.
[49] Aderholt, M., Vogelien, D. L., Koether, M., & Greipsson, S. (2017). Phytoextraction of contaminated urban soils by Panicum virgatum L. enhanced with application of a plant growth regulator (BAP) and citric acid. *Chemosphere*, *175*, 85-96.