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EUROPEAN COMMISSION
Horizon 2020
EUROPEAN UNION FUNDING FOR RESEARCH & INNOVATION

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H2020-TWINN-2015

Osmotic and heavy metal stress – impacts and responses

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Contents

- Short overview of common plant responses to stress
- Osmotic stress
- Heavy metal stress

Plant tolerance to various stress - inducing agents

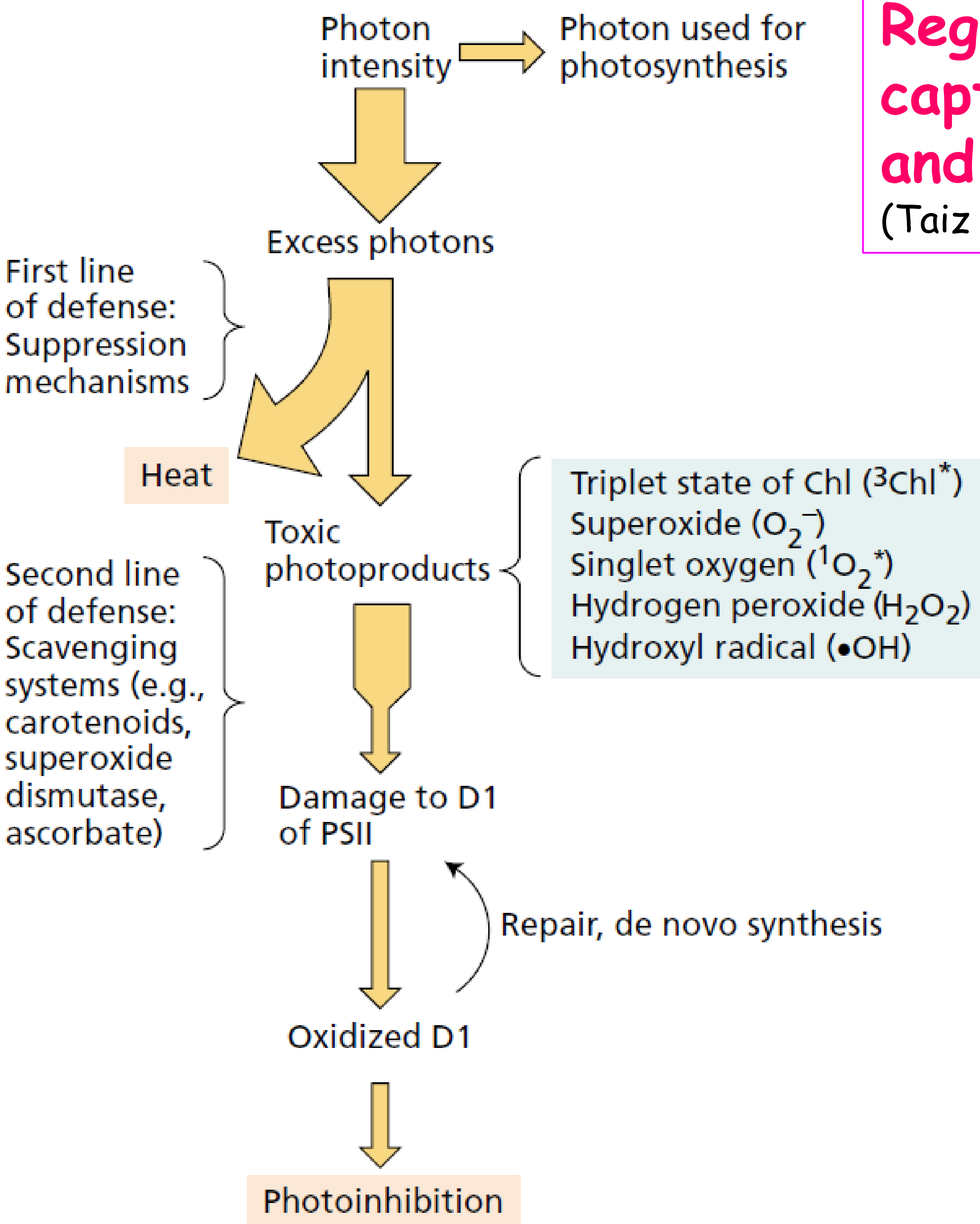
- Plants imploy similar mechanisms when exposed to different kinds of stress
- There is high genotype specificity with respect to stress tolerance

Compounds involved in stress response

- ROS
- Antioxidative enzymes and compounds
- ABA, ethylene
- Jasmonic (JA) and methyl-jasm. acid
- Brasinosteroids - 40 compounds
- Salicilic acid (SA)
- ....

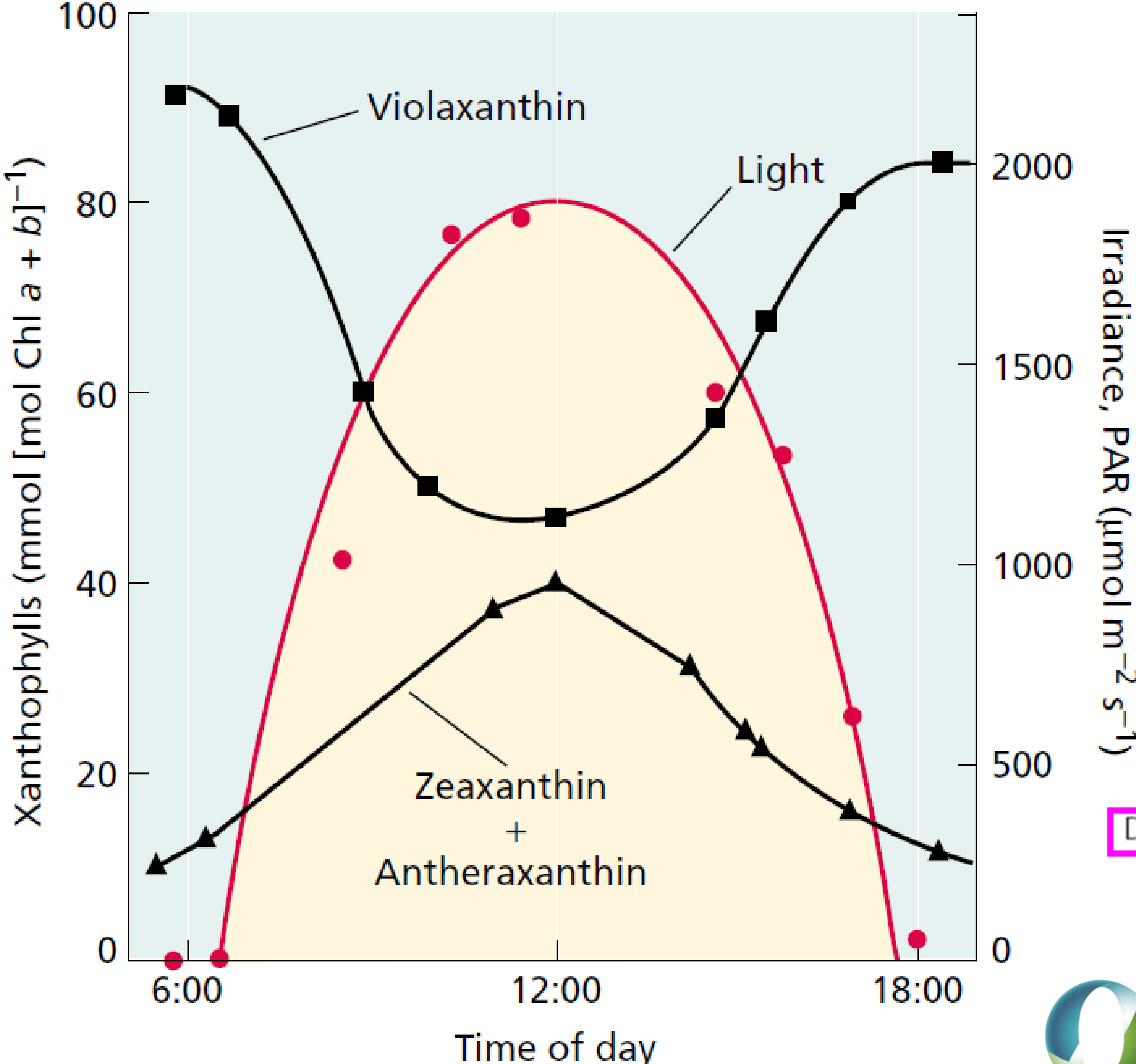
Regulation of photon capture and the protection and repair of photodamage.

(Taiz and Zeiger 2014, after Asada 1999.)



Protection against photodamage is a multilevel process.

1. Suppression of damage by **quenching of excess excitation as heat**. If this defense is not sufficient and toxic photoproducts form,
2. **a variety of scavenging systems** eliminate the reactive photoproducts. If this second line of defense also fails, the photoproducts can damage the D1 protein of photosystem II. This damage leads to photoinhibition. The D1 protein is then excised from the PSII reaction center and degraded.
3. A **newly synthesized D1** is reinserted into the PSII reaction center to form a functional unit.



Diurnal changes in xanthophyll content as a function of irradiance in sunflower (*Helianthus annuus*).

Demmig-Adams et al. 1996.

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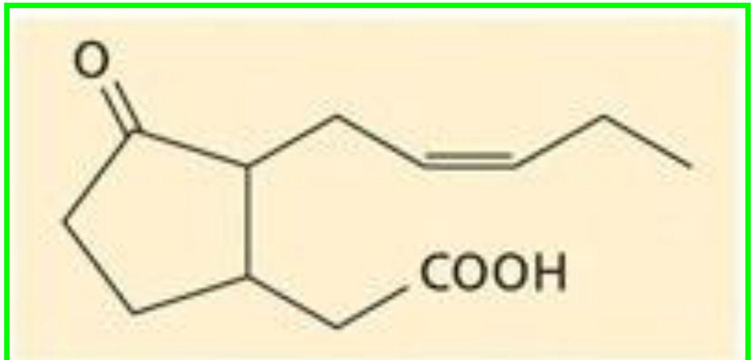
Jasmonic and methyl-jasmonic acid



Jasmonic acid

Jasmonic acids - a class of lipidic plant hormones

Synthesized from linolenic acid present in the chloroplast membrane

Involved in development, abiotic stress responses and plant-microbes interactions in defence and symbiosis.

Derivates such as methyl-jasmonate are volatile and participate in long range signalling between plants.

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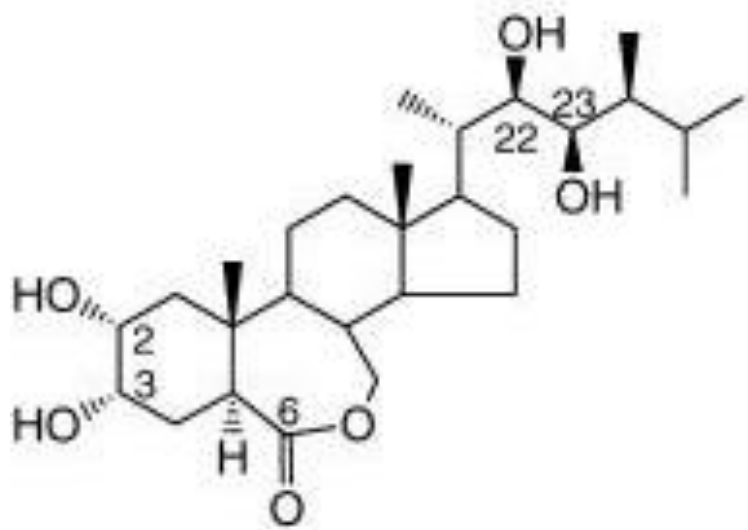
Brassinosteroids



Brassinolide, frstly extracted from *Brassica napus*

A group of plant steroid hormones

Regulate growth and development

Structurally similar to cholesterol-derived animal steroid hormones and insect ecdysteroids.

Involved in cell expansion, biotic and abiotic stress tolerance, vascular differentiation, pollen tube formation, and other important processes during the life of the plant.

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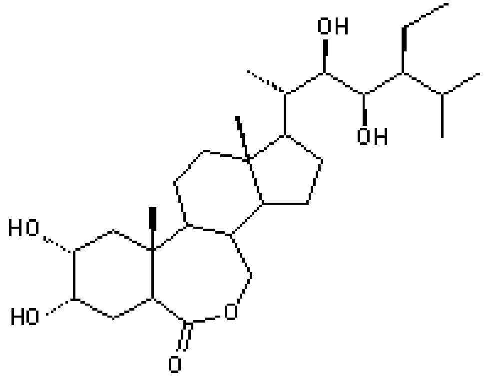
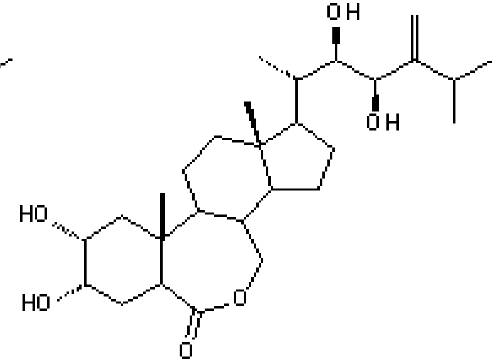
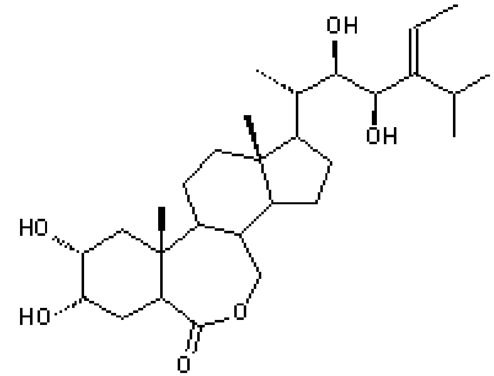
Brassinosteroids



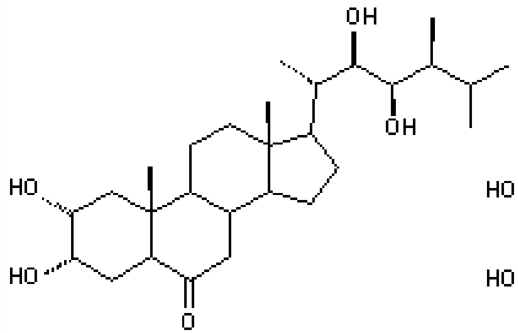
Homobrassinolide



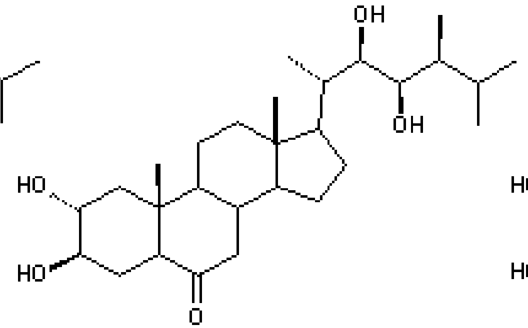
Dolicholide



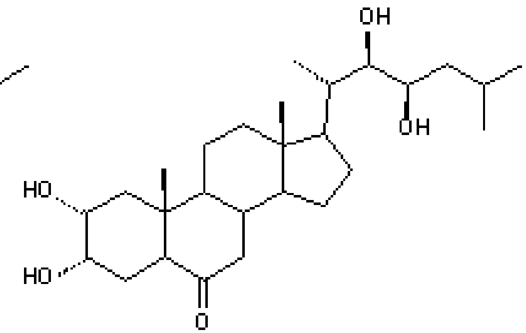
Homodolicholide



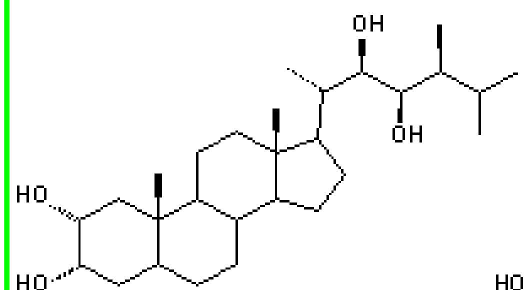
Castasterone



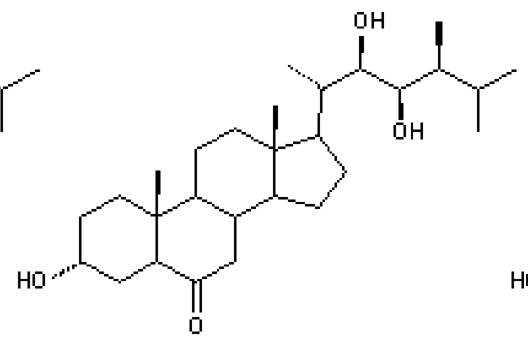
3-Epicastasterone



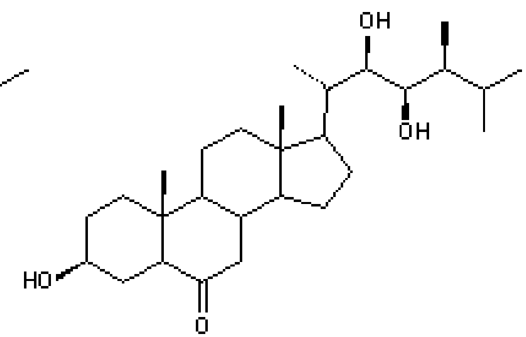
Brassinone



6-Desoxocastaterone



Typhasterol



Teasterone

EXCESS OF SALTS AS A FACTOR CAUSING OSMOTIC STRESS IN PLANTS

NaCl in the soil solution 0.001-0.01%

Salinization of soils

High concentration of salts in the soil solution impairs uptake of nutrients and water and may have toxic effects on cultivated plants

Factors contributing to soil salinization:

- Quality of irrigation water
- High level of underground water with high solt content and salty waste waters
- Excessive application of mineral fertilizers
- Absence of drainage (dewatering), especially on primarily salty soils (secondary salinization)

Soil salinization

Besides advantages, irrigation in the long run can pose a great danger from the standpoint of preserving the soil structure

Uncontrolled use of water for irrigation, even of good quality, may leed to:

- **Secondary soil salinization**
- **Acute and hidden salinization**

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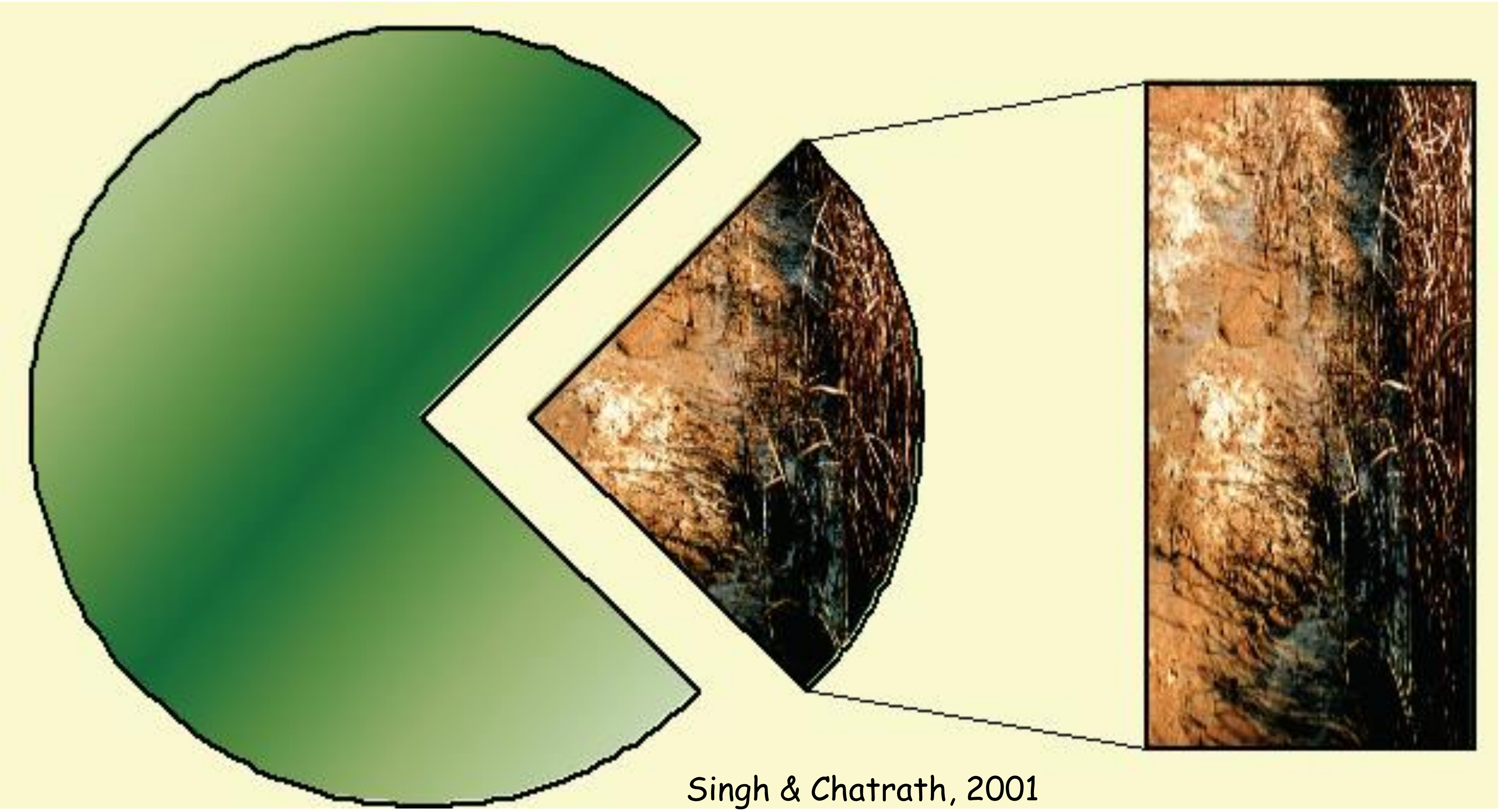
Irrigated soils damaged due to excess of salts



Total irrigated soil surface

Already damaged soils

Osmotic stress

Restoration of saline soils is expensive and uncertain, and to maintain the ionic balance of the soil it is necessary:

- That conditions for moving water down the depth below the root system are present - percolation
- That input of salts into the soil and rinsing of soil with water are balanced

Soils containing excessive salts can be improved by more frequent watering, higher irrigation rates and by "plastering"

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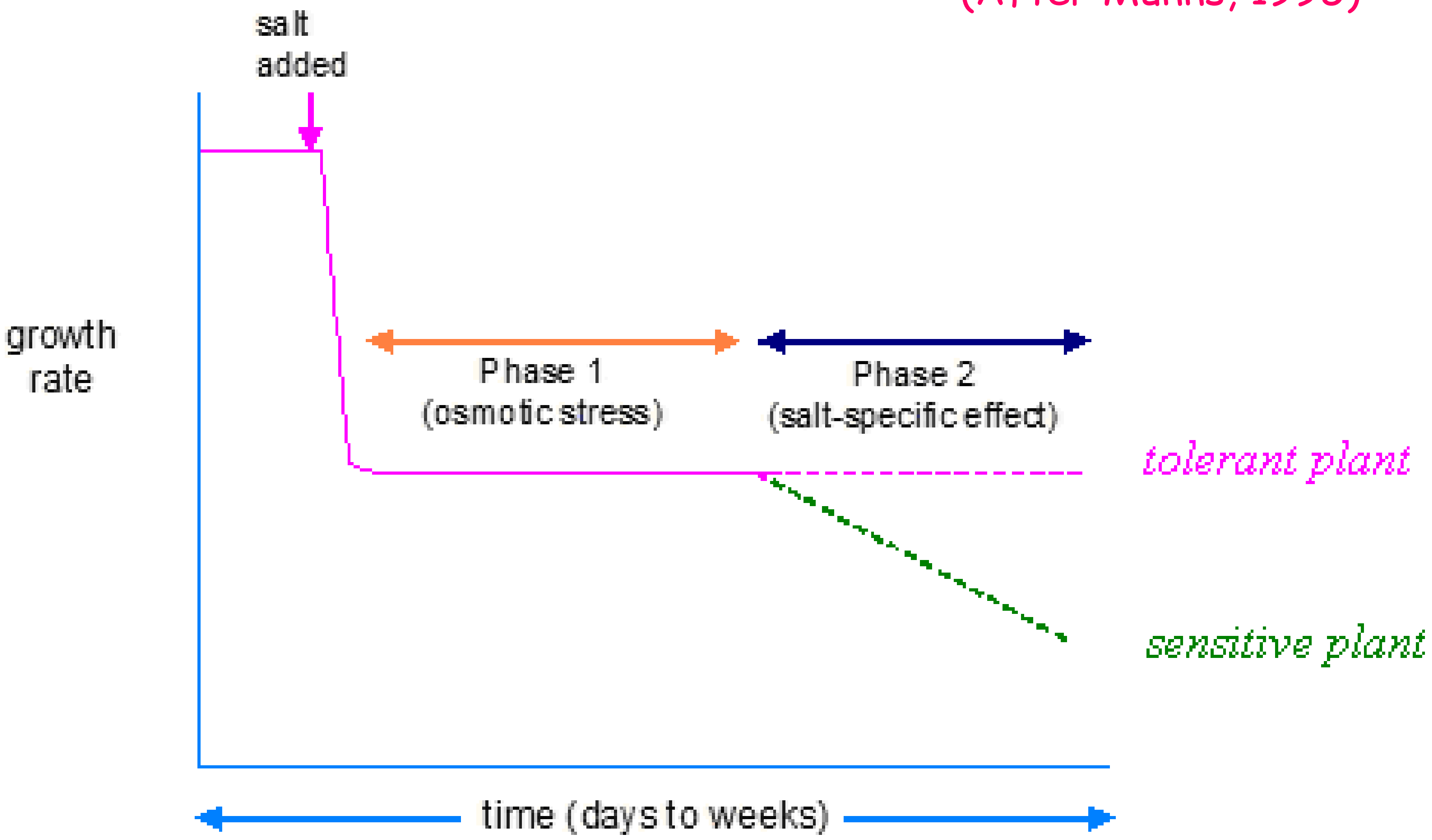
Scheme of the two-phase growth response to salinity

(After Munns, 1995)



Ecological groups of plants according to tolerance to excess of salts:

- **Halophytes**
- **Glycophytes**

Adaptation:

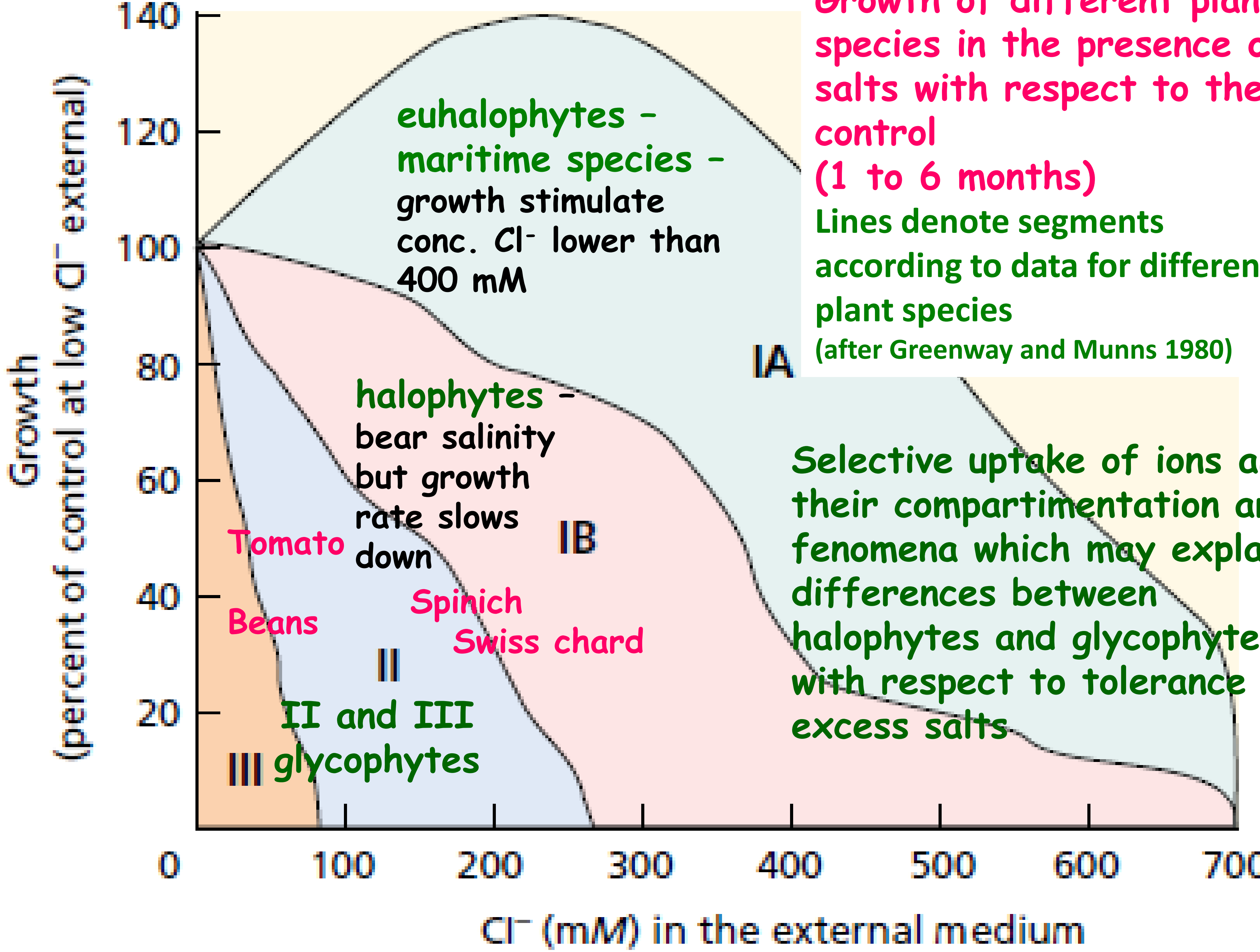
Active transport of salts from the cells
Uptake of water – dillution of salt conc. in the cells

Halophytes – high content of minerals, low osmotic potential, high suction power, low biomass production

Among crop plants there are no halophytes!

Growth of different plant species in the presence of salts with respect to the control (1 to 6 months)

Lines denote segments according to data for different plant species (after Greenway and Munns 1980)



Selective uptake of ions and their compartimentation are fenomena which may explain differences between halophytes and glycophytes with respect to tolerance to excess salts

Impact of salt stress on plants

Primary

Lack of water
Ionic disbalance – NaCl, dominant salt: Na^+ impairs uptake of K^+

Secondary

Reduced cell growth
Reduced photosynthesis
Reduced intensity of metabolic reactions
Production of ROS

Glycophytes also have mechanisms for adaptation to increased concentrations of salts

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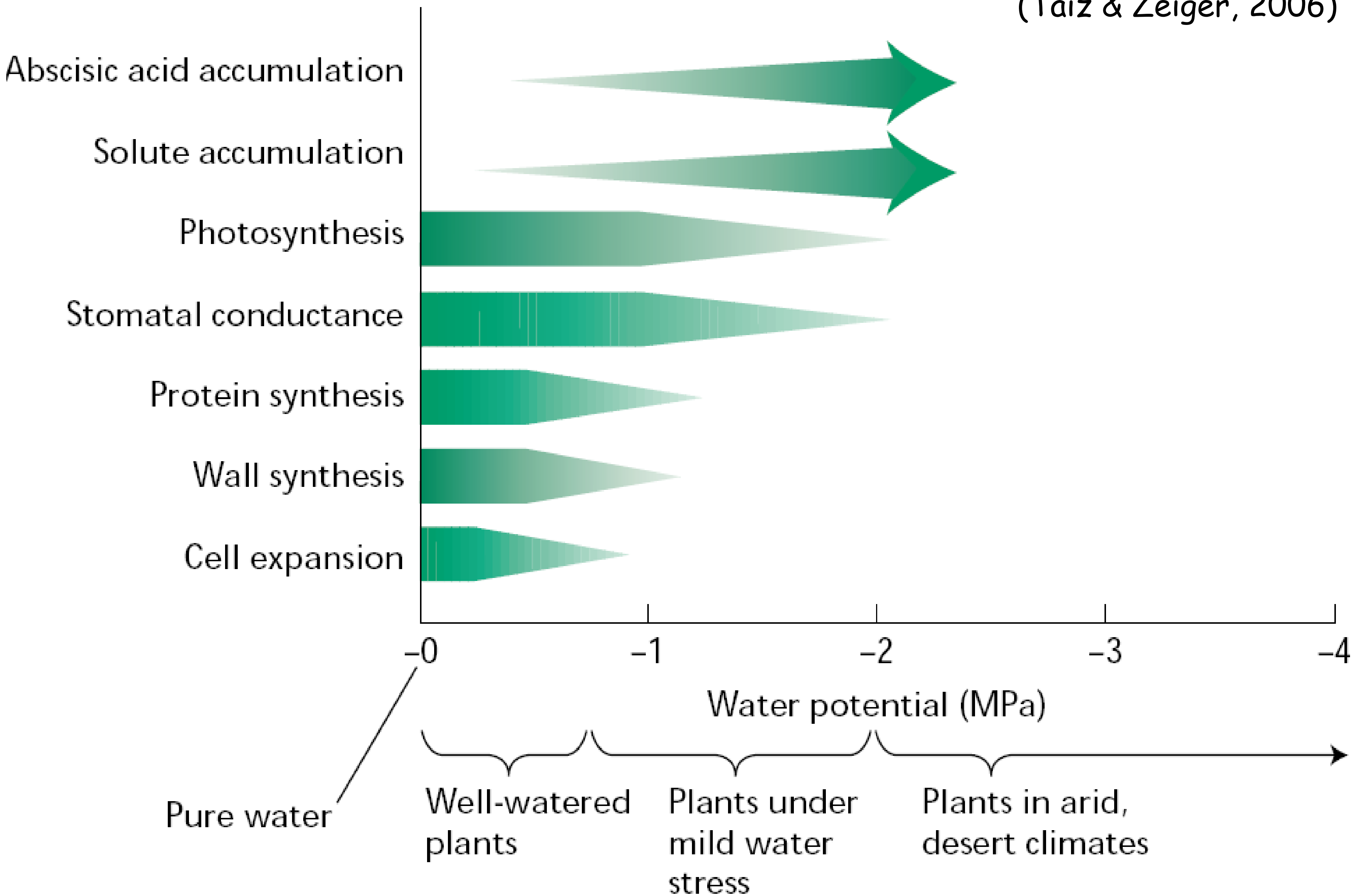
Osmotic potential increases with salinity

- High concentration of salts
- Reduced growth due to impaired uprake of water
- Visible already at germination
- Efect depends on phenophase

Osmotic stress – effects

(Taiz & Zeiger, 2006)



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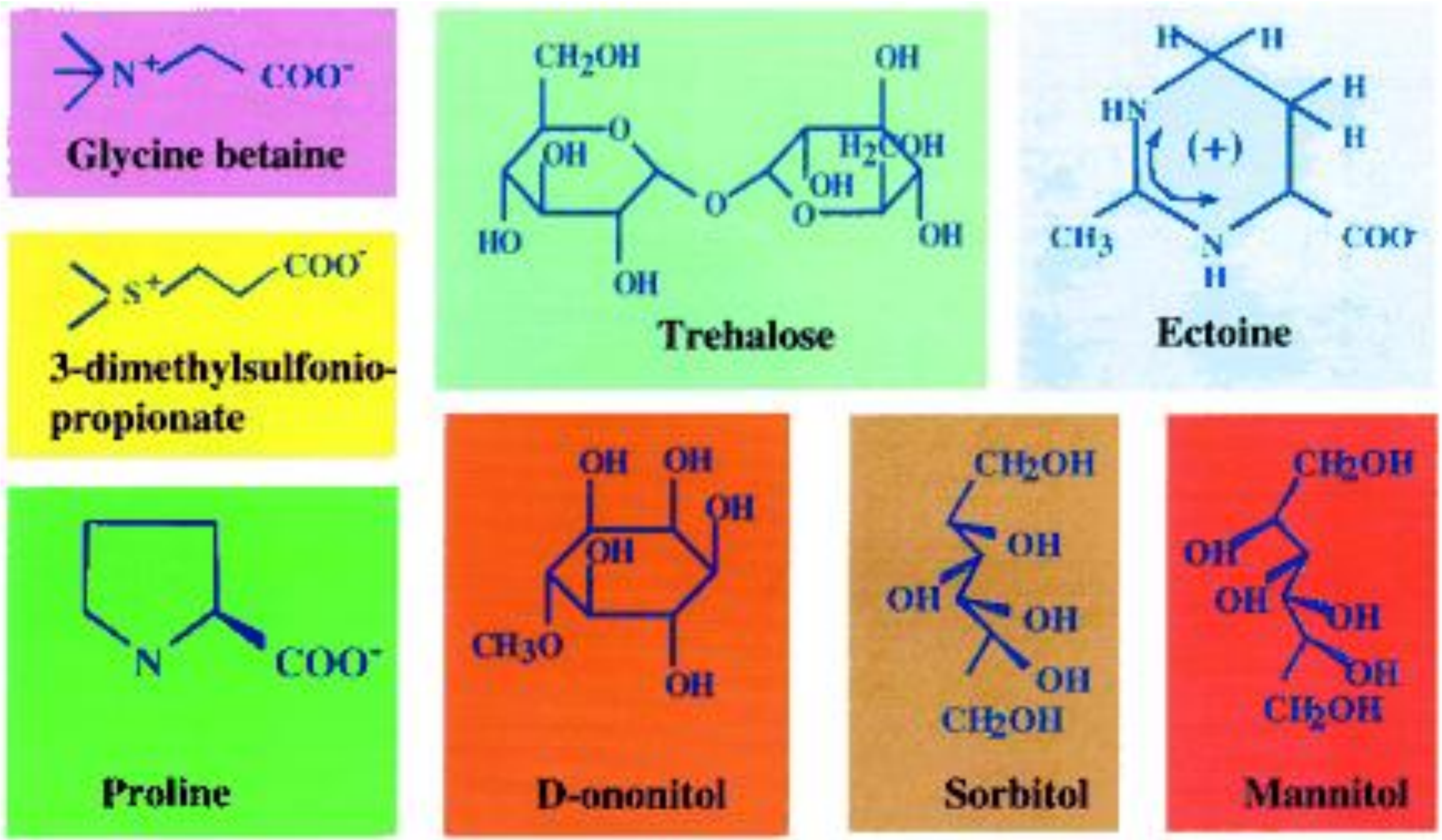
Compatible osmolytes – osmoprotectants – allow osmotic adjustment of plants



Hasegawa et al., 2000

Retarded growth and dark green leaves caused by too high osmotic value of the nutrient solution

PLANT RESPONSE TO SALINITY AT DIFFERENT TIME SCALES. The effects on a salt-tolerant plant are basically identical to those due to soil water deficit (Munns, 2002)

Time	Water stress effects (Observed effect on growth of a salt-tolerant plant)	Salt-specific effects (Additional effects on growth of a salt-sensitive plant)
Minutes	Instant reduction in leaf and root elongation rate, than rapid partial recovery	
Hours	Steady but reduced rate of leaf and root elongation	
Days	Leaf growth more affected than root growth; Reduced rate of leaf emergence	Injury visible in older leaf
Weeks	Reduced final leaf size and/or number of lateral shoots	Death of older leaves
Months	Altered flowering time, reduced seed production	Younger leaves dead, plant may die before seed matures

Type of water	Total soluble salts (ppm)	EC (dS m^{-1})	Plant species	Trashhold EC (dSm^{-1})	Degree of tol.
Sweet water	< 500	< 0.6	all		
A little brackish	500-1000	0.6-1.5	Beans	1.0	S
			Carrot	1.0	S
			Onion	1.0	S
			Eggplant	1.1	MS
			Melon	1.2	MS
			Radish	1.2	MS
			Lettuce	1.3	MS
			Pepper	1.5	MS
Brackish	1000-2000	1.5-3.0	Garlic	1.7	MS
			Potato	1.7	MS
			Cabbage	1.8	MS
			Celery	1.8	MS
			Spinich	2.0	MS
			Squash	2.5	MS
			Tomato	2.5	MS
Moderatly saline	2000-5000	3.0-8.0	Peas	3.4	MS
			Red beet	4.0	MT
			Asparagus	4.1	T
Saline	5000-10000	8.0-15.0	-		
Very saline	10000-35000	15.0-45.0	-		

Genotype features which affect uptake of ions

<u>ROOT</u> MORPHOLOGY: root type (primary, secondary), mass, length, topography, absorption surface, cortex thickness

<u>LEAF</u> MORPHOLOGY: size, shape, thickness, position

<u>STEM</u> MORPHOLOGY: diameter, length, number of elements of conductive vessels and their sturcture

SHOOT/ROOT RATIO

PHYSIOLOGICAL PROCESSES: photosynthesis, transpiration, respiration, distribution and reutilization of inorganic and organic compounds

BIOCHEMICAL PROCESSES: enzymatic activity, direction of synthesis of organic compounds (sugar, protein, fat), phytochrome content, amino acids and organic acids

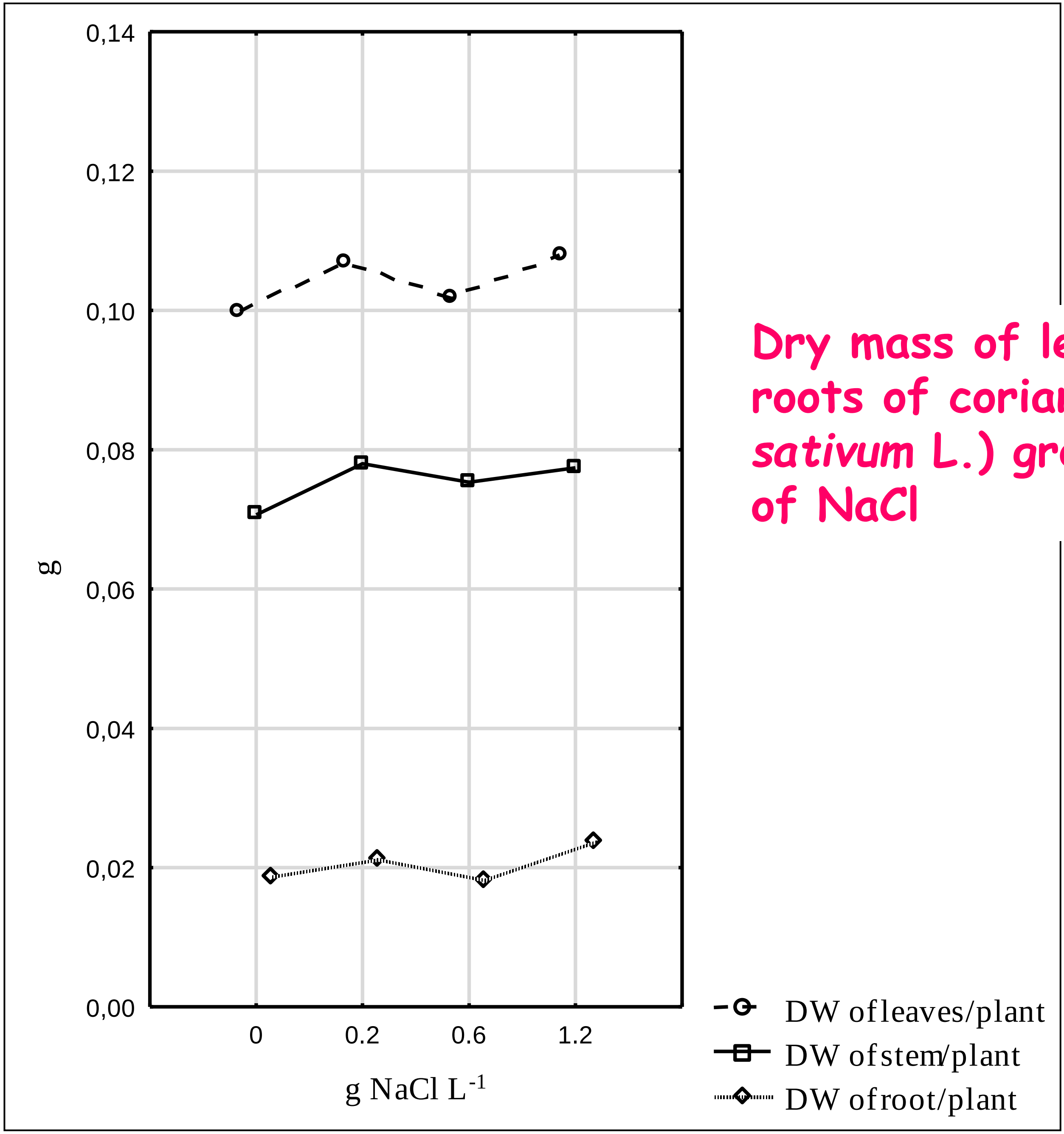
LEVEL OF PLOIDY AND HYBRIDITY

The fect of low concentrations of NaCl on physiological and biochemical features and chemical composition of coriander (*Coriandrum sativum* L.)

Spice and medicinal plant – antioxidant, antiseptic, diuretic.

g NaCl L^{-1}	mS cm^{-1}
0	1.10
0.2	1.50
0.6	2.26
1.2	3.39

½ Hoagland
14 d old plants
Treatment 21 d



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Dry mass of leaves, stems and roots of coriander (*Coriandrum sativum* L.) grown in the presence of NaCl

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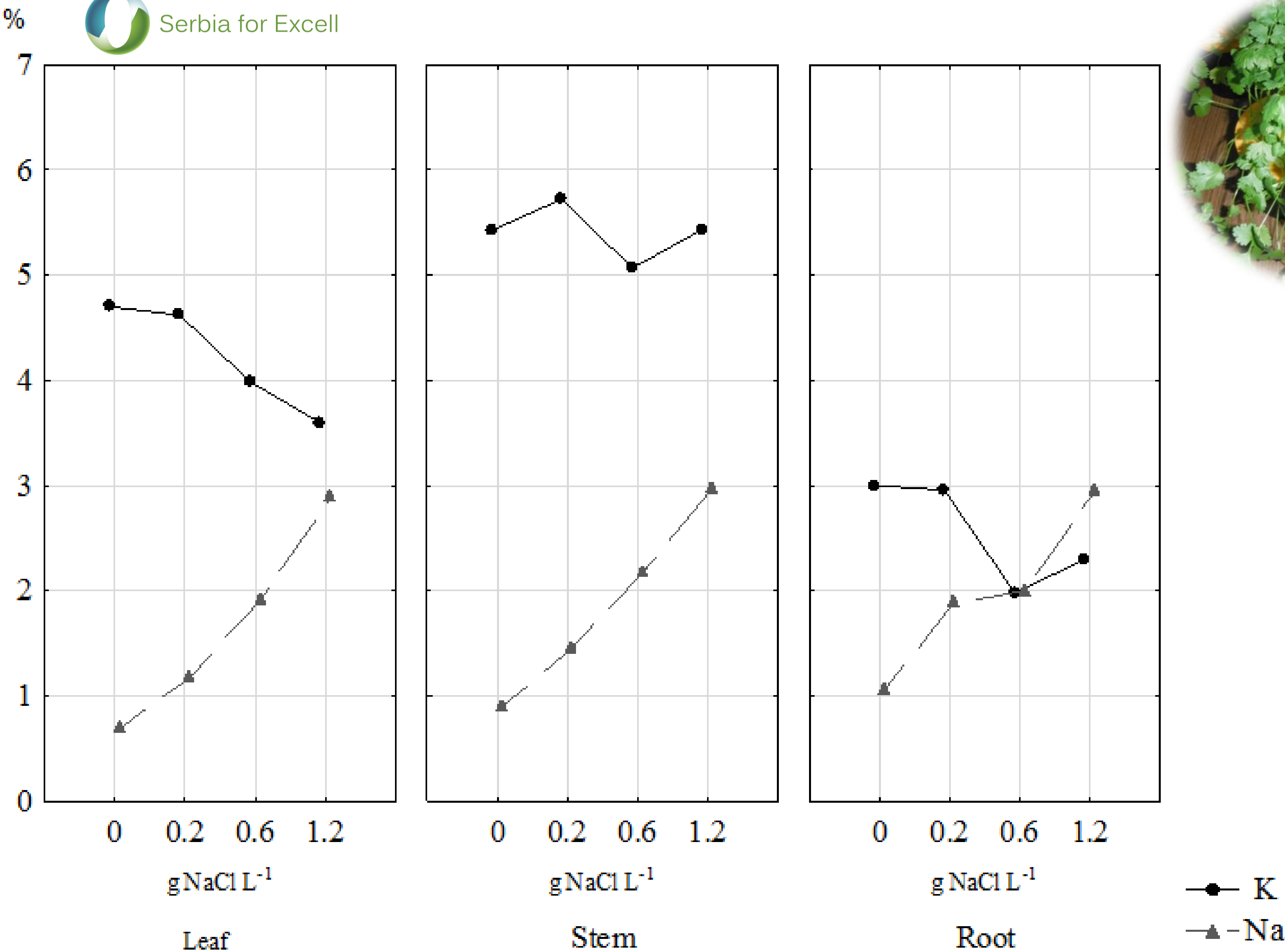
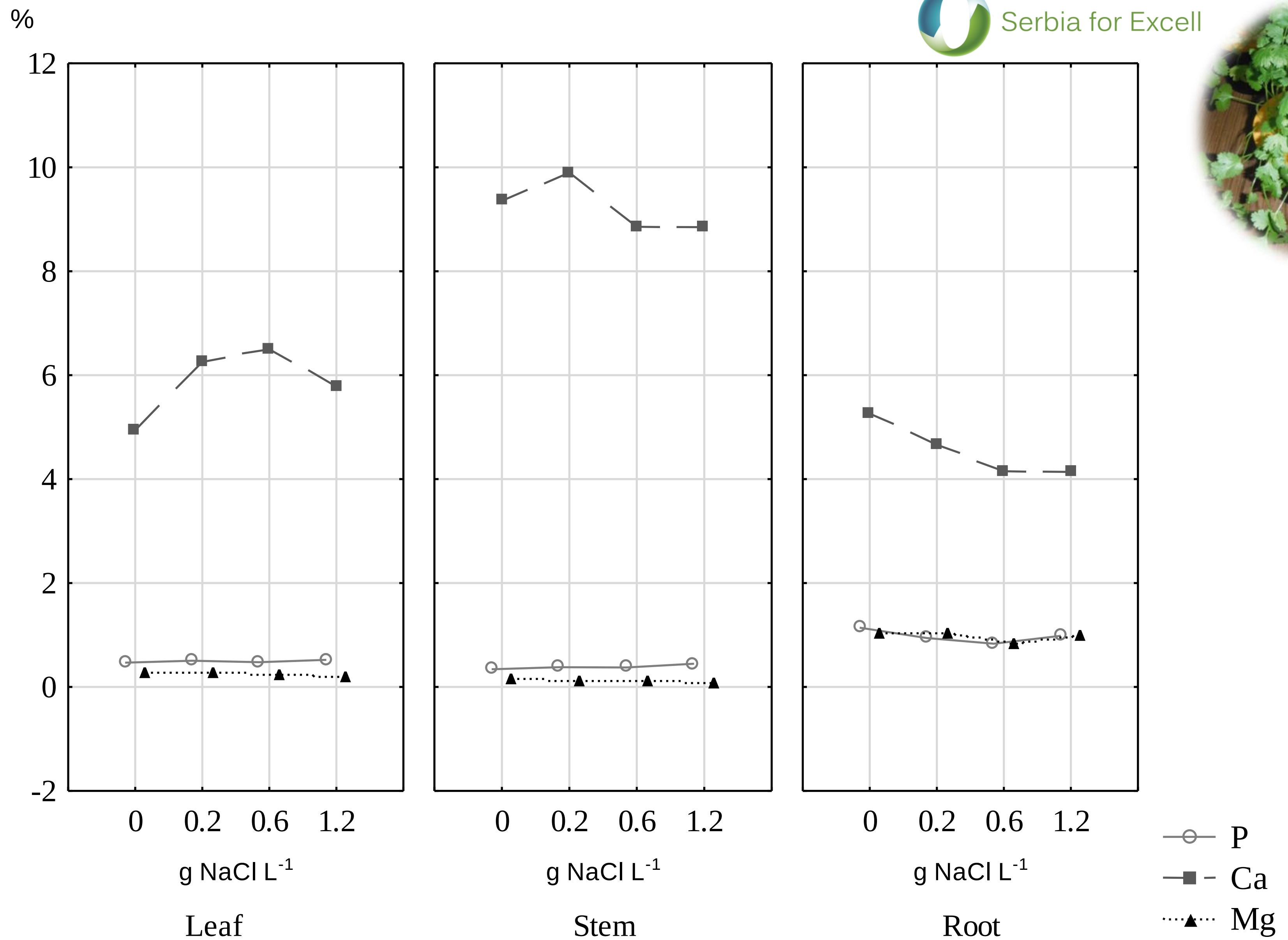
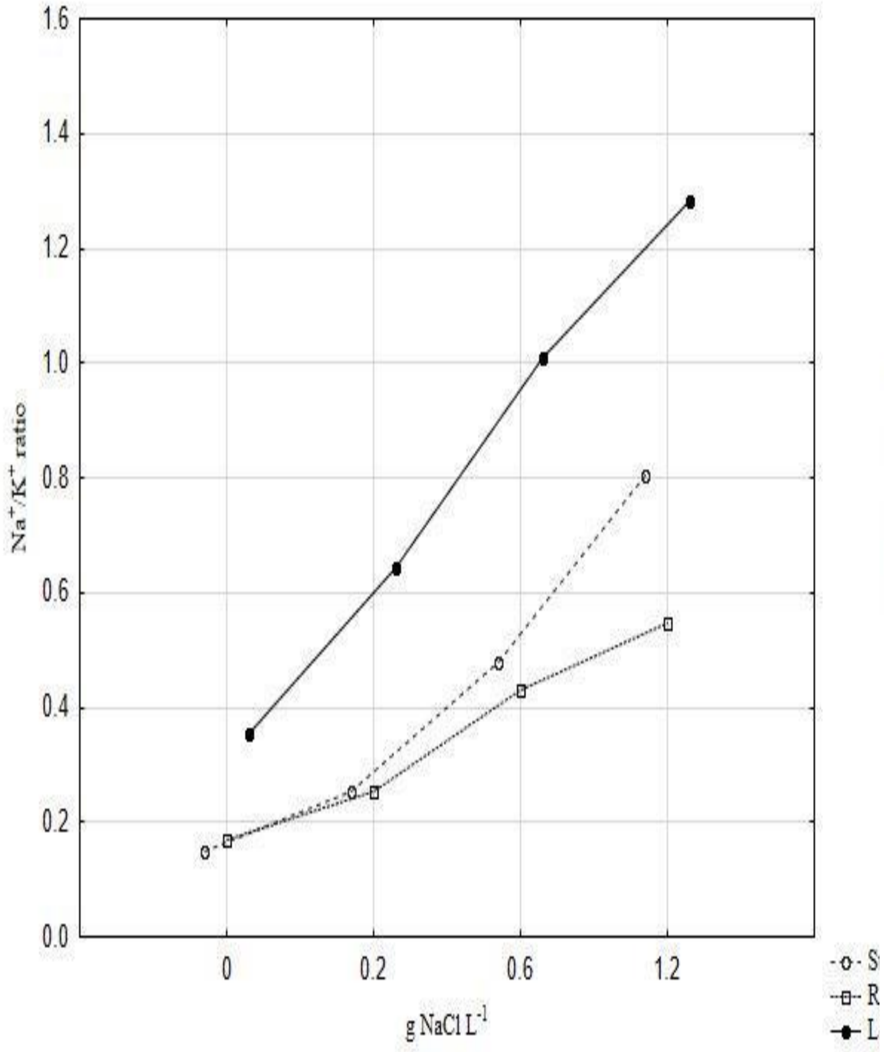


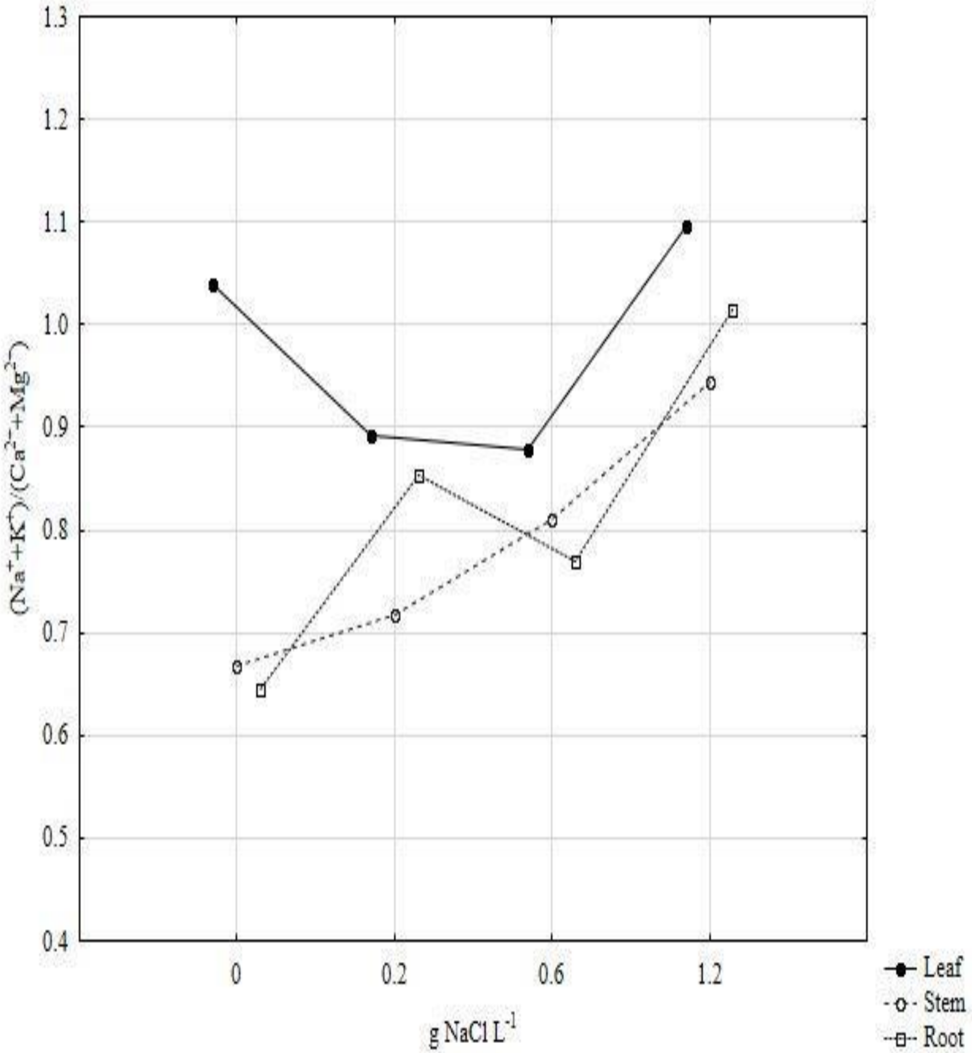
Concentration of Na and K in leaves, stems and roots of coriander (*Coriandrum sativum* L.) grown in the presence of NaCl



Serbia for Excell

Concentration of Ca, P and Mg in leaves, stems and roots of coriander (*Coriandrum sativum* L.) grown in the presence of NaCl





Na^+/K^+

$(Na^++K^+)/(Ca^{2+}+Mg^{2+})$

in leaves, stems and roots of coriander (*Coriandrum sativum* L.) grown in the presence of NaCl

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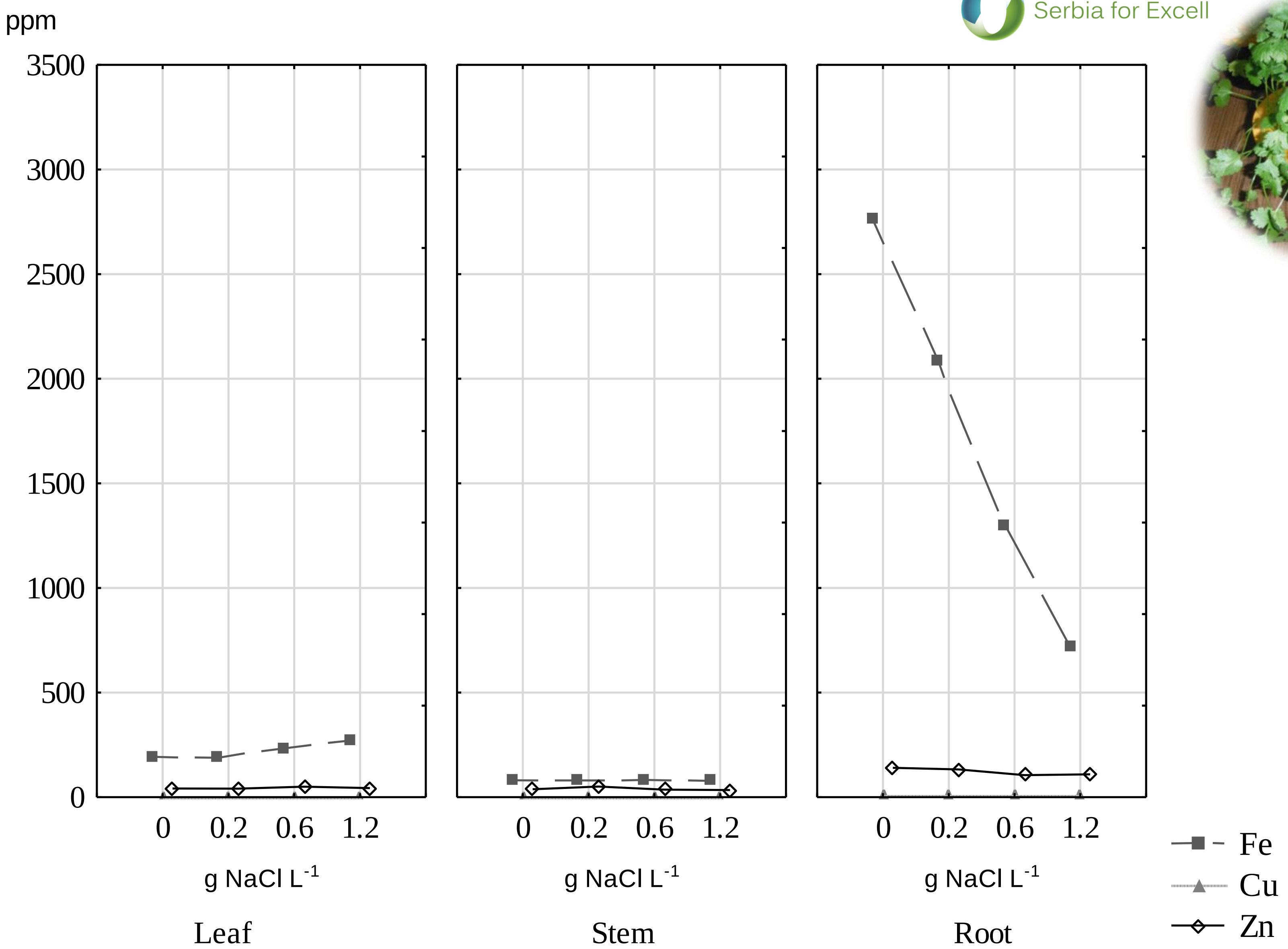

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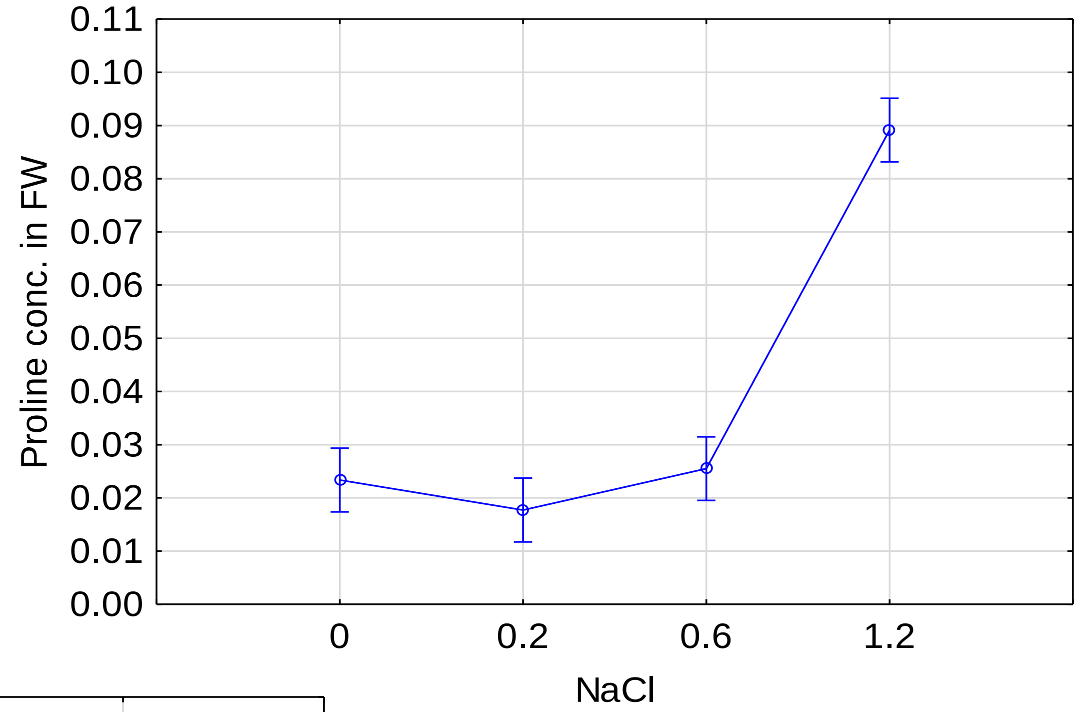
Concentrations of Fe, Cu and Zn in leaves, stems and roots of coriander (*Coriandrum sativum* L.) grown in the presence of NaCl

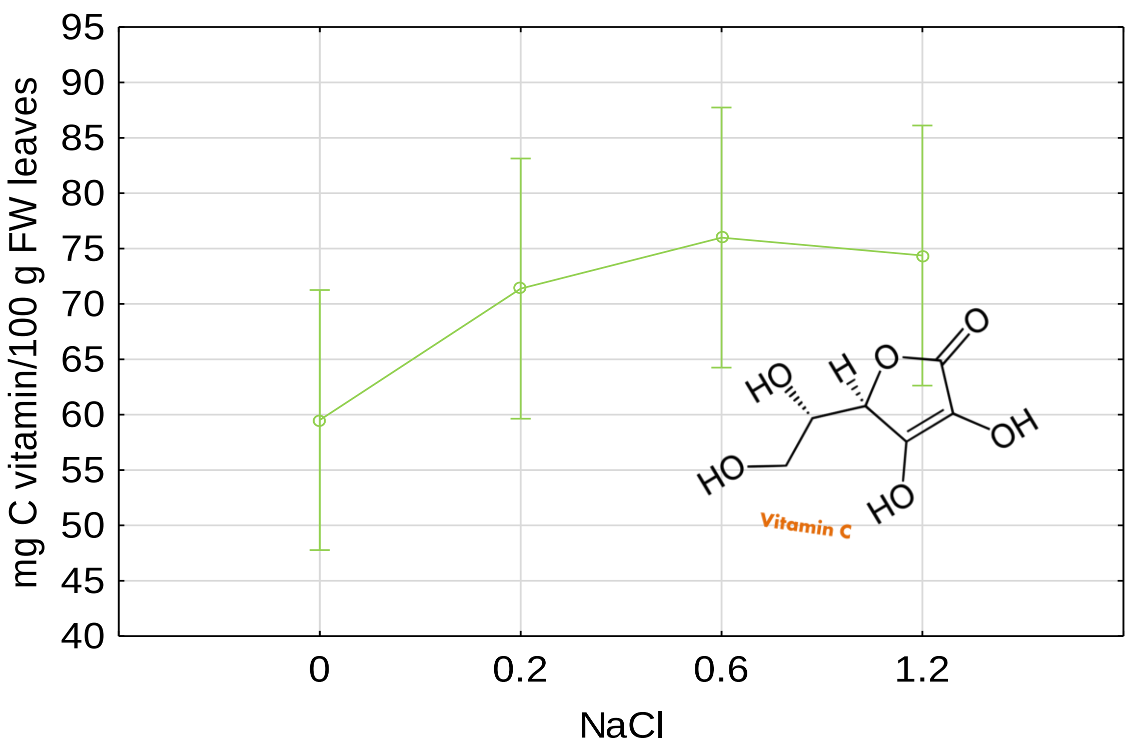
Antagonism and synergism of ions and osmotic stress

Specific effects of excess of particular nutrients on the content of the other nutrients

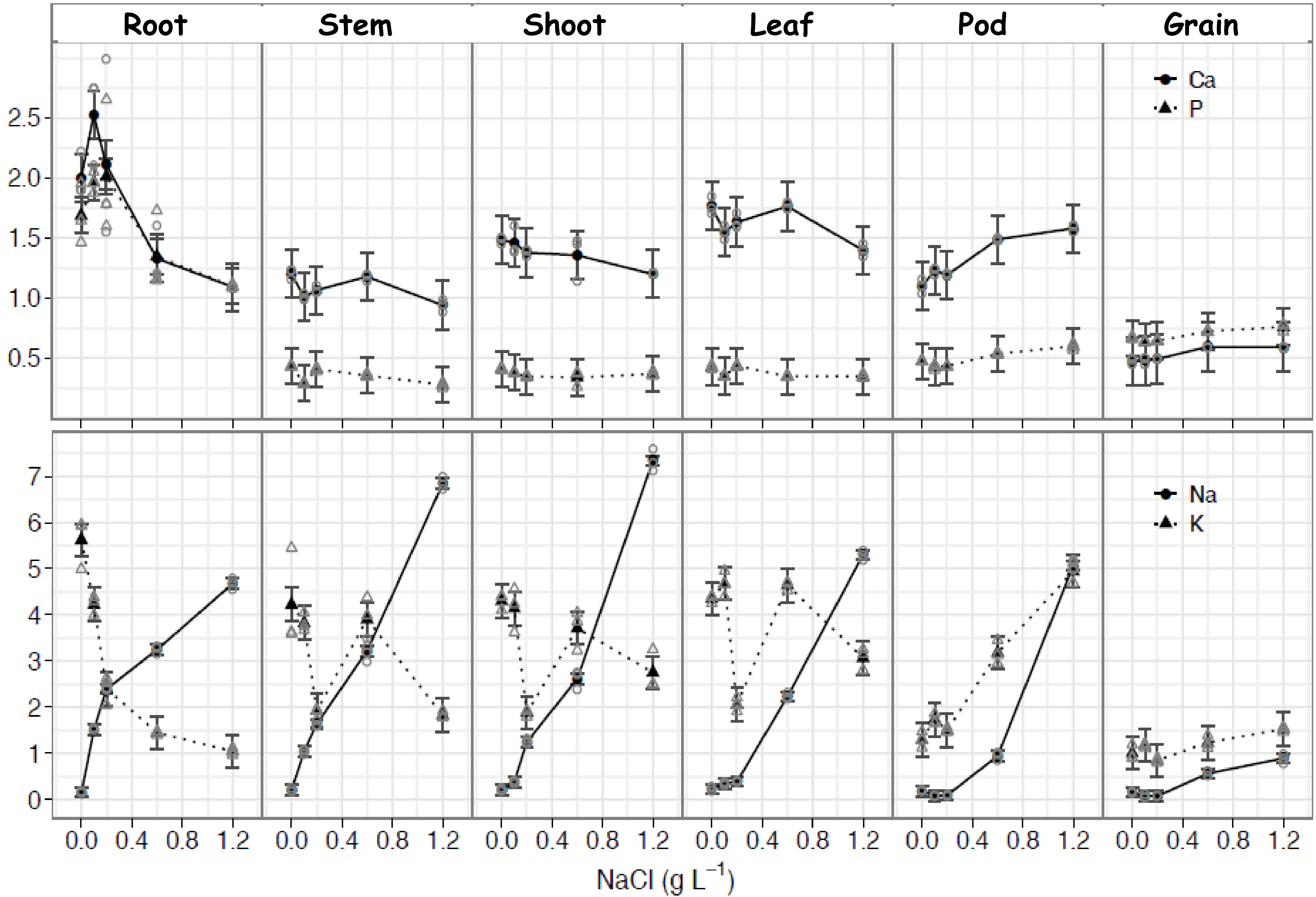
Element in excess	Type of effect	
	Positive (increase in content 15% and more)	Negative (reduction in content 15% and more)
N	-	Mg, Co, Mo, B
P	-	N, Ca, Mg, Co, B
K	Mo	N, P, Ca, Mg, Cu, Zn, Mn, Co
Mg	-	P, K, Ca, Mn, Co, B
Cu	Mg, Co, Mn	Mo
Zn	Ca, Mg, Co	-
Mn	K, Zn, Co	Mg, Mo
B	Cu	-

Concentration of free proline and vitamine C in the leaves of coriander (*Coriandrum sativum* L.) grown in the presence of NaCl





Impact of NaCl on the concentration and distribution of Ca, P, K and Na in pea



Salt (osmotic) stress - conclusion

Soil salinization is important problem in agricultural production

Harmful efect of salts is visible on the entire plant; even when it is not obvious it may leed to yield reduction and deterioration of quality - losses

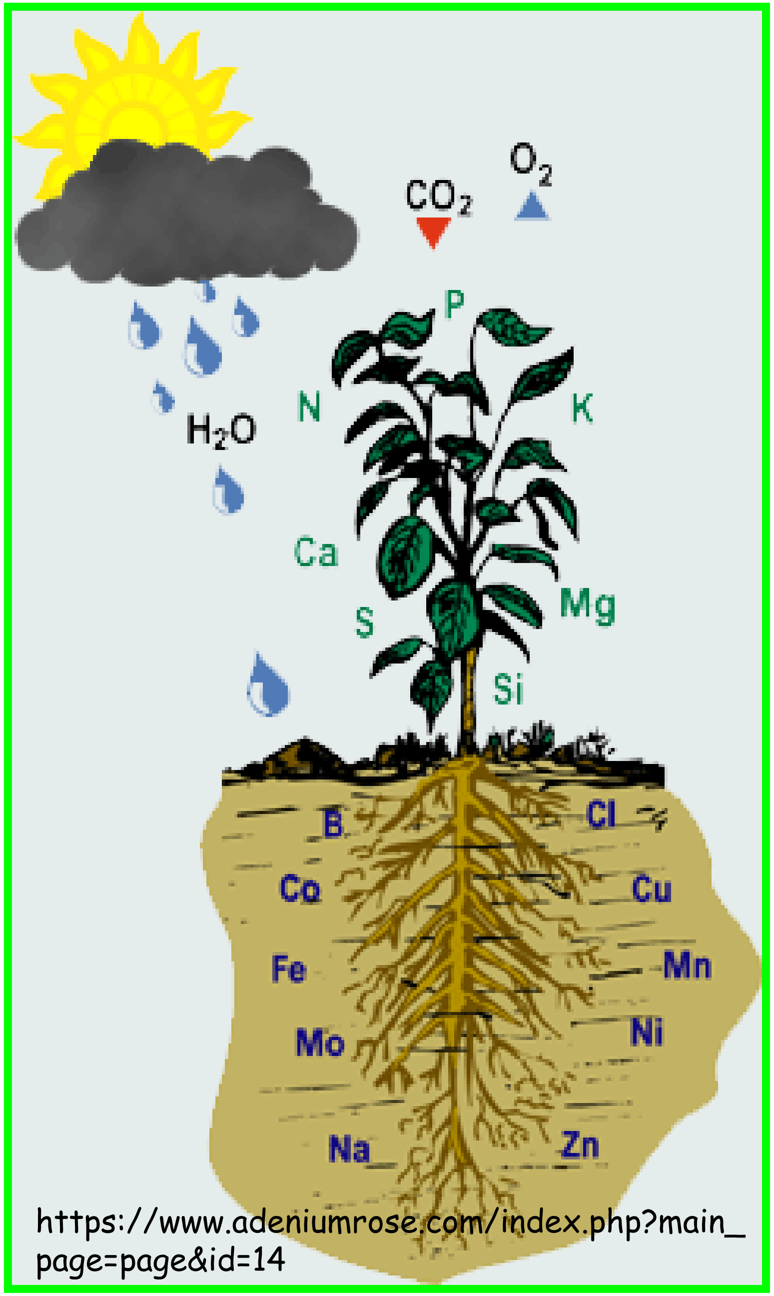
Plant species differ with respect to tolerance to excessive concentrations of salts

Understanding of responses of plant cells and whole plants to salt stress is crucial for stimulation of mechanisms leading to adaptation

Selection and breeding – both by classic and molecular methods will contribute to beter adaptation of plants and increased tolerance to salt stress

HEAVY METALS

- Metals whose specific weight is above 5 g/cm^3
- High concentrations are <u>extremely toxic</u>
  - Soluble in water
  - Living organisms uptake them easily (plants and animals)
    - Concentrate in tissues
  - React with biomolecules
    - Proteins
    - Nucleic acids



https://www.adeniumrose.com/index.php?main_page=page&id=14

Cu Mo Co Hg Pb Cd Mn Ni Fe Zn Mo

Sources of HM pollution

Smelters

Exhaust of automobiles

Additives in paints

HM in the environment

Urban wastes

Industry

Effluents from battery industry

Fertilizers, pesticides

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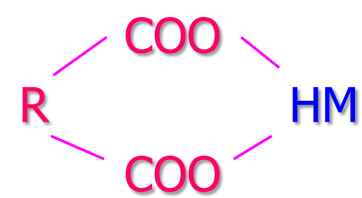
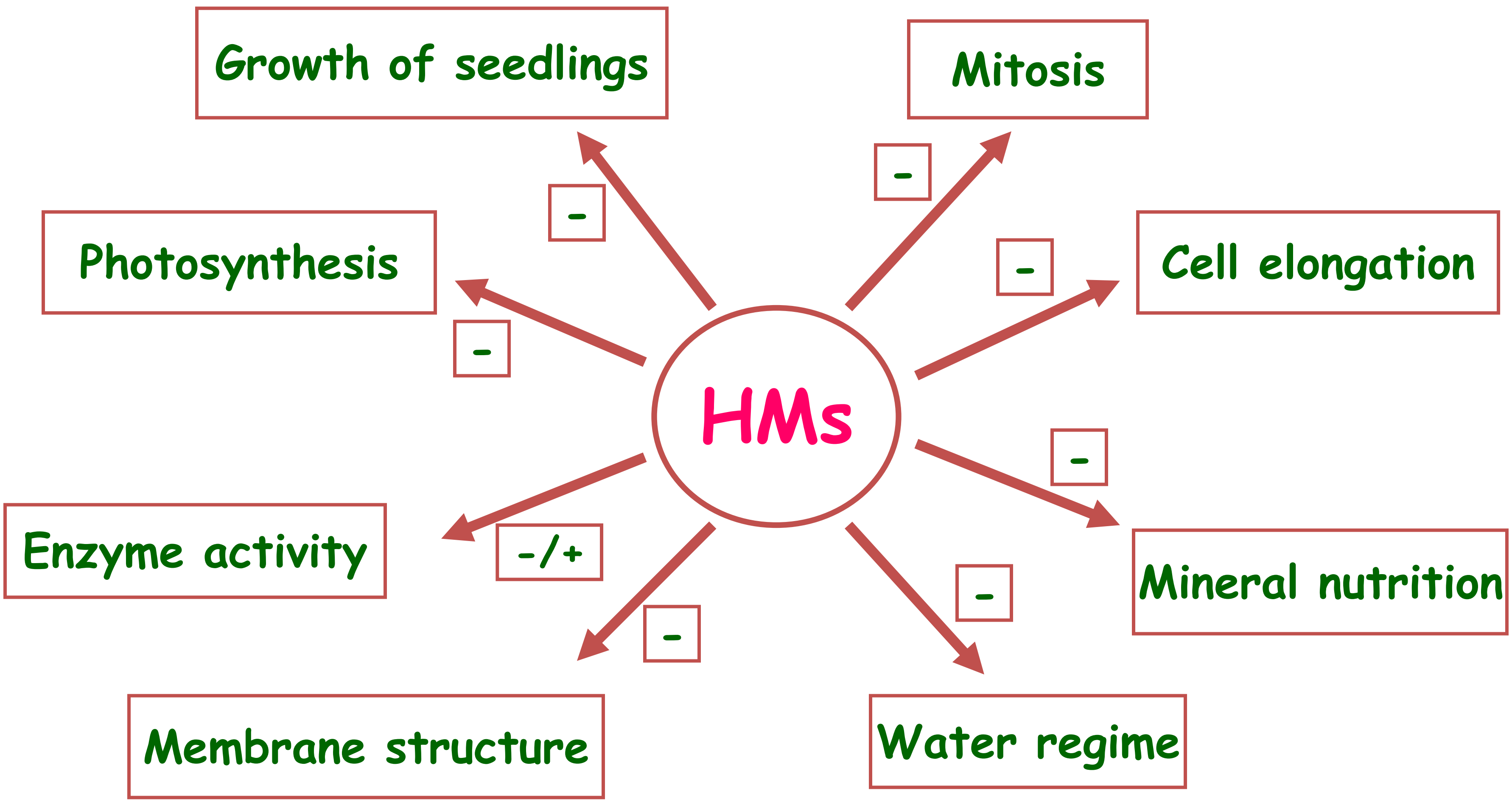
Ways of HM binding in the soil

Phase	Forms of binding	Examples
Fluid phase	1. Free hydrated ions	$[Ca(aq)]^{2+}$, $[Na(aq)]^{+}$, $[Cu(aq)]^{2+}$, $[Fe(aq)]^{3+}$
	2. Associations of ions and inorganic complexes	$CaHCO^{3+}$, $CaSO_4^{0}$, $CdCl^{+}$, $AlSO_4^{+}$, $CuOH^{+}$, $AlOH^{2+}$
	3. Water-soluble complexes	 HM – Fulvo acid; HM – lipid
	4. Dispersed coloids	$Fe(OH)_3 n\ H_2O$; $Mn(OH)_4$; Fe OOH
Solid phase	5. Sediment	CsS, FeS, $PbCO_3$, $CdCO_3$, $CuCO_3$
	6. Replacable and adsorbed specifically for colloids	HM – humate; HM – clay minerals; HM – hydrated sediment
	7. Ions forming net in silicates	Primary silicates, clay minerals

Effects of HMs on plants



Generalized pattern of partitioning of HMs in roots and shoots

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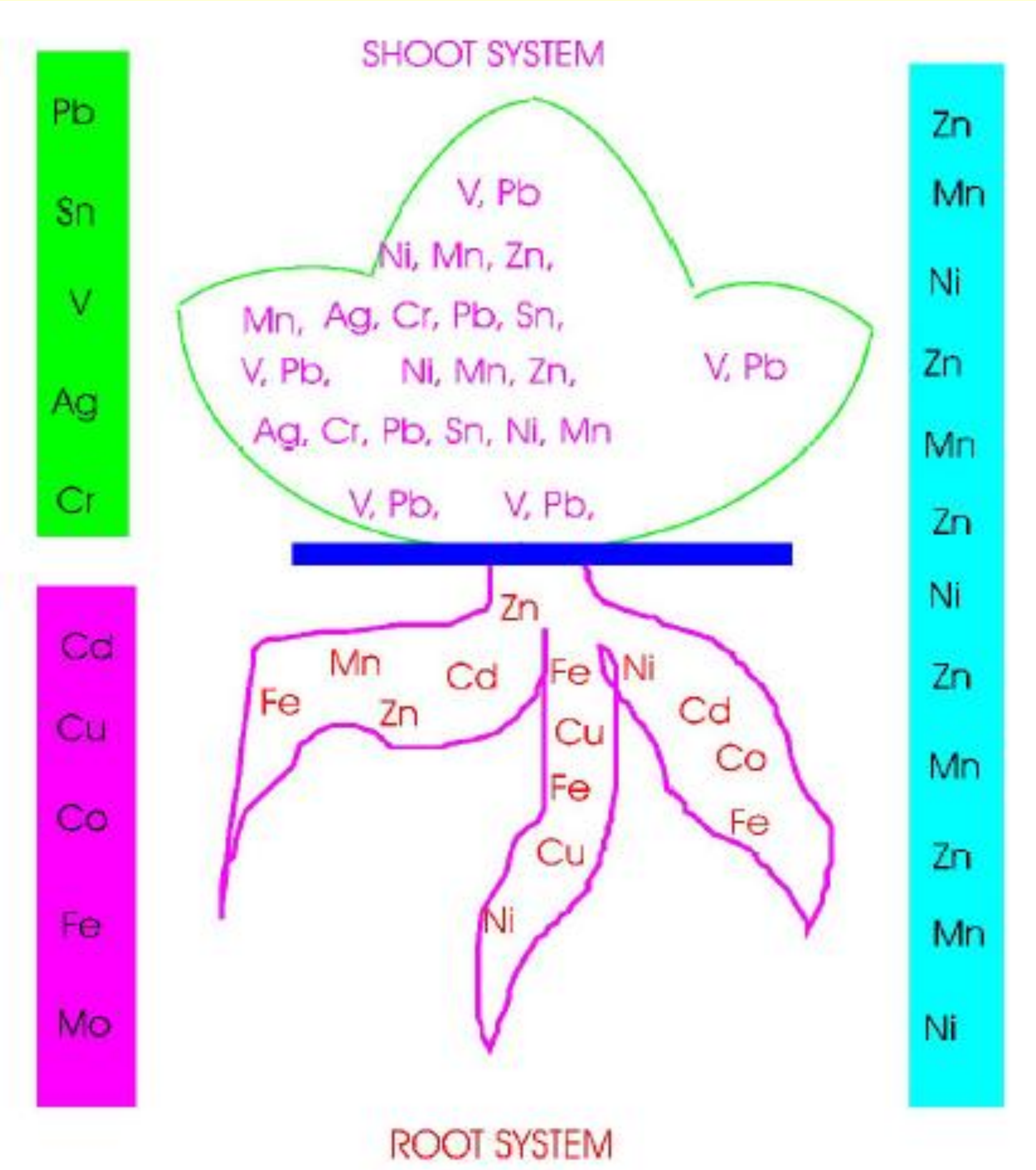

Ag, Cr, Pb, Sn and **V** accumulate more in **shoots** (stems and leaves) compared to roots and rhizomes.

Cd, Co, Cu, Fe and **Mo** acumulate more in **roots** and **rhizomes** than in shoots (stems and leaves).

Ni, Mn and **Zn** are distributed more or less **uniformly** in root/shoot of the plant

(Prasad and De Oliveira Freitas, 1999).

Effect of pH on solubility of HMs

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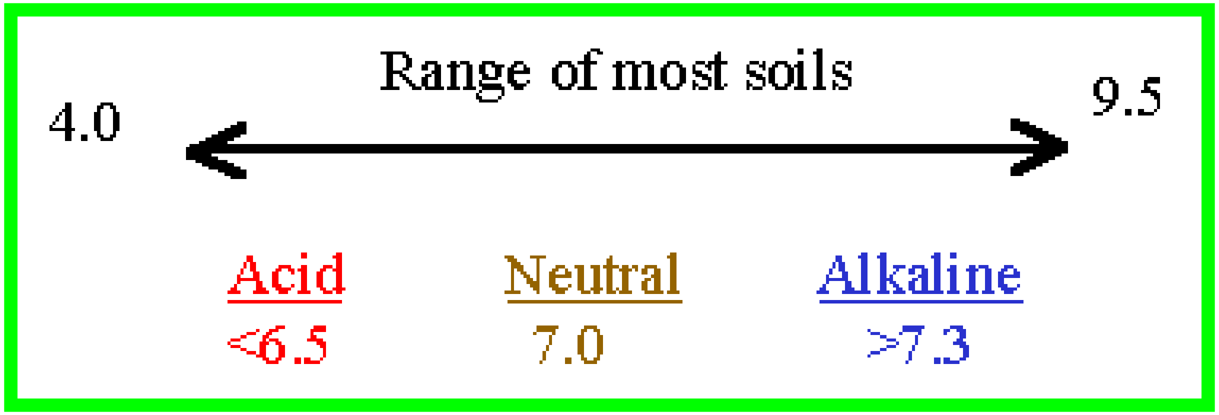
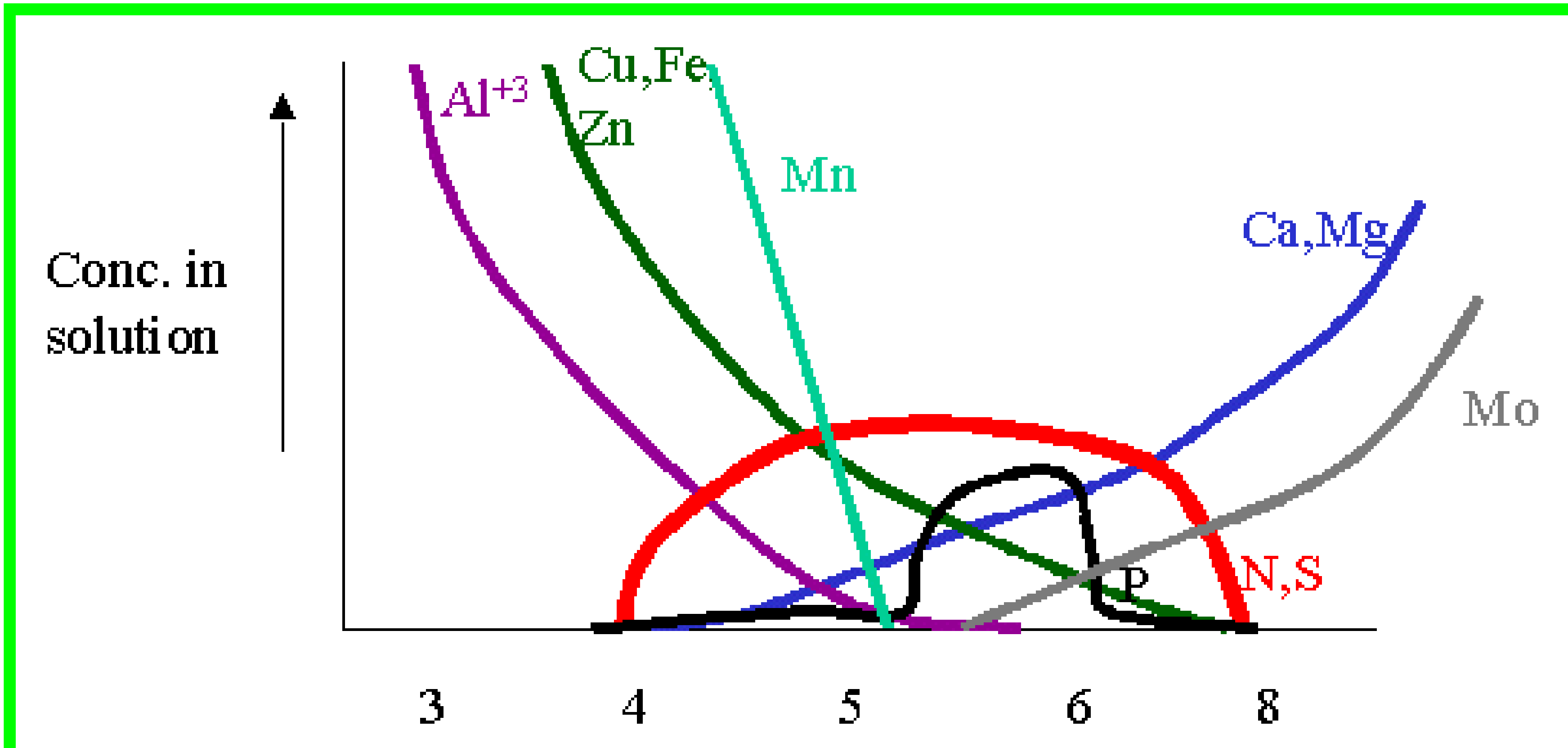
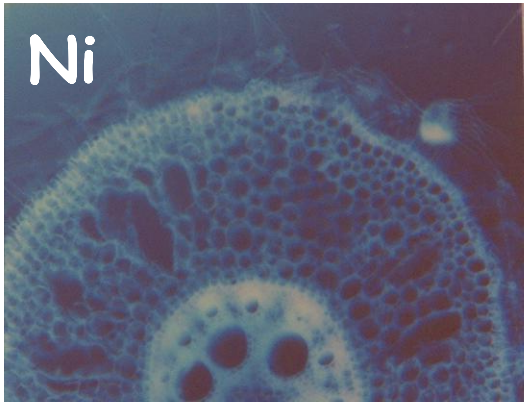
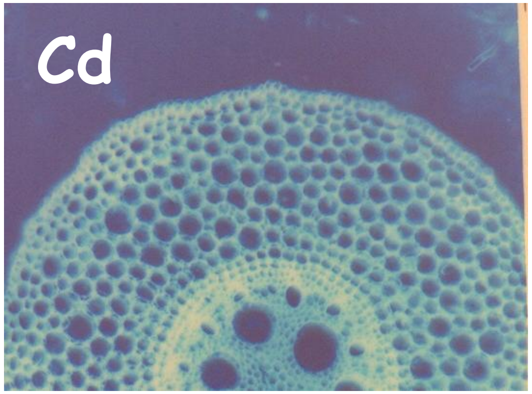
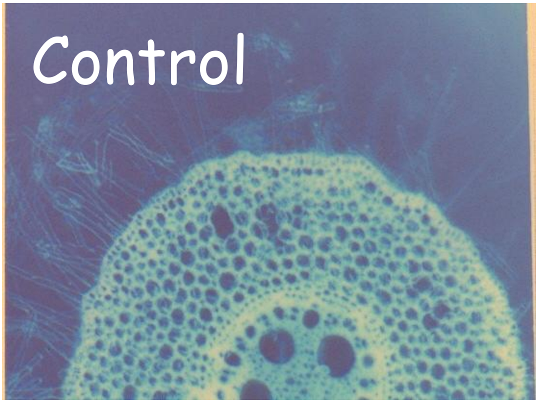




HMs may affect plant anatomy







0.1 mM $CdCl_2$
0.1 mM $NiSO_4$
demineralized water (control)

Steady presence of Cd and Ni affects young maize root anatomy and accumulation and distribution of essential metals.
Maksimović et al, Biologia plantarum

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Uptake and distribution of Cd in soybeans (*Glycine max* (L.) Merr.)

T. Teklić, I. Maksimović, M. Špoljarević, M. Putnik-Delić, M. Lisjak, M. Mirosavljević, M. Živanov, R. Kastori

Uptake and distribution of Cd in soybeans (*Glycine max* (L.) Merr.) – biomass production

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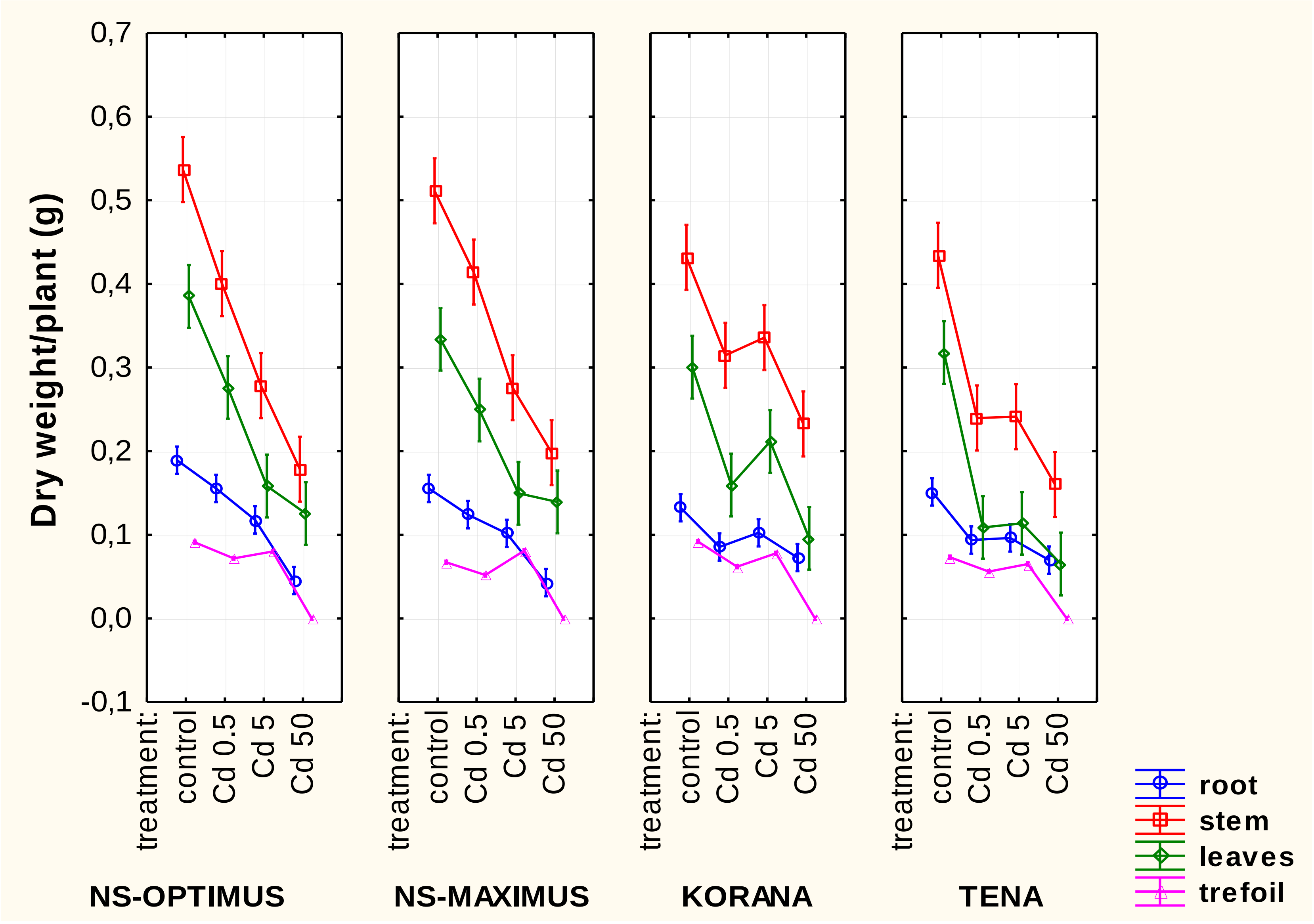
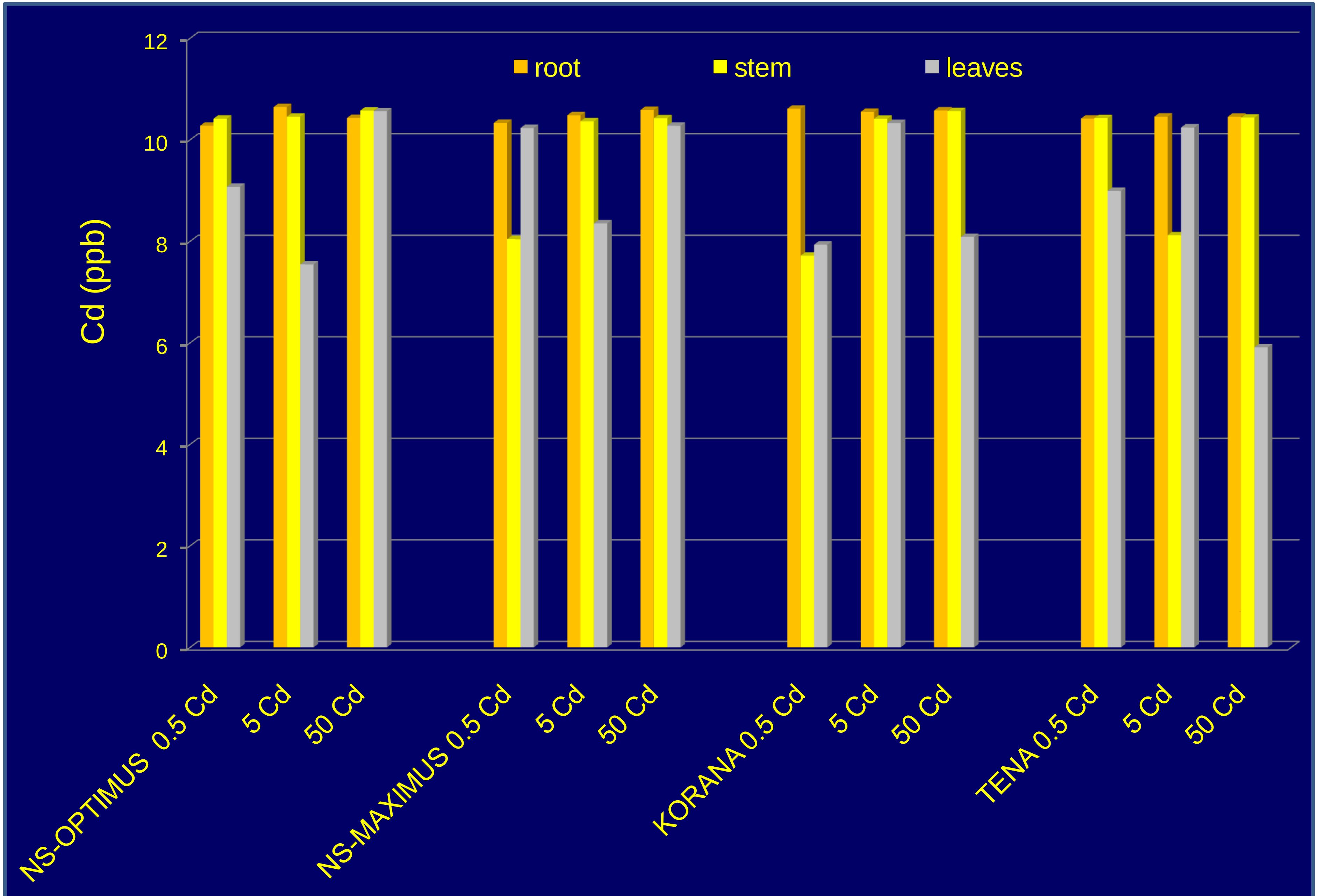


Concentration of Cd in soybean (*Glycine max* (L.) Merr.)



Impact of HMs on chemical composition and growth of camelina (gold-of-pleasure, false flax) (*Camelina sativa* L.)

Oil crop (40% oil in the seed – similar to sunflower, soybeans, oil seed rape, ...)

High content of omega 3 fatty acids and proteins

Suitable for food and feed, for biodisel, cold pressed oil, marginal soils.

Accumulation of HMs – 1) food safety and 2) phytoremediation

Experimental setup

- Seed 24 h imbibid in deionized water (control), 1 μM Cd ($CdCl_2$) or Cu ($CuSO_4 x 5H_2O$) and 10 μM Ni ($NiSO_4$) or Zn ($ZnSO_4 x 7H_2O$) in deionized water.
- ½ Hoagland to which were added Cd or Cu to final conc. 1 μM and Ni or Zn to final conc. 10 μM
- 5 replications, 8 plants per replication.
- Plants grown 30 days

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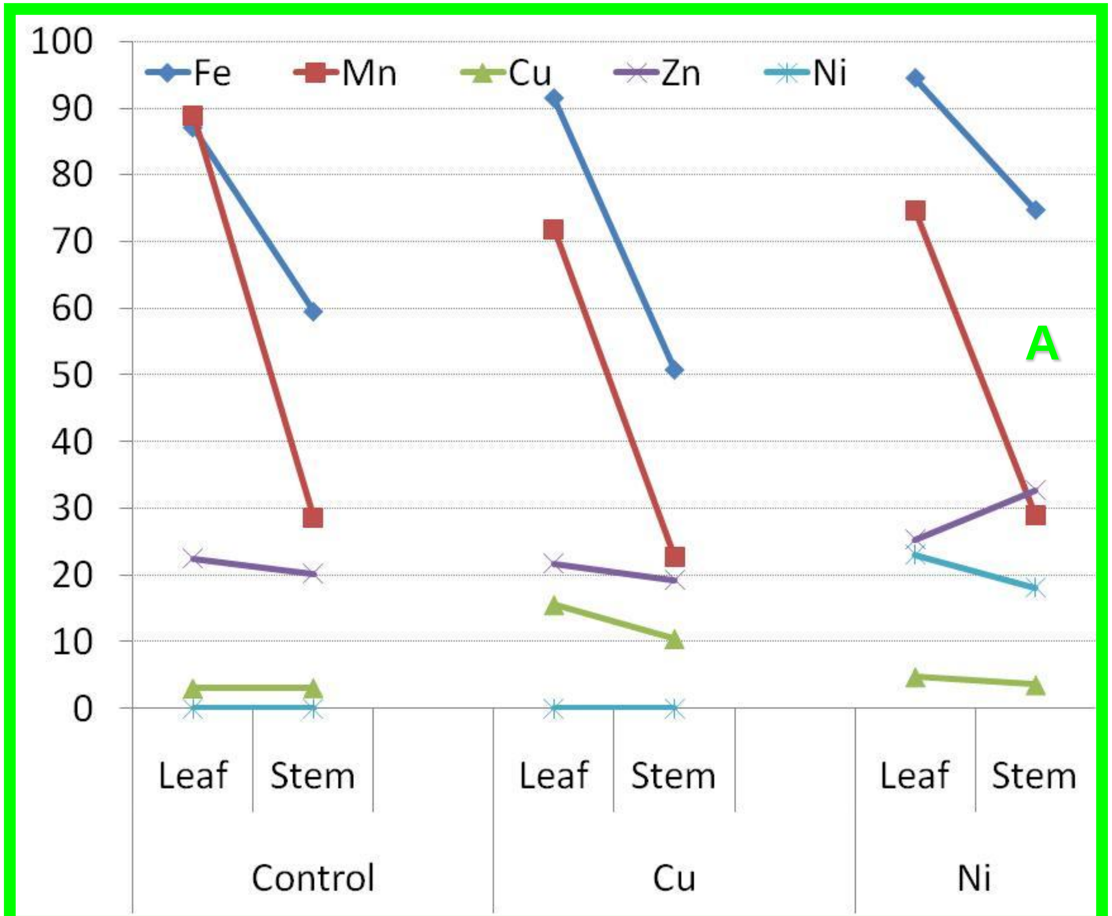
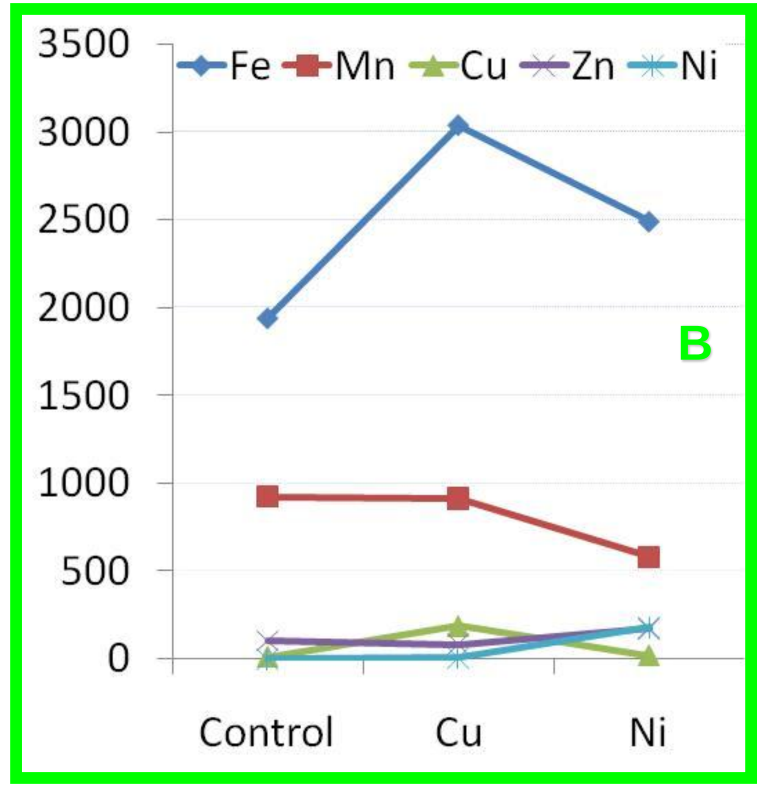
Concentration of photosynthetic pigments, IT, ANR, conc. free proline in camelina grown in the presence of Cu or Ni

Treatment	Concentration of chloroplast pigments (mg g^{-1} FW)				Transpiration intensity (g H_2O dm^{-2} h^{-1})	Activity of nitrate reductase (μM NO_2^- g^{-1} h^{-1})	Concentration of free proline (μg g^{-1} FW)
	Chl a	Chl b	Car	Chl a+b			
Control	0.71	0.26	0.17	0.97	1.06	0.08	31.25
Cu	0.78	0.26	0.19	1.04	1.01	0.03	17.71
Ni	0.76	0.23	0.18	0.99	1.11	0.09	38.18

Concentration (mg kg^{-1} DM) of Fe, Mn, Cu, Zn and Ni in leaves, stem (A) and root (B) of camelina grown in the presence of Cu or Ni





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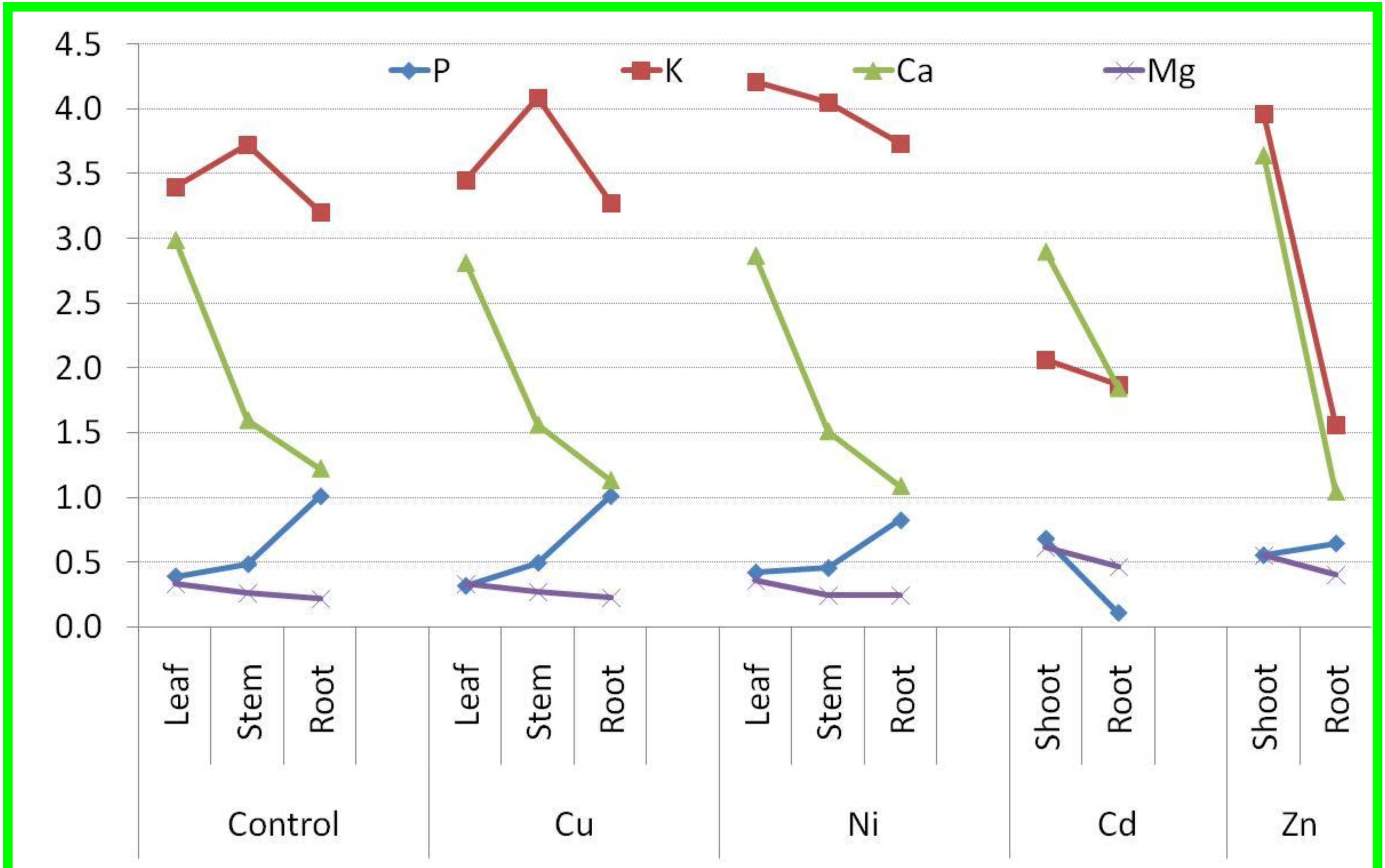
Concentrations (%) of P, K, Ca and Mg in shoots and roots of camelina grown in the presence of Cu, Ni, Cd or Zn



Conclusion - camelina

Zn and Cd exerted toxic effects in the applied concentrations.

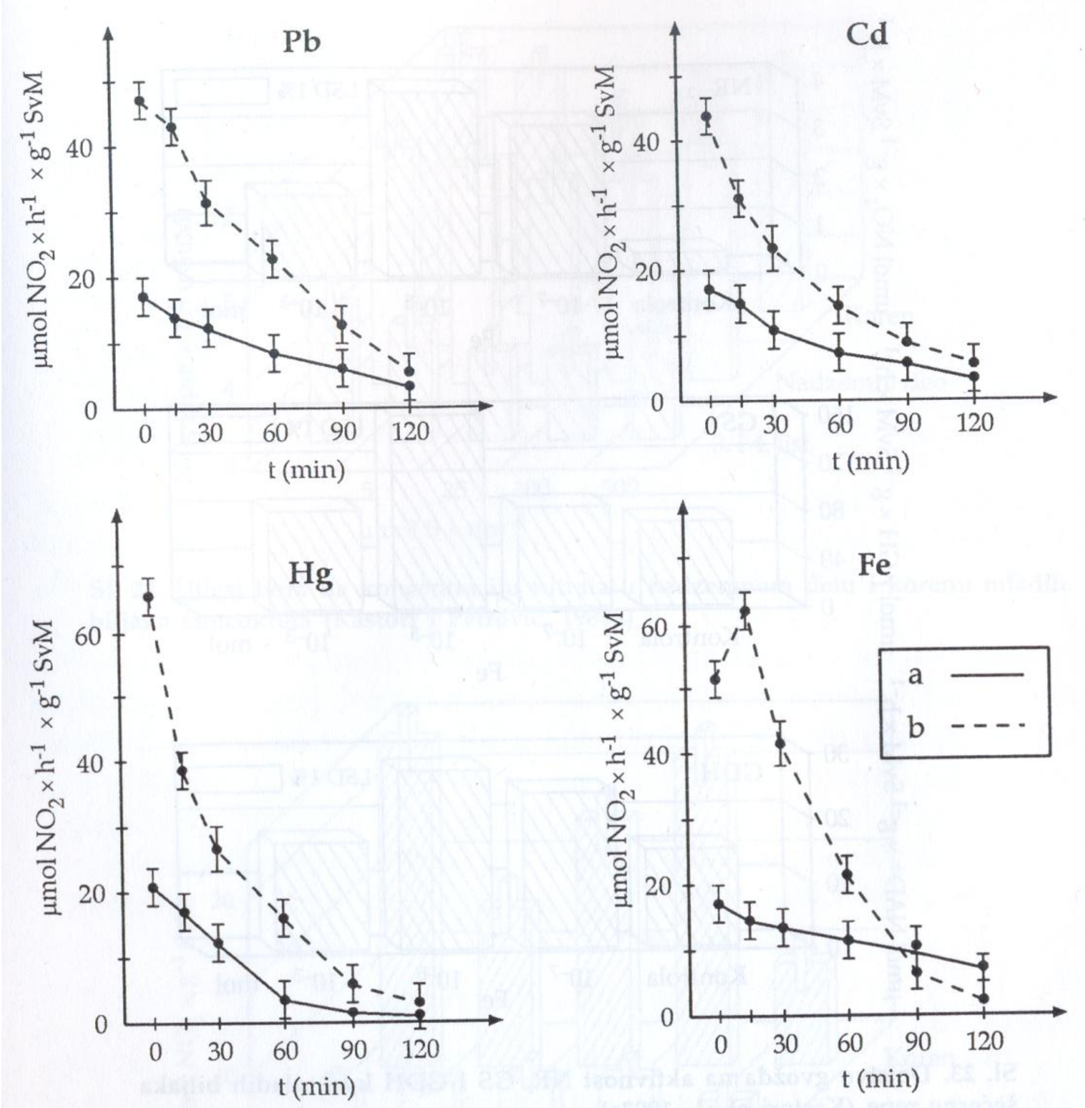
In the presence of Cu and Ni DW was reduced, but water content, IT and concentration of photosynthetic pigments werw not significantly changed.

Concentration of free proline and ANR declined in the presence of Cu – impairement of N metabolism.

Effect of Pb, Cd, Hg, and Fe on the dynamics of nitrate reductase activity in roots (a) and leaves (b) of sugar beet

NR - the most sensitive enzyme in the cytoplasm to higher concentrations of microelements, especially heavy metals

NRA decreases especially in the presence of mercury or cadmium, and to a smaller extent in the presence of essential microelements



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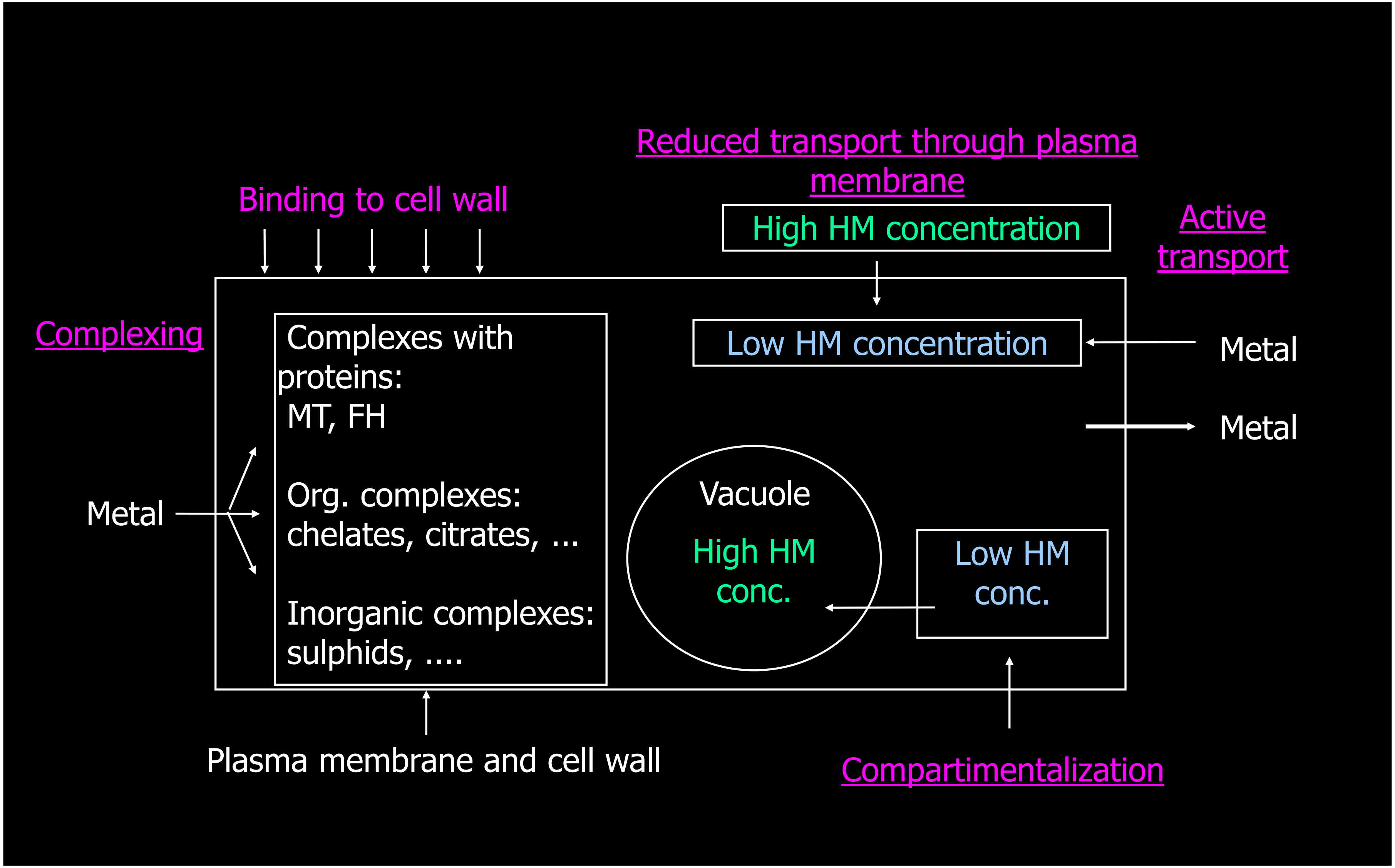
Mechanisms of tolerance to HMs



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Mechanisms of tolerance to HMs

Exogenous mechanisms (*apoplastic*)

- immobilization in the cell wall
- efflux of chelates
- establishment of the pH barrier
- ectotorphic mycorrhizae

Endogenous mechanisms (*symplastic*)

- formation of chelates by metal-binding proteins and polypeptides – phytochelatins in the cytoplasm
- compartimentation and formation of complexes with organic and inorganic acids in the vacuole
- binding by phytic acid
- heat shock proteins

Other protective mechanisms

- lesser permeability of plasma membrane to HM
- reduced uptake
- binding in the root
- presence of other ions in the soil (Si, Ca - Mn; P - Pb; S - Cd)

Phytoremediation → the use of green plants to remove pollutants from the environment or to render them harmless (Cunningham i Berti, 1993; Raskin i sar., 1994).

Aim → Find the plant species and genotype with characteristics suitable for phytoextraction

Fundamental processes involved in phytoremediation of contaminated and polluted soils

Process	Effect on pollutant	Target pollutants[a]
Phytostabilization	Inactivation	**HM**, MO, HA, **RA**, OR
Phytoimmobilization	"	**HM**, MO, HA
*Phytoextraction	Removal	**HM**, **MO**, **HA**, **RA**, OR
Phytovolatilization	"	HM, **MO**, HA, **OR**
Phytodegradation	"	**OR**

[a]**HM**-heavy metals, **MO**-metalloids, **HA**-halides, **RA**-radionuklids, **OR**-organic pollutants
*Phytoextraction includes phytomining

Efficiency of HM accumulation can be expressed as

phytoextraction coefficient:

$$\frac{\text{mg HM / g tissue dry weight}}{\text{mg HM / g substrate dry weight}}$$

Examples of field trials for the phytoremediation of HMs

HM	Plant	Location	Method[a]	Comments	Ref.
Pb	***Brassica juncea***	Trenton, NJ	PE-CA	EDTA-enhanced uptake over one cropping season resulted in a 28% reduction in the Pb contamination area	Brown et al., 1995
Cd Zn	***Thlaspi caerulescens*** ***Silene vulgaris***	Beltsville MD	PE-C	Phytoextraction of sludge-amended soils. Cd accumulation was similar in both species. Zn accumulation in *T. caerulescens* was 10-fold higher then in *S. vulgaris*	
Zn Cd Ni Cu Pb Cr	***Brassica oleracea*** ***Raphanus sativus*** ***Thlaspi caerulescens*** ***Alyssum lesbiacum*** ***Alyssum murale*** ***Arabidopsis thaliana***	Rothamstead, UK	PE-C	Sludge-amended soil	Baker et al., 1991

PE, phytoextraction
CA, chelate-assisted phytoextraction
C, continuous phytoextraction

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Features of plants suitable for phytoextraction

- Tolerance of the presence of higher HM concentrations
- Capacity for intensive translocation of HM from the root to the above-ground parts (metal-specific)
- Accumulation and multitolerance of HMs
- Rapid growth and high biomass production
- Adaptability to concerned edaphic and climatic conditions
- Short growing season
- No need for special cultural practices – keep low costs
- Able to withstand monoculture

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Conclusions on phytoremediation

- Many laboratory and field studies have confirmed that the **practical application of plants for soil HM clean-up is possible**.
- The success of phytoremediation depends first and foremost on the **plant species used**, its capacity for HM accumulation and translocation to shoots, its biomass production and measures used to promote HM accumulation in plants.
- Results suggest that **some crop plants could be used for clean-up of HM-polluted soils**.

Impact of stress factors an plant responses

Light

Temperature stress

Ionic stress

Drought

Salinity

Heavy metals

Secondary stresses (osmotic stress, oxidative stress)

Disruption of osmotic and ionic homeostasis and damage to structural and functional proteins

Stress signal sensors Kinases, Secondary messengers

Transcriptional factors

Activation of stress-responsive genes

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Acknowledgement

Project SERBIA FOR EXCELL has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 691998.

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Thank you for yout attention ☺