# PHOSPHORUS AMENDMENT REDUCES HEMATOLOGICAL, HEPATIC AND RENAL TOXICITY OF LEAD-CONTAMINATED SEDIMENT TO MALLARDS

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## ABSTRACT

**Ingestion of lead-contaminated sediments has resulted in lead poisoning of waterfowl for decades in the Coeur d'Alene River Basin in Idaho. This study examined whether the addition of phosphoric acid to contaminated sediments would reduce the bioavailability and toxicity of lead to mallards (*Anas platyrhynchos*). Mallards received diets containing 12% clean sediment (controls) or 12% sediment from each of three different sites containing up to 6990 ug/g lead (dw) with or without phosphoric acid amendment for 8 weeks. Amendment resulted in reductions in tissue lead concentrations of up to 64% for blood, 57% for liver, and 77% for kidney. Unamended lead-contaminated sediment resulted in the following hematological and plasma alterations: 90% or greater depression of red blood cell ALAD activity, elevated free erythrocyte protoporphyrin (FEP) concentration, lower hematocrit and hemoglobin concentrations (as much as 30%), elevated plasma enzyme activities (ALT, CK and LDH-L) and creatinine concentration. Hepatic effects included: 1.6 fold elevation of liver GSH concentration, higher GSH S-transferase and GSSG reductase activities, and lower PBSH concentration. Renal effects included 2.1 fold elevation of kidney GSH concentration with resulting lower GSSG to GSH ratios, elevated GGT activity, and 1.7 fold increase in lipid peroxidation (TBARS). Phosphorus amendment restored hematocrit, hemoglobin and plasma enzyme activities so that they did not differ from controls and lowered elevated FEP concentrations by up to 80%. Amendment restored all hepatic variables as well as the renal variables TBARS concentration and GGT activity so they did not differ from controls. Although amendments of phosphorus substantially reduced the bioavailability of lead and some of the toxic effects, lead concentrations in the tissues of mallards fed the amended sediments were still above those believed to be harmful to waterfowl under the present conditions.**

## INTRODUCTION

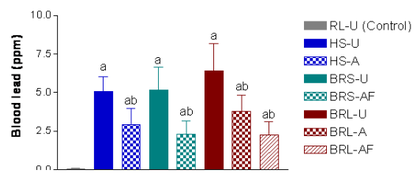
Waterfowl in the Coeur d'Alene River Basin (CDARB) of Idaho have been dying from lead poisoning since the early 1900s as a consequence of many decades of mining and smelting operations. Species affected have included mallards, Canada geese, and tundra swans. Field and laboratory studies have demonstrated that the ingestion of lead-contaminated sediment can poison waterfowl (Blus *et al.*1999; Henny *et al.* 1999; Beyer *et al.* 1998). Removal of lead-contaminated sediments would be costly and might not be practical. Therefore, another option has been to add a material such as phosphoric acid ($H_3PO_4$) to the sediment that has been shown to bind the lead into biologically unavailable forms.

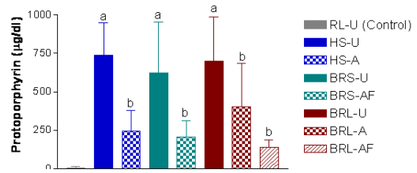
Clinical and biochemical effects of ingestion of lead-contaminated CDARB sediments in waterfowl include pronounced hematological effects with over 90% depressions of red blood cell ALAD activity accompanied by highly elevated free erythrocyte protoporphyrin (FEP) concentration, lower hematocrit and hemoglobin concentrations, and alterations in plasma and organ biochemistries including oxidative stress (Hoffman *et al.* 2000). Heinz *et al.* (2004) recently reported that both lab and field amendments of phosphorus to CDARB sediments substantially reduced the bioavailability of ingested lead to mallards, resulting in lower concentrations of lead in tissues of mallards, with up to 64% reduction of lead in the blood. It was the purpose of the present study to determine whether amendments of phosphorus to CDARB sediment would reduce the extent of adverse hematological effects and oxidative stress in the liver and kidney caused by ingestion of lead-contaminated sediments in mallards.

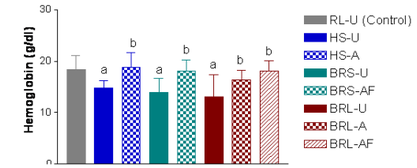
## METHODS

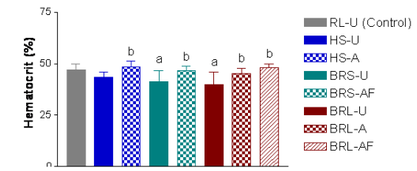
Ten mallards were randomized to each of 8 different diets containing 12% clean control unamended sediment (Round Lake; RL) or 12% sediment unamended (U) or amended (A) with phosphoric acid from each of three different CDARB sites (Harrison Slough; HS, Black Rock Slough; BRS, and Bull Run Lake; BRL) containing up to 6990 ug/g lead (dw). After 8 weeks on the experimental diets, a sample of blood was taken from each bird for lead analysis and plasma, the birds were euthanized with $CO_2$, and samples of liver and kidney taken from each bird for biochemical and lead analysis. Blood was analyzed for free erythrocyte protoporphyrin (FEP), hemoglobin, hematocrit, and ALAD.

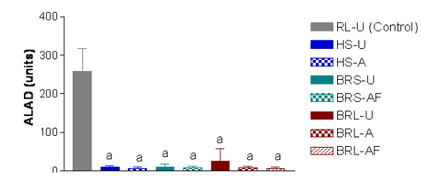
Tissues for biochemical assays were immediately frozen and stored at –80°C until assayed. Basic methods and assay conditions are described by Hoffman and Heinz (1998; Environ. Toxicol. Chem. 17:161-166). Measurements included: Plasma - glutathione peroxidase (GSH-peroxidase), ghtathione reductase (GSSG - reductase), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine kinase (CK), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), cholinesterase (ChE), uric acid (UA), creatinine (CRN), glucose (GLU), total plasma protein (TPP), albumin (ALB), cholesterol (CHL), triglycerides (TRG), calcium and inorganic phosphorus; Liver - GSH-peroxidase, GSSG-reductase, glutathione-S-transferase (GSH-S-transferase), glucose-6-phosphate dehydrogenase (G-6-PDH), reduced glutathione (GSH), oxidized glutathione (GSSG), total sulfhydryl concentration (TSH), and thiobarbituric acid reactive substances (TBARS); Kidney – GGT and enzymes related to glutathione metabolism, oxidative stress and TBARS as for liver assays. All clinical, biochemical, and residue measurements were compared among treatment groups using a combination of analysis of variance ($p \leq 0.05$) followed by Dunnett's multiple comparison test ($p \leq 0.05$) in order to separate all other means from the control group mean (Round Lake unamended, lab-aged sediment) and either the same procedure or two-tailed t-tests ($p \leq 0.05$) were used to separate unamended from the corresponding location amended means. Where homogeneity of variance was lacking, log transformation of data was conducted prior to analysis.




[a]Significantly different from the RL-U (control) group (≤ 0.05).
[b]Significantly different from the corresponding unamended group for that site (≤ 0.05).
U= Unamended
A= Amended
AF= Amended in the field

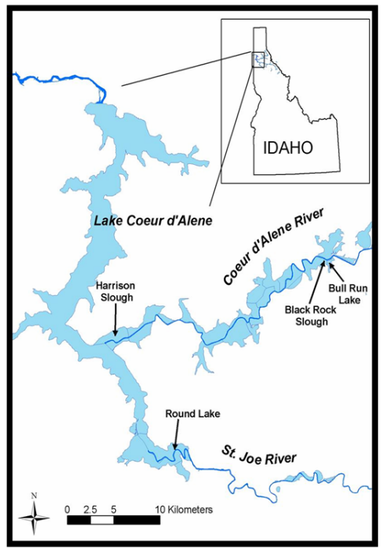



**Table 1a.** Measurements of Hepatic Oxidative Stress in Mallards Receiving Unamended and Amended Sediments.[a]

| Location/Treatment of sediment | RL-U | HS-U | HS-A | BRS-U | BRS-AF | BRL-U | BRL-A | BRL-AF |
|---|---|---|---|---|---|---|---|---|
| GSH-peroxidase[b] | 689 | 728 | 646 | 672 | 743 | 636 | 651 | 712 |
| GSSG-reductase[b] | 65 | 76 | 61 | 65 | 61 | 78[c] | 79 | 68 |
| GSH S-Transferase[b] | 576 | 645 | 627 | 646 | 599 | 717[c] | 730[c] | 673 |
| G-6PDH[b] | 107 | 88 | 95 | 88 | 110 | 91 | 101 | 108 |
| GSH (µmol/g) | 3.3 | 4.3 | 4.1 | 4.0 | 3.7 | 5.4[c] | 5.4[c] | 4.3 |
| GSSG (µmol/g) | 0.39 | 0.58[c] | 0.53 | 0.52 | 0.57[c] | 0.55 | 0.47 | 0.46 |
| GSSG/GSH | 0.12 | 0.17 | 0.13 | 0.14 | 0.17 | 0.10 | 0.09 | 0.12 |
| PBSH (µmol/g) | 15.9 | 14.9 | 15.3 | 14.6 | 15.3 | 13.6[c] | 15.3[d] | 15.7[d] |
| TSH (µmol/g) | 19.2 | 19.1 | 19.5 | 18.5 | 19.1 | 19.0 | 20.7 | 20.1 |
| TBARS (nmol/g) | 32.3 | 35.9 | 35.4 | 36.8 | 34.6 | 36.4 | 33.7 | 32.7 |
| Liver lead (ug/g WW) | 0.17 | 21.3[c] | 12.6[cd] | 18.2[c] | 8.5[cd] | 22.5[c] | 14.5[cd] | 10.1[cd] |

**Table 1b.** Measurements of Renal Oxidative Stress in Mallards Receiving Unamended and Amended Sediments.[a]

| Location/Treatment of sediment | RL-U | HS-U | HS-A | BRS-U | BRS-AF | BRL-U | BRL-A | BRL-AF |
|---|---|---|---|---|---|---|---|---|
| GSH-peroxidase[b] | 577 | 616 | 541 | 553 | 625 | 535 | 440 | 494 |
| GSSG-reductase[b] | 70 | 73 | 70 | 68 | 77 | 71 | 69 | 74 |
| GSH S-Transferase[b] | 448 | 471 | 449 | 487 | 483 | 501 | 491 | 444 |
| G-6PDH[b] | 14.0 | 13.3 | 12.7 | 13.5 | 12.2 | 14.3 | 11.9 | 12.1 |
| GGT[b] | 33.9 | 36.5 | 31.6 | 42.4 | 34.9 | 44.5[c] | 39.8 | 35.9 |
| GSH (µmol/g) | 1.2 | 2.2[c] | 2.2[c] | 2.3[c] | 1.9[c] | 2.5[c] | 2.5[c] | 2.2[c] |
| GSSG (µmol/g) | 0.12 | 0.09 | 0.12 | 0.13 | 0.12 | 0.10 | 0.13 | 0.12 |
| GSSG/GSH | 0.12 | 0.04[c] | 0.06[c] | 0.07 | 0.08 | 0.05[c] | 0.06[c] | 0.06[c] |
| PBSH (µmol/g) | 15.6 | 14.6 | 15.0 | 14.7 | 15.2 | 14.0 | 14.7 | 14.9 |
| TSH (µmol/g) | 16.8 | 16.8 | 17.1 | 17.0 | 17.1 | 16.4 | 17.2 | 17.2 |
| TBARS (nmol/g) | 16.3 | 17.2 | 18.7 | 19.4 | 18.5 | 27.4[c] | 21.2[d] | 17.6[d] |
| Kidney lead (ug/gWW) | 0.32 | 36.0[c] | 15.0[cd] | 28.7[c] | 10.7[cd] | 45.6[c] | 21.0[cd] | 12.3[cd] |

[a]Mean; n = 10
[b]nmol/min/mg of 10,000 g supernatant protein.
[c]Significantly different from the RL-U (control) group ($p \leq 0.05$).
[d]Significantly different from the corresponding unamended group for that site ($p \leq 0.05$).

## SUMMARY and CONCLUSIONS

**Unamended lead-contaminated sediment effects included:**

- Hematological and plasma alterations: 90% or greater depression of red blood cell ALAD activity, elevated free erythrocyte protoporphyrin (FEP) concentration, lower hematocrit and hemoglobin concentrations (as much as 30%), elevated plasma enzyme activities (ALT, CK and LDH-L) and creatinine concentration.
- Hepatic effects: 1.6 fold elevation of liver GSH concentration, higher GSH S-transferase and GSSG reductase activities, and lower PBSH concentration.
- Renal effects: 2.1 fold elevation of kidney GSH concentration with resulting lower GSSG to GSH ratios, elevated GGT activity, and 1.7 fold increase in lipid peroxidation (TBARS).

**Phosphoric acid amendment of lead-contaminated sediment resulted in:**

- Reductions in tissue lead concentrations of up to 64% for blood, 57% for liver, and 77% for kidney.
- Restoration of hematocrit, hemoglobin and plasma enzyme activities so that they did not differ from controls and lowered elevated FEP concentrations by up to 80%.
- Restored all hepatic variables as well as the renal variables TBARS concentration and GGT activity so they did not differ from controls.
- Although amendments of phosphorus substantially reduced the bioavailability of lead and some of the toxic effects, lead concentrations in the tissues of mallards fed the amended sediments were still above those believed to be harmful to waterfowl under the present conditions.