Activities of glutamate dehydrogenase and aspartate and alanine aminotransferases in freshwater snails Helisoma duryi and Lymnaea natalensis exposed to copper.

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ACTIVITÉS OF GLUTAMATE DEHYDROGENASE, ALANINE AND ASPARTATE AMINOTRANSFERASES IN FRESH WATER SNAILS, HELISOMA DURYI AND LYME A NATALENSI EXPOSED TO COPPER

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Snails are known to accumulate metal ion pollutants in their tissues this being attributed to activity of metal binding proteins (Petering and Fowler, 1986). A consequence of the presence of pollutants in water inhabited by aquatic organisms maybe the induction of enzymes required to metabolise or degrade the pollutants in such organisms. Other enzymes may also be induced in response to toxic effects of these pollutants on metabolic pathways in which these enzymes are involved. We are investigating the potential use of key enzymes of amino acid metabolism as markers of pollution due to metal ions in fresh water snails, Helisoma duryi and Lymnea natalensis.

Experimental snails were drawn from concrete breeding tanks where they were regularly fed on lettuce. The snails were exposed for 96 hours to 0.01, 0.1 and 1 ppm concentrations of copper as a chloride salt. After exposure, snails were shelled excluding any dead snails. The tissue was homogenised and centrifuged at 500 x g for 10 minutes at 4°C to pellet nuclei and unbroken cells. The post nuclear supernatant was centrifuged at 10 000 x g for 10 minutes at 4°C. After suspension of the resulting pellet in buffer, both the pellet fraction (“mitochondrial” fraction) and the supernatant (cytosolic fraction) were aliquoted and stored at -82°C. The samples were assayed for the activities of glutamate dehydrogenase, and aspartate and alanine aminotransferases. The concentration of cadmium in breeding waters and in shells and tissues (not homogenised) was also determined.

The concentration of copper in tissues rose with increasing concentration of Cu in breeding waters. In the absence added Cu i.e. 0.13 and 0.14 µg/ml Cu concentration in breeding waters of Helisoma and Lymnea respectively, the concentration of Cu was 7.04 µg/g in Helisoma tissues and 6.1µg/g in Lymnea tissue. These concentrations rose respectively to 15.3 and 21.4 µg/g at 1 ppm showing 2.1 and 3.4 fold increases over the initial concentrations. The biggest increase in tissue concentrations of Cu occurred between 0.01 and 0.1 ppm added metal ion. There were similar accumulations of Cu in shells of the two snail species. Generally the activities of glutamate dehydrogenase (GDH), Aspartate and Alanine aminotransferase (AST and ALT) increased with concentration of copper but then decreased at 1 ppm. The most consistent increases in GDH activity were in the 10 000 x g pellet. At 0.1 ppm added copper the increase was 1.8 and 1.6 fold over initial activities for Helisoma and Lymnea respectively. AST showed consistent increases in the 10 000 x g supernatant with 2.5 and 1.6 fold increases over initial activities for Helisoma and Lymnea respectively, at 0.1 ppm added copper. The pellet ALT activity was very sensitive to higher concentrations of copper and its activity disappeared at 1 ppm added Cu in Helisoma and at 0.1 ppm in Lymnea. Since alanine and aspartate aminotransferase are known to be dually localised in the mitochondrion and cytosol in a number of species, a possibility that the enzymes could have redistributed due to organelle damage is unlikely considering the low initial activities in the pellets. Further the rise in enzyme activity with metal ion concentration is also seen in the homogenates. The increases in enzyme activities were therefore likely to be due to induction of enzymes but this breaks down as the concentration of Cu becomes lethal at 1 ppm. Pellet GDH and ALT, and supernatant AST could therefore be sensitive indicators of copper pollution these snail species.