

Given the physiological and metabolic traits of the used strains, the alcohol-free isolation method was structurally optimized and adapted to the specific technological challenges encountered during the experiments. In particular, an additional post-dialysis centrifugation step was introduced due to the coagulation of destabilized structural peptides. These protein molecules lost their solubility and formed aggregates as a result of a rapid decrease in the ionic strength of the solution. The critical evaporation stage also underwent optimization. To protect the EPS from thermal degradation, this process was carried out at a temperature not exceeding 45 °C, with a reduction in solution volume of no more than 5-fold. Additionally, a filtration step using activated charcoal was performed to remove hydrophobic impurities and pigments, as both strains proved to be active producers of riboflavin.

Conclusions:

1. An alcohol-free scheme for the isolation of EPS from *B. velezensis* strains UCM B-7242 and UCM B-7243 was implemented and optimized.
2. It was established that under the proposed alcohol-free isolation method, the specific EPS yield was 0.021 g/L for *B. velezensis* UCM B-7242 and 0.084 g/L for the UCM B-7243. The content of residual peptide components in the concentrated extracts was strictly limited to 0.005 %, while that of nucleic acids was 0.016 %.
3. The most significant results of the methodological optimization included the enhancement of the deproteinization stage, the determination of the optimal evaporation conditions, and the implementation of an adsorption purification step.

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INFLUENCE OF STRESS-TOLERANT BACTERIA ON THE MORPHOMETRIC AND BIOCHEMICAL PARAMETERS OF SPRING WHEAT GROWN IN SOIL WITH COPPER(II) CHLORIDE

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Abstract. This study examines the influence of inoculating spring wheat (*Triticum aestivum* L., cv. Tybalt) seeds with stress-tolerant bacterial strains isolated from Antarctic substrates, Lviv

municipal solid waste landfill leachates, and war-affected soils on seedling growth under exposure to copper(II) chloride (300 mg/kg). Copper toxicity was shown to cause a 4.5-fold reduction in root length, a 15 % decrease in the *chl a/chl b* ratio, and an accumulation of flavonoids and proline in the leaves of non-bacterized seedlings. Inoculation of wheat seeds with the strains *Ochrobactrum* sp. K3, *Microbacterium* sp. EO1, *Rhodococcus* sp. 37, *Bacillus* sp. S304, *Pseudomonas* sp. EO2, and *Paenibacillus* sp. 5A-101 stimulated root growth of 14-day-old seedlings. Inoculation of seeds with all studied bacterial strains mitigated adverse effects in wheat seedling leaves: the *chl a/chl b* ratio and proline levels were restored to those of the metal-free control, whereas flavonoid content decreased compared with non-bacterized plants grown under copper(II) chloride influence. These results confirm the potential of using stress-tolerant plant growth-promoting bacteria to enhance wheat tolerance to copper-induced stress.

Keywords: chlorophyll, flavonoids, plant growth-promoting bacteria, proline, *Triticum aestivum* L.

Introduction. Soil contamination with heavy metal ions is a severe environmental problem that threatens the stability of agroecosystems and food safety. Heavy metal input into the environment primarily stems from industrial effluents, transport, as well as the intensive application of pesticides and mineral fertilizers in farming (Adrees et al., 2015; Briffa et al., 2020). The problem has become especially critical as a result of full-scale warfare: ammunition detonation, wildfires, and the destruction of military hardware result in severe soil contamination (Solokha et al., 2024; Krainiuk et al., 2025). Copper, derived from brass and bronze projectile components, explosives, and anticorrosion coatings, enters the soil as Cu^{2+} ions, accumulating persistently in topsoil layers (Krainiuk et al., 2025). An excess of Cu^{2+} ions induces phytotoxic effects, leading to root growth inhibition, photosynthetic apparatus impairment, and oxidative stress (Adrees et al., 2015). A promising, eco-friendly approach to enhancing crop stress tolerance and mitigating the negative impact of metals is the application of plant growth-promoting bacteria isolated from extreme environments.

This study aimed to evaluate how seed bacterization with stress-tolerant bacterial strains affects the morphometric parameters, photosynthetic pigment levels, non-enzymatic antioxidant content, and osmoprotectant accumulation in spring wheat (*Triticum aestivum* L., cv. Tybalt) during early ontogenesis under exposure to copper(II) chloride.

Methods. Bacterial strains isolated from extreme habitats at the Department of Microbiology of Ivan Franko National University of Lviv during 2020–2024 were used in this study: *Ochrobactrum* sp. K3 isolated from the leachate of the Lviv municipal solid waste landfill; *Microbacterium* sp. EO1, *Pseudomonas* sp. 3B-In-57, *Lysinibacillus* sp. Km8, *Bacillus* sp. RT1, *Arthrobacter* sp. E35, and *Paenibacillus* sp. 5A-101 from various Antarctic substrates collected during the 23th–27th Ukrainian Antarctic Expeditions; and *Streptomyces* sp. S253 and *Bacillus* sp. S304 from soil samples damaged by military actions. Bacteria were grown in Luria–Bertani broth for 2 days, harvested by centrifugation (6000 rpm, 10 min), and resuspended in 0.9 % NaCl solution to a density of 5.0 McFarland standard. Wheat seeds were inoculated by soaking in the bacterial suspension for 18 h and subsequently sown in prepared soil. Copper(II) chloride was applied to the soil as a solution to achieve a final concentration of 300 mg/kg. Wheat seeds soaked in 0.9 % NaCl and grown in soil with or without $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ served as controls. After 14 days of growth, root and shoot lengths, photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and *chl a/chl b* ratio), alongside levels of phenolic compounds, flavonoids, and proline in leaves, were measured.

Results. In non-bacterized 14-day-old wheat seedlings, copper accumulation in leaves reached up to 20 mg/kg of dry weight during growth in soil with copper(II) chloride.

Copper(II) chloride exposure caused substantial alterations in the morphometric and biochemical parameters of non-bacterized wheat seedlings. The root length of non-bacterized seedlings exposed to copper(II) chloride was 4.5-fold shorter compared to uninoculated control plants grown in metal-free soil. Copper(II) chloride exposure increased total chlorophyll content in the leaves of non-bacterized seedlings, accompanied by a 15 % reduction in the chl a/chl b ratio, a twofold increase in flavonoid content, and a 30 % increase in proline accumulation compared to plants grown in unamended soil.

Accumulation of Cu in leaves of 14-day-old seedlings was lower following seed inoculation with *Paenibacillus* sp. 5A-101 and *Ochrobactrum* sp. K3, while other evaluated strains did not significantly alter leaf Cu levels compared to the non-bacterized control under copper(II) chloride exposure. Seedlings inoculated with *Ochrobactrum* sp. K3, *Microbacterium* sp. EO1, *Rhodococcus* sp. 37, *Bacillus* sp. S304, *Pseudomonas* sp. EO2, or *Paenibacillus* sp. 5A-101 and grown in copper(II) chloride-amended soil exhibited longer roots than non-bacterized control plants exposed to copper(II) chloride. Seed inoculation with all tested bacterial strains led to a lower leaf chlorophyll content in 14-day-old seedlings compared to non-bacterized plants under copper(II) chloride exposure; nevertheless, the chl a/chl b ratio remained comparable to that of non-bacterized seedlings grown in metal-free soil. Under copper(II) chloride exposure, the leaf flavonoid content in bacterized seedlings was lower than that in non-bacterized seedlings. Proline content in the leaves of bacterized seedlings was lower compared to that in non-bacterized seedlings grown under copper(II) chloride exposure, and did not differ from levels in non-bacterized plants grown in metal-free soil.

Conclusions. Inoculation of wheat seeds with the stress-tolerant bacterial strains promoted plant development at early developmental stages, positively modulating both morphometric traits and physiological status under copper(II) chloride exposure.

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