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
- Brassinosteroids – 40 compounds
- Salicilic acid (SA)
- .............
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.

Regulation of photon capture and the protection and repair of photodamage.
(Taiz and Zeiger 2014, after Asada 1999.)
Diurnal changes in xanthophyll content as a function of irradiance in sunflower (*Helianthus annuus*).
Jasmonic and methyl-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.
Brassinosteroids

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.

Brassinolide, firstly extracted from Brassica napus
Brassinosteroids

Homobrassinolide
Dolicholide
Homodolicholide
Castasterone
3-Epicastasterone
Brassinone
6-Desoxocastasterone
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 salt 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 lead to:

- Secondary soil salinization
- Acute and hidden salinization
Irrigated soils damaged due to excess of salts

Total irrigated soil surface

Already damaged soils

Singh & Chatrath, 2001
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"
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 – dilution 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 compartmentation are phenomena which may explain differences between halophytes and glycophytes with respect to tolerance to excess salts.

- **Euhalophytes** - maritime species - growth stimulate conc. Cl$^-$ lower than 400 mM
- **Halophytes** - bear salinity but growth rate slows down
- **Tomato**
- **Beans**
- **Spinach**
- **Swiss chard**
- **II and III glycophytes**
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
Osmotic potential increases with salinity

- High concentration of salts
- Reduced growth due to impaired uptake of water
- Visible already at germination
- Effect depends on phenophase
Osmotic stress - effects

(Taiz & Zeiger, 2006)

Abscisic acid accumulation
Solute accumulation
Photosynthesis
Stomatal conductance
Protein synthesis
Wall synthesis
Cell expansion

Water potential (MPa)

Pure water
Well-watered plants
Plants under mild water stress
Plants in arid, desert climates
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)

<table>
<thead>
<tr>
<th>Time</th>
<th>Water stress effects</th>
<th>Salt-specific effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Observed effect on growth of a salt-tolerant plant)</td>
<td>(Additional effects on growth of a salt-sensitive plant)</td>
</tr>
<tr>
<td>Minutes</td>
<td>Instant reduction in leaf and root elongation rate, than rapid partial recovery</td>
<td></td>
</tr>
<tr>
<td>Hours</td>
<td>Steady but reduced rate of leaf and root elongation</td>
<td></td>
</tr>
<tr>
<td>Days</td>
<td>Leaf growth more affected than root growth; Reduced rate of leaf emergence</td>
<td>Injury visible in older leaf</td>
</tr>
<tr>
<td>Weeks</td>
<td>Reduced final leaf size and/or number of lateral shoots</td>
<td>Death of older leaves</td>
</tr>
<tr>
<td>Months</td>
<td>Altered flowering time, reduced seed production</td>
<td>Younger leaves dead, plant may die before seed matures</td>
</tr>
<tr>
<td>Type of water</td>
<td>Total soluble salts (ppm)</td>
<td>EC ( (dS \text{ m}^{-1}) )</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Sweet water</td>
<td>&lt; 500</td>
<td>&lt; 0.6</td>
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<tr>
<td>A little brackish</td>
<td>500–1000</td>
<td>0.6–1.5</td>
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<tr>
<td>Brackish</td>
<td>1000–2000</td>
<td>1.5–3.0</td>
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<tr>
<td>Moderately saline</td>
<td>2000–5000</td>
<td>3.0–8.0</td>
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<td></td>
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<tr>
<td>Saline</td>
<td>5000–10000</td>
<td>8.0–15.0</td>
</tr>
<tr>
<td>Very saline</td>
<td>10000–35000</td>
<td>15.0–45.0</td>
</tr>
</tbody>
</table>
Genotype features which affect uptake of ions

**ROOT MORPHOLOGY:** root type (primary, secondary), mass, length, topography, absorption surface, cortex thickness

**LEAF MORPHOLOGY:** size, shape, thickness, position

**STEM MORPHOLOGY:** diameter, length, number of elements of conductive vessels and their structure

**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 effect 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.

<table>
<thead>
<tr>
<th>g NaCl L⁻¹</th>
<th>mS cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.10</td>
</tr>
<tr>
<td>0.2</td>
<td>1.50</td>
</tr>
<tr>
<td>0.6</td>
<td>2.26</td>
</tr>
<tr>
<td>1.2</td>
<td>3.39</td>
</tr>
</tbody>
</table>

½ Hoagland
14 d old plants
Treatment 21 d
Dry mass of leaves, stems and roots of coriander (*Coriandrum sativum* L.) grown in the presence of NaCl
Concentration of Na and K in leaves, stems and roots of coriander (*Coriandrum sativum* L.) grown in the presence of NaCl
Concentration of Ca, P and Mg in leaves, stems and roots of coriander (*Coriandrum sativum* L.) grown in the presence of NaCl.
in leaves, stems and roots of coriander (*Coriandrum sativum* L.) grown in the presence of NaCl
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

<table>
<thead>
<tr>
<th>Element in excess</th>
<th>Type of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (increase in content 15% and more)</td>
</tr>
<tr>
<td>N</td>
<td>-</td>
</tr>
<tr>
<td>P</td>
<td>-</td>
</tr>
<tr>
<td>K</td>
<td>Mo</td>
</tr>
<tr>
<td>Mg</td>
<td>-</td>
</tr>
<tr>
<td>Cu</td>
<td>Mg, Co, Mn</td>
</tr>
<tr>
<td>Zn</td>
<td>Ca, Mg, Co</td>
</tr>
<tr>
<td>Mn</td>
<td>K, Zn, Co</td>
</tr>
<tr>
<td>B</td>
<td>Cu</td>
</tr>
</tbody>
</table>
Concentration of free proline and vitamin 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 effect of salts is visible on the entire plant; even when it is not obvious it may lead 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 better adaptation of plants and increased tolerance to salt stress
HEAVY METALS

- Metals whose specific weight is above 5 g/cm³
- High concentrations are extremely toxic
  - Soluble in water
  - Living organisms uptake them easily (plants and animals)
    - Concentrate in tissues
  - React with biomolecules
    - Proteins
    - Nucleic acids

Cu  Mo  Co  Hg  Pb  Cd  Mn  Ni  Fe  Zn  Mo

Sources of HM pollution

- Smelters
- Exhaust of automobiles
- Additives in paints
- Urban wastes
- Effluents from battery industry
- Industry
- Fertilizers, pesticides

HM in the environment
### Forms of binding

<table>
<thead>
<tr>
<th>Forms of binding</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Free hydrated ions</td>
<td>[Ca(aq)]^{2+}, [Na(aq)]^{+}, [Cu(aq)]^{2+}, [Fe(aq)]^{3+}, CaHCO_{3}^{+},</td>
</tr>
<tr>
<td>2. Associations of ions and inorganic complexes</td>
<td>CaSO_{4}^{0}, CdCl^{+}, AlSO_{4}^{+}, CuOH^{+}, AIOH^{2+}</td>
</tr>
<tr>
<td>3. Water-soluble complexes</td>
<td></td>
</tr>
<tr>
<td>4. Dispersed coloids</td>
<td></td>
</tr>
<tr>
<td>5. Sediment</td>
<td></td>
</tr>
<tr>
<td>6. Replacable and adsorbed specifically for colloids</td>
<td></td>
</tr>
<tr>
<td>7. Ions forming net in silicates</td>
<td></td>
</tr>
<tr>
<td><strong>Fluid phase</strong></td>
<td><strong>Solid phase</strong></td>
</tr>
<tr>
<td></td>
<td></td>
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</tbody>
</table>
Effects of HMs on plants

- Growth of seedlings
- Photosynthesis
- Enzyme activity
- Membrane structure
- Mitosis
- Cell elongation
- Mineral nutrition
- Water regime

HMs

-/-/+
Generalized pattern of partitioning of HMs in roots and shoots

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

Cd, Co, Cu, Fe and Mo accumulate 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

Range of most soils:

- Acid: < 6.5
- Neutral: 7.0
- Alkaline: > 7.3

Conc. in solution vs. pH graph:
- Al³⁺, Cu, Fe, Zn, Mn, Ca, Mg, Mo, N, S, P
HMs may affect plant anatomy

Steady presence of Cd and Ni affects young maize root anatomy and accumulation and distribution of essential metals. Maksimović et al, Biologia plantarum
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
Concentration of Cd in soybean (Glycine max (L.) Merr.)

The graph shows the concentration of Cd in different parts of soybean plants (root, stem, and leaves) under various Cd treatments (0.5 Cd, 5 Cd, 50 Cd) for different cultivars (NS-OPTIMUS, NS-MAXIMUS, KORANA, TENA).

- The concentration of Cd in the root, stem, and leaves is measured in parts per billion (ppb).
- The x-axis represents the cultivar and the Cd treatment level, while the y-axis represents the Cd concentration in ppb.
- The bars indicate the average concentration of Cd in each part of the plant under the specified conditions.
Impact of HMs on chemical composition and growth of camellina (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 biodiesel, 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 (CdCl₂) or Cu (CuSO₄·5H₂O) and 10 μM Ni (NiSO₄) or Zn (ZnSO₄·7H₂O) 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

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of chloroplast pigments (mg g⁻¹ FW)</th>
<th>Transpiration intensity (g H₂O dm⁻² h⁻¹)</th>
<th>Activity of nitrate reductase (μM NO₂⁻ g⁻¹ h⁻¹)</th>
<th>Concentration of free proline (μg g⁻¹ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Chl a 0.71, Chl b 0.26, Car 0.17, Chl a+b 0.97</td>
<td>1.06</td>
<td>0.08</td>
<td>31.25</td>
</tr>
<tr>
<td>Cu</td>
<td>Chl a 0.78, Chl b 0.26, Car 0.19, Chl a+b 1.04</td>
<td>1.01</td>
<td>0.03</td>
<td>17.71</td>
</tr>
<tr>
<td>Ni</td>
<td>Chl a 0.76, Chl b 0.23, Car 0.18, Chl a+b 0.99</td>
<td>1.11</td>
<td>0.09</td>
<td>38.18</td>
</tr>
</tbody>
</table>

Serbia for Excell
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.
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 were not significantly changed.

Concentration of free proline and ANR declined in the presence of Cu - impairment 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
Mechanisms of tolerance to HMs

Complexing
- Complexes with proteins: MT, FH
- Org. complexes: chelates, citrates, ...
- Inorganic complexes: sulphids, ....

Binding to cell wall

Reduced transport through plasma membrane
- High HM concentration
- Active transport
- Metal

Vacuole
- Low HM concentration

Plasma membrane and cell wall

Compartimentalization
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

<table>
<thead>
<tr>
<th>Process</th>
<th>Effect on pollutant</th>
<th>Target pollutants&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytostabilization</td>
<td>Inactivation</td>
<td>HM, MO, HA, RA, OR</td>
</tr>
<tr>
<td>Phytoimmobilization</td>
<td>“</td>
<td>HM, MO, HA</td>
</tr>
<tr>
<td>*Phytoextraction</td>
<td>Removal</td>
<td>HM, MO, HA, RA, OR</td>
</tr>
<tr>
<td>Phytovolatilization</td>
<td>“</td>
<td>HM, MO, HA, OR</td>
</tr>
<tr>
<td>Phytodegradation</td>
<td>“</td>
<td>OR</td>
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</table>

<sup>a</sup>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:

\[
\frac{\text{mg HM}}{\text{g tissue dry weight}} \quad \quad \frac{\text{mg HM}}{\text{g substrate dry weight}}
\]
## Examples of field trials for the phyto Remediation of HMs

<table>
<thead>
<tr>
<th>HM</th>
<th>Plant</th>
<th>Location</th>
<th>Method</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>Brassica juncea</td>
<td>Trenton, NJ</td>
<td>PE-CA</td>
<td>EDTA-enhanced uptake over one cropping season resulted in a 28% reduction in the Pb contamination area</td>
</tr>
<tr>
<td>Cd</td>
<td>Thlaspi caerulescens</td>
<td>Beltsville MD</td>
<td>PE-C</td>
<td>Phytoextraction of sludge-amended soils. Cd accumulation was similar in both species. Zn accumulation in T. caerulescens was 10-fold higher than in S. vulgaris</td>
</tr>
<tr>
<td>Zn</td>
<td>Silene vulgaris</td>
<td></td>
<td>PE-C</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>Brassica oleracea</td>
<td>Rothamstead, UK</td>
<td>PE-C</td>
<td>Sludge-amended soil</td>
</tr>
<tr>
<td>Cd</td>
<td>Raphanus sativus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>Thlaspi caerulescens</td>
<td></td>
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<td></td>
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<tr>
<td>Cu</td>
<td>Alyssum lesbiacum</td>
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<td></td>
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<tr>
<td>Pb</td>
<td>Alyssum murale</td>
<td></td>
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<tr>
<td>Cr</td>
<td>Arabidopsis thaliana</td>
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</table>

Ref. Brown et al., 1995

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

Project SERBIA FOR EXCELL has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 691998.
Thank you for your attention 😊