ANNOTATION

of the dissertation work for a degree of Doctor of Philosophy (Ph.D)
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Restoration of the soil contaminated with organochlorine pesticides using the second-generation biofuel crops with following cascading use of biomass

General description of the research. This Ph.D. dissertation is devoted to studying the phytoremediation potential of promising energy plants concerning soil pollution of organic and inorganic nature and the development of approaches to improve phytoremediation and increase biomass production for the subsequent conversion into bioproducts.

Significance of the research. Environmental monitoring studies regularly reveal sites contaminated by various pollutants that restrict their use. Thus, the search for cost-effective and eco-friendly methods to clean the environmental matrices is a crucial direction worth to be researched. However, only a limited number of researches are focused on the ecosystems’ mixed contamination while in natural conditions, it is the most common environmental issue. Such mixed contaminations of the environment are an acute ecological problem of the Republic of Kazakhstan. It occurs in different regions due to the development of the various economic areas: oil & gas industry, metal mining and smelters, uranium mining, or the Baikonur cosmodrome operation. Despite the huge contribution of these activities to the national economy, their emissions create different hotspots with problematic mixed contaminations in soils needing management strategies to face such a situation and protect the local population. Moreover, the very intensive practices in agriculture and especially the disposal facilities and former warehouses of obsolete pesticides dating from to the 1960s were reported to endanger very seriously the environment in Kazakhstan [1,2], Ukraine [3], and Moldavia [4].

One of the necessary steps to prevent the toxic effects of pollutants on the environment and human health is the remediation of contaminated soils. Nowadays, there are two widely used areas of soil remediation: extraction of xenobiotics and physicochemical treatment. These technologies are extremely energy-intensive and require a large capital investment. Therefore, as an alternative to physical or chemical methods of soil remediation, phytoremediation is a highly promising technology harmoniously interacting with the ecosystem. This technology is based on the use of plants to remediate the soils contaminated by toxic trace elements (TTEs), hydrocarbons, pesticides, oil products, and also radionuclides. However, phytoremediation has limitations such as a long period of remediation, the necessity to utilize contaminated biomass, as well as the slow decomposition of organic xenobiotics in the soil.

Nowadays, the application of bioenergetic plants in the phytoremediation process is gaining popularity following the bioeconomy strategy focused on the search for alternative raw materials to be converted into energy products to achieve sustainable growth. Such plants have to easily grow with rapid production of biomass sufficient to evacuate notable amounts of pollutants from the soil. Undoubtedly, the nature of remediation would directly depend on the plant's ability to uptake and accumulate the concerned pollutant. In long term, the important property of bioenergetic plants that has to be taken into account is the high contents of lignin, cellulose, and lignocellulose that are responsible for the quality of the obtained biomass and effects their application.

The purpose of the research. The purpose of the research is to elucidate the physiological features of the organochlorine pesticides (OCPs) and TTEs effect on energy plant species for optimization of the phytoremediation technology and to develop the methods of conversion the biomass produced during the phytoremediation into the bioproduct.

The main tasks of the research to accomplish purpose are as following:

1. To identify second-generation energy plants suitable for application in phytoremediation of OCPs or TTEs contaminated soils producing the high yield of biomass and being able to accumulate and/or degrade of OCPs and TTEs.
2. To study the energy crops tolerance to the high concentrations of OCPs and TTEs in soil.
3. To optimize the energy crops growth, biomass production and phytoremediation potential incorporating to the OCPs or TTEs contaminated soils the organic and inorganic amendments.
4. To develop a method for utilization of contaminated energy crops biomass obtained as a result of the phytoremediation process.

The research objects and materials. Soils: a) historically contaminated with OCPs; b) artificially contaminated with TTEs. Second-generation energy crops: a) *Miscanthus sinensis* And.; b) *Miscanthus* × *giganteus* Greef et Deu.

Research methods. Physiological (visual observation of plants for the presence of stress indicators; measuring the biomass productivity), chemical (agrochemical profile of contaminated soil; Atomic absorption spectrometry with electrothermal atomization; Gas Chromatography with an Electron Capture Detector), biotechnological (clonal micropropagation), Adobe Illustrator image analysis & edition, and R statistical analysis software. The experimental data had a sufficient number of replicates and were statistically reliable.

The scientific novelty of the research. During the study, it was first discovered the ability of *Miscanthus sinensis* to tolerate the high concentrations of numerous OCPs including 15 POP pesticides in soils, and the potential of biochar production from contaminated rhizomes of *Miscanthus* × *giganteus* to achieve a zero-waste cycle approach in phytoremediation.

The theoretical and practical significance of the research. The dissertation contributes to fundamental aspects of the morphological and physiological parameters of second-generation energy crops' tolerance to organochlorine pesticides and toxic trace elements contamination in order to solve the theoretical concepts of plant adaptation patterns to the xenobiotics' impact.

Thus, the thesis has theoretical and practical significance.

The main provisions for the defense are as follows:

1. The soil around the former obsolete pesticide warehouse is contaminated with 23 OCPs including 15 POP pesticides (aldrin, chlordane, 2.4-DDD, 4.4-DDD, 4.4-DDE, 4.4-DDT, dicofol, dieldrin, endrin, endosulfan α and β, HCB, α-, β-, and γ-HCH, and heptachlor) in concentrations exceeding the MPC up to 1000 times that pose a threat to the environment and human health.

2. *M. sinensis* can tolerate soil heavy pollution (145 mg kg^{-1}) by numerous OCPs, unlike *M.* × *giganteus*. The plant tolerance index of 0.99 confirms the above statement. The plants' height and aboveground biomass dry weight decreased by 5% and 23% while the length and dry weight of roots increased by 16% and 11%, respectively. It was found that OCPs influence the content of chlorophyll pigments: Chl_a decreased by 30%, Chl_a – by 37%, and carotenoids – by 29%.

3. *M. sinensis* can uptake OCPs from contaminated soil and hyperaccumulate dicofol, chlordane, dieldrin, endosulfan sulfate, β-HCH and HCB with BCFs of 66.8, 35.9, 21.1, 21.7, 6.6 and 28.7. The energy crop has the potential to bioconcentrate 4.4-DDE, 4.4-DDT, methoxychlor, *γ*-HCH, aldrin, heptachlor, endosulfan β, endrin aldehyde and hexabromobenzene with BCFs being higher than 1 and equal to 2.1, 1.5, 3.9, 1.1, 1.6, 2.7, 2.6, 1.2 and 2.1, respectively, to phytostabilize 4.4-DDE, dicofol and chlordane with TLFs of 0.49, 0.07 and 0.47, respectively, and to phytoextract β-HCH, *γ*-HCH and heptachlor with TLFs of 4.04, 84 and 2.01, respectively. 4.4-DDT, methoxychlor, aldrin, dieldrin, endosulfan β, endosulfan sulfate and endrin aldehyde are equally distributed within the plant. The correlation analysis confirmed that the uptake of OCPs from the soil depends on their hydrophobicity: the higher the hydrophobicity, the lower the accumulation in plant tissues. It was found that the *M. sinensis* growing on the contaminated soil collected near the former pesticide warehouse developed a physiological resistance mechanism during adaptation to OCPs, that is OCPs accumulation and translocation in “soil – root – aboveground biomass” system using phytostabilization and phytoextraction mechanisms. *M. sinensis* can be used to remediate the OCPs-contaminated soils.

4. Optimizing the conditions of *M. sinensis* cultivation in OCPs-contaminated soil revealed that incorporation of Tween 20 enhances the plant height and roots length by 16.6% and 20.8%, respectively; increases the OCPs accumulation and provides a phytostabilizing effect concerning aldrin, chlordane, dieldrin, endosulfan β, endosulfan sulfate, endrin aldehyde, heptachlor, hexabromobenzene, methoxychlor, 4.4-DDT, and 4.4-DDE increasing their uptake and decreasing translocation to AGB by 2.2 and 10.4, 1.4 and 6.0, 2.2 and 11.3, 1.8 and 6.1, 2.5 and 8.2, 2.3 and 10.1, 1.3 and 8.5, 1.3 and 16.0, 1.2 and 13.6, 2.1 and 17.5, 1.6 and 3.9 times, respectively; reduces the accumulation of HCB, β-HCH and γ-HCH by 2.7, 1.3 and 23.1 times, respectively; ensures the phytoextraction of dicofol increasing its migration by 2.2 times: the use of Tween 20 increases the efficiency of OCPs uptake and phytostabilization.

Amending OCPs-contaminated soil by AC revealed that it decreases the uptake of OCPs, particularly, the accumulation of aldrin, chlordane, dieldrin, endosulfan β, endosulfan sulfate, endrin aldehyde, HCB, heptachlor, hexabromobenzene, methoxychlor, β-HCH, γ-HCH, and 4.4-DDT decreased by 1.6, 3.0, 1.6, 1.1, 1.4, 1.5, 3.0, 1.4, 2.0, 1.5, 2.4, 23.1, and 1.4 times, respectively; and enhances the phytostabilization of all OCPs in the range of 1.9 to 115 times, except for chlordane its translocation to AGB increased by 1.8 times.

5. *M.* × *giganteus* can tolerate (TI up to 2.0) high concentrations of V (by 11.7 times higher than MPC), Sr (59.8 × MPC), Cr (2.7 × MPC), Ni (2.1 × MPC), and especially Pb (4.6 × MPC and 33.9 × MPC), which was the most bioavailable due to spiked soil pollution, consequently, the absence of soil aging. Energy crop able to accumulate TTEs from contaminated soil: under the multiple TTEs contamination condition, *M.* × *giganteus* can bioconcentrate and phytoextract Mn with BCFs for AGB and roots of 1.2-1.6 and 0.5-0.7, respectively; with increasing Pb concentrations in soil, Mn accumulation in AGB and roots reduced by 25.6% and 31.8%, respectively. In relation to four remained TTEs, namely, Cu, Zn, Sr, and Pb, *M.* × *giganteus* has no potential to bioconcentrate them (BCFs are less than 1, even 0.1 except Zn) under the multiple TTEs contamination conditions. Moreover, *M.* × *giganteus* can not uptake and accumulate V, Cr, and Ni in soil contaminated with multiple TTEs.

6. Optimizing the conditions of *M.* × *giganteus* cultivation in TTEs-contaminated soil revealed that inoculation of rhizomes by PGPB *B. altitudinis* KP-14 improves the physiological parameters such as height and dry weight of leaves, stems, and roots by 28.2%, 49.1%, 85.9%, and 76.0%, respectively; provides a phytostabilizing effect concerning Cu, Sr, and Pb increasing their uptake by 30.6%, 30.3%, and 39.7%, respectively.

7. Contaminated biomass of *M.* × *giganteus* can be used for biochar production in order to reach a zero-waste approach in phytoremediation. The average yield of biochar produced either from Miscanthus straw (aboveground biomass) or rhizomes is 31.2%. Biochar has good thermochemical and physicochemical properties to be applied in the process of improving soil quality and phytoremediation: contaminated biomass can be converted to biochar for assisting the phytoremediation.

The levels of research organization. The research described in this dissertation was performed on tissue, organ, organism, and ecosystem levels.

Relationship of the research with the scientific project. This Ph.D. research on examining different energy plants for the potential to remediate soils contaminated by xenobiotics of organic and inorganic origin was supported by the program BR05236379 "Comprehensive assessment of the impact of unutilized and prohibited pesticides on the genetic status and health of the Almaty region population" provided by Ministry of Education and Science of the Republic of Kazakhstan, and partly performed at the Institute of Genetics and Physiology, MES RK. The part of the research work focused on studying the conversion of the contaminated biomass to the bioproduct was supported by CORNET German-Czech project “MiscanValue” and performed at the Faculty of the Environment, Jan Evangelista Purkyně University in Usti nad Labem, the Czech Republic.

The contribution of the author for the results described in this dissertation. All the main results described here are performed and collected by the author. In addition, main research results, analyses, tables, data, and figures are generated by the author, and all the new observations and conclusions are made from the results derived from the Ph.D. candidate’s work and research.

Research approbation. The main results and observations are presented and confirmed by the publication in prestigious international scientific journals included in the Scopus database, Web of Science, journals recommended by the CCES, and in three chapters of the “Phytotechnology with Biomass Production: Sustainable Management of Contaminated Sites” Taylor & Francis. The results were discussed at the Institute scientific seminars, and reported at international and republican scientific conferences:

- at an international scientific conference for students and young scientists “Farabi Alemi” (2019, Almaty, Kazakhstan);
- at the international scientific conference for students and young scientists “Farabi Alemi” (2020, Almaty, Kazakhstan).

Publications. The majority of this dissertation content was published in 17 scientific works, including 7 research articles with impact factors (IF = 5.65; IF = 5.26; IF= 4.07; IF = 3.06 (2); IF = 2.60; IF = 1.697) according to the *SCOPUS* database, 5 articles in scientific journals recommended by Education and Science Monitoring Committee of the Ministry of Education and Science of the Republic of Kazakhstan (CCESF MES RK), 3 abstracts in the materials of international conferences, 3 chapters in the book “Phytotechnology with Biomass Production: Sustainable Management of Contaminated Sites” of the Taylor & Francis publisher, 1 chapter in “Key Questions on Climate Change and Sustainability. Toward the Make-or-Break Years” of the Printeko publisher, 1 brochure, and 1 cadaster.

Dissertation structure. This dissertation is written in 96 pages, and contains notations and abbreviations, introduction, literature review, materials and methods, results and discussions, conclusions, and references from 231 sources, contains 14 tables, and 20 figures.