

Cadmium Accumulation and Distribution in *Xenopus Laevis* after Long-Term Cadmium Treatment

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Abstract. The distribution of cadmium in *Xenopus laevis* was studied polluted by cadmium. *X. laevis* was exposed to 0.5 mg/L, 1 mg/L and 5 mg/L cadmium respectively. After 30 days of cultivation, the kidney, muscles and reproductive organs of *X. laevis* were dissected and measured to investigate the accumulation and distribution of cadmium in *X. laevis*. The results showed that the cadmium content in kidney, genital organ and muscle was significantly different ($p < 0.05$), after long-term treatment with cadmium. Compared with the control group, in 0.5, 1 and 5 mg/l treatment groups, the content of cadmium increased by 3.83, 5.34 and 3.60 times in muscle respectively, by 5.45, 8.99 and 9.04 times in kidney respectively, and by 3.86, 5.23 and 7.69 times in reproductive organs respectively. This study confirmed that the accumulation of cadmium in *X. laevis* increased significantly after cadmium treatment, but the distribution of cadmium in various organs was uneven.

1. Introduction

Cadmium (Cd) is a silver-white lustrous metal with toxicity [1]. The air, water environment and food polluted by cadmium are harmful to human and animals [2, 3]. Cadmium compounds are not easily digested and absorbed by the intestines of organisms, but they can be absorbed by organisms through respiration and accumulated in the liver or kidneys, especially in the kidneys. Kidney and reproductive organs are some parts of the body that cause cadmium accumulation, the rest are distributed in the lungs, pancreas, thyroid, testis and ovaries. The content of Cd in organs and tissues varies greatly with different regions and environmental pollution.

Cadmium is also highly toxic to aquatic organisms [4, 5]. It is an unnecessary and highly toxic element of aquatic animals. It has the characteristics of strong lipophilicity, easy enrichment and refractory degradation, and may have long-term adverse effects on the water environment. Due to the unique life history of amphibians and the high permeability of their skin at various stages of development, heavy metals, especially cadmium, can easily enter their bodies, thereby harming aquatic organisms [6, 7]. In this paper, *Xenopus laevis* was used as the research object to study the distribution and concentration of cadmium by long-term treatment in Cd-contaminated water environment.



2. Materials and methods

2.1. Experimental animal

X. laevis is an aquatic frog, and it is an important model organism. Complete aquatic organisms, whether tadpoles or frogs, basically live in water.

2.2. Instruments and reagents

Drying box, temperature control electric heating plate, electronic balance (Shanghai Yueping Science Instrument Co., Ltd., model: FA2204B Max220g) for sample pretreatment and standard solution preparation. Graphite Furnace Atomic Absorption Spectrophotometer (Perkin Elmer, USA, model: PinAAcle 900) with cadmium hollow cathode lamp were to detect the concentration of cadmium standard solution. Picking gun, other necessary utensils, such as bowl, crucible, capacity bottle, centrifugal tube, glass rod, beaker, measuring barrel, rubber head dropper, etc were used.

CdCl₂ samples (purity 99.99%, CAS: 10108-64-2) were used to prepare standard reserve solution with concentration of 1000 mg/ml. The standard storage solution was diluted to cadmium at a concentration of 0.5, 1, 5 mg/L.

2.3. Poisoned way

X. laevis (tadpole stage) was randomly divided into four groups: A, B, C and D. There were three dosage groups and one control group, and each group had 10 individuals including five females and five males (Table 1). They were placed in an aquarium with 53.5 cm × 39.0 cm × 29.0 cm. Tap water needs to be purified and dechlorinated. The temperature of water environment is about 17.5 ± 0.5 °C.

Table 1. Treatment methods of *Xenopus laevis* in cadmium polluted water environments

Group	Cadmium concentration (mg/l)	Average weight of tadpoles (g)	Water capacity (ml)	Group name
A	0	3.9933	5000	Control group
B	0.5	4.0701	5000	Experience group
C	1.0	3.8726	5000	Experience group
D	5.0	4.0182	5000	Experience group

2.4. Determination of heavy metals

The *X. laevis* was sampled in serial numbers after 30 days of treatment. After each *X. laevis* was dissected, its reproductive organs, lungs, liver, kidneys, pancreas, thyroid gland and muscle were taken out, grouped and labeled.

1% nitric acid (made of super pure reagent and deionized water), nitric acid and hydrogen peroxide mixture (HNO₃: H₂O₂=2:1). 1% nitric acid, 0.2% magnesium nitrate and 1% ammonium dihydrogen phosphate are all of high grade purity. All glass and plastic containers are soaked in 2% HCL for 2 hours, cleaned with deionized water for many times, then soaked in 2% HNO₃ for 2 hours, and cleaned with deionized water for many times.

Add 3ml fresh HNO₃: H₂O₂=2:1 to the selected samples. The mixture was kept overnight or digested until the solution was clear and transparent. If a sample is not digested completely, some mixed digestive solution can be added until the sample is digested completely. The digested sample is placed on a temperature-controlled electric heating plate to drive acid to a wet salt state (solution volume should be less than 0.2 ml). The content of metal in acid tissue fluid was determined by graphite furnace atomic absorption spectrometry (GFAAS). The content of heavy metals in *X. laevis* (μg/g) = (determination of concentration of sample solution × dilution multiple × solution volume) / the quality of sample. By using PinAAcle 900 atomic absorption spectrophotometer, graphite furnace was selected for determination of Cd elements. The working conditions of the instrument are shown in Table 2 below:

Table 2. Test conditions for graphite furnace atomic absorption spectrophotometer

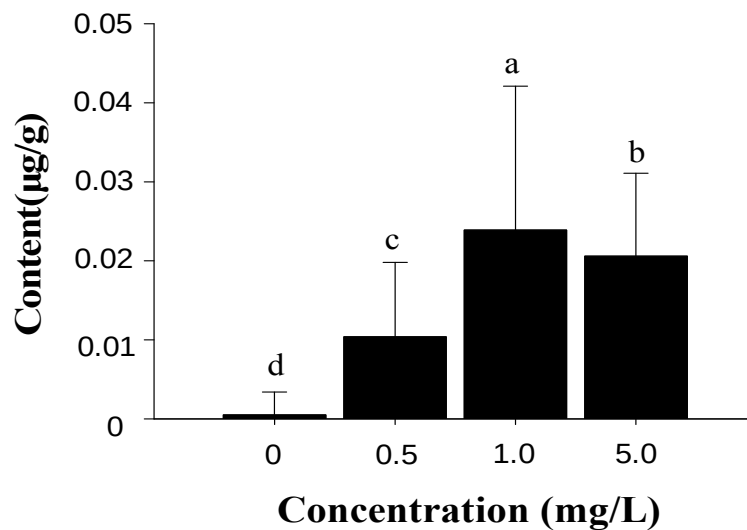
Dry1		Dry2		Ashing		Atomization		Clean	
temperature /°C	time /s	temperature /°C	time /s	temperature /°C	time /s	temperature /°C	time /s	temperature /°C	time /s
110	90	130	30	850	20	1700	5	2450	3

3. Results

After long-term living in cadmium-contaminated environment, cadmium was found in the kidneys, muscles, liver, reproductive organs, lungs, pancreas and thyroid glands of *X. laevis*.

3.1. Cadmium content in muscle

After long-term treatment with cadmium, *X. laevis* had no obvious changes in external morphology, but the content of cadmium in muscle was relatively high. The cadmium content in muscle of different treatment groups was significantly different ($p < 0.05$). With the increase of cadmium concentration, the cadmium content in muscle increased first and then decreased. In the 1.0 mg/l treatment group, cadmium content was the highest, while in the 5.0 mg/l treatment group, cadmium content in muscle decreased. Compared with the control group, the content of cadmium in muscle of 0.5, 1, 5mg/l treatment group increased by 3.83, 5.34 and 3.60 times respectively.

**Figure 1.** Distribution of cadmium in muscle of *X. laevis* after 30 d treatment

3.2. Cadmium content in kidney

Cadmium has the most distributed in the kidneys of *X. laevis*, indicating that cadmium is easily accumulated in the kidneys and can not be easily discharged directly from the body. With the increase of cadmium content, it was found that the metamorphosis of *X. laevis* delayed and the development of *X. laevis* was slow. In different treatment groups, there was a significant difference in the content of cadmium in the kidney ($p < 0.05$). With the increase of cadmium concentration, the content of cadmium in kidney increased gradually. When the concentration began to increase, the content of cadmium increased rapidly, then slowed down gradually and maintained within a certain concentration range. Compared with the control group, the cadmium content in kidney of 0.5, 1, 5mg/l treatment group increased 5.45, 8.99, 9.04 times respectively.

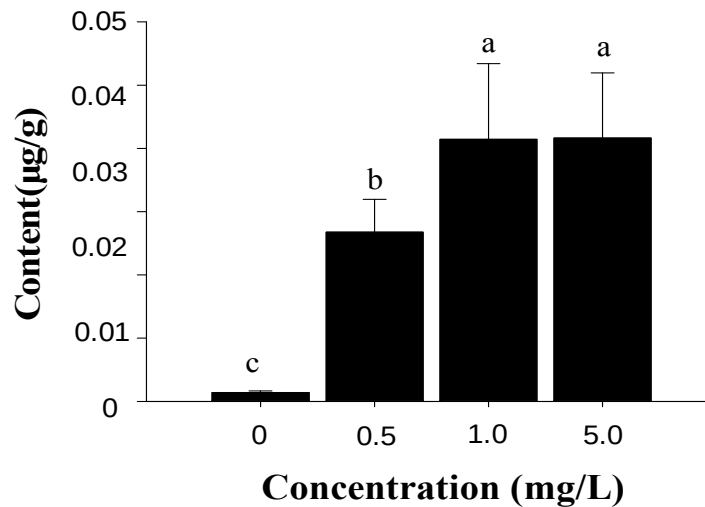


Figure 2. Distribution of cadmium in kidneys of *X. laevis* after 30 d treatment

3.3. Cadmium content in Reproductive organ

Several ovarian cavities, testicular atrophy and interstitial abnormalities were found on the unilateral side of the gonad after treatment with cadmium, suggesting that cadmium may affect sperm activity, may lead to difficult fertilization or abnormal fertilized eggs, and affect the survival rate of the next generation. After treatment, no morphological changes were found in the ovary. In different treatment groups, there was a significant difference in the cadmium content in the genital organs ($p < 0.05$). With the increase of cadmium concentration, the content of cadmium in kidney increased gradually. Compared with the control group, the content of cadmium in genitals of 0.5, 1, 5mg/l treatment group increased by 3.86, 5.23 and 7.69 times respectively.

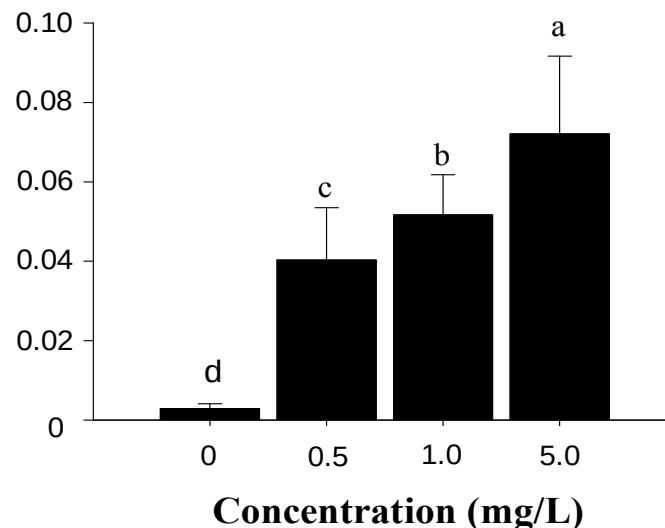


Figure 3. Distribution of cadmium in the reproductive organs of *X. laevis* after 30 d treatment

4. Conclusion

Cadmium is mainly distributed in the kidneys, muscles and reproductive organs of *X. laevis*. The accumulation of cadmium in the kidneys is much higher than that in other parts of *X. laevis*. The kidney is a very important organ in the organism. Its main function is to remove metabolites and some waste and toxic substances in the body. These functions ensure the stability of the internal

environment of the body. If the kidney is poisoned by heavy metals such as cadmium, its effects can not be restored. The harm of cadmium to the reproductive system is still serious. Cadmium alters the morphology of the testicle and changes the physical and chemical properties of sperm, such as reducing sperm production and survival. In recent years, heavy metal pollution has become more and more serious. It is necessary to take necessary measures to reduce pollution [8, 9]. For example, industrial sewage should be treated strictly to meet the discharge standards, and people's awareness of environmental protection should also be enhanced.

Acknowledgments

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