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Dissertation on the topic:

"Development of Biotechnology for obtaining a soil amendment based on lignite to increase the yield of potatoes"

Submitted for the Degree of Doctor of Philosophy (Ph.D.)
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"8D05105 - Biotechnology"

ANNOTATION

General description of the work. This scientific study is devoted to the development of an organo-mineral soil additive that is important for agriculture and the assessment of its potential use for stimulating growth and increasing potato yields. The study examines the physicochemical properties of a composite mixture based on fly ash and humic acid, as well as its effect in combination with rhizobacteria on soil mechanical structure, chemical parameters, and microbiological activity. The study established the effectiveness of the composite in improving soil fertility and assessed its effect on the growth, development, and quality indicators of potatoes. The results obtained can be used in the development and implementation of organo-mineral soil additives aimed at optimizing soil quality and increasing agricultural productivity.

Relevance of the Topic: Currently, the deterioration of the ecological condition of soils worldwide and the growing demand for agricultural products require the search for new, effective, and environmentally safe methods of restoring soil fertility. For many regions of Kazakhstan, problems such as soil erosion, a decrease in organic matter content, and a deterioration in their physical and chemical properties are particularly relevant.

Kazakhstan's coal industry is highly developed, with enterprises producing significant volumes of fly ash and coal waste every year. Most of this waste is currently used inefficiently, which leads to environmental problems. At the same time, the processing of such industrial waste for use in agriculture as soil additives has significant potential. Fly ash and humic acids extracted from coal can improve the physical and chemical properties of soils, increase their fertility, have a beneficial effect on plant growth and development, and enhance the activity of soil microbiota. Fly ash contains elements necessary for plants, such as potassium, calcium, magnesium, and phosphorus. Ash helps reduce soil acidity, improves soil structure, and stimulates microbiological activity.

Humic acids are a group of high-molecular organic compounds (65–70%) formed as a result of the decomposition of plant and animal residues. They improve soil structure, regulate nutrient exchange, increase the buffering properties of soils, reduce the toxicity of heavy metals, and reduce the intake of harmful substances by plants. The humic acid content in brown coal can range from 30 to 80%.

The combined use of coal fly ash and humic acids can be an effective, environmentally friendly, and affordable source of soil additives for agriculture. The use of this technology is particularly promising in the cultivation of potatoes, which are one of the most important agricultural crops in Kazakhstan's food market.

Rhizobacteria are a group of beneficial microorganisms that live in the rhizosphere of plants, promoting their growth, fixing nitrogen, converting phosphorus and potassium into forms accessible to plants, suppressing the development of phytopathogenic microorganisms, and increasing plant resistance to stressful conditions. A complex based on fly ash, humic acids, and rhizobacteria helps restore the soil ecosystem and activate the microbiocenosis.

The use of soil additives based on fly ash, humic acids, and rhizobacteria to increase potato yield and quality, as well as to optimize the biological and physicochemical properties of the soil, is a pressing issue for agricultural science and practice. This study aims to solve one of the environmental problems of the coal mining industry in Kazakhstan and to develop effective methods for restoring soil fertility.

Purpose of research: To develop a composite soil additive based on coal fly ash, humic acid, and rhizobacteria, as well as to investigate its effect on the physicochemical and biological properties of soil, as well as on the growth and yield of potatoes.

Research objectives:

1. Prepare and characterize the starting materials, including coal fly ash and humic acid isolated from brown coal.
2. Isolate and identify rhizobacteria, determine their nitrogen-fixing and phosphorus-solubilizing activity.
3. Obtain a composite soil additive based on coal fly ash and humic acid and study its physicochemical properties, including elemental composition, X-ray structural analysis, scanning electron microscopy, and study its sorption properties.
4. Study changes in the physicochemical and microbiological properties of soil under the influence of the composite additive and rhizobacteria, including nutrient content, enzymatic activity, and microorganism abundance.
5. Evaluate the effect of the composite additive and rhizobacteria on potato growth and yield.

Research objects. Fly ash samples were obtained from a coal-fired power plant in Aksu (Pavlodar region, Kazakhstan, coordinates: 52°07'04.9“N 76°52'30.7”E).

Humic acid was isolated from brown coal mined in open pits in the Ekibastuz basin (coordinates: 51°41'08.0“N 75°27'26.0”E).

Rhizobacteria cultures were isolated from the roots of plants growing on coal-contaminated sites in the Ekibastuz area.

Research methods. Both traditional and modern microbiological methods were used in the study. In particular, molecular genetic identification using Sanger sequencing of the 16S rRNA gene was used to identify rhizobacterial strains. The properties of fly ash from coal, brown coal, and soil composite additives were studied using scanning electron microscopy (SEM), thermogravimetric and differential scanning calorimetric analysis, X-ray fluorescence analysis, X-ray diffraction, the Brunauer-Emmett-Teller method, elemental analysis, and Brunauer porosimetric analysis. The physical (moisture, density, water permeability, porosity) and chemical (pH, nitrogen, phosphorus, and potassium content) characteristics of

soil samples were determined along with enzymatic activity. For potatoes, the following biochemical indicators were determined: starch, vitamin C, reducing and total sugars, nitrate content, as well as protein content and moisture.

Scientific novelty. For the first time, a composite additive based on coal fly ash, humic acid, and rhizobacteria was developed, and a comprehensive study of its complex effect on soil and potatoes was conducted. The positive effect of the composite components on the physicochemical properties of the soil and the biological activity of its microbiota has been scientifically proven. The proposed comprehensive approach can become an important tool for increasing soil fertility and crop yields. The study lays the foundation for the development of environmentally safe and effective technologies for processing coal waste and forming a sustainable scientific and practical base.

Theoretical and practical significance. The study scientifically substantiates the role of a composite additive based on fly ash, humic acid, and rhizobacteria in improving the agrochemical properties of soil. The data obtained expand theoretical understanding of the mechanisms of action of organo-mineral soil additives and are important for the development of biotechnologies in agriculture and for taking into account the ecological and economic aspects of soil use. From a practical point of view, the possibilities of using coal waste to create environmentally safe, inexpensive, and effective soil additives have been identified.

Main points to be defended:

1. The study shows that fly ash has a high capacity to interact with humic acid, which is accompanied by an increase in its adsorption activity at neutral pH. At the same time, humic acid adsorbed on fly ash is capable of gradual release (desorption), which should be taken into account when developing biofunctional soil additives.

2. In smart agriculture, along with effective organo-mineral additives enriched with macro- and microelements, methods of plant growth biostimulation based on the use of sources of biologically active compounds are necessary. Rhizobacteria are known as bioinoculants and are an important component of the soil microbiome. The use of rhizobacteria in the creation of composites based on coal fly ash and humic acid contributes to the preservation and improvement of soil fertility. As part of this study, two strains of rhizobacteria were isolated and used: Pseudomonas koreensis and Achromobacter sp., capable of nitrogen fixation and phosphate mobilization, which enhances the biological activity of soils.

3. Coal fly ash obtained from the combustion of coal from the Ekibastuz coal deposit (Aksu Thermal Power Plant, Pavlodar Region) and humic acid isolated from the same coal were used as raw materials. The conversion of ash and organic components into a soil additive provides an ecological effect (reducing the risk of environmental pollution) and agronomic value, promoting plant growth.

Connection with scientific research programs. The work was carried out within the framework of projects funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (AP13268305) and the Ministry of Agriculture of the Republic of Kazakhstan (BR10764907).

Personal contribution of the dissertation author. All stages of the work—creation of the composite, determination of its properties, analysis of soil and

microbiota, monitoring of potato growth, processing of results, and development of conclusions—were performed by the author independently. The methods and reliability of the data obtained have been comprehensively analyzed and scientifically substantiated.

Approval of the work. Individual sections of the work were discussed at scientific seminars of the department. Comments and suggestions were taken into account when finalizing the dissertation research.

Publications:

The main results of the work were published in 5 scientific papers, 3 of which were included in the authoritative international database Scopus: Biology (71%, SJR 0.815), ES Materials & Manufacturing (98%, SJR 0.621), International Journal of Design & Nature and Ecodynamics (55%, SJR 0.230). Two scientific theses were published in conference proceedings.

Scope and structure of the dissertation. The work is presented on 115 pages and contains 26 figures and 23 tables. The dissertation consists of an introduction, a literature review, a description of the research objects and methods, results, conclusions, and appendices. The list of references includes 320 sources.

Conclusions:

1. As a result of the physicochemical characterization of the source materials, it was established that coal fly ash (CFA from the Aksu Thermal Power Plant) contains the following main components: SiO2 – 59.74% and Al2O3 – 27.44%, and its particles have the following dimensions: d10 – 4.3 μm, d50 – 31.5 μm, and d90 – 86.0 μm. Humic acid isolated from brown coal contains 75.1% humic substances and is characterized by the presence of functional groups (C=O, C-O, COOH, etc.), confirmed by IR spectroscopy. The established properties confirm the suitability of the materials for creating soil composites.

2. Rhizobacteria were isolated and identified from the rhizosphere of plants growing in coal-polluted areas of the Ekibastuz deposit (Pavlodar region, Republic of Kazakhstan): – Pseudomonas koreensis Pas1 and Achromobacter sp. Jps2. It was determined that they have nitrogen-fixing and phosphorus-solubilizing activity, which allows them to be used as biofunctional components in soil additives.

3. A composite soil additive based on coal fly ash and humic acid was obtained. A comprehensive characterization of the physicochemical properties was carried out: elemental composition, X-ray structural analysis, scanning electron microscopy. A study of sorption properties using Freundlich isotherms showed a high affinity for humic acids, confirming its effectiveness as a sorption and structural component for soil improvement.

4. The joint application of the K-G composite (fly ash + humic acid, 1 g/kg) as a dry additive and rhizobacteria as a suspension (1×108 CFU/kg, 1:50 w/w) contributed to the improvement of the physicochemical properties of the soil. Moisture retention capacity increased from 45.3% to 54.2%, water permeability from 1.93×10-5 cm/s-1 to 3.35×10-5 cm/s-1, and P_2O_5 content increased by 10% and K_2O by 23% compared to the control. Enzymatic activity for dehydrogenase increased from 16.2 to 28.1 μg TPF g-1 · day-1, and for polyphenoloxidase from 32.3 to 38.9 mg purpurgallin g-1 · 2 h -1

5. The use of composite and rhizobacteria had a positive effect on potato growth and yield. Depending on the application method, plant height increased by an average of 18.35%. The use of composite increased the total tuber weight by an average of 8.16%. The use of coal soil improvers also contributed to the improvement of tuber quality indicators: the starch content increased from 16.55±0.01% (in the control) to 16.65%±0.01%, and the vitamin C content increased from 13.750.01% to 16.250.01%. Soil treatment contributed to the activation of the morphophysiological processes of potatoes, which led to an improvement in phenological indicators and, as a result, to an increase in yield.