ASSESSMENTS OF THE CONTENT OF 21 ELEMENTS IN BRYOPHYTE SPECIES (*Hypnum cupressiforme*, *Scleropodium purum* AND *Camptotecium lutescens)* FOR ENVIRONMENTAL POLLUTION IMPACT OF LEAD-ZINC FLOTATION PLANT

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MOSS Biomonitoring – real measurement or semi quantitative approach?

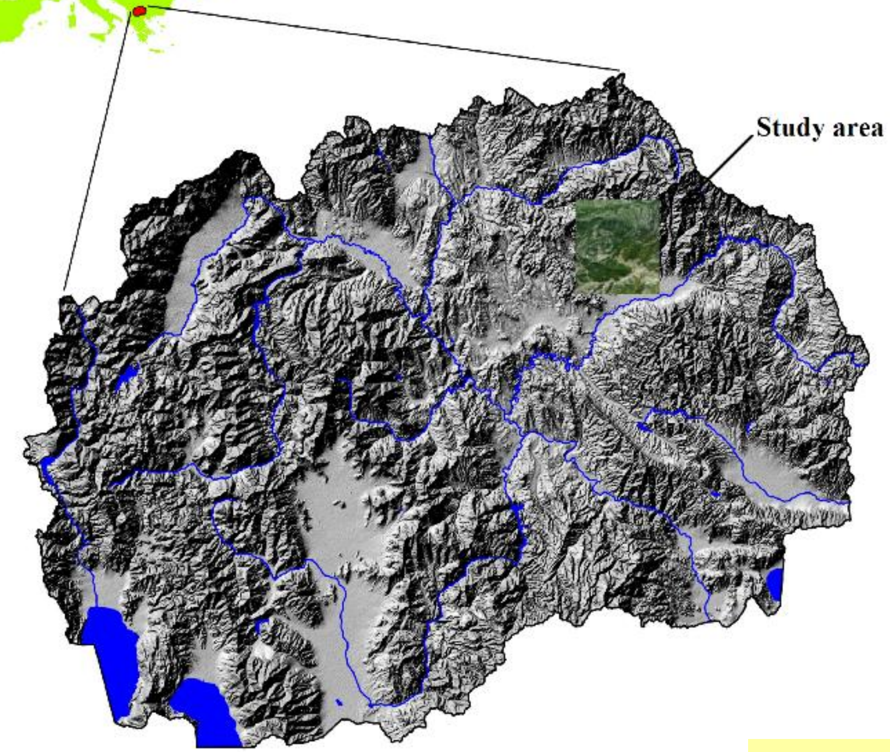
- “How well" they can measured?
- How much measurable information their measurement scale can provide?
- “Type of measurement scale“ – efficiency in small scale?
- Statistical multivariate approach for quantified values for elements contents in moss species (interchangeable using of FA; PCA and CA)

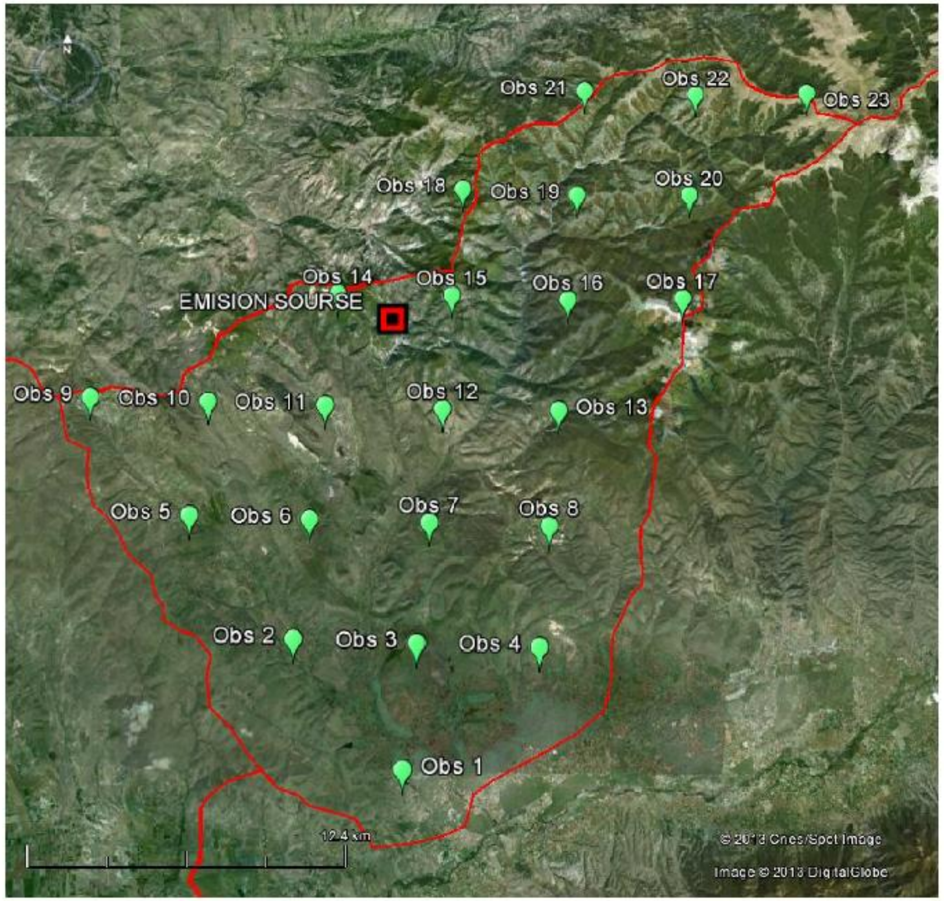
AIMS AND SCOPES IN THE INVESTIGATION

The investigated area (Pb-Zn mine environ)

The Pb-Zn mine is located 5 km NW from the Zletovo village and about 7 km from the city of Probistip

Geographic coordinates:
N: 41°51'– 42°08'
E: 22°01' – 22°25'





<u>The Zletovo Pb–Zn mine</u> have annual capacity of 300,000 tons **(9% Pb and 2% Zn)** and significant concentrations of Ag, Bi, Cd, and Cu

CHARACTERISTIC MOSS SPECIES FOR THE TERRITORY OF THE R. MACEDONIA (Eastern part-Probishtip region)

Hypnum cupressiforme
(Hedw.)

Camptothecium lutescens
(Hedw.) B.S.G.

Scleropodium purum
(Hedw.) M. Fleisch.

Dominant species with the 39% for each specie

Sampled 22 % from all collected moss species

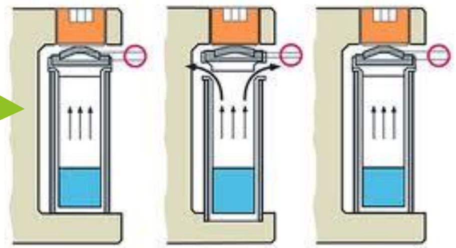
Sampling and sample preparation

- Digestion method was performed in to two steps for total dissolving of moss tissue using closed-pressure attack digestion:

Step	Ramp to: Temperature/ºC	Hold: Time/min	Absorbed Power/W	Applied Pressure/bar
1	180	5	500	20
2	180	15	500	20

cleaned moss tissue

Microwave digestion vessels-using the pressure and temperature control



0.5 g of moss samples
5ml concentrated HNO_3 and
2ml H_2O_2 (30%, m/V) were added

Microwave digestion operating system
CEM, model Mars

Instrumental techniques

- Inductively coupled plasma atomin emission spectrometer, **ICP-AES** (Varian, 715ES), for Al, Ba
  Ca, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sr, V, Zn;

- Electro-thermal atomic absorption spectrometer, **ETAAS** (Varian, SpectrAA 640Z) was applied for analysis of As, Co, and Cd;

The QC/QA of the applied techniques was performed by
standard addition method:

recovery for the investigated elements ranges:
for ICP-AES 98.5–101.2%,
for ETAAS 96.9–103.2%.

Basic statistics

Element	Dis	X_a	Md	Range
Ag	log	0.12	0.09	0.03 - 0.4
Al	log	3925	3969	1530 - 7169
As	log	2.35	1.67	0.5 - 8.35
Ba	log	68.4	58.8	21.3 - 226
Ca	log	10871	10585	3708 - 17838
Cd	log	0.31	0.21	0.08 - 1.73
Cr	N	3.47	3.44	0.51 - 7.43
Cu	log	9.28	6.82	4.11 - 21.4
Fe	N	3795	3624	1345 - 8269
K	log	3850	3490	2231 - 7178
Li	log	2.01	1.99	0.604 - 3.62
Mg	log	2127	1602	1130 - 4367
Mn	N	180	169	55.5 - 376
Mo	log	0.23	0.18	0.07 - 0.6
Na	log	45.7	44.4	22.8 - 78.2
Ni	N	4.07	3.75	0.94 - 11.1
P	log	1499	1511	597 - 2930
Pb	log	33.8	15.4	4.01 - 200
Sr	log	44.3	36.5	18.6 - 123
V	N	8.20	8.11	2.16 - 15.8
Zn	log	46.5	33.8	12.8 - 186

NORMAL distributions for Cr, Fe, Mn, Ni, and V

Plant-biogenic elements: **Ca, K, Na, P, Sr,** undergoes with the contents of macro and micro element nutrients in moss tissue.

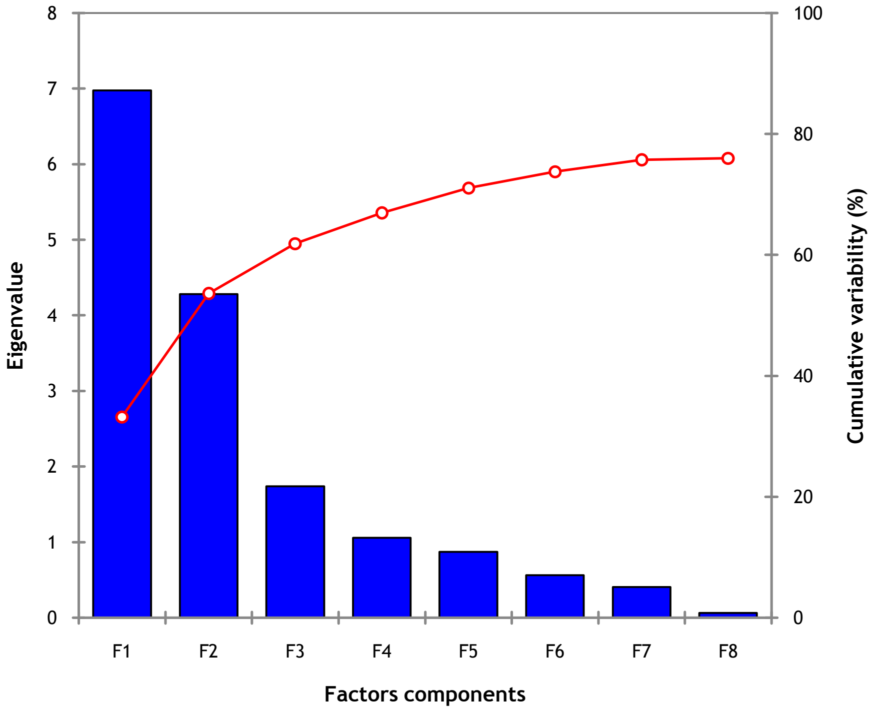
The contents for the lithogenic elements **Al, Cr, Fe, Mo, Ni-** relays on the geology of the region.

Significant enrichments found for As, Cd, Pb and Zn

Factor analysis

Kaiser criterion

	F1	F2	F3	F4	F5	F6	F7	F8
Eigenvalue	**6.98**	**4.28**	**1.74**	**1.06**	0.87	0.56	0.41	0.06
Variability (%)	33.2	20.4	8.28	5.05	4.15	2.69	1.93	0.31
Cumulative (%)	33.2	53.6	61.9	66.9	71.1	73.8	75.7	76.0



Scree plot for Eigenvalues

How many factors to retain?

Find the place where the smooth decrease of eigenvalues appears to level off to the right of the scree plot (as graphical method)

Factor analysis-varimax rotation

Element	Before Varimax rotation		After Varimax rotation	
	F1	F2	D1	D2
Ag	**0.78**	0.49	**0.94**	-0.09
Al	0.45	**-0.68**	0.05	**0.85**
As	**0.49**	**-0.37**	**0.25**	**0.58**
Ba	**0.39**	**-0.31**	**0.19**	**0.48**
Ca	**0.27**	**-0.45**	**0.01**	**0.55**
Cd	**0.73**	0.35	**0.82**	0.02
Cr	0.21	**-0.60**	-0.11	**0.66**
Cu	**0.83**	0.46	**0.97**	-0.03
Fe	**0.77**	-0.22	**0.57**	0.57
K	**0.62**	-0.18	0.46	**0.46**
Li	0.40	**-0.75**	-0.02	**0.89**
Mg	**0.81**	0.26	**0.84**	0.14
Mn	0.52	-0.12	**0.40**	0.35
Mo	**0.60**	0.29	**0.68**	0.02
Na	**0.71**	0.27	**0.77**	0.08
Ni	**-0.08**	**-0.45**	**-0.29**	**0.38**
P	**0.30**	**-0.34**	**0.10**	**0.46**
Pb	**0.66**	0.20	**0.69**	0.12
Sr	**0.07**	**-0.48**	**-0.18**	**0.49**
V	**0.62**	**-0.62**	0.24	**0.88**
Zn	**0.72**	0.35	**0.82**	0.02

F1: Ag, Cd, Cu, Fe, Mg, Mn, Na, Pb, Zn

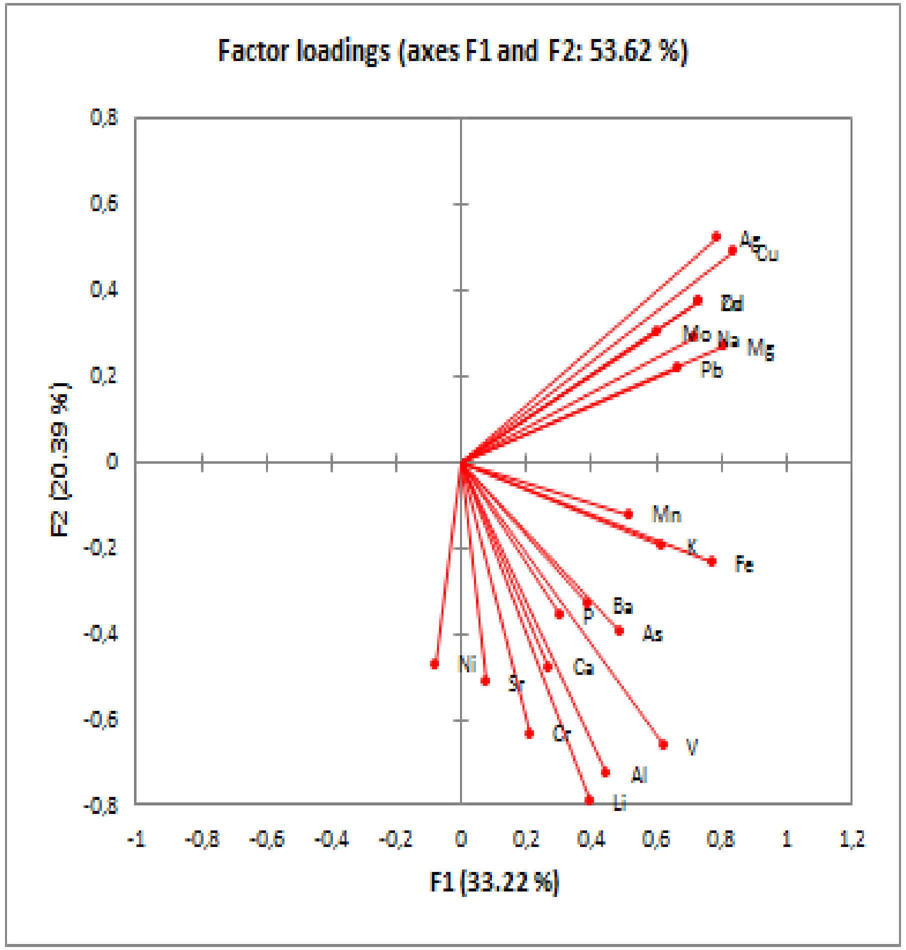
F2: Al, As, Ba, Ca, Cr, K, Li, Ni, P, Sr, V

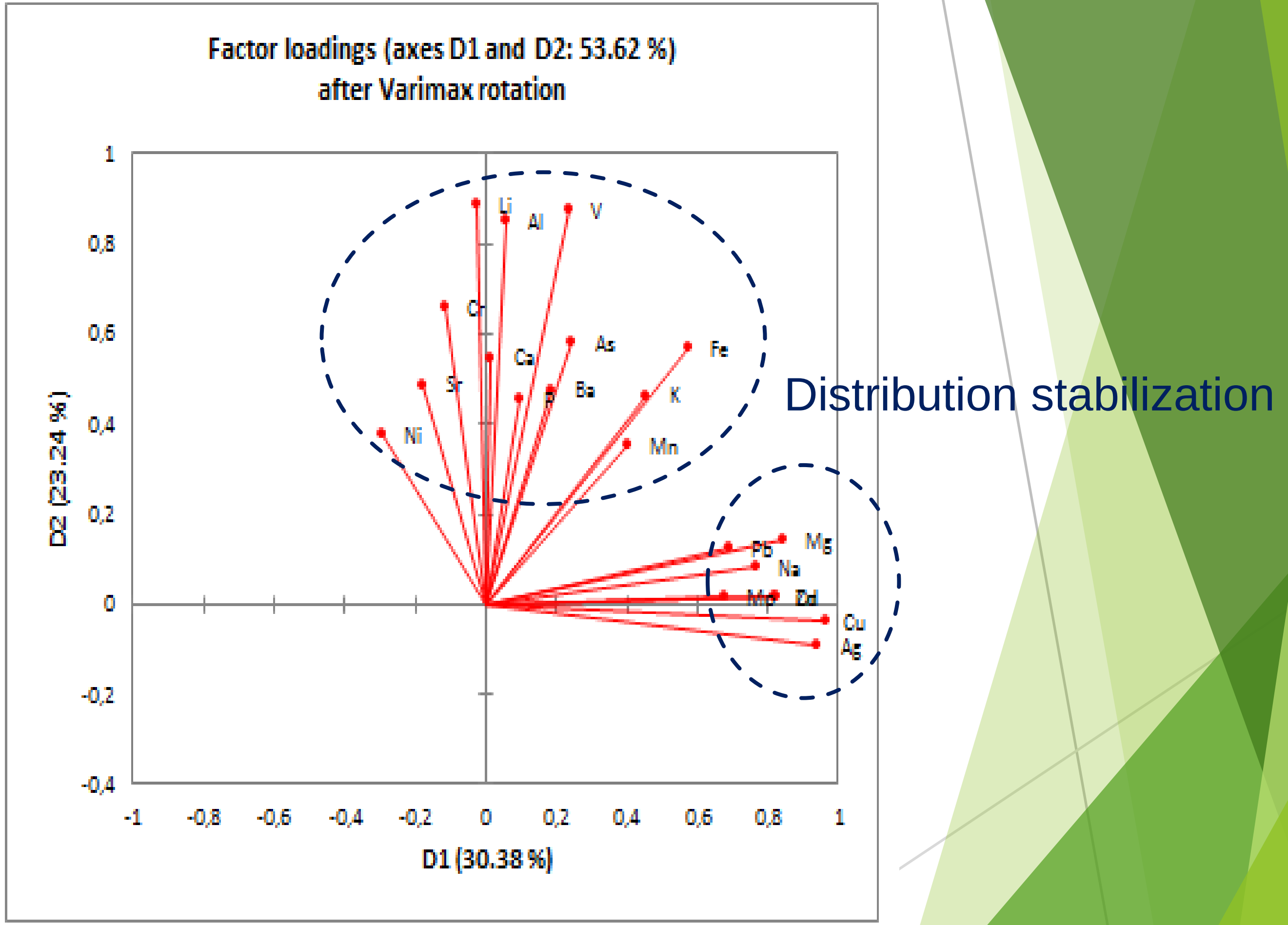
Retention of elements with factor loadings <0.60 assumed for:

As, Ba, Ca, Fe, K, Mn, Ni, P, Sr

VARIABLE DISTRIBUTION IS REDUCED

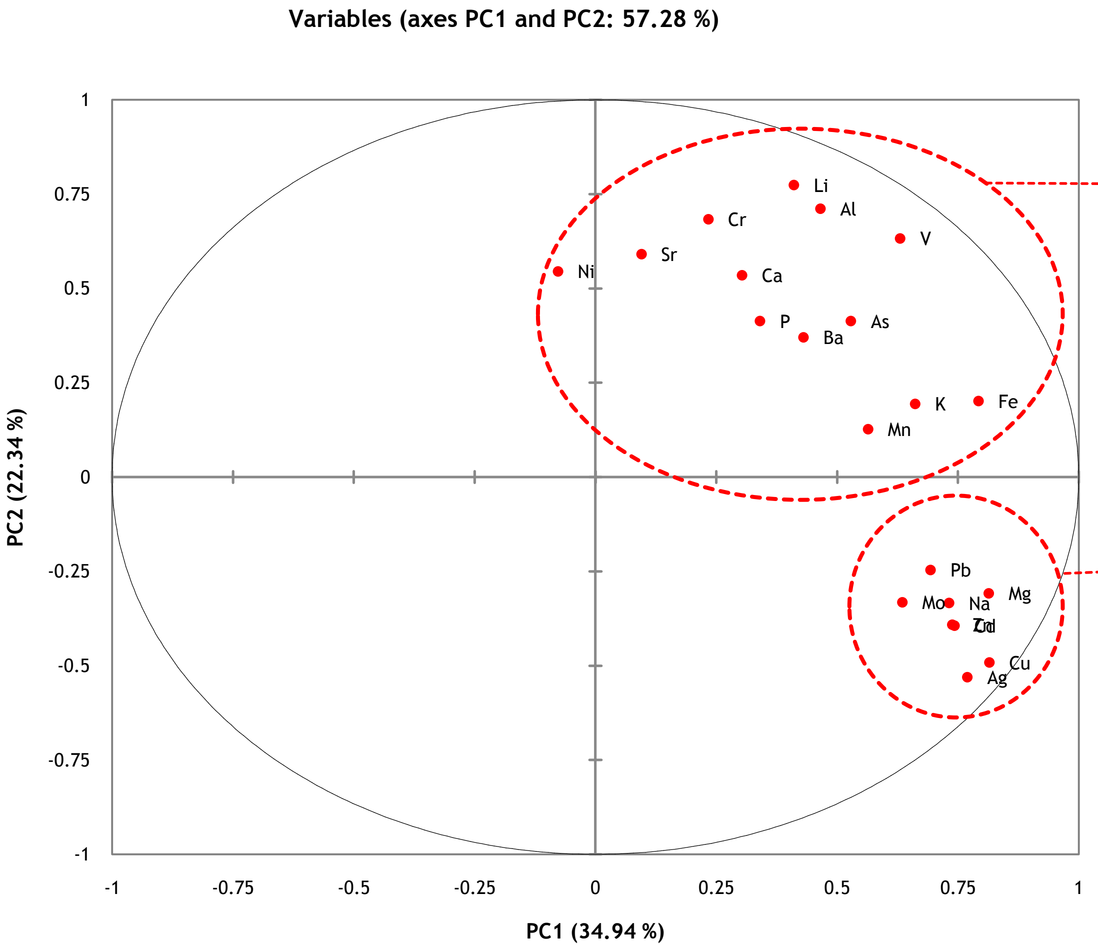
Factor analysis-varimax rotation





Effects of varimax rotation on elements contents *vs.* F1/F2 (left) and D1/ D2 after the varimax rotation (right)

Principal component analysis (PCA)



PC2: **Al, As, Ba, Ca, Cr, Fe, Li, Mn, Mo, K, Ni, Sr, P, V**

GEOGENIC DISTRIBUTION

PC1: **Ag, Cd, Cu, Mg, Mo, Na, Pb, Zn**

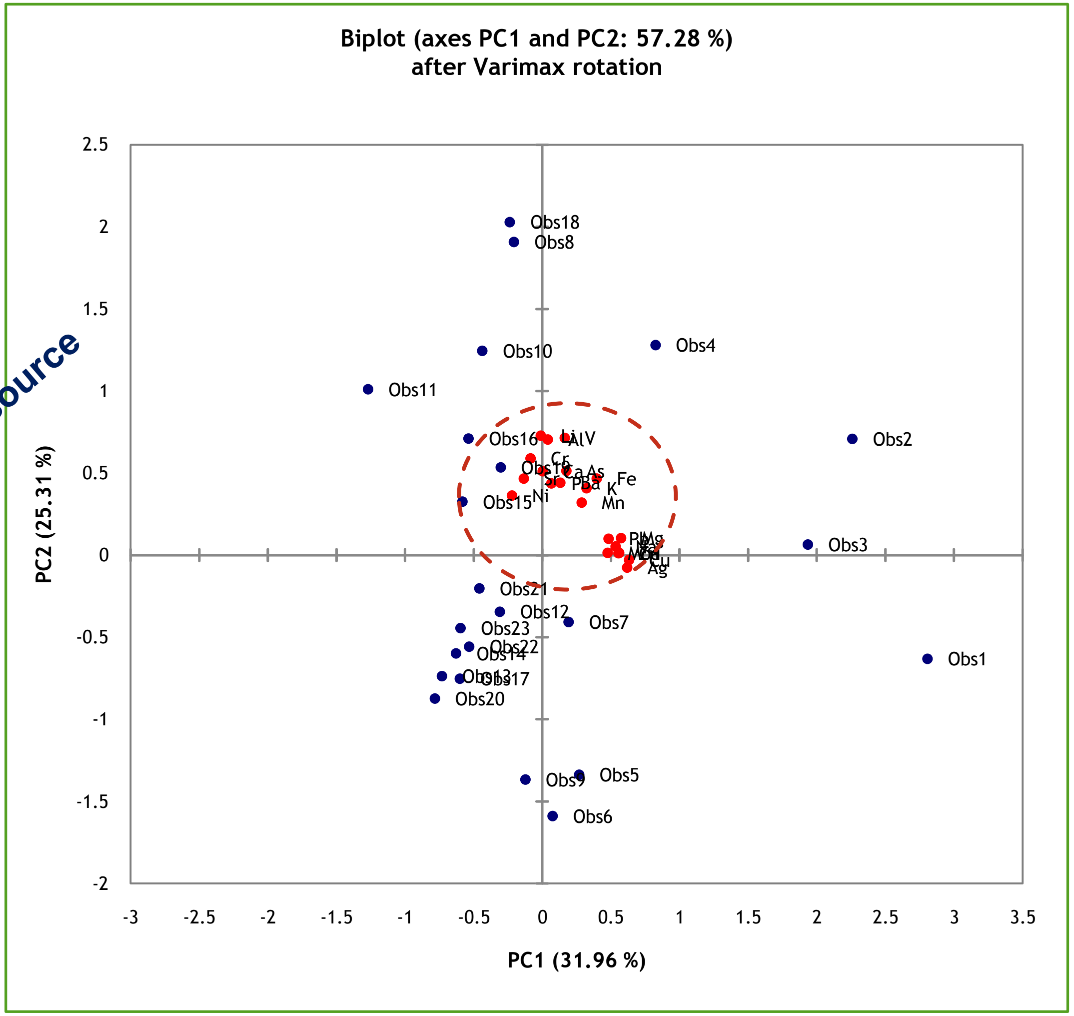
ANTHROPOGENIC IMPACT

PCA for moss species vs. metals contents

Observation	Moss species	PC1	PC2
Obs1	*Hypnum cupressiforme*	**2.716**	-0.546
Obs2	*Hypnum cupressiforme*	**2.299**	0.592
Obs3	*Hypnum cupressiforme*	**1.561**	0.226
Obs4	*Scleropodium purum*	**1.012**	0.558
Obs5	*Campthotecium lutescens*	0.819	**-1.635**
Obs6	*Campthotecium lutescens*	-0.322	**-0.978**
Obs7	*Scleropodium purum*	-0.131	**0.216**
Obs8	*Scleropodium purum*	-0.532	**2.518**
Obs9	*Campthotecium lutescens*	0.069	**-0.812**
Obs10	*Campthotecium lutescens*	-0.292	**0.930**
Obs11	*Campthotecium lutescens*	-0.980	**0.993**
Obs12	*Hypnum cupressiforme*	-0.379	**-1.005**
Obs13	*Hypnum cupressiforme*	-0.514	**-0.843**
Obs14	*Campthotecium lutescens*	**-1.003**	-0.465
Obs15	*Hypnum cupressiforme*	**-0.149**	0.111
Obs16	*Hypnum cupressiforme*	**-0.457**	0.328
Obs17	*Hypnum cupressiforme*	**-0.898**	-0.775
Obs18	*Hypnum cupressiforme*	-0.541	2.491
Obs19	*Campthotecium lutescens*	0.129	**-0.278**
Obs20	*Campthotecium lutescens*	-1.050	**-1.120**
Obs21	*Scleropodium purum*	**-0.827**	0.035
Obs22	*Campthotecium lutescens*	-0.015	**-0.246**
Obs23	*Scleropodium purum*	**-0.514**	-0.296

Obs 15, Obs 16, Obs 19 showing stronger correlations with the higher contents of almost all analyzed elements

Very close to the emission source

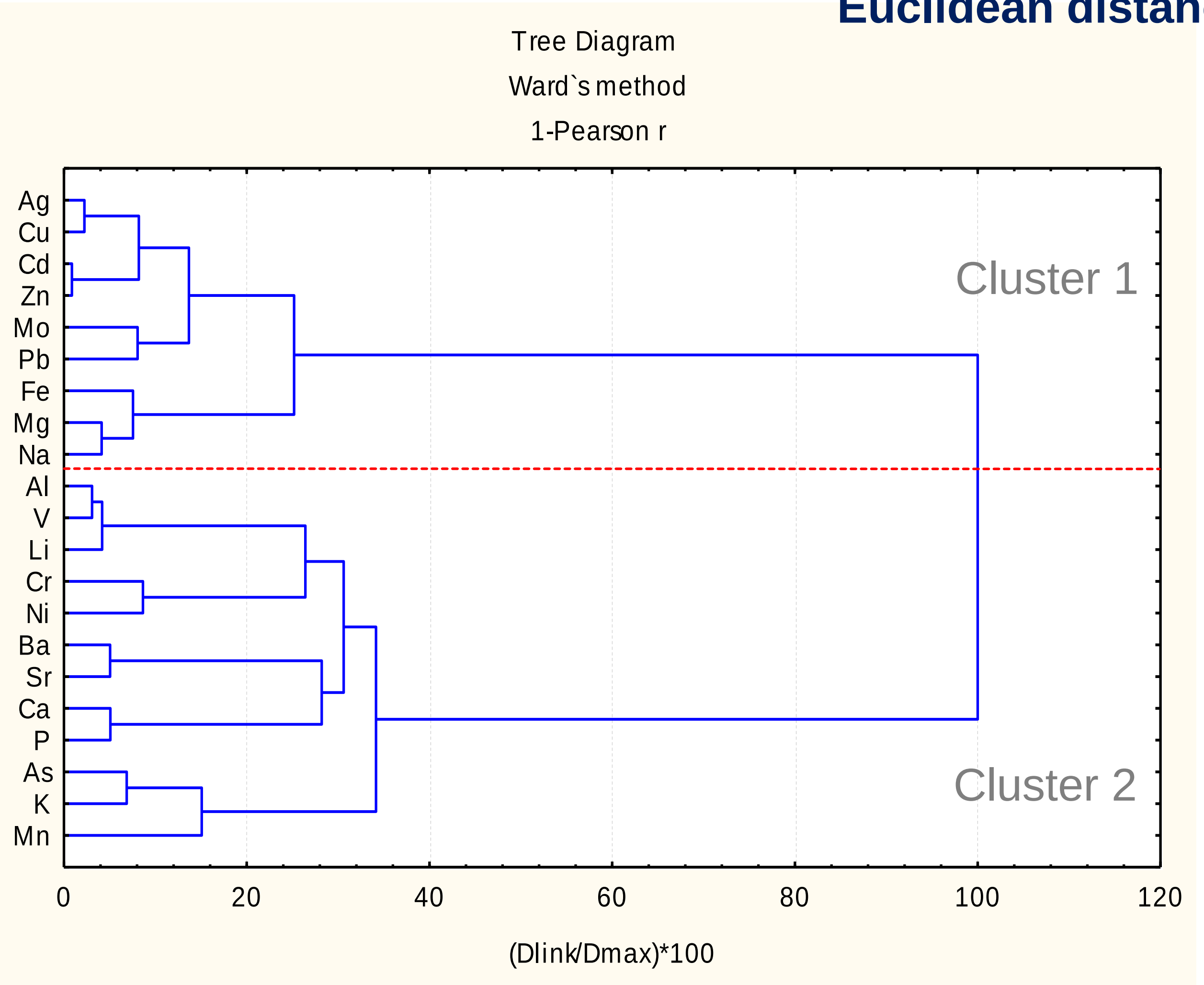


Observations are not correlated to the principle components, in aspects of better usefulness for moss species

Cluster analysis

Euclidean distance *v.s.* Pearson correlation distance measuring

revealed better expression in the data similarity



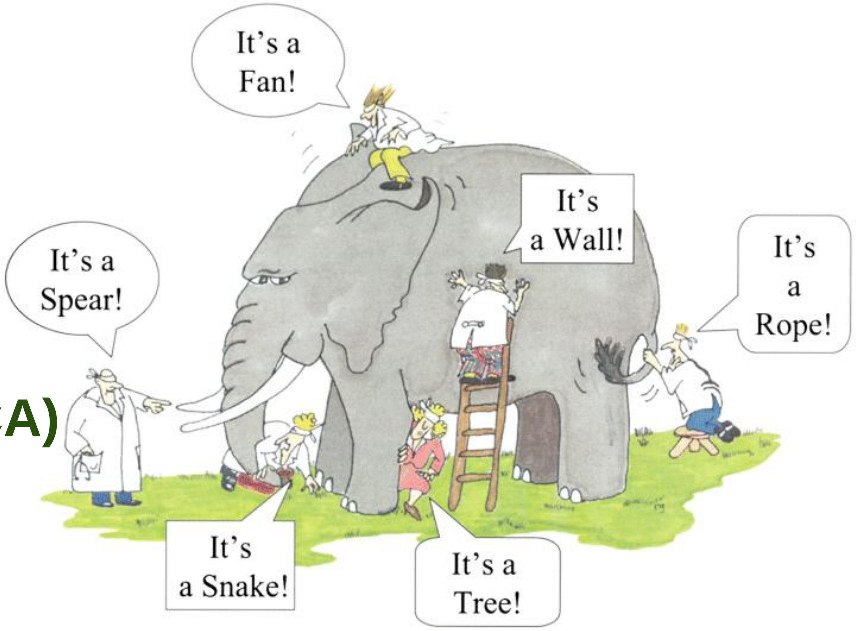
C1: Ag-Cu-Cd-Zn-Mo-Pb-Fe-Mg-Na

C2: Al-V-Li-Cr-Ni-Ba-Sr-Ca-P-As-K-Mn

CONCLUSIONS

- Distribution of Ag, Cd, Cu, Pb and Zn relays on anthropogenic introduction from Pb-Zn mine
- PCA and CA showed better expressions in elements association v.s FA
- FA – not very efficient for small scale monitoring investigations
- PCA for moss species v.s. elements contents localize “hot spots” – very close emission source environ
- Lithogenic elements association Al-As-Ba-Ca-Cr-Fe-Li-Mn-Mo-K-Ni-Sr-P-V undergoes with the soil surface dusting (geogenic impact)

PERSPECTIVIES.......



Interchangeable using of multivariate techniques (FA, PCA and CA) finds the "most significant solution possible" in:

Quantified data from moss samples

Visualization of "hot spots" make possible without spatial distribution mapping

Small scale area monitoring requires denser sampling networks

Denser sampling will enable closer identification of anthropogenic impacts of pollution source

THANK YOU FOR YOUR ATTENTION!