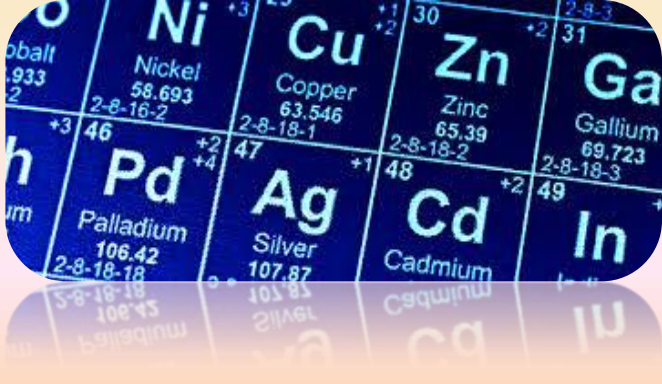


# COMPARATIVE ANALYSIS FOR MACRO AND TRACE ELEMENTS CONTENT IN GOJI BERRIES BETWEEN VARIETIES FROM CHINA AND R. MACEDONIA

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# Chemical elements and Goji Berries

## WHY IS GOJI BERRY (*Lycium barbarum)* A SUPERFOOD?

## Size does not matter!

**Benefits for human health**

- Goji Berry contains ~500 times more vitamin C than oranges!
- It packs ~15 times more iron than spinach!
- Contains 22 minerals including Zn, Fe, Ca….. and 11 amino acids!!

**Essential nutrients in human dietary**

# *Chemical elements and Goji Berries*

**Ag, B, Co, Cr, Ni, Se, Rb, Ge , Ti, Tl, Si, P**

**Identification of the GEOGRAPHICAL ORIGIN of Goji berry, their purity or their adulteration**

**Certain forms of some metals/semimetals can be TOXIC, even in relatively small amounts, and therefore pose -**

**RISK TO THE HUMAN HEALTH**

**As, Cd, Pb, Hg**

# ANALITICAL PROCEDURES

## SAMPLE PRETREATMENT

## DETERMINATION OF ELEMENTS CONTENTS

**Hard organic content of the matrix**

**Multi-element mass spectrometry**
with ICP as introduction system

### Microwave digestion system


- Closed-high-pressure decomposition
- Mixture of reagents ($HNO_3$, $H_2O_2$)

**Model MARS 5, CEM Corporation, USA**

**Model 7500 cx Agilent, USA**

# SAMPLES DIGESTION

**Dry SAMPLE 0.5 g**

**5 mL NITRIC acid - $HNO_3$** (trace select)
**2 mL Hydrogen peroxide - $H_2O_2$** (trace select)

**Placed in Teflon vessel**

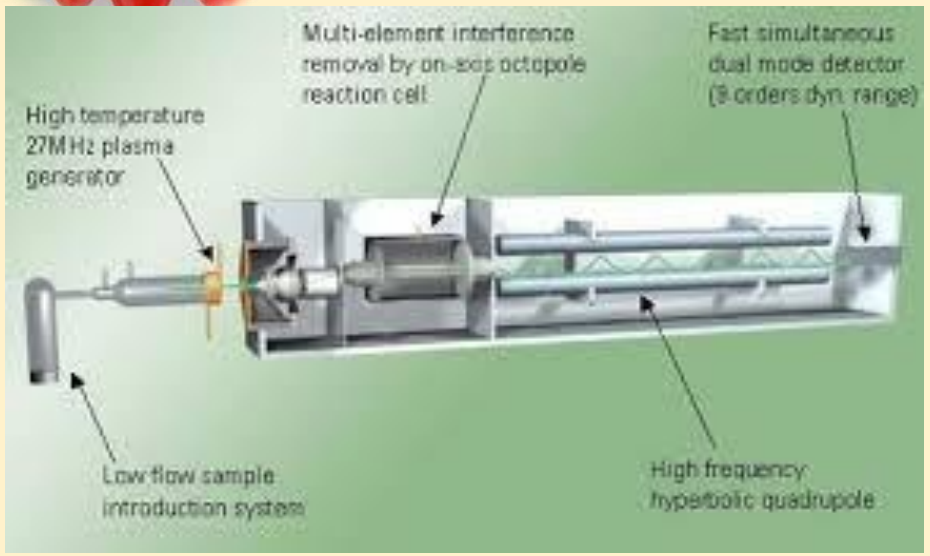


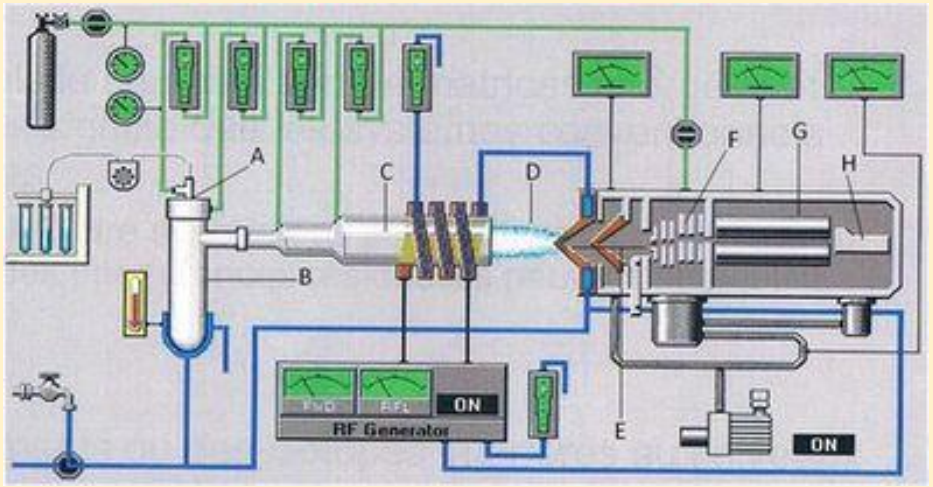
**Closed-high-pressure decomposition**

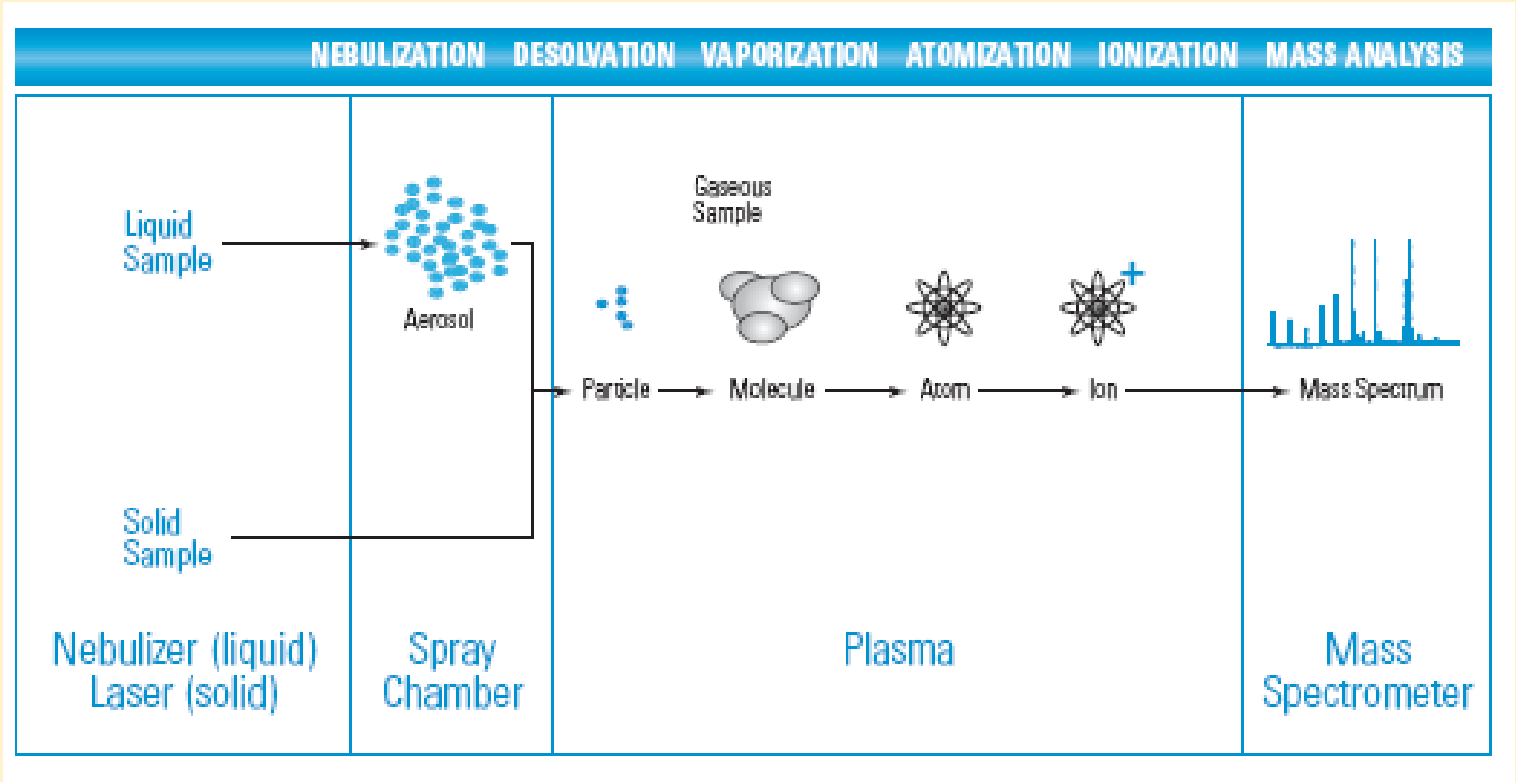
**MICROWAVE DIGESTION PROGRAM**

| Step | Initial temperature | Final temperature | Applied power (W) | Time (min.) |
|---|---|---|---|---|
| 1 | 25 | 150 | 600 | 15 |
| 2 | 150 | 150 | 600 | 5 |
| 3 | 150 | 180 | 800 | 10 |
| 4 | 180 | 180 | 800 | 10 |

# General processes in ICP-MS



# DETERMINATION OF ELEMENTS CONTENTS

| Element | Isotope | LOQ (mg/kg) |
|---|---|---|
| Na | 23 | 0.1 |
| Mg | 24 | 0.1 |
| P | 31 | 0.1 |
| K | 39 | 0.1 |
| Ca | 42 | 0.1 |
| Fe | 57 | 0.05 |
| Ni | 60 | 0.0008 |
| Cu | 63 | 0.0008 |
| Zn | 66 | 0.0009 |
| Ge | 72 | 0.0002 |
| As | 75 | 0.0002 |
| Se | 77 | 0.00015 |
| Cd | 111 | 0.0004 |
| Hg | 202 | 0.00005 |
| Pb | 208 | 0.00001 |
| Bi | 209 | 0.0005 |

## QA/QC

**Certified reference material:**
**SRM 3287 (blueberry)**
(National Institute of Standrds & Technology, NIST, Charleston, CS, USA)
For: Ag, Ca, Cu, Fe, Mg, Ni, P, Pb, Zn

**Recoveries ranges in 87.5-109%**

**t- test (p>0.05)**
**no-significant differences were found**

**Standard addition method:**

**10 ppb**; R (88.1-112%)

**50 ppb**; R (85.1-102%)

**150 ppb**; R (92-105%)

**1 ppm**; R (75.1-114%)

# ICP-MS

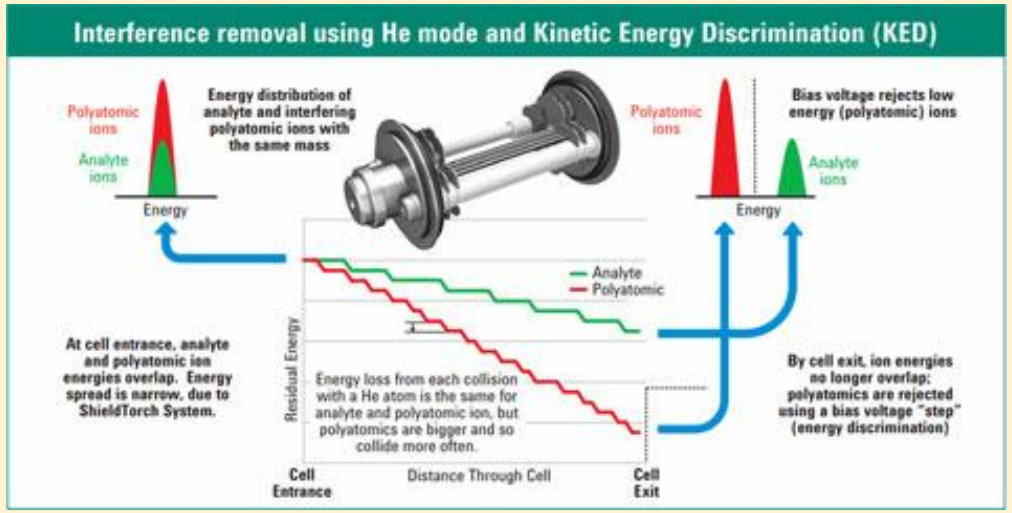
**INTERFERENCES** in ICP-MS - MOLECULAR (POLYATOMIC)
ion at the same nominal mass as the isotope of interest

**PLASMA-BASED:** such as $^{40}Ar$, $^{40}Ar^{16}O$, and $^{40}Ar^{38}Ar$

**MATRIX-BASED:** such as $^{35}Cl^{16}O$, and $^{32}S^{32}S$

## COLLISION / REACTION CELL (CRS) with optional He gas

**$^{51}V$, $^{53}Cr$, $^{57}Fe$, $^{60}Ni$, $^{63}Cu$, $^{66}Zn$, $^{72}Ge$, $^{75}As$, $^{77}Se$**




# Determination of elements contents

| INSTRUMENT | (ICP introduction system) |
|---|---|
| Sampler | Cu (standard) |
| Skimmer | Ni (standard) |
| Nebulizer | MicroMist (standard) |
| Plasma torch | Quartz, 2.5 mm (standard) |
| **Integration Time** (for all analyzed elements) | 0.3 sec x 1 point |
| Replication | 3 |
| **Tune parameters** | |
| RF power | 1500 W |
| Sample depth | 8.5 mm |
| Carrier gas | 0.80 L/min |
| Makeup gas | 0.23 L/min |
| Extract lens 1 | -3 V |
| Extract lens 2 | -150 V |
| Energy discriminator | 2 V |
| Reaction gas | He 5.0 mL/min |
| Cerrtificate | **0.58%** (ref. value ~ 0.65 %) |

# BASIC STATISTICS

Values given in mg/kg

| Elements (Ch) | Na | Mg | P | K | Ca | Fe | Ni | Cu | Zn | Ge | Se | As | Cd | Hg | Pb | Bi |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| min | 406 | 1009 | 1846 | 2261 | 507 | 63.3 | 1.67 | 8.91 | 10.4 | 0.12 | 0.025 | 0.007 | 0.003 | <LOQ | 0.015 | 0.001 |
| max | 816 | 1452 | 2541 | 3877 | 1034 | 82.6 | 4.87 | 14.3 | 17.6 | 0.34 | 0.061 | 0.036 | 0.033 | 0.00024 | 0.096 | 0.046 |
| **Average** | **638** | **1209** | **2258** | **3004** | **751** | **75.6** | **3.67** | **11.5** | **14.3** | **0.20** | **0.042** | **0.018** | **0.012** | **0.00008** | **0.054** | **0.012** |
| SD | 180 | 120 | 261 | 573 | 265 | 10.0 | 1.63 | 1.90 | 3.69 | 0.12 | 0.011 | 0.015 | 0.017 | 0.00014 | 0.028 | 0.025 |
| Elements (Mk) | Na | Mg | P | K | Ca | Fe | Ni | Cu | Zn | Ge | Se | As | Cd | Hg | Pb | Bi |
| min | 15.2 | 632 | 2059 | 3823 | 269 | 20.9 | 0.21 | 7.45 | 7.50 | 0.12 | 0.005 | 0.006 | 0.055 | <LOQ | 0.008 | 0.015 |
| max | 27.2 | 825 | 2781 | 5047 | 478 | 60.0 | 0.60 | 10.8 | 13.3 | 0.32 | 0.018 | 0.18 | 0.093 | 0.00025 | 0.028 | 0.060 |
| **Average** | **21.4** | **707** | **2234** | **4310** | **331** | **38.9** | **0.40** | **9.31** | **9.44** | **0.25** | **0.010** | **0.06** | **0.079** | **0.00006** | **0.022** | **0.039** |
| SD | 4.22 | 66.7 | 160 | 493 | 51 | 16.3 | 0.16 | 1.06 | 1.59 | 0.09 | 0.005 | 0.08 | 0.016 | 0.00012 | 0.010 | 0.014 |

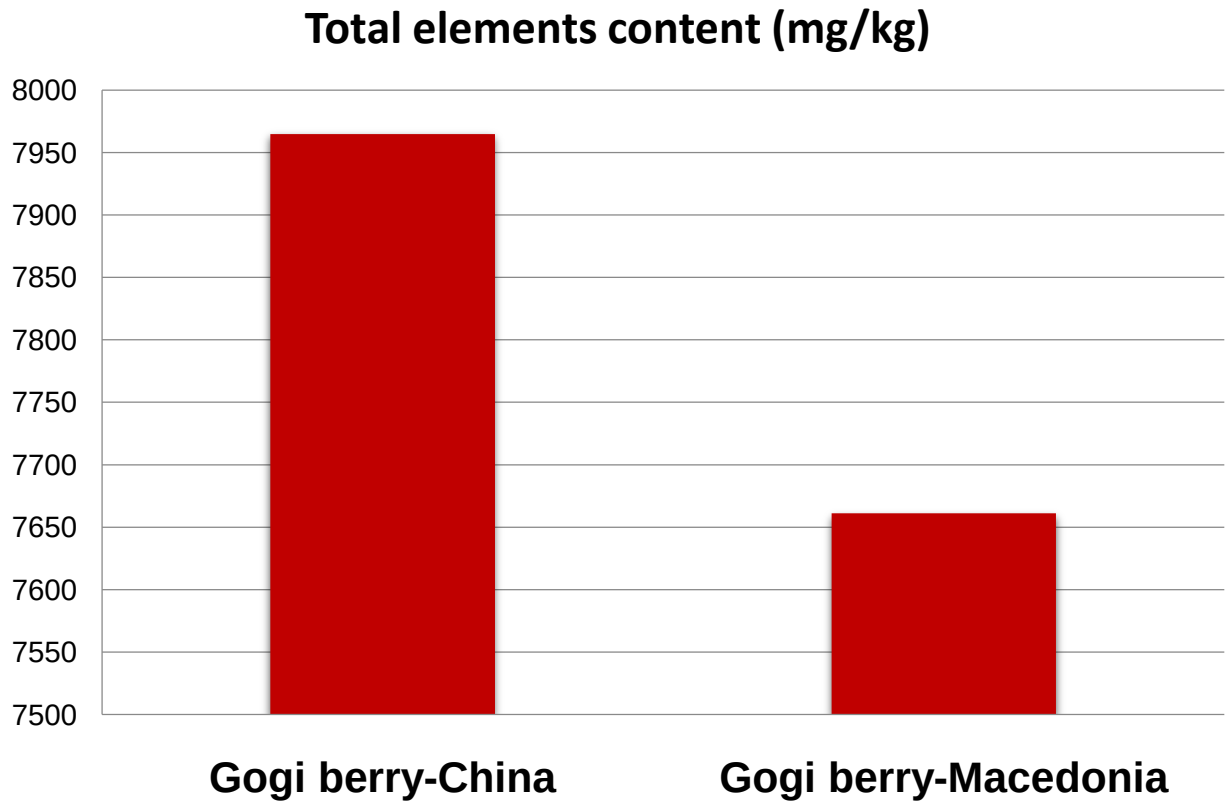
Maximum permitted content for Pb and Cd (**0.1 mg/kg**)

# Comparative analysis

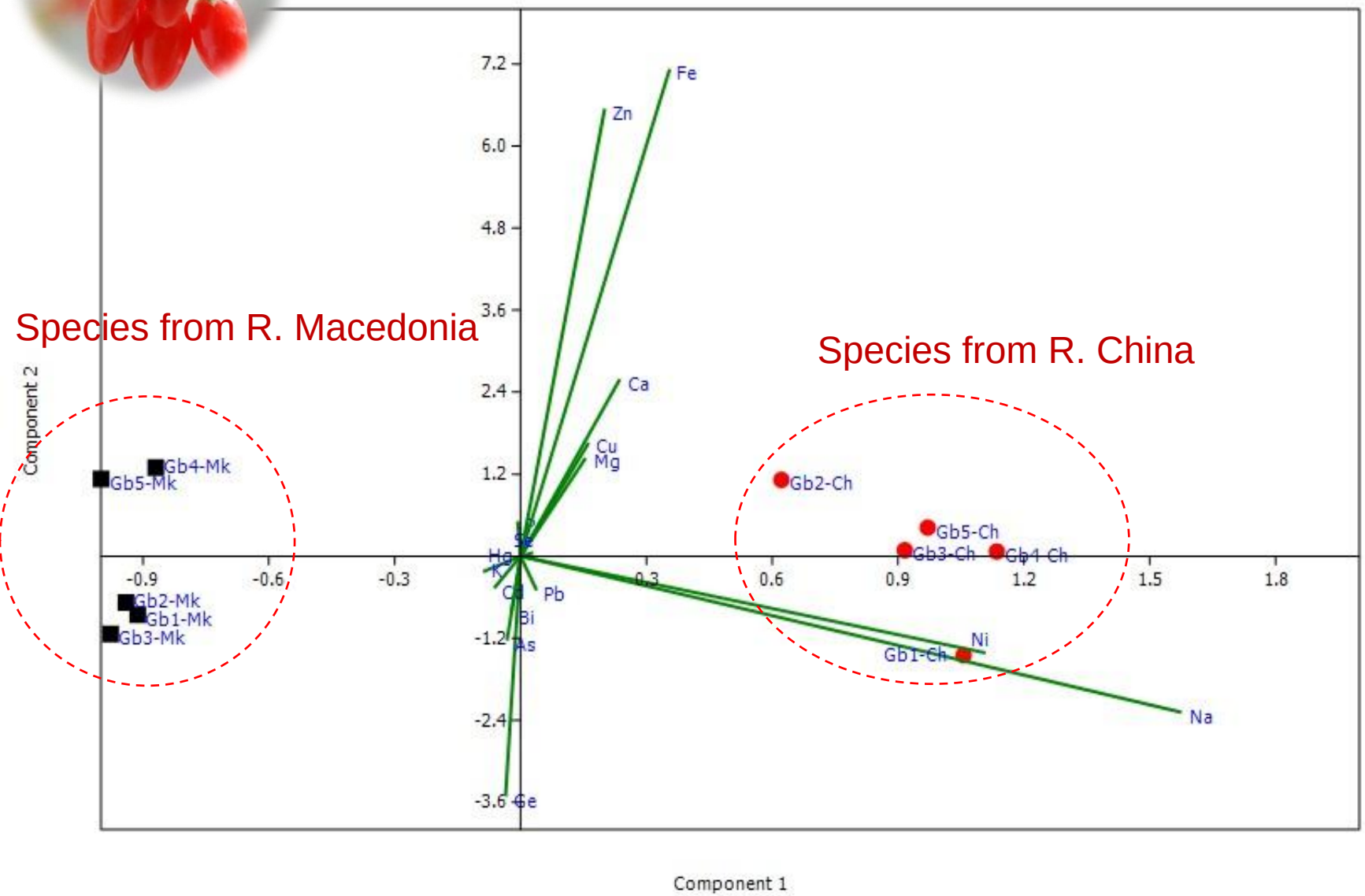
**T-test for Dependent Samples**

| Element | t | p |
|---|---|---|
| Na | 7.849 | 0.001 |
| Mg | 8.617 | 0.001 |
| P | -0.700 | 0.523 |
| K | -4.836 | 0.008 |
| Ca | 3.960 | 0.017 |
| Fe | 4.973 | 0.008 |
| Ni | 5.526 | 0.005 |
| Cu | 3.435 | 0.026 |
| Zn | 2.659 | 0.056 |
| Ge | -0.923 | 0.408 |
| Se | 4.490 | 0.011 |
| As | -1.044 | 0.356 |
| Cd | -5.928 | 0.004 |
| Hg | 0.991 | 0.378 |
| Pb | 3.088 | 0.037 |
| Bi | -1.952 | 0.123 |

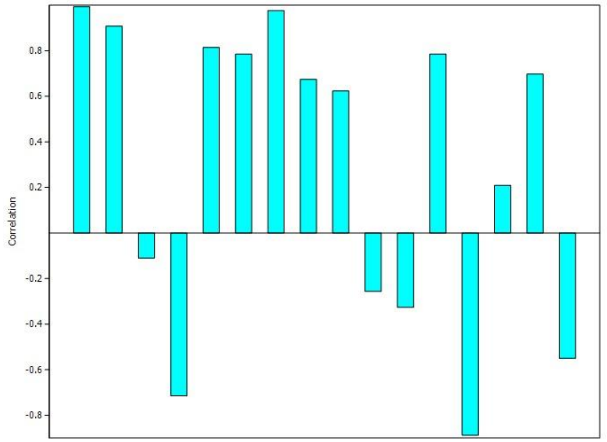
Marked differences are significant at p < .05000



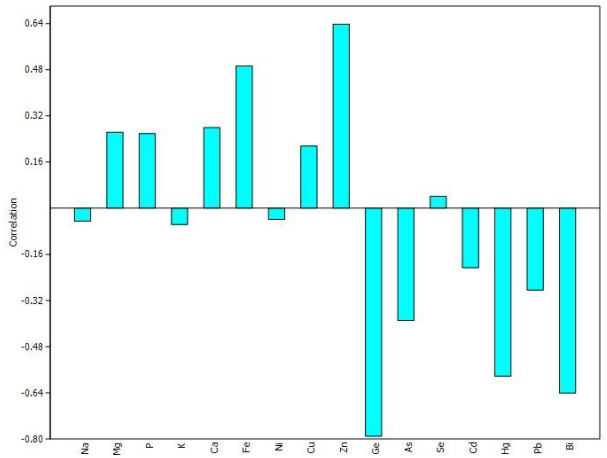
# Principal component analysis




PC1




PC2




# FACTOR ANALYSIS

**Most expressed association:**

**F1: Na-Mg-Fe-Ni-Se-Bi**

**F2: Zn-Hg-Pb**

**F3: P-K**

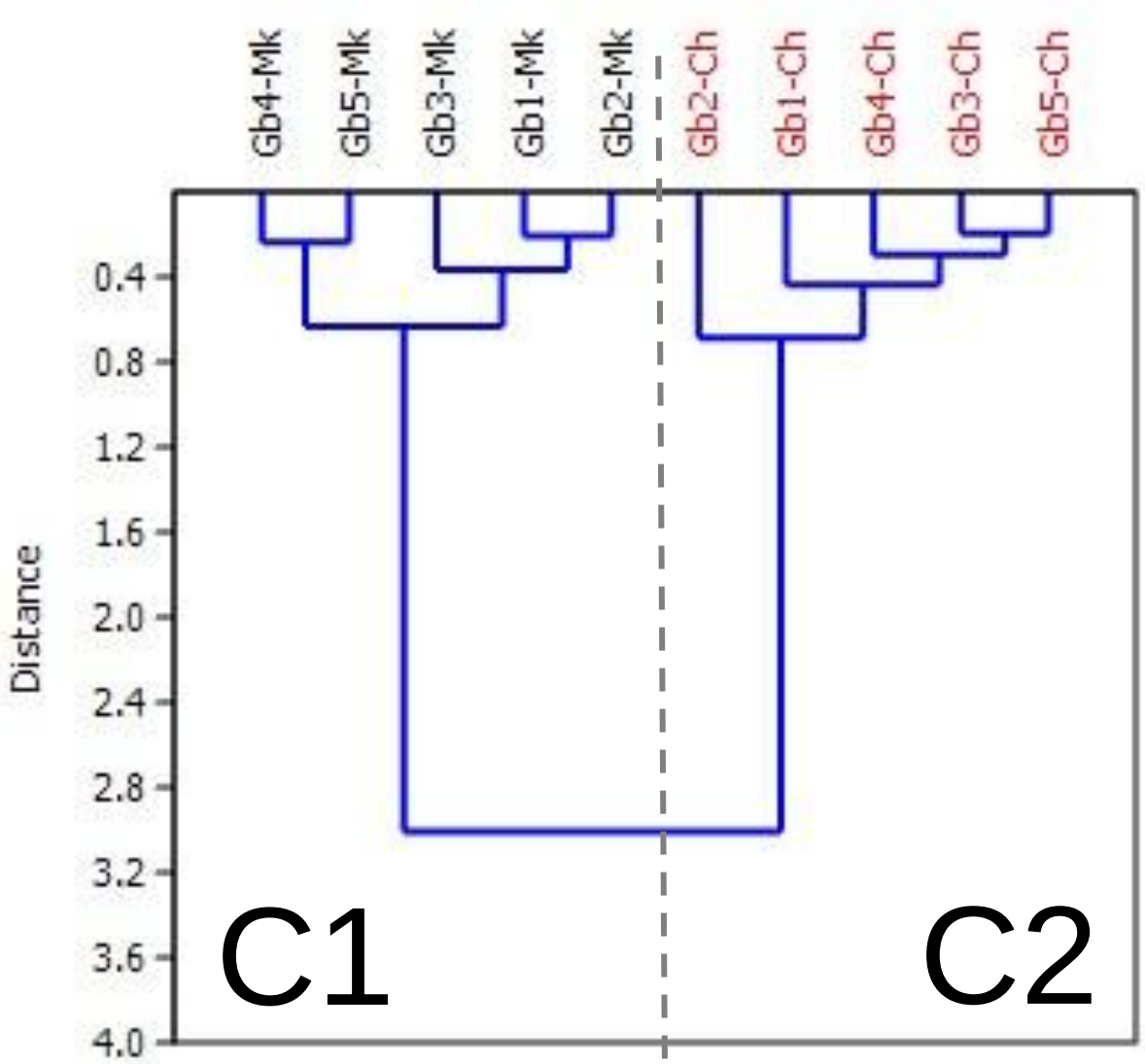
**F4: Ca-Ge-Cd**

**F5: As**

| Element | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|
| Na | **0.71** | 0.32 | 0.07 | 0.58 | 0.11 |
| Mg | **0.78** | -0.11 | 0.15 | 0.49 | 0.20 |
| P | 0.04 | -0.01 | **0.99** | -0.05 | 0.09 |
| K | -0.31 | 0.01 | **0.60** | **-0.70** | 0.04 |
| Ca | 0.20 | -0.08 | 0.06 | **0.93** | 0.15 |
| Fe | **0.74** | -0.15 | -0.10 | 0.56 | 0.18 |
| Ni | **0.94** | -0.15 | -0.13 | 0.04 | 0.12 |
| Cu | 0.32 | -0.39 | 0.15 | 0.70 | 0.42 |
| Zn | -0.32 | **0.79** | 0.18 | -0.20 | -0.34 |
| Ge | 0.22 | 0.25 | -0.28 | **0.87** | 0.03 |
| Se | **0.76** | 0.41 | 0.02 | 0.44 | 0.11 |
| As | -0.17 | 0.02 | -0.09 | -0.13 | **-0.97** |
| Cd | -0.54 | 0.05 | 0.15 | **-0.77** | -0.09 |
| Hg | 0.03 | **0.92** | -0.05 | 0.01 | 0.12 |
| Pb | 0.44 | **0.68** | -0.18 | 0.40 | -0.05 |
| Bi | **-0.59** | 0.49 | -0.02 | -0.45 | -0.06 |
| | | | | | |
| Variance | 0.27 | 0.17 | 0.10 | 0.29 | 0.09 |

**Total variance of factor loadings=85.9%**

# CLUSTER ANALYSES




**SIGNIFICANT LOADING VALUES >60% OF THE LINKAGE**

Distance measuring:
Ward's method
1-Pearson correlations

# *CONCLUSIONS*

**1. The proposed method shows advantages of using :**

•Small amount of sample

•The shorter treatment time

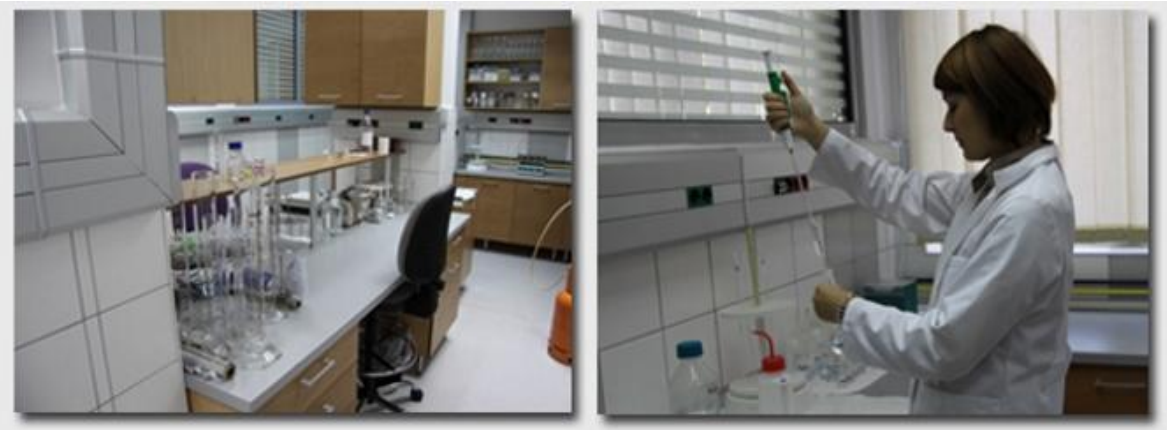
•Sensitive method for determination of 16 elements –under same instrumental condition (tune conditions)

**2.** Monitoring the toxic elements contents (As, Cd, Hg, Pb) is ESSENCIAL – preventing excessive build up of these metals in the human chain

**3.** Determination of the geographical origin

# Acknowledgment

**LABORATORY FOR PLANT AND ENVIRONMENT PROTECTION**
(Faculty of Agriculture - “GOCE DELČEV” University – Štip, R. Macedonia)



# Thank you for your attention!