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DYNAMICS OF SOIL PROPERTIES UNDER A POLLUTION GRADIENT IN URBAN AREAS (PLOVDIV, BULGARIA)

Slaveya PETROVA[1], Bogdan NIKOLOV[1], IlianaVELCHEVA[1], Mariya YANKOVA[1], Emiliya KOGAN[1], Elena ZHELEVA[2], Ekaterina VALCHEVA[3], Alexandar ALEXANDROV[4], Mariana MARHOVA[4], Marinela TSANKOVA[4], Ivan ILIEV[4]

[1] Department of Ecology and Environmental Conservation, Faculty of Biology, University of Plovdiv "Paisii Hilendarski", 24 Tzar Assen Str., 4000-Plovdiv, Bulgaria
[2] Department of Ecology, Environmental Conservation and Restauration, Faculty of Ecology and Landscape Architecture, University of Forestry, 10 Kliment Ohridski Str., 1756-Sofia, Bulgaria
[3] Department of Agroecology and Environmental Conservation, Agricultural University, 2 Mendeleev Boul., 4000-Plovdiv, Bulgaria
[4] Department of Biochemistry and Microbiology, Faculty of Biology, University of Plovdiv "Paisii Hilendarski", 24 Tzar Assen Str., 4000-Plovdiv, Bulgaria

Corresponding author email: sl.petrova@abv.bg

Abstract

Urban ecosystems are comprised of diverse land areas, resulting in different habitats for plants, animals and human within urban landscape. Urban habitat quality results the integration of different abiotic and biotic components, such as air, soil and water quality, microclimate and vegetation. Urban soils differ from the other components by the prolonged retention and accumulation of pollutants. As the traffic is rising as the most serious emitter of harmful substances in the environment, we aimed to assess its effect to some soil properties under a pollution gradient. Soil samples were collected on the 7.5 m, 25 m, and 50 m distance from two of the main boulevards in the city of Plovdiv (Bulgaria), using the transect method. Content of some heavy metals and toxic elements in soils was analyzed using ICP-MS. Data revealed that soil contamination is strongly influenced by the distance from the road, followed by the wind rose and urban gradient. Regarding the microbial soil communities, this study confirms that the anthropogenic pressures (building and road infrastructure, deforestation) are the most important factors affecting the soil quality in the urban areas.

Key words: *urbanization, pollution, gradient, traffic, microbial communities.*

INTRODUCTION

Urban ecosystems are comprised of diverse land areas, resulting in different habitats for plants, animals and human within urban landscape. Urban habitat quality results from the integration of different abiotic and biotic components, such as air, soil and water quality, microclimate and vegetation. There is a clear relationship between urbanization processes and anthropogenic transformation of the landscapes structure and functioning, revealing to an increment of trace elements content in environment and modifying of their load (Petrova et al., 2014 a, b).

Urban soils are a complex and heterogeneous biogeochemical system with both natural-anthropogenic genesis. Various products from anthropogenic sources fall on the soil surface with dry and wet atmospheric deposition, accumulate into the surface horizons and cause significant changes in their chemical content before to be reintegrated in the natural or technogenic migration cycles.

Urban soils differ from the other components by the prolonged retention and accumulation of pollutants. Some studies have shown that concentrations of technogenic elements in urban soils could reflect the intensity of contamination in the past 20-50 years as the soils are more static and resistant in comparison with the other components such as the air, water, biota etc. (Penin, 1989, 1997, 2003).

Anthropogenic pressure results in pollution of all components of the urban environment, damaging the main soil properties. Microorganisms in soil ecosystems are ubiquitous, abundant, diverse and essential for many soil functions such as carbon and nitrogen cycling and plant productivity. Soil microbiology is a key component in urban ecosystems. Bacterial communities take part in different soil processes as mineralization of the organic matter, humus synthesis, nutrient supply and nitrogen fixation (Beare et al., 1994). They are of primary importance for soil quality and natural productivity.

Many European city environments have a long history of industrialization and urbanization, resulting in elevated concentrations of potentially harmful elements, including heavy metals such as arsenic (As), mercury (Hg), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), vanadium (V) and zinc (Zn), derived from industrial and mineral processing, and the atmospheric depositions from traffic fumes and power generation (Reimann et al., 2005; Wang et al., 2008).

In this context, as the car traffic is rising and is the most important and constant emitter of harmful substances in the urban environment, we aimed to: i) assess its effect on both chemical and biological soil properties; ii) assess the significance of factors "Distance from the road", "Wind rose", "Background level" and "General urban activity" for the elements concentrations in soils near road network.

MATERIALS AND METHODS

Research site description

City of Plovdiv (N42°9', E24°45') is one of the biggest and most densely populated settlements in Bulgaria-338,000 inhabitants on 102 km^2.

Soil samples are taken in June 2017 from two of the main boulevards (Hristo Botev Boul. and Vassil Aprilov Boul.) along a pollution gradient using the transect method (Figure 1).

Method of transects

Systematic soil sampling was made along transects, where a sampling line is set up across areas with clear environmental gradients (Buckland et al., 1993). Transects started from the road towards the four directions (N, E, S, W) in order to allow the effect of the wind rose on soil properties be assessed. Twelve samples were collected at the 7.5 m, 25 m, and 50 m distance from the road (Table 1) on the depth of 0-20 cm. Each sample was formed by 5 subsamples (Petrova et al., 2013).

Soil chemical properties

Soil pH was measured using pHotoFlex Set, 2512000, WTW-Germany, and the soil conductivity was measured using Multiset, F340, WTW-Germany.

Content of Cd, Cr, Cu, Mn, Pb and Zn was determined by inductively coupled plasma mass spectrometry (ICP–MS) (Agilent 7700). Data were presented as mean values in mg/kg and residual standard deviation (RSD) in %.

Table 1. Sampling plots description

Boulevard	Sampling plot direction	Distance from the road	Soil samples symbol
Hristo Botev	North	7.5 m	HB-N-7.5
		25 m	HB-N-25
		50 m	HB-N-50
	South	7.5 m	HB-S-7.5
		25 m	HB-S-25
		50 m	HB-S-50
Vassil Aprilov	East	7.5 m	VA-E-7.5
		25 m	VA-E-25
		50 m	VA-E-50
	West	7.5 m	VA-W-7.5
		25 m	VA-W-25
		50 m	VA-W-50

Soil microbiology

Microbiological samples were collected in sterile containers and stored at 4°C in the dark until analysis for no longer than 24 h. Prior to the analysis, the samples were passed through 1 mm stainless steel sieve. A total of 1 g (dry weight equivalent) of each was suspended in 99 ml of sterile saline solution. The suspensions were homogenized by mixing at 200 rpm for 20 min for cells extraction (Goto and Yan, 2011). We used culturing techniques to compare the aerobic heterotrophic bacterial population`s total viable count (TVC) at 22°C and 37°C and fungi in soils (Zuberer, 1994). The presence of *Escherichia coli*, faecal coliforms (FC) and faecal streptococci (FS) was used as a pollution indicator. A volume of 100 ml soil solution was filtered through a membrane filter (Membrane Solutions) with pore size 0.45 μm. The filter was transferred and cultivated on selective growth medium for 24 h (ISO 9308-1:2014; ISO 7899-2 2000).

Multi ANOVA and Student/Fisher test were used for testing the differences of elemental concentrations, both between the soils samples from different road distance and also between the studied sampling sites ($p<0.05$).

Relationships between the element content in soil samples were tested using Pearson correlation ($p<0.05$). Data were also processed with Principal Components Analysis (PCA) in order to find the main factors affecting urban soils quality. All statistical analyses were made with STATISTICA 7.0 statistical package (StatSoft, 2004).

Figure 1. Map of Plovdiv and location of sampling plots

RESULTS AND DISCUSSIONS

According to their genesis, the soils in the Plovdiv area are classified as Fluvisol according to the FAO World Reference Base for Soil Resources (2014). Due to the prolonged human presence on the studied territory (more than 8000 years) and the increasing temps of urbanization in the last decades, soils properties are significantly influenced and now it is more appropriate to discuss them as Technosols.

The pH values of the studied soil samples from the surface layer (0-20 cm) varied between 6.22 and 7.08 (in the neutral zone), and the conductivity was in the range of 102-121 $\mu S/cm^2$. The neutral soil reaction makes difficult the migration of many heavy metals and of other elements into the soil profile so they remain immobilized into the topsoils.

Heavy metals and toxic element such as As, Cd, Cr, Cu, Ni, Pb, V and Zn are derived into the urban environment by many anthropogenic processes and activities, such as mining, smelting, industrial engineering, metal processing and plating, ceramics and electronics, domestic activities, residential heating, incinerators, petrol and diesel vehicles, (Johnson et al., 2011).

Manganese is a natural element in soils and its content generally varies from 0.01% to 0.4% (Kabata-Pendias and Pendias, 2001) both as a result from the rocks layer and the atmospheric deposition. Anthropogenic origin of this element into the air is related with such as mining, smelting, industrial activities (especially heavy engineering, metal processing). In our study, Mn was the most abundant from the studied elements but the content is half less average concentration in Bulgarian and European soils (Table 2). In this study, contrary to the case of other metals, manganese concentrations found at different points did not varied much.

Zinc content in our study varied between 64 and 130 mg/kg with a mean value of 90 mg/kg (Table 2). So, some increment was found in the city of Plovdiv in comparison with Bulgarian and European soils, more pronounced in the soils near the Vasil Aprilov Boul.

Table 2. Content of heavy metals and toxic elements in soil samples [[1]Penin (1989, 2003) and [2]Salminen (2005)]

Soil sample	Mn		Zn		Pb		Cr		Cu		Cd	
	Conc. mg/kg	RSD, %	Conc. mg/kg	RSD %	Conc. mg/kg	RSD, %	Conc. mg/kg	RSD, %	Conc. mg/kg	RSD, %	Conc. mg/kg	RSD, %
HB-N-7.5	490	2.3	85	4.2	40	1.5	35	3.2	28	1.6	0.41	6.1
HB-N-25	471	1.8	88	3.1	38	2.1	40	2.8	26	2.5	0.38	5.1
HB-N-50	435	2.4	88	1.3	26	1.9	32	2.4	22	5.5	0.26	7.3
HB-S-7.5	453	2.7	98	4.7	37	1.9	32	2.6	34	3.0	0.34	12
HB-S-25	457	2.4	95	1.8	45	1.9	32	2.0	27	2.5	0.47	8.5
HB-S-50	447	2.0	66	3.7	30	2.3	35	3.6	21	4.4	0.32	8.2
VA-E-7.5	528	1.5	108	3.0	63	1.6	39	3.1	40	3.3	0.56	6.1
VA-E-25	511	1.7	64	4.4	23	1.9	18	4.2	26	3.0	0.19	12
VA-E-50	469	2.2	71	4.0	33	1.6	14	5.1	28	2.7	0.32	3.9
VA-W-7.5	335	3.7	130	3.1	67	2.0	26	3.6	38	3.4	0.72	8.0
VA-W-25	451	2.3	94	2.4	41	1.8	37	1.1	29	4.8	0.4	6.7
VA-W-50	457	2.4	98	1.5	117	1.5	48	2.8	34	3.6	0.5	9.1
Average in Plovdiv	458	-	90	-	47	-	32	-	29	-	0.41	-
Soils in Bulgaria[1]	1000	-	75	-	35	-	70	-	30	-	0.32	-
Soils in Europe[2]	810	-	68,1	-	32.6	-	94.8	-	17.3	-	0.28	-

As a whole, lead and cadmium content was also quite elevated in comparison with the average level in Bulgarian and European soils (up to 1/3 higher), with both maximums into soils surrounding the Vasil Aprilov Boul. Copper content was very close to the average of Bulgarian value while the chromium content was half less the Bulgarian soil concentration and 2/3 times lower from the European level (Table 2).

Table 3. Correlation between studied elements, $p<0.05$

	Mn	Zn	Pb	Cr	Cu	Cd
Mn	1.00					
Zn	-0.54	1.00				
Pb	-0.18	0.56	1.00			
Cr	0.08	0.33	0.57	1.00		
Cu	-0.12	**0.78**	**0.63**	0.18	1.00	
Cd	-0.48	**0.88**	**0.67**	0.33	**0.76**	1.00

Statistical evaluation of the elements relationships showed strong positive correlation between Cd, Zn, Pb and Cu (Table 3). Many authors have shown that there is a strong relationship between many of these contaminants and the use of motor-vehicles – leaded fuels for Pb, tyre wear for Zn and Cd, brake pads for Sb, and catalytic converters and exhaust systems for platinum group elements (Kelly et al., 1996; De Miguel et al., 1997; Zereini et al., 1997; Schafer et al., 1998; Angelone et al., 2002; Cichella et al., 2003).
This relationship was also pointed by the cluster analysis (Figure 2).

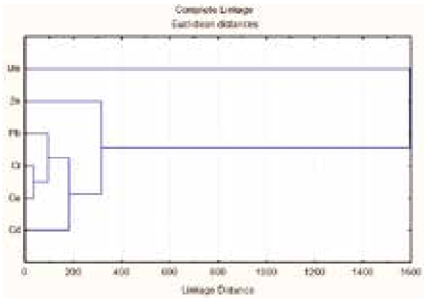

Figure 2. Cluster analysis of studied elements, $p<0.05$

The strongest similarity was proved between Cu and Cr, and then Pb, Cd and Zn have joined. Mn was found to be quite different from the other elements which fact was not surprising as Mn is naturally more abundant in soils.
Regarding the "Distance from the road" as a factor affecting the soil pollution, our data confirmed the statements of other authors that the concentrations generally decrease away from the main road network and with increasing depth from the ground surface (Johnson et al., 2011).
No significant differences were found between samples at the West direction transect on Vasil Aprilov Boul., but the opposite transect (Vasil Aprilov, South) showed very strong gradient of decreasing pollution from 7.5 m to 25 m and to 50 m samples (VA-E-7.5, VA-E-25 and VA-E-50) ($p<0.05$). Considering the effect of the wind direction on the dispersion of pollutants

in the environment, this fact is obviously due to the prevailing winds from west to east - 75% of all winds in Plovdiv through the year. That is the reason for the lack of pollution gradient into the windward transect too.

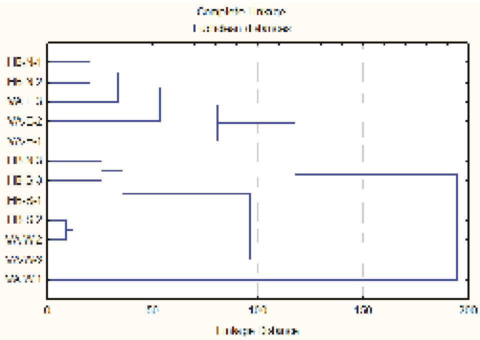

Figure 3. Cluster analysis of studied transects and soil samples, p<0.05

The second boulevard, Hristo Botev Boul., is oriented into the W-E direction (as the wind rose) and the distribution of traffic related elements was quite complex. We could not find strong significant relations in their content with the distance from the road network. Both transects (North and South) showed low correlation gradients, as follows: p=0.13-0.21 for 7.5 m and 50 m samples and p=0.09-0.13 for 25 m and 50 m samples.

Cluster analysis divided the studied soil samples in two main clusters (Figure 3). First one consisted from the first two samples in North direction from the Hristo Botev Boul. and all the three samples in East direction from the Vasil Aprilov Boul. Second cluster included the aggregation of the rest of samples from Hristo Botev Boul. (3 to the South and one to the North) and the three samples in the West direction from the Vasil Aprilov Boul.

Next step of the statistical evaluation of our results was the Principal Component Analysis in order to test the significance of several factors ("Distance from the road", "Background level", "Wind rose" and "General urban activity") for the elements concentrations in soils near road network (Figure 4). Main factor (Factor 1=58.59%), contributing to the element content in soil, was found to be the "Background level" in the area, followed by the factor "Distance from the road" (Factor 2=20.51%) which affected mainly Cd and Zn. "Wind rose" as factor (Factor 3=12.48%) played an important role of the dispersion of Cu, Cd and Zn, while the "General urban activity" contributed to the behaviour of almost all studied elements without Pb (Factor 4= 5.58%).

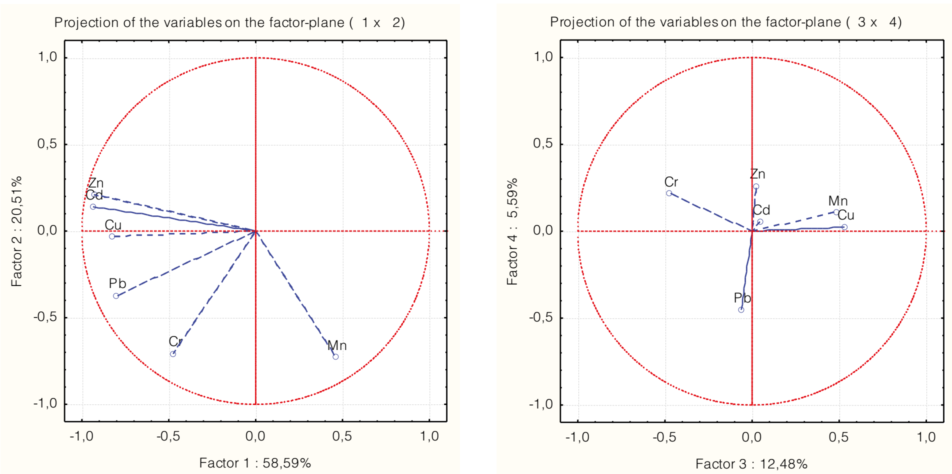

Figure 4. Results from the Principal Component Analysis (PCA), p<0.05

For chromium and lead we could not prove some dependence of the studied factors in PCA. However, Pb showed strong synergism with Cu and Cd, so we can suppose that it is influenced but in lower extent. Cr and V are considered indicative of vehicle emissions (Garty et al., 1996) and partly associated with tire and brake abrasion, as we have shown in our previous studies (Petrova, 2011; Petrova et al., 2013; Petrova et al., 2014 a,b; Petrova et al., 2015). So, this lack of correlation with other traffic-related elements could be a result of some other mechanisms and processes, i.e. higher mobility, phytoextraction etc.

Urban infrastructure and development are some of the most severe threats for microbial communities' structure and well-being (O`Donnell et al., 2001). The results from the microbiological analysis are shown in Table 4. The total numbers of heterotrophic microorganisms cultured at 22°C (TVC 22) and 37°C (TVC 37) were used as an indicator for the soil-forming capacity and anthropogenic pressure respectively. The study showed a strong positive correlation between the parameters (Table 5).

The values for TVC 22 and TVC 37 in the soil samples differed significantly between transects with higher numbers of heterotrophic bacteria near the Hristo Botev Boul. Our data showed a consistency in the distribution of the heterotrophic microorganisms. Their numbers decreased from 7.5 to 50 m (going away from the road) in each transect. This can be explained by the presence of planted trees and shrubs along the road which could have considerable influence on soil microbial communities (Xu et al., 2013), and with the care of the municipality for the seasonal forestry maintenance of these green areas. The observed differences in the bacterial numbers could also be driven by the environmental parameters topology, and site`s geological characteristics.

There is considerable evidences documenting the negative effect of the long-term heavy metal exposure on the soil bacterial community (Sobolev and Begonia 2008; Xie et al., 2016). However, the parameters TVC 22 and TVC 37 showed no significant negative correlation with the tested heavy metals. This is probably due to the relatively low level of contamination compared to the other studies.

The cultivation-based detection and enumeration of microbiological indicators remain the gold standard in the environment protection (Rodgers et al., 2011). We have established clearly defined differences in the distribution of the sanitary state indicators between the two studied areas (Table 4).

The number of *E. coli* correlate positively with the number of FC and FS (Table 5). Studies of fecal coliforms didn`t show statistically significant differences between the soil samples from the Hristo Botev Boul. Fecal streptococci and *E. coli* were unevenly distributed. Their amounts in the northern transect increased in the direction away from the road, with the FC: FS ratio in these samples > 4:1 which indicates the presence of nonpoint source of surface soil contamination. Similar trend was observed for the VA-W transect. In general, the total number of the indicators is not high, and our conclusion, based on their decay speed (Rodgers et al., 2011) is that the pollution in the surveyed areas is significantly low.

Table 4. Average values of the studied microbiological indicators

Soil sample	TVC 22 (cfu.10^5/g)	TVC 37 (cfu.10^5/g)	FC (cfu/g)	*E. coli* (cfu/g)	FS (cfu.g)	Fungi (cfu.10^3/g)
HB-N-7.5	417±15	69±6	18905±8200	115±9	78±7	20±3
HB-N-25	256±15	46±3	8360±3000	467±23	108±10	96±6
HB-N-50	143±10	18±2	7384±1460	952±61	330±26	107±3
HB-S-7.5	521±14	79±2	10860±3040	1100±111	823±225	42±2
HB-S-25	337±11	79±2	11930±4040	300±10	287±23	45±8
HB-S-50	184±18	45±9	7485±1854	4±3	0	204±5
VA-E-7.5	86±8	8±0.1	480±120	0	8±0	4±0.2
VA-E-25	37±2	8±0.2	650±320	0	18±0	9±0.1
VA-E-50	15±2	2±1	540±200	0	8±1	10±0.4
VA-W-7.5	159±11	41±0.9	5000±160	900±114	1080±44	46±2
VA-W-25	94±5	41±5	18100±2100	1320±98	1340±84	56±2
VA-W-50	31±6	16±2	18300±3400	3240±249	2100±200	34±1

Table 5. Correlation between element concentrations and the studied microbiological indicators, $p<0.05$

Variable	Mn	Zn	Pb	Cr	Cu	Cd	TVC22	TVC37	FC	FS	*E. coli*	Fungi
Mn	1.00											
Zn	-0.54	1.00										
Pb	-0.18	0.56	1.00									
Cr	0.08	0.33	0.57	1.00								
Cu	-0.12	0.78	0.63	0.18	1.00							
Cd	-0.48	0.88	0.67	0.33	0.76	1.00						
TVC22	-0.06	0.15	-0.25	0.17	-0.03	0.01	1.00					
TVC37	-0.22	0.16	0.07	0.25	-0.04	0.15	0.86	1.00				
FC	-0.10	0.14	0.33	0.62	-0.07	0.11	0.36	0.42	1.00			
FS	-0.46	0.50	0.65	0.50	0.31	0.30	-0.06	0.08	0.58	1.00		
E. coli	-0.28	0.37	0.83	0.54	0.36	0.34	-0.21	0.06	0.58	0.91	1.00	
Fungi	-0.14	-0.30	-0.20	0.30	-0.48	-0.09	0.32	0.39	0.29	-0.20	-0.17	1.00

Fungi fulfill a range of important ecological functions particularly those associated with nutrient and carbon cycling processes in soil. The bacteria-to-fungi ratio tends to be lower in acidic, low nutrient soils and high C-to-N ratio, whereas bacteria are increasingly prominent in high N+P, saline, and alkaline soils. Previous studies demonstrated the higher resistance of the fungi to heavy metals (Hiroki, 1992; Ashraf and Ali, 2007). Despite that our research show low numbers of fungi indicating that soil-forming capacity of the studied areas is low and strongly influenced by the urbanization of the territory.

CONCLUSIONS

Our study revealed that the contamination of soils along the road network with Cd, Pb, Cu and Zn is in direct relationships both with the distance from the road and the location of the boulevard itself according to the wind rose. This situation reflected also on the soil microbial community structure, leading not only to the damages in the physico-chemical properties of soils but to the decrease into their quality and functions. As a consequence of the data obtained, we could make some recommendations to use some plant species with high bioaccumulation capacity for green casting the road surrounding.
Finally, it can be added that the data provided are part of a project covering the whole territory of the city of Plovdiv and all the results obtained by us will show a more complete and detailed picture of the anthropogenic impact in the urbanized area.

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