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Individual and combined effects of Copper and Lead on the fresh water crab *Sesarma boulengeri* from Shatt Al-Arab , Basrah . Iraq

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Abstract

The acute toxicity by single or combined of Copper and Lead on the fresh water crab *Sesarma boulengeri* (Calman , 1920) was evaluated by static bioassays, calculating the values of LT_{50} (median lethal time) and LC_{50} (median lethal concentration), after exposure to concentrations (50,100,150,175 and 200) µg l^{-1}.Each bioassay tested up to 96 hours, the survival time was decreased with increasing concentrations of Copper and Lead in the laboratory circumstances. Copper was more toxic to *S. boulengeri* than Lead, when tested individually. When tested in combination, the toxicity increased, and the expected mortalities were not similar to those observed mortalities, the effect of paired metals is synergistic and the two metals acted interactively. Toxicities of mixtures of Copper and Lead were assessed using the toxic-unit concept.

Key words: Copper, Lead, median lethal time, median lethal concentration, toxic , *Sesarma boulengeri* .

1- Introduction

Crustaceans is frequently used as bio indicators tend to concentrate various toxic and non toxic trace metals in their bodies with no evident danger to themselves. Crustaceans were contaminated by trace metals may lead to bioaccumulation in the food chain including macro invertebrates (Ahsanullah *et al.,*1988).Trace metals are taken up and accumulated by aquatic organisms, both from the surrounding medium and via food sources (Wang *et al.,*2005). Many trace metals play essential roles in metabolism, and all have the potential to cause eco-toxicological effects (Morrisey *et al*., 2003). The presence of these metals in the aquatic ecosystem has far-reaching implications directly to the

biota and indirectly to man (Ravera *et al.,* 2007).Trace metals have been referred to as common pollutants, which are widely distributed in the environment with sources mainly from the weathering of minerals and soils (O' Neil, 1993). The United States Environmental Protection Agency has classified Pb as being potentially hazardous and toxic to most forms of life (USEPA, 1986). It has been found to be responsible for quite a number of ailments in humans such as chronic neurological disorders especially in fetuses and children. Automobile exhaust fumes have been reported to account for about 50% of the total inorganic Pb absorbed by human beings Other inputs of Pb into the environment are from used dry-cell batteries, sewage effluent, runoff of wastes and atmospheric deposition (Mohan and Hosetti, 1998). Effect of lead in the aquatic environment increase with the effect of fluctuating temperature (weiner, 2000) .

Copper is classified as essential metal to life due to their involvement in certain physiological processes. Elevated levels of Copper, however, have been found to be toxic (Spear, 1981). Copper form the essential group of metals required for some metabolic activities in organisms (Teo and Chen, 2001). Cu is found in natural water as a trace metal usually at concentration < 5μg l^{-1} but can also be present at much higher concentration as a result of industrial processes (MAFF, 1995) . Relatively little is known about the combined effect of mixture of metals which may be dangerous. The most basic model is the Toxic Unit Model which involves determining the toxic strength of an individual compound , expressed as a toxic-Unit (Landis and Ya, 1995). S. *boulengeri* occurs along the intertidal zone of Hammadan canal which is a branch from Shatt Al-Arab . It tolerates a wide range of temperatures and salinities. It is important for small-scale commercial fisheries in ShattAl-Arab .This crab eat a wide variety of food both plant (like *Ceratophyllum* spp) and animal food (like *Gambusea affinnis*), and are preyed up on extensively by fish and birds. Local studies were done on the concentration of trace metals in the Shatt Al-Arab River (Abaychi and DouAbul ,1985; Abaychi and Mustafa ,1988 ; Abaychi and Al Saad ,1988). They showed contamination with the trace metals in the water, sediment and the organisms in the habitat of the Shatt Al-Arab River .The water lied on the permitted level in spite of the domestic agriculture and industrial pollutants beside the oil pollution. Al- Higag (1997) assessed the concentration of Cd , Cu, Pb and Zn in Al- Ashar and in Al- khandak canals. Al-Mansoori (1999) determined the

toxicity of (Pb, Cu, Cd, and Zn on fresh water shrimp *Atyaephyra desmaresti mesopotamica* Al- Imarah *et al*. (1997) assessed the concentration of Cd , Cu,Fe,Pb,Fe,Ni,V and Ni in tow species of commonly edible shrimp *Metapenaeus affiinis* and *Penaeus semisulcatus* from Iraq and Kuwait . Local studies were done on Crab *S. boulengeri*, Al-Yaseri (2007) measured the toxicity of Lead and the concentration of some trace metals (Pb, Cu, Mn, Fe, Ni and Zn) in a whole body of *S. boulengeri* from Shatt Al-Arab River, Sultan (2004) measured the toxicity of Selenium on Crab *S. boulengeri*, Al-Samer (1989) measured the toxicity of some trace metals on Crab *S. boulengeri*.

2- The aim of the study

The aim of this study is to assess the acute lethality of Copper and Lead individually and paired combinations to crab *S.boulengeri* using the method of Stratton and Cork described by Aoyama *et al*., (1987) to understand the kind of effect whether synergistic or antagonistic.

3- Material and Methods

The crab *S .boulengeri* were collected from the intertidal zone of Hammdan canal (Fig 1).The study was carried out in May 2009 on individuals of which is located in Abou Al-Khaseeb as a branch from Shatt Al- Arab River. The test individuals male and female of the crabs were transferred to an aquarium (60x30x30) cm for acclimation period of seven days prior the toxicity experiment, under laboratory temperature of 20 ± 2º C with light cycle of (12= 12) light and dark period. After the acclimation period the dead and week animals were discharged. Stock solution of BDH grade chemicals, Cu (II) sulphate ($CuSO_4.3H_2O$) and Pb $(NO_3)_2$ were prepared in fresh water from Shatt Al-Arab and diluted as required.The concentrations of 50, 100,150,175,and 200 µg l^{-1} of Cu and Pb were used besides the control (fresh biota water).Three replicate series of experiments were carried out simultaneously for Cu and Pb. The test solutions were then slowly added to each experimental aquarium ,10 crabs were added to each aquarium *S .boulengeri* may survive in this manner for 96 hours the mean body weight was 2.566 gm ±0.124 gm. Female crab differ by their broad abdomen. Animals were checked daily for mortality. Death was considered in the absence of movement when the test organisms were prodded. Dead individuals were removed after each observation. The experiments were conducted at 26.5ºC ± 2,2. 2.65%0 ± 0.5 salinity,7.70 ± 0.2 pH for each of the two metals separately and combined. LT50 is the time elapsed from the beginning of the experiment to the death of the half of individuals tested.

Stratton and Cork found in Aoyama *et al*., (1987) in order to describ the kind of effect whether synergistic or antagonistic according to formula :

$$Pe = P(a)+P(b)\ (100-P(a))\ /\ 100$$

Where :

Pe = expected effect of the two pollutants ; Pb and Cu (a and b) when they were added together.

Po= observed effect of the two pollutants ; Pb and Cu (a and b) when they were added together.

P(a) = LC50 for Pb after 96 hours(pollutant a) .

P(b) = LC50 for Cu after 96 hours(pollutant b).

From compare the expected (Pe) and the observed (Po) values of the two pollutants, When :

Pe $\leq$ Po this mean that the effect is synergistic ,and if Pe > Po this means that the effect is antagonistic.

4-Results and Discussion

The toxicity test was done by recognizing the survival after exposure to contaminated and uncontaminated (control) water (ASTM, 1990 and U.S. COE, 1991). None of the control animals were died. Mortality of *S. boulengeri* increased with increasing Copper and Lead concentrations .

96 hours LC50 and LT50 values of Copper and Lead of *S. boulengeri* are shown in Tables 1 and 2. No mortalities were observed at the end of the 96 hours exposure at the concentration of 50 µg l^{-1} Cu and 50 µg l^{-1} Pb. The results of 96 hours LC50 determination showed that Copper was more toxic than Lead in male and female *S.boulengeri* and this was agree with Abdullah,(1986) in his study on isopod *Asellus.aquaticus* ,while the combined effect was the most toxic .

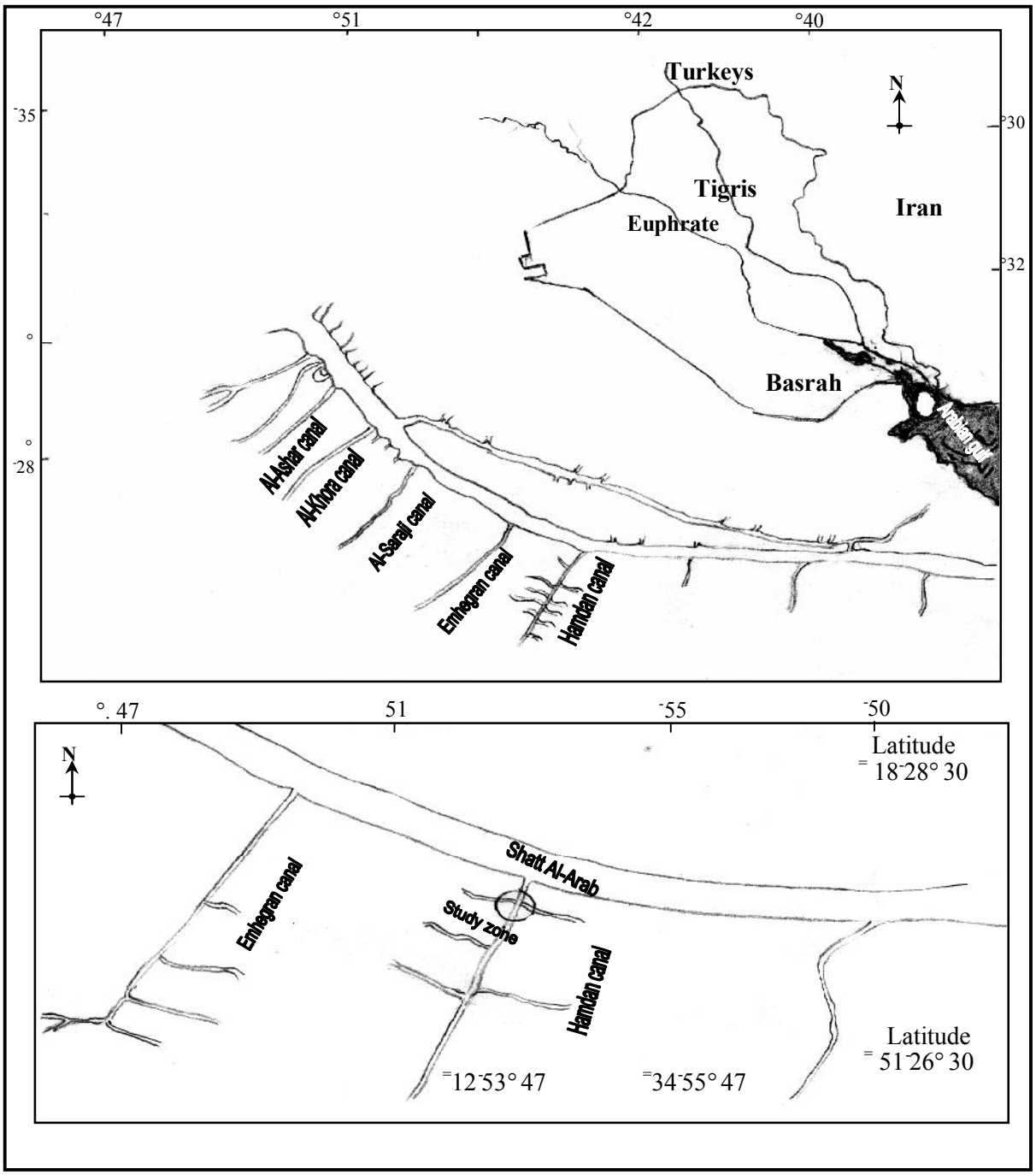

Fig .1: Shatt Al-Arab and Hammadan canal (the sampling site).

Table 1.The 96 hours LC_{50} values (µg l^{-1}) with standard deviation of the freshwater crab *S..boulengeri* exposed to Copper and Lead and combinations of the two metals

Sex	Cu LC_{50}	Pb LC_{50}	Cu+Pb LC_{50}
Male	15.25 ± 0.321	22.50 ±0.295	36.25 ±0.964
Female	14.50 ±0.865	18.25 ±0.731	32.55 ±0.686

Table 2. The LT_{50} values (hours) of the freshwater crab *S..boulengeri* exposed to Copper and Lead and combinations of the two metals exposed to lower and higher concentrations.

Sex	Pb LT_{50}	Cu LT_{50}	Cu+pb LT_{50}	Pb LT_{50}	Cu LT_{50}	Cu+pb LT_{50}
Male	>96	>96	>96	103.2	72	62.4
Female	>96	>96	>96	79.2	74.8	47.6

When the combined effect of trace metals means applied with the summation of parts of single compounds toxicity this is known the (additive effect), when the combined effect higher than expected , this effect is known (more than additive effect), or less than expected this effect is known (less than additive effect), (Spragu,1970). The combined effect of two trace metals give a clear idea about the effect of trace metals upon the organisms, Moraltou-Apostolopoulos and Verriopoulos(1٩82) was illustrated that when the two metals were added together they give synergistic effect when the mortality ratio were high in copypod *Tisbe holothuriae* (39%, 42%, and70%) to (Cu+Cd, Cu+Cr and Cd+Cr) respectively

The values of trace metals toxicity depends upon the kind of species, Murti and Shukla (1984) was illustrated that the value of 96h LC_{50} for shrimp *Macrobrachium lamarrei* (0.246 and 3.188) mg l^{-1} for (Cu and Zn), while Correa (1987) was illustrated that the value of 96h LC_{50} for shrimp *Macrobrachium carcinus* (0.1 and 0.2) mg l^{-1} for (Cu and Zn) ,while Break *etal.,*(1976) was found synergistic effect of the two metals (Cd+Zn) on the diatoms *Thalassiasira psedonana*, *Amhidrium carteri* and antagonism on the diatoms *Phaeodactylum tricornutum*

The toxicity curves of Cu, Pb and Cu + Pb of the crab *S .boulengeri* are shown in Figure 1. Lead, which is a non-essential metals, is significantly less toxic to the crab than Copper, which is an essential

metal, these results agree with the toxicity studies on other invertebrate ,Shrimp *Callianassa australiensis* (Ahsanullah *et al.,*1981;Negilski et al.,1981) .this may be due to the increasing of accumulation of Copper concentration in blood and hepatopancreas gland, while Zn accumulated in flashy tissues in their study whereas the combined effect of the two metals were the most toxic. This may be partly due to the geographical situation of the sites which have a high background concentrations of metals such as Copper. (Bryan, 1976). It is expected, therefore, that invertebrates of the region may have developed either a physiological or genetic adaptation or a combination of both to some metals (De Nicola *et al*., 1992 a and b).It is concluded that the differences between the observed percentage mortality and that expected by independent dissimilar action is insignificant .

Mortalities occurred at all concentrations (Table ٣and 4) In this study, the toxic – units which calculated for 96 hours indicated that the Cu and Pb in paired mixtures acted in an interactive manner. Moreover, the toxicity of Pb was less than of Cu at lower and higher concentrations of Cu and Pb, indicating that more than 50% of exposed population of *S .boulengeri* would survive in lower concentrations, while at higher concentrations of single and paired metals, more than 50% of population would die. The results showed from Stratton and Cork equation ,the expected effect (Pe) > the observed effect (Po) , this mean that the effect is synergistic when the two metals were added together, and this applied with (Abdullah and King 2004) in their study, the effect of Cu and Zn in crustacean *Asellus aquaticus* .

The aquatic organisms may be exposed in the field for many different combinations of pollutants like different trace metals, most acute toxicity tested have considered only the individual effects of these pollutants. There is little information about the combined effects of mixture of metals which may be most dangerous. Therefore, the acute toxicity of Copper and Lead to the crab *S.boulengeri* were assessed in paired combinations. In some studies the effects of mixture of some heavy metals (Cu and Pb) have been reported as synergistic effect on shrimp *Atyaephyra desmaresti mesopotamica* (Al-Mansoori, 1999)

The results of the present study showed that Lead, which is a non-essential metals, is significantly less toxic to the crab than Copper, which is an essential metal, the toxicity of combination of two metals (Pb + Cu) was different from that of individual metal , The mixture of two metals had synergistic effect on the crab *S. boulengei*

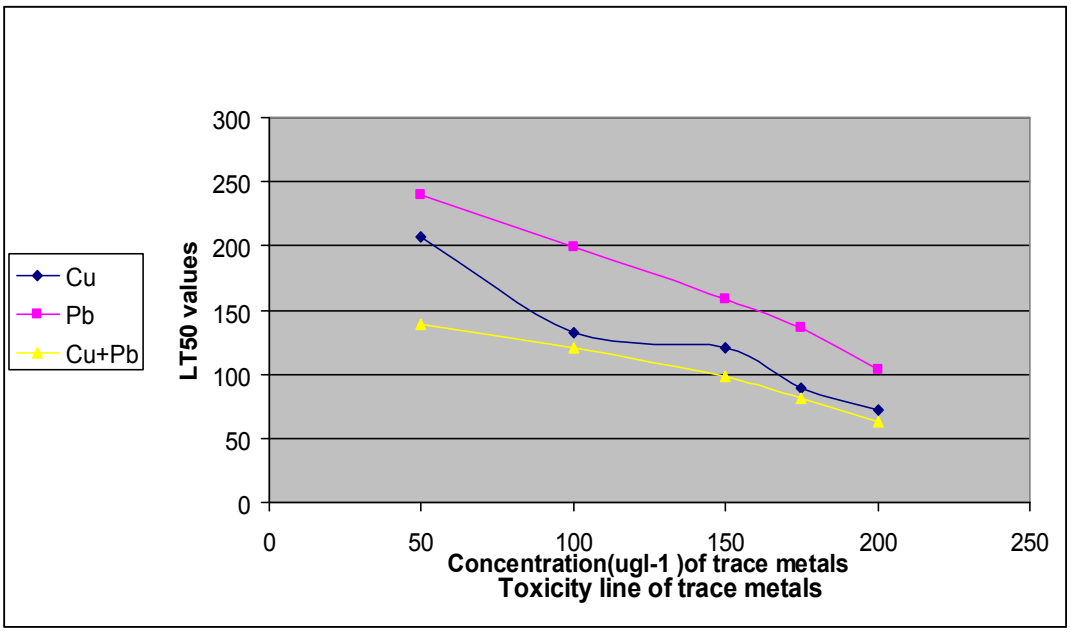

Fig .2 : The toxicity curves of *S .boulengeri* for Cu , Pb and Cu + Pb

Table 3. Concentrations of Cu and Pb with expected percentage mortalities, expected and observed mortalities and toxic units for mixture concentrations for 96 hours of male *S. boulengeri*.

concentrations of metals mg l^{-1}		No, of animals	% mortality observed		% mortality expected		% observed mortality of mixture	% expected mortality of mixture
Cu	**Pb**		**Cu**	**Pb**	**Cu**	**Pb**		
control	Control	10	----	---	---	----	---	---------
50	50	10	20	13.3	21	12	30	29
100	100	10	33.33	20	32	22	36.66	36
150	150	10	40	26.66	38	27	50	38
175	175	10	53.33	36.66	55	35	66.66	60
200	200	10	70	46.66	66	47	77	73.33

Table 4. Concentrations of Cu and Pb with expected percentage mortalities, expected and observed mortalities and toxic units for mixture concentrations for 96 hours of female of *S .boulengeri*.

concentrations of metals mg l^{-1}		No, of animals	%mortality observed		%mortality expected		% observed mortality ofmixture	% expected mortality of mixture
Cu	Pb		Cu	Pb	Cu	Pb		
ontrol	control	10	----	---	---	-----	---	---------
50	50	10	23.33	26.66	21	29	26.66	22
100	100	10	26.66	36.66	28	38	33.33	30
150	150	10	43.33	43.33	40	45	56.66	54
175	175	10	63.33	63.33	64	53	70	66
200	200	10	76.66	66.56	78	58	88	83.33

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التأثير المفرد والمشترك للنحاس والرصاص في السرطان النهري *Sesarma boulengeri* المستجمع من شط العرب , البصرة . العراق .

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الخلاصة

اختبرت السمية الحادة لعنصري النحاس والرصاص كل على انفراد واختبرت السمية للعنصرين سوياً , في التأثير على السرطان النهري (Calman , 1920) *S. boulengeri* حسبت السمية باستخدام الإحصاء الإحيائي , قيم متوسط الزمن المميت (LT_{50}) بالساعات وقيم التركيز المميت LC_{50} جزء بالمليون حسبت بعد التعريض للتراكيز(٥٠و ١٠٠ ١٥٠و١٧٥ و ٢٠٠) جزء بالمليون مختبرياً لفترة ٩٦ ساعة . حسبت أعداد الذكور والإناث الحية الباقية بعد التعريض للعنصرين مفردين ومجتمعين . إن زيادة التراكيز تؤدي إلى وفيات أكثر في الظروف المختبرية . وجد إن النحاس (وهو من العناصر النزرة الأساسية) أكثر سمية من الرصاص (وهو من العناصر النزرة السامة الغير اساسية) , ووجد إن السمية تزداد عند مزجهما معاً أي أن التأثير ترافقي وليس تضادي في هذه الدراسة .

كلمات مفتاحية : النحاس , الرصاص , متوسط الزمن المميت , متوسط التركيز المميت , السمية , السرطان النهري