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Molecular Acute Toxicity of Eight Heavy Metals To Three Marine Fish Species

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ABSTRACT

Marine water solutions of aluminum chloride, cadmium chloride, lead nitrate, nickel nitrate, potassium chromate, stannous chloride, copper sulphate and zinc sulphate were compared for their 96hr acute toxicity LC_{50} against the three marine fish species *Dicentrarachus labrex; Sparus auratus;* and *Solea vulgaris.* Larvae of 25 days old of each fish species were used for the bioassay. Molar corretion was needed to reach a more precise order of relative acute hazard. Accordingly, aluminum was found to be the most acute toxic to *Dicentrarachus labrex;* while tin was the most acute toxic to *Sparus auratus,* and lead was the most toxic to *Solea vulgaris.* The three marine fish species differed in their susceptibilities to the tested heavy metals. *Solea vulgaris* was shown generally to be the highly susceptible fish of the three tested species. It was concluded that multispecies screening will be more reliable to evaluate ecotoxicological hazards of heavy metals. Besides, molecular correction will be required to reach an accurate picture of relative acute toxic hazard in relation to chemical structure.

INTRODUCTION

Aquatic toxicology is stimulated by the concern for evaluation of the quality of the environment as a whole and in particular the ecotoxicological safety in water bodies for aquaculture fisheries and areas for recreation. Using fish as a bio monitoring tool for the quality of the aquatic environment is becoming more developed especially in marine pollution monitoring programmes (Wester and Vos 1994). The use of acute toxicity tests for assessing the potential hazard of chemical contaminants to aquatic organisms is well documented (Johnson and Finley, 1980). Tin and aluminium cations were found to be highly toxic to both shrimp and artemia followed by cadmium and lead (Elsebae 1993, and 1994). Madigosky *et al*., (1991) reported that lead, cadmium and aluminum are cumulative poisous in both aquatic and terrestrial ecosystems.

Lau (1991) reported that the antifouling agent, tributyltin is severely toxic to nontarget marine or fresh water organisms including microscopic phytoplankton, crab, shrimp, oyster and fish. Ruiz *et al.* (1995) studied the effect of tributyltin on the survival and growth of the bivalve *Scrobicularia plana*. Stromgren and Bongard (1987) demonstrated that tributyl tin fluoride reduced growth rate and shell deposition of the oyster *Mytilus edulis*. According to Pynnonen (1991), high Al^{3+} and H^{+} concentrations cause different physiological disturbances in freshwater organisms in fish (Wood and McDonald, 1982); in crustacea (Havas 1985); and in molluscs (Malley *et al.* 1988).

Bhamra and Costa (1991) reported that elevated Al^{+3} concentration is considered one of the main causes of death in fish especially in acid rain areas and low pH water bodies. The Atlantic salmon exposed to aluminum cation showed a significant reduction in growth and survival particularly at higher concentrations at pH 5.5. RNA and DNA biosynthesis was significantly reduced. (Mckee *et al.* 1989). WHO, IPCS (1995) reported that significant effects have been observed at monomeric inorganic Al^{3+} levels as low as 25 ug/litre. Eisler (1985), reported that sublethal effects to marine organisms recorded at cadmium Cd^{+2} concentrations of 0.5 to 10 ppb (ug/l) included decreased growth, respiratory disruption, altered enzyme levels and abnormal muscular contractions. These adverse effects were most obvious at low salinities and high temperature. Spehar *et al.* (1978) studied the toxicity and bioaccumulation of cadmium and lead in aquatic invertebrates. (Anderson *et al.*, (1986).

Lalande and Pinel-Allool (1986) studied the acute toxicity of cadmium, copper, mercury, and zinc to the invertebrate *Tropocyclops prasinus.* Survival and growth of the aquatic insect *Tanytarsus dissimilis* (Chironomidae) exposed to copper, cadmium, zinc and lead was evaluated by Anderson *et al.* (1980). Mckim and Benott (1971) studied the effects of copper on survival, reproduction, and growth of brook trout fish and concluded that 9.5 ug Cu/l was a safe concentration, for this species in Lake Superior water.

Monmt (1964) developed an autopsy tecluique for zinc-caused fish mortality at higher levels. Besides the effect of copper on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* Bate (Crustacea, Decapoda), was investigated.

In the present investigation, the more abundant heavy metal aquatie pollutants: aluminum, cadmium, chronium, copper, lead, nicked, tin and zinc were compared for their acute toxicity hazard to three marine fish species.

MATERIALS AND METHODS

Tested Heavy Metal Salts

The selected chemically pure water soluble salts of the eight tested heavy metals were:- aluminum chloride, cadmium chloride, lead nitrate, nickel nitrate, potasium chromate, stannous chloride, and zin sulphate. They were available from Sigma Company.

Bioassay Organisms:

Larval stage of age 25 days fry of each of the three species of the marine fish, (sea bass) *Dicentrarachus labrex*, (sea bream) *Sparus auratus* ,and *Solea vulgaris*, were made available by the Marine Culture Center at Alareesh.

<u>Acute Toxicity Bioassay :</u>

The used sea water samples were with total settable solids range 105-116 and pH levels of 6.4-6.8 range, and of average temperature 24.6°C.

Sea water solutions with series of concentrations of each of the tested heavy metal salts were prepared and then were used for acute toxicity testing in three replicates each of 200 ml water solution in 500 ml glass beaker and for each concentration 10 fry larvae of each species of 25 days old fry were added. Control tests were included and kept at room temerature. The tests were repeated twice and the average mortality counts were recorded after 96 hrs. By using the concentration / mortality probits, relationships were expressed in log/probit regression lines. The corresponding 96 hrs LC_{50}'s with their confidense limits and slope of regression lines were computed according to Finney (1971).

The adopted acute toxicity procedure complies with all the specifications stated by the standard practices for conducting toxicity tests reported by the American Society for Testing and Materials (ASTM) (Anonymous, 1980).

RESULTS AND DISCUSSION

Tables 1,2, and 3 present the acute 96 hr LC_{50}'s of the tested heavy metals on the three marine fish species - *Diacentrarachus labrex*, *Sparus auratus* and *Solea volgaris* respectively. According to the 96hr LC_{50} values, the marine fish *Solea vulgaris* proved to be more susceptible to the tested eight heacy metals, followed by *Sparus auratus* and then *Diacentrarachus labrex* in a descending order. Generally, in the three fish species, Aluminum was the more toxic heavy metal tested and zinc was the least toxic. From Tables 1, and 2, the potent toxicity of aluminum

Table (1) :Acute Toxicity of Eight Heavy Metals To the 25 days old fry larvae of *Dicentrarachus labrex* (Sea bass) fish.

Heavy Metal	96 hr LC_{50} $\mu g/l^{-1}$ p.p.b.	Slope	Confidence limits
Aluminum	27	0.329	$4.4 x 10^{-2}$ - $2.2 x 10^{-2}$
Tin	60	0.404	$7.3 x 10^{-2}$ - $4.9 x 10^{-2}$
Copper	78	0.373	$9.5 x 10^{-2}$ - $6.4 x 10^{-2}$
Cadmium	80	0.451	$9.8 x 10^{-2}$ - $6.5 x 10^{-2}$
Lead	85	0.307	$0.4 x 10^{-1}$ - $6.9 x 10^{-2}$
Nickel	600	0.497	$6.9 x 10^{-1}$ - $5.2 x 10^{-1}$
Chronium	800	0.433	$9.2 x 10^{-1}$ - $6.9 x 10^{-1}$
Zinc	1400	0.400	1.6 —1.2

was followed by tin, copper, cadmium, lead, nickel, chromium and zinc in a descending order. However from Table (3), the highly susceptible *Solea vulgaris* fish showed some variations in its response to the acute toxication of the tested heavy metals. The highly toxic aluminum was followed by copper, tin, cadmium, chromium, lead, nichel, and zinc in a descending order. The WHO, IPCS (1995) report, stated that at monomeric inorganic aluminum levels as low as 25 ug/l (25 ppb), acute toxicity of Al^{+3} was demonstrated. Bharma and Costa (1991) reported that Al^{+3} contanination is considered to be one of the main causes of death in fish, especially in lakes with acid rain. Tin was generally the second candidate in its acute hazard to the three marine fish species. Tin was reported to be highly toxic to marine -d fish water orgasism ranging from the microscopic phytoplankton to crab, shrimp, oyster and fish even at very low concentrations in the order of parts per trillion (Lau, 1991). Recently, Ruiz *et al.*, (1995) showed that Tributyl tin (TBT) in the range of 37-102 ng Sn/l^{-1} caused substantial mortalities and negligible shell growth in the bivalve *Scrobicularia plana*. This is why TBT was banned as an antifouling agent. Bambang *et al.*, (1995) demonsteated the acute toxicity of copper on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* Bate (Crustacea, Decapoda) at levels as low as 17ng/l^{-1} (ppt.). Anderson *et al.*(1980) concluded that the acute LC_{50} concentration of cadmium, copper, and zinc, to the invertebrate *Tanytarsus dissimilis* were 3.8, 16.3 and 36.8 ug/l respectively, while the LC_{50} for lead was 258 ug/l^{-1}. This range of toxicity is similar and parallel to the present data. Putte *et al.*, (1981) evaluated the effect of pH on the acute toxicity of hexavalent chromium to rainbow trout fish. Decreased pH increased toxic potency and this implies that the relative acute hazard of these heavy metal cations in the present study is anticipated to be enhanced in highly acidic media.

Table (2) :Acute Toxicity of Eight Heavy Metals To the 25 days old fry larvae of *Sparus auratus* (Sea breem) fish.

Heavy Metal	96 hr LC_{50} µg/l^{-1} p.p.b.	Slope	Confidence limits
Aluminum	9.5	0.578	$1.6x10^{-2}$ - 2.2×10^{-2}
Tin	10.0	0.362	$1.22 x10^{-2}$ - $4.9 x10^{-2}$
Copper	11.0	0.320	$1.3x10^{-2}$ - $6.4 x10^{-2}$
Cadmium	24.0	0.370	$2.9 x10^{-2}$ - $6.5 x10^{-2}$
Lead	37.0	0.459	$4.8 x10^{-2}$ - $6.9 x10^{-2}$
Nickel	37.0	0.333	$4.5 x10^{-2}$ - $5.2 x10^{-1}$
Chronium	43.0	0.325	$5.3 x10^{-2}$ - $6.9 x10^{-1}$
Zinc	270.0	0.342	$3.1x10^{-1}$ - $2.3x10^{-1}$

Table (3) :Acute Toxicity of Eight Heavy Metals To the 25 days old -fry larvae of *Solea vulgaris* fish.

Heavy Metal	96 hr LC_{50} µg/l p.p.b.	Slope	Confidence limits
Aluminum	3.7	0.466	$3.8x10^{-3}$ - $3.6x\ 10^{-3}$
Tin	5.0	0.361	$5.2x10^{-3}$ - $4.8\ x10^{-3}$
Copper	4.8	0.264	$5.1\ x10^{-3}$ - $3.0\ x10^{-3}$
Cadmium	5.4	0.366	$5.6\ x10^{-3}$ - $5.2\ x10^{-3}$
Lead	7.0	0.358	$7.2\ x10^{-3}$ - $6.7\ x10^{-3}$
Nickel	8.4	0.351	$8.9\ x10^{-3}$ - $7.9\ x10^{-3}$
Chronium	6.0	0.281	$0.62\ x10^{-2}$ - $0.58x10^{-2}$
Zinc	30.0	0.322	$3.1\ x\ 10^{-2}$ - $2.8\ x\ 10^{-2}$

Molar Correction of LC_{50}'s:-

The molar conparative susceptibilty of the three marine fish species to the eight tested heavy metals was carried out using the mol. weight of the used inorganic salts as shown in Tables 4 and 5. Molar correction was needed to give equal chances for correlating chemical structure with acute toxicity.

Table (4), indicates the comparative LC_{50} values of the eight heavy metals of the three marine fish species, and their corresponding molar LC_{50} values which are considered more precise and accurate in correlating the toxicaction with molecular structure as reported by El-Sebae (1963) The importance of such molecular correction is most obvious when comparing lead nitrate with nickel nitrate, the two has equal LC_{50} of 37 ug/l to the fish *Sparus auratus*, however with molecular LC_{50}'s lead nitrate was shown to be almost double the acute toxicity of nickel , this is simply because the atomic weight of lead is 207.2 while that of nickel is 58.71.

Table (4): Molar Comparative Susceptibility of The Three Marine fish species To The tested Heavy Metals

Heavy Metal Salts	96 hr LC_{50} µg/l (ppb)			Mol wt	Molar LC_{50} X 10^3		
	Dicentrara -chus labrex	*Sparus auratus*	*Solea vulgaris*		*Dicentrara -chus labrex*	*Sparus auratus*	*Solea vulgaris*
Aluminum Chloride	27	9.5	3.7	133.5	20.22	7.12	2.77
Stannous chloride	60	10.0	5.0	189.0	31.75	5.29	2.64
Copper Sulphate	78	11.0	4.8	159.5	48.80	6.90	3.01
Cadmium Chloride	80	24.0	5.4	182.5	43.84	13.15	2.96
Lead nitate	85	37.0	7.0	331.0	25.68	11.18	2.11
Nickel Nitrate	600	37.0	8.4	182.5	328.76	20.27	4.6
Potasium Chromate	800	43.0	6.0	195.0	412.97	22.16	3.1
Zinc Sulphate	1400	270.0	30.0	161.0	869.57	167.77	18.63

Table (5): Malar Comparative Susceptibility of The Three Marine fish species To The Acute Hazard of The Tested Heavy Metal Salts

Heavy Metal Salts	Mol wt.	*Dicentrarachus labrex*		*Sparus auratus*		*Solea vulgaris*	
		acute hazard rank	Molar acute hazard rank	acute hazard rank	Molar Acute hazard rank	acute hazard rank	Molar acute hazard rank
$AlCl_3$	133.5	*1st*	1st	*1st*	*3rd*	1st	*3rd*
$Sn(Cl)_2$	189.0	*2nd*	3rd	*2nd*	*1st*	3rd	*2nd*
$CuSO_4$	159.5	*3rd*	5th	*3rd*	*2nd*	2nd	*5th*
$Cd(NO_3)_2$	182.5	*4th*	4th	*4th*	*5th*	4th	*4th*
$Pb(NO_3)_2$	331.0	*5th*	2nd	*5th*	*4th*	6th	1st
$Ni(NO_3)_2$	182.5	*6th*	6th	*6th*	*6th*	7th	7th
K_2CrO_4	194.0	*7th*	7th	*7th*	*7th*	5th	6th
$ZnSO_4$	161.0	*8th*	8th	*8th*	*8th*	8th	8th

Ranking the relative acute toxicity hazard of the eight heavy metal salts to the three marine fish species was presented in Table 5, where the molecular correction indicates the more precise order of relative hazards. Aluminum lost its first rank against *Sparus auratus* to tin, and also against *Solea vulgaris* to lead. Thus it can be concluded that aluminum, tin, and lead were of the first order of acute toxic hazard to the three marine fish species *Dicentrarachus labrex*, *Sparus auratus* and *Solea vulgaris* respectively. Such variation in the three marine species reflects the specificity in susceptibility of fish to various pollutants. This suggests that ecotoxicological management requires broad screening of the environmental pollutants against a great number of the economic fish species. Reliance on one species will not be indicative for the actual hazard and thus will be very deceiving. Such multi species acute toxicity screening is requiered for ecotoxicological risk assessment.

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الملخص العربى

السمية الحادة الجزيئية لثمانية املاح من المعادن الثقيلة على ثلاثة انواع من الاسماك البحرية

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تم عمل دراسة مقارنة بين ثمانية املاح للمعادن الثقيلة الاتية المذابة فى عينات من مياه البحر:- كلوريد الومنيوم – كلوريد كادميوم – نترات رصاص – نترات نيكل – كرومات بوتاسيوم – كلوريد قصديروز – وكبريتات الزنك وكبريتات النحاس. وقد تم تقدير الجرعة المتوسطة للموت بعد ٩٦ ساعة بعد تعريض يرقات زريعة كل من انواع السمك الثلاثة لسلسلة تركيزات من املاح المعادن الثقيلة الثمانية. وكانت انواع السمك البحرى الثلاثة هى :- *Dicenrarachus labrex, Sparus auratus* , *Solea vulgaris*. وقد اظهرت النتائج تفاوتا فى السمية الحادة للمعادن الثقيلة المختبرة ولكى يتم الربط بين التركيب الكيميائى والتأثير السام تم تعديل الجرعات المتوسطة للموت الى السمية النسبية والتى يمكن ربطها بالتركيب الكيميائى عن طريق التركيزات الجزيئية . ومن ذلك فيمكن التوصية بضرورة اجراء اختبارات السمية البيئية على اكثر من نوع من الاسماك والكائنات لتفاوت حساسيتها كما أن التركيزات الجزيئية المتوسطة للموت لكل من المعادن الثقيلة الثمانية ضد الانواع الثلاثة للاسماك البحرية.

وقد اوضحت أن الالومنيوم هو أشد المعادن الثقيلة سمية ضد النوع الاول من الاسماك البحرية *Dicentraraclus labrex* – بينما كان القصدير هو أشد المعادن الثقيلة سمية ضد النوع الثانى *Sparus auratus* فى حين كان الرصاص هو اشدها سمية ضد النوع الثالث Solea vulgaris. كما تبين ان هذا النوع من الاسماك (*Solea vulgaris*) هو اكثرها حساسية بصفة عامة ضد المعادن الثقيلة وبينما كان اقلها حساسية النوع الاول *Dicentraraclus labrex* .