Article

Biodiagnostics of Resistance to the Copper (Cu) Pollution of Forest Soils at the Dry and Humid Subtropics in the Greater Caucasus Region

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Abstract: Forest ecosystems perform important forestry and ecological functions. However, mining and processing companies cause significant soil contamination by heavy metals, in particular, copper (Cu). The resistance of nine types and subtypes of forest soils of the dry and humid subtropics in the Greater Caucasus region to Cu contamination at concentrations of 100, 1000, and 10,000 mg/kg was evaluated for the first time following the most sensitive and informative biological (microbiological, biochemical, and phytotoxic) indicators via a laboratory simulation study. Contamination was simulated under laboratory conditions. The series of forest soils was established following their resistance to Cu pollution: brown leached soils (Haplic Cambisols Eutric) = brown typical soils (Haplic Cambisols Eutric) > brown carbonate soils (Haplic Cambisols Eutric) ≥ yellow soils (AlbicLuvisols Abruptic) ≥ leached sod-carbonate soils (Rendzic Leptosols Eutric) > brown forest slightly unsaturated soils (Haplic Cambisols Eutric) > acid brown forest soils (Haplic Cambisols Eutric) > acid brown forest podzolized soils (Haplic Cambisols Eutric). Regional environmentally safe standards for the Cu content in forest soils of the dry and humid subtropics of the Greater Caucasus were proposed: for brown typical soils, brown leached soils, brown carbonate soils, brown forest slightly unsaturated soils, sod-carbonate typical soils, leached sod-carbonate soils, and yellow soils, the rMPC was 100 mg/kg; for acid brown forest soils and acid brown forest podzolized soils, the rMPC was 70 mg/kg.

Keywords: pollution; copper (Cu); Haplic Cambisols Eutric; Rendzic Leptosols Eutric; Albic Luvisols Abruptic; biological activity of soil

1. Introduction

The territory of the wet and dry subtropics of the Greater Caucasus region is a unique natural region with valuable forest soils: brown forest soils, brown soils, yellow soils, and others [1,2]. The terrain is mostly mountainous, with geographical depressions in relation to the Black, Azov, and Caspian Seas. There are many springs with healing mineral waters and mud in the territory of the region. All these factors make the forests of the North Caucasus a recreational region and quite attractive for tourists. Even though this area is mostly virgin forests, there are many roads and industrial enterprises located in its territory. Forest soils are often contaminated with heavy metals [3], which has a negative impact on the growth and development of deciduous and coniferous trees [4].

Copper (Cu) ores are found in an enormous amount in both the Transcaucasia and Greater Caucasus regions, and until recently, ores from Transcaucasia (Azerbaijan, Armenia, and Iran) were almost exclusively developed [5,6]. The annual production of Cu ore in the Caucasus region in the middle of the 20th century accounted for up to one-third of all Cu mined in Russia [7]. Currently, there are several enterprises for the extraction and processing
of Cu and other metals in the Caucasus region. One of these enterprises is the Urup Mining and Processing Plant, which has been operating since 1968, located on the territory of the Karachay–Cherkess Republic (Mednogorsky Village). About 46% of Cu ore from the reserves is mined by the mining method in the territory of this plant [8]. Mining enterprises have a significant negative impact on the environment, in particular causing chemical pollution of adjacent areas, an increase in waste, and soil and ecosystem degradation [9,10]. Soil contamination with heavy metals is widespread in the vicinity of the tailings of gold and Cu mining enterprises in South Africa, Chile, and other countries [11–13]. Copper (Cu) is one of the essential elements necessary for the human body. However, when clarke concentrations of Cu in the environment are exceeded, the agroecological and recreational potential of soils decreases [14–19].

Intensive mining of Cu leads to an excess of its regional clarkes in the soil. This requires the environmental regulation of the content of heavy metals in environmental objects near pollution sources [20–24]. It is advisable to carry out ecological regulation of the Cu content in soils according to the response of soil biological indicators to pollution: total number of bacteria, catalase activity, dehydrogenase activity, cellulolytic activity, and phytotoxicity [25,26]. The total number of bacteria in the soil reflects the state of reducers in the ecosystem [27]. Oxidoreductases (catalase, dehydrogenase, peroxidases, and polyphenoloxidases) are functionally necessary for the decomposition of pollutants, the transformation of organic matter, and the maintenance of the metabolism of microorganisms [28,29]. Catalase activity decreases with petroleum hydrocarbon and heavy metal soil contamination [30–32]. Cellulolytic activity reflects the processes of the decomposition of organic matter in the soil under various types of pollution [33,34]. All used biological indicators are sensitive to and informative for heavy metal contamination. A similar approach was previously studied in the numerous studies on various types of soils in the Ciscaucasia, the Caucasus, and Crimea contaminated with oil, gasoline [35–37], cadmium [38], and other metals [34,39].

The objective of this paper is to assess the resistance of forest soils of the dry and humid subtropics in the Greater Caucasus region to Cu pollution.

2. Materials and Methods
2.1. Study Site

Soil samples for the model experiments were selected from the top layer of 0–10 cm, as most of the pollutants are retained in non-arable soils (Table 1 and Figure 1). The main soils of the wet and dry subtropics were selected as the objects of this research. In this territory, there is a wide variety of soil types, which can probably be distinguished by their resistance to anthropogenic influences. Yellow soils were found only in the Sochi region and made up 0.05% of the country’s area [40,41].

<table>
<thead>
<tr>
<th>No.</th>
<th>Types of Soil</th>
<th>World Reference Base (WRB, 2015)</th>
<th>Sampling Place</th>
<th>Organic Content, %</th>
<th>pH</th>
<th>Grain Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Brown typical soil</td>
<td>Haplic Cambisols Eutric</td>
<td>Anapsky District, State nature reserve “Utrish”</td>
<td>9.3</td>
<td>7.2</td>
<td>HL</td>
</tr>
<tr>
<td>2.</td>
<td>Brown carbonate soil</td>
<td>Haplic Cambisols Eutric</td>
<td>Anapsky District, State nature reserve “Utrish”</td>
<td>15.0</td>
<td>7.0</td>
<td>ML</td>
</tr>
<tr>
<td>3.</td>
<td>Brown leached soil</td>
<td>Haplic Cambisols Eutric</td>
<td>Anapsky District, State nature reserve “Utrish”</td>
<td>6.8</td>
<td>7.1</td>
<td>HL</td>
</tr>
<tr>
<td>4.</td>
<td>Acid brown forest soil</td>
<td>Haplic Cambisols Eutric</td>
<td>Tuapsinsky District, Gorskoe Village</td>
<td>1.3</td>
<td>4.4</td>
<td>HL</td>
</tr>
</tbody>
</table>
Table 1. Characteristics of the soils of the wet and dry subtropics.

<table>
<thead>
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<th>Sampling Place</th>
<th>Organic Content, %</th>
<th>pH</th>
<th>Grain Composition</th>
</tr>
</thead>
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<td>Anapsky District, State nature reserve &quot;Utrish&quot;</td>
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<td>7.2</td>
<td>HL</td>
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<td>Haplic Cambisols Eutric</td>
<td>Anapsky District, State nature reserve &quot;Utrish&quot;</td>
<td>6.8</td>
<td>7.1</td>
<td>HL</td>
</tr>
<tr>
<td>4.</td>
<td>Acid brown forest soil</td>
<td>Haplic Cambisols Dystric</td>
<td>Tuapsinsky District, Gorskoe Village</td>
<td>1.3</td>
<td>4.4</td>
<td>HL</td>
</tr>
<tr>
<td>5.</td>
<td>Acid brown forest podzolized soil</td>
<td>Haplic Cambisols Dystric</td>
<td>City Sochi, Lazarevsky City District, Sochi National Park</td>
<td>1.7</td>
<td>4.1</td>
<td>LL</td>
</tr>
<tr>
<td>6.</td>
<td>Brown forest slightly unsaturated soils</td>
<td>Haplic Cambisols Eutric</td>
<td>Tuapsinsky District, Dzhubga Village</td>
<td>1.9</td>
<td>5.1</td>
<td>HL</td>
</tr>
<tr>
<td>7.</td>
<td>Sod-carbonate typical soil</td>
<td>Rendzic Leptosols Eutric</td>
<td>Tuapsinsky District, Dzhubga Village</td>
<td>5.4</td>
<td>7.5</td>
<td>HL</td>
</tr>
<tr>
<td>8.</td>
<td>Leached sod-carbonate soil</td>
<td>Rendzic Leptosols Eutric</td>
<td>City Sochi, Khostinsky City District, Caucasus reserve, Thysosamshitovaia Grove</td>
<td>4.8</td>
<td>6.9</td>
<td>HL</td>
</tr>
<tr>
<td>9.</td>
<td>Yellow soil</td>
<td>Albic Luvisols Abruptic</td>
<td>City Sochi, Adlersky City District</td>
<td>3.2</td>
<td>5.2</td>
<td>HL</td>
</tr>
</tbody>
</table>

Note: HL—heavy loamy; ML—middle loamy; LL—light loamy.

Figure 1. Selection sites for different soil types.

2.2. Copper

Copper (Cu) pollution was simulated under laboratory conditions. Contamination with this element is most often found because of anthropogenic activity. In the present study, Cu was introduced for contamination into the soil in the form of its oxides i.e., CuO, at concentrations of 100, 1000, and 10,000 mg/kg (1, 10, and 100 MPC). Soil contamination with metal oxides occurs more often than contamination with other chemical forms [17]. The use of heavy metal oxides eliminates the effect on the properties of the accompanying anion, which is observed when metal salts have been introduced into the soil. For this purpose, oxides, as compounds that are practically insoluble in water, were previously ground with a lesser quantity of soils and then mixed with the rest of the soil mass.

2.3. Experimental Details

Three samples of contaminated soils were incubated in plastic vessels at 20 ± 2 °C and 60% water field capacity. A 30-day period is the most informative for assessing the chemical effect on soil as the maximum decrease in values is observed within this period for most biological indexes. Methods common for soil biology were used to determine the biological properties of the soils [42]. The most sensitive and informative biological indicators were investigated, such as the total bacterial count, determined by direct luminescent microscopy; catalase activity, determined by the Galstyan method; dehydrogenase
activity, determined by Khaziev et al.’s [43] modification of the Galstyan method; cellulolytic activity, determined by the degree of cotton decomposition on Ashby’s medium; and phytotoxicity of soils, determined by the length of radish (*Raphanus sativus* L.) roots [44] (Table 2).

**Table 2. Methods for the measurement of biological indicators.**

<table>
<thead>
<tr>
<th>No</th>
<th>Biological Indicator</th>
<th>Methods</th>
<th>Measurement Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total number of bacteria</td>
<td>Luminescent microscopy with a solution of acridine orange, 40×</td>
<td>$10^9$ bacteria in 1 g of soil dry weight</td>
</tr>
<tr>
<td>2</td>
<td><em>Azotobacter</em> sp. abundance</td>
<td>Fouling lumps on Ashby medium</td>
<td>% fouling lumps</td>
</tr>
<tr>
<td>3</td>
<td>Catalase activity</td>
<td>Rate of decomposition of hydrogen peroxide</td>
<td>mL O$_2$ per 1 g of soil dry weight in 1 min.</td>
</tr>
<tr>
<td>4</td>
<td>Dehydrogenases activity</td>
<td>Rate of conversion of triphenyl tetrazolium chloride (TPC) to triphenyl formazan (TPF)</td>
<td>mg of TPF per 1 g of soil dry weight for 24 h</td>
</tr>
<tr>
<td>5</td>
<td>Cellulolytic activity</td>
<td>Percentage of decomposed cotton (30th day) to the initial weight of the cotton on the 1st day of incubation.</td>
<td>%</td>
</tr>
<tr>
<td>6</td>
<td>Seed germination rate</td>
<td>Seed germination (<em>R. sativus</em> L.) on the contaminated soils in Petri dishes for 7 days</td>
<td>%</td>
</tr>
<tr>
<td>7</td>
<td>Length of radish roots</td>
<td>Length of the roots (<em>R. sativus</em> L.) after 7 days of the experiment</td>
<td>mm</td>
</tr>
</tbody>
</table>

The reasons for choosing these biological indicators are described below. The total number of bacteria in the soil reflects the state of decomposers in the ecosystem. The activity of oxidoreductases, i.e., catalase, and dehydrogenases indicates the rate of mineralization of organic substances in soil. Moreover, oxidoreductases are highly sensitive to chemical contamination in comparison with other classes of enzymes. The enzyme activity of soils determines the potential biological activity of soil, and cellulolytic activity determines the actual activity. The seed germination rate and the length of the radish roots aid in the determination of soil phytotoxicity, and the intensity of the initial growth and development of plants.

### 2.3.1. Measurement of the Total Number of Bacteria

The total number of bacteria in the soils was determined by the luminescence microscopy method considering the number of soil bacteria present after staining with acridine orange dye. Acridine orange is a fluorochromatic dye that binds to nucleic acids in bacteria and other cells. Under the influence of ultraviolet radiation, acridine orange stains ribonucleic acid (RNA) and single-stranded DNA with an orange color (as soil particles) and double-stranded DNA with green (as bacterial cells). After incubation, the fresh soils were dried and a soil suspension (soil:water, 1:100) was prepared. On prepared glasses (defatted and sterilized), 10 μL of soil suspension was placed, air-dried (air temperature—22–24 °C), and dried on a burner flame (duration 3–5 s). Then, the glasses were stained with a solution of acridine orange dye (dilution of the solution of acridine orange dye—1:10,000) for 20 min. The glasses were washed to remove excess dye and dried in the air. The glasses were observed under a Carl Zeiss Axio Lab A1 microscope at a magnification of 40× (20 bacterial cells of counting fields).

### 2.3.2. Measurement of *Azotobacter* sp. abundance

*Azotobacter* sp. abundance has traditionally been used to indicate chemical pollution in soils, which is determined by the method of fouling lumps on Ashby’s medium. To assess the number of bacteria, Ashby’s medium was prepared and then poured into Petri dishes. Lumps of moistened soils were added with 25 pieces per Petri dish, and incubated at a
temperature of 22–25 °C. These operations were performed in a BAVnp-01-“Laminar-S”
bacterial air-box. The number of fouling lumps was counted after 14 days of incubation.
The counting of soil lumps overgrown with the *Azotobacter* sp. abundance relative to the
control was carried out.

2.3.3. Evaluation of Catalase and Dehydrogenases Activity

The catalase and dehydrogenase activities were used to estimate potential biological
activity in soils. Oxidoreductases (catalase and dehydrogenases) were more sensitive
to chemical pollution than other enzymes. Catalase activity was determined according
to Galstyan’s method. The enzyme activity was determined by the gasometric method
based on the rate of the decomposition of 5% hydrogen peroxide after contact with the soil
(temperature—20–22 °C). Dehydrogenases were determined according to the modification
of Galstyan’s method by Khaziev [43]. The activity of dehydrogenases was determined by
the conversion of triphenyl tetrazolium chloride (TPC) to triphenyl formazan (TPF). The
optical density of the colored solutions was spectrophotometrically determined on a PE
5800VI spectrophotometer at a wavelength of 540 nm.

2.3.4. Evaluation of Cellulolytic Activity

The indicator of the cellulose activity of soils was the volume of cotton cloth decom-
posed after 30 days of incubation. The size of the canvas matched the size of the bottom
of the model box. Under laboratory conditions, the cotton cloth was laid in the soil at
the beginning of incubation, and after 30 days, it was removed. After that, solid soil impurities
were removed from the cotton cloths. The cotton cloths were washed and dried in air.
Finally, the cotton clothes were weighed. The volume was calculated from the difference in
the weights of the cloths before and after incubation, expressed as a percentage. Cellulolytic
activity was estimated as the percentage of the initial weight of the cotton cloth.

2.3.5. Measurement of Seed Germination Rate and Length of Roots of Radish
(*Raphanus sativus*)

Soil phytotoxicity was investigated by the seed germination rate of radish (*R. sativus* L.)
and length of roots in a Binder KBW 240 growth chamber. To assess soil toxicity, garden
radish (*R. sativus* L) was used. Compared with other plant test objects, radish had a fast
response to soil nutrients and moisture. The seed germination rate and root length were the
most informative of the many indicators of soil phytotoxicity [45–47]. After the incubation
of the soil with CuO for 30 days, the soils were placed in a Petri dish. Radish seeds were
sown in the Petri dishes for germination in the soil mass, with 25 seeds per Petri dish,
moisture of 60%, and a temperature of 24 ± 2 °C. After 7 days of incubation, the radishes
were removed from the soils and the percent seed germination and length of the radish
roots were determined. The seed germination rate was assessed by the number of seeds
that germinated after 7 days of the experiment (after the appearance of 2 or more leaves).

2.4. Data Analysis

The total number of soil bacteria was determined by the luminescence microscopy
method considering the number of soil bacteria after staining with acridine orange [44].
The results were expressed in $10^9$ bacteria in 1 g of soil dry weight, as given in Equation (1):

$$M = \frac{b \times A \times H \times T}{P}$$

(1)

where $M$—number of cells per 1 g of fresh soil; $b$—coefficient magnification factor ($b = 4$);
$A$—average number of cells within one field of vision; $H$—dilution index; $T$—conversion
factor in billions of bacteria per 1 g of soil ($T = 10^{10}$); and $P$—area of the field of vision, µm².
The indices of the intensity of the initial growth of radish (length of shoots and roots) were calculated as an average for biological samples [36]. The indicator seed germination (G) was calculated using Equation (2):

\[ G = \frac{n_1 + n_2}{2} \]  

(2)

where G—Seed germination; \( n_1 \)—number of seeds in 1st replicate; and \( n_2 \)—number of seeds in 2nd replicate.

The cellulolytic activity (CA) was calculated according to the difference in the masses of the web before and after incubation, expressed as a percentage, by Equation (3) given below

\[ CA = \left( \frac{m_k - m_1}{m_k} \right) \times 100 \% \]  

(3)

where CA—cellulolytic activity, \( m_k \)—mass of the control web; and \( m_1 \)—mass of the decomposed web.

To establish the general patterns of change in the biological properties of soils using the indicators mentioned above in Table 2, we determined the integral index of the biological state (IIBS) of soils [42]. For this purpose, the indices of the unpolluted soils (control) were taken as 100%. The values of biological characteristics in unpolluted soils (control) were taken as 100%. The values of the indices in contaminated soils (variants of the experiment) were expressed as a percentage relative to the above values (Equation (4)).

\[ IIBS = \left( \frac{V_1 + V_2 + V_3 + \ldots + V_n}{N} \right) \]  

(4)

where IIBS—Integral Index of the Biological State; \( V_1, V_2, V_3, \) and \( V_n \)—percentage values for each biological parameter of the control; and \( N \)—number of biological indicators.

Furthermore, for each variant of the experiment, the mean value for the six indicators was calculated. This technique allows the integration of the relative values of different indicators, the absolute values of which cannot be combined into a single indicator, as they have different units of measurement.

2.5. Statistical Analyses

The analysis of the rate of variation i.e., standard deviation at \( p \leq 0.05 \), was calculated to determine the reliability of the results. Data were taken as average of triplicate samples. Statistical data processing was carried out using the software Statistica 12.0 and Python 3.6.5 Matpolib package. The nonparametric Spearman’s correlation coefficient was calculated between the concentration of heavy metals and petroleum carbohydrates as an average of the biological indicators.

3. Results

3.1. Azotobacter sp. Abundance and the Total Number of Bacteria

A significant decrease in Azotobacter sp. abundance was found in the soil contaminated with one maximum permissible concentration (MPC) of Cu: for sod-carbonate typical soil, a decrease of 12% occurred; sod-carbonate eluviated and brown forest podzolic soils—23%; brown forest slightly unsaturated soil—26%; and brown typical soil—27% of control (Figure 2). While at 10 MPC of Cu, decreases in Azotobacter sp. abundance were observed for brown carbonate, brown forest slightly unsaturated, brown typical, sod-carbonate typical, brown forest podzolic soils, and yellow soil of 36, 40, 43, 45, 46, and 48%, respectively.
For sod-carbonate eluviated soil, the decrease was found to be 52%, and for brown forest acid soil, it was 68%. At 100 MPC of Cu, the abundance of bacteria of the *Azotobacter* genus decreased by 50–63% in brown carbonate, brown forest slightly unsaturated, brown typical, and brown eluviated soils. In yellow, sod-carbonate typical, sod-carbonate eluviated, brown forest podzolic, and brown forest acid soils, the decrease in the abundance of *Azotobacter* sp. was found to be 71–100%.

The introduction of one MPC of Cu into the soil significantly reduced the total number of bacteria in brown forest acid, sod-carbonate eluviated, brown forest slightly unsaturated, brown forest podzolic, brown carbonate, and brown eluviated soils by 9–16%, and by 32% in yellow soil. Additionally, with the introduction of 10 MPC of Cu, the total number of bacteria decreased by 19% in brown typical soil, 24% in brown carbonate soil, 26% in brown eluviated soil, 27% in brown forest podzolic soil, 29% in brown forest slightly unsaturated soil, 37% in sod-carbonate typical soil, 39% in brown forest acid soil, 42% in yellow soil, and 53% in sod-carbonate eluviated soil. At 100 MPC of Cu, suppression of the total number of bacteria by 59–80% was observed in the studied soils.

3.2. Catalase and Dehydrogenases Activity

The catalase activity with the introduction of one MPC of Cu significantly decreased by 12% in brown soil, by 16% in brown forest slightly unsaturated soil, and by 31% in brown forest podzolic soil. In the other types of soils, the changes in catalase activity did not differ from that of the control, only in the brown forest acidic and sod-carbonate typical soils, the effect of hormesis resulted in increase of 11 and 12%, respectively.

The introduction of 10 MPC of Cu significantly reduced the catalase activity only in brown carbonate (by 12%), brown forest slightly unsaturated (by 17%), and brown forest podzolized (by 28%) soils. At 100 MPC of Cu, the catalase activity was observed to be decreased by 11% for brown typical soil, 14% for brown eluviated soil, 18% for yellow soil, 30% for brown forest slightly unsaturated soil, 32% for brown carbonate soil, 42% for sod-carbonate typical soil, 45% for brown forest podzolic soil, 48% for sod-carbonate eluviated soil, and 51% for brown forest acid soil.

The activity of dehydrogenases was significantly decreased by 46% only in brown forest acid and podzolic soils, and by 48% in brown forest slightly unsaturated soil with the introduction of one MPC of Cu. At 10 MPC of Cu, a decrease in the activity of dehydrogenases of 16% was recorded in brown eluviated soil, 29% in brown typical soil, 37% in yellow soil, 39% in sod-carbonate typical soil, 40% in brown carbonate soil, 46% in sod-carbonate eluviated soil, and 50% in brown forest acid soil and in brown forest acid soil.
podzolic soil, while in the brown forest slightly unsaturated soils, it was found to be decreased by 59% of control. Contamination of the soils with 100 MPC of Cu decreased the dehydrogenase activity in yellow soils by 49%, by 54% in sod-carbonate typical soil, by 62% in brown forest slightly unsaturated soil, by 63% in brown typical and eluviated soils, by 64% in brown forest podzolic soil, by 66% in sod-carbonate eluviated soil, by 72% in brown forest acid soil, and by 74% in the brown carbonate soil, as shown in Figure 3.

![Figure 3](image-url)

**Figure 3.** Changes in the catalase (A) and dehydrogenases (B) activity of the forest soils: 1, brown leached soil; 2, brown typical soil; 3, brown carbonate soil; 4, yellow soil; 5, acid brown forest soil; 6, sod-carbonate typical soil; 7, leached sod-carbonate soil; 8, acid brown forest podzolized soil; and 9, brown forest slightly unsaturated soils.

### 3.3. Cellulolytic Activity

The cellulolytic activity with the introduction of one MPC of Cu was decreased by 11% in sod-carbonate typical soil, by 12% in brown eluviated soil, by 13% in sod-carbonate eluviated soil, by 16% in brown forest slightly unsaturated soil, by 19% in brown carbonate soil, by 24% in yellow soil, by 27% in brown forest acid soil, and by 34% in brown forest podzolic soil (Figure 4).

![Figure 4](image-url)

**Figure 4.** Changes in the cellulolytic activity of the forest soils: 1, brown leached soil; 2, brown typical soil; 3, brown carbonate soil; 4, yellow soil; 5, acid brown forest soil; 6, sod-carbonate typical soil; 7, leached sod-carbonate soil; 8, acid brown forest podzolized soil; 9, brown forest slightly unsaturated soils.
The introduction of 10 MPC of Cu caused a decrease in the cellulolytic activity by 22% in brown eluviated soil, 25% in sod-carbonate typical soil, 34% in sod-carbonate eluviated soil, 37% in brown carbonate soil, 40% in brown forest slightly unsaturated soil, 45% in brown typical soil, 53% in yellow soil, 65% in brown forest podzolic soil, and 68% in brown forest acid soil. At 100 MPC of Cu, a decrease in cellulolytic activity of 45% was observed in brown eluviated soil, 48% in brown carbonate soil, 49% in brown forest slightly unsaturated soil, 55% in brown typical soil, 58% in sod-carbonate typical soil, 72% in sod-carbonate eluviated soil, 78% in brown forest acid soil, 89% in brown forest podzolic soil, and 98% in yellow soil.

3.4. Length of the Roots of Radish (Raphanus sativus)

Copper contamination with a dose of one MPC negatively affected the length of plant roots significantly: the root length was decreased by 14% in brown typical soil, 22% in yellow soil and sod-carbonate typical soil, 31% in brown eluviated soil, 33% in sod-carbonate eluviated soil, 43% in brown forest slightly unsaturated soil, 45% in brown carbonate soil, 64% in brown forest acid soil, and 88% in brown forest podzolic soil (Figure 5).

![Figure 5. Changes in the length of the radish (Raphanus sativus) roots in forest soils: 1, brown leached soil; 2, brown typical soil; 3, brown carbonate soil; 4, yellow soil; 5, acid brown forest soil; 6, sod-carbonate typical soil; 7, leached sod-carbonate soil; 8, acid brown forest podzolized soil; 9, brown forest slightly unsaturated soil.](image)

The introduction of 10 MPC of Cu caused a decrease in root length of 16% for brown typical soil, 29% for brown carbonate soil, 30% for brown eluviated soil, 41% for yellow and sod-carbonate typical soils, 51% for sod-carbonate eluviated soil, 83% for brown forest acid and slightly unsaturated soils, and 91% for brown forest podzolic soil. At 100 MPC of Cu, decreases in root length of 28% for brown typical soil, 47% for brown carbonate soil, 61% for brown eluviated soil, 73% for sod-carbonate eluviated soil, 80% for yellow soil, 83% for sod-carbonate typical soil, 87% for brown forest acid soil, 93% for brown forest podzolic soil, and 95% for brown forest slightly unsaturated soil were observed.

3.5. Integral Index of the Biological State (IIBS) Ecotoxicity of Copper (Cu)

The highest resistance to Cu contamination with respect to the changes in IIBS was found in southern chernozem, brown leached, and brown typical soils—IIBS was lowered by 18, 20, and 21% relative to the control, respectively (Figure 6).
were observed, mainly in the concentration range of 100 mg metal per kg soil. Such phenomena are widely known in ecotoxicology as hormesis. A series of heavy metal ecotoxicities with respect to the soils of the wet and dry subtropics was obtained.

Acid brown forest and acid brown forest podzolized soils were found to be least resistant to the effects of Cu contamination. For these soils, notable decreases of 38% and 42% in IIBS were observed relative to their respective controls. The average resistance indicators in brown carbonate soil, sod-carbonate typical soil, yellow soils, and leached sod-carbonate soils decreased by 25, 25, 26, and 30% relative to the control, respectively.

Regarding Cu contamination: brown leached soils (73) = brown typical soils (73) > brown carbonate soils (67) = sod-carbonate typical (67) ≥ yellow soils (61) ≥ leached sod-carbonate soils (60) > brown forest slightly unsaturated soils (56) > acid brown forest soils (50) > acid brown forest podzolized soils (44).

IIBS had a close relationship with the content of copper in the soil, with the following correlation coefficients: brown leached soil \( r = -0.97 \); brown typical soil \( r = -0.88 \); brown carbonate soil \( r = -0.87 \); yellow soil \( r = -0.86 \); acid brown forest soil \( r = -0.82 \); sod-carbonate typical soil \( r = -0.92 \); leached sod-carbonate soil \( r = -0.87 \); acid brown forest podzolized soil \( r = -0.76 \); and brown forest slightly unsaturated soils \( r = -0.76 \).

4. Discussion

It was found that the Cu pollution/contamination in the soils of the wet and dry subtropics led to decreases in their biological parameters. It is noted that the change in the biological state of the soils directly depended on the nature of the soils themselves, the concentration in the soils, and the nature of the pollutant.

However, in some cases, stimulating effects of heavy metal on soil biological properties were observed, mainly in the concentration range of 100 mg metal per kg soil. Such phenomena are widely known in ecotoxicology as hormesis. A series of heavy metal ecotoxicities with respect to the soils of the wet and dry subtropics was obtained.

The presented series of stability was determined by the ecological–genetic properties of the studied soils (as shown in Table 1), such as the granulometric composition, alkaline–acid and redox conditions, biological activity, and organic matter content. The brown and sod-carbonate soils were less resistant to pollution with heavy metals. Typically, these soils are characterized by a heavy-grained granulometric composition, high humus content, neutral or slightly alkaline reaction of the medium, and other properties that contribute to the retention of heavy metals.

Brown forest soils showed the least resistance to chemical contamination. They were distinguished by a relatively low humus content, acidic medium reaction, and lower enzymatic activity; therefore, high mobility of heavy metals and a low rate of petroleum hydrocarbon decomposition could be observed. Yellow soils were less resistant to heavy
metal contamination than brown and sod-carbonate soils, but more resistant than brown forest soils. Yellow soils were characterized by intermediate humus content values and a medium response. The relatively high biological activity of yellow soils and their good structure make them relatively resistant to petroleum hydrocarbon pollution. The results of the present study suggest that the soil biological indicators used in this work can be recommended for use in the diagnosis of the chemical contamination of soils in the wet and dry subtropics.

Earlier, in the chernozems [30], it was found that high concentrations of organic matter provided soils a buffer to chromium (Cr) pollution to a greater extent, and high pH values more determined the soils’ resistance to Cu. The study of the soils of the wet and dry subtropics confirmed this pattern.

Based on results of this study, regression equations were calculated to characterize the relationship between IIBS and the content of chemicals in the soil. Using these equations, we calculated the concentrations of pollutants that led to the degradation of various ecological functions of soils (Table 3). The disruption of soil ecological functions occurred in a certain order, and it is advisable to use the soils’ IIBS as an indicator of the degradation of the soils’ ecological functions [21]. If the IIBS was reduced by less than 5%, the soils’ function was not affected. A decrease in the IIBS value of 5%–10% indicated the degradation of information functions; a decrease in the IIBS value of 10%–25% indicated the degradation of biochemical, physicochemical, chemical, and holistic functions; and a decrease in the IIBS value of more than 25% indicated the deterioration of the physical functions of the soils.

<table>
<thead>
<tr>
<th>Decrease in Soils’ IIBS</th>
<th>&lt;5%</th>
<th>5%–10%</th>
<th>10%–25%</th>
<th>&gt;25%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deterioration of Soils’ Ecological Functions</strong></td>
<td></td>
<td>Informative</td>
<td>Chemical, Physico-Chemical, Biochemical, Holistic</td>
<td>Physical</td>
</tr>
<tr>
<td><strong>Soil Contamination Degree</strong></td>
<td>Not</td>
<td>Little</td>
<td>Moderate</td>
<td>High</td>
</tr>
<tr>
<td>Brown typical soils</td>
<td>&lt;55</td>
<td>55–100</td>
<td>100–300</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Brown leached soils</td>
<td>&lt;50</td>
<td>50–100</td>
<td>100–275</td>
<td>&gt;275</td>
</tr>
<tr>
<td>Brown carbonate soils</td>
<td>&lt;50</td>
<td>50–100</td>
<td>100–250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Acid brown forest soils</td>
<td>&lt;50</td>
<td>50–70</td>
<td>70–200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Acid brown forest podzolized soils</td>
<td>&lt;50</td>
<td>50–70</td>
<td>70–200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Brown forest slightly unsaturated soils</td>
<td>&lt;50</td>
<td>50–100</td>
<td>100–220</td>
<td>&gt;220</td>
</tr>
<tr>
<td>Sod-carbonate typical soils</td>
<td>&lt;60</td>
<td>60–100</td>
<td>100–250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Leached sod-carbonate soils</td>
<td>&lt;50</td>
<td>50–100</td>
<td>100–250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Yellow soils</td>
<td>&lt;50</td>
<td>50–100</td>
<td>100–220</td>
<td>&gt;220</td>
</tr>
</tbody>
</table>

From Table 3, if, for example, the Cu content in brown typical soils does not exceed 55 mg/kg, then the soils function normally. A copper concentration from 55 to 100 mg/kg could degrade the information ecological functions of the soils. A copper concentration from 100 to 300 mg/kg could degrade chemical, physicochemical, biochemical, and holistic functions in addition to the information function. Cu concentrations above 300 mg/kg could lead to the degradation of physical functions of soils. It is obvious that the degradation of the chemical, physicochemical, biochemical, and, most importantly, holistic functions of the soil, ensuring soil fertility, is unacceptable. Consequently, a Cu concentration of 100 mg/kg should be considered as the MPC of Cu in the brown typical soils of the wet and dry subtropics, or the regional MPC (rMPC). According to the author’s method, Kolesnikov et al. [21] calculated the rMPCs of Cu for the soils of the Great Caucasus—for brown typical soils, brown leached soils, brown carbonate soils, brown forest slightly unsaturated soils, sod-carbonate typical soils, leached sod-carbonate soils, and yellow soils, the
rMPC is 100 mg/kg; and for acid brown forest soils and acid brown forest podzolized soils, the rMPC is 70 mg/kg. According to the degree of pollution and the response of biological indicators, methods for restoring soil health were proposed [21] for the contaminated soils. Therefore, with insignificant pollution by heavy metals, it is possible to carry out phytoremediation and soil leaching. With medium pollution, moderate chemical reclamation is possible, such as the introduction of organic substances, ion-exchange resins, phosphorus fertilizers, lime, zeolites, etc. In the case of severe pollution, in which there is a violation of the physical properties of the soil, it is necessary to remove the contaminated soil layer and replace it with ecologically effective and agricultural soils.

5. Conclusions

The contamination of forest soils in the dry and humid tropics of the Greater Caucasus region with Cu affects the number of Azotobacter sp., cellulolytic activity, catalase and dehydrogenase activity, and plant growth and development. The change in the biological parameters depends on the Cu concentration in the soil and the ecological and genetic properties of the soils: brown leached soils (73) = brown typical soils (73) > brown carbonate soils (67) = sod-carbonate typical (67) ≥ yellow soils (61) ≥ leached sod-carbonate soils (60) > brown forest slightly unsaturated soils (56) > acid brown forest soils (50) > acid brown forest podzolized soils (44). Brown forest soils showed the lowest resistance to chemical pollution. They were distinguished by a relatively low humus content, acidic reaction of the medium, low enzymatic activity, and, therefore, high mobility of heavy metals. Regional norms of the maximum permissible content (rMPC) of Cu in soils of wet and dry subtropics were proposed, determined based on the degradation of the ecological functions of soils.

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References

2. Yusuf, Y.; Panagiotis, P.; Luca, M. Soil Resources of Mediterranean and Caucasus Countries. Geography 2013, 134, 03247. [CrossRef]
4. Sidor, C.G.; Vlad, R.C.; Popa, I.; Semeniuc, A.; Apostol, E.N.; Badea, O. Impact of Industrial Pollution on Radial Growth of Conifers in a Former Mining Area in the Eastern Carpathians (Northern Romania). *Forests* 2021, 12, 640. [CrossRef]


16. D’yachenko, V.V.; Matasova, I.Y. Regional clarke of chemical elements in soils of southern European Russia. *Eurasian Soil Sci.* 2016, 49, 1091–1098. [CrossRef]


26. Yáfuez, C.; Verdejo, J.; Moya, H.; Donoso, P.; Rojas, C.; Dovletyarova, E.A.; Shapoval, O.A.; Krutyakov, Y.A.; Neaman, A. Microbial responses are unreliable indicators of copper ecotoxicity in soils contaminated by mining activities. *Chemosphere* 2022, 300, 134517. [CrossRef]


