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Alkaline Technosol contaminated by former mining activity and its culturable autochthonous microbiota

A. Šimonovičová [a,*], P. Ferianc [b], H. Vojtková [c], D. Pangallo [b], P. Hanajík [a], L. Kraková [b], Z. Feketeová [a], S. Čerňanský [d], L. Okenicová [e], M. Žemberyová [e], M. Bujdoš [f], E. Pauditšová [g]

[a] Department of Soil Science, Faculty of Natural Sciences, Comenius University, 842 15 Bratislava, Slovak Republic
[b] Institut of Molecular Biology, Slovak Academy of Sciences, 845 51 Bratislava, Slovak Republic
[c] Institute of Environmental Engineering, Faculty of Mining and Geology, VŠB – Technical University of Ostrava, Czech Republic
[d] Department of Environmental Ecology, Faculty of Natural Sciences, Comenius University, 842 15 Bratislava, Slovak Republic
[e] Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University, 842 15 Bratislava, Slovak Republic
[f] Institute of Laboratory Research on Geomaterials, Faculty of Natural Sciences, Comenius University, 842 15 Bratislava, Slovak Republic
[g] Department of Landscape Ecology, Faculty of Natural Sciences, Comenius University, 842 15 Bratislava, Slovak Republic

HIGHLIGHTS

- Alkaline and strongly alkaline Technosol contaminated by As, Cu, Mn, Pb, Zn caused changes in autochtonous microbiota.
- *Variovorax pantotropha* and *Staphylococcus pasteuri* Sp-12 were isolated.
- 20 fungal species belonged to 13 genera were recovered.
- The isolated microorganisms showed their high adaptation abilities.
- Different isolates can be useful for various industrial and remediation processes.

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ABSTRACT

Technosols or technogenic substrates contaminated by potentially toxic elements as a result of iron mining causes not only contamination of the surrounding ecosystem but may also lead to changes of the extent, abundance, structure and activity of soil microbial community. Microbial biomass were significantly inhibited mainly by exceeding limits of potentially toxic metals as arsenic (in the range of 343–511 mg/kg), copper (in the range of 7980–9227 mg/kg), manganese (in the range of 2417–2670 mg/kg), alkaline and strong alkaline pH conditions and minimal contents of organic nutrients. All of the 14 bacterial isolates, belonged to 4 bacterial phyla, *Actinobacteria*, *Firmicutes*; β- and γ-*Proteobacteria*. Thirteen genera and 20 species of microscopic filamentous fungi were recovered. The most frequently found species belonged to genera *Aspergillus* (*A. clavatus*, *A. niger*, *A. flavus*, *A. versicolor*, *Aspergillus* sp.) with the dominating *A. niger* in all samples, and *Penicillium* (*P. canescens*, *P. chrysogenum*, *P. spinulosum*, *Penicillium* sp.). Fungal plant pathogens occurred in all surface samples. These included *Bjerkandera adustata*, *Bionectria ochloleuca* with anamorph state *Clonostachys pseudochloleuca*, *Lewia infectoria*, *Phoma macrostoma* and *Rhizoctonia* sp.

1. Introduction

According to World Reference Base for Soil Resources (WRB; 2006) Technosols include soils from wastes, mine spoils or ashes and they are often referred to as urban or mine soils. Such soils are formed during re-cultivation of overburdens, tailings and other spoils and wastes resulting from mining and other industrial activities (Levyk et al., 2007; Baran et al., 2014). According to Hafeez et al. (2012), Technosols could support soil functions, including primary production, but the knowledge about other ecosystemic role is limited. Mining of mineral resources results in extensive soil damage, altering microbial communities and affecting vegetation

* Corresponding author.
E-mail address: asimonovicova@fns.uniba.sk (A. Šimonovičová).

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leading to destruction of environment (Sheoran et al., 2010). Mining activities, disposal of metals or metal containing material inevitable cause contamination in the surrounding ecosystem together with changes in the extent, structure and activity of soil microbial community (Ge and Zhang, 2011). Many Technosols have to be treated carefully as they may contain toxic substances resulting from industrial processes and represent severe danger. Mine soils are often physically degraded, and so they hinder plants development (Asenio et al., 2013).

Technosols at the site of Slovinky (Slovak Republic) presents fine-grained industrial material originated from iron ore mining (Petrák et al., 2011; Tóth et al., 2013). At the site, the flotation sludge was deposited during 1969–2009 reaching more than 4.8 million m^3 upto now. The decanting plant, as high as 113 m, is the highest in Slovakia. The material contains elevated concentrations of various elements: arsenic (As), cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb) and zinc (Zn) carrying potential risk in the case of leaching from substrate and being released into the surface and ground water. This mining waste site is bounded by broadleaf mixed forest with formed mostly by *Betula pendula*, *Fagus sylvatica*, *Coryllus avelanea*, *Larix decidua* and *Picea abies*. The substrate surface is occasionally moss-grown (Krokusová, 2005). Such a substrate clearly represents an extreme environment for microorganisms (Roadcap et al., 2006; Levyk et al., 2007; Wang et al., 2011; Gostinčar and Turk, 2012; Gostinčar et al., 2012; Šimonovičová et al., 2013a, 2013b, 2016). Anyway, those are well-adapted to specific conditions at the level of their micromorphological structures and metabolic activities as well.

The aim of this study was to characterize Technosol from former mining site contaminated by high amounts of arsenic and other potentially toxic elements, such as Cd, Cu, Mn, Pb, Zn, and minimal contents of organic nutrients. The aim was also to show the ability of its culturable autochthonous bacteria and microscopic fungi to grow and produce biomass despite of exceeding metal contamination and lack of organic nutrients of this particular substrate. All organisms living in Technosol showed their high adaptation abilities to stress conditions, which allow them not only to survive but to build a basis for future settlements of other organisms. In addition, it would be possible to select interesting microorganisms able to be used in different biotechnology applications such as bioleaching and bioremediation.

2. Material and methods

2.1. Study sites and sampling

The samples were picked up from the places evidenced in Fig. 1. Samples of substrate were collected in the middle of the decanting plant (site 1) from the depths of 0–10 cm (sample 1a–e) and 20–30 cm (sample 2a–e) and in marginal parts nearby the birch bush and high grasses (site 2) from the same depths (samples 3a–e and 4a–e), respectively. The substrate from the depth of 0–2 cm (site 3, sample 5a–e), was collected below the crust of root system of mosses (Fig. 2). The humidity of soil samples was measured. Each samples had five replicates; in laboratory all samples were homogenized by quartering, passed through a 2 mm sieve and stored at 4 °C in darkness until all microbiological analyses were performed.

2.2. Basic chemical analyses

Values of pH_{H2O} and pH_{KCl} were measured potentiometrically and $\%C_{ox}$ (oxidizable organic carbon) content was determined by oxidimetry under laboratory conditions. The value of humus content was calculated from $\%C_{ox}$ multiplied by a conversion factor of 1.724 (Hrivňáková et al., 2011). Soil microbial biomass carbon (C_{mic}) was detected by fumigation extraction method according to Jenkinson and Ladd (1981). Soil respiration, it means the amount of carbon dioxide measured according to Schinner et al. (1993) as basal respiration (B-CO_2) in the original sample and as potential respiration (P-CO_2) with addition of 1% glucose in the same sample. There were three replicated runs for each analytical determination and average values are showed as results.

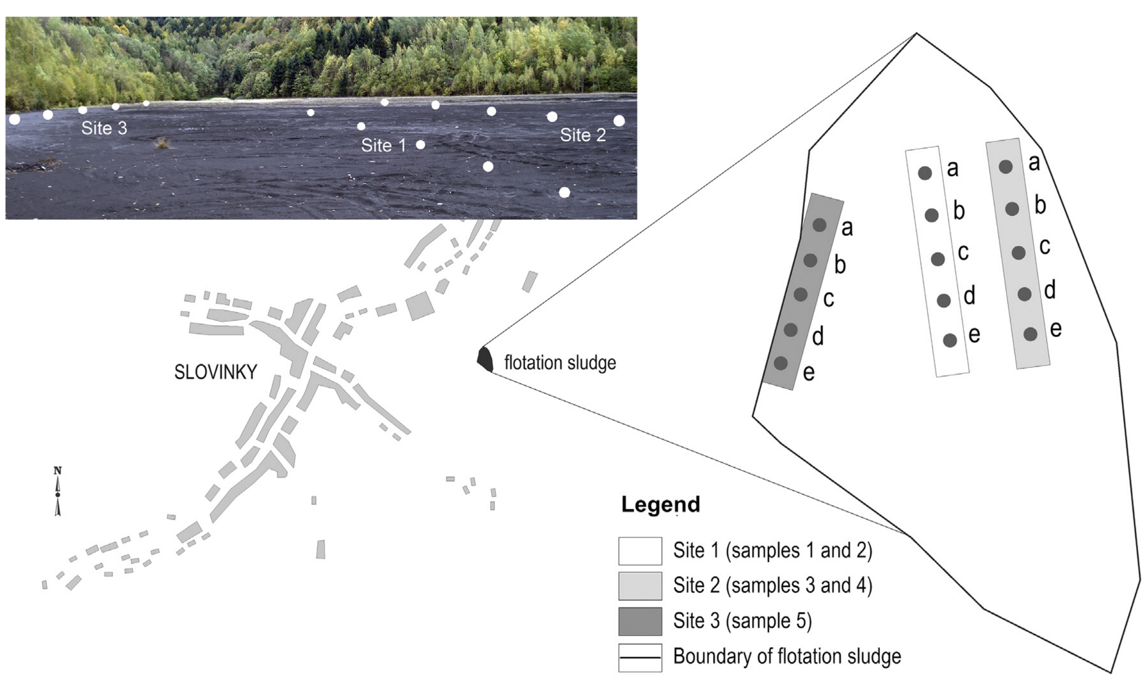

Fig. 1. Location of the sampling sites.