Oxidation State of Manganese Determines Toxicological Responses in Marine Amphipods: Evidence from Acute Toxicity and Transcriptomics

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According to the Planetary Boundaries framework, several boundaries are beyond their thresholds. Trace heavy metals play essential roles in various physiological and biochemical processes. However, the release of excessive amounts of these substances into the environment is likely to lead to adverse effects on nature. Manganese (Mn) can exist in various oxidation states. In particular, oxidation states +2 and +7 are important for human activities. Manganese (II) chloride ($MnCl_2$) has been widely used as an agricultural fertilizer because Mn (II) functions as an essential trace element. In addition, potassium permanganate ($KMnO_4$) has been used as a therapeutic agent for fish diseases due to its strong oxidative properties. Although it is known that the toxicity and physiological responses to Mn vary depending on its oxidation state, experimental validation under the same species and identical experimental conditions remains limited. *Ptilohyale barbicornis*, a marine amphipod, is widely distributed from coastal regions to the deep sea. Thus, *P. barbicornis* is useful fortoxicity experiments.
In this study, we conducted the exposure experiment for marine amphipods, *Ptilohyale barbicornis*, to evaluate the biological impacts of $MnCl_2$ and $KMnO_4$. (1) By estimating the 96 h LC_{50} values and (2) assessing internal manganese accumulation. (3) Furthermore, we performed RNA-seq–based transcriptomic analysis to gain deeper insights into the underlying physiological responses and to establish an integrated framework for evaluating metal toxicity.
After estimating the values of 96 h LC_{50} of $MnCl_2$ and $KMnO_4$ in rearing experiments, exposure experiments with $MnCl_2$ and $KMnO_4$ for transcriptomic analysis were conducted at concentrations corresponding to 1/5, 1/10, and 1/20 of 96 h LC_{50}.
In RNA-seq analysis, adapter trimming was performed using cutadapt. The adapter-trimming reads were *de novo* assembled by Trinity. Coding sequences were predicted using TransDecoder, and redundant amino acid sequences were removed using CD-HIT. Putative amphipod protein-coding sequences were identified and annotated using BLASTp (E-value < 1e-5) based on SwissProt protein database. Transcripts Per kilobase Million (TPM) was calculated by kallisto, and differentially expressed genes (DEGs) were identified using edgeR. Expression patterns were visualized by PCA, heatmaps, and hierarchical clustering (dendrograms). To elucidate the mechanisms of metal stress responses occurring within the organism, Gene Ontology (GO) analysis was performed using g:GOSt program of g:Profiler, using *Hyalella azteca* as reference database.
The 96 h LC_{50} indicate that $KMnO_4$ exhibits much greater toxicity than $MnCl_2$. At 1/10 and 1/20 of the 96 h LC_{50}, GO terms related to responses centered on metal binding and transcriptional regulation were significantly enriched, indicating that homeostatic mechanisms against metal stress were preferentially activated. At 1/5 concentration of 96 h LC_{50}, primary metabolic process and cellular response to stimulus, Biological Process GO terms, were significantly enriched. The findings suggest a transition from early responses centered on metal binding and transcriptional regulation to a phase characterized by the maintenance and regulation of cellular mechanisms and metabolic homeostasis required for sustaining vital biological functions with increasing Mn concentrations. Based on the 96 h LC_{50} values, $KMnO_4$ exhibited much higher toxicity than $MnCl_2$, whereas both treatments showed similar concentration-dependent stress response patterns. Only $MnCl_2$ exposure group, the GO term of mitochondrion (GO:0005739) was significant. In the $KMnO_4$ treatment groups, the body surface was

noticeably brown. This difference may be attributable to the distinct chemical properties of Mn (II) and Mn (VII).
To evaluate the biological impacts of different chemical forms, we conducted exposure experiments using *Ptilohyale barbicornis* with Mn (II) and Mn (VII). As a result, $KMnO_4$ exhibited much high biological toxicity than $MnCl_2$. Furthermore, RNA-seq analysis revealed molecular-level biological responses.

Keywords: Marine amphipod, Manganese, Oxidation state, RNA-seq

