Soil diagnosis of an urban settlement with low levels of anthropogenic pollution (Stepnoe, Saratov region)

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Abstract. A soil diagnosis of an urban territory Stepnoe (Saratov region) was conducted within the framework of soil research monitoring of inhabited localities with low levels of anthropogenic impact using chemical and microbiological analysis. Excess over maximum permissible concentration (MPC) of mobile forms of Cr, Zn and Cd were not observed within the researched territory. A universal excess over MPC of mobile forms of Ni, Cu and Pb was established which is most likely connected with anthropogenic contamination. It was discovered that, at the territory of the Stepnoe settlement, mobile forms of heavy metals compounds (HM) in most cases formed paragenetic associations with high correlation coefficient and despite this, an excess over MPC was not significant. This point to a common mineralogical origin of the elements inherited from the parent rock. The values of the total index of chemical contamination were not above 16, which puts the researched samples in a category with permissible contamination. The indices of the total number of heterotrophic bacteria, iron-oxidizing and hydrocarbon-oxidizing bacteria in most samples corresponded to normal indices for chestnut solonetsous and saline soils. In some samples, a deviation from the normal indices was observed justifying the impact of specific contaminants on the soil.

1. Introduction
In the modern world, compounds of heavy metals (HM) have been known to be part of universal pollutants of soil cover [1]. They influence soil biological properties while inhibiting soil enzyme activities [2]. This is connected with repression of microbial biochemical activity and also a qualitative and quantitative reduction in their structure [3]. Problems of soil pollution with HM in districts of large cities have been well studied [4-6] but for settlements with a population less than 50 thousand with less industrial activities, this problem has practically not been studied.

Within the framework of soil research monitoring in settlements which serve as administrative centers in Saratov region we carried out soil diagnosis of an urban village Stepnoe using chemical and microbiological analysis. According to Aleksenko’s classification [7] the researched territory falls under a group of small settlements, communities and small villages that represent a transition from agricultural, grassland and forest landscapes to city landscapes of inhabited locality.

In the course of this work, the content of mobile forms of copper, zinc, lead, cadmium, chromium and nickel was determined, the ecologically harmful heavy metals hazard ratio and the total index of chemical contamination $Z_c$ by HM were calculated. Microbiological indices of the soil samples were also evaluated for the total number of heterotrophic microorganisms (HMs), number of iron-oxidizing bacteria (IOMs) and hydrocarbon-oxidizing bacteria (HOMs). Soils are often exposed to mixed pollution. Oil hydrocarbons and HM are known to be widely distributed and dangerous soil pollutants [8]. In connection with the above said, the aim of this research was to biogeochemically analyze soils
of a territory with low levels of anthropogenic impact (for example, the settlement Stepnoy, Saratov region).

2. Material and methods
Objects of this research were soil samples obtained from the territory of the urban village Stepnoe (figure 1) with a size of 8 km² and a population of about 12,340 inhabitants (according to data obtained in 2015). This settlement is located within the Syrtian Plain, belonging to the East European Plain. The relief is gently sloping. Soils are chestnut solonetsous and saline soils.

Figure 1. Map showing soil sample positions of the urban settlement territory Stepnoe.

Selection and preparation of samples was carried out in accordance with State Standards [9]. Soil sample points were distributed taking into consideration the wind rose, peculiarities of the microtopography, distribution of the building plans and basic amenities. In accordance with State Standards, a test was conducted on the upper layers of the soil horizon «A» to the depth of 5 cm where basic pollutants from the atmosphere are usually accumulated. The size of the sample area varied from 2-3 to 10 m². Soil sampling was carried out using the envelope method – one sample obtained from the center and 4 samples from the corners of the sample point and also from 2-3 samples points around the top of the envelope. The weight of the combined samples varied in a scale of about 0.5-1.0 kg.

Determination of HM concentration in the soil samples was conducted using atom-adsorption spectrometric method (Kvant-2AT). The mobile acid-soluble form of the metals (Cu, Zn, Ni, Cd, Pb) was determined in 1 M HNO₃ extracts [10]. To determine the ecologically dangerous concentrations of HM in the soil cover, the actual concentration of each HM was compared with its maximum permissible concentration (MPC), while calculating the heavy metal hazard ratio \( K_o \) using the formula (1):

\[
K_o = \frac{C_i}{MPC}
\]

(1)

Where \( C_i \) – content of HM forms in the sample, [mg kg⁻¹];
**MPC** – maximum permissible concentration, [mg kg⁻¹].

The total index of chemical contamination \( Z_c \) was determined to help evaluate the level of geochemical transformation by the mobile forms of HM using the formula (2):

\[
Z_c = \sum K_{o_n} \cdot (n - 1)
\]

where \( Z_c \) – total index of chemical contamination by HM in the sample;
\( n \) – number of determined elements;
\( K_{o_n} \) – heavy metal hazard ratio in the sample.

For calculating \( Z_c \), an excess over of MPC was used (\( K_{o_n} \)).

The total number of cultivable heterotrophic microorganisms in the soil (1 g, wet weight) was determined by plate dilution technique with a beef-extract agar medium. The number of hydrocarbon-oxidizing microorganisms was evaluated on Mills et al. [11] modified mineral agar medium, g 1⁻¹: Na₂HPO₄ – 6.0; KH₂PO₄ – 3.0; NaCl – 0.5; NH₄Cl – 1.0 with vaseline oil (1%) as a sole carbon and energy source. An account of the number of neutrophilic iron-oxidizing microorganisms was carried out on a selective agar medium with the following composition, g 1⁻¹: FeSO₄•7H₂O – 5.9; (NH₄)₂SO₄ – 0.5; NaNO₃ – 0.5; K₂HPO₄ – 0.5; MgSO₄•7H₂O – 0.5; lemon acid – 10.0, sucrose – 2.0, peptone – 1.0, pH 7.0 [12].

Plating on beef-extract agar and on selective media for an account of the number of hydrocarbon-oxidizing and iron-oxidizing bacteria was carried out from dilutions 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵ in replicates. The plates with inoculants were incubated at 28-30°C for 2-5 days at a temperature of 28-30°C in an incubator and colony-forming units (CFU) were counted per g of dry soil [13].

Data were analyzed statistically using the software (Microsoft Excel 2010). The differences in probable errors \( p<0.05 \) (95% confidence interval) was considered the confidence. The interrelationships between the chemical and microbiological indices of soil were evaluated using pair correlation coefficients for \( p<0.01 \).

### 3. Results

The concentrations of mobile forms of Cu, Zn, Pb, Cd, Cr and Ni were determined in the 30 soil samples selected from the territory of the urban settlement Stepnoe (table 1). Concentrations of mobile forms of Cr observed in all soil samples were between 1.33 to 4.22 mg kg⁻¹ which were less than the maximum permissible concentration (MPCₜₐₚ = 6.0 mg kg⁻¹) [14]. Mobile forms of Zn were noticed in all soil samples in concentrations between 4.38 to 15.74 mg kg⁻¹ and was also below the maximum permissible concentration (MPCₜₐₚ = 23.0 mg kg⁻¹) [15].

Concentrations of mobile forms of Cd observed in all soil samples were between 0.08 to 0.11 mg kg⁻¹ which were less than the maximum permissible concentration (MPCₜₐₚ = 1.0 mg kg⁻¹) [14]. The heavy metal hazard ratio varied from 0.08 to 2.19 and the MPC was not exceeded. Mobile forms of Ni were noticed in all soil samples in concentrations between 7.66 to 14.20 mg kg⁻¹, MPCₜₐₚ = 4.0 mg kg⁻¹ [14]. The heavy metal hazard ratio varied at a scale of 1.92 to 3.55. An excess over MPC was established in all the soil samples with the highest value observed in sample №3 (3.55 MPC). Concentrations of mobile forms Pb of observed in all soil samples were between 3.64 to 7.62 mg kg⁻¹, MPCₜₐₚ = 6.0 mg kg⁻¹ [14]. The heavy metal hazard ratio was at a range of 0.61 to 1.27. An excess over MPC was observed in 4 samples with sample №3 having a maximum value of 1.27 MPC. Mobile forms Cu of were noticed in all soil samples in concentrations between 3.05 to 7.82 mg kg⁻¹, MPCₜₐₚ = 3.0 mg kg⁻¹ [16]. The heavy metal ratio varied from 1.02 to 2.61. Excess over MPC were stated in all samples with sample №3 having a maximum value of 2.61 MPC.

A geochemical order for mobile forms of HMs in the territory of the urban settlement Stepnoe was as follows: Ni>Zn>Pb>Cu>Cr>Cd (based on average concentrations) and Ni>Cu>Pb>Cr>Zn>Cd (based on increments above normal indices). Analyzing the research results, the elements were distributed into 2 groups: Group 1 – Ni, Pb, Cu, soil samples in which an excess over MPC was observed; Group 2 – Cr, Zn and Cd, soil samples in which an excess over MPC was not noticed. A calculation of the total index of contamination showed the following – in the researched territory Z,
varied at a scale from -0.8 to 3.92. All of the soil samples (100%) were put in a category with permissible levels of pollution (Zc, from 0 to 16).

**Table 1.** Results of soil chemical analysis of the settlement Stepnoe, Saratov region.

| Soil Sample Identifier | Concentration of heavy metals, mg kg\(^{-1}\) of soil | Heavy metals hazard ratio (K\(_{h}\)) | Zc |
|------------------------|---------------------------------------------|----------------------------------|-----|
|                        | Cr  | Ni  | Pb  | Zn  | Cd  | Cu  | Cr  | Ni  | Pb  | Zn  | Cd  | Cu  |     |     |
| 1                      | 2.89| 10.90| 5.69| 9.24| 0.09| 5.74| 0.48| 2.73| 0.95| 0.40| 0.09| 1.91| 1.56|
| 2                      | 3.60| 11.60| 6.41| 10.49| 0.09| 6.86| 0.60| 2.90| 1.07| 0.46| 0.09| 2.29| 2.40|
| 3                      | 4.22| 14.20| 7.62| 15.74| 0.11| 7.82| 0.70| 3.55| 1.27| 0.68| 0.11| 2.61| 3.92|
| 4                      | 3.44| 13.41| 6.14| 10.34| 0.11| 5.63| 0.57| 3.35| 1.02| 0.45| 0.11| 1.88| 2.39|
| 5                      | 3.70| 13.13| 6.49| 12.08| 0.11| 6.19| 0.62| 3.28| 1.08| 0.53| 0.11| 2.06| 2.68|
| 6                      | 2.80| 11.08| 5.82| 9.59| 0.10| 5.58| 0.47| 2.77| 0.97| 0.42| 0.10| 1.86| 1.58|
| 7                      | 1.49| 9.68| 4.30| 9.43| 0.10| 3.85| 0.25| 2.42| 0.72| 0.41| 0.10| 1.28| 0.17|
| 8                      | 1.83| 9.25| 4.64| 7.57| 0.09| 4.34| 0.31| 2.31| 0.77| 0.33| 0.09| 1.45| 0.26|
| 9                      | 1.33| 8.58| 3.99| 6.34| 0.09| 3.63| 0.22| 2.15| 0.67| 0.28| 0.09| 1.21| 0.40|
| 10                     | 1.40| 7.99| 4.15| 6.47| 0.08| 3.64| 0.23| 2.00| 0.69| 0.28| 0.08| 1.21| 0.50|
| 11                     | 1.60| 8.14| 4.82| 5.64| 0.08| 4.55| 0.27| 2.04| 0.80| 0.25| 0.08| 1.52| 0.05|
| 12                     | 1.63| 8.53| 4.90| 5.57| 0.09| 4.33| 0.27| 2.13| 0.82| 0.24| 0.09| 1.44| 0.01|
| 13                     | 1.81| 8.99| 5.50| 5.87| 0.09| 4.40| 0.30| 2.25| 0.92| 0.26| 0.09| 1.47| 0.28|
| 14                     | 1.94| 8.77| 5.10| 5.98| 0.08| 5.16| 0.32| 2.19| 0.85| 0.26| 0.08| 1.72| 0.43|
| 15                     | 1.51| 7.66| 4.66| 6.46| 0.08| 3.94| 0.25| 1.92| 0.78| 0.28| 0.08| 1.31| 0.38|
| 16                     | 1.69| 8.45| 4.90| 5.70| 0.08| 4.06| 0.28| 2.11| 0.82| 0.25| 0.08| 1.35| 0.11|
| 17                     | 1.58| 9.10| 4.74| 5.73| 0.10| 4.12| 0.26| 2.27| 0.79| 0.25| 0.10| 1.37| 0.05|
| 18                     | 1.64| 8.42| 4.95| 6.48| 0.09| 4.22| 0.27| 2.10| 0.82| 0.28| 0.09| 1.41| 0.02|
| 19                     | 1.74| 8.32| 5.01| 5.92| 0.09| 4.51| 0.29| 2.08| 0.84| 0.26| 0.09| 1.50| 0.06|
| 20                     | 1.64| 8.12| 5.23| 5.52| 0.09| 4.73| 0.27| 2.03| 0.87| 0.24| 0.09| 1.58| 0.09|
| 21                     | 1.83| 10.78| 5.02| 6.28| 0.10| 4.46| 0.31| 2.70| 0.84| 0.27| 0.10| 1.49| 0.70|
| 22                     | 1.76| 10.10| 4.41| 5.99| 0.10| 4.09| 0.29| 2.52| 0.73| 0.26| 0.10| 1.36| 0.28|
| 23                     | 1.54| 9.56| 4.10| 5.25| 0.10| 3.80| 0.26| 2.39| 0.68| 0.23| 0.10| 1.27| 0.07|
| 24                     | 1.67| 10.36| 4.20| 5.45| 0.10| 3.57| 0.28| 2.59| 0.70| 0.24| 0.10| 1.19| 0.10|
| 25                     | 1.55| 8.83| 4.78| 6.00| 0.10| 4.30| 0.26| 2.21| 0.80| 0.26| 0.10| 1.43| 0.05|
| 26                     | 1.42| 8.28| 3.64| 4.38| 0.08| 3.05| 0.24| 2.07| 0.61| 0.19| 0.08| 1.02| 0.80|
| 27                     | 1.54| 9.36| 4.66| 5.90| 0.10| 3.99| 0.26| 2.34| 0.78| 0.26| 0.10| 1.33| 0.06|
| 28                     | 1.81| 10.74| 4.56| 5.97| 0.10| 4.15| 0.30| 2.69| 0.76| 0.26| 0.10| 1.38| 0.49|
| 29                     | 1.51| 9.40| 4.89| 6.02| 0.10| 4.16| 0.25| 2.35| 0.81| 0.26| 0.10| 1.39| 0.17|
| 30                     | 1.42| 9.12| 4.50| 4.93| 0.09| 4.01| 0.24| 2.28| 0.75| 0.21| 0.09| 1.34| 0.09|

Results of a correlation analysis conducted to identify possible paragenetical associations of elements showed the presence of strong correlation connections (values of \(r\) from \(\pm 0.7\) to \(\pm 1.0\)) in most cases, this is typical of associations such as: Cr-Ni, Cr-Pb, Cr-Zn, Cr-Cu, Ni-Pb, Ni-Zn, Ni-Cd, Ni-Cu, Pb-Zn, Pb-Cu, Zn-Cu (table 2). Average correlation interconnections (values of \(r\) from \(\pm 0.3\) to \(\pm 0.699\)) which is usual for the pairs: Cr-Cd, Pb-Cd, Zn-Cd, Cd-Cu. On the basis of these results and also considering the fact there was no excess over MPC, it can be proposed that, these elements most probably have the same mineralogical origin inherited from their parent rock.
Table 2. Correlation coefficients between the numbers of different microorganisms and chemical parameters of soil.

| Parameters | $C_{Cr}$ | $C_{Ni}$ | $C_{Pb}$ | $C_{Zn}$ | $C_{Cd}$ | $C_{Cu}$ | $Z_c$ | HMs  | HOMs  | IOMs  |
|------------|----------|----------|----------|----------|----------|----------|-------|-------|--------|--------|
| $C_{Cr}$   | 1        | 0.89**   | 0.92*    | 0.91*    | 0.56     | 0.94*    | 0.98* | -0.24 | -0.26  | -0.41  |
| $C_{Ni}$   |          | 1        | 0.75*    | 0.83*    | 0.82*    | 0.76*    | 0.93* | -0.14 | 0.02   | -0.35  |
| $C_{Pb}$   |          |          | 1        | 0.46     | 0.96*    | 0.93*    | -0.27 | -0.38 | -0.36  |
| $C_{Zn}$   |          |          |          | 1        | 0.56     | 0.86*    | 0.92* | 0.02  | -0.12  | -0.30  |
| $C_{Cd}$   |          |          |          |          | 1        | 0.43     | 0.65* | -0.01 | 0.37   | -0.27  |
| $C_{Cu}$   |          |          |          |          |          | 1        | 0.95* | -0.24 | -0.36  | -0.36  |
| $Z_c$      |          |          |          |          |          |          | 1     | -0.19 | -0.20  | -0.04  |
| HMs        |          |          |          |          |          |          |       | 1     | 0.50   | 0.63*  |
| HOMs       |          |          |          |          |          |          |       |       | -0.02  |        |
| IOMs       |          |          |          |          |          |          |       |       |        | 1      |

*Statistically significant differences for $p<0.01$

Abbreviations: HMs – heterotrophic microorganisms; HOMs – hydrocarbon-oxidizing microorganisms; IOMs – iron-oxidizing microorganisms

It is well known that the total number of heterotrophic microorganisms serves as a reliable sensitive monitoring indicator in soil analysis [17]. Iron-oxidizing soil microorganisms carry out the dissociation of iron compounds and iron-organic complexes [18], which can be as a consequence of technogenic soil contamination and in this case with HM. It is based on this the indices of the number of iron-oxidizing bacteria was chosen for the microbiological monitoring analysis of the soil samples. In most of the researched soil samples the total number of heterotrophic microorganisms was between 24.8 to 99.4×10^5 CFU g⁻¹ of soil (figure 2).

Figure 2. Number of microorganisms in soil samples of the urban settlement territory Stepnoe.

The number of iron-oxidizing microorganisms ranged from 4.6 to 34.7×10^5 CFU g⁻¹ of soil. The number of hydrocarbon-oxidizing microorganisms in 18 samples was between 29.5 to 86.0×10^5 CFU g⁻¹ of soil. These indices of the number of bacteria corresponded with the normal indices for chestnut solonetous and saline soils. In three samples (№ 1, 2 and 3) obtained at the boarder of the researched
In the territory, the total number of heterotrophic microorganisms and the number of hydrocarbon-oxidizing microorganisms was low, comprising of 4.3 of 12.4×10⁵ and 2.7 to 15.8×10⁵ CFU g⁻¹ soil respectively. In samples №1 and 2 the number of iron-oxidizing microorganisms was also low (0.04 and 0.01×10⁵ CFU g⁻¹ soil). Sample № 11, 12, 13 and 14 differed with low values for the number of hydrocarbon-oxidizing microorganisms. Sample №7 was observed to possess a high number of heterotrophs, hydrocarbon-oxidizing and iron-oxidizing microorganisms, while in sample № 11 – the number of heterotrophs and iron-oxidizing microorganisms was very significant. This could be connected with the peculiarity of the contaminants and the level of soil pollution in that given sample point, which was located at the central part of the settlement. As a whole, a significantly positive correlation between the total number of heterotrophic microorganisms and the number of iron-oxidizing microorganisms was observed r = 0.63 (p<0.01) (table 2).

An increased hydrocarbon-oxidizing microbial content was observed in soil samples № 21, 22, 23 and 24. This can serve as a proof of fresh hydrocarbon contamination, as it is well known that the presence of fresh hydrocarbon soil pollution leads to an increase in the content of hydrocarbon-oxidizing microorganisms [19].

Between the amount of bacteria of the researched physiological group and the concentrations of HM in the soil samples from the settlement, strong correlation connections were not discovered. A positive correlation coefficient (r =0.37, p<0.01) was noticed between the number of hydrocarbon-oxidizing microorganisms and the concentration of Cd. It is a well known fact that Cd is quite often present in petroleum products [20, 21], which explains why when volatile hydrocarbons vanish from contaminated soils in the course of time or hydrocarbons undergo biodegradation, Cd which had been accumulated in soil continues to show an influence on soil biocenosis.

**Conclusion**

An examination of the geochemical peculiarities, in this case the presence of mobile forms of HM in the soils of the urban settlement Stepnoe brought about the following conclusions. A universal excess over MPC of mobile forms of the following elements was established within the boundaries of the researched territory – nickel, copper and lead. This increment most probably was due to anthropogenic contamination. An analysis of the correlation interconnections showed a significant correlation relationship practically between all the elements, which most likely indicates their belonging to one of the mineralogical associations.

Results of the soil microbiological analysis of the urban settlement Stepnoe showed: Three soil samples were distinct and showed a low soil microbial content with regards to the researched physiological groups, these samples were also characterized by high values of the total index of chemical contamination. A high microbial content of all the researched groups was observed in one of the samples (№ 7), while a maximum value for iron-oxidizing microorganisms and a high heterotrophic microbial content was noticed in sample № 11 justifying the influence of a specific contaminant on the soil.

In general, the identified geochemical anomalies (content of mobile forms of heavy metals) indicate favorable ecological and geochemical conditions in the territory of the urban settlement Stepnoe. Values of total index of chemical contamination were not above 16, which help place the 30 soil samples in a category with permissible contamination. According to the results of microbiological analysis, 25 soil samples can be assigned to the category with permissible contamination.

The absence of significant correlation between the number of heterotrophic, hydrocarbon-, and iron-oxidizing microorganisms and the indicators of chemical soil contamination also speaks well of the favorable ecological situation in the territory of the settlement Stepnoe.

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