Comet assay of green and brown hydra treated with aluminium

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Hydras are simple aquatic organisms, members of the phylum Cnidaria, usually found in unpolluted freshwater. Green hydra (*Hydra viridissima* Pallas, 1766) is an endosymbiotic and brown hydra (*Hydra oligactis* Pallas, 1766) is a non-symbiotic species [1]. Aluminium is one of the most abundant elements in the Earth's crust, but its effects upon living organisms have not been much studied. The aim of this research was to explore the effects of aluminium in two closely related hydra species, and to trace the extent of damage caused.

For the first time, a modified alkaline comet assay [2] was used to study primary DNA damage in green and brown hydra cells. Five animals of each species were treated under laboratory conditions (21.5°C) with aluminium-sulphate ($Al_2(SO_4)_3.18H_2O$; 25, 250, 475 mg/L) in aerated aquarium water in glass dishes, 6 cm in diameter, 3.5 cm in height (photoperiod 10 hrs light/15 μmol/m^2s, 14 hrs dark) and compared with the control groups. After 72 hrs, animals from each of the concentrations of Al were transferred into a micro-test-tube containing 10 μl of chilled distilled water. Hydras were homogenized with a 100 μl micropipette by sucking the animals in and out several times. Fully frosted slides were coated with 1% and 0.6% normal melting point (NMP) agarose. Homogenized hydras were mixed with 0.5% low melting point (LMP) agarose and placed on the slides. The slides were immersed for 1.5 hrs in freshly prepared ice-cold detergent lysis solution pH 10. Alkaline denaturation was carried out in freshly prepared buffer pH 13.0 for 15 min and electrophoresis for another 10 min at 0.8 V/cm. Slides were neutralized with Tris-HCl buffer pH 7.5, stained with ethidium bromide and examined using epifluorescence microscope Orthoplane (Leitz Wetzlar, Germany), equipped with immersion objective 170/0.17 63/1.30, an excitation filter of 515–560 nm and a barrier filter of 590 nm (Leitz Wetzlar, Germany). A total of 100 comets were scored per slide. Using a black and white high performance CCD camera 4900 Series (COHU), the microscope image was taken and analysed by Comet Assay II software (Perceptive Instruments Ltd.). Tail length (μm) and tail intensity were used as a measure of DNA damage. Entire DNA damage evaluation was performed in duplicate. Means of repeated experiments were used to test the difference of the tail length and intensity values between treated and control animals applying the Student's *t*-test (Statistica 5.0, StatSoft, USA). The dependence of the comet assay endpoints on the Al concentration was evaluated by calculating the Spearman correlation coefficient. Multiple regression was performed to test the predictivity of the Al-dose-response. Significance level was set to 0.05.

Aluminium toxicity triggered DNA changes. DNA tail length and intensity changes were greater in brown than in green hydras. Since the comet assay detects DNA lesions at a single cell level [3], it enables to observe intercellular differences in the genomic susceptibility. Control cells appear as compact orange spheres, whereas cells bearing the genome damage exhibit more or less dense tail of orange spots resembling the comets in the sky (Fig. 1). Brown hydra was more susceptible to aluminium than green hydra, confirming the evolutionary advantage provided by symbiosis. Due to its higher genetic susceptibility,

brown hydra appears to be a better biomarker for aquatic toxicology studies compared with green hydra. Due to its higher correlation and predictivity, the tail intensity was shown to be a more relevant comet assay parameter for DNA damage than tail length. Biomonitoring protocols using hydra and the comet assay could be developed to provide a valuable and rapid method for determining the quality of freshwater.

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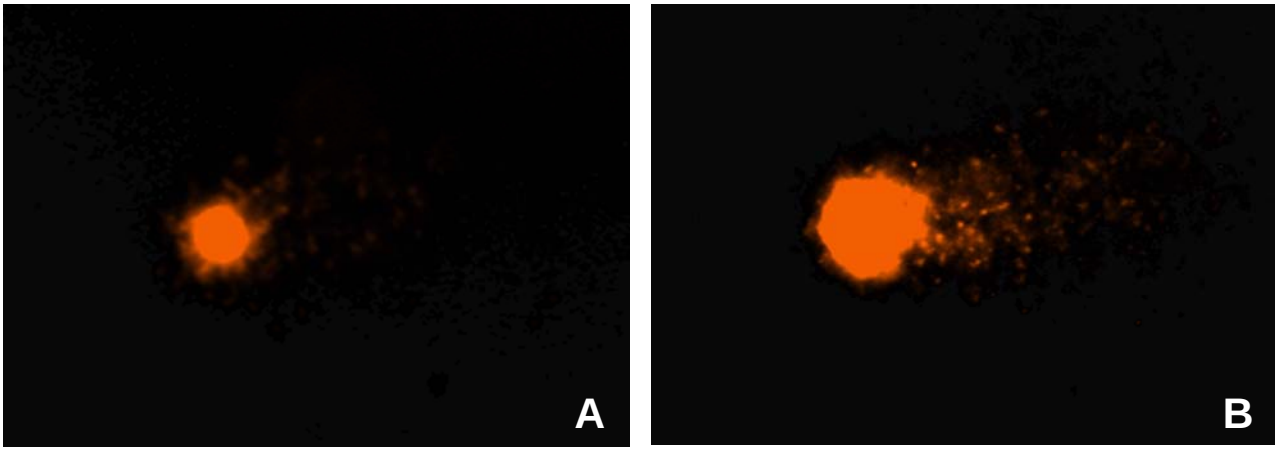

Figure 1. Micrographs of green hydra cell (a) and brown hydra cell (b) with highly damaged DNA. Hydras were exposed to the 250 mg/L $Al_2(SO_4)_3.18H_2O$ for 72 hours. Micrographs were taken using Comet Assay II software and COHU CCD camera 4900 Series. Magnification 400x.