Sublethal Effect of Zinc Sulphate Concentrations on Lake Qarun Brine Shrimp (Artemia parthenogenetica) on Survival, Maturity, Reproductive and Life Span Characteristics

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ADDITIONAL INDEX WORDS: Zn so4, Artemia parthenogenetica, Qarun, Survival, Maturity, Reproductive and life sapn.

ABSTRACT

Inland Artemia strain (Artemia parthenogenetica) from Qarun Lake was assayed to their response to stress by using 4 sublethal zinc sulphate (Zn so₄) concentrations (0.1, 0.25, 0.5 and 1mg Zn so₄ /I) and control under laboratory conditions. The effects of zinc stress on survival, maturation, reproductive and life span characteristics of Lake Qarun brine shrimp (A. parthenogenetica) were investigated. The results revealed that, there was inversely proportional relation between the survival and elevated zinc sulphate (Zn so₄) concentrations after certain time according to the concentrations and time of exposure.

At low Zn so₄ concentrations (0.1 and 0.25 mg/l Zn so₄) the animals were able to detoxify the accumulated Zn so₄ and proceed with its biological development. High survival % and maturation were obtained at these low Zn so₄ concentrations in such a way there were no significant differences among treated organisms and control. With the progressive days Zn so₄ has been found to have very negative effect on the reproductive and life span characteristics (reproductive period from 25.05 to 13.65 day, post reproductive period from 2 to 1.6 days and total life span from 45.8 to 39.35 day) especially cysts hatching percentage. The present study indicated that Zn so₄ has high negative effects on the maturity and reproductive and life span characteristics of Lake Qarun brine shrimp (*A. parthenogenetica*) Highly negative effects of sublethel Zn so₄ doses were also observed on its₄ cyst hatchability.

INTRODUCTION

Lakes among most important food sources, are subjected to high pollutant loadings from a wide variety of sources, including municipal waste, industrial wastewater discharges, storm sewer overflow, runoff from agriculture and atmospheric deposition (Chow and patterson, 1966 and Langford, 1971). Aquatic organisms and flora break down organic molecules to carbon dioxide and water. However non-biodegradable metal are gradually concentrating in these water bodies (Allen, 1994).

Lake Qarun is a closed saline lake in the northern part of El-

Fayoum Depression (Middle Egypt) at the margin of the Great Western Desert. It is almost entirely sustained by inflow from the Nile River and, during the 20th century, lake water salinity has increased strongly (Fathi and Flower, 2005) and became polluted with heavy metals and a wide variety of pesticides (e.g. lindane, aldrin, some DDT analogues, malathion) (Ali et al., 2008). The effluents of The Egyptian Company for Salts and Mineral (EMISAL) discharged into the lake also caused increasing in most of the pollution parameters (Authman, and Abbas, 2007). The identified phytoplankton species from Qarun lake during 2001 indicate a tendency towards eutrophy but total crop densities were relatively low compared with eutrophic lakes elsewhere (Fathi and Flower, 2005). Mohamed et al., (2007); Hussein et al., (2008) Fatma and Mohamed (2009) were concluded that the environmental contamination of Lake Qarun induced several histopathological alterations in the tissues of Tilapia zillii and Solea vulgaris. Gad (2008) showed that the abundance of metals in lake Qarun water followed the order of Zn>Pb>Fe>Hg>Mn>Cu, with concentrations of 0.626 ± 0.08 ; 0.384 ± 0.04 ; 0.346 ± 0.001 ; 0.311 ± 0.003 ; 0.051 ± 0.005 and 0.040 ± 0.003mg/l respectively. Aquatic organisms accumulate metals to concentrations many times higher than present in water or sediment (Oloja et al., 2005). The results of Gad (2008) revealed that zinc concentration in lake Qarun water was 0.626 mg/l, however, it was 55.88 and 127.07; 60.38 mg/kg in muscles and 65.94; 46.38 and 51.75 mg/kg in liver (dry weight) of Tilapia zillii; Solea volgaris and Mugil capito respectively.

The economical importance acquired by the cyst of Artemia for feeding fish and shellfish larvae has been increased during the last 20 years (Abatzopoulos *et al.*, 1998). Both cyst and nauplii size variation appears to be first criterion that at least with some predator species determines the ingestibility of species Artemia nauplii (Beck *et al.*, 1980). The size of cyst and nauplii from Qarun seems to be comparable to the Artemia franciscana cyst, which is one of the main sources of brine shrimp cysts for use in aquaculture (El-Bermawi, 2003). This feature makes the Artemia population from Qarun very attractive for commercial use. But low cyst hatchability is one of main obstacle limiting its use in aquaculture. Zinc is one of the metal that exist in Qarun lake water (El-shabrawi, (2001); Mohamed *et al.*, (2007); Gad, (2008); Hussein *et al.*, (2008); and Fatma and Mohamed (2009).

A. parthenogenetica from Qarun is the most qualified Egyptian Artemia strain to be used in the marine hatcheries (El-Bermawi, 2003; El-Bermawi, et al., 2004). The present study aimed to investigate the potential of Qarun A. parthenogenetica strain under Zn so₄ chronic stress, which is the highest element in the lake water.

MATERIAL AND METHODS Artemia strains

Parthenogenetic Artemia (A. parthenogenetica) cysts population were collected from the Egyptian Company for Salts and Mineral (EMISAL-Fig. 1) located on the southern coast of Qarun Lake, and processed according to Lavens and Sorgeloos (1996). Different sieves were used (different mesh sizes) in the beginning to remove all foreign materials from the collected cysts. Density separation was conducted in brine (>200g/l) with strong aeration for one day using Instant Ocean[®] (synthetic sea salt, Aquarium Systems, Inc, Mentor, Ohio, USA) salt mixture. The aeration was stopped for 2 hrs in order to allow heavy debris and full cysts to sink, empty cysts and light debris to float. Cysts and light debris were collected and washed with fresh water for 10 min using a 150µm mesh sieve to remove all the salt. For full Artemia cysts separation cysts were collected from the bottom of the cone. Fast and homogeneous drying of the cysts in oven at temperature <40°C was provided until 10% water content in order to stop the metabolic activity in the cysts. Dried cysts were vacuum packed and stored at 4°C.

Toxicity tests

A. parthenogenetica cysts were hatched under the optimal conditions according to the method of Sorgeloos *et al.* (1986). Artemia of 24 hours old freshly hatched nauplii were transferred to one liter cylindro-conical recipients with an initial density of 1 nauplii per 3 ml at $22\pm1^{\circ}$ C. Five concentrations of Znso₄ were used (0.0, 0.1, 0.25, 0.5 and 1 mg Znso₄/l). The hatching of the cysts and experiments were performed in artificial seawater with a salinity of 36 g/l (Instant Ocean[®] Synthetic Sea Salts, Aquarium Systems).

Three replicate experiments were set up for each Znso₄ concentration. The animals were cultured in cones and fed with a mixed diet of the yeast-based formulated feed LANSY PZ (INVE Aquaculture NV Belgium), and *Dunaliella salina* (from El-Max saline company) following the feeding schedule suggested by Triantaphyllidis *et al.* (1995). Mortality was monitored on a daily basis and nauplii were considered death if no movement of the appendages was observed. The rate of sexual maturation was determined on the basis of the occurrence of well-developed egg sacs in females. Water was renewal every 3 days after the animals matured.

Reproductive performance

Randomly twenty females from the mature A. parthenogenetica were taken from the mass culture and placed in 50 ml plastic cylindro-conical tubes and examined daily for mortality and offspring production under the same exposure to Znso4 exposure concentrations at room temperature treatments, i.e. 22 1°C. The remaining animals were further cultured under the same conditions and samples were collected to determine the performance. reproductive reproductive Six and four life-span characteristics were according to Browne et al. (1988): offspring per brood, number of broods per females, percent of the encysted offspring, total offspring per female, days between broods, pre-reproductive period in days, reproductive period in days, post-reproductive period in days, total life span in days and hatching percentage of cysts. Offspring were removed and counted, nauplii were counted and cysts, which had been produced, were filtered, kept in brine of 200 g/l in the refrigerator for 4°C for three weeks, and re-hydrated under identical hatching conditions to determine the hatchability of the cysts.

The results were statistically analyzed by one-way ANOVA (Sokal and Rohlf, 1981)

RESULTS

Survival of A. parthenogenetica

The survivals of A. parthenogenetica affect by exposure to four different Znso₄ concentrations treatments are presented in Table (1) and Fig (2). There are significant (P<0.01) differences in survival between the control treatment and Znso₄ treatments over the higher exposure range (0.5 and 1 mg/l Znso₄) especially at days 3, 6, and 9. It was clear that more than 50% and 35% of the organisms died within18 days of exposure to 1 and 0.5 mg/l Znso₄ respectively. There were no significant differences among the control and the low level of Znso₄ (0.1to 0.25 mg/l Znso₄) for the entire exposure period. The highest tolerance of the organisms was (P<0.01) in the range of 0.1to 0.25 mg/l Znso₄.

Maturity of A. parthenogenetica

Numbers of animals reaching maturity during $Znso_4$ exposure after hatching is presented in Table 2 and Fig. 3. The required time to reach maturation was (P<0.05) significantly affected by $Znso_4$ treatment with the progressive days. No maturation occurred at $1mg/I Znso_4$ within 24 days. Under the three other $Znso_4$ concentrations (0.1, 0.025 and 0.5) used in the present study, the required period to reach maturity significantly (P<0.05) increased and maturation was slower compared to the control group. In control group the first maturity was observed after 9 days and it was significantly (P < 0.05) different from $0.1 \text{mg/l} \text{ Znso}_4$ at the same period of time. After 24 days 100% of the survived control organisms had reach maturity while 55.11, 44.11, and 33.64% of the treated animals with Znso₄ at the same period of time. It was clear that the effect of Znso₄ on maturity was even stronger at higher concentrations.

Reproductive performance and life span of A. parthenogenetica

Effects of different Znso4 concentrations on A. parthenogenetica from Qarun Lake on reproductive and life span characteristics are summarized in Table 3. It should be noted that there were no data for 1mg/l Znso4 treatment due to high mortality. Statistical analysis of the results indicated significant (P<0.05) differences exist between the Znso₄ levels treatments and the control treatment for most of the studied characteristics. Mean reproductive and post-reproductive period were significantly (P<0.05) reduced compared to control (P<0.05). The longest reproductive period was recorded at 0.1 mg/l Znso₄ among remaining treatments (25.05 days). First offspring production at low Znso₄ level was after 18.75 days and it was longer with increasing Znso₄ levels 21.25 and 24.1 days for 0.25 and 0.5mg/l Znso₄ respectively. There was no effect of Zn so₄ (*P*>0.05) on number of offspring per broods with elevated concentrations. Further more there were not significantly differences among all the treatment and the control for number of broods per female and adult post-productive period. Intrapopulation comparison revealed that Znso4 above 0.1 mg/l Znso4 has a negative effect on reproductive output. This is most obvious when considering the number of offspring per brood (36.94 ±4.97, 31.77 ±3.12 and 20.21 ±5.71) and total offspring per female (199.13 ±23.43, 156.40 ±28.24 and 93.46 ±31.53).

Cyst hatchability of A. parthenogenetica

During the experimental period Artemia cyst percentage (14.60 ±11.31; 14.25 ±7.67 and 17.27 ±13.01) were deposited by only a small number of females in each treatment group especially at high Znso4 level $(0.5 \text{ mg/l Znso}_4)$. The majority of cysts were produces from the 1st to 3rd broods, all the cysts which has been produced during the reproductive period has been scored for hatchability after storing in the refrigerator for 3 weeks then dehydrated. Artemia cysts from all Znso4 treatment were (P<0.01) viable (less than 30% significantly less with elevated concentrations of Znso₄) than control cysts. There were significant differences (P<0.01) between the control (91.96 ±3.83) and all the treated organisms in cyst hatching percentage (27.12 ±17.58, 24.53 ±8.18 and 22,54 ±4,61); it was clear the negative effect of Znso4 on the A. parthenogenetica hatching percentage.

DISCUSSION

Modern civilization together with increased industrial activities brought gradual redistribution of a number of toxic elements. The presence of these elements in tissues reflects the contact of organisms with their environment (Nováková et al., 2007 and Dvořák et al., 2005). Essential metals such as zinc (Zn) and Copper are necessary for proper growth, but the optimum concentrations are presumably those normally found in the environment and higher concentrations lead to inhabitation. Although in lobster Homarus gammarus zinc readily absorbed from the gut and is probably largely obtained from its food, the starving animals lose very little Zn and appear to be able to equilibrate with water containing about 0.004 ppm of the metal. The blood flowing through the gills contains about 7ppm (wet) of Zn, which is 103-104 times greater than the amount in the water. However, this does not mean that the concentration gradient across the gills is maintained by active transport because, in the blood, Zn is so tightly bound to proteins such as haemocyanin that the concentration gradient for unbound Zn usually favors its penetration from the water through the gill into the blood. Thus, a dialysis sac containing blood will exchange Zn with seawater until the concentration inside is more than 2000times of the water (Bryan, 1971).

Parthenogenetic Artemia species have been described to be more broadly tolerant of environmental stress of heavy metals. (Forbes *et al.*, 1995). In general Artemia species (sexual and a sexual) are among those crustaceans that most tolerant to many toxicants (Bryan 1976). Zinc toxicity depends on the uptake and accumulation rate and the intrinsic sensitivity of the organisms for the metal and is capable of inducing detoxifying systems (McLuskey *et al.*, 1986 and Chou *et al.*, 1987). In the present study it was clear that the inversely proportional relation between the survival and elevated Zn concentrations after certain time were in accordance to the concentrations and time of exposure.

At low Zn so₄ concentrations (0.1and 0.25 mg/l Znso₄) the animals were able to detoxify the accumulated zinc and proceed with its biological development. High survival % and maturation were obtained at these low zinc concentrations in such way there were no significant differences among treated organisms and control with the progressive days. At high Zn concentrations (0.5 and 1mg/l Znso₄) the survival (55.16 and11%) and maturation (32.64 and 0%) retardation was more pronounced. Such phenomenon has been explained by Tedengren *et al.* (1999) with mussels. McFarlane and Franzin (1980) stated that zinc concentration of 10mg/l reduced survival of cod, they concluded that fish from Hamell Lake (where high Znso₄ concentration is similar to toxicity tests) were experiencing

reduced spawning success, reduced larval and egg survival, smaller egg size, and reduced survival. Also Toppin *et al.* (1987) recorded reductions in *Porcello* progeny numbers in Zn-nich diets.

Toxicant induced disruption in somatic physiology may include changes in behaviors well as in appetite, feeding, assimilation, neurosecretory function, heart beat, excretory efficiency, and other somatic aspects, which may influence reproductive performance (Grosch, 1976). The affinity of Zn for binding with sulphydryl group of many enzymes (Mouneryrac *et al.*, 1998) may result in inhibition of many enzymes-mediated steps in metabolism. Perturbation in reproductive performance, which may disrupt reproductive performance might include genetic damage, dis-function of secretory cells associated with nutrition, cell wall formation of the cyst. The effect of Zn on fathead minnows was studied over a period of 10 months by Bryan (1966), who found that reproduction was almost totally inhibited at concentrations, which had no effect on survival, growth or maturation of the fish. At a concentration of 0.18 ppm of Zn the number of eggs produced was 83% lower that at the control concentration of 0.03ppm. Our results in Table 3 revealed that Zn has a marked effect on the life cycle performance of the A. parthenogenetica. This can explain in terms of the examined reproductive and life span characteristics the negative effect of elevated Zn level on reproductive period from 25.05 to 13.65 day, post reproductive period from 2 to 1.6 days and total life span from 45.8 to 39.35 day. This agree with Toppin *et al.*, (1987) who reported that the response of *F. eroclitus* to heavy metal stress involved changes in life history characteristics; fish from contaminated sites were smaller, and had shorter life-spans. On the other hand elevated Zn level has a great effect on the offspring per brood (34.67 to 20.21 offspring) and total offspring during the reproductive period (207.90 to93.46 offspring). This may agree with finding of Joosse et al., (1981) how recorded reductions in progeny numbers, growth and food consumption in Zn-rich diets, Zn is shown to have a synergistic effect, reducing brood size and extending gestation time in *Porcello*. Cysts are the resistant (over wintering) zygotes encapsulated within a thick shell, which are avoided directly into the environment (Nakanishi et al., 1962). But nauplii are thinshelled zygotes that hatch within the female's brood sac. Since cysts are the only zygote type affected by Zn perhaps shell dis-function is responsible for the reduction in viability. The tertiary envelope (outer cell layer) of the cyst is secreted by the shell gland (Anderson *et al.*, 1970). Cysts produced by some of the treated females exhibited a sever reduction in hatchability and this agree with results by Cunningham (1976) and El-Bermawi (2003). The chemical or structural modifications in the cell wall Vol. 14 (2), 2009 383

might reduce the resistance of the cysts to desiccation, and environmental stresses, thus reducing hatchability (El-Bermawi *et al.*, 2004). This is explaining cyst low hatchability (27.12, 24.53 and 22.54%) percentage with tested Zn treatments (0.1, 0.25 and 0.5 mg/l Zn). In contrast, whether offspring are produced as cysts or nauplii has low habitability.

The present results revealed that *A. parthenogenetica* were less resistance to high $Znso_4$. The results showed low survival percentage especially at high $1mg/l Znso_4$ after 24 days and maturity has been affected at the same $Znso_4$ level. Reproductive and life-span characteristics has been affected with the elevated $Znso_4$ levels especially cyst hatching percentage, which can explain cysts low hatchability from Qarun Lake, because zince is exist in the lake water in such high levels.

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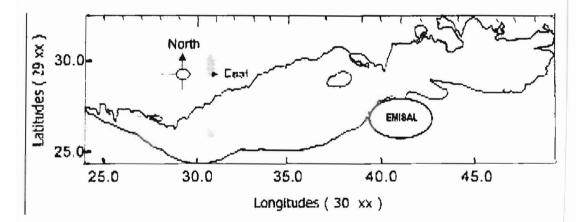


Fig. (1). Qarun Lake and the location of the Egyptian Co. for Salts and Mineral (EMISAL)

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Table (1). Effect of sublethal concentrations of zinc sulphate (Znso₄) on survival (%) of Lake Qarun *A. parthenogenetica.* (Data presented as mean ±s.d.)

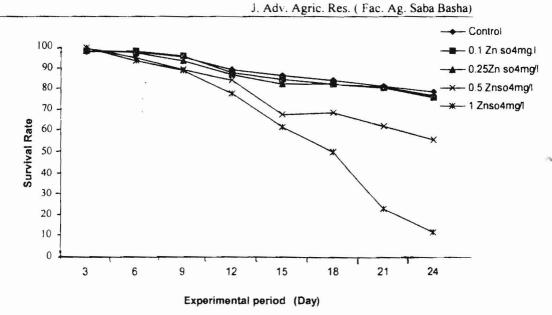
				./					
Znso₄	Time after hatching (in days)								
mg/l	3	6	9	12	15	18	21	24	
0	97 ^a	97 ^a	95 ^ª	88.83 ^{sbc}	86 ^{ab}	83.9 ^{ab}	80.83 ^{ab}	78.33 ^{8b}	
	±1	±1	±0.50	±0.57	±3.05	±2.11	±1.89	±1.04	
0.1	97.5 ^ª	97.66 ^a	95.16 ^ª	87.5 ^{#b}	84.16 ^{ab}	81.83 ^{ab}	80 ^{ab}	75.5 ^{ab}	
	±0.50	±0.75	±1.04	±1.00	±2.64)	±2.1	±2.17	±1.00	
0.25	98.83 [⊅]	96.66 ^ª	93.16 ^ª	86.50 ^{8b}	82.16 ^{ab}	81.83 ^{ab}	80.5 ^{ab}	76.5 ^{ab}	
	±0.76	±0.76	±2.51	±0.50	±1.52)	±2.08	±1.73	±1.73	
0.5	99 ^b	93.5 ^b	88.66 ^b	84 ^{cde}	67 ^{ac}	67.83 ^{ac}	61.66 ^{ac}	55.16 ^{ac}	
	±0.86	±0.5	±1.25	±0.86	±0.86)	±4.25	±1.75	±3.51	
1	99 ^b	93 ^b	88.33 ^b	77.33 ^{bcd}	61.33 ⁶⁰	49.5 ^{bc}	22.83 ^{2C}	11 ^{bc}	
	±0	±1	±1.15	±2.25	±0.76)	±7. 5 4	±4.5	±2.29	

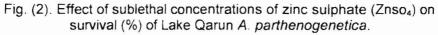
For every character values with the same superscript are not significantly different.

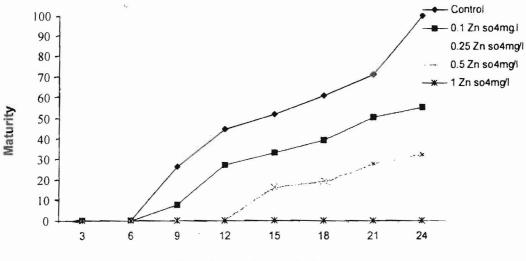
Table (2). Effect of sublethal concentrations of zinc subplate (Znso₄) on maturity (%) of Lake Qarun *A. parthenogenetica.* (Data presented as mean ±s.d.)

Znso ₄	Time after hatching (in days)								
mg/l	3	6	9	12	15	18	21	24	
0	0.00	0.00	26.33 ^{ab} ±3.51	45 ^{abc} ±6.08	52 ^{abcd} ±3.60	61 ^{abc} ±6.24	71 ^{abc} ±4.58	100 ^{abcd} ±0.00	
0.1	0.00	0.00	7.72 ^{ac} ±3.96	27.56 ^{abd} ±1.13	33.53 ^{abc} ±4.70			55.11 ^{abc} ±7.78	
0.25	0.00	0.00	0.00 ^{bc}	10.15 ^{acd} ±1.11	26.77 ^{abde} ±2.59	36.16 ^{abd} ±3.76	43.71 ^{abd} ±4.56	44.11 ^{abde} (±3.00	
0.5	0.00	0.00	0.00 ^{bc}	0.00 ^{bcd}	16.01 ^{acde} ±3.05	19.26 ^{acd} ±3.45	27.71 ^{acd} ±2.05	32.64 ^{acde} ±2.95	
I	0.00	0.00	0.00 ^{bc}	0.00 ^{bcd}	0.00	0.00 ^{bcd}	0.00	0.00 ^{bcde}	

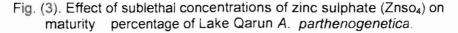
For every character values with the same superscript are not significantly different.







Experimental period (Day)



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Table (3). Effect of sublethal concentrations of zinc sulphate (Znso ₄) on											
reproductive and life span characteristics of Lake Qarun A.											
parthenogenetica. (Data presented as mean ±s.d.)											
Znso₄	A	В	С	D	E	F	G	Н	1		
(mg/l)											
0	19.45 ^{6cd}	29.55 ^{bcd}	2.45 ^b	51.45bcd	6.35	34.67 ^b	40.14 ^b	207.90 ^b	91.96 ^b		
0	±1.84	±6.68	±0.88	±6.46	±1.26	±11.52	± 3.88	±30.38	±3.83		
0.1	18.75 ^{bd}	25.05 ^{abd}	2	45.8 ^{abc}	5.45	36.94 ^{bd}	14.6 ^ª	199,13 ^b	27.12 ^ª		
	±1.20	±4.66	±0.91	±4.87	±0.75	±4.97	±11.31	±23.43	±17.58		
0.25	21.25 ^{abc}	19.4 ^{abc}	1.75 ^ª	42.4 ^{abc}	4.9	31.77 ^{bc}	14.25 [°]	156,40°	24.53 ^a		
0.23	±1.25	± 2.21	±0 .71	±2.96	±0.64	±3.12	±7.67	±28.24	±8.18		
0.5	24.1 ^{acd}	13.65 ^{acd}	1.6"	39.35 ^{acd}	5.79	20.21 ^{acd}	17.27 ^a	93.46 ^{ac}	22.54ª		
	±1.88	±2.64	±0.75	±2.97	±7.04	±5.71	±13.01	±31.53	±4.61		
1	-							-			

N=20 in all cases.

For every character values with the same superscript are not significantly different.

Reproductive Criteria:

A: pre reproductive period (in days),

- B: reproductive period (in days),
- C: post reproductive period (in days),
- D: total life span (in days),
- E: number of broods per females,
- F: offspring per brood,
- G: percentage offspring encysted,
- H: total offspring per female,

I: hatching percentage of cysts.

الملخص العربى

تأثير التركيزات تحت المميتة من كبريتات الزنك على معدل الإعاشة و البلوغ الجنسى و الخواص الإنتاجية و مواصفات الحياة لأرتيميا Artemia بحيرة قارون

ناجى منصور البرماوى

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دراسة الثر ٤ تركيزات تحت ممينة من كبريتات الزنك(١ , و٢٠, و٥ , و٥ مو ا مليجرام لكل لتر)بالأضافة الى الكنترول (بدون اضافة) على معدل الإعاشة و البلوغ الجنسى و المواصفات الإنتاجية و الحياتية لارتيميا من بحيرة قارون. أشارت النتائج إلى وجود علاقة عكسية بين معدل الإعاشة و المستويات المتزايدة من كبريتات الزنك مع زيادة فترة التعرض تمكنت الحيوانات من تحمل مستويات كبريتات الزنك المنخفضة المتراكمة (١ , و٢٠٠ مليجرام لكل لتر من كبريتات النحاس) و عندها كانت معدلات الأعاشة و البلوغ الجنسى فى احسن مستوياتها بشكل متساوى مع الكنترول حتى مع زيادة فترة التعرض و لكن كان لها تاثير سلبى على المواصفات الأنتاجية و الحياتية للحيوانات (تاثرت الفترة الأنتاجية من٥ ، ٢٥ الى ٢٥ ، ١٢ يوما و فترة ما قبل الأنتاج من ٢ الى ١٦. الكنتريات الزنكان لها تاثير سلبى على المواصفات الأنتاجية و الحياتية للحيوانات (تاثرت الفترة الأنتاجية من٥ ، ٢٥ الى ٢٥ ، ١٢ يوما و فترة ما قبل الأنتاج من ٢ الى ١٠٠

الدراسة توضح التأثير السيء للتلوث بالزنك على البلوغ الجنسى والمواصفات الإنتاجية والحياتية للأرتيميا وبخاصة فى التركيزات المرتفعة إضافة إلى التأثير السيء على معدل الفقس لبيض الأرتيميا.