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# OCCURRENCE OF SELECTED SOIL MICROORGANISMS IN POINTS LOCATED BY THE MAIN TRANSPORT ROUTES OF KRAKOW

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***Summary***

The research was conducted to determine the number and species composition of microorganisms isolated from the soils collected in the vicinity of seven largest transport nodes and roundabouts in Krakow. Moreover, the investigations aimed at verifying the differences in the occurrence and biodiversity of the researched microorganism population between four calendar seasons of the year. The soil samples were collected 4 times during the period from November 2013 to August 2014 and then analysed using serial dilutions method. A great microorganism biodiversity was found in the analysed samples. Microorganisms were the most numerous in the autumn-winter period. The most numerous isolated group were vegetative bacteria and ammonifiers. Filamentous fungi were less numerous, which may have been caused by the neutral or slightly alkaline soil pH. Presence of dormant bacteria forms may result from unfavourable environmental conditions caused by a toxic effect of the substances from road transport. Less numerously isolated were actinomycetes and *Azotobacter* bacteria, regarded as bioindicators of soil fertility. It was found, that the differences in the numbers of the analysed microorganisms over the year are statistically significant for the vegetative and ammonifying bacteria, phytopathogenic fungi and actinomycetes. No significant differences in the microorganism number were stated between the research points and the control (meadow).

**Keywords**: road transport, microorganisms, soil, Krakow, biodiversity

## INTRODUCTION

Soil is the habitat of many microorganisms. The number, biodiversity and metabolic activity of microorganisms directly affect improvement of soil fertility and soil structure. Soil microorganisms participate in element cycling in nature and in the organic matter mineralization process, providing plants with nutrients necessary for life (Libudzisz and Kowal 2000, Libudzisz *et al*. 2007).

Due to increasing intensity of road traffic in large cities, the soils situated in the immediate vicinity of transport routes are exposed to many pollutants. Over the recent years this issue became one of major ecological problems. It is estimated that the dusts originating from fossil fuels burning have the most serious influence on the reduction or loss of biological activities in soil. The process results in emission into the atmosphere of large amounts of exhaust gases, dusts, ashes or soot. These are among the main causes of reduced numbers and activity of the microorganisms which positively affect the soil and the processes occurring in it (Potarzycki and Apolinarska 2000, Badyda 2010).

Road transport pollution comprises exhaust emission from car engines, various kinds of spills from leaking installations or pollutions resulting from streets sprinkled with salt in winter. Pollution emission into the atmosphere may lead to mixing pollutants with atmospheric precipitation resulting in acid rains (Chłopek 2002). Solid or liquid particles coming out of the engine exhaust system contain compounds of carbon, sulphur, nitrogen and heavy hydrocarbons. They persist in the environment for a long time and are easily absorbed from the soil surface. Another component of the suspension are dusts from abrasion of the road surface and car tyres. All these compounds become deposited on the soil surface as soot, smokes or ashes causing reduction or loss of microorganism activity (CSO 2009). In 2003 the index of registered vehicles in Poland increased to 653 cars per 1000 inhabitants. The highest increase was noted for passenger cars – reaching the level of 502 vehicles. It denotes that Poland exceeded the average EU number of 484 cars registered per 1000 inhabitants.

Another problem is soil salinity in the vicinity of transport routes, occurring mainly in winter. Roads are sprinkled with salt to melt the ice accumulating on the road surface. Mainly sodium chloride (NaCl) with an admixture of calcium chloride ($CaCl_2$) is used for this purpose. High content of salt may cause destruction of the soil structure, disturb the ionic balance, diminishing its permeability and cause changes in pH value leading to the ground alkalinisation. Salt toxicity to a great extent depends also on the soil moisture level and organic matter content. The higher the salt concentration, the lower the water content. Increase in the osmotic pressure caused by salinity makes impossible water uptake thus diminishing cell activity of microorganisms, which may lead to cell growth inhibition or their destruction (Silva and Fay 2012).

Another cause of soil pollution are oil derivative spills from leaking car installations which may find their way to deeper soil layers with rain, which leads to changes in soil physio-chemical composition. Oil products are composed of over 120 various hydrocarbons, including aliphatic and aromatic hydrocarbons, toxic for the environment. These among others include the compounds forming BTEX group: benzene, toluene, ethylbenzene and xylene. These compounds are water soluble, therefore they persist in soil for a long time, also because of their poor biodegradability. Oil derivatives influence an increase in acidity and disturb the soil organic composition, which in result affects quantitative and qualitative changes in microorganism species settling the soils (Ziółkowska and Wyszkowski 2010).

Only a small part was isolated and identified from among a large number of genera and species of soil microorganisms. Some fix atmospheric nitrogen making it more available to plants, other are decomposing dead organic matter.

Microorganisms may produce substances of a secondary metabolic character (antibiotics or growth hormones), which penetrating into the soil participate in soil forming processes. Moreover, some microorganisms produce toxic compounds (mycotoxins) which may inhibit development of other organisms. Microorganisms may also participate in the decomposition of oil derivatives, at the same time contributing to bioremediation of contaminated areas. They may be also indicator organisms because changes in their number and species composition are often caused by petrol fumes penetration into the soil.

The paper aimed at determining the number and species composition of selected microorganisms isolated from the soils situated in close vicinity to main roundabouts and transport nodes in Krakow. Moreover, the aim of the article was to verify the differences in the occurrence and biodiversity of the investigated microorganism populations between four calendar seasons of the year.

## MATERIAL AND METHODS

Soils samples for analyses were collected from 7 points located in the vicinity of the largest transport routes in Krakow, characterized by a considerable intensity of car and tram traffic. The control was soil sample collected from a meadow located on the city outskirts (Table 1).

Soil samples of 100g were collected to sterile containers on the following dates: 7.11.2013 (autumn), 5.02.2014 (winter), 7.04.2014 (spring) and 27.07.2014 (summer) to consider the phenomenon of seasonality, following the principles of Polish Standard (PN-ISO10381-6:1998). The air temperature was measured by means of a Biowin electronic thermometer on the days of soil sampling. A serial dilutions method by Koch was applied to determine the general number and species composition of selected microorganisms in the analysed samples. The analysis comprised determining a general number of bacteria (vegetative and dormant forms, phytopathogenic fungi, actinomycetes, amonifiers and *Azotobacter* spp.) (Table 2). Dry matter and moisture content of the analysed soil samples were determined using MB45 Ohaus moisture analyser and the soil reaction was measured using CP-105 Elmetron pH meter.

**Table 1.** Location of soil sampling points – GPS coordinates.

| No. | Sampling point | GPS |
|---|---|---|
| 1. | Matecznego roundabout | N 50°2’10” E 19°56’24” |
| 2. | Grunwaldzki bridge | N 50°2’57” E 19°55’55” |
| 3. | Inwalidów Square | N 50°4’9” E 19°55’35” |
| 4. | Central Railway station | N 50°3’53” E 19°56’43” |
| 5. | Mogilskie roundabout | N 50°3’55” E 19°57’32” |
| 6. | Czyżyńskie roundabout | N 50°4’24” E 20°1’0” |
| 7. | Central Square | N 50°4’20” E 20°2’18” |
| 8. | Meadow (control) | N 50°3’58” E 20°2’25” |

Source: Author’s own elaboration

**Table 2.** The microbiological media and conditions for microorganisms cultivation.

| No. | Microorganisms | Medium | Incubation temperature [°C] | Incubation time |
|---|---|---|---|---|
| 1. | Vegetative and endospores bacteria | MPA (bacteriological agar, BTL) | 37 | 24-48h |
| 2. | Fungi | worty agar (own medium) | 28 | 7 days |
| 3. | Ammonifiers | agar Rougieux (Pochon and Tardieux 1962) | 37 | 24-48h |
| 4. | *Azotobacter* spp. | Ashby agar (Atlas and Parks 1997) | 28 | 7 days |
| 5. | Actinobacteria | Pochon agar (BTL) | 28 | 7 days |

When the incubation was finished, the grown up fungi colonies were counted and the results were stated as colony forming units per one gram of soil mass (cfu/g d.w.). Also initial identification of microorganisms was conducted using diagnostic keys (Gilman 1957, Domsch *et al*. 1980, Bergey and Holt 1994, Macura 2008).

The number of vegetative and dormant bacteria, particularly the ratio of these two values will provide information about the soil microbial activity, but also about its abundance in organic matter, which is the source of food for microorganisms. In order to survive in unfavourable environment conditions, to which undoubtedly may be counted, the soils close to main transport routes of Krakow, bacteria enter a dormant state (as spores or cysts). Therefore, in the analysed soil we may expect a large number of dormant in relation to vegetative bacteria forms. Presence of fungi may, but does not have to correspond with the soil reaction, which was also determined. It is commonly thought, that phytopathogenic fungi prefer slightly acid or acid pH of the environment. On the other hand, the number of actinomycetes, amonifiers and *Azotobacter* bacteria is the indicator of soil fertility, therefore a low number of the above mentioned microorganism groups may evidence strong soil degradation (Domsch *et al*. 1980, Bergey and Holt 1994, Paul and Clark 2000).

Statistical analysis was also conducted to calculate an average number of microorganisms in the analysed soil samples, and was applied to test the significance of the time and space diversification of selected microorganism group numbers. Pearson's correlation coefficient $r$ was computed between the number of isolated microorganisms and the air temperature and moisture content, and the reaction of sampled soils.

## RESULTS AND DISCUSSION

Conducted analyses revealed a considerable microorganism diversity in the soils sampled in points located by the main transport routes in Krakow. The data concerning number of isolated microorganisms, pH, moisture content and dry matter of samples and the air temperature were compiled in Tables 3-6.

In autumn, vegetative and ammonifier bacteria were the most numerous in the sampling points. Great numbers of dormant forms were isolated by Mateczny and Mogilskie roundabouts, at Grunwaldzki bridge and by the Central Railway Station. *Azotobacter* spp. were impossible to isolate in many places, they were present only in the samples taken at Inwalidów square and by Mogilskie roundabout. Actinomycetes were present almost in all sampling points, whereas phytopathogenic fungi proved impossible to isolate at the Central Square. Microorganism number, except actinomycetes, was higher in most samples than in the control (meadow). The soil pH ranged between slightly acid – 5.69 (Grunwaldzki bridge) and neutral – 7.20 (Central Square). The average assessed moisture content of the analysed soils was on the level of 16.6%.

**Table 3.** Evolution of the number of examined groups of microorganisms [cfu/g d.w.] in selected locations in Krakow – AUTUMN 2013.

| Sampling point | Vegetative bacteria | Bacterial endospores | Fungi | Ammonifiers | *Azotobacter* spp. | Actinobacteria | pH | d.w. | Moisture [%] | Air temperature [°C] |
|---|---|---|---|---|---|---|---|---|---|---|
| Matecznego roundabout | 2117931 | 5321 | 4888 | 1416904 | 0 | 1064 | 5.83 | 8.081 | 19.11 | 11 |
| Grunwaldzki bridge | 792075 | 1555 | 4604 | 729491 | 0 | 365 | 5.69 | 8.362 | 16.50 | 11 |
| Inwalidów Square | 413823 | 0 | 5806 | 1699988 | 389 | 3831 | 6.52 | 8.353 | 16.57 | 11 |
| Central Railway station | 2693761 | 2951 | 2519 | 2238122 | 0 | 80 | 6.82 | 8.735 | 12.95 | 8 |
| Mogilskie roundabout | 421329 | 2951 | 25247 | 3332104 | 480 | 0 | 6.83 | 8.133 | 18.82 | 8 |
| Czyżyńskie roundabout | 568668 | 0 | 2864 | 763825 | 0 | 1031 | 7.16 | 8.728 | 13.40 | 8 |
| Central Square | 2759499 | 0 | 0 | 1053604 | 0 | 1460 | 7.20 | 8.115 | 18.81 | 8 |
| **Average** | **1395298** | **3195** | **7655** | **1604863** | **435** | **1305** | **6.6** | **8.3** | **16.6** | **9.3** |
| Meadow (control) | 655267 | 0 | 4123 | 972593 | 0 | 5397 | 6.66 | 8.246 | 17.84 | 8 |

**Table 4.** Evolution of the number of examined groups of microorganisms [cfu/g d.w.] in selected locations in Krakow – WINTER 2014.

| Sampling point | Vegetative bacteria | Bacterial endospores | Fungi | Ammonifiers | *Azotobacter* spp. | Actinobacteria | pH | d.w. | Moisture [%] | Air temperature [°C] |
|---|---|---|---|---|---|---|---|---|---|---|
| Matecznego roundabout | 1567427 | 2643 | 51289 | 1892042 | 0 | 3826 | 7.47 | 7.188 | 28.33 | 8 |
| Grunwaldzki bridge | 5922166 | 846 | 81876 | 1841982 | 0 | 9447 | 7.42 | 7.098 | 29.12 | 8 |
| Inwalidów Square | 2936887 | 0 | 16973 | 1345235 | 0 | 2699 | 7.51 | 7.954 | 20.52 | 8 |
| Central Railway station | 1715102 | 5657 | 13697 | 4037637 | 476 | 0 | 7.54 | 8.396 | 16.63 | 0 |
| Mogilskie roundabout | 3669843 | 3992 | 32192 | 3090394 | 1056 | 0 | 7.48 | 7.766 | 22.83 | 0 |
| Czyżyńskie roundabout | 2817663 | 475 | 119 | 826102 | 0 | 1070 | 7.82 | 8.413 | 15.97 | -3 |
| Central Square | 2693646 | 0 | 278697 | 1716298 | 0 | 2434 | 7.52 | 7.858 | 21.87 | -3 |
| **Average** | **3046105** | **2727** | **67835** | **18262659** | **766** | **3895** | **7.5** | **7.8** | **22.1** | **3.6** |
| Meadow (control) | 2416268 | 0 | 8814 | 3512969 | 0 | 5162 | 7.03 | 7.942 | 20.81 | -3 |

In winter, ammonifiers and vegetative bacteria were the most numerous. A big number of phytopathogenic fungi was also isolated. Dormant forms of bacteria were also present in many places. *Azotobacter* spp. bacteria were present only in the samples collected at the Central Railway station and Mogilskie roundabout. No actinomycetes were isolated on these sites, whereas they were present in large numbers in the other sampling points. No significant differences in pH were noted among the analyzed samples, which ranged from 7.03 to 7.82, whereas the sample moisture content was 22.1%.

**Table 5.** Evolution of the number of examined groups of microorganisms [cfu/g d.w.] in selected locations in Krakow – SPRING 2014.

| Sampling point | Vegetative bacteria | Bacterial endospores | Fungi | Ammonifiers | *Azotobacter* spp. | Actinobacteria | pH | d.w. | Moisture [%] | Air temperature [°C] |
|---|---|---|---|---|---|---|---|---|---|---|
| Matecznego roundabout | 94579 | 698 | 979 | 773830 | 176 | 610 | 6.97 | 8.529 | 14.97 | 21 |
| Grunwaldzki bridge | 417455 | 1656 | 603 | 437714 | 56 | 416 | 7.42 | 8.453 | 15.97 | 21 |
| Inwalidów Square | 343360 | 0 | 545 | 587248 | 0 | 2168 | 7.72 | 8.344 | 17.83 | 21 |
| Central Railway station | 295842 | 420 | 1404 | 597213 | 14 | 1020 | 7.08 | 9.042 | 9.77 | 16 |
| Mogilskie roundabout | 247092 | 1959 | 641 | 311016 | 125 | 101 | 7.49 | 8.424 | 16.00 | 15 |
| Czyżyńskie roundabout | 287479 | 225 | 2392 | 393037 | 31 | 434 | 7.45 | 8.905 | 11.35 | 15 |
| Central Square | 1006344 | 193 | 2687 | 304532 | 0 | 338 | 7.50 | 8.275 | 17.92 | 15 |
| **Average** | **384593** | **859** | **1322** | **486370** | **80** | **727** | **7.4** | **8.6** | **14.8** | **17.7** |
| Meadow (control) | 127885 | 3492 | 754 | 783941 | 143 | 517 | 7.22 | 8.419 | 16.24 | 15 |

Ammonifying bacteria were the most numerous in the analyzed samples in spring, whereas vegetative bacteria composed the next microorganism group regarding the number. Dormant forms of bacteria were isolated in all sampling points, except Inwalidów square. Low number of fungi might have been caused by a neutral pH assessed in the analyzed soils. *Azotobacter* spp. bacteria were absent in the samples from the Inwalidów square and Central Square, whereas actinomycetes occurred in all sampling spots. The soil reaction in the collected samples ranged from 6.97 to 7.72 and average moisture content was 14.8%.

Analysis conducted in summer 2014 revealed the most numerous occurrence of vegetative bacteria in collected soil samples. Small quantities of dormant forms were isolated from Mateczny, Mogilskie and Czyżyńskie roundabouts and from the meadow (control). The second numerous group were ammonifying bacteria. *Azotobacter* spp. bacteria and phytopathogenic fungi were present in each analyzed sample, while actinomycetes were registered in all sampling points except the Central Railway station. No greater differences were noted in pH values of the samples, which fluctuated from 8.17 to 8.59 and the average soil moisture was assessed on the level of 14%.

**Table 6.** Evolution of the number of examined groups of microorganisms [cfu/g d.w.] in selected locations in Krakow – SUMMER 2014.

| Sampling point | Vegetative bacteria | Bacterial endospores | Fungi | Ammonifiers | *Azotobacter* spp. | Actinobacteria | pH | d.w. | Moisture [%] | Air temperature [°C] |
|---|---|---|---|---|---|---|---|---|---|---|
| Matecznego roundabout | 390882 | 96 | 3123 | 347930 | 620 | 817 | 8.25 | 8.335 | 16.96 | 20 |
| Grunwaldzki bridge | 113619 | 0 | 587 | 262861 | 201 | 1267 | 8.42 | 8.684 | 13.52 | 20 |
| Inwalidów Square | 308877 | 0 | 4853 | 688619 | 24 | 370 | 8.59 | 8.655 | 13.69 | 20 |
| Central Railway station | 772577 | 0 | 5963 | 810081 | 249 | 0 | 8.20 | 8.888 | 11.40 | 22 |
| Mogilskie roundabout | 1365958 | 496 | 870 | 164867 | 6 | 563 | 8.45 | 8.613 | 14.23 | 22 |
| Czyżyńskie roundabout | 1505833 | 67 | 3381 | 480823 | 441 | 302 | 8.17 | 8.943 | 10.90 | 22 |
| Central Square | 1356425 | 0 | 6794 | 239796 | 399 | 848 | 8.34 | 8.257 | 17.40 | 22 |
| **Average** | **830596** | **220** | **3653** | **427854** | **277** | **695** | **8.4** | **8.6** | **14** | **21.1** |
| Meadow (control) | 471048 | 1375 | 4074 | 260023 | 578 | 185 | 8.24 | 8.730 | 13.03 | 22 |

It was found, that the differences in the number of investigated microorganisms over the year are statistically important for vegetative and ammonifying bacteria, phytopathogenic fungi and actinomycetes. On the other hand, no statistically significant differences were registered among the investigated soil sampling sites. On the basis of the statistical analysis of the dependence of selected microorganism group number on the air temperature, soil pH and moisture content, a considerable diversification was stated for the distribution of correlation coefficient values. However, no full positive or very high correlation between the analyzed values were registered in any case. A high positive correlation was obtained only for the influence of moisture content on the number of vegetative bacteria and actinomycetes (Table 7).

Microorganism number in soil is conditioned by a number of factors, i.e. soil pH and soil moisture content, air access, presence of nutrients, temperature and soil structure. Moreover, the number and species composition of soil microorganisms may be indirectly affected also by both environmental and anthropogenic factors, such as pollutants from the road transport discussed in this paper (Zwolinski 2005, Lenart and Wolny-Koładka 2013).

**Table 7.** Results of variance analysis of the spatial and temporal differences in the number of microorganisms and the value of the Pearson *r* correlation coefficient.

| Microorganism | F statistics | | r | | |
|---|---|---|---|---|---|
| | Sampling point | Season | pH | Moisture | Air temperature |
| Vegetative bacteria | 0.267 | 12.58* | -0.075 | 0.642 | -0.591 |
| Bacterial endospores | 1.839 | 1.355 | -0.374 | 0.217 | -0.294 |
| Fungi | 0.776 | 3.027* | 0.006 | 0.457 | -0.431 |
| Ammonifiers | 0.719 | 11.69* | -0.337 | 0.467 | -0.736 |
| *Azotobacter* spp. | 0.69 | 1.515 | 0.251 | -0.023 | 0.056 |
| Actinobacteria | 1.127 | 3.054* | -0.182 | 0.655 | -0.334 |

* values are significant at significance level <0.05

The microorganisms most numerously isolated during the research period were vegetative and ammonifying bacteria. A lower number of fungi might have been caused by the soil pH, which was neutral or slightly alkaline. The soil reaction is an important factor regulating qualitative and quantitative composition of microorganisms because it affects mineral substances solubility and therefore their availability (Górska and Russel 2004).

The presented research had a seasonal character and aimed to assess the differences in the number of selected microorganism groups between the four seasons of the year. It was found that the researched microorganisms were most numerous in the autumn-winter season, which may evidence a wide range of their tolerance to the temperature. The number of dormant forms in relation to vegetative forms was growing in winter, which is connected with possible forming cysts and spores by the microorganisms allowing them to survive under unfavourable environmental conditions. Presence of dormant bacteria forms in all seasons of the year may also evidence a toxic effect of substances penetrating into the soil from the road transport. *Azotobacter* spp. bacteria and actinomycetes, regarded as indicators of soil fertility, were isolated in large numbers from all sampling points, therefore it may demonstrate their adaptability to unfavourable environment conditions (Lenart and Wolny-Koładka 2013). However, it should be noticed that *Azotobacter* spp. growth was affected by the temperature, because in spring and summer these bacteria occurred in many more sampling points than in autumn and winter.

Investigations conducted by Bis (2006) on the number of fungi in the soils under anthropopressure in the area of Krakow revealed that they were the most numerous on the Mateczny roundabout (234000 cfu/g d.w.). The authors obtain similar results in their own research while analyzing the number of fungi in the soils sampled from Mogilskie roundabout in autumn 2013 (167000 cfu/g d.w.). Much lower fungi numbers on the other sampling sites might result from neutral soil pH. Matthies *et al*. (2007) and Rousk *et al*. (2009) stated that fungi reveal a higher growth under conditions of acid soil reaction. On the other hand, bacteria prefer pH from 6.5 to 7.5, therefore the conditions in the analyzed soils are favourable for them, as evidenced by their large numbers. Czarnowska (1997) and Sitarski (2008) demonstrated that soils originating from the centres of large Polish cities reveal pH from neutral to slightly alkaline. These observations are convergent with the Author's own research results. According to Bielinska and Młocek (2010), the soil pH may be also influenced by alkaline dust depositions, application of chemicals during snow-clearing of streets, or wastes from road repairs.

The research on heavy metals and oil derivatives effect on the number of selected microorganism groups is quite frequent. An example of such research may be works conducted by Lenart and Chmiel (2008), Młocek-Płóciniak (2011) or Kaczyńska *et al*. (2014), who analyzed heavy metal effect on the growth of selected soil microorganisms. Moreover, papers by Michalcewicz (1995), Przybulewska *et al*. (2004) and Hawrot-Paw (2012) provide information about microorganism response to various doses of diesel fuel. On the other hand plenty of information about lead petrol effect on soil microorganism activity may be found in the works by Ziółkowska and Wyszkowski (2010) and Wyszkowska and Wyszkowski (2011). However, considering still increasing vehicle traffic in Krakow and its unfavourable effect on the environment, the subject discussed in presented paper seems purposeful and requires further investigations.

## CONCLUSIONS

1. A high biodiversity of soil microorganisms was observed in selected measurement points located by the main transport routes in Krakow.
2. Vegetative and ammonifying bacteria were the most frequently isolated from the investigated soils.
3. The least numerous microorganisms in the analyzed samples were *Azotobacter* bacteria.
4. No significant differences were stated in the microorganism numbers between the test points and the control (meadow), as evidenced by the statistical analysis.
5. The air temperature affected the number of some microorganisms, i.e. *Azotobacter* spp. and dormant bacteria forms, whose number in the winter period, respectively decreased and increased.

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