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Taxonomic Position of Copper-resistant Microorganisms of the Extreme Ecosystems

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Background

Environmental contamination by copper

Copper pollution of the environment destroys natural ecosystems. The particular sources of copper contamination are industrial wastewater, metal mines, and tailing sites

<u>The aim of the work was:</u>

-to predict theoretically and confirm experimentally the possibility of microorganisms to grow at the presence of Cu(II) at high concentrations (up to 1.0 M);

- to determine the main pathways of microbial interaction with toxic Cu(II) compounds;

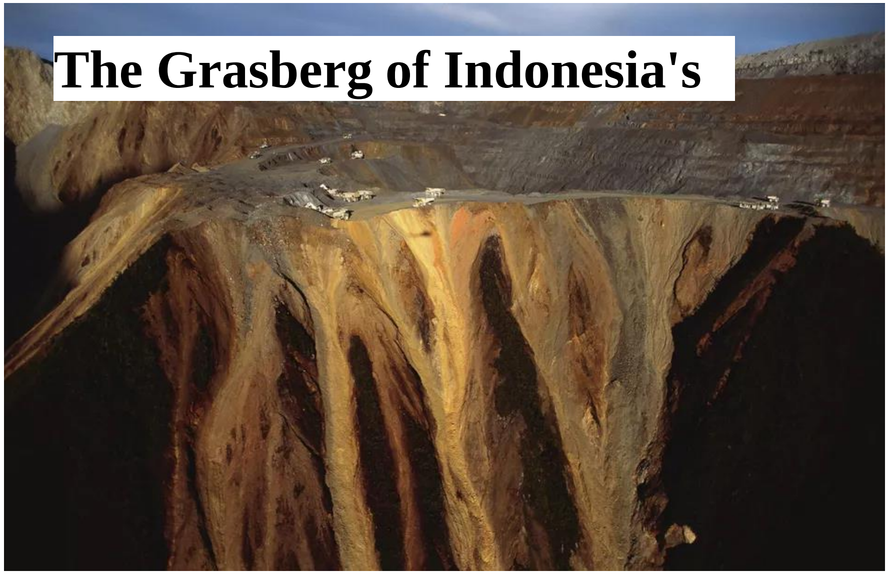
to isolate industrially promising copper resistant strains from natural ecosystems and determine their taxonomic position.

Environmental Pollution by Copper

Copper mines as a sources of toxic copper compounds contamination

Cerro Verde mine in Peru

contains 13 million metric tons of copper



The Grasberg of Indonesia's

Bingham Canyon in USA

Los Bronces in central Chile

Environmental Pollution by Copper

Excessive use of pesticides

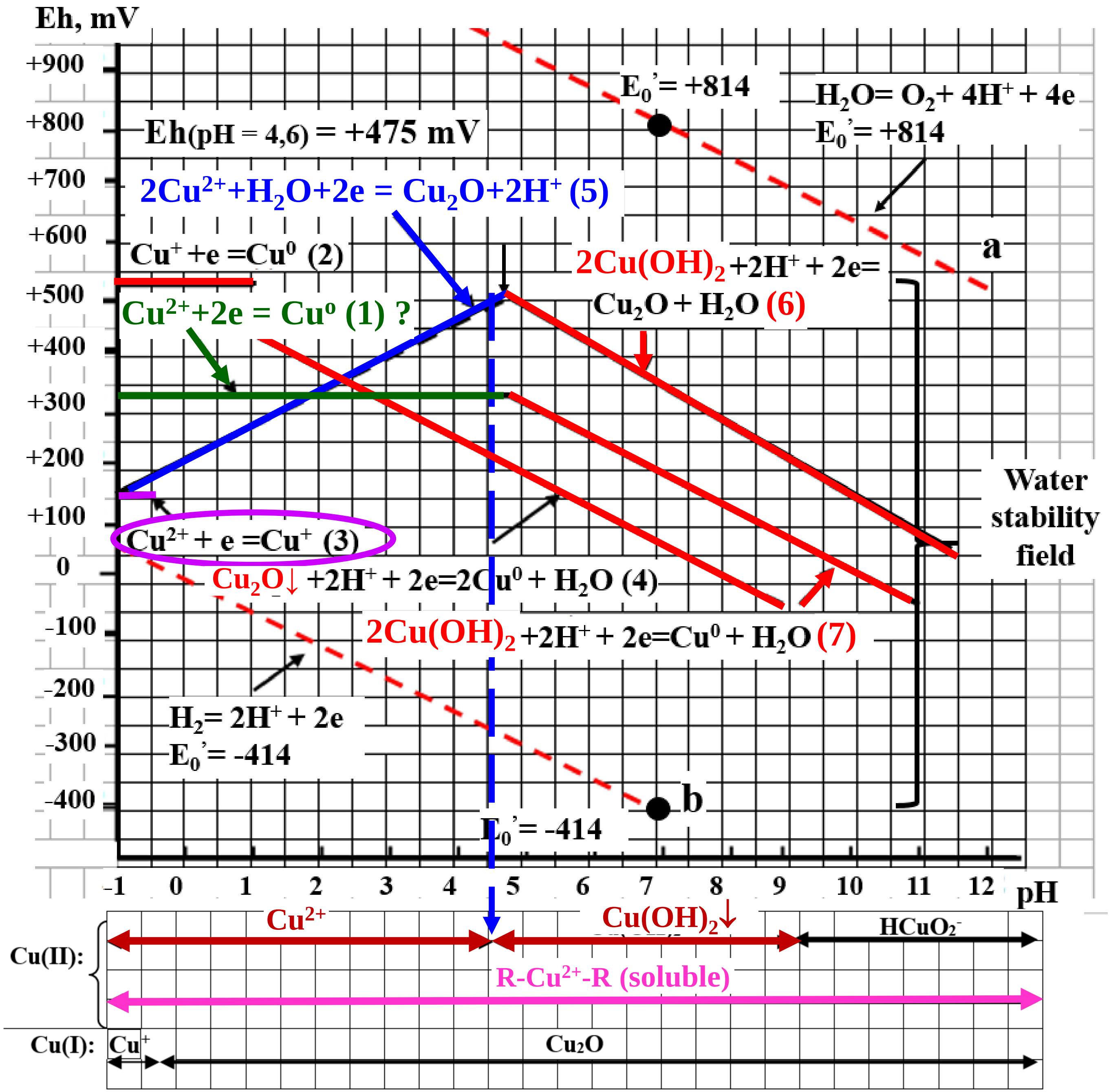
Zambia pollution by copper

Lemhi County, Idaho, US

Outline of The Presentation

- ✓ The characterization of theoretically positions of thermodynamic prognosis of the interaction of microorganisms with copper(II) compounds;
- ✓ The experimental confirmation of capability of microorganisms to grow at the presence of Cu(II) at high concentrations (up to 1.0 M);
- ✓ Determination of taxonomic position and genome sequencing of super resistant to Cu(II) microorganisms;
- ✓ The specific copper resistant genes screening in genomes of isolated strains.
- ✓ The experimental confirmation of mechanisms of microbial interaction with Cu(II);

Thermodynamic Prognosis of Microbial Interaction with Cu(II)

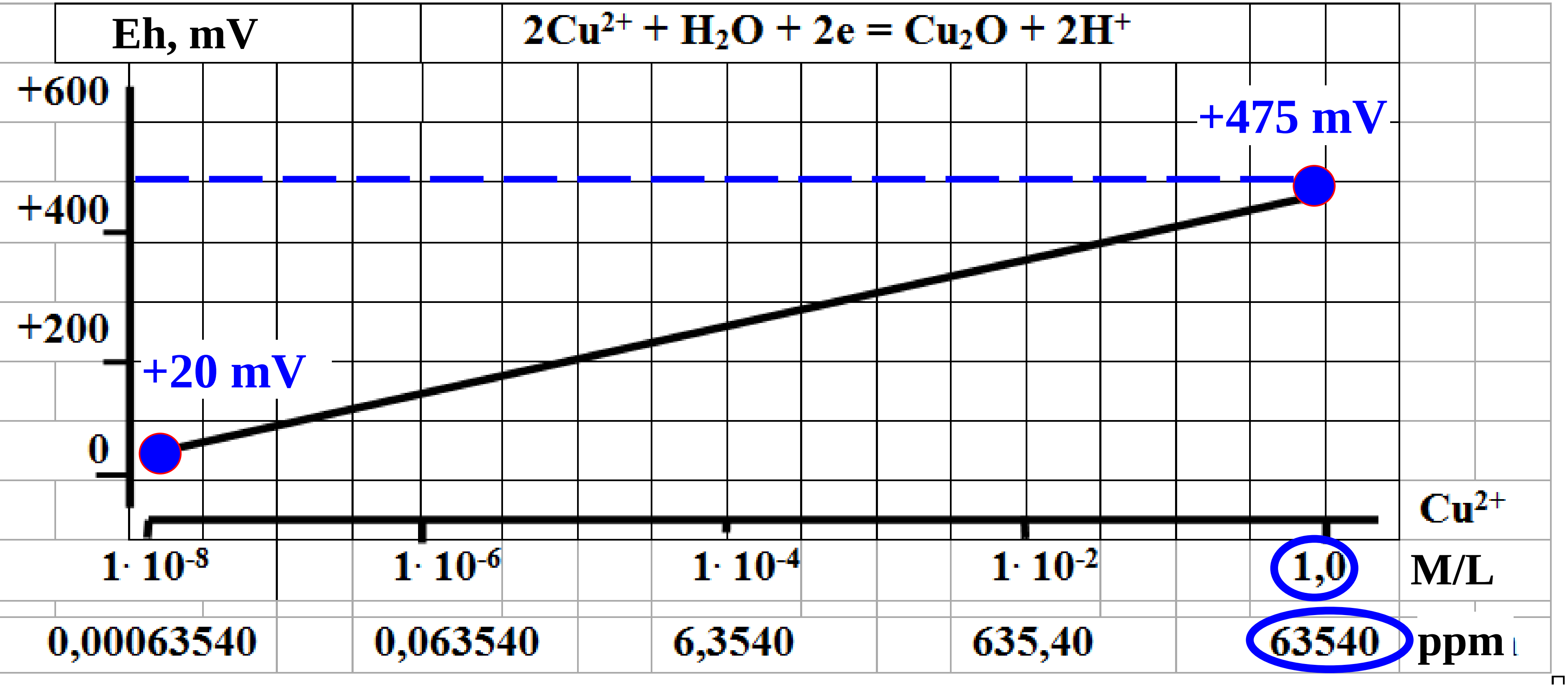


Thermodynamically available pathways of microbial interaction with Cu(II) compounds:

1. Cu^{2+} reduction to $Cu_2O\downarrow$
2. Cu^{2+} immobilization
3. $Cu(II)\downarrow$ mobilization

Thermodynamic confirmation of possibility of microbial grows at super high Cu^{2+} concentrations

The value of the standard redox potential Cu^{2+} (E_o`) is located in the zone of thermodynamic stability of water (from - 414 to + 814 mV) in the range of concentrations from $1 \cdot 10^{-8}$ to 1,0 M/L



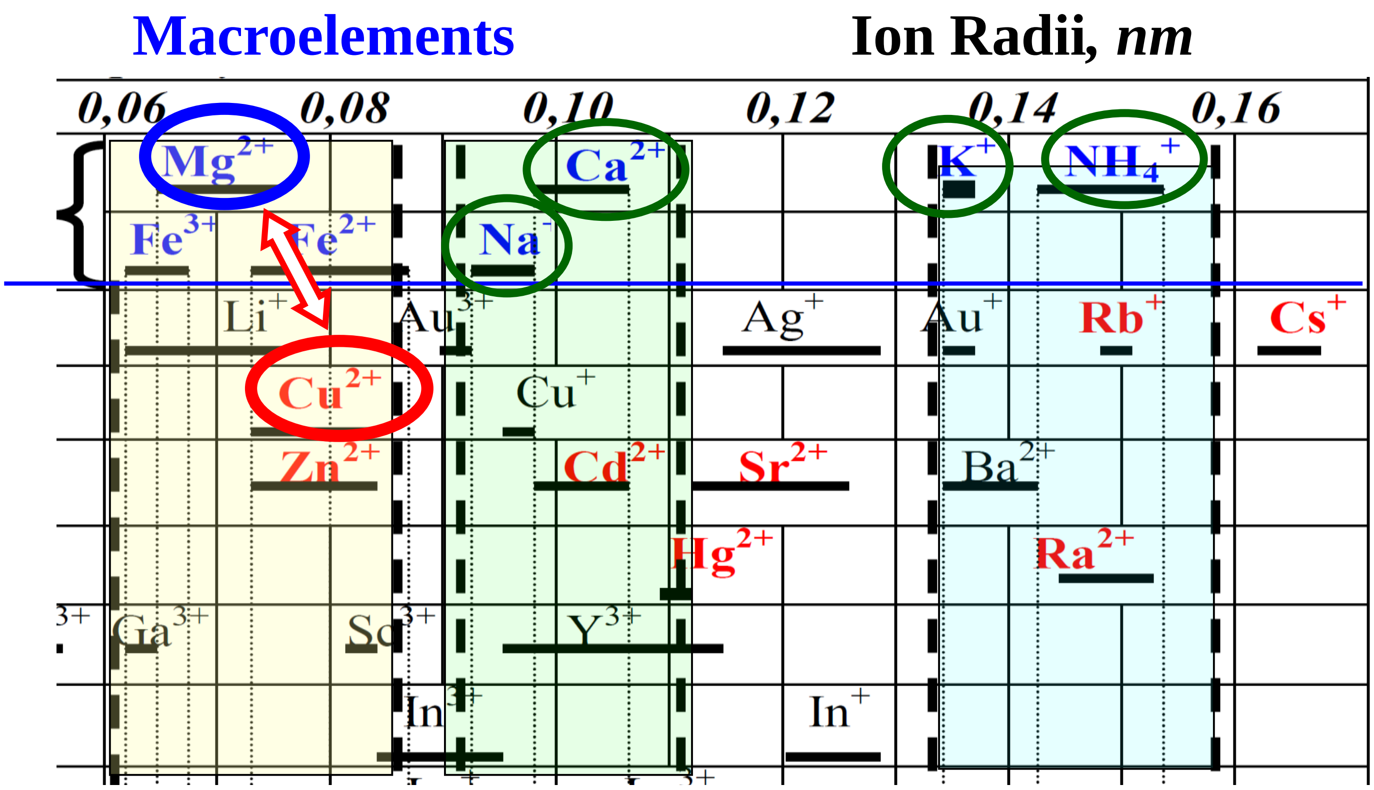
Changing the RedOx Potential in The Range of Concentration of Cu(II) from $1 \cdot 10^{-8}$ to 1.0 M/L

So we made an attempt to isolate super resistant to Cu^{2+} microorganisms

Active transport of metals inside microorganisms due to stereochemical analogy of toxic metals and macroelements (Mg^{2+}, K^{+}, NH_4^{+} etc.)

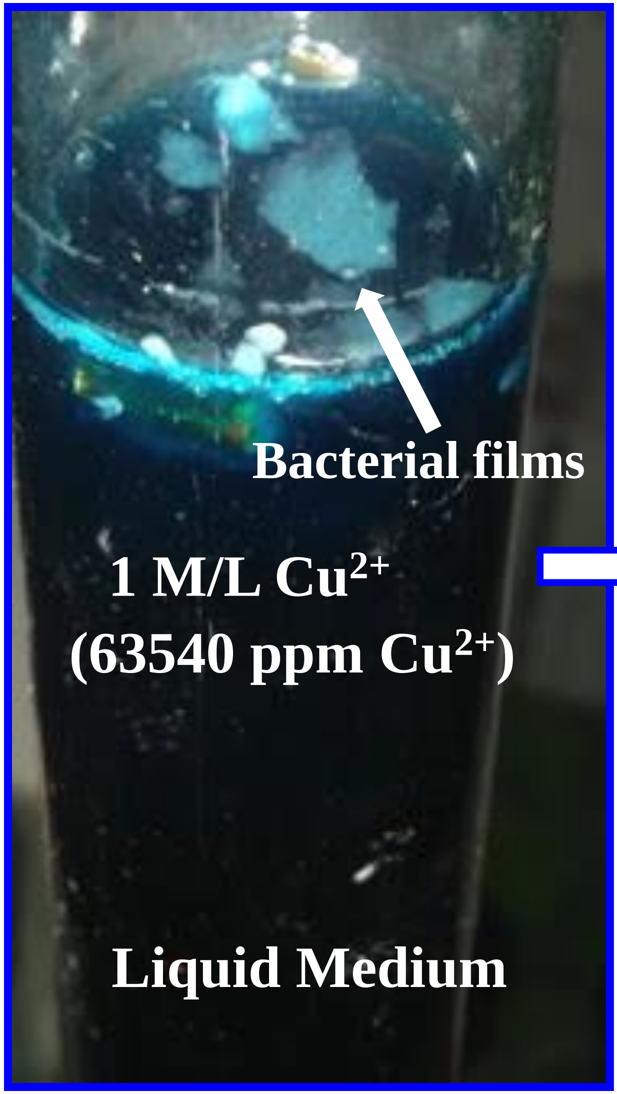
In this case stereochemical analogy – is the proximity of ion radii of Mg^{2+} and Cu^{2+} (is about 0,075 nm)

We expect that microbial transport systems will "mistake" and transport Cu^{2+} inside cells together with Mg^{2+}

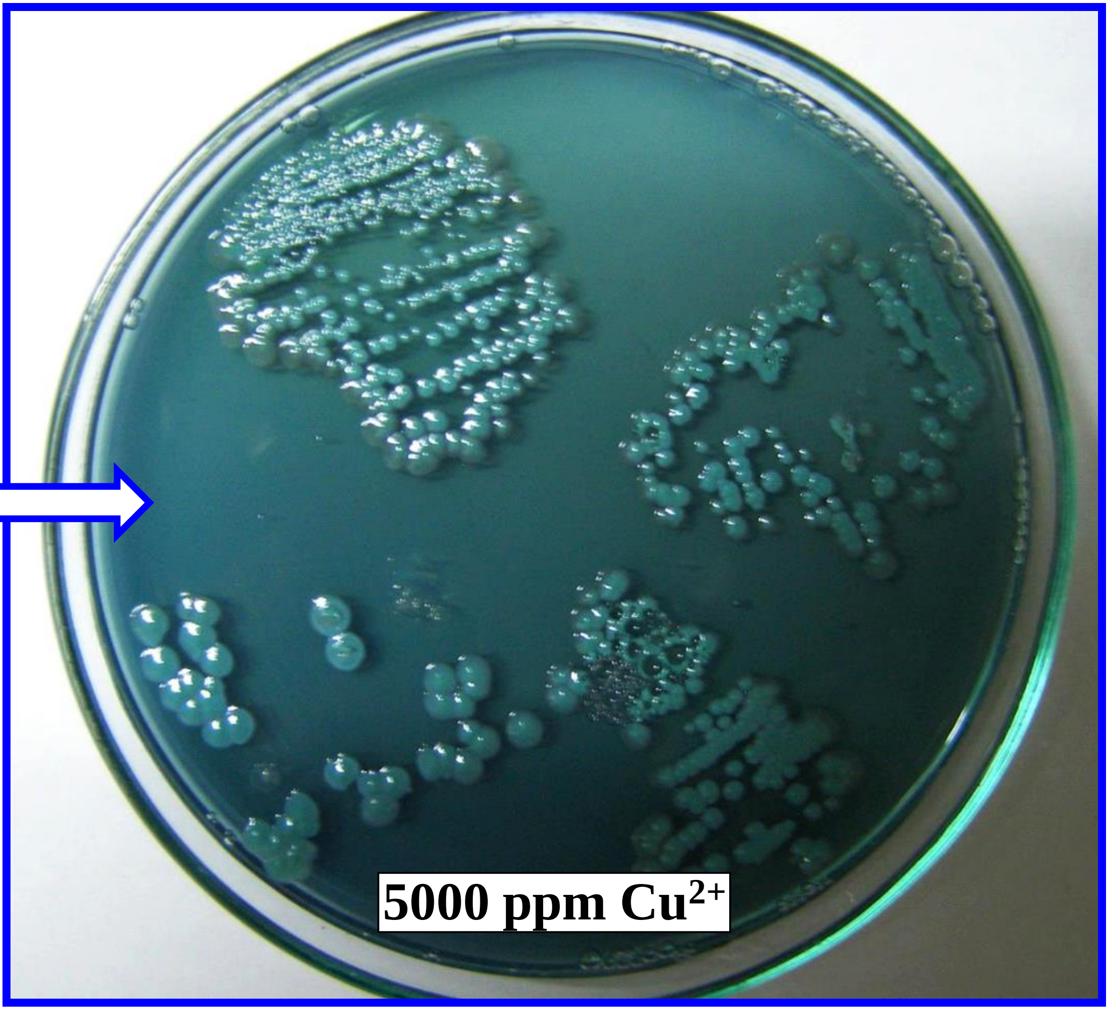


Experimental confirmation of Thermodynamic Prognosis

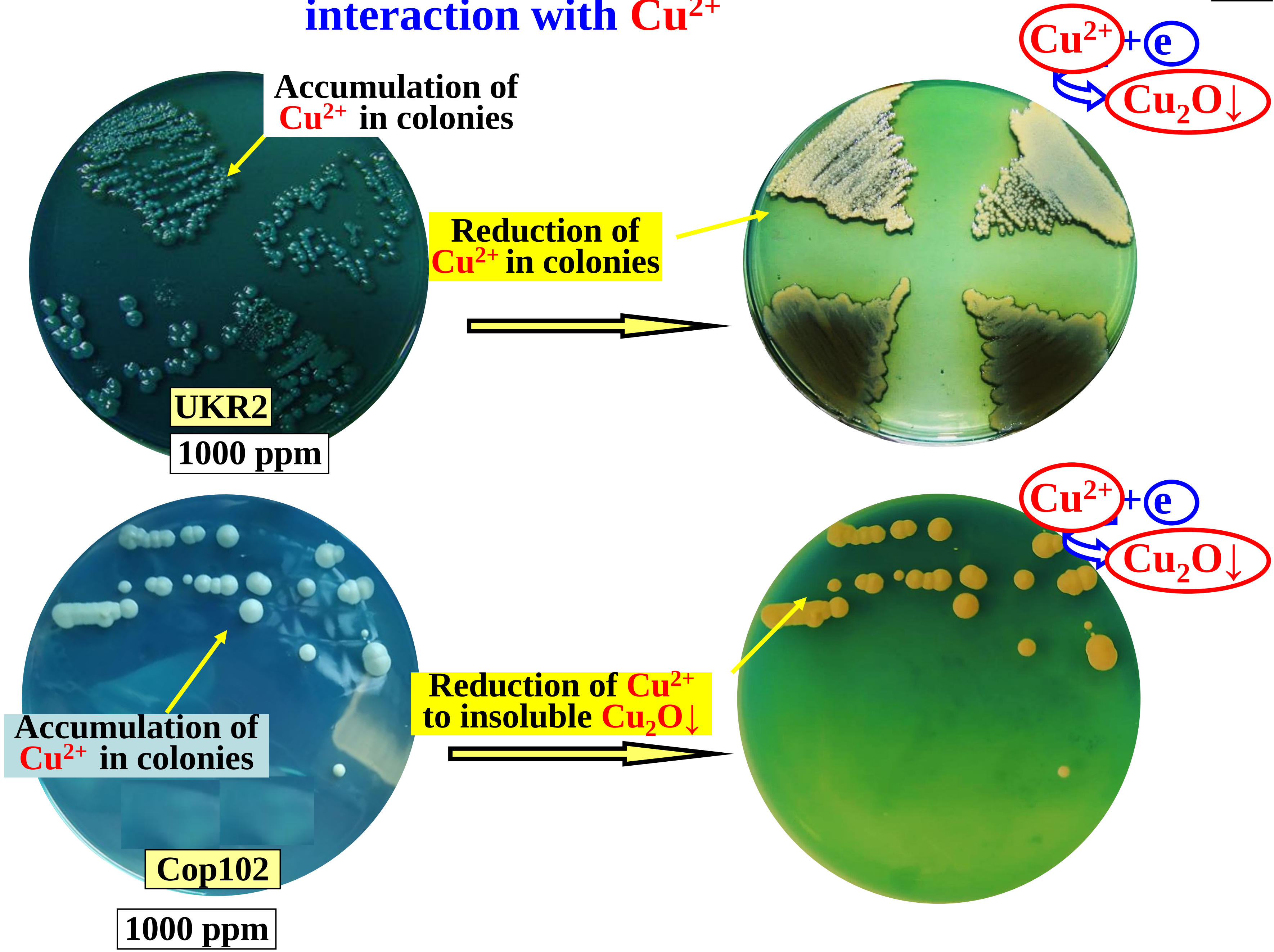
Growth and isolation of super resistant to Cu^{2+} strains



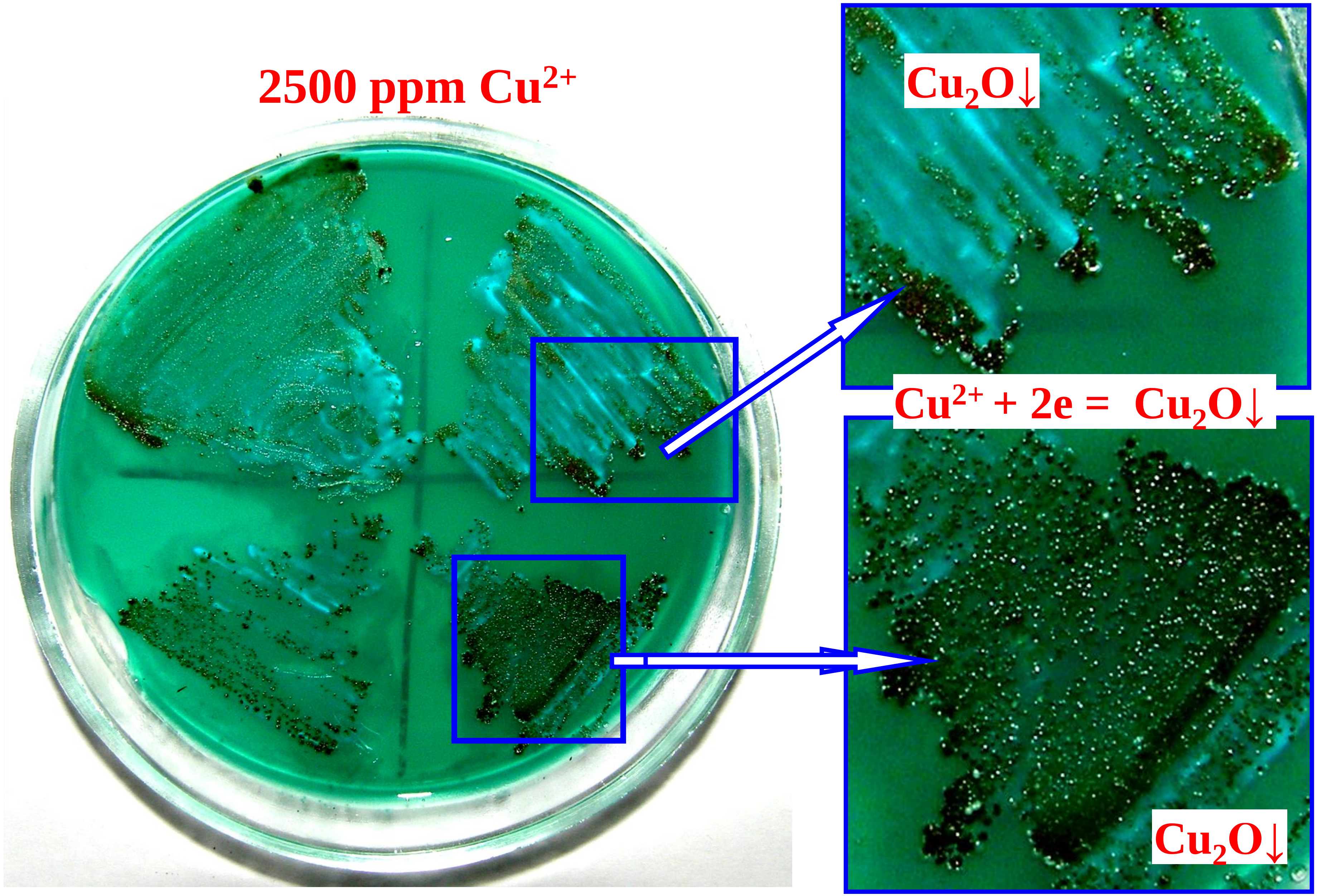
Isolation of Cu^{2+} resistant Strains



Experimental confirmation of types of microbial interaction with Cu^{2+}

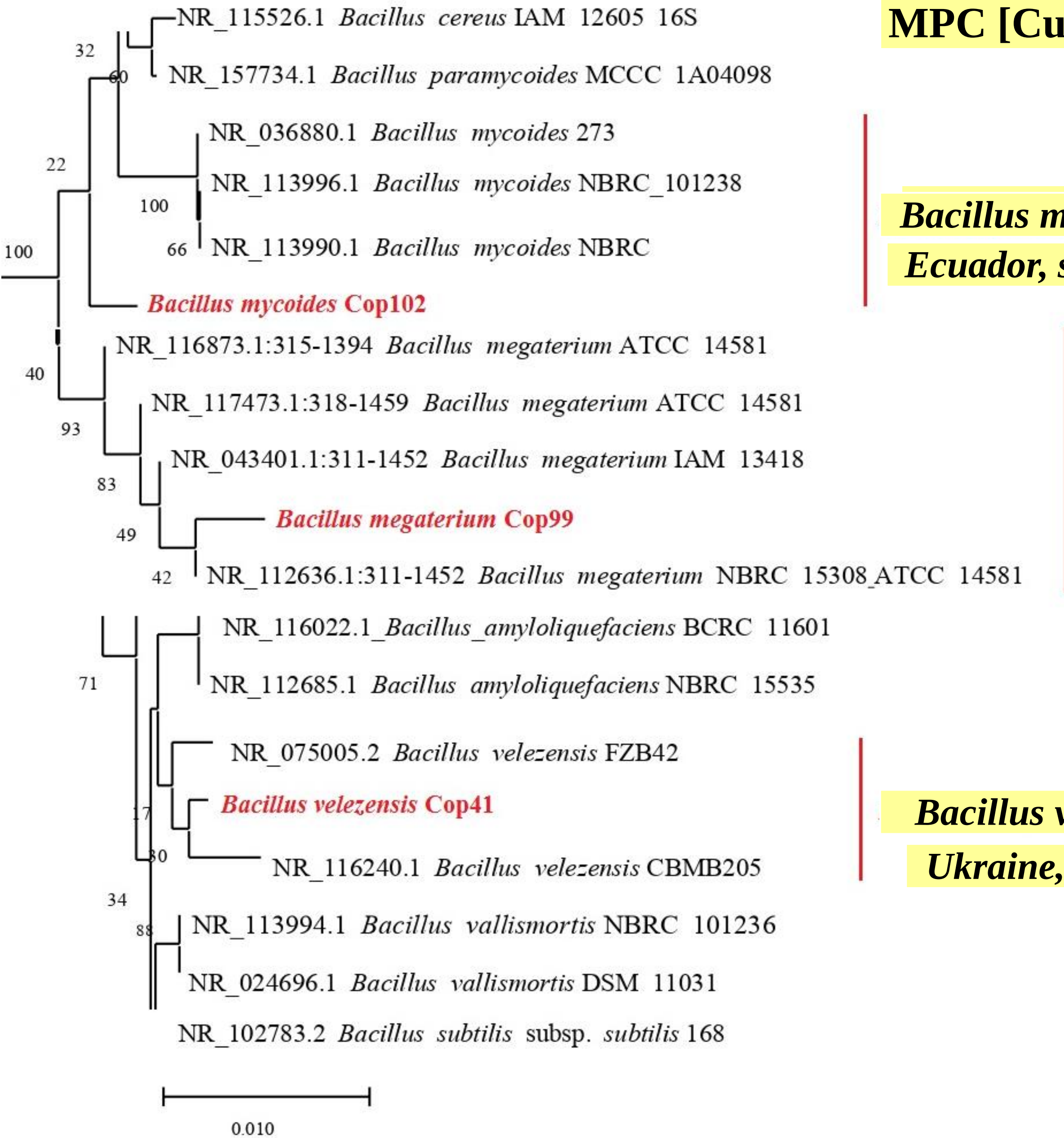


Reduction of Cu^{2+} to insoluble Copper(I) Oxide by UKR4



Taxonomic Position of Super Resistant to Copper(II) Microorganisms

MPC [Cu^{2+}] = 63 546 ppm = 1 M/L



***Bacillus mycoides* Cop102**

Ecuador, soil

***Bacillus megaterim* Cop99**

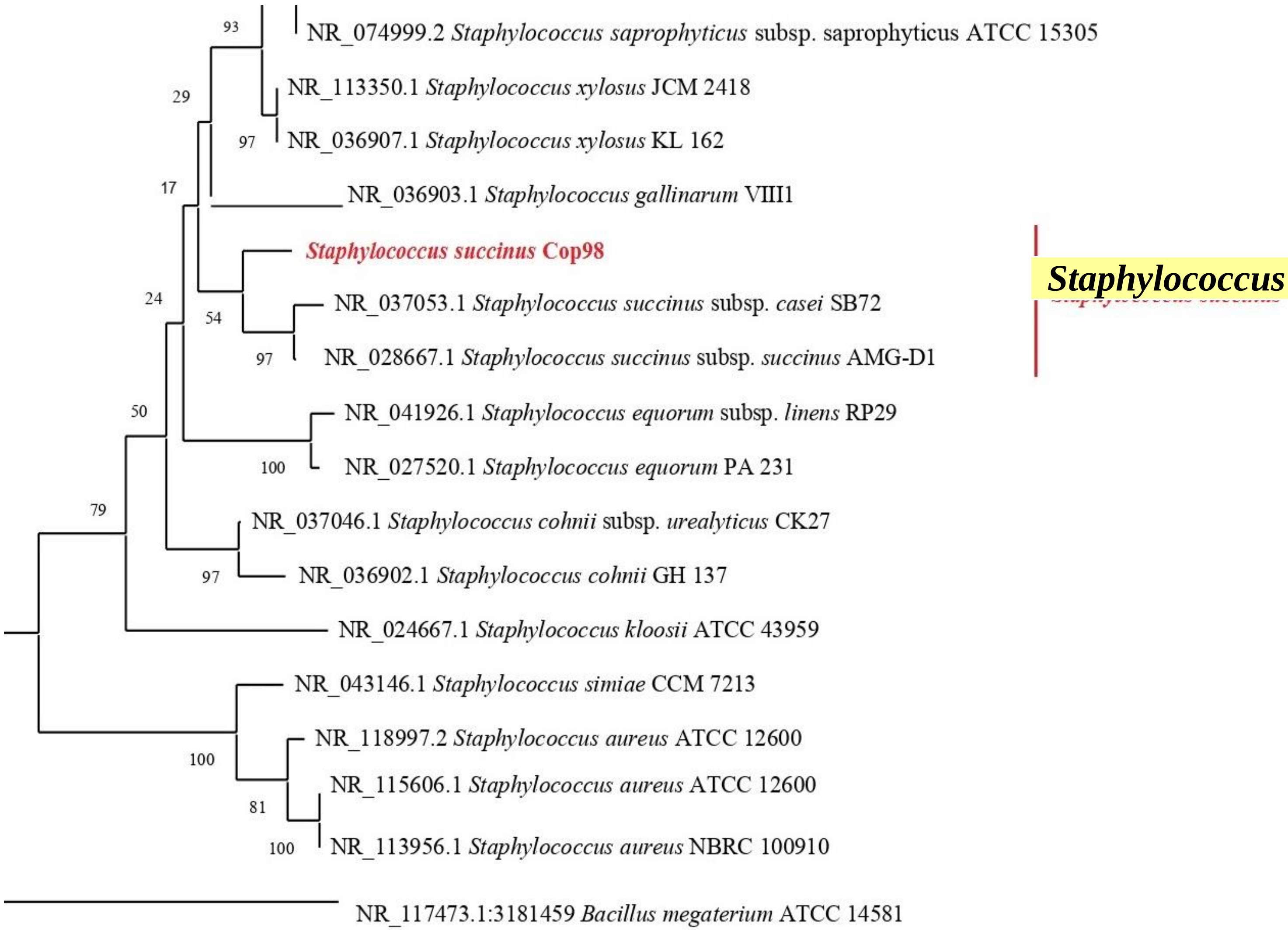
Ukraine, «Optymistychna» cave, clay

***Bacillus velezensis* Cop41**

Ukraine, soil

Taxonomic Position of Super Resistant to Copper(II) Strain from Dead Sea

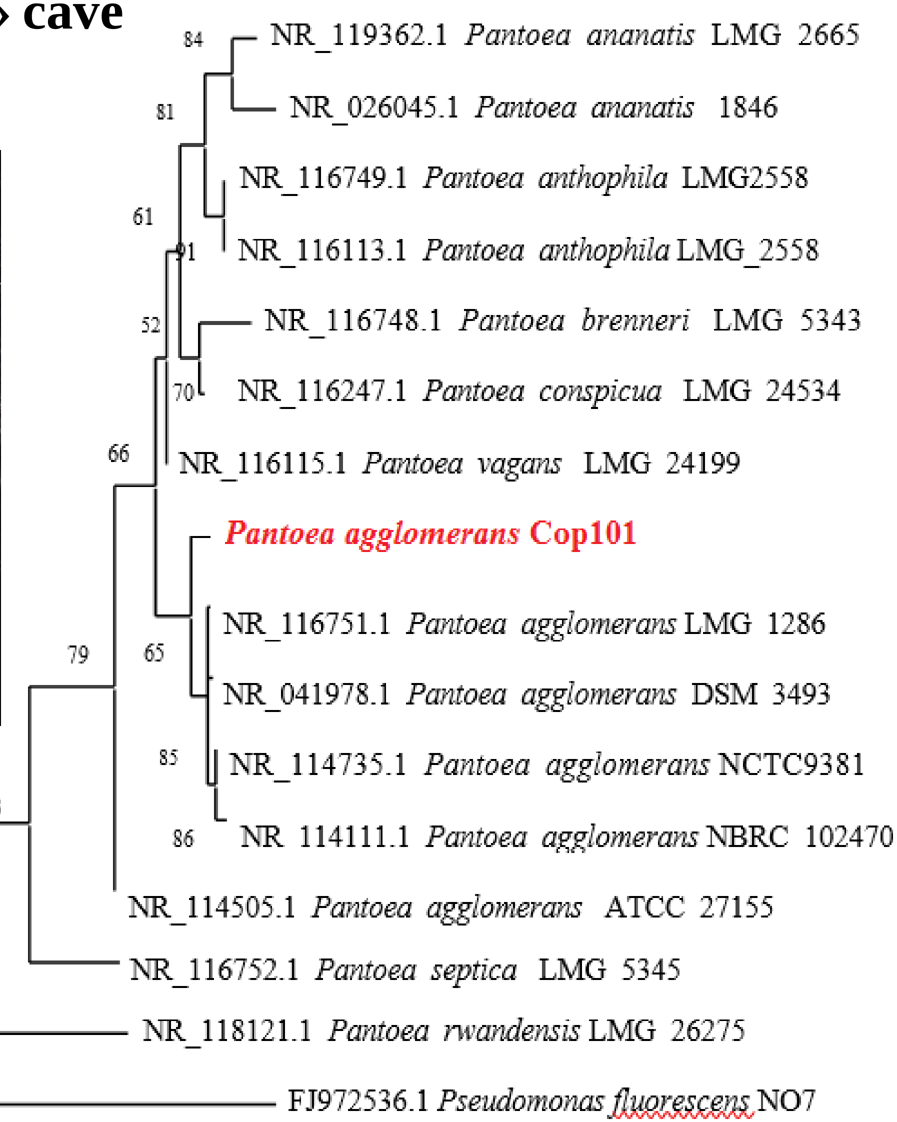
MPC [Cu^{2+}] = 63 546 ppm = 1 M/L



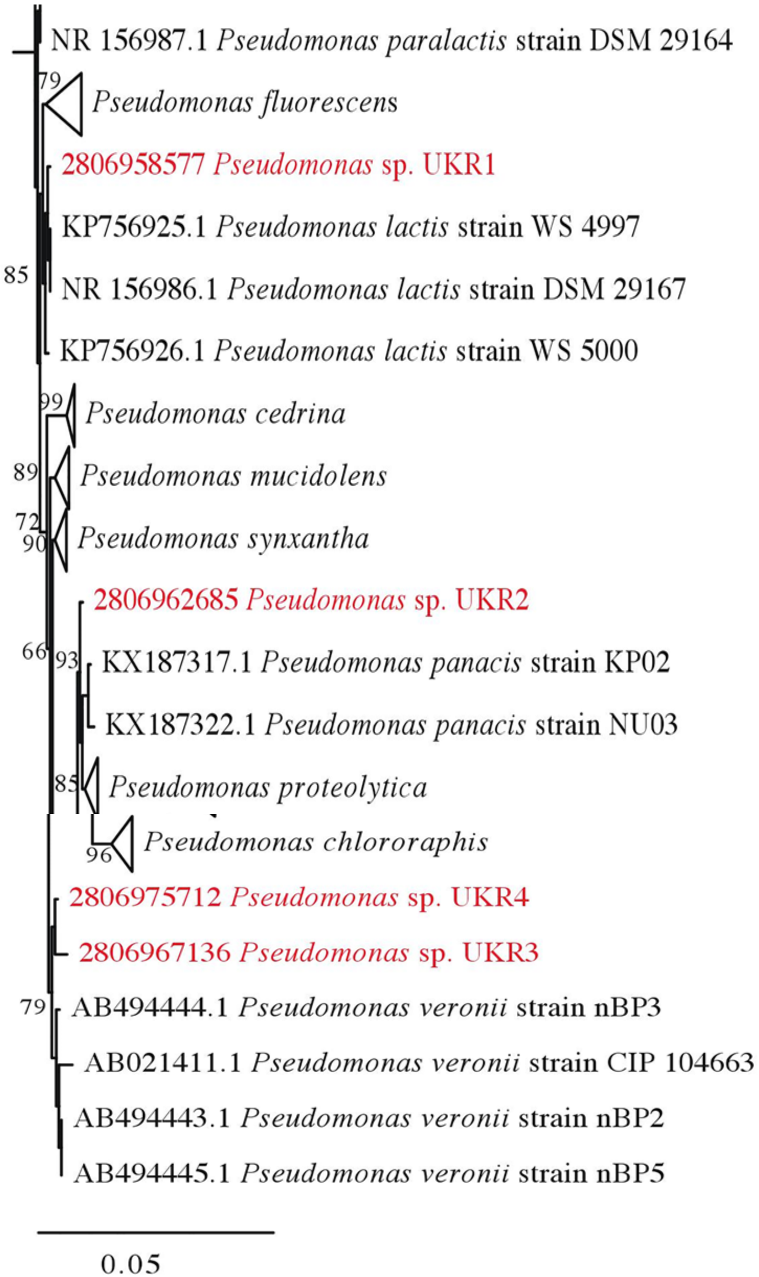
***Staphylococcus succinus* Cop98**

Taxonomic Position of Super Resistant to Copper(II) Strain from «Atlantida» cave

MPC [Cu^{2+}] = 2400 ppm



Taxonomic Position of Super Resistant to Copper(II) Microorganisms



MPC [Cu^{2+}] = 63 546 ppm = 1 M/L

Pseudomonas lactis **UKR1**

Genome accession number VWXW00000000

Ukraine, Kyiv region, soil

Pseudomonas panacis **UKR2**

Genome accession number WXV00000000

Ukraine, Kyiv region, soil

Pseudomonas veronii **UKR3**

Genome accession number VWXU00000000

Arctic, soil

Pseudomonas veronii **UKR4**

Genome accession number VWXT00000000

Antarctic, soil

The Screening of Specific Copper Resistant Proteins in Genomes of Isolated *Pseudomonas* Strains

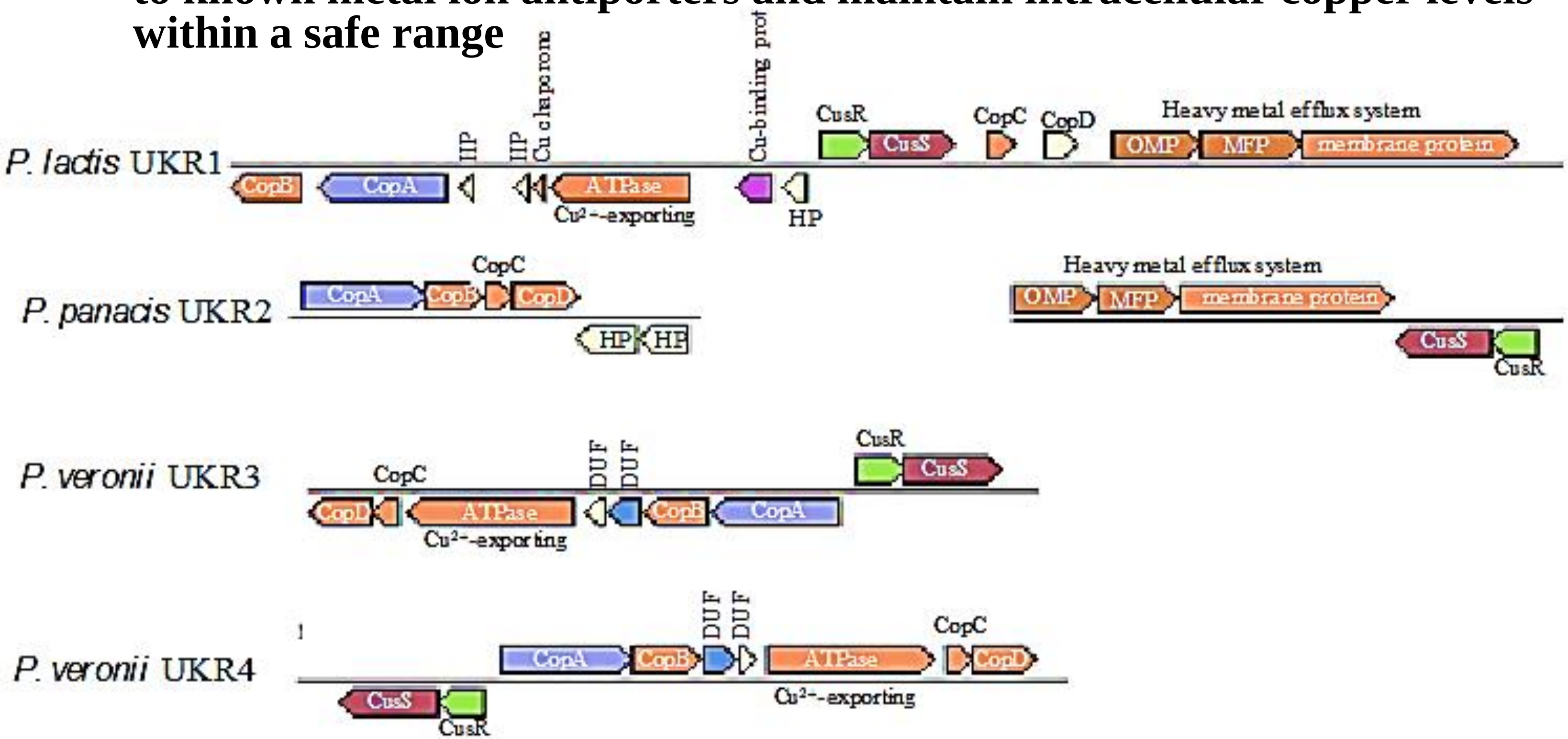
CopA — Copper binding protein (periplasmic multicopper oxidase) uses ATP to pump Cu^{+} and Cu^{2+} across cell membranes.

CopB — Outer membrane protein or P-type ATPase is exhibite high copper transport activity. CopB exports Cu^{+} and Cu^{2+} from the cytoplasm.

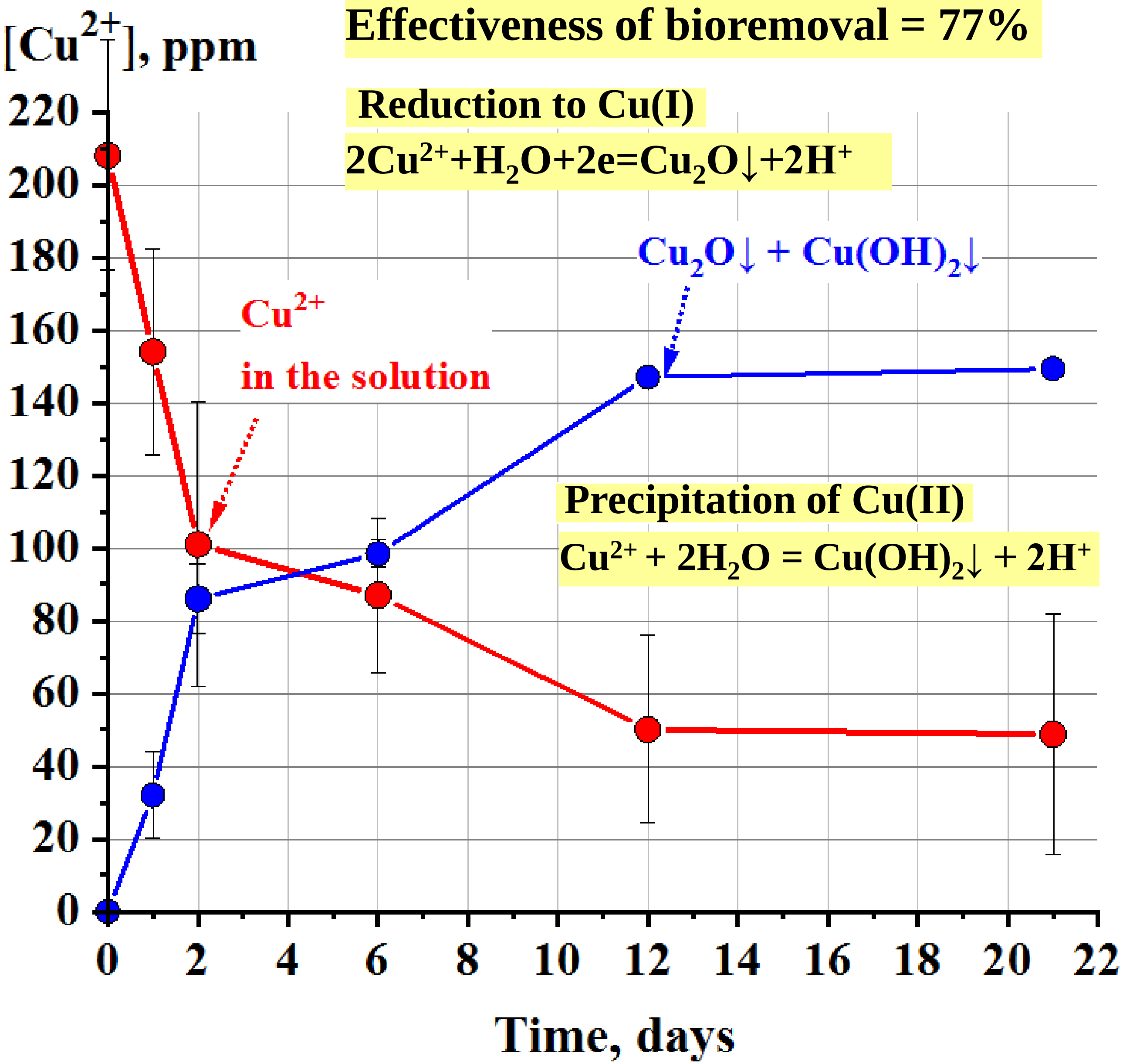
Cop D — Internal membrane protein executes copper uptake to the periplasm.

CopZ — Chaperone that serves for the intracellular sequestration and transport of Cu^{2+}. Delivers $Cu^{2+}$to the copper-transporting ATPase CopA.

CusRS — Chromosomal two-component genes system that encode proteins homologous to known metal ion antiporters and maintain intracellular copper levels within a safe range

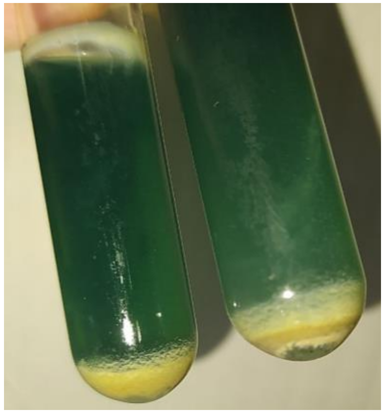


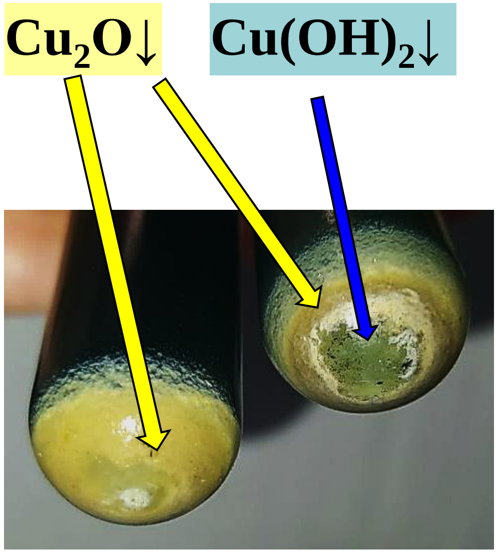
Bioremoval of Cu^{2+} by *Pseudomonas lactis* UKR1



LB + 5 ppm $C_6H_{12}O_6$

200 ppm Cu(II)



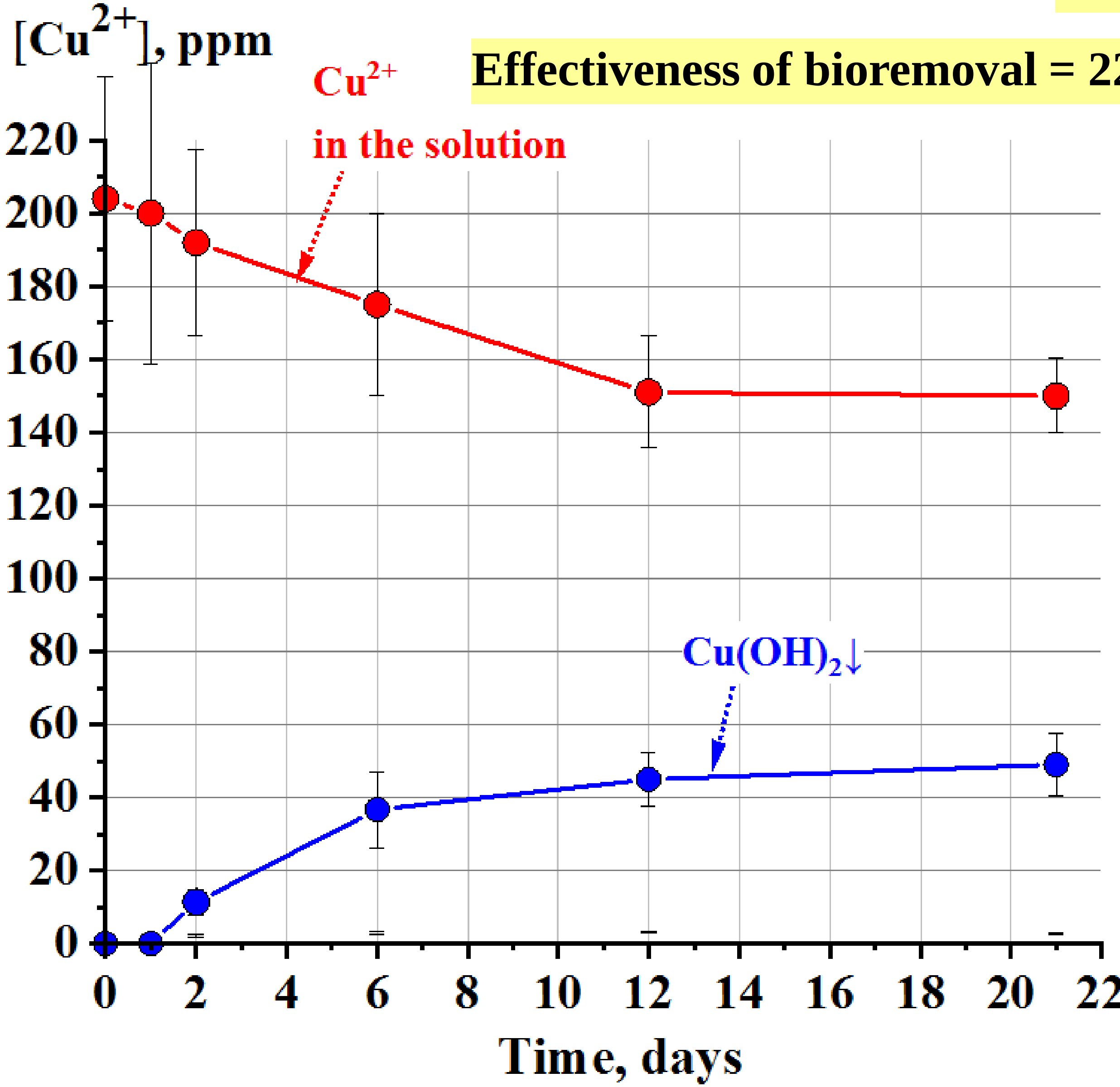


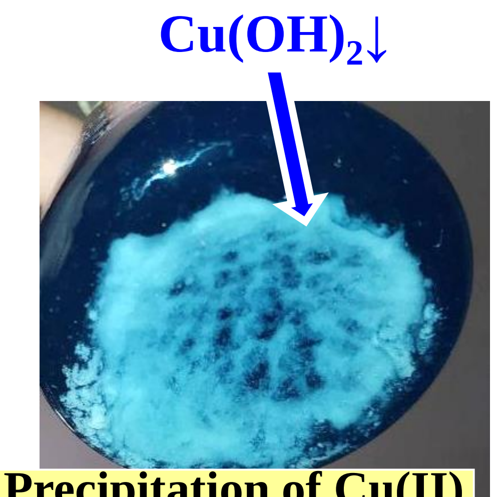
Immobilization of Cu^{2+} by *Pseudomonas lactis* UKR1

Mineral medium with citrate

200 ppm Cu(II)

Effectiveness of bioremoval = 22%





Precipitation of Cu(II)

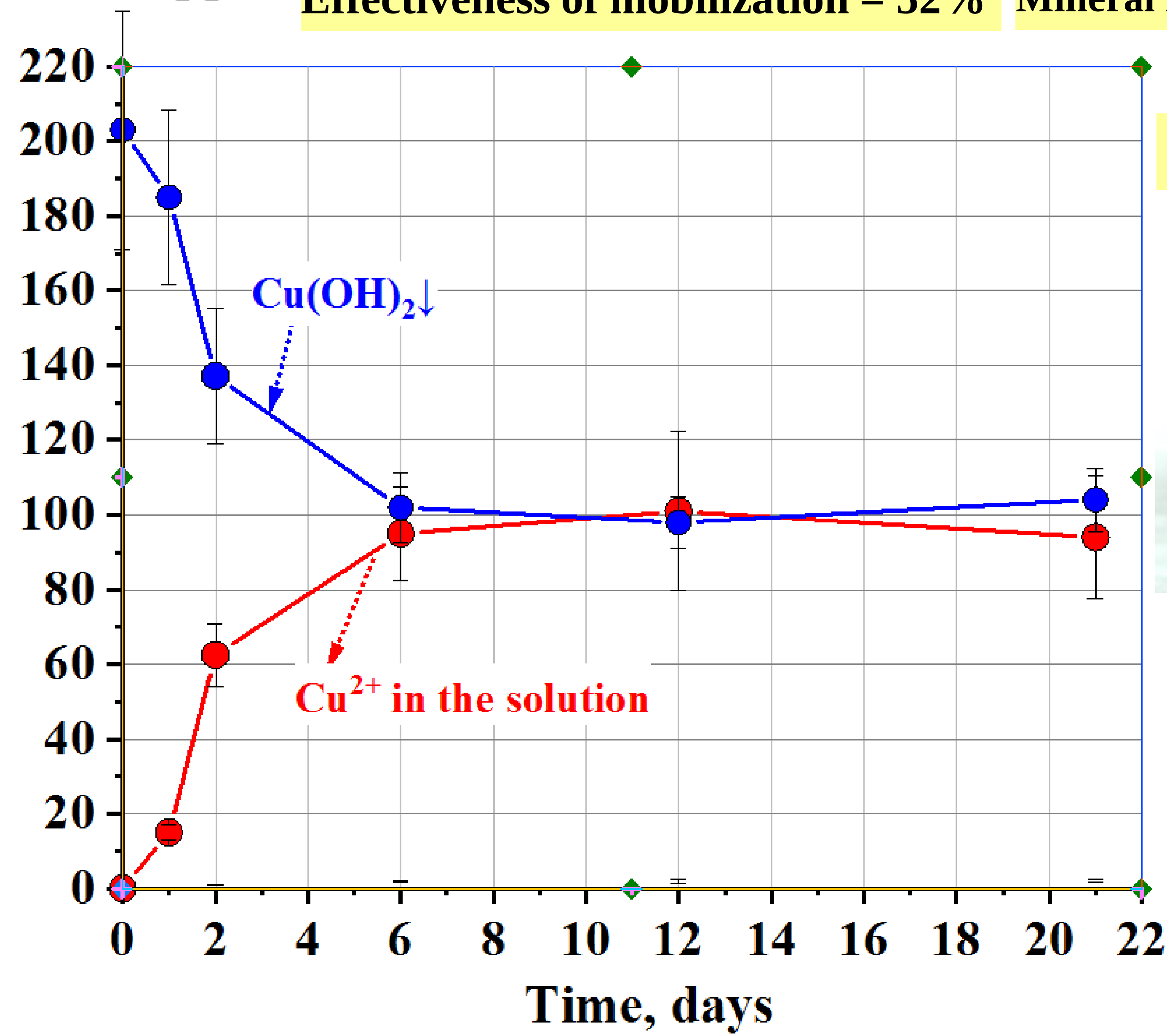
$Cu^{2+} + 2H_2O = Cu(OH)_2\downarrow + 2H^+$

Mobilization of Cu^{2+} by *Pseudomonas lactis* UKR1

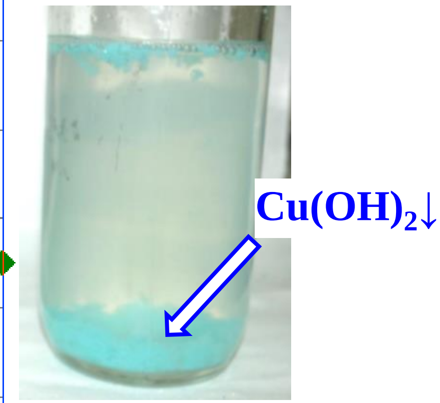
Effectiveness of mobilization = 52%

Mineral medium with $C_6H_{12}O_6$
200 ppm Cu(II)

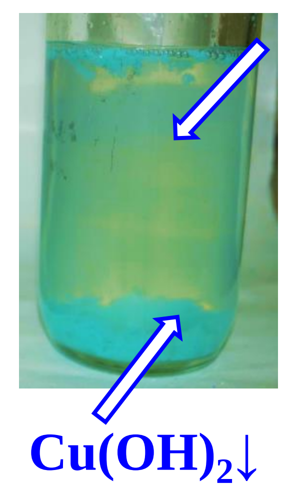
$[Cu^{2+}]$, ppm



Mobilization of Cu(II) by organic acids



Cu^{2+} in medium



Conclusions

1. **The key points of the thermodynamic prognosis about growth and interaction of microorganisms at the presence of Cu(II) in super high concentrations (up to 1 M), in particular, the thermodynamically permissible types of its interaction were experimentally confirmed:**
   - **Cu^{2+} reduction to Cu_2O↓;**
   - **Cu^{2+} immobilization;**
   - **Cu(II)↓ mobilization.**

2. **Nine bacterial copper-resistant strains were isolated from various extreme ecosystems and identified using molecular biological and physiological-biochemical methods (*Pseudomonas lactis* UKR1, *P. panacis* UKR2, *P. veronii* UKR3 and UKR4, *Bacillus mycoides* Cop102, *B. velezensis* Cop 41, *B. megaterium* Cop99, *Staphylococcus succinuss* Cop98, *Pantoea agglomerans* Cop101.**

3. **Copper-resistant microorganisms and their interaction with copper compounds are promising for development biotechnologies of copper-containing wastewater purification and bioremediation of copper-contaminated ecosystems.**

Thank You for attention!