

Lead (Pb) Contamination Improved by Cyanidiales

Molecular mechanisms for the removal of Pb by Cyanidiales: a potential biomaterial applied in thermo-acidic conditions.

Yu-Ting Liu (National Chung Hsing University) and her collaborators recently revealed molecular mechanisms for the removal of Pb by Cyanidiales. Such releases of heavy metals due to anthropogenic activity have threatened the health of ecosystems. Lead (Pb) is a particularly dangerous chemical substance as it accumulates in not only organisms but entire food chains. Once human beings become exposed to Pb, the brain and kidneys, of both adults and children, might be damaged. To improve the situation caused by release of Pb into environments, especially in acid wastewater and soil systems, the team of Liu and her coworkers developed Cyanidiales as a potential biomaterial for the removal of Pb. Cyanidiales (red microalgae) are capable of surviving in extreme environments (20–56 °C; pH 0.5–5.0) with concentrated metals, allowing them to serve as promising green materials applied in metal remediation. An understanding of their mechanisms of metal detoxification has been limited. This work is hence the first to determine the capacity and related mechanisms of sorption of Pb(II) ions on Cyanidiales including *Galdieria maximum*

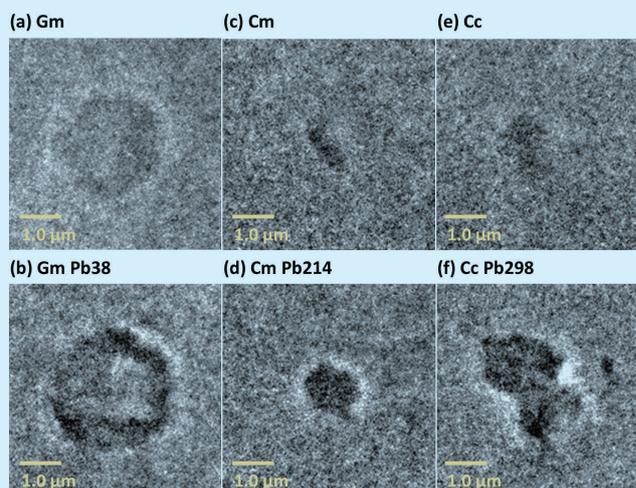


Fig. 1: 2-D images for (a,b) Gm, (c,d) Cm, and (e,f) Cc with and without sorbed Pb. Sorbed Pb was 38, 214 and 298 mg g⁻¹ on Gm, Cm and Cc. [Reproduced from Ref. 1]

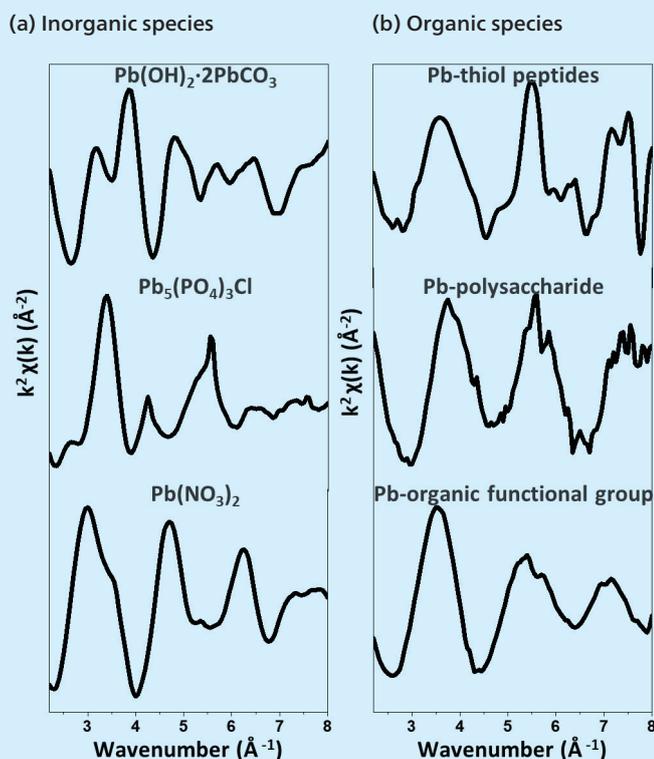
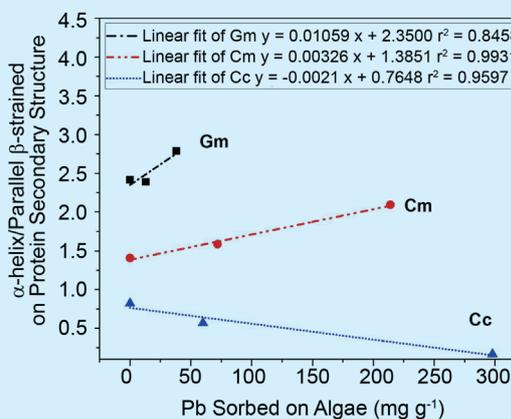


Fig. 2: Pb L_{III}-edge X-ray absorption spectra of (a) inorganic and (b) organic Pb species as standard references used in linear combination fitting (LCF). (c) Results of LCF analyses to determine the Pb species on Gm, Cm and Cc on which Pb was sorbed at 38, 214 and 298 mg g⁻¹, respectively. (d) Ratios of signal intensity for an α-helix (1652 cm⁻¹) to a parallel β-strand (1631 cm⁻¹) on protein secondary structure as a function of amount of Pb sorbed on Gm, Cm and Cc. [Reproduced from Ref. 1]

(c) Results of LCF analyses

	Gm Pb38	Cm Pb214	Cc Pb298
Pb-PO ₄ precipitate	35.3%	16.5%	18.7%
Pb-polysaccharide	12.6%	13.2%	17.4%
Pb-thiol peptide	8.4%	3.0%	17.9%
Pb-organic functional group	43.7%	67.2%	46.0%

(d) Protein secondary structure in vivo of cell



(Gm), *Cyanidioschyzon merolae* (Cm) and *Cyanidium caldarium* (Cc), which belong to varied genera. The maximum observed sorption of Pb(II) ions Cc, Cm and Gm were 298.4, 214.0 and 38.2 mg g⁻¹, respectively.

Employing a transmission X-ray microscope (TXM) at **TLS 01B1**, the team revealed that the darker shadows along with the cell outline in Gm with sorbed Pb implied that Pb generally distributed around the cell surface (**Fig. 1(b)**). In contrast, TXM images for Cm and Cc with sorbed Pb showed darker shadows that were scattered around the cell (**Figs. 1(d) and 1(f)**), indicating a plausible accumulation *in vivo* for Pb. These significant variations in the tomography of Cyanidiales upon sorption of Pb(II) ions indicated that alternative mechanisms for Pb tolerance might be adopted by individual Cyanidiale genera.

Utilizing X-ray absorption spectra (XAS) at **TLS 17C1** and **SP 12B1**, the team found that the Pb inventory in all samples was dominated by both inorganic [Pb₅(PO₄)₃Cl] and organic [Pb-polysaccharide, Pb-thiol peptide and Pb-organic functional group] Pb species. Whereas Pb₅(PO₄)₃Cl was the major specie on Gm, the proportion of the Pb-organic functional group was the major specie on Cm. Noteworthily, the great proportion of Pb-thiol peptide on Cc plausibly implies a unique mechanism of defence against the toxicity of heavy metals, leading to the greatest capacity of Pb(II) ion sorption on Cc.

To determine the changes in functional groups of Cyanidiales after Pb(II) ion sorption, the team used synchrotron-based Fourier-transform infrared (FTIR) spectra at **TLS 14A1**. Due to the heterogeneous morphology of algae surfaces, changes of the α-helix were normalized to that of a β-strand to obtain accurate trends caused by sorption of the Pb(II) ion. The metallothionein protein that regulates metal homeostasis and imparts a defence against heavy-metal toxicity through intracellular sequestration contains N-terminal and C-terminal motifs joined by α-helix and β-strand structures. It is thus reasonable to postulate the α-helix/β-strand ratio as the major indicator responsible for metal stress tolerance in Cyanidiales. These structural changes in the secondary structure of proteins implied the denaturation of existing proteins or the variation in protein distribution during apoptosis. An inverse relation hence indicated the disruption or modification of the α-helix by the Pb stress (**Fig. 2(d)**). Among tested Cyanidiales, Cc showed a unique response for the metal tolerance, wherein the α-helix was prone to unfold as the sorbed Pb increased. Such protein denaturation *in vivo* agreed with the greatest proportion of unordered structure in the sample of Cc.

In summary, Cyanidiales generally perform four mechanisms against Pb toxicity (**Fig. 3**); individual defence responses were highlighted by specific Cyanidiales species. The knowledge provided here could improve the application of Cyanidiales in environmental remediation as an innovative green technology. (Reported by Yu-Ting Liu, National Chung Hsing University)

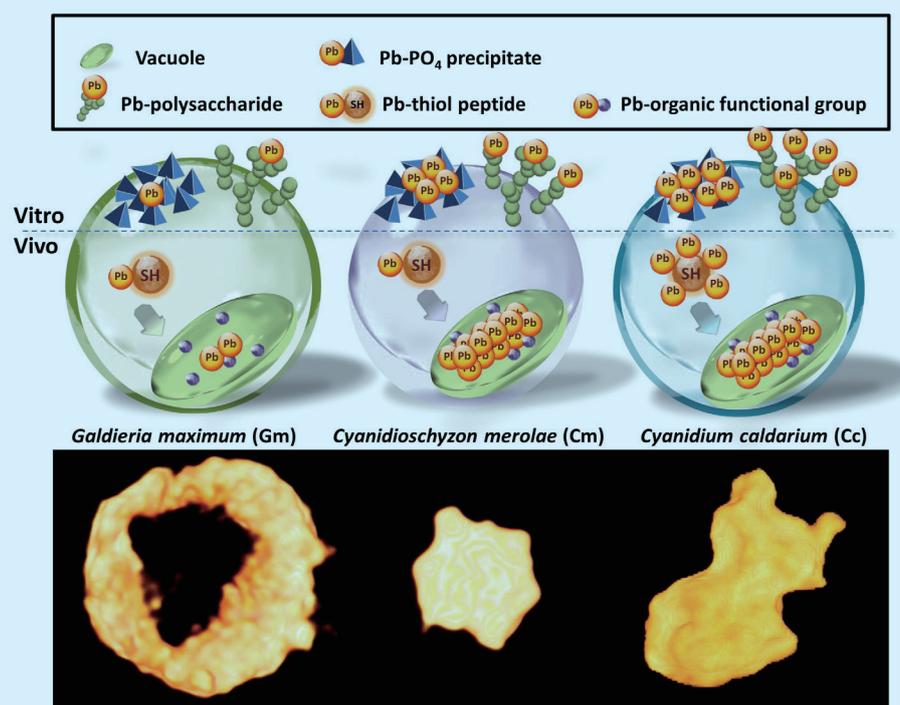


Fig. 3: Conceptualization of four mechanisms of Pb retention on Gm, Cm and Cc: the defence line provided by polysaccharide, inorganic Pb-PO₄ precipitation, organic Pb complexation concomitant with transport to cell vacuoles and specific thiol-Pb chelation involved in disruption of protein secondary structures. [Reproduced from Ref. 1]

This report features the work of Yu-Ting Liu and her collaborators published in *Chem. Eng. J.* **401**, 125828 (2020).

TLS 14A1 BM – IR Microscopy
TLS 17C1 W200 – EXAFS
TLS 01B1 SWLS – X-ray Microscopy
SP 12B1 BM – Materials X-ray Study

- IR, TXM, XAS
- Environmental and Earth Science, Biological Science, Chemistry

Reference

1. Y.-L. Cho, Y.-C. Lee, L.-C. Hsu, C.-C. Wang, P.-C. Chen, S.-L. Liu, H.-Y. Teah, Y.-T. Liu, Y.-M. Tzou, *Chem. Eng. J.* **401**, 125828 (2020).