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Effect of Heavy Metals Ions on Enzyme Activity in the Mediterranean Mussel, *Donax trunculus*

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The toxic effects of heavy metals on organisms have recieved considerable scientific interest in the last decade. Heavy metal ions are waste products of many industrial processes and in many places are released into the ocean. They are important causes of environmental pollution.

Heavy metal ions strongly are bound by sulfhydryl groups of proteins (Viarengo, 1985). Sulfhydryl binding changes the structure and enzymatic activities of proteins and causes toxic effects evident at the whole organism level (Hodson 1988). Heavy metal ions like Cd, Cu, Hg, Zn, and Pb in sufficiently high concentrations might kill organisms or cause other adverse effects that changing aquatic community structures. (Martin and Holdich 1986).

The bivalve *Donax trunculus* is found along sandy beaches of the Mediterranean Sea. Haifa Bay on the northern part of the coast of Israel supports a dense population of *D. trunculus*. This area is polluted by effluents from oil refineries, chlor-alkali plants, fertilizer plants and other industrial plants.

Bivalves are known to be heavy metal accumulators. Roth and Hornung (1977) showed that in Haifa bay, the concentration of heavy metals in sediment and in certain organisms (including *D. trunculus*) is higher than in an unpolluted area. El-Rayis (1986) pointed out that *D. trunculus* in relation to other marine organisms is a good accumulator of Cd.

The aim of the present study was to examine the effects of different concentrations of each of five heavy metal ions on the activity of four enzymes in *D. trunculus*. As it is known that heavy metals inhibit the activity of a wide range of enzymes (Dixon and Webb 1964), we chose representative examples of dehydrogenases (lactate and malate dehydrogenases), respiratory enzyme (cytochrome oxidase) and digestive enzyme (α-amylase). The acute effects of different concentrations of selected metals

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were examined. These concentrations were higher than those found usually in the locality where the animals occur (Roth and Hornung 1977), but might be encountered during a given event of pollution.

MATERIALS AND METHODS

Specimens of *Donax trunculus* were collected at Hof Haargaman near Akko (Acre) and kept at $20\pm1^{o}C$ in aquarium with circulated sea water. Its bottom was covered with 5cm. layer of carbonate gravel, on top of which a layer of sand, collected in Akko, was placed.

Five specimens of *D. trunculus* (shell lengh 30mm, wt 0.14g) were placed in a 1 L beaker with 0.5 L of aerated sea water and were exposed seperately to elevated concentrations (given in table 1) of Hg, Zn, Cu, Cd or Pb (added as cloride salts). The animals were kept in the solution for 24h at $20\pm1^{o}C$. Enzyme activity was estimated and recorded at the end of that period. Each experiment was repeated 5 times.

The crystalline styles of these animals were removed and used for the determination of amylase activity. The remainder of the body was used for the determination of the activity of lactate dehydrogenase, malate dehydrogenase and cytochrome oxidase. Enzyme activity was related to 1 mg of protein estimated by the folin phenol reagent (Lowry et al. 1951).

The crystalline styles of 5 specimens were removed pulled together and homogenized at $4^{o}C$ in 2 ml of 20mM phosphate buffer, pH 6.9, containing 6.7mM NaCl. The suspension was then exposed to an ultrasonic vibrator for 30 sec and centrifugation at 15000xg for 5 min. α-amylase activity was determined by the Bernfeld (1951) method and was expressed in terms of mg glucose released in 8 min by 1 mg of protein. The liberated glucose was measured by 2,5 dinitrosalicylate reagent and was determined spectrophotometrically at 540 nm. The reaction mixture contained 0.1 ml of crystalline style extract; 0.4ml of phosphate buffer and 0.5 ml of 1% soluble starch.

Bodies of 5 specimens were removed and homogenized at $4^{o}C$ in 5ml of distilled water. The suspension was then centrifuged at 15000xg for 5 min. The supernatant was used for the enzyme assay.

Lactate dehydrogenase activity was determined as described by Kornberg (1955). The reaction mixture contained 0.1 ml of pyruvate 0.02M; 0.1 ml of NADH (0.5mg/ml); 0.2 ml of tris buffer 0.5M pH 7.5 and 0.5ml homogenate. The decrease in pyruvate concentration after 30 min was determined spectrophotometrically at 540nm by 2,4 dinitrophenyl-hydrazine reagent (Friedemann 1957).

Malate dehydrogenase activity was estimated as described by Roodyn et al.(1962) by following the reduction of NAD by malate

at 340 nm. The reaction mixture contained 2 ml of 0.1M glycine-NaOH buffer pH9.9; 0.3 ml of 0.18M sodium-l-malate; 0.3 ml of NAD (4.5 mg/ml). The reaction was started by the addition of o.1 ml of the body extract (1 volume of the suspension was pretreated with 10 volumes of 0.2% triton X 100 in 0.3M sucrose for 30 min at $0^{o}C$ before assay).

Cytochrome oxidase was measured by following the oxidation of reduced cytochrome C at 550 nm, as described by Freeman (1965). The results are expressed by activity units. An activity unit is defined as oxidizing cytochrome C at the rate of 1 μM per minute, per 1mg protein. The reaction mixture contained 2.6 ml of 17μM reduced cytochrome C in 0.03M phosphate buffer pH7.4 and 0.1 ml of the body extract. The examined suspension was the same as that prepared for malate dehydrogenase estimation.

RESULTS AND DISCUSSION

Our results (Table 1) demonstrate a wide variation in the acute toxicities of different metals. Enzymes were affected differently in the presence of the ions studied. α-amylase which is an important digestive enzyme of bivalves was only slightly affected by any ion.

α-amylase acts primarily in the stomach which also contains organic particles which are able to bind metal ions (Simkiss and Taylor 1981) and cause them to be unavailable for interaction with the enzymes. Wolfe (1970) found that in *Crassostrea virginica* nearly all the Zn was bound to soluble high molecular weight protein or to structural protein.

The other enzymes tested by us were more sensitive then α-amylase to heavy metals ions.

At the test concentrations of 0.05 and 0.1ppm, most of the metals we tested did not influence the enzymes studied. The only metal ion that caused an effect at 0.1ppm was Cd, which slightly inhibited the activity of cytochrome oxidase and lactate dehydrogenase. It is apparent that the respiratory enzymes cytochrome oxidase and lactate dehydrogenase were the most sensitive to the heavy metal ions studied.

Cd and Hg were found to be high inhibitors of lactate dehydrogenase and cytochrome oxidase of *Donax* at 1 and 10ppm. With Pb and Cu, even at the high concentrations, there was only a slight inhibition in the activity of these two respiratory enzymes. About 50% of the activity of cytochrome oxidase was inhibited by 10ppm Zn, and it was hardly effective on the lactate dehydrogenase.

Earnshow et al. (1986) pointed out that the motility of mussel's spermatozoa was slowed down by heavy metals, which is explained by these authors as a consequence of respiratory inhibition by these ions. Crespo and Sala (1986) showed that chloride cell

Table 1. Effect of heavy metals on enzyme activity. (Results are expressed in: μmole pyrovate/ mg protein for lactate dehydrogenase; μmoleNADH/mg protein for malate dehydrogenase; units/mg protein for cytocrome oxidase and mg glucose/mg protein for α-amylase.)

Metal	Concentration ppm	Lactate dehydrogenase	Malate dehydrogenase	Cytocrome oxidase	α-amylase
Control		13.17±0.32	2.07±0.09	0.170±0.005	1.82±0.03
Cd	10	2.63±0.28	1.60±0.02	0.060±0.002	1.62±0.07
	1	6.20±0.35	2.00±0.06	0.090±0.008	1.75±0.05
	0.1	9.15±0.25	2.10±0.05	0.140±0.007	1.80±0.06
	0.05	11.95±0.31	2.10±0.09	0.140±0.005	1.80±0.04
Hg	10	5.35±0.28	0.53±0.02	0.057±0.007	1.81±0.08
	1	10.49±0.33	1.47±0.08	0.110±0.004	1.80±0.04
	0.1	12.37±0.29	1.83±0.06	0.160±0.006	1.80±0.05
	0.05	12.85±0.32	2.20±0.05	0.180±0.008	1.90±0.03
Zn	10	12.95±0.32	1.06±0.03	0.090±0.004	1.65±0.06
	1	13.50±0.36	1.58±0.05	0.180±0.005	1.70±0.05
	0.1	13.45±0.42	1.98±0.05	0.182±0.003	1.80±0.04
	0.05	13.15±0.35	2.02±0.03	0.175±0.007	1.79±0.06
Cu	10	12.94±0.41	1.48±0.02	0.150±0.007	1.67±0.07
	1	13.50±0.35	1.70±0.06	0.180±0.004	1.71±0.09
	0.1	13.23±0.37	2.05±0.07	0.180±0.006	1.85±0.07
	0.05	13.02±0.29	1.97±0.08	0.178±0.008	1.80±0.04
Pb	10	11.42±0.43	2.03±0.08	0.130±0.004	1.70±0.06
	1	13.00±0.49	2.04±0.05	0.160±0.007	1.85±0.09
	0.1	13.00±0.38	2.05±0.07	0.175±0.005	1.80±0.06
	0.05	12.87±0.42	2.10±0.07	0.180±0.007	1.80±0.09

mitochondria is a target of Zn contamination, and treatment with Zn caused ultrastructural alteration of the chloride cell of the dogfish *Scyliorhinus canicula*. They suggested that these alterations are related to an impairment of oxidative phosphorylation.

The activity of malate dehydrogenase was most strongly inhibited by Hg. The inhibition increased from 30% at 1ppm to 70% at 10ppm. This enzyme was partially inhibited by the other ions, except Pb which did not affect the activity.

Brown and Newell (1972), working with Zn and Cu, reported that a solution of 500 ppm of Cu exerted an inhibitory effect on the oxygen consumption of the whole animal *Mytilus edulis*. Cu, but not Zn, exerted an inhibitory effect on gill-tissue respiration. Cronin and Flemer (1967) pointed out that chemicals which slow down physiological processes may result in slow down of energy transfer in the community and change the structure of it.

The results presented here show that inhibition of enzyme activity was exerted by heavy metals at concentrations higher than those found in the area where the animals were collected (Roth and Hurnung 1977). However constant exposure to heavy metals at low concentration can be different from acute toxicity of the pollutant. Brown (1976) claimed that although a sublethal dose does not kill the animal within a short time, it affects the ability of the organisms to respond to the environment and likely will shorten its life. Prolonged exposure result also in bioaccumulation of the contaminant or genetic changes in the population (Lavie and Nevo 1986).

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REFERENCES

Bernfeld P (1951) Enzymes of starch degradation and synthesis. In: Nord FF (ed) Advances in Enzymology, vol 12. Interscience, New-York. pp 379-428

Brown BE, Newell RC (1972) The effect of copper and zinc on the metabolism of the mussel *Mytilus edulis* Mar Biol 16:108-118

Brown GW (1976) Biochemical aspects of detoxification in the marine environment. In: Malins DC Sargent JR (eds) Biochemical and Biophysical Perspectives in Marine Biology, vol 3. Academic Press, New York. prp 319-406

Crespo S, Sala R (1986) Chloride cell mitochondria are target organelles in acute zinc contamination. Mar Poll Bull 17:329-331

Cronin LE, Flemer DA (1967) Energy transfer and pollution. In: Olson TA and Burgess FJ (eds) Pollution and marine ecology. Interscience, New York. pp 171-183

Dixon M, Webb EC (1964) Enzyme inhibitors. In: Enzymes. London: Longmans and Green. pp 345-347

Earnshow MJ, Wilson S, Akaberly HB, Butler RD, Marriott KRM (1986) The action of heavy metals on the gametes of the marine mussel *Mytilus edulis* III. The effect of applied copper and zinc on sperm motility in relation to ultrastructure damage and intracellular localisation. Mar Environ Res 20: 261-278

El-Rayis OAM (1986) Bioaccumulation of cadmium in plankton, bivalves, crustacea and in six different fish species from Mex Bay, west of Alexandria. FAO Fisheries Report 334 (suppl.). pp 50-56

Freeman KB (1965) Preparation and properties of mitochondria from Krebs II mouse ascites tumour cells. Biochem J 94:494-499

Friedemann TE (1957) Determination of keto acid. In: Clowick SP, Kaplan NO (eds) Method Enzymology , vol.3. Academic Press, New York. pp 414-418

Hodson PV (1988) The effect of metal metabolism on uptake, disposition and toxicity in fish. Aquat Toxicol 11:3-18

Kornberg A (1955) Lactic dehydrogenase in mussel. In: Clowick SP, Kaplan NO (eds) Method Enzymology, vol. 1. Academic Press, New York. pp 441

Lavie B, Nevo E (1986) The interaction effects of cadmium and mercury pollution on allozyme polymorphisms in the marine gastropod *Cerithium scabridum*. Mar Poll Bull 17:21-23

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265-275

Martin TR, Holdich DM (1986) The acute lethal toxicty of heavy metals to peracarid crustaceans (with particular reference to fresh-water asellids and gammarids). Wat Res 20:1137-1147

Murphy CB, Spiegel SJ (1982) Bioaccumulation and toxicity of heavy metals and related trace elements. J Water Pollut Control Fed 54:849-854

Roodyn DB, Suttie JW, Work TS (1962) Rate of incorporation in vitro of radioactive amino acids into soluble proteins in the mitochondrial fraction including catalase, malic dehydrogenase and cytochrome C. Biochem J 83:29-38

Roth I, Hornung H (1977) Heavy metal concentrations in water, sediments and fish from Mediterranean coastal area, Israel. Envir Sci & Tech 11:265-26

Simkiss K, Taylor M (1981) Cellular mechanisms of metal ion detoxification and some new indices of pollution. Aquat Toxicol 1:279-290

Viarengo A (1985) Biochemical effects of trace metals. Mar Poll 16:153-15

Wolfe DA (1970) Levels of stable Zn and ^{65}Zn in *Crassostrea virginica* from North Carolina. J Fish Res Bd 27:47-57

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