INNOVATIVE APPROACH TO CONTAMINATED SOIL PHYTOREMEDIATION: HEAVY METAL PHYTOEXTRACTION USING ENERGY CROPS

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Abstract: *Heavy metal accumulation potential in the biomass and different plant parts of the selected species of energy crops cultivated on contaminated soil was evaluated. Phytoextraction potential, biomass yield and qualitative parameters of bioenergy plants grown on heavy metal contaminated soil has been measured. Finally heavy metal influence on biomass utilization possibilities, energy recovery and further safe use of the rest products has been evaluated. Demonstrated possibilities to grow bioenergy plants on moderately contaminated soil could increase the use of marginal lands, decrease land use competition between food and liquid biofuels and provide options for a gentle and cost-effective remediation.*

Keywords: HEAVY METALS, SOIL CONTAMINATION, PHYTOEXTRACTION, ENERGY CROPS, BIOMASS

1. Introduction

Land contamination is now recognized as a problem capable to affect ecosystems and human health in a range of different ways. Perception of soil as a vital natural element as well as an essential resource for human survival and development raises public awareness of soil contamination as an important environmental issue worldwide. It is reported that more than 10 mln contaminated sites exist on our planet, with half of the sites contaminated with heavy metals *(Khalid et al., 2017)*. Every site identified as a potential contamination source has information about ongoing activities or the ones that were carried out in the past; loads and origins of the chemicals used during the activities; role and impact to the ecosystem and hazards to human health. Most of such activities were carried out before 1990. Historical pollution is very troublesome in Lithuania. In the absence of person, responsible for creating the pollution, expenses for remediation are paid from the state budget. Lithuanian Geological Survey states that every third identified pollution source pose risk to the environment, and every tenth source has a very high negative impact to the environment. The Survey says that area of the contaminated territory might be about 280 km^2. Main contaminants are oil products, but every fifth object is contaminated with pesticides, heavy metals and polycyclic aromatic hydrocarbons *(Ministry of Environment, 2012)*. Increased public awareness about health risks posed by heavy metals triggers pressure for the academic society to look for more efficient ways how to solve this problem. If most of organic contaminants are easily biodegradable, heavy metals (HM) are highly resistant to either biologically or chemically induced degradation. It is stated in the *Plan for the management of contaminated sites in 2013-2020*, that soil contaminated with heavy metals should be excavated and further treated *ex situ* or landfilled *(Ministry of Environment, 2012)*. Although, excavation being one of the cheapest options to “treat” heavy metal-contaminated soil so far, it is always very harsh for the soil micro- and macro-environment, it has low public acceptance and finally, the problem is not solved, but only transported elsewhere. Thus search for alternative cost-effective and environmentally friendly technologies and materials for clean-up of heavy metal contaminated sites is extremely important.

The EU countries agreed upon increasing the use of renewable energy, which strongly stimulates usage of biomass. Beneficial biomass ash on-land recycling or, alternatively, safe disposal of ashes will become more and more important in the future because of the increasing volume of the produced ashes worldwide. Removal of HM using phytoextraction followed by energy and/or HM recovery could be applied in the nearest future as an attracting closed cycle green technology.

This study aimed at gaining better understanding on heavy metal accumulation in different plant parts, estimating biomass yield and utilization possibilities focusing on phytoextraction using bioenergy crops.

2. Bioenergy plants for phytoextraction

The main aim of *soil remediation* is to reduce risks posed to humans and environment. A need for remediation is based on various legislative and normative documents as well as assessment of existing risks. Such parameters as price, long-term stability, technological efficiency, applicably, public acceptance, etc. has to be well thought through when selecting possible remediation technique.

Selection of the remediation method is influenced by contaminant’s speciation, concentration and distribution. Therefore, thorough characterization of the contaminated territory is always needed prior taking further steps. Depending on the remediation technology, contaminants can be immobilized in the soil or they can be mobilized and removed from soil. Thus, target establishment has a major impact on technology type.

Remediation techniques for the soil contaminated with HM can be categorized into three groups: 1) gentle *in situ* remediation; 2) rigorous tools that limit further contaminant’s dispersion *in situ* and 3) rigorous tools that remove contaminant *in situ* or *ex situ (Wuana and Okieimen, 2011)*. Gentle remediation is applied when it is desired to restore soil functions and improve its quality. While rigorous tools are necessary to prevent humans and environment from direct hazard, especially in the case of heavy point-source contamination. United States Environmental Protection Agency (USEPA) classifies remediation technologies into two large groups: 1) contamination source control and 2) isolating measures to detain contaminants and prevent them from dispersion. Another classification is based on different technological principles: isolation, stabilization, mobility and toxicity reduction, physical separation and extraction. In order to reduce costs and achieve maximum results, various combinations of latter technologies are used in practice *(USEPA, 2008)*. However, it is widely acknowledged that many of the traditional remediation methods can cause adverse secondary impacts to the environment, e.g., heavy machinery powered by diesel fuel, emit large amounts of greenhouse gases. Furthermore, clean soil is required to replenish excavated site in case when a contaminated soil is treated *ex situ*. For excavation and transporting of soil, heavy machinery is used once again *(USEPA, 2008)*.

Phytoremediation is as an environmental clean-up biotechnology, incorporating selection of HM accumulating plant species. It can be seen as an alternative to the above mentioned process-based methods. Basic model of phytoextraction is not complicated. Metal tolerant plants that have a capability to accumulate high levels of HM are seeded or transplanted into a contaminated soil and are cultivated using relevant agricultural procedures. Plants absorb compounds containing HM through root system and transport them to the aboveground green parts for accumulation. When desired biomass is reached, vegetation of plants is stopped by harvesting them as well as removing accumulated HM permanently *(Prasad et al., 2003)*.

Natural phytoextraction should be considered as a long-term technology for HM removal from vast contaminated territories. However, it can be applied only to the sites that contain low to moderate levels of pollution as vegetation cannot persist in highly contaminated media. Moreover, such sites should have suitable landscape for agricultural machinery and other equipment. Plants suitable for phytoextraction should ideally have the following characteristics: high growth rate, production of more aboveground biomass, widely distributed and highly branched root system, more accumulation of the target HM from soil, translocation of the accumulated HM from roots to shoots, tolerance to the toxic effects of the target HM, good adaptation to prevailing environmental and climatic conditions, resistance to pathogens and pests, easy cultivation and harvest, repulsion to herbivores to avoid food chain contamination *(Wuana and Okieimen, 2011)*. However, among all known plant families such ideal *hyperaccumulator* simply does not exist. Still there are reported about 400 species that can accumulate one HM or in very rare occasion several, and mainly they are slow growing and produce low biomass, thus metal accumulation is limited *(Lasat, 2000; Peer at al., 2004; Reeves et al., 2017)*.

Plant-based technologies are low-cost not only at establishment phase but also from a future maintenance perspective. Costs of growing plants are significantly lower in comparison to costs of physico-chemical technologies, like excavation (replacement), vitrification, soil washing, etc. Moreover, green biotechnologies such as phytoextraction always has a better public acceptance as it is less disruptive to ecosystems and therefore, it is easier to make ready a clean-up project.

The potential of a plant species for a phytoextraction is determined by two key factors: HM concentration in aboveground part and biomass yield. Comparatively less biomass is produced when growing HM hyperaccumulating plants on contaminated soil, but they tend to accumulate target HM to a greater extent. On the other hand, bioenergy plants grown on contaminated soil, accumulate target HM to a lesser extent, but produce more aboveground biomass, so that overall accumulation is comparable to that of hyperaccumulators due to the production of more biomass. Thus, hyperaccumulator will yield a HM-rich, low-volume biomass, which is economically easy to handle in case of both HM recovery and safe disposal. While use of non-accumulators will yield a HM-poor, large-volume biomass, which can be economically unfeasible for metal recovery and also costly for safe disposal *(Ali et al., 2013)*. So what are the advantages of growing bioenergy plants on contaminated soil?

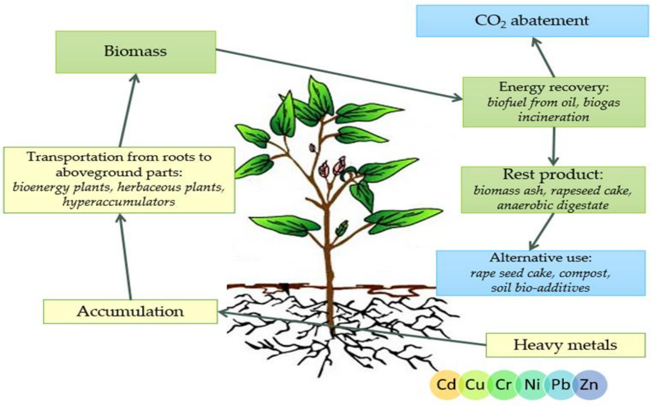

Fig. 1. *Conceptual model for phytoextraction technology using bioenergy plants*

Growing high biomass yielding energy crops on HM contaminated land could be rewarding for several reasons. Firstly, competition for a food-fuel land could be avoided, as contaminated soil is not suitable for growing conventional crops anyway *(Tilman et al., 2006)*. Secondly, polluted runoff, soil erosion and HM percolation to groundwater could be decreased due to the closed vegetation layer. All this leads to risk reduction, which is the main goal of phytoremediation and any other remediation technology. Such approach is well compatible with an alternative land use and, furthermore, generation of an additional income for land owner because of the produced energy. Figure 1 presents a conceptual model, how phytoextraction can be combined with energy recovery from high biomass yielding plants.

Harvested plant biomass can be transformed into energy through two main paths: thermo-chemical conversion including pyrolysis, gasification, and direct combustion; and bio-chemical conversion including fermentation and anaerobic digestion. Heavy metals after biomass combustion will be concentrated in slag, ash (bottom and fly ash), and dust particles. The quantity and quality of residual products is strongly dependent on the biomass properties as well as combustion technology. *Ministry of the Environment (2011)* has set rules for the management and use of ash from woody biomass. Maximum permissible values are established for HM in the ash to be used for agricultural and land reclamation purposes: 30 mg/kg dry weight (DW) for Ni and Cr, 5 mg/kg DW for Cd, 3 mg/kg DW for As, 50 mg/kg DW for Pb, 200 mg/kg DW for Cu, and 1500 mg/kg DW for Zn. Although, this document applies to ash from woody biomass, somewhat similar MPC values should serve for the ash from herbaceous plants as well

3. Materials and Methods

Historically HM-contaminated soil was used in the phytoextraction experiment. Composite sample of the technogenic soil was taken from three different spots at the Molainiai former septic drain fields. This contaminated territory is located in the northern Lithuania in the city of Panevėžys with a total area of about 96 ha. According to local drinking and wastewater management company JSC “Aukstaitijos vandenys”, sewage sludge containing various amounts of HM was pumped into the Molainiai septic drain field by the companies that did not have their own wastewater treatment plants. Ltd “Ekranas”, Ltd “Elektrotechnika” and state-run factories of compressors for motor vehicles and of precise electrotechnics involved HM compounds in processes like tin dipping and galvanization. Septic drain fields with the biological filtering purpose were installed and started its activity in 1961. Their exploitation was finished in 1978. Green recreational areas were designed to be built in this territory in the general city plan. According to previous investigations *(DGE Baltic Soil and Environment, 2010; Geotestus, 2013)*, the concentration of more than four HM exceeded the MPC values in the selected sampling places. Contaminated soil was excavated using a stainless-steel sampling shovel from 0-0.2 m depth and placed into plastic buckets, transported to the university greenhouse, mixed thoroughly, sieved to pass a 20-mm mesh screen, homogenized, and then used for the below-described vegetative phytoextraction experiment. Subsamples were taken from a thoroughly homogenized bulk soil to determine plant nutrients and HM content.

Uncontaminated soil (without known contamination) was taken from an agricultural field at a local experimental station of a former Aleksandras Stulginskis University and was used as a control for the comparison of further results. Soil samples were also taken from three different spots, mixed together, sieved to pass a 20-mm mesh screen, and subsamples from a thoroughly homogenized bulk soil were used for physico-chemical analysis as well.

Rapeseed *(Brassica napus)* Jerusalem artichokes *(Helianthus tuberosus)* were further selected as energy crops for HM phytoextraction experiment. Both contaminated and uncontaminated soils were separately divided into subsamples and placed into the plastic buckets of 26 l volume. One hundred seeds of summer rapeseed cultivar “Fenja” were seeded into each bucket. Pot-experiment was carried out under greenhouse conditions where temperature was kept at 25°±2° C and tap water used to maintain stable moisture content. The experiment was implemented in triplicates. Throughout the 13 weeks of experiment plants were thinned on three occasions: at the initial stem growth phase, at the bud formation phase and at the flowering phase until 21±3 plants in each bucket were left. Stem height and root length of uprooted plants were measured to compare the growth of rapeseed on HM-contaminated and uncontaminated soils. Pods with seeds were cut using scissors and shucked, stems with leaves were separated from the roots. Vegetative parameters of rapeseed: dry weight, stem height, root length, and weight of 100 seeds were recorded after harvesting the plants. At least three randomly selected plants from each bucket were measured for this purpose.

Jerusalem artichoke plants were also grown under controlled greenhouse conditions. Uncontaminated and contaminated soils were separately divided into subsamples averaging 21 kg each and placed into plastic buckets. Two seedlings of the Jerusalem artichoke cultivar “Sauliai” were planted in each bucket. During the repot seedlings were ~0.7 m height. The length of tubers together with smaller rootlets was 0.10-0.12 m. Each plant had 8 to 10 fully-formed leaves. Before the repot Jerusalem artichoke was grown in an open-air agricultural (without known contamination) field for four weeks. Tap water was used to keep moisture and the temperature in the greenhouse chamber was maintained automatically at 25±2º C. This vegetative experiment was also carried out in triplicates.

In order to assess the effects of potential HM toxicity throughout the 22-week pot experiment, selected vegetative characteristics of Jerusalem artichoke plants including stem height, number of leaves and blossoms were measured 5 times. When plants were reaped and tubers dug-out, wet weight was recorded as well. Separate parts of air-dry energy plants: stem with leaves, roots, pods, seeds, were grinded into fine powder using laboratory grinding mill and preserved for HM analysis.

Soil samples were dried at 40±5° C temperature in an oven, homogenized with mechanical ball-mill, sieved to pass 2 mm mesh. For total HM concentration, sub-samples were wet-digested with *aqua regia*. *Plant* parts were dried out after harvesting, milled with lab grinder and wet-digested with a mixture of HNO_3 and H_2O_2. Extraction from *ashes* after combustion experiment was carried out through the application of strong mineral acids. After cooling and diluting with deionised water, HM concentrations in all digestates were determined by ICP-OES. Acid digestions and other analytical procedures were always performed at least in triplicate. Calibration using standard solutions was performed before all instrumental analysis.

All HM phytoextraction/accumulation vegetative pot experiments were carried out during 2014-2016 at Aleksandras Stulginskis University mainly in the newly constructed greenhouse of the Faculty of Agronomy.

4. Results and Discussions

Vegetative pot experiments were used as the main tool to evaluate phytoextraction efficiency using energy crops. Two species of high biomass yielding plants, Jerusalem artichoke and rapeseed were cultivated on historically HM-contaminated soil; biomass was obtained and used for further combustion experiment. Ash qualitative parameters at the end of the experiment were compared with qualitative characteristics of ashes originated from biomass combustion plants in Lithuania.

In general, rapeseed plants from contaminated soil at the end of the vegetation produced more biomass, larger seeds as well as exposed higher resistance to pests in comparison to plants grown on uncontaminated soil (Fig. 2). Similar findings were obtained by *Marchiol et al.* (2004) and *Brunetti et al.* (2011) as both studies indicate that rapeseed exhibited diminutive symptoms of toxicity when grown on HM contaminated soil under greenhouse conditions. *Ghnaya et al*., (2009) tested the resistance of four rapeseed cultivars to Cd and Zn stress and concluded that the response depends both on cultivar and metal.

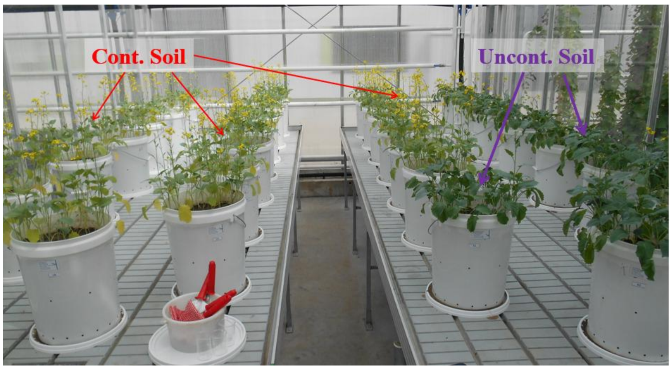

Fig. 2. *Rapeseed development on contaminated (taller, yellowish plants with first flowers) and uncontaminated (dark green plants without flowers) soil*

Despite of manifold higher than background Cd, Cu and Zn content in the soil, rapeseed development was similar to the control samples grown on uncontaminated soil. Rapeseed produced even heavier seeds when cultivated on contaminated soil. This is a very important characteristic for oil-bearing plants, such as rapeseed. Our experiment showed that rapeseed can be successfully cultivated on HM contaminated soil as an energy crop with moderate phytoextraction performance and subsequent liquid biofuel production from rapeseed oil – rapeseed not only matured high weight seeds, nor seeds did accumulate dangerous amounts of heavy metals.

Significantly increased Cd, Cu and Zn concentrations were detected both in aboveground and belowground biomass/plant parts of rapeseed grown on contaminated soil. In case when plants were grown on contaminated soil, Cd concentration in roots was higher by 62 times, in stem and leaves by 24 times, in pods and seeds by 10 times than in the same parts of rapeseed grown on uncontaminated soil. The highest Cd concentration in plants from contaminated soil was detected in the roots, while in stem and leaves of plants from uncontaminated soil. Concentration of Cu in the roots was higher by 27 times, in stem and leaves by 12 times, in pods by 4 and in seeds by 2 times in comparison to their counterparts from the uncontaminated soil. In both cases, the highest concentration was recorded in the roots. Differences between Zn accumulation in plants grown on contaminated and uncontaminated soil were the smallest among the three analyzed elements. Concentrations of Zn in roots and stem with leaves from plants grown on contaminated soil were higher by 7 times than in the corresponding parts of rapeseed plants from uncontaminated soil; while in pods and seeds accumulated Zn amount was almost the same.

The total biomass of Jerusalem artichoke grown on contaminated soil was again 1.6 times greater than the biomass of plants grown on uncontaminated soil. Plants grown on contaminated soil started to blossom one week earlier than those grown on uncontaminated soil. Leaf and stem color of Jerusalem artichoke from uncontaminated soil was dark green, whereas leaves of plants from contaminated soil were a lighter green color.

Values of heavy metal bioconcentration (BCF) and translocation (TF) factors for Jerusalem artichoke plants are presented in Table 1. A BCF is a ratio between element concentration in the tubers (roots) and element concentration in the soil. When the value is greater than unity, it may be considered as an indication of a plant’s potential to be a hyperaccumulator and thus to effectively uptake HMs from the soil. Translocation factor is a shoot-to-root ratio. Therefore, it can also be an indicator for the presence of hyperaccumulation, because non-hyperaccumulator plants tend to hold bigger concentrations of HM in the root zone rather than transfer them to aboveground parts *(Martinez-Sanchez et al., 2012)*. According to *Baker and Brooks* (1989), hyperaccumulators tend to accumulate >1000 mg/kg of Cu, Co, Cr, Ni, or Pb, and >10 000 mg/kg of Mn or Zn.

Table 1. *Heavy metal bioconcentration and translocation factors for Jerusalem artichoke*

Element	**Contaminated soil**		**Uncontaminated soil**	
	BCF	**TF**	**BCF**	**TF**
Cd	0.03	7.0	0.66	2.2
Cu	0.03	1.3	0.78	1.1
Zn	0.06	4.5	0.57	1.5

According to the obtained results, Jerusalem artichoke cannot be identified as a hyperaccumulator, as none of BCF values exceeded unity. Higher Cu and Zn transfer from roots to shoots coefficient can be explained by both Cu and Zn being essential micronutrients for plant metabolic processes *(Alloway, 2013)*. Translocation factor for Cd was the highest amongst the other elements.

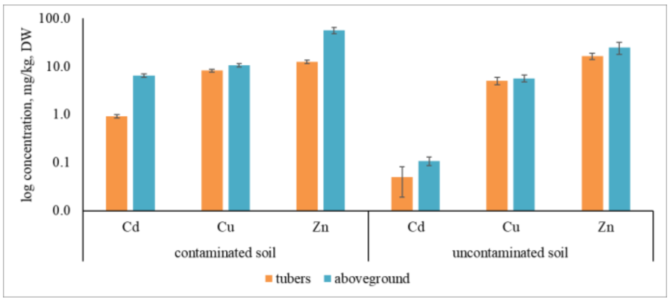

Fig. 3. *Heavy metal accumulation in Jerusalem artichoke tubers and aboveground biomass*

Results on HM accumulation in Jerusalem artichoke biomass are given in Fig. 3. As expected, plants grown on contaminated soil accumulated higher amounts of metals when compared to plants grown on uncontaminated soil.

Combustion experiment showed that straw ash from agricultural waste exhibited similar concentrations of Cu as ash of Jerusalem artichoke from uncontaminated soil; whereas Cd and Zn concentrations were significantly higher in straw ash but similar to those in Jerusalem artichoke ash from contaminated soil. Cadmium content was very similar in rapeseed from contaminated and uncontaminated soils, but Cu and Zn differed significantly.

5. Conclusions

Obtained results indicate that both tested bioenergy plant species: rapeseed and Jerusalem artichoke can be grown on low to moderately heavy metal-contaminated soil and give equally high yield as their counterparts from uncontaminated soil. Both species were identified as HM excluders, i.e., they have rather low potential for HM extraction but may be efficient for phytostabilization purposes, when plants are grown on marginal land to avoid soil erosion and percolation of contaminants to groundwater.

Jerusalem artichoke and rapeseed grown on heavy metal contaminated soil in most cases produced more aboveground biomass, heavier seeds and larger tubers, and plants were less sensitive to pest attacks in comparison to plants grown on uncontaminated soil. This proved that bioenergy plants can be successfully cultivated on moderately contaminated soil both for phytoextraction and for energy recovery purposes and mitigate competition for arable land.

Biomass produced on moderately contaminated soil does not necessarily mean that rest products of energy recovery will have high metal content. Ashes from Jerusalem artichoke biomass produced on contaminated soil exhibited similar heavy metal concentrations to the ones in ashes obtained from biomass power plants operating in Lithuania. None of the metals exceeded maximum permissible values according Lithuanian legislation and such ashes could be used as fertilizers both in forestry and agriculture.

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