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Amphibians in metal-contaminated habitats

Wolfram Adlassnig [1], Stefan Sassmann [1], Anja Grawunder [2], Markus Puschenreiter [3], Amadea Horvath [1] & Marianne Koller-Peroutka [1]

[1] Universität Wien, Core Facility Cell Imaging and Ultrastructure Research, Althanstr. 14, 1090 Wien, Austria

[2] Friedrich-Schiller-Universität Jena, Institut für Geowissenschaften, Burgweg 11, 07749 Jena, Germany

[3] Universität für Bodenkultur, Institut für Bodenforschung, Konrad-Lorenz-Str. 24, 3430 Tulln an der Donau, Austria

Corresponding author: Wolfram Adlassnig, e-mail: wolfram.adlassnig@univie.ac.at

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Abstract. Mining for heavy metals usually has a strong impact on the environment, including the formation of spoil heaps, mine tailings and mine drainage, all of which are heavily contaminated. Heavy metals are generally regarded as toxic for most organisms, including amphibians, although the effects of heavy metals may be extremely complex and sometimes even positive. This study presents a survey of observations of amphibians in habitats severely contaminated by mining for heavy metals in Central and Eastern Europe. Rocky spoil heaps and sandy mine tailings were generally found to be devoid of amphibians. In moist habitats, especially streams, puddles and ponds fed by drainage water, however, six species of amphibians were observed, i.e., *Bombina variegata*, *Rana ridibunda*, *R. temporaria*, *Bufo viridis*, *Salamandra salamandra* and *S. atra*. All six species were found in habitats superficially similar to their typically preferred habitats, e.g., *Bombina variegata* in small puddles, *Salamandra salamandra* larvae in a swiftly running stream. Moderately increased concentrations of copper, arsenic, antimony and other elements and an acidic pH of soil and water did not keep amphibians away. Highly contaminated or extremely acidic water bodies are usually devoid of amphibians even though they may be present in the surroundings, suggesting that amphibians may be capable of recognising and avoiding extreme degrees of contamination. With the uptake of the pollutants being highly probable, some amphibians appear to possess a limited tolerance against heavy metals.

Key words. Amphibia, acidic mine drainage, heavy metals, metalloids, mining, mine tailings, pollution, *Bombina variegata*, *Bufo viridis*, *Rana temporaria, Rana ridibunda, Salamandra salamandra, Salamandra atra.*

Introduction

Many populations and species of amphibians are seriously endangered throughout the world (Brito 2008). Among others, the reasons include both environmental pollution and the loss of habitats. Mining for heavy metals affects huge areas by the deposition of solid mine waste and production of drainage water, both contaminated with elevated concentrations of toxic metals. Solid mine waste can be classified into rocky spoil heaps and mine tailings consisting of fine-grained material. Drainage water can emanate from spoil heaps, mine tailings or mine pits and may influence whole river systems downstream of the mine (Nordstrom 2011). Both spoil heaps and mine tailings are usually rich in toxic metals and metalloids and exhibit an acidic pH; these conditions lead to an inhibition of soil development, or to the degradation of existing soils. Thus, mining areas usually lack vegetation cover and are inhabited only by specially adapted communities of metal-resistant plants, especially Caryophyllaceae (carnation family), Brassicaceae (cabbage familiy), mosses and lichens. In addition to the toxic substrate, the open vegetation and the undeveloped soils lead to extreme ecological conditions including aridity, high surface temperatures, extreme temperature spans, low net productivity, etc.

These extreme conditions have significant consequences on animal abundance and diversity. Spoil heaps are generally avoided by millipodes and isopodes (Tajovsky 2001), and fish are negatively affected by mine drainage (Arnekleiv & Storset 1995). Little is known about the occurrence of amphibians in mining areas. At a first glance, the conditions for their occurrence seem to be extremely unfavourable. Data on how amphibians respond to heavy metal stress in general, however, are contradictory and often surprising. On the one hand, Blaustein et al. (2003) consider heavy metal pollution as one of the major causes of the worldwide decrease in amphibians. Several studies, including those by Blem & Blem (1991) or Dorchin & Shanas (2010), found significant negative effects on the levels of population and individuals. In *Rana esculenta*, e.g., exposition to heavy metals leads to inflammations and an increase in parasitic cysts (Fenoglio et al. 2011). On the other hand, some surprising responses of amphibians to heavy metal stress have been described (Freda 1991): In

Rana sphenocephala, exposition to cadmium showed both positive or negative effects, depending on other environmental parameters (James & Semlitsch 2011). Concentrations of up to 1 mg × l^{-1} of arsenic in the water only led to reduced motility in *Lithobates pipiens* (Chen et al. 2009).

The present study describes observations of amphibians in heavily contaminated mining areas in Central Europe. It aims to provide evidence that at least some species of amphibians exhibit a sufficient ecological plasticity to colonise habitats that, at first sight, could not be less favourable.

Materials and methods

From 2007 to 2013, habitats affected by heavy metal mining in Austria, Germany, Slovakia, Hungary and Romania were repeatedly visited. Only such spoil heaps, mine tailings and water bodies were considered where heavy metal contamination led to an unambiguous impoverishment of the vegetation or to the occurrence of metallophytes, i.e., plant species that indicate the presence of elevated concentrations of bio-available heavy metals in the soil (e.g., *Arabidopsis halleri*, *Noccea ceaerulescens*, *Asplenium cuneifolium*, *Armeria maritima* div. ssp., etc. (Ernst 1965, 1974).

All amphibians found in these habitats were recorded. Amphibians were tracked down at different times of the day and under different weather conditions, according to the habitat: aquatic habitats were studied in sunny or rainy conditions and in daylight. The same was true for putative habitats of *Bombina variegata*, which is preferably active under sunny conditions (Kapfberger 1982). Terrestrial habitats were preferably visited in rainy weather. The specimens were checked for visible deformations. The contamination of their habitat was quantified by analysis of soil and water. If not available from the literature, the total heavy metal content of soils was determined by digestion in boiling *aqua regia* and subsequent quantification by ICP-MS, ICP-OES, atomic absorption, or neutron activation analysis. Bio-available heavy metals were estimated by means of 1:10 extraction in 1 M NH_4NO_3; the same solution was used for the determination of soil pH. Reagents were obtained from Merck and Sigma-Aldrich in p. a. quality. Water samples were analysed for their pH and content of soluble heavy metals. Data on geological and historical backgrounds are taken from the literature.

Results

No amphibians were found in the majority of habitats affected by mining for heavy metals, including all of the 16 investigated rocky spoil heaps such as, e.g., Knappenberg (Edlach an der Rax, Lower Austria), where *Salamandra salamandra* is common in the surrounding forest, Špania Dolina (Horerhonie, Slovakia), Čechy (Podunajsko, Slovakia, the large spoil heaps in the Mansfeld Basin (Mansfelder Land, Saxony-Anhalt, Germany), and several others. Eight mining sites with amphibians present are described in more detail below.

In order to facilitate the understanding of the extent of pollution encountered at these sites, Table 1 shows selected guideline levels for toxic elements for soil and water.

Dos Valley / Apuseni Mountains

In the Apuseni Mountains around the city of Zlatna in Alba County, Romania, a large number of gold and silver deposits were exploited from Roman times to the post-communist period. Especially the gold mine Roşia Montană was of worldwide importance. In modern times, various other metals like copper and mercury were extracted as well. (Tóth et al. 2006)

In the Dos Valley (46°09.975' N, 23°10.706' E, 612 m a.s.l., next to Izvoru Ampoiului), the visible remains of past mining activity are found in the shape of slag, covered with yellow Hg-precipitations, a rocky spoil heap, and several small water bodies fed by wells draining the spoil heap. These waters contain floccose masses of amorphous manganese and iron oxides, which may lead to a gel-like consistence of the water. Due to the comparatively high pH of 7.4–8.4, the concentrations of dissolved metals in the water were rather low with < 1 µg × l^{-1} arsenic, 0.4–0.5 µg × l^{-1} copper and < 0.1 mg × l^{-1} manganese. The soil around these water bodies exhibited a circumneutral pH and contained 365 ± 456 mg × kg^{-1} arsenic (0.6% bio-available), 182 ± 201 mg × kg^{-1} (9.0% bio-available) chromium, 122 ± 15 mg × kg^{-1} (5.8% bio-available) copper, and 8,466 ± 1,975 mg × kg^{-1} (0.1% bio-available) manganese as well as traces of other heavy metals.

Several adult specimens of *Bombina variegata* were found in these water bodies (Figure 1) on 06 December

Table 1. Selected guidance levels for total concentrations of toxic elements and ions in water and soil. Exact guidance levels for soils depend on soil pH and texture. Sources: (1) WHO (2011); (2) Council of the European Union (1998); (3) WHO (2003); (4) Umweltbundesamt (2003); (5) Mergenthaler & Richner (2002); (6) Hein et al. (2013).

Parameter	Water [µg × l^{-1}]	Soil [mg × kg^{-1}]
Arsenic	10[1]	15–20[4]
Antimony	20[1]	30–150[5]
Cadmium	3[1]	1–3[5]
Chromium	50[1]	50–100[4]
Copper	2,000[1,2]	50–140[6]
Lead	10[1]	50–300[6]
Manganese	50[2]	–
Nickel	70[1]	30–75[6]
Zinc	3,000[3]	150–300[6]
Sulphate	250,000[2]	–
pH	6.5–9.5[2]	–

2010 during sunny weather. The animals did not appear to avoid contact with the manganese precipitations, but used them as perches or for hiding like they would use algal mats in a more natural habitat. As manganese and iron oxides adsorb high amounts of arsenic and other elements (Gopal et al. 2004), a higher degree of contamination can be expected in these precipitations than in the water.

Haneş / Apuseni Mountains

The mining site at Haneş (46°07.279' N, 23°07.463' E, 704 m a.s.l., next to Almaşu Mare) belongs to the same ore body as Dos Valley, but the site has a very different appearance. The main source of pollution here is acidic drainage (pH 3.0) from the mine adit. The drainage water forms a small stream devoid of any animal or plant life except Cyanobacteria, and exhibits colourful yellow to orange ferric precipitations rich in heavy metals. The water contains elevated concentrations of multiple elements, including lead, cadmium, copper and zinc (Tab. 2). This drainage flows into a tributary of the Ampoi River, which shows signs of heavy metal contamination for at least ten kilometres downstream. Furthermore, several large mine tailings, consisting of sandy material and partly overgrown by resistant trees (mainly *Populus tremula*), are found next to the mine entrance. No amphibians were observed both in the contaminated streams and on the mine tailings.

The facilities of the mine incorporate several concrete basins that used to be used for ore processing and are now filled with rainwater. Although increased concentrations of zinc and copper were noted here as well, the metal concentrations were considerably lower than in the drainage water, and the pH was circumneutral (Tab. 2). Here, several specimens of *Bombina variegata* were found on 12 June 2010 in sunny weather; at least two couples were mating (Figure 2). The soil in the surroundings is heavily contaminated as well, but it is questionable whether the animals are able to leave the basin at all due to the vertical walls. Stays outside the water may, however, be facilitated by construction wood and other objects forming ramps.

Aurul Plant / Baia Mare

In the surroundings of the city of Baia Mare in Maramureş, Romania, huge mine tailings have been heaped up from the extraction of gold from hydrothermal deposits (Grancea et al. 2003). Fine-grained solid mine waste was deposited on two large spoil heaps, whereas liquid waste was stored in a huge pond, the Aurul Reservoir. In the year 2000, a breach in the dike led to the liberation of some 200,000 m^3 of water contaminated with cyanide and heavy metals (Macklin et al. 2003), an event that is thought of by some as the worst environmental disaster in Europe since the Chernobyl accident (Cunningham 2005). Even though most of the mine spillage flew into the Săsar river, and on into the Tisza and Danube (Soldán et al. 2001), part of the material was less mobile and is still clearly evident in the surroundings of the Aurul plant. At present, the most contaminated areas comprise (1) mine tailings consisting of sand and coarse silt enclosing; (2) the Aurul Reservoir and a few minor water bodies in the centres of or below mine tailings; and (3) heavy metal-rich sand suspension leaking from a pipeline that cause increased heavy metal concentrations in the soil below. The concentrations of pollutants in selected water bodies, the mine tailing, and below the pipeline are shown in Tab. 3. The concentrations and bioavailabilities of pollutants do not differ between the mine tailing and the soil below the pipeline with the exception of cyanide, which occurs only at the mine tailing.

A visit on 15 June 2010 did not yield any amphibians in the Aurul Reservoir (47°38.732' N, 23°28.670' E, 168 m a.s.l.), which was devoid of all plant or animal life.

Figure 1. *Bombina variegata* perched amidst amorphous Fe/Mn-precipitations in a water body fed by mine drainage in the Dos Valley.

Figure 2. *Bombina variegata* mating in a basin formerly used for ore processing at Haneş.

Table 2. Multiple element contamination at Haneş (partly after Grawunder et al., in press). Soil samples were taken around the water bodies, not from the mine tailings (n.d. = not determined).

Parameter	Mine Drainage Water [μg × l^{-1}]	Water from Basins [μg × l^{-1}]	Soil [mg × kg^{-1}]
Arsenic	289 ± 300	3.3 ± 4.7	440 ± 268 (0.6% bio-available)
Cadmium	206 ± 107	Not detected	2.6 ± 1.3 (18.7% bio-available)
Cobalt	206 ± 126	0.1 ± 0.1	12.1 ± 5.4 (8.6% bio-available)
Copper	270 ± 106	1.6 ± 1.1	151 ± 32 (10.7% bio-available)
Lead	54.9 ± 19.3	0.3 ± 0.1	886 ± 650 (7.8% bio-available)
Manganese	190,170 ± 128,230	240 ± 310	2.374 ± 1.343 (0.8% bio-available)
Nickel	390 ± 166	Not detected	21.8 ± 5.9 (10.0% bio-available)
Zinc	107,533 ± 78,318	29.1 ± 1.2	1,472 ± 1,539 (23.1% bio-available)
Sulphate	3,786 ± 2,215	20.0 ± 24.9	n. d.
pH	2.9 – 3.0	7.6 – 7.8	3.9 – 6.3

Table 3. Contamination of the mine waste of the Aurul Plant, Baia Mare (n.d. = not determined).

Element	Pond A (water) [μg × l^{-1}]	Pond B (water) [μg × l^{-1}]	Pond C (water) [μg × l^{-1}]	Mine Tailing (soil) [mg × kg^{-1}]	Pipeline (soil) [mg × kg^{-1}]
Arsenic	12±10	1±2	22±14	478±309 (1.2% bio-available)	1,267±1,430 (0.6% bio-available)
Cadmium	Not detected	Not detected	Not detected	1.4±1.2 (29.4% bio-available)	7.6±5.4 (15.7% bio-available)
Copper	464±49	74±17	42±6	335±139 (10.0% bio-available)	349±174 (9.9% bio-available)
Lead	198±14	96±16	175±20	2,188±1,947 (9.0% bio-available)	2,352±1,067 (7.2% bio-available)
Manganese	28,400±392	53,000±2,470	1,726±108	417±291 (10.6% bio-available)	1,768±1,566 (3.9% bio-available)
Zinc	8,560±74	9,810±59	48±59	1,700±2,35 (23.3% bioavailable)	1,092±1,111 (23.1% bioavailable)
Cyanide	n.d.	n.d.	n.d.	0.1±0.2	n.d.
pH	3.8	4.9–5.4	2.9	2.8–3.6	2.6–6.3

However, several specimens of *Rana ridibunda* were observed in a pond (Pond A) next to the base of the heap (47°28.610' N, 23°28.764' E, 188 m a.s.l.) that is influenced by runoff water from the spoil heap. This observation was repeated on 26 July 2012 and 03–04 July 2013, each time during sunny weather. The vegetation surrounding the pond consisted of *Phragmites australis* (Poaceae) and *Equisetum fluvatile* (Equisetaceae), both of which are well known to be resistant to heavy metal contamination.

On 03 July 2013, more than 20 freshly metamorphosed specimens of *Bufo viridis* and about ten specimens of *Rana ridibunda*, one of them an albino, were observed in another pond (Pond B) in the centre of a mine tailing (47°37.973' N, 23°27.684' E, 185 m a.s.l.) in sunny weather. The vegetation was dominated by *P. australis* and *E. fluvatile* as well. A third pond in the centre of another mine tailing (Pond C) lacked amphibians during all three visits from 2010 to 2013. Remarkably, Pond C exhibited significantly lower concentrations of several heavy metals than either Pond A and B, but its pH was highly significantly lower (2.9 vs. 3.8 and 5.2). Data on the water chemistry of these ponds are shown in Tab. 3.

On 15 June 2010, several small ponds and puddles filled with rainwater were found on the side of a road paralleling

Table 4. Concentrations of selected metals at the Gyögyösoroszi zinc-lead mine. Data after Földessy et al. (2005) (n.d. = not determined).

Parameter	Acid mine drainage [mg × l^{-1}]	Neutralised mine drainage [mg × l^{-1}]	Lime sludge [mg × kg^{-1}]	Flotation tailings [mg × kg^{-1}]
Arsenic	236	n.d.	n.d.	325
Cadmium	n.d.	< 0.02	102	17
Lead	< 25.0	< 0.05	146	1,212
Zinc	17.5	17	29,000	2,898

the pipeline that contaminates the soil below. At least three of these were inhabited by one or two apparently healthy *Bombina variegata* each (47°38.966' N, 23°29.434' E, 177 m a.s.l.). This observation could not be repeated in 2012 and 2013 due to drier weather conditions,.

Gyögyösoroszi Mine

The Gyögyösoroszi zinc-lead mine is located in the Matra Mountains in northeastern Hungary (Heves province, 47°51.929' N, 19°52.376' E, 400 m a.s.l.). From 1952 through 1986, up to 150,000 t of low-grade mesothermal and epithermal ores were excavated here every year. Since 1979, the acid mine drainage produced by the mine is neutralised by the addition of lime. Thus, the surroundings of the mine are affected by (1) acid mine drainage before neutralisation, (2) mine drainage after neutralisation, (3) lime sludge as the by-product of neutralisation, and (4) flotation tailings. All these contain increased concentrations of heavy metals as shown in Tab. 4 (Földessy et al. 2005). Recently, extensive measures have been undertaken to immobilise or extract the metals by the company, Mecsek-Öko Zrt/Pécs (pers. comm. E. Mühlmann).

In spite of the contamination still present, numerous subadult specimens of *Bufo viridis* were observed on spoil heap material next to the entrance of the mine on 24 July 2012, in the late evening of a sunny day (Figure 3). The animals appeared on the excavated material at dusk; highly contaminated areas without vegetation were not avoided, but no animals were found on mud soaked with acid mine drainage.

Smolník River

At the mining villages of Smolník and Smolnícka Huta (Slovakian Ore Mountains, Košický Kraj, Slovakia), the Smolník River shows signs of heavy metal pollution, and

Figure 3. Subadult specimen of *Bufo viridis* perched on mine waste without vegetation at the Gyögyösoroszi mine.

the same is true for the alluvial soils at its banks. The pollution stems from different sources, including acid mine drainage, remnants of ore processing plants, and historic smelters next to the river. Soil contamination varies along the river; maximum values were 1,000 mg × kg^{-1} copper, 28 g × kg^{-1}arsenic, and 270 mg × kg^{-1} cadmium.

Concrete canals used to be used to channel mine drainage towards the river (48°44.08' N, 20°44.145' E, 561 m a.s.l.). Since the discontinuation of mining activities, the water in these canals flows only slowly. Lasting contamination by heavy metals is evident from the acidic pH of 2.3 and the formation of red ferric precipitates. Furthermore, domestic waste can be found in these canals. In these waters, two subadult specimens of *Bombina variegata* were found on 19 May 2007 in sunny weather. One of them exhibited strongly stunted fingers and toes, but was apparently swimming and diving without handicap, the other one appeared healthy.

Schlaining

Between the villages of Burgschlaining and Stadtschlaining in Burgenland, Austria, an antimony deposit carrying the antimony mineral stibnite, as well as arsenopyrite and pyrite, was exploited for 200 years (Pollak 1955). Due to the depletion of the deposit, mining was ended in 1999. Afterwards, most of the mine waste was covered with fresh soil and renaturated, but in 2008, elevated concentrations of arsenic and antimony were found in several habitats, which has had visible effects on soil and vegetation (Steinhauser et al. 2009).

A small stream (47°20'59.0" N, 16°16'00.5" E, 330 m a.s.l) is contaminated with sulphide, arsenic and especially antimony by an afflux arising from a spoil heap and entering the main stream 50 m below its source. The vegetation on the banks of the creek is significantly impoverished and dominated by mosses like *Pellia epiphylla* and *Plagiomnium undulatum*. In the water below the afflux, only a crustacean, *Gammarus* sp., and masses of filamentous, *Thiothrix nivea*-like bacteria were found (Steinhauser et al. 2009) whereas Trichoptera larvae, Ephemeroptera larvae, and water snails occurred exclusively upstream of the afflux; 250 m below the afflux, one larva of *Salamandra salamandra* was found in June 2008. Data on the water chemistry at this spot are shown in Tab. 5. The larva was captured for further observation, developed normally under aquarium conditions, and was released after metamorphosis. On 23 July 2013, another single larva was observed in exactly the same spot. Furthermore, a large number (> 50) of adult and subadult *Rana temporaria* and a single adult specimen of *Bombina variegata* were observed in and around the stream.

Table 5. Contamination of the forest stream at Sauerbrunn/Schlaining. Data after Steinhauser et al. (2009) (n.d. = not determined).

Parameter	Creek	Alluvial Soil
Arsenic	26 µg × l^{-1} (water) 4,500 mg × kg^{-1} (sediment)	810 mg × kg^{-1} (0.13% available)
Antimony	27 µg × l^{-1} (water) 6,380 mg × kg^{-1} (sediment)	36,400 mg × kg^{-1} (0.15% available)
Sulphide	3,880 mg × kg^{-1} (sediment)	n.d.
pH	7.8	6.2

Schwarzwand / Großarl Valley

The Großarl Valley near Salzburg (Austria) hosts a series of ore deposits dominated by pyrite and chalcopyrite, which were cut open in several places by the Großarl River and its tributaries (Derkmann 1976). Wherever feasible, these deposits were exploited from the 16th through the 19th century. The spoil heaps of these mines are still present today and host very different sets of heavy metal-tolerant plant species as a result of hydrological differences (Adlassnig et al. 2011).

The Schwarzwand (47°09'37.0" N, 13°13'12.1" E, 1,500–1,800 m a.s.l.) was the most productive of these deposits. Today, an area of more than 100.000 m^2 is contaminated with copper in concentrations of more than 10 g × kg^{-1}. Streams fed by circumneutral mine drainage continue to precipitate secondary copper minerals. Although the Schwarzwand is devoid of closed forest cover, the habitat is not impoverished in plant species but hosts a great diversity, especially of mosses and lichens (Adlassnig et al. 2013, Saukel 1980). On 11 June 2007, an adult specimen of *Salamandra atra* was observed in the larch woodland in the periphery of the contaminated area (47°09'37.3" N, 13°13'11.4" E, 1,450 m a.s.l.) during light rain. Here, 189 ± 111 mg × kg^{-1} copper were found in the soil, 6.7% of which are bio-available. This observation was repeated on 17 July 2013 in exactly the same spot during a thunderstorm. In the more heavily contaminated central part of the Schwarzwand, with up to 10.3 g × kg^{-1} copper, no amphibians were observed so far, although frequent visits during all seasons and all weather conditions have been taking place since 2001. So far, no *S. atra* were observed in the uncontaminated *Picea abies* forest in the surroundings of the Schwarzwand where understorey vegetation is virtually absent.

Tofernalm / Großarl Valley

At the Tofernalm (47°09'42.0" N, 13°10'57.6" E; 1,600 m a.s.l.), the same ore body as on the Schwarzwand was exploited until 1752. Today, only one small spoil heap remains where the soil contains up to 4,056 mg × kg^{-1} copper; other heavy metals are present only in trace concentrations (Sissolak 1984). The high copper concentration leads to an open vegetation of a few tolerant species of vascular plants, mosses and lichens, including *Silene rupestris* (Caryophyllaceae), *Saxifraga stellaris* (Saxifragaceae), *Pohlia* spp. (Bryaceeae), or *Thamniola vermicularis*

(Icmadophilaceae). Below the spoil heap, a little swamp has formed. Due to spillage from the spoil heap, the water contains 7–10 mg × l^{-1} copper (Adlassnig et al. 2011).

On 05 September 2012, two subadult specimens of *Rana temporaria* were observed in and around the swamp below the heap, which is the only body of stagnant water in the area (Figure 4). On 17 July 2013, two subadult and one adult specimen were found at the same place. Both observations took place during cloudy weather with occasional precipitation. *R. temporaria* is very common in the mountain pastures and woodlands of the Großarl Valley and was frequently observed in similar but uncontaminated habitats.

Discussion

Heavy metal tolerance of amphibians

Amphibians were observed at eight locations that were found to be heavily contaminated with heavy metals and metalloids. Other mining habitats lacked evidence of an occurrence of amphibians. Six different species could be identified, i.e., *Bombina variegata* at five locations, *Bufo viridis* and *Rana temporaria* at two, and *Rana ridibunda*, *Salamandra salamandra* and *S. atra* at one location each.

Although the heavy metal content of animal tissues was not determined in this study, it may be supposed that amphibians incorporate heavy metals from their habitat. Three different pathways of uptake are suggested: (1) Amphibians of all habitats described here are in constant contact with heavy metal-rich soil or water. The glandular skin cannot be expected to form a perfect barrier against the heavy metals, especially if it is irritated by an extremely acidic pH of soil or water. For *Rana ridibunda*, the skin was found to be permeable to copper (Papadimitriou & Loumbourdis 2003); in *Anaxyrus americanus*, prolonged contact with cadmium-contaminated soil led to reduced fitness (James et al. 2004). (2) Spiders, ants and other potential prey animals are common in most contaminated habitats; some contain increased amounts of heavy metals (M. Weidinger, unpublished data). Furthermore, contaminated soil particles may be ingested during prey capture, as happens frequently in amphibians, e.g., in *Ambystoma* (Allmeling et al. 2012). Bulog et al. (2002) found that *Proteus anguinus* may absorb and accumulate significant amounts of heavy metals from the sediments of its habitat even if the water itself is clean. (3) In some of the studied habitats, including Haneş, Baia Mare, Gyögyösoroszi and Tofernalm, wind can easily raise contaminated dust, as the mine waste partly consists of fine-grained material and is not covered by vegetation. At least in humans, incorporation of heavy metals after inhalation may be relevant (Pruvot et al. 2006).

Some observations indicate that heavy metals have a negative effect on amphibians. For *Bufo viridis*, heavy metals like copper and lead have been shown to increase the frequency of morphological malformations at concentrations that were far lower than those measured in this study

Figure 4. Subadult specimen of *Rana temporaria* at Tofernalm on the copper-tolerant moss *Pohlia* sp. (Bryaceae) and the copper-tolerant saxifrage *Saxifraga stellaris* (Saxifragaceae).

(Dorchin & Shanas 2010; see also Blem & Blem 1991 and Fenoglio et al. 2011). In the investigated mining habitats, however, only one subadult specimen of *Bombina variegata* exhibited deformed hands and feet, and one *Rana ridibunda* was an albino; all other animals were inconspicuous. These results are in accordance with other reports on an astonishing high resistance to heavy metals in some amphibians. *Lithobates pipiens*, e.g., tolerates extremely high concentrations of arsenic (Chen et al. 2009), and similar reports have been provided by Freda (1991) and James & Semlitsch (2004). Dobrovolj et al. (2003) found metallothioneins that are capable of detoxifying copper, zinc and cadmium in two species of Caudata. It can be expected that similar mechanisms are present in the observed species as well.

Selection of habitats

Even though some amphibians occurred in contaminated mining habitats, it is obvious that not all mining sites are suitable for colonisation. No amphibians were found on dry and rocky spoil heaps or dry and sandy mine tailings. Wet habitats, on the other hand, were occasionally inhabited. At Haneş, circumneutral and moderately contaminated water bodies were preferred over acidic and heavily contaminated ones by *B. variegata*. At Aurul Plant, *Bufo viridis*, *Rana ridibunda* and *Bombina variegata* were found in heavily contaminated yet circumneutral water bodies but appeared to be avoiding highly acidic ones. This matches nicely an observation made by Grosse & Wawrzyniak (2013) that *Lissotriton vulgaris*, *L. helveticus*, *Triturus cristatus* and *Ichthyosaura alpestris* are able to colonise a pond on serpentine bedrock where slightly elevated concentrations of nickel and chromium can be expected.

In most cases, the contaminated sites had a very similar appearance to the typical habitat of the respective species. In Smolník, at Aurul Plant, Haneş and Dos Valley, *Bombina variegata* was found in very small water bodies like puddles or small artificial basins, which all came very close to the preferred habitat of this species (Diesener et al. 1985, Gollmann & Gollmann 2012). In Schlaining, the forest stream is a typical summer habitat for adult *B. variegata* (Seidel 1988). It is well known that *B. variegata* tolerates contaminated waters and other extreme conditions if no better suitable habitats are available (Feldmann & Sell 1981, Kapfberger 1982). *Rana ridibunda* was found in much larger ponds with a dense vegetation of reeds, which were also very similar to the typical habitat (Nöllert & Nöllert 1992). *R. temporaria* was not restricted to the heavy metal-contaminated Tofernalm, but occurred frequently in the subalpine meadows of the Großarl Valley; it may have been driven to the puddles at the base of the spoil heap by the lack of other, uncontaminated bodies of stagnant water in the surroundings. Larvae of *Salamandra salamandra* are frequently found in streamlets originating from the openings of mining adits and caves (Thiesmeier 2004), as was the case in Schlaining. The concentrations of arsenic and antimony in this water exceeded the guideline of the WHO (2011); however, considerably higher concentrations of arsenic had no serious effects on the larvae of *Lithobates pipiens* (Chen et al. 2009). *Salamandra atra* can be expected to be common in a light, subalpine woodland like the Schwarzwand. The moist microclimate (Saukel 1980) and the large number of moss cushions providing moist shelters may additionally attract *S. atra*.

It can be concluded that some species of amphibians are able to colonise habitats affected by mining, if they are moist and superficially similar to their preferred habitat. Enhanced concentrations of metals and an acidic pH are tolerated to an astonishing extent both in the soil and in the water. Among the moist mining sites, only such habitats were completely avoided that exhibited extreme degrees of contamination or an extremely acidic pH and are virtually devoid of higher organisms. The uptake of heavy metals from soil and water and the effects of lasting exposition, however, still need to be clarified.

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