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Yeasts associated with an abandoned mining area in Pernek and their tolerance to different chemical elements

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Abstract Four plants, *Cirsium arvense* (creeping thistle), *Equisetum arvense* (field horsetail), *Oxalis acetosella* (wood sorrel) and *Phragmites australis* (common reed), which grew in an abandoned Sb-mining area in Pernek (Malé Karpaty Mts., Slovakia), were investigated for the yeast species. Yeasts were isolated from both the leaves of the plants and the soil adjacent to the plants. In total, 65 yeast cultures, belonging to 11 ascomycetous and 5 basidiomycetous yeast species, were isolated. The species most frequently isolated from both the soil and leaf samples were *Trichosporon porosum*, *Galactomyces candidus* and *Candida solani*, whereas *Aureobasidium pullulans*, *Candida tsuchiyae* and *Sporidiobolus metaroseus* were isolated exclusively from the plant leaves. All the yeast species isolated were tested for their tolerance to two heavy metals (Cd, Zn) and three metalloids (As, Sb and Si). The yeasts isolated from both the leaves and soils exhibited a high tolerance level to both As and Sb, present in elevated concentrations at the locality. Among the yeast species tested, *Cryptococcus musci*, a close relative to *Cryptococcus humicola*, was the species most tolerant to all the chemical elements tested, with the exception of Si. It grew in the presence of 200 mmol/L Zn, 200 mmol/L Cd, 60 mmol/L As and 50 mmol/L Sb, and therefore, it can be considered as a multi-tolerant species. Some of the yeast species were tolerant to the individual chemical elements. The yeast-like species *Trichosporon laibachii* exhibited the highest tolerance to Si of all yeasts tested, and *Cryptococcus flavescens* and *Lindnera saturnus* showed the same tolerance as *Cryptococcus musci* to Zn and As, respectively. The majority of the yeasts showed a notably low tolerance to Cd (not exceeded 0.5 mmol/L), which was present in small amounts in the soil. However, *Candida solani*, isolated from the soil, exhibited a higher tolerance to Cd (20 mmol/L) than to As (2 mmol/L).

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Introduction

A wide diversity of bacteria, archaea, yeasts and filamentous fungi occupy various natural ecosystems. Yeasts and yeast-like organisms are important members of all parts of plants as well as soil environments. In these habitats, yeasts consume nutrients, stimulate plant metabolism, decompose low and high molecular compounds, act as antagonists of diverse microorganisms, form a symbiotic or mutualistic system and serve as a nutrient source for various organisms (Lindow and Brandl 2003; Glushakova and Chernov 2007; Botha 2011; Lachance 2011). The diversity and density of yeasts in the phyllosphere are linked to geographical locality, climatic conditions, season, plant species and plant organs. Plant age is also an important factor which affects the community structure (Teixidó et al. 1999; Marschner et al. 2004). Yeasts present in soil reflect more or less the yeast population associated with the plants, animals and fungi living above ground, but many yeast species are typical inhabitants of soil (Winding et al. 2005; Botha 2006; Sláviková et al. 2009; Yurkov et al. 2012).

Metal industries, mining activities and agriculture are main sources of elevated amounts of various chemical compounds, predominantly toxic metals, in environments. Contamination of soil, water and stream sediments by large amounts of these compounds is a serious problem as it poses a significant risk to public health and ecosystems. Moreover, toxic metals and

elevated concentrations of essential elements can affect the size, activity, diversity and both physiological and morphological properties of the microbiota present in such habitats. However, individual bacterial, fungal and yeast species possess mechanisms which eliminate the adverse effects of toxic compounds on their cells, and as a result, these organisms thrive in polluted environments (Balsalobre et al. 2003; Raspor and Zupan 2006; Kavamura and Esposito 2010; Muñoz et al. 2012; Singh et al. 2013).

The forest in Pernek is a former mining area with antimony deposits, abandoned mine shafts and piles. There are excessive concentrations of antimony and arsenic present in the soil.

The purpose of this work was to isolate yeasts colonizing both the leaves of *Phragmites australis*, *Cirsium arvense*, *Equisetum arvense* and *Oxalis acetosella* grown in Pernek as well as the soil in which they grew, with the aim of finding out the yeast diversity and the tolerance of yeast isolates to As, Sb, Zn, Cd and Si.

Material and methods

Isolation of yeast cultures

Four plants: *Cirsium arvense* (creeping thistle), *E. arvense* (field horsetail), *O. acetosella* (wood sorrel) and *P. australis* (common reed) were investigated for the yeast species. All the plants examined grew in the same contaminated locality in the forest in Pernek. The seeds of creeping thistle, originated from another Sb-mining site in Slovakia (Medzibrod, Nízke Tatry Mts.), germinated and grew in a greenhouse in an uncontaminated soil for 2 months. Subsequently, the plants were transferred into the forest soil in Pernek for a further 3 months. The other three plant species are autochthonous in this area.

The samples of leaves and soils were collected into sterile plastic bags, transported to a laboratory, and processed within 2 h after harvesting. The soil samples were taken at a depth up to 10 cm. The samples of both plants and soil were collected in August 2011. Plant or soil samples (5 g) were placed in 250-mL flasks containing 50 mL of sterile distilled water and shaken on a rotary shaker for 2 h at 25 °C. The washings were serially diluted and 0.1 mL of each dilution was spread on the malt agar (Merck) containing 0.1 g/L of antibiotic CEFZIL (Cefprozilum monohydricum). Different colonies were picked up after 3, 5 and 10 days, and the representatives of them were purified according to Slávikoá et al. (1992). The yeast cultures were maintained on the malt agar slants at 4 °C.

Characterization of yeast cultures

The morphological and physiological characteristics of yeast cultures were examined by the methods described by Kurtzman et al. (2011a). Strains were identified according to Kurtzman et al. (2011b). The molecular identification of yeasts was carried out using the sequence analysis of the D1/D2 domains of the 26S rRNA gene.

DNA was isolated from yeast cultures (48 h) using UltraClean Microbial DNA Isolation kit (MO BIO Laboratories) in accordance with the manufacturer's instructions. The extracted DNA was stored at −20 °C. Amplification of DNA primers NL-1 (5′-GCATAT CAATAAGCG GAG GAA AAG-3′) and NL-4 (5′-GGT CCG TGT TTC AAG ACG G-3′) (Elisabeth Pharmacon) were used for the amplification (Kurtzman and Robnett 1997). PCR was performed in 0.2-mL thin wall tubes in a total reaction volume of 50 μL, consisting of 5 μL Taq polymerase buffer A (1.5 mmol/L) (Kapa Biosystems), 1 μL of dNTPs (10 mM/L) (Kapa Biosystems), 0.2 μL of Taq DNA polymerase (Kapa Biosystems), 2 μL of primer NL-1 (10 μmol/L), 2 μL of primer NL-4 (10 μmol/L), 1 μL of template DNA and sterile distilled water up to 50 μL.

The amplification products were purified using StrataPrep PCR Purification Kit (Agilent Technologies) according to the supplier's instructions. PCR products were analysed by automated capillary electrophoresis using the Agilent 2100 Bioanalyzer (Agilent Technologies) and the Agilent DNA 7500 LabChip kit according to the manufacturers' protocols. The purified PCR product was sequenced using primers NL-1 and NL-4 on ABI Prism3130x1 DNA Genetic Analyser (BITCET SR, Bratislava, Slovakia). The sequences obtained were compared with those found in the BLAST network service of the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST).

The strains with the acronym CCY are deposited in the Culture Collection of Yeasts, Bratislava, Slovakia.

Tolerance of yeasts to chemical elements

The strains were cultured in a medium that consisted of 1 % $(NH_4)_2SO_4$, 0.1 % KH_2PO_4, 0.05 % $MgSO_4$, 2 % glucose and 0.3 % yeast autolysate. The medium was sterilized by autoclaving at 121 °C for 20 min.

Sterilized stocks of $CdSO_4$, $K_2(SbO)_2C_8H_4O_{10}$, $HAsNa_2O_4$, $ZnCl_2$ or sodium silicate solution (27 % SiO_2 dissolved in 14 % NaOH) were aseptically added to reach the final concentration from 0.001 to 200 mmol/L (1 mmol/L concentrations of the five chemical compounds and corresponding mg/L values are given in Table 1).

Strains were cultivated in L-shaped tubes containing 9.5 mL of sterile medium, 0.2 mL of suspensions (10^8 cells/mL) and 0.3 mL of the individual solution of chemical elements tested. Yeasts were grown aerobically at their optimal temperature (20 °C for the yeasts of the genera *Cryptococcus* and *Sporidiobolus*, 28 °C for another yeast species) on a shaker (100 rpm). The cell biomass was measured by the absorbance at 660 nm at regular intervals for a period of 14 days. The absorbance of strains grown in metal-containing solutions

Table 1 Concentrations of chemical compounds used in the study (corresponding mg/L values to 1 mmol/L)

Chemical substance	mg/L	Individual chemical element (mg/L)
Silicon compound	960	28
$ZnCl_2$	136	65
$HAsNa_2O_4$	312	75
$K_2(SbO)_2C_8H_4O_{10}$	614	244
$CdSO_4$	208	112

was compared to that of strains grown in the solution without the chemical element (control). The growth of yeasts was simultaneously determined by dry biomass (at 105 °C to constant mass). The strain was regarded as tolerant towards metal in the relevant concentration if its biomass in the metal-containing medium reached at least 80 % of the growth of control. All experiments were repeated three times.

Determination of chemical elements in soil

The soil samples were collected directly from the localities where the plants were harvested. The collected soil samples were air-dried at <40 °C and sieved to the <0.125-mm fraction. The concentrations of selected elements (As, Cd, Sb, Zn) were measured by atomic absorption spectrometry with hydride generation (HG-AAS; device 3100 HIAS PE 100) in the fraction <0.125 mm after extraction with the mixture HF/$HClO_4$ in ACME Analytical Laboratories Ltd. (Vancouver, Canada).

Results

Four plants, *Cirsium arvense* (creeping thistle), *E. arvense* (field horsetail), *O. acetosella* (wood sorrel) and *P. australis* (common reed), were investigated for the yeast species. All the plants examined grew at the same locality in the forest in Pernek. Seeds of the creeping thistle originated from the Sb-mining site Medzibrod, whereas the other plants grew naturally in the Sb-mining site in the forest in Pernek. The creeping thistle was used in the present study because it exhibited a high tolerance level to As and Sb (Jurkovič et al. 2010). Yeasts were isolated from both the leaves of plants and the soil in which the plants grew. In total, 65 yeast cultures, belonging to 11 ascomycetous and 5 basidiomycetous yeast species, were isolated. The ascomycetous species *Candida solani* and *Galactomyces candidus* together with the basidiomycetous species *Trichosporon porosum* were present in about half of the samples examined (Table 2). *Candida solani* and *T. porosum* were isolated from the soil samples associated with all plants, whereas *G. candidus* was found in the soil in which *P. australis* grew. The highest diversity of the yeast species was linked to the soil in which *P. australis* and *E. arvense* grew. Nine yeast species were isolated from it, whereas only two species were isolated from the soil in which *O. acetosella* grew (Table 2).

Four yeast species were exclusively found in the soil in which the plants grew. *Candida sake* was linked to *E. arvense*, *Candida odintsovae* and *Candida pseudolambica* to *P. australis* and *Cryptococcus musci* to *Cirsium arvense*. However, *Candida tsuchiyae* and the red-pigment-producing *Sporidiobolus metaroseus* were associated only with the leaves of *P. australis* and *O. acetosella*, respectively (Table 2).

All the yeast species isolated were tested for their tolerance to the five chemical elements: Zn, Cd, Sb, As and Si. The concentrations of chemical elements in the soil and their permissible limits are given in Table 3.

The highest tolerance of yeast cultures was found with Zn. *Cryptococcus flavescens* and *Cryptococcus musci* tolerated 200 mmol/L Zn and were the most resistant species, whereas *Aureobasidium pullulans*, *Candida odintsovae*, *Debaryomyces hansenii* and *S. metaroseus* exhibited the lowest tolerance (5 mmol/L).

The tolerance of yeast cultures to sodium silicate was tested in a liquid and on a solid medium. The majority of the yeast strains grew at concentrations of 2 and 10 mmol/L of sodium silicate in the liquid and solid medium, respectively (Table 4). Two species of the genus *Trichosporon*, *D. hansenii* and *Cryptococcus musci* exhibited the highest tolerance (15 mmol/L). The yeasts associated with the soil were more tolerant to Si than those found on the leaves (Table 4).

The greatest variations among the strains were found with cadmium. The tolerance levels ranged between 0.01 and 200 mmol/L. *D. hansenii* was the most sensitive species. The majority of the strains did not tolerate more than 0.5 mmol/L Cd. However, *Cryptococcus musci*, isolated from the soil, grew at a concentration of 200 mmol/L Cd (Table 4).

About half of yeasts tested tolerated arsenic concentration above 10 mmol/L. *Lindnera saturnus* and *Cryptococcus musci*, associated with the soil samples, exhibited the highest tolerance, whereas the strain of *Candida solani*, inhabiting the same environment, was the most sensitive culture. The latter species was also the most sensitive to antimony. The vast majority of the yeast cultures tested grew at a concentration of 50 mmol/L Sb (Table 4).

Discussion

In total, 16 yeast species belonging to 10 families were isolated from both the four plant species and soil. The basidiomycetous yeasts belonged to three genera, whereas the ascomycetous yeasts covered eight genera. *T. porosum* was the most frequently isolated species. This basidiomycetous yeast-like species, and *Trichosporon laibachii*, which was also found,

Table 2 Yeasts and yeast-like species isolated from the leaves and soils

Species	No.[a]	Family	*Phragmites australis*		*Equisetum arvense*		*Oxalis acetosella*		*Cirsium arvense*	
			Leaves	Soil	Leaves	Soil	Leaves	Soil	Leaves	Soil
Aureobasidium pullulans	2	Dothioraceae	x		x					
Barnettozyma californica	1	Wickerhamomycetaceae		x						
Candida odintsovae	1	Wickerhamomycetaceae		x						
Candida tsuchiyae	1	Metschnikowiaceae			x					
Candida sake	1	Saccharomycetaceae				x				
Candida solani	8	Wickerhamomycetaceae		x		x		x	x	x
Cryptococcus flavescens	2	Tremellaceae	x							x
Cryptococcus musci	2	Tremellaceae								x
Debaryomyces hansenii	2	Debaryomycetaceae				x				x
Geotrichum candidum	7	Dipodascaceae		x		x	x		x	
Meyerozyma guilliermondii	2	Debaryomycetaceae		x					x	
Lindnera saturnus	2	Wickerhamomycetaceae		x						x
Pichia fermentans	1	Pichiaceae		x						
Sporidiobolus metaroseus	1	Sporidiobolaceae					x			
Trichosporon laibachii	2	Trichosporonaceae		x		x				
Trichosporon porosum	9	Trichosporonaceae		x		x	x	x	x	x

[a] Number of samples positive for the species

have been reported as common inhabitants of both forest and grassland soils (Wuczkowski and Prillinger 2004; Mestre et al. 2011; Yurkov et al. 2012). Moreover, Middelhoven et al. (2001) noted the ability of *T. porosum* and related species to assimilate hemicelluloses as well as some typical plant compounds, and therefore posited their active role in the mineralization of plant material decaying.

G. candidus is also involved in the mineralization of plant material decaying, as well as in nitrification processes in soil (Wainwright and Falih 1996; Middelhoven et al. 2001; Waqas et al. 2014). This ascomycetous yeast-like species was isolated in small quantities from the soil of both flooded and non-flooded forests in Austria (Wuczkowski and Prillinger 2004). In our previous reports, *G. candidus* was isolated from the leaves of willow trees, but it was not linked to the forest soil in which the trees had grown (Sláviková and Vadkertiová 2000; Sláviková et al. 2007).

Table 3 Concentrations of chemical elements in the soil and permissible limits

Chemical element	Concentration of the element in the soil in Pernek (mg/kg)	Permissible limits in soil (mg/kg)[a]
As	236.8±23.50	29
Cd	0.3±0.04	0.8
Sb	396.5±42.40	Not determined
Zn	85.2±12.90	140

Values are means±SD, $n=3$

[a] According to Slovak Act No. 531/1994-540

Our present results show that *G. candidus* was associated with the leaves of the same plants as *T. porosum* and was also found in the soil. Both species are representatives of soil-inhabiting yeasts and probably enter leaf surfaces via dirt and debris accumulated from wind action (Yaghmour et al. 2012).

Candida solani was the second most abundant species. It was associated with both soil and plant samples. *Candida solani* is a fermentative yeast belonging to the family Wickerhamomycetaceae, together with *Barnettozyma californica*, *Candida odintsovae* and *L. saturnus*. These latter species were present only rarely and were related to the soil in which *P. australis* was grown. The species *L. saturnus* (synonym *Williopsis saturnus*) and *B. californica* (synonym *Williopsis californica*) are soil-related yeasts (Botha 2006). However, only small quantities of them have been associated with grasslands in Germany, forests in Austria and agricultural soil in Slovakia (Sláviková and Vadkertiová 2003a; Wuczkowski and Prillinger 2004; Yurkov et al. 2012). The abilities of *W. californica* to solubilize phosphates and *W. saturnus* to promote plant growth by the production of auxin have been reported (Wainwright and Falih 1996; Nassar et al. 2005).

The black yeast *A. pullulans* and species of the genera *Cryptococcus*, *Rhodotorula* and *Sporobolomyces* have been recognized as typical constituents of the yeast community on leaves, but their association with forest, grassland and

Table 4 The maximum tolerance of the individual yeast and yeast-like strains to chemical compounds

No. CCY	Yeast species	Origin	Plant species	Maximum tolerance levels (mmol/L)					
				Si–L[a]	Si–S[b]	Zn	Cd	As	Sb
027-001-129	*Aureobasidium pullulans*	Leaves	*Phragmites australis*	2	5	5	0.05	10	50
038-006-013	*Barnettozyma californica*	Soil	*Phragmites australis*	5	10	20	0.5	10	50
029-174-002	*Candida odintsovae*	Soil	*Phragmites australis*	2	10	5	0.5	0.2	50
029-189-003	*Candida pseudolambica*	Soil	*Phragmites australis*	2	10	10	0.1	15	50
026-015-003	*Candida sake*	Soil	*Equisetum arvense*	2	10	20	0.1	30	50
029-023-019	*Candida solani*	Soil	*Phragmites australis*	2	10	20	20	0.1	5
029-023-018	*C. solani*	Soil	*Cirsium arvense*	5	5	10	20	2	50
029-187-001	*Candida tsuchiyae*	Leaves	*Equisetum arvense*	2	10	20	0.1	10	50
017-027-004	*Cryptococcus flavescens*	Leaves	*Phragmites australis*	2	10	30	1	0.2	40
017-027-007	*C. flavescens*	Soil	*Cirsium arvense*	2	10	200	15	10	40
017-026-001	*Cryptococcus musci*	Soil	*Cirsium arvense*	2	15	200	200	60	50
041-006-022	*Debaryomyces hansenii*	Soil	*Cirsium arvense*	5	15	5	0.01	30	50
016-001-032	*Galactomyces candidum*	Leaves	*Oxalis acetosella*	5	10	30	0.5	30	50
038-007-004	*Lindnera saturnus*	Soil	*Phragmites australis*	5	10	20	0.5	60	50
039-023-012	*Meyerozyma guilliermondii*	Leaves	*Cirsium arvense*	2	10	20	0.1	40	50
019-006-018	*Sporidiobolus metaroseus*	Leaves	*Oxalis acetosella*	1	0.5	5	0.5	30	50
005-002-001	*Trichosporon laibachii*	Soil	*Phragmites australis*	10	15	30	0.1	30	50
030-018-001	*T. porosum*	Soil	*Phragmites australis*	2	15	20	0.5	40	50
030-018-004	*T. porosum*	Leaves	*Cirsium arvense*	2	10	20	0.5	30	50
030-18-003	*T. porosum*	Leaves	*Oxalis acetosella*	2	15	20	0.1	30	50

[a] Tolerance tested in a liquid medium

[b] Tolerance tested on a solid medium

agricultural soil samples has also been found (Sláviková and Vadkertiová 2003a, b; Fonseca and Inácio 2006; Yurkov et al. 2012). Glushakova and Chernov (2004) have noted that *Sp. roseus* (a synonym of *Sporobolomyces metaroseus*) and *Rhodotorula glutinis* are common inhabitants of *O. acetosella*. Our results show a low incidence of phylloplane yeast species on the leaves. *A. pullulans* and *Cryptococcus flavescens* (belonging to *Cyptococcus laurentii* group) were associated with *P. australis* and *E. arvense*, whereas the only *S. metaroseus* was isolated from *O. acetosella*. Although our previous study showed that *Cryptococcus laurentii* (a close relative of *Cryptococcus flavescens*) was the most abundant species in the forest soil (Sláviková and Vadkertiová 2000), our present results show only a rare occurrence of *Cryptococcus flavescens*.

The Debaryomycetaceae family was represented by *D. hansenii* and *M. guilliermondii*. Both species are commonly associated with soils and plants (Middelhoven 1997; Botha 2006). In our previous studies, they were not found in the forest soil, and only a few samples of different plant organs of fruit trees were positive for *M. guilliermondii* (Sláviková and Vadkertiová 2000; Vadkertiová et al. 2012). The present study shows that *M. guilliermondii* was isolated from both soil and leaf samples, whereas *D. hansenii* was associated only with the soil. Similar to *W. californica* and *L. saturnus*, the plant-growth-promoting properties of *M. guilliermondii* have been noted (Nakayan et al. 2013).

Various plants grow naturally in polluted areas and are resistant to excessive concentrations of chemical substances present in the soil. The concentrations of chemical substances in plants which inhabit contaminated sites are higher than those which occupy uncontaminated areas. Some of the plants are able to take enormous amounts of chemical compounds present in the environment, whereas others can take only small quantities. The uptake and the concentration of chemical compounds in plants depend on the type and plant species as well as the plant organs. The roots usually take much higher concentrations of chemical compounds than the shoots and leaves (Baroni et al. 2004; Vaculík et al. 2013). In the present work, four plant species were examined for the yeasts. *P. australis* is a common species of wet habitats and is also associated with abandoned Sb-mining areas. It was found to accumulate up to 688 mg/kg of As and around 1310 mg/kg of Zn into the roots whereas the uptake into the leaves and shoots was insignificant (4 and 68 mg/kg, respectively) (Stoltz and Greger 2002; Baroni et al. 2004). Massa et al. (2010) noted low level of Cd (0.1–0.9 mg/kg), As (0–4.3 mg/kg) and Zn (18.7–133.9 mg/kg) in autochthonous *Cirsium arvense* which grow in a multi-metal-contaminated area in Italy. Jurkovič

et al. (2010) reported differences in the tolerance of two species of the genus *Equisetum*, which are autochthonous for the Sb-mining area examined. *E. arvense* contained lower concentration of As in its shoots (10.1 mg/kg) than *Equisetum palustre* (45.8 mg/kg). Antosiewitz et al. (2008) noted that an arsenic-tolerant species *O. acetosella*, which grows in an area around an old arsenic/gold mine, was able to accumulate 44–69 mg/kg of Zn and 14–34 mg/kg of As into its shoots, respectively.

Arsenic and antimony are commonly associated in the environment. The toxicity of arsenic (V) is caused by its similarity to phosphorus (Tamaki and Frankenberger 1992). Individual species of bacteria and fungi are able to methylate arsenic and antimony present in the environment. Of the yeasts, *Cryptococcus humicola* (synonym *Apiotrichum humicola*) exhibits a high tolerance to arsenic and the ability to convert chromated copper arsenate to trimethylarsine (Bentley and Chasteen 2002). In our study, *Cryptococcus musci* exhibited a high tolerance to As(V). This species belongs to humicola clade and its physiological similarity to *Cryptococcus humicola* has been reported (Fonseca et al. 2011). Our results also show the similarity of both species in their high tolerance to arsenic. A strain of the black yeast *Exophiala sideris*, isolated from an arsenic mine, exhibited a tolerance up to 10 g/L of As (Seyedmousavi et al. 2011). The authors pointed out that such a remarkable tolerance is probably related to the high melanin content of the yeast culture. Our results show that also *A. pullulans*, another melanin-producing species, exhibits a relatively high tolerance to arsenic, but it did not belong to the most resistant yeasts of the species tested.

The concentrations of Sb in uncontaminated soils are low (about 0.2 mg/kg) whereas in contaminated areas can reach up to 6700 mg/kg (Baroni et al. 2000; Vaculík et al. 2013). Antimony potassium tartrate was used in our study of the tolerance of yeasts to Sb(V). Although Filella et al. (2002) reported Sb to be as toxic as As, our results show that the yeast strains were less tolerant to As than to Sb. These findings are in agreement with Sigel et al. (2010), who reported low toxicity of potassium antimony tartrate. Moreover, the presence of arsenic in the environment enhances the methylation of antimony as the enzymes involved in arsenic methylation also catalyze the methylation of antimony compounds (Bentley and Chasteen 2002; Hartmann et al. 2003).

Although zinc is a micronutrient required for normal growth and the metabolism and physiology of yeast cells, yeast cultures differ from each other considerably in their tolerance to this metal. The lowest inhibitory concentration for the yeast strains, isolated from the plant wastewater treatment in Spain, ranged between 28 and 32 mmol/L of Zn for *Geotrichum candidum* (synonym of *G. candidus*) and 16–18 mmol/L for *Trichosporon* sp. (Muñoz et al. 2012). Our results show similar results. *G. candidus* and *T. porosum* strains did not exceed tolerance levels of 30 and 20 mmol/L Zn, respectively. Another yeast-like species, *T. laibachii*, showed a similar tolerance like *G. candidus*. The carotenoid yeast *Sp. metaroseus*, associated with *O. acetosella*, tolerated a 50-fold higher concentration than the strain of the same species isolated in our previous study from a freshwater lake located in an unpolluted area (Vadkertiová and Sláviková 2006). It belonged to the most sensitive species of the yeasts tested.

Cadmium is a non-essential element with a detrimental effect on cells. This heavy metal reaches up to 2 mg/kg in Sb-mining sites (Jurkovič et al. 2010; Massa et al. 2010), a finding which is also confirmed by our data. Our results also show that the majority of the strains tested tolerated only a maximum of 0.5 mmol/L Cd. Balsalobre et al. (2003) noted a maximum tolerance level up to 1.5 mmol/L of Cd, whereas our previous findings (Vadkertiová and Sláviková 2006) reported a tolerance of *R. glutinis* up to 25 mmol/L. Although Balsalobre et al. (2003) found that *D. hansenii* tolerated 1.5 mmol/L of Cd, our results show a more than 100-fold lower tolerance. *Cryptococcus laurentii*, isolated from sewage sludge, tolerated 0.5 mmol/L (Balsalobre et al. 2003), and *Cyptococcus laurentii*, isolated from the forest soil, 0.05 mmol/L of Cd (Vadkertiová and Sláviková 2006). Deng et al. (2012) reported that the endophytic yeast strain *Cryptococcus* sp., associated with *Brassica* sp., was resistant up to 20 mmol/L of Cd. Our results show the strain *Cryptococcus flavescens* has a high tolerance to Cd but *Cryptococcus musci* has a 10× higher resistance. Breierová et al. (2002) found the species *Cyptococcus laurentii* was capable of absorbing Cd^{2+} ions by its extracellular polymers produced into the environment and Andreeva et al. (2014) have suggested that the accumulation of polyphosphates may be one of the factors responsible for the tolerance of *Cryptococcus humicola* to heavy metals.

Silicon is the second most abundant element present in soil. It participates in essential structures and plays functional roles in a wide variety of organisms. The solubility of the Si compound depends on its concentration. This compound is in the form of soluble silic acid at a concentration up to 2.0–2.3 mmol/L, and in higher concentrations polymerizes to silica. Plants take Si in the form of silicic acid by their roots, transport it to the shoot and polymerize and accumulate it in the cells to form silica-cuticle double layer and silica-cellulose double layer. Plants are able to accumulate much higher amounts of Si than that present in the environment (up to 18 mmol/L) (Yoshida 1965; Mitani et al. 2005). Silicon affects positively the growth of plants suffering from abiotic stress, supports the plant protection against phytopathogenic fungi and alleviates the inhibitory effects of toxic metals. Therefore, it has been commonly used for plant treatment in various studies. Silicon concentrations generally range between 0.2 and 1 mmol/L (Kidd et al. 2001; Nwugo and Huerta 2008),

although Vaculík et al. (2012) reported that the most suitable concentration, for the plants growing in the presence of Cd, is 35 mmol/L Si.

The influence of silicon compounds on the growth of fungi and yeasts has been only rarely studied up to now. Qin and Tian (2005) and Farahani et al. (2012) reported a synergistic effect of a yeast culture and silicon against the development of diseases caused by phytopathogenic fungi. However, a silicon substance alone, at a concentration of 0.6 %, restricted the growth of both fungi and yeasts. Brasser et al. (2006) found that a silicon compound did not influence the growth rate of *Saccharomyces cerevisiae* at concentrations up to 10 mmol/L; only an 11 % inhibition of growth was observed at 100 mmol/L of the silicate. Our results did not confirm such a high tolerance of the yeasts tested. In the liquid medium, the majority of strains tested grew at 2 mmol/L of Si compound, present in the form of silica acid. However, some of the yeast cultures tolerated also higher concentrations of Si (5 and 10 mmol/L) which was present in a form of polymerized substance. The yeast cultures exhibited higher tolerance to Si at the solid medium in which agar was used as a solidifying agent.

The contamination of polluted sites is usually caused by several chemical elements. Therefore, microbiota present in such an environment could exhibit a tolerance to more than one chemical element. However, elevated concentrations of chemical compounds affect the population size, diversity and activity (Gadd and Sayer 2000; Kavamura and Esposito 2010; Muñoz et al. 2012). Our results show that the soil and plants were more occupied by ascomycetous than basidiomycetous yeasts. The species most frequently isolated from both the soil and leaf samples were *T. porosum*, *G. candidus* and *Candida solani*, whereas *A. pullulans*, *Candida tsuchiyae* and *S. metaroseus* were isolated exclusively from the plant leaves. Only a few yeast species were found on the leaves of the plants. The yeast strains isolated from soil exhibited a high tolerance level to both As and Sb, present in elevated concentrations in the environment. Although the autochthonous plants contain significantly lower concentrations of chemical elements in their shoots and leaves than in the roots, the yeasts isolated from the leaves also exhibited a high tolerance level to both chemical elements. However, some of the yeasts, associated with both leaves and soil, tolerated only low concentrations of both metalloids, whereas other strains showed a high tolerance level to Cd which was present in this environment in insignificant concentrations. Moreover, the species *Candida solani* exhibited a higher tolerance to Cd than to As and *Cryptococcus musci*, a close relative to *Cryptococcus humicola*, was the species most tolerant to all the chemical elements tested, with the exception of Si. It grew in the presence of 200 mmol/L Zn, 200 mmol/L Cd, 60 mmol/L As and 50 mmol/L Sb, and therefore, it can be considered a multitolerant species. Some of the yeast species were tolerant to the individual chemical elements. The yeast-like species *T. laibachii* exhibited the highest tolerance to Si of all yeasts tested, and *Cryptococcus flavescens* and *L. saturnus* showed the same tolerance as *Cryptococcus musci* to Zn and As, respectively.

In conclusion, 16 yeast species, belonging to 11 genera, were isolated from the four plant species and the soil adjacent to these plants. Some of the species isolated by us have been reported to exhibit plant-growth-promoting characteristics, take part in the mineralization process in soil, accumulate toxic compounds into their cell structures and produce protective substances. However, knowledge of these topics is still limited. Our results also showed that the yeast strains tested exhibited a high tolerance level to chemical elements. To isolate resistant strains is one of the most important steps to find microorganisms with the capability to accumulate these elements (Roepke et al. 2011; Malik 2004). Therefore, diverse yeasts and yeast-like organisms isolated from plants and the soil adjacent to plants, as well as a high tolerance of yeast cultures to chemical elements tested, raise the possibility for further investigations of biosorption capability and beneficial properties of yeasts towards plants and soil originating from a contaminated area.

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