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A COMPARATIVE STUDY OF ECOLOGICAL AND GENETICAL ADAPTATION OF THREE IRAQI FRESH WATER SNAILS IN RESPECT TO HEAVY METAL POLLUTION

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ABSTRACT

A comparative study was carried out on ecological and genetical adaptation of three Iraqi freshwater snails, *Physa acuta, Melanopsis buccinoidea* and *Melanoides tuberculata,* in respect to acute toxicity of heavy metals (Zn, Cd and Hg). Longevity are used as poisoning tolerance criterion. LT 50 and LT 100 were determined for the studied snails at (0.5, 1, 5, and 10 ppm), for the three metals. Results indicated that *Physa acuta* had a higher tolerance than *Melanopsis buccinoidea* and *Melanoides tuberculata,* which was the lower one. Previous exposure to heavy metals in the original habitat was affecting on experimental tolerance and no relationships of physical and chemical factors (total hardness, temperature, D. O. and sulphate) between original and experimental exposuring has been occurred. Results indicated no ability of the three species for genetical adaptation on experimental bioassays, except for lower concentrations (0.5, 1 ppm), since *Physa acuta* managed to give more than two healthy new generations, whereas *Melanopsis buccinoidea* was disabled to give more than one weakling generation.The species *Melanoides tuberculata* showed a complete disability to give any generation . The present study suggested a new wide experiment to be design, not only by acute toxicity but with chronic toxicity, in order to determine the interference between the ecological and genetical adaptation and the previous exposure to many environmental pollutants.

INTRODUCTION

Little attention has been paid to the adaptation of Iraqi mollusca, especially the freshwater snails. Najim (1959) gave an important notes on the distribution of molluscs in Iraq. Further notes on distribution of molluscs in Iraq with a first record of *Physa* has been given also by Najim (1961). Most of these studies were focusing on larval trematode parasites of freshwater molluscs in Iraq (Watson, 1950). One of the best and pioneer studies on ecology of the freshwater mollusca of Iraq has been given by Harris (1965). New attention appears recently on distribution and dispersal of freshwater snails in respect to some heavy metals pollution (Sheriff, 1992; Sheriff and Delool, 1993).

The biological effects of increasing amount of metal ions in aquatic system have been, in recent years, the concern of large number of environmentalists (Patrick, 1973). The degree or extent of the effects of the environmental changes on aquatic life varies with the type and amount of pollutant and the character of the receiving water. In most waters the concentrations of heavy metals are very low (Riley and Chester, 1971).

Mortality studies of adult organisms over a given period of time have generally been the only standard bioassay techniques in pollution studies. Consequently, such techniques have played a major role in setting standards (Vernberg *et al*., 1971). Tolerance experiments conducted in connection with water pollution research or control usually have one or both of

two immediate objectives. One is to establish the relationship between survival time, usually median survival time of a species and lethal factor level. This relationship can be used to estimate the length of time the species could tolerate any given level of the lethal agent, or the level that could be tolerated for any given exposure period (Warren, 1971).

The aims of the present study is to compare the effectes of Zinc, Cadmium and Mercury on ecological and genetical adaptation of three Iraqi freshwater snails, *Physa acuta, Melanopsis buccinoidea* and *Melanoides tuberculata*. The first species is from heavily polluted drainage canal, the second species is from sulferous springs habitat and the third is from slightly polluted drainage canal. Longevity experiments were carried out to evaluate the LT 50 and LT 100 of these three freshwater snails in respect to heavy metal pollutants, such criteria represents the poisoning resistance. We usually know very little about the concentration of toxic substances that may in nature have deleterious effects on the survival, reproduction, growth and ecogenetical adaptations of living organism.

MATERIALS AND METHODS

All experiments were conducted during 1992. Test organisms were the adults of three Iraqi freshwater snails, *Physa acuta* snails were collected from Saklawiya drainage canal which was heavily polluted. *Melanopsis buccinoidea* were collected from Shathatha (Ain altamor) springs (50 km southwest of Karbalaa) which were slightly sulferous water and *Melanoides tuberculata* from Hikmat drainage canal (2 km northeast of Baghdad), it was representing a slightly polluted water.

Aqueous stock solutions (10 ppm of metal ion) of Zinc ($ZnSO_4.H_2O$), Cadmium ($CdCl_2.2$ ½ H_2O) and Mercury ($HgCl_2$) have been prepared. In order to obtain the experimental concentrations of 5ppm, 1 ppm and 0.5 ppm, diluted concentrations from the stock solutions were prepared also. All concentrations refer to ppm of the metal ion in solution at the start of the experiments, and were calculated by atomic weights. It was not possible to monitor the concentrations of heavy metals during the course of an experiment because of the lack of suitable recording equipment, but it was assumed that these initial concentrations of metal would not alter significantly over a 5-day period as well-washed pyrex containers were used throughout.

After an acclimation period for at least 2 weeks, samples each consisting of 100 standard-sized individuals were placed in 5 liter experimental media. These were maintained at constant temperature of 15±1°C. Glass aquaria containing 5 liter distilled water alone were used as controls. Three replicates of each solution were used. A photoperiod of natural daylight was employed throughout the bioassays. Experimental animals were checked at ½ , 1, 3, 6, 12 and 24 hours and any dead specimen removed and recorded. Since all three species live in lotic water, the tanks were aerated in all experiments. All specimens were starved throughout the bioassays, so that solutions were changed every 24 hours. The toxicity results are plotted as the total mean percentage mortality at the end of 96 h of three replicate experiments, together with the standard deviations, where 50% mortality occurred, the LT50, LT100 values have also been calculated. Any specimen which failed to respond when touched with perspex needle were considered to be dead and were removed. The number of dead animals found at each inspection was recorded.

Standard methods for the examination of water and waste water (A. P. H. A., 1976) were used for D.O, $SO_4^{=}$ and water hardness. Temperature was recorded at natural habitats. Heavy metals (Zn, Cd and Hg) were analysed using SP9 spectrophotometer. Since o.5 ppm and 1 ppm Zinc concentrations were almost nearest to natural concentrations in three species habitats, only for this metal further breeding experiments conducted to determine the genetic adaptation by testing the number of generations succeeded on the three species.

RESULTS AND DISCUSSION

Table 1 shows the longevity of the three treated species with different metals poluttants. The results indicated that *Physa acuta* had a highest tolerance for the three experimental metals. Concerninig the concentrations of these three metals in natural habitats (Table 3), we found that all three concentrations were higher in Saklawiya drainage canal (the natural habitat of this species) than the other two habitats (Ain altamor and Hikmat drainage canal).

Literature on development of tolerance to metals reported that in same species increased tolerance to the toxic effectes of some metals can be acquired by previous exposure to sublethal concentrations. Several examples of this have been given by Sprague (1970), for fish. Llolyed (1960) found that rainbow trout *Salmo gairdneri* were more resistant to lethal concentrations of zinc following exposure to sublethal concentrations, and in the same species Sinley *et al*. (1974) showed that more tolerance fish were produced from Zinc-treated eggs. When adults brine shrimp *Artemia salina* were exposed to 0.1 ppm of copper for three weeks, the median lethal time in 1ppm was approximately double that of untreated animals (Saliba and Ahsanullah, 1973).

Data from table 3 shows that zinc concentration in Aim altamor was higher than in Hikmat drainage canal (mean concentration 0.55 ppm and 0.16 ppm respectively). This could explain the higher resistance of *Melanopsis buccinoidea* to zinc pollution in contrast with *Melaneides tuberculata* to (Table 1). The same data showed a higher original concentrations of Cd and Hg in Hikmat drainage canal in contract with the same metals concentration in Ain altamor (0.023 ppm and 0.000224), (0.00034 ppm and 0.00019 ppm) for Cd and Hg respectively, these differences may explain the higher resistance of *Melanoides tuberculata* in contract with *Melanopsis buccinoidea,* (See table 1). In conclusion, resistance order for the three species can be arranged in respect to the metals as following:

acuta > *buccinoidea*> *tuberculata* for Cd and Hg concentreations together.

Data from table (2). Shows the differences of some chemical and physical factors between water of the original habitats and the experimental water. Water hardness, temperature and water sulphate were lower than experimental water, whereas dissolved oxygen was higher in experimental water than original habitats. Results showed that there was no effect of previously exposure to these factors on altering the tolerance of all three studied species. In conclusion the response for poisoning concentrations constricted only with previously metals exposures and depending also on ecological and genetical adaptations criteria.

Results from further breeding of the three studies species in 0.5 ppm and 1 ppm of zinc media showed a successful genetical adaptation of *Physa acuta,* since it yield two active generations or may be more, whereas *Melanopsis buccinoidea* yield only one weakling generation, No successful breeding or culture occurred with *Melaneides tuberculata* in these two concentrations. Although the effects of metals on the genetic materials of cells is still unclear, but no doubt, certain metals have an effect on DNA and RNA . (Passe *et al,* 1961).

There was a suggestion of genetic adaptation to heavy metals in many kinds of fauna. (Vernberg and Vernberg, 1974). Genetic changes need to be more fully investigated with a large series of metals under a wide spectrum of condition in the freshawater organisms.

The high tolerance and successful of ecological and genetical adaptation of *Physa acuta* from the present study could give a possible precaution on wide levels of distribution and new habitats invasion by this species, especially in those habitats which have a little heavy metals contaminations.

Bioassays data showed a low concentration of Cd and Hg in Ain altamor water in comparison with higher concentration of zinc, which could explain the large abundance of *Melanopsis buccinoidea,* in regarding of zinc role on growth rater and its importance in animal nutrition and enzymes. (Macan, 1980) (table 3).

Results obtained from such acute toxicity still confirming the poisoning order of the three studied metals as following: (Hg > Cd > Zn). Freshwater snails as a part from invertebrates

are considered excellent indicator organisms because of their capability in concentrating metals, among other pollutants. Beside measuring the concentrations of those metals in aquatic ecosystems, there is a need not only for acute toxicity but also for effects of chronic toxicity on such important molluscs.

In conclusion, one can say that although there has been a great deal of interest in and considerable research on metal pollution during the last few year as a result of human and other fauna poisoning, there is still much to be learned about the effects of metals on aquatic organisms and our ecosystems. If we are to be able to manage our renewable resources properly in the face of growing technology and industrial production, such new learning must be acquired soon.

Table 1: Longevity of the three studied species on different metal pollutants.

Species	pollutant	LT hours	Concentrations (ppm)			
			0.5	1	5	10
Physa acuta	Zn	LT50	>96	>96	63	47
		LT100	>96	>96	>96	93.5
	Cd	LT50	90	74	52	36
		LT100	96	96	63	54
	Hg	LT50	44	33	28	22
		LT100	67	41	35	28.5
Melanopsis buccinoudea	Zn	LT50	>96	>96	60	40.5
		LT100	>96	>96	>96	78.5
	Cd	LT50	45	39	30.5	27
		LT100	67	50	42.5	30
	Hg	LT50	22	16	12.5	9.5
		LT100	29	21	18	14.5
Melanoides turberculata	Zn	LT50	>96	>96	48	35.5
		Lt100	<96	>96	72	54
	Cd	LT50	57	53	48	30.5
		LT100	74	66	56.5	44
	Hg	LT50	38	27.5	21	17.5
		LT100	52.5	35	30	24

Table 2: Some chemical and physical factors in different habitats

Habitat Factors		Ain altamor springs	Saklawiya D. Canal	Hikmat D. Canal	Experimental media
Hardness mg/l as $CaCO_3$	Average	955	563	346	215
	Range	327-1302	484-622	292-466	-
Dissolved Oxygen mg/l	Average	5.4	3.9	6.2	6.8
	Range	4.6-6.5	3.2-5.9	5.1-6.8	-
Temperature C°	Average	24.5	21.4	18.8	15±1
	Range	20-28	14.6-26.3	9.6-25.2	-
Sulphate $SO_4^{=}$	Average	6071	3112	2156	78.5
	range	6022-6080	1675-3860	1842-2332	-

Table 3: Metal concentrations in the original habitats.

Metals		Ain altamor springs	Saklawiya D. Canal	Hikmat D. Canal
Zinc ppm	Average	0.55	0.74	0.16
	Range	0.35-0.62	0.52-0.83	0.10-0.22
Cadmium ppm	Average	0.0024	0.046	0.023
	Range	0.0018- 0.0040	0.031-0.063	0.015-0.028
Mercury ppm	Average	0.00019	0.0030	0.00034
	range	0.00027-0.00010	0.0017 – 0.0044	0.00022-0.00055

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دراسة مقارنة للتكيفات البيئية والوراثية لثلاثة أنواع من قواقع البيئة المائية العراقية بالعلاقة مع ملوثات المعادن الثقيلة

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الخلاصة

تم اجراء الدراسة المقارنة للتكيف البيئي والوراثي لثلاثة أنواع من قواقع المياه العذبة العراقية *Physa acuta* و *elanopsis buccinoidea* و *Melanoides tuberculata* بالعلاقة مع التعرض الحاد (قصير الأمد) لملوثات المعادن الثقيلة (الزنك والكادميوم والزئبق). وقد تم استخدام معيار مقاومة السمية بدلالة التعمير (Longevity) واحتساب الوقت المميت النصفيLT50 والكلي LT100. أظهرت الدراسة وجود التباين في سمية العناصر الثقيلة تنازلياً، الزئبق فالكادميوم فالزنك، والتي استخدمت بتراكيز (0.1, 1, 5, 10 ppm). وقد كان اكثر الأنواع مقاومة وأفضلها في التكيف البيئي والوراثي النوع *Physa acuta* يليه *Melanopsis buccinoidea* ثم *Melanoides tuberculata* . ولقد أظهرت الدراسة وجود علاقة بين التعرض السابق للكائن الى المعادن الثقيلة في بيئته الأصلية وبين المقاومة التجريبية، ولم يكن لاختلاف بعض العوامل الفيزياوية والكيمياؤية التجريبية مثل (العسرة الكلية والحرارة والاوكسجين المذاب والكبريتات) عن التعرض السابق لمثيلاتها في البيئة الأصلية أي تأثير يذكر في المقاومة. وقد أظهرت النتائج عدم قدرة الأنواع الثلاثة للقواقع على التكيف الوراثي في التراكيز التجريبية باستثناء التراكيز الواطئة للزنك (0.5, 1 ppm) حيث تمكن النوع *Physa acuta* من انتاج اكثر من جيلين من الأفراد الأصحاء بينما عجز النوع *Melanopsis buccinoidea* عن انتاج اكثر من جيل واحد ضعيف. أما النوع

Melanoides tuberculata فقد فشل تماماً في انتاج أي جيل بذكر. لقد اقترحت الدراسة التوسع في اجراء البحوث الخاصة ليس بالتعرض الحاد (قصير الأمد) فقط وانما بالتعرض المزمن (طويل الأمد) وذلك لتحديد التداخل بين التكيف البيئي والوراثي المسبق للكائن ومديات تحمله لمختلف الملوثات في البيئة.