Cytogenotoxicity evaluation of a heavy metal mixture, detected in a polluted urban wetland: Micronucleus and comet induction in the Indian green frog (*Euphlyctis hexadactylus*) erythrocytes and the *Allium cepa* bioassay

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Abstract

Heavy metal contamination in wetland ecosystems is a serious environmental and health concern. This study evaluated the cytogenotoxicity of a previously evidenced heavy metal contamination (Cd, Cr, Cu, Pb and Zn ~5 ppm each) in a polluted urban wetland, the Bellanwila-Attidiya sanctuary (BAS) in Sri Lanka, using a battery of cytogenotoxic assays. Micronucleus and comet assays evaluated the genotoxicity in erythrocytes of a common amphibian, the Indian green frog (*Euphlyctis hexadactylus*), under natural metal exposure in the wetland, and *in vitro* exposure, respectively.The *Allium cepa* bioassay assessed the cytogenotoxicity of the heavy metal mixture and of the individual metals, under laboratory exposure. Although *in vivo* natural exposure showed no significant induction of micronuclei in frog erythrocytes ($P > 0.1$), a significant and dose dependent elevation of comets was evident with *in vitro* exposure to the metal mixture ($P < 0.001$). Field controls did not show significant impacts in the *A. cepa* bioassay, whereas individual exposure to heavy metals reported lower effects than their combined exposure under laboratory conditions; $Pb^{2+}$was the most toxic metal, with the highest mitotic inhibition (Pb^{2+}>Cd^{2+}>Zn^{2+}>Cr^{6} >Cu^{2+}), mutagenic potential as evaluated in the percentage incidence of chromosomal aberrations (Pb^{2+}> Zn^{2+}> Cu^{2+}> Cr^{6+}> Cd^{2+}) and cytotoxicity evaluated by the incidence of cell apoptosis and necrosis (Pb^{2+}>Cr^{6+}>Cu^{2+}>Cd^{2+}>Zn^{2+}). Thus, the test battery of micronucleus, comet and *A. cepa* assays that reveal differential aspects of cytogenotoxicity may serve as a valuable tool in environmental monitoring, primarily to screen for complex environmental mixtures of heavy metals that may impact ecological health.