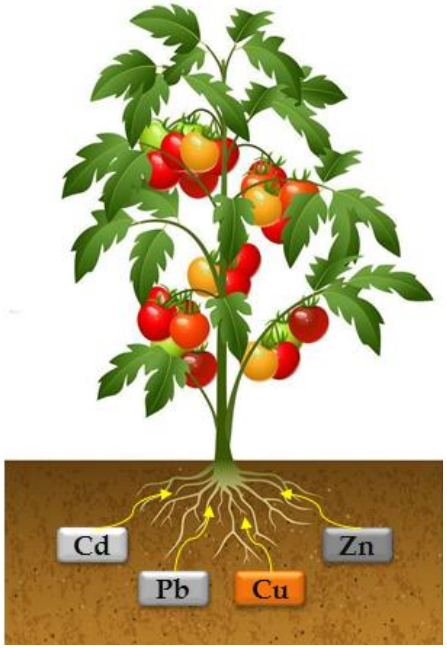
INTERNATIONAL SYMPOSIUM „ENVIRONMENT AND INDUSTRY"

UNIVERSITY OF AGRONOMIC SCIENCES AND VETERINARY MEDICINE – BUCUREȘTI

An overview of methods used for quantification of heavy metal contents in vegetal samples

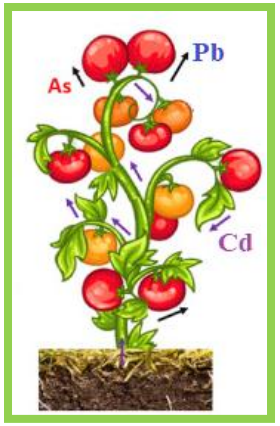


Lecturer PhD. Gina **VASILE SCĂEȚEANU**
Assoc. Prof. PhD. Roxana Maria **MADJAR**
PhD. student Andrei **MOȚ**

24th September 2021
Bucharest, Romania

Which was the purpose of our contribution?

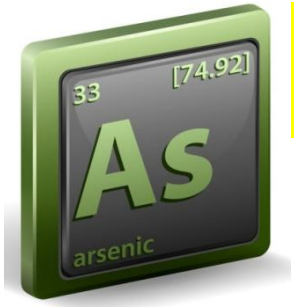
Continuous monitoring of heavy metals content in vegetal products **is a priority** for food control and a risk assessment strategy for human health.



Why is this action a priority

Heavy metals

- harmful to the health of general public
- cumulative poisons

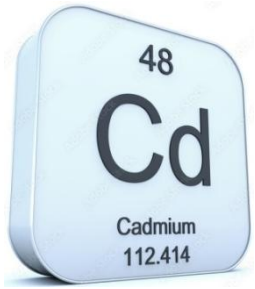


neurologic, behavioral, hematologic **disorders**

cardiovascular **diseases**

cancers

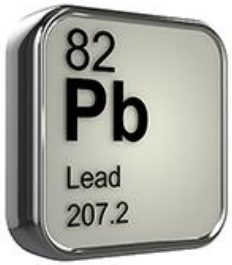
mechanism by which it produces tumors in humans **is not completely understood**



-pulmonary and gastrointestinal **irritant** → **can be fatal if inhaled or ingested**;

-affects reproduction in mammals;

-can affect the skeletal system → skeletal demineralization → **Itai-itai disease**



- the most vulnerable target of lead poisoning is **nervous system**;

-for **adults**: reproductive effects, brain damage, kidney damage;

- for **children**: lower IQ, decreased ability to pay attention and underperformance at school.

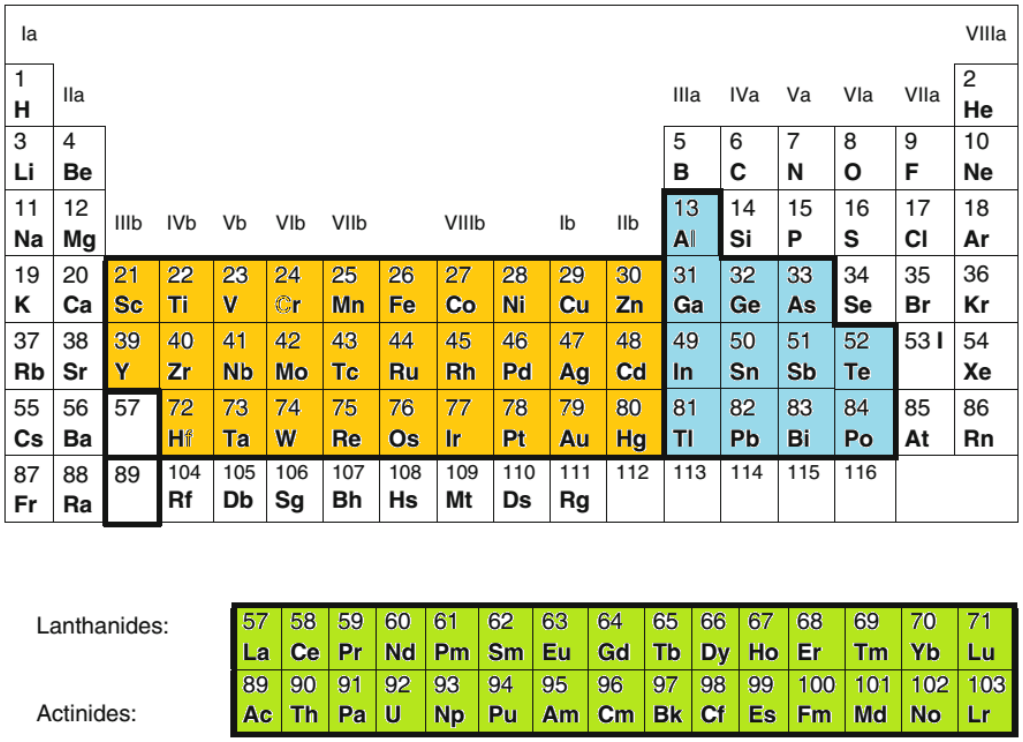
Rafati Rahimzadeh M., Rafati Rahimzadeh M., Kazemi S., Moghadamnia A.A. Cadmium toxicity and treatment: An update. *Caspian J Intern Med*. **2017**, 8(3):135-145. doi:10.22088/cjim.8.3.135

Tchounwou P.B., Yedjou C.G., Patlolla A.K., Sutton D.J. Heavy metal toxicity and the environment. *Exp Suppl*. **2012**, 101,133-164. doi:10.1007/978-3-7643-8340-4_6

Which chemical elements are considered *heavy metals*?

Duffus (2002): Chemical elements defined as "*heavy metals*" has density between 3.5-7 g/cm^3.

Appenroth (2010): "*heavy metals*" are represented by metals and some metalloids that are framed in three groups.



In our contribution, the term "*heavy metals*" is used from environmental point of view and is related to their effects on human health.

Duffus J.H., Heavy metal – a meaningless term? *Pure Appl.Chem.* **2002**, 74, 793-807

Appenroth K.-J. What are heavy metals in Plant Sciences? *Acta Physiol. Plant.* **2010**, 32,615-619.

Which are the environmental sources of heavy metals?

agriculture
(fertilizers and pesticides)

phosphate fertilizer

Cd, As, Pb, Cr, Hg, Ni, V – variable levels depending of the phosphate rocks sources

natural causes
(volcanic activity, soil erosion, etc)

Heavy metals sources

industrial activities

atmospheric deposition

irrigation with wastewater

preservation practices
of vegetal samples

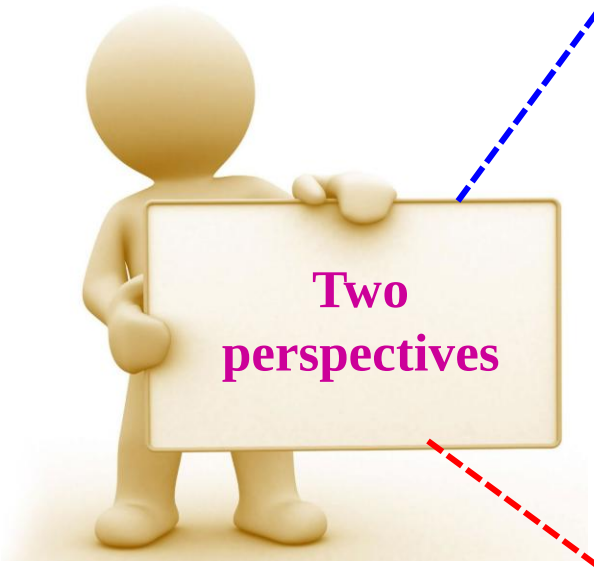
What about legislation?

Regulation (EC) No 1881/2006

Table 1. Maximum admitted levels for lead and cadmium in vegetal products and provisional tolerable weekly intake (PTWI)

Heavy metal	Foodstuffs	Maximum level (mg/kg fresh weight)
Lead (Pb)	Cereals, legumes and pulses	0.20
	Vegetables, excluding brassica vegetables, leaf vegetables, fresh herbs and fungi. For potatoes the maximum level applies to peeled potatoes.	0.10
	Brassica vegetables, leaf vegetables and cultivated fungi	0.30
	Fruit, excluding berries and small fruit	0.10
	Berries and small fruit	0.20
PTWI for lead, mg/kg body weight		0.025
Cadmium (Cd)	Cereals excluding bran, germ, wheat and rice	0.10
	Bran, germ, wheat and rice	0.20
	Soybeans	0.20
	Vegetables and fruit, excluding leaf vegetables, fresh herbs, fungi, stem vegetables, pine nuts, root vegetables and potatoes	0.050
	Leaf vegetables, fresh herbs, cultivated fungi and celeriac	0.20
	Stem vegetables, root vegetables and potatoes, excluding celeriac. For potatoes the maximum level applies to peeled potatoes.	0.10
PTWI for cadmium, mg/kg body weight		0.007

Heavy metals accumulation in vegetal products



Elevated levels of heavy metals produce:

a) ***physiological changes in plants***:

-inhibit the ability of the plant to synthesize chlorophyll;

-high oxidative stress;

-suppression of plant growth;

b) ***modification of biochemical parameters:***

- increase of sugar, phenol and ascorbic acid contents;

- decline of protein soluble levels.

Related to **negative effects** on consumers' health:

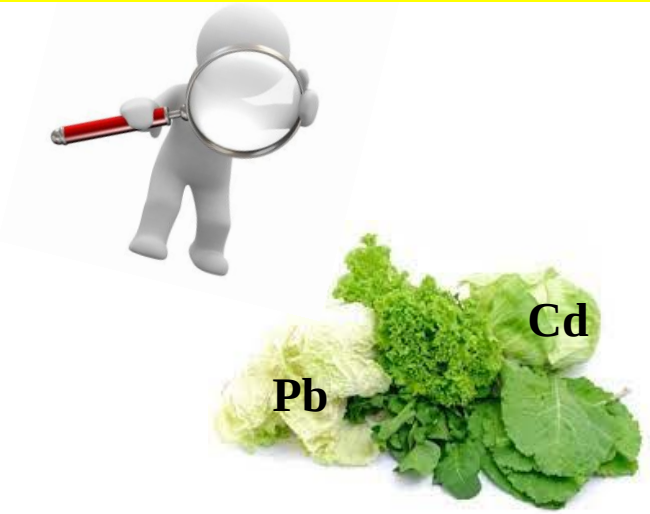
-chronic diseases

-disturbances at central nervous system level

-renal dysfunctions

Different studies demonstrated that arsenic, lead and cadmium:

- were **highly enriched in leafy vegetables** and barely accumulated in fruit vegetables;
- the levels in leafy vegetables were **three times higher** than those in non-leafy vegetables;
- the ability of vegetables to uptake and accumulate follows variation: leafy vegetables>stalk vegetables>root vegetables.



Zhou, H., Yang, W.T., Zhou, X., Liu, L., Gu, J.F., Wang, W.L., Zou, J.L., Tian, T., Peng, P.Q., Liao, B.H., *International Journal of Environmental Research and Public Health*, **2016**, 13, 289, doi:10.3390/ijerph13030289.

Yang, J., Guo H., Ma, Y., Wang, L., Wei, D., Hua, L., Journal of Environmental Sciences, **2010**, 22(8), 1246-1252.

Methods for heavy metals quantification in vegetal products

First step

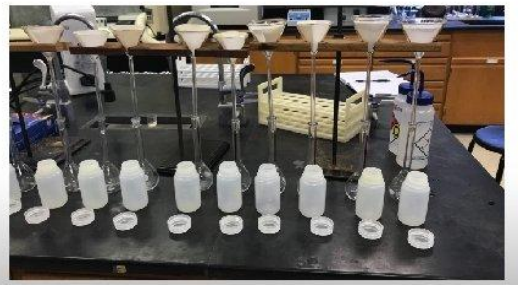
Sample digestion

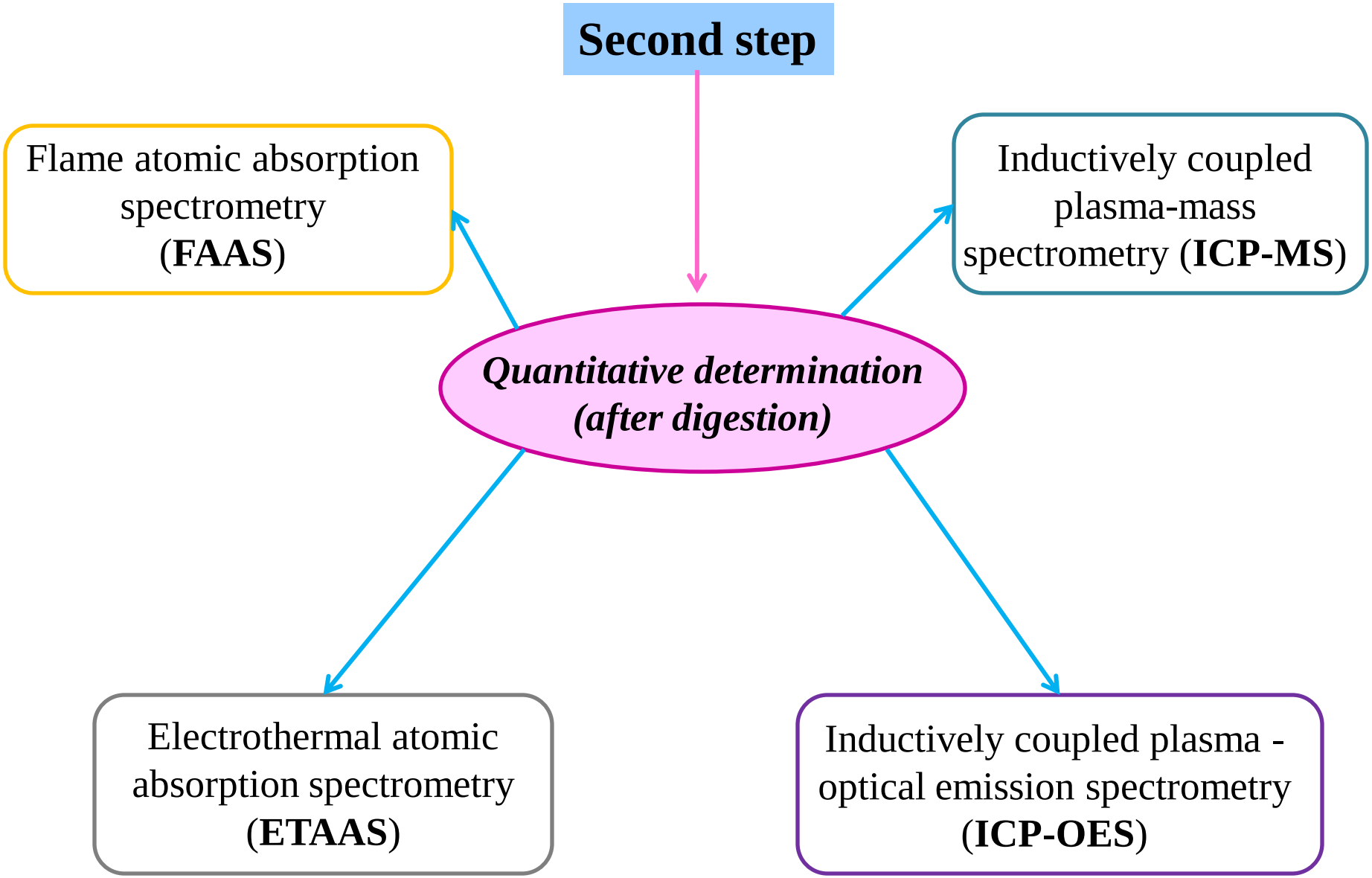
calcination

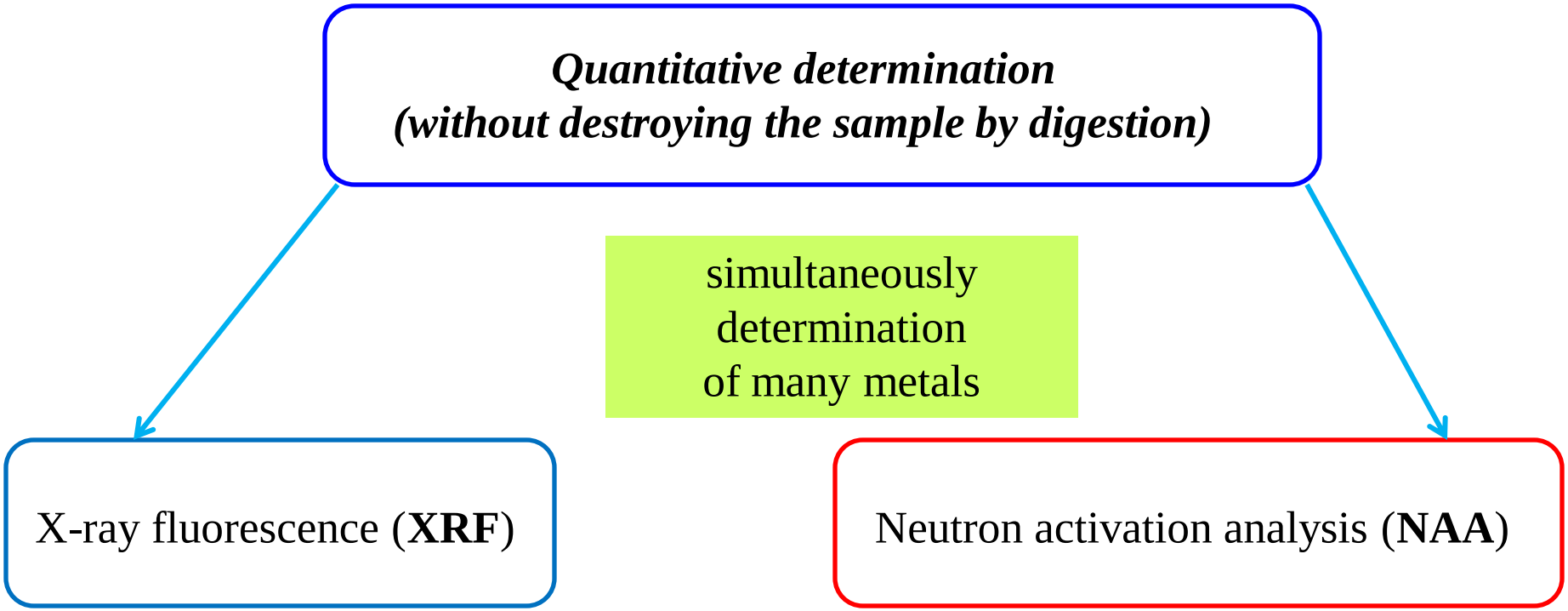
digestion with mixtures of concentrated acids

digestion with acids under heating

microwave digestion in acidic medium




Second step
Flame atomic absorption spectrometry (FAAS)
Inductively coupled plasma-mass spectrometry (ICP-MS)
Quantitative determination (after digestion)
Electrothermal atomic absorption spectrometry (ETAAS)
Inductively coupled plasma - optical emission spectrometry (ICP-OES)



Haidu, D., Parkanyi, D., Moldovan, R.I., Savii, C., Panzaru, I., Dehelean, C., Kurunczi, L., Journal of Analytical Methods in Chemistry, **2017**, article ID 9748413, https://doi.org/10.1155/2017/9748413.

Overview of procedures used for heavy metals determination by AAS technique (*selection of literature data*)

Sample	Metals	Digestion	Analytical technique
fruits, vegetables	Cd, Pb	samples are dried at 105°C and **calcinated** at 450°C; residue was treated with 5 mL **HNO_3 65%** and heated on a sand bath at 150°C	FAAS
leafy and fruit vegetables	Pb, Cd, Fe, Zn, Cu	10 g sample + 10 mL **HNO_3 conc**., digestion at 90°C for 45 minutes and at 130°C for 3 hours	FAAS
leafy, fruit and root vegetables	Mn, Fe, Cu, Zn, Pb, Cd	1 g dry sample + 15 mL triacid mixture (**HNO_3 70%, $HClO_4$ 65%, H_2SO_4 70%,** 5:1:1) + digestion at 80°C	FAAS
legumes, leafy vegetables, stems, roots, fruits	Fe, Mn, Cu, Zn, Pb, Cd, Hg	drying at 100°C, grinding 1 g dry sample + 15 mL triacid mixture (**HNO_3 65%, $HClO_4$ 65%, H_2SO_4 70%,** 5:1:1) + digestion at 80°C	ETAAS HGAAS
vegetables	Ni, Zn, Cu	sample is oven-dried at 105°C for 24 hours; 3 g dried sample **dry-ashed** 3 hours at 450°C; ash is treated with 10 mL **HNO_3** conc.	FAAS
vegetables	Cd, Pb, Ni, Co, Cr	drying at 65°C; 1 g sample was digested in Pyrex tubes with **HNO_3:$HClO_4$** = 3:1	FAAS
vegetables	Cu, Zn, Pb, Cd	10 g sample is dried at 105°C, then is **calcinated** at 450°C; the ash is treated with 5 mL **$HClO_4$** and 10 mL **HNO_3**	FAAS (Cu, Zn) GFAAS (Cd, Pb)

Overview of procedures used for heavy metals determination by ICP-MS technique (*selection of literature data*)

Sample	Metals	Digestion
apple, carrots	Cd, Pb, Cu, Zn	digestion at 200°C with 8 mL **HNO_3** and 2 mL **H_2O_2**
corn, mint, eggplant, pepper, tomato	Cd, Co, Cr, Cu, Mo, Ni, Pb, Zn	0.5 g sample digested with **HNO_3** conc. and the resulted residue treated with aqua regia
fruits, vegetables	Pb, Cd, Hg, V, Cr	1 g sample was treated with 5 mL **HNO_3** 69% and 1 mL **H_2O_2** 30%, microwave digested
fruits, vegetables, nuts	Co, Cr, Cu, Mn, Mo, Ni, Sr, Tl, U, V	2 g sample is treated with 6 mL **HNO_3** 68% and 2 mL **H_2O_2** 30%; microwave assisted digestion
vegetables	Pb, Zn, Cd, Cu	0.5 g dried sample microwave digested with 5 mL **HNO_3** conc., heated at 100°C for 30 minutes; after cooling was added 2 mL **H_2O_2** and microwave digested for other 21 minutes
vegetables (fruits, leaves, tubers, bulbs)	As, Cd, Pb	microwave digestion with **HNO_3** 65% and **H_2O_2** 30%

Overview of procedures used for heavy metals determination by ICP-OES technique (*selection of literature data*)

Sample	Metals	Digestion
cereals, pulses, tuber, nuts, dried fruit	Cd, Cr, Cu, Pb	0.25 g dried sample treated with 5 mL **HNO_3 conc.**; after an hour was **microwave digested**
endive	Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Zn	0.5 g dried plant was **microwave digested** with 2 mL **HNO_3** conc. and 2 mL **H_2O_2**
fruit juices	Cr, Cd, Pb	0.5 mL sample is treated with 5 mL **HNO_3** 69.5%, 2 mL **H_2O_2** 35% and heated in oven at 200°C for 20 minutes, close to dryness
fruits	As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Sb, Se, Tl, Zn	0.5 g sample treated with 15 mL **HNO_3** and 5 mL **H_2O_2**, left overnight; digestion 2 hours at 130°C
fruits	As, Cd, Pb	0.5 g of dried and ground sample was **microwave digested** using 6 mL **HNO_3** 65%, 2 mL **H_2O_2** 30%
lettuce, broad bean	Cd, Pb	samples are dried at 70°C; digestion by wet oxidation with **HNO_3** conc. under pressure in a microwave oven
medicinal plant species	As, Cd, Co, Cr, Ni, Pb	plants were fried at 105°C, grounded, digested with **HNO_3** and **H_2O_2**
perennial plants	As, Cd, Pb	1 g plant sample is wet digested with 16 mL mixture **HNO_3** and **H_2O_2** (6:2) on a hot plate



Conclusions

Heavy metals monitoring in vegetal products, especially in those destined for human consumption **is a priority for food control**, therefore, *selection of the most appropriate method of analysis to obtain accurate results is a must.*

For quantification of heavy metals in plants, *literature studies present a large range of analytical techniques*, the most used of them being addressed in this paper.

Selection of one technique or other must be correlated with type of sample, the metal of interest and its' concentration in the sample, time of analysis, the financial possibilities of the laboratory.

It is important to evaluate advantages and disadvantages of each method and to document before each analysis, moreover some available analysis protocols depicted in literature must be amended and adapted to the subjected sample to analysis.

ACKNOWLEDGEMENTS

This work is consistent with research directions and guidelines specified by Ministry of Agriculture and Rural Development in project ADER 1.4.4. *Identification, evaluation, testing, development and validation of analysis methods of nutrients and contaminants from inputs usable in organic agriculture*.

The authors of this contribution thank you for listening to this presentation!

For further information, please fell free to contact us:
Gina VASILE SCĂEȚEANU – email: ginavasile2000@gmail.com
Roxana Maria MADJAR – email: rmadjar@gmail.com
Andrei MOȚ - email: mot_andrei@yahoo.com