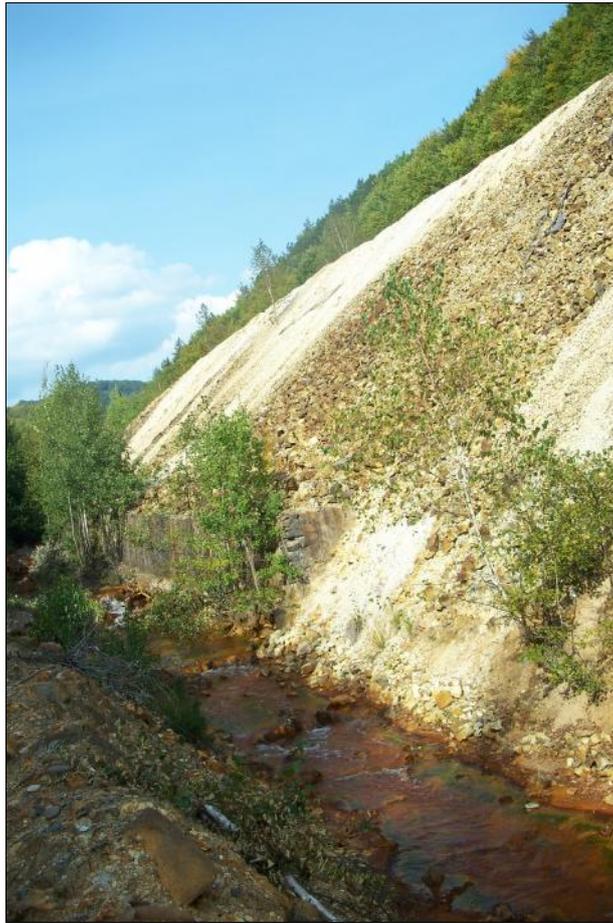


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**Environmental impact of gold and polymetallic mining in the
Nistru-Băița, North Romania**

– Ph.D. Thesis abstract –



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Contents

CHAPTER 1. INTRODUCTION	4
CHAPTER 2. THE NEOGENE MAGMATISM AND ASSOCIATED ORE DEPOSITS IN THE BAIA MARE AREA	6
CHAPTER 3. THE NISTRU AND BĂIȚA ORE DEPOSITS	7
CHAPTER 4. SAMPLES, SAMPLE PREPARATION AND ANALYTICAL METHODS ..	8
CHAPTER 5. THE GEOCHEMISTRY OF THE ENVIRONMENT AFFECTED BY ORE MINING – AN OVERVIEW OF THE THEORETICAL FACTORS AND PROCESSES	9
CHAPTER 6. THE MINERALOGY AND GEOCHEMISTRY OF MINING-RELATED ZONES IN THE NISTRU AREA	10
6.1. The pH, EC, and the redox potential measurements along the Nistru creek.....	11
6.2. The mineralogy of the Nistru Creek sediment	11
6.3. The water geochemistry in the Nistru Creek.....	12
CHAPTER 7. THE MINERALOGY AND GEOCHEMISTRY OF MINING RELATED ZONES IN THE VALEA ROȘIE-BĂIȚA VALLEYS AREA	13
7.1. The pH, EC, Eh measurements along the Valea Roșie and Băița creeks.....	13
7.2. Mineralogy of the layered precipitates from the Valea Roșie and Băița river beds as inferred from thin sections and XRPD	14
7.3. Chemistry of the Valea Roșie and Băița precipitates as inferred from EMPA data	16
7.4. SEM-EDX study of the Valea Roșie precipitates	17
7.5. Mössbauer type spectroscopy of the Valea Roșie precipitates	18
7.6. PGAA of the bulk samples of Valea Roșie precipitate	20
7.7. ICP-AES of the inflowing AMD and related creek sections.....	20
7.8. TEM study of the AMD precipitate	22
CHAPTER 8. THE MINING-RELATED ENVIRONMENT IN THE NISTRU-VALEA ROȘIE-BĂIȚA AREA: A BIOLOGICAL APPROACH	24
8.1. Toxicology test with ice lettuce	24
8.2. Toxicology tests with duckweed	25
8.3. Biodiversity studies in the Valea Roșie and Băița rivers	26
8.4. Microbiology of the Valea Roșie river affected by AMD.....	28
CHAPTER 9. DISCUSSIONS	29
CHAPTER 10. CONCLUSIONS	35
REFERENCES	37

Keywords: Acid mine drainage, Neogene ore deposits, environmental pollution, ecotoxicology, Nistru, Băița, Valea Roșie, Romania.

CHAPTER 1. INTRODUCTION

It is known that various thermodynamic processes interacting synergistic and antagonistic lead to the element cycling in the universe. Along the history, the man, driven by his curiosity, developed a complex image in order to understand the processes that created and kept this balance of the element cycling. He learned how to use for his own advantage these processes and the elements and how to create new phases and chemicals, creations that could exist for thousands of years. However to build something new or to transform something existing is also a great responsibility. He has to obey the rules of nature, and respect the harmony that was undisturbed prior to the appearance of mankind.

The humans fail to fulfil the duty towards nature generally results in environmental pollution. Within it, the sulphide oxidation related to acid mine drainages (AMD) pollutes the environment at numerous places around the world. Beside the low pH (1–4) and high electric conductivity, an extremely increased concentration of Fe^{3+} and SO_4^{2-} and other potentially environmentally toxic elements characterise this ore/coal mining connected acid waters (Schwertmann, 1985; Bigham et al., 1996; Regenspurg et al., 2004; Accornero et al., 2005; Blowes et al., 2005; Nagy et al., 2006; Nagy-Korodi et al., 2009, 2011).

The Nistru-Valea Roşie-Băiţa area (NBA) is located in the Gutâi Mts., within the Maramureş county (NW Romania), well known for the base metals (Cu, Pb, Zn) and gold ore mining and processing (Fig. 1.1a). Contrary to other ore deposits in Romania, here the mining activity is still ongoing. The NBA represents an environmental „hot spot” due to intense sulphide alteration, acid mine drainage and significant pollution with heavy metals related to old and actual mining (see also Cordoş et al., 2003; Macklin et al., 2003; Osán et al., 2004; Rauta et al., 1995; Sárkány-Kiss and Sîrbu, 1999). These processes are mostly evident in the mining spoils in the surroundings of the Nistru village and in the alluvial deposits of the Nistru (NR), Valea Roşie (VR) and Băiţa (BR) rivers (Fig. 1.1.b).

Some time ago, the hydrated lime [$\text{Ca}(\text{OH})_2$] was used for neutralizing the acid waters of the Valea Roşie and Băiţa rivers, but due to an inadequate use and some technical problems this procedure was stopped. Actually, without any previous treatment, the yellowish-brown, Fe-rich waters of the Băiţa mine flow directly into the Valea Roşie Creek and then they are transported into the Băiţa River and the Someş River and further on into Tisza River. The

latter recorded several pollution events during the last decade (Macklin et al., 2003; Osán et al., 2004).

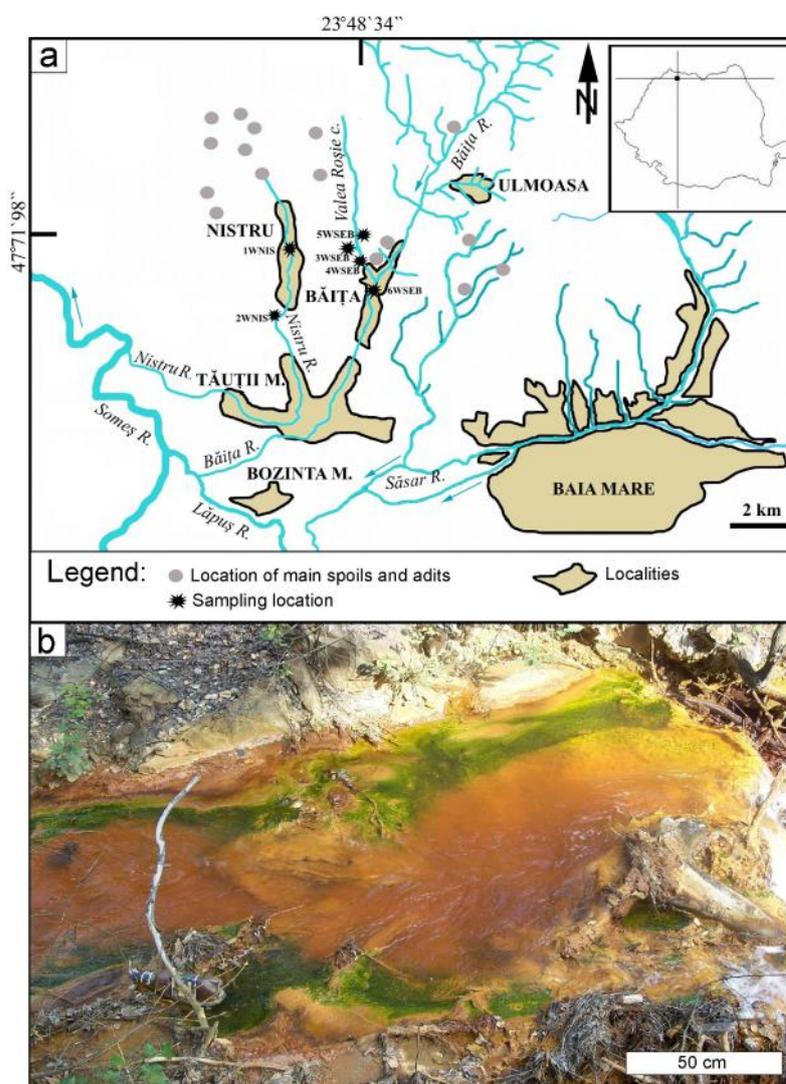


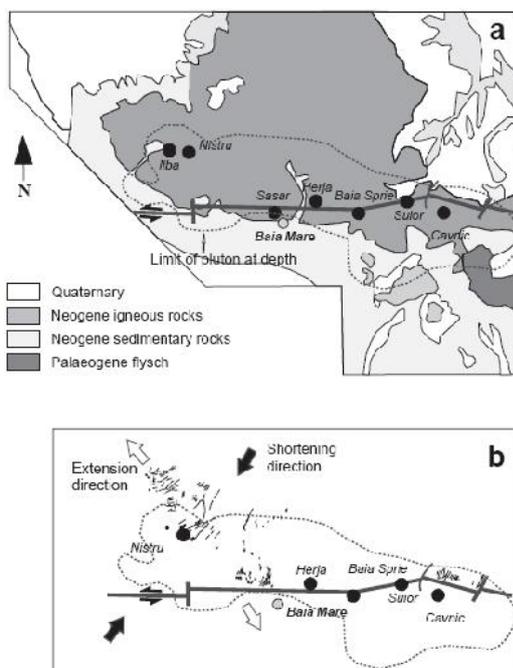
Fig. 1.1 a) The Nistru-Băița mining area, with the sampling location (black stars); b) Appearance of the Valea Roșie riverbed downstream the water treatment station, with green algae coating the alluvial sediments (4WSEB measurement point). The insert in upper right (b) represents the position of the studied area within the Romanian territory.

The Gutâi Mts. are located in the north-western end of the Eastern Carpathians, an area consisting of a pre-Neogene basement, Neogene sediments and magmatic rocks (Borcoș et al., 1980, 1981, 1984; Săndulescu, 1984). The latter belong to the Carpathian Neogene-Quaternary volcanic chain (Seghedi et al., 2004a,b) and are dominated by intermediate to rare acidic lava flows, lava breccias, pyroclastics and some subvolcanic bodies. The associated Pb-Zn-Cu-Au-Ag hydrothermal ore deposits were subject of exploitation since antiquity (Lang,

1979). The long lasting mining activity in area resulted in numerous spoils spread over the whole region, in particular around the galleries (Fig. 1.1a).

CHAPTER 2. THE NEOGENE MAGMATISM AND ASSOCIATED ORE DEPOSITS IN THE BAIJA MARE AREA

Within Romanian territory, the latest magmatic event is the Cenozoic one. It comprises calc-alkaline to alkaline magmatism, especially volcanism. This magmatic event lasted since Neogene (Miocene) till Quaternary (Pliocene) times and can be traced from SE Austria, to Western Carpathians, Eastern Carpathians, as well as in the Apuseni Mts. (Seghedi et al., 2004a,b). At larger scale, it “was controlled by the Cretaceous to Neogene subduction and collision of the Africa with Eurasia” (Seghedi & Downes, 2011). The Baia Mare district in NW Romania (Fig. 2.1, 2.2) represents a complex segment of the volcanic chain in the Eastern Carpathians. The calc-alkaline volcanism and associated intrusives (mainly subvolcanic) ranges in age from 13.4 to 6.9 Ma and are partly contemporaneous with the deposition of the Upper Badenian to Pannonian sedimentary rocks (Pécskay et al., 1995, 1997, 2006; Seghedi et al., 2004a). A high number of ore deposits are related to this magmatic



event. Several studies (Bailly et al., 1998; Grancea et al., 2002) proposed a five-stage evolution of mineralization in the Baia Mare area: (1) an initial Fe precipitation stage; (2) Cu-(Bi)-W stage; (3) Pb-Zn stage; (4) Sb stage, and (5) Au-Ag stage. Alteration assemblages include quartz–illite/sericite–adularia for Au–Ag systems and quartz–calcite–rhodochrosite–rhodonite for Pb-Zn mineralization.

Fig. 2.1 a) Structural map of central sectors of the Baia Mare district, including distribution of mineralization; b) Interpretation of vein systems of the Baia Mare district (from Neubauer et al., 2005).

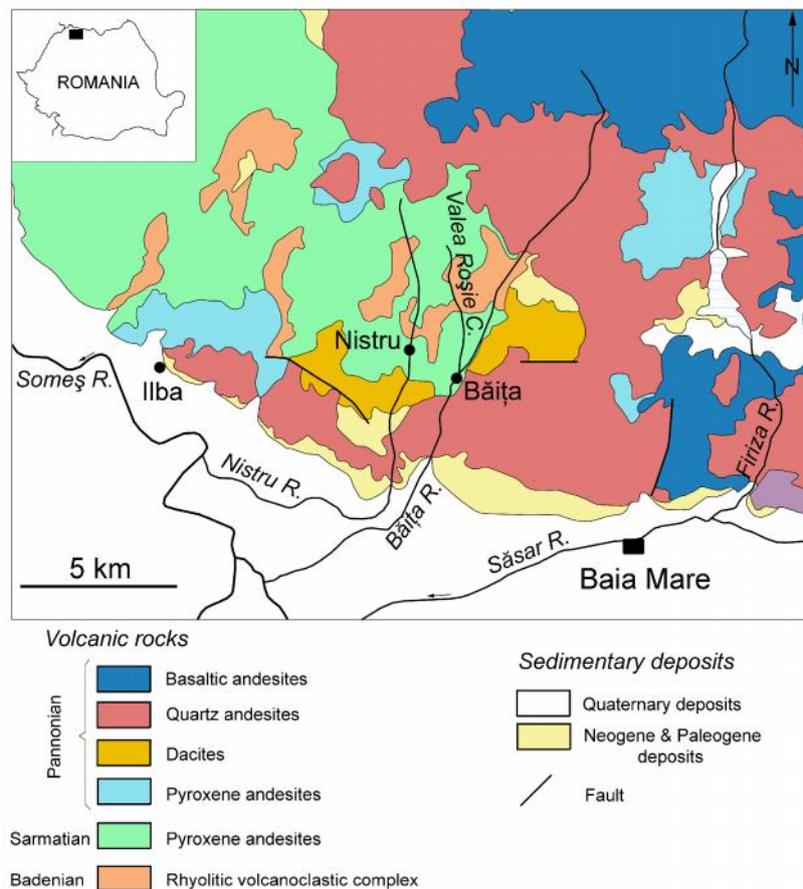


Fig. 2.2 Simplified geological map of the southern part of the Gutâi Mts. based on Jurje et al. (2012). The insert in upper left represents the position of the map within the Romanian territory.

CHAPTER 3. THE NISTRU AND BĂIȚA ORE DEPOSITS

The rich Pb-Zn-Cu-Au-Ag hydrothermal mineralizations and ore deposits associated to the Neogene volcanics in the Gutâi Mts. were subject of exploitation since antiquity (Lang, 1979). Among them, one important is the Nistru–Băița deposit, located at the convergence of the Cu-Pb-Zn veins associated to the Sarmatian pyroxene andesites and the Au-Ag veins connected to the Pannonian quartz andesites (Borcoș et al., 1972a,b, 1974a,b; Kovacs et al., 1997a,b; Damian, 1999a,b, 2003; Kovacs, 2001; Kovacs and Fülöp, 2003). The Băița mine is still subject of an ongoing activity and release significant amount of polluted water into the river system nearby.

CHAPTER 4. SAMPLES, SAMPLE PREPARATION AND ANALYTICAL METHODS

Basically, the study includes two different approaches, a geological and a biological one. Through the separate sets of methods it was possible to highlight the features of both, the geological and biological impacts, caused by the acid mine drainages at Nistru-Valea Roşie-Băiţa river system.

A total of 2719 samples were used during the study. The detailed list of the analytical methods is displayed in Table 4.1; 423 samples were used for physical-chemical (including mineralogical) analyses, and 2296 samples for biological investigation.

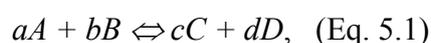
Table 4.1 Method used in the study and the number of samples studied with each method. The sample quantity involved, depends on each specific method standards.

Crt.№	Analytical method	Number of samples studied
1.	Optical microscopy in polarized light (OM) (thin sections)	16
2.	pH measurement	50
3.	Redox potential (Eh) measurement	50
4.	Electric conductivity (EC) measurement	50
5.	X-Ray Powder Diffraction (XRPD)	120
6.	Scanning electron microscopy (SEM)	30
7.	Electron microprobe analyses (EMPA)	30
8.	Qualitative EDX measurements (associated to SEM)	60
9.	Mössbauer spectroscopy (MSP)	3
10.	Prompt Gamma Activation Analyses (PGAA)	1
11.	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	13 (water samples)
12.	Transmission electron microscopy (TEM)	3
13.	Toxicity test with ice-lettuce (seeds)	1300
14.	Toxicity test with duckweed (plantlet)	975
15.	Biodiversity test	20
16.	Denaturing gradient gel electrophoresis (DGGE) - microbiota	1
TOTAL		2719

The biological experiments included germination test with ice-lettuce (*Lactuca sativa* L. var. *Great Lakes 118*), growth tests with duckweed (*Lemna minor*, L.), biodiversity studies of the Valea Roşie and Băiţa shores and microbiology studies – the microbiota of Valea Roşie.

CHAPTER 5. THE GEOCHEMISTRY OF THE ENVIRONMENT AFFECTED BY ORE MINING – AN OVERVIEW OF THE THEORETICAL FACTORS AND PROCESSES

The study of chemical equilibrium is based on the law of mass action, which states that the rate of a chemical reaction is proportional to the active masses of the participating substances (Hem, 1961; Fetter, 1994). This principle was proposed by Guldberg and Waage in the mid-19th century (Hem, 1985). A basic hypothetical reaction between substances A and B to produce products C and D, in a closed system, can be written (Eq. 5.1):



where lower case letters represent multiples required to balance the equation. The rates of forward and reverse reaction, according to the mass law, will be, respectively Eq. 5.2 (Hem, 1985),

$$R_1 = k_1' [A]^a [B]^b, \text{ (Eq. 5.2)}$$

and

$$R_2 = k_2' [C]^c [D]^d, \text{ (Eq. 5.3.)}$$

bracketed terms represent active masses. The quantities k_1' and k_2' are proportionality constants for the forward and reverse reactions (Hem, 1985). When $R_1=R_2$, the system will be in a state of dynamic equilibrium and no change in active concentrations (represented by the bracketed quantities) will occur. This leads to the expression Eq. 5.4 (Hem, 1985):

$$[C]^c [D]^d / [A]^a [B]^b = k_1' / k_2' = K, \text{ (Eq. 5.4)}$$

The quantity K is referred to as the equilibrium constant (Eq. 5.4). It has a characteristic value for any given set of reactants and products, and many experimentally determined values are available in published chemical literature (Hem, 1985). The value of the equilibrium constant is influenced by temperature and pressure. Standard thermodynamic conditions (25 °C and 1 atmosphere of pressure) are usually specified, but K values for many reactions have been determined at other temperatures, or over a temperature range (Hem, 1985).

Acid mine drainage and mine spoils contaminate surface water bodies, groundwater, soils, and sediments at innumerable locations around the world. The principal sulphide minerals in mine wastes are pyrite, pyrrhotite, and chalcopyrite, but others are susceptible to

oxidation, releasing elements such as As, Al, Cd, Co, Cu, Hg, Ni, Zn, and Pb to the water flowing through the mine waste.

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The theoretical factors and processes could be classified and studied as the following:

5.1. The geochemical processes of the spoil weathering

5.1.1. Basic terminology

5.1.2. The characteristics of the spoils

The location and the construction of the spoils

The geochemical fluxes of the waste rock spoils

The geochemical fluxes of the mill tailings

5.1.3. The weathering processes of the spoils and mill tailings

Waste rock spoils and mill tailings and their control factors

5.1.4. The alteration of the Fe, Cu, Pb and Zn sulphides (weathering processes of: pyrite, marcasite, pyrrhotite, chalcopyrite, sphalerite, galena, and the secondary minerals formed through the weathering of the sulphides: goethite, lepidocrocite, akaganéite, amorphous ferrihydroxide, ferrihydrite, jarosite minerals, schwertmannite, other Fe-oxy-hydroxide-sulphates).

CHAPTER 6. THE MINERALOGY AND GEOCHEMISTRY OF MINING-RELATED ZONES IN THE NISTRU AREA

As a consequence of hundreds of years of ore-mining, in the Nistru area there are numerous old and recent spoils. Their material might contaminate the surface water bodies, the groundwater, the soils, and river sediments. As mentioned in the “Introduction” chapter, our study was focused on the largest spoil body, i.e. the Nistru Old Spoil (NOS), as well as on the characteristics of the Nistru river, flowing nearby. The latter included not only the geochemistry of the water observed in a 3 years period of time but also the sediments from the river bed.

The material constituting the NOS has a heterogeneous granulometry, ranging from very fine (lutitic) material to larger boulders of rocks. The later are heavily weathered fragments of Sarmatian pyroxene andesites – the host rock of the ore. Optical microscopy on thin sections shows, besides relics of quartz, feldspars transformed into clay minerals and pyroxenes and amphiboles replaced by chlorite±opaque minerals. Pyrite and Fe-oxides-hydroxides and rare ilmenite are visible as well.

6.1. The pH, EC, and the redox potential measurements along the Nistru creek

The physico-chemical parameters measured in the Nistru creek in 2009 and 2010 show a circum-neutral pH (6.38 to 7.19), a slightly variable but low electric conductivity (between 215 and 409 $\mu\text{S}/\text{cm}$, with 290 $\mu\text{S}/\text{cm}$ average value), and a slightly variable redox potential (from 172 to 242 mV and an average value of 210 mV).

6.2. The mineralogy of the Nistru Creek sediment

The XRPD reveals the predominance of quartz and the presence of jarosites (K- and hydronium-jarosite), goethite and some albite (table 6.1) in the bulk sample 1SPN and in the coarser fraction (≤ 100 to > 63 μm). The finer fractions, i.e. ≤ 63 to > 20 μm and ≤ 20 μm , respectively, kaolinite and schwertmannite were identified as well. The decreasing order of frequency of minerals in the 1SPN sample is: quartz, hydronium- and K-jarosites, goethite, albite, schwertmannite and kaolinite.

The samples collected from the Nistru creek alluvial sediments are mainly composed of minerals of the host rock, such as quartz, muscovite and feldspar-albite. Ore minerals phases such as pyrite, sphalerite, galenite, antimonite or wurtzite were not identified by XRPD.

The absence of diffraction lines which could be assigned to secondary minerals such as ferrihydrite, schwertmannite, akaganéite is due either to the local physico-chemical parameters which do not favour their formation or to their low amount/low crystallinity.

The presence of primary minerals such as fresh feldspar or/and muscovite among the detrital phases shows short distance of transport and the immaturity of sediment (the feldspar and muscovite did not break down to clay minerals).

Table 6.1 Mineralogical composition of the Nistru Old Spoil samples as determined by XRPD. Abbreviations: Jar – jarosite; Schw – schwertmannite; Gt – goethite; Qtz – quartz, Ab – albite; Kln – kaolinite.

Sample description	Sample no.	Identified mineral phases					
		Jar	Schw	Gt	Qtz	Ab	Kln
Bulk sample	1SPN1107a	+			+		
	1SPN1107b	+		+	+		
≤100 to >63 μm Fraction	1SPN1107a63				+	+	
	1SPN1107b63	+		+	+		
≤63 to >20 μm Fraction	1SPN1107a20		+		+		+
	1SPN1107b20				+	+	
≤20 μm Fraction	1SPN1107aF	+			+		
	1SPN1107bF				+	+	
Samples treated with ammonium oxalate	1SPNaAO	+			+		
	1SPNbAO	+			+		

6.3. The water geochemistry in the Nistru Creek

The chemical and physical data, obtained by ICP-AES for the Nistru Creek water, show that the alkalis (Na^+ and K^+) values were lower in 2010 (down to 4.25 mmole/l and 1.69 mmole/l, respectively) compared with 2009 (12.50 mmole/l and 4.27 mmole/l, respectively). This holds true also for Ca^{2+} (from 53.9 to 24.3 mmole/l) and Mg^{2+} (from 12.8 to 5.29 mmole/l), as well as for anions like Cl, SO_4^{2-} . The total dissolved solids decreased, from 314 to 169 mg/l. Only Mn^{2+} and some anions (HCO_3^- and PO_4^{3-}) show a slight increase.

By contrast, the heavy metal concentration in the Nistru waters, in particular for Zn, Co, Al, Cu and Cd increased significantly. For example, Zn had 171 $\mu\text{g/l}$ in 2009 and increased up to 675 $\mu\text{g/l}$ in 2010. Co, from below 1 $\mu\text{g/l}$ in 2009, showed 5.69 $\mu\text{g/l}$ in 2010. The most spectacular increase regards Al, from 33.1 $\mu\text{g/l}$ in 2009 up to 923 $\mu\text{g/l}$ in 2010 and Cu, from 6.93 $\mu\text{g/l}$ to 84.7 $\mu\text{g/l}$.

CHAPTER 7. THE MINERALOGY AND GEOCHEMISTRY OF MINING RELATED ZONES IN THE VALEA ROȘIE-BĂIȚA VALLEYS AREA

As mentioned in the Samples and methods chapter, besides the spoil material, alluvial sediments and water of the Nistru creek, similar materials (i.e. alluvial sediments/layered precipitate and water) from the nearby Băița and Valea Roșie (its tributary) creeks, were included in the study. The complex analytical investigation, by X-Ray powder diffraction, electron microprobe, ⁵⁷Fe Mössbauer spectroscopy, inductively coupled plasma-atomic emission spectroscopy, ion chromatography and transmission electron microscopy was performed in order to outline the speciation of the main cations (Fe²⁺ and Fe³⁺) and anions (SO₄²⁻), to identify the secondary minerals, possibly related to the mining activity in the area. The physical parameters of the waters were also investigated.

7.1. The pH, EC, Eh measurements along the Valea Roșie and Băița creeks

The chemistry of the Valea Roșie creek waters shows the influence of the acid mine drainage inflowing at Valea Tirsă. The approximately neutral value (pH of 6 at the springs – sample 3WSEB10) decreases down to pH of 3.14, with a simultaneously 36 times increased electric conductivity (from 77 to 2790 μS/cm) (table 7.1). Downstream to the confluence with the Valea Roșie creek in the Băița creek the pH only slightly increases to 3.6, however the increasing trend of the electric conductivity still relates to the AMD.

Table 7.1 The pH, electric conductivity (EC, in μS/cm), redox potential (Eh, in mV) and temperature (t, in °C) parameter (P.) values as measured in the Valea Roșie-Băița Creeks waters in 2009 (samples 3WSEB09; 4WSEB09; 5WSEB09 & 6WSEB09) and 2010 (samples 3WSEB10; 4WSEB10; 5WSEB10, 6WSEB10 & 7WSEB10).

Crt. no.	P.	3WSEB09 (2009)	3WSEB10 (2010)	4WSEB09 (2009)	4WSEB10 (2010)	5WSEB09 (2009)	5WSEB10 (2010)	6WSEB09 (2009)	6WSEB10 (2010)	7WSEB10 (2010)
1.	pH	3.32	6.90	3.39	2.78	3.21	3.13	3.31	3.14	3.60
2.	EC	2160	76.8	2290	1536	1020	3120	1540	2790	1110
3.	Eh	411	216	416	477	516	369	470	370	325
4.	t	16.5	19.0	16.8	18.9	18.1	16.6	17.6	17.8	21.6

7.2. Mineralogy of the layered precipitates from the Valea Roşie and Băița river beds as inferred from thin sections and XRPD

In the Valea Roşie river bed, many alluvial boulders and pebbles are covered by a more or less thick yellowis-red, ochreous precipitate (Fig. 1.1). This sediment has a stratified texture, being composed of more compact dark red-brown layers alternating with of less compact and friable yellow-red layers (Fig. 7.1). Sometimes the less compact layers show plant remnants. When dry (above the water), the precipitate material shows intense fissuring.

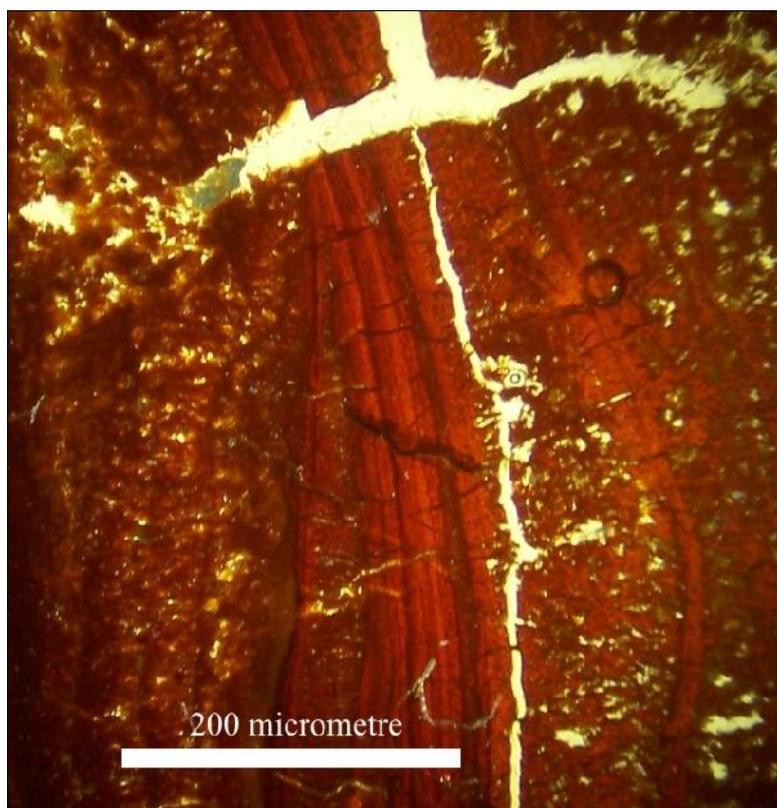


Fig. 7.1 Optical microphoto in plane polarized light of the Valea Roşie layered precipitate (IP; sample 5SEDSEB07).

The mineralogy of the Valea Roşie precipitate sampled in 2007 (including the granulometric fractions as described in chapter Samples and methods) and investigated by XRPD at the University of Salzburg, is shown in Table 7.2 (see also Nagy-Korodi et al., 2012). Quartz, akaganéite and goethite are present in all samples, the latter obviously being concentrated in the finer fractions. Ferrihydrite occurs only randomly, whereas jarosites was identified only in bulk samples of compact and less compact layers, respectively.

Table 7.2 Mineralogical composition of the precipitates as determined by XRPD. Abbreviations: Jar – jarosite; Schw – schwertmannite; Fh – ferrihydrite; Gt – goethite; Ak – akaganéite; Gp – gypsum; Qtz – quartz; Hal – halloysite.

Sample description	Sample no.	Identified mineral phases							
		Jar	Schw	Fh	Gt	Ak	Gp	Qtz	Hal
Bulk samp.	5SEB1107				+		+		
	8SEB1107		+						+
Loose layers	6SEBaLY	+				+	+		
	6SEBbLY		+		+	+			
	7SEBLY		+				+	+	
Compact layers	6SEBaDA	+	+						
	6SEBbDA		+	+					+
	7SEBDA		+		+	+			
≤100 to >63 μm Fraction	5SEB110763		+				+	+	
	6SEB1107a63				+	+			
	6SEB1107b63		+		+	+			+
	7SEB110763				+				+
	8SEB110763		+						+
≤63 to >20 μm Fraction	5SEB110720		+				+	+	
	6SEB1107a20				+				+
	6SEB1107b20			+	+	+			
	7SEB110720	+			+				+
	8SEB110720				+		+	+	+
<20 μm Fraction	5SEB1107F	+		+		+	+		+
	6SEB1107aF				+				+
	6SEB1107bF				+				+
	7SEB1107F				+				+
	8SEB1107F				+				+
Samples treated with ammonium oxalate	5SEBAO			+		+	+		
	6SEBAO		+		+	+			
	6SEBbAO		+		+				+
	7SEBAO				+				+
	8SEBAO				+				+

The mineralogy of the Valea Roșie precipitate sampled in 2009-2010

The **3SEDSEB09** sample was collected from the main inflowing AMD close to the water treatment/purification station. It is composed of poorly crystallized phases, the certainly identified minerals being akaganéite and goethite. The **3SEDSEB10** sample, collected from the unpolluted Valea Roșie river bed sediment shows the presence of quartz and detrital mica (muscovite). The **4SEDSEB10** sample was collected from the first inflowing AMD, at the Valea Tirsa riverbed sediment; beside the detrital quartz, poorly crystallised schwertmannite

is present. The latter mineral may be the most common direct precipitate of iron from acidic effluents at pH 2–4 (Bigam & Nordstrom, 2000). Should be noted, the mineral is difficult to identify because of its poor crystallinity and the almost invariable presence of associated iron oxyhydroxides and jarosite (Blowes et al., 2005). In 2010, beside the above mentioned phases at the centre of Băița village two more additional minerals were found: epsomite and alunogen.

7.3. Chemistry of the Valea Roșie and Băița precipitates as inferred from EMPA data

The chemistry of Valea Roșie precipitate (samples 6SEB1107a; 6SEB1107b and 7SEB1107) obtained at Salzburg University by EMPA is presented in Table 7.3.

Table 7.3 EMPA data (in wt.%) obtained for 6SEB1107a, 6SEB1107b and 7SEB1107 layered precipitate samples. Abbreviation: MP – analyzed micropoint.

MP	SiO ₂	Al ₂ O ₃	MgO	Na ₂ O	CaO	K ₂ O	Fe ₂ O ₃	MnO	As ₂ O ₃	SO ₄ ²⁻	ZnO	Cu ₂ O	P ₂ O ₅	Total
Sample 6SEB1107a														
1	0.20	0.20	0.00	0.04	0.18	0.07	69.82	0.00	0.23	14.24	0.42	0.00	1.13	86.52
2	0.26	0.24	0.01	0.04	0.25	0.06	70.34	0.00	0.11	13.99	0.59	0.00	1.14	87.04
3	0.18	0.25	0.03	0.01	0.22	0.00	70.54	0.00	0.10	14.17	0.46	0.00	1.15	87.10
4	0.19	0.29	0.03	0.00	0.23	0.08	69.06	0.04	0.15	13.45	0.59	0.00	1.19	85.29
5	0.21	0.33	0.04	0.02	0.18	0.01	71.25	0.02	0.17	13.22	0.53	0.00	1.34	87.33
6	0.30	0.58	0.02	0.02	0.17	0.12	71.50	0.03	0.31	12.46	0.41	0.10	1.65	87.68
7	0.28	0.53	0.01	0.00	0.15	0.02	67.73	0.02	0.29	11.68	0.39	0.26	1.55	82.91
8	0.42	0.63	0.03	0.08	0.16	0.04	67.82	0.00	0.37	13.21	0.55	0.00	1.82	85.10
9	0.34	0.58	0.02	0.01	0.17	0.01	71.51	0.01	0.30	12.37	0.28	0.00	1.40	87.00
10	0.34	0.63	0.04	0.00	0.20	0.08	73.58	0.01	0.44	12.52	0.52	0.00	1.56	89.93
Sample 6SEB1107b														
1	0.33	0.30	0.06	0.01	0.29	0.03	68.65	0.04	0.18	13.33	0.74	0.00	1.21	85.16
2	0.20	0.23	0.04	0.06	0.29	0.08	68.53	0.01	0.21	13.34	0.77	0.00	1.42	85.19
3	0.21	0.23	0.04	0.00	0.26	0.02	70.96	0.00	0.09	13.54	0.63	0.03	1.70	87.70
4	0.19	0.26	0.05	0.00	0.24	0.06	70.34	0.04	0.16	13.31	0.58	0.06	1.50	86.79
5	1.55	0.66	0.09	0.06	0.25	0.11	69.55	0.03	0.16	13.45	0.51	0.00	1.42	87.84
6	1.18	0.53	0.12	0.03	0.17	0.04	75.40	0.01	0.11	11.16	0.53	0.04	1.89	91.23
7	0.19	0.31	0.00	0.04	0.22	0.02	73.37	0.00	0.18	12.42	0.73	0.05	1.56	89.10
8	0.28	0.33	0.07	0.00	0.27	0.03	70.60	0.05	0.13	13.44	0.73	0.00	1.37	87.30
9	0.23	0.40	0.03	0.00	0.28	0.01	70.01	0.00	0.05	12.29	0.64	0.00	1.27	85.20
10	0.58	0.37	0.07	0.06	0.24	0.04	71.74	0.00	0.14	12.07	0.62	0.00	1.46	87.39
Sample 7SEB1107														
1	0.45	0.62	0.06	0.02	0.29	0.04	72.66	0.00	0.23	14.24	0.42	0.00	1.13	86.52
2	1.24	0.62	0.09	0.02	0.28	0.04	68.43	0.00	0.11	13.99	0.59	0.00	1.14	87.04
3	0.88	0.66	0.08	0.00	0.26	0.07	71.78	0.00	0.10	14.17	0.46	0.00	1.15	87.10
4	0.62	0.45	0.02	0.00	0.21	0.03	71.09	0.00	0.15	13.45	0.59	0.00	1.19	85.29
5	0.45	0.40	0.09	0.07	0.20	0.05	72.01	0.04	0.17	13.22	0.53	0.00	1.34	87.33
6	0.38	0.33	0.01	0.07	0.19	0.02	70.54	0.01	0.31	12.46	0.41	0.10	1.65	87.68
7	0.46	0.63	0.05	0.00	0.13	0.03	75.47	0.00	0.29	11.68	0.39	0.26	1.55	82.91

The materials looks relatively homogeneous and very fine grained. Only few scattered grains of quartz (<10 µm in size) were identified.

From the EMPA data the following relationship could be inferred: Fe >> S > Si > Al > P > Zn > As > Ca > Cu > Na > Mg > K > Mn. Thus, Fe is the dominant whereas Na, Mg, K and Mn are in very low amounts, sometimes below the detectiona limit. S occurs most likely as sulphate than sulphide.

The presence of metals such as Fe, Zn, As and Cu in the precipitate reflects the mineralogy of the ore deposit, with mostly pyrite, sphalerite, arsenopyrite and chalcopyrite (Borcoş et al., 1974b). Zn and As are trapped in the Fe-rich minerals, most likely in the poor/crystalline phases (colloids?).

7.4. SEM-EDX study of the Valea Roşie precipitates

The secondary electron (SE) images of the yellowish red precipitate sampled in the Valea Roşie riverbed and in the bed of the AMDs show a highly porous texture (Figs. 7.2) and layered structure.

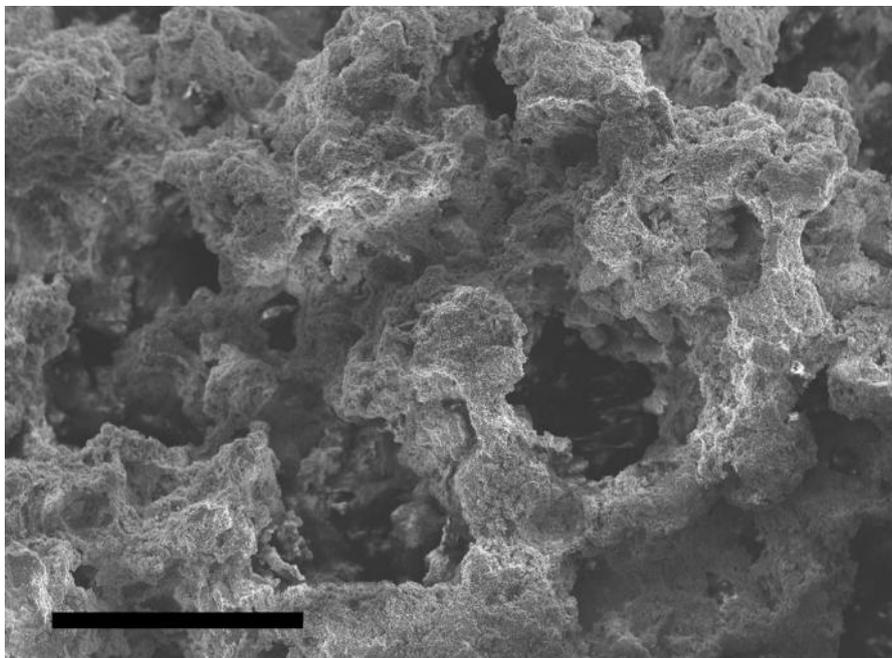


Fig. 7.2 SE image of the 9SEB09 porous precipitate collected in the bed of the main AMD. Scale bar = 1 mm.

The SE image of the 6SEB10 precipitate from Valea Roşie riverbed showing alternating compact and porous layers. Early formation of gypsum is possible because of cation (Ca^{2+} and SO_4^{2-}) availability through the hydrated lime treatments and acid mine drainages. In the layered and porous structures of the precipitate spheric to pin-cushion-like phases form, which can be assigned to schwertmannite (Loan et al., 2004; Asta et al., 2010) are visible. In the vicinity of the hedgehog bacteria alike filamentous formations can be find.

7.5. Mössbauer type spectroscopy of the Valea Roşie precipitates

Three samples were measured by MSP: 3SEDSEB09 – from the main inflowing AMD (collected in September 2009), 5SEDSEB10A – the loose light yellowish red layer from the inflowing second AMD (collected in August 2010) and 5SEDSEB10B – the compacted dark reddish-brown layers from the inflowing second AMD (collected in August 2010)

The envelope of the room temperature spectrum of each sample exhibits two lines. The simplest decomposition of the spectrum allows the evaluation into a doublet. No magnetically split subspectra occur. The Mössbauer parameters are shown in Table 7.4. The isomer shift, $\delta = 0.37$ mm/s for all doublets, reveals solely Fe(III) state. No Fe(II) valence state occurs in our samples.

Table 7.4 The 293°K and 80°K Mössbauer parameters. The relative errors are ± 0.01 mm/s, ± 0.02 mm/s, ± 0.03 mm/s and 0.5 T for isomer shift, quadrupole splitting, line-width and internal magnetic field, respectively.

Spectrum No.	KEU3128B	KEA93C	KEU3124V	KEA94C	KEU3116V	KEA95C
Meas. temp. [°K]	80°K	293°K	80°K	293°K	80°K	293°K
Sample No.	5SEDSEB10B	5SEDSEB10B	3SEDSEB09	3SEDSEB09	5SEDSEB10A	5SEDSEB10A
Mixed M+Q (1)			14.5 %		9.6 %	
Isomer shift [mm/s]			0.41		0.43	
Magn. field [T]			47.85		47.66	
Q. splitting [mm/s]			0.01		-0.27	
Line width [mm/s]			0.66		0.59	
Doublet (1)	100.0 %	100.0 %	30.3 %	100.0 %	40.2 %	100.0 %
Isomer shift [mm/s]	0.47	0.37	0.47	0.37	0.46	0.37
Q. splitting [mm/s]	0.74	0.71	0.80	0.72	0.77	0.70
Line width [mm/s]	0.50	0.57	0.66	0.58	0.59	0.55
Magn. relaxation (1)			55.2 %		50.2 %	
Isomer shift [mm/s]			0.50		0.50	
Magn. field [T]			48.41		48.08	
Jump up rate			8.49		8.38	
Line width [mm/s]			0.70		0.75	

Based on the 293°K spectra no different iron microenvironments can be distinguished. However, the Mössbauer spectra recorded at 80°K exhibit significant differences compared to those obtained at 293°K (Tab. 7.4). For the samples 3SEDSEB09 and 5SEDSEB10A the 80°K spectra can be decomposed into a doublet, a well resolved magnetically split sextet and a “relaxation” component. In the latter sub-spectrum the magnetic splitting is partially collapsed. Based on the Mössbauer parameters of the components (Tab. 7.4) it may be assumed that the doublet corresponds to the Fe(III) doublet collected at the room temperature (Stevens, 1975–2006; Kuzmann et al., 2010).

The average hyperfine field is about 48 T for both sextets. A slightly lower absolute value of quadrupole splitting is more characteristic for the sample 3SEDSEB09 than for sample 5SEDSEB10A. The relaxation component is dominant in both spectra, namely is about 55% for sample 3SEDSEB09 and 50% for sample 5SEDSEB10A, respectively.

The doublet parameters at the room temperature for the investigated samples can be assigned to either: akaganéite, lepidocrocite or ferrihydrite. Additionally, the occurrence of very small grains of goethite is also possible, although the spectrum of well crystalline goethite is magnetically split at 293°K (Murad & Johnston 1987; Stevens et al., 1983).

The comparison of our Mössbauer spectra taken 293°K and 80°K shows that the iron-bearing minerals constituting the samples are superparamagnetic at room temperature at least in 70% in the sample 3SEDSEB09 and 60% in the sample 5SEDSEB10A (since their 80°K Mössbauer spectra reflect the occurrence of paramagnetic phases via doublet in such amounts; see table 7.4). The well resolved magnetically split components can be assigned to both akaganéite and goethite, and the presence of both minerals is possible. More probably, the sextet might be assigned to akaganéite in sample 3SEDSEB09 and to goethite in sample 5SEDSEB10B (Murad & Cahsion, 2004), which is in agreement with the XRPD results.

In the samples where XRPD showed the presence of ferrihydrite, its fingerprint in the 80°K Mössbauer spectra can be the doublet which remains besides the dominant magnetic components. In the case of sample 5SEDSEB10B the 80°K spectrum shows only paramagnetic doublet without magnetic components, similarly to its 293°K spectrum. The XRPD of this sample reveals the presence of ferrihydrite as dominant component. Therefore the doublet in the Mössbauer spectrum of sample 5SEDSEB10BA can mainly be assigned to ferrihydrite. However, superparamagnetic components of akaganéite or goethite with very small grain size cannot be entirely excluded.

7.6. PGAA of the bulk samples of Valea Roşie precipitate

By means of PGAA, the major elemental components (Si, Al, Fe, K, S and H) and some minor and trace elements (Zn and Cl) in the 5SEDSEB10 sample of precipitates collected in the Valea Roşie riverbed were analysed. The PGAA data are given in table 7.5, with major components expressed in oxides weight percentages, and the trace elements in ppm. Besides the detrital phases (feldspar, quartz, clay minerals) the overall geochemical composition reflects the presence of the Fe-rich phase.

Table 7.5 PGAA data for the layered precipitate (5SEDSEB10 sample). Major elements and relative errors are given in %, trace elements in ppm. FeO_{TOT} as Fe_2O_3 .

Oxide	Composition %	Relative error (%)	Detection limit (%)
Al_2O_3	1.0	4.3	1.0
Fe_2O_3	47.0	1.1	0.51
MgO	0.30	27.0	0.3
K_2O	0.51	8.0	0.1
H_2O	1.62	1.0	0.09
S	5.7	2.2	0.84
Total (wt.%)	56.73	-	-
Zn	0.6	18.0	1.79
Cl	0.01	44.0	0.03

7.7. ICP-AES of the inflowing AMD and related creek sections

The geochemical composition of the surface waters in downstream the main inflow of AMD might reveal which and how much potentially ecotoxicological element reaches the creeks and approximately how far they are transported. The analytical data show that the total dissolved solids significantly increased in 2010 compared with 2009 in both the Valea Roşie (samples: 3WSEB10, 4WSEB09, 5WSEB09, 6WSEB10), and Băiţa Creeks (samples: 6WSEB09, 7WSEB10), and inflowing acid mine drainages (samples: 3WSEB09, 4WSEB10, 5WSEB10) as well (Table 7.6). The main acid mine drainage inflow changed the geochemistry of the Valea Roşie creek. The measured amount of cations and anions show positive correlation with the water electric conductivity and the pH parameters.

Table 7.6 Water chemistry and physical parameters values in the Valea Roşie and Băiţa Creeks in September 2009 and August 2010 (in mg/l). Abbreviations: EC – Electric conductivity (in $\mu\text{S}/\text{cm}$); Eh – Redox potential (in mV); t – temperature (in $^{\circ}\text{C}$); Tot.Dis.Sol. – Total Dissolved Solids (in mg/l); Tot. Hard. – Total water hardness (in mgCaO/l); Carb.Hard. – Carbonate hardness (in mgCaO/l); Phen.Alk. – Phenolphthalein Alkalinity (in mmole/l).

Measurement point/Element concentrations ($\text{mg}\cdot\text{l}^{-1}$)	3WSEB09	3WSEB10	4WSEB09	4WSEB10	5WSEB09	5WSEB10	6WSEB09	6WSEB10	7WSEB10
Na ⁺	10.90	1.59	10.3	2.54	2.69	9.19	12.5	8.01	6.57
K ⁺	8.93	3.6	9.26	6.45	4.03	8.88	7.26	7.34	4.21
Ca ²⁺	199	5.9	228	57.9	39.5	200	145	196	78.5
Mg ²⁺	99.8	2.29	88.6	24	16	121	48.3	103	32.6
Fe ²⁺	92.7	0.3	98.8	59.4	14.2	307	73.9	220	45.9
NH ₄ ⁺	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Mn ²⁺	36.20	0.091	31.9	5.05	3.33	42.4	16.6	36.4	10.9
Cl ⁻	3.22	<1.0	<1.0	<1.0	9.02	<1.0	<1.0	<1.0	<1.0
NO ₃ ⁻	<0.1	0.47	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
NO ₂ ⁻	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
HCO ₃ ⁻	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
CO ₃ ²⁻	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
PO ₄ ³⁻	0.08	0.02	0.13	0.17	0.01	0.1	0.31	0.06	0.16
SO ₄ ²⁻	1780	21.2	1750	853	539	2150	716	1910	513
OH ⁻	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
H ₂ SiO ₃	71.20	24.9	65.80	82	61.50	75.5	48.80	70.10	38.80
Tot.Dis.Sol.	2310	67.9	2290	1100	696	2920	1080	2560	739
Tot.Hard.	510	13.6	525	137	92.4	561	315	513	186
Carb.Hard.	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Phen.Alk.	<0	<0	<0	<0	<0	<0	<0	<0	<0

The heavy metal concentration exceeds with several ten or hundreds of times the unpolluted spring's level and the maximum admissible values – MAC, based on the STAS 4706/88 Romanian National Standards and laws (Law 458/2002; Law 311/2004) - Table 7.7.

Table 7.7 The dissolved heavy metal concentrations in the Nistru, Valea Roşie and Băiţa Creeks in September 2009 and August 2010 (in $\mu\text{g}/\text{l}$). Abbreviation: n.a. – not available; MAC** – Maximum admissible concentration based on the STAS 4706/88 Romanian National Standard.

Measurement point/Element concentrations ($\mu\text{g}\cdot\text{l}^{-1}$)	Cr	Zn	Co	Ni	Ba	Al	Cu	Sr	Mo	B	Pb	Cd	Li	As
MAC (in $\mu\text{g}\cdot\text{l}^{-1}$)**	50	30	n.a.	n.a.	n.a.	n.a.	50	n.a.	n.a.	n.a.	50	3	n.a.	10
3WSEB09	6.12	96900	258	148	3.2	84700	523	280	<1	53.3	41.7	200	98.6	<3
3WSEB10	<1	8.38	<1	<2	60.1	166	1.93	25.3	<1	7.81	<3	<0.25	<5	<3
4WSEB09	5.32	84630	232	134	5.45	77560	474	276	<1	49.1	43.5	178	91.8	<3
4WSEB10	9.82	21090	151	91.9	31.7	74200	798	63.8	<1	13.3	271	276	39.5	<3
5WSEB09	4.59	15930	94.8	60	33.4	42880	323	53.8	<1	17.4	108	157	24.4	3.53
5WSEB10	11.8	13690	375	262	5.61	128500	1494	256	<1	46.8	59.5	451	126	9.98
6WSEB09	3.04	39380	119	69.3	15.7	33180	231	237	<1	112	37.8	88.1	67.4	85.3
6WSEB10	10.4	108090	324	227	11.9	107560	1297	232	<1	40.7	57.3	391	110	9.18
7WSEB10	2.27	36630	95.7	67.6	17.7	30154	375	145	<1	50.3	37.1	112	40.3	19.9

7.8. TEM study of the AMD precipitate

Sample 5SEDSEB10A

The less compact and porous light brown precipitate sampled in August 2010 from the bed of the main AMD (sample 5SEDSEB10A) was investigated by TEM (Table 7.8). The selected area electron diffraction (SAED) pattern of the sample 5SEDSEB10A –, refers to 2-line ferrihydrite (2L fy), with broad diffraction rings. Measured d -values at 2.5–3 and 1.5–1.7 Å of F317 measurement point of 2L fy are in agreement with data published for ferrihydrites by Janney et al. (2000a,b), this result is supported by the Mössbauer spectroscopy, Cu- anode X-Ray Powder diffraction as well. Beside the 2L fy, the radial intensity profile pattern of the sample 5SEDSEB10A – at F327 measurement point (Table 7.8), refers to 6L-ferrihydrite (6L fy).

Sample 3SEDSEB09

The sample 3SEDSEB09 represents the bulk highly porous yellowish red material collected in 2009 from the inflowing acid mine drainage (AMD) at Valea Roşie. The Mössbauer spectroscopy shows the presence of akaganéite but the diffraction spots at 6.2 Å suggest other secondary precipitate, the lepidocrocite (Table 7.8). The Fe-(oxi)hydroxide phase is poorly crystalline, typical crystal size (from the dark field image) is few tens of nanometres.

Sample 5SEDSEB10B

The sample 5SEDSEB10B represents the compacted dark reddish brown layers from the largest inflowing AMD collected at Valea Roşie in 2010. The Mössbauer spectroscopy showed goethite as main phase in the sample 5SEDSEB10B. The diffraction spots at 4.2 and 2.5 Å confirmed the presence of goethite (Table 7.8). According to EDS measurements they contain sulphur, up to 5 at % (Table 7.8).

Table 7.8 TEM EDS weight (wt.%) and atomic percentages (at.%) of the measuring points (MP).

Sample	<i>5SEDSEB10A</i>						<i>3SEDSEB09</i>						<i>5SEDSEB10B</i>					
MP	F317		F321		F323		F325		F327		F331		F332		F337		F340	
Element	at.%	wt.%	at.%	wt.%	at.%	wt.%	at.%	wt.%	at.%	wt.%	at.%	wt.%	at.%	wt.%	at.%	wt.%	at.%	wt.%
O	58.68	30.34	61.55	32.81	61.63	39.50	61.48	32.57	67.04	37.71	67.75	48.52	64.05	36.27	64.20	36.73	58.68	30.34
Mn	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.12	0.00	0.00	0.02	0.05	0.00	0.00	0.01	0.02	0.00	0.00
Si	1.06	0.96	0.26	0.24	0.69	0.78	1.34	1.25	0.40	0.40	0.82	1.03	1.84	1.83	2.56	2.57	1.06	0.96
Al	0.82	0.71	0.60	0.54	0.50	0.54	1.22	1.09	0.33	0.31	0.07	0.08	0.24	0.23	0.28	0.27	0.82	0.71
P	0.22	0.22	0.00	0.00	12.23	15.18	0.25	0.26	0.35	0.38	0.00	0.00	0.14	0.15	0.05	0.05	0.22	0.22
Zn	0.00	0.00	0.03	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
As	0.00	0.00	0.03	0.07	0.01	0.03	0.00	0.00	0.13	0.33	0.00	0.00	0.13	0.34	0.00	0.00	0.00	0.00
Pb	0.18	1.21	0.00	0.00	0.05	0.41	0.11	0.77	0.09	0.65	0.01	0.09	0.02	0.12	0.01	0.08	0.18	1.21
Cd	0.06	0.23	0.03	0.13	0.00	0.00	0.02	0.09	0.00	0.00	0.00	0.00	0.06	0.24	0.00	0.00	0.06	0.23
S	4.54	4.70	4.30	4.59	0.56	0.72	2.09	2.22	2.23	2.52	17.26	24.78	6.26	7.11	6.22	7.13	4.54	4.70
Fe	33.61	60.67	32.89	61.19	6.04	13.52	33.18	61.35	29.26	57.45	0.33	0.83	27.01	53.38	26.48	52.88	33.61	60.67
Ca	0.00	0.00	0.00	0.00	18.10	29.07	0.08	0.11	0.13	0.19	13.68	24.54	0.09	0.13	0.14	0.21	0.00	0.00
Cl	0.83	0.95	0.31	0.37	0.18	0.25	0.15	0.17	0.05	0.06	0.05	0.08	0.16	0.20	0.05	0.06	0.83	0.95
<i>Total</i>	100.00	99.99	100.00	100.00	99.99	100.00	99.98	100.00	100.01	100.00	99.99	100.00	100.00	100.00	100.00	100.00	100.00	99.99
<i>Phases identified</i>	2L ferrihydrite		2L ferrihydrite		apatite		polycrystal Fe-phase(s)		6L ferrihydrite		gypsum		lepidocrocite		goethite		goethite	

CHAPTER 8. THE MINING-RELATED ENVIRONMENT IN THE NISTRU-VALEA ROȘIE-BĂIȚA AREA: A BIOLOGICAL APPROACH

Life forms and the chemical processes associated with them are intimately related to water and to the solutes contained in water. Aquatic plants, as well as the terrestrial plants require nutrient elements, especially nitrogen and phosphorus, collected through roots from the bottom soil/sediment or directly assimilated from the water. The photosynthesizing biota provides food and oxygen for other life forms in the water where they grow, thus a water containing harmful elements, e.g. heavy metals, might have negative effects on a whole habitat.

The waters of the rivers in the Nistru-Valea Roșie-Băița area were sampled for biological purposes in September 2009 and August 2010. Two types of ecotoxicological testings were carried out: with ice-lettuce (*Lactuca sativa* L. var. *Great Lakes 118*) and the duckweed (*Lemna minor* L.), respectively. Our study showed (Chapter 7) an unpolluted environment in the Nistru river. By contrast, the Valea Roșie-Băița area shows highly polluted waters (Chapter 8), with pH of 3.21–3.39 in 2009 and 3.14 to 3.60 in 2010 and EC from 1,020 to 2,290 $\mu\text{S}/\text{cm}$ in 2009 and 1110 to 2790 $\mu\text{S}/\text{cm}$ in 2010).

8.1. Toxicology test with ice lettuce

The close observations of the behaviour of the seeds showed that at the beginning the germination energy was higher in the case of unpolluted waters (94% for the Nistru samples) and significantly lower, up to 75%, in the polluted water of Băița, in particular in the water sampled in the village centre (Fig. 8.1). In time, the germination capacity increased in the Valea Roșie-Băița samples up to the same level as in the Nistru waters indicating that pollution with heavy metals might delay but not inhibit the germination process. However, the toxicity obviously affected the roots, in particular damaging their growing apex. The apical meristem tissue changed from white-light green to brown or dark brown, and in many cases exhibited severe necrosis. In the presence of the polluted water, the roots of the plantlets were shorter than 0.2–0.4 cm. maintained with water sampled in the Nistru river, the lettuce plantlets were fresh and green and developed healthy roots, up to 6–8 cm in length.

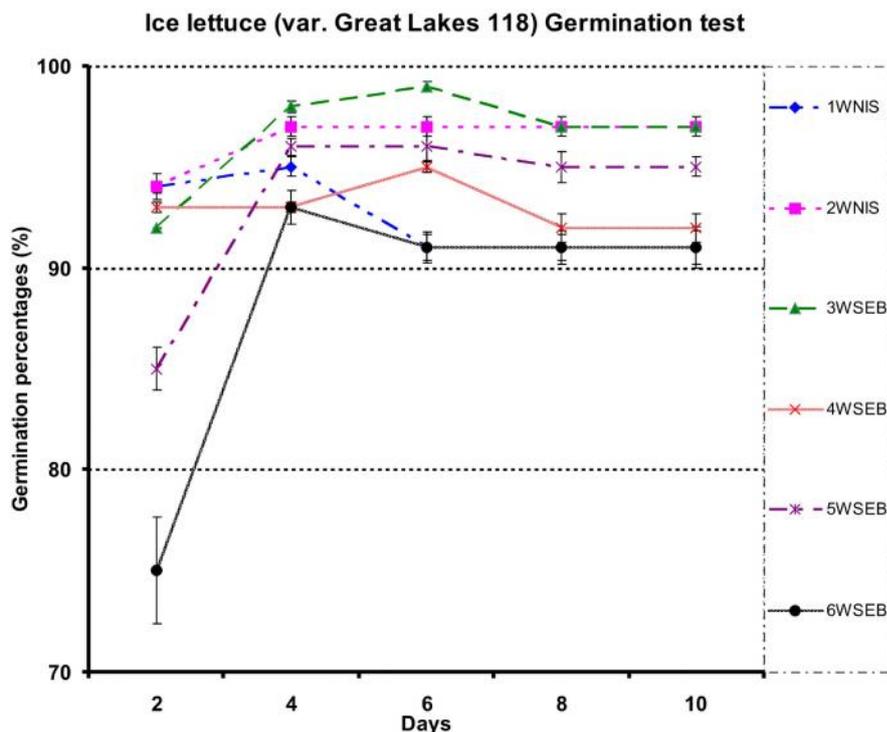


Fig. 8.1 Effect of water samples from Nistru (samples 1WNIS09 and 2WNIS09), main inflowing AMD (sample 3WSEB09), Valea Roşie (samples 4WSEB09 and 5WSEB09) and Băiţa (sample 6WSEB09) waters on ice lettuce seeds germination.

In time, the germination capacity increased in the Valea Roşie-Băiţa samples up to the same level as in the Nistru waters indicating that pollution with heavy metals might delay but not inhibit the germination process (Fig. 8.1).

8.2. Toxicology tests with duckweed

The ecotoxicological test performed with Duckweed (*Lemna minor* L.) is often used as a bioindicator of water quality (Naumann et al., 2007) and might reveal the effects of ecotoxic waters on aquatic organisms. The ecotoxicological test performed with water samples collected in September 2009 showed that almost all plants died after 2 days of experiment when set in samples 3WSEB (main inflowing AMD) and 4WSEB (Valea Roşie river, downstream AMD) – Fig. 8.2. Nevertheless, the duckweed plants resisted up to 8 days in the 5WSEB (downstream the main AMD in VR) and 6WSEB samples (downstream the confluence with VR, at the Băiţa village centre). Even more, the favourable conditions created by the 1WNIS (unpolluted Nistru spring) and 2WNIS (200 downstream the Nistru village) water samples allowed to increase the biomass production of duckweed. It reached almost 180% of the initial control number, in the case of water sample from downstream Nistru (2WNIS).

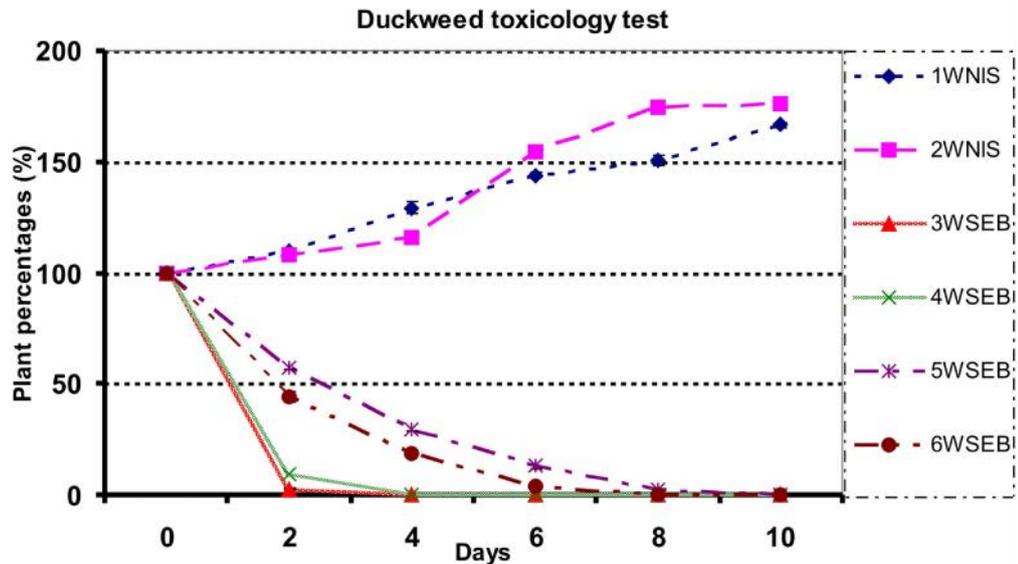


Fig. 8.2 Effect of water samples from Nistru (samples: 1WNIS and 2WNIS), main inflowing AMD (sample: 3WSEB), Valea Roşie (samples: 4WSEB and 5WSEB) and Băiţa (samples: 6WSEB) rivers on duckweed cultures observed in a period of 10 days.

8.3. Biodiversity studies in the Valea Roşie and Băiţa rivers

The microorganisms contribute to a vast array of biogeochemical cycles, among which are oxygenic photosynthesis, ammonia oxidation, sulphur oxidation/reduction, methanogenesis, fermentation, and respiration (Blowes et al., 2005). This allows the microorganisms to survive in an environment containing low or finite nutrients or even in at low pH, as the case of AMD.

The bed river of the Valea Roşie and Băiţa displays along several kms green biofilms, i.e. continuous coatings or mats consisting of *Euglena mutabilis*, Schmitz (Fig. 8.3). They occur only close to the shores of the river because the strong water current in the middle of the streams. The Valea Roşie-Băiţa *E. mutabilis* occurrence is might be one of the largest reported in the Eastern Carpathians. *E. mutabilis* is a microalgae (protist) which indicates acidic water (acidophil) and prefers a pH of 3-3.5, high electric conductivity and high redox potential (Fig. 8.3) (Brake et al., 2001; Forray, 2002a,b; Nagy et al., 2006).



Fig. 8.3 Typical fusiform shape of *Euglena mutabilis* algae in the Băița River waters (6WSEB09 measurement point in 2009).

The extreme physical and chemical conditions which encourage the growth of *E. mutabilis* (forming a sort of acidic niche) are not favourable to most of aquatic organisms (Allan, 1995; Fang et al., 2007). However, in the literature several other microbiotic species are mentioned to live in this acidic niche (Fig. 8.4).

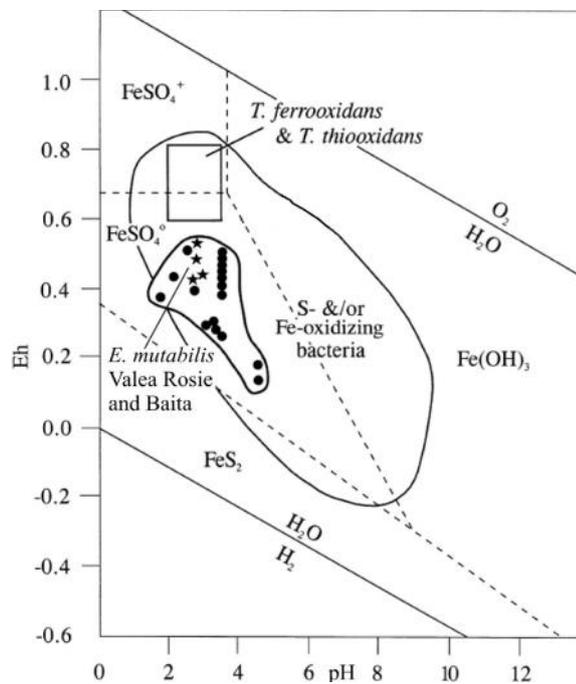


Fig. 8.4 Variation Eh-pH diagram for the Fe-S-O₂-H₂O system at 25 C° (from Brake et al., 2001, with modifications). $\Sigma\text{Fe}=10^{-1}$ m, and $\Sigma\text{S}=10^{-1}$ m. Stability fields for Fe phases are shown as dashed lines. Overlapping the Fe stability fields are the Eh and pH conditions supporting S- and/or Fe-oxidizing bacteria. *Euglena mutabilis* at Valea Roșie-Băița Valleys in 2009 (marked with stars) and at other locations referred by Brake et al (2001) marked by dots and *Thiobacillus ferrooxidans* and *T. thiooxidans*; for physico-chemical parameters see Chapter 7.

8.4. Microbiology of the Valea Roşie river affected by AMD

As we mentioned in the chapter 4 (Samples and methods) the applied technique was the denaturing gradient gel electrophoresis (DGGE). DGGE has emerged as a powerful method (Bernard et al., 2001; Kawai et al., 2002) for its sensitivity is close to 100% in resolving DNA fragments differing by as little as a single nucleotide (Dolinsky et al., 2002).

The sample 5WSEB10MA1 was collected in 2010 (Fig. 8.5), from the inflowing main acid mine drainage (upstream the confluence with the Valea Roşie creek), from approximately 5 cm water depth, from the right part of the bank. Bryophyte communities were macroscopically visible at the site as well, however at the bank, not in the creek's water.



Fig. 8.5 Location of sample 5WSEB10MA1 (in the main inflowing AMD at Valea Roşie). The Bryophyte communities form a greenish mat (in the image, above the plastic Eppendorf). The yellowish-green mass of *Euglena mutabilis* (in the centre of the image) covers cm-sized iron oxide-hydroxide-sulphate spherules.

The denaturing gradient gel electrophoresis (DGGE) patterns of the sample 5WSEB10MA1 confirms the presence of *Acidocella facilis* and *Bryophyta* (most likely: *Dicranella* and *Bartramia*) genera.

CHAPTER 9. DISCUSSIONS

The different methods gave an insight into the speciation of the secondary minerals, on the possible transport ways for elements. The main physico-chemical parameters i.e. pH, EC, Eh, and t, provide a better understanding of the ongoing processes, mainly the influence of the AMD upon natural waters characteristics.

As shown in the results chapters (chap. 6–8), the river sediments (yellowish-red-brown precipitates) consist of Fe-oxide-hydroxide phases which have a high adsorbance capacity, in particular for the heavy metals transported by AMDs. This is proved by the precipitate content in heavy metals, such as: Zn, As and Cu. This Fe-ric precipitate acts as a specific carrier matrix with “trapping – effect”. Its chemistry reflects the sphalerite, arsenopyrite and chalcopyrite bearing ore, as well as the specific trace elements accompanying these minerals. The increase of Ca and SO_4^{2-} amount shows only that the purification station is still working, hydrated lime being irregularly added into the creek water.

The way of primary sulphide minerals alteration and formation of secondary minerals is shown in fig. 9.1 (Jambor & Dutrizac, 1998). Dissolved types of $\text{Fe}(\text{OH})_x^{3-x}$ through hydrolysis, nucleation and crystallisation lead to formation of goethite, akaganéite and ferrihydrite – the latter through thermal transformation and dehydration leads to the stable hematite. By following different pathways other $\text{Fe}(\text{OH})_x^{3-x}$ and $\text{Fe}(\text{OH})_y^{3-y}$ phases through precipitation and rapid oxidation result in the formation of green-rusts, feroxyhyte [δ -FeOOH], lepidocrocite and magnetite [Fe_3O_4]. The latter at higher temperatures and oxidation may change its crystal structure to maghemite which through thermal transformation ends in the stable hematite.

The meta-stable secondary oxihydroxide precipitates (i.e. ferrihydrite, akaganéite) generally have large surface area (in the case of ferrihydrite $>340 \text{ m}^2/\text{g}$) and high reactivity, which enable them to adsorb high amount of various elements. Among these, heavy metals, some anions and organic species have an important role in the characteristics of surface water, groundwater, soil or mine tailings systems (Jambor & Dutrizac, 1998). The absorbance and subsequent release of the entrapped metals depend on the solution pH, thermal and biogeochemical parameters. These processes interact in synergistic and antagonistic ways to facilitate the transformation of various metastable iron-oxide-hydroxides in continuously fluctuating environmental conditions.

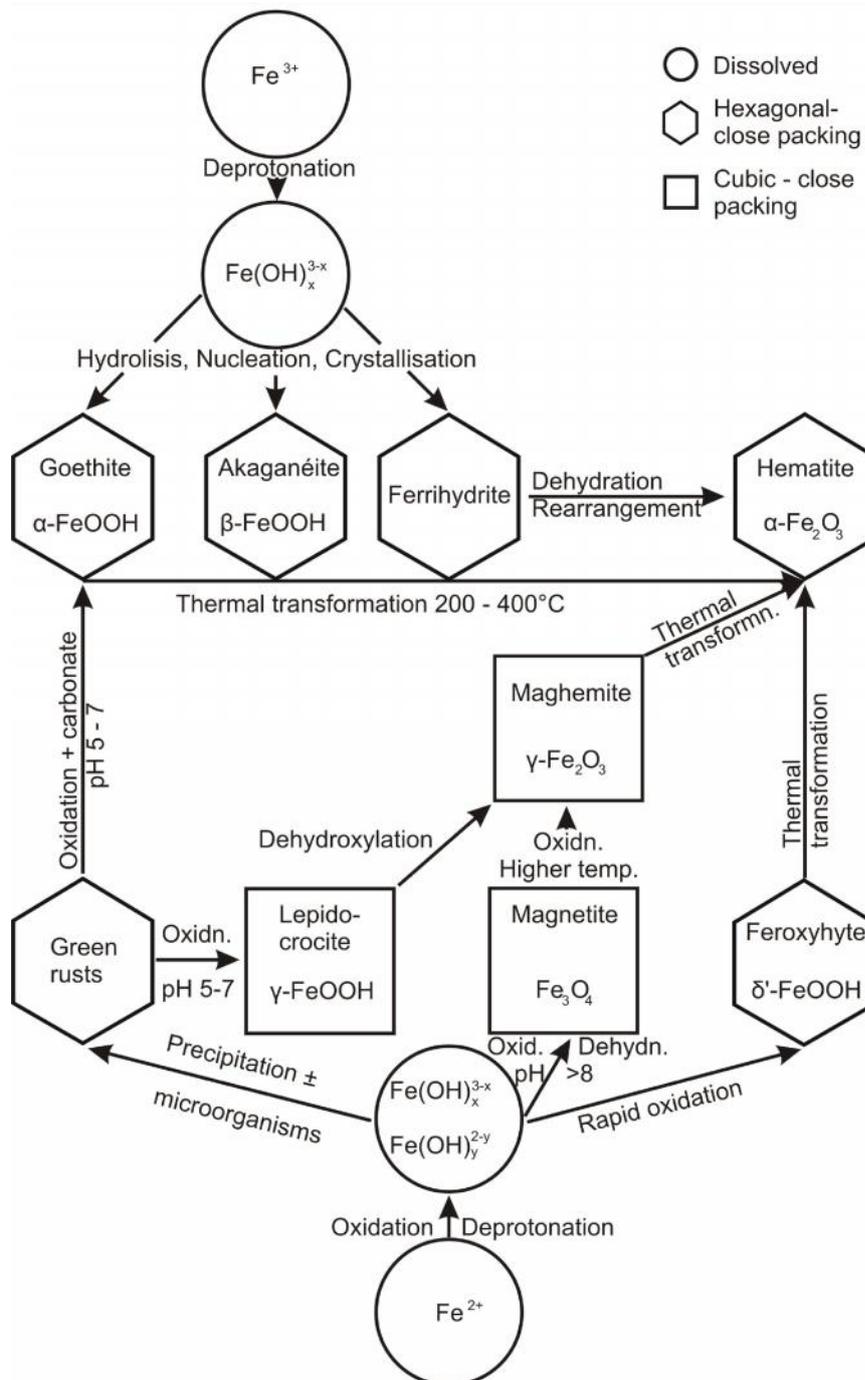


Fig. 9.1 Schematic representation of formation and transformation pathways of common iron-oxides (with modification, from Jambor & Dutrizac, 1998).

As the acid mine drainages originate from a base-metal rich ore and the host rocks, various ions are present. The dissolution and weathering of silicates results in high concentration of Mg^{2+} , Ca^{2+} , Mn^{2+} , Na^+ , K^+ and Al^+ , while the oxidation of the ore minerals (such as: pyrite, sphalerite, galena, tetrahedrite, arsenopyrite and chalcopyrite) increases the amount of Fe^{2+} , Zn^{2+} , Cu^{2+} , Pb^{2+} ions, or oxyanions as SO_4^{2-} , H_2AsO_4^- , as well as protons (H^+). The breakdown of silicates (muscovite, feldspar, amphibole, pyroxene etc.) results in formation

of clay minerals and chlorite, respectively. The ions generated by the sulphide oxidation interact with minerals and might facilitate the dissolution processes and the formation of secondary phases, such as jarosite, goethite, schwertmannite and akaganéite.

Jarosites formation ($\text{pH} < 3$, Dold & Fontboté, 2001) is one of the characteristics of the Nistru spoil. It is related not only with sulphides weathering but also with the release of K^+ by feldspars and muscovite transformation (Fig. 9.2). In a pH window of 2–4 and high concentration of Fe^{3+} and SO_4^{2-} , the schwertmannite, the most frequent AMD Fe oxi-hydroxy-sulphate may form (Bigham & Nordstrom, 2000). It is present in the Nistru spoil but mainly in the alluvial sediments of Valea Roşie-Băiţa, including the AMDs. The transformation of schwertmannite into goethite increases the acidity of the solutions, thus inhibiting sulphate reduction (Regenspurg et al., 2004). Schwertmannite can therefore should to be regarded as a key intermediate in the transition process from iron reducing to iron oxidising conditions (Blodau, 2006). Schwertmannite could be formed bacterially as well, e.g by *Acidithiobacillus ferrooxidans* (Egal et al., 2009). Even schwertmannite forms in lower pH (2–4) than ferrihydrite (pH 5–8), our study shows that these minerals can coexist (see also Majzlan et al., 2004).

The transformation of schwertmannite may lead also to the formation of ferrihydrite (Majzlan et al., 2004). Because of its surface area, ferrihydrite has a large adsorption capacity for the cations and anions present in natural systems (Antelo et al., 2010) as is the case at Valea Roşie-Băiţa.

The most abundant and thermodynamically stable Fe-mineral in our area is goethite (Fig. 10.2), which play an important role as adsorbent of ions, including heavy metals. The formation of the secondary phases is lead by thermodynamic processes. The pH, Eh, electric conductivity, dissolved oxygen, dissolved element concentration are amongst the most important factors.

In the acid mine drainages, together with physico-chemical processes, the biological factor might increase the system complexity. Various life-forms such as bacterias and algae developed mechanisms that allow metals uptake and excretion, to maintain tissue concentrations within controllable ranges, and obviously to prevent toxicity (Kapustka et al., 2004). In the case of low concentrations – where organisms suffer nutritional deficiency – increased uptake and retention of metals occur to serve nutritional needs. However at the concentrations above the nutritional expectation, the organisms maintain a limit for the element uptake (Kapustka et al., 2004). When these mechanisms can not cope with the increased concentrations of metals, a toxic situation will result (Salazar-Camacho & Villalobos, 2010).

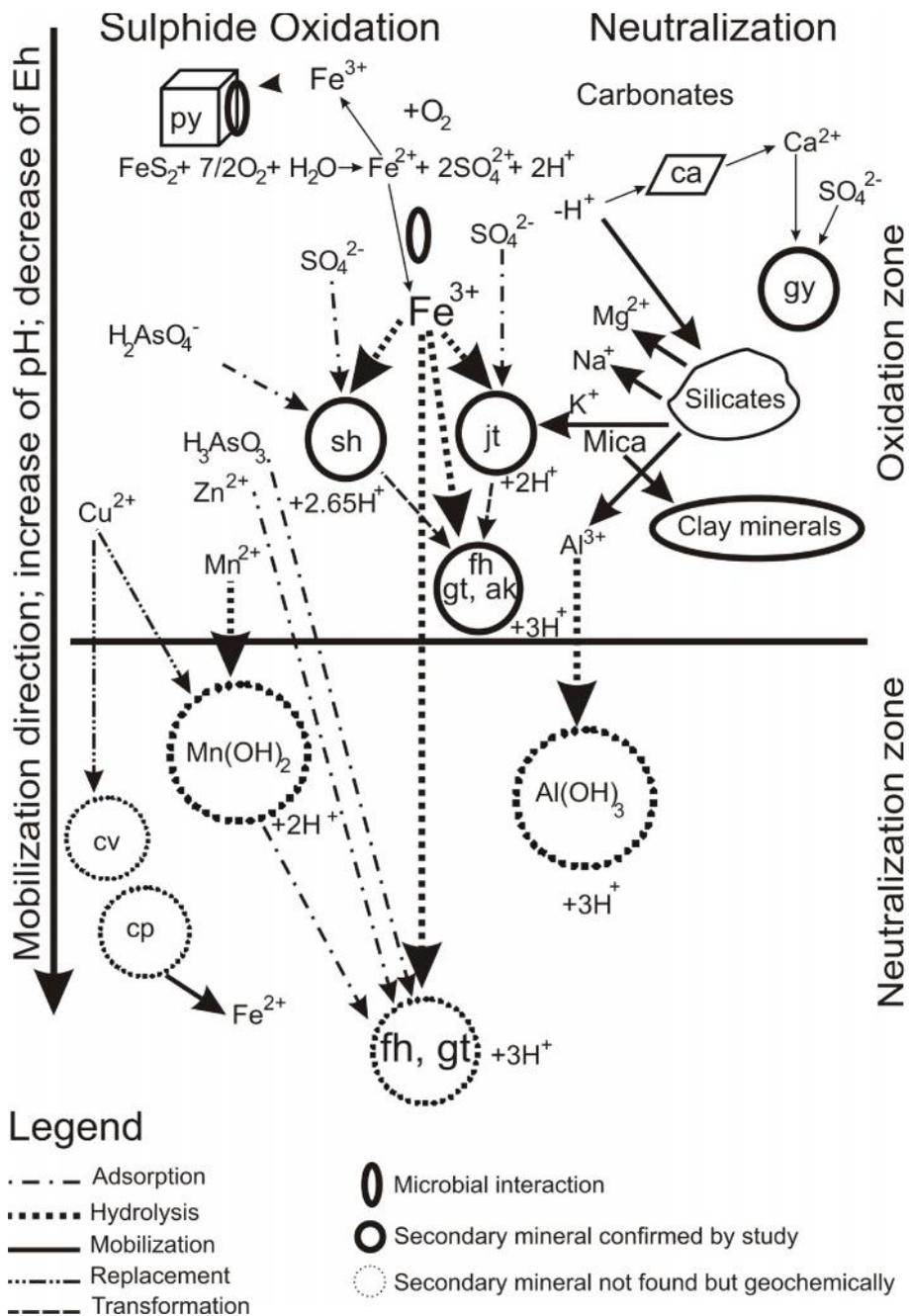


Fig. 9.2 Summary of the element cycling and mineral forming in sulphidic tailing of Nistru, and the acid mine drainage affected Valea Roşie-Băița creeks (diagram adopted, with modifications, after Dold & Fontboté, 2001 and Dold, 2005).

The acid mine pollution in the Valea Roşie-Băița area is marked by the presence of *Euglena mutabilis* Schmitz microalga, indicator of acidic water, which is populating the creek as green coatings. Beside the *Euglena mutabilis* the bacteria are among the few forms of life that can tolerate these extreme environments. The Eh-pH conditions supporting *E. mutabilis*

fall well within the Eh-pH range for S- and/or Fe-oxidizing bacteria (Brake et al., 2001). *Euglena mutabilis* does, however, prefer less oxidizing conditions than that which support *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*, bacteria that also resides in AMD systems. The presence of *E. mutabilis* has the potential to greatly influence water chemistry, particularly Fe concentrations (Brake et al., 2001, 2002; Fang et al., 2007).

The acidophilic bacteria play an important role in the specification of the Fe-related reactions in the acidic environment. Coupland & Johnson (2008) studying stromatolites from an acid mine drainage (Green Valley, USA) found that the specific ecological niche with *Euglena mutabilis* is composed from five groups of microorganisms: a) phototrophic micro-eukaryotes (algae, protozoa, cyanobacteria), b) gram-positive and other anaerobic bacteria, c) sulphate reducing bacteria, d) aerobic gram-negative, acid producing bacteria and e) fungi. In our sediments (precipitate) samples collected from the main inflowing AMD at Valea Roşie the *Acidocella facilis* and *Bryophyte* were found.

The plants are primary producers in supporting all other life-forms, thus their role in stabilisation and nutrient cycling in aquatic and terrestrial environments is very important (Schultz & Joutti, 2007). The plant tests developed to study single chemical and mixed chemical effects and to evaluate water and soil contaminations (Eisler, 1993; Khan et al., 2000; Memon et al., 2001; Cai & Ma, 2003; Kapustka et al., 2004; Göhre & Paszkowski, 2006; Garrido et al., 2010; Shah et al., 2010) are necessary in any environmental research. Among the heavy metals there are essential elements for the normal development of plants, such as (Kapustka et al., 2004; Shah et al., 2010): cobalt, copper, iron, manganese, molybdenum, nickel and zinc. Due to their high reactivity, these metal elements can directly affect growth, development, senescence and energy generating processes (Shah et al., 2010). Regulation of metal accumulation by organisms complicates the interpretation and application of bioaccumulation data for aquatic and terrestrial organisms; living forms have evolved homeostatic mechanisms that allow metals, as naturally occurring substances, to be stored for later use (Kapustka et al., 2004). These mechanisms regulate the uptake and excretion of metals to maintain tissue concentrations within desirable ranges, as well as to prevent toxicity (Kapustka et al., 2004). However, in case of increased (potentially toxic) concentrations, in order to survive plants must develop efficient mechanisms to transform metals into a physiologically tolerable form, providing the essential elements for the metabolic functions of plants. On the other hand, the excess of any elements, whether essential or toxic, has to be metabolically inactivated (Cai & Ma, 2003). The different heavy metals induce various effects on the plant germination and development. The seed germination and young plantlet

development are very sensitive to harsh environmental conditions (Kapustka et al., 2004), such as AMD or rivers affected by AMD (e.g. Valea Roşie – Băiţa). Mahmood et al. (2007) found that Cu, Zn and Mg inhibit seed germination and early growth of rice, wheat seeds. The slow grow in the first days followed by subsequent recovery, as noticed in our tests, can be related to the root tissues and specific barrier mechanisms. Such a mechanism was described by Verbruggen et al. (2009);

The metallic elements adversely affect height and growth of plants. The reduction in plant height might be mainly due to reduced root growth and regulation of lesser nutrients and water transport to the aerial parts of the plant (Shah et al., 2010). Peralta et al. (2000) by studying heavy metal effects on alfalfa (*Medicago sativa*, L.) plantlet growth found that 5 ppm Cd reduced the shoot size by about 16% as compared with shoot length of the control group; on the other hand, a dose of 5 ppm of Cr, Cu, Ni, and Zn increased the shoot length with 14%, 60%, 36%, and 7%, respectively. However, Cd and Cr, in an amount of 10 ppm, significantly reduce the shoot growth. A concentration of 40 ppm of these metals has a deadly effect on plants (Peralta et al., 2000). Our tests confirmed the negative effect of the AMD waters on the ice-lettuce growth rate.

Garrido et al. (2010) studied the effect of water with heavy metal content similar to that measured at Valea Roşie-Băiţa and found that even 80 ppm of Cd and several thousand ppm of Zn can already accumulate in potato leaves. Memon et al. (2001), Fargašova & Szárazová (2007), John et al. (2009) and Benzarti et al. (2010) showed also that most of the heavy metals are entrapped in the roots, and in less extent in stems.

When the plants metabolism can not cope with the extreme doses of heavy metals (or even with macro-nutrients), one of the following processes might happen (Hall, 2002; Kapustka et al., 2004; Göhre & Paszkowski, 2006): a) the heavy metals modify protein structure or replace a vital element, b) reactive oxygen is generated, damaging plant tissue. Similar consequences were noticeable in our biological tests, by root meristem damaging, growth retardation. The Valea Roşie and Băiţa water samples containing high metal concentrations had obviously a lethal effect on most of the duckweed and ice-lettuce plantlets.

However, during the ecotoxicological tests it seemed the ice-lettuce seeds resisted longer in all water samples whether polluted or not. Contrary, only a few of the duckweed plantlets survived but only in less polluted Băiţa waters. It is possible that the significantly larger surface exposed to metal-bearing solutions (water) by duckweed might increase the toxicity potential. Besides the wide range of variation in heavy metals content in the polluted waters, one should take into account the specific genetic tolerance of tested species, stage of growth, and age and

type of tissue (Treshow, 1978). The most sensitive stage of growth differs somewhat for each heavy metal, but generally, the young, just recently matured tissues are most sensitive (Treshow, 1978).

CHAPTER 10. CONCLUSIONS

The investigation in the Nistru-Valea Roşie-Băiţa area in NW Romania provided a complex image of the consequences related to old (ceased) and actual (still ongoing) ore exploitation. The spoil material, the alluvial sediments (precipitates) and the river waters display clear evidence of the pollution. New phases form on the expenses of metallic minerals, in particular sulphides originating in the hydrothermal ore deposit related to the Neogene volcanics. The primary minerals which are subject of transformation are pyrite, sphalerite, chalcopyrite and galena. Among these minerals, the most important polluting factor is pyrite. It undergoes complex alteration processes which take place all along from the exploitation place deep inside the earth, up to the heaping the spoils. Thus, new Fe-rich phases such as goethite, ferrihydrite, jarosite (hydronium- and K), akaganéite, lepidocrocite and schwertmannite are formed. They are often poorly crystallized and may be responsible for the adsorption of metals.

The Nistru Old Spoil material is consists of primary minerals as well as products of their weathering. Quartz is the main mineral but most of the samples contain large amounts of jarosites, some kaolinite, but also akaganéite, goethite and gypsum. As the valley damp impoundment is combined with high potential energy of the relief, the stability of the tailings dam is highly questionable, even the area is partially remediated. The water-chemistry of the rivulet flowing nearby shows that the partial remediations of the Nistru spoil does not affect the surface/underground water.

Comparing the Nistru and Valea Roşie-Băiţa sediments, our study showed a bed river detritus composed mainly of quartz, muscovite and feldspar for the first. The second area, i.e. the Valea Roşie-Băiţa shows much finer, more or less compact sediment (precipitate) with a layered structure and biological fingerprint. The iron dominates the layers enclosing plant remnants. The laminated stromatolite structures might be due the *Euglena mutabilis* acidophil protist. The layers show different mineralogies, the dark yellow-brown compact precipitate consists of schwertmannite, akaganéite and ferrihydrite, while the loose, light yellow layers are composed of jarosite, goethite, ferrihydrite, akaganéite and gypsum. The ratio between the intensity of the acid-producing processes (e.g. the decomposition of pyrite and the formation of

secondary Fe-rich minerals) and the neutralizing processes (e.g. the treatment with hydrated lime, which leads to the gypsum formation), highly influences the actual features of the Valea Roşie-Băiţa alluvial sediments.

The chemistry of the Valea Roşie -Băiţa river water shows also the negative effect of the mining, part of the potentially ecotoxic elements such as Zn, Cd and As exceeding the maximum concentrations allowed by Romanian standards and laws. The absorbance and release of the entrapped metals is mainly dependent on the solution pH, among the thermal and bio-geo-chemical parameters; these processes interact in synergistic and antagonistic ways to facilitate the transformation of the different meta-stable iron-oxide-hydroxides in the continuously fluctuating environmental conditions. The inflowings of the AMDs into the Valea Roşie decrease the pH from alkaline (~7) to acidic (~3) and increase the electric conductivity from cca. 100 to 3000 $\mu\text{S}/\text{cm}$.

The negative effect of acid mine drainages on terrestrial plant was studied by ecotoxicological test with seeds of ice-lettuce (*Lactuca sativa* L. var. Great Lakes 118). The toxicity of the high concentration of heavy metals in the AMD-affected water samples of Valea Roşie-Băiţa rivers, is visible on the plantlets roots. The apical meristem tissue becomes dark brown, and in many cases exhibits severe necrosis. In the same time, the ice-lettuce plantlets treated with the unpolluted Nistru and Valea Roşie spring waters were fresh green, and showed no negative response. The toxic cations e.g. Al, Zn and Cu might inhibit the germination and the metabolism of enzymes by connecting S ions to the proteins.

The ecotoxicological test performed with duckweed (*Lemna minor* L.) as bioindicator of water quality revealed the effects of the acid mine drainage on aquatic organisms. The consequences of the toxicity induced by the polluted Valea Roşie-Băiţa were visible by root meristem damaging, growth retardation, leaves chlorosis and plant death. Similar to the response of ice-lettuce seeds, the duckweed cultivated in the unpolluted Nistru and Valea Roşie spring water samples were fresh green, and showed no negative response.

Our results show that despite the large size and mineral/ chemical-composition of the Nistru Old Spoil, the area of Nistru, in particular the Nistru river waters are not much influenced by the ceased mining activity. The same hold true for the springs of the Valea Roşie river.

By contrast, the waters of both Valea Roşie (downstream the AMD inflows from the active mine), and the Băiţa river (which takes the Valea Roşie waters) show highly contaminated features, with some of the heavy metals content exceeding the national limits.

The quality of these water might negatively influence the germination and grown of the terrestrial and aquatic plants.

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