Antagonistic effects of mercury exposure with phosphorus depletion and excess nitrogen supply reveal the provenance-specific phytoremediation potential of black locust–rhizobia symbiosis

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Abstract: Plants are subjected to a gradual increase in the concentrations of many man-made contaminants, as well as of different environmental and industrial pollutants. Thus, global climate change and environmental pollution present plants with unique combinations of different abiotic and environmental stresses. These byproducts of human activities include, among others, such as heavy metals and nutrition fertilizers. In this context, interaction of different environmental constrains pose severe threats to plants that cannot be predicted from individual stress exposure. Mercury (Hg) as toxic and dangerous heavy metal poses an enormous threat to living organisms on a global scale and, therefore, has attracted particular attention. In this context, biological nitrogen (N_2)-fixing (BNF) leguminous can be used for phytoremediation of Hg contaminated soils, whereas N availability could greatly affect its N_2-fixation efficiency. Moreover, N_2-fixation of leguminous also highly relies on phosphorus (P) availability for nodule formation and functioning. However, information on the consequences of P deficiency and/or excess N availability combined with Hg exposure of plants, particularly for woody legume species have not been reported yet. Here, we conducted two sets of greenhouse experiments for investigation for the interactions of rhizobia inoculation, Hg exposure (+Hg) with (i): low P (-P), as well as with (ii): excess N (+N) supply, individually and in their combination (-P*Hg and +N*Hg, respectively), on photosynthesis as well as biochemical traits in seedlings of *Robinia pseudoacacia* L. provenances originating from two contrasting climate and soil background, *i.e.,* the Gansu Province (GS) in northwest China and the Dongbei region (DB) in northeast China. Our results from greenhouse experiment (i) indicated an antagonistic effect of combined Hg exposure and P depletion. Provenance-specific responses were observed under the combined -P*Hg treatment in inoculated DB plants including significantly increased biomass accumulation, increased root total P content and reduced Hg accumulation. Reduced malondialdehyde (MDA) contents in leaves and roots further indicated less oxidative damage under these conditions. Rhizobia inoculation significantly improved the performance of both *Robinia* provenances under individual and combined exposure to Hg and low P by promoting photosynthesis, increasing foliar N and P contents, and reducing MDA accumulation despite enhanced Hg uptake. DB plants developed more nodules, had higher biomass, and accumulated higher Hg amounts than GS plants.

The results from our greenhouse experiment (ii) showed similar antagonistic effects of combined +N*Hg exposure compared to the individual treatments that were provenance-specific. Compared to individual Hg exposure,

combined +N*Hg stress significantly increased foliar gas exchange of inoculated DB seedlings and resulted in more negative $\delta^{15}N$ abundance in the roots. Furthermore, combined +N*Hg stress greatly increased amino acid N content and nitrate reductase (NR) activity, and decreased MDA content in roots of inoculated GS seedlings. Inoculation with rhizobia significantly promoted plant Hg uptake, reduced MDA contents, enhanced photosynthesis and maintained the nutrient balance in leaf and root tissues of both investigated *Robinia* provenances. Among the two *Robinia* provenances investigated, DB seedlings formed more nodules, had higher biomass and Hg accumulation than GS seedlings. For example, total Hg concentrations in leaves and roots of inoculated DB seedlings were 1.3 and 1.9 times higher than in inoculated GS seedlings under combined +N*Hg stress, respectively. Therefore, from the present results of both sets of experiments, the DB provenance is considered to possess a higher potential for phytoremediation of Hg contamination compared to the GS provenance in environments subjected to P depletion and N deposition condition in future.

Key words: excess nitrogen availability, mercury exposure, phosphorus depletion, phosphorus and nitrogen partitioning, photosynthesis, *Robinia*-rhizobia association, stress combination