The Effect of Heavy Metal Concentration and Soil pH on the Abundance of Selected Microbial Groups Within ArcelorMittal Poland Steelworks in Cracow

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Abstract The present study aimed to identify the effect of heavy metal concentration and soil pH on the abundance of the selected soil microorganisms within ArcelorMittal Poland steelworks, Cracow. The analysis included 20 soil samples, where the concentration of Fe, Zn, Cd, Pb, Ni, Cu, Mn, Cr and soil pH were evaluated together with the number of mesophilic bacteria, fungi, Actinomycetes and Azotobacter spp. In the majority of samples soil pH was alkaline. The limits of heavy metals exceeded in eight samples and in one sample, the concentration of Zn exceeded 31-fold. Chromium was the element which most significantly limited the number of bacteria and Actinomycetes.

Keywords Heavy metals · pH · Soil · Soil microorganisms

Soil environment in industrial areas where the metallurgical plants are located, as well as in the agricultural areas surrounding these facilities is often heavily polluted with various xenobiotics, such as: polycyclic aromatic hydrocarbons (Sofilić et al. 2008) and heavy metals, mainly: Cu, Mn, Zn, Cd and Pb (Rodella and Chiou 2009; Ettler et al. 2004). Increased heavy metal content negatively affects soil microbial population, which may have direct negative effect on soil fertility (Ahmad et al. 2005). Environmental pressure resulting from the contamination may reduce the biodiversity of microorganisms and disturb the ecological balance. However, there are reports stating that soil microorganisms may adapt to the increased, even toxic heavy metal and other xenobiotics’ concentration in soil (Kozdrój 1995) by developing various mechanisms to resist heavy metal contamination (Rathnayake et al. 2010).

Undoubtedly, soil microorganisms are essential for proper functioning of ecosystem and soil fertility. Chemical analyses often measure the particular amounts of contaminants but they do not reflect the environmental consequences resulting from their effect on key processes of soil metabolism. Biological methods can measure the actual impact of contaminants on soil organisms and they show the growth and activity inhibition under stress conditions (Šmejkalová et al. 2003) Given the above described relationships, the research was undertaken to assess the heavy metal contamination of soils in the area of ArcelorMittal Poland steelworks in Cracow and its effect on the abundance of the selected groups of soil microorganisms.

Materials and Methods

Based on the analyses conducted previously (Lenart 2011), 20 soil sampling sites were selected in the area of ArcelorMittal Poland steelworks in Cracow. The exact location of the sampling sites are given in Table 1. The samples were collected in September 2011 into sterile polypropylene containers from the depth up to about 20 cm (ISO 1038). After being transported to the laboratory of the Department of Microbiology, University of Agriculture in Cracow, the samples were passed through a 2 mm sieve and analyzed by the serial dilutions method for the abundance of mesophilic bacteria (Trypticase Soy Agar, 48 h at 37°C), fungi (Malt Extract Agar—MEA, 3 days at 28°C), Actinomycetes (Pochon Agar, 5–7 days at 28°C) and Azotobacter spp. (Ashby’s agar, 5 days at 26°C). The
The concentration values are presented. Additionally, soil moisture and pH analyses were performed in three replications and average was calculated per one gram of the soil dry weight. The Table 1

| Sample no. | Location               | GPS coordinates | Soil pH | Soil moisture |
|-----------|------------------------|-----------------|---------|---------------|
| 1         | Gate no. 2             | N 50°04.958' E 20°04.423' | 8.6     | 14.92         |
| 2         | Gate no. 3             | N 50°05.286' E 20°05.160' | 8.0     | 12.37         |
| 3         | Welded tube rolling mill | N 50°05.450' E 20°05.735' | 7.7     | 18.32         |
| 4         | Cold rolling mill      | N 50°05.050' E 20°05.135' | 8.24    | 14.85         |
| 5         | Strip mill, below the pipeline | N 50°05.354' E 20°05.887' | 8.37    | 1.27          |
| 6         | Hearth steel plant     | N 50°04.674' E 20°04.835' | 8.65    | 23.40         |
| 7         | Converter steel plant  | N 50°04.216' E 20°04.790' | 8.20    | 24.87         |
| 8         | Slabing rolling mill   | N 50°03.956' E 20°05.090' | 8.59    | 12.24         |
| 9         | Agglomerating plant 2  | N 50°04.305' E 20°06.047' | 8.63    | 19.41         |
| 10        | Cement plant           | N 50°04.495' E 20°07.018' | 8.43    | 15.44         |
| 11        | Coking plant           | N 50°04.537' E 20°06.042' | 8.22    | 14.84         |
| 12        | Biological treatment plant | N 50°05.098' E 20°06.833' | 9.20    | 14.10         |
| 13        | Slag heap 1            | N 50°05.000' E 20°07.130' | 9.40    | 10.61         |
| 14        | Slag heap 2            | N 50°04.450' E 20°07.410' | 9.10    | 16.89         |
| 15        | Slag heap 3            | N 50°03.639' E 20°07.781' | 8.78    | 5.28          |
| 16        | Slag heap 4            | N 50°03.657' E 20°06.687' | 11.40   | 23.60         |
| 17        | Slag heap 5            | N 50°03.896' E 20°06.817' | 8.60    | 13.05         |
| 18        | Settling tank          | N 50°03.681' E 20°05.588' | 8.20    | 11.09         |
| 19        | Ash and sludge settling tank | N 50°03.450' E 20°05.588' | 8.46    | 39.45         |
| 20        | Port channel           | N 50°03.738' E 20°05.687' | 8.23    | 20.92         |

Based on the data it was found that in 8 out of the 20 sites, the concentration of various heavy metals in the studied soil samples exceeded the limit values (Journal of Laws of the Republic of Poland 2002). The concentration of zinc exceeded the admissible values in samples: Gate no. 3 (the concentration of zinc in this sample exceeded 31-fold the limit value), Welded tube rolling mill (11-fold transgression) and Slag heap 2 (Table 2). Additionally the concentrations of cadmium, lead, copper and chromium exceeded the admissible values in 8 soils sampled from: Gate no. 3, Welded tube rolling mill, Coking plant, Slag heap 2, 3, 4 and 5, and Ash and sludge settling tank. The heavy metal concentrations varied significantly between the samples—e.g. the highest concentration of zinc recorded in the sample no. 2 was over 31,000 mg kg⁻¹, while the lowest, recorded in the sample no. 8 was 37.46 mg kg⁻¹. The mean values of the majority of heavy metals were higher than those reported by other Authors (Akoto et al. 2008; Kaszubkiewicz and Kawalko 2009), however the chromium concentration, even in the slag heaps, was lower than the one recorded by Huang et al. (2009) in samples collected from slag heaps of the steel alloy factory in China.

The water extractable and exchangeable, and organically bound fractions are considered as the most toxic fractions of Cd, Pb and Zn in soils in terms of the food chain input (Šmejkalová et al. 2003). The available forms of heavy metals varied between the analyzed sites and they were low as compared to the total heavy metal concentration (Table 3). Only in the case of Zn the concentrations were relatively high in three sampling sites.

In most samples, the soil pH fluctuated in the range from 7.7 to 9.40 and in one of the samples, (collected from the Slag heap 4) the pH was strongly alkaline: 11.40 (Table 1). This site is located in the old part of the metallurgical slag heap, which is currently being re-operated. The material at this site largely consists of slag, which may indirectly

number of colony forming units (CFU) of microorganisms was calculated per one gram of the soil dry weight. The analyses were performed in three replications and average values are presented. Additionally, soil moisture and pH were measured (ISO 1039; ISO 1146). The concentration of heavy metals (Fe, Zn, Cd, Pb, Ni, Cu, Mn and Cr) was determined using atomic absorption spectroscopy (AAS) following the procedure described by Akoto et al. (2008) and the evaluation of heavy metals’ availability was performed using Inductive Coupled Plasma Atomic Emission Spectrometry (ICP-AES) after extraction with CaCl₂, as described by Galfati et al. (2011). The results were compared with Polish regulations for the maximum concentrations of heavy metals allowed in soils. Statistical analysis of the results was performed using Statistica software (StatSoft, USA), Pearson correlation (r) was applied to test relationships between the number of microorganisms and soil pH and heavy metal concentration at the level of significance equal to 0.05.

Results and Discussion

Based on the data it was found that in 8 out of the 20 sites, the concentration of various heavy metals in the studied soil samples exceeded the limit values (Journal of Laws of the Republic of Poland 2002). The concentration of zinc exceeded the admissible values in samples: Gate no. 3 (the concentration of zinc in this sample exceeded 31-fold the limit value), Welded tube rolling mill (11-fold transgression) and Slag heap 2 (Table 2). Additionally the concentrations of cadmium, lead, copper and chromium exceeded the admissible values in 8 soils sampled from: Gate no. 3, Welded tube rolling mill, Coking plant, Slag heap 2, 3, 4 and 5, and Ash and sludge settling tank. The heavy metal concentrations varied significantly between the samples—e.g. the highest concentration of zinc recorded in the sample no. 2 was over 31,000 mg kg⁻¹, while the lowest, recorded in the sample no. 8 was 37.46 mg kg⁻¹. The mean values of the majority of heavy metals were higher than those reported by other Authors (Akoto et al. 2008; Kaszubkiewicz and Kawalko 2009), however the chromium concentration, even in the slag heaps, was lower than the one recorded by Huang et al. (2009) in samples collected from slag heaps of the steel alloy factory in China.

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Table 2  Total heavy metal content in the analyzed soil samples within ArcelorMittal steelworks [mg kg\(^{-1}\)]

| Site no. | Fe  | Zn   | Cd   | Pb   | Ni  | Cu  | Mn   | Cr  |
|----------|-----|------|------|------|-----|-----|------|-----|
| 1        | 15,830 | 46.17 | 1.31 | 25.75 | 19.60 | 56.23 | 582.5 | 24.25 |
| 2        | 23,150 | 43.08 | 16.07 | 275.80 | 19.55 | 120.13 | 809.5 | 65.70 |
| 3        | 24,140 | 1165 | 8.20 | 16.07 | 275.80 | 19.55 | 120.13 | 809.5 | 65.70 |
| 4        | 18,170 | 3100 | 1.35 | 25.75 | 19.60 | 56.23 | 582.5 | 24.25 |
| 5        | 41,365 | 670.03 | 3.96 | 197.99 | 10.05 | 56.30 | 1378.5 | 25.80 |
| 6        | 27,225 | 322.31 | 3.73 | 168.59 | 20.40 | 81.00 | 933.5 | 67.75 |
| 7        | 28,270 | 186.10 | 1.30 | 110.44 | 12.70 | 70.39 | 886.5 | 26.65 |
| 8        | 18,200 | 37.46 | 0.09 | 8.63 | 12.90 | 29.11 | 634.5 | 17.65 |
| 9        | 69,700 | 252.64 | 0.07 | 110.44 | 12.70 | 160.13 | 1210.5 | 35.75 |
| 10       | 15,950 | 157.86 | 1.89 | 60.87 | 12.30 | 27.09 | 889.5 | 29.95 |
| 11       | 23,800 | 408.93 | 1.50 | 1073.99 | 35.25 | 891.40 | 116.50 | 29.25 |
| 12       | 32,405 | 279.11 | 1.46 | 50.06 | 81.10 | 933.5 | 67.75 | 29.25 |
| 13       | 41,220 | 824.5 | 2.65 | 104.40 | 48.35 | 61.45 | 5685 | 67.0 |
| 14       | 46,485 | 1600.5 | 8.70 | 316.60 | 49.15 | 145.75 | 2637 | 498.3 |
| 15       | 122,150 | 150.15 | 0.37 | 52.12 | 11.05 | 30.55 | 25665 | 862.00 |
| 16       | 101,400 | 49.28 | 0.43 | 2.80 | 4.30 | 23.35 | 584 | 54.70 |
| 17       | 75,800 | 245.60 | 0.90 | 80.75 | 13.30 | 70.39 | 886.5 | 26.65 |
| 18       | 149,200 | 813.12 | 7.90 | 451.47 | 27.20 | 83.52 | 18345 | 76.70 |
| Mean     | 45698.00 | 2496.45 | 5.31 | 212.95 | 21.65 | 141.86 | 3728.35 | 200.64 |
| SD       | 38511.59 | 7173.77 | 9.67 | 262.53 | 12.30 | 218.55 | 6215.68 | 274.48 |

Heavy metal contents which exceeded the admissible values (Regulation of the Minister of Environment of September 9th 2002) in bold letters.

Table 3  Content of available forms of heavy metals in the analyzed soil samples within ArcelorMittal steelworks [mg kg\(^{-1}\)]

| Site no. | Fe  | Zn   | Cd   | Pb   | Ni  | Cu  | Mn   | Cr  |
|----------|-----|------|------|------|-----|-----|------|-----|
| 1        | 0.105 | 0.395 | 0.015 | 0.115 | 0.075 | 0.130 | 0.125 | 0.030 |
| 2        | 2.195 | 24.198 | 0.210 | 0.135 | 0.055 | 0.070 | 0.130 | 0.025 |
| 3        | 1.705 | 281.86 | 0.095 | 0.495 | 0.085 | 0.185 | 0.595 | 0.040 |
| 4        | 0.255 | 22.870 | 0.075 | 0.115 | 0.070 | 0.155 | 0.640 | 0.0305 |
| 5        | 1.265 | 8.295 | 0.085 | 0.145 | 0.085 | 0.425 | 1.055 | 0.045 |
| 6        | 0.215 | 22.470 | 0.085 | 0.130 | 0.070 | 0.155 | 0.640 | 0.035 |
| 7        | 0.305 | 5.970 | 0.020 | 0.075 | 0.075 | 0.120 | 0.180 | 0.005 |
| 8        | 0.110 | 0.175 | 0.025 | 0.130 | 0.075 | 0.055 | 0.120 | 0.010 |
| 9        | 0.365 | 0.615 | 0.030 | 0.035 | 0.060 | 0.290 | 0.175 | 0.055 |
| 10       | 0.160 | 0.235 | 0.025 | 0.045 | 0.095 | 0.125 | 0.105 | 0.045 |
| 11       | 2.205 | 1.615 | 0.020 | 0.055 | 0.120 | 0.130 | 1.054 | 0.025 |
| 12       | 0.125 | 0.050 | 0.010 | 0.130 | 0.085 | 0.155 | 0.085 | 0.030 |
| 13       | 7.875 | 0.415 | 0.035 | 0.140 | 0.055 | 0.080 | 0.745 | 0.045 |
| 14       | 1.045 | 0.355 | 0.085 | 0.195 | 0.045 | 0.125 | 0.125 | 0.020 |
| 15       | 1.003 | 0.165 | 0.025 | 0.120 | 0.080 | 0.045 | 0.710 | 0.030 |
| 16       | 0.075 | 0.810 | 0.035 | 0.095 | 0.080 | 0.035 | 0.685 | 0.010 |
| 17       | 0.110 | 0.115 | 0.055 | 0.115 | 0.055 | 0.060 | 0.090 | 0.105 |
| 18       | 3.020 | 0.640 | 0.020 | 0.070 | 0.070 | 0.055 | 0.145 | 0.055 |
| 19       | 0.095 | 0.375 | 0.030 | 0.110 | 0.085 | 0.055 | 0.225 | 0.065 |
| 20       | 0.130 | 1.420 | 0.060 | 0.115 | 0.075 | 0.100 | 0.195 | 0.030 |
| Mean     | 1.118 | 18.652 | 0.052 | 0.128 | 0.075 | 0.128 | 0.369 | 0.071 |
| SD       | 1.819 | 62.497 | 0.046 | 0.094 | 0.017 | 0.093 | 0.333 | 0.146 |

The highest concentrations of metals in bold letters.
affect the distribution and abundance of microorganisms in this area (Huang et al. 2009), which was confirmed by our observations (Table 4). The number of mesophilic bacteria varied between the collected samples—from 10,435,950 in the sample collected at the Port channel to none in the sample Slag heap 4 (Table 4), which was characterized by the exceeded concentration of chromium and strongly alkaline pH (11.40). Moreover, none of the analyzed microbial groups were detected in this sample. This situation could have been caused mainly by very highly alkaline pH of the soil (Martyn and Skwaryło-Bednarz 2005), and by the increased chromium concentration (Megharaj et al. 2003). The highest abundance of fungi was observed in the sample Slag heap 2. It is the oldest metallurgical heap (mixture of production wastes without selective storage), also currently re-operated. These microorganisms were absent in two samples—the previously mentioned Slag heap 4 and Ash and sludge.

Table 4  Abundance of the analyzed microorganisms in 20 soil samples within ArcelorMittal Poland steelworks [CFU g⁻¹]

| Analyzed microorganisms       | Soil samples |
|-------------------------------|--------------|
|                               | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   |
| Mesophilic bacteria           | 3,105,400 | 1,215,930 | 1,314,890 | 291,640 | 136,740 |
| Fungi                         | 1,070   | 5,700   | 1,600   | 1,590   | 14,450  |
| Actinomycetes                 | 68,700  | 1,480   | 15,700  | 7,200   | 99,500  |
| Azotobacter spp.              | 1,656   | 0      | 0      | 0      | 0      |

Table 5  Correlation coefficients between microbial characteristics: total number and presence of the tested microorganisms and heavy metal content in soil and soil pH

|                 | Fe   | Zn   | Cd   | Pb   | Ni   | Cu   | Mn   | Cr   | pH   |
|-----------------|------|------|------|------|------|------|------|------|------|
| **Mesophilic**  |      |      |      |      |      |      |      |      |      |
| bacteria        | -0.27 | -0.05 | -0.02 | -0.10 | 0.008 | -0.17 | -0.20 | -0.31 | -0.08 |
| Fungi           | -0.10 | -0.06 | 0.05  | 0.19  | 0.42  | 0.04  | -0.12 | 0.09  | 0.004 |
| Actinomycetes   | -0.33 | -0.24 | -0.26 | -0.24 | -0.27 | -0.21 | -0.32 | -0.40 | -0.12 |
| Azotobacter spp.| -0.19 | -0.18 | -0.19 | -0.25 | -0.10 | -0.20 | -0.23 | -0.30 | -0.05 |
| **Available**   |      |      |      |      |      |      |      |      |      |
| heavy metal      |      |      |      |      |      |      |      |      |      |
| content          |      |      |      |      |      |      |      |      |      |
| Mesophilic bacteria | -0.21 | -0.05 | -0.11 | -0.19 | 0.07  | -0.02 | -0.29 | -0.17 | 0.17  |
| Fungi            | -0.09 | -0.14 | 0.15  | 0.06  | -0.27 | 0.17  | -0.07 | -0.20 | -0.20 |
| Actinomycetes    | -0.11 | -0.12 | -0.24 | -0.15 | 0.20  | 0.70  | 0.06  | -0.24 | -0.24 |
| Azotobacter spp. | -0.27 | -0.16 | -0.23 | -0.14 | -0.16 | 0.05  | -0.38 | -0.017 | -0.17 |
settling tank, where the increased copper concentration was
detected. The number of Actinomycetes in the investigated
samples was also varied and ranged from 99,500 CFU g⁻¹
of soil in the sample Strip mill to 0 in the sample Slag heap 4.
In general, this was low in comparison to the numbers
recorded by Martyn and Skwaryło-Bednarz (2005) in non-
contaminated soils and by Ahmad et al. (2005) in soils
amended with various heavy metals. The analyzed soil
samples differed also in terms of the abundance of
*Azotobacter* spp. Populations of these bacteria in soils of
neutral and alkaline pH rarely exceed several thousand cells
per one gram of soil (Martyniuk and Martyniuk 2003). The
results obtained in this study confirm this observation. The
highest observed number of *Azotobacter* spp. was
2,890 CFU g⁻¹ of soil in the sample with pH 8.59. These
bacteria are also very sensitive to soil acidification and are
rarely detected in acid soils (Martyniuk et al. 2007). It is
impossible to verify this relationship in the studied area due
to the fact that the majority of the analyzed samples was
alkaline.

The analysis of correlation between the soil pH and the
abundance of the analyzed microorganisms indicated weak
relationship between these values (Table 5). Moreover, the
statistical analysis revealed that the soil pH did not affect
the number of fungi. Although the correlation was not
statistically significant, it needs to be stressed that the
presence of the tested microbial groups was not detected in
the soil sample collected from the Slag heap 4, whose pH
was 11.4. The statistical analysis of the effect of soil pH on
the abundance of microorganisms may be hindered in this
case due to the fact that the soil pH was generally alkaline,
while the samples with acidic or neutral pH were not found
among the studied ones.

At the site gate no. 3, despite the recorded high
concentrations of zinc, cadmium and lead (Zn—the allowable
concentration exceeded 31-times; Cd-3- and Pb—the
allowable concentration exceeded by 86 mg kg⁻¹) a relatively
high number of mesophilic bacteria and fungi were
observed. The presence of Actinomycetes was also recorded
at this site (1,480 CFU g⁻¹), whereas the bacteria of the
genus *Azotobacter* were not detected.

Based on the statistical analysis of correlation between
the heavy metal content in soils and the abundance of the
studied microbial groups, a weak negative correlation was
found between the concentration of the majority of heavy
metals and the number of *Azotobacter* spp. Only in the case
of chromium this correlation was moderate. The same
relationship was found in the case of mesophilic bacteria.
Similarly for the fungi—the relationship between their
numbers in soil samples and heavy metal content was very
weak. Also the abundance of Actinomycetes was weakly
correlated with the concentration of the heavy metals in
soils. Only with regard to iron, manganese and chro-
nium the moderate negative correlation was observed.
These results are contrary to the findings of Šmejkalová
et al. (2003) or Ahmad et al. (2005), who reported a sig-
nificant decrease in CFU of most microbial groups with the
increase of heavy metal concentration. Based on the
described relationships it may be concluded that the ana-
alyzed microorganisms presented the potential resistance to
toxic concentrations of heavy metals in the environment.
The obtained results of statistical analysis suggest that
chromium was the only element which most significantly
limited the number of the microorganisms in soils. This
metal presents strong toxic effect towards not only
microorganisms, but also towards higher organisms (Shi
et al. 2002).

In conclusion, the majority of soil samples were alkaline
and in over one-third of them the heavy metal concentra-
tions exceeded the allowable limits, however the observed
differences in pH and the concentration of heavy met-
als only slightly affected the number of the analyzed
microorganisms. Chromium was the element which most
significantly limited the number of bacteria and Actino-
mycetes in soils of ArcelorMittal Poland steelworks in
Cracow. The fact that the heavy pollution of some of the
samples did not decrease the number of bacteria, Actino-
mycetes and fungi suggests that these microorganisms may
present the resistance to increased or even toxic concen-
tration of some heavy metals in soils and therefore may be
used in future research on the bioremediation of contami-
nated areas.

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