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Individual and Combined Toxicity of Manganese and Molybdenum to Mussel, *Mytilus edulis*, Larvae

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During toxicity testing of mine tailings from the proposed Quartz Hill molybdenum mine, which will be situated near Ketchikan, Alaska, the metals molybdenum (Mo) and manganese (Mn) showed evidence of leaching from the tailings (Mitchell et al. 1985). In order to determine whether these metals could be responsible for toxicities observed by Mitchell et al. (1985), Mytilus edulis mussel larvae bioassays (ASTM 1984) were conducted with these metals, both individually and in combination. The results of the bioassays showed that these metals could account for only a very small proportion of the tailings toxicity (Mitchell DG, Vigers GA, Morgan JD, Nix PG, Chapman PM, E.V.S. Consultants Ltd., North Vancouver, B.C., Canada, unpublished report). Since a literature review indicated that there was a paucity of published data on the toxicities of these two metals, our data are reported herein.

MATERIALS AND METHODS

Adult bay mussel stocks (M. edulis) were collected from Woodlands, Indian Arm, British Columbia. Mussels were placed in continuous-flow conditioning trays to permit gonadal maturation, and were thermally conditioned for 1-4 weeks in unfiltered sea water at 15±1°C. Prior to spawning, 40 mussels were scraped free of adherent organisms and stored moist at 4°C for 22 h.

Spawning was induced by placing the chilled mussels in individual Pyrex dishes containing 250 mL of 5 µm filtered, UV-sterilized seawater (26 ppt salinity) at 22°C. Female and male mussels began to produce gametes after about 30 min and were allowed to spawn for 30-60 min before being removed from the spawning dishes. Fertilization was accomplished within 1 h of spawning initiation by combining eggs and sperm in a 1 L Nalgene beaker. The fertilized eggs were then washed through a 250 µm Nitex screen to remove excess gonadal material and suspended in 2 L of treated seawater at incubating temperature. The embryos were kept suspended prior to testing by frequent agitation with a perforated plunger. When microscopic examination of fertilized eggs revealed the formation of polar bodies, egg density was determined from triplicate counts of the number of eggs in 1.0 mL samples of a 1:99 dilution of homogeneous egg suspension.

Testing was conducted in clean, distilled water-rinsed 1 L polyethylene bottles; final test solution volume was 750 mL per container. Stock solutions of Mn were prepared at 3000 mg/L in distilled water using $MnSO_4$,

and for Mo these were prepared at 4000 mg/L using $(NH_4)_6Mo_7O_{24}\ 4H_2O$, the ammonia being driven off by rapid boiling. A 1:1 mixture of Mn and Mo was also tested to detect possible synergistic/antagonistic effects of the two metals. Test concentrations were confirmed by direct flame atomic absorption spectrophotometry, using a Perkin-Elmer Model 2380 with HGA-400 Graphite Furnace and MHS-20 Hydride Generator. Instrument calibration was performed using a certified reference standard for seawater (NASS-1) obtained from the National Research Council of Canada. Instrument settings were optimized in accordance with the manufacturers' recommendations and anticipated concentrations.

Test concentrations were prepared by volumetric dilution of stock solutions of metals with treated seawater. Bioassay concentrations were determined following results of range-finder tests. Twelve concentrations of each metal were run in duplicate. Clean seawater controls were also prepared and run concurrently. The test concentrations were well mixed by vigorous agitation for 5 sec and were allowed to settle at incubation temperature for 1 h prior to adding the embryos. No additional agitation was provided after inoculation.

Within 2 h of fertilization, each container was inoculated by automatic pipette with developing mussel embryos to give a concentration of about 15 per mL. The containers were covered and air-incubated for 48 h at $19\pm1^{\circ}C$. Test vessels were not aerated during the bioassay. After 48 h, larvae were concentrated using a 38 µm sieve, washed into a 100 mL graduated cylinder, quantitatively transferred to 8 mL screw-cap vials and preserved with 5% buffered formalin. Preserved samples were examined in Sedgewick-Rafter cells under 100X magnification. As bivalve larvae sink after preservation (ASTM 1984) half of the water was discarded from the vials before examining the residual volume containing the larvae.

Normal and abnormal prodissoconch I larvae were enumerated to determine percent survival and percent abnormality. Percent survival was determined as the number of larvae surviving in each test container relative to the seawater control. Larvae which failed to transform to the fully shelled, straight hinged, 'D' shaped prodissoconch I stage were considered abnormal. The bioassay results were analyzed to express abnormal development in terms of a 48-h EC50 by computational methods (Binomial Probability, Moving Average or Probit analysis) and confirmed by graphical log-probit analysis (APHA 1980).

Water quality parameters were monitored at bioassay initiation and termination. The tests were conducted at 26 ppt salinity, pH 8.4 ± 0.2 and dissolved oxygen levels greater than 70% saturation.

RESULTS AND DISCUSSION

Salinity, pH and dissolved oxygen values determined at the beginning and termination of the bioassays remained within acceptable levels as defined by ASTM (1984).

The results of the mussel larvae bioassays are presented in Table 1. Mussel larvae response was monitored as mean percent relative survival (compared to controls), and mean percent abnormal larvae. The latter provided more

Table 1. Mussel Larvae Bioassay Results

Nominal Concentration (mg/L)	Manganese Mean Values[a]			Molybdenum Mean Values[a]			Manganese: Molybdenum Mean Values[a]		
	Number of Larvae	Percent Abnormal	% Relative Survival[b]	Number of Larvae	Percent Abnormal	% Relative Survival[b]	Number of Larvae	Percent Abnormal	Relative Survival[b]
560	0	-	0	1	100.0	1	0	-	0
320	1	100.0	1	13	76.9	7	3	100.0	2
180	34	86.6	18	20	55.0	11	67	70.9	36
130	55	70.6	29	34	39.7	18	29	53.4	15
100	45	76.7	24	57	38.1	30	73	43.4	39
75	108	67.6	57	62	26.6	33	57	49.1	30
56	61	56.2	32	94	23.9	50	71	30.5	38
32	73	53.4	39	102	21.6	54	123	22.8	65
18	80	25.6	43	85	16.6	45	88	25.7	47
10	118	20.0	63	59	15.4	31	89	22.5	47
3.2	153	14.1	81	109	13.8	58	119	12.2	63
1	190	10.3	101	97	9.3	52	248	8.5	132
Seawater Control	188	7.6	100	188	7.6	100	188	7.6	100

[a] n=2

[b] In terms of mean seawater control survivals which are assigned a survival value of 100%, following standard procedures (ASTM 1984).

consistent data and was used to determine 48-h EC50 values although the survival values generally agreed with the data on abnormalities. The mussel embryos used in the bioassays exhibited an abnormal larvae value of 7.6% in the seawater control which was well within the 10% abnormality standard suggested as an acceptable value for bivalve larvae bioassay controls (ASTM 1984). The 48-h EC50 values for mussel larvae exposed to Mn, Mo and a 1:1 mixture of these metals are shown in Table 2.

Table 2. Mussel Larvae 48-h EC50 Valves

Toxicant	48-h EC50 (mg/L)	95% Confidence Limits
Mn	30	20-46
Mo	147	127-169
Mn/Mo*	100	61-128

*1:1 mixture by weight

The acute lethal value (96-h LC50) of manganese to rainbow trout was reported by Lewis et al. (1979) to be 16.0 mg/L. A 48-h LC50 for manganese of 9.8 mg/L was reported by Bengtsson (1978) for Daphnia magna. These freshwater values are comparable to the 48-h EC50 for mussel larvae of 30 mg/L. The apparent lower toxicity of the metal in seawater is consistent with the decreased toxicity of most metals in seawater (Chapman et al. 1982).

No acute lethal value of molybdenum was found in the literature. However, Goettl and Davies (1975) reported that molybdenum concentrations up to 18.5 mg/L had no adverse effects in long-term bioassays with rainbow trout eggs and juveniles, which is consistent with our 48-h EC50 value of 147 mg/L for mussel larvae.

Based on our EC50 values, a 1:1 mixture of Mn and Mo has a theoretical 48-h EC50 value of 88.5 mg/L (50% of Mn 48-h EC50 + 50% of Mo 48-h EC50). The actual 48-h EC50 of 100 mg/L was not significantly different from this, and the acute lethal toxicity of a mixture of manganese and molybdenum can therefore be described as additive (Sprague 1970).

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