SOIL IN THE ENVIRONMENT

CONFERENCE REGISTRATION FORM
3rd International Conference of Young Scientists:
Soil in the Environment
16-19 September 2018, Kraków-Poronin, Poland

IUNG Instytut Uprawy Nawożenia i Gleboznawstwa Państwowy Instytut Badawczy

IDENTIFICATION OF NITROGEN-FIXING BACTERIA FROM THE RECLAIMED SMELTER WASTELAND USING 16S RDNA SEQUENCING METHOD AND GENOTYPING OF STRAINS USING PCR-MP.

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Smelter wastelands with extreme contents of zinc, lead, cadmium and arsenic constitute a serious problem in the area of Upper Silesia. Soil contamination with metals can cause toxicity to plants and soil microorganisms, limiting the land ability to perform basic functions.

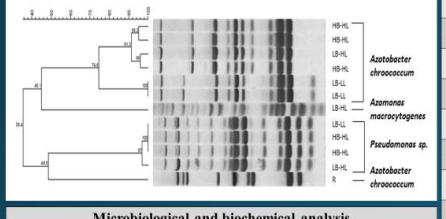
The activity of N-binding bacteria is extremely important for the development of the plant cover in these areas. In 2016, samples taken from a long-term field experiment (reclamation in 1997) at a waste heap in Piekary Śląskie were collected from a depth of 0-20 cm in three replications.

The material was collected from 6 transects representing different combinations of sewage sludge and by-product lime rates. The method of plate dilutions was used to determine the total number of Azotobacter which was expressed as the number of colonies (CFU) in 1 gram of air dried soil matter. Then bacterial species identification was performed by 16S rDNA sequencing and PCR genotyping of strains.

As a result of the genotyping of the diazotroph strains, 7 different electrophoretic profiles were obtained.

The dimension of the whole experimental site was 40mx100m. Six different treatments of the top layer. Were applied in the plot experiment and compared withthe untreated control:

- **LB – lower biosolids (150 t ha -1),**
- **HB – higher biosolids rate (300 t ha-1),**
- **LB-LL – lower biosolids (150 t ha-1) and lower lime (100 t ha-1) rates,**
- **HB-LL – higher biosolids (300 t ha-1) and lower lime (100 t ha-1) rates,**
- **LB-HL – lower biosolids (150 t ha-1) and higher lime 1000 t ha-1) rates,**
- **HB-HL – higher biosolids (300 t ha-1) and higher lime (1000 t ha-1) rates.**



	H_2O - extractable						Total content									
	Cd		Zn		Pb		Cd		Pb		Zn		As		Hg	
	mean	STD	mean	STD	mean	STD	mean	STD	mean	STD	mean	STD	mean	STD	mean	STD
Control	0.016^{abc}	0.010	0.07^{a}	0.04	0.07^{a}	0.04	163^{abc}	114	4876$^{abc d}$	2953	19859^{d}	9781	1286^{d}	195	0.52^{a}	0.45
LB	0.021^{bc}	0.015	0.74^{cd}	0.29	0.41^{bc}	0.13	607^{d}	412	16394^{e}	8164	24040^{f}	7003	1219^{d}	162	2.15^{bc}	0.91
HB	0.027^{c}	0.013	1.49^{d}	0.49	0.76^{c}	0.36	242^{d}	109	6351^{cde}	2840	7613^{b}	2525	273^{ab}	111	2.70^{c}	0.29
LB-LL	0.009^{b}	0.002	0.95^{d}	0.25	0.28^{b}	0.06	334^{d}	92	9483de	2761	26274^{g}	8056	672^{c}	5	2.09^{bc}	0.56
HB-LL	0.011^{bc}	0.005	1.19^{d}	0.47	0.32^{bc}	0.10	224^{c}	14	6503^{bcd}	472	17521^{e}	3957	677^{c}	200	1.71^{b}	0.47
LB-HL	0.002^{a}	0.000	0.18^{b}	0.05	0.04^{a}	0.02	134^{b}	11	3052^{ac}	396	16203^{e}	102	213^{a}	1	0.98^{a}	0.23
HB-HL	0.003^{a}	0.001	0.82^{c}	0.48	0.08^{a}	0.03	95^{a}	4	2303^{a}	175	13425^{b}	456	173^{a}	19	1.86^{b}	0.21

Microbiological and biochemical analysis

In order to characterize biochemical activity of the amended wasteland, activities of three enzymes (dehydrogenases, acidic and alkaline phosphatases) were measured using standard protocols (Tabatabai, 1994). The determination of dehydrogenases was performed according to Casida et al. (1964) by the colorimetric method, using TTC (triphenyltetrazole chloride) as a substrate, after 24 hours incubation at 37°C. Alkaline and acid phosphatase activities were measured by colorimetric method using PNP (sodium p-nitrophenylphosphate) after 1 hour incubation at 37 ° C at 410 nm wavelength (Tabatabai and Bremner 1969).

The total count of bacteria and actinobacteria (Wallace and Lockhead, 1950), ammonification bacteria (Rodina, 1968), bacteria of *Azotobacter* (Fenglerowa, 1965) and total count of fungi (Martin 1950) were determined by the plate dilution method. The plates were incubated in temperature 28°C and the number of colonies was counted after 3 – 5 days of growth. All biochemical and microbiological measurements were done in triplicate.

	Dehydrogenases		Alkaline phosphatase		Acidic phosphatase	
	μg TPF g soil DM^{-1} h^{-1}		μg p-nitrophenyl g soil DM^{-1} h^{-1}		μg p-nitrophenyl g soil DM^{-1} h^{-1}	
	mean	STD	mean	STD	mean	STD
Control	0.12^{ab}	0.04	27.9^{ab}	10.4	26.0^{a}	5.7
I	0.09^{a}	0.02	22.1^{a}	6.0	39.4^{a}	10.3
II	0.16^{bc}	0.00	36.5^{b}	0.9	67.8^{b}	3.5
III	0.22^{d}	0.05	52.5^{c}	11.3	86.8^{b}	18.8
IV	0.27^{de}	0.08	71.0^{cd}	29.6	118.9^{bc}	38.8
V	0.20^{cd}	0.03	47.4^{c}	5.5	85.2^{b}	8.2
VI	0.40^{e}	0.05	91.3^{d}	8.0	154.7^{c}	18.0

	Total number of bacteria		Total number of bacteria *Azotobacter*		Total number of ammonification bacteria		Total number of fungi	
	10^7 CFU g^{-1} d.m. of soil		10^1 CFU g^{-1} d.m. of soil		10^7 CFU g^{-1} d.m. of soil		10^4 CFU g^{-1} d.m. of soil	
Control	2.35^{a}	1.14	0.00^{a}	0.00	0.25^{a}	0.15	12.76^{a}	3.86
I	6.32^{b}	2.60	0.00^{a}	0.00	1.80^{ab}	1.16	12.31^{a}	2.99
II	42.93^{c}	17.02	0.00^{a}	0.00	0.81^{a}	0.59	121.44^{c}	21.40
III	100.38^{cd}	63.57	207.64^{bc}	102.60	2.97^{b}	0.28	62.52^{abc}	45.48
IV	95.38^{cd}	35.58	180.61^{bc}	76.91	5.16^{bc}	4.67	44.47^{b}	16.28
V	47.63^{c}	13.81	221.17^{d}	19.20	5.94^{bc}	3.77	55.70^{b}	27.40
VI	155.43^{d}	34.40	111.91^{b}	15.14	9.44^{c}	2.56	73.61^{bc}	37.20

NARODOWE CENTRUM NAUKI

Research was performed within the project "Role of microorganisms in colonization of smelter wastelands by plants and their impact on bioavailability of trace elements "; Preludium 9, UMO-2015/17/N/ST10/03182