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Effect of zinc on zoeal development of the estuarine hermit crab *Clibanarius olivaceus* (Henderson)

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Abstract. The 96 h LC_{50} value for zinc was 100 ppb. Larvae reared in 7 sublethal (1, 5, 10, 30, 50, 70, 90 ppb) and 96 h LC_{50} level concentrations (100 ppb) showed survival rates more than 10%, thus perfectly fitting in with definition of chronic concentration. Survival rate of larvae decreased with increase in test concentration but in the different chronic test concentrations, the overall time required for the completion of zoeal development did not differ significantly from that of control.

Keywords. *Clibanarius olivaceus*; chronic influence; zinc; survival rate; mean days of moulting.

1. Introduction

On both the sides of Vellar estuary (lat. 11°29′ N; long. 74°46′ E), cultivation of paddy is extensively practiced. To increase the yield, different types of pesticides are used which are ultimately drained into the estuary. These chemicals are non-degradable in nature and are persistent in the environment and can affect the larva, juvenile and adult of both endemic and migratory organisms in the estuary. Most of the pesticides in use contain heavy metals like zinc in their composition (organophosphorous and carbamates). Sewage released into the estuary from Portonovo town also contains zinc (Kumaraguru 1980). The present study aims to bring out the effect of this heavy metal on the larval stages of the most abundant hermit crab *Clibanarius olivaceus*.

2. Materials and methods

C. olivaceus for toxicological study was collected from the Vellar estuary. Ovigerous females were separated, offered new shells and kept in aquarium containing filtered sea water until hatching of larvae occurred. As soon as the larvae were liberated by the ovigerous crabs, they were separated in clean 500 ml beakers containing filtered sea water. The salinity of the sea water used in the present study was $35 \pm 1\%$ and the water temperature during the larval rearing period was $29 \pm 1°C$. 4·43 g of zinc sulphate ($ZnSO_4$ $5H_2O$—analar grade) dissolved in 1 litre of deionized water was used as the stock solution and the strength of this solution was 1 ppt. Serial dilutions were made to get required test concentrations.

2.1 *Test procedure*

In order to find out the 96 h LC_{50} value, 30 larvae each were used in different concentrations of zinc following the method of Ahsanullah and Arnott (1978). The

acute toxicity test was run in 11 concentrations (1, 5, 10, 30, 50, 70, 90, 100, 125, 150 and 200 ppb). During the 96 h experimental period, no mortality was observed upto 70 ppb and the mortality increased after that. The 96 h LC_{50} value for zinc was found to be 100 ppb (figure 1). Three replicate beakers each containing 10 larvae were used for each test concentration. All larvae for each test came from a single berried crab and were less than 12 h old at the initiation of an experiment. Test beakers contained 100 ml of test water and were not aerated. Larvae were transferred daily to clean beakers containing fresh medium by means of a pipette and were fed with freshly hatched *Artemia* nauplii. The criterion for determining death was the absence of movement, when the larvae were prodded. Dead larvae were removed on each observation. Presently the larval development was studied in 7 sublethal concentrations (1, 5, 10, 30, 50, 70 and 90) besides the 96 h median lethal concentration.

3. Results

3.1 *Larval survival rate*

The survival rate of *C. olivaceus* zoeal stages at different test concentrations of zinc is given in figure 2. The overall survival rate was 80% in control. Mortality occurred in all the zoeal stages except zoeal stage II.

Survival rate in other test concentrations was lower than in the control and as the concentration increased the survival rate decreased and the lowest survival rate (46·6%) was found in 100 ppb concentration. Mortality occurred in all the stages except zoeal stage II.

3.2 *Mean days of moulting*

The mean days of moulting of each zoeal stage in different test concentrations is given in table 1 and figure 3.

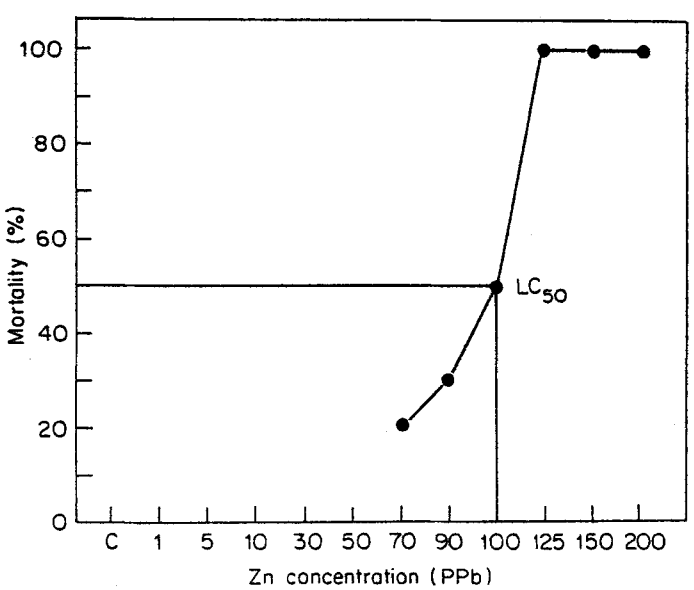

Figure 1. Toxicity of zinc on I zoea of *C. olivaceus* (96 h LC_{50}).

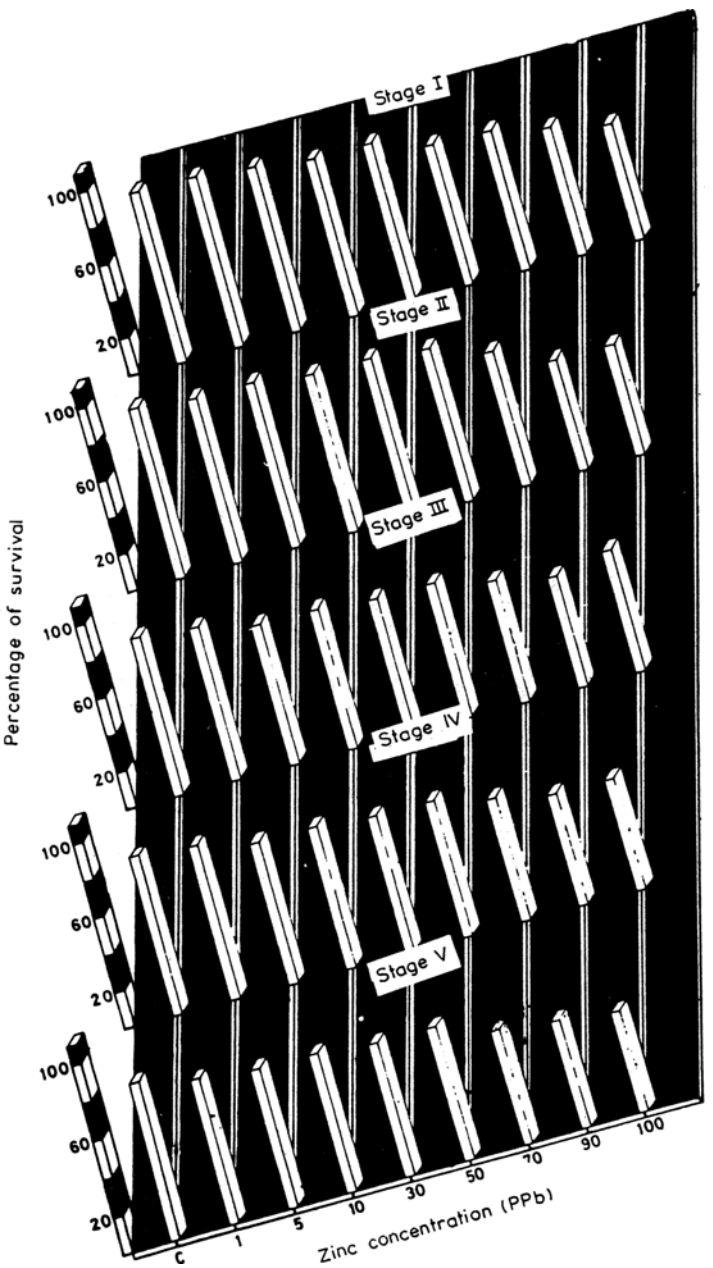

Figure 2. Average per cent survival of *C. olivaceus* zoeal stages I–V reared from hatching to glaucothoe in different concentrations of zinc.

3.3 *I Zoea* (*Carapace length* 0·9 *mm*)

Here, as the test concentration increased, intermoult duration also increased. Only in two test concentrations the intermoult duration was shorter than that of the control and in other concentrations the duration was more. The maximum of 10·37 days was

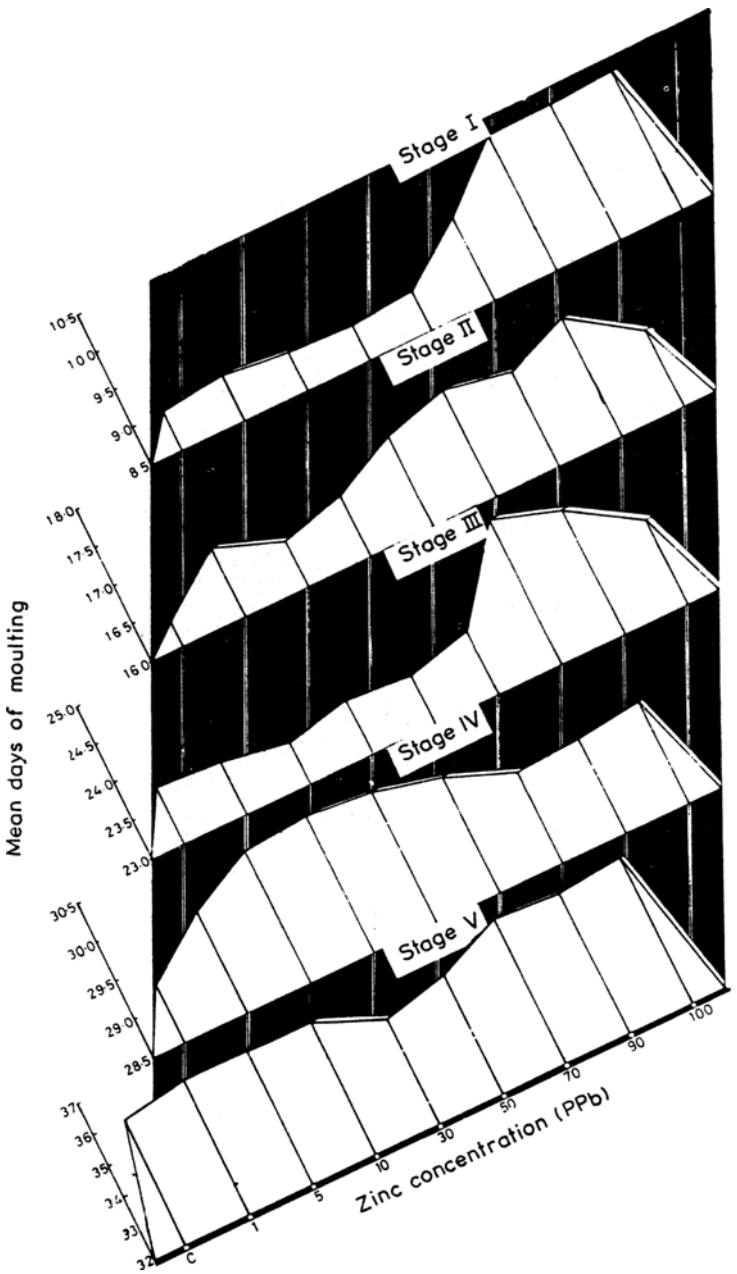

Figure 3. Mean days of moulting of zoeal stages I–V of *C. olivaceus* in different test concentrations of zinc.

encountered in 100 ppb concentration. T test was used to find out the differences in intermoult duration between different concentrations (table 2). Intermoult duration in higher concentrations differed significantly from that of control and other lower concentrations.

Table 1. Mean days of moulting of zoeal stages I–V of *C. olivaceus* (along with standard deviation and variance) exposed to different test concentrations of zinc.

Stage	Control	1 ppb	5 ppb	10 ppb	30 ppb	50 ppb	70 ppb	90 ppb	100 ppb
I Zoea Mean	9·04 ±	9·11 ±	9·00 ±	8·96 ±	9·12 ±	9·61 ±	10·30 ±	10·33 ±	10·37 ±
SD	1·00	0·83	0·88	0·82	0·86	0·99	0·82	0·73	0·68
Variance	1·00	0·69	0·77	0·68	0·75	0·98	0·88	0·53	0·47
Number	28	28	27	26	26	23	23	21	19
II Zoea Mean	16·32 ±	16·88 ±	16·59 ±	16·81 ±	17·19 ±	17·44 ±	17·23 ±	17·56 ±	17·00 ±
SD	1·06	1·59	1·62	1·58	0·75	0·92	0·97	1·25	1·19
Variance	1·12	2·53	2·62	2·50	0·56	0·84	0·95	1·56	1·42
Number	28	25	27	26	26	25	22	18	18
III Zoea Mean	23·75 ±	23·68 ±	23·5 ±	23·70 ±	23·64 ±	23·82 ±	24·90 ±	24·61 ±	24·06 ±
SD	1·37	1·38	1·38	1·40	1·50	1·53	1·52	1·33	1·30
Variance	1·88	1·90	1·90	1·96	2·25	2·34	2·31	1·77	1·69
Number	26	25	23	23	22	22	20	18	18
IV Zoea Mean	29·24 ±	29·83 ±	30·23 ±	30·27 ±	30·19 ±	29·95 ±	29·67 ±	29·71 ±	29·81 ±
SD	1·69	1·64	1·48	1·58	1·57	1·50	1·50	1·65	1·56
Variance	2·86	2·69	2·19	2·50	2·46	2·25	2·25	2·72	2·43
Number	25	23	22	22	21	21	18	17	16
V Zoea Mean	36·00 ±	36·30 ±	36·24 ±	36·24 ±	35·38 ±	35·80 ±	36·65 ±	36·50 ±	36·71 ±
SD	1·85	1·92	1·73	1·89	1·86	2·24	2·18	2·16	2·23
Variance	3·39	3·69	2·99	3·57	3·46	5·02	4·75	4·67	4·97
Number	24	23	21	21	21	20	17	16	14

Table 2. *T* values for the differences in mean intermoult duration of I zoea of *C. olivaceus* in control and different test concentrations of zinc.

Control	1 ppb	5 ppb	10 ppb	30 ppb	50 ppb	70 ppb	90 ppb	100 ppb
Control	0·2849	0·1574	0·2701	0·2755	2·0346[a]	4·8384[d]	5·1013[d]	5·1040[d]
1 ppb		0·4775	0·6590	0·0432	1·9617	5·1072[d]	5·3590[d]	5·4635[d]
5 ppb			0·1707	0·5008	2·3097[b]	5·3667[d]	5·6026[d]	5·6866[d]
10 ppb				0·6822	2·5069[b]	5·6770[d]	5·9623[d]	6·0712[d]
30 ppb					1·8483	4·8674[d]	4·7957[d]	5·2063[d]
50 ppb						2·5683[c]	2·7261[c]	2·8297[c]
70 ppb							0·1274	0·2950
90 ppb								0·1783
100 ppb								

[a]$P < 0.05$; [b]$P < 0.02$; [c]$P < 0.01$; [d]$P < 0.001$.

Table 3. *T* values for the differences in mean intermoult duration of II zoea of *C. olivaceus* in control and different test concentrations of zinc.

Control	1 ppb	5 ppb	10 ppb	30 ppb	50 ppb	70 ppb	90 ppb	100 ppb
Control	1·5239	0·7347	1·3471	3·4631[c]	4·1613[c]	3·0547[c]	3·6137[c]	2·0246[a]
1 ppb		0·6508	1·5750	0·8962	1·4771	0·8941	1·5079	0·2697
5 ppb			0·5003	1·7208	2·3047[b]	1·6278	2·1487[a]	0·9198
10 ppb				1·1076	1·6595	1·0829	1·6801	0·4314
30 ppb					1·0505	0·1607	1·6302	0·6502
50 ppb						0·7609	0·4142	1·3693
70 ppb							0·9797	0·6718
90 ppb								1·3763
100 ppb								

[a]$P < 0.05$; [b]$P < 0.02$; [c]$P < 0.001$.

3.4 *II Zoea (Carapace length 1·08 mm)*

The mean days of moulting for control was 16·32 days. This duration increased as the test concentration increased and the highest (17·56 days) was in 90 ppb concentration. However in the highest concentration of 100 ppb there was a fall and the mean days of moulting was lower than in 30, 50, 70 and 90 ppb concentrations. Mean days of moulting in higher concentrations (30, 50, 70, 90 and 100 ppb) differed significantly from that of control, and the difference between these concentrations was not statistically significant (table 3).

3.5 *III Zoea (Carapace length 1·20 mm)*

Trend in mean days of moulting in this stage differed from that of the previous two zoeal stages. With increase in test concentration upto 30 ppb, the mean day of moulting declined from that of control. But in 50 and 70 ppb concentrations it increased slightly and again in 90 and 100 ppb concentrations there was a decline but mean days of moulting in these two concentrations was more than that of control.

Mean days of moulting in 70 and 90 ppb concentrations differed significantly from that of control (table 4).

3.6 *IV Zoea (Carapace length 1·73 mm)*

Trend in mean days of moulting resembled more or less that of the previous zoeal stage. It increased from control upto 10 ppb concentration and then declined in 30, 50 and 70 ppb concentrations and again increased slightly in 90 and 100 ppb concentrations. The lowest was recorded in control (29·24 days) and the highest (30·27 days) in 10 ppb concentration. Mean days of moulting of only 5 and 10 ppb concentrations differed significantly from that of control (table 5).

3.7 *V Zoea (Carapace length 2·19 mm)*

As the test concentrations increased mean days of moulting also increased and declined. Shortest duration (35·38 days) was encountered in 30 ppb concentration and the highest (36·71 days) in the highest concentration. *T* values did not suggest significant difference between control and test concentrations and among test concentrations (table 6).

Table 4. *T* values for the differences in mean intermoult duration of III zoea of *C. olivaceus* in control and different test concentrations of zinc.

Control	1 ppb	5 ppb	10 ppb	30 ppb	50 ppb	70 ppb	90 ppb	100 ppb
Control	0·1817	0·5845	0·1261	0·2652	0·2865	2·7804[c]	2·0702[a]	0·6774
1 ppb		0·4016	0·0498	0·0952	0·3300	2·8188[c]	2·2142[b]	0·9129
5 ppb			0·4393	0·2796	0·6917	3·1221[d]	2·5511[c]	1·2759
10 ppb				0·2746	0·5617	2·7250[c]	2·1104[a]	0·8427
30 ppb					0·3940	2·7017[c]	2·1900[a]	0·9346
50 ppb						2·2921[b]	1·7214	0·8253
70 ppb							0·6226	1·8203
90 ppb								1·2544
100 ppb								

[a] $P < 0·05$; [b] $P < 0·02$; [c] $P < 0·01$; [d] $P < 0·001$.

Table 5. *T* values for the differences in mean intermoult duration of IV zoea of *C. olivaceus* in control and different test concentrations of zinc.

Control	1 ppb	5 ppb	10 ppb	30 ppb	50 ppb	70 ppb	90 ppb	100 ppb
Control	0·5466	0·4486	0·4309	1·1215	0·3251	1·0319	0·7848	1·0614
1 ppb		1·1084	0·1045	1·6108	0·7880	0·5380	0·3038	0·5926
5 ppb			1·7891	1·2824	0·7059	0·6470	0·4067	0·7015
10 ppb				1·4863	0·6809	0·6207	0·3897	0·7609
30 ppb					0·6543	1·9326	1·6920	1·9142
50 ppb						1·1644	0·9461	0·8983
70 ppb							0·1984	0·0755
90 ppb								0·2616
100 ppb								

Table 6. *T* values for the differences in mean intermoult duration of V zoea of *C. olivaceus* in control and different test concentrations of zinc.

Control	1 ppb	5 ppb	10 ppb	30 ppb	50 ppb	70 ppb	90 ppb	100 ppb
Control	1·2249	2·1218*	2·1474*	1·9611	0·9287	0·8615	0·9932	1·0846
1 ppb		0·8576	0·9154	0·7424	0·2523	0·3216	0·2281	0·0382
5 ppb			0·0866	0·8694	0·6160	1·1834	1·0353	0·8447
10 ppb				0·1498	0·6801	1·2216	1·0764	0·8906
30 ppb					0·5067	1·0530	0·9170	0·7320
50 ppb						0·5811	0·4220	0·2765
70 ppb							0·9214	0·6918
90 ppb								1·7870
100 ppb								

*$P < 0.05$.

4. Discussion

Acute and sublethal concentrations of pollutants are defined by Epifanio (1979). Acute concentrations are those, in which less than 10% of the larvae reach the crab stage, whereas sublethal concentrations are those, in which there is significant mortality or delay in moult, but more than 10% of the larvae reach the crab stage. However, these definitions are valid, when survival to the crab stage is high in the control but not when the survival is low (Bookhout and Costlow 1975). Presently the overall survival rate of larvae in control was more and also in different concentrations of bioassay used the survival rate was more than 10%. Thus the sublethal concentrations of the present study perfectly fit in with the definition of Epifanio (1979).

4.1 *Survival rate*

In the present study, larvae of *C. olivaceus* were reared in 7 sublethal and 96 h LC_{50} level concentrations from hatching to glaucothoe stage. With increase in test concentrations of zinc survival rate of larvae decreased. Similar findings have been reported elsewhere also. Epifanio (1971) studied the effect of dieldrin (organochloride pesticide) on the larval development of the crabs *Leptodius floridanus* and *Panopeus herbstii* and found that as the test concentrations increased, the survival rate of larvae decreased. Christiansen *et al* (1977) studied the effect of hydroprene on the larval development of mud crab *Rhithropanopeus harrisii* and found that as the test concentrations increased, the number of larvae reaching the megalopa stage decreased. The study of Bookhout *et al* (1976) about the effect of methoxychlor on larval development of mud crab and blue crab *R. harrisii* and *Callinectes sapidus* also showed similar results. They also noted that the sharpest reduction in survival took place in the megalopa stage and they attributed that this was possibly due to transformation of zoeal tissues to adult tissues. During this period the pesticides, which had been stored in fat during zoeal development were released into the blood stream and this caused mortality in some unknown way. Presently the survival rate during post larval stage (glaucothoe stage) was not found, as these hermit crab larvae require minute shells for occupation before moulting into the first crab instar (Reese

1963). But in another species of crab *R. harrisii*, megalopa stage was less sensitive to hydroprene than were the zoeal stages and thus different species of crabs show differential sensitivity to the same pesticide at different stages of development (Christiansen *et al* 1977). Moreover to compare the sensitivity of different zoeal stages, it will be better if each zoeal stage is introduced to the test medium freshly. The above observations including the present one show the prolonged influence of test concentrations during development.

4.2 *Mean days of moulting*

Presently, mean days of moulting under different test concentrations of heavy metal, zinc, showed interesting results. With increase in test concentration up to 10 ppb, the mean day of moulting also increased. In 30 and 50 ppb concentrations it was found to be less than that of control. Again it was more in the highest concentrations of 70, 90 and 100 ppb. But statistically the overall time taken for completion of zoeal development in different test concentrations did not differ significantly from that of control. Buchanan *et al* (1970) observed delay in the moulting of larvae reared in sublethal concentrations of sevin from zoeal stages I to II. Similar results have been reported for the zoeal stages of *R. harrisii*, *Menippe mercenaria* and *C. sapidus* by Bookhout *et al* (1972, 1976) caused by different pollutants.

Stress in higher concentrations of pollutant may act in two different ways. First an alteration in normal development i.e., an increase in number of zoeal stages. Bookhout *et al* (1972) found the mirex to interpose an additional zoeal stage (VI zoea) in the normal development of 5 zoeal stages. As most of the crabs consistently have a definite number of zoeal stages perhaps both larval moulting and rate of development are controlled by the same mechanism. There are two exceptions to the above and those are the larvae of *C. sapidus* and *M. merceneria*. Costlow (1968) suggested that atleast two mechanisms may be involved—one that controls moulting and a second normally synchronized with first that regulates the rate of morphological development in larval stages. The site for the regulation of morphological development appears situated in the larval eyestalk for when both eyestalks of *R. harrisii* were removed the larvae passed through an extra zoeal stage without morphological development. Secondly stress in higher concentrations of pollutants may exert an action on the mechanism, which regulates morphological and moulting changes thus speedy development of larvae (Bookhout *et al* 1972). Toxicity studies of pesticides on crab larvae support the above view. Many authors suggested that toxicity of pesticides like sevin was associated with the moulting process (Epifanio 1979). Moulting in crab larvae is known to be a neuroendocrine function (Costlow 1963, 1966a, b) and naturally sublethal toxicity of neurotoxins might be expected to interfere, with moulting process. Just like pesticides sevin, hydroprene and dieldrin heavy metal zinc may also be a neurotoxin and it may also interfere with moulting. Presently in some sublethal concentrations of zinc, the mean days of moulting was found to be shorter.

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