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# Bioaccumulation of Pb (Lead) in the Muscles of *Danio rerio* and its Effect on the Anti-oxidant Enzyme Catalase in the Mining Vicinity of Kolar Gold Fields

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## ABSTRACT

**Background:** The study has a bifold objectives, firstly to determine the concentration of lead (Pb) in the pond water and bioaccumulation of the lead in the muscles of *Danio rerio* raised with the same pond water. Secondly, the study seeks to investigate its effect on the anti-oxidant enzyme catalase in *Danio rerio*, within the vicinity of a historical gold mine region located in the Kolar Gold fields (KGF), Karnataka, India.

**Methods:** To achieve the objectives, the study employed Thermo Scientific's Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and ICP-AES using Iteva software to evaluate and statistically analyze the distribution of lead in the pond water and and ICP-OES to evaluate the concentration of lead in the muscles sample. Four sample sites (A, B, C and D) were selected based on their proximity to residential area. Pond water samples were analyzed between April and October 2022 following a southwest monsoon rain.

**Result:** During the study in the pond water, sample site B and D exhibited an exceedingly high Pb concentration during October with 0.98 mg/L against 0.21 and 0.16 mg/L in April 2022 against the maximum permissible value of 0.05 mg/L by WHO 2008 and USEPA 2009, followed by site A and C. The control sample had a normal range of 0.01 mg/L. The concentration of Pb in the muscles on day 30 of October was exceedingly high in the sample site D with 1.9 mg/Kg against 1 mg/Kg on day 30 of April followed by the sample sites B,C and A, which is far above the threshold value as per the regulatory bodies (WHO 2008). The increase in the concentration of the lead decreases the catalase enzyme in the brain region of *Danio rerio*, the value goes down drastically from 112.3 (µM $H_2O_2$ utilized/min/mg protein) to 105.8 in sample site D between day 30 of April and October months, to that of the control value being stable from 151.7 to 150.94 (µM $H_2O_2$ utilized/min/mg protein). The outcome of this experiment will facilitate the assessment of pond water contamination levels and the likelihood of contaminants entering the aquatic environment, resulting in the bioaccumulation of heavy metals in the aquatic organisms. The above results clearly indicates the effect of south-west monsoon rain in increasing the levels of lead concentration in the pond water during the month of October to that of April, which indirectly affects the physiology and metabolism of the fishes raised in the contaminated habitat of Kolar Gold Fields.

**Key words:** Antioxidant enzyme, Catalase, *Danio rerio*, Gold mining, ICP-OES, Lead toxicity.

## INTRODUCTION

The town of Kolar Gold Fields, often known as K.G.F., is located in the Bangarpet taluk of the Kolar district in the Indian state of Karnataka. In order to take over the mining operations of the mines situated at latitude 12°53′12″N and longitude 78°15′03″E, at the southernmost point of a short schist strip of the township, KGF, whose residents are primarily the families of gold mine workers, the Government of India established the public company (Bharat Gold Mines Limited), or BGML. For more than a century, underground gold mining has been practiced at KGF. 65 kilometers of tunnel construction have been used to mine gold to a depth of 3 kilometers below the surface and 40 million tonnes of mill tailings have accumulated Subbaraman, (2006). Tailings, a by-product of gold extraction that is heavily polluted with heavy metals, are the main product (HM). These metals seep out into the environment uncontrollably when they come into touch with water or are dispersed by the wind (Singh and Ansari, 2017).

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Heavy metals are defined in the literature as naturally occurring elements with large atomic weights and densities that are five times greater than those of water (Tchounwou *et al*., 2012); (Banfalvi, 2011). Due to their hazardous nature, environmental scientists have given heavy metals the most attention among all contaminants. As more industries pour their untreated, metal-rich effluents directly into our drinking water, the amount of heavy metals in our resources is causing great anxiety (Ganeshmurthy *et al.,* 2008).

Heavy metals turn harmful when they accumulate in soft tissues without being digested by the body Fu and Xi (2020). They may come into contact with individuals in residential, industrial, pharmaceutical, or agricultural settings and enter the body by inhalation of food, water, or air or skin absorption (Mishra *et al*., 2019); (Grover and Pandit, 2015).

**Lead**

Widespread lead consumption has caused serious problems for the environment and human health in many regions of the world. Unfortunately, lead is one of the most toxic metals available. Lead is a metal that, when exposed to air, takes on a brilliant silvery blue hue. Upon coming into contact with air, it starts to tarnish and, depending on the circumstances, forms a complicated variety of chemicals. Heavy metals are extremely toxic and pose major health risks to both people and other living creatures, even though they are only present in small amounts in water sources. This is because metal's level of toxicity can differ depending on a variety of factors, including its nature, its biological function and the length of time that organisms are exposed to it. As a result, all species are impacted by the heavy metal poisoning of water. Humans, an example of an organism that feeds at the top of the food chain, are more susceptible to serious health problems because heavy metal concentrations grow in the food chain (Musilova *et al*., 2016). Heavy metals cause several fish abnormalities in natural and lab-grown specimens. For decades, contaminants, notably heavy metals, in fish's aquatic surroundings (seas, rivers, lagoons) have caused severe harm to the organisms.

**Impact of heavy metals on muscles**

Green *et al.* (2018) have evaluated the role of aquatic models, particularly zebrafish, in recent years in shedding light on heavy metal toxicity and the operation of the nervous system (Green and Planchart, 2018). Shahjahan *et al*., (2022) claim that heavy metal pollution endangers aquatic life and organisms if it exceeds the permitted limits. Heavy metal exposure strongly affects each of the indicators, making them important biomonitoring approaches for heavy metal toxicity. Many fish species exposed to heavy metals exhibit cellular, nuclear, hematological and metabolic problems (Shah *et al.,* 2020).

Catalase is an important enzyme found in all vertebrates that come across free radicals, the most important function of this enzyme is to breakdown hydrogen peroxide into simpler forms like water and oxygen. The tetramer catalase is made up of four polypeptide chains, each over 526 amino acids long. The iron-containing heme groups in the enzyme's four subunits allow it to interact with hydrogen peroxide. In order to prevent damaging cells and tissues, hydrogen peroxide must be converted quickly into less dangerous molecules since it is a harmful by-product of many frequent metabolic activities (Gaetani *et al.,* 1996).

Lead is dangerous to organisms, including the brain, liver, ovary, kidney and fish gills, at greater quantities in freshwater. Singh *et al*. (2017) explored how the heavy metal exposure affected the antioxidant enzyme catalase (CAT) (Singh and Ansari, 2017).

The outcome of this experiment will facilitate the assessment of pond water contamination levels and the likelihood of contaminants entering the aquatic environment, resulting in the bioaccumulation of heavy metals in the aquatic organisms.

## MATERIALS AND METHODS

During this research work, the general methodology was devised based on spatiotemporal factors like place and time. So, the four places selected based on the minimal distance from the abandoned gold mining sites were Oorgaum, Tenants, Champion and Balghat and named A, B, C and D which are located 50, 100, 200 and 300 meters from the mining sites respectively.

**Collection of pond water sample**

The pond water samples from sampling sites A, B, C and D were collected to assess the levels of heavy metal contamination from tailings and compare against the pond water located 55 km away from the KGF mining site as the control site during the month of April and October 2022. The above-mentioned months April and October were considered based on two contrasting seasons. April is the pre-monsoon season when predominantly heavy metals tend to stay in soil tailings and during the onset of south-west monsoon season between June to September, heavy rainfall results in the runoff of heavy metals from soil and reaches the pond water present in low altitude. The two months were selected based on the contrasting amount of rainfall received based on the Indian Meteorological Department (IMD) data of the past 100 years.

Pond water samples from the affected region and the control samples were analyzed for the presence and increase in heavy metal concentration during April and October 2016 using ICP- AES (Mounia *et al*., 2013).

**Sample preparation-muscles**

Adult and healthy zebrafishes were purchased from the authorized vendor in Bengaluru during March and September 2022. Zebrafishes were acclimatized and fed as per standard protocols formulated by CPCSEA (2021). The fish were then euthanized and dissected to extract muscle tissues along with brain for estimation of catalase antioxidant enzymes.

A total of 12 adult fishes were used to acquire 1 gram of muscle tissue for digestion. The fish organ samples were compared between April and October on day 1, 15 and 30 against fish grown in control water to assess the assimilation of heavy metal concentrations using ICP- OES.

Each specimen had a sample of 1g collected in 3 repetitions. All samples were dried in an oven for two hours at 70°C. After the drying process was finished, the samples were placed in a muffle furnace and heated to between 450 and 500°C for four hours, with the temperature being raised by 50°C increments so that all of the samples could be reduced to ash.

The stage digesting process was initiated once all of the materials had been reduced to ash. In tubes, one gram of powdered material was obtained and digested using HCl and $HNO_3$ at a ratio of 1:3. 2 ml HCl and 6 ml $HNO_3$ were heated for an hour in a water bath at 70°C to guarantee full digestion. The filtrate was then collected in polyethylene tubes after each tube had been allowed to cool to room temperature and been filtered using filter paper. Finally, de- ionized water was used to dilute each filtrate to a final amount of 25 ml.

Before being subjected to an ICP-OES analysis for heavy metal concentration, tubes were sealed with polyethylene films and maintained at room temperature.

Standard solutions made from high purity material provided by Rankem chemicals had been utilised for the estimation of lead concentration. Additionally, a control sample was created using 2 ml HCL and 6 ml $HNO_3$ without the addition of meat powder. De-ionized water was then used to get the amount up to 25 ml. Prior to measuring the study sample samples, the device was calibrated using the control sample.

### Test for catalase enzyme activity

The process is based on the fact that, when heated in the presence of $H_2O_2$, dichromate in acetic acid reduces to chromic acetate, with the creation of per chromic acid as an unstable intermediate. At 570-610 nm, the chromic acetate that is thus created is calorimetrically measured. Dichromate has no absorbance in this range, thus adding it to the test mixture has absolutely no effect as to how much chromic acetate is determined calorimetrically. The catalase preparation is allowed to split $H_2O_2$. After the reaction mixture has been heated, the reaction is stopped at a certain point by adding a dichromate mixture and the residual is quantified by calorimetrically measuring chromic acetate Sinha (1972).

## RESULTS AND DISCUSSION

According to the Table 1, the average lead concentration in April across all sample sites was 0.2 mg/L, which is four times higher than the legal limits. Site C had the highest lead concentration, at 0.25 mg/L, while site D had the lowest, at 0.16 mg/L, on day 1 of April, following the dry season. In contrast, the average concentration of lead in all four locations increased and the average concentration in all 4 sampling sites was 0.67 mg/L in October, after the southwest monsoon season, which resulted in HM being transferred to the pond water from tailings. Site B and D have the highest concentration of lead, at 0.98 mg/L, followed by C and A.

According to Table 2, there is a negative and non-significant correlation between lead levels in the pond water during April and October ($r = -.224$, $p>0.05$).

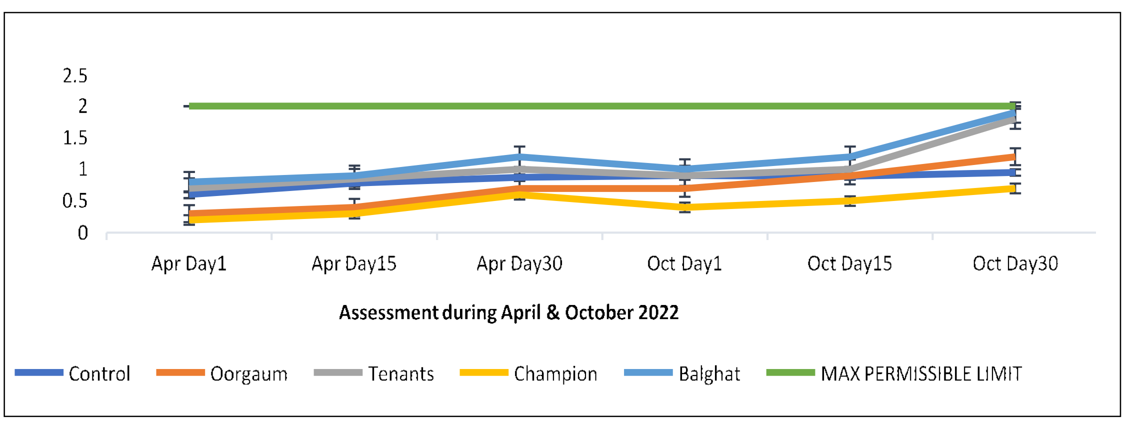
According to Graph 1, the maximum permissible limit of lead in various parts of the fish is 2 mg/Kg as per WHO (2008). The average concentration of lead on day 1 of April

**Table 1:** Concentration of Lead in pond water in mg/L in sampling sites during April and October 2022.

| Sampling site | April | October |
|---|---|---|
| Control | 0.001 | 0.001 |
| Oorgaum (A) | 0.18 | 0.24 |
| Tenants (B) | 0.21 | 0.98 |
| Champion (C) | 0.25 | 0.49 |
| Balghat (D) | 0.16 | 0.98 |
| Max permissible limit | 0.05 | 0.05 |
| Average | 0.2 | 0.6725 |

**Table 2:** Correlation of lead concentration in pond water in April and October 2022.

| During April and October 2022 | Correlation | Sig. |
|---|---|---|
| April and October | -.224 | .776 |




**Graph 1:** Concentration of Lead in the muscles of zebrafish in sampling sites during April and October 2022.

was observed as 0.5 mg/ kg. The highest concentration of lead was seen at site D with 0.8 mg/Kg within 24 hours of treatment subsequently increasing on days 15 and 30 which is much higher than day 1 in April during the dry season. Post South-West monsoon season, the amount of lead in muscles was observed to be 1 mg/Kg in site D on day 1 of October and 1.9 mg/Kg on day 30 in site D, which is below the maximum permissible limit. The average concentration of lead in the muscles of zebrafish in all 4 sampling sites were 0.75, 0.9 and 1.4 mg/Kg on days 1, 15 and 30 of October 2022.

According to Table 3, there is a positive and significant correlation between lead concentration in the muscles of zebrafish in pond water on April day 15 and Oct day 15 and April day 30 and October day 30 since the p-value <0.05.

Metals have been speculated to be concentrated in aquatic animals at amounts several times higher than in their natural environments. Fish are widely recognized as a reliable biological indicator of heavy metals in water bodies and have been employed for this purpose for decades. Although heavy metals occur naturally in the aquatic environment, their concentrations have grown as a result of human activities such as mining, manufacturing and agriculture. As these metals build up in fish tissues, they alter the body's physiological, pathological and biochemical processes. While heavy metals are necessary for life, excessive amounts in freshwater can be harmful to a fish's nervous system, liver, ovaries, kidneys and gills. The purpose of this study was to examine the effects of heavy metals on the antioxidant enzyme Catalase (CAT) in the brain of zebrafish after 1, 15 and 30 days of exposure.

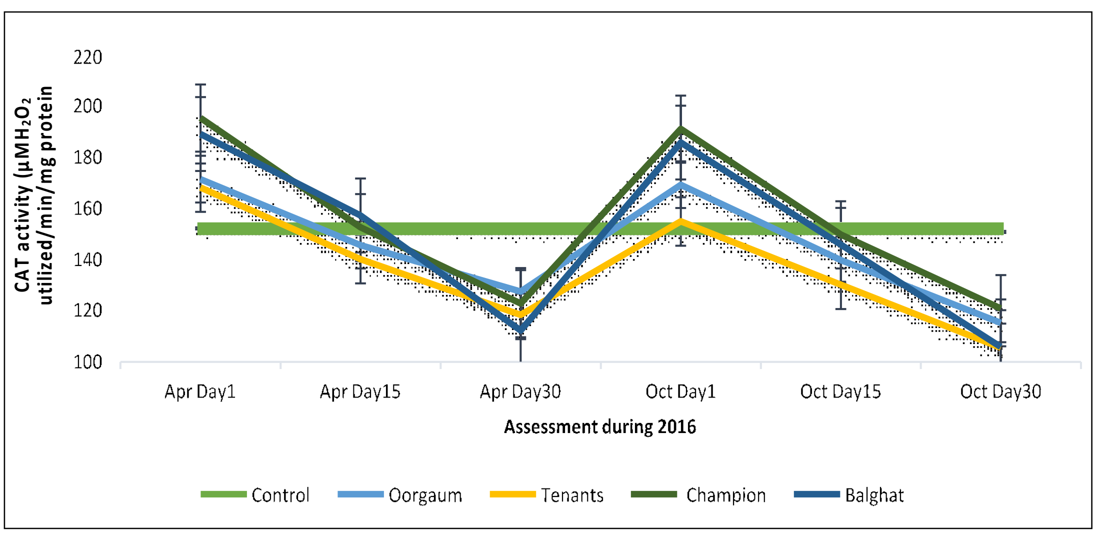
Table 4, shows a pattern of decreasing CAT activity in the brain of zebrafish in the month of April from day 1 to day 30. This implies that the longer the fish are raised in the heavy metal- contaminated water, these metals are surely impacting on the CAT activity of the zebrafish. A similar pattern is observed in the CAT activity in the month of October 2022. The control values showed in the range of 152.35-151.75 during April day 1- day 30. And in graph 2, during October month the values ranged from 151.47-150.94,

**Table 3:** Correlation of lead concentration in the muscles of zebrafish in pond water in April and October 2022.

| Assessment days | Correlation | Sig. |
|---|---|---|
| Apr day 1 and Oct day 1 | 0.854 | .065 |
| Apr day 15 and Oct day 15 | 0.891 | .042 |
| Apr day 3 0 and Oct day 30 | 0.923 | .025 |

**Table 4:** Effect of different pond water samples on CAT activity (μM $H_2O_2$ utilized/min/mg protein) in the Brain of zebrafish during April and October 2022.

| Days | Treatment period (days) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | | 15 | | 30 | |
| Location | April | October | April | October | April | October |
| Control | 152.35 | 151.47 | 153.54 | 152.89 | 151.75 | 150.94 |
| Oorgaum | 171.65 | 169.45 | 145.8 | 139.9 | 127.62 | 115.3 |
| Tenants | 168.32 | 155 | 140.23 | 130.2 | 118.35 | 105.5 |
| Champion | 195.61 | 191.23 | 152.62 | 149.8 | 122.82 | 120.87 |
| Balghat | 189.34 | 185.97 | 157.38 | 145.89 | 112.32 | 105.8 |




**Graph 2:** CAT activity in the brain of zebrafish during April and October 2022.

so even the control zebra fishes with normal water also show declining activity with CAT activity. Against control values, zebrafishes raised in site D- Balghat showed the highest decline from 185.97-105.8, wherein there was a decrease of almost 80 units, followed by sites C, A and B with 74-, 54.15- and 49.5-units during October day 1- 30 day treatment.

According to Table 5, there is a positive and significant correlation between CAT activity in the brain of zebrafish during Apr Day 1 and Oct Day 1 and Apr Day 30 and Oct Day 30 since the p-value<0.05.

Constant monitoring of soil trace metal concentrations has been advocated for by several studies. To this goal, numerous scientific investigations into the detection of heavy metals in manufacturing settings have been conducted. Most of these investigations, however, were conducted in formerly industrialized cities. Therefore, research of this kind is essential. This research will provide important context to the current worldwide record on soil pollution by heavy metals in industrial areas and highlight the necessity for more frequent monitoring of soil heavy metal content in these zones.

Since it affects every component of an ecosystem, soil contamination from substances like heavy metals is particularly harmful. Despite the fact that heavy metals are a naturally occurring part of soil, toxic pollution can frequently occur at industrial and mining areas. Despite their importance to plant development, many heavy metals play rather minor roles in plant physiology. These dangerous metals may pass via plants on their way to the human food chain.

The average lead concentration in April across all sample sites was 0.2 mg/L, which is four times higher than the legal limits. Site C had the highest lead concentration, at 0.25 mg/L, while site D had the lowest, at 0.16 mg/L, on day 1 of April, following the dry season. In contrast, the average concentration of lead in all four locations increased and the average concentration in all 4 sampling sites was 0.67 mg/L in October, after the southwest monsoon season, which resulted in HM being transferred to the pond water from tailings. Site B and D have the highest concentration of copper, at 0.98 mg/L, followed by C and A. In a similar study done to assess the lead concentrations in water ponds fish farm in Zator, South Poland using graphite furnace Atomic Absorption Spectroscopy. Sayadi *et al*. (2014) reported that the concentration of lead drastically increased during the month of October with 7.57 µg/L, which corresponds to the results obtained.

Gradually the heavy metals lead enters the muscles of zebrafish raised in the pond water of four different sampling points. The highest concentration of lead in muscles was found at site D with 0.8 mg/Kg within 24 hours of treatment Post South-West monsoon season, the amount of lead in muscles was observed to be 1 mg/Kg in site D on day 30 April and 1.9 mg/Kg on day 30 of October in site D, which is below the maximum permissible limit. According to similar research conducted in Turkey, (Keskin *et al.,* 2007) the range of 0.822 mg/Kg was found in witting (*Merlangius merlangus*), piceral (*Macna smaris*)., which is within the allowable limits.

**Table 5:** Correlation of heavy metal contaminated water on CAT activity of zebrafish in April and October 2022.

| Assessment days | Correlation | Sig. |
|---|---|---|
| Apr day 1 and Oct day 1 | .961 | .009 |
| Apr day 15 and Oct day 15 | .865 | .058 |
| Apr day 30 and Oct day 30 | .966 | .008 |

Our observation shows a pattern of decreasing CAT activity in the brain of zebrafish in the month of April from day 1 to day 30. This implies that the longer the fish are raised in the heavy metal-contaminated water, these metals are surely impacting on the CAT activity of the zebrafish. A similar pattern is observed in the CAT activity in the month of October 2016. The control values showed in the range of 152.35-151.75 during April day 1- day 30. Against control values, zebrafishes raised in site D- Balghat showed the highest decline from 185.97- 105.8, wherein there was a decrease of almost 80 units, followed by sites C, A and B with 74, 54.15- and 49.5-units during October day 1-30-day treatment. In a similar study in Gorakhpur, (Singh and Ansari, 2017). observed declining CAT activity in the brain of fish with values of 159.33±1.25, when treated with lead and cobalt heavy metals. In a similar study in Portugal, The GSH activity in the brain of fish was 171.6±69, when treated with microplastics and copper-induced toxicity on anti-oxidant enzymes in the brain of zebrafishes (Santos *et al*., 2017) which also justifies our results.

One of the world's most destructive practices is gold mining. Over thirty hazardous substances can be found in toxic mining waste, often known as tailings. Kolar Gold Mines (KGM) alone has generated around 32 million tons of gold ore tailings contaminating every sphere of the environment. The present work thus attempted to explore the level of contamination in pond water and aquatic organisms during April and October 2022. Based on the baseline data that we have obtained during this research, we ascertain that there is an elevated range of HM contamination of water bodies. Using this as a baseline data further study would be conducted to reduce the amount of heavy metal using micro-organisms and plants through phytoremediation in the future. Also further recommend related government bodies and regulatory authorities to take appropriate measures to contain and reduce the persistence of HM's in soil and water bodies in Kolar Gold Fields.

## CONCLUSION

This study found that sample sites had exceedingly high lead concentrations in both the pond water and the muscles of Danio rerio, exceeding the maximum permissible values set by WHO 2008 and USEPA 2009.

The concentration of lead in the muscles increased over time and this increase was associated with a decrease in catalase enzyme activity in the brain region of Danio rerio. This study's findings highlight the impact of lead contamination in pond water on the physiology of fish in the Kolar Gold Fields region.

The study recommends further research on reducing heavy metal contamination using microorganisms and plants through phytoremediation, as well as measures to contain and reduce the persistence of heavy metals in soil and water bodies.

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**Conflict of interest**

All authors declare that they have no conflict of interest.

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