( 133 )

# Phagocytosis as an immunological biomarker to detect aquatic heavy metal pollution in *Euphlyctis hexadactylus (Ranidae)*: an in depth *in vitro* study

S. Priyadarshani*, Deepthi D. Wickramasinghe and Preethi V. Udagama

Department of Zoology, University of Colombo, Sri Lanka.
*uoc.priyadarshani@yahoo.com

**Abstract**

In a previous, pilot study conducted *in vitro* for the first time in Sri Lanka, we observed immunomodulation of the frog species, *Euphlyctis haxadactylus* (Indian Green Frog), stimulated by selected heavy metals, where ability to use phagocytosis as a biomarker in Ecoimmunotoxicology was apparent to detect aquatic heavy metal pollution. The present study, aimed to further substantiate our previous findings by carrying out an in depth study using a wider range of concentrations of the selected heavy metals, using the functional test, *in vitro* phagocytic assay, based on Nitro Blue Tetrazolium dye reduction.

In the present study too, *Euphlyctis hexadactylus* (N=6) were collected from the Bolgoda South wetland (reference site) where negligible amounts of heavy metals, Copper (Cu:0 ppm), Zinc (Zn: 0.05 ppm), Lead (Pb: 0 ppm) and Cadmium (Cd: 0 ppm), were previously detected by Atomic Absorption Spectrophotometry (AAS) as compared to the polluted test site, Bellanwila Attidiya Sanctuary (Zn - 2.71 ppm, Pb - 0.955 ppm, Cu - 0.04 ppm, Cd - 0.019 ppm). Assuming these frogs had minimal aquatic heavy metal exposure, their blood leukocytes, splenocytes and peritoneal macrophages after *in vitro* exposure to selected heavy metals, Cu, Zn, Pb and Cd were used to measure the phagocytic capacity by the NBT assay, by calculating the stimulation index (SI). The four heavy metals were used at concentrations ranging from $10^{-2}$ to $10^{-10}$M, increasing in two fold dilutions.

The assays resulted in similar shaped dose-response curves; at low concentrations all metals were observed to have the potential to stimulate phagocytosis, and as metal concentrations increased the trend was towards immunosuppression. Dose related responses, resulted in a significant linear trend ($p<0.05$) for all cell types with the exception of blood leukocytes for Cadmium.

The concentration for each metal which induced 50% suppression of phagocytosis (IC 50) was calculated, for all different cell type used. Accordingly, Cadmium was the most potent inhibitor of phagocytosis followed by Zinc and Copper, while Lead was the least immunotoxic.

In conclusion, this *in vitro* study unequivocally reiterated that phagocytosis may be considered as a sensitive immunological biomarker for aquatic heavy metal pollution due to its capability of demonstrating immunomodulation of all selected cell types of the frog species, *E. haxadactylus*.

***Key words:*** Aquatic pollution, heavy metals, immunotoxicity, *Euphlyctis hexadactylus* , phagocytosis, immunological biomarker