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ORIGINAL PAPER

Soil organic matter and biological activity under long-term contamination with copper

Inna V. Zamulina · Andrey V. Gorovtsov · Tatiana M. Minkina · Saglara S. Mandzhieva · Marina V. Burachevskaya · Tatiana V. Bauer

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Abstract Organic matter (OM) and enzymes activity can act as indicators of the time and level of soil contamination with heavy metal. The goal of this study is evaluation of the effect of chronic long-term soil contamination with Cu on OM and biological activity in Spolic Technosols. The monitoring plot is located in the zone of industrial wastewater storage and sludge reservoirs in the Seversky Donets River flood plain. The total amount of Cu in the investigated soils varied greatly from 52 to 437 mg/kg. The results of Cu sequential fractionation the contaminated soil have shown that the chemical fraction composition of metal changed when the soil contamination level increased. The amount of Cu compounds associated with OM and Fe and Mn oxides was also higher. Fractions of OM from the humic and fulvic acids groups were studied. Soil was subjected to extraction with cold and hot water, and the content of water-soluble OM (WSOM) was determined. An increased solubility of humic and fulvic acids as well as elevated content of cold and hot extraction WSOM was established. The cold-extracted amount of WSOM increased with an enhance in the Cu content. The long-term contamination of soil with Cu leads to an adaptation of microorganisms to this adverse environmental factor, and this adaptation is manifested in the WSOM content increase. The effect of Cu contamination on microbiological activity was assessed by plate-counting culturable microorganisms and determining urease and dehydrogenase enzymatic activity. A high level of soil contamination with Cu showed a noticeable negative effect on the number of soil bacteria; however, active and potentially active bacteria were observed even in the highly contaminated soils. The changes in soil OM and microbial communities caused by Cu pollution can lead to disruption of ecosystem functioning.

Keywords Contaminated soil · Dehydrogenase · Urease · Humic and fulvic acids · Fractional composition of organic matter · Sequential fractionation of Cu

Introduction

Soil contamination with heavy metals is among the world's most important environmental hazards, existing primarily due to the human activities (Zhao et al., 2017). Once in the environment, heavy metals persist for a long time, accumulating in components of ecosystems (Doyi et al., 2018). The accurate assessment of the pollution effects in different areas requires the data on the contamination level, as well as the

I. V. Zamulina (✉) · A. V. Gorovtsov ·
T. M. Minkina · S. S. Mandzhieva ·
M. V. Burachevskaya · T. V. Bauer
Southern Federal University, Rostov-on-Don, Russian Federation 344090
e-mail: inir@mail.ru

mobility of metals and the main properties of the soil, which are related to the adsorption and retention of pollutants (Barsova et al., 2019). Speciation of metals determines the degree of environmental hazard of landscape pollution, the migration ability of their compounds (Liu et al., 2019). Soil organic matter (SOM) acts as an important agent in these processes. Studying the relationship of metals with SOM components is important in order to evaluate the bioavailability heavy metals (Lasota et al., 2020).

The SOM has a large impact on biogeochemistry and the environment in general (Escalona et al., 2021). The main components of SOM are humic and fulvic acids, organic compounds with a complex structure, including carbon chains, polycondensed aromatic rings, amine and saccharide residues, heteroatoms and various functional groups. The reactions of metals complexation with organic ligands affect their mobility, increasing or decreasing their sorption on mineral surfaces (Pinskii et al., 2018). The formation of stable complexes with humic substances that contain a large number of functional groups with high affinity for metals contributes to a decrease in the availability of metals (Liu et al., 2019; Wang et al., 2017). The maturation of humic acids molecules in the soil can be hampered by contamination with heavy metals and their structure can be changed (Yakovets, 2021). These changes influence the most labile part of OM which are represented by water-soluble organic matter, root exudates, nonspecific organic compounds, free fulvic and humic acids and partially humified substances (Bezuglova, 2019; Zhang & Zhang, 2010). Metal ions adsorbed on the soil particles surface can interact with fulvic acids, and resulting complex compounds can pass into the soil solution, increasing their bioavailability (Liu et al., 2019; Wang et al., 2017). Labile compounds of the organic matter (OM) are readily mineralized and used by microorganisms as a food source (Akinwole et al., 2021).

Microbiota plays a key role in ecosystem processes like transformation of OM, turnover of elements, arrangement of soil structure and plant nutrition (Stefanowicz et al., 2020). Several groups of bacteria are actively involved in fresh organic matter decomposition and SOM formation. Many species of aerobic spore-forming bacteria are known for their capability to decompose cellulose (Dobrzyński, et al., 2021). The process of specific soil organic substances formation, including fulvic and humic acids, is mediated by hydrolytic and oxidative enzymes, produced by soil microorganisms. The changes in microbial community structure can lead to different organic carbon decomposition patterns (Yan et al., 2018), and the balance can shift from humification and SOM accumulation to mineralization and SOM decomposition. The models linking SOM decomposition patterns with specific groups within microbial communities are yet to be developed. This is a challenging task, as different soils and land use types should be studied and the use of culture-independent methods is needed. Nowadays, most of the studies in this field are focused on agricultural practices and the use of organic fertilizers. At the same time, the effects of soil biological activity on SOM composition in polluted soils are largely neglected. The negative effects of heavy metal pollution on soil microbial communities can lead to disruption of ecosystem functioning (Enya et al., 2020; Gorovtsov et al., 2019; Minkina et al., 2006; Stefanowicz et al., 2020). Enzymatic activity is one of the sensitive indicators of changes occurring in soils (Stefanowicz et al., 2020). Under pollution conditions, SOM mineralization and transformation can be reduced. This decrease is especially notable for, WSOM due to the reduction of microbial activity and partial inactivation of redox and hydrolytic enzymes (Enya et al., 2020). Dehydrogenases characterize the intensity of the dehydrogenation processes of organic substrates and the metabolic activity of microorganisms (Stefanowicz et al., 2020). High reliability, specificity, sensitivity and the widespread use of methods for the determination of these enzymes make it possible to use them as key biochemical indicators of heavy metals soil pollution (Datta et al., 2021). The purpose of this study was to evaluate the effect of long-term soil contamination with Cu on the OM and soil biological activity.

Materials and methods

Study area

The object of the study was the soils of the territory adjacent to dried Lake Atamanskoe in the Kamensky district of the Rostov region (Russia) (Fig. 1). In the 50s- mid-90s of the last century, this lake is considered as sludge collector chemical plants in the area. The sediments in the lake are contaminated with heavy metals, and there are highly hazardous contamination degree of the organometallic compounds (Bauer et al., 2018). Most of the pollutants are in the upper layer (till 2.5 m thick); Cu reserves exceed 176 tons (Privalenko et al., 2000). Over the past 30 years, hydrological conditions have changed. Industrial runoffs discharges have ceased. Due to the longer-term dry period, active soil formation began (Fig. 2).

Soil sampling

Soil samples of Spolic Technosols were taken from the monitoring plots located at the southern, western and northern edges of the dried Atamanskoe Lake (plots 2–5) with high degree of anthropogenic contamination of soil (Minkina et al., 2018). Soil samples of Fluvisol were collected at approximately 2 km from the lake (plot 1). This soil has similar bioclimatic soil formation conditions and was chosen as background.

Sampling was done based on ISO 18400-104 (2018). In each monitoring plot, five subsamples were collected from the top layer (0–20 cm depth) with a drill. Five subsamples were mixed to obtain a combined soil sample. All soil samples were taken in triplicate. The weight of the mixed sample from each plot was 1500 g.

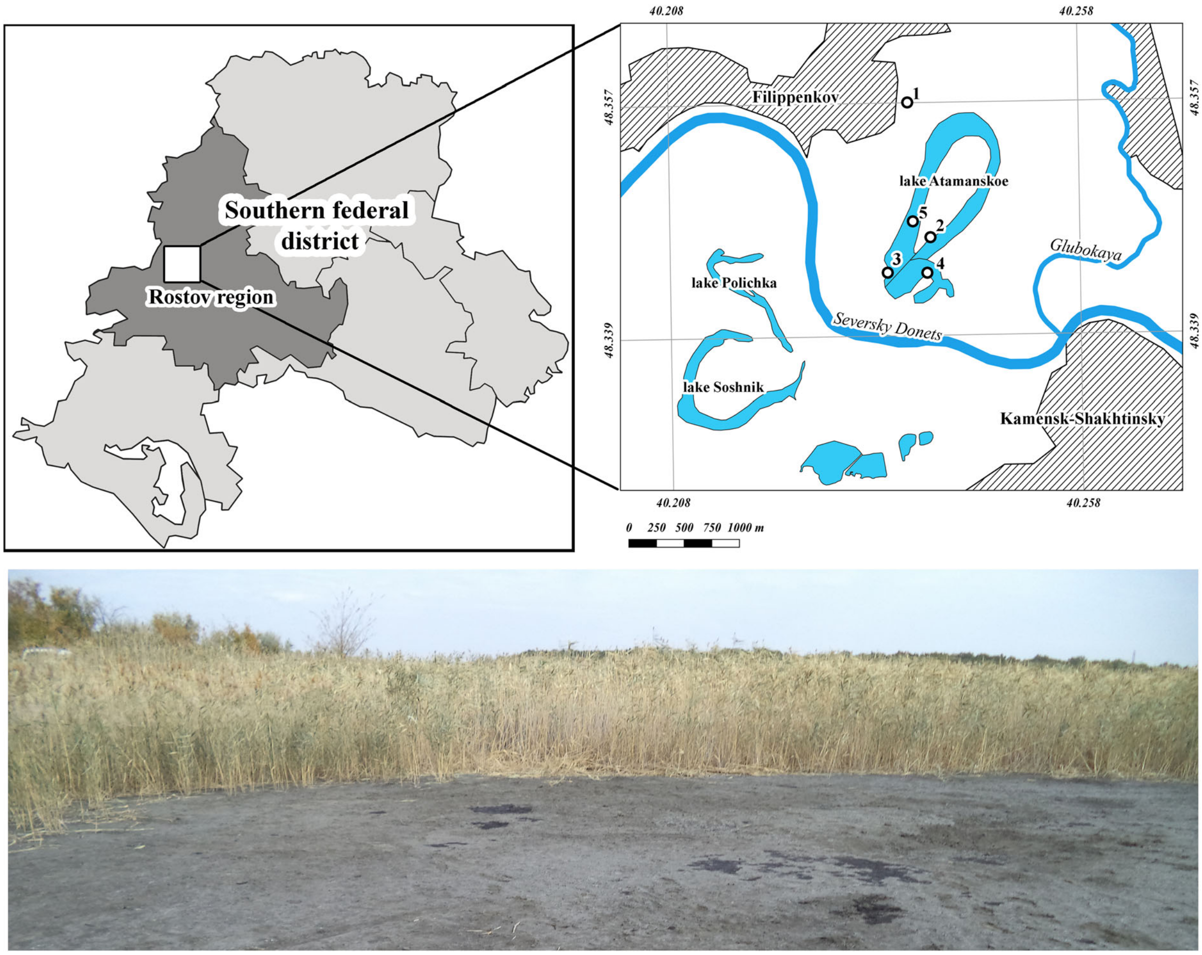

Fig. 1 Dried Atamanskoe lake and surrounding area in the vicinity of Kamensk-Shakhtinskii city, Rostov region, Russian federation (the location of the monitoring plots is denoted with circles)

Fig. 2 Scheme change in hydrological conditions of the Lake Atamanskoe for the past 30 years

Laboratory soil analysis

Soil OM measurement

SOM (Corg) content was conducted by sulfochromic oxidation, ISO 14235 (1998). The OM group and fractional analyses were determined as by Ponomareva and Plotnikova (1980). Three sequential extractions were performed. The free in colloidal humic substances (HS) and the HS that are connected with labile iron oxides comprise the first fraction. The «Ca-connected humic acids» making up the second fraction are obtained by extraction with 0.1 M sodium hydroxide after the removal of carbonates. The third fraction consists of HS bound with clay minerals and stable sesquioxides. It is obtained by boiling the sample for 6 h in 0.02 M sodium hydroxide. The humic acids (HA) that are present in these three fractions are designated HA-1, 2 and 3, and the fulvic acids (FA) are designated FA-1, 2 and 3. An additional fraction of «aggressive fulvic acids» which is extracted with 0.5 M sulfuric acid is designated 1-a-FA (Abakumov et al., 2013). The types of OM are determined by C_{HA}:C_{FA} ratio: humic (C_{HA}:C_{FA} > 2.0), fulvic–humic (C_{HA}:C_{FA} = 1.0–2.0), humic–fulvic (C_{HA}:C_{FA} = 0.5–1.0) and fulvic (< 0.5) (Bondareva & Fedorova, 2020).

The labile SOM components extractable by cold water and hot water were selected to describe SOM quality. Cold extraction WSOM (C_{cold}) was extracted with distilled water at 22–24 °C, soil/water ratio 1:5, followed by filtration through paper filters (pore diameter 1–2 µm) (Arinushkina, 1970). The organic carbon content of cold-extracted WSOM was evaluated by the permanganate (C_{cold1}) and bichromate (C_{cold2}) oxidation of aqueous extracts. The first method gives information on the content of easily oxidized substances, the second—more resistant to oxidation. The method of permanganate oxidation is based on the oxidation of organic substances in an acidic medium by 0.05 N $KMnO_4$. The method of bichromate oxidation is based on the oxidation of organic substances by potassium dichromate (Arinushkina, 1970).

Hot extraction WSOM was obtained (C_{hot}) by extraction at 70 °C for 20 h soil/water ratio 1:20, followed by filtration through paper filters (pore diameter 1–2 µm) (Sekaran et al., 2021). Hot-water extracts were assessed only using the method of bichromate oxidation.

Determination of Cu

The Cu sequential fractionation in the soil was carried out according to Tessier et al. (1979). Five fractions of Cu can be separated by this procedure: exchangeable (1 M $MgCl_2$, pH 7.0), carbonate-bound (1 M $NaCH_3COO$, pH 5.0 (with CH_3COOH)), Fe–Mn oxides bound (0.04 M $NH_2OH \cdot HCl$ in 25% CH_3COOH), bound to OM (0.02 M HNO_3 + 30% H_2O_2, pH 2.0 (with HNO_3), then 3.2 M NH_4CH_3COO in 20% HNO_3), silicates-bound (residual) (extract of $HF + HClO_4$ from the residual fraction). After the extraction, the atomic absorption spectroscopy (AAS) method was used to determine Cu concentrations in the extracts. The measurement of total Cu in the samples was performed by X-ray fluorescence (XRF) method on SPECTROSCAN MAX-GV spectrometer. The chemical analysis of each sample was done in triplicate relative to the control for analytical precision. The accuracy of metal content was evaluated with a reference state standard soil sample no. 9288-2009 (Federal State Unitary Enterprise Ural Research Institute of Metrology) to control measurement error methods of total content of certified components in the soil. The accuracy of the obtained data was repeatedly confirmed by international intercalibrations. Estimates of the degree of heavy metals contamination in the soils are compared to the maximum permissible concentration for Cu (55 mg/kg) (Bauer et al., 2018).

Determination of soil properties

The particle size distribution of the studied soils was determined according to ISO 13317-2 (2001). The methods were used for the measurement of pH of water soil suspension using a glass electrode in a 1:5 ISO 10390 (2005); calcium carbonate by using a Scheibler apparatus, ISO 10693 (1995). Mineralogical composition of soils was measured microscopically according to (Methodical Recommendation…, 2008).

Determination of enzymatic activity (urease and dehydrogenase)

To measure urease activity, the concentration of ammonium released during urea decomposition was measured by the modified indophenol method (Sekaran et al., 2021). Five grams of soil was placed in a conical flask and supplemented with 20 ml borate buffer and 2.5 ml of 0.72 M urea solution. One ml toluene was added to each flask; they were sealed and placed in an incubator for 2 h at 37 °C. Separate flasks were prepared as controls: they were supplemented with the same mixture, but instead of urea solution 2, 5 ml borate buffer was added. After the incubation, 30 ml of 1 M KCl + 0.01 HCl solution was added, and the flasks were placed on a rotary shaker at 200 rpm. After centrifugation, 1 ml of supernatant was collected and 1 ml of salicylate–nitroprusside reagent, 1 ml hypochlorite solution were added. The mixture was left for 1 h for color development, and absorbance was measured at λ = 690 nm on Beckman Coulter DU800 spectrophotometer.

For the determination of soil dehydrogenase activity, a modified procedure of Galstyan (1974) was used. Six grams of soil was added to sterile screw-cap tubes, and supplemented by 1.5 ml of 2% glucose solution, 2.0 ml 3% triphenyl tetrazolium chloride solution. Control tubes were supplemented with 3.5 ml sterile water. The contents of each tube were thoroughly mixed and placed in an incubator for 24 h at 30 °C. Following the incubation, the extraction of the reaction product, triphenyl tetrazolium formazan, was performed by washing the soil with portion of ethanol on Whatman No. 5 filter paper, until the filtrate was colorless. Resulting extracts were adjusted to equal volumes in volumetric flasks with pure ethanol. The concentration of triphenyl tetrazolium formazan was measured by absorbance at λ = 490 nm on Beckman Coulter DU800 spectrophotometer.

Determination of the abundance of culturable bacteria

Culturable bacteria were enumerated by plate counting on several solid media (Gorovtsov et al., 2019). The numbers of copiotrophic bacteria utilizing organic nitrogen, prototrophic bacteria, capable of using mineral nitrogen, aerobic spore-forming bacteria and actinomycetes were determined. These groups of bacteria participate actively in soil OM decomposition and turnover. Fresh OM entering the soil, i.e., plant residues, is actively degraded by aerobic spore-forming bacteria (bacilli) due to their ability to produce extracellular hydrolytic enzymes (proteases and peptidases, amylases, cellulases, etc.). Aerobic spore-forming bacteria were enumerated on nutrient agar with addition of 50% barley wort (final carbohydrate content 3%). To eliminate vegetative cells of other bacteria, the soil suspension was heated to 80 °C and kept for 20 min at this temperature (Gorovtsov et al., 2019). Copiotrophic bacteria growing on nutrient agar also take part in fresh OM decomposition. As the main C and N source in the nutrient agar is a mixture of peptides, most microorganisms that are enumerated are capable of ammonification and release ammonium to the soil. To determine the abundance of prototrophic bacteria that utilize mineral nitrogen and do not need exogenic amino acids plate counting on starch–ammonium agar (ISP-3) was performed. The actinomycetes were counted on the same medium, and their colonies were distinguished by characteristic colony morphology and aerial mycelium formation. This group of bacteria takes part in transformation of hardly decomposable compounds (chitin, lignine, etc.) and plays a significant role in humification process.

Statistical analysis

All data obtained by chemical analyses were presented as mean of three replicates. The descriptive statistics, including minimum and maximum values, means and confidence intervals for mean values, was carried out in the STATISTICA 10.0 software.

Results

The particle size distribution analysis of soil has shown that the soils of the investigated area were loams (Table 1). In Fluvisol, the content of soil particles (< 0.01 mm) was higher than in Spolic Technosols. The results of Fluvisol (plot 1) mineralogical analysis are presented in Table 2. In the Spolic Technosols mineral phases, the authigenic minerals were prevailed indicating their high degree of transformations. Sulfate's content was as high as 47–61% (Table 2). The industrial wastes dumped in the study area contained high sulfide's concentration (Privalenko et al., 2000; Bauer et al. 2018).

The reaction of the medium was also variable at different plots and characterized as strongly acidic 4.4 (plot 5), which was related to buried pollution sources; neutral pH 6.9–7.3 (plots 1, 3), strongly alkaline 7.7–8.0 (plots 2, 4). The content of carbonates in Spolic Technosols varied greatly (0–18.5%), while the carbonate content in Fluvisol was 1.7% (Table 1).

The total content of Cu (52 mg/kg) in Fluvisol (plot 1) is not higher than the maximum permissible concentration (55 mg/kg). In Spolic Technosols, Cu concentration reaches 437 mg/kg that is up to 10 times higher than the maximum permissible concentration (Table 2). There is a high variation in the Cu content, indicating an uneven distribution of pollutant in the Spolic Technosols.

Sequential fractionation of Cu compounds in Fluvisol (Table 3, Fig. 3) has revealed that the residual fraction associated with silicates is dominant in the soil (83% of the sum of fractions). The mobility of Cu in Fluvisol is very low (Fig. 3). The fractional distribution of Cu compounds in Fluvisol was following: residual > bound to organic matter > bound to Fe and Mn (hydr)oxides. The fractionation of Cu in Spolic Technosols revealed a high degree of metal fixation with OM (Table 3, Fig. 3), which is consistent with the previously reported data (Minkina et al., 2016). However, despite the high affinity of Cu with organic matter, the metal content in the residual fraction associated with silicates is dominant. The fixation of Cu fractions in the studied Technosols is the following: residual > bound to OM ≥ bound to Fe and Mn (hydr)oxides > exchangeable > bound to carbonates.

The average content of SOM in Spolic Technosols is 1.84%, and in Fluvisol – 2.03%. The Fluvisol indicated the lowest WSOM content, both easily oxidized (C_{cold1}) and more recalcitrant to oxidation (C_{cold2}): 49 and 85 mg/l, respectively (Table 4). In the contaminated soils, the average content of easily oxidized WSOM (C_{cold1}) in the contaminated soil is enhanced to 70 mg/l and recalcitrant to oxidation (C_{cold2}) 96 mg/l. The content of hot water extracted WSOM (C_{hot}) is an order of magnitude higher: in Fluvisol, the content of WSOM is the highest

Table 1 Physical and chemical properties of Fluvisol (plot 1) and Spolic Technosol (plots 2–5)

Plot	pH	$CaCO_3$, %	SiO_2, %	Fe_2O_3, %	Al_2O_3, %	Particle size, %		Exchangeable cations cmmol(+)/rg	
						Σ < 0.01 mm	< 0.001 mm	Ca^{2+}	Mg^{2+}
1	7.3 ± 0.2	1.7 ± 0.02	62.8 ± 7.1	7.5 ± 0.3	13.0 ± 1.1	53.1 ± 2.7	32.4 ± 1.8	31.0 ± 2.4	4.5 ± 0.3
2	7.8 ± 0.2	18.5 ± 1.4	66.7 ± 4.9	8.9 ± 0.1	11.9 ± 0.9	33.4 ± 1.6	2.8 ± 0.5	25.8 ± 2.1	3.5 ± 0.2
3	6.9 ± 0.2	1.1 ± 0.01	26.0 ± 1.3	9.1 ± 0.2	6.4 ± 0.3	48.3 ± 2.4	20.2 ± 1.6	30.0 ± 2.1	4.1 ± 0.4
4	7.9 ± 0.1	12.9 ± 0.1	18.9 ± 0.9	9.5 ± 0.3	6.1 ± 0.1	23.0 ± 0.9	10.8 ± 0.9	19.1 ± 1.5	4.8 ± 0.3
5	4.4 ± 0.1	n.d	62.0 ± 4.1	9.6 ± 0.2	10.8 ± 0.7	31.2 ± 1.5	9.6 ± 0.6	21.2 ± 1.8	3.9 ± 0.1

n.d not detected

Table 2 Mineralogical composition of Fluvisol (plot 1) and Spolic Technosol (plots 2–5), %

Group minerals	Minerals	Plot				
		1	2	3	4	5
Terrigenous minerals						
Stable	Rutile	4.0	Traces	Traces	Traces	Traces
	Zircon	8.0	Traces	Traces	Traces	Traces
	Ilmenite	5.0	7.0	12.0	8.0	14.0
Intermediate	Kyanite	Traces	Traces	Traces	Traces	Traces
	Staurolite	4.0	n.d	n.d	n.d	n.d
Unstable	Amphibole	n.d	3.0	n.d	n.d	n.d
	Muscovite, biotite	n.d	2.0	n.d	n.d	n.d
	Magnetite, hematite	57.0	1.0	4.0	Traces	Traces
	Pyroxene	3.0	2.0	n.d	n.d	n.d
Authigenic minerals						
	Iron hydroxides	13.0	11.0	10.0	26.0	25.0
	Pyrite, marcaplot	1.0	9.0	11.0	8.0	14.0
	Carbonate	Traces	9.0	2.0	6.0	Traces
	Sulfates	5.0	56.0	61.0	52.0	47.0

n.d. not detected

Table 3 Fractional composition of Cu in the soils of Fluvisol (plot 1) and Spolic Technosol (plots 2–5) (mg/kg) (*n* = 3)

Plot	Total	Fraction					Sum of fraction (total)
		Exchangeable	Bound to carbonates	Bound to Fe and Mn (hydr)oxides	Bound to organic matter	Residual	
1	52.4 ± 2	0.10 ± 0.0	0.20 ±	2.10 ±	6.80 ± 0.9	44.60	53.80 ± 4.6
2	132.4 ± 5	6.30 ± 0.4	4.20 ± 0.2	10.80 ± 0.8	45.90 ± 8.9	68.00	135.20 ± 9.0
3	437.6 ± 18	25.46 ± 2.3	13.15 ± 2.2	29.08 ± 2.7	168.46 ± 6.4	207.54	443.69 ± 35.1
4	391.8 ± 14	32.90 ± 2.8	8.10 ± 2.1	23.00 ± 0.9	127.00 ± 2.0	203.90	394.90 ± 23.1
5	285.6 ± 8	16.67 ± 1.3	9.81 ± 3.3	20.10 ± 1.5	90.67 ± 9.2	163.33	300.57 ± 18.0

*According to data (Minkina et al., 2017)

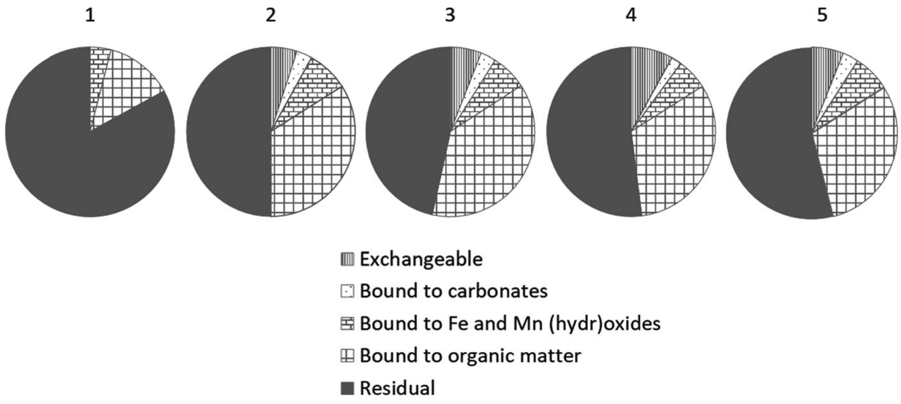

Fig. 3 Loosely bound Cu compounds in the soils of Fluvisol (plot 1) and Spolic Technosol (plots 2–5)

Table 4 The fractional and group composition of organic matter of Fluvisol (plot 1) and Spolic Technosol (plots 2–5)

Plot	C_{tot}, %	HA, % of C_{tot} in the soil			∑HA	FA, % of C_{tot} in the soil				∑FA
		1	2	3		1a	1	2	3	
1	2.03 ± 0.1 16.3 ± 0.7	2.3 ± 0.1	28.6 ± 1.3	4.9 ± 0.3	35.8 ± 1.7	1.4 ± 0.1	5.6 ± 0.2	4.9 ± 0.3	4.4 ± 0.1	
2	1.57 ± 0.2 20.6 ± 1.0	5.1 ± 0.3	26.1 ± 1.5	7.6 ± 0.4	38.8 ± 2.2	1.8 ± 0.2	4.9 ± 0.1	6.3 ± 0.4	7.6 ± 0.3	
3	1.16 ± 0.2 29.0 ± 1.3	7.3 ± 0.3	18.3 ± 2.2	10.6 ± 0.5	36.2 ± 3.0	4.2 ± 0.3	6.5 ± 0.2	9.8 ± 0.5	8.5 ± 0.3	
4	1.04 ± 0.2 24.4 ± 1.1	5.8 ± 0.3	20.8 ± 1.3	10.9 ± 0.4	37.5 ± 2.0	3.2 ± 0.3	5.7 ± 0.2	7.9 ± 0.4	7.6 ± 0.2	
5	3.60 ± 0.2 26.3 ± 1.4	6.2 ± 0.5	20.1 ± 1.9	10.6 ± 0.6	36.9 ± 2.3	2.6 ± 0.4	6.0 ± 0.5	9.7 ± 0.3	8.0 ± 0.6	

Plot	C_{tot}, %	∑HA + ∑FA	$\frac{C_{HA}}{C_{FA}}$	HC	C_{water}-soluble, mg/l		
					C_{cold1}	C_{cold2}	C_{hot}
1	2.03 ± 0.1	52.1 ± 2.4	2.20	47.9 ± 2.6	49 ± 3	85 ± 1	960 ± 20
2	1.57 ± 0.2	59.4 ± 3.2	1.88	40.6 ± 2.7	68 ± 3	90 ± 1	710 ± 40
3	1.16 ± 0.2	65.2 ± 4.3	1.25	34.8 ± 2.5	70 ± 3	93 ± 2	780 ± 20
4	1.04 ± 0.2	61.9 ± 3.1	1.54	38.1 ± 2.3	68 ± 2	96 ± 3	750 ± 20
5	3.60 ± 0.2	63.2 ± 3.6	1.40	36.8 ± 3.1	65 ± 2	89 ± 1	850 ± 20

C_{tot} total organic carbon content, *HA* the humic acids content, *FA* the fulvic acids content, *HC* the humic content, C_{cold1} carbon content of cold water extracts by permanganate oxidation, C_{cold2} carbon content of cold water extracts by dichromate oxidation, C_{hot} carbon content of hot water extracts

– 960 mg/l; for Spolic Technosols, the average content of WSOM is 780 mg/l.

In the Fluvisol, the contents of free and sesquioxide-bound HAs (HA-1) were 2.3% and free FA (FA-1a) was 1.4% and were characterized as low (Table 4). A decrease in the amount of humic acids was found with pollution increasing. In Spolic Technosols, the magnification of the HA-1 and FA-1a amount was established. Moreover, the content of the fraction bound to Ca (HA-2) is higher than other fractions. The contamination led to increase in the mobile HA and FA bound to sesquioxides (HA-3) content. The type of SOM for Fluvisol was characterized as humic, for Spolic Technosols—fulvic–humic. In the SOM composition, HA presumably bound to Ca (HA-2) prevail over FA.

The determination of Fluvisol enzymatic activity (plot 1) has shown that it was higher than in all studied Technosols. The activity of dehydrogenases in Fluvisol was 70.7 µg TPF/g soil/h, which corresponds to high activity based on D.G. Zvyagintsev scale (1978). At the same time, only the Technosols from the plot 2 exhibited high dehydrogenase activity, while in the other plots it ranged from 2.18 to 26.6 µg TPF/g soil/h, corresponding to low or very low activity. Urease activity in Fluvisols is as high as 283.39 µg N-NH_4/g soil/h, and estimated as high activity, whereas in Technosols, it is only 4.65–41.69 µg N-NH_4/g soil/h (Fig. 4). The results demonstrate that long-term pollution has led to a dramatic decrease in the studied

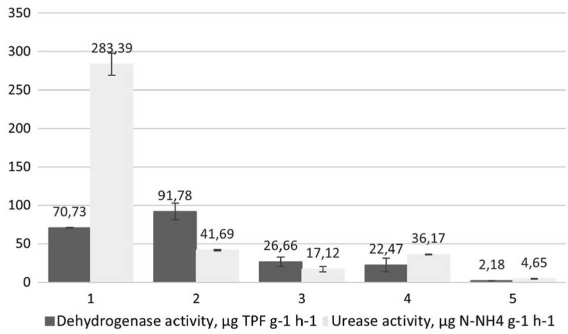

Fig. 4 Dehydrogenase and urease activity in the Fluvisol (plot 1) and Spolic Technosol (plots 2–5)

Technosols enzymatic activity as compared to their natural intrazonal analogues.

High levels of soil contamination with Cu had a noticeable negative impact on the number of soil bacteria. The culturable microorganisms numbers varied significantly across the studied plots: copiotrophic bacteria from 1.9 to 85.1 million CFU/g of dry soil and prototrophic bacteria from 3.8 to 78.1 million CFU/g. The decrease in spore-forming bacteria number was observed from 7,669,000 to 789,000 CFU/g oven-dried soil and for the actinomycetes from 13,552,000 to 129,000 CFU/g oven-dried soil. In Spolic Technosols, a sharp decrease in the number of bacteria from all studied groups was observed (Fig. 5). However, even in the most contaminated soils a large number of culturable bacteria were found, which indirectly prove the adaptation abilities of the microbial community (Gorovtsov et al., 2019).

Discussion

With increase in pollution, the amount of mobile Cu compounds increases. However, the residual metal fraction in Spolic Technosols is 47–54%. The process of metal compounds accumulation by crystal lattices of clay minerals is slow, but due to a long period of pollution, it is possible (Minkina et al., 2016). The spectra of the X-ray absorption fine structure were shown that the Cu^{2+} cations absorbed by the soil can replace some of the Al^{3+} ions in crystal lattices of clay minerals (Strawn & Baker, 2008). Specific adsorption and ion exchange are the main mechanisms of Cu retaining in the solid phase. Under the acidic conditions, the adsorption is due to a constant charge on clay minerals, whereas at neutral reaction, the formation of hydrolyzed varieties and organic complexes plays the key role (Pinskii et al., 2018).

In a low concentration range (unpolluted soils), Cu is specifically adsorbed by the surfaces of various soil colloids in amounts exceeding their exchange capacity (Bauer et al., 2020). The ion-exchange adsorption with an increase in metal concentration prevails over the specific. The metals in solution react with different components. The physicochemical characteristics of soils and the amount of SOM limit the number of active surface functional groups. Certain places of adsorption are already occupied, and the centers of ion exchange become smaller (Petruzzelli et al., 1997). Humic and fulvic acids and Fe and Mn (hydr)oxides are recognized as important colloidal structures due to their high affinity for contaminants. Interaction with them also controls the availability and lability of metals (Sokolova et al., 2013). The specific ligand and metal ion electrochemical and structural properties determine the selectivity of sorption. The binding force of interactions of SOM with metal is often expressed in terms of stability constants, which are higher for humic acids (7.8) than for fulvic acids (4.0) (Bauer et al., 2020). An increase in the proportion of the fraction associated with OM in contaminated soils is associated with the ability of Cu forms to become more stable organomineral compounds, represented by intraspheric complexes of the chelate type (Minkina et al., 2006). Cu^{2+} ions can participate in the formation of octahedral inner-sphere coordination chelate complexes with humic acids of the Chernozem. The study of such compounds' fine structure was performed by the X-ray absorption method (Bauer

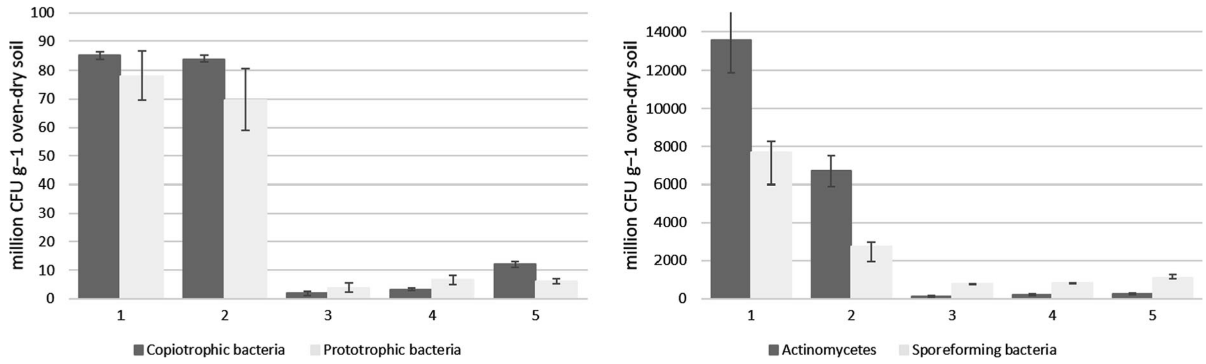

Fig. 5 Abundance of culturable bacteria in the Fluvisol (plot 1) and Spolic Technosol (plots 2–5)

et al., 2020). Chernozems and Fluvisols were developed in similar climatic conditions, which contributes to the formation of similar physical and chemical properties, especially SOM (Minkina et al., 2018).

It was established that there was close relationship between total Cu and clay contents (particle size $<$ 0.01), content ($r = 0.48$, $p > 0.05$), exchangeable Cu fraction ($r = 0.53$), Cu fraction bound to Fe and Mn (hydr)oxides ($r = 0.49$), residual Cu fraction ($r = 0.50$). The Cu fraction bound with carbonate correlated with pH ($r = 0.43$) and carbonate content ($r = 0.59$). A correlation was found between OM with total content of Cu ($r = 0.51$) and Cu fraction bound to OM ($r = 0.65$). The level of carbonate content in Fluvisol formed as a result of hydromorphic conditions, properties of rocks and the process of biomineralization (Bauer et al., 2018). The carbonate content in Spolic Technosols depends on the composition of industrial waste (Privalenko et al., 2000). A correlation was found between OM with total content of Cu ($r = 0.51$) and Cu fraction bound to OM ($r = 0.65$). Complexation of Cu with organic ligands is one of the mechanisms that determine its mobility. The formation of complexes with poorly soluble OM can increase the proportion of metals in the solid phase (Gao et al., 1997). Therefore, the retention of trace metals in soils and rocks can be substantially affected by the content of organic matter.

According to the data obtained, a high level of pollution led to a magnification in the content of the amount of WSOM and free fractions of HA and FA. Under the influence of metal ions, disturbances in the structure of humic acid molecules, breaking of bonds between constitutional atoms and the creation of new centers for the formation of organomineral complexes can occur (Bondareva & Fedorova, 2020; Minkina et al., 2006; Yakovets, 2021).

At the initial stage of HA complexation with metal ions, one covalent bond is formed and one H^+ cation is released (Sokolova et al., 2013). The potential of the complexing agent is not completely implemented by the formation of chemical bonds in this case. Ions (anions of metal salts) present in the solution can act as counter ions, compensating the second charge of the metal cation. The formation of less stable metal–organic complexes can be a reason for the increase in the content of free HA (HA-1) and FA (FA-1a) in soil pollution. Compared to humic acids, fulvic acids are usually characterized by a lower content of total and aromatic carbon (Bezuglova, 2019). They are more dispersed, hydrophilic and more reactive. As one of the most accessible to microorganisms groups of SOM, fulvic acids can accumulate in relatively larger quantities than humic acids in metal-contaminated soil with a decrease in microbiological activity (Yakovets, 2021), which is noted in Spolic Technosols. However, the content of most reactive fraction fulvic acid (FA 1a) can change due to its rapid consumption. The organic carbon in this fraction redistributed in other fractions fulvic acids or decompose with the release of CO_2 (Bondareva & Fedorova, 2020).

Conclusion

The long-term soil pollution with Cu affected on the SOM. The amount of readily oxidized water-soluble organic matter, as the most labile group of SOM compounds, magnificated along with the increasing level of Cu content. The changes in the SOM fractional composition were established: the amount of mobile free fractions of HA and FA increased. The amount of humic acids bound to Ca decreases, while the total amount of fulvic acids increases. The SOM fractional composition shifts from humic to fulvic–humic. With increase in pollution, the content of Cu mobile fractions, exchangeable and associated with organic matter, increased. The labile part of SOM is a sensitive indicator of Cu interaction with organic matter. Pollution reduces the activity of urease and dehydrogenase and affects the number and species composition of microorganisms. However, despite the decrease in the number of bacteria in Spolic Technosols, the presence of active and potentially active bacteria was observed even in the most polluted soils, which indicates the adaptation of the microbial community. The bacteria that decompose fresh OM are the most resistant to pollution. These include copiotrophic bacteria and aerobic bacteria forming endospores. The study of heavy metals relationship with the OM and the activity of soil enzymes is of great significance for evaluation of contaminated ecosystems.

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Author contributions IVZ and AVG were involved in data curation, formal analysis, methodology, investigation, conceptualization, supervision, visualization, writing—original draft preparation—review & editing; TM helped in conceptualization, supervision, writing—review & editing; SM contributed to supervision, formal analysis, methodology, writing—review & editing; MVB and TB were involved in investigation, data curation, visualization, formal analysis.

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Data availability The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

Declarations

Conflict of interest The authors have no financial or proprietary interests in any material discussed in this paper.

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