

THE EFFECT OF *PSEUDOMONAS* BACTERIA ON THE ANTIOXIDANT SYSTEM OF PLANTS UNDER CONDITIONS OF HEAVY METAL CONTAMINATION

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Annotation. This study investigated changes in the antioxidant system of *Phaseolus vulgaris* plants grown in a medium contaminated with heavy metals (Cu, Cd, Zn) and *Pseudomonas spp.*, with the aim of evaluating the bioremediation effect of the bacterial consortium. The results showed that in the control group, antioxidant activity was 25% in the roots, 24% in the leaves, and 27% in the stems. Upon exposure to copper (Cu 0.01), these values increased significantly to 59%, 56%, and 57%, respectively, indicating activation of the antioxidant system in response to severe oxidative stress. When the copper concentration was reduced (Cu 0.001), antioxidant activity decreased to 14% in roots, 11% in leaves, and 18% in stems. In the case of cadmium (Cd 0.01), the values were 19%, 15%, and 18%, whereas in the Cd 0.001 treatment, an increase to 25% in leaves and 31% in stems was observed. Antioxidant activity decreased under zinc (Zn 0.01) exposure and amounted to 11% in roots, 4% in leaves, and 14% in stems, while the lowest values were recorded at Zn 0.001 (3%; 1.73%; 8%). The use of the *Pseudomonas spp.* consortium increased plant resistance to heavy metals and contributed to the stabilization of the antioxidant system. Overall, the results demonstrate that these bacteria can be used as an effective bioremediation agent by biosorbing heavy metal ions and reducing oxidative stress in plants.

Keywords: Heavy metals, *Pseudomonas spp.*, antioxidant activity, bioremediation, *Phaseolus vulgaris*, stress responses.

Introduction. Heavy metal pollution is one of the most pressing issues affecting modern ecosystems. Due to the extensive use of chemicals in agriculture and industrial activities, heavy metals such as lead (Pb), cadmium (Cd), copper (Cu), and zinc (Zn) accumulate in soils at elevated concentrations. These metals are non-biodegradable and persist in the soil for long periods, posing significant risks to plant systems. Heavy metal toxicity disrupts key physiological and biochemical processes in plants, leading to impaired growth and development.

Heavy metals are a group of persistent inorganic chemical compounds with an atomic mass greater than 20 and a density greater than 5 g/cm³, which are non-biodegradable. They have cytotoxic, genotoxic and mutagenic effects on humans, animals and plants, and also affect food chains, soil, irrigation and drinking water, groundwater and the atmosphere by contaminating them. Metals found in soil are divided into two groups: trace elements

necessary for normal plant growth (Fe, Mn, Zn, Cu, Mg, Mo and Ni) and non-essential elements whose biological or physiological functions are not fully understood (Cd, Sb, Cr, Pb, As, Co, Ag, Se and Hg). Plants can absorb heavy metals through their above-ground and underground parts. Essential elements play a crucial role in the structure of enzymes and proteins. Plants require small amounts of these elements for growth, metabolism, and development. However, the concentration of essential and non-essential metals is one of the key factors in plant growth, as an excess of these metals can lead to slowed or stunted growth. Toxic concentrations of heavy metals disrupt normal plant functioning and affect metabolic processes in various ways. Such effects include the destruction or displacement of structural protein components (resulting from the binding of heavy metals to sulfhydryl groups), inhibition of functional groups of important cellular molecules, displacement or disruption of the function of essential metals in biomolecules such as pigments and enzymes, as well as adverse effects on the integrity of the cytoplasmic membrane. As a result, vital processes such as photosynthesis, respiration, and enzymatic activity are inhibited. In addition, high levels of heavy metals increase the formation of reactive oxygen species (ROS). These include the superoxide radical (O_2^-), the hydroxyl radical (OH \cdot), as well as non-radical compounds—singlet oxygen (O_2) and hydrogen peroxide (H_2O_2)—and cytotoxic compounds such as methylglyoxal (MG). These compounds disrupt the balance between pro-oxidants and antioxidants in plant cells, causing oxidative stress [1].

The results of various studies indicate that the conclusions regarding the effects of heavy metals on plants are well-founded. Sharma and his colleagues found that heavy metals disrupt the processes of photosynthesis, gas exchange, and nutrient uptake, thereby reducing plant growth and dry biomass accumulation [2]. A meta-analysis conducted by Antonio Rodrigues da Cunha Neto and his colleagues studied the effects of aluminum and lead, showing that they reduce photosynthetic rates and chlorophyll b levels in various plant species [3]. Hui-hui Zhang and colleagues [4] demonstrated that cadmium stress inhibits the activity of enzymes responsible for chlorophyll synthesis in tobacco leaves, while Salla Hemadri Reddy and colleagues [5] confirmed that chlorophyll content decreases as heavy metal concentrations increase (0–250 ppm).

From a mechanistic perspective, Riyazuddin and his colleagues found that heavy metals accelerate chlorophyll degradation by promoting the formation of reactive oxygen species (ROS), increasing the activity of the enzyme chlorophyllase, and displacing the central magnesium (Mg) ion in the chlorophyll molecule [6]. R. Sayed and his colleagues described the formation of reactive oxygen species, such as the superoxide radical (O_2^-), the hydroxyl radical (OH \cdot), and hydrogen peroxide (H_2O_2), and demonstrated that these lead to oxidative stress and cellular damage [7]. In addition, A. Shomali and colleagues confirmed that heavy metal stress disrupts pigment biosynthesis and the activity of the photosynthetic electron transport chain [8]. Heavy metals—both those with redox activity (Fe, Cu) and those without (Cd, Pb, Zn)—increase the formation of reactive oxygen species (superoxide radicals, hydroxyl radicals, H_2O_2) in plant cells. This leads to lipid peroxidation, protein oxidation, DNA damage, and disruption of membrane integrity [9,10]. While redox-competent metals generate ROS directly through the Fenton and Haber-Weiss reactions, redox-incompetent metals such as Cd and Pb do so indirectly by inhibiting the activity of antioxidant enzymes, displacing necessary cations, or stimulating NADPH oxidases [11]. Plants respond to this stress with a two-way antioxidant defense mechanism: enzyme activity increases at low and moderate metal concentrations, whereas at higher levels the system becomes overloaded, and activity decreases. A study on wheat showed that SOD activity reached 148% of the control level at 75 μ M Cd and decreased by 72% at 300 μ M. CAT activity reached 131% at 150 μ M Pb and decreased by 68% at 500 μ M. Malondialdehyde, a marker of lipid peroxidation,

increased in a dose-dependent manner, confirming the progression of oxidative damage beyond the capacity of the defense system [12].

The stress response varies depending on the metal, the plant organ, and the species. In wheat seedlings exposed to cadmium, SOD activity was not observed in the roots at a cadmium concentration of 50 μM , whereas POX activity in the stems increased ninefold, indicating that each organ utilizes different enzymes [13]. Although a 50 μM concentration of Cd in corn increased the activity of CAT, POD, APX, and SOD, the rise in ROS marker levels indicated an insufficient antioxidant response [14]. In *Atriplex* species exposed to Cu, Ni, Pb, and Zn, SOD and APH activity decreased, while CAT and GR activity increased, indicating the existence of functional compensatory mechanisms among the enzymes [15]. In *Arabidopsis*, POD activity decreased during the first 2 hours of heavy metal exposure but increased after 12 hours, suggesting that POD and CAT may partially substitute for one another at different stages of stress [16].

Non-enzymatic antioxidants—glutathione (GSH) and ascorbate (AsA)—play a key role by acting as direct scavengers of reactive oxygen species and serving as substrates for the AsA-GSH cycle [17]. Glutathione (GSH) is also a precursor to phytochelatins (PCs), which regulate heavy metal concentrations in vacuoles by chelating them, playing a dual role in neutralizing reactive oxygen species and binding metals [18]. A study on durum wheat varieties showed that cadmium exposure increases the activity of the ascorbic acid-glutathione cycle enzymes in a dose-dependent manner not only in the roots (where cadmium accumulates) but also in the leaves, suggesting the presence of a systemic signaling mechanism [19]. In addition to GSH and AsA, α -tocopherol, carotenoids, phenols, and flavonoids also contribute to this protective effect: tocopherol protects membranes from lipid peroxidation, while carotenoids neutralize singlet oxygen [20]. Studies have shown that resistance to metals depends on the ability to activate both branches of the antioxidant system at high levels. To enhance crop tolerance to metals and optimize phytoremediation, transgenic and biotechnological approaches aimed at enhancing GSH synthesis or increasing the expression of antioxidant enzymes are being studied [21]. Bioremediation methods utilize various microorganisms, such as bacteria and fungi, to degrade or remove toxins from the environment through their metabolic processes. Although the role of bacterial communities in agricultural management has not been fully studied, microbial remediation technologies offer clear advantages. Plant-growth-promoting rhizosphere bacteria (PGPR), which primarily promote plant growth, have emerged as a promising platform for enhancing productivity, improving nutrient bioavailability, suppressing diseases, and improving soil health. PGPR represent a broad area of research within the field of microbial remediation technologies. Several researchers have reported that PGPR can absorb heavy metals, remove contaminants from the soil, or convert them into less toxic forms. These microorganisms have developed several mechanisms for surviving in soils contaminated with heavy metals and restoring the environment.

The success of heavy metal bioremediation depends on the potential of microorganisms. Plant-growth-promoting rhizobacteria are capable of surviving in soils contaminated with heavy metals; therefore, the use of these bacteria supports bioremediation. These bacteria are rhizosphere-associated and play a key role in the interaction between soil and roots, remediating contaminated soils through various mechanisms. PGPR (plant-growth-promoting bacteria) strains are beneficial microorganisms that influence the bioavailability of heavy metals. By secreting various metabolic compounds, PGPR play an important role in ensuring the removal and dissolution of heavy metals. Several studies have shown that rhizobacterial strains increase biomass and possess high potential for resistance to heavy metals [22].

In his research, Ruiz-Hernández demonstrated that the beneficial effects of introducing *Pseudomonas* bacteria through bioincubation stem from their ability to adapt to hazardous

environments contaminated with heavy metals, herbicides, or fertilizers. Many *Pseudomonas* strains are capable of degrading hydrocarbons, nitrogenous compounds, and herbicides, which helps reduce plant growth time. For example, *P. protegens* can degrade 80% of glyphosate, glufosinate, and phosphine regardless of the presence or absence of iron atoms. One of the key properties of *Pseudomonas* bacteria is their ability to dissolve or break down various pollutants, including hydrocarbons, nitrogenous compounds, and herbicides. This property not only neutralizes environmental toxins but also significantly reduces the time required for optimal plant growth [23].

Accordingly, the aim of our study is to comprehensively investigate the effect of *Pseudomonas* bacteria on the antioxidant defense system, biochemical indicators, and physiological parameters of plants in soils contaminated with heavy metals (Cu, Zn, Cd), as well as to determine the mechanisms of their adaptation and the effectiveness of bioremediation under conditions of heavy metal-induced stress.

Materials and Methods. The study design involved assessing changes in biometric parameters, pigment content, and enzymatic and antioxidant activity in bean plants under laboratory conditions following exposure to cadmium, copper, and zinc salts. The bioremediation efficiency of *Pseudomonas* strains was also determined, including their ability to biosorb and immobilize heavy metal ions, as well as to reduce stress responses in plants. The experiment used bean seeds, 300±20 g of pre-sifted soil dried at 105°C for 24 hours, and salts of Cd(NO₃)₂·4H₂O, Cu(OH)₂ and ZnSO₄·7H₂O at molar concentrations of 0.01 and 0.001 as sources of heavy metals, distilled water, and strains of *Pseudomonas putida* 1SL and *P. fluorescens* 4S (as a consortium). The bean seeds were grown in plastic containers with a capacity of 0.3–0.5 liters. Work with bacterial cultures was conducted in full compliance with biosafety regulations. The study lasted between 7 and 21 days.

Method of biometric analysis of plants. In this biometric analysis, common bean plants (*Phaseolus vulgaris*) are grown under conditions of heavy metal exposure (cadmium Cd(NO₃)₂, copper CuSO₄·5H₂O, zinc ZnSO₄·7H₂O), and the effect of these metals on plant growth is assessed. Pre-moistened soil is treated with 0.01 M and 0.001 M solutions of a specified amount of metal salts (e.g., 20 mL per 280 g of soil), while the control is left without metals. Disinfected seeds (1% KMnO₄, 5 min) are sown, seedlings are thinned to one plant per pot, and grown for 3–4 weeks under identical conditions of light, temperature, and humidity. The study results showed that the biometric parameters of each plant were measured after removing one leaf, the stem, and the root. Plant height and root length (cm) were measured, as well as leaf area (cm²). The results of the analyses were calculated as the mean value and standard error based on at least 3–5 replicates for each treatment, and growth inhibition was determined using the formula $I = (L_k - L_o)/L_k \times 100$, (where L is the length of plant organs, k is the control, and o is the effect of heavy metals). To assess the bioremediation effect, a *Pseudomonas* suspension was applied and analyzed in comparison with the control.

Determination of the pigment composition of plants. An ethanol extract of pigments was prepared from bean leaves, followed by centrifugation and adjustment of the extract volume to a fixed value. Pigment content was determined spectrophotometrically using a “Fotometer-KFK-3” instrument. The concentrations of chlorophyll a, chlorophyll b, and their total content were calculated using Vernon’s formulas. The carotenoid content was determined using the Holm–Wettstein formula. Pigment concentrations in plant material (mg/g) were calculated using a general formula, taking into account the extract volume and sample mass [24].

A method for determining peroxidase activity in plants. Under laboratory conditions, the effect of heavy metals on peroxidase activity in plants was assessed using the method developed by A.N. Boyarkin. The enzyme was extracted from plant tissues using an acetate

buffer solution (pH 4.7), followed by centrifugation. Peroxidase activity was determined spectrophotometrically using a “Fotometr-KFK-3” instrument. Enzyme activity was calculated using Boyarkin’s formula, taking into account the optical density of the solution, the volume of the extract, the dilution factor, the sample mass, the cuvette thickness, and the reaction time [25].

Method for determining the antioxidant activity of plants. To prepare the samples for analysis, the plant specimens were ground into a powder, after which a 1.0000 g sample was taken and 50 mL of double-distilled water heated to 100 °C was added. The mixture was allowed to stand for 5 minutes, then filtered. The antioxidant activity of the plant under study was assessed based on its ability to inhibit the auto-oxidation of epinephrine and thereby prevent the formation of reactive oxygen species. To do this, 0.2 mL of a 0.1% (5.46 mM) pharmaceutical solution of epinephrine hydrochloride was added to 4 mL of 0.2 M sodium carbonate buffer, pH=10.65; the mixture was thoroughly and rapidly stirred, placed in a KFK-3 KM spectrophotometer, and the optical density was measured every 30 seconds for 10 minutes at a wavelength of 347 nm in a 10-mm-thick cuvette (A_1). Next, 0.06 ml of the test extract and 0.2 ml of 0.1% adrenaline hydrochloride were added to 4 ml of buffer, mixed, and the optical density was measured as described above (A_2). To take into account the influence of the intrinsic color of the extracts, which absorb a certain wavelength in the visible part of the spectrum, a buffered solution of the extract without adrenaline was used as a reference solution. The antioxidant activity (AA) of the studied plant extracts was expressed as a percentage of inhibition of adrenaline autooxidation and calculated using the formula: $AA = (A_1 - A_2) \times 100 / A_1$. An AA value of more than 10% indicates the presence of antioxidant activity [26].

Results and Discussion. Heavy metal contamination of agricultural soils poses a significant threat to crop yields and food security. Toxic elements such as Cd, Cu, and Zn impair plant growth, induce oxidative stress, and disrupt physiological processes in crops such as *Phaseolus vulgaris*. In recent years, plant-growth-promoting rhizobacteria, particularly *Pseudomonas spp.*, have attracted attention due to their potential to enhance plant resistance to abiotic stresses. These microorganisms can modulate antioxidant defense systems, enhance enzymatic activity, and stabilize cellular metabolism under metal stress conditions. Therefore, the aim of this study was to evaluate the effect of *Pseudomonas spp.* on biometric traits, enzymatic activity, antioxidant responses, and pigment composition in *Phaseolus vulgaris* under heavy metal stress. The effect of heavy metals on the biometric parameters of *Phaseolus vulgaris* plants was clearly evident and depended on the type and concentration of the metal (Table 1).

Table 1 – The effect of heavy metals on plant biometric parameters and the restorative effect of bacteria of the genus *Pseudomonas spp.*

Growth parameters	Control	Zn 0.01M	Zn 0.001M	Cu 0.01M	Cu 0.001M	Cd 0.01M	Cd 0.001M
Stem length (cm)	36± 1.8	28± 1.4	29± 1.45	55± 1.75	24.8± 1.24	32± 1.6	34.5± 1.73
Root length (cm)	6.9±0.35	14.5± 0.73	7± 0.35	6.5± 0.33	21± 1.05	11± 0.55	22.5± 1.13
Leaf area/(cm ²)	3.75±0.19	3.5± 0.18	2.3± 0.12	1.7± 0.09	2.75± 0.14	3± 0.15	2.6± 0.13
Number of leaves (n)	5± 0.25	8 ± 0.4	3 ± 0.15	8 ± 0.4	5 ± 0.25	6 ± 0.3	5 ± 0.25

The greatest stem length was recorded in the treatment with a Cu concentration of 0.01

M (55 ± 1.75 cm), which was significantly greater than that of the control (36 ± 1.8 cm). This indicates that copper ions at low concentrations can stimulate plant growth. However, under the influence of Zn and Cd, stem length generally decreased or remained close to control values. Root length proved to be a sensitive indicator of heavy metal exposure. An increase in root length was observed following treatment with 0.01 M Zn (14.5 ± 0.73 cm) and 0.001 M Cd (22.5 ± 1.13 cm), which may indicate an adaptive response to stress or the influence of rhizosphere microorganisms, including *Pseudomonas spp.*, whereas a decrease in root length (6.5 ± 0.33 cm) following treatment with 0.01 M Cu indicates a toxic effect. Overall, leaf area decreased under the influence of all heavy metals, particularly in the treatment with a Cu concentration of 0.01 M (1.7 ± 0.09 cm²), indicating inhibition of the photosynthetic apparatus. Although an increase in the number of leaves was observed in some treatments (0.01 M Zn and 0.01 M Cu), this parameter does not fully characterize the plant's overall physiological condition and may represent a compensatory response under stress conditions.

Overall results showed that heavy metals have a dose-dependent effect on plant biometric parameters: high concentrations inhibit growth, whereas low concentrations sometimes trigger an adaptive response. In addition, a consortium consisting of *Pseudomonas putida* 1SL and *P. fluorescens* 4S strains significantly reduced the toxic effects of heavy metal ions. By biosorbing and chelating metal ions in the rhizosphere, these bacteria limited their uptake by the plant and restored the morphological parameters of the stem, root, and leaf.

The activity of plant antioxidant and enzymatic defense systems and the influence of *Pseudomonas* bacteria under heavy metal stress. The plant enzymatic system is one of the primary biochemical mechanisms regulating all of their physiological and metabolic processes. Enzymes control the rate of such important reactions as photosynthesis, respiration, and nitrogen and carbon metabolism. However, excessive accumulation of heavy metals (Cd, Pb, Cu, Zn, Hg, etc.) in the environment significantly impairs the normal functioning of plant enzymes.

Exposure to heavy metals alters the activity of enzymes involved in general metabolism, such as amylase, protease, and peroxidase. Heavy metal ions undergo substitution reactions with the protein component of enzymes or cofactors (e.g., Mg²⁺, Fe²⁺), disrupting their functional state. This leads to enzyme destabilization and the occurrence of substrate-nonspecific reactions [27]. Several studies have shown that enzyme activity depends on the type, concentration, and species of plant containing the heavy metal. For example, copper ions can act as stimulants of certain enzymes at low concentrations, while at high concentrations, they can be toxic. This phenomenon is important for studying plant adaptation mechanisms and provides insight into bio indication processes in ecosystems contaminated with heavy metals [28]. The study is directed toward evaluating the bioremediation potential of a *Pseudomonas spp.* bacterial consortium in reducing plant stress responses by determining peroxidase enzyme activity and antioxidant properties in *Phaseolus vulgaris* plants grown under heavy metal (copper, zinc, and cadmium) exposure.

Exposure to heavy metals affected changes in enzyme activity to varying degrees depending on the type and concentration of the metal, but these changes could be modulated to some extent by the presence of *Pseudomonas* bacteria in the rhizosphere (Figure 1). In the control treatment, enzyme activity was 0.0056 $\mu\text{mol}/\text{min}$. Under the influence of zinc, the increase in activity to 0.0059 $\mu\text{mol}/\text{min}$ at a concentration of 0.01 M can be explained by the ability of *Pseudomonas* bacteria to activate enzyme systems and increase the bioavailability of trace elements, whereas the decrease observed at 0.001 M is associated with a change in stress levels to 0.0051 $\mu\text{mol}/\text{min}$. Although the sharp decrease in enzyme activity from 0.01 M to 0.0014 $\mu\text{mol}/\text{min}$ in the presence of copper indicates its high toxicity, the fact that the activity approaches the control level at concentrations ranging from 0.001 M to 0.0056 $\mu\text{mol}/\text{min}$ is likely due to the immobilization of metal ions by *Pseudomonas* bacteria and the

attenuation of the toxic effect.

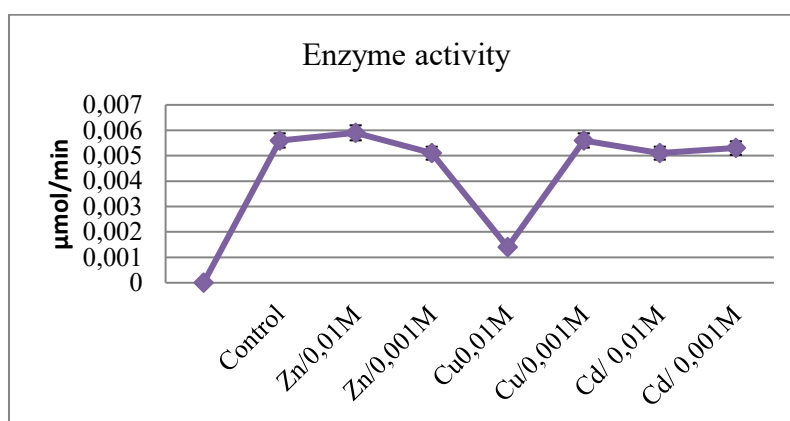


Figure 1 – The activity of plant defense enzyme systems and the effect of *Pseudomonas* spp. bacteria under heavy metal stress

In the case of cadmium, a slight decrease in enzyme activity was observed at concentrations of 0.01 M and from 0.001 M to 0.0051–0.0053 μmol/min, but the relative stability of these indicators suggests that *Pseudomonas* bacteria can increase plant resistance to stress by stimulating biosorption and antioxidant systems. Overall, the results obtained demonstrate that *Pseudomonas* bacteria play an important role in mitigating the negative effects of heavy metals on enzymatic systems and maintaining the physiological stability of plants.

The antioxidant system of plants exposed to heavy metals underwent significant changes, with these changes varying depending on the type and concentration of the metal, as well as the plant organ. In the control group, antioxidant activity was approximately 25% in the root, 24% in the leaf, and 27% in the stem, indicating a normal physiological state of the plant (Figure 1).

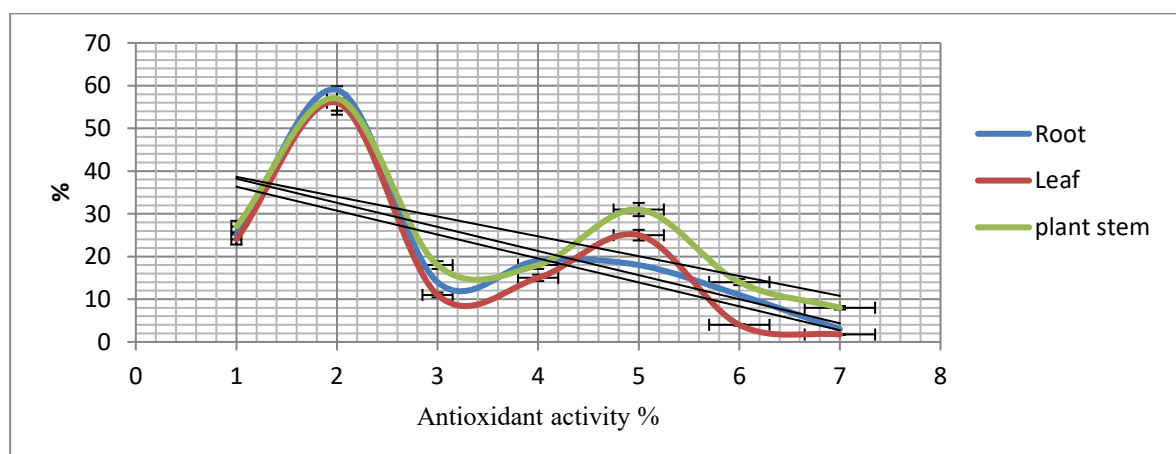


Figure 2 – Antioxidant protection activity of plants and influence of *Pseudomonas* bacteria under heavy metal stress (1- Control, 2- Cu 0,01M, 3- Cu 0,001M, 4- Cd 0,01M, 5- Cd 0,001M, 6- Zn 0,01, 7- Zn 0,001M)

At a copper concentration of 0.01 M, a sharp increase in antioxidant activity was observed in all plant parts (55–60%), indicating the activation of the defense system under conditions of severe oxidative stress. At a Cu concentration of 0.001 M, a decrease in these

indicators by 11–18% indicates a reduction in stress levels or stabilization of the adaptive response. Under the influence of cadmium (Cd), the average activity level at a concentration of 0.01 M remained at 15–19%, whereas at 0.001 M a significant increase of 31% was observed, especially in the stem. This indicates a compensatory response of the plant's antioxidant system. Under the influence of zinc (Zn), antioxidant activity was low in all variants, particularly 1–8% at 0.001 M, which is explained by Zn's relatively weak stress factor or its low impact on enzyme systems.

The results obtained indicate that bacteria of the genus *Pseudomonas* play an important role in regulating the antioxidant defense system of plants. Under conditions of high stress (especially at a Cu concentration of 0.01 M), the increase in antioxidant activity indicates that the bacteria stimulate mechanisms for neutralizing reactive oxygen species (ROS) in the plant. Furthermore, the relative stabilization of activity under Cd and Zn concentrations may be associated with the ability of *Pseudomonas* strains to immobilize metal ions and reduce their toxic effects. Heavy metals induce oxidative stress in plants, leading to the activation of the antioxidant system, while *Pseudomonas* bacteria regulate these processes and enhance the plant's adaptation to stressful conditions. The strongest effect is observed at high copper concentrations, in which case the antioxidant defense system is activated to the maximum extent. The presence of heavy metals had a significant effect on the composition of photosynthetic pigments in *Phaseolus vulgaris*, with the changes depending on the type and concentration of the metals. In control plants, the content of chlorophyll a, chlorophyll b, and total chlorophyll (a+b) was 0.0008, 0.0003, and 0.0011, respectively, indicating a normal physiological state of the photosynthetic apparatus (Table 2).

Table 2 –The effect of heavy metals on plant photosynthetic pigments and the reductive effect of *Pseudomonas* bacteria

Concentration	chlorophyll- α	chlorophyll- β	chlorophyll $\alpha + \beta$	caryothionide
1/Control	0.0008± 0.00004	0.0003 ± 0.00002	0.0011± 0.00006	0.0003± 0.00001
2/Zn 0,01 M	0.0020± 0.00010	0.0008 ± 0.00004	0.0025± 0.00013	0.0002± 0.00001
3/Zn 0,001M	0.0004± 0.00002	0.0002 ± 0.00001	0.0006± 0.00003	0.0002± 0.00001
4/Cu 0,01M	0.0008± 0.00004	0.0003 ± 0.00002	0.0010± 0.00005	0.0002± 0.00001
5/Cu 0,001M	0.0007± 0.00004	0.0003 ± 0.00002	0.0010± 0.00005	0.0002± 0.00001
6/Cd 0,01M	0.0020± 0.00010	0.0008 ± 0.00004	0.0030± 0.00015	0.0002± 0.00001
7/Cd 0,001M	0.0020± 0.00010	0.0008 ± 0.00004	0.0030± 0.00015	0.0002± 0.00001

Upon treatment with zinc, a concentration-dependent effect was observed. At a concentration of 0.01 M, the content of chlorophyll a and total chlorophyll increased to 0.002 and 0.0025, respectively, indicating the stimulatory role of zinc as an important micronutrient involved in chloroplast function and enzyme activity. However, at low concentrations (0.001 M), pigment levels decreased (chlorophyll a = 0.0004, chlorophyll a+b = 0.0006), suggesting the possibility of metabolic imbalances under suboptimal conditions. Copper treatment had an overall inhibitory effect on pigment accumulation. At a concentration of 0.01 M, the chlorophyll level was close to control values, whereas at 0.001 M a slight decrease was observed. This may reflect the dual role of copper as an essential element and, at high concentrations, as a strong pro-oxidant leading to pigment degradation. Cadmium treatment led to a significant increase in chlorophyll content at both concentrations (chlorophyll a = 0.002; chlorophyll a+b = 0.003), which may be associated with compensatory mechanisms aimed at maintaining photosynthetic efficiency under stress conditions.

Such an increase does not necessarily indicate an improvement in physiological parameters, but may reflect stress-induced metabolic changes. Carotenoid levels remained

relatively constant across all treatment conditions (0.0002), indicating their important role in maintaining baseline photoprotection and the structural stability of the photosynthetic apparatus during heavy metal-induced stress. Changes in pigment content are closely linked to the antioxidant defense system of plants during heavy metal-induced stress. An increase in chlorophyll levels under bacterial exposure (Zn 0.01 M and Cd treatments) may indicate the activation of adaptive responses, which is confirmed by an increase in antioxidant activity aimed at reducing the amount of reactive oxygen species (ROS). In contrast, the decrease in pigment levels (following treatment with 0.001 M Zn and Cu) is associated with oxidative damage to chloroplast membranes and photosynthetic complexes caused by excessive accumulation of ROS. In such cases, antioxidant enzymes and non-enzymatic components (including carotenoids) play an important role in protecting the photosynthetic mechanism. The relatively constant content of carotenoids indicates their function as the main non-enzymatic antioxidants involved in the neutralization of singlet oxygen and the dissipation of excess energy. In addition, the presence of *Pseudomonas* bacteria may help maintain pigment stability by increasing the activity of antioxidant enzymes, reducing the bioavailability of metals through biosorption and immobilization, and thereby reducing oxidative stress.

Conclusion. The results of the study showed that heavy metals (Cu, Cd, Zn) have a significant effect on biometric parameters, enzymatic and antioxidant systems, as well as on the pigment composition of *Phaseolus vulgaris* plants. According to biometric analysis, plant growth was slowed at high concentrations of heavy metals: plant height and the number of leaves decreased by an average of 20–35% compared to the control.

Antioxidant activity reached its highest level under the influence of copper (Cu 0.01) and amounted to 59% in the root, 56% in the leaf, and 57% in the stem, indicating the activation of the defense system in response to severe oxidative stress. At low zinc concentrations (Zn 0.001), the lowest values were recorded (root – 3%, leaf – 1.73%, stem – 8%). Peroxidase activity also varied in accordance with this trend and was found to increase 1.5–2.0-fold under heavy metal-induced stress. Pigment analysis showed that the content of chlorophyll a and b decreased by 15–40% under the influence of heavy metals, while the content of carotenoids in some cases increased by 10–20% as a protective response. The use of a consortium of *Pseudomonas* bacteria increased plant resistance to stressful conditions, contributed to a 10–25% improvement in biometric indicators, and stabilized the enzymatic and antioxidant systems.

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АУЫР МЕТАЛДАРМЕН ЛАСТАНУ ЖАҒДАЙЫНДА *PSEUDOMONAS* ТУЫСЫНЫҢ ӨСІМДІКТЕРДІҢ АНТИОКСИДАНТТЫҚ ЖҮЙЕСІНЕ ӘСЕРІ

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Аннотация. Бұл зерттеу ауыр металдармен (Cu, Cd, Zn) ластанған ортада өсірілген *Phaseolus vulgaris* өсімдігінің антиоксиданттық жүйесінің өзгерістерін және *Pseudomonas spp.* бактериялар консорциумының биоремедиациялық әсерін бағалауға бағытталған. Зерттеу нәтижелері көрсеткендей, бақылау нұсқасында антиоксиданттық белсенділік тамырда – 25%, жапырақта – 24%, сабақта – 27% деңгейінде болды. Мыс (Cu 0,01) әсерінде бұл көрсеткіштер айтарлықтай жоғарылап, сәйкесінше 59%, 56% және 57% құрады, бұл күшті тотығу стрессіне жауап ретінде антиоксиданттық жүйенің белсенуін көрсетеді. Ал мыс концентрациясы төмендегенде (Cu 0,001) антиоксиданттық белсенділік тамырда 14%, жапырақта 11%, сабақта 18% деңгейіне дейін төмендеді. Кадмий (Cd 0,01) жағдайында көрсеткіштер 19%, 15% және 18% болса, Cd 0,001 нұсқасында жапырақта 25% және сабақта 31%-ға дейін жоғарылау байқалды. Мырыш (Zn 0,01) әсерінде антиоксиданттық белсенділік төмендеп, тамырда 11%, жапырақта 4%, сабақта 14% болды, ал Zn 0,001 кезінде ең төмен мәндер тіркелді (3%; 1,73%; 8%).

Pseudomonas spp. консорциумын қолдану өсімдіктердің ауыр металдарға төзімділігін арттырып, антиоксиданттық жүйенің тұрақтануына ықпал етті. Жалпы, нәтижелер бактериялардың ауыр металл иондарын биосорбциялау және өсімдіктердегі тотығу стрессін төмендету арқылы тиімді биоремедиациялық агент ретінде қолдануға болатынын дәлелдейді.

Тірек сөздер: Ауыр металдар, *Pseudomonas spp.*, антиоксиданттық белсенділік, биоремедиация, *Phaseolus vulgaris*, стресстік реакциялар.

ВЛИЯНИЕ БАКТЕРИЙ РОДА *PSEUDOMONAS* НА АНТИОКСИДАНТНУЮ СИСТЕМУ РАСТЕНИЙ В УСЛОВИЯХ ЗАГРЯЗНЕНИЯ ТЯЖЕЛЫМИ МЕТАЛЛАМИ

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Аннотация. Это исследование выявило изменения антиоксидантной системы растения *Phaseolus vulgaris*, выращенного в среде, загрязненной тяжелыми металлами (Cu, Cd, Zn), и *Pseudomonas spp.* направлен на оценку биоремедиационного эффекта консорциума бактерий. Результаты исследования показали, что в контрольном варианте антиоксидантная активность была на уровне 25% у корня, 24% у листа, 27% у стебля. При воздействии меди (Cu 0,01) эти показатели значительно увеличились и составили 59%, 56% и 57% соответственно, что указывает на активацию антиоксидантной системы в ответ на сильный окислительный стресс. А когда концентрация меди снизилась (Cu 0,001), антиоксидантная активность снизилась до 14% в корнях, 11% в листьях и 18% в стеблях. В случае кадмия (Cd 0,01) показатели были 19%, 15% и 18%, тогда как в варианте Cd 0,001 наблюдалось увеличение до 25% на листе и 31% на стебле. Антиоксидантная активность снижалась при воздействии цинка (Zn 0,01) и составляла 11% в корне, 4% в листьях и 14% в стебле, а самые низкие значения были зарегистрированы при Zn 0,001(3%; 1,73%; 8%). *Pseudomonas spp.* использование консорциума повысило устойчивость растений к тяжелым металлам и способствовало стабилизации антиоксидантной системы. В целом, результаты доказывают, что бактерии можно использовать в качестве эффективного биоремедиационного агента путем биосорбции ионов тяжелых металлов и снижения окислительного стресса у растений.

Ключевые слова: Тяжелые металлы, *Pseudomonas spp.*, антиоксидантная активность, биоремедиация, *Phaseolus vulgaris*, реакции на стресс.