

## HEPATORENAL ENZYMATIC ALTERATIONS IN PIGEONS (*COLUMBA LIVIA*) EXPOSED TO COPPER AND ZINC TOXICITY

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### ABSTRACT

Heavy metals are very deleterious to an organism's health in concentrated quantities. The damage caused by heavy metals on organs is revealed by abnormal levels of enzymes like Aspartate aminotransferase (ASAT) and Alanine aminotransferase (ALAT). In the present research work ALAT and ASAT of the liver and kidney of pigeons under the influence of heavy metals copper and zinc have been studied. Pigeons were divided into control and experimental groups. Experimental groups were divided into heavy (20µg/g) and light (10µg/g) doses of copper and zinc. Enzymatic assessment was done after given 9 doses with interval of 15 days in between each dose. The excessive concentration of copper and zinc resulted in significant (P<0.001) elevation in levels of ASAT and ALAT of liver and kidney in pigeons as a result of organ destruction by heavy metals.

**Key Words:** Heavy metals, Copper, Zinc, Aspartate aminotransferase (ASAT), Alanine aminotransferase (ALAT), Liver, Kidney.

### INTRODUCTION

The present world is facing numerous problems related to the environment. The biggest factor of these issues humanity has to deal with today is environmental pollution (Ma and Wang, 2021). Heavy metals can be defined as "a metal which has a high atomic mass and has a specific gravity that is more than the specific gravity of water by five or more times at 4 degrees Celsius". The heavy metals included are Mercury (Hg), Cadmium (Cd), Chromium (Cr), Thallium (Tl), Lead (Pb), Copper (Cu) and Zinc (Zn) (Yadav *et al.*, 2019; Raychaudhuri *et al.*, 2021). Heavy metals pass on in organisms by food chain and accumulated by biomagnification (Bharti and Sharma, 2022).

The importance of pigeons as a bioindicator has been accepted for a long time and their frequent use in environmental studies has been documented due to the cheap cost, easy availability and human friendly nature (Tong *et al.*, 2022). Heavy metals have immense biological roles in organisms, but they have also been categorized as environmental pollutants because excessive levels disturb the biological system (Sharma *et al.*, 2005). Transaminase enzymes like Aspartate aminotransferase (ASAT) and Alanine aminotransferase (ALAT) have a tremendous role in various biomolecule metabolism, like carbohydrates and proteins and acts as biomarkers. Abnormal high levels of these biomarkers show organ damage (Rasool *et al.*, 2020).

Zinc is considered as a trace element, and it is a very essential component for a number of different organs and more or less every biochemical pathway requires zinc in some proportion (Chasapis *et al.*, 2020). Hyper consumption of zinc supplementation also destroys the structure and stability of vitamins and other important nutrients. Excess zinc can also cause resistance in pathogenic gut bacteria and help in the severity of gut bacterial infection (Bortoluzzi *et al.*, 2020). Zinc in a hyperaccumulated state produces maximum cell death and cytotoxicity in major organ systems, including nervous system (Tyszka-Czochara *et al.*, 2014). Excess exposure to zinc also disturbs ALAT, ALAP and ASAT enzymes (Abdel-Khalek *et al.*, 2015).

Copper is a cofactor for many enzymes like superoxide dismutase and cytochrome C oxidase. Absence of copper results in compromised activities of such enzymes (Buse, 2018). The common manifestations of chronic copper toxicity involve irritation in the nasal, oral and corneal epithelium along with headaches, abdominal disturbances, diarrhea and vomiting. Intentional high consumption of copper may cause severe damage to the hepatic and renal tissues, hepatic necrosis, and cholestatic liver disease, which results in death of the individual. Copper also changes enzymatic parameters like ALAT, ASAT and ALAP in the liver and kidneys in chronic courses of its toxicity. Problems like jaundice and hemolytic crises are also correlated with the uptake of high copper (Ejaz *et al.*, 2020; Barber *et al.*, 2021). In the present study, hepatorenal enzymes ALAT and ASAT have been studied under the influence of copper and zinc.

## MATERIALS AND METHODS

### EXPERIMENTAL BIRDS

The pigeon (*Columba livia*) was selected as a tool in this research work. The healthy pigeons were checked physically and selected for purchase from local Empress Market of Karachi, and they were kept in a cage. The pigeons were allowed to have fresh air, light, water and free access to move in the cage.

### EXPERIMENTAL DESIGN

The zinc and copper were given in their light (10 µg/g) and heavy (20 µg/g) doses to pigeons through intramuscular injections after every 15-day interval. So, the work was based on an active monitoring method in which animals directly absorb heavy metals in the body. Total 9 doses were given. The pigeons were divided into two batches. Each batch had 5 groups with 4 pigeons in each. One group was designated as the control and 4 batches were the experimental groups. So, the total pigeons were 20 (4×5) in each batch. To ensure the hygienic conditions, the cage was cleaned daily and washed with detergent weekly. The pigeons were given 100gm of feed thrice a day, i.e. morning, noon and evening. Mortality of birds was also checked carefully. Animals were used and all procedures performed in accordance with the guidelines of committee of ethics on animal use by the Department of Zoology, University of Karachi (No. 1034545).

The details of the groups were as follows:

**Control group:** The pigeons of this group were not subjected to heavy metal injections.

**Experimental groups:** Pigeons designated as experimental groups were divided into four groups.

- 1) **Copper Sulphate (Low dose):**- This group was given CuSO<sub>4</sub> (10 µg/g).
- 2) **Copper Sulphate (High dose):**- This group was given of CuSO<sub>4</sub> (20 µg/g).
- 3) **Zinc Sulphate (Low dose):**- This group was given ZnSO<sub>4</sub> (10 µg/g).
- 4) **Zinc Sulphate (High dose):**- This group was given ZnSO<sub>4</sub> (20 µg/g).

## ANALYSIS OF ENZYMATIC ACTIVITIES OF THE KIDNEY AND LIVER

### PREPARATION OF TISSUE SERUM

Firstly, slaughtering of pigeons was done, and fresh liver and kidney were taken out. Then, 1gm of tissue was taken. Thirdly, crushing of tissues in chilled mortar was done properly so that it converted into a paste-like consistency. Lastly, 100 µl of distilled water was added and all the content was poured into the micro centrifuge test tube carefully. Centrifugation was done at 1000rpm for 10 minutes.

### SPECTROPHOTOMETRY

The solution, which underwent spectrophotometry, was made up of three important components. R1 and R2 solutions from the respective enzyme's kit and tissue's supernatant, which was prepared by centrifugation. The volume of R1 was 400 µl and the volume of R2 was 100 µl along and after that immediately a tissue sample of volume 50 µl was put in the test tube. After that, immediately put the prepared solution in the cuvette. Total volume of the solution was 550 µl and three readings were recorded at a wavelength of 340 nm each after an interval of 1 minute. Mean value was calculated from three absorbance values by  $\Delta A$  nm/min.

### ENZYMATIC ANALYSIS

The activities of ALAT and ASAT in the supernatant of tissue serum have been measured by practicing the legitimate protocols of the International Federation of Clinical Chemistry (IFCC). The commercially available kit ALAT (Merck, Catalog# 5.17531), ASAT (Merck, Catalog# 5.17521) were used for enzymatic analysis.

This formula was used to assess the ASAT/ALAT activity.  $ASAT/ALAT \text{ Activity (u/l)} = (\Delta A \text{ nm/min.}) \times 1746$ . Normal value of ALAT and ASAT in kidney of pigeon is 11.640-18.624 uL and 11.058-19.788 UI, respectively (Hussain and Tabassum, 2019)

### STATISTICAL ANALYSIS

All the data were statistically analyzed by using IBM SPSS (version 21.0). The (Mean ± S.D) values of all parameters were used. Significance of data was checked out by using ANOVA (Analysis of One- Way Variance). Tuckey HSD test was used to compare the mean of different groups. (P < 0.001) were considered significant.

## RESULTS

The ALAT (alanine aminotransferase) and ASAT (aspartate aminotransferase) activities in hepatic and renal tissues of pigeons exposed to CuSO<sub>4</sub> and ZnSO<sub>4</sub> in their heavy (20µg/g) and light (10 µg/g) doses indicated elevated levels compared to the control group (Table 1-2).

### ALANINE AMINOTRANSFERASE (ALAT) ACTIVITY

In the kidney, the level of ALAT is increased significantly ( $P < 0.001$ ) in all treated groups as compared to control group. This marked elevation was dose-dependent and the highest level was recorded in the CuSO<sub>4</sub> (20 µg/g) group- about six times more than control – followed by the ZnSO<sub>4</sub> (20 µg/g) group. This pattern is similarly followed by the ALAT of liver which was increased about five folds by CuSO<sub>4</sub> (20 µg/g), showed advance liver deterioration. Zinc doses also accelerated ALAT level but to a lesser extent than copper, showing relatively lower hepatotoxic ability (Table 1).

### ASPARTATE AMINOTRANSFERASE (ASAT) ACTIVITY

The ASAT activity in kidney and liver also showed the similar pattern like ALAT. The CuSO<sub>4</sub> (20 µg/g) group reflected more significantly increased ( $P < 0.001$ ) enzyme activity about seven times higher than control in the liver and six times elevated in the kidney. Zinc has also induced elevated enzymatic activity, but again emphasizing that it is less toxic than copper (Table 2).

**Table 1.** Descriptive statistics of enzyme ALAT in kidney and liver for control group pigeons and experimental groups' pigeons.

Variables	Groups	N	Min	Q1	Q2	Q3	Max	Mean	Std	p-JB	Significance vs Control	
ALAT (Kidney)	Control	16	11.29	12.47	13.89	18.22	19.20	14.97	2.92	0.07	--	
	Groups	CuSO <sub>4</sub> 10 µL/b.wt	16	37.91	42.12	49.36	51.01	59.98	48.43	6.60	0.2	***
		ZnSO <sub>4</sub> 10 µL/b.wt	16	22.47	29.59	37.72	39.43	42.43	34.78	6.00	0.02	***
		CuSO <sub>4</sub> 20 µL/b.wt	16	79.23	82.67	93.80	96.00	99.45	90.66	6.64	0.02	***
		ZnSO <sub>4</sub> 20 µL/b.wt	16	71.98	74.65	80.08	86.78	89.98	80.96	6.34	0.2	***
ALAT (Liver)	Control	16	10.90	12.40	13.25	16.67	19.23	14.35	2.82	0.061	--	
	Groups	CuSO <sub>4</sub> 10 µL/b.wt	16	40.13	51.92	54.87	56.73	59.89	53.61	4.88	0.044	***
		ZnSO <sub>4</sub> 10 µL/b.wt	16	25.32	32.12	35.48	38.20	52.95	35.39	6.32	0.200	***
		CuSO <sub>4</sub> 20 µL/b.wt	16	80.66	82.20	88.14	92.41	98.90	87.99	6.01	0.20	***
		ZnSO <sub>4</sub> 20 µL/b.wt	16	73.82	77.12	81.98	89.13	89.84	82.69	5.70	0.20	***

\* Jarque-bera JB: H0: the data is normally distributed

**Table 2.** Descriptive statistics of enzyme ASAT in kidney and liver for control group pigeons and experimental groups' pigeons.

Variables	Groups	N	Min	Q1	Q2	Q3	Max	Mean	Std	p-JB	Significance vs Control	
ASAT (Kidney)	Control	16	10.22	10.99	12.32	14.30	19.24	12.81	2.62	0.005	--	
	Groups	CuSO <sub>4</sub> 10 µL/b.wt	16	21.40	42.20	44.19	46.71	49.98	43.40	6.53	0.001	***
		ZnSO <sub>4</sub> 10 µL/b.wt	16	23.09	30.01	35.47	38.04	40.00	33.49	5.32	0.006	***
		CuSO <sub>4</sub> 20 µL/b.wt	16	74.92	83.10	89.87	93.75	96.98	88.32	8.84	0.001	***
		ZnSO <sub>4</sub> 20 µL/b.wt	16	67.41	74.90	79.16	82.84	88.23	78.73	5.64	0.007	***
Variables	Groups	N	Min	Q1	Q2	Q3	Max	Mean	Std	p-JB	Significance vs Control	
ASAT (Liver)	Control	16	10.22	11.21	12.41	14.06	15.97	12.71	1.69	0.20	--	
	Groups	CuSO <sub>4</sub> 10 µL/b.wt	16	25.87	31.20	36.18	43.47	48.92	36.98	7.63	0.08	***
		ZnSO <sub>4</sub> 10 µL/b.wt	16	22.90	26.91	33.03	37.97	42.87	32.67	6.26	0.2	***
		CuSO <sub>4</sub> 20 µL/b.wt	16	80.49	83.33	88.13	97.07	99.21	90.02	6.74	0.2	***
		ZnSO <sub>4</sub> 20 µL/b.wt	16	66.78	70.24	73.97	78.53	79.91	74.28	4.34	0.2	***

\* Jarque-bera JB: H0: the data is normally distributed

**Table 3.** ANOVA analysis of enzymes ASAT and ALAT of liver and kidney of pigeons of control and treated groups.

		GROUP P-VALUE
Enzyme ALAT (KIDNEY)	CONTROL	464.076(0.000)
	CuSO <sub>4</sub> 10 µg/g	
	ZnSO <sub>4</sub> 10 µg/g	
	CuSO <sub>4</sub> 20 µg/g	
	ZnSO <sub>4</sub> 20 µg/g	
Enzyme ALAT (LIVER)	CONTROL	555.437(0.000)
	CuSO <sub>4</sub> 10 µg/g	
	ZnSO <sub>4</sub> 10 µg/g	
	CuSO <sub>4</sub> 20 µg/g	
	ZnSO <sub>4</sub> 20 µg/g	
Enzyme ASAT (KIDNEY)	CONTROL	509.008(0.000)
	CuSO <sub>4</sub> µg/g	
	ZnSO <sub>4</sub> 10 µg/g	
	CuSO <sub>4</sub> 20 µg/g	
	ZnSO <sub>4</sub> 20 µg/g	
Enzyme ASAT (LIVER)	CONTROL	491.853(0.000)
	CuSO <sub>4</sub> 10 µg/g	
	ZnSO <sub>4</sub> 10 µg/g	
	CuSO <sub>4</sub> 20 µg/g	
	ZnSO <sub>4</sub> 20 µg/g	

**COMPARATIVE ANALYSIS**

Overall, both enzymes' activities indicated this trend: Control < ZnSO<sub>4</sub> (10 µg/g) < CuSO<sub>4</sub> (10 µg/g) < ZnSO<sub>4</sub> (20 µg/g) < CuSO<sub>4</sub> (20 µg/g).

It has been reflected that a clear dose and metal-dependent marked elevation occurred in liver and kidney transaminases. These changes were statistically significant (P<0.001) according to one-way ANOVA analysis, establishing the fact that tissue injury happened due to metal toxicity. (Table 3)

Box-plots further displayed the above-mentioned differences, with the elevated enzyme activities in copper exposed pigeons and least concentrations in control group, thus suggesting strong association between metal exposure and transaminase activities (Fig. 1-4).

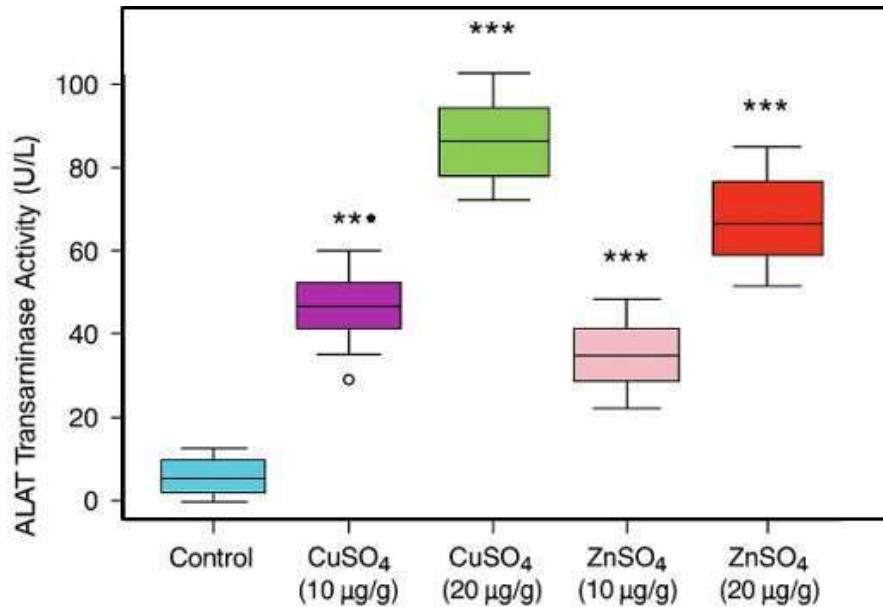


Fig. 1. Box plots showing ALAT activity (U/L) in liver in pigeons exposed to different concentrations of CuSO<sub>4</sub> and ZnSO<sub>4</sub>. Data indicate median±IQR. Asterisks show significant differences from control (\*\*\*)p<0.001).

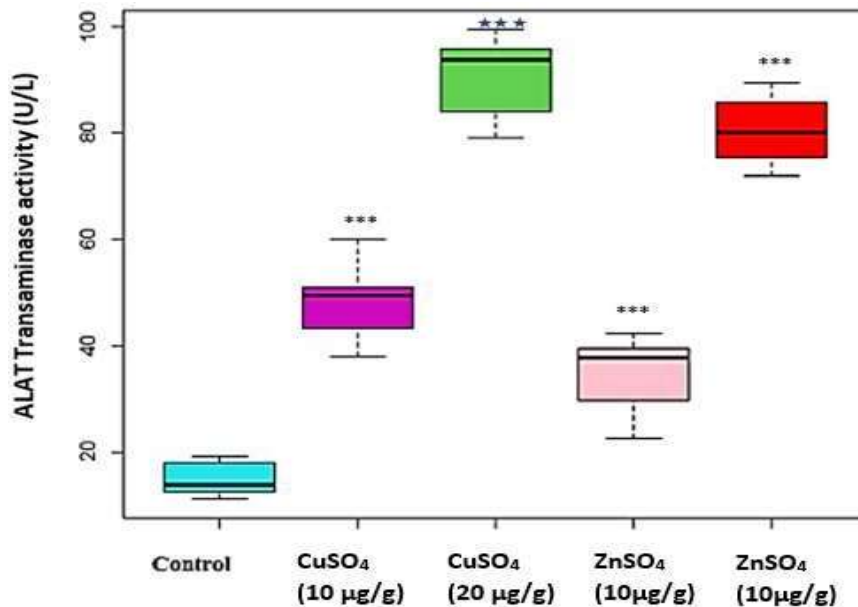


Fig. 2. Box plots showing ALAT activity (U/L) in kidney in pigeons exposed to different concentrations of CuSO<sub>4</sub> and ZnSO<sub>4</sub>. Data indicate median±IQR. Asterisks show significant differences from control (\*\*\*)p<0.001).

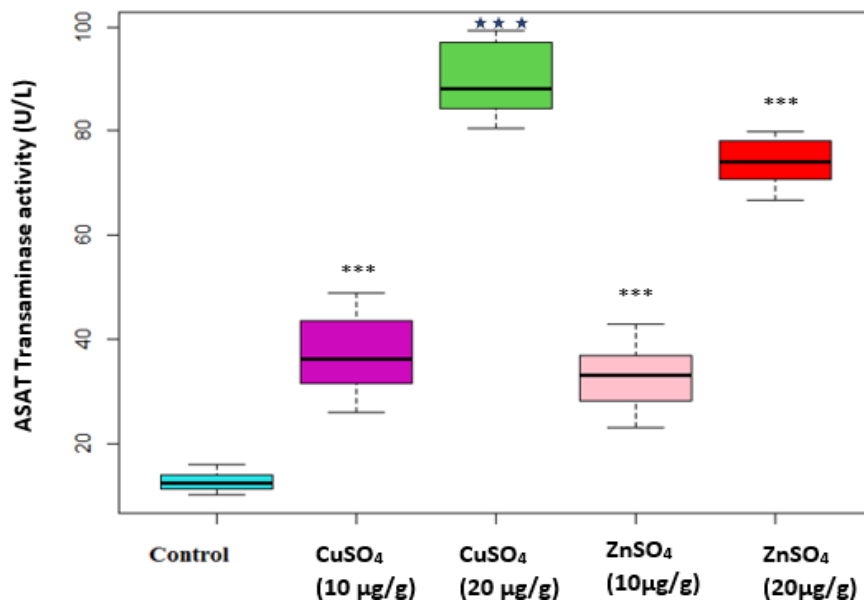


Fig. 3. Box plots showing ASAT activity (U/L) in liver in pigeons exposed to different concentrations of CuSO<sub>4</sub> and ZnSO<sub>4</sub>. Data indicate median±IQR. Asterisks show significant differences from control (\*\*\*) $p < 0.001$ .

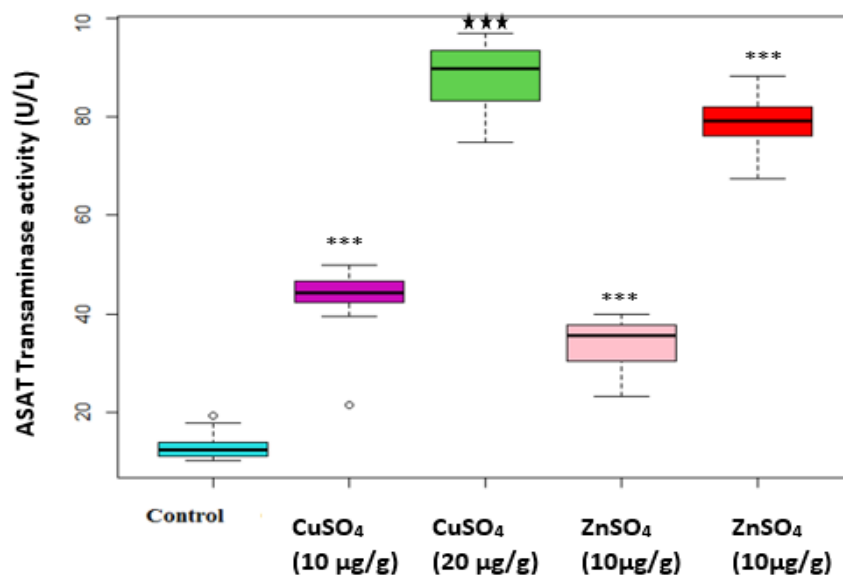


Fig. 4. Box plots showing ASAT activity (U/L) in kidney in pigeons exposed to different concentrations of CuSO<sub>4</sub> and ZnSO<sub>4</sub>. Data indicate median±IQR. Asterisks show significant differences from control (\*\*\*) $p < 0.001$ .

## DISCUSSION

The transaminases like alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) are well-known biomarkers for assessing the damage caused in the organ by heavy metals. These enzymes are present in many organs like liver, kidney, lungs, muscles and brain. The high concentrations of these biomarkers are a quick reflector of organ damage as reported by Kwiecien *et al.*, (2006). The elevation in enzymes shows cellular injury, disruption of plasma membrane, as transaminases are usually present in the cytoplasm of renal tubular cells and hepatocytes and come in circulation after cellular damage (Botros and Sikaris, 2013). CuSO<sub>4</sub> treated pigeons showed

much high concentration of enzymes than ZnSO<sub>4</sub> treated pigeons among all treated groups indicating that copper posed more hepatotoxic and nephrotoxic effects than zinc.

The high level of ALAT and ASAT advocated that Cu and Zn damage cellular homeostasis by oxidative stress and lipid peroxidation processes. Copper can form reactive oxygen species (ROS) because it is a transition metal which performs redox cycling between Cu<sup>+</sup> and Cu<sup>2+</sup> states (Yang *et al.*, 2019). The amassing of ROS results in peroxidation of membrane lipids, malfunctioning of mitochondria and finally leakage of intracellular enzymes into the bloodstream (Tsang *et al.*, 2021). Zinc is an essential trace element, but in accumulated state it becomes toxic by interfering with metallothionein management and antioxidant enzyme balance. However, because zinc has no redox potential like copper, so it has less ability to form ROS which may describe the less elevation of enzymes in zinc treated pigeons (Nordberg *et al.*, 2022). The present results are agreeing with previous findings reported high serum concentration of ALAT and ASAT after heavy metal exposure in various birds and mammals. Kar *et al.*, (2018) documented similarly high levels of hepatic enzymes in hens after exposure to copper, while El-Amawy *et al.* (2022) and Melila *et al.* (2022) provided consistent results in rats and humans, respectively, confirming that elevated levels of enzymes occurred due to oxidative damage of cells. In aquatic species, Yancheva *et al.* (2014) documented that fish from heavy metal polluted water possessed significant increase in ALAT and ASAT, confirming the heavy metals induced oxidative stress results in cellular damage.

However, not all studies consistent with present findings. Elejaz *et al.* (2011) reported no significant elevation in transaminase levels in rock pigeons inhabiting polluted sites. The inconsistency in results could be due to difference in exposure pattern and duration of heavy metal exposure. Active monitoring (in which heavy metals directly injected in the body) vs passive monitoring (chronic environmental accumulation). Present study involves active monitoring methods as performed by Hussain and Tabassum (2019) which cause rapid systemic absorption and acute hepato-renal cells damage while Elejaz *et al.* (2011) observed it through passive monitoring where compensatory antioxidant mechanisms may have been switched on.

Higher enzyme activity is reported in the current study by Cu treated groups supported the concept that copper has a higher hepatotoxic ability than zinc. This difference occurs because copper has more redox reactivity and potential to catalyze ROS synthesis which are responsible for pronounced oxidative changes of proteins, lipids and nucleic acids. In contrast, zinc toxicity basically comes from displacement of important metal ions and blockage of enzymatic reaction rather than oxidative damage. The higher values of enzymes in Cu treated pigeons also suggests dose-dependent renal and hepatic stress, aligned with the manifestation of metal-induced oxidative damage.

Similar results have been documented in other avian fauna exposed to heavy metals. Binkowski *et al.* (2013) reported histopathological lesions in hepatic and renal tissues of mallards from metal-polluted sites, and Sadoon *et al.* (2022) reported raised hepatic enzymes in geese from metal-contaminated areas in Basrah city. These studies, along with other researches, show that avian fauna is specifically susceptible to heavy metal stress due to rapid metabolism and restricted detoxification capacity.

Overall, the higher levels of ALAT and ASAT in Cu and Zn treated pigeons indicated that hepatic and renal tissues damage occurs by membrane disruption and oxidative stress induced by heavy metals. Copper showed more redox toxicity and potential to produce reactive intermediates compounds that alter tissue structure. These findings highlighted the importance of transaminases as reliable biomarkers for assess metal induced organ damage in birds. Furthermore, the present study gives the basis of Cu and Zn induced hepato-renal toxicity, accentuated the urge for controlling environmental metal exposure to protect ecosystem.

## CONCLUSION

It could be concluded that the control group had not received any heavy metal dose, so, liver and kidney were not damaged so the levels of enzymes remaining normal. The elevation in enzymes by heavy metals showed the toxicity of metals and deterioration of liver and kidney as a result of damage caused by the ions of heavy metals copper and zinc which probably disrupt the normal structure of cells and caused them to lyse and probably due to cellular lysis the stored enzymes released and caused elevated levels of ASAT and ALAT which was the indicator of organ deterioration.

## CONFLICT OF INTEREST

The authors have no conflict of interest.

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