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# EFFECTS OF HEAVY METALS (CADMIUM, COPPER, AND MERCURY) ON REPRODUCTION, GROWTH, AND SURVIVAL OF BRINE SHRIMP (ARTEMIA SALINA) FROM THE GREAT SALT LAKE

by

Karl A. Gebhardt

A thesis submitted in partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE

in

Civil and Environmental Engineering

Approved:

UTAH STATE UNIVERSITY Logan, Utah

37842

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Of course without her I would not be here.

Kad a. gebhardt

## TABLE OF CONTENTS

								Page
ACKNOWLEDGMENTS								ii
LIST OF TABLES								iii
LIST OF FIGURES								v
ABSTRACT								ix
INTRODUCTION							•	1
LITERATURE REVIEW								3
Great Salt Lake								3
Great Salt Lake description Lake biology and ecology .								3 5
Bioassay	•	•	•		•	•	•	9
	•	•	•	•	•	•	•	
Toxicology		:	:	:	:	:	:	9
Heavy Metals								12
Mercury in the environment								13
Mercury toxicology								16
Cadmium in the environment.								19
Cadmium toxicology							•	20
Copper in the environment								21
Copper toxicology Analysis of cadmium, copper,								22
	an	d m	erc	ury	٠			25
Brine Shrimp	٠	•	٠	٠	•	•	•	26
Biology and ecology								26
Morphology								29
			-			-		
METHODS AND PROCEDURES	•		•					34
Experimental Procedure								34
Introduction								34
Sampling								34
	-	-	-	-	-			34

## TABLE OF CONTENTS (continued)

																		Page
		Algae c	ultur	es														36
		Shrimp (	cultu	ire														36
		Prepara	tion	and	ha	ndl	ing	of	me	tal	so	lut	ions	3				39
	Bioas	say Pro	cedur	es														40
		Acute b	ioass	av														40
		Growth 1	cate	and	ch	ron	ic :	toxi	ici	tv 1	oio	355	avs					42
		Hatching											•					43
RESU	LTS .		έ.															44
	Acute	Toxicit	у.															44
		Cadmium																44
				-	•	•	•		•			•	•		•	•	•	48
		Copper .										•	•		•			54
		Mercury	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	54
	Growt	h and Re	prod	ucti	on									•	٠			60
		Effects Effects																60
		ductio					•			•								65
	Emerg	ence and	Hat	chin	ıg													65
		Emergenc									s c	of c	adm	iu	n			
		copper	, or	mer	cur	У	•	•	•	•	٠	٠	•	•	•	•		65
DISC	JSSION	, CONCLU	SIONS	S, A	ND	REC	OMM	END	ATI	ONS								70
	Discus	ssion .	Ĭ.															70
	(	Cadmium																70
	(	Copper .							Ī							•		73
		Mercury			:					:	•	•	•	•	•	•	•	75
		Implicat									ovi	· cit	inc	ir			•	
		the Gr												•				77
	Conclu	sions .																80

## TABLE OF CONTENTS (continued)

															Page
	Re	cc	omn	nei	nda	tic	ns							٠	81
REFE	EREN	CE	S	CI	TE	D									82
APPE	ENDI	X	Α												88
APPE	ENDI	X	В												111
VITA	A														114

## LIST OF TABLES

Table		Page
1.	Metal concentrations in the Bear, Weber, and Jordan Rivers	6
2.	Mineral composition of the north arm of Great Salt Lake (Post, 1975).	7
3.	Mineral composition of the south arm of Great Salt Lake	8
4.	Acute toxicity of mercury to various marine organisms .	17
5.	Acute toxicity of copper to various marine organisms	24
6.	Simulated Great Salt Lake water, north area	37
7.	Algae culture media	38
8.	Summary of cadmium toxicity to adult brine shrimp, showing time to mortality, statistical analysis at various	
	concentrations	45
9.	Statistical analysis testing for equal means among pairs of acute cadmium tests using adult brine shrimp	46
10.	Summary of copper toxicity to adult brine shrimp, showing time to mortality, statistical analysis at various concentrations	49
11.	Copper analysis of media containing precipitate	51
12.	Statistical analysis testing for equal means among pairs of acute copper tests using adult brine shrimp	52
13.	Comparison of copper time to mortalities in salinities of 150 grams per liter total dissolved solids (TDS) and 320 grams per liter total dissolved solids	53
14.	Summary of mercury toxicity to adult brine shrimp, showing time to mortality, statistical analysis at various	
	concentrations	55

## LIST OF TABLES (continued)

Table			Page
15.	Statistical analysis testing for equal means among pairs of acute mercury tests using adult brine shrimp		57
16.	Comparison of mercury time to mortalities in salinities of 150 grams per liter total dissolved solids (TDS) and		
	320 grams per liter total dissolved solids	•	58
17.	Toxicity on adult brine shrimp from algal cells containing mercury		59
18.	Time until first observed mating and production of nauplii in cadmium, copper, or mercury. This data is a continuation of growth experiments		66
19.	Minimum concentrations (in millimoles per liter and milligrams per liter) of cadmium, copper, and mercury producing 100 percent mortality among adult brine shrimp at 150 and 170 grams per liter total dissolved solids.		71
			, _
20.	Concentrations of cadmium, copper, and mercury in the Great Salt Lake and experimental concentrations producing toxic effects		79
21.	Data summary: Acute toxicity from mercury as mercuric chloride; 150, 170 grams per liter total solids		89
22.	Data summary: Acute toxicity from cadmium as cadmium sul fate; 150, 170 grams per liter total solids		90
23.	Data summary: Acute toxicity from copper as copper chloride; 150, 170 grams per liter total solids		90
24.	Acute copper toxicity data, 320 grams per liter total solids		91
25.	Acute mercury toxicity data, 320 grams per liter total solids		92
26.	Methyl mercuric chloride compared to mercuric chloride in distilled water		93

## LIST OF TABLES (continued)

Table	Pag	е
27.	Postmortem analysis of brine shrimp for mercury content	4
28.	Copper and cadmium excystment data 9	5
29.	Data for mercury hatch experiment 9	6
30.	Acute copper toxicity data 9	7
31.	Acute cadmium toxicity data	1
32.	Acute mercury toxicity data	4
33.	Coefficient of variation for metal analysis techniques	
	used in Great Salt Lake and artificial brines 11:	2

## LIST OF FIGURES

'igur	ne	1	Page
1.	Map of the Great Salt Lake showing relative size of the freshwater inflows and possible heavy metal sampling points		4
2.	Brine shrimp nauplius, 1 - 2 days old, third instar, 0.5 - 1 millimeters long		31
3.	Brine shrimp nauplius, 5 - 6 days old, seventh instar, 1.6 - 1.9 millimeters long		32
4.	Juvenile brine shrimp, 10 days old, eleventh or twelfth instar, 2.4 - 2.7 millimeters long		33
5.	Map of the Great Salt Lake showing location of inflows and sampling points		35
6.	Relationship between cadmium concentration and time to 50 percent mortality among adult brine shrimp		47
7.	Relationship between copper concentration and time to 50 percent mortality among adult brine shrimp		50
8.	Relationship between mercury concentration and time to 50 percent mortality among adult brine shrimp		56
9.	Postmortem mercury content of brine shrimp used in toxicity experiments		61
10.	Brine shrimp growth in copper		62
11.	Brine shrimp growth in mercury		63
12.	Brine shrimp growth in cadmium		64
13.	Hatching of brine shrimp eggs in solutions of cadmium sulfate		67
L4.	Hatching of brine shrimp eggs in solutions of cupric chloride		68
L5.	Hatching of brine shrimp eggs in solutions of mercuric chloride		69

#### ABSTRACT

Effects of Heavy Metals (Cadmium, Copper, and Mercury) on

Reproduction, Growth, and Survival of Brine Shrimp

(Artemia salina) from the Great Salt Lake

by

Karl A. Gebhardt

Utah State University, 1975

Major Professor: Dr. Donald B. Porcella Department: Civil and Environmental Engineering

The purpose of this paper is to report findings concerning the effects of cadmium, copper, and mercury on the brine shrimp, Artemia salina, of the Great Salt Lake. Metal toxicity was observed in relation to acute susceptibility, growth, reproduction, and hatching of the brine shrimp.

Heavy metal concentrations such as cadmium, copper, and mercury are known to be considerably higher in the Great Salt Lake than those in both freshwater and seawater. No published study has been concerned with heavy metal effects on organisms in salinities greater than seawater (35 grams per liter total dissolved solids). The experiments reported in this paper were carried out in salinities approximating the Great Salt Lake (150-320 grams per liter total dissolved solids).

Results of this study indicate that cadmium, copper, and mercury toxicities to the brine shrimp may not be comparable at varying salinities. Findings of acute toxicity experiments were compared to other heavy metal studies on marine organisms. The brine shrimp was found to be very resistant to cadmium and copper poisoning and moderately resistant to mercury.

Neither cadmium nor copper inhibited hatching of the brine shrimp eggs although mercury caused severe inhibition at concentrations of 0.3 milligrams per liter. Only cadmium at concentrations between 1.0 and 33 milligrams per liter significantly suppressed growth rate and reproduction. Mercury and copper were not found to affect growth and reproduction below concentrations causing acute poisoning.

Mercury was found to be the most lethal to the adult brine shrimp with a range of times to 50 percent mortality from 126 to 8.5 hours at mercury concentrations of 0.01 to 100 milligrams per liter respectively. Copper caused mortalities at concentrations of 1 to 67 milligrams per liter with respective times to 50 percent mortality of 124 and 12 hours. Copper was shown to precipitate out at concentrations near 12 milligrams per liter. Cadmium was found to be the least lethal with a range of times to 50 percent mortality from 94 to 320 hours with respective cadmium concentrations of 100 and 3.3 milligrams per liter.

#### INTRODUCTION

The Great Salt Lake is a relatively simple ecosystem and is very susceptible to change brought about by man. The purpose of this study was to determine the sensitivity of the brine shrimp <a href="Artemia salina">Artemia salina</a> of the Great Salt Lake to the inorganic forms of the metals cadmium, copper, and mercury.

Past studies (Corner et al., 1956, 1957, 1958; Brown and Ahsanullah, 1971; Saliba and Ahsanullah, 1973; Wisely and Blick, 1966) have shown that the brine shrimp is sensitive to mercury, cadmium, and copper with mercury being the most toxic and cadmium the least. Current published aquatic studies concerning heavy metals have been concerned with freshwater and marine environments.

This study focuses on salinities much greater than that of seawater, since the Great Salt Lake ranges in salinity from 120 g/l to 330 g/l total dissolved solids.

The Great Salt Lake is a sink to most liquid wastes discharged near the Wasatch Front. Many industries such as mining operations are sources for many heavy metals as are the Weber, Bear, and Jordan Rivers which empty into the Great Salt Lake.

Because of the vulnerability of the lake's ecosystem and the potential threat due to heavy metals, a study to evaluate the brine shrimp's susceptibility is needed.

The objectives of this study were:

- 1. To bioassay adult brine shrimp <u>Artemia salina</u> from the Great Salt Lake for acute susceptibility to mercuric chloride, cupric chloride, cadmium sulfate, also some comparative work was done with an organo-mercurial, methylmercuric chloride.
- 2. To determine chronic effects on reproduction and growth by the compounds mercuric chloride, cupric chloride, and cadmium sulfate on brine shrimp from the Great Salt Lake.
- 3. To determine detrimental effects on hatching of  $\underline{A}$ .  $\underline{\text{salina}}$  eggs collected from the Great Salt Lake by mercuric chloride, cupric chloride, and cadmium sulfate.
- 4. To speculate on criteria concerning concentrations of copper, cadmium, and mercury salts that will be safe for the brine shrimp in the Great Salt Lake.

#### LITERATURE REVIEW

#### Great Salt Lake

#### Great Salt Lake description

The Great Salt Lake is the largest "sink" type of lake in the Western Hemisphere and is the remnant of ancient Lake Bonneville. The lake covers 5180 km² (over 2000 square miles) and has a maximum depth of about 10.7 m (35 feet). In 1957-1959 a railroad causeway was constructed connecting Promontory Point and Lakeside. The causeway prevented the "normal" circulation in the lake. Since 95 percent of the freshwater inflow is on the southern side of the causeway, there is now a great salinity discontinuity at the causeway. North of the railroad causeway the salinity is near saturation being on the order of 320 g/1 TDS (grams per liter total dissolved solids). South of the causeway the brine is fresher being between 120 and 170 g/1 total dissolved solids.

Three major inflows, the Bear, the Weber, and the Jordan rivers, transport 90 percent of the freshwater and 80 percent of the solids to the lake (Figure 1). Possible contributors of cadmium, copper, and mercury in the lake are natural weathering and industries which may use the rivers directly or indirectly as disposal sites. More specifically, electroplating industries may be the largest contributors of cadmium. There are at least ten such industries located in the Great Salt Lake drainage areas.

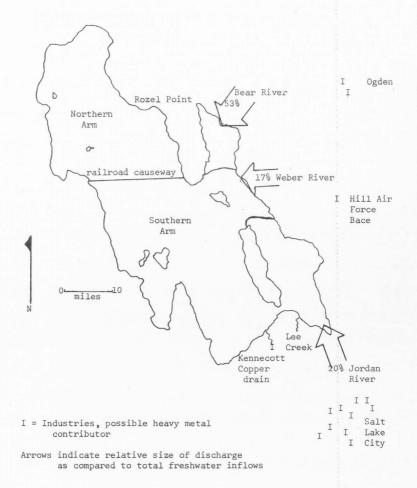


Figure 1. Map of the Great Salt Lake showing relative size of the freshwater inflows and possible heavy metal sampling points.

Copper can be expected from such industries as the Kennecott
Copper smelting operations which are located near the lake. Kennecott
Copper is permitted to discharge approximately 147 pounds copper/day
through its treatment facility and nearly 37 pounds copper/day from its
tailings pond (Environmental Protection Agency, 1975). Other significant copper inputs are as a result of runoff due to natural weathering. The largest source of mercury is due to natural weathering since
there are many known geologic sites within the Great Salt Lake
drainage area having high concentrations of mercury-containing
minerals. The metal concentrations of the Bear, Weber, and Jordan
Rivers, as well as other parameters, are given in Table 1.

#### Lake biology and ecology

The Great Salt Lake is now, due to the causeway, considered as two separate bodies because of the salinity difference between the north and south arms (Wirick and Gillespie, 1972). The lake is a harsh environment because of the salinity and as a result very few types of organisms have adapted to the environment. The chemical composition of the northern arm is given in Table 2 and the southern arm in Table 3. The temperature ranges from below -5 C to 40 C in the shallowist margins of the lake. The ecosystem in the southern arm has been modeled by Stephens and Gillespie (1972). They found two basic functioning systems: the first consisted of algae <u>Dunaliella viridis</u> as primary producer, the brine shrimp <u>A. salina</u> as primary consumer, and a number of protozoa and algae; the second is a benthic system composed of the blue-green algae <u>Coccochloris elebans</u>, detritus and larvae of the brine fly <u>Ephydra</u> spp. The two systems are linked by

Table 1. Metal concentrations in the Bear, Weber, and Jordan Rivers

Location	Date	Discharge CFS	Total Cadmium mg/l	Total Copper mg/l	Total Mercury mg/l
Bear River					
at Corinne	10-74	1370	10	10	0
Jordan River					
2100 South	10-75	NA	10	40	0
	10-75	·NA	10	10	0.1
	7-75	NA.	10	60	0.1
	4-75	117	10	50	NA
	1-75	97	10	30	0.1
Red Butte	9-75	NA	10	10	0.0
	6-75	20	10	10	0.1
Magna	8-75	NA	0	8	0
Lee Creek	7-75	NA	4	200	0.0
Magna	4-75	39	3	190	0.0
8	1-75	8.8	8	49	0.0
	12-74	NA	6	300	0.1
	10-74	2.2	1	360	2.0
Kennecott Drain	7-75	NA	2	10	0.1
	4-75	114	. 8	50	0.1
	1-75	100	10	50	0.0
	12-74	NA	18	42	0.1
	11-74	107	0	29	0.1
Weber, Middle	10-72	NA	NA	5	0.1
Fork	10-72	NA	NA	8	0.1
Weber, Plain City	10-72	NA	10	20	NA
CILY					

NA = Not Available
Data obtained from U.S. Geological Survey, open files, Salt Lake City,
Utah

Table  $^2\cdot$  Mineral composition of the north arm of Great Salt Lake (Post, 1975).

Chemical	Concentration mg/l	
Boron	13	
Calcium	312	
Magnesium	11,124	
Lithium	66	
Potassium	6,690	
Sodium	105,386	
Sulfate	27,000	
Chloride	181,000	
Fluoride	16	
Carbonate	270	
Bicarbonate	454	
Oxygen	0.1-1.7	
Nitrate and nitrite	0 µg/l	
Ammonia	10 µg/l	
Total phosphate	500 µg/l	
Total solids	332,480 mg/l	
Specific gravity (field) pH	1.220	

Aluminum, Barium, Iron, Manganese, Silica, and Vanadium > 1 mg/l

Antimony, Arsenic, Beryllium, Cadmium, Chromium, Cobalt, Copper, Lead, Mercury, Molybdenum, Nickel, Silver Thallium, Titanium, Tungsten, Zinc, Bromine, and Selenium < 1 mg/l

Data from August-November 1974 samples except Calcium and Lithium were estimated from other sources.

Table 3. Mineral composition of the south arm of Great Salt Lake

Bulk Components	Concentration mg/l
Sodium	55,000
Chloride	78,000
Magnesium	6,200
Calcium	150
Potassium	3,200
Lithium	28
Boron	20
	Total <u>142,598</u>

Data estimated from Utah Geological Survey files on Great Salt Lake.

the brine fly larvae feeding on detritus and the brine shrimp feeding on its own fecal pellets and blue-green algae. This author would tend to diminish the importance of the fecal pellet feeding. The ecosystem of the northern arm has not been modeled, but it probably consists of the algae <u>Dunaliella salina</u>, the brine shrimp, and the brine fly <u>Ephydra spp.</u> with a few protozoans, bacteria, and at least three viruses (Post, 1975).

Both the brine shrimp and brine fly live in the lake even though its harsh environment prevents other types of animal life. Birds have been observed feeding on the brine fly and brine shrimp from the southern arm of the Great Salt Lake. Other types of biomass removal consist of the brine shrimp harvesting companies that use the lake as a source for both adult brine shrimp and eggs.

## Bioassay

## Toxicology

Toxicity studies are used to evaluate the effects of a suspected residual on an organism. Hassall (1969) reports two distinct types of toxic effects. Acute toxic effects occur shortly after application of a single dose of a poison, and oftentimes the substance can be traced to a specific biochemical effect. Acute toxicity is generally measured in terms of the dose at which 50 percent mortality is observed, commonly seen as  ${\rm LD}_{50}$  (unit toxicant weight/unit body weight).  ${\rm LD}_{50}$  refers to a directly applied dose. When the toxicant cannot be applied topically, then it must be placed in the organisms' immediate environment at known concentrations. In these cases toxicity measurements are

expressed as the concentration at which 50 percent mortality occurs in a known time increment, reported as  $LC_{50}$  in X hours, or the time at which 50 percent mortality occurs at a known concentration ( $LT_{50}$  at X concentration). This type of expression is used in aquatic work. Acute symptoms may be noticed immediately and sometimes will respond to an antidote or removal of the toxicant.

Chronic toxic effects are more difficult to quantify depending on the parameter observed, and result from repeated small, non-lethal doses of toxic material (Hassall, 1969). Sublethal expressions are ED, ET, or EC meaning effective dose, time, or concentration respectively.

For a toxicant to act it first must reach a physiologic site and be able to interfere with some function that is important to the organism. These physiologic sites may be part of the central nervous system, or they may be critical enzyme systems responsible for respiration, membrane transport, etc. In order for a toxicant to reach a critical site of action it oftentimes must penetrate the organism's protective covering (e.g. the chitinous layers found on insects and crustaceans). The ability of a substance to pass this barrier may be dependent on its temperature, liposolubility, volatility, stability, etc. Once inside the organism the toxicant may not be able to act on a critical site of action due to inert storage by indifferent tissues, excretion, detoxification, other internal barriers, or competition with a normal enzyme substrate. If, however, the toxicant does arrive at an important site of action, the organism will react to the interference and symptoms will generally be present (Winteringham, 1969).

The reactions of the organism may be spasms followed by a moribund state and eventually total motor paralysis. The loss of movement of an organism is used as a death criterion in many bioassays with invertebrates.

#### Bioassay methods

Bioassays are controlled tests to evaluate the effects of certain toxic or suspected toxic materials upon the metabolism, growth rate, reproduction, or some other physiologic function of an organism. Bioassays can be used to evaluate the toxicity of effluents, determine quality standards, or establish organism sensitivity to various substances (American Public Health Association, 1971).

In actual bioassays organisms are subjected to various concentrations of the material being tested under adequately controlled conditions. Toxicant concentrations are generally selected so that they have an even logarithmic distribution (American Public Health Association, 1971). When testing chronic effects, the Standard Methods Draft (American Public Health Association, 1975) recommends that the highest concentrations of toxicant used should be that of the  $LC_{50}$  at 96 hours, since functions such as reproduction may be impaired by some toxicants at much lower levels than the acute toxicity values.

Controls are carried out in exactly the same manner as those containing the toxicant to assure valid interpretations of the results. It is advised that no more than 10 percent of the control organisms should die and 90 percent should appear healthy (American Public Health Association, 1971).

The bioassay bases its credibility on the suitability of the control. If the control should be in question, then the entire experiment is subject to criticism. The measurements of toxicity are basically a comparison of the control organisms to the response of the organisms exposed to toxic substances. The measurements should at least be triplicated so statistical analyses can be performed.

#### Heavy Metals

Heavy metals such as zinc, cadmium, copper, chromium, and mercury are used extensively by industry or are byproducts of such processes as electroplating, smelting, refining, etc. In many instances they could present serious problems since they are known to have toxic properties.

The United States Public Health Service (1962) has set limits on various heavy metals in drinking water: cadmium 0.01 ppm, chromium 0.05 ppm, copper 1 ppm, zinc 5 ppm. The Utah State Division of Health has set limits on Class C surface water for selected heavy metals: cadmium 0.01 ppm, chromium 0.05 ppm, copper 1 ppm, and zinc 5 ppm.

Heavy metals in seawater are said to be present in four forms: as part of a living organism, as a colloid, adsorbed on colloidal particles, and in a true ionic solution. In seawater heavy metals concentrations are not controlled by the low solubility of the compounds; rather, they are controlled by the presence or absence of the four forms mentioned above (Krauskopf, 1956).

Heavy metals are known to have at least two interactions with proteins. The first is in the presence of specific ligands, particu-

larly carboxyls, imidazole, and sulfhydryl groups. The second are those associated with special arrangements of amino acid residues important in enzyme reactions. Because of these interactions heavy metals can be potential inhibitors to both extra- and intracellular enzymes. The cell membrane may be the active site of toxic action for heavy metals if there are physiologically important functions located on the membrane that the metals can interfere with. On the other hand the cell membrane may act as a barrier to the cell's interior if membrane sites are physiologically inert (Rothstein, 1959).

#### Mercury in the environment

Mercury is now realized to be one of the most toxic elements in our environment. Mercury in many forms is known to occur in trace amounts in soil, water, atmosphere, and organisms (Hammond, 1971). Concentrations in the environment have been reported from only a few nanograms to more than 7 mg/l (Anonymous, 1970). The oceans have been estimated to contain 10<sup>8</sup> metric tons of mercury of which the great majority is due to natural weathering (Hammond, 1971). Of the 9000 metric tons of mercury produced worldwide in 1966, approximately 27 percent was processed in the United States. About 4000 tons of the worldwide total were released into the environment (Klein and Goldberg, 1970).

The Environmental Protection Agency (Personal communication, 1975) has suggested a 5 ppb (parts per billion) limit on drinking water.

The United States Geological Survey reports that surface waters are usually below 0.1 ppb (Hammond, 1971).

Even though many epidemiologic studies have been carried out (West and Lim, 1968; Rentos and Segliman, 1968; Smith et al., 1974; Skerfuing et al., 1970; Aberg et al., 1969), Harriss (1970) states that a lack of information of biological effects of mercury prevents the establishment of adequate water quality standards. The long-term effects of mercury pollution below 1 ppb must be determined.

Since 1960 over 450 cases of human mercury poisonings have been reported (Hammond, 1971). For example, 111 cases of mercury poisonings occurred, with 41 deaths in Kyushu, Japan between 1960 and 1965.

These cases were attributed to contaminated seafood (Gavis and Ferguson, 1972; Klein and Goldberg, 1970). Marine organisms, mainly fish, are known to concentrate mercury due to direct uptake and/or uptake through trophic levels (Holm and Cox, 1974). Mercury concentrations in fish have been reported to be over 1000 times greater than seawater (Klein and Goldberg, 1970).

Mercury retention by organisms and toxicity is increased when inorganic mercury is converted into an organic form. Various microorganisms can convert inorganic mercury into organic mercury compounds (Hammond, 1971; Jensen and Jernelov, 1969; Imura, 1971; Lindstrom, 1964; Wood et al., 1968). This conversion can occur anaerobically but is more efficient in an aerobic environment (Hammond, 1971). Wood (1968) has also shown that mercury inhibits methane formation. Once in man the biological half-life for methyl mercury has been reported as 70 days (Hammond, 1971) and 36-95 days for inorganic mercury (Aberg et al., 1969). Hammond (1971) reports that the biological half-life for methyl mercury in fish is 200 days.

Harriss (1970) supports the theory that mercury toxicity is dependent on the chemical nature of the compound (inorganic or organic). Mercury toxicity is also dependent upon its reaction with important sulfhydryl groups (Rothstein, 1956, Goodman and Gilman, 1970). Enzyme interference resulting in cellular malfunction can occur even at low levels of mercury.

The action of inorganic mercury appears to occur in two phases, one rapid, one slow. The rapid phase of binding has a half-life of only a few minutes with completion in less than one half hour. Mercury in the rapid binding phase is easily removed with various complexing agents. The slow binding phase seems to occur with higher concentrations of mercury and binding is essentially permanent (Rothstein, 1959). The slow binding appears to affect cellular structure rather than enzyme properties. Mercury has been shown to interfere with respiration, potassium metabolism, and other cellular processes.

The methylation of mercury always presents a more serious threat to the biosphere than the inorganic form. It has been shown that methylation can take place abiotically in the presence of methylcobalamin, a vitamin  $\mathrm{B}_{12}$  analogue (Imura, 1971). The proposed steps for this methylation process are as follows:

$$2CH_3 - -[Co] + HgCl_2 + (CH_3)2Hg + Cl_2 - -[Co]$$

$$(CH_3)_2Hg + HgCl_2 \rightarrow 2CH_3HgCl$$

Methylcobalamin has been isolated in many marine organisms (Lindstrom, 1964), and the methyl transfer may be a non-reducing step in mild reducing conditions (Wood et al., 1968).

Holm and Cox (1974) report that in a sediment-water system the methylmercury content was always less than one percent of the total mercury concentration, and aeration stimulated release of elemental mercury from the sediment-water system. Mercury was reported to occur mainly as HgCl<sub>4</sub> and HgCl<sub>3</sub> ions (Krauskopf, 1956; Garrett, 1939) since chloride ions complex strongly with mercury in seawater (Feick et al., 1972). However mercury in water is most likely to be found adsorbed on organic particles such as plankton (Krauskopf, 1956).

#### Mercury toxicology

Mercury, a well-known pollutant, has been studied extensively in the marine environment (Barnes and Stanbury, 1948; Brown and Ahsanullah, 1971; Calabrese et al., 1973; Corner and Rigler, 1958; Corner and Sparrow, 1957; Davies, 1974; Karbe, 1972; Portmann, 1968). Portmann (1968) reported results of mercuric chloride toxicities as shown in Table 4.

Corner (1956, 1957, 1958) studied the toxicity of various mercury compounds on two-day old A. salina nauplii maintained in filtered seawater. The criteria for death was loss of activity since A. salina are usually very active. The reported LT<sub>50</sub> value at 800 mg Hg/l (as HgCl<sub>2</sub>) was 2.5 hours. Toxicities of two other mercury compounds were calculated relative to that of mercuric chloride (given a value of 1); ethylmercuric chloride was given a value of 24 showing the greater toxicity of organic mercury. Corner et al. (1956), relating LD<sub>50</sub>, concentration, and time, reported the following values for mercuric chloride: Mercuric chloride effects on A. salina nauplii 20 hours at 3 ppm, 10 hours at 5 ppm, 4 hours at 10 ppm, and 2 hours at 20 ppm.

Table 4. Acute toxicity of mercury to various marine organisms.

	LC <sub>50</sub> mg/l	Time	Author
Crangon crangon	3.3-10	48h	Portmann 1968
Pandalus montagui	0.075	48h	"
Cardium edule	3.3-10	48h	11
Watersipora cucullata (1)*	0.1	2h	Wisely & Blick 1966
Bugula neritina (1)	0.14	2h	n .
Spirorbis lamellosa (1)	0.14	2h	n .
Galeolaria caespitosa (1)	1.2	2h	"
Mytilus edulis (1)	13	2h	11
Cassostrea commercialis	180	2h	n
Elminus modestus	0.3	2.5h	Corner & Sparrow
cartia clausi	0.3	2.5h	1958
fitocera spinipes	0.7	24h	Barnes & Stanbury 1948

<sup>\* (1) =</sup> larvae

Corner (1958) used radioactive labeled  ${\rm HgCl}_2$  to determine uptake rates of <u>A. salina</u> nauplii. The following data was taken from the text figure: 1-hour uptake, 1.5 x  $10^{-4}{\rm g-Hg/g}$  dry weight of nauplii; 3-hour uptake, 4 x  $10^{-4}{\rm g}$  Hg/g dry weight. In this paper Corner et al. (1958) state that:

Differences between the toxicities of certain organomercury poisons and that of mercuric chloride are far greater when a highly resistant test animal like Artemia is used; that, in general, poisons which are very toxic are also highly lipoid-soluble; and that no correlation exists between the toxicities of these compounds and their abilities to inactivate enzymes. Corner & Sparrow considered these observations to be consistent with the view that Hg poisons act by penetrating the test animal and assumed that the extreme resistance of Artemia was a direct result of this animal's impermeability (p 93).

Wisley and Blick (1966) studied 24-hour-old nauplii in filtered seawater. Mercury was used in the form of  ${\rm HgCl_2}$  and stored in  $10^{-1}$ -  $10^{-6}$  molar solutions. Criterion of death was loss of movement as in Corner's (1956, 1957, 1958) experiments. Ten to twenty larvae were concentrated to 0.2 - 2 ml by pipetting the seawater; then the toxic solution was applied. These tests were done to determine  ${\rm LD_{50}}$  by direct application. The  ${\rm LD_{50}}$  in 2 hours was 9.0 x  $10^{-3}$  molar Hg.

Brown and Ahsanullah (1971) studied mortality on adult Artemia salina. Six batches of ten 16-day-old A. salina were placed in 250 ml beakers with varying concentrations of mercuric chloride. The media used was filtered seawater and Farex (Glaxo), renewed on alternate days. Mortalities were recorded every two hours. The results of the mercury tests showed the LT $_{50}$  increased linearly with increasing log concentration (ppm). The following points were taken from the text figure: 17 hours at 0.1 ppm, 17 hours at 0.5 ppm, 25 hours at 1.0 ppm,

27 hours at 2.5 ppm, 30 hours at 6 ppm, 50 hours at 10 ppm. An explanation of possible instability of mercuric chloride in seawater was given for these inverse results.

#### Cadmium in the environment

Cadmium is used as a corrosion protectant for many metals and is therefore used extensively in the electroplating industry. Other uses include pigment and plastic stabilizers (National Academy of Science, 1974).

Cadmium has a relative high potential for poisoning and has been reported as being a toxic element in some cases of food poisoning (American Public Health Association, 1971).

Cadmium is fairly common and the following general estimates of occurrence have been reported by Van der Weyden (1973): Earth's crust 170 ppb, rivers 0.03 ppb, oceans 0.11 ppb, marine sediments 450 ppb. Drinking water ranges from 0.4-60 ppb cadmium (American Public Health Association, 1971).

The metal is known to occur in the presence of zinc sulfide deposits and other zinc related compounds. The concentration of cadmium in seawater is generally dependent on the organo-particulate concentration and is not necessarily dependent upon the pH or temperature (Krauskopf, 1956). Dominant forms of cadmium in seawater are reported as CdCl<sup>+</sup>, Cd<sup>++</sup>, and CdCl<sub>2</sub> (Van der Weyden, 1973; Krauskopf, 1956).

Cadmium is known to cause proteinuria, emphysema, anoxemia, hypertension, and adverse arterial changes in the kidneys (Pfitzer, 1972).

Some epidemiologic studies have been carried out which include those concerning cadmium fume intoxification (Lehnert et al., 1969; Zavon and Meadows, 1970).

Cadmium has no recognized biological function (National Academy of Science, 1974; McKee and Wolf, 1971) and is considered as dangerous as mercury in the environment (Van der Weyden, 1973). Cadmium accumulates and probably behaves in an organism like most heavy metals, but its mode of action is not well understood.

The drinking water standards for cadmium as set by the United States Public Health Service is 0.01 ppm (United States Public Health Service, 1962). The United States Public Health Service reports that 4 percent of the surface water in the United States exceeds the 10 ppb level and 42 percent are between 1 and 10 ppb (National Academy of Science, 1974).

## Cadmium toxicology

Few studies concerning the toxicity of cadmium on marine organisms have been reported (Brown and Ahsanullah, 1971; Calabrese, Collier, Nelson, and MacInnes, 1973; Karbe, 1972; Thurburg, Dawson, and Collier, 1973).

Calabrese (1973), using  $CdCl_2$ , found the 48 hour  $LC_{50}$  on embryos of the American oyster <u>Crassostrea</u> <u>virginica</u> to be 3.8 ppm. No mode of action was reported. However, Collier et al. (1973) showed that cadmium caused a decrease in gill tissue oxygen uptake in the mud crab <u>Eurypanopeus depressus</u>. The 72 hour  $LC_{50}$  was 4.9 ppm, the  $LC_{0}$  was 1 ppm, and the  $LC_{100}$  was 11.0 ppm. Thurburg (1973) found that the gill tissue consumption rates of the green crab Carcinus maenas and

the rock crab <u>Cancer irroratus</u> were reduced by 20-25 percent. He also found an elevated serum level above its normal hyperosmotic state in the green crab. Gardner and Yevich (1970) and Eisler (1971) showed that the killfish <u>Fundulus heteroclitus</u> suffered hypertrophy of the gill filaments and hyperplasia of the epithelial surface of respiratory lamellar in 50 ppm cadmium.

To my knowledge, Brown and Ahsanullah (1971) performed the only cadmium toxicity tests on Artemia salina. They reported LT<sub>50</sub> values at 100 ppm being 7 days, 50 ppm being 8 days, 10 ppm being 11 days, 5 ppm being 13 days, and 1 ppm being 16 days. In these experiments cadmium was used as cadmium sulfate in seawater, and shrimp were 15-day-old adults. No symptoms were reported, no criterion of death was reported, and the animals were not fed during the duration of the experiments. Batches of 10 adults in 250 ml vessels were used at concentrations of 100, 50, 10, 1, and 0 ppm cadmium. Mortalities were checked and recorded every two hours. No growth rate experiments were attempted with the cadmium. The seawater used during the experiment was filtered with graphite and maintained at 20 C. Medium was exchanged on alternate days.

## Copper in the environment

Copper is a necessity for life and conversely considered a very toxic substance (Steemann-Neilsen, 1970). Coastal waters are said to contain 10 microgram/1 (Atkins, 1932) while the open sea contains 3 ppb (Goldberg, 1963).

Copper, like mercury, acts as a heavy metal in that it can bind directly to cellular structures and possibly interfere with physiological

functions. Two stages or steps in copper binding also occur, with physiological inhibition typified by rapid blockage of glucose uptake and a diminution of respiration (Rothstein, 1959).

Copper is known to occur in seawater in the following forms: Cu<sup>++</sup> (most common), CuCl<sup>+</sup> (common), CuCl<sub>2</sub> (small, CuCl<sub>3</sub>, CuCl<sub>4</sub> (negligible). The insoluble compound of copper in seawater is mostly CuCO<sub>3</sub>. Copper is adsorbed more strongly and consistently than either cadmium or mercury, although mercury is adsorbed more strongly by plankton (Krauskopf, 1956). The concentration of copper in seawater is controlled mainly by the particulate matter in the water. The United States Public Health Service has limited the concentration of copper in drinking water to 1 ppm as has the Utah Division of Health for Class C waters.

## Copper toxicology

Copper toxicity was shown in a number of studies (Baker, 1969;
Barnes and Stanbury, 1948; Brown and Ahsanullah, 1971; Brown and Newell,
1972; Calabrese et al., 1973; Corner and Sparrow, 1956; Hubschuman,
1967; Jones, 1942; Karbe, 1972; Kerkut and Munday, 1962; Saliba and
Ahsanullah, 1973; Wisely and Blick, 1966).

Calabrese et al. (1973) found that copper as CuCl<sub>2</sub> gave a 48 hour LC<sub>50</sub> of 0.103 ppm in the American cyster <u>Crassostrea virginica</u> embryos. Brown and Newell (1972) showed that at low concentrations of copper sodium citrate damage to the gills occurred in the mussel <u>Mytilus</u> edulis. It was also reported that copper sodium citrate inhibited oxygen consumption in the same mussel. Thurburg et al. (1973) found that crabs exposed to copper lost osmoregulatory functions, and as

copper concentration increased, the normally hyperosmotic serum became isotonic with the medium. Copper showed no effect on gill-tissue oxygen constumption. Table 5 shows the  $LC_{50}$  for some selected marine organisms.

Wisely and Blick (1967) experimented with A. salina nauplii with direct application of copper sodium citrate. The molarity of the copper sodium citrate is given with LD $_{50}$ : 1.0 x 10 $^{-1}$ m, 27 min; 5 x 10 $^{-2}$ m, 33 min; 1.0 x 10 $^{-2}$ m, 44 min; 5.0 x 10 $^{-3}$ m, 55 min; 4.0 x 10 $^{-3}$ m, 163 min. The toxicity of sodium citrate was also tested, the molarities and time to LD $_{50}$  given as: 1.0 x 10 $^{-1}$ , 26 min; 5.0 x 10 $^{-2}$ , 36 min; 1.0 x 10 $^{-2}$ , 50 min; 5.0 x 10 $^{-3}$ , 61 min; 4.0 x 10 $^{-3}$ , 168 min. No apparent difference in toxicities exist between sodium citrate and copper sodium citrate (Corner and Sparrow, 1956). Copper sodium citrate was used because it was the only copper salt to give lethal concentrations in seawater. The toxicity of copper sodium citrate was .005, as compared to that of mercuric chloride, which was given a value of 1. Copper appears to be far less toxic to A. salina nauplii than mercury.

The effects of copper and mercury when mixed were also studied and the following data reported in terms of synergism: (Synergism =  $(LT_{50}$  theoretical -  $LT_{50}$  experimental)/ $LT_{50}$  theoretical): Cu/Hg, Synergism 1:20, 11; 1:1, 26; 25:1, 32:50:1,22:100:1,18:400:1,26. The copper had a marked and immediate effect reducing respiration, but showed little effect on motility.

Brown and Ahsanullah (1971) reported on the effects of copper sodium citrate on the mortality and growth of  $\underline{A}$ ,  $\underline{salina}$ . Using ten shrimp and 250 ml of filtered seawater, the following  $LT_{50}$  data was

Table 5. Acute toxicity of copper to various marine organisms.

	LC <sub>50</sub> mg/l	Time Hours	Author
Crangon crangon	10-33	48	Portmann 1968
Carcinus maenas	100	48	
Pandalus montagui	0.14	48	n -
Cardium edule	1.0	48	n .
Mytilus edulis (1)*	22	2	Wisley & Blick 1966
Bugula neritina (1)	4	2	"
Sipora cucullata (1)	0.16	2	11
Galeolaria caespitosa (1)	3	2	m .
Spirorbis lamellosa (1)	0.5	2	п
Crassostrea virginica	0.1-0.5	96	Galtsoff 1932

<sup>\* (1) =</sup> larvae

given: 100 ppm, 42 hours; 50 ppm, 50 hours; 10 ppm, 70 hours; 5 ppm 90 hours; 1 ppm, 168 hours. The copper showed no significant suppression of growth at 0.1, 0.5, or 1 ppm copper.

Saliba and Ahsanullah (1973) also worked with copper sulfate and  $\underline{A}$ . Salina in experiments on growth rate suppression, acclimation, and tolerance. No significant difference was observed in nauplii growth rate with copper sulfate at 0.025 ppm as compared to the seawater controls. However significance was shown after 8 days at 0.05 ppm. Acclimation had some effect on the  $LT_{50}$  values for copper at 1 ppm. The  $LT_{50}$  of the control at 1 ppm copper was 30 hours. The  $LT_{50}$  increased to 49 hours, 51 hours, and 85 hours, when 2 week old animals were first acclimated with 0.025, 0.05, and 0.1 ppm copper respectively. The acclimation resistance was also reported to be an acquired trait rather than an inherited one. Adult  $LT_{50}$  at 100 ppm and 1 ppm were 36 hours and 49 hours respectively. Symptoms included an initial increase in activity followed by shrimp dropping to the bottom of the container and exhibiting spasmodic motions.

# Analysis of cadmium, copper, and mercury

Analyses for heavy metals are performed almost exclusively by atomic absorption spectrophotometry although other methods do exist such as colorimetric, polarographic, etc. Atomic absorption spectrophotometry is the best method for cadmium determination according to Standard Methods and the Environmental Protection Agency. The dithizone method may also be used if atomic absorption analysis is unsuitable. I have found the dithizone method as described in Standard Methods (American Public Health Association, 1971) to be adequate for

highly saline water. Friberg et al. (1971) criticized some data obtained by atomic absorption because of sodium interference. The Varian Corporation (1975) recommended saltwater be treated with ammonium nitrate prior to analysis to help lessen the effects of the sodium interference.

Copper is best analyzed by atomic absorption spectrophotometry according to both the Environmental Protection Agency and Standard Methods. Colorimetric methods do exist for copper but are highly susceptible to error.

In the determination of mercury levels the Environmental Protection Agency (1974) recommends acidification of field samples with nitric acid and stored in polyethlene bottles. A wet digestion is followed by a cold-vapor analysis by atomic absorption spectrophotometry. This recommended procedure is currently in use by the United States Geological Survey (Gunnell, 1975, personal conversation).

# Brine Shrimp

# Biology and ecology

The brine shrimp Artemia salina belongs to the subclass Branchiopoda, order Anostraca, and family Artemidae.

The brine shrimp has almost worldwide distribution, living and reproducing in natural and artificial brine pools and lakes (Jennings and Whitaker, 1941; Needham, 1959). A salina is easy prey for fish and other marine animals and therefore only exists in abundance in harsh environments (Jennings and Whitaker, 1941). The brine shrimp in the Great Salt Lake may serve as food for some birds but are primarily unmolested due to the high salinity.

The general salinity range for A. salina is between 35 percent and 150 percent total solids although they are known to live and reproduce in saturated salt solutions (Post, 1975). The optimum salinity seems to be 35 percent (reeve, 1963). The optimum temperature is near 30 C (Galen, 1969) however A. salina are known to live at temperatures from 6 C (Wirick, 1972) to 37 C (Needham, 1959). The life span of a male A. salina appears to be near 60 days (Squire and Grosch, 1974).

Nutrition requirements are met from both micronutrients as solutes and bulk nutrients as particles, and they are therefore considered obligate phagotrophs (Provasoli and D'Agostino, 1969). In most community systems of <u>A. salina</u> algae serve as the organisms' main source of food. The primary food for <u>A. salina</u> in the southern arm of Great Salt Lake is the algae <u>Dunaliella viridis</u> and in the northern arm, the algae <u>Dunaliella salina</u>.

Oviparous reproduction is accomplished by eggs which may or may not be fertilized. Parthenogenetic reproduction, or reproduction without the aid of the male, is not uncommon to members of Branchio-poda (Waterman, 1960). Parthenogentic reproduction has been reported by Von Siebold (1912), Jensen (1918) and Reylea (1939) and has been contradicted by Bowen (1962).

Copulation takes place when the male fastens his claspers around the female's abdomen just above the ovisac. During copulation the male and female swim as a synchronized unit, and may do so for a few minutes to a few hours. The male can attach himself to a female in a matter of seconds. It is also interesting to note that this author has observed two males appearing to mate the same female, two males

grasping as if mating, and a male grasping another male which was mating a female. This has been observed at population densities between 1 and 30 shrimp/l and salinities from 120 to 330 g/l total solids.

Copulation is accomplished when the male bends and genital appendages come into contact with the oviduct (Jensen, 1918). The females produce and carry eggs in the ovisac until deposition occurs. During the development of eggs the female A. salina expends twice as much energy as during metabolism and molting for a 30 day period prior to reproduction (Khmeleva, 1967). Squire (1974) reports that a single female may produce 169± 329 gametes in a lifetime.

Two types of eggs are produced. One type (summer eggs) are soft-shelled and usually hatch in a few hours. Winter eggs are hard, chitinous cysts and will hatch at various rates depending mainly on temperature, osmotic pressure and chemical makeup of media (Garrett, 1960; Jensen, 1918; Boone and Bass-Becking, 1931; Jennings and Whitaker, 1941; Clegg, 1965).

Two stages in the excystment occur. These are initial emergence from the shell and final hatching from an enclosed membranous sac. Free glycerol is the major carbohydrate of cysts (Clegg, 1965) and may be used by the excysted nauplii for a few days to avoid starvation (Jennings and Whitaker, 1941). The glycerol also seems to play an important role in overcoming osmotic pressure differences.

The populations of  $\underline{A}$ . Salina are highly dependent upon algal concentrations and fluctuate accordingly (Wirick, 1972). In the southern arm of the Great Salt Lake winter eggs hatch during April and May due

to the freshwater inflow and the rise in temperature. The subadults that hatch from the winter eggs eventually mature and produce summer eggs which sustain the population through the summer.

In the northern arm hatching is not as simple as in the south.

The salinity is above the level which allows hatching to occur. Three possible mechanisms exist for the appearance of brine shrimp in the northern arm. First, there may be a movement of brine shrimp from the southern arm through the small openings in the railroad causeway. Second, there may be the presence of a salinity layer tolerable to hatching upon the higher density brine of the northern arm. Third, there may be hatching near freshwater springs located in the northern arm.

Brine shrimp of the northern arm usually appear in late June or July and once acclimated to the high salinity can live and reproduce by means of summer eggs. The population, like that of the southern arm, is highly dependent on algal concentrations and seems to be in active competition for food with the brine fly larvae (Post, F. J., Utah State University, personal communication, 1975).

# Morphology

The brine shrimp has a number of distinctive life stages after hatching which are referred to as instars. Provasoli and D'Agostino (1969) have classified twenty-four instars for the male and twenty-five for the female. During the first few instars the nauplius (baby brine shrimp) is very unlike the adult. A nauplius has only the central occllus (simple eye) and three pairs of appendages. As molting progresses more appendiges develop until eleven pairs are present.

These appendages serve as swimming and respiratory organs. Stalked compound eyes also develop and in the male large claspers are formed which are used in mating. During the first fourteen instars the features are easily distinguishable, but after that time the instars can only be differentiated by size. Three stages in the brine shrimps development are shown in Figures 2, 3, and 4.

Osmoregulation is the process which allows a marine organism to survive in varying salinities. In the case of A. salina it has been shown to survive in solutions from 3.5 to 35 percent salt. Osmoregulation in A. salina is related to haemolymph (body fluid) and its ability to maintain the hypotonic haemolymph in a highly saline water (Croghan, 1958a). The ionic composition and osmotic pressure of the cherry-red haemolymph can vary greatly from that of the environment, yet the ionic ratio of the haemolymph remains fairly constant.

A. salina has also been shown to be permeable at the branchiae and the gut epithelium. A permeable animal whose haemolymph is not at the same osmotic pressure as its environment must have mechanisms to control the NaCl in the haemolymph and its water balance. A. salina has developed a branchiae excretory mechanism for maintaining the NaCl balance and a gut mechanism for maintaining the water balance.

This along with other evidence shows that  $\underline{A}$ .  $\underline{salina}$  was once a freshwater organism that has evolved these mechanisms for survival in saline environments (Croghan, 1958a).

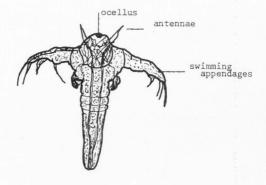


Figure 2. Brine shrimp nauplius, 1 - 2 days old, third instar, 0.5 - 1 millimeters long.

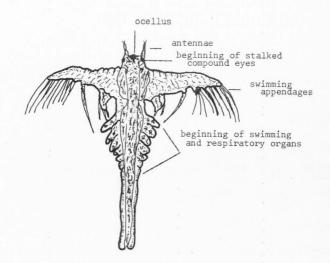


Figure 3. Brine shrimp nauplius, 5-6 days old, seventh instar, 1.6 - 1.9 millimeterslong.

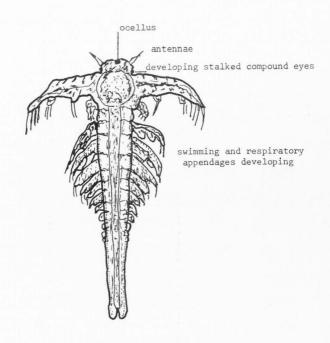


Figure 4. Juvenile brine shrimp, 10 days old, eleventh or twelfth instar, 2.4-2.7 millimeters long.

#### METHODS AND PROCEDURES

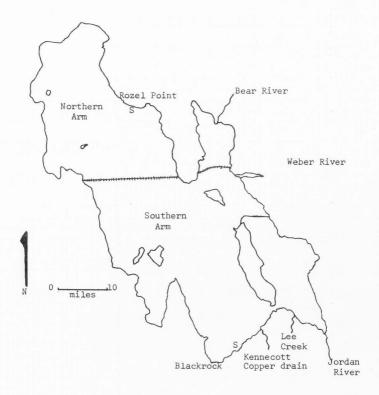
## Experimental Procedure

#### Introduction

Brine shrimp cultures were initiated with brine from the Great Salt Lake. The shrimp were raised on algae which were cultured from an isolated strain of a Great Salt Lake alga. Adult brine shrimp were used in mortality experiments, newly hatched nauplii were used in growth and reproduction experiments, and brine shrimp eggs were used in hatching experiments. Metal solutions were made up with mercuric chloride, cupric chloride and cadmium sulfate. Solutions were added to the brine shrimp's environment at known concentrations. Results were recorded periodically and statistical analyses performed.

## Sampling

Brine from the Great Salt Lake south of the causeway was collected at Blackrock near Silver Sands Beach about nine miles west of Salt Lake City. Brine from the northern part of the lake was collected at Rozel Point about twenty miles southwest of Golden Spike National Monument (Figure 5). The brine along with shrimp if they were present was placed in 5 gallon plastic containers. They were taken to the Utah State University Insect Culture Laboratory where the brine was placed in 10 gallon glass aquariums. The aquariums were covered with plastic sheeting to prevent evaporation and aeration was provided at a rate of



S = sampling points

Figure 5. Map of the Great Salt Lake showing location of inflows and sampling points.

about 1 liter per minute. The temperature was held at 27 C  $^{\pm}$  2 C. Northern arm shrimp were collected over a period of two months and were individually transferred to the previously collected brine.

Artificial brine was developed using the chemical analysis of the Great Salt Lake brine for simulation of Great Salt Lake environment (Table 6).

## Algae cultures

The alga <u>Dunaliella viridis</u> was the primary food for the brine shrimp in the culture aquariums. A strain of <u>D</u>. <u>viridis</u> was collected from the Great Salt Lake and isolated by Dr. F. J. Post, Utah State University. The algae was placed in medium composed of a 200 g/l NaCl base and various essential nutrients (Table 7). An aquarium containing the algae and the medium was lighted by two twenty-watt cool white flourescent lamps with an intensity of 400-foot candles <u>†</u> 20 percent as recommended by the Environmental Protection Agency (1971). The aquarium was covered with plastic and maintained at room temperature. Alga cells were collected with a pipette from the aquarium bottom at cell concentrations on the order of 5 x 10<sup>6</sup> cell per mm<sup>3</sup>.

## Shrimp culture

Eggs of the brine Shrimp Artemia salina were obtained from the Saunders Brine Shrimp Company which collects eggs from the shores of the Great Salt Lake. The eggs were placed in a jar containing 5.0 percent NaCl and gently aerated. After hatching (usually 48 hours) the medium was raised to the desired salinities by either of the following methods: The first method (evaporation) gave good results but took a longer period of time. A few milliliters of the concen-

Table 6. Simulated Great Salt Lake Water, north area

	g/l	
NaCl	255	
KCl	15	
MgSO <sub>4</sub>	71	
CaCl <sub>2</sub>	1.3	
MgCl <sub>2</sub>	36.6	
NaHCO3	1.5	
LiCl	0.4	
н <sub>з</sub> во <sub>з</sub>	0.3	
	μg/l_	
MnCl <sub>2</sub>	264	
ZnCl <sub>2</sub>	33	
CoCl <sub>2</sub>	0.78	
CuCl <sub>2</sub>	0.01	
NaMoO <sub>4</sub> • 2H <sub>2</sub> O	7.26	
FeCl <sub>3</sub>	96	
Na <sub>2</sub> EDTA•2H <sub>2</sub> O	300*	
H <sub>3</sub> PO <sub>4</sub>	1	
NaF	13	
Total dissolved solids	381.1	

<sup>\*</sup> Not used for metal test media

Table 7. Algae culture media

Base	g/1
VaC1	200
Macronutrients	mg/l
NaNO <sub>3</sub>	76.5
K <sub>2</sub> HPO <sub>4</sub>	3.132
MgCl <sub>2</sub>	17.1
MgSO <sub>4</sub> •7H <sub>2</sub> O	44.1
CaCl <sub>2</sub> •2H <sub>2</sub> O	13.23
VaHCO3	45.0

Micronutrients	Hg/l
H <sub>3</sub> BO <sub>3</sub>	556.56
MnCl <sub>2</sub>	792.792
ZnCl <sub>2</sub>	98.127
CoCl <sub>2</sub>	2.34
CuCl <sub>2</sub>	0.027
NaMo0 <sub>4</sub> • 2H <sub>2</sub> 0	21.78
FeCl <sub>3</sub>	288
Na <sub>2</sub> EDTA•2H <sub>2</sub> O	900

trated algae solution were added to the water and then the water was left to evaporate to the desired salinity. Salinity was measured using a hydrometer. The second method used successive increases in salinity; it was a faster method than the first, but was found to be hazardous to the nauplii if not done properly. Salinity differences in each increment did not exceed the transfer solution by more than 10 percent, and shrimp were left in the solution for at least 12 hours. During the acclimation, algae were added in sufficient quantities (10-20 cells/mm<sup>3</sup>) to prevent starvation. The first method described was used in most instances for acclimating the brine shrimp.

Shrimp survival was better if the acclimation medium was slightly higher in salinity than the culture aquarium to which the shrimp was being placed. The initial shrimp culture contained at least 100 individuals to assure an adequately large population for shrimp production. Shrimp were not used for any experiments until a first generation was produced (usually 3 to 4 weeks).

Aquaria were aerated, lighted and covered with plastic as in algae cultures. A photoperiod of 16 hours light was provided. The temperature was maintained at 27 C.

Shrimp from the actual lake water were treated in the same way, except acclimatization was not necessary. The lake brine was left unfiltered in the culture aquarium to simulate closely the actual lake environment.

# Preparation and handling of metal solutions

The following metal salts were used in preparing stock metal solutions: mercuric chloride, cupric chloride, and cadmium sulfate.

The reagent grade salts were dried to a constant weight, and enough of each weighed to give 1 gram of metal. Each gram equivalent was dissolved in a liter of deionized distilled water. The metal stock solutions were stored in polyethylene bottles. Stock solutions were made up every thirty days.

In the case of methyl mercuric chloride, 1 gram of HgCH<sub>3</sub>Cl was dissolved in 1 liter. Solutions were made up during the week of testing and stored in polyethylene bottles (see appendix concerning the precautions that should be taken when handling organic mercury).

Dilutions of the stock metal solutions were made up daily using deionized distilled water. All metal solutions were handled with individually labeled glassware to prevent contamination. Glassware was washed in a low phosphate detergent, rinsed in distilled water, rinsed in nine normal nitric acid solution and finally rinsed seven times in distilled water. All mercury solutions were saved and evaporated for proper disposal.

## Bioassay Procedures

## Acute Bioassay

No standard procedure for toxicity bioassays on the brine shrimp has been published, however many individuals have reported methods for bioassays on similar organisms. A number of bioassay procedures recommend media volumes of around 150 ml with ten individuals being used in each; however, the possible surface adsorption of the metals being used makes a high surface to volume ratio undesirable. Filip and Lynn (1972) showed mercury at a concentration of 0.1 ppm was reduced by 50 percent in 50 hours by surface absorption. For this reason a Pyrex

number 3250 specimen jar was selected for its low surface area to volume ratio (0.6) which is about two thirds of the surface to volume ratio of a beaker.

The total working volume for medium and toxicant was 300 ml, 270 ml of which was the desired culture medium (filtered and reaerated). The 30 ml were reserved for the addition of metal solution and distilled water. Algae were added to the specimen jars to concentration of 0-250 cells/mm<sup>3</sup> in most experiments. Concentrations of 40-50 cells/mm<sup>3</sup> were used in most cases because these are more comparable to the brine shrimp's natural environment.

The test shrimp were selected in one of the following ways: first, by specific age (adults at least 16 days old, usually 21 days old) (juveniles 5-8 days old) (nauplii 1-5 days old); second, by sex; and third, by a combination of age and sex which was used in most of the tests. Five shrimp per specimen jar were used as this concentration is most like that of the southern arm of the lake. The entire medium including metal and food were replaced every third day if necessary.

Controls were used with all tests and treated in the same way, except 30 ml of distilled water were added in lieu of the 30 ml of metal solution. At least one control for every three specimen jars was used. No experiment was accepted when controls showed mortality.

Test medium was saved periodically for analysis to assure the designed concentrations were being maintained. Acute mercury test shrimp were washed thoroughly and analyzed for metal content. The mercury bioassays were the most comprehensive since mercury was expected to be the most toxic metal tested.

Mercury and copper bioassays were checked every 2 hours, and cadmium bioassays were checked every 12 hours. Shrimp were classified as dead when no movement of the thoracic appendages was noted, and as moribund when ciliary movement was noticeably slow. All observed symptoms were recorded. Algae counts were taken periodically to assure that the cell concentrations were near desired level and to possibly estimate algal consumption. All algae counts were carried out using a hemacytometer and counting at least five fields of 1.0 mm<sup>2</sup>. Light intensity upon the covered specimen jars was 400 foot candle ± 20 percent using cool white flourescent lamps and a photoperiod of 16 hours light. The temperature of the laboratory was maintained at 27 C ± 2 C.

## Growth rate and chronic toxicity bioassays

Growth rate and chronic toxicity tests were done in the same manner as the acute bioassays, except metal concentrations were usually below that of the lowest observed LC<sub>50</sub>. Lengths of shrimp were measured visually in a small chamber and also by photographic methods. Handling of the brine shrimp was avoided to insure that no injury would occur. Growth rate tests were started with 24- to 48-hour-old nauplii and continued until the control produced viviparous nauplii (the second generation). Reproduction was classified as severely inhibited, moderately inhibited, or uninhibited. "Severely inhibited" indicated that very few nauplii or no nauplii were produced within normal reproduction times. Normal reproduction generally occurs in 23 to 33 days, although controls will establish "normal" for each reproduction experiment. A delay in nauplii production by more than three days was considered moderate inhibition of reproduction.

Growth rates were compared to a standard growth curve, and a probability value was computed for each growth test in relation to the standard curve.

## Hatching

Hatching tests were carried out in 150 ml beakers using 0.1 gm of Great Salt Lake brine shrimp eggs. Because of the high concentrations of metals to be used (10-1000 ppm) surface adsorption of the metals was considered negligible.

One hundred milliliters (m1) of 3.5 percent NaCl served as the hatching media for each beaker with the metal salts being added directly. Controls were duplicated with every metal. Three units of measurement were recorded: emergence, hatching, and deaths, all reported in concentrations per ml. Emergence was defined as when the egg first cracked and the membranous sac appeared. Hatching was said to be complete when the nauplius was free from the sac, and death was recorded when no motion was shown by the hatched nauplius. Results were recorded every 12 hours and plotted in comparison to results from controls to show significant effects.

#### RESULTS

#### Acute Toxicity

Experiments determining acute toxicity were completed at various concentrations in each metal to determine  ${\rm LT}_{50}$  values and for future comparison with other aquatic organisms. All recorded measurements are listed in Appendix B.

#### Cadmium

Cadmium was found to be least toxic of the three metals tested. At 3.3 mg Cd/l, the lowest concentration producing 100 percent mortality, the LT<sub>50</sub> was 320 hours ± 13.3. Other time to mortality values are given in Table 8 along with equations for lines and corresponding regression coefficients. Individual pairs of cadmium tests were statistically analyzed to test the hypothesis that their means were equal at a 95 percent level of confidence. Table 9 lists the F values calculated with the 0.05 F values taken from statistical tables. Concentrations at 17 and 33 mg/l, and 67 and 100 mg/l were shown to have equal means. Figure 6 shows the relation between cadmium concentration and the amount of time to 50 percent mortality.

The major symptom of cadmium toxicity appeared to be the slowing of the beating rate of the thoracic appendages. In the moribund state, adult shrimp appeared to lose control of their orientation ability,

Table 8. Summary of cadmium toxicity to adult brine shrimp, showing time to mortality, statistical analysis at various concentrations.

Cadmium				to indi		**		X percent		D
Concentration mg/l	20%	40%	60%	ortalit 80%	100%	50%	a (LI	(x) = aX + b	R*	Regression Coefficien
1.0										
3.3	308	314	323	337	337	320	0.36	302	13.3	0.85
17	142	160	166	214	283	180	1.18	121	76	0.97
33	136	148	176	213	229	176	1.15	119	31.5	0.99
67	71	87	102	149	164	115	1.24	53	48	0.98
100	75	80	93	114	126	94	0.675	63.6	50	0.98

<sup>\*</sup> R = limits for 95% level of confidence

<sup>\*\* 50% =</sup>  $LT_{50}$  calculated from equations

Table 9. Statistical analysis testing for equal means among pairs of acute cadmium tests using adult brine shrimp

dmium tests compared ncentrations in mg/l		Fcalculated	F(.05)		
					· · · · · · · · · · · · · · · · · · ·
33	and	17	80		4.2
17	and	33	0.1		4.2
33	and	50	0.7		4.3
50	and	67	11		4.2
67	and	100	1.36		4.1

<sup>\*</sup> If  $F_{calculated}$  is less than  $F_{(.05)}$ , statistically there is no difference between pairs of tests at a 95 percent level of confidence.

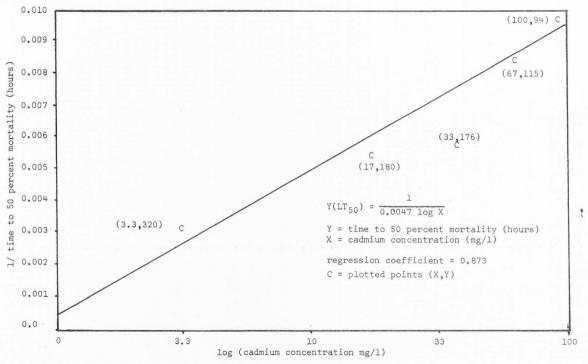


Figure 6. Relationship between cadmium concentration and time to 50 percent mortality among adult brine shrimp.

swimming with their ventral side downward. Typically normal brine shrimp swim with their ventral side upwards. No other symptoms were noted with the cadmium experiments.

### Copper

The  $LT_{50}$  for copper at 1 mg/l was 143 hours  $\overset{+}{.}$  43.  $LT_{50}$  dropped (drastically) to 21 hours  $\overset{+}{.}$  7.2. Results and statistical analysis are presented in Table 10. Figure 7 shows the concentrations of copper added, plotted against time to 50 percent mortality.

Precipitation of copper occurred in all tests of 33 mg Cu/l or greater. The medium was filtered (0.45  $\mu$  filter) an analyzed by atomic absorption for copper in solution. The concentration of copper in the test solutions were found to have a mean of 12.3 mg Cu/l at the various test concentrations (Table 11).

Data from the 10 mg Cu/l, 33 mg/l, 67 mg/l and 100 mg/l tests were statistically analyzed, testing for equality of means. Table 12 presents the calculated F values with corresponding F values taken from statistical tables.

All four of the tests showed equal means at 99 percent confidence level. All tests analyzed, including the 33 mg Cu/l test, showed equality of means at a 95 percent confidence level. Tests in higher saline water (320 g/l TDS) were not significantly different from the 150 g/l TDS, 170 g/l TDS experiments. Equality of means was shown in both 1.0 mg Cu/l and 0.05 mg Cu/l experiments (Table 13).

Symptoms appeared to be the same as cadmium with the slowing down of normally fast-moving thoracic appendages. The brine shrimp would eventually loose all swimming capabilities and enter a moribund

Table 10. Summary of copper toxicity to adult brine shrimp, showing time to mortality, statistical analysis at various concentrations.

Copper		Time in	hours t	to indi	cated			X percent		
Concentration		per		ent mortality	У	**	$(LT_{\star}) = aX + b + R$			Regression
mg/l	20%	40%	60%	80%	100%	50%	a	ь	R*	Coefficient
1.0	-81	122	140	170	178	124	1.57	46.3	43	0.98
3.3	20.4	20.4	21.6	23	25.6	21	0.05	19.1	7.2	0.98
10.0	12.3	14	14.3	16	16	15	0.03	13.3	5.7	0.35
33	10.9	11.4	12.8	13.1	14.6	12	0.05	9.9	3.6	0.99
67	14	14	14	14	16	14.4	0.02	13.4	1.5	0.707

<sup>\*</sup>R + limits for 95% level of confidence

<sup>\*\* 50% =</sup> LT<sub>50</sub> calculated from equations

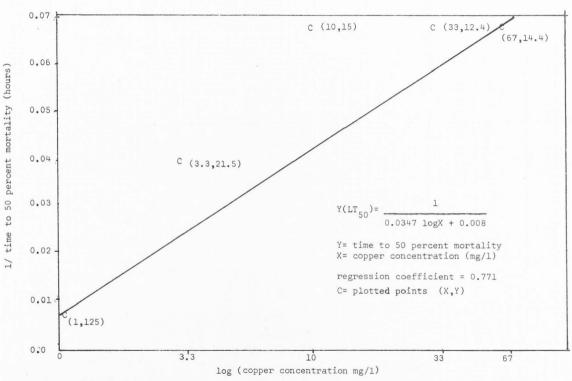


Figure 7. Relationship between copper concentration and time to 50 percent mortality among adult brine shrimp.

Table 11. Copper analysis of media containing precipitate  $% \left( 1\right) =\left( 1\right) \left( 1\right) \left($ 

Total copper added (mg to 1 liter of media	
100	11.2
100	8.2
100	8.4
67	14.8
67	14.1
67	8.1
33	7.0
33	17.2
33	15.0
33	18.0
33	13.8
	X = 12.3 standard deviation 3.9

\* Analysis by atomic absorption

Table 12. Statistical analysis testing for equal means among pairs of acute copper tests using adult brine shrimp.

Copper 1	tests ration	compared *	Fcalculated **	F(.05)	F(.01)
10	and	33	4.82	4.21	7.68
10	and	100	0.0007	4.6	
10	and	67	0.05	4.26	

 $<sup>^{*}</sup>$  Concentration in solution is limited to near 12 mg Cu/l Table

is less than F(.05) or F(.01) then there is no
difference between pairs of tests at a 95% and 99% level of
confidence respectively.

Table 13. Comparison of copper time to mortalities in salinities of 150 grams per liter total dissolved solids (TDS) and 320 grams per liter total dissolved solids.

Copper Concentration mg/l	LT <sub>50</sub> (Hours)	Fcalculated	F(.05)
1.0	124	0.28	4,1
3.3	21	0.34	4.04

If F is less than F (.05) then tests are equal at a 95 percent level of confidence.

state at the air-water-glass interface along the perimeter of the specimen jar. Some of the brine shrimp had copper precipitate accumulating between their appendages and these appeared to be the first to succumb.

## Mercury

Mercury was found to be the most toxic of the three metals tested. The LT $_{50}$  at 1.0 mg/l was 18.1  $^+_-$  4.2 hours although concentrations of mercury above 0.1 mg/l produced LT $_{50}$ 's below 25 hours. Table 14 summarizes the mercury testing at 150 g/l and 170 g/l total dissolved solids. Table 14 gives the statistical analysis, equations and the resulting LT $_{50}$  values. Figure 8 was plotted using the data from Table 14.

Mercury tests at 0.1 mg Hg/l and 0.33 mg Hg/l showed equal means at 95 percent significance as did tests at 1.0 mg Hg/l and 3.3 mg Hg/l. Tests at 10 mg/l and 33 mg/l, also had equality of means at the 95 percent level (Table 15).

Tests conducted at 320 g/l total solids (0.05 mg Hg/l) showed no significant differences at a 95 percent level of confidence with tests at 150 a/l and 170 g/l total solids (Table 16).

Mercury was placed in an algal culture at concentrations of 25 mg/l. The algae was then centrifuged and washed before being placed in the brine shrimp's immediate environment. The time to  $LT_{50}$  at 0.1 mg/l was 19 hours. The medium was analyzed for mercury before and after filtration. The mercury concentration before filtration was 0.107 mg/l, and 0.002 mg/l afterwards (Table 17).

Table 14. Summary of mercury toxicity to adult brine shrimp, showing time to mortality, statistical analysis at various concentrations.

Mercury	7		hours			**			mortality	
Concentration mg/l	20%	pe 40%	rcent m 60%	ortalit 80%	100%	50%	a (LT	x) = aX + b	D ± R R*	Regression Coefficient
.005										
0.01	72	.84	144		7 -	126	1.8	3.6	32	
0.03	58	61								
0.05	22	29	46	53	53	36	0.43	14.8	8.7	0.89
0.1	18	21	22	26	28	22	0.124	15.8	11.2	0.66
0.33	21	22	22	24	27	23	0.07	19.8	13.6	0.96
1.0	17	17.5	18	19	20	18	0.037	16.2	4.2	0.97
3.3	15	15.4	16	17	18.5	16.5	0.049	14.1	6	0.99
10	12	12.7	14	16	18	14	0.083	9.75	4.4	0.96
33	10	12.6	15	15	15	12	0.057	9.15	13.4	0.94
100	6.8	7.4	9.6	9.6	9.6	8.5	0.039	6.5	2.8	0.99

<sup>\*</sup> R = limits for 95% level of confidence

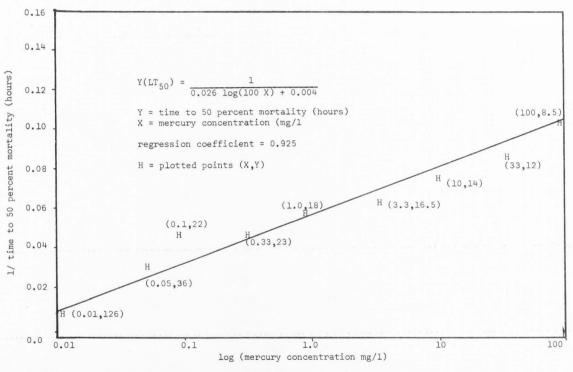


Figure 8. Relationship between mercury concentration and time to 50 percent mortality among adult brine shrimp.

Table 15. Statistical analysis testing for equal means among pairs of acute mercury tests using adult brine shrimp.

Mer	rcury to	ests compared tions in mg/l	Fcalculated*	F(.05)
	0.1	and 0.33	0.14	3.98
	0.33	and 1.0	9.74	4.03
	1.0	and 3.3	3.69	4.02
	3.3	and 10	6.24	4.03
	10	and 33	0.21	4.02
	10	and 100	21	4.21

<sup>\*</sup> If  $F_{calculated}$  is less than  $F_{(.05)}$ , statistically there is no difference between pairs of tests at a 95 percent level of confidence.

Table 16. Comparison of mercury time to mortalities in salinities of 150 grams per liter total dissolved solids (TDS) and 320 grams per liter total dissolved solids.

Mercury Concentra- tion	(hours)	LT <sub>50</sub> (hours) 150 & 170	Statistical Analysis*			
mg/l		g/l TDS	Fcalculated	F(.05)	F(.01)	
0.01	17.8	22	5.4	4.025	7.14	
0.05	44.0	36	1.5	4.025	7.14	

 $<sup>^{\</sup>rm *}$  If F\_calculated is less than F(.05) then tests are equal at a 95 percent level of confidence.

Table 17. Toxicity on adult brine shrimp from algal cells containing mercury.

Parameters	Numerical Values
Total Mortalities/Total Brine Shrimp	10/10
Time to 50 percent mortality (LT <sub>50</sub> ) hours	19
Standard Deviation	1.35
Mercury Concentration	
before filtration	0.11
after filtration	0.002
Statistical analysis	
Fcalculated	2.5
F(.05)	4.04

Mercury was placed in algal cultures then algae were separated by centrifugation and washed, then used as food for adult brine shrimp.

Postmortem analysis of 61 adult brine shrimp used in mercury tests showed mercury content was 0.195 (.12 - .34) micrograms per shrimp (0.39 - 0.65 micrograms Hg/mg dry weight of brine shrimp), and the concentration in the shrimp seemed independent of the mercury concentration in the medium (Figure 9).

The symptoms prior to death included a spasmotic twitching of the brine shrimp's abdomen with a slowing of the thoracic appendage beating rate.

#### Growth and Reproduction

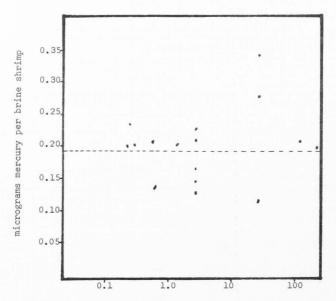
#### Effects of copper, cadmium, and mercury on growth

Copper and mercury showed no significant effect on the growth of newly hatched brine shrimp below the acute toxicity levels of the adults. The maximum length to which the brine shrimp nauplii matured was about 8 mm.

The means of the copper, mercury, and control tests were compared and found to be equal at a 95 percent confidence interval. Points are plotted on Figure 10 for copper and Figure 11 for mercury. All of the copper and control nauplii reached maturity (Figure ); however only nauplii from the 0.001 mg/l mercury test reached full length (Figure

). Nauplii mortality was complete at 0.003 mg/l mercury (Figure 10).

Cadmium was found to suppress the rate and extent of growth in solutions at and below that of acute toxicity levels of adults. At a 95 percent level of confidence brine shrimp reaching 25 days old at 33, 3.3 and 1.0 mg Cd/l showed unequality of means with that of the control (Figure 12).



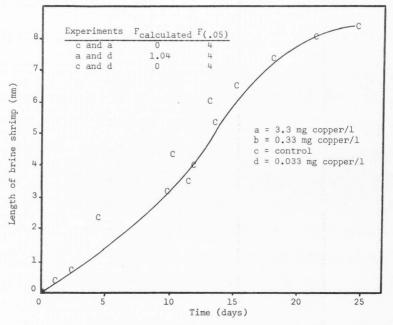
Mercury in brine shrimp's immediate environment (mg/l)

See Table 27 in Appendix for data.

The mercury concentration seems to be independent of the concentration of  ${\tt mercury}$  in shrimp's environment.

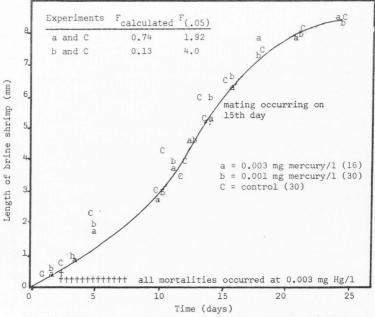
Dashed line represents mean value

Figure 9. Postmortem mercury content of brine shrimp used in toxicity experiments.



Control and copper growth data were identical. Copper concentrations were 0.3, 0.03, and 0.003  $\mbox{mg/l}$ 

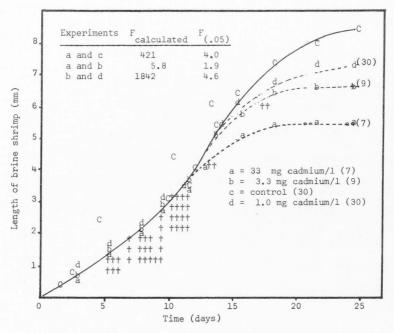
Figure 10. Brine shrimp growth in copper.



t indicates mortality, test concentrations were 0.001 and .003

() indicates number of shrimp remaining from the original thirty animals

Figure 11. Brine shrimp growth in mercury.



- t indicates mortality
- () indicate number of shrimp remaining out of original thirty Test concentrations were 1.0, 3.3, and 33 mg Cd/l

Figure 12. Brine shrimp growth in cadmium.

# Effects of copper, mercury, or cadmium on reproduction

Neither copper nor mercury caused a delay in mating or production of nauplii. Mating occurred in the control experiments as well as in the mercury and copper media when the brine shrimp were about 15 days old (Table 18). Nauplii were produced about 15 days later.

Brine shrimp in the cadmium solutions were not observed mating until about two days after the controls. No mating occurred in the 33 mg/l cadmium solution, and no nauplii were produced during the experiment (Table 18).

# Emergence and Hatching

# Emergence and hatching in solutions of cadmium, copper, or mercury

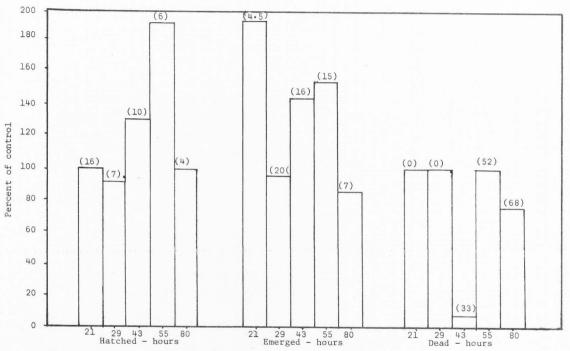
The excystment process from dessicated eggs proceeded normally in cadmium concentrations of 100 and 1000 mg/l and in copper concentrations of 10 mg/l. Figures 13, 14, and 15 summarize the excystment data, with emergence and hatching values also given.

Mercury had no apparent effect on excystment. However hatching never occurred in mercury concentrations at or above 0.33 mg/l. In mercury concentrations of 0.03 and 0.003 mg/l hatching was uninhibited. Figure 15 shows the control and mercury concentration for the excystment experiments.

Table 18. Time until first observed mating and production of nauplii in cadmium, copper, or mercury. This data is a continuation of growth experiments.

Metal	Concentration mg/l	Time until nauplii production (days)	Time until first mating observed (days)
Controls	0	29	15
Cu	0.3	31	15
Cu	0.03	30	15
Cu	0.003	30	15
Cd	33.		(a)
Cd	3.3	<del></del>	17 (ъ)
Cd	1.0		17
Hg	0.05	-	(c)
Hg	0.003	32	17 (d)
Hg	0.001	30	15

- (a) 7 of 30 test animals survived the duration of the experiment (35 days)
- (b) 9 of the original 30 test animals survived the duration of the experiment (35 days)
- (d) None of the test animals survived
- (d) 16 of 30 test animals survived. The remaining organisms appeared to function normally



Light areas indicate experiments at 100 mg cadmium/1. () indicate concentration of nauplii per ml in control.

Figure 13. Hatching of brine shrimp eggs in solutions of cadmium sulfate.

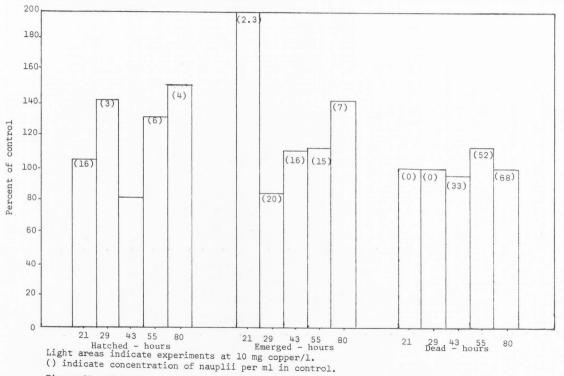


Figure 14. Hatching of brine shrimp eggs in solutions of cupric chloride.

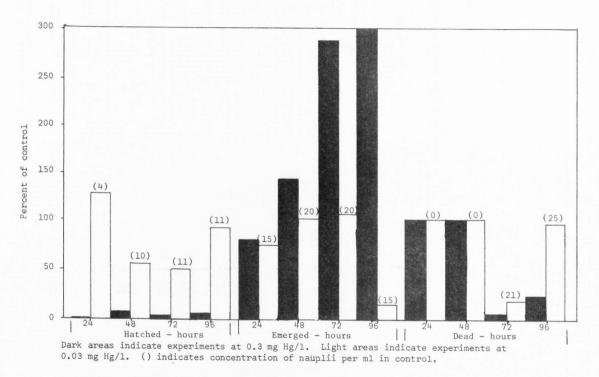


Figure 15. Hatching of brine shrimp eggs in solutions of mercuric chloride.

## DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

#### Discussion

Table 19 lists the minimum concentrations of cadmium, copper, and mercury producing 100 percent mortality to adult brine shrimp. In comparing the moles of each metal causing 100 percent mortality, it can be seen that mercury requires fewer molecules in solution. A more detailed study of mercury was made because (1) it was suspected as being the most toxic, and (2) analysis techniques for mercury in brine is well understood.

The discussion will present the metals (cadmium, copper, and mercury) separately when compared to other toxicity studies and together when discussing the implications of the metals in the Great Salt Lake.

# Cadmium

In the Great Salt Lake cadmium has been reported to be near 0.167 mg Cd/l (Ford Laboratory Certificate of Analysis 74-34034), but more data is needed to obtain a better estimate to assure its accuracy.

Marine and freshwater systems are reported to have concentrations between 0.03 and 0.11 mg Cd/l which is on the order of 1000 times less than found in the Great Salt Lake. This could be expected because (1) the Great Salt Lake is not as vast as the ocean, and (2) it does not

Table 19. Minimum concentrations (in millimoles per liter and milligrams per liter) of cadmium, copper, and mercury producing 100 percent mortality among adult brine shrimp\* at 150 and 170 grams per liter total dissolved solids.

	Conce	Concentration				
Metal	m moles/l	mg/l	Mortality (hours)			
Cadmium	0.03	3.3	320			
Copper	0.016	1.0	124			
Mercury	0.00025	0.05	36			

<sup>\*</sup> Combined male and female data.

have an outlet as do most freshwater systems. As a result metals can concentrate above ranges normally found in aquatic systems.

No great difference concerning the toxicity of cadmium between marine and Great Salt Lake brine shrimp was noticed. Brown and Ahsanullah (1971) reported the only cadmium biological assay on the brine shrimp known to this author. They found a 100 mg Cd/l LT $_{50}$  to be 7 days as compared to this study's findings of 4 days in Great Salt Lake salinity. At 10 mg Cd/l the LT $_{50}$  for the brine shrimp was 11 days in seawater and 10 days at the Great Salt Lake's salinity. At low concentrations of cadmium (<30 mg Cd/l) no difference was noticed in the toxicity cadmium at the two salinities Great Salt Lake (150+ g/l total dissolved solids), seawater (35 g/l total dissolved solids).

The brine shrimp appears very resistant to cadmium poisoning as compared to other marine organisms. The crab <u>Eurypanopeus depressus</u> was shown to have an LT<sub>50</sub> of 3 days at 4.9 mg Cd/l (Calabrese, 1973) as compared to 13 days for the brine shrimp (Brown and Ahsanullah, 1971). Calabrese (1973) also reported an LT<sub>50</sub> of 2 days at 3.8 mg Cd/l for the embryos of the American oyster <u>Cassostiea virginica</u>. For the brine shrimp at the same concentration the LT<sub>50</sub> was estimated to be 14 days (Brown and Ahsanullah, 1971).

Cadmium inhibited growth and reproduction of the brine shrimp at levels near 1 mg Cd/l in the Great Salt Lake study. Collier (1973) experimented with cadmium effect on oxygen uptake in the mud crab. It was found that cadmium did inhibit oxygen uptake. Although no attempt was made to test growth rates of brine shrimp at various oxygen levels, it could be considered a possible cause of growth inhibition since

oxygen levels in the Great Salt Lake are below 3 mg/l. No cadmium growth study was found in the literature.

Emergence and hatching occurred as normal in very high solutions of cadmium (100 mg/l) therefore it can be overlooked as an important aspect of cadmium toxicity towards the life cycle of the brine shrimp.

#### Copper

Copper in the Great Salt Lake has been reported to be near 0.1 mg Cu/l (Ford Laboratory), although it is possible that copper concentrations could be higher. As seen in Table 1 there is a great deal of copper inflow to the Great Salt Lake and no outflow. Marine and freshwater systems range around 0.003 mg Cu/l to 0.01 mg Cu/l which is over 10 times less than concentrations in the Great Salt Lake. During copper toxicity experiments with Great Salt Lake water a blue-green precipitate occurred (probably CuCO<sub>3</sub>). The highest concentration of copper in solution was around 12 mg Cu/l, although it is not known if all the copper was in true solution or in colloidal form small enough to pass the filter. In the Great Salt Lake copper concentrations should increase toward the inflows of copper and are probably sediment bound. Copper does bind rapidly to suspended matter, but no testing was done to substantiate this in the Great Salt Lake brine.

Many studies have been concerned with copper poisoning although none have been conducted in extremely high salinities. Table 2 gives values of copper  ${\rm LC}_{50}$  data for various marine organisms. The range of  ${\rm LC}_{50}$  is between 0.14 and 100 mg Cu/l in 48 hours and between 22 and 0.1 mg Cu/l in 2 hours for the experiments. The brine shrimp was reported by Brown and Ahsanullah (1971) to have  ${\rm LC}_{50}$  of 30 hours at 1 mg

Cu/l. These comparisons show that the brine shrimp is extremely tolerable to copper poisoning as compared to other marine organisms.

In the Great Salt Lake brine shrimp have an  $LT_{50}$  of less than 15 hours. Decreases in copper concentrations from between 3.3 mg Cu/l and 1.0 mg Cu/l caused the  $LT_{50}$  to increase from 21 hours to 124 hours indicating a very small threshold in which copper is poisonous and nonpoisonous. Brown and Ahsanullah (1971) reported a 168 hour  $LT_{50}$  at 1 mg Cu/l in seawater as compared to 124 hours in the Great Salt Lake. They also found a 90 hour  $LT_{50}$  at 5 mg/l as compared to this study's 21 hour  $LT_{50}$ .

Saliba and Ahsanullah (1973) reported that the brine shrimp can acquire a resistance to copper poisoning at continuous low level exposures (0.1 mg Cu/l). This may be a possible explanation of the large difference in LT50's at 1.0 and 3.3 mg Cu/l. However, the same reactions were noticed in the experiments from artificial Great Salt Lake culture medium where no low level copper was present. Perhaps the chemistry of the high saline water is causing this effect. There does seem to be a difference in copper toxicity when results in seawater are compared to those in the Great Salt Lake.

Although brine shrimp were observed with the copper precipitate among their thoracic appendages, statistical analysis has shown that the  $\mathrm{LT}_{50}$ 's with and without the precipitate are the same (10 and 33 mg Cu/l). This effect could be interpreted to say that it is important for the copper to be in solution in order for toxic action to occur.

Growth and reproduction was not inhibited in the Great Salt Lake below copper concentrations that caused adult mortality (0.3, 0.03,

0.003 mg Cu/1). Brown and Ahsanullah (1971) showed no significant suppression of growth at concentrations of 0.1, 0.5 or 1 ppm copper in seawater. Saliba and Ahsanullah (1973) however, showed a significant suppression of nauplii growth at a copper concentration of 0.05 ppm in seawater. Brown and Ahsanullah (1971) used copper in the form of copper sodium citrate while Saliba and Ahsanullah experimented with copper sulfate. The differences in the two copper compounds could explain the opposing results of the two studies. Behavior of copper compounds in varying salt concentrations is not well understood, yet this may be one factor regulating brine shrimp growth in the Great Salt Lake.

Copper had no apparent effect on emergence and hatching of the brine shrimp eggs. A possible explanation for the non-effect of copper may be an inability for the copper to penetrate the membranous sac containing the nauplius. The emergence and hatching of brine shrimp eggs can be overlooked as an important aspect of copper toxicity towards the life cycle of the brine shrimp.

# Mercury

In the Great Salt Lake mercury appears to be well below 0.001 mg Hg/l, yet most surface waters are limited to 0.1 ppb (Hammond, 1971). The higher than usual mercury content of the Great Salt Lake is consistent with the fact that the lake is a sink to surrounding discharges.

Brown and Ahsanullah (1971) published the only known mercury toxicity data concerning the brine shrimp. Their results showed a decreasing mortality rate with increasing mercury concentrations. How-

ever their results were near the same range as those reported in the Great Salt Lake. Brown and Ahsanullah's (1971) range of toxic concentrations was between 0.1 and 10 ppm mercury and corresponding  $\rm LT_{50}$ 's were between 17 and 60 hours. The range of the data presented in Great Salt Lake study for toxic concentrations was between .05 and 100 mg Hg/l with corresponding  $\rm LT_{50}$  values between 36 and 8.5 hours. Brown and Ahsanullah (1971) state:

A possible explanation is the instability of mercuric chloride in seawater, resulting in a reduced concentration of metal in the test solutions (Corner and Rigler, 1957), or alternatively, some factor affecting the penetration of mercuric chloride at low concentrations (p 187).

I experimented with mercury in high concentrations of algal (200 cells/mm<sup>3</sup>) and found a similar relationship to that of Brown and Ahsanullah (1971), although other tests at lower algal concentration (0-100 cells/mm<sup>3</sup>) showed an expected increase in mortality with increasing mercury concentration. Perhaps the factor Brown and Ahsanullah (1971) are hypothesizing is the concentration of organic particles (such as algae) in the test medium.

The results in high algal concentrations prompted the experiment in which mercury was placed in algal cultures, and the algae was then used for food by the brine shrimp. No significant difference in time to mortality was shown even though the mercury content of filtered media was negligible. This fact coupled with that of varying ingestion rates in the filter-feeding brine shrimp (Reeve, 1963d) may indicate that a complex relationship exists concerning mercury toxicity, algae concentration, and the behavior of mercury in saline water. In any event, organic particle content should be considered an important factor concerning toxicity tests in aquatic systems.

In examining the mercury toxicity data shown in Table 4 it can be seen that the range of mercury concentrations producing the same  $\rm LT_{50}$  varies greatly (.015-10 ppm mercury with an  $\rm LT_{50}$  of 48 hours). The brine shrimp's  $\rm LC_{50}$  in the Great Salt Lake at 48 hours is below 0.05 mg Hg/l which is among the lower values presented in Table 4 Acute mercury toxicity in Great Salt Lake concerning the brine shrimp does not appear to differ greatly from other marine organisms.

Growth and reproduction was not significantly affected in mercury concentrations at or below those causing acute results. Hatching was not significantly inhibited in high mercury concentrations (0.3 mg/l) although emergence was practically stopped. This indicates that the mercury is able to act on or through the membranous sac enclosing the nauplius and prevent its emergence. Although no studies have been published concerning the action of mercury upon brine shrimp eggs, it may be important when considering that mercury tends to concentrate on or in organic particles.

# Implications of results to metal toxicities in the Great Salt Lake

Nearly all of the cadmium, copper, and mercury sources to the Great Salt Lake are found south of the railroad causeway. Therefore any problem concerning these metals would become evident in the southern arm first. Although very few analysis of heavy metals have been conducted on the Great Salt Lake, I have found that cadmium, copper, and mercury concentrations are higher in the southern arm.

A metal reaching the southern arm of the Great Salt Lake probably will either become a part of another suspended particle or become sediment bound. If the metal does stay suspended, factors such as circulation and time will control the metal's distribution in the lake. It would appear that the only way a metal in the southern arm could reach the northern arm is by passing through the railroad causeway. Ultimately, the fate of cadmium, copper, and mercury depends on the metal remaining suspended or in solution and on the circulation patterns in the Great Salt Lake.

It is not understood which factors in the Great Salt Lake control the concentration of metals in solution, however in seawater it has been shown (Krauskopf, 1956) that solubility is not the major controlling factor. In seawater, absorption of the metals on various particles is the major factor regulating their concentration. When mercury was placed in algal cultures (Table 17 ) and the algae fed to the brine shrimp, it was shown that mercury passing a filter was negligible as compared to that which was suspended. This may indicate that mercury sorption on organic particulates is a controlling factor for free and suspended mercury concentrations in the Great Salt Lake.

Once cadmium, copper, or mercury enters the brine shrimp's environment, the shrimp may be affected by the metal. It is estimated that cadmium concentrations would have to exceed 1.0 mg Cd/l in the Great Salt Lake before direct effects upon the brine shrimp would be noticeable. Copper concentrations currently in the Great Salt Lake appear safe (0.1 mg Cu/l) for the brine shrimp, however, an increase could be detrimental. Mercury levels seem to be low (0.001 mg Hg/l) in the lake, but it has been shown that mercury even at low concentrations can accumulate in the brine shrimp. Serious concern should be taken if mercury levels in the Great Salt Lake rise any higher than

0.05 mg Hg/l. Table 20 summarizes the concentrations of metals in the lake with experimental levels producing toxic effects.

Table 20. Concentrations of cadmium, copper, and mercury in the Great Salt Lake and experimental concentrations producing toxic effects.

	Estimated tration i Salt Lake south arm	n Great	Minimum concentration causing mortality to adults		Minimum concen- tration causing toxicity to eggs
	mg/l	mg/l	hours	mg/l	mg/l
cadmium	0.17	0.1	320	3.3	
copper	0.3	0.1	124	1.0	
nercury	0.001	0.001	126	0.01	

In the past two years the Saunders Brine Shrimp Company has experienced a reduction in hatching percentage among eggs collected from the southern shores of the Great Salt Lake (personal communication, 1974, 1975). Eggs unable to hatch appear to be either empty shells or eggs in which emergence was not completed. In experiments with the eggs (Gebhardt, 1975, unpublished) it appeared that approximately 90 percent of the eggs were below the usually normal density of 1.15. This may be explained from the facts that the southern arm of the lake has experienced a freshening in the past ten years. This freshening has caused the density of the lake water to decrease, and eggs which in past years have floated on the surface are now submerged. Only eggs and

fragments of eggs with densities below that of the lake are floating, therefore eggs washing upon the shore are mostly nonviable. It may be that mercury is also a cause of this problem since eggs were found with incomplete emergence.

Mercury should receive more attention in the Great Salt Lake since brine shrimp and apparently brine fly larvae (Gebhardt, 1975, unpublished) can accumulate mercury. It is known that waterfowl feed on both the brine shrimp and brine fly in the southern arm of the lake, and shrimp are harvested from the lake for use as tropical fish food. Further study is needed to find if a possible threat does exist.

Heavy metals in freshwater, seawater, and highly saline waters can be poisonous to organisms. It seems that toxiological data on these three types of environments are not interchangeable even though the same organisms may exist in each. It is important to know, however, that factors controlling the toxicities and concentrations of heavy metal in different aquatic systems may be the same. These factors need to be identified as to their importance in each type of system. Once a behavioral understanding of heavy metals is established, applications can be directed towards useful gains.

### Conclusions

- Cadmium, copper, and mercury concentrations in the Great Salt
   Lake are much greater than those in most freshwater and marine systems.
- No great difference in acute toxicity of cadmium to the brine shrimp in seawater and Great Salt Lake water was noticed.
  - Cadmium greatly inhibited growth at levels near 1 mg Cd/l.

- 4. Cadmium had no effect on hatching or emergence.
- 5. Copper had no effect on hatching or emergence.
- 6. Copper had no effect on growth and reproduction.
- 7. Mercury was the most toxic to adult brine shrimp.
- 8. Mercury had no effect on growth and reproduction.
- 9. Mercury was found to concentrate to nearly the same level irrespective of the mercury concentration causing mortality.
- 10. Concentrations of organic particles should be considered when evaluating the toxicity of mercury in aquatic systems.

#### Recommendations

- Bioassays on all organisms in the Great Salt Lake should be conducted with the metals.
- Brine shrimp harvested from the lake should be analyzed periodically for mercury content.
- 3. Uptake rates for mercury should be determined for Great Salt Lake algae and brine shrimp.
  - 4. Circulation in the lake should be modeled.
- Behavior of heavy metals in the Great Salt Lake should be studied.
- 6. Tropical fish should be bioassayed using brine shrimp containing mercury.

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APPENDIX A

Table <sup>21.</sup> Data summary: Acute toxicity from mercury as mercuric chloride; 150, 170 grams per liter total solids.

Concentra	ation	Total	Mean time to	Standard	Number of
m moles/l	mg/l	Mortalities		Deviation	Experiments
.000025	0.005	0/30			3
.00005	0.01	6/30	100	35	3
.00015	0.03	4/30	84	30	3
.00025	0.05	10/10	41	13.5	2
.0005	0.1	40/40	22.5	6.1	8
.0016	0.33	33/33	23	6.8	7
.005	1.0	20/20	18.1	2.1	5
.0164	3.3	38/38	16.5	3.5	8
.05	10	15/15	13.9	3.3	3
.1645	33	21/21	8.3	3.7	5

Table 22. Data summary: Acute toxicity from cadmium as cadmium sulfate; 150, 170 grams per liter total solids.

Concentr	ration	Total	Mean time to	Standard	Number of
moles/l	mg/l	Mortalities	Mortality	Deviation	Experiments
.03	3.3	15/15	324	4.4	3
.15	17	20/20	182	48	4
.29	33	15/15	180	40	3
.596	67	15/15	115	59	3
. 89	100	20/20	98	25	4

Table 23. Data summary: Acute toxicity from copper as copper chloride; 150, 170 grams per liter total solids.

d Number of	Standard	Mean time to	Total	Concentration				
n Experiments	Deviation	Mortality	Mortalities	mg/l	moles/l			
3			3/15	0.5	.0079			
5	39	143	24/24	1.0	.016			
7	3	22 3		22	35/35	35/35	3.3	.052
3	2.8	14.6	13/13	10	.157			
7	2.2	12.6	35/35	33 <b>*</b>	.52			
1	0.89	14.4	5/5	67 <b>*</b>	1.05			
	0.89	14.4	5/5	67 <b>*</b>	1.05			

<sup>\*</sup> Copper in solution less due to precipitation. (See Figure for concentration in solutions.

Table 24. Acute copper toxicity data, 320 grams per liter total solids.

Media	Algae	Mercury concentration mg/l	Percent Mortality	Time to Death (hours)	Sex
GSL-320	0	1.0	20	95	m
020		1.0	40	144	m
			60	156	m
			80	156	m
			100	160	m
			20	95	f
			40	144	f
			60	144	£
			80	156	£
			100	160	f f f
			20	120	m
			40	120	m
			60	120	m
			80	144	m
			100	144	m
	0	3.3	20	18	m
	0	5.5	40	20	m
			60	20	m
			80	22	m
			100	24	m
			20	20	f
			40	20	f
			60	20	f
			80	20	f
			100	24	f
			20	20	m
			40	22	m
			60	22	m
			80	24	
			100	28	m m

Note: Statistical analysis has shown the means to be equal with their corresponding concentrations at 150, 170 g/l total solids at a 95 percent level of confidence.

Table 25. Acute mercury toxicity data, 320 grams per liter total solids.

Media	Algae	Mercury concentration mg/l	Percent Mortality	Time to Death (hours)	Sex
GSL-320	0	0.05	20	40	m
		0.00	40	40	m
			60	48	m
			80	52	m
			100	60	m
			20	36	
			40	400	f
			60	40	f
			80	40	f f f f
			100	52	f
			20	42	m
			40	42	m
			60	48	m
			80	52	m
			100	52	m
		0.1	20	16	m
			40	16	m
			60	18	m
			80	21	m
			100	23	m
			20	15	f
			40	18	f
			60	19	f
			100	22	f
			20	15	m
			40	16	m
			60	19	m
			80	20	m
			100	22	m

Table <sup>26</sup>. Methyl mercuric chloride compared to mercuric chloride in distilled water

		_
Time to death in 0.004M HgCH3Cl	Time to death in 0.005M HgCl <sub>2</sub>	
26 min.	4	
28	5	
28	5	
30	5	
31	5	
Mean = 28.6	Mean = 4.8	
Standard Deviation = 1.95	Standard Deviation = 0.45	

Table 27. Postmortem analysis of brine shrimp for mercury content

Ig Hg	Number of * Shrimp	µg/shrimp	Mercury Concentration in environment mg/l
0.4	2	0.2	0.1
0.8	4	0.2	0.1
0.65	3	0.22	0.1
0.45	3	0.15	0.33
1.0	5	0.20	0.33
0.65	3	0.22	0.67
0.70	4	0.175	1.0
0.45	3	0.15	33.3
0.6	3	0.20	3.3
0.8	5	0.16	3.3
0.5	3	0.17	3.3
0.65	3	0.21	3.3
0.5	4	0.125	10
1.4	5	0.28	33.3
1.0	4	0.25	33.3
0.62	3	0.21	83.3
0.8	4	0.20	100.0

<sup>\*</sup> Note: 48 male, adult brine shrimp

Table 28. Copper and cadmium excystment data

Metal	Time	Hatch	ned		Emerg		Dead			
	Hours				X				n	Sx
0.0	21	16	4	1	2.3	4	1			
11	29	7	4		20		2.4			
11	43	10		4					3	3.5
11	55	6		1			5		-	5
11	80	4		_	7		1		3	
Cadmium										
100	21	16	4	2	4.5	4	3			
11	20	6.2	4	2	19	4	1.2			
**	43	13			23	4	2.6	2.3	3	1.5
11	55	11.7	4	3.1	9.8	4	. 9	52.7	3	5
"	80	4	3	. 7	6	3	.7	52	3	7
Cadmium										
	21	14 7	14	2	4.7	LL.	1		-	
11					22.4					
11					17.3		1000		3	1.2
11	55	6		2.6						.6
11	80	4			9	3	2000			1.8
Copper										
	21	17	4	.6	5	4	1			-
**	29	10	4	1	17	4	1			
11	43	8		. 5	18	4	. 6	31	3	3.6
11	55	8	4	1	17	4	1	-		5.1
. 11	80	6		.6	10		. 8	68	3	

Table 29. Data for mercury hatch experiment

		Hat	ched	Eme	erged	De	ead
	time hours	mean	standard deviation	mean	standard deviation	mean	standard deviation
	24	0	0	11.2	1.0	0	0
ation 3	48	0.5	0.6	28	1.6	0	0
Mercury Concentration mg/l 0.3	72	0.25	0.5	56	5.5	1	0
	96	0.5	0.6	55	5.3	4	.5
	24	5	0.5	10	0.6	0	0
Mercury Concentration mg/l 0.03	48	5	0	20	2	0	0
	72	5	0.6	21	1	3	3
Mercury Concent mg/l	96	10	0.8	2	2	24	3
	24	4	0.5	15	1	0	0
ition 103	48	10	1	20	2	0	0
iry entra 0.0	72	8	1	15	. 5	15	1
Mercury Concentration mg/l 0.003	96	5	• 5	10	0	24	3
	24	4	0	15	1	0	0
uo	48	10	• 5	20	1	0	0
trati	72	11	. 5	20	.5	21	.6
Concentration mg/l 0.0	96	11	.5	15	2	25	1

Note: N = 4

Table 30. Acute copper toxicity data

Data	Media	Algae Concen- ctration Cells/mm <sup>3</sup>	Metal Concen- tration	Percent Mortality	Time to Mortality	Sex
.0-28	GSL-150	0	0.5	20 40 60	144 288	m m f
			80 100		m f	
			0.5	20	144	m
				40		m
				60		m
				80 100		f
0-28	GSL-150	0	1.0	20	96	m
				40	120	m
			60	144	f	
			80	168	m	
			100	168	f	
			1.0	20	120	m
				40	120	m
				60	168	f
				80 100	168	m f
-20	GSL-150	100aa	1	20	24	m
				40	74	m
				60	94	m
				80	156	m
				100	200	m
-20	GSL-150	100	1	20	46	m
				40 60	140 140	m m
				80	156	m
				100	156	m
	101 15					
-2	ASL-170	10	1	20	120	m
				40	156	m
				60	156	f
				80	200	m
				100	200	f

Table 30 Continued

Data	Media	Algae Concen- tration Cells/mm <sup>3</sup>	Metal Concen- tration	Percent Mortality	Time to Mortality	Sex
7-24	GSL-150	50	3.3	20	20	m
				40	20	m
				60	20	f
				80	22	f
				100	30	m
-4	GSL-150	40	3.3	20	20	m
				40	20	f
				60	22	m
				80	22	m
				100	22	f
-2	ASL-170	10	3.3	20	20	m
				40	20	f
				60	22	m
				80	23	f
				100	23	m
-20	0 GSL-150	100aa	3.3	20	25	m
				40	25	f
				60	28	m
				80	28	m
				100	30	f
-24	ASL-170	60	3.3	20	20	m
				40	20	f
				60	22	m
				80	25	f
				100	30	f
-20	GSL-150	100	3.3	20	20	m
				40	20	m
				60	22	f
				80	24	f
				100	24	m
-30	GSL-150	200	3.3	20	18	m
				40	18	f
				60	18	m
				80	18	f
				100	20	m

Table 30 Continued

Data	Media	Algae Concen- tration Cells/mm <sup>3</sup>	Metal Concen- tration	Percent Mortality	Time to Mortality	Sex
6-30	GSL-150	200	10	20	11	m
				40	14	f
				60	14	m
				100	14	f
5-2	GSL-150	50	10	20	16	m
				40	18	m
				60	18	m
				80	18	f
				100	18	f
-24	ASL-170	60	10	20	10	m
				60	11	m
				80	14	m
				100	14	m
-20 GSL-150	100	33	20	4	m	
				40	8	f
				60	12	f
				80	12	m
				100	16	m
-20	GSL-150	100aa	33	20	12	m
				40	12	m
				60	14	f
				80	14	m
				100	16	f
-30	GSL-150	200	33	20	12	m
				40	12	m
				60	12	f
				80	12	f
				100	12	m
-24	ASL-170	60	33	20	12	m
				40	12	m
				60	14	m
				80	14	m
				100	14	m

Table 30 Continued

Data	Media	Algae Concen- tration Cells/mm <sup>3</sup>	Metal Concen- tration	Percent Mortality	Time to Mortality	Sex
6-4	GSL-150	40	33	20	12	m
				40	12	f
				60	12	m
				80	14	m
				100	16	f
7-24	GSL-150	50	33	20	12	f
				40	12	m
				60	14	m
				80	14	f
				100	16	m
5-2	ASL-170	10	33	20	12	m
				40	12	f
				60	12	m
				80	12	m
				100	12	f
5-4	GSL-150	40	67	20	14	m
				40	14	f
				60	14	m
				80	14	m
				100	16	f

Table 31. Acute cadmium toxicity data

Data	Media	Algae Concen- tration Cells/mm <sup>3</sup>	Metal Concen- tration	Percent Mortality	Time to Mortality	Sex
5-4	GSL-150	.40	3.3	20	300	f
				40	320	f
				60	320	f
				80	340	f
				100	340	f
-13	GSL-150	50	3.3	20	312	m
				40	312	m
				60	312	m
				80	336	m
				100	336	m
-13b	GSL-150	50	3.3	20	312	m
				40	312	m
				60	336	m
				80	336	m
				100	336	m
-21	21 GSL-150	50	17	20	216	m
				40	216	m
				60	216	f
				80	216	f
				100	216	m
-21b	GSL-150	50	17	20	113	m
				40	113	f
				60	135	m
				80	209	m
				100	221	f
-4	GSL-150	40	17	20	120	m
				40	120	f
				60	120	m
				80	192	f
				100	240	m
-23	GSL-150	0	17	20	120	m
				40	192	m
				60	192	m
				80	240	m
				100	240	m

Table 31 Continued

Data	Media	Algae Concen- tration Cells/mm <sup>3</sup>	Metal Concen- tration	Percent Mortality	Time to Mortality	Sex
6-4	GSL-150	SL-150 40	33	20	144	m
				40	144	m
				60	168	f
				80	192	f
				100	240	m
i-4	GSL-150	40	33	20	144	m
				40	144	m
				60	168	f
				80	240	m
				100	240	f
-23	GSL-150	0	33	20	120	m
				40	156	m
				60	192	m
				80	206	m
				100	206	m
-11 GSL-150	80	50	20	216	m	
				40	240	m
				60		f
				80		f
				100		m
-21b	GSL-150	40aa	50	20	312	m
				40	312	f
				60		f
				80		m
				100		m
-21	GSL-150	50	50	20	216	m
				40	216	m
				60	240	m
				80	240	f
				100	240	f
-21	GSL-150	20	50	20	72	m
				40	96	m
				60	96	m
				80	144	m
				100	144	m

Table 31 Continued

Data	Media	Algae Concen- tration Cells/mm <sup>3</sup>	Metal Concen- tration	Percent Mortality	Time to Mortality	Sex
5-4	GSI-150	40	67	20	56	m
				40	56	f
				60	96	f
				80	168	m
				100	168	m
3-30	GSL-150	200	67	20	120	m
				40	156	m
				60	156	f
				80	206	f
				100	206	m
-23	GSL-150	0	67	20	36	m
				40	48	m
				60	56	m
				80	72	m
				100	120	m
-21	1 GSL-150	40	100	20	66	m
				40	84	m
				60	84	f
				80	144	m
				100	144	f
-21b	GSL-150	50aa	100	20	66	m
				40	66	f
				60	96	m
				80	120	m
				100	120	f
-11	GSL -150	80	100	20	72	f
				40	72	m
				60	96	f
				80	96	f
				100	96	m
-11b	GSL,-150	80aa	100	20	96	m
				40	96	m
				60	96	m
				80	96	f
				100	144	f

Table 32. Acute mercury toxicity data

Data	Media	Algae Concen- tration Cells/mm <sup>3</sup>	Metal Concen- tration	Percent Mortality	Time to Mortality	Sex
10-28	GSL-150	0	.5	20	22	m
				40	29	f
				60	46	f
				80	53	m
				100	53	f
				20	22	m
				40	29	m
			60	46	m	
				80	53	f
				100	53	f
			.01	20	72	m
				40	72	m
				60	144	f
				80		m
				100		f
				20	72	m
				40	96	f
				60	144	m
				80		m
				100		f
-13	GSL-150	20-30	.033	20	58	m
				40	61	m
				60		m
				80		m
				100		m
-23	GSL-150	0	0.1	20	15	m
				40	16	m
				60	16	m
				80	20	m
				100	22	m
-30	GSL-150	200aa	0.1	20	20	m
				40	30	m
				60	30	f
				80	35	f
				100	48	m

Table 32 Continued

Data	Media	Algae Concen- tration Cells/mm <sup>3</sup>	Metal Concen- tration	Percent Mortality	Time to Mortality	Sex
6-17	ASL-170	50	0.1	20	16	m
				40	18	m
				60	20	m
				80	20	f
				100	20	f
7-14	GSL-150	6aa	0.1	20	16	m
				40	18	m
				60	20	m
				80	20	m
				100	22	m
7-21	GSL-150	40aa	0.1	20	16	m
				40	18	f
				60	18	m
				80	20	f
				100	22	m
7-21	GSL-150	10	0.1	20	24	f
				40	24	f
				60	24	m
				80	24	m
				100	24	m
-24	GSL-150	50	0.1	20	24	m
				40	24	m
				60	28	m
				80	28	f
				100	28	f
-21	ASL-170	10	0.1	20	20	m
				40	22	m
				60	22	m
				80	22	m
				100	24	m 
-24	GSL-150	50	0.33	20	16	m
				40	18	f
				60	18	m
				80	18	f
				100	20	m

Table 32 Continued

Data	Media	Algae Concen- tration Cells/mm <sup>3</sup>	Metal Concen- tration	Percent Mortality	Time to Mortality	Sex
6-23	GSL-150	10	0.33	20	16	m
				40	20	m
				60	22	m
				80	22	m
				100	24	m
6-24	GSL-170	60		20	30	m
				40	30	m
				60	30	m
				80	42	m
				100	48	m
7-21a	GSL-150	10	0.33	20	20	m
				40	24	f
				60	24	m
				80	24	f
				100	24	f
7-21b	lb GSL-150	40aa	0.33	20	24	m
				40	24	f
				60	24	f
				80	24	m
				100	24	m
5-19	ASL-170	250	0.33	20	18	m
				40	18	m
				60	20	m
				80	20	m
				100	20	m
-18	ASL-170	250	0.33	30	18	m
				60	18	m
				80	18	m
				100	18	m
-17	ASL-170	50	1.0	20	18	m
				40	20	f
				60	20	m
				80	22	m
				100	24	f

Table 32 Continued

Data	Media	Algae Concen- tration Cells/mm <sup>3</sup>	Metal Concen- tration	Percent Mortality	Time to Mortality	Sex
6-30	GSL-150	200	1.0	20	16	m
				40	18	m
				60	18	m
				80	18	f
				100	18	f
7-14	GSL-150	6aa	1.0	20	16	m
				40	16	m
				60	18	m
				80	18	m
				100	18	m
7-24	GSL-150	50	1.0	20	16	m
		*		40	16	m
				60	16	f
				80	18	f
			100	18	m	
-18 ASL-170	250	3.3	30	18	m	
				60	18	m
				80	18	m
				100	18	m
-19	ASL-170	250	3.3	20	10	m
				40	18	m
				60	20	m
				80	20	m
				100	20	m
-21	GSL-150	40aa	3.3	20	10	m
				40	10	m
				60	10	m
				80	16	f
				100	16	f
-17	ASL-170	50	3.3	20	20	m
				40	20	m
				60	20	f
				80	20	m
				100	20	f

Table 32 Continued

Data	Media	Algae Concen- tration	Metal Concen- tration Cells/mm <sup>3</sup>	Percent Mortality	Time to Mortality	Sex
6-23	GSL-150	0	3.3	20	12	m
				40	16	m
				60	16	m
				80	16	m
				100	18	m
6-24	ASL-150	8	3.3	20	12	m
				40	16	m
				60	16	m
				80	24	m
				100	24	m
7-21b	GSL-150	80aa	3.3	20	12	m
				40	14	f
				60	14	f
				80	14	m
				100	16	m
7-21	1 GSL-150	50	3.3	20	14	m
				40	14	m
				60	14	f
				80	16	m
				100	16	f
5-21	ASL-170	10	10	20	12	m
				40	16	m
				60	16	m
				80	16	m
				100	20	m
-30	GSL-150	200	10	20	10	m
				40	12	f
				60	12	m
				80	16	m
				100	20	f
-14	GSL-150	6	10	20	10	m
				40	10	m
				60	12	m
				80	12	m
				100	14	m

Table 32 Continued

Data	Media	Algae Concen- tration	Metal Concen- tration Cells/mm <sup>3</sup>	Percent Mortality	Time to Mortality	Sex
7-24	GSL-150	50	33	20	10	m
				40	10	m
				60	10	m
				80	12	f
				100	12	f
7-21b	GSL-150	40aa	33	20	8	m
				40	8	m
				60	10	f
				80	10	f
				100	12	m
7-21	GSL-150	10	33	20	7	m
				40	7	m
				60	10	f
				80	10	m
				100	12	f
5-24	ASL-170	8	33	20	10	m
				40	10	m
				60	10	m
				80	14	m
				100	14	m
6-23	GSL-150	0	22	20	10	m
				40	12	m
				60	12	m
				80	12 14	m
				100		m
5-30	GSL-150	200	33	20	18	m
				40	18	m
				60	20	m
				80 100	20 20	f
				100		I
5-17	ASL-170	50	33	20	8	m
				40	8	f
				60	10	f
				80	16	m
				100	16	m

Table 32 Continued

Data	Media	Algae Concen- tration	Metal Concen- tration Cells/mm <sup>3</sup>	Percent Mortality	Time to Mortality	Sex
6-19	ASL-170	250	33	20 40	20	m
				60 80	24	m
				100	28	m
6-18	ASL-170	250	33	20	24	m
				40	28	m
				60	28	m
				80	28	m
				100	33	m
6-23	GSL-150	0	100	20	2	m
				40	3	m
				60	8	m
				80	8	m
				100	8	m
6-24	ASL-170	8	100	20	14	m
				40	14	m
				60	16	m
				80	16	m
				100	16	m
7-24	GSL-150	50	100	20	6	m
				40	6	m
				60	8	f
				80	8	f
				100	8	m
6-17	ASL-170	50	100	20	6	m
				40	8	f
				60	8	m
				80	8	m
				100	8	f
5-21	ASL-170	10	100	20	6	m
				40	6	m
				60	8	m
				80	8	m
				100	8	m

APPENDIX B

Table 33. Coefficient of variation for metal analysis techniques used in Great Salt Lake and artificial brines.

Metal	Method	Concentration mg/l	Coefficient of Variation (percent)
Cadmium	Dithizone (Colorimetric)	0.2	3.2
		0.4	12
		0.04	22
Copper	Atomic Absorption Spectrophotometry	10	1.2
		8	11
		3	8
		1	29
Mercury	Cold-Vapor, Wet- Digestion	0.05	6.7
	(Atomic Absorption)	0.001	33

# RECOMMENDATIONS CONCERNING THE HANDLING OF METHYLMERCURIC CHLORIDE

- Do not let compound come in contact with skin. Use mask, rubber gloves, lab coat, etc.
  - 2. Always handle under ventilation (hood).
  - 3. Do not mouth pipette, use labeled glassware.
- 4. In case of skin contact, wash with water and types of binding compounds.

#### VITA

#### Karl A. Gebhardt

## Candidate for the Degree of

### MASTER OF SCIENCE

Thesis: Effects of heavy metals cadmium, copper, and mercury on reproduction, growth, and survival of brine shrimp Artemia salina from the Great Salt Lake

Major Field: Environmental Engineering

Biographical Information:

Personal Data: Born at Salt Lake City, Utah, March 2, 1952, son of Louis Philipp and Johann Else Gebhardt; married Joyce Marie Polanshek, Salt Lake City, Utah, June 10, 1972.

Education: Attended elementary school in Salt Lake City, Utah; graduated from Highland High School in 1970; received the Bachelor of Science degree from the University of Utah with a major in Civil Engineering, 1974; did graduate work in Environmental Engineering, specializing in water quality, Utah State University, 1974-1975. Bureau of Land Management Watershed and Water Quality Training Workshop, August, 1975, Salt Lake City, included two days in the field with practical experience.

Honors: Tau Beta Pi (National Engineering Society); Chi Epsilon (National Civil Engineering Society); President American Society of Civil Engineers; received the Robert Ridgeway Award for outstanding ASCE Student Chapter while its president at University of Utah; received ASCE Senior Award; received John Call Scholarship, University of Utah; received Environmental Protection Agency Training Grant; received Utah State University Project Grant; member University of Utah College Council; Chairman, University of Utah Student Advisory Committee; Editor, Chi Epsilon Newsletter; graduated cum laude.

VITA (continued)

Professional Societies: Tau Beta Pi; Chi Epsilon, American Society of Civil Engineers (ASCE); American Water Works Association (AWWA); Water Pollution Control Federation (WPCF); The Wildlife Society (TWS) Utah Chapter.

Professional Experience: Created and produced slide presentation
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Research Assistant, Utah State University, September, 1974
to present; part-time staff engineer for Call Engineering,
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