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Assessment of the Functional State of Soils in Moscow Zoo on the Basis of Microbiological Parameters

N. E. Yurkova, A. M. Yurkov, and A. V. Smagin

Garden–Park Department, Moscow Zoo, Moscow, Russia
Institute of Ecological Soil Science, Moscow, Russia
Faculty of Soil Science, Moscow State University, Moscow, 119991 Russia

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Abstract—The functional state of soils in the Moscow Zoo was assessed on the basis of the indices of intensity of the main biological processes. Studies of the carbon and nitrogen cycles in the soils, the soil enzyme activity, the microbial biomass, and the functional diversity of soil microorganisms (using the method of multisubstrate testing) were performed in aviaries, open-air animal enclosures, and on public parts of the Moscow Zoo. Against the background of relatively favorable physical properties of the soils (soil density and soil air, water, and temperature regimes), their biological activity was very low. The highest values of the biological activity were found in the soils of enclosures with the white-tailed gnu (*Connochaetes gnou*) and the cassowary (*Casuarius casuarius*). No significant differences in the biological activity of the soils within the aviaries and animal enclosures and on the public territories were found.

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INTRODUCTION

The intensive human activity in megalopolises leads to significant, often irreversible changes in the environment. Human-induced disturbances of biological cycles in an urban ecosystem depend on the source and character of human intervention, technogenic factors, and the quality of the environment. Usually, they are accompanied by adverse consequences. Urban ecosystems are of lower recreation importance as compared with undisturbed natural ecosystems (for instance, forests). Their biological cycle is disturbed, the biodiversity is reduced with respect to the species composition and structural–functional characteristics, and the number of pathogenic microorganisms is higher in comparison with those in undisturbed natural ecosystems [2].

In urban landscapes, territories with green plantations (public gardens, parks, boulevards and, especially, botanical and zoological gardens) are of great importance. Traditionally, the territories of zoological gardens are not considered separately from other anthropogenic urban landscapes, though they represent very specific human-modified landscapes. Apart from the anthropogenic impact common for urban territories, animals introduced into this environment also affect it. The permanent presence of animals on a limited territory urges researchers to consider the soil cover of zoological gardens from the viewpoints of sanitary microbiology and the presence of specific pathogenic microflora. Investigations of the microbial communities in soils of zoological gardens (specific artificially created habitats) have yet to be performed. The microbial community ensures the stable functioning of biogenic element cycles and determines the ability of soils to support their productivity and homeostasis and to preserve the admissible quality of water and air.

In essence, soils of the Moscow Zoo have not been studied. There is only fragmented data on some of the chemical properties of the soils obtained in 1991–1992 [5]. After the reconstruction of the zoo in 1997, no soil studies were performed. The territory of the zoological garden is located in the center of Moscow and is subjected to strong technogenic impacts.

The aim of this work was to investigate a number of characteristics widely used in microbiology for evaluation of the state of soils in the Moscow Zoo.

OBJECTS AND METHODS

The field studies were conducted in the territory of the Moscow Zoo from August 2005 to September 2006. The Moscow Zoo is located on a gentle slope of the Moskva River valley near the confluence of the Presnya and Moskva rivers. In the past, this area was covered with natural linden, maple, ash, oak, and elm forests. The present soil cover is represented by urban soils; the majority of the soils are of artificial (filled) origin. According to the existing classification of urban soils [2], the soils of Moscow Zoo belong to constructozem types (artificially constructed soils) [2].

The soil samples were collected in open-air enclosures of musk ox (*Ovibos moschatus*), white-tailed gnu (*Connochaetes gnou*), guanaco (*Lama guanicoe*), black antelope (*Hippotragus niger*); in aviaries of cassowary (*Casuarius casuarius*) and various species of ducks

(*Anas* sp.); and within public places. Mixed samples taken from the depth of 10–20 cm were analyzed.

The following microbiological characteristics were studied: soil respiration, the microbial biomass and its group composition, the activity of some soil enzymes, the intensity of nitrogen cycle and methanogenesis, and the functional diversity of soil biota (using the method of multisubstrate testing). Also, some parameters of the water and air regimes of soils (soil porosity and soil water contents) and the soil temperature regime were studied.

Physical characteristics. To determine the soil bulk density and porosity, undisturbed soil samples were taken with a special cylinder of 100 cm^3 in volume. The porosity (%) was estimated as the ratio of the difference between the soil solid phase density and the soil bulk density to the solid phase density. The latter was taken as a constant (2.63 g/cm^3), which is acceptable for tentative assessments [11].

The soil air and water regimes were assessed using the index of water saturation W/Ws, where W is the soil water content, %; and Ws is the total water capacity, % [11].

Programmable temperature loggers Thermochron were used for temperature measurements [11]. They were installed in the shadow and in the sun. Air temperatures and soil temperatures at depths of 5, 10, and 20 cm were measured every four hours for a year.

Biological characteristics. The intensity of biochemical processes in the soils was judged from the activity of redox (catalase and dehydrogenase) and hydrolytic (urease and invertase) soil enzymes. The enzyme activity was analyzed according to standard schemes [14], and the results were calculated per unit weight or unit volume of soil. The values obtained were ranged into several classes reflecting the quality (favorable conditions) of the environment [10].

The microbial biomass was determined by the method of substrate-induced respiration [15] and by direct counting with the help of luminescent microscopy [9]. Fungal mycelium, bacterial cells, and fungal spores were separately counted.

Gas chromatography was used to determine the intensities of the nitrogen cycle (nitrogen fixation and denitrification) and the methanogenesis. The analysis was performed according to the standard scheme [7].

The functional diversity of the microbial community was investigated by the method of multisubstrate testing (the Ecolog system) [3]. On the basis of a standard set of substrates, indices of the rank distribution (b, d) reflecting the state and stability of a system were calculated, as well as the functional diversity expressed by the Shannon index [4].

Statistical processing of the results. The water–air and temperature regimes were analyzed according to the criteria proposed for urban soils [11]. The biological activity was assessed using a scale based on the amount of oxygen consumed for respiration; it was calculated on the basis of data on the carbon dioxide emission determined by the method of substrate-induced respiration [11]. The intensity of enzyme processes proceeding in the soils was estimated in points [10]. The stability of the microbial system (coefficient d) was assessed according to [4]. All the indices used to characterize the microbial community were ranked from one to five.

The statistical processing of the data was performed using the Statistica 6.0 software (StatSoft Inc., 1996–2006). The ordination of the data by the method of principal components was performed using point estimates (from one to five) as dependent variables.

RESULTS AND DISCUSSION

Physical characteristics. Bulk density and porosity are the most important soil properties that characterize the capacity of soils to accumulate available moisture and air that are necessary for the development of plants and the functioning of soil microflora. As a rule, urban soils are highly compact in the surface horizons [11]. The strong compaction of these soils ensures the development of anaerobic conditions in the root zone, especially during the rainy periods in spring and autumn. In urban forest-parks, gardens, and boulevards, where the soils are not exposed to strong compaction, the porosity ranges from 45 to 75%. The soil compaction leads to worsening of the soil water and air regimes, and the soil porosity decreases to 25–45%. In the soil samples collected in the Moscow Zoo, the soil porosity ranged from 48.2% (in the enclosures of white-tailed gnu (*Connochaetes gnou*)) to 65.4% (in the aviaries of cassowary (*Casuarius casuarius*).

The monitoring of the water regime of the root layer in relation to the degree of soil water saturation (W/Ws) shows that it remained within optimum values during a larger part (66%) of the growing season. In some cases (10%), an insignificant moisture deficit (W/Ws = 0.2–0.3) was observed; in 24% of the cases, the soil was excessively moistened (Fig. 1). A seasonal decrease in the soil's water content during the precipitation deficit is generally typical of urban soils in Moscow [11]. However, this is not a limiting factor for the soils of the Moscow Zoo, where the conditions of soil moistening are generally favorable for the development of the root system and soil microflora.

The results of monitoring of the temperature regime showed that the temperature of the root layer during the growing season was relatively favorable. Very low temperatures (0–5°C) were only observed in 5% of measurements; in 16% of measurements, the root layer temperature was relatively cold (5–10°C). The high temperature was recorded in 12% of measurements. The air temperature only reached extreme values (30–45°C) in 1% of the measurements. In general, the soil

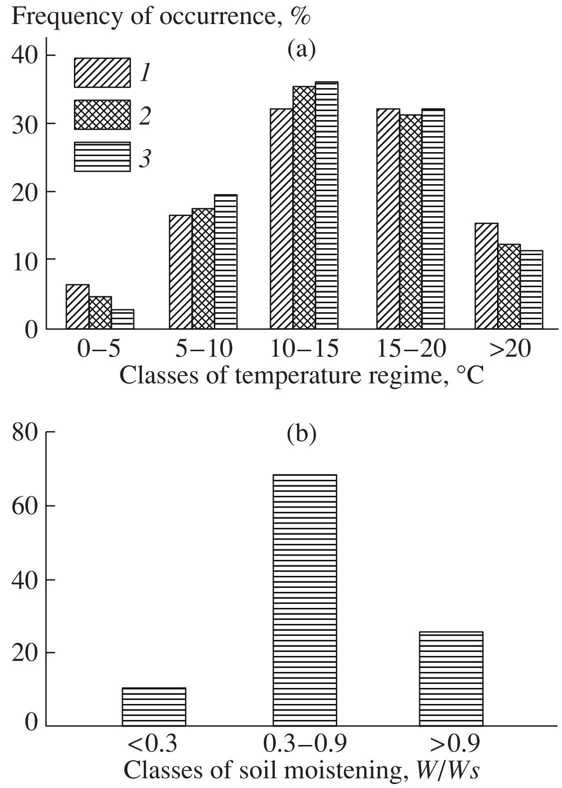

Fig. 1. Distribution of the indices characterizing the ecological state of soils in the Moscow Zoo: (a) the temperature regime in the root layer at the depths of (*1*) 5, (*2*) 10, and (*3*) 20 cm and (b) the degree of soil moistening.

temperature conditions were favorable for the functioning of the microflora (Fig. 1).

Biological characteristics. The microbial biomass is an important living and labile component of soil organic matter. The carbon of microbial biomass represents a part of easily available soil organic matter. The portion of the carbon of microbial biomass in the total pool of soil organic matter is an important index used in microbiological studies. The value of microbial respiration is used to assess the functional (mineralization) activity of the soil microbial community.

The microbial biomass and the intensity of soil respiration in all the soils were close and averaged 850 μg C/g soil and 2.29 mg O_2/kg soil/h, respectively. The maximum values were measured in the soil samples taken from the enclosures with guanaco and musk ox, i.e., 907 and 926 μg C/g soil and 2.95 and 3.27 mg O_2/kg soil/h, respectively. The minimum values were recorded in the soils of the enclosure of white-tailed gnu: 785 μg C/g soil and 1.54 mg O_2/kg soil/h (table).

The use of luminescent microscopy showed that fungi predominate in the microbial communities of all the samples. The portion of fungal mycelium and spores exceeds 95% in all the cases. The portion of bacteria is usually no higher than 0.5%; the highest value (3.7%) was found in the soil of the enclosure of white-tailed gnu.

The value of the microbial biomass in the soils of the Moscow Zoo corresponded to the lower limit of the values typical of undisturbed natural zonal soils in central Russia and to the average values in cultivated soils [13]. However, in the latter soils, the portion of prokaryotes is higher and may reach 25% [9]. Our data on the microbial biomass and on the sharp predominance of fungi that can efficiently utilize trace nutrients suggest that the biological activity of studied soils is low, especially if we take into account considerable amounts of organic matter entering the soils from the animals. The low values of the biological activity, as well as the high ratio between the fungal and bacterial biomasses, may indicate the presence of pollutants in soils [16]. The data on soil respiration (calculated according to the consumption of oxygen) are also indicative of low biological activity in the soils. The very

Absolute values (numerator) and point estimates (denominator) of the biological activity of the soils

Animal species	Respiration		Enzyme activity				Multisubstrate testing
	biomass	respiration	catalase	urease	dehydrogenase	invertase	
	μg C/g	mg O_2/kg/h	cm^3 O_2/g/min	mg NH_3/g/24 h	mg TPhF/g/24 h	mg glucose/g/24 h	coefficient d
Ovibos moschatus	926	3.27/3	2.60/2	4.9/2	0.40/1	1.37/1	1.2/1
Connochaetes gnou	785	1.54/2	4.75/3	50/4	2.15/2	0.17/1	1.5/1
Lama guanicoe	907	2.95/3	2.35/2	5.75/2	2.60/2	0.77/1	0.9/2
Hippotragus niger	838	2.04/3	3.06/3	2.5/1	0.80/1	0.70/1	1.4/1
Casuarius casuarius	829	1.95/2	5.30/3	50/4	1.35/2	1.58/1	1.0/1
Anas sp.	801	1.68/2	1.45/3	8.9/2	2.40/2	1.35/1	0.9/2
Beyond animal enclosures	886	2.63/3	2.45/2	3.55/2	1.45/2	0.67/1	1.8/1

low biological activity characterized 43% of the soil samples, and the low biological activity was determined in 57% of the samples. According to the criteria proposed for urban soils [11], the soil cover of the zoo is poorly suitable for the growth and development of plants.

Another well-known and commonly used indicator of soil quality and the intensity of soil biochemical processes is the enzyme activity. In the studied soils, all the indices of the soil enzyme activity were low. The catalase and invertase activities were close to the values typical of poor soils under primitive undeveloped vegetation (takyric and saline soils, primitive weathering crusts, etc.) [6]. The maximum values of the enzyme activity were as follows: 5.3 cm^3 O_2/g soil/min (for catalase) and 1.6 mg glucose/g soil/24 h (for invertase) in the soil from the aviary of cassowary (table). As known, the activity of these enzymes positively correlates with the content of available organic matter and the intensity of mineralization processes. In the soils of European Russia, the dehydrogenase activity reflects the intensity of humification, whereas the catalase activity reflects the intensity of mineralization [1]. Our data on the activity of these metabolites make it possible to suppose that mineralization processes somewhat predominate over humification processes in the soils of the Moscow Zoo. Contrary to the expectations, the urease activity (5.1 mg NH_3/g soil/24 h) was not high in the studied soils. Its values were comparable with those typical of the soddy-podzolic soils. The high values of the urease activity (exceeding the rest by 10 times) were only found in the soils of the enclosures of white-tailed gnu and cassowary.

Among the ranked estimates, points 1 and 2 (very poor and poor soils, respectively) prevailed. The urease activity of the soils in the enclosures of white-tailed gnu and cassowary had the highest estimate (point 4, rich soil). The lowest enzyme activity values were found in the soils of the enclosures of *Hippotragus niger* and musk ox, whereas the highest values were found in the soils of the enclosures of white-tailed gnu and cassowaries due to the high urease activity (table).

The analysis of the indices characterizing the nitrogen cycle obtained with the use of gas chromatography attests to a predominance of denitrification over nitrogen fixation in the soils of most of the animal enclosures and aviaries. This may be explained by the fact that nitrogen fixation is inhibited by the high amounts of nitrates and NH_3 ions—products of animals' vital functions—entering the soils. The excess of nitrogen fixation over its removal from the soil was noted for an area outside the aviaries and animal enclosures, the area near a pond with ducks, and in the enclosure of black antelope. The intensity of nitrogen fixation in the soil samples taken from a footpath and in the enclosure of black antelope was several times higher than in the rest of the samples and amounted to 0.15 and 0.21 nmol C_2H_2/g/h, respectively. These values of nitrogen fixation are typical of eutrophic peat soils in central Russia [12]. In the other soil samples, the rate of nitrogen fixation was comparable with that in poor soils (burozem, oligotrophic peat gley soil) and varied from 0.01 nmol C_2H_4/g/h (the enclosure of musk ox) to 0.06 nmol C_2H_4/g/h (the area near the pond). The process of denitrification is the most intense in the soils of enclosures of large animals. The results of the factor analysis show that the rate of denitrification is higher in the soils of enclosures with cloven-hoofed animals that in the soils of aviaries with birds ($F = 6$, $p = 0.06$).

The low values of nitrogen fixation in the majority of studied soils of the Moscow Zoo are related to the low portion of prokaryotes in the microbial community and to the small number of anaerobic/microaerobic zones necessary for nitrogen fixation. The latter suggestion well agrees with the data on the porosity of the soils in the Moscow Zoo.

In almost all of the soils, the low intensity of methane emission was found, the mean value of which (0.10 nmol/g/h) was close to that in alpine meadow soils [12]. This fact indicates good aeration of the soils. The maximum rate of methane emission was determined in the soil of cassowary's aviary—2.24 mol/g/h; it was several times higher than in the other soil samples and comparable with the level of methanogenesis in peat soils. The minimum (0.03 nmol/g/h) rates were in the soil of the enclosure of musk ox. The high rate of methane emission from the soil of cassowary's aviary might be caused by the locally excessive moistening with corresponding changes in the redox conditions.

The functional diversity of the microbial community was studied by the method of multisubstrate testing based on the analysis of the intensity of utilization of 48 different substrates (the Ecolog system). The spectra of substrate utilization were very similar in all the soils. To characterize the state of the soil microflora, some parameters of the distribution of substrate utilization were analyzed. The coefficient of flexibility and sustainability (d) of the community is considered to be the most informative parameter characterizing the state of the natural systems [4]. In the studied samples, the values of this coefficient approach 1.0 or even exceed it, which attests to the unfavorable conditions for the development of the soil microflora.

The functional microbial diversity was assessed on the basis of the Shannon index (H) and the H/d ratio. The maximum biodiversity was in the soils of the enclosures with black antelope, white-tailed gnu, and guanaco (the Shannon index 5.2 to 5.3); the minimum biodiversity was in the area near the pond with ducks (4.7). The ability to absorb many substrates (45 from 48) was characteristic of all the samples. Probably, the results of the multisubstrate testing may be understated, as the sampling was made in the late spring. There is information that the minimum levels of the functional

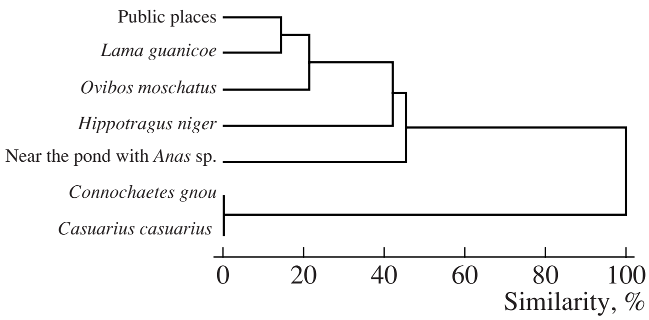

Fig. 2. Dendrogram of similarity of the soils of animal enclosures, aviaries, and public places of the Moscow Zoo with respect to the estimates of various biological parameters.

diversity are observed in natural ecosystems in the early summer (in June) [4]. On the whole, these results support the conclusions about the low biological activity of soils in the zoo despite the quite favorable physical conditions for the development of microbial communities.

The microbiological indices characterizing the functional state of the soils are irregularly distributed in the soils of studied enclosures and aviaries; it is impossible to give an unambiguous estimate of the good or bad conditions for the development and functioning of microorganisms. We have grouped (using cluster analysis and ordination) the soil samples collected in different enclosures with respect to the estimates of soil quality suggested by different researchers. The results of both methods of grouping are very similar. The results of ordination (Fig. 2) plotted on the basis of the calculated point estimates of the soil quality (table) show that the highest estimates are typical of the soils sampled in the enclosures of white-tailed gnu and cassowary. A relatively good soil quality is characteristic of the area around the pond with different species of ducks. The microbiological characteristics of the soils of other aviaries and animal enclosures and the territories open for visitors are similar and relatively poor.

Among the plots studied in the Moscow Zoo, the area near the pond with ducks may be considered as the control plot. This area is closed to visitors but, at the same time, the ducks are not strictly attached to it and can move freely over the whole territory. We suggest that the nonspecific external impact on this territory is the same as on the other areas of the zoo, but the specific effect of animals (compaction, trampling, manuring with products of vital activity) is lower. As compared with this background area, white-tailed gnu and cassowary positively affect the soil characteristics. On other plots, the degradation of soil properties is observed, and, probably, some reclamation measures should be performed to ensure the efficient soil functioning on these plots.

CONCLUSIONS

(1) The physical conditions of soils in the Moscow Zoo are relatively favorable for the development of plants and microflora; unfavorable conditions arise sporadically and the probability of their appearance does not exceed 30–40%.

(2) All the soils of the Moscow Zoo are poor and very poor with respect to their microbiological parameters.

(3) The highest values of the biological activity were found in the soils of enclosures with white-tailed gnu and cassowary.

(4) The features of the main microbial processes in the soils of animal enclosures and the areas available for visitors do not principally differ.

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