



## Subcellular Localization, Isolation, and Partial Purification of Mercury-binding Biomolecules in *Chromolaena odorata* (L.f.)

R.M. King *et al.* H. Robinson

Gilda C. Rivero<sup>1,2</sup> and Hannah Joy P. Alcantara<sup>\*1</sup>

<sup>1</sup>*Institute of Biology, University of the Philippines, Diliman*

<sup>2</sup>*National Research Council of the Philippines, DOST, Bicutan*

*\*Author for correspondence: hannahdyoi@gmail.com, mobile no: (+63920)5471005*

### ABSTRACT

*Chromolaena odorata* (L.f.) R.M. King *et al.* H. Robinson plants were grown in Hoagland's solution modified with 1.00 ppm Hg(NO<sub>3</sub>)<sub>2</sub>. Cold Vapor-Atomic Absorption Spectrophotometry (CV-AAS) analyses for Hg<sup>2+</sup> contents established the presence of Hg<sup>2+</sup> in 3 out of 4 of the subcellular components obtained from the leaves of the Hg-treated *C. odorata* plants. Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) analyses of the isolated protoplasts and vacuoles revealed that the ultimate localization of Hg<sup>2+</sup> was in the vacuoles.

The Hg-binding, SH-containing biomolecules, which were initially detected through the 5,5'-dithiobis(2-nitro-benzoic acid) (DTNB) assay, manifested as a predominant peak in the chromatographs of both the control and Hg-treated plants, obtained through Reverse Phase-High Performance Liquid Chromatography (RP-HPLC), with their retention times falling within the ranges of reduced glutathione, metallothionein, and cysteine standards. However, the concentrations of the glutathione- and/or metallothionein-like, cysteine-containing biomolecules detected in the leaves of Hg-treated *C. odorata* plants were ten-fold higher than those detected in the control.

The findings of this study provided evidence that the enhanced production of Hg-binding biomolecules and the localization of Hg<sup>2+</sup> ions are ultimately in the vacuoles of the leaves and that these are the mechanisms which bring about Hg<sup>2+</sup> tolerance and homeostasis in *C. odorata* plants exposed to mercury. These results indicate that *C. odorata* is a hyperaccumulator and hence, a potentially effective phytoremediator for Hg<sup>2+</sup> ions.

**Keywords:** *Chromolaena odorata*, mercury, phytoremediation, phytochelatin, glutathione, metallothionein, cysteine, subcellular localization, DTNB assay, RP-HPLC