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Toxicology

CADMIUM BIOACCUMULATION AND ITS EFFECTS ON SOME HEMATOLOGICAL AND HISTOLOGICAL ASPECTS IN CARP, *CYPRINUS CARPIO* L., AT SELECTED TEMPERATURE

BIOAKUMULACJA KADMU W KARPIU, *CYPRINUS CARPIO* L. PRZY WYBRANEJ TEMPERATURZE ORAZ JEJ WPŁYW NA ZMIANY HEMATOLOGICZNE I HISTOLOGICZNE

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Cadmium bioaccumulation and its effects on the changes of some hematological parameters in carp, *Cyprinus carpio* L., was studied. Carp was exposed 24 hr to acute concentration of cadmium (0.5 mg Cd/dm^3 water) at 27°C. Particularly the greatest accumulation of cadmium was in gills, kidneys, alimentary canal, hepatopancreas, and with lesser degree in spleen and vertebral column; while in skin and muscles accumulated only low levels of cadmium. Hematologically, cadmium bioaccumulation significantly rised erythrocytes count, hemoglobin content, hematocrite value and blood glucose, but decreased leukocytes count in comparison to control samples. Histologically, cadmium caused pathological alterations in the gill filaments and respiratory lamellae, hepatopancreas and kidney but did not affecting the skin. In addition, cadmium disturbed the metal contents (Cu, ZN, Fe and Mg) in organs in which it accumulated.

INTRODUCTION

Anthropogenic contamination of the aquatic environment by cadmium has increased substantially in the last several decades and resulted in the elevation of Cd in the tissues of aquatic organisms at all trophic levels. Although acute toxically testes are used to safe concentration of toxicants in the environment, they provide little informations on the

mode of toxic action or environmental situations where accessory factors affect toxicity. By combining physiological and histological studies with more traditional acute toxicity test one can gain insight into the mode and site of toxic action, as well as determine environmentally safe concentrations of toxicants. Cadmium is highly able to accumulated in the living organisms. In fish, the gill, kidney, alimentary canal, and liver are the primary target organs for cadmium (Rowe and Massaro, 1974; Sangalang and Freeman, 1979; Giles, 1988). Due to its ability to accumulated in organs, several pathobiochemical and histological alterations appeared (Axelson and Piscator, 1966; Gardner and Xevich, 1970; Giles and Pant, 1983; Lowe-Linde and Niimi, 1984; Donald and Giesy, 1986). Many of the observed toxic effects of cadmium are though to be the results of induced secondary deficiencies in such essential trace elements as copper, iron and zinc, since the uptake of cadmium both antagonizes and antagonized by the uptake of these metals (Bremner, 1974). Cadmium induced disturbance in copper, iron and zinc in organs that accumulated it (Stonard and Webb, 1976; Ashby et al., 1980). The present study was conducted to compare the results of histopathological and hematological diagnostis procedures relative to elucidating the mode and sites of cadmium accumulation and toxicity in carp., *Cyprinus carpio* L., that exposed to cadmium.

MATERIALS AND METHODS

Healthy carp, *Cyprinus carpio L.*, was collected from Fish Farming Station at Nowe Czarnowo in Poland. Fishes were acclimized in the laboratory three weeks at 19°C in a large well-aired aquaria. Fish average weight was 450∓50 gramms. The sample size for each experiment was four fishes. Fishes were divided into two groups, the first one (control) kept in cadmium free water at 27°C, while the second group exposed 24 hr to acute concentration of cadmium in water (0.5 mg Cd/dm^3 water) at 27°C. After the exposure period, fish samples were removed from the aquaria. Blood was collected from the caudal vessel by means of heparinized cold syringe (Johanson-Sjobeck and Larsson, 1978), fishes were killed and dissected quickly to extract gills, kidneys, alimentary canal, muscles, hepatopancreas, spleen, vertebral column, and skin for bioaccumulation and histological studies.

HEMATOLOGICAL ANALYSIS: Erythrocyte count was determined by spectrophotometric method; while leukocyte count was determined by mixing method and counting took place by Thoma-slide. Hemoglobin concentration was determined by cyanmethemoglobin method (Wintrobe, 1956) using Drabkin's reagent. Hematocrite value was determined by using heparinized microhematocrite tubes that filled with blood samples and sealed on one end, then centrifuged 15 minutes at 11000 r.p.m. in microhematocrite centrifuge, and the ratio of erythrocytes to plasma in percents was measured with an hematocrite reader. Blood indices were computed from Hb, Ht values and erythrocytes count:

$$MCHC\ (\%) = \frac{Hb\ (gm/100\ ml\ blood) \times 100}{Ht\ (\%)}$$

$$MCH\ (pg) = \frac{Hb\ (mg/100\ ml\ blood) \times 10}{RBC\ in\ millions/ml\ blood}$$

$$MCV\ (\mu m^3) = \frac{Ht\ (\%)}{RBC\ in\ millions/ml\ blood}$$

blood glucose was determined by o-toluidine method.

HISTOLOGICAL STUDIES, were done by manual routine method and staining was in hematoxylin and eosin.

CHEMICAL ANALYSIS. Cadmium and other metals (Cu, Fe, Zn, and Mg) were determined by atomic absorption spectrophotometric metod after complete oxidation by a mixture of HNO_3+$HClO_4$ (4:1) (Protasowicki, 1985). The elements concentration were represented in $\mu g/g$ wet organ. Statistically, data were analysed by F-test for variance and Student's t-test or Welch's and Aspin's test at 0.01 and 0.05 level of significance.

RESULTS AND DISCUSSION

Cd, Cu, Zn, and Mg concentrations in different organs of carp following acute exposure to Cd are listed in table (1). Gills, kidneys, alimentary canal, and hepatopancreas accumulated the greatest amount of Cd, but spleen and vertebral column accumulated a lesser degree, as well as muscle and skin accumulated only very low levels of Cd in comparison to control samples. Cadmium is selectively localized in tissues and the investigations designed here to determine the fate of Cd in the body of carp after exposure to cadmium indicated that the metal is normally accumulated in the following order: gill > kidneys > alimentary canal > hepatopancreas > remainder of the body > the muscle. This selectivity was similarly mentioned in previous studies. In present study, muscle and skin accumulated only low levels of cadmium supporting other observations of other (Sangalang and Freeman, 1979; Wilson et al., 1981; Giles 1988; Protasowicki and Chodyniecki, 1988) species of fresh water fishes.

EFFECT OF CADMIUM ON TRACE METALS: In present study there is no significance correlation between cadmium and other metals after the exposure to cadmium, but there is a significant differences in comparison to control samples.

Copper increased in skin, ileum, and liver, but decreased in gills, muscle, and vertebral column. These results are parallel to data reported by some authors who suggested that Cd may disturb Cu metabolism by inhibiting the biliary excretion of Cu resulting in

Table 1

Cadmium, copper, zinc, iron, and magnesium concentrations in organs of carp (μg/g wet organ)

Organs		Cd	Cu	Zn	Fe	Mg
Gill	C	0.213±0.009	1.06±0.08	333.1±50.7	143.4±11.5	517.1±16.6
	T	7.229±4.048**	0.72±0.20*	34.8±3.50**	142.8±26.4	446.1±14.5
HP	C	0.330±0.029	16.43±9.6	209.4±69.0	174.3±27.8	280.3±95.2
	T	0.711±0.011**	37.41±2.9*	35.3±20.2**	87.4±14**	257.7±56.1
Kid.	C	0.709±0.267	1.42±0.19	917.5±56.3	113.5±48.2	201.8±50.7
	T	1.738±0.314**	1.70±0.22	272.4±25.6*	118.6±10.8	211.8±47.8
AC.	C	0.548±0.106	1.79±0.05	454.5±17.8	16.3± 3	247.5±48.1
	T	1.048±0.150**	8.21±1.69**	409.7±13.7	72.3±15.7*	404.2±45.9*
Sp.	C	0.214±0.053	3.12±1.73	153.4±12.7	163.6±15.6	267.3±30.5
	T	0.306±0.007**	1.13±0.14*	195.0± 6**	233.7±20.8	254.1±15
VC.	C	0.230±0.083	1.59±0.19	70.12±3.4	011.9±0.8	999.9±39.9
	T	0.575±0.291	0.78±0.14	54.59±10.9*	36.0±12**	918.6±42.2
Skin	C	0.047±0.004	0.48±0.08	118.5±24.6	13.82±2.6	115.7±12.7
	T	0.060±0.008*	0.74±0.04**	51.6±11.0**	7.98±0.8*	148.5±21.5*
Mus.	C	0.049±0.011	0.32±0.02	8.48±1.700	3.83±0.7	344 ±32.4
	T	0.051±0.009	0.25±0.04*	3.89±0.210**	6.19±0.8*	773.8±65.6**

Note, each value represents the mean concentration in organs (μg/g) wet organ) ± SD. Asterisks denote significance differences between test' and control groups; * $P < 0.05$ and ** $P < 0.01$. (C) denotes control samples; and (T) the toxicated. HP (hepatopancreas), AC (alimentary canal), Kid. (kidney), Sp. (spleen), VC. (vertebral column), Mus. (muscles).

accumulation of liver copper which stimulates ceruloplasmin synthesis. Since one function of ceruloplasmin is a copper donor to extrahepatic tissues (Owen, 1965).

Iron is decreased significantly in liver and skin, while increased in ileum, muscle and vertebral column. The disturbance in Fe concentrations after exposue to acute Cd conc. suggested that Cd also interfers with Fe metabolism. The decreased in hepatic Fe may be due to increased the lost of Fe as ferritin in the process of erythropeiosis to increase RBC production, as supported in present results (table 2), and that confirmed by Stonard and Webb (1976) who – reported that diminished ferritin iron in liver after cadmium administration. Zinc was decreased markdely in all organs; and that may be due to increase Zn excretion in bile. Magnesium, in present results, was not affected significantly, except in ileum, muscle, and skin Mg increased significantly, i.e. Cd is synergtic to magnesium absorption in ileum.

HISTOPATHOLOGY: Gross examination revealed that gill filaments were thicker three to four times the size of those in controls. No other significant gross pathology was obvious. Microscopic examinations, stricking histopathology was observed in gill filaments and respiratory lamellae, kidney, and hepatopancreas of carp after cadmium exposure.

Gill filaments and respiratory lamellae: Alterations of gill filaments and respiratory lamellae were represented in figure 1b. Microscopic examination revealed hypertrophy of gill filaments, and hyperplasia of the epithelial surface of respiratory lamellae and interlamellar filament epithelium. The hyperplasia was accompanied by a marked increase of mitotic figures, and the lamellae fused together and appeared as club-shaped lamellae. Necrotic and sloughed respiratory epithelium was observed. Our present observations are parallel to opinion of some authors that suggested respiratory system of teleost may be damage by cadmium, since acute concentration of cadmium was found to cauterize the gill lamellae of several fresh water fishes (Karlsson et al., 1985). In the instance of necrotized gill epithelium, the underlying basal cells were also destroyed. The impairment of these cells, from which the gill epithelium generates, might essentially nullify external activity of the gills. Further, the respiratory and external functions of the gill would appear to be impaired, following hypertrophy and hyperplasia of the internal epithelia, by more reduction of the respiratory surface in comparison to control (fig. 1a).

Kidneys: The damage appeared limited to the proximal tubules of the kidney after exposure to Cd (figure 2b) in comparison to controls (figure 2a). Many of the proximal tubules exhibited pink – staining granules casts with nuclear debris, as well as cloudy swelling and hypertrophy of proximal tubules epithelial cells. The investigations designed to determine the fate of Cd in he body indicated that Cd is normally accumulated in the proximal tubules of kidney, where renal damage is considered to appear first in the case of intoxication (Axelsson and Piscator, 1966; Protasowicki and Morsy, in press).

Hepatopancreas: The alterations in hepatopancreas, due to cadmium exposure, were represented in the damage, atrophy, and necrosis of hepatic cells that decreased in size, and their outline became indistingushable, and their nuclei and nucleoli became small in size (figure 3b) in comparison to controls (figure 3a).

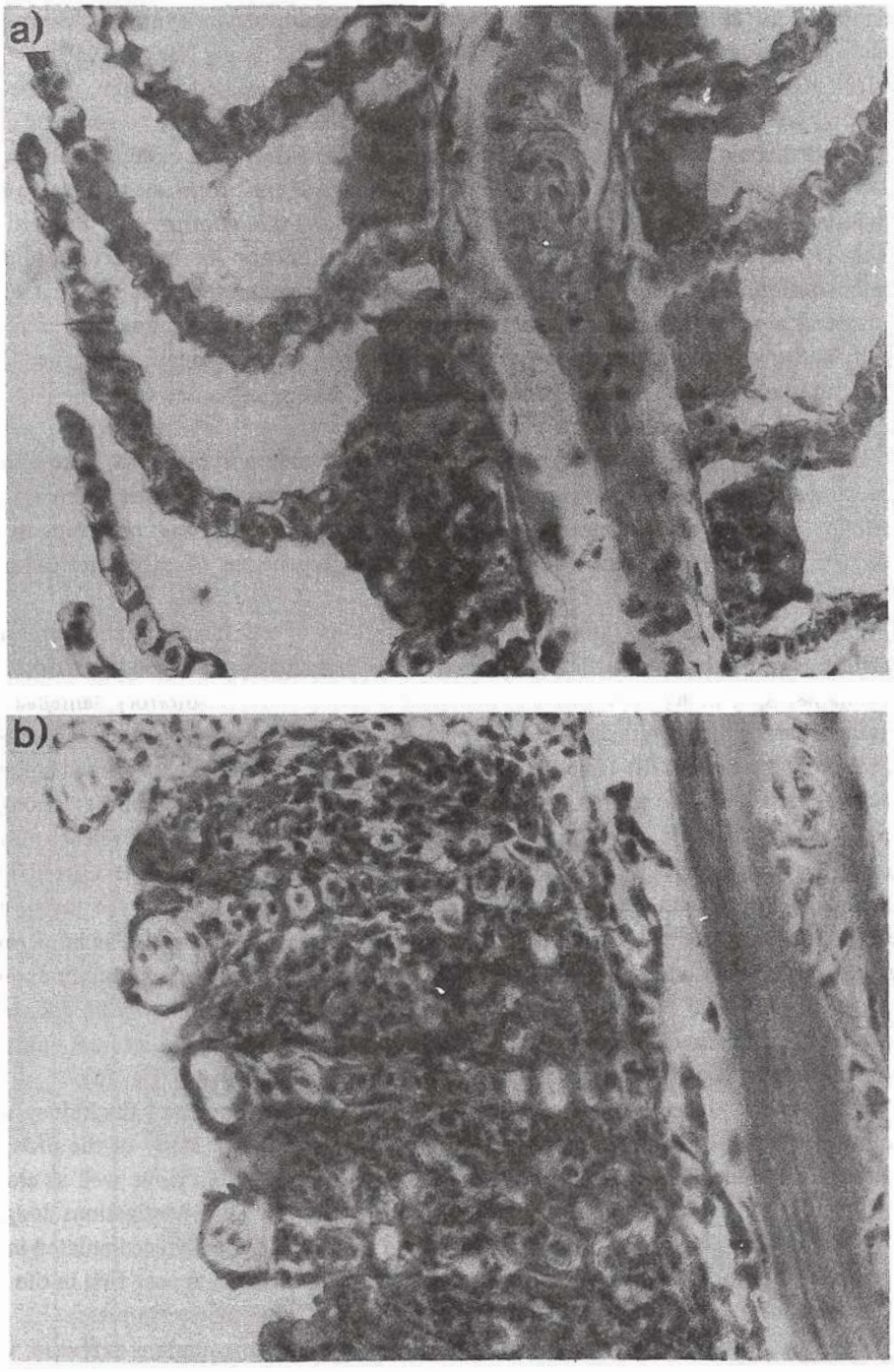

Fig. 1. Gill filaments, (a) represents the typical structure; while (b) indicates the toxic effect of cadmium (x1000)

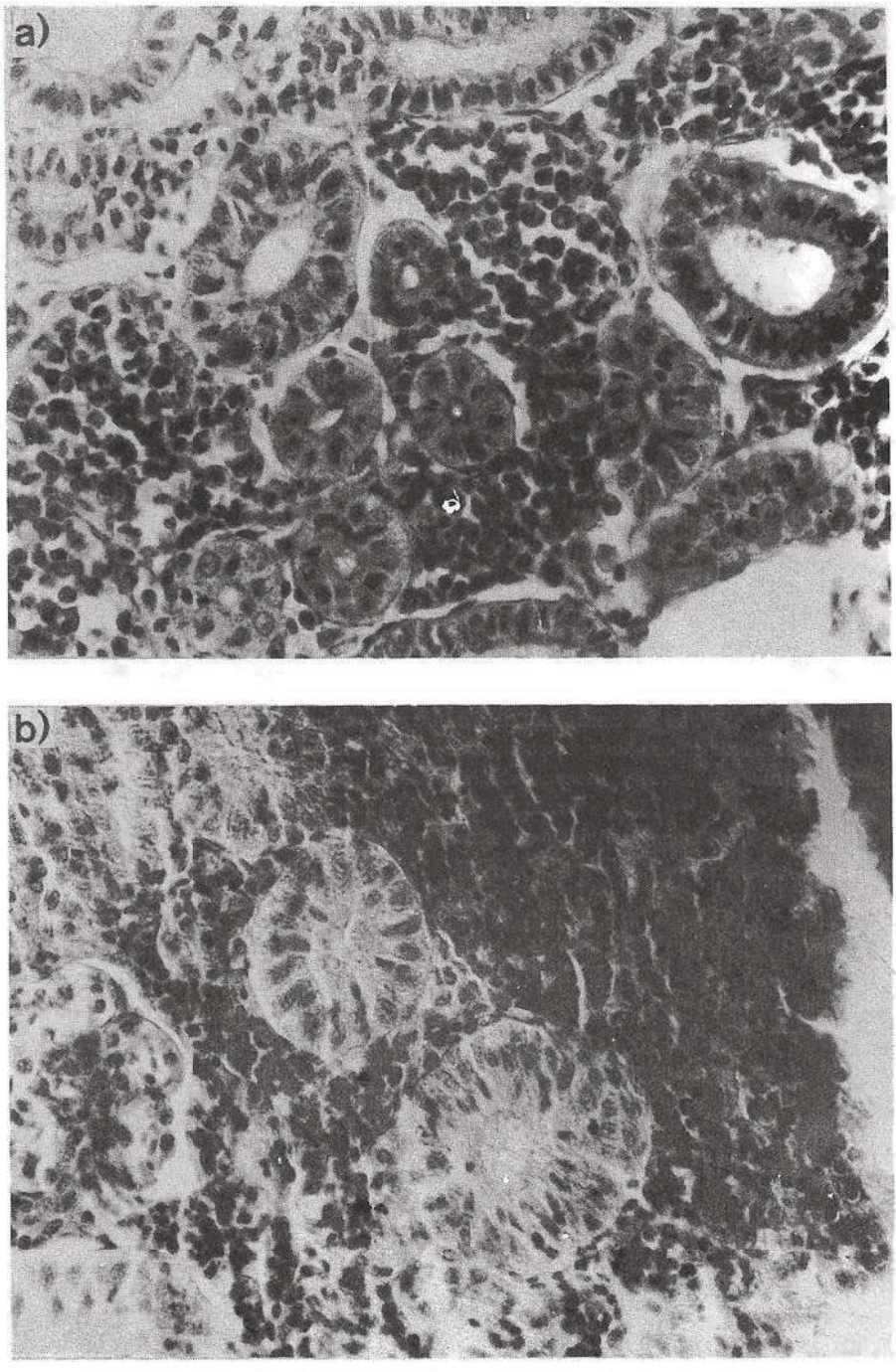

Fig. 2. Kidney, (a) represents normal architecture, whereas;
b) show cadmium induced damage to renal tubules after 24 hr of exposure (x945)

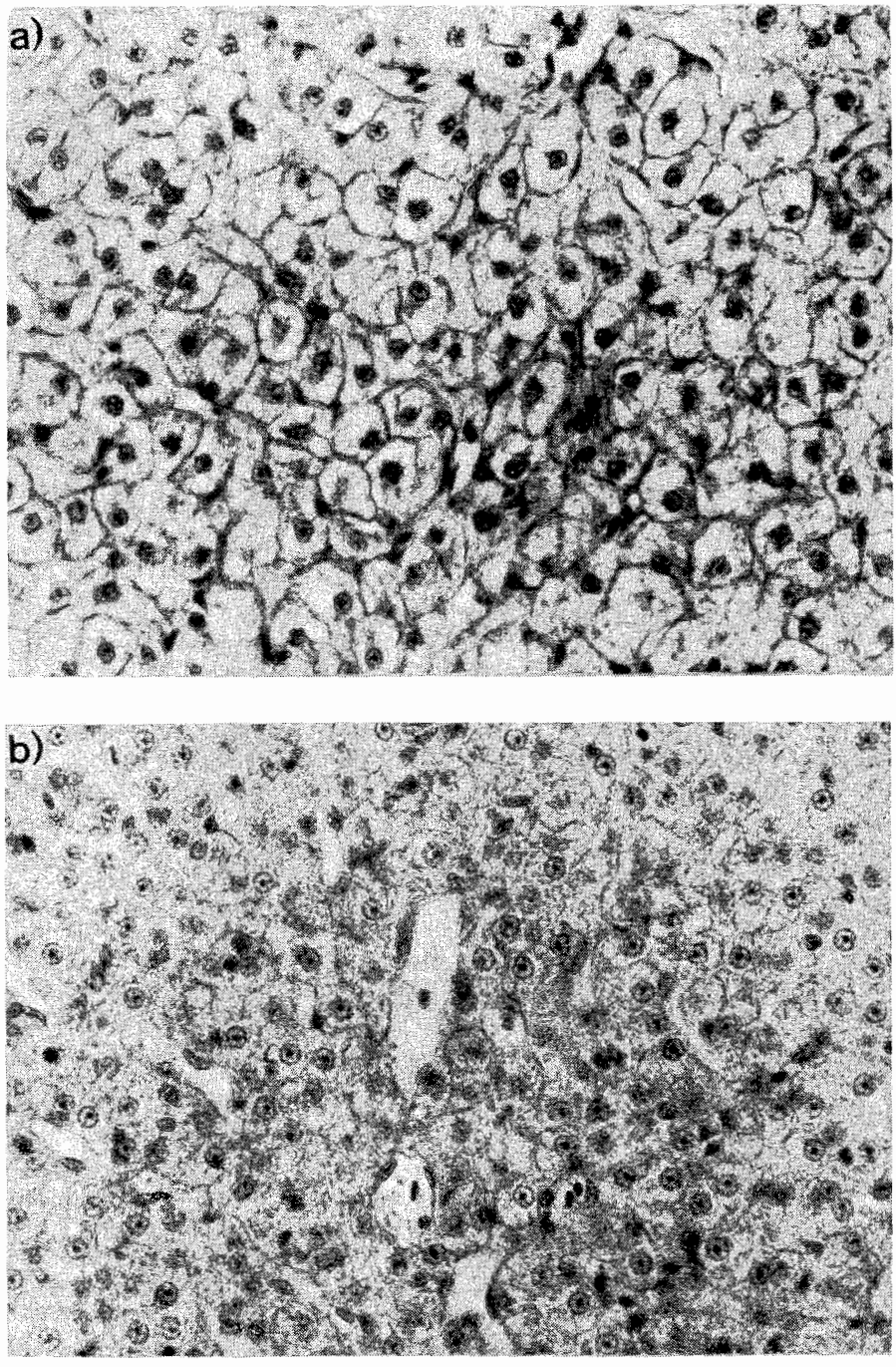

Fig. 3. Liver (a) represents the typical appearance of the hepatic cells, while (b) indicates stage of cadmium effect (x630)

Table 2

RBCC, Hb, Ht, MCHC, MCH, MCV, WBCC, and blood glucose of carp after exposure to cadmium (T), and the controls (C)

	RBCC	Hb	Ht	MCHC	MCH	MCV	WBCC	Glucose
C	2.36∓.04	10.8∓.4	42.0∓1.0	26∓.28	44∓4.6	183∓1.8	9.4∓.7	57.5 ∓8
T	2.74∓.20**	12.2∓.4**	47.0∓2.0**	26∓.33	44∓4.9	181∓1.9	4.5∓.7**	143∓30**

Each value represents the mean of four fishes ∓ SD. Asterisks denote differences between test' and control groups: * $P < 0.05$ and ** $P < 0.01$.

Skin: There was no histological changes for skin after exposure to cadmium, and that may be due to low level of Cd accumulated by the skin that increased the mucous secretion during exposure period, and that fixed Cd on the surface as a protection against toxicants.

HEMATOLOGICAL EFFECT: RBCC, Hb, Ht, and blood glucose were increased significantly, while leukocytes decreased; but did not caused any statistical significance for MCHC, MCH, and MCV after exposure to Cd, in comparison to controle (table 2). The increased RBCs, Ht, and Hb may be as indirect effect of cadmium on gills, since as observed above, Cd impairment in respiration and increased oxygen consumption (Bishope and Alan, 1981) due to gill destroying, and this caused shortage in oxygen supply for fish. Oxygen shortage acts as activating factor for RBCs and Hb production to capture more amount of oxygen (Wagh et al., 1985). Hyperglycemia may be due to alternations in physiological functions of α- and β-cells in Islet's of Langerhans as a result of Cd accumulation in hepatopancreas, and that caused disturbance in blood glucose level (Giles and Pant, 1983).

CONCLUSION

We have been detected cadmium – induced damagae of tissues during acute exposure which to be able to produce histopathological effects on the internal organs that accumulated a greatest amount of cadmium, and this caused some hematological alternations in carp, i.e. it is possible to combining histological and hematological alternations to indicate the site and mode of acute cadmium toxicity. In addition, acute exposure to cadmium significantly alter the tissue disposition of other elements; but the mechanism by which cadmium exerts its influences on other metals are not, as yet, fully understood.

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BIOAKUMULACJA KADMU W KARPIU – *CYPRINUS CARPIO* L. PRZY WYBRANEJ TEMPERATURZE ORAZ JEJ WPŁYW NA ZMIANY HEMATOLOGICZNE I HISTOLOGICZNE

STRESZCZENIE

Badano bioakumulację kadmu i jej wpływ na zmiany niektórych parametrów hematologicznych oraz histologicznych u karpia – *Cyprinus carpio* L. Karpie eksponowano na wysokie stężenie kadmu (0,5 mg Cd/dm^3 wody) przez 24 godz. w temperaturze 27°C. Najwyższa akumulacja kadmu miała miejsce w skrzelach, nerkach, przewodzie pokarmowym i wątrobotrzustce. W mniejszym stopniu kumulowały ten pierwiastek śledziona i kości kręgosłupa. Natomiast w skórze i mięśniach jego zawartość była najmniejsza.

Bioakumulacja kadmu w porównaniu z próbą kontrolną powodowała istotny wzrost liczby erytrocytów, zawartości hemoglobiny, hematokrytu i poziomu glukozy we krwi przy równoczesnym spadku liczby leukocytów.

W obrazie histologicznym, u ryb poddanych intoksykacji, kadm powodował patologiczne zmiany w listkach skrzelowych, wątrobotrzustce i nerkach, przy braku widocznych zmian w skórze.

Ponadto kadm powodował zaburzenia poziomu innych metali (Cu, Zn, Fe i Mg) w narządach w których odkładał się w znacznych ilościach.

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