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***Exposure of Microalgae to Heavy Metals: Effects on Growth,
Bioremediation Strategies and Identification of New Methods to Identify
Physiological Damages***

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Abstract

Microalgae are currently considered promising candidates for heavy metal (HM) removal, offering a viable alternative to conventional methods - often relying on costly chemicals and producing hazardous by-products - due to their high surface-to-volume ratios and consequent sorption capacity, rapid growth rates and high biomass yields. HM sequestering can employ either dead biomass, which passively adsorbs metals via cell-wall interactions, or living organisms that actively transport HMs into cells. While existing microalgal-based devices primarily exploit non-viable biomasses with lower maintenance needs, their removal capacity is less effective than their living counterparts with the added benefit of active transport leading to more stable internal sequestration. Microalgae HM tolerance and sorption ability varies by species and metal type, with higher concentrations adversely affecting physiological processes and impairing cell morphology.

The thesis investigates two pivotal roles of microalgae under HM pollution: their ability to adsorb/compartmentalize pollutants and their potential as biosensors for rapid HM detection. In light of potential *in situ* bioremediation applications, two endemic species were tested for their capacity to remove copper (Cu) and cadmium (Cd), widely distributed HMs in contaminated coastal waters in the Gulf of Naples.

Non-destructive methodologies, including X-ray fluorescence (XRF) and X-ray photoelectron spectroscopy (XPS), complemented traditional techniques like Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in an innovative approach to evaluate microalgal-mediated HM removal and discriminate adsorbed/internalized HM quotas. Additionally, the combination of lab-based methods specific for detailed investigation of HM oxidative stress like enzymatic assays and molecular analyses on target antioxidant enzymes and rapid, high-throughput label-free Quantitative Phase Microscopy (QPM) techniques for the early detection of HM-driven alterations in microalgal morphology and behavior furnished a comprehensive picture on HM-mediated stress on microalgae.

Overall, the study supports the use of these microalgal strains for bioremediation and as bio-probes for fast detection of HM-induced stress.