Chapter 13
Ecoservice Role of Earthworm (*Lumbricidae*) Casts in Grow of Soil Buffering Capacity of Remediated Lands Within Steppe Zone, Ukraine

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13.1 Introduction

Protecting environment, managing natural resource, and ensuring environmental safety of human life are essential conditions for the sustainable economic and social development of European countries (Sklenicka et al. 2004; Pecharová et al. 2011; Behnassi et al. 2014). In this regard, solving the environmental problems of anthropogenic disturbed areas within Eastern Europe is on the front burner (Thassitou and Arvanitoyannis 2001; Pecharová and Hrabankova 2006; Böhm et al. 2009). Coal industry activity is considered to be one of the most powerful factors leading to deterioration of natural landscapes variety (Strzyszcz 1996).

Soil contamination with heavy metals affects primarily the soil biological and ecological conditions; it can change the conservative soil signs such as humus status, structure, acidity, and other characteristics. Soil contamination leads to partial and, in some cases, complete loss of soil fertility that reduces soil economic cost (Orlov 1994; Orlov et al. 2005; Truskavetskiy 2003). While investigating the man-made contamination, such contamination brings up the challenge of soil protective capacity under the elevation of heavy metals concentrations. The better soil protective properties, the more heavy metals can be insolubilized and no plant-available. As a result, over amounts of chemicals in food chains are restricted, and their migration to surrounded environment ecosystems is limited (Ilyin 1995; Cooke and Johnson 2002).

As a result of coal mining, the lands intended for economic purpose are withdrawn from agricultural use. Thus, such lands are replaced by man-made landscapes, i.e. dumps and open-cuts, which are characterized by subsidence, highly

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M. Behnassi et al. (eds.), *Climate Change, Food Security and Natural Resource Management*, https://doi.org/10.1007/978-3-319-97091-2_13

mineralized groundwater table rise, acid mine drainage, toxic contamination (Jachimko 2012; Pecharová et al. 2001). These factors affect species wealth and biological diversity within territories disturbed by production activities (Ripl et al. 1994). Disrupted lands developed during coal mining can be partially restored by remediation (Wang et al. 2001; Pecharová et al. 2001; Cooke et al. 2002).

Main agro-chemical characteristics determining remediated lands productivity and suitability for biota living are actual acidity (pH) and salinity (Arranz-González 2011). Scientists notice that mine spoil often has high density, low coefficient of structure, as well as high salinity of water extracts. Such characteristics determine extremely low suitability of such substrates for biota existence (Skousen et al. 1998; Cooke and Johnson 2002). The next remediation stage is covering with topsoil, such as compost mass or humus layer. Humus topsoil of ordinary chernozem or humus-free subsoil is usually used as remediated layers under conditions of Ukrainian Steppe, particularly within Western Donbass. Final stage of damaged lands remediation is a biological stage. The most using type is phyto-remediation by herbal, arboreal and shrubbery plantations (Böhm et al. 2011; Singh and Singh 2006; Pakhomov et al. 2009).

Among soil invertebrates, earthworms have a leading role in formation of stability mechanisms in arboreal (forest) plantations. As a result of their life activity, earthworms make a significant ecological contribution to transformation of soil characteristics and properties (Lavelle et al. 2006; Didur et al. 2011; Aira et al. 2003; Choosai et al. 2010; Bottinelli et al. 2010; Kul'bachko et al. 2011). Earthworms so called 'ecosystem engineers' are the animals effecting on environment and communities of soil invertebrates, and resulting in ecosystem successions (Jones et al. 1994; Aira et al. 2010; Eisenhauer 2010). Such earthworm environmental-forming function does not duplicate by any groups of living organisms; it plays a crucial role in protecting and improving the fertility in remediated soils (Didur et al. 2013; Kul'bachko et al. 2014).

Tropho-metabolic activity of saprophages is considered to be an important element in the formation of numerous environmental properties (Brygadyrenko 2016); that causes maintaining of buffer properties in artificial soil against copper contamination within remediated areas. Copper among such metals as zinc, molybdenum, cadmium, and lead – is one of the main man-made environmental pollutants (Pokarzhevsky 1985; Safonov 2005).

Mainly, buffering capacity is a soil capability to resist changes under impact of various factors. It may be acid-alkaline buffering capacity (pH-buffering capacity) (Truskavetskiy 2003), or buffering capacity to contamination by heavy metals (Ilyin 1995; Pampura et al. 1993). Some studies examining the buffering capacity of Ukrainian soils are agriculturally orientated (Truskavetskiy 2003); they are devoted to study of buffering capacity of different soil genetic types, and not associated with a soil-zoological component (Gamkalo 2005, 2001). There is no information about the participation of soil saprophages in buffering properties formation and maintenance of remediated lands under arboreal plantations.

The study goal is determining the tropho-metabolic effect of earthworm (*Lumbricidae*, as zoogenic component of ecosystem services) to maintenance stability of remediated soil against some negative effects such as soil solutions with acid pH values and copper contamination.

13.2 Materials and Methods

The present research was carried out within Steppe zone, in Western Donbass as a part of Donetsk Coal Basin (Fig. 13.1). The western part of this basin located in Dnipropetrovsk region is called Western Donbass. In the basin area, it was found about 40 layers with a working capacity 0.6–1.6 m, which underlies 400–1800 m depth. Coal production is performed by open-cut mining. Flat and conical piles at eleven mines of Western Donbass contain more than 70 million tons of phytotoxic sulfur-containing carbon-bearing and argillaceous shale, argillite with a high content of pyrite (FeS_2), troilite (FeS), chalcopyrite (FeCuS), etc. When deeply buried deposits of Cretaceous period are moved onto surface, it is initiated the processes of physical weathering, oxidation, dissolution, hydrolysis, and burning. A number of other negative factors are also determined, such as high concentration of soluble toxic salts, alkalinity level rise, low absorbency and permeability, high spoil density, low carbon and plant-available nitrogen (Novitskiy 2011; Mtui et al. 2006).

Soil samples are selected within remediated areas in Norway maple plantations, on two different remediated sites that differ in stratigraphic structure (location of layers from top to bottom): Variant 1: loess loam 0–50 cm, tertiary sand: 50–100 cm;

Fig. 13.1 Location of Donbass Coal Basin in Ukraine: (1) Western Donbass; (2) Central Donbass

mine spoil: 100–700 cm; Variant 2: humic layer of ordinary black soil: 0–50 cm, loess loam: 50–100 cm, tertiary sand: 100–150 cm, mine spoil: 150–700 cm.

Samples of fresh cast of earthworm *Aporrectodea caliginosa* (Savigny 1826) are selected from abovementioned remediated lands. It is the only species of worm found on the investigated remediated site. *Aporrectodea caliginosa* (Savigny 1826) is saprophage, secondary destructor, humificator (Milcu et al. 2006; Striganova 1980). According Bouché classification it belongs to endogeic morpho-ecological group of earthworms (Butt and Lowe 2011).

Sampling plots in the study site were selected on the basis of occurrence of fresh worm casts. The fresh earthworm casts were represented by small soil conglomerates weighting 0.8–13.4 g. Collected fresh worm casts were combined into a common sample for each study variant weighing approximately 1 kg. The samples were taken to the laboratory and weighed, air-dried, powdered with mortar and pestle, and passed through a fine sieve (1 mm-mesh). For each test, the sample of 10 g was taken from the combined sample in triplicate. For each experiment was calculated mean and standard error.

Differences in the physico-chemical properties of worm casts and adjacent soil were tested by Student's t-test. All statistics were calculated using Statistic software Statistica version 6.0.

13.2.1 Evaluation Method of Earthworm Casts and Soil Buffering Capacity Against Different pH Values

To assess the buffering capacity of earthworm casts, soil and subsoil, we used an approach based on determination of changes in their pH level after adding of acids or alkalis solutions. The experimental design for acid and alkaline exposure ranges is shown in Table 13.1.

A series of weights of soil samples, earthworm casts and heat-treated sand (reference) were prepared for analysis. Fixed amounts of acid and alkali solutions were added to all samples, while the total amount of solution (water-acid or water-alkali) was constant (25 mL). Ratio soil (or earthworm cast): solution was amounted to 1:2.5. Results of actual acidity measuring were plotted. X-axis indicates the number

Table 13.1 Design of experimental study in determination of pH-buffering capacity in soil samples and earthworm casts

Reagent	**Volume of reagent added, mL**						
	Acid exposure range						
0.1 M HC1	–	1.5	3	4.5	6	7.5	9
H_2O	25	23.5	22	20.5	19	17.5	16
	Alkaline exposure range						
0.1 M NaOH	–	1.5	3	4.5	6	7.5	9
H_2O	25	23.5	22	20.5	19	17.5	16

of milliliters of acid (or alkali) added, and Y-axis indicates the corresponding pH values. Obtained plotted curves allow buffering capacity of samples by the buffering area calculation to be estimated in acid and alkaline ranges. Buffering area is defined as area between titration curves of sample and reference (quartz sand), and expressed in conditional units.

To calculate buffering capacity, method of numerical integration was applied. The task was solved by means of Simpson formula (Atkinson 1989; Chapra 2012):

$$\int_a^b f(x)\,dx = \frac{b-a}{6n}\begin{bmatrix}(y_0 + y_{2n}) + 2(y_2 + y_4 + \ldots y_{2n-2}) + \\ +4(y_1 + y_3 + \ldots + y_{2n-1})\end{bmatrix},$$

where

a and *b* are the lower and upper integration limits, respectively;

n is a number of pairs of parabolic trapezia, corresponding to a number of ordinates (points) excluding one, taken by half;

y are integration variables (values of a function at the corresponding points).

In square brackets, the extreme ordinates (y_0 and y_{2n}) are taken with a coefficient of “1”; other ordinates with even indices are taken with a coefficient of “2”, and odd indices are taken with a coefficient of “4”. To calculate area by Simpson formula an odd number of ordinates is required. If there are five ordinates, then $n = 2$ because they form only two pairs of curved (parabolic) trapezia (the first pair: $[y_0,y_1]$, $[y_1,y_2]$, the second pair: $[y_2,y_3]$, $[y_3,y_4]$); if there are seven ordinates, then $n = 3$, etc. Measurement of actual acidity (pH) in samples was performed in triplicate. Mean, its standard error and significant difference of the mean were calculated (Van Emden 2008; Zar 2010; Weiss 2012).

13.2.2 *Evaluation Method of Earthworm Casts and Soil Buffering Capacity Against Different Levels of Copper Contamination*

To determine zoogenic environmental-forming function in soil resistance formation against copper contamination, we studied immobilization (immobility) – mobilization (mobility) of copper in earthworm casts and bulk soils, and participation of earthworm casts in formation of resistance against contamination with copper. Previously prepared air-dry soil and casts samples were weight, transferred in 150 mL flasks and added copper sulphate pentahydrate solution in 1:10 ratio, which contains copper at concentrations 5–40 mg/L with 5 mg/L grade. Flasks were shaking at 2 h and leaved during a day. Then a suspension was stirred and mixed again, only the funnels with filter content were used in further assay. Matter on the filter was transferred in glass weighing bottle and air-dried. Sample weights were analyzed on moving forms of copper compounds by atomic absorption method.

To assess the impact of earthworms' tropho-metabolic activity for maintaining resistance of their habitats to copper pollution we used effect and toxicant immobilization efficiency. Effect (E. inf.) is a difference of areas between reference and sample buffering capacity in nominal units. Effect value indicates the soil material stability to contaminant. Than it is higher, soil is the more stable to such effect. Effectiveness of toxicant immobilization (E. imm.) (binding) is calculated as effect to reference ratio. Reference is presented as a curve made by points relevant to logarithm of initial metal concentrations in solution. Immobilization effectiveness is expressed as relative dimensionless units (in percentage). When effectiveness values increase, soil stability to heavy metal impact growth.

13.3 Results and Discussion

13.3.1 Effect of Earthworm Casts on Soil Buffering Capacity Against Different pH Levels

For Variant No. 1, where loess loam is represented, changes in pH values in the analyzed samples within acid range are shown in Table 13.2. Note that loess loam has conditionally neutral reaction, and casts have neutral one. By adding small amount of acid solution (3 mL), reaction of loess loam was changed from

Table 13.2 Results of buffering capacity measurements in acid and alkaline ranges for Variant No. 1 (Loess Loam, Earthworm Casts) and sand (Reference)

Amount of reagent added, mL	Acidity (pH)		
	Sand	Loess loam	Casts
	Acid range (Reagent 0.1 M HCl)		
0	6.95 ± 0.05	6.60 ± 0.05	7.16 ± 0.06
1.5	3.25 ± 0.10	6.09 ± 0.07	6.78 ± 0.08
3	2.80 ± 0.05	5.07 ± 0.09	6.55 ± 0.05
4.5	2.52 ± 0.04	4.64 ± 0.07	6.42 ± 0.07
6	2.45 ± 0.05	4.43 ± 0.08	6.33 ± 0.11
7.5	2.36 ± 0.06	3.93 ± 0.11	6.24 ± 0.09
9	2.29 ± 0.04	3.73 ± 0.08	6.10 ± 0.08
	Alkaline range (Reagent 0.1 M NaOH)		
0	6.95 ± 0.05	6.60 ± 0.05	7.16 ± 0.06
1.5	11.45 ± 0.05	6.78 ± 0.08	7.43 ± 0.07
3.0	11.65 ± 0.10	7.20 ± 0.10	8.01 ± 0.06
4.5	11.72 ± 0.07	7.72 ± 0.07	8.55 ± 0.05
6.0	11.87 ± 0.06	8.25 ± 0.10	8.87 ± 0.07
7.5	12.05 ± 0.05	8.75 ± 0.05	9.01 ± 0.10
9.0	12.05 ± 0.10	9.35 ± 0.07	9.20 ± 0.07

Here and below, mean and its reference error are given conditionally neutral (6.60 ± 0.05) to acid one (5.07 ± 0.09), while casts having initially neutral reaction (7.16 ± 0.06) will have conditionally neutral one (6.55 ± 0.05), which can be maintained at adding a larger amount of acid.

By adding a small amount of alkaline solution (1.5 mL), reaction of loess loam remains conventionally neutral (6.78 ± 0.08), while casts get clear slightly alkaline reaction (7.43 ± 0.07) instead original neutral reaction (7.16 ± 0.06) (see Table 13.2). By adding larger amount of alkaline solution (6 mL), reaction of loam and cast becomes alkaline (8.25 ± 0.10 and 8.87 ± 0.07, respectively) and both samples develop strong alkaline reaction by adding maximum amount of alkaline solution (9 mL).

Buffering capacity curves within acid range for Variant No. 1 (loess loam, earthworm casts) and sand (reference) are shown in Fig. 13.2. Buffering capacity area of remediated soil samples (Fig. 13.2a) lies between titration curve of sand and titration curve of bulk soil; buffering capacity area of earthworm casts (Fig. 13.2b) lies between titration curve of sand and titration curve of casts. In acid range, location of titration curves of the studied samples indicates that buffering capacity area of casts is significantly larger than that of loess loam.

Figure 13.3 shows curves of buffering capacity in alkaline range for Variant No. 1 (loess loam, earthworm casts) and sand. Location of titration curves of the studied samples indicates that buffering capacity area of casts is smaller than one of loess loam.

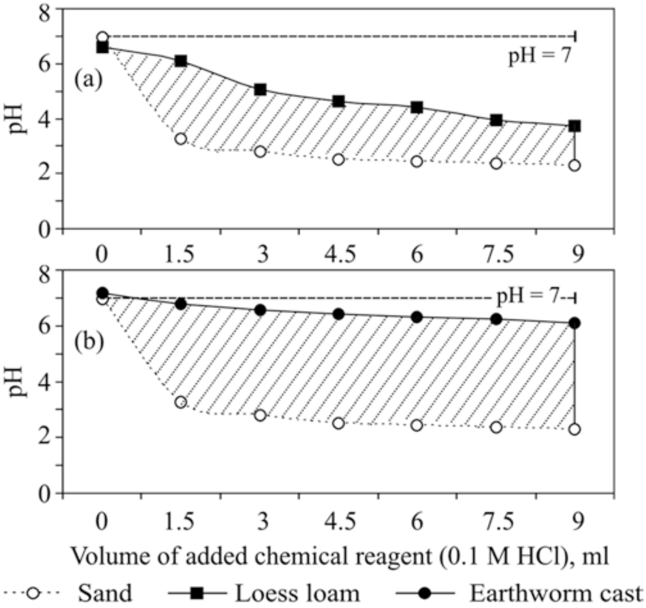


Fig. 13.2 Buffering capacity area of the studied samples in remediated sites within acid exposure range (Variant No. 1): (**a**) loess loam; (**b**) earthworm casts

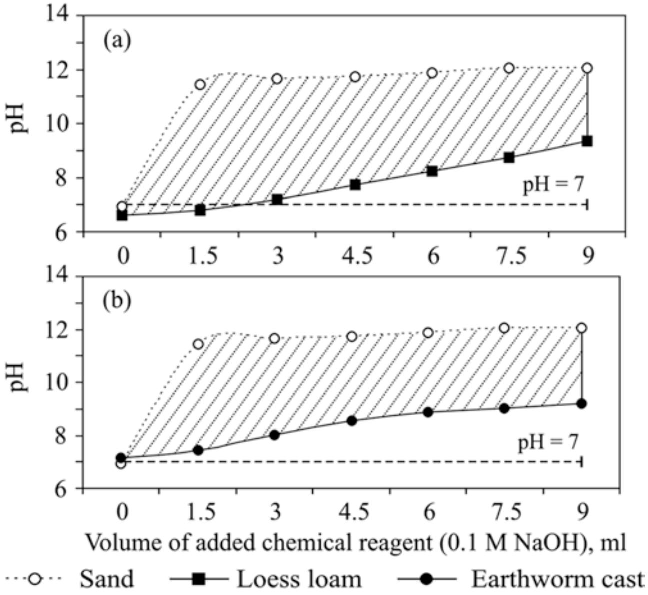


Fig. 13.3 Buffering capacity area of the studied samples in remediated sites within alkaline exposure range (Variant No. 1): (**a**) loess loam; (**b**) earthworm casts

Table 13.3 Estimation of soil buffering capacity indexes in Variant No.1 (loess loam, earthworm casts)

Range	Buffer capacity area, nominal cm^2	
	Loess loam	Casts
Acid	18.1 ± 0.51	32.5 ± 0.52**
Alkaline	33.8 ± 0.43	28.7 ± 0.06*
Acid-Alkaline(Total)	51.9 ± 0.60	61.1 ± 0.53***

Significant difference of mean with level of significance: * – ≤ 0.05, ** – ≤ 0.01, *** – ≤ 0.001

Table 13.3 shows the calculated values of the buffering capacity areas of loess loam and earthworm cast, reflecting buffering capacity of soil of Variant No. 1 and their statistical evaluation. The total area of casts buffering capacity is statistically higher by 9.2 nominal cm^2, i.e. by 17.9% higher than the total area of loess loam buffering capacity. However, their buffering capacity is shown more in the acid range.

For Variant No.2, the upper layer of which consists of humic topsoil filling, changes in pH of the remediated soil samples, earthworm casts and sand (reference) in acid range are shown in Table 13.4. Humic topsoil has conditionally neutral reaction, and casts have neutral one. By adding small amount of acid solution (1.5 mL), reaction of humic topsoil was changed from conditionally neutral in soil solution (6.82 ± 0.12) to subacid reaction (5.86 ± 0.11), while casts having initially neutral reaction (7.16 ± 0.04) get conditionally neutral one (6.33 ± 0.07). By adding large amounts of acid solution (from 4.5 to 9 mL), reaction of humic topsoil solution and casts is the same (acidic).

Table 13.4 Results of buffering capacity measurements within acid and alkaline ranges, Variant No. 2 (humic topsoil, earthworm casts) and sand (reference)

Volume of reagent added, mL	Acidity (pH)		
	Sand	Humic topsoil	Casts
	Acid range (Reagent 0.1 M HCl)		
0	6.95 ± 0.05	6.82 ± 0.12	7.16 ± 0.04
1.5	3.25 ± 0.10	5.86 ± 0.11	6.33 ± 0.07
3	2.80 ± 0.05	5.37 ± 0.10	5.77 ± 0.07
4.5	2.52 ± 0.04	4.86 ± 0.09	5.34 ± 0.08
6	2.45 ± 0.05	4.38 ± 0.08	4.97 ± 0.07
7.5	2.36 ± 0.06	4.12 ± 0.12	4.65 ± 0.10
9	2.29 ± 0.04	4.00 ± 0.10	4.43 ± 0.08
	Alkaline range (Reagent 0.1 M NaOH)		
0	6.95 ± 0.05	6.82 ± 0.12	7.16 ± 0.04
1.5	11.45 ± 0.05	7.68 ± 0.08	7.50 ± 0.06
3.0	11.65 ± 0.10	8.67 ± 0.12	8.23 ± 0.09
4.5	11.72 ± 0.07	9.42 ± 0.09	8.90 ± 0.10
6.0	11.87 ± 0.06	9.94 ± 0.08	9.32 ± 0.07
7.5	12.05 ± 0.05	10.31 ± 0.05	9.56 ± 0.08
9.0	12.05 ± 0.10	10.46 ± 0.13	9.90 ± 0.05

In order to get same changes in pH of the studied samples by means of adding a small amount of alkaline solution (1.5 mL), reaction of humic topsoil and casts was changed to weakly alkaline (7.68 ± 0.08 and 7.50 ± 0.06, respectively) (see Table 13.4). By adding further amounts of alkali, soil and cast developed alkaline and strongly alkaline reaction. Thus, casts in comparison to the humic topsoil represent lower values of pH.

Figure 13.4 shows curves of buffering capacity within acid range for Variant No. 2 (humic topsoil, earthworm casts) and sand (reference). Location of titration curves of the studied samples in this range indicates that area of casts buffering capacity is more than area of humic topsoil buffering capacity.

Figure 13.5 shows curves of buffering capacity within alkaline range for Variant No. 2 (humic topsoil, earthworm cast) and sand. Location of titration curves of the studied samples (curve of cast buffering capacity is lower than one of humic topsoil buffering capacity) indicates that area of casts buffering capacity exceeds area of humic topsoil buffering capacity.

In Variant No.2, values of buffering capacity areas in humic topsoil, earthworm casts and their statistical evaluation are given in Table 13.5. It was found statistically significant difference within each exposure range (see Table 13.5). Thus, casts in this variant of remediation have a large buffering capacity area in the acid and alkaline ranges than that of original humic topsoil. It was found that total area of buffering capacity of casts in Variant No. 2 was significantly larger by 8.5 nominal cm^2 (20.8%) than that of humic topsoil. Both within acid and alkaline ranges, earthworm casts increased values of buffering area.

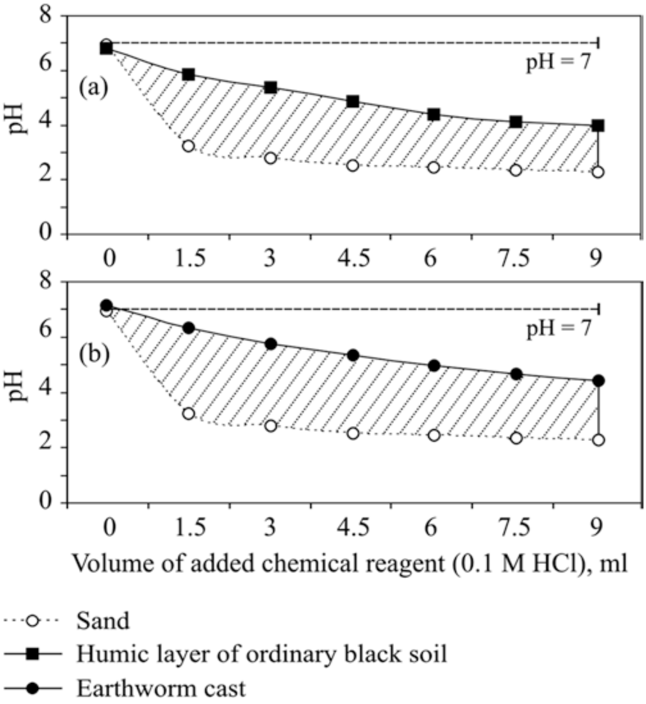


Fig. 13.4 Buffering capacity area of the studied samples in remediated sites within acid exposure range (Variant No. 2): (**a**) humic topsoil; (**b**) earthworm casts

13.3.2 Effect of Earthworm Cast on Soil Buffering Capacity to Different Levels of Copper Contamination

Earthworm casts effect on humus-free loess loam within copper concentration range from 5 to 40 mg Cu/L is less then effect of casts on humus layer of ordinary black soil (197.5 nom. units – humus-free loess loam, 336.1 nom. units – humic topsoil, Table 13.6). Effectiveness of immobilization reflected a degree of resistance to copper contamination was increased from 23.1% to 39.2%, respectively. It is explained by the fact that earthworm casts on humus-free loess loam basically presented as a loam, significantly organic-poor matter, while casts on humus soil are enriched by organic components. Therefore, presence of organic matter in casts is an additional agent of remediated soil resistance to copper toxic concentrations. Areas of toxicant influence ($p \leq 0.01$) between casts of humus-free loess loam and casts of humus layer are different significantly: this difference is more in casts of humus-free loess loam (659.6 nom. units), and smaller in casts of humus soil (426.1 nom. units). Statistical difference between areas of toxicant influence in casts on humus soil (521.0 nom. units) and humus level of soil is missing (534.5 nom. units).

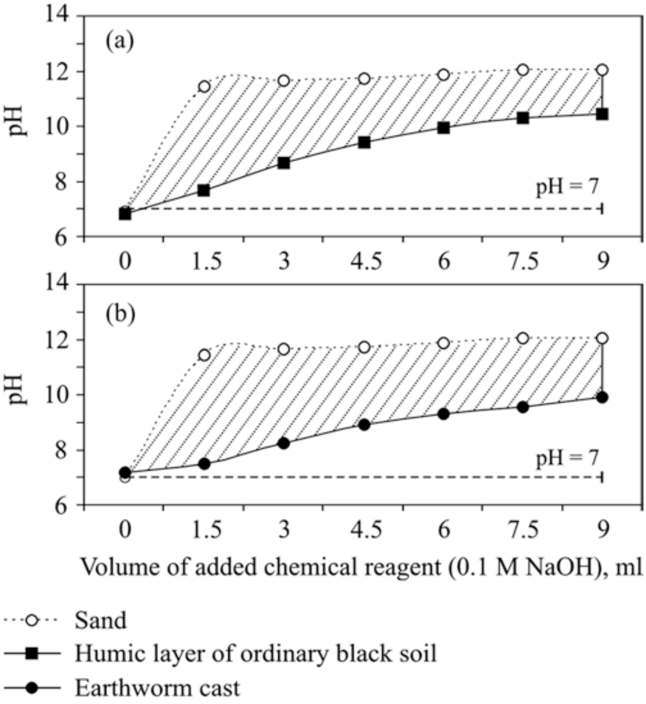


Fig. 13.5 Buffering capacity area of the studied samples in remediated sites within alkaline exposure range (Variant No. 2): (**a**) humic topsoil; (**b**) earthworm casts

Table 13.5 Estimation of buffering capacity values in Variant No. 2 (humic topsoil, earthworm casts)

External exposure range	Buffer capacity area, nominal cm^2	
	Humic topsoil	Casts
Acid	18.9 ± 0.38	23.3 ± 0.52(*)
Alkaline	21.6 ± 0.54	25.7 ± 0.45*
Acid-Alkaline(Total)	40.5 ± 0.57	49.0 ± 0.62***

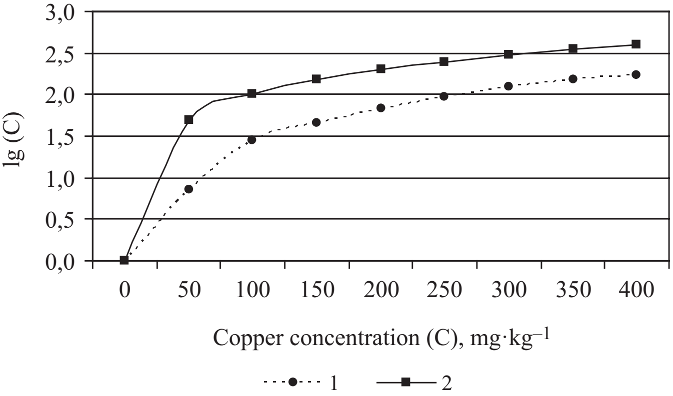
Significant difference of the mean with significance level: (*) ≤ 0.07, * ≤ 0.05, *** ≤ 0.001

Graphic model of earthworm casts resistance to copper contamination (Variants No. 1, 2) are represented in Figs. 13.6 and 13.7. It indicates higher buffering capacity of casts in humus variant.

Thus, earthworm tropho-metabolìc activity within different variants of forest remediation sites affects the soil immobilization capacity maintenance (buffering capacity to heavy metals, including copper). Resistance to elevated copper concentrations increased to casts in range: humus-free loess loam – humic layer of remediated soil.

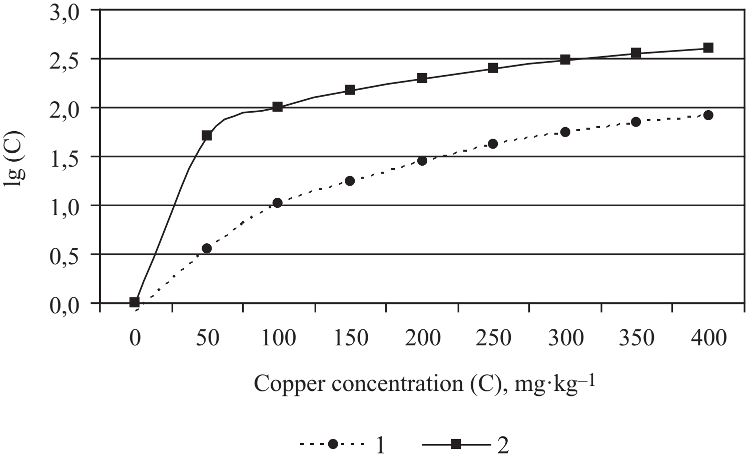
Table 13.6 Quantitative resistance assessment of earthworm casts and soil to copper contamination

Characteristics	Reference area, nom. units ($S_{reference}$)	Sample area, nom. units (S_{sample})	$\frac{S_{sample}}{S_{reference}} \cdot 100\%$	Effect ($S_{ref} - S_{sam}$) nom. units	Effectiveness of Toxicant Immobilization $\frac{S_{sample}}{S_{reference}} \cdot 100\%$
Earthworm casts on humus-free loess loam (Variant 1)	857.1	659.6 ± 1.55	77.0	197.5	23.0
Earthworm casts on humus layer of ordinary black soil (Variant 2)	857.1	521.0 ± 8.80	60.8	336.1	39.2
Humus layer of ordinary black soil	857.1	534.5 ± 4.23	62.4	322.6	37.6



Note. 1 – Earthworm casts (humus-free loess loam, Variant No 1); 2 – Reference.

Fig. 13.6 Graphic model of earthworms casts resistance to copper contamination (Variant No. 1, humus-free)



Note. 1 – Earthworm casts (humic layer, Variant No 2); 2 – Reference.

Fig. 13.7 Graphic model of earthworm casts resistance to copper contamination (Variant No. 2, humic layer)

13.4 Conclusion

Ecosystem effectiveness of soil saprophages (earthworms, *Lumbricidae*) was shown to be effected on increase of acid-alkaline (pH-buffering) buffering capacity in remediated soil. The study proves that acid-alkaline buffering capacity of earthworm casts was significantly higher than that of initial remediated soil and subsoil by 17.9% and 20.8%, respectively.

Resistance to copper concentrations increased in casts within follow range: humus-free loess loam – humus layer of remediated soil. Effectiveness of copper immobilization reflecting resistance to copper contamination was increasing from 23.1% to 39.2%, respectively.

That brings positive changes to soil and environmental conditions of remediated soil and naturalization of artificial soil in remediated lands within Steppe zone. Thus, efficiency of remediated land restoration increases with enrichment by earthworm casts; it leads to improvement of ecological quality in remediated soil. Earthworm ecoservice activity had positive changes to environmental conditions of remediated soil and naturalization of artificial edaphotopes within remediated lands in Steppe zone.

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