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Effect of some heavy metals synergism on the longevity and bioaccumulation inside the muscles of frog ridibunda species under the laboratory conditions

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Effect of some heavy metals synergism on the longevity and bioaccumulation inside the muscles of frog ridibunda species under the laboratory conditions

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Abstract. Rana ridibunda frogs (Amphibians) are examined under laboratory conditions (10-18 C°) of the aquarium water (tap water), oxygen saturation, non-saturation and starvation. Body weight for two groups of frogs were (260-285 gr) and (58-115 gr). Two types of heavy metals effect were examined (single type and mixed types) of Cdcl2 and HgSo4 used. Longevity as LT50% and LT100% were determined by single heavy metals effect and synergism effect for both oxygen saturated and oxygen non-saturated waters experiments were conducted, then the bioaccumulation inside the legs muscles of the frog were examined for the heavy metals poisoning media (2PPT) concentration. Synergism effects were very clear and differences of O_2 saturation were giving clear different results.

1. Introduction

The fresh water marsh frog Rana ridibunda occurs in the largest part of Europe and in Asia from Afghanistan to Iran, Iraq and then distribution through all Middle East [1,2]. It prefers a rivers and marshes even any irrigated areas [9]. This species is often referred to as a green frog to distinguish them from the more European Rana species which are known as (brown frog). The diet of the marsh frog consists of dragon flies and other insects spiders, earth worms and slugs, larger frogs also eat small rodents and sometimes smaller amphibians and fish, For this reason it was very important for the food chain, and when it will be poisoned by any heavy metals, there was a dangerous hazards problems [6],

Heavy metal pollutants are a major problem in aquatic environment because of their toxicity, their persistency and tendency to accumulate inside the organisms and undergo food chain amplification. Heavy metals from man-made pollution sources are continually released into aquatic ecosystems in trace amounts although some of them are important for their physiological functions and use them as structural components. Other heavy metals such as Cadmium, Lead and Mercury have known beneficial effect and their consumption over the time in animal body can cause illness [10].

The present investigation was undertaken to know the effect of heavy metals in the frog leg's muscles tissues and determine the potential use of the fresh water frog Rana ridibunda as a bio accumulative indicator of Mercury and Cadmium pollution in aquatic ecosystem.

2. Materials and Methods

Frog sampling were collected from the share of the Dijlah arm river (North of Baghdad, were the highest density of frogs occupied this area between the aquatic planes. More than 500 individual frogs (250 large samples), weight (260- 285 gr) and (250 gr small samples weight), (58-115 gr) were collected for the two types of the present experiments.

All samples were acclimated inside a big aquarium for one week with the laboratory normal conditions and feeding with the earth worms for this period. The experiment was conducted as three aquariums replicate with another one aquarium as a control. Ten samples were put in each tank for a starvation experiment with the large size and small size frogs.

Stock solution of the poisoning heavy metals as HgCl2 and CdSO2 (2PTT) were prepared and used singly and mixing. Acute toxicity was tacking for 96 hours. Results were recorded as LT 50% and LT 100%. Then it was reading within 2, 6, 8, 12, 18, 24 hours. (Table 1-6).

At the end of the mortality experiments, three samples from each experiments dissected and legs muscle (5gr) were tacking from each specimen for the bio accumulate experiments using the procedure of atomic absorption spectrometer to determine the heavy metals concentration inside the muscle by PPM and changing it into µg/g dray weight by using a calculation from the formula main shined by [1]. The results of the bioaccumulations recorded on (Table 7). Statistical analysis was calculated to have the significance variation of each experiment.

3. Results and discussion

The results from the present investigation showed the differences between the toxicity of the mercury and Cadmium on their effects on the mortality time LT 50 % and LT 100%. The mercury poisoning was higher than Cadmium (Table 1-6) (Figures 1-6). Many other researchers fixed such case in fact [3][5][11].

The results showed the synergism of the two wed heavy metals were very clear from the low time of the LT50% and LT 100%. The differences of O2 saturation and non-saturation were very clear in their effects on the results obtained. Oxygen saturation have a good effect on increasing the time of both LT 50% and 100% (Tables 1-6), this case was known from many researchers before [4], this fact was coincided for the small and large specimens of experimental frogs. Small sizes frogs were less tolerance to the toxicity of the heavy metals even they are not like that with another types of organisms when it shows the opposite [13].

Bioaccumulation experiments showed that the Cadmium had the bigger values than the mercury bioaccumulation at all experiments, whereas small sizes of the frogs showed a less values from the large sizes and for the mixed poisoning the values of the two poisoning metals showed a less values than total theoretical for both metals (Table 7). These results were coincided values with other studies mentioned before [2].

Bioaccumulation experiments showed that the values for each element alone was less than the total of mercury and Cadmium together, results for mercury was at value as 96.120 µg/g dry weight, inside the leg's muscles of the frog. Whereas for the Cadmium was a value as 172.434 µg/g dry weight so that the total values must be as 268.560 µg/g dry weight theoretically for the large sizes of the frogs, and the value from the mixed experiment for the same sizes was 162.412 µg/g dry weight (Table 7). The same was occurred with the small sizes, so the bioaccumulation for the mercury alone was 72.115 µg/g dry weight whereas it was 103.257 µg/g dry weight for the Cadmium alone so theoretically it must be as 175.372 µg/g dry weight, but experimentally was 148.377 µg/g dry weight, so this is showed the synergism effect of the two metals on each other.

It conclusion it can be found that big sizes frogs were accumulates the metals more than the small sizes, this is may be due to the large weight although they had more bioaccumulation, but the reason may be due to the different on the tolerance when small sizes did not have the enough tolerance.
The results here were coincided with another recorded investigates [8] (Table 7).

There were a clear significances variation between the results from experiments of oxygen saturated or non-saturated and between large and small sizes of the experimented frogs, also between the two metals used ($P \leq 0, 05$).

Table 1-a Mortality by HgCl2 of the frogs (small size) O2 saturated
(LT: Lethal dose; S.D: Standard Deviation)

No of specimens	LT 50% hours ± S.D.	LT 100% hours ± S.D.
10	26	38
10	18 ± 2	42 ± 2
10	28	40
Total 30	Mean 24	Mean 40

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Table1-b Mortality by HgCl2 of the frogs (small size) O2 non-saturated

No of specimens	LT 50% hours ± S.D.	LT 100% hours ± S.D.
10	12	28
10	18 ± 1	32 ± 1
10	18	30
Total 30	Mean 16	Mean 30

Table 2-a Mortality by CdSO4 of the frogs (small size) O2 saturated

No of specimens	LT 50% hours ± S.D.	LT 100% hours ± S.D.
10	46	56
10	48 ± 1	60 ± 1
10	44	58
Total 30	Mean 46	Mean 58

Table 2-b Mortality by CdSO4 of the frogs (small size) O2 non-saturated

No of specimens	LT 50% hours ± S.D.	LT 100% hours ± S.D.
10	40	50
10	36 ± 2	42 ± 2
10	32	40
Total 30	Mean 36	Mean 40

Table 3-a Mortality by mixed HgCl2 and CdSO4 of the frogs (small size) O2 saturated

No of specimens	LT 50% hours ± S.D.	LT 100% hours ± S.D.
10	10	20
10	14 ± 1	20 ± 2
10	10	14
Total 30	Mean 12	Mean 18

Table 3-b Mortality by mixed HgCl2 and CdSO4 of the frogs (Small size) O2 non-saturated

No of specimens	LT 50% hours ± S.D.	LT 100% hours ± S.D.
10	4	12
10	8 ± 1	10 ± 1
10	6	8
Total 30	Mean 6	Mean 10

Table 4-a Mortality by mixed HgCl2 of the frogs (large size) O2 saturated

No of specimens	LT 50% hours ± S.D.	LT 100% hours ± S.D.
10	30	40
10	30 ± 1	54 ± 2
10	34	45
Total 30	Mean 32	Mean 48

Table 4-b Mortality by mixed HgCl2 of the frogs (Large size) O2 non-saturated

No of specimens	LT 50% hours ± S.D.	LT 100% hours ± S.D.
10	18	38
10	22 ± 1	40 ± 2
10	20	36
Total 30	Mean 20	Mean 38

Table 5-a Mortality by CdSO4 of the frogs (large size) O2 saturated

No of specimens	LT 50% hours ± S.D.	LT 100% hours ± S.D.
10	66	75
10	60 ± 1	69 ± 2
10	66	72
Total 30	Mean 64	Mean 72

Table 5-b Mortality by CdSO4 of the frogs (large size) O2 non-saturated

No of specimens	LT 50% hours ± S.D.	LT 100% hours ± S.D.
10	51	5
10	54 ± 1	63 ± 2
10	57	70
Total 30	Mean 54	Mean 66

Table 6-a Mortality by mixed HgCl2 and CdSO4 of the frogs (large size) O2 saturated

No of specimens	LT 50% hours ± S.D.	LT 100% hours ± S.D.
10	22	34
10	22 ± 1	30 ± 2
10	25	29
Total 30	Mean 23	Mean 31

Table 6-b Mortality by mixed HgCl2 and CdSO4 of the frogs (large size) O2 non-saturated

No of specimens	LT 50% hours ± S.D.	LT 100% hours ± S.D.
10	7	14
10	10 ± 1	12 ± 2
10	10	13
Total 30	Mean 9	Mean 13

Table 7-a Bio-accumulation µg/g dry weight of small size frog legs muscle (5 gr) after LT 100% for the studied metals small size, O2 saturated

Bio-accumulation µg/g dry weight	Metal	Metal	Total
	HgCl2	CdSO4	
Separated metals	72.115	103.257	175.372
Mixed metals	54.646	39.731	148.377

Table 7-a Bio-accumulation µg/g dry weight of small size frog legs muscle (5 gr) after LT 100% for the studied metals small size, O2 saturated

Bio-accumulation µg/g dry weight	Metal	Metal	Total
	HgCl2	CdSO4	
Separated metals	72.115	103.257	175.372
Mixed metals	54.646	39.731	148.377

Table 7-b Bio-accumulation µg/g dry weight of large size frog legs muscle (5 g) after LT 100% for the studied metals large size, O2 saturated

Bio-accumulation µg/g dry weight	Metal	Metal	Total
	HgCl2	CdSO4	
Separated metals	96.126	172.434	268.560
Mixed metals	80.179	112.233	192.412

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