In-vitro, in-vivo and spectroscopic assessment of the reduction of lead exposure via ingestion and inhalation pathways

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Lead (Pb) exposure is a significant global concern due to its negative impact on neurological and cognitive development in children. Particularly, in many historic mining sites, high levels of lead (Pb) in the ingestible soil (<250 µm) and inhalable dust (<10 µm) have prompted the investigation into techniques for *in-situ* Pb immobilization using soil amendments. Although the efficacy of Pb immobilization using phosphates has been demonstrated via the ingestion pathway (e.g. by promoting the formation of insoluble Pb-phosphates in the soil), its efficacy in reducing Pb exposure via the inhalation pathway has received little attention. This is particularly relevant because 90% of the inhaled dust may be swallowed within 24 h and reach the gastro-intestinal (GI) tract where the Pb may be subjected to the same conditions as ingested soil. This study compared Pb immobilisation efficacies of phosphorus (P) and iron (Fe) amendments via ingestion and inhalation pathways using *in-vitro* and *in-vivo* assays in conjunction with Pb speciation and dynamics via X-ray based spectroscopic methodologies. Mining/smelting impacted soil from Broken Hill, Australia was amended with Phosphoric Acid (PA), Hydroxyapatite, Mono-Ammonium Phosphate (MAP), Triple Super Phosphate (TSP), bonemeal biochar and Fe_2O_3 at a molar ratio of Pb:P/Fe=1:5. Pb *in-vitro* bioaccessibility (IVBA) was conducted in the <250 µm ingestible soil particle fraction (ingestible fraction) using the solubility bioaccessibility research consortium assay, and in the <10 µm inhalable soil particle fraction (dust fraction) using inhalation-ingestion bioaccessibility assay and artificial lysosomal fluid assay. *In-vivo* bioassays were conducted in the mouse model [relative bioavailability (RBA) in the ingestible fraction and blood and tissue bioavailability via instillation in the dust fraction over a 24 h exposure period]. Spectroscopic methodologies utilised included assessment of Pb-phosphate formation by X-ray Absorption Spectroscopy (XAS) in the pre and post-IVBA residuals, 2D mapping of Pb in the mice lungs and GI tracts by X-ray Fluorescence Microscopy (XFM) and X-ray Absorption Near Edge Spectroscopy (XANES) in the 24 h mice lungs. Despite no *in-situ* Pb-phosphate formation was observed using XAS analysis, a significant reduction ($p<0.05$) in Pb IVBA was observed in all P amended ingestible fraction [Treatment effect ratio (TER): 0.02-0.1] and in PA, MAP and TSP amended dust fraction (TER 0.6-0.8). A 21.1% reduction in Pb RBA and 56.4% reduction in blood Pb concentration were observed via the ingestion and inhalation pathways respectively. 2D images of lungs and GI tracts via XFM indicated that Pb started clearing from the lungs within 4-8 h and reached the stomach, while XANES assessment of Pb speciation indicated that anglesites and organic matter bound Pb were the predominant residual species in the lungs 24 h post instillation.