Risultati del Progetto STOPP

Un approccio multidisciplinare al monitoraggio ambientale multi-scala attraverso bioindicatori vegetali

Monitoraggio metabolico dello stress ambientale: marker metabolici in piante di mais coltivate su suoli inquinati

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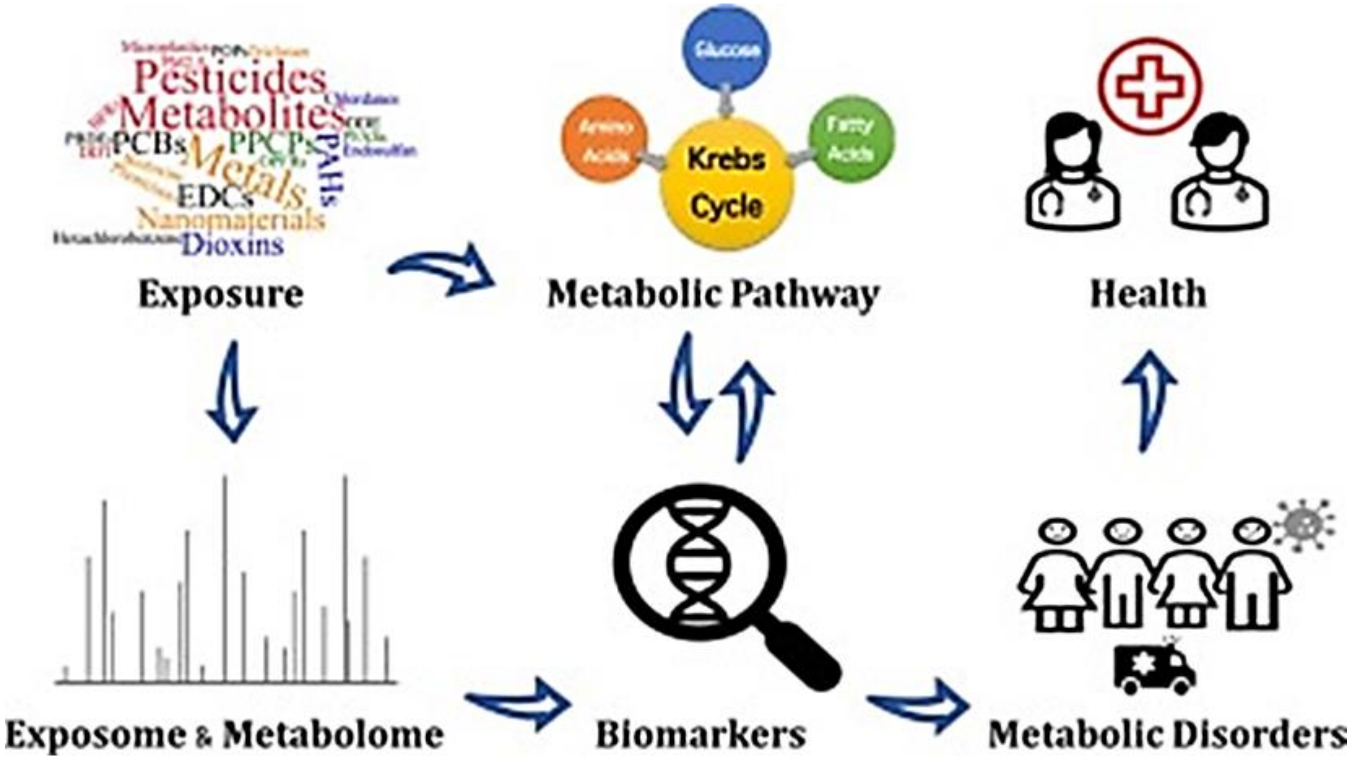
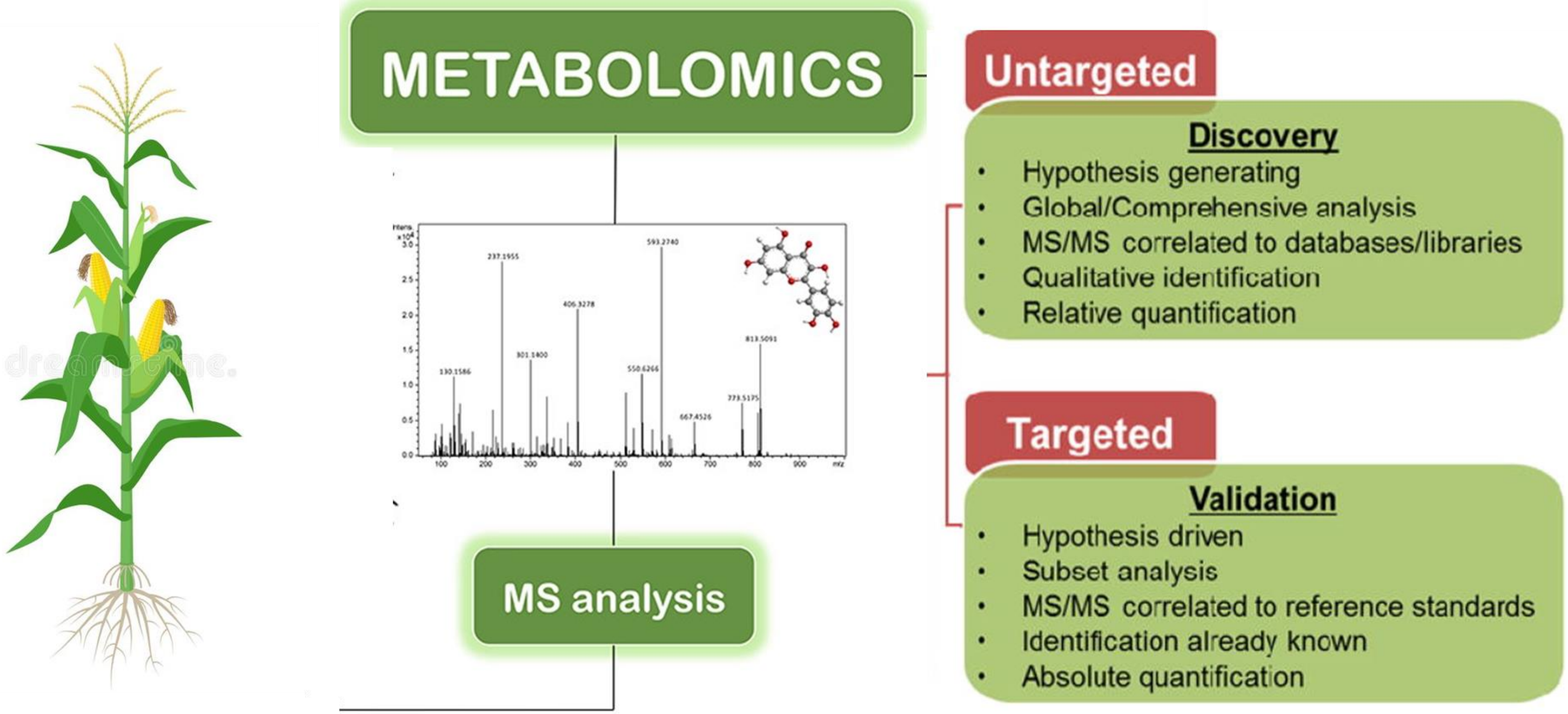
D3.3 Characterization of primary and secondary metabolites in plant tissue subjected to increasing doses of contaminants by mass spectrometry and NMR spectroscopy

- Monitoring the levels of inorganic and organic pollutants in plants is crucial for exploring their bio-accumulation and toxicity.



✓ **Different metabolomic approaches to biomonitoring environmental condition**

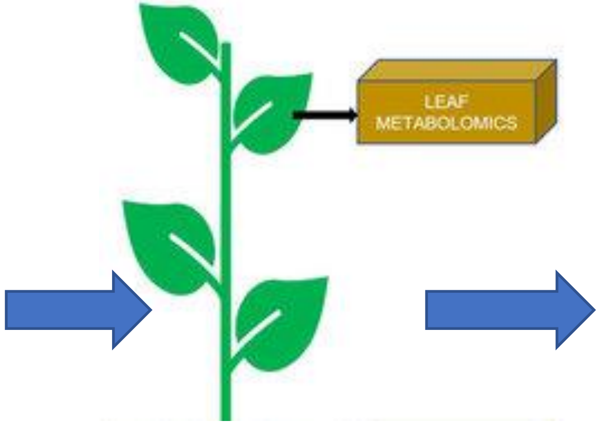
- Metabolomics can be used to evaluate the toxicity of heavy metals and develop new toxicity biomarkers through models constructed in the laboratory, which creates a new vision and approach for heavy metal exposure research

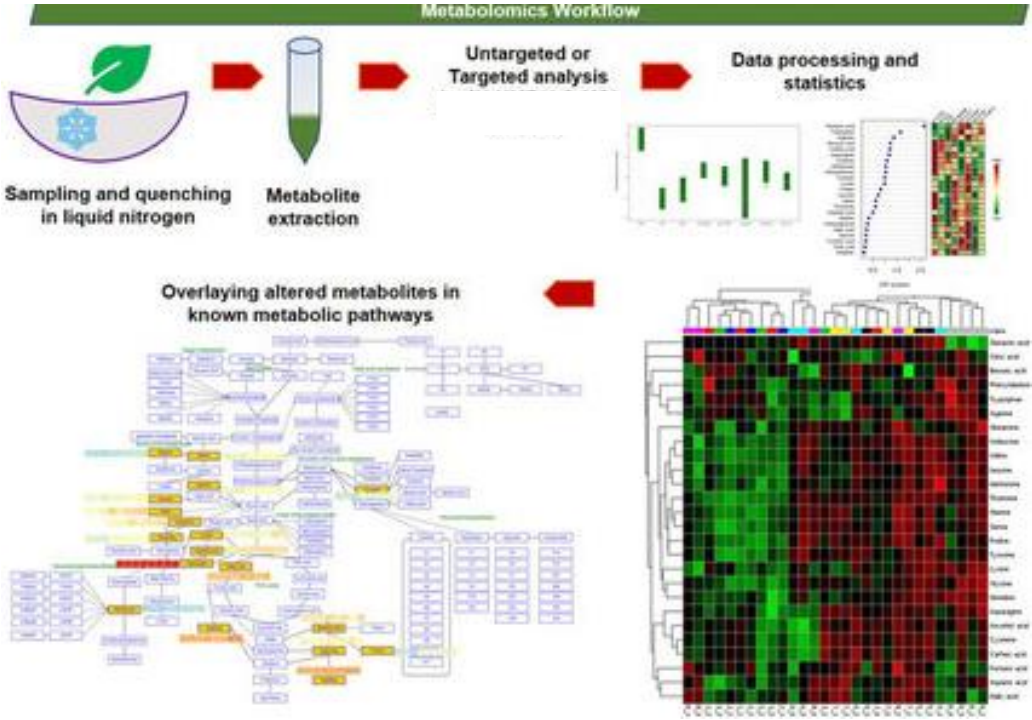


Materials and Methods

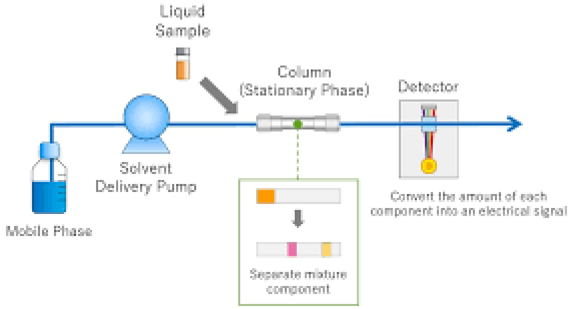
- ✓ The leaves collected during the **FIRST EXPERIMENTAL FIELD IN POTS** to Department of Agriculture (UNINA) (2022) were frozen in nitrogen to perform different metabolomic analyses.
- ✓ Samples leaves were analyzed applying a combined metabolomic approach.
- ✓ Amino acid profile has been carried out by HPLC analysis whereas polyphenol profiles have been performed using LC-MS-IT-TOF.

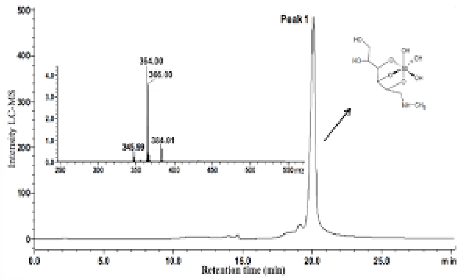
DIPARTIMENTO DI AGRARIA





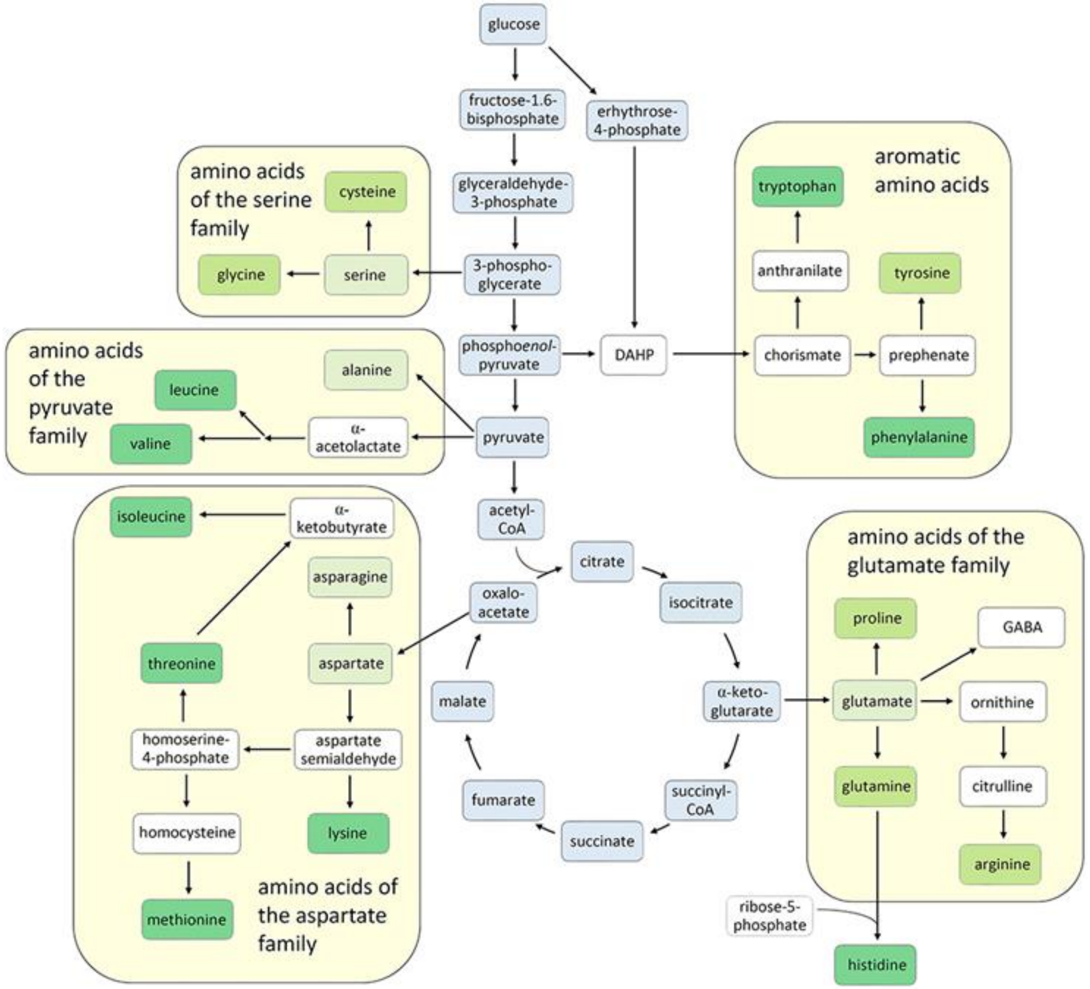
- HPLC's versatility, sensitivity, and adaptability make it an invaluable tool in plant metabolomics, allowing researchers to obtain a detailed view of amino acid profiles and their roles in adaptive and stress-related responses in plants





- LC-MS-IT-TOF provides a robust platform for accurately identifying and quantifying phenolic compounds, allowing researchers to profile complex phenolic mixtures and detect low-abundance compounds often present in plant tissues affected by pollution. The high resolution and accuracy of LC-MS-IT-TOF provide specific benefits for phenolic analysis under heavy metal stress.

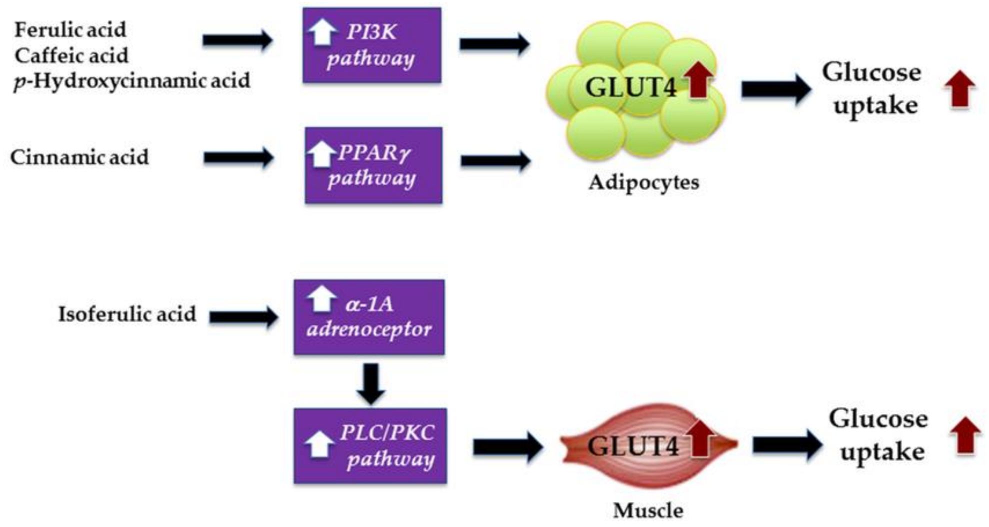
Results

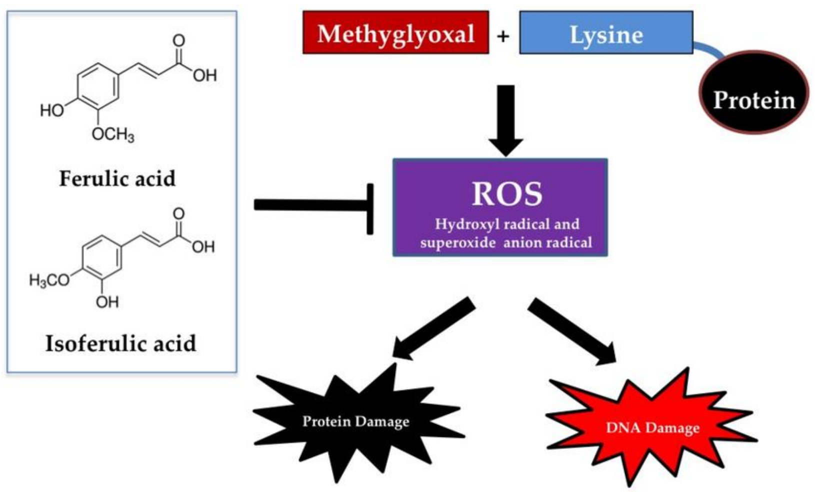


- ✓ The preliminary experiment results revealed varying concentrations of specific amino acids across different treatments, including aspartate, glutamine, asparagine, glycine, threonine, alanine, leucine, and lysine.
- ✓ Overall, we observed either an abundance or a shortage of these amino acids, which may indicate that the plants are experiencing stress due to metal exposure or unfavorable growth conditions.

Results

1. Aspartate can contribute to the synthesis of antioxidants, helping to mitigate oxidative damage. This protective role can drive its accumulation as the plant attempts to counteract stress-induced oxidative stress (Rafi, 2023).
2. Furthermore, aspartate is a key component in the biosynthesis of essential amino acids such as lysine, methionine, threonine, and isoleucine that are important in the production of compounds involved in the stress responses.



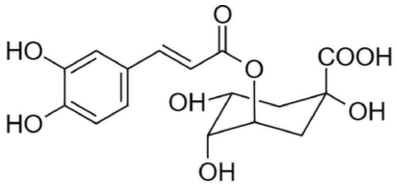


3. Asparagine plays several important roles in the metabolic pathways of maize leaves, particularly in nitrogen metabolism and amino acid synthesis. During stress conditions, such as drought or nutrient deficiency, asparagine levels can increase, indicating its role in nitrogen remobilization and adaptation to environmental challenges.

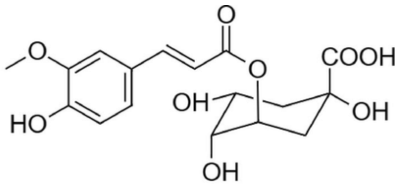
❑Secondary metabolites profiling

- Heavy metal exposure often triggers the synthesis of secondary metabolites like flavonoids and phenolic compounds. These compounds play a role in plant defense against oxidative stress and can help mitigate damage caused by heavy metals (Hayford et al. 2024).

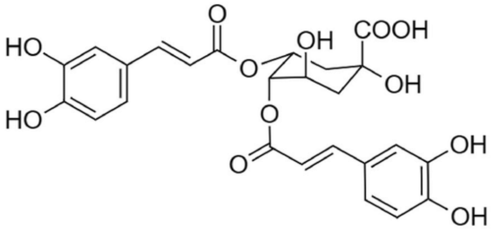
1. Caffeoylquinic acid
2. Feruloylquinic acid
3. Hydroxycinnamic acid
4. Feruloylhydroxycitric acid
5. Dillenetin glucoside-glucuronide
6. Caffeoylhydroxycitric acid



5-caffeoylquinic acid



5-feruloylquinic acid



3,4-dicaffeoylquinic acid

✓ These compounds play significant roles in plant growth and development, particularly in response to environmental stresses

Conclusions

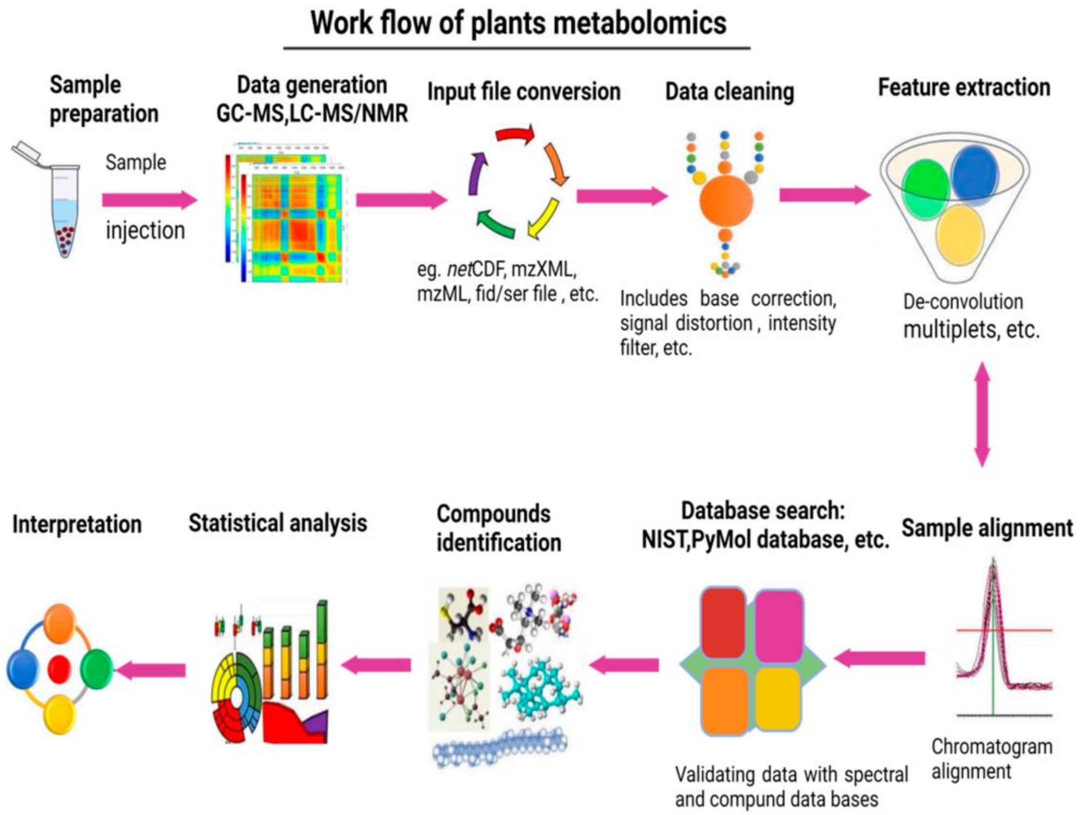
1. Metabolomics is an emerging field of “omics” that characterizes small molecule metabolites in biological systems.
2. Metabolomics analyses reflect both the steady-state physiological equilibrium of cells or organisms as well as their dynamic metabolic responses to environmental conditions.

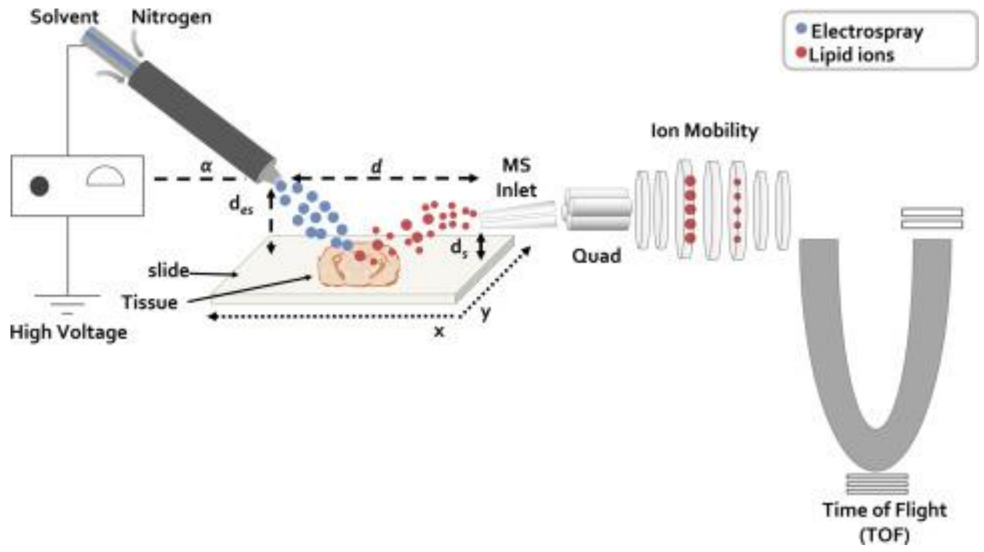
✓ In this work, we focus our attention on identification of primary or secondary metabolites in maize leaves treated by heavy metals to identify several biomarkers related to remote sensing acquisitions in environmental pollution conditions combined untarget and target metabolomic approaches.

- D3.6 “Target analysis results in pot experiments”

Introduction

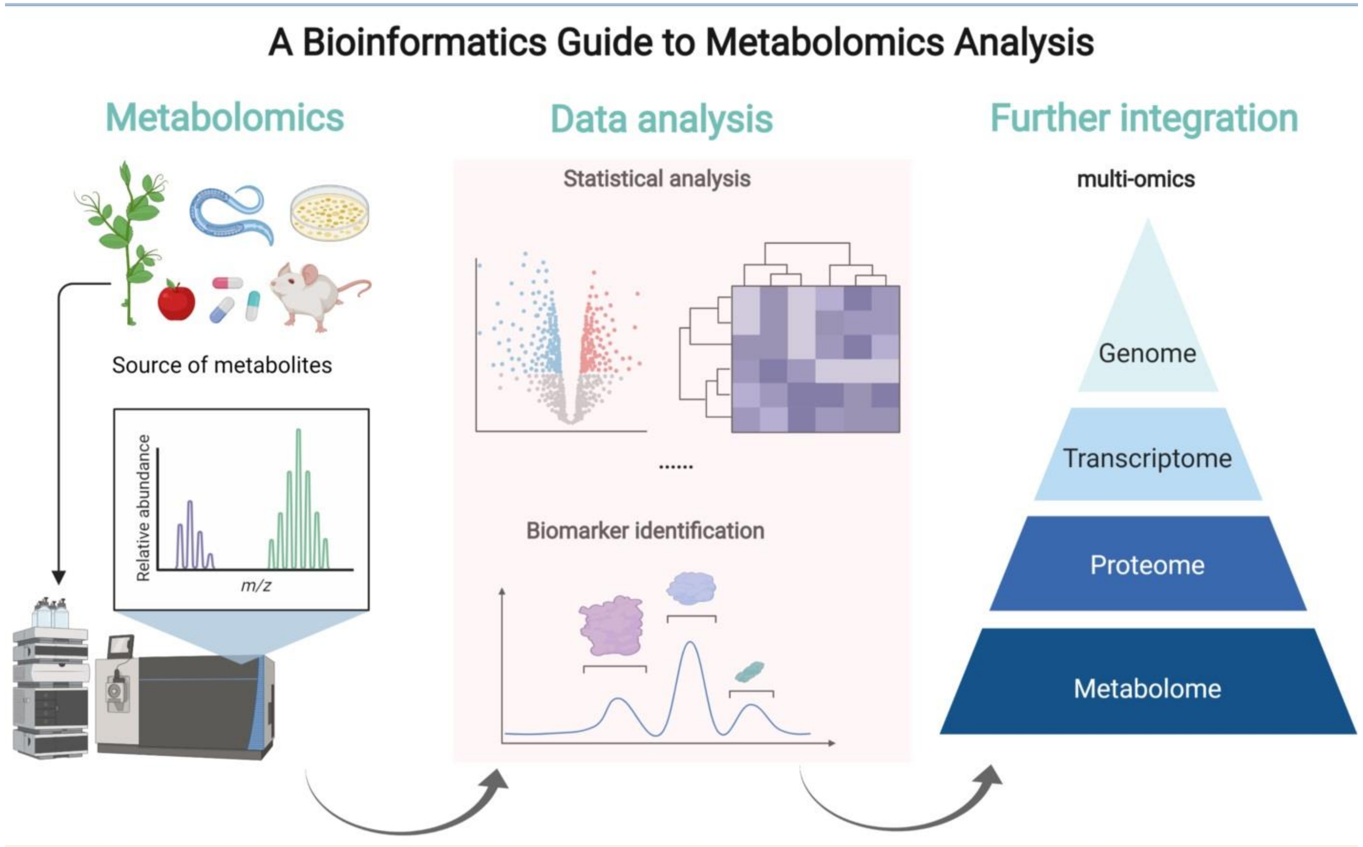
- Metabolomic approaches using Quadrupole Time-of-Flight Mass Spectrometry (Q-TOF-MS) have become central to understanding plant responses to various environmental and biological stresses, including heavy metal exposure, drought, pathogen attack, and nutrient deficiency





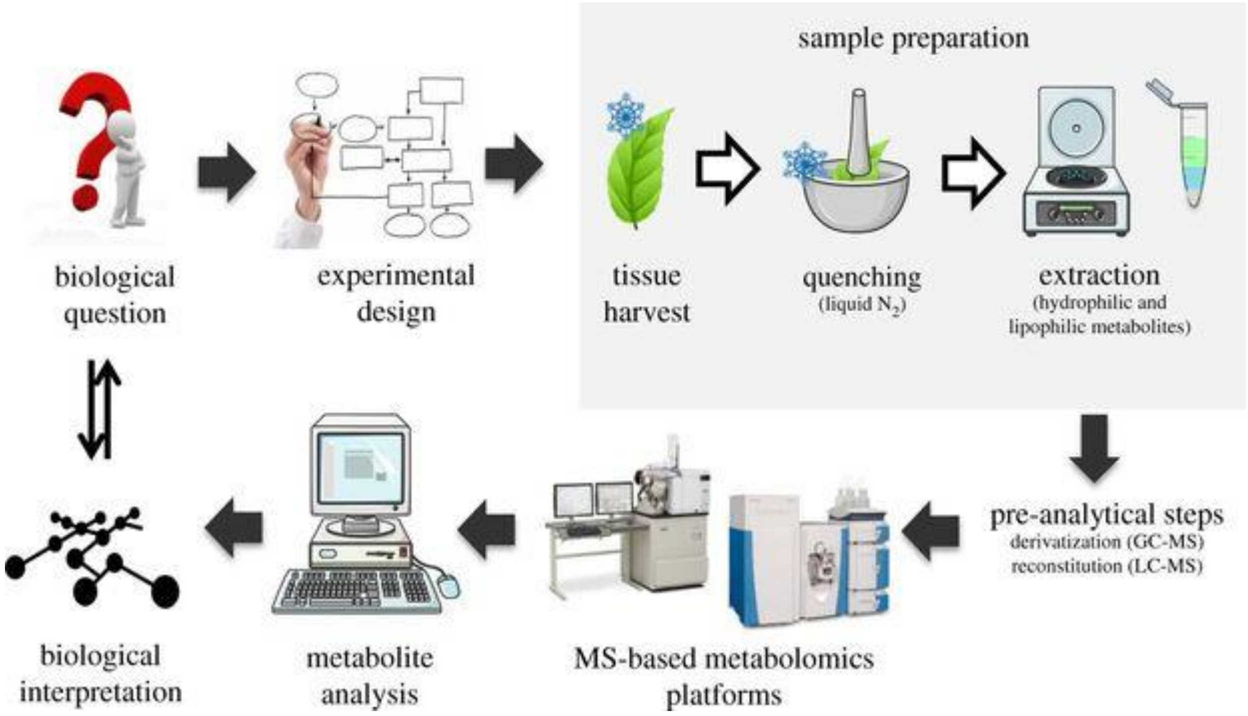
Materials and Methods

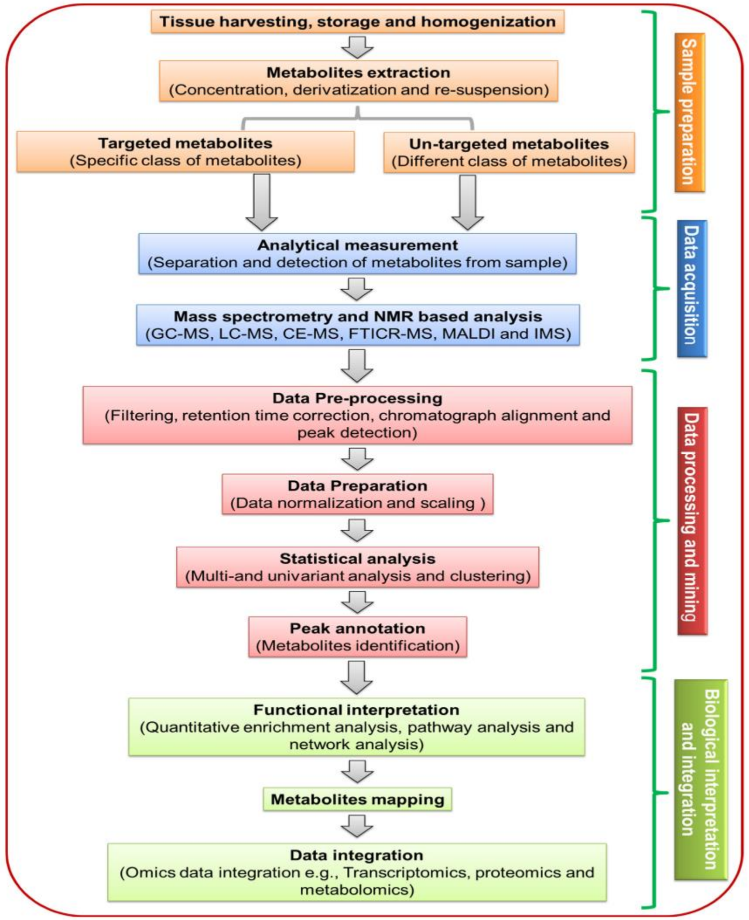
- Metabolomic approaches using Quadrupole Time-of-Flight Mass Spectrometry (Q-TOF-MS) have become central to understanding plant responses to various environmental and biological stresses, including heavy metal exposure, drought, pathogen attack, and nutrient deficiency.



Materials and Methods

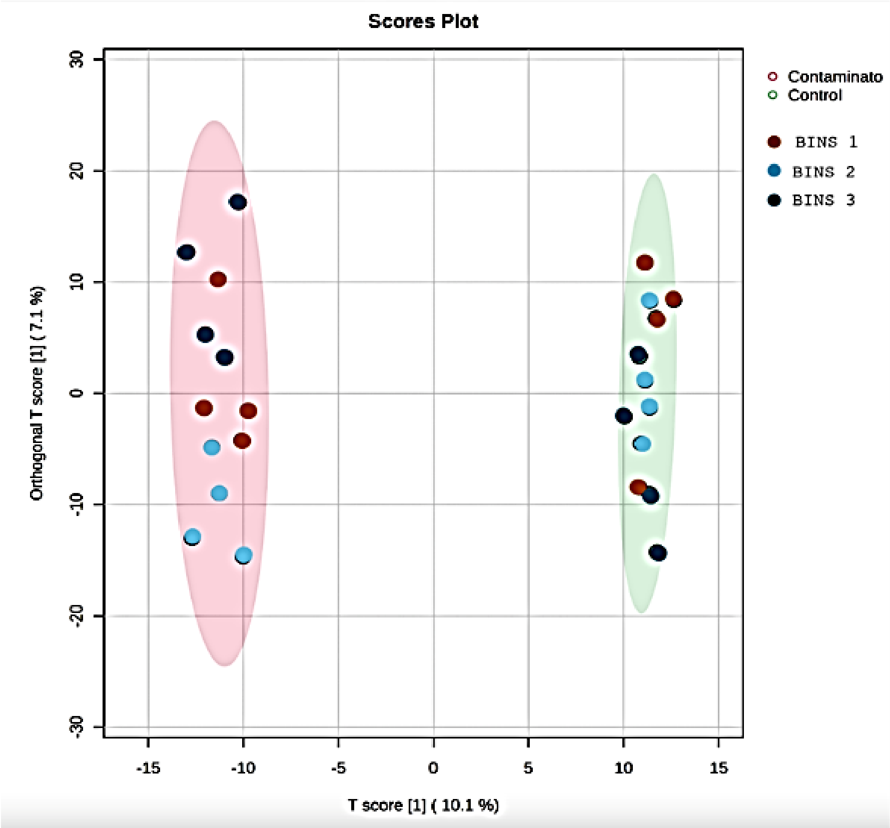
- In a typical Q-TOF-MS workflow for biomarker detection, plant samples (often leaf or root tissue) are first prepared through a metabolite extraction process. Solvents like methanol, ethanol, or acetonitrile are used to extract a broad spectrum of metabolites, both polar and nonpolar, enabling a comprehensive view of the metabolome.

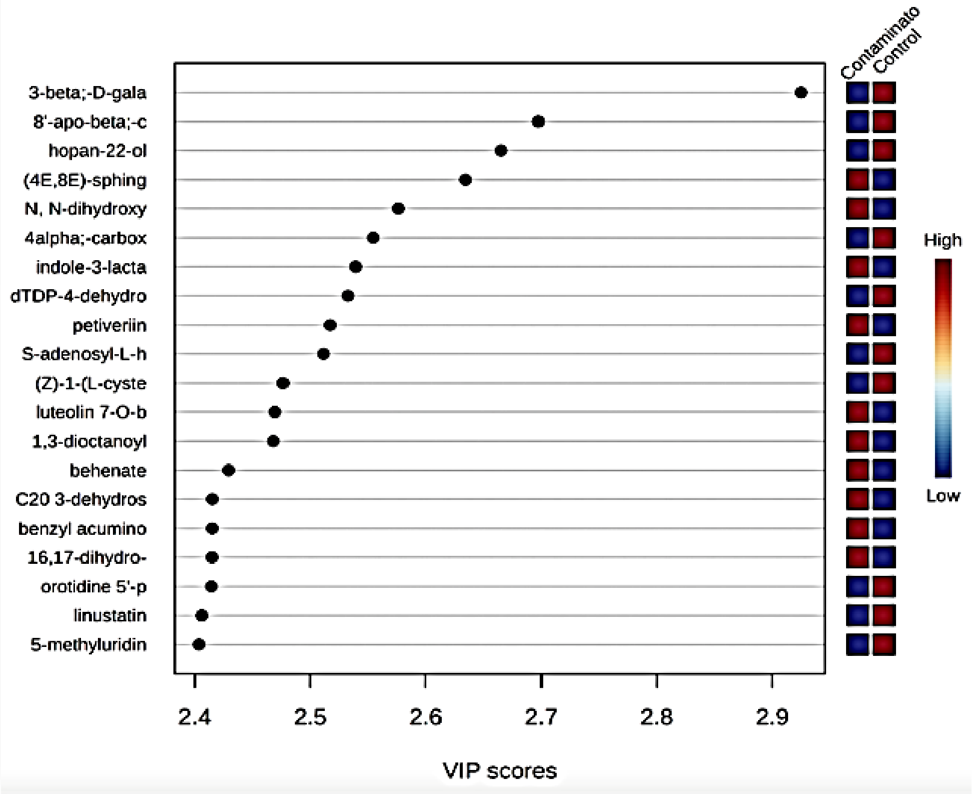




Results

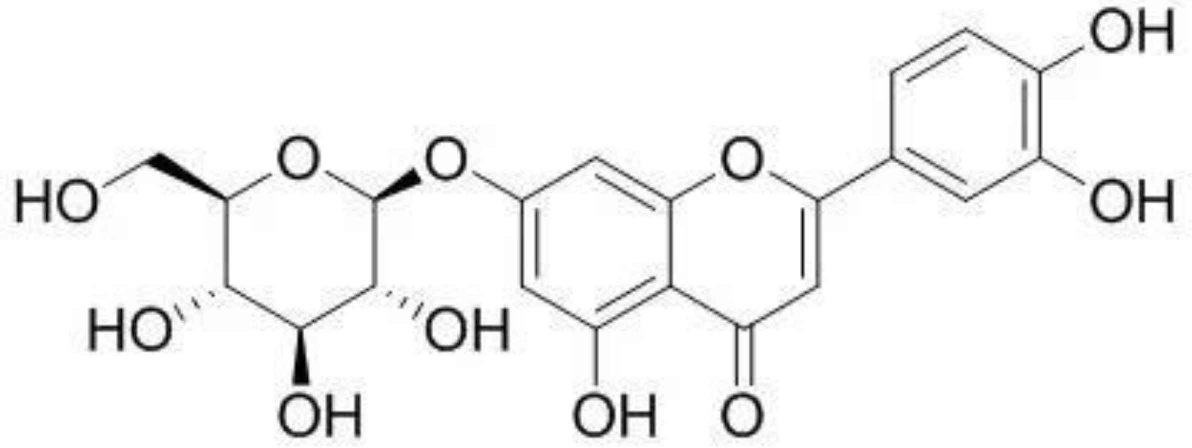
- Metabolomic analysis of our samples by Q-TOF identified among 1400 metabolites applying an untargeted approach. Conversely, statistical validation by OPLSA combined by Volcano analysis allowed the identification of 211 metabolites showing a fair significance between samples grown on contaminated or control soil





Results

1. 3-β-D-Galactosyl-sn-glycerol
2. 8’-Apo-β-carotenal
3. N, N-Dihydroxytrihomomethionine
4. Hopan-22-ol
5. Indole-3-lactate
6. Luteolin 7-O-β-D-glucoside
7. Behenate
8. Petiveriin

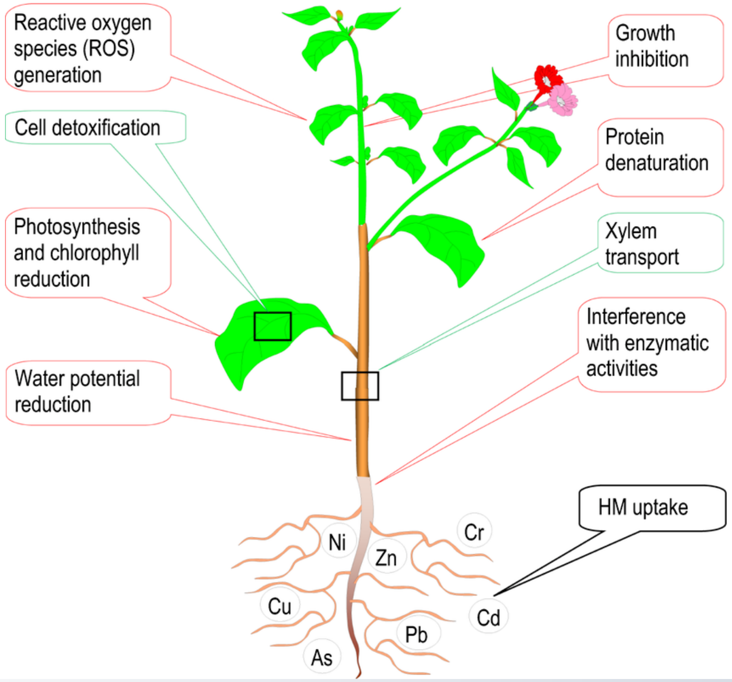


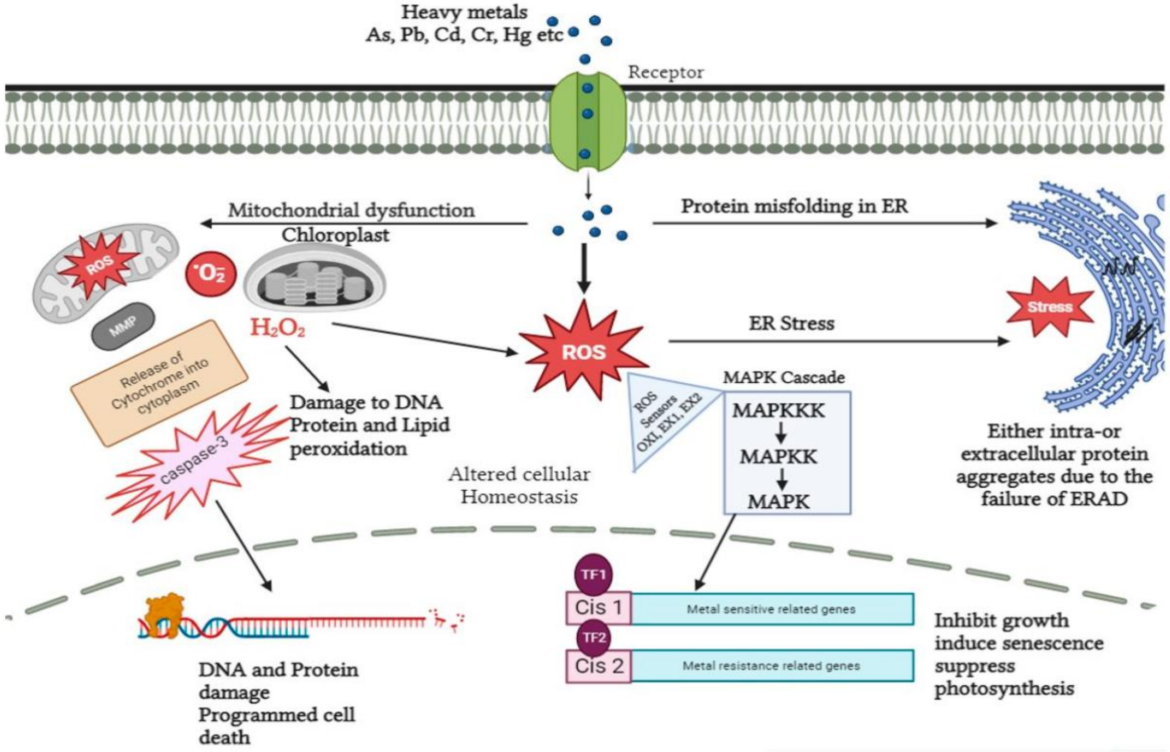
These metabolites have been identified as potential biomarkers, reflecting the plant’s antioxidant activity, metal-binding capabilities, and overall stress response.

Conclusions

- In maize (*Zea mays*), **indole-3-lactate** has been implicated in the plant's response to heavy metal stress due to its involvement in the tryptophan biosynthesis pathway and its potential role in modulating plant defense mechanisms.

- Heavy metal contamination, particularly from metals such as cadmium (Cd), lead (Pb), arsenic (As), and copper (Cu), induces oxidative stress in plants, leading to the production of reactive oxygen species (ROS) that can damage cellular components like proteins, lipids, and DNA





Conclusions

- **These biomarkers hold great promise for assessing the impact of heavy metal contamination on maize growth and can be utilized for screening purposes in environmental monitoring programs.**

- **As heavy metal contamination continues to pose a threat to agricultural productivity and food security, there is a growing need for sustainable solutions to mitigate its effects.**

✓The integration of metabolomic data with other technologies, such as remote sensing, can provide a comprehensive understanding of the plant stress responses across different spatial and temporal scales

✓Metabolomic Results → Physiological analyisis

❖Metabolites→ Spectral Signatures (Unique Fingerprint) :
Cellular Changes and Environmental Responses

❖Future presentations will explore physiological and spectroscopic aspects, integrating our systems approach in order to investigate cellular damage and plant responses