6. SUMMARY

Environmental contamination by numerous chemicals and non- essential elements, such as heavy metals (lead and arsenic), is an unfortunate byproduct of a complex, industrialized, high-tech society. Lead and arsenic have been associated with various forms of diseases in aquatic organisms including fishes. Lead and arsenic are absorbed through gills and gastrointestinal tract and thus enter the blood. Blood is a good medium for bringing in contact such heavy metals with immune cells present in blood itself, as well as those present in resident lymphoid organs.

Heavy metals are potent immunomodulators that have been shown to antagonize macrophage, lymphocyte differentiation and negatively regulate numerous macrophage functions as well as antioxidant defence mechanisms. Lead and arsenic induced increased production of ROS, lipid peroxides, protein carbonyls and increased percentage of DNA fragmentation which suggests marked oxidative stress in fish macrophages. Lead and arsenic exposed cells have a marked compromised antioxidant defence system characterized by reduced CAT, GST, GSH, GR and GPx activity associated with significant increase in SOD release. Lead and arsenic interferes with the ability of macrophages not only in modulating immune response, but by also inhibiting macrophage inflammatory function. Lead and arsenic exposed cells poorly recognizes foreign pathogenic organism and are unable to eradicate pathogens from the intestine and liver of exposed fishes leading to prolonged survival of bacteria.

Oxidants can be produced within cells by multiple enzymes that use molecular oxygen as a substrate. Two classic phagocytic ROS-generating enzymes, the multisubunit NADPH oxidase and myeloperoxidase (MPO), were first studied because of their essential role in host defence. Oxygen radicals and other toxic species produced by the NADPH

oxidase and MPO were thought to be directly responsible for killing microorganisms; however, new evidence suggests that it is the release of proteases from within the neutrophil and not ROS per se that is required for bacterial destruction (Finkel, 2003). Protease release, however, requires an NADPH-oxidase-dependent influx of ROS within the phagocytes endocytic vacuoles. In this new model, oxidants therefore serve more as a signal than an ultimate effector. Similarly, a new role for MPO was uncovered by the observation that during endotoxemia, MPO localised near endothelial cells. The ROS generated by the enzyme in this acute setting of heavy metal toxicity, in turn significantly alters the bioactivity of another oxidant species, nitric oxide. Other recent observations also support the notion that enzymes, classically thought to generate ROS solely for destructive purposes, might actually use oxidants for important signalling functions.

The present study suggests that lead (Pb) and arsenic (As) exert oxidative stress and adversely alters both the defense mechanisms of innate immunity as well as antioxidant system in fish. A variation in morphology and functional activities of macrophages including enzyme release suggests that both the antioxidant and immune defense systems of *C. punctata* intestine are compromised by metals (Pb and As) exposure at low concentrations, probably by compromising the molecular cross-talk between signaling molecules. Suppression of proinflammatory cytokine release (TNF and IL-1b) implicates the involvement of MAPK cascades and downregulation of NFkB genes. The study also reports that a simultaneous exposure to lead and arsenic has a synergistic effect as compared to the effects of independent exposure to them. Thus, the alterations in the antioxidant status, innate immune functions and associated oxidative stress parameters can be used as potential biomarkers for risk assessment in aquatic ecosystems.