

Use Fry of *Cyprinus Carpio* as Biomarker for Lead and Cadmium

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ABSTRACT

Fishes are considered a good biomarker and bioindicator of pollution, Our current research aims to investigate the magnification of (Pb and Cd) and how both affect antioxidants and use fry carp *Cyprinus carpio* as a bioindicator. The samples were analyzed to find the possible biomagnification of metals in fish. The highest concentration in fry fish fed with Artemia used *Dunaliella salina* as food treated with Pb in Food type 1 for 28 days, with a significant difference. The highest concentration of Cd in fry fish fed with Artemia used *D. salina* as food treated with Cd in food type 1 for 28 days, with a significant difference. The activity of the enzyme (SOD - GSH - CAT- GST) U/gm is affected by toxicity in fry fish mussels. The investigation has proved that exposure of fish to (lead and cadmium) induced a significant increase in content and increased activity of GST U/gm. For this purpose, an aquarium-based trial was conducted with two different types of food treated with Cd and Pb. The highest concentration of SOD U/gm enzyme of fry fish fed with Artemia used *D.salina* treated with Pb in food type 1 for 28 days when fry fish fed on Artemia used food treated with Pb the results show a strong positive significant correlation between (GPX with SOD and CAT) U/gm and (SOD with CAT) U/gm. While the concentration has a positive significant correlation with all enzymes, With Cd the highest concentration was in the SOD U/gm enzyme in fry fish fed with Artemia that used *D. salina* as food treated with Cd in food type 2 for 28 days.

Keywords: *Cyprinus carpio*, heavy metal, pollution, oxidative stresses, accumulation, magnification.

INTRODUCTION

Aquatic environments are polluted by metals that are produced from different activities such as mining, industrial, and domestic wastewater discharge, In addition to natural activities such as volcanic. The most common heavy metal pollutants are Cd, Pb, etc., The metals can accumulate in fauna like fish, and stabilize in water and sediments (Muhammad *et al.*, 2022).

Lead is bioaccumulated in fish organs, such as the gill, liver, and spleen, as well as in both systems urine and the digestive (Abdel-Warith *et al.*, 2020). Pb affects the fish's blood parameters (Ikeogu *et al.*, 2016) Additionally, Pb toxicity causes changes in enzyme activity in the liver and the plasma, which leads to damage in the cell membrane and shreds the liver cells in fish (Farhan *et al.*, 2023). Also negatively affects growth by reducing weight gain, rate of growth, and food intake (Zulfahmi *et*

al., 2021). Fish are an important type in aquatic reservoirs. They are one of the best types of diets for humans, and the heavy metals presence in them can cause a health danger. (Ullah *et al.*, 2017) the higher bioaccumulative fish capability and is very sensitive to chemicals, toxicant materials, and any change in their niche; fish is a good model that can be used in toxicology, drug or chemical risk determination, safety studies, and aquatic or environmental toxicology. (Ullah *et al.*, 2021).

The heavy metals can stabilize in the environment, they accumulate in organisms and are transferred through the food chains. Their accumulation in biota depends on their rate of collection and their removal from their bodies. According to Kalisinska *et al.* (2017). Metals bioaccumulation depends on factors of biotic(species, mass, dimensions of body, age, sex, metabolism, feeding types, and their level in the trophic pyramid) in addition to the abiotic factors (metals distribution

in the environment, temperature of water, pH, salinity and interactions with other metals) The heavy metal toxicity stimulate both of the oxidative stress and the antioxidant enzymes which are work as a protective mechanism. recently there has been discovered relationship between oxidative lesions in different organs of the common carp (*Cyprinus carpio*) and tumors in the liver that result from polluted environments (Medhat *et al.*, 2017).

The aim of this study determination the biomagnification of some heavy metals (Pb and Cd) through food chain determination of the effect of these heavy metals on some antioxidants in fry fish, for example, CAT - SOD- GPX –GST)U/gm.

MATERIAL AND METHODS

Fry of *Cyprinus carpio* fish were used as bio-markers. They were acclimatized for 3 days before we started the experiments, and then they were fed with two types of food that had previously been treated with heavy metals: food type (F1), which is only *Artemia* fed with two species of algae *D. salina* and *T. suecica* treated with lead and cadmium, and food type2 (F2), which is the same food mixed with aquarium industrial fish food 1:1. Then we measured the biomagnification concentrations in the fry fish muscles according to Schmitt and Finger (1987) after exposure for 28 days using an automatic spectrophotometer, while measuring the number of enzymes: CAT according (Clariborn, 1985) and (Aebi, 1974, 1984), SOD by (Marklund and Marklund, 1974), GPX by (Rotruck *et al.*, 1973) and (Hafemann *et al.*, 1974), and GST determined by (Habig *et al.*, 1974) for days 4 and 28 of exposure. Statistical analysis – the SPSS V 23 program was used for calculating the significant difference by using the Duncan test and correlation.

RESULTS AND DISCUSSION

The results of biomagnification of Table 1 showed the highest concentration in fry fish fed with *Artemia* used *D. salina* as food treated with Pb in F1: 194.5 ppm for 28 days, with a significant difference, and *T. suecica* when using F1: 153 ppm for 28 days with no significant difference. The highest concentration of Cd in fry fish fed with *Artemia* used *D. salina* as food treated with Cd in F1: 208.97 ppm for 28 days, with a significant difference, and for *T. suecica* 96.32 ppm in F2 for 28 days. Research has shown that both metals pose destroying effects on different fish systems such as hormonal, physiological, biochemical, histopathological, and hematological systems (Atli and Canli, 2011). However, the metal's toxicity is different based on its structure and essential or non-essential nature (Atli *et al.*, 2016). Olatunji-Ojo *et al.* (2020) stated that the causes of lead bioaccumulation may be caused by chronic exposure which may lead to death or damage to the central nervous system, brain, kidney, liver, and genotoxic. The residue of heavy metals may affect fish growth.

In Table 2. Results of the current study showed the highest concentration of the CAT enzyme in fry fish fed with *Artemia* used *D. salina* as food treated with Pb in F1: 20.52 U/gm for 28 days, with a significant difference, and *T. suecica* when using F1: 18.59 U/gm for 28 days with no significant difference. While the highest concentration of the SOD enzyme in fry fish fed with *Artemia* used *D. salina* as food treated with Pb in F1: 70.76 U/gm for 28 days, with a significant difference, and SOD for *T. suecica* 55.95 U/gm in F2 for 28 days. While with Cd The highest concentration in *D. salina* of CAT enzyme 16.76 U/gm in F 1 for 28 days and for

Table 1. Mean and Stander deviation of the concentrations for biomagnification in Fry fish that fed on *Artemia* which us (*D. salina*, *T. suecica*) as food, after treated with lead and cadmium

Metal	Alge	Metal concentrations (M±SD)				
		Control without metals	Food type 1 for 4 days	Food type 1 for 28 days	Food type 2 for 4 days	Food type 2 for 28 days
Pb ppm	<i>D. salina</i>	0 (ND) a	42.6 ± 1.65 b	194.5±1.04 c	13 ± 2.01 d	81.23 ± 0.856 e
	<i>T. suecica</i>	0 (ND) a	20.85 ± 0.9 b	153.48 ± 1.69 c	10.9 ± 0.33 d	74.02 ± 1.3 e
Cd ppm	<i>D. salina</i>	0 (ND) a	49 ± 1.55 b	208.97 ± 2.72 c	9.90 ± 0.87 d	41.73 ± 1 e
	<i>T. suecica</i>	0 (ND) a	11.97 ± 0.51 b	96.32±0.55 c	4.45±0.28 d	56.89±1.12 e

Note: * The similar letter for each row meanings are not significantly different ($p < 0.05$).

Table 2. Mean and standard deviation for CAT U/gm and SOD U/gm in Fry fish that fed on *Artemia* which us (*D. salina*, *T. suecica*) as food, after treated with lead and cadmium

Metal	Alge	Metal concentrations (M±SD)								
		Control	CAT U/gm				SOD U/gm			
			Food type 1 in 4 days	Food type 1 in 28 days	Food type 2 in 4 days	Food type 2 in 28 days	Food type 1 in 4 days	Food type 1 in 28 days	Food type 2 in 4 days	Food type 2 in 28 days
Pb	<i>D. salina</i>	0 (ND) a	14.79±3.43 a	20.52±0.82 b	16.56±0.13 a	9.66±8.45 a	64.02±2.8 d	70.76±1.71 e	53.74±0.1 c	56.04±3.00 c
	<i>T. suecica</i>	0 (ND) a	16.55±0.30 a	18.59±1.55 a	12.61±0.20 a	16.11±2.8 a	41.34±1.2 a	43.09±1.6 a	35.68±30. a	55.95±1.84 b
Cd	<i>D. salina</i>	0 (ND) a	4.80±4.16 a	16.76±2.14 b	8.90±1.37 a	13.77±3.4 b	45.18±2.5 c	51.63±1.24 d	62.54±0.2 e	72.05± 0 f
	<i>T. suecica</i>	0 (ND) a	15.83±2.31 a	19.61±0.27 b	15.26±1.38 a	23.66±0.9 c	16.41±0.2 a	30.58±0.23 b	56.58±1.7 e	52.47±2.12 d

Note: * The similar letter for each row meanings are not significantly different (p < 0.05).

T. suecica 23.66 U/gm in F 2 for 28 days, while the highest concentration of the SOD enzyme in fry fish fed with artemia used *D. salina* as food treated with Cd in F2: 72.05 U/gm for 28 days, for *T. suecica* in F2: 56.58 U/gm for 4 days, all have a significant difference.

Tanhana *et al.* (2023) record that increased CAT U/gm activity of the enzyme is a response to increasing in the accumulation of lead in the liver, so, The response of antioxidative is indicated by SOD activity in the liver which decreases when Pb con, increases. Sevcikova *et al.* (2011) indicated for the change in the activity of SOD U/gm after heavy metal exposure has been. The response of the antioxidant system differs depending on the redox (active or inactive) nature of metals (Pinto *et al.*, 2003).

Table 3 shows the highest concentration for GPX U/gm enzyme in fry fish fed with Artemia used *D. salina* as food treated with Pb in F1: 35.51 for 4 days. In F2: *T. suecica* 25.50 for 28 days. The GST U/gm enzyme in the *D. salina*: 23.27, and *T. suecica*: 15.53 in F2 for

28 days, all have significant differences. However, The highest concentration of GPX U/gm enzyme in fry fish fed with Artemia used *D. salina* as food treated with Cd 34.82, and *T. suecica* 25.50 both in F1 for 4 days, with a significant difference. With GST U/gm enzyme in *D. salina* 24.20 with F2 at 4 days with a significant difference. And *T. suecica* 22.32 both in F1 for 4 days, with no significant difference. One of the major effects of lead at low levels is interfering with the biosynthesis of haemei (Jun-Hwan K. and Ju-Chan K. 2015). There is more research on various biomarkers and the toxic effects of metal on fish. one of the biomarkers is reactive oxygen species, which is a good sign of oxidative stress when fish exposure to heavy metals. ROS shares with the biochemical aspects of the fish, where attacking the lipids in the membrane and converting them to lipid peroxide (Ullah *et al.*, 2021). In Table 4 The results show there is a strong positive significant correlation between (GPX with SOD - CAT) also (SOD with CAT) While the

Table 3. Mean and Stander deviation of GPX U/gm and GST U/gm in fish that fed on Artemia which us (*D.salina* – *T.suecica*) as food, after being treated with lead and cadmium

Metal	Alge	Metal concentrations (M±SD)								
		Control	GPX U/gm				GST U/gm			
			F1 in 4 days	F1 in 28 days	F2 in 4 days	F2 in 28 days	F1 in 4 days	F1 in 28 days	F2 in 4 days	F2 in 28 days
Pb	<i>D. salina</i>	0 (ND) a	35.51±2.1 e	31.78±1.03 cd	28.15±2.0 ab	29.45±0.7 bc	18.72±1.9bcd	22.29±1.5 de	19.63± 0.20 cde	23.27±3.2e
	<i>T. suecica</i>	0 (ND) a	19.33±1.2a	21.67±0.99 ab	23.57±2.5 b	25.50±2.6 c	8.49±0.94 a	12.96±2.4 cde	11.92±1.83 bcd	15.53±2.3e
Cd	<i>D. salina</i>	0 (ND) a	34.82±3.3 c	25.96±1.79 bc	22.64±2.5 a	32.92±2.7 c	21.98±3.2 cd	23.31±2.3cd	24.20±3.16 c	21.17±1.8 b
	<i>T. suecica</i>	0 (ND) a	22.64±2.0 bc	15.39±13.3 ab	21.96±1.6b	18.18±0.8 ab	22.32±1.8 a	15.39±13. a	13.63±0.56 a	20.87±1.9 a

Note: * The similar letter for each row meanings no significantly different (P>0.05).

Table 4. Correlations between fish enzymes and concentration of Pb and Cd in food type *D. salina* and *T. suecica* treated with Pb and Cd

<i>D. salina</i>		Pb				<i>D. salina</i>		Cd			
		GPX	SOD	CAT	concentration			GPX	SOD	CAT	concentration
GST	Pearson correlation	.213	.087	.049	.638	GST	Pearson correlation	.874	-.196	.026	.025
	Sig. (2-tailed)	.787	.913	.951	.362		Sig. (2-tailed)	.126	.804	.974	.975
GPX	Pearson Correlation		.990**	.974*	.875	GPX	Pearson correlation		.197	.454	.081
	Sig. (2-tailed)		.010	.026	.125		Sig. (2-tailed)		.803	.546	.919
SOD	Pearson correlation			.975*	.799	SOD	Pearson correlation			.384	-.530
	Sig. (2-tailed)			.025	.201		Sig. (2-tailed)			.616	.470
CAT	Pearson correlation				.800	CAT	Pearson correlation				.563
	Sig. (2-tailed)				.200		Sig. (2-tailed)				.437
<i>T. suecica</i>		Pb				<i>T. suecica</i>		Cd			
		GPX	SOD	CAT	concentration			GPX	SOD	CAT	concentration
GST	Pearson correlation	.902	.685	.057	.408	GST	Pearson correlation	.228	-.478	.296	-.125
	Sig. (2-tailed)	.098	.315	.943	.592		Sig. (2-tailed)	.772	.522	.704	.875
GPX	Pearson correlation		.532	-.366	-.008	GPX	Pearson correlation		-.056	-.700	-.982*
	Sig. (2-tailed)		.468	.634	.992		Sig. (2-tailed)		.944	.300	.018
SOD	Pearson correlation			.403	.261	SOD	Pearson correlation			.288	-.133
	Sig. (2-tailed)			.597	.739		Sig. (2-tailed)			.712	.867
CAT	Pearson correlation				.800	CAT	Pearson correlation				.649
	Sig. (2-tailed)				.200		Sig. (2-tailed)				.351

concentrations have a positive correlation with all enzymes when used food treated with Pb in *D. salina*. And positive correlation between (GST with GPX) when food treated with Cd in *D. salina*, and positive correlation between (GST with GPX) also (CAT with concentration) when food was treated with Pb in *T. suecica*, and a strong negative significant correlation between (GPX with concentration) when food was treated with Cd in *T. suecica*. While in the study of Tanhana *et al.* (2023), no correlation was found between metal concentrations in the muscle and SOD activity of all fish species, while a significant positive correlation was found for Pb concentration and the liver’s CAT activity. there is a strong negative significant correlation between GPX and Cd concentration in *D.salina*. Cadmium is one of the most toxic contaminants of polluted water that causes toxicity in every ecological layer (Rashed, 2001). It has been indicated that Cd does not generate ROS directly and can alter GSH levels (Ercal *et al.*, 2001). The study of Vardi and Chenji (2020) showed that the fry

had low resistance against heavy metals even at low concentrations, which reduced of population in all treatments compared with the controls, which was expected from the exposure to heavy metals.

CONCLUSIONS

The heavy metals lead and cadmium are transported in the food chain through food, using two types of algae as food and using Artemia as the primary consumer in laboratory conditions for 4–28 days. Accumulation increased with an increase in the exposure period, as it was found that exposure experiments to heavy elements for 28 days were always significantly higher than exposure for 4 days for the same food and concentrations. The enzymes GST, GPX, SOD, and CAT were affected by heavy metal concentrations, type of food, and exposure period, Therefore, it can be considered as a good biomarker for contamination with these heavy metals.

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