Comparative lethal and sub-lethal toxicity of hexavalent chromium in selected faunal species in Sri Lanka

A thesis submitted for the

Degree of Doctor of Philosophy

H. K. A. Vindhya Ashvinie Kulasena Fernando

Faculty of Science

University of Colombo

May 2018

Abstract

Hexavalent chromium (Cr(VI)) is a heavy metal that is well known as a health hazard to human and animals. The majority of the empirical studies conducted to determine the ecotoxicity of Cr(VI), exceed levels generally recorded in the environment. The current study was therefore undertaken to assess lethal and sub-lethal toxicity of environmentally-relevant levels of Cr(VI) on three faunal species, representing three taxa, inhabiting urbanized landscapes which are prone to Cr(VI) pollution. The three selected taxa were earthworms (*Eudrilus eugeniae*; Kinberg, 1867), fish (*Labeo dussumieri,* Valenciennes, 1842) and toads (*Duttaphrynus melanostictus,* Schneider, 1799).

Lethal toxicity was evaluated using LC_{50} values after short (4 days) and long term (21/ 28 days) exposures. Sub-lethal toxicity, both in the short and long term, were evaluated using alterations in growth, activity, development, histology, haematology and genetic structure. Adult earthworms, fingerlings and adult fish, and tadpoles and adult toads were exposed to five concentrations of Cr(VI) ranging from 0.002 to 20 mg/L, while maintaining a control group in each case. The exposure concentrations used in the present study were determined based on the analysis of dissolved Cr concentrations in water samples collected from 94 sampling locations in 18 major water bodies in Colombo and within a radius of 20 km around Colombo. It is noteworthy that Cr levels in many of the water bodies exceeded the permissible levels for aquatic life in Sri Lanka provisioned by the Central Environmental Authority. Chromium levels in some water bodies in Colombo (e.g. Kelani River) which supplies drinking water, also exceeded the safety levels for drinking water recommended by WHO (i.e. 0.05 mg/L).

Comparative toxicity assessment based on mortality revealed that amphibian larvae were the most vulnerable to Cr(VI) exposure. It must be reiterated however that environmentally-relevant levels did not induce significantly high lethality in any of the three species. Contrary to lethal effects, sub-lethal toxicity was evident in all three study species exposed to field levels of Cr. Histological, haematological and genotoxicity effects were observed at environmentally relevant concentrations and hence provided better monitoring tools than changes in growth, development and activity. The latter three was not significantly altered by exposure to field levels of Cr. However, development of amphibian larvae was seen to be affected at levels marginally higher than typical field levels, which may occur at points of effluent discharge. Histological analysis was revealed to be the most sensitive method to detect Cr(VI) toxicity in the three study species and so could be used as an effective biomarker. Body wall and intestinal epithelium of earthworms, and gills, liver and muscle tissue of fish and amphibian larvae were comparatively evaluated in a qualitative and quantitative manner using several histological indices. Amphibians in comparison to other two taxa, were more prone to histological damage at environmentally relevant Cr(VI) concentrations. Similarly, haematological assessments were done using standard parameters (RBC and WBC counts, PCV, haemoglobin concentration, MCV, MCHC, MHC, differential WBC counts and RBC morphometry). With the exception of RBC morphometry, all parameters could be used as suitable biomarkers since alterations occurred in a dose-dependent manner. Genotoxic effects were investigated *in vivo* by means of micronucleus test and *in vitro* by assessing tail DNA percentage and Olive Tail Moment (comet assay), in earthworm coelomocytes and fish and amphibian RBCs. The present study shows that amphibians are more susceptible to genotoxicity from Cr(VI) exposure. No published

information is available to date on the use of the micronucleus test to demonstrate heavy metal induced genotoxicity and the present study is the first to show Cr(VI) induced genotoxicity in an amphibian. The present study also investigated the production of superoxide (a Reactive Oxygen Species - ROS) as a result of exposure to Cr(VI) in both earthworm (using coelomocytes) and amphibians (using RBC). The study demonstrates that ROS production correlated strongly and positively with Cr(VI) levels and that genotoxic effects (tail DNA percentage and Olive Tail Moment) also correlated with ROS levels in the respective cell types. These results provides evidence to strengthen the claim that ROS production is a possible mechanism through which damage is elicited when exposed to Cr(VI).

This present study is the only systematic evaluation of comparative toxicity in three local taxa exposed to environmentally realistic levels of Cr(VI) in Sri Lanka. The results of the present study showing differential susceptibility of the taxa highlights the limitation of focusing on individual species to set safety standards. The results of the study can be effectively utilized in making judgement for the selection of suitable species for biomonitoring for the re-assessment of water quality standards in the country with regards to Cr pollution. Overall it was evident that the amphibians are comparatively more sensitive to Cr(VI) exposure and therefore better suited for toxicity assessments over fish and earthworms. The study is the first to cover all main waterbodies in Colombo and suburbs in determining levels of dissolved Cr, which has provided a direct comprehensive assessment of Cr pollution in the Colombo city and suburbs. Although lethal effects were not prominent on the study species, sub-lethal impacts will lead to reduction in fitness in the long term. Therefore it is important to closely monitor the sub-lethal effects to avert Cr(VI) induced mortality.