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Metagenomic Insight into the Microbial Diversity of the World Unique Lorandite Mine Allchar

Vaso Taleski[1], Ivica Dimkić[2], Blazo Boev[1], Ivan Boev[1], Sanja Živković[3], Slaviša Stankovic[2]

[1] University "Goce Delchev" Shtip, North Macedonia

[2] University of Belgrade, Faculty of Biology, Serbia

[3] University of Belgrade, Institute of Nuclear Sciences, Vinča, Serbia

Key words in this talk:

- Allchar (mine, deposit ore)
- Thallium
- Lorandite
- Neutrino
- Metagenomics & Diversity (bacterial and fungal)

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Librazhd
Struga
Allchar deposit
Gevgelija
Lake Kerkini
Elbasan
Shkumbin
Prrenjas
Lake Ohrid
Ohrid
Resen
Bitola
Lake Prespa
Crna
Medzitlija
Pogradec
Gramsh
Edessa
Florina
Devoll
Lake Vegoritida
Small Prespa Lake
Maliq
Korce
21
22
23
42
41
Republic of North Macedonia
International Boundary
Road
River
National Capital
City or Town
0 10 20 30 KM
0 10 20 30 Miles
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Allchar history:
A myth or truth?

THE SECRET POWER OF ALEXANDER THE GREAT?

Published on June 29, 2015

Christopher MacDonald

LONDON

Allchar history (truth):

- Others estimates that mine was known from 12 or 13 century.

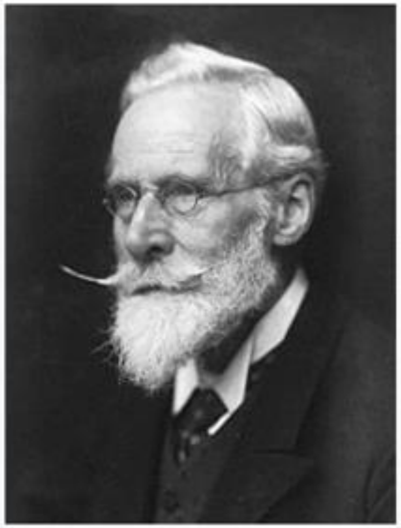
- In documents of Ottoman's Empire mine is mentioned from 1481

– Sale of **arsenic** made great income for Turkish sultan.

- Deposit was mined extensively between 1881 and 1908 for: **Antimony, Arsenic and Thallium,** when British-French company got a concession from Ottoman’s Empire.

Thallium is a chemical element with atomic number 81. that is **not found free in nature**.

Chemists William Crookes and Claude-Auguste Lamy discovered thallium independently in 1861.



Sir William Crookes

Born	17 June 1832 London, England, UK
Died	4 April 1919 (aged 86) London, England, UK

Claude Auguste Lamy

Born	15 June 1820 Ney, Jura, France
Died	20 March 1878 (aged 57) Paris, France

Thallium

Pronunciation	/ˈθæliəm/ (THAL-ee-əm)
Appearance	silvery white
Standard atomic weight $A_{r,\ std}$(Tl)	[204.382, 204.385] conventional: 204.38

Thallium in the periodic table

mercury ← thallium → lead

Atomic number (Z)	81
Group	group 13 (boron group)
Period	period 6
Block	p-block
Element category	Post-transition metal
Electron configuration	[Xe] $4f^{14}\ 5d^{10}\ 6s^2\ 6p^1$

The Periodic Table

1 H																	2 He
3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba	57-71	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
87 Fr	88 Ra	89-103	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg	112 Cn	113 Nh	114 Fl	115 Mc	116 Lv	117 Ts	118 Og
		57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu	
		89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr	

Thallium in this story?

- Thallium and its compounds are **extremely toxic**.

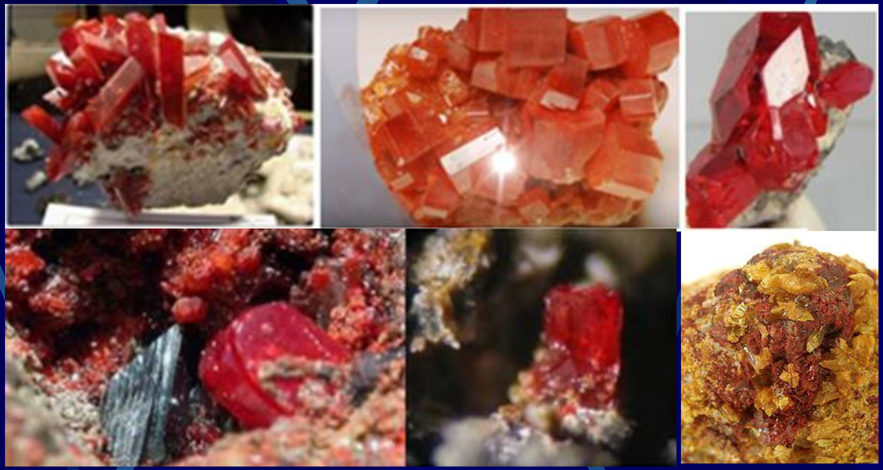
There are numerous recorded cases of fatal thallium poisoning.

- Thallium has **25 isotopes** whith **atomic masses** in range from **184 to 210.**

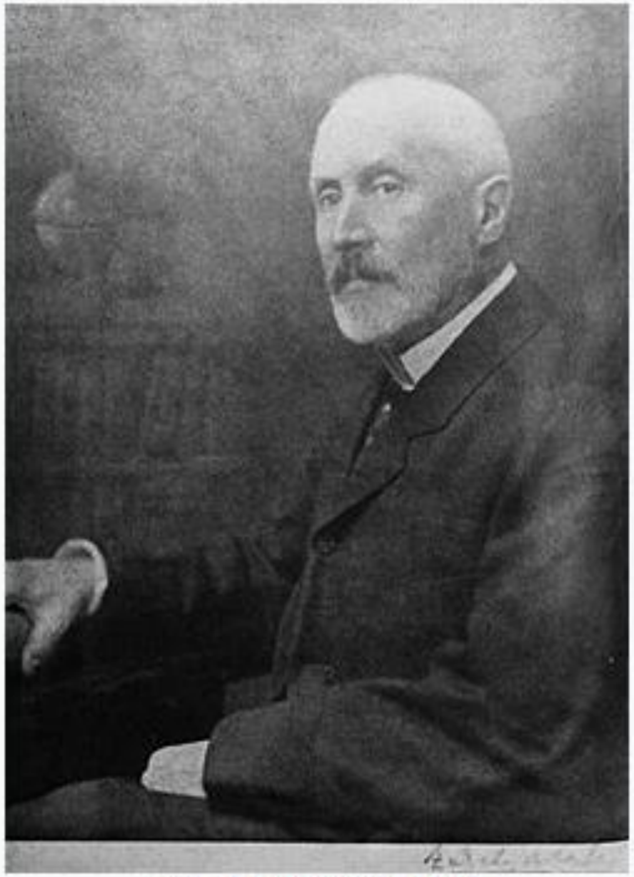
- **^{203}Tl and ^{205}Tl are the only stable isotopes and make up nearly all of natural thallium.**

From 47 worldwide registered **minerals** containing **thallium (Tl)**, **13 exists in Allchar**:

- Bernardite,
- Fangite,
- Jankovicite,
- **Lorandite (TlAsS2)**
- Parapierrotite,
- Pictopaulite,
- Raguinite,
- Rebulite,
- Simonite,
- Thalliumpharmacosiderite,
- Weissbergite,
- Vrbaite,
- Dollcharite.



Lorándite was first discovered at the **Allchar deposit**, in **1894** by **Krener**, and named after **Loránd Eötvös**, a prominent Hungarian **physicist**.



Loránd (Roland) Eötvös

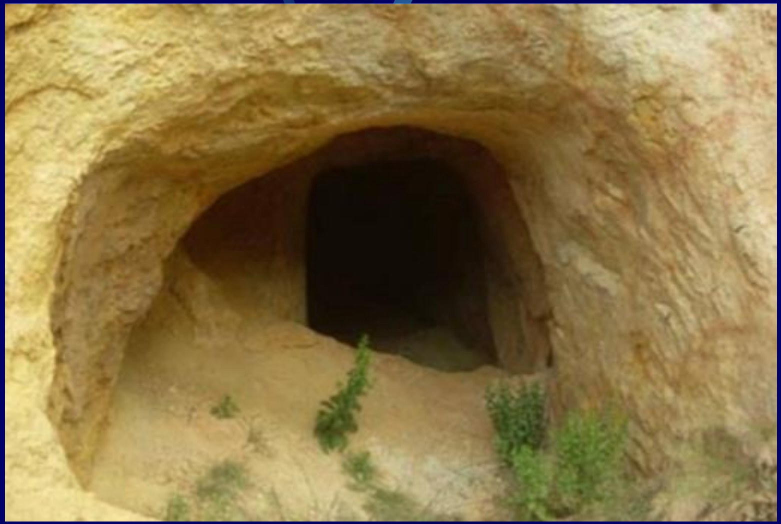
Born	27 July 1848 Buda
Died	8 April 1919 (aged 70) Budapest

Allchar is as a unique mine in the **world:**

- **Richest in the world-** deposits of **lorandite**

The “Crven Dol”, holds estimated amount of **500 t** of Tl.

- **Purest lorandite in the world** (contains only traces of K, Cr, Fe, Cu, Pb and Zn).

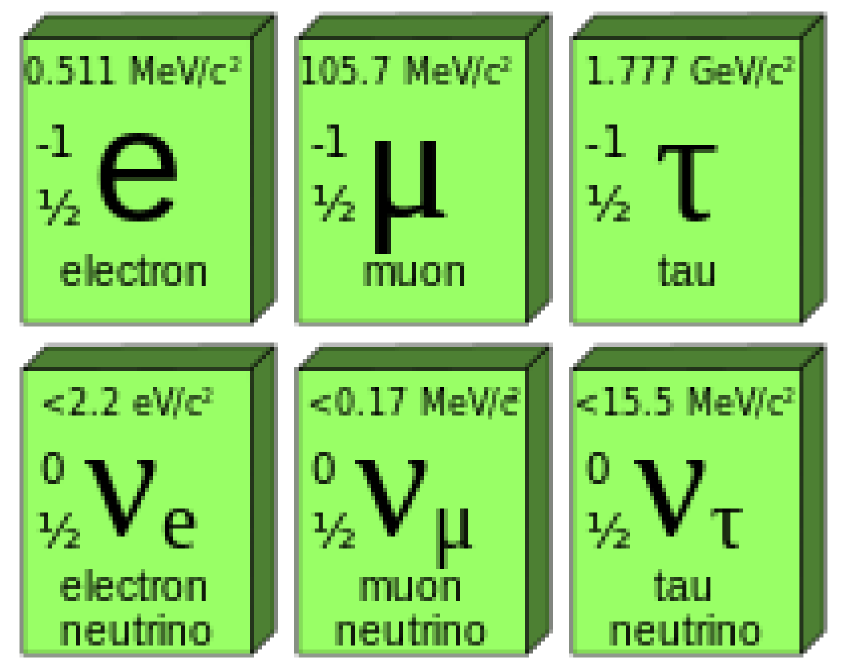


Entrance to “Crven Dol”, part of Allchar complex

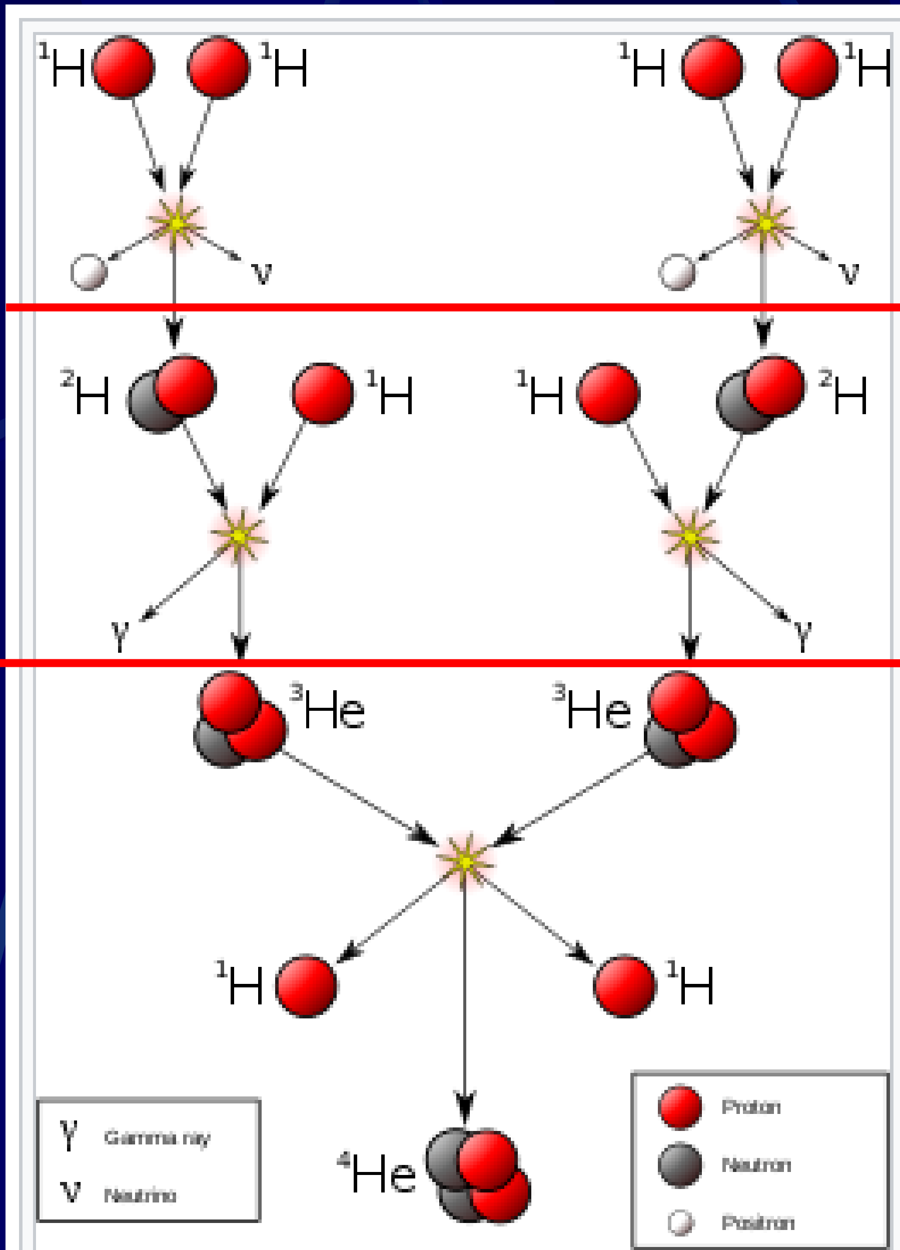
Neutrinos (V) in this story?

Three kinds:

- Electron neutrinos (Ve)
- Muon neutrinos (Vμ)
- Tau neutrinos (Vτ)



Six flavours of leptons



Fusion of Hydrogen to Helium

1 billion years

4 seconds

400 years

Electron (solar) neutrinos travel

149,597,870 km

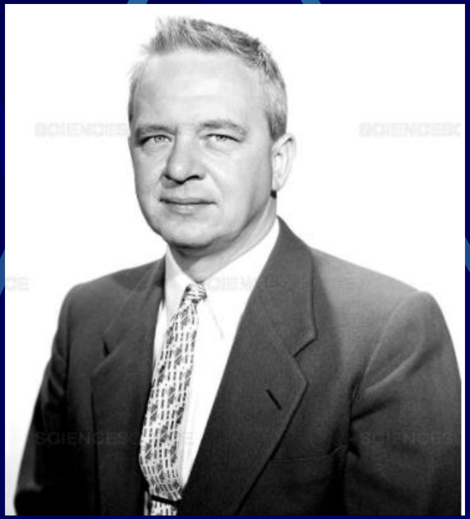
8 min 20 sec

2,3 sec

At the surface of the Earth, the flux is about 65 billion solar neutrinos, per second per square centimeter.

In 1956, Frederick **Reines** and Clyde **Cowan** detected neutrinos and prove their existence.
Both, awarded with Nobel prize in Physics 1995.

Frederick Reines
(1918 – 1998)



Clyde Cowan
(1919 – 1974)

Neutrinos:

They are impossible to see and incredibly difficult to detect, because they are:

- **neutral, has no electrical charge,**
- **has a very small mass, which might even be zero and**
- **have very little interaction with matter.**

Detection techniques

Scintillators

Radiochemical methods

Cherenkov detectors

Radio detectors

Tracking calorimeters

Coherent Recoil Detector

Neutrino detectors

- Different reactions using Gallium (**40 t**), Lithium, Manganese etc.

- Chlorine atoms transfers to Argon (**100.000 gallons** of liquid chlorine, in 1 week produces: expected 10 atoms of Argon observed 3 atoms of Argon.

- The Super-K, 1,000 m underground in the Mozumi Mine in Hida's Kamioka area, consists of a cylindrical stainless steel tank that is 41.4 m tall and 39.3 m in diameter holding **50,000 tons of ultrapure water**.

Neutrino telescopes

Underwater neutrino telescopes:

- DUMAND Project (1976–1995; cancelled)
- Baikal Deep Underwater Neutrino Telescope (1993 on)
- ANTARES (2006 on)
- KM3NeT (future telescope; under construction since 2013)
- NESTOR Project (under development since 1998)
- "P-ONE". (prospective telescope; path finders deployed in 2018, 2020)

Under-ice neutrino telescopes:

- AMANDA (1996–2009, superseded by IceCube)
- IceCube (2004 on)[3][g]
- DeepCore and PINGU, an existing extension and a proposed extension of IceCube

Underground neutrino observatories:

- Baksan Neutrino Observatory, Russia, site of SAGE, GGNT and the future BLVSD.
- Gran Sasso National Laboratories (LNGS), Italy, site of Borexino, CUORE, and other experiments.
- Soudan Mine, home of Soudan 2, MINOS, and CDMS[19][h]
- Kamioka Observatory, Japan
- Underground Neutrino Observatory, Mont Blanc, France / Italy

His idea was to analyse thallium-containing lorandite from Allchar and examines the quantity of lead in it as a basis for calculation of the number of neutrinos which over the millennia have passed through the lorandite, enabling the calculation of the Sun's age.

Freedman M.S., Stevens C.J., Horwitz E.P., Fuchs L.H., Lerner J.L., Goodman L.S., Childs D.J., Hessler J.
Solar neutrinos: Proposal for a new test
Science, 193 (1976), pp. 1117-1118

neutrino-capture reaction $^{205}Tl + \nu_e \rightarrow {}^{205}Pb + e^-$

Estimated age of lorandite is around 10 million years, and predicted trapped ^{205}Pb concentration of 132 atoms per gram of lorandite.

It is estimated that, 10kg of lorandite contains about (3.5 – 11.6) x 10^5 atoms of ^{205}Pb.

Half life of ^{205}Pb is 15,3 million years.

LOREX (LORandite EXperiment) is long-term project (running between 2008 and 2010) and still actively pursued, using lorandite from Allchar, to determine the solar neutrino flux.

Science-fiction or reality?

ELSEVIER

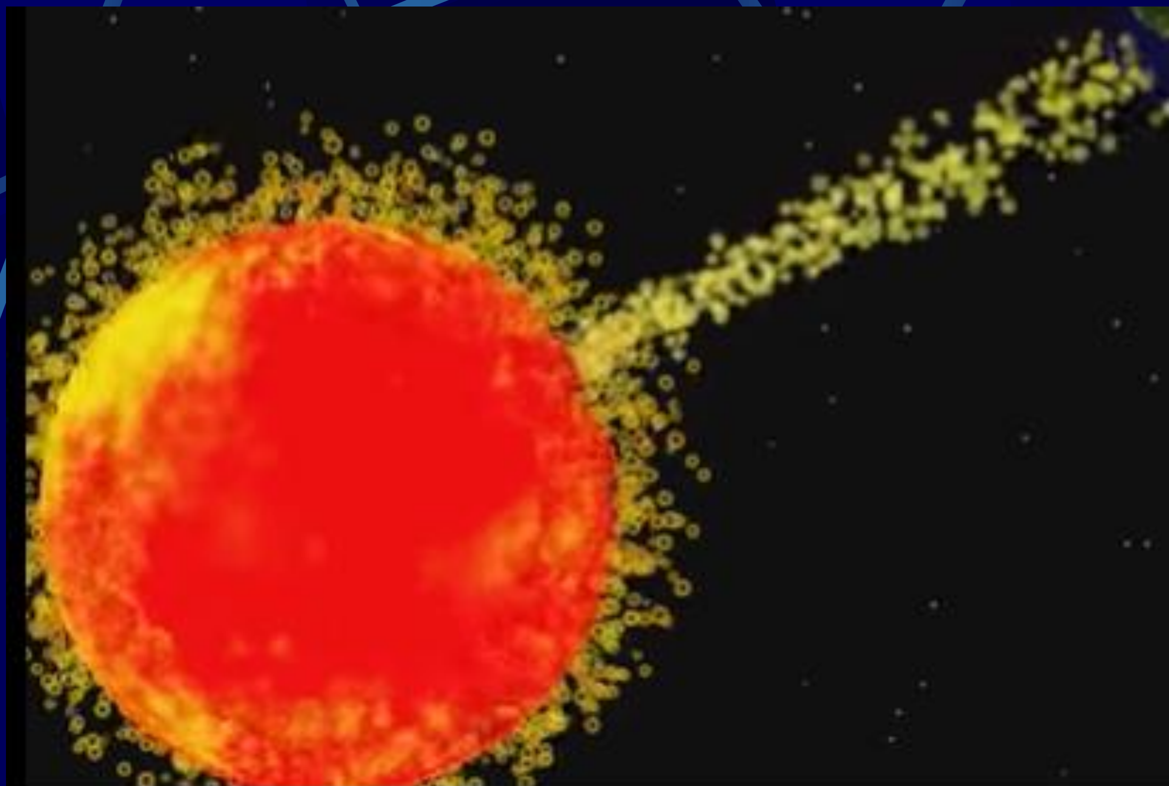
Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment

Volume 895, 1 July 2018, Pages 62-73

Lorandite from Allchar as geochemical detector for pp-solar neutrinos

Miodrag K. Pavićević [a], Georg Amthauer [a], Vladica Cvetković [b], Blazo Boev [c], Vladan Pejović [d], Walter F. Henning [e], Fritz Bosch [f, 1], Yuri A. Litvinov [f], Reinhard Wagner [a]

https://doi.org/10.1016/j.nima.2018.03.039



Project to Connect:
Sun – Earth – Neutrinos - Allchar – Lorandite – Thallium & Microbial diversity

Microbial diversity in various mine sites are connected with the environment conditions and **usually remain undervalued**.

The huge bio diversity and uncultivable nature of certain m.o. make it immensely difficult for accurate representation of microbial communities in a particular ecological niche.

The majority of microorganisms are **unculturable**, and therefore **culture-independent approaches** are sought to investigate the soil microbial communities.

Metagenomic analysis revealed significant microbial biomass and abundance changes in metal-contaminated sites without significant variations in microbial diversity (Feng et al. 2018).

Although heavy metals are **toxic** for various microorganisms, many **metal-tolerant microbes** inhabit metal-polluted environments.

Several authors identified **arsenic-resistant bacteria** (Katsnelson 2010; Wolfe-Simon et al. 2011; Cressey 2012; Erb et al. 2012; Scheirmeier 2012).

Sklodowska and Matlakowska (2004) showed that bacteria isolated from heavy metal contaminated post-flotation and smelt wastes were **resistant to Tl at very high concentrations**, while

Bao et al. (2006) isolated nine groups of **Tl-tolerant culturable Alphaproteobacteria** from heavy metal polluted river water.

Metagenomics is a revolutionary concept in the aspect of studying microbial bio diversity, their adaptation to the ecological niches and their evolution.

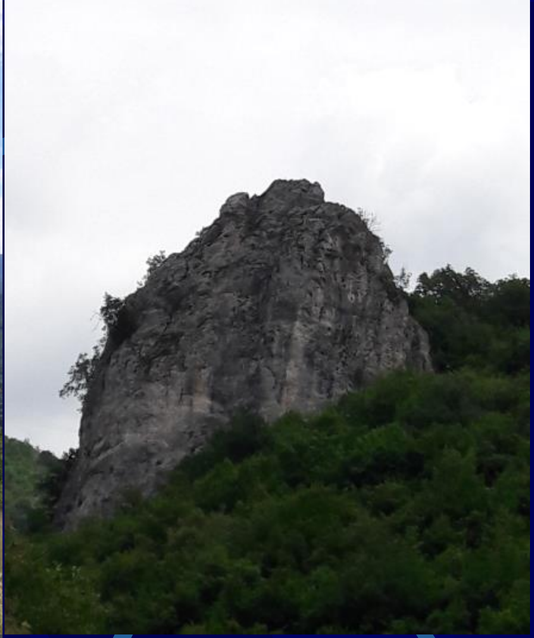
Metagenomic data are obtained by high-throughput **sequencing** of environmental samples provides an aggregation **of all the genetic materials** of the studied environment.

This strategy **overcomes** the problems associated with conventional molecular methods of retrieving genetic information for a particular environment.

High throughput **bioinformatic analysis enables the accurate exploration of a gene of interest**.

The geological and geobiochemical environment, metagenomic approaches have enabled straight forward **investigation of the microbiome in deep mining deposits** (Turnbaugh, *et al., 2007,* Brazelton *et al*., 2012).

Some studies have also provided **novel genes, metabolic processes, the evolutionary history of the dwelling microorganisms, the mechanism of their metal tolerance and solubilation abilities** (Delmont *et al*., 2011, Li *et al*. 2015).



Allchar – Kozuf mountain

- 110 km from Skopje to Kavadarci
- 40 km off-road from Kavadarci
- 1 h walking

June 23rd 2018

Dangerous and…

Very exciting

Entrance– “Crven Dol” Allchar

Entrance – “Crven Dol” Allchar
















New flora and fauna???

MATERIAL

Samples were collected on June 23rd 2018 at the location "Crven Dol", at mine Allchar.

Sampling sites were divided on three areas :

1. **Floor** (water present, sample VT1)
2. **Side Beams** (with biofilm, sample VT2) and
3. **Side walls** of the tunnel (sample VT3).

Samples were collected from all areas **in triplicates.**

Tunnel at CRVEN DOL (Allchar)

Tunnel at CRVEN DOL (Allchar)

Supporting beams

Tunnel walls – Crven Dol Allchar

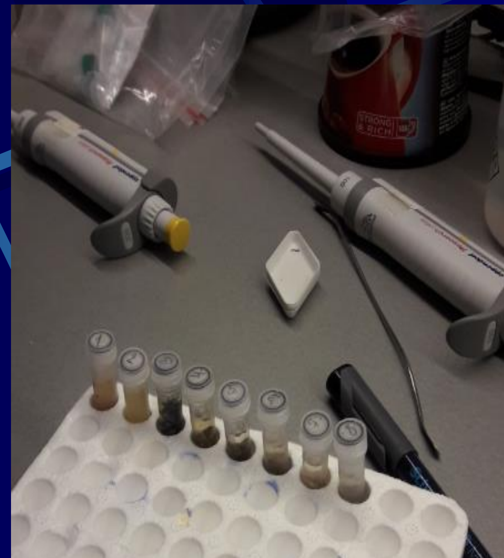
Sampling

Sampling

Sampling

June 27th 2018


ZYMO RESEARCH
ZymoBIOMICS™ DNA Miniprep Kit
(50 preps.)
Catalog No: D4304
Storage: Room Temperature
Lot No: ZRC200778




CH-100
Heating/Cooling Dry Block
Digital


TIME (MIN)
SPEED (RPM)
20
2440
SPEED ALARM
START STOP
DISRUPTOR GENIE
Digital
Scientific Industries


ZYMO RESEARCH

METHODS

1. DNA extraction, library preparation and NGS sequencing

- The extraction of ultra-pure DNA was done using the **PowerSoil® DNA Isolation Kit** (MO BIO Laboratories, Inc., Carlsbad, USA) in triplicates, following manufacturer's protocol.

- **Total DNA from each replica was pooled into single sample for each sampling site.**

1. DNA extraction, library preparation and NGS sequencing

- **The DNA yield of analyzed samples was measured using Qubit Fluorometric Quantitation** (Qubit 4 Fluorometer, Invitrogen™, USA).

- The amplicons were amplified following the target gene Metagenomic Sequencing Library Preparation **Illumina protocol** (Cod. 15044223 Rev. A).

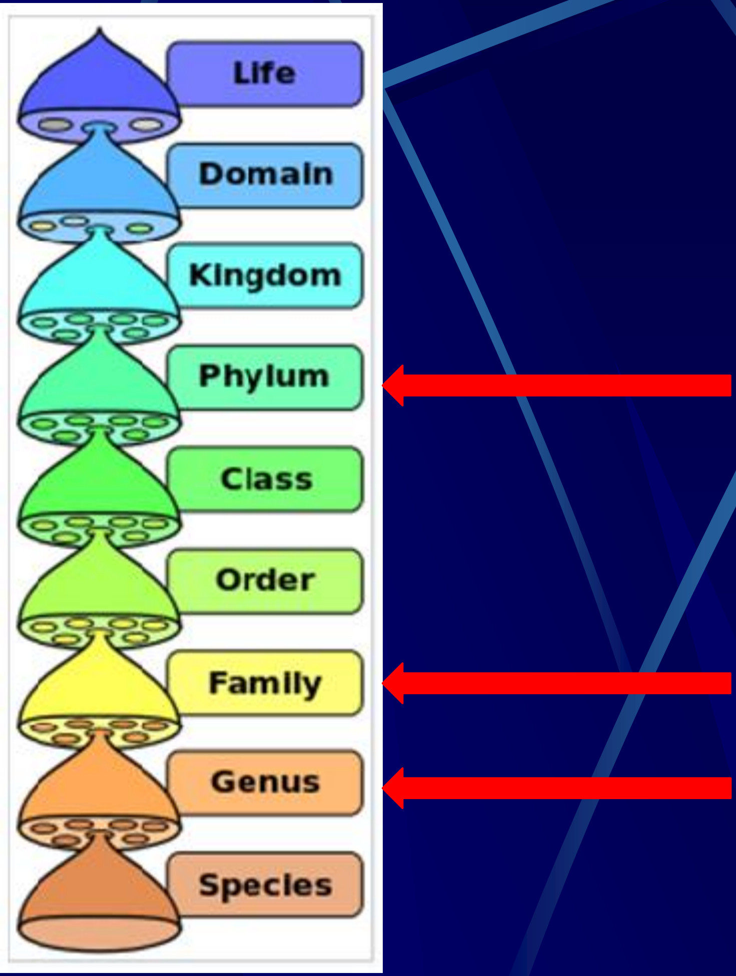
- The gene-specific sequences used in **this protocol target the 16S rRNA gene V3 and V4 region**, with the defined primers for 16S forward (5′-CCTACGGGNGGCWGCAG-3′) and reverse sequences (5′-GACTACHVGGGTATCTAATCC-3′).

2. Sequence data process and taxonomy annotation

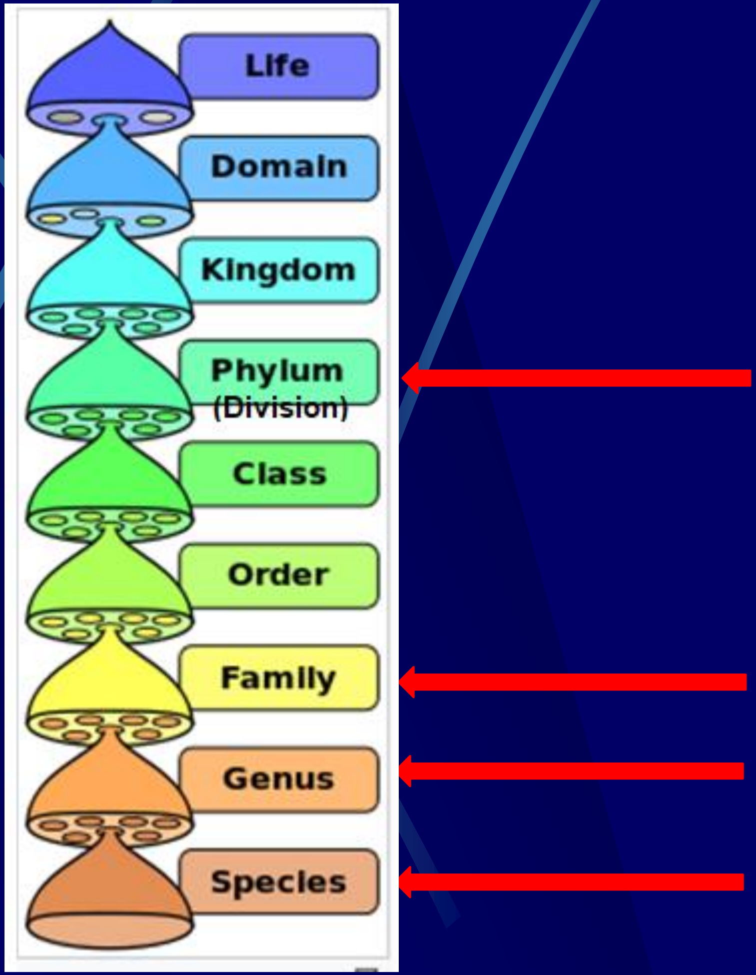
Quality assessment was performed by the use of ***prinseq-lite*** **program.**

Taxonomic annotation tables are summarised using **Krona** tool as an **interactive viewer** for taxa distributions by samples.

3. Bioinformatic analysis



Bacteria



Fungi

4. Sample preparation for ICP-OES analysis (inductively coupled plasma optical emission spectrometry)

According to the **EPA Method** 3051, using Thermo Scientific™ iCAP™ 7400 ICP-OES analyser.

Results

ICP-OES sample analysis

Results given in table 3, showed content of 25 elements and high concertation of As and Tl.

Table 3. ICP-OES analysis of three samples of the solid texture from the mine

Concentration (mg/kg)	VT1	VT2	VT3
Al	4600 ± 470	4300 ± 450	2700 ± 300
Sb	6.6 ± 0.3	5.2 ± 0.2	0.8 ± 0.1
As	7500 ± 300	3300 ± 130	1100 ± 50
Ba	112 ± 5	684 ± 30	21.9 ± 0.9
Be	5.6 ± 0.3	0.9 ± 0.1	5.4 ± 0.3
B	27 ± 3	39 ± 5	13 ± 2
Cd	<2	<2	<2
Ca	63000 ± 3000	72000 ± 3000	60000 ± 3000
Cr	11.2 ± 0.4	11.9 ± 0.5	13.3 ± 0.5
Co	27.0 ± 1.0	7.1 ± 0.3	35.4 ± 1.4
Cu	27.4 ± 1.2	15.6 ± 0.7	25.6 ± 1.1
Fe	33000 ± 1500	9600 ± 500	36900 ± 2000
Pb	23.4 ± 0.9	25.3 ± 1.0	23.3 ± 0.9
Mg	18000 ± 1000	7900 ± 400	19600 ± 1000
Mn	4400 ± 200	1700 ± 100	4600 ± 200
Hg	2.9 ± 0.2	2.8 ± 0.2	< 1
Mo	9.1 ± 0.4	2.6 ± 0.1	9.4 ± 0.4
Ni	77 ± 3	41 ± 2	89 ± 4
Se	< 0.2	< 0.2	< 0.2
Ag	< 1.4	< 1.4	< 1.4
Tl	276 ± 11	222 ± 9	70 ± 3
Ti	31.0 ± 1.4	43.0 ± 1.9	8.3 ± 0.4
V	33.3 ± 1.3	17.3 ± 0.7	29.0 ± 1.2
Zn	129 ± 5	98 ± 4	132 ± 5
Sr	82 ± 4	253 ± 12	29 ± 2

EPA Method 3051a: Microwave assisted acid digestion of sediments, sludges, soils, and oils

Diversity of microbial communities

Biodiversity measures of obtained taxa trough microbial richness and alpha diversity indexes.

	Phylum/Family/Genus - bacteria								
	Shannon	Simpson	invSimpson	FisherAlpha	OBS*	CHAO1	CHAO1.SE	ACE	ACE.SE
VT1_16S	2.10	0.79	4.82	3.73	37.00	37.33	0.92	37.88	2.68
	4.26	0.97	32.10	40.84	307.00	324.40	8.41	331.70	8.38
	4.56	0.97	33.96	109.29	714.00	870.20	34.83	850.25	14.55
VT2_16S	2.04	0.81	5.16	2.65	26.00	27.50	2.58	27.66	2.24
	4.12	0.97	33.70	27.28	204.00	219.55	9.45	217.59	6.69
	4.51	0.97	39.75	64.18	425.00	470.38	15.38	471.16	10.58
VT3_16S	1.97	0.78	4.50	2.84	28.00	28.00	0.25	28.58	1.98
	3.93	0.96	26.14	27.56	209.00	217.05	5.15	221.73	6.90
	4.18	0.96	28.18	56.52	388.00	441.33	17.38	445.95	10.51

	Phylum/Family/Genus/Species - fungi								
	Shannon	Simpson	invSimpson	FisherAlpha	OBS*	CHAO1	CHAO1.SE	ACE	ACE.SE
VT1_ITS	1.08	0.61	2.58	0.55	7.00	7.00	0.00	7.00	0.93
	3.26	0.94	16.28	22.28	202.00	205.24	2.83	207.52	6.58
	3.36	0.94	16.60	41.84	353.00	369.00	7.45	370.86	9.39
	3.28	0.91	11.43	70.33	514.00	546.41	11.24	543.23	11.40
VT2_ITS	0.66	0.35	1.53	0.48	6.00	6.00	0.00	NA	NA
	2.08	0.77	4.31	12.02	112.00	148.25	17.83	154.49	7.30
	2.14	0.77	4.34	17.82	159.00	206.30	19.02	211.38	7.66
	1.65	0.62	2.61	24.88	206.00	286.50	25.79	315.27	10.21
VT3_ITS	0.95	0.55	2.23	0.49	6.00	6.00	0.00	6.00	0.91
	1.85	0.71	3.42	7.96	76.00	83.20	6.44	82.33	4.23
	1.87	0.71	3.43	11.31	104.00	123.43	12.65	119.00	5.41
	1.38	0.52	2.10	15.51	133.00	154.43	11.33	152.77	6.13

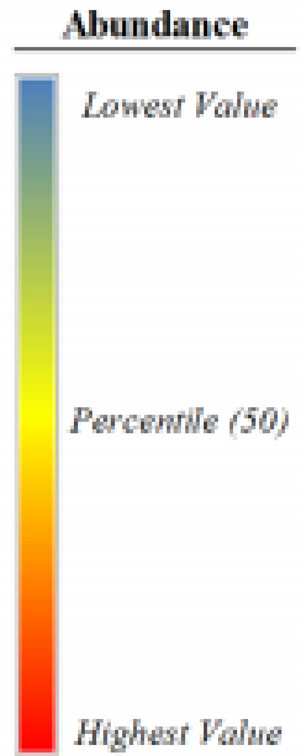
*OBS – observed species richness

Bacterial diversity

(A)

Phyla	VT1_16S	VT2_16S	VT3_16S
Proteobacteria	40.68	36.83	41.43
ud-Bacteria	12.60	9.74	14.05
Bacteroidetes	11.01	16.88	11.80
Actinobacteria	5.67	4.71	3.98
Acidobacteria	5.67	7.10	9.49
Parcubacteria	4.30	0.68	2.47
Verrucomicrobia	4.13	5.98	2.74
Chloroflexi	3.74	2.18	0.71
Planctomycetes	3.42	8.94	6.01
Firmicutes	2.43	2.04	0.12
Candidatus Saccharibacteria	1.94	0.80	2.46
Chlamydiae	1.53	1.51	1.44
Ignavibacteriae	0.50	0.00	0.00
Nitrospirae	0.46	1.59	1.32
Gemmatimonadetes	0.44	0.25	1.03

Samples



Relative abundance of bacterial taxa as assessed by 16S rDNA gene sequences on phylum level (A)

Scientific classification

Domain: **Bacteria**

Currently there are 29 phyla accepted by
List of Prokaryotic names with Standing in Nomenclature (LPSN)

- Acidobacteria
- Actinobacteria
- Aquificae
- Armatimonadetes
- Bacteroidetes
- Caldiserica
- Chlamydiae
- Chlorobi
- Chloroflexi
- Chrysiogenetes
- Coprothermobacterota[2]
- Cyanobacteria
- Deferribacteres
- Deinococcus-Thermus
- Dictyoglomi
- Elusimicrobia
- Fibrobacteres
- Firmicutes
- Fusobacteria
- Gemmatimonadetes
- Lentisphaerae
- Nitrospirae
- Planctomycetes
- Proteobacteria
- Spirochaetes
- Synergistetes
- Tenericutes
- Thermodesulfobacteria
- Thermotogae
- Verrucomicrobia

(B)

Families			
ud-Betaproteobacteria	5.07	2.78	4.01
Parcubacteria_genera_incertae_sedis	4.30	0.68	2.47
ud-Gammaproteobacteria	4.27	2.85	5.20
Planctomycetaceae	3.29	8.48	5.32
ud-Rhizobiales	2.77	3.09	3.15
Anaerolineaceae	2.62	0.32	0.25
Comamonadaceae	2.27	1.88	1.22
Gp6	2.10	1.70	3.39
ud-Alphaproteobacteria	1.99	3.11	2.78
Subdivision3_genera_incertae_sedis	1.98	1.79	1.22
Flavobacteriaceae	1.97	2.75	3.36
Saccharibacteria_genera_incertae_sedis	1.94	0.80	2.46
ud-Bacteroidetes	1.90	2.53	1.18
Sphingomonadaceae	1.80	1.36	1.45
Chryseolinea	1.72	1.14	1.99
Hydrogenophilaceae	1.68	0.00	0.21
Pseudomonadaceae	1.65	1.72	1.69
Chitinophagaceae	1.38	3.69	1.33
Opitutaceae	1.22	0.78	0.64
ud-Deltaproteobacteria	1.12	1.05	2.15
ud-Acidimicrobiales	1.06	0.47	1.47
Parachlamydiaceae	1.05	1.25	0.37
Moraxellaceae	0.99	0.00	0.05
Rhodobacteraceae	0.94	0.85	0.40
Geobacteraceae	0.92	0.04	0.04
Hyphomicrobiaceae	0.90	1.79	0.57
Helicobacteraceae	0.87	0.00	0.00
Desulfobulbaceae	0.85	0.00	0.00
ud-Proteobacteria	0.84	1.23	1.64
Rhodocyclaceae	0.80	0.22	0.38
ud-Myxococcales	0.79	1.20	1.61
ud-Bacteroidales	0.77	0.01	0.00
Carnobacteriaceae	0.76	0.00	0.00
Gp16	0.74	0.23	1.53
ud-Actinobacteria	0.74	0.35	0.49
Gp3	0.72	0.70	0.53
Xanthomonadaceae	0.65	1.69	2.46
Sphingobacteriaceae	0.55	0.64	0.24
ud-Sphingobacteriales	0.54	0.56	0.81
Prolixibacteraceae	0.53	0.01	0.01
Nocardioidaceae	0.53	0.40	0.15

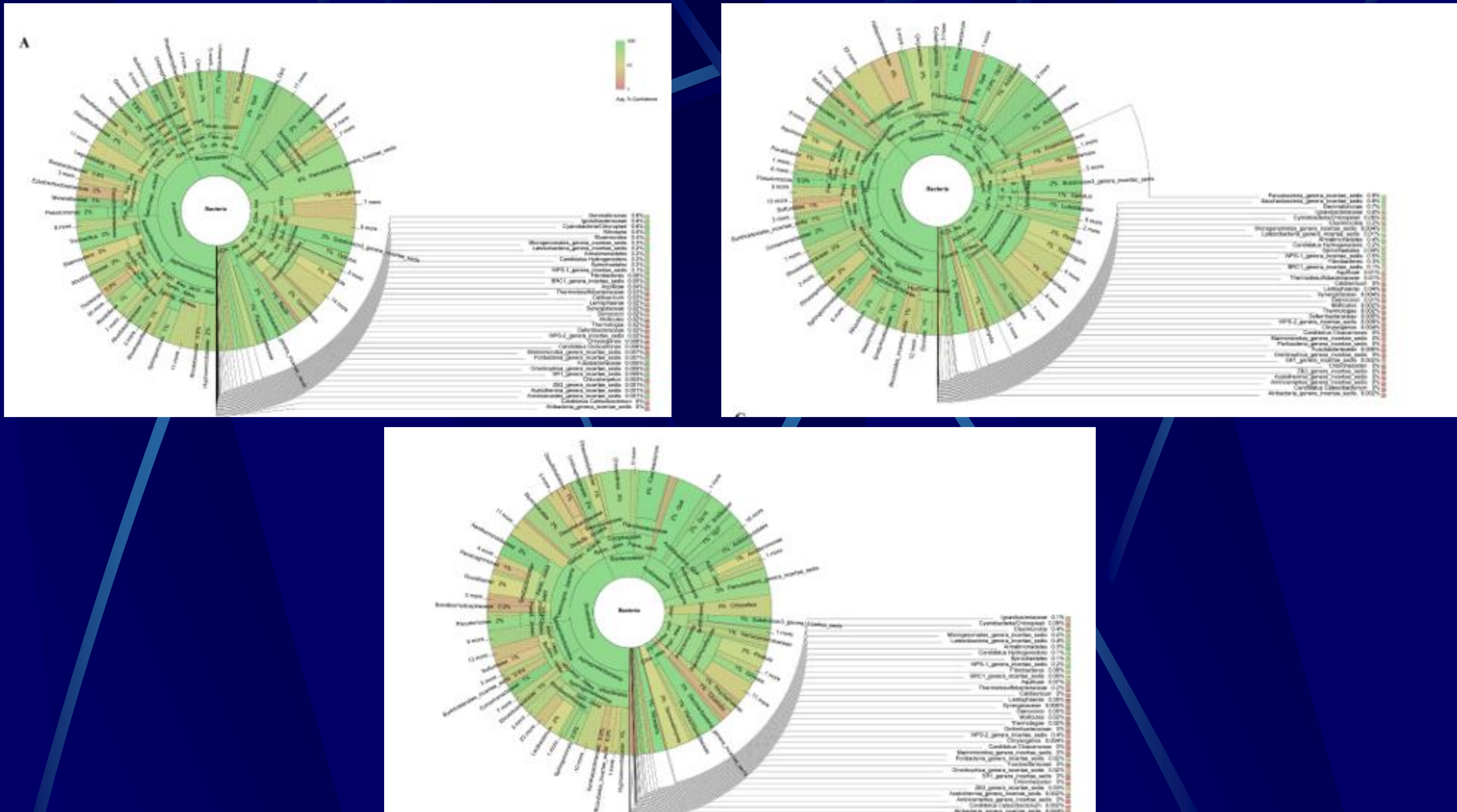
Abundance

Lowest Value

Percentile (50)

Highest Value

Relative abundance of bacterial taxa as assessed by 16S rDNA gene sequences on family (B) level.



Relative abundance of bacterial taxa at the genus level
across the VT1 (A), VT2 (B) and VT3 (C) samples

Fungal diversity

(A)

Phyla	VT1_ITS	VT2_ITS	VT3_ITS
Ascomycota	49.91	79.37	36.65
Basidiomycota	34.57	5.99	55.83
ud-Fungi	13.6	14.04	5.13
Zygomycota	1.16	0.12	1.65
Fungi_unidentified	0.63	0.43	0.73

Samples

Abundance
Lowest Value
Percentile (50)
Highest Value

Division
Ascomycota
Basidiomycota
Blastocladiomycota
Chytridiomycota
Glomeromycota
Microsporidia
Neocallimastigomycota
Zygomycota
Total: 8

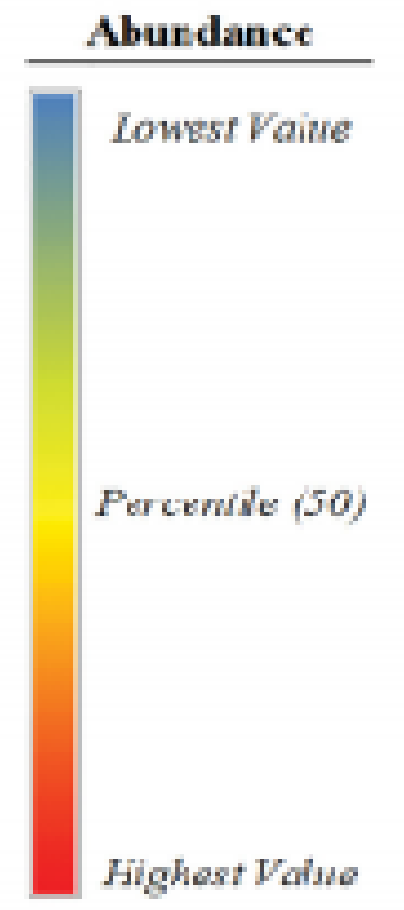
Phillum level

Fungal diversity

(B)

Families

	Ascomycota		
ud-Ascomycota	10.67	13.51	10.23
Incertae_sedis_2	8.64	0.65	1.70
Incertae_sedis_16	4.69	0.00	0.00
ud-Hypocreales	3.96	3.68	0.17
ud-Sordariomycetes	3.88	5.56	15.74
Ascomycota_unidentified	2.76	0.73	0.07
Microascaceae	2.43	0.48	0.03
Nectriaceae	2.13	4.73	0.32
Microascales_unidentified	1.37	0.43	0.11
Hypocreaceae	1.24	3.15	0.19
ud-Sordariales	0.86	0.60	0.04
Incertae_sedis_13	0.73	0.01	0.01
Sordariomycetes_unidentified	0.65	0.70	1.98
Pseudeurotiaceae	0.62	0.10	0.07
Helotiaceae	0.55	0.05	0.12
Xylariaceae	0.42	43.03	0.39
Lasiosphaeraceae	0.46	0.20	0.62
ud-Lectiomycetes	0.03	0.00	0.98
Herpotrichiellaceae	0.19	0.83	3.18
	Basidiomycota		
Strophariaceae	8.03	0.00	0.00
Psathyrellaceae	5.46	2.33	50.09
ud-Agaricomycetes	5.42	0.71	1.75
Bondarzewiaceae	4.24	0.00	0.34
Agaricaceae	3.69	0.74	2.76
ud-Basidiomycota	1.03	0.63	0.00
Agaricales_unidentified	1.48	0.04	0.00
Mycenaceae	0.91	0.01	0.13
Stereaceae	0.69	0.01	0.00
Trechisporales_unidentified	0.65	0.45	0.52
Physalacriaceae	0.10	0.71	0.00
	Zygomycota		
Mortierellaceae	0.60	0.11	1.62
Mucoraceae	0.51	0.01	0.00



Family level

Fungal diversity

(C)

Genera	VT1_ITS	VT2_ITS	VT3_ITS
Ascomycota			
Pilidium	8.15	0.00	0.00
Dendroclathra	4.67	0.00	0.00
Microascaceae_unidentified	2.43	0.48	0.03
Nectriaceae_unidentified	1.78	4.00	0.26
Phoma	0.62	0.00	0.00
Neobulgaria	0.55	0.04	0.12
ud-Hypocreaceae	0.53	1.36	0.18
Pseudogymnoascus	0.51	0.10	0.02
Rosellinia	0.41	43.02	0.39
Tetracladium	0.33	0.65	1.70
ud-Nectriaceae	0.32	0.62	0.05
Hypomyces	0.18	1.71	0.00
Herpotrichiellaceae_unidentified	0.04	0.04	3.16
Cladophialophora	0.02	0.67	0.02
Basidiomycota			
Hypholoma	8.03	0.00	0.00
Coprinellus	5.44	2.33	50.09
Heterobasidion	4.24	0.06	0.34
Coprinus	3.69	0.74	2.76
Mycena	0.81	0.01	0.13
Stereaceae_unidentified	0.63	0.01	0.00
Armillaria	0.00	0.71	0.00
Zygomycota			
Mortierella	0.60	0.11	1.62

Samples

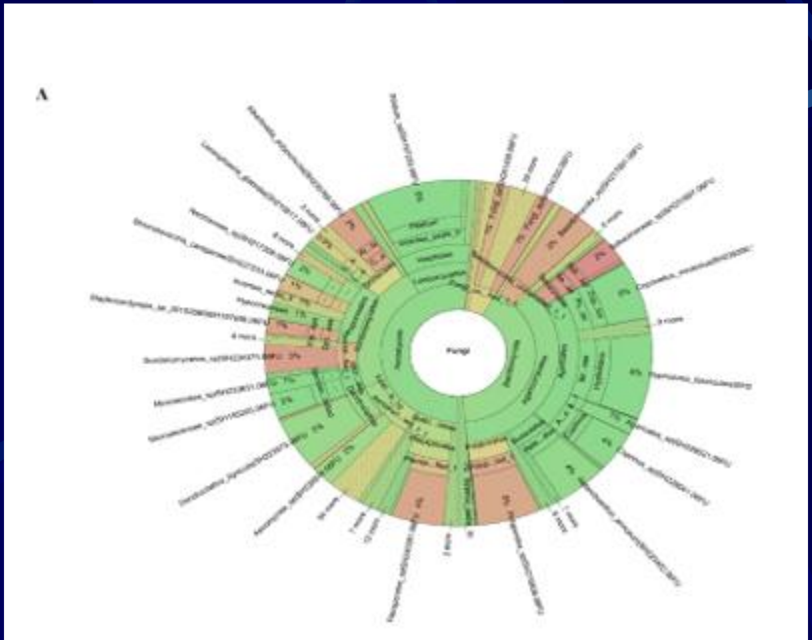
Abundance

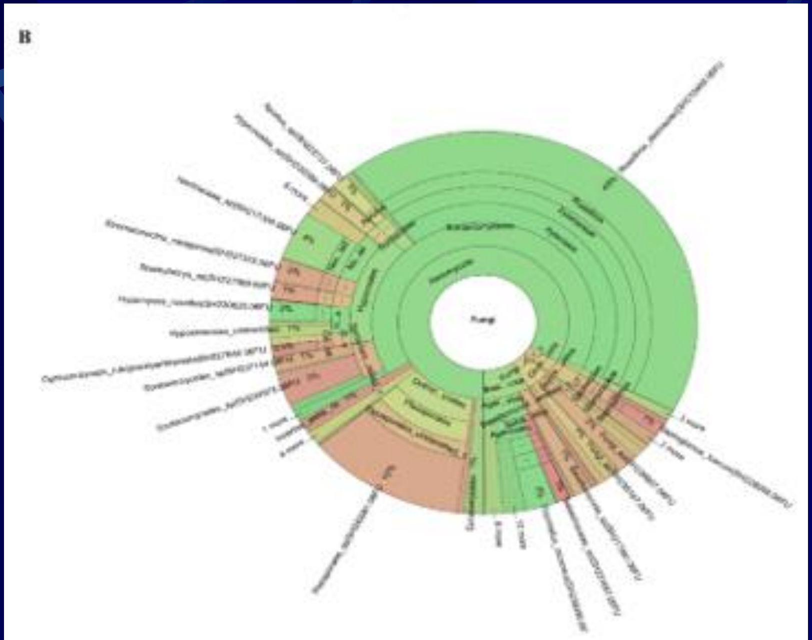
Lowest Value

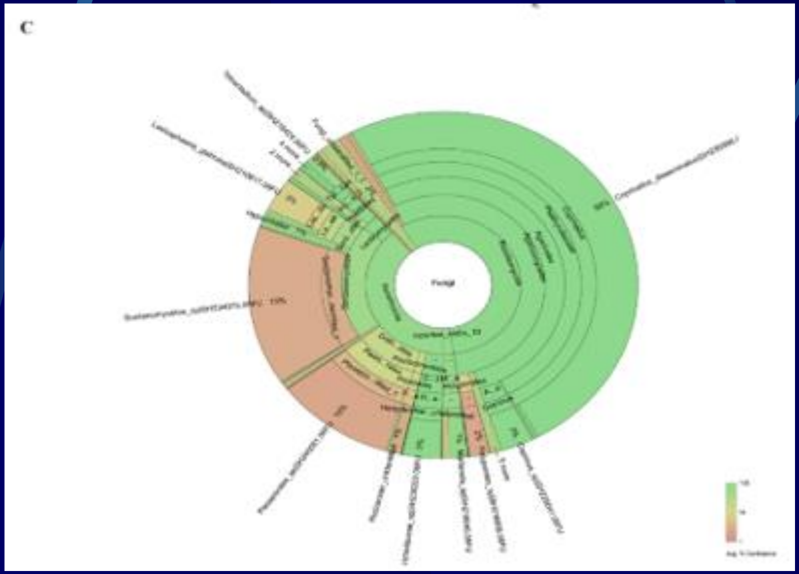
Percentile (50)

Highest Value

Relative abundance at genus level







Fungal taxa at the **species level**, according **Krona interactive viewer**

CONCLUSION

- This is the **first study on the microbial diversity** of Allchar mine

We confirmed presence of:

- **25 different elements** and **high concentration of As and Tl**
- **Huge bacterial and fungal diversity**

- The **metagenomics approach** allowed us to evaluate the community structure, diversity and microbial relationships in Allchar sites

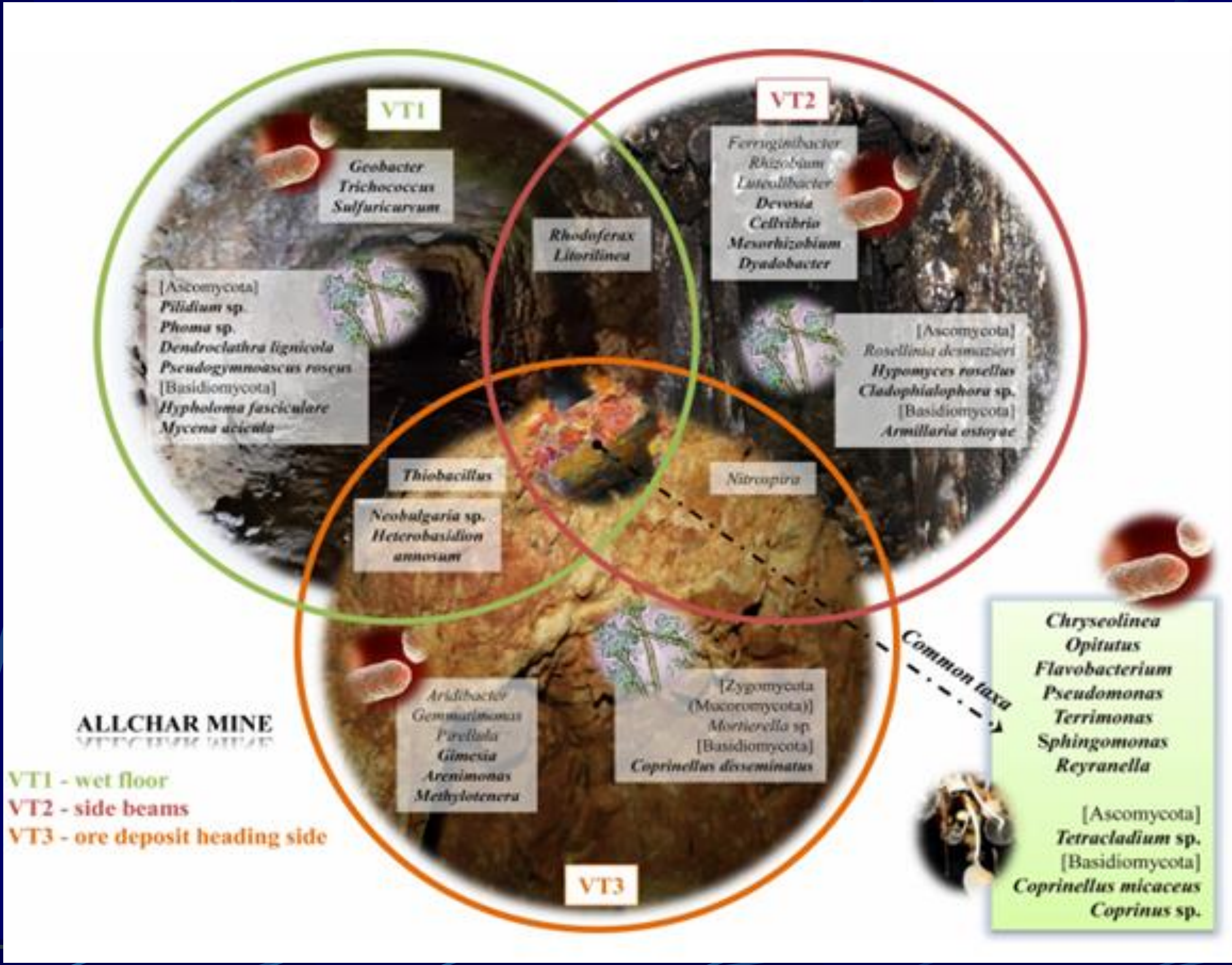
- Our results showed a **positive correlation** between the **diversity and richness** of the observed communities at all taxonomic levels

- We report, for the **first time**, the presence of **bacterial** genera such as *Trichococcus, Devosia, Litorilinea, Gimesia* and *Chryseolinea* or

- pathogenic **fungi** *Cladophialophora* sp., *Hipomyces rosellus* and *Rosellinia desmazieri.*

- This is the **first report** of **As- and Tl-tolerant *Pilidium* sp., *Neobulgaria* sp. and *M. acicula* sp.**

- This study revealed the **significance of geomicrobiology** in Allchar sites, indicating community resilience and their potential role in bioremediation strategies and industrial applications.



Venn diagram

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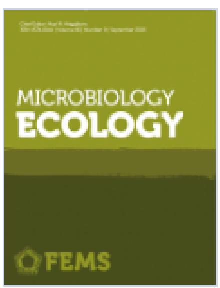

Volume 96, Issue 9

Bacterial and fungal diversity in the lorandite ($TlAsS_2$) mine 'Allchar' in the Republic of North Macedonia

Vaso Taleski ✉, Ivica Dimkić, Blazo Boev, Ivan Boev, Sanja Živković, Slaviša Stanković

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Allchar "Diversity research Team"

Vaso Taleski
Prof. d-r

Ivica Dimkic
Associate Research Prof.

Ivan Boev
Assistant prof.

Blazo Boev
Academic
Prof. d-r

Slavisa Stankovic
Prof. d-r

Sanja Zivkovic
Research Associate


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