The bioavailability of metals is due to the pH of the medium, temperature, toxi-
cation of these mechanisms are necessary for the normal functioning of organisms (Barra
ducers (Ayangbenro & Babalola, 2017; Presentato et al., 2019). The toxicity of metals
ctivity of specific membrane transporters), disappearance of the trans-
ometric methods, and statistical processing of the results was performed using two-way ANOVA and factor analysis. It was found that
ferric chloride at a concentration of 1–12 mM causes inhibition of the accumulation of biomass of bacteria Rh. yavorovii IMV B-7620 up to
nuclear, carbonyl groups in proteins, which are closely linked by a direct bond and inversely related to the content of lipid hydroperoxides and catalase activity. The second latent factor included duration of cultivation of bacteria, biomass accumulation, and superoxide dismutase activity, which are inversely related to lipid hydroperoxide content and catalase activity. Under the influence of cobalt (II) chloride, the first latent factor included the content of lipid hydroperoxides, carbonyl groups in proteins, as well as catalase and superoxide dismutase activities, which are inversely related to bacterial biomass.

Keywords: free radical damage; lipid peroxidation; heavy metals; oxidative modification of proteins; antioxidant defense system; co-
balt ions; ferric ions.

Introduction

Most heavy metals (Fe, Zn, Mn, Ni, Cr, Co etc.) in appropriate concentrations are necessary for the normal functioning of organisms (Barra Caracciolo & Terenzi, 2021). In addition to natural processes (weathering of minerals, etc.), sources of heavy metal pollution include industry, agricultural fields, sewage sludge, and waste treatment plants (Kapahi & Sachdeva, 2019). Accumulating in the environment, heavy metals cause negative effects for biocenoses (Li et al., 2022). Toxicity of heavy metals to living organisms is due to participation in oxidation reactions, catalysis of Fenton-type reactions (which involve transition metals such as copper, nickel, iron), Haber–Weiss reaction (with the formation of reactive oxygen species (ROS)), inhibition of transport processes (due to influence on activity of specific membrane transporters), disappearance of the transmembrane proton potential of membranes (Prabhakaran et al., 2016) etc. Toxicity of metals depends on their bioavailability and concentration. The bioavailability of metals depends on the medium, temperature, redox potential of the medium and the presence of various organic sub-
stances (Ayangbenro & Babalola, 2017; Presentato et al., 2019). The toxicity of some metals is due to the displacement of biologically essential metals from their native binding sites or the interaction of ligands (Prabhakaran et al., 2016).

Increasing the concentration of unbound iron ions causes oxidative stress mediated by the Fenton reaction (Wang et al., 2004; Vasyliv & Hnatush, 2013). Fe²⁺ can damage photosystems and inhibit photosynthesis. Fe⁺ and Fe³⁺ can affect the transport of other elements for cells (Prabhakaran & Barra Caracciolo & Terenzi, 2021). The mechanism of cobalt toxicity is unknown. This metal is characterized by a high affinity for sulfhydryl groups, which can lead to inhibition of important enzymes. Also, the toxicity of cobalt for different organisms may be due to the substitution of divalent cations in the ionic center of metal-activated enzymes; its antagonistic effect on Ca²⁺ channels; with probable inhibition of Ca²⁺ entry, Ca²⁺ signaling and competition with Ca²⁺ ions for intracellular Ca²⁺-binding proteins; by generating ROS in cells (e.g. by Fenton-like reactions) that cause oxidative damage to DNA, proteins and lipids (Simonsen et al., 2012). Cobalt ions alter the conformation of biomolecules (proteins or nucleic acids) and metabolic reactions by binding to reactive groups – hydroxyl, carbonyl and sulfhydryl (Singh et al., 2016). In addition, metals at high concentrations can damage cell membranes, alter enzyme specificity, disrupt cellular functions, and damage DNA structure (Mohammed et al., 2011). Mechanisms of resistance of microorganisms to metals are: intra- and extracellular binding, active efflux, enzymatic reduction and alteration of sensitivity of cellular targets to metal ions, etc. (Mohammed et al., 2011; Prabhakaran et al., 2016; Yin et al., 2018). Different combinations of these mechanisms give preference to microorganisms in metal-contaminated environments (Mohammed et al., 2011). Microorganisms interact with metal ions through binding to the cell wall, intracellular accumulation,

Heavy metals that enter the environment due to natural processes or industrial activities, when accumulated, have a negative impact on organisms, including microorganisms. Microorganisms have developed various adaptations to heavy metal compounds. The aim of our work was to investigate the influence of ferric citrate and cobalt (II) chloride on biomass accumulation, indicators of free radical damage and activity of enzymes of the antioxidant defense system of bacteria Rhodopseudomonas yavorovii IMV B-7620, that were isolated from the water of Yavorivske Lake (Ukraine, Lviv region), which was formed as a result of flooding of a sulfuric quarry. We used cultural, pho-
tometrics, and histological methods, and statistical processing of the results was performed using two-way ANOVA and factor analysis. It was found that


This page is a part of the publication: Influence of cobalt chloride and ferric citrate on purple non-sulfur bacteria Rhodopseudomonas yavorovii. Biosystems Diversity, 30(1), 38–45. doi:10.15421/012205

S. O. Hnatush, O. D. Maslovska, S. Y. Komplikevych, I. V. Kovbasa

Ivan Franko National University of Lviv, Lviv, Ukraine
synthesis of siderophores, extracellular transformation reactions, extracel-
lular mobilization or immobilization of metal ions, conversion of metals
into volatile forms (Ayangbenro & Babalola, 2017; Yin et al., 2018). De-
position of metals on the surface of bacteria is associated with the interac-
tion of metal cations with negatively charged groups of the cell wall sur-
face of microorganisms (hydroxyl, amine, carboxyl, sulfhydryl, phos-

Some microorganisms use metals for respiration, some bacteria in the
process of evolution have formed mechanisms of detoxification of heavy
metals. Microbial interactions with metals play a significant role in the
biogeochemical cycles of toxic heavy metals, as well as in cleaning the
environment from metal pollution. The transformation of metals by mic-
roorganisms can be divided into two broad categories: redox transforma-
tions of inorganic forms and transformations from inorganic to organic
form and vice versa. Microorganisms can produce energy by oxidizing
iron, manganese or arsenic. Dissimilative reduction is also possible with
the use of metals as ultimate electron acceptors for anaerobic respiration
(Mohammed et al., 2011).

Purple non-sulfur bacteria, which are common in waters of various
types, including wastewater, are characterized by flexible metabolism
(Monroy & Buitrón, 2020), which is a prerequisite for the use of individu-
al representatives for biotechnological purposes (Delgado-Sarmiento,
2020; Asif et al., 2021). Strains of genera Rhodospseudomonas, Rhodobac-
ter, Rhodocyclus are producers of single cell protein (Garimella et al.,
2017), hydrogen (Sagar & Alipour, 2021), carotenoids (Wang et al., 2017;
Realspti et al., 2019), polyhydroxyalkanoates (Monroy & Buitrón, 2020;
Montiel-Corona & Buitrón, 2021), 5-aminovaleric acid (Bunraksa et al.,
2020). It is known that Rhodobacter sp. GSKRLMBKU-03 are resistant to
0.05 mM chrome ions and reduce 0.014–0.045 mM, depending on conditions of reaction (Rajyalaxmi et al., 2019). Arsenic-resistant strains of
Rhodospseudomonas and Rhodobacter species are resistant to 0.5 mM
CoCl₂, PbCl₂, NiCl₂ under aerobic and anaerobic growing conditions, up
to 0.5 mM Na₂SO₄ under anaerobic growing conditions; individual strains are resistant to 0.5 mM CuCl₂ (Mohsin et al., 2019). In recent years,
bio-technologies for treatment of wastewater of various origins with the
involvement of purple non-sulfur bacteria have been developed (Bunraksa
et al., 2020; Cernutí et al., 2020; Lu et al., 2021). Because industrial pro-
cesses are sources of various toxic substances that are by-products of pro-
duction, it is important to understand the effects of these compounds on
the cells of microorganisms. One of the main components of wastewater are
heavy metal compounds (Changpipat et al., 2010; Huang et al., 2019;
Malovany et al., 2019) and organic complexes (Kasukih et al., 2018; da
Silva Brito et al., 2019; Li et al., 2020; Yang et al., 2021). Therefore, it is
advisable to study the adaptations of bacterial strains to the effects of such
compounds.

The aim of our work was to investigate the effect of ferric citrate and
cobalt (II) chloride on biomass accumulation, indicators of free radical
damage and activity of enzymes of the antioxidant system of bacteria
Rhodospseudomonas yavorovskii IMV B-7620 (Tarabas et al., 2021) which
were isolated from the water of Yavorivske Lake (Ukraine, Lviv region),
which was formed as a result of flooding of a sulfur quarry.

Materials and methods

Bacteria Rhodospseudomonas yavorovskii IMV B-7620 were grown in
ATCC 1449 medium (g/L): NH₄Cl – 0.4, MgSO₄•7H₂O – 0.32,
K₂HPO₄ – 0.6, CaCl₂ – 0.05. Sodium citrate at a concentration of 12 mM
was added as a carbon source. To study the effect of heavy metal ions, 1,
5, 10, 15 mM cobalt (II) chloride or 1, 3, 6, 9, 12 mM ferric citrate were
added to the medium. The control was a medium without metal salts.
When ferric citrate was added, the concentration of sodium citrate in the
medium was proportionally reduced.

To obtain cell-free extracts, the precipitated cells were washed twice
with 0.9% NaCl solution and resuspended in 0.05 M potassium phosphate
buffer (pH 7.0) with phenylmethylsulfonyl fluoride (10⁻⁵ M) and ethyle-
nediaminetetraacetic acid (10⁻⁵ M). Cells were disrupted with ultrasonic
disintegrant UZDN-27 (22 kHz, 5 min, 0°C). Cell fragments were precipi-
tated by centrifugation (8000 g, 30 min, 4°C). The protein concentration
was determined by the Bradford method (Bradford, 1976).

The processes of lipid peroxidation were studied by changes in the
content of diene conjugates, lipid hydroperoxides, thiobarbiturate reactive
species (TBARS) in the cell-free extract of bacteria during cultivation.
To determine the content of diene conjugates, to 0.2 mL of cell-free ex-
tract 1.8 mL of a mixture of n-heptane and isopropl alcohol was added in a
ratio of 1:1. The obtained mixture was shaken and left in closed tubes for
15 min, then centrifuged (2000 g, 10 min). The supernatant was collected
in tubes, which were previously made 0.05 mL of double-distilled water.
To 0.5 mL of the obtained heptane phase 2.0 mL of ethanol was added and
the absorbance measured at λ = 233 nm. The control was a solution
containing 0.5 mL of n-heptane and 2.0 mL of ethanol.

To 0.2 mL of cell-free extract 2.8 mL of ethanol and 0.05 mL of 50%
threochloroacetic acid solution were added, the obtained mixture was shaken
for 5 minutes. 1.5 mL of the supernatant was taken and 1.2 mL of ethanol,
and 0.02 mL of concentrated HCl, and 0.03 mL of a 1% solution of Mol's salt in a 3% solution of HCl were added, the obtained mixture was
shaken and 0.2 mL of 20% ammonium thiocyanate solution was added after
30 s. Absorption was measured at a wavelength of 480 nm on a spectrophotometer SF-46. The content of lipid hydroperoxides was de-
termined by the difference between the test sample and the control, in
which instead of the cell-free extract the appropriate amount of double-
distilled water was added. The concentration of lipid hydroperoxides was
expressed in conventional units per 1 g of protein.

To determine TBARS 1 mL of trichloroacetic acid was added to 1 mL of
cell-free extract to a final concentration of 10% and centrifuged
(2500 g, 10 min). The resulting supernatant was mixed with 1.5 mL of
saturated thiobarbituric acid solution in 0.1 M HCl solution (pH 2.5).
The mixture was boiled in a water bath for 20 minutes. In the control
sample, double-distilled water was added instead of the supernatant. After
rapid cooling, 3 mL of butanol was added to the samples, was stirred vigorously and centrifuged in the previous mode. The concentra-
tion of TBARS in the butanol layer was determined at a wavelength of
535 nm.

Carbonyl groups (CG) in proteins were investigated by reaction with
2,4-dinitrophenylhydrazine. To 0.5 mL of cell-free extract 1 mL of trichlo-
roacetic acid was added to a final concentration of 10% and centrifuged
(5000 g, 5 min). To the resulting precipitate 1 mL of a 0.10 mM solution
of 2,4-dinitrophenylhydrazine in 2 M HCl solution was added. 1 mL of 2 M
HCl solution was added to the control solution instead. The mixture was
stirred and incubated for 1 h at room temperature, then centrifuged
(7000 g, 5 min). The precipitate was washed twice with 1 mL of a mixture of
ethan and ethyl acetate (1:1) and centrifuged (7000 g, 5 min). The washed
precipitate was dissolved for 30 min in 6 M gauadin hy-
drocchloride solution. Undissolved material was separated by centrifuga-
tion (8000 g, 15 min). In the supernatants, the extinction of CG in proteins
was determined at a wavelength of 370 nm.

To determine the specific superoxide dismutase activity, reagent C
was prepared (100 mL of 0.08 mM EDTA solution and 100 mL of 0.1 M
potassium phosphate buffer (pH 7.8) and the pH was adjusted to 10 with
concentrated N₂N₂N₂tetramethylammonium solution). Quercetin at a
concentration of 1.4 μM was dissolved in dimethyl sulfoxide and
brought to a liquid state by immersion in hot water. Immediately before
determination, the resulting quercetin solution was diluted 10 times with
distilled water. 1 mL of reagent C, 2.4 mL of H₂O, 0.1 mL of quercetin
were added to the test tube. To the test tube was added 1 mL of reagent C,
2.3 mL of H₂O, 0.1 mL of cell-free extract, 0.1 mL of quercetin. Mea-
surements were performed using DS-11+ (DeNovix Inc., USA, 2018)
spectrophotometer at a wavelength of 406 nm at zero time (immediately
after the addition of quercetin) and after 20 minutes.

The reaction mixture for determining catalase activity contained
2.8 mL of 0.5% H₂O₂ solution and 0.1 mL of cell-free extract diluted n
times. After 5 min of incubation, the reaction was stopped by adding
1.0 mL of 6% (NH₄)₂MoO₄ solution. The control was a sample that con-
tained H₂O instead of cell-free extract. Measurements were performed on
a DS-11+ spectrophotometer (DeNovix Inc., USA, 2018) at a wavelength of
410 nm immediately after the addition of (NH₄)₂MoO₄.

Statistical processing of the results was performed in OriginPro 8.5
(OriginLab Corporation, USA, 2010). The values of different groups were
compared using ANOVA followed by Bonferroni test. The two-way
Results

Ferric citrate at concentration of 1–6 mM did not cause inhibition of *Rh. yavorovii* IMV B-7620 biomass accumulation during the 7th day of culture growth. Reduction of the biomass accumulation by 10–15% under the influence of these concentrations of ferric citrate occurred by increasing of the duration of cultivation of bacteria up to 14 days. The intensity of the damaging action also increased with an increase in the concentration of metal salt in the cultivation medium. Ferric citrate at concentration 9–12 mM caused inhibition of biomass accumulation up to 44.7%, compared with control.

In order to study the influence of cobalt ions, cobalt (II) chloride in concentrations 1, 5, 10, 15 mM was added into the medium. Cobalt (II) chloride in concentration of 1 mM did not significantly influence the accumulation of biomass of *Rh. yavorovii* IMV B-7620 for 14 days of growth. It was found that 5–15 mM cobalt (II) chloride affected inhibition of biomass accumulation by 60.0–70.4% for 14 days of bacterial growth.

Investigation of indicators of lipid peroxidation, oxidative modification of proteins, catalase and superoxide dismutase activity was performed at 7th, 10th and 14th days of cultivation of bacteria.

At all studied concentrations of ferric citrate, the content of diene conjugates in the cells of *Rh. yavorovii* IMV B-7620 was higher than in the control. The highest content of diene conjugates under the influence of all studied concentrations of ferric citrate was found for the 10th day, which was 1.6–7.5 times higher than the content of diene conjugates in the cells of bacteria not exposed to this metal (Fig. 2a). High levels of diene conjugates were found in the cells of bacteria grown with 10 and 15 mM cobalt (II) chloride on the 14th day of cultivation (197 and 295 nmol/mg protein, accordingly, Fig. 2b).

The highest content of lipid hydroperoxides (18.1 ± 0.01 conditional units/mg protein) in the cells of *Rh. yavorovii* IMV B-7620 under the influence of ferric citrate was on the 7th day of cultivation at a concentration of 12 mM (Fig. 3a). Under the influence of cobalt (II) chloride, the content of lipid hydroperoxides also increased compared to the control. When 5–15 mM cobalt (II) chloride was added into the culture medium of *Rh. yavorovii* IMV B-7620, the maximum content of lipid hydroperoxides was detected on the 10th day of cultivation (Fig. 3b). Under the influence of ferric citrate in concentrations of 1–9 mM, the content of primary and secondary products of lipid peroxidation differed slightly or was lower compared to the control. An increase in the content of lipid peroxidation products was observed under the influence of 12 mM ferric citrate (Fig. 4a). At all studied concentrations of cobalt (II) chloride, the content of TBARS in the cells of bacteria was significantly higher than in the control and exceeded the content of these products when exposed to 12 mM ferric citrate (Fig. 4b).

Ferric citrate and cobalt (II) chloride in selected concentrations caused the formation of carbonyl groups in proteins, indicating free radical dia-
mage to proteins of *Rh. yavorovii* IMV B-7620 (Fig. 5). The dynamics of formation of carbonyl groups in proteins under the influence of the investigated salts of metals was different. Under the influence of ferric citrate, the content of carbonyl groups in proteins was the highest during the 7th day of growth at all investigated concentrations of metal salt, except for a concentration of 12 mM. Under the influence of cobalt (II) chloride, the content of carbonyl groups in proteins of *Rh. yavorovii* IMV B-7620 increased with an increase in the duration of cultivation of bacteria and metal concentration in the medium. We assume that free radical damage to proteins of *Rh. yavorovii* IMV B-7620 under the influence of the iron or cobalt can affect the processes of cell metabolism and adaptation reactions under these conditions.

Under the influence of the studied metal salts, the catalase activity of *Rh. yavorovii* IMV B-7620 was higher than in the control. Under the influence of ferric citrate, catalase activity was highest on the 7th day of bacterial cultivation and exceeded the activity in the control 41 times. During further cultivation, the catalase activity of *Rh. yavorovii* IMV B-7620 differed slightly from the control (Fig. 6a). Under the influence of cobalt (II) chloride, catalase activity increased up to 10 days of *Rh. yavorovii* IMV B-7620 culturing. Under the influence of 5 and 10 mM cobalt (II) chloride, the maximum values of catalase activity were almost the same as under the influence of 12 mM ferric citrate, when the concentration of cobalt (II) chloride increased to 15 mM, catalase activity increased 1.3 times (Fig. 6b).

**Fig. 3.** The content of lipid hydroperoxides in the bacteria *Rhodopseudomonas yavorovii* IMV B-7620 under the influence of ferric citrate (*a*) and cobalt (II) chloride (*b*): x ± SD, n = 3, * – P < 0.05 – probable changes compared to control

**Fig. 4.** Content of thiobarbituric acid reactive species in bacteria *Rhodopseudomonas yavorovii* IMV B-7620 under the influence of ferric citrate (*a*) and cobalt (II) chloride (*b*): x ± SD, n = 3, * – P < 0.05 – probable changes compared to control

**Fig. 5.** The content of carbonyl groups in proteins of bacteria *Rhodopseudomonas yavorovii* IMV B-7620 under the influence of ferric citrate (*a*) and cobalt (II) chloride (*b*): x ± SD, n = 3, * – P < 0.05 – probable changes compared to control
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Fig. 6. Catalase activity of bacteria *Rhodopseudomonas yavorovii* IMV B-7620 cells under the influence of ferric citrate (a) and cobalt (II) chloride (b): x ± SD, n = 3, * – P < 0.05 – probable changes compared to control

Fig. 7. Superoxide dismutase activity of *Rhodopseudomonas yavorovii* IMV B-7620 cells under the influence of ferric citrate (a) and cobalt (II) chloride (b): x ± SD, n = 3, * – P < 0.05 – probable changes compared to control

The addition of ferric citrate and cobalt (II) chloride into the culture medium of *Rh. yavorovii* IMV B-7620 caused an increase in superoxide dismutase activity by 1.25–176 times. Under the influence of all studied concentrations of ferric citrate, the values of superoxide dismutase activity were higher than under the influence of cobalt (II) chloride at concentrations of 1–15 mM (Fig. 7, a, b).

Under the influence of ferric citrate, the increase in the concentration of metal salts caused statistically significant changes in the accumulation of biomass, the content of hydroperoxides of lipids and TBARS, superoxide dismutase activity. The content of diene conjugates and catalase activity did not depend on the concentration of the metal salt, but changed with increasing duration of bacterial cultivation. There are also relationships between changes in the concentration of ferric citrate and the duration of cultivation on the studied indicators (Table 1). Increasing the concentration of cobalt (II) chloride from 1 to 15 mM caused statistically significant changes in all studied indicators. Also, significant changes in the accumulation of biomass, indicators of lipid peroxidation and oxidative modification of proteins, the activity of antioxidant enzymes were caused by prolongation of the duration of bacterial cultivation. Relationships between these two factors have been identified (Table 1).

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Factor</th>
<th>F (DFn, DFd) value under the influence of ferric citrate</th>
<th>P value under the influence of</th>
<th>F (DFn, DFd) value under the influence of cobalt (II) chloride</th>
<th>P value under the influence of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>concentration</td>
<td>F(5, 13) = 273.77</td>
<td>&lt;0.0001*</td>
<td>F(4, 10) = 923.94</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>day of cultivation</td>
<td>F(2, 6) = 103.97</td>
<td>*</td>
<td>F(2, 6) = 71.92</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>interactions</td>
<td>F(10, 43) = 42.76</td>
<td>&lt;0.0001*</td>
<td>F(8, 36) = 38.04</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Lipid hydroperoxides</td>
<td>concentration</td>
<td>F(5, 13) = 1062.59</td>
<td>&lt;0.0001*</td>
<td>F(4, 10) = 1818.07</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>day of cultivation</td>
<td>F(2, 6) = 257.26</td>
<td>&lt;0.0001*</td>
<td>F(2, 6) = 967.64</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>interactions</td>
<td>F(10, 43) = 128.52</td>
<td>&lt;0.0001*</td>
<td>F(8, 36) = 648.14</td>
<td>&lt;0.0001*</td>
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<tr>
<td>Diene conjugates</td>
<td>concentration</td>
<td>F(5, 13) = 807.96</td>
<td>&lt;0.0001*</td>
<td>F(4, 10) = 1233.80</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>day of cultivation</td>
<td>F(2, 6) = 1934.33</td>
<td>&lt;0.0001*</td>
<td>F(2, 6) = 1808.59</td>
<td>&lt;0.0001*</td>
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<tr>
<td></td>
<td>interactions</td>
<td>F(10, 43) = 259.39</td>
<td>&lt;0.0001*</td>
<td>F(8, 36) = 741.18</td>
<td>&lt;0.0001*</td>
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<tr>
<td>Thiobarbiturate reactive species</td>
<td>concentration</td>
<td>F(5, 13) = 748.22</td>
<td>&lt;0.0001*</td>
<td>F(4, 10) = 1854.46</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>day of cultivation</td>
<td>F(2, 6) = 1207.82</td>
<td>&lt;0.0001*</td>
<td>F(2, 6) = 2197.05</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>interactions</td>
<td>F(10, 43) = 560.13</td>
<td>&lt;0.0001*</td>
<td>F(8, 36) = 768.13</td>
<td>&lt;0.0001*</td>
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<tr>
<td>Carbonyl groups in proteins</td>
<td>concentration</td>
<td>F(5, 13) = 789.01</td>
<td>&lt;0.0001*</td>
<td>F(4, 10) = 1966.23</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>day of cultivation</td>
<td>F(2, 6) = 2537.88</td>
<td>&lt;0.0001*</td>
<td>F(2, 6) = 850.42</td>
<td>&lt;0.0001*</td>
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<tr>
<td></td>
<td>interactions</td>
<td>F(10, 43) = 877.84</td>
<td>&lt;0.0001*</td>
<td>F(8, 36) = 417.91</td>
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<td>Catalase activity</td>
<td>concentration</td>
<td>F(5, 13) = 570.13</td>
<td>&lt;0.0001*</td>
<td>F(4, 10) = 1128.63</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>day of cultivation</td>
<td>F(2, 6) = 1464.70</td>
<td>&lt;0.0001*</td>
<td>F(2, 6) = 1268.46</td>
<td>&lt;0.0001*</td>
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<tr>
<td></td>
<td>interactions</td>
<td>F(10, 43) = 520.85</td>
<td>&lt;0.0001*</td>
<td>F(8, 36) = 606.91</td>
<td>&lt;0.0001*</td>
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<td>Superoxide dismutase activity</td>
<td>concentration</td>
<td>F(5, 13) = 772.94</td>
<td>&lt;0.0001*</td>
<td>F(4, 10) = 707.79</td>
<td>&lt;0.0001*</td>
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<tr>
<td></td>
<td>day of cultivation</td>
<td>F(2, 6) = 2251.04</td>
<td>&lt;0.0001*</td>
<td>F(2, 6) = 902.64</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>interactions</td>
<td>F(10, 43) = 624.59</td>
<td>&lt;0.0001*</td>
<td>F(8, 36) = 1095.60</td>
<td>&lt;0.0001*</td>
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</table>

Note: * – significant differences within one of three variables — concentration of ferric citrate or cobalt (II) chloride, day of cultivation, or their interactions.
To establish the relationship between the processes of free radical damage to lipids and proteins, the activity of enzymes of the antioxidant system, the accumulation of *Rh. yavorovii* IMV B-7620 biomass and the concentration of ferric citrate or cobalt (II) chloride in the environment the factor analysis of principal components was performed. As a result of the analysis, the data were reduced, where 8 variables were combined into two factors, the variance of which according to the Kaiser and Cattell criteria was greater than 1 (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Salt of metal</th>
<th>Value</th>
<th>Eigenvalue</th>
<th>Total variance, %</th>
<th>Cumulative eigenvalue</th>
<th>Cumulative, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferric citrate</td>
<td>1</td>
<td>4.86</td>
<td>60.81</td>
<td>4.86</td>
<td>60.81</td>
</tr>
<tr>
<td>Cobalt (II) chloride</td>
<td>2</td>
<td>2.76</td>
<td>34.54</td>
<td>7.63</td>
<td>95.35</td>
</tr>
</tbody>
</table>

Analysis of the matrix of factor loadings of the studied indicators under the influence of ferric citrate revealed two latent factors that explain 95.4% of the total data variance, and under the influence of cobalt (II) chloride – 99.2% (Table 2). This result indicates that a large number of parameters that significantly affect the functionality of the system are taken into account.

Under the influence of ferric citrate on *Rh. yavorovii* IMV B-7620 cells, the first latent factor included diene conjugates, TBARS, carbonyl groups in proteins, which are closely linked by a direct bond and inversely related to the content of lipid hydroperoxides and catalase activity (Table 3, Fig. 8a). The variance of the first factor is 51.5%, which indicates the high importance of these indicators. The second latent factor included the duration of bacterial cultivation, biomass accumulation, content of lipid hydroperoxides, catalase and superoxide dismutase activity. In particular, duration of cultivation of bacteria, biomass accumulation, and superoxide dismutase activity are inversely related to lipid hydroperoxide content and catalase activity. The variance of this factor is 43.8%.

### Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ferric citrate</th>
<th>Cobalt (II) chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor 1</td>
<td>Factor 2</td>
<td>Factor 1</td>
</tr>
<tr>
<td>Day</td>
<td>–</td>
<td>0.978</td>
</tr>
<tr>
<td>Biomass</td>
<td>–</td>
<td>0.845</td>
</tr>
<tr>
<td>Lipid hydroperoxides</td>
<td>–0.817</td>
<td>0.965</td>
</tr>
<tr>
<td>Diene conjugates</td>
<td>0.997</td>
<td>–</td>
</tr>
<tr>
<td>Thiobarbiturate reactive species</td>
<td>0.982</td>
<td>–</td>
</tr>
<tr>
<td>Carbonyl groups in proteins</td>
<td>0.961</td>
<td>–</td>
</tr>
<tr>
<td>Catalase</td>
<td>–0.692</td>
<td>0.713</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>–0.953</td>
<td>0.861</td>
</tr>
<tr>
<td>Exp. Var</td>
<td>4.12</td>
<td>3.51</td>
</tr>
<tr>
<td>Pp. Tol%,</td>
<td>51.5</td>
<td>43.8</td>
</tr>
</tbody>
</table>

Under the influence of cobalt (II) chloride, the first latent factor included the content of lipid hydroperoxides, carbonyl groups in proteins, as well as catalase and superoxide dismutase activities, which are inversely related to bacterial biomass accumulation (Table 3, Fig. 8b). The variance of this factor is 49.0%. The obtained results show that under the influence of cobalt (II) chloride the influence of both factors on the system is equal and important.

### Fig. 8

Distribution of factor loadings of the studied parameters of *Rhodopseudomonas yavorovii* IMV B-7620 cells under the influence of different concentrations of ferric citrate (a) and cobalt (II) chloride (b): LH – lipid hydroperoxides, DC – diene conjugates, TBARS – thiobarbiturate reactive species, CG – carbonyl groups in proteins, SOD – superoxide dismutase

### Discussion

The effect of metal compounds on the cells of microorganisms is due to the chemical properties of ions, which are formed as a result of the dissociation of these compounds. Because sodium citrate was used as a carbon source for the growth of *Rh. yavorovii* IMV B-7620 in the study, the citrate ion concentration was left unchanged (12 mM) for the study of oxidative processes under the influence of ferric citrate, which led to the dissociation of these compounds. Because sodium citrate was used as a carbon source for the growth of *Rh. yavorovii* IMV B-7620 in the study, the citrate ion concentration was left unchanged (12 mM) for the study of oxidative processes under the influence of ferric citrate, which led to the dissociation of these compounds.

Despite the large number of studies on the effects of cobalt ions on the cells of microorganisms, the mechanism of damaging effects of cobalt ions is debatable. It is known that cobalt ions are involved in the formation of ROS because they are in the redox states, mainly Co (II) and Co (III), but also under certain circumstances Co (I) (Barras & Fontecave, 2011). In addition to catalyzing the reactions in which ROS are formed, cobalt ions affect the homeostasis of iron and sulfur ions, disrupt the organization of FeS clusters in enzymes and the electron transport chain, have genotoxic effects, etc. (Barras & Fontecave, 2011; Kumar et al., 2017; Liu et al., 2020). Kumar et al. (2017) found that the toxicity of cobalt ions to *Escherichia coli* BW25113 is due to ROS-independent DNA damage, decelerating the replication fork progression, inhibition of the activity of enzymes involved in the SOS response, in particular inhibition of RecBCD exonuclease functions to suppress SOS response. In response to the stress caused by cobalt ions, the cells of sulfur-reducing bacteria *Geobacter sulfurreducens*, that accumulate significant concentrations of cobalt ions, undergo extensive transcriptional reprogramming, during which, along with overexpression of metal efflux systems from cytoplasm and periplasmatic space, specific histidine kinases and transcriptional regulators of metabolism, there is an increase in the level of transcripts of periplasmic glutaredoxin, which restores oxidized cysteine residues in proteins, and digenic cytochrome c peroxidase, which restores H2O2 (Duday et al., 2020). One of the reasons for the toxicity of LiCoO2 nanoparticles to *Bacillus subtilis* is the accumulation of cobalt ions in cells, which is accompanied by ROS generation and increased expression of genes whose products provide antioxidant protection, transport and DNA repair (Garti et al., 2021).
In cells of *Rh. yavorovii* IMV B-7620 at high concentrations of ferric citrate and all studied concentrations of cobalt (II) chloride there is an increase in the content of diene conjugates and lipid hydroperoxides, indicating a free radical mechanism of damage to cellular lipids of *Rh. yavorovii* IMV B-7620 under the influence of these salts. Under these conditions, oxidative damage to protein molecules also occurs, which reflects the increase in the content of carboxyl groups in protein molecules. After increasing the duration of cultivation of bacteria, the content of carboxyl groups in proteins decreases, which may be due to proteolysis of oxidized proteins or, conversely, the formation of a significant number of carboxyl groups in protein molecules, and makes their detection impossible (Gruke et al., 2004). To determine the reasons for reduction in the content of carboxyl groups in proteins of *Rh. yavorovii* IMV B-7620 under the influence of ferric citrate during different phases of culture growth, further studies are needed. However, the formation of these groups in proteins under the influence of heavy metal compounds is an indicator of free radical damage to the proteins of *Rh. yavorovii* IMV B-7620. Catalase and superoxide dismutase provide neutralization of the formed free radical compounds in *Rh. yavorovii* IMV B-7620 cells under the influence of ferric citrate and cobalt (II) chloride. Bacteria of the genus *Rhodopseudomonas* are known to have two types of catalases: manganese (WP_114358207.1) and catalase/peroxidase (WP_092615581.1). There are *Rhodopseudomonas palustris* (ADU42423.1), *Rh. pseudopalustris* (SEP24300.1), *Rh. pentosanotetraoxigenes* (RED38428.1), *Rh. thermotolerans* (REGEO00313.1), *Rh. rhodocyclus* (WP_1844258921.1) amino acid catalase sequences in GenBank. Cu/Zn (WP_114359541.1) and Fe/Mn (WP_013503506.1) superoxide dismutases were detected in cells of the bacteria of the genus *Rhodopseudomonas*. Amino acid sequences of Fe/Mn-SOD *Rh. palustris* (ADU45394.1, WP_011406573.1, WP_011459508.1, WP_012495261.1, WP_011665231.1, WP_011504010.1, ACP004161.1) and Cu/Zn-SOD *Rh. palustris* (RIA03292.1, WP_011437085.1, WP_011665231.1, WP_011504010.1, ACP004161.1) and catalase/peroxidase (WP_092615581.1).

The studied concentrations of ferric citrate and proteins (II) chloride caused free radical damage to lipids and proteins of *Rh. yavorovii* IMV B-7620. As a result of two-way ANOVA we found that under the influence of ferric citrate statistically significant changes in biomass accumulation, lipid hydroperoxides and thiobarbiturate reactive species content, superoxide dismutase activity were predetermined by increasing the concentration of metal salts as well as increasing the duration of cultivation of bacteria, while the content of diene conjugates and catalase activity changed with increasing duration of cultivation. Under the influence of cobalt (II) chloride, statistically significant changes in all studied indicators were found both due to the increase in the concentration of metal salts and with increasing duration of bacterial cultivation. The studied parameters of *Rh. yavorovii* IMV B-7620 cells under the influence of ferric citrate and cobalt (II) chloride are combined into two factors, that explain 95.4% and 99.2% of the total data variance, respectively. Under the influence of ferric citrate, the first latent factor included diene conjugates, thio-barbiturate reactive species, carboxyl groups in proteins, which are closely linked by a direct bond and inversely related to the content of lipid hydroperoxides and catalase activity. The second latent factor included duration of cultivation of bacteria, biomass accumulation, and superoxide dismutase activity, which are inversely related to lipid hydroperoxides content and catalase activity. Under the influence of cobalt (II) chloride, the first latent factor included the content of lipid hydroperoxides, carboxyl groups in proteins, as well as catalase and superoxide dismutase activities, which are inversely related to bacterial biomass.

**References**


