Toxic effects of selected heavy metals, as xenobiotics on *Euphlyctis hexadactylus* (Indian green frog) in a polluted urban wetland, Bellanwila Attidiya sanctuary in Sri Lanka

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U. A. Jayawardena

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Abstract

Consequential implications of heavy metal contamination on wetland biota, including man is a prevailing ecotoxicological concern globally. As environmental sentinels of wetland ecosystems, amphibians provide a cluster of biomarkers for xenobiotic induced stress mechanisms elicited as intoxication and detoxication signals involving general physiological, immunological, nueroendocrinological, reproductive and stress responses.

Severe immunotoxic effects demonstrated in *Euphlyctis hexadactylus* (Indian green frog) with heavy metal exposure in the Bellanwila Attidiya sanctuary, a polluted urban wetland in Sri Lanka, through a pilot study conducted from 2010-2011, provided the rationale for the current study. This study investigated the prevailing metal contamination in water and sediment of this wetland from 2013-2015; Comparisons were drawn with a pristine reference site, the Labugama reservoir catchment area, while *E. hexadactylus* of the two sites served as the animal model. Metal mediated stress signals were comprehensively evaluated by cytogenotoxicity, haematotoxicity, immunotoxicity, histopathologic and biochemical alterations, and neuro-endocrine and reproductive toxicity. Field experiments were validated by laboratory exposure of reference site adult *E. hexadactylus* to an equivalent heavy metal mixture as present in the polluted wetland.

Water samples of the polluted site were contaminated with significantly high levels (18.39-3.75 ppm) of heavy metals, Cu>Cr>Pb>Zn>Cd, while the metal accumulation in the sediment followed a dissimilar trend with Zn >Pb>Cu>Cd>Cr, both exceeding relevant water and sediment quality criteria established by authorities. Bioaccumulation of metals, particularly Cr and Cu, in frog liver was significantly higher compared to that of the muscle tissue, following a universal trend. Comet and *Allium cepa* assays reported cytogenotoxic potential of a heavy metal mixture (Cu, Cr, Pb, Zn and Cd) with significant genetic damage at ecologically relevant concentrations (5 ppm), while the micronulcues assay substantiated its cytotoxic potential at higher concentrations. Reduced RBC counts and corpuscular volume signified haematotoxity ($p<0.05$) while immunotoxicity was demonstrated by significantly ($p<0.05$) abated total WBC counts and elevated neutrophil/lymphocyte ratio. Significantly elevated Th1 cytokines, IFNγ and TNFα, positively correlated ($p<0.05$) with neutrophil counts in blood, and melanomacrophage centres in the liver that signified both systemic and hepatic inflammation. Basal levels of IFNγ, TNFα, IL6 and IL10 cytokines reported for *E. hexadactylus* were first time records for any amphibian species locally and globally. A novel, semi quantitative scoring method was validated to evaluate histopathological impairment of liver, kidney, lung and skin tissue. Significant ($p<0.05$) aggregation of melanomacrophages, bile secretion, haemorrhages and sinusoidal dilations of the liver, and distortions of alveolar sacs in the lungs manifested as major impairments in metal exposed frogs. Liver injuries complemented significantly elevated levels ($p<0.05$) of aspartate transaminase, alanine transaminase γ-glutamyltransferase and alkaline phosphatase in frog liver homogenates. Elevated levels of malondialdehyde indicated increased lipid peroxidation. Neither neuroendocrine nor reproductive effects were observed; Yet, Significantly elevated ($p<0.05$) oxidative stress hormones, cortisol and thyroxin, revealed an endocrine disruptive role for heavy metals. A skewed sex ratio towards female *E. hexadactylus* was apparent.

In conclusion, this study revealed significantly high contamination of heavy metals in the Bellanwila Attidiya sanctuary, with considerable bioaccumulation and related toxic effects in *E. hexadactylus*. An array of potential toxicity measures based on cytogenotoxicity, haematotoxicity, immunotoxicity, and histopathological aberration associated biochemical alterations, were corroborated as biomarkers of aquatic health, predominantly linked with heavy metal toxicity of a sentinel amphibian species in a wetland ecosystem. (Key words- Heavy metals, xenobiotics, immunotoxicity, cytogenotoxicity, haematotoxitiy, histopathology)