The Effect of Salicylic Acid on Garlic (*Allium sativum*) in Response to Elevated Levels of Heavy Metals in the Hackensack Meadowlands

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Introduction

Garlic (*Allium sativum*) is a profitable cash crop species that is valued as an essential flavoring or medicinal herb in a variety of cultures. The rich history of garlic and the awareness of garlic as natural medicine is celebrated in the United States. Around 400 million pounds of garlic are produced in US annually, and New York is among the top five producing states.

While garlic is known for its biological elasticity when acclimating to various growth conditions, it requires a steady supply of irrigated water and heavy fertilization to reach marketable size. When high levels of solutes are present in the soil or irrigation, water and nutrient uptake via the roots can be impacted due to elevated osmotic stress.Which, in turn, inhibits garlic growth.

New Jersey Meadowlands, also known as the Hackensack Meadowlands, is a salt marsh located a few miles west of New York City. The sediments contain high levels of heavy metals, including chromium (Cr), Copper (Cu), Zinc (Zn) and Lead (Pb). To prevent from inhibition, plants release a defense hormone called Salicylic acid (SA) to maintain its healthy growth and development through morphological, physiological, and biochemical means.

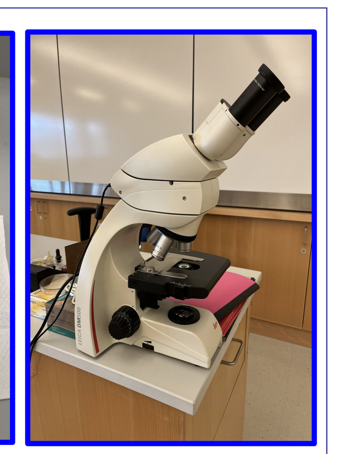
In this study, we examined the effects of elevated levels of copper and zinc (2 mM), and salicylic acid (1 mM) on the growth and physiology of garlic. The results indicated salicylic acid intercepts heavy metal inhibitory effect as it maintains growth, leaf and root functions, and protein metabolism closer to control. Overall, our findings emphasized that salicylic acid facilitates resistance to ecological stressors, such as heavy metals, on the life cycle of non-halophytic crop species.

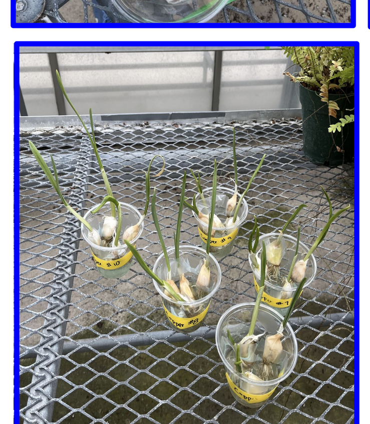
Materials and Methods

Twenty-three garlic cloves were germinated in deionized water and divided into five groups to receive one of the following treatment solutions: 1) Control (di- water); 2) 2mM $CuCl_2$; 3) 2 mM $ZnCl_2$; 4) 1 mM SA + 2 mM $CuCl_2$; 5) 1 mM SA + 2 mM $ZnCl_2$.

Garlic plants were monitored in the greenhouse on campus for two weeks. Weekly measurements included leaf and root counts, lengths of the longest leaf and root, and microscopic imaging of leaf stomata. Photoreceptor levels (chlorophyll and carotenoids) in leaves, along with soluble protein contents in leaves and roots, were quantified using a spectrophotometer. Antioxidant enzyme activity, including catalase, and lipid peroxidation levels of cell membrane were analyzed using a BioRad spectrophotometer.

Facilities and Instruments





Results

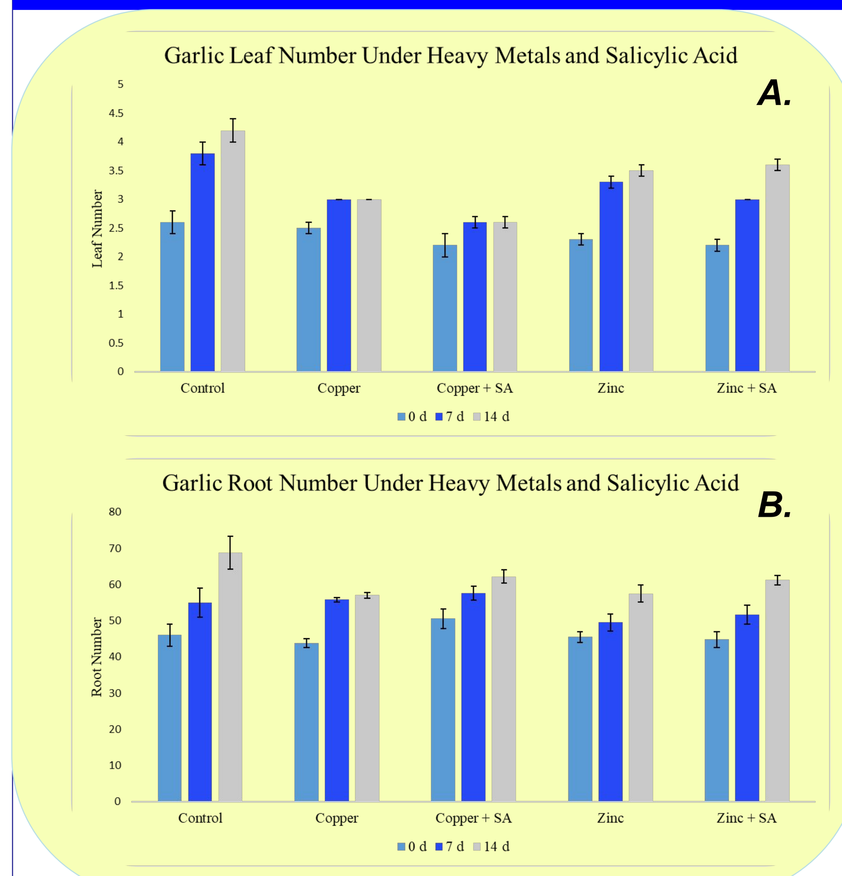


Figure 1 Leaf (*A.*) and Root (*B.*) Numbers of Garlic Plant Under Heavy Metals and SA

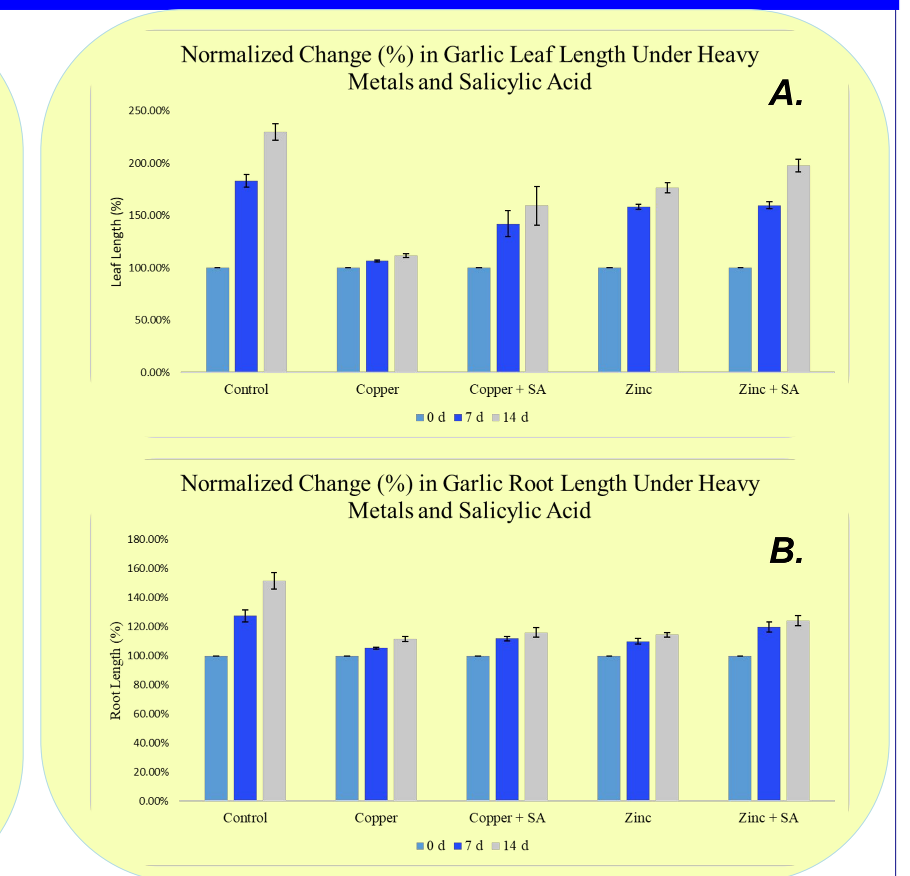


Figure 2 Leaf (*A.*) and Root (*B.*) Length of Garlic Plant Under Heavy Metals and SA

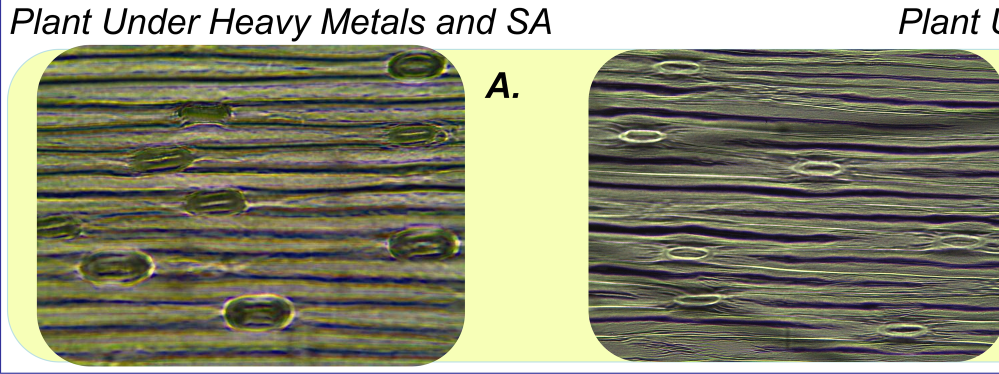


Figure 3 Stomata in Garlic Leaf Under Zinc Treatment (*A.*) and Zinc + SA Treatment (*B.*)

Results

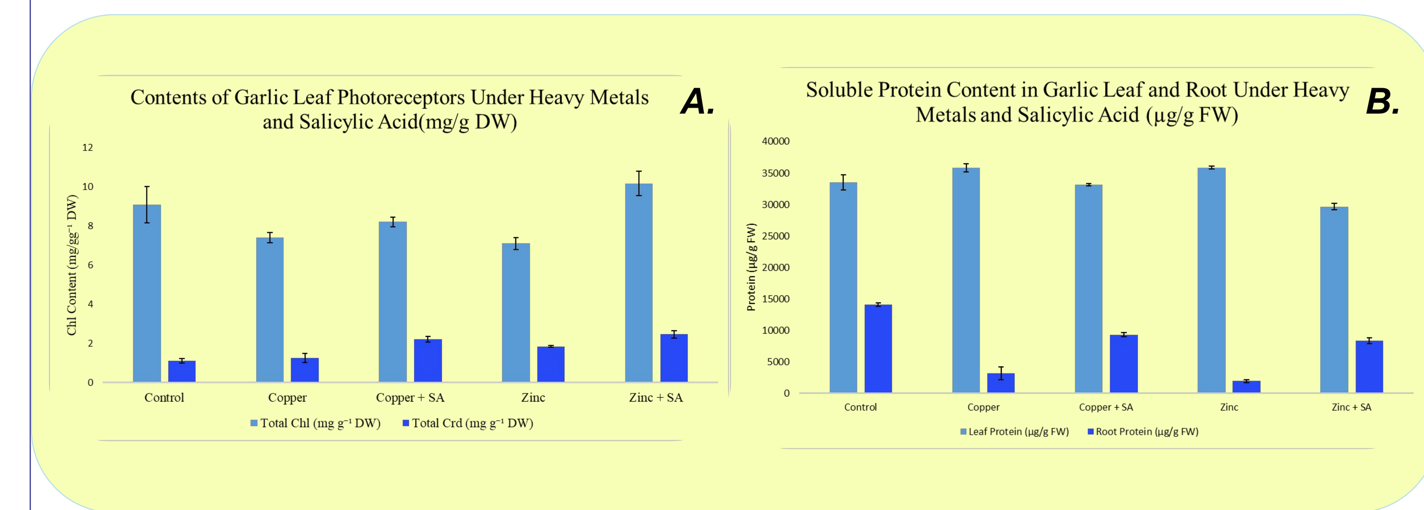
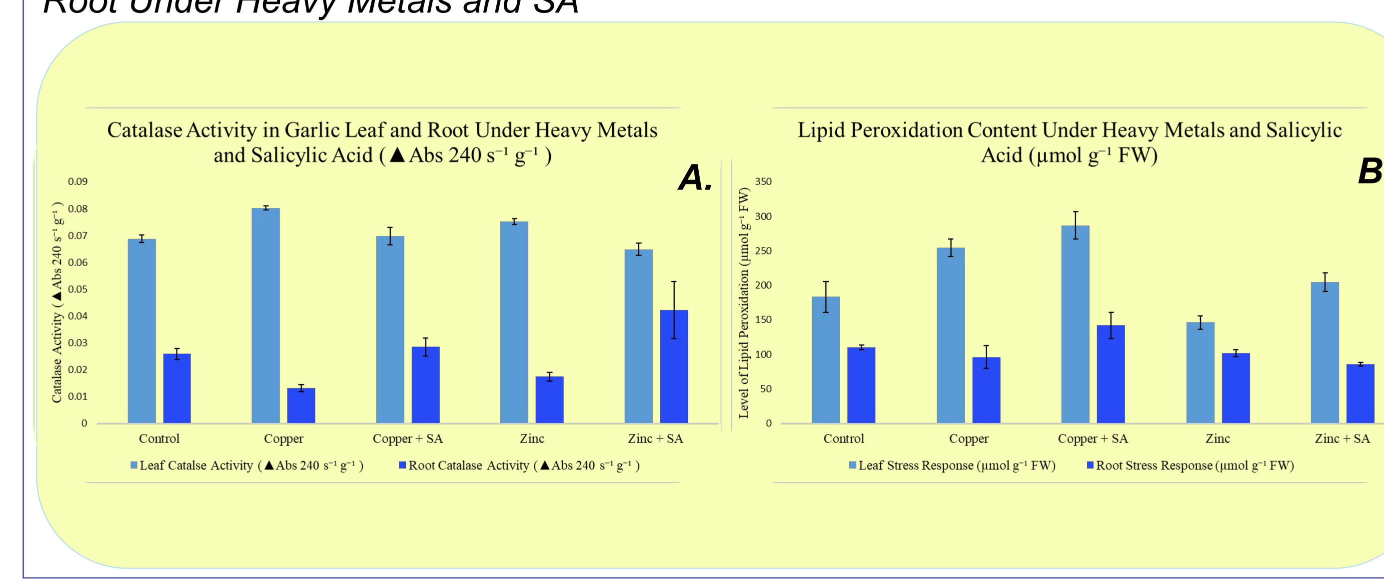


Figure 4 Leaf Chlorophyll and Carotenoid (*A.*) Protein Content in Garlic Leaf and Root (*B.*) Under Heavy Metals and SA

Figure 5 Catalase Activity (*A.*) and Lipid Peroxidation Content(*B.*) in Garlic Leaf and Root Under Heavy Metals and SA



Discussion

- New garlic leaves, except Cu-treated, and roots (Figure 1A and 1B) were steadily added under SA treatments than isolated Cu or Zn treatments.
- Garlic leaves (Figure 2A) grew longer under SA than heavy metals, and roots appeared to have slight more growth in SA (Figure 2B).
- Cu and Zn-treated garlic (Figure 3A) displayed predominantly closed stomata, whereas garlic treated with SA (Figure 3B) showed a higher proportion of open stomata, indicating increased resistance to heavy metal inhibition.
- In fact, garlic treated with SA, appeared to have more photoreceptors than both the control group and heavy metal-treated samples, as observed in chlorophyll and carotenoids (Figure 4A). The increase in photoreceptors enabled the plant to exhibit higher levels of photosynthetic activity
- In terms of protein content, Cu and Zn surpassed protein levels in leaves but exhibited the lowest in roots. In SA treatment, the hormone reduced leaf protein content while increasing root protein closer to the control levels (Figure 4B), suggesting that most heavy metals absorbed by garlic roots tended to accumulate there rather than translocated to the leaves.
- The catalase activity of antioxidant enzymes appeared to increase in roots under SA treatment but was reduced in leaves (Figure 5A). This suggested that SA treatment enhanced detoxification, reducing oxidative damage primarily in the roots, where they were most affected, contrasting with leaves, where there was less ROS (reactive oxygen species).
- Lipid peroxidation products were more pronounced in SA treatment (Figure 5B) in both leaves and roots, except in Zn + SA-treated roots. This indicated significant membrane damage, suggesting that SA may have aggravated toxicity and influenced both uptake and/or accumulation in plant organs.

Summary

- Application of salicylic acid (1 mM) enhances the health and development of garlic plants under heavy metal stress.
- Salicylic acid (1 mM) is more effective under copper (2mM) than zinc (2 mM), potentially due to copper having more of an inhibitory effect and heavy toxicity.
- Both SA and heavy metals can modulate signaling pathways involved in stress responses. The combined effect of SA and heavy metals may down-regulate these pathways, leading to excessive lipid peroxidation and membrane damage.

Future Work

- Conduct additional biochemical and molecular analysis to reveal the mechanisms of garlic plants to cope with harsh environments.
- Examine the synergistic and antagonistic effects of multiple plant hormones on the response of garlic to environmental factors.

Acknowledgements

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