D-5869
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RESEARCH ARTICLE Agricultural Science Digest

Spatial Evaluation of the Heavy Metal Iron in Soil, Pond Water and its Mobility into the Muscles of Zebrafish using ICP-OES

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10.18805/ag.D-5869

ABSTRACT

Background: The study has a bifold objectives, firstly to determine the concentration of iron (Fe) in the soil and pond water within the vicinity of a historical gold mine region located in the Kolar Gold fields (KGF), Karnataka, India. Secondly, the study seeks to investigate the process of bioaccumulation of the heavy metal, iron in the muscles of Zebrafish inhabiting the same area.

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 iron in the soil, pond water and muscles samples. Four soil sample sites (A, B, C and D) were selected based on their proximity to residential area. Soil samples were examined from January to December, 2022, while pond water samples were analyzed between April and October, 2022 following a southwest monsoon rain.

Result: During the study, the analysis of soil samples taken from the four mine landfills revealed elevated levels of heavy metals, with the highest mean concentration of 41915.17 mg/kg found in Site A. In the pond water, sample site B exhibited an exceedingly high Fe concentration during October with 5.56 mg/L followed by sample site D, A and C with 4.89, 3.89 and 3.54 mg/L, respectively against 0.98 mg/L in the control site. The concentration of Fe in the muscles was exceedingly high in the sample site B with 10457 mg/Kg on day 30 of October to that of 8219 mg/Kg on day 30 of April month, which is far above the threshold value of 100 mg/kg as per the regulatory bodies (WHO 1993). The outcome of this experiment will facilitate the assessment of surface soil contamination levels and the likelihood of contaminants entering the aquatic environment, resulting in the bioaccumulation of heavy metals in the aquatic organisms.

Key words: Bioaccumulation, Heavy metal toxicity, ICP-OES, Iron, Kolar gold fields.

INTRODUCTION

Kolar Gold Fields, or K.G.F., is a town in Karnataka's Kolar district's Bangarpet taluk. The KGF gold mines lie at latitude 12°53′12″N, longitude 78°15′03″E, on a short schist strip. It comprises KGF, a township populated by gold mine workers' families. KGF has mined gold underground for almost 120 years (Rao *et al.,* 2006). The problem of heavy metal contamination at KGF became more severe over time as mining operations progressively increased in depth. Currently, gold mining activities have reached a depth of about 3.3 km. Additionally, approximately 40 million tons of mill tailing have been generated and spread across 4 square km Srinivasan *et al.* (1999). After the valuable gold ore is extracted, the processing water and finely powdered rock are mixed to make tailings having a characteristic chemical and physical makeup. Factors such as ore type, local geochemistry, extraction method, pulverized material size and chemical process all play a role in determining the final product (Franks *et al*., 2011). Determining acceptable metal ions in soil and water is critical. Trace metals can enter the water system by leaching rocks and forest fires, due to the high sulfide content, groundwater around tailings is acidic and contains several non-essential metals Mitileni *et al.,* (2011) and Harish *et al.,* (2015) recorded pH values of 3.25-6.28 and 3.48-8.12, respectively. Vegetables grown in contaminated soil and water can be harmful to people and other higher organisms because they absorb and store non-essential heavy metals including arsenic, cadmium and nickel. For the metabolism of plants, higher animals and microorganisms, the most important heavy metals are iron, cobalt and copper. Osmotic pressure is also regulated by electron transport pathways and enzyme components. Arsenic (As), cadmium (Cd), nickel (Ni), chromium (Cr) and lead (Pb) are non-essential heavy metals that are harmful to ecosystems but have no biological use. Plants absorbing these metals may cause them to enter the food chain (Ndeddy Aka *et al.,* 2016) and also it may impact the physical and socio-economic wellness of people residing in the affected region (Verma *et al.,* 2012; and Ashok *et al.,* 2022).

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How to cite this article: Ashok, D. and Harini, B.P. (2023). Spatial Evaluation of the Heavy Metal Iron in Soil, Pond Water and its Mobility into the Muscles of Zebrafish using ICP-OES. Agricultural Science Digest. doi: 10.18805/ag.D-5869.

Submitted: 24-08-2023 **Accepted:** 02-11-2023 **Online:** 25-11-2023

MATERIALS AND METHODS

In the course of this research, a comprehensive methodology was developed, considering spatiotemporal factors such as location and time. The study focused on four specific places, based on the minimal distance from the abandoned gold mining sites were Oorgaum, Tenants, Champion and Balghat. These locations were assigned the names as A, B, C and D, corresponding to their respective distances of 50, 100, 200 and 300 meters from the mining sites.

During this research, a 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.

The four sampling seasons were

Season 1: Winter season during January-February.

Season 2: Spring season during March-May.

Season 3: Southwest Monsoon season during June-September.

Season 4: Post or Northeast monsoon season during October-December. (http://cgwb.gov.in/District_Profile/karnataka/KOLAR.pdf).

Within the time frame of January-December, 2022, a total of 48 soil samples were examined (12 per season) to determine the quantity of certain heavy metals. The results of soil contaminants in the form of heavy metals in mg/Kg were compared against the maximum permissible limits devised by USEPA (U.S. Environmental Protection Agency 2002) and WHO (World Health Organization 1993).

Collection of soil samples

In the research conducted during January to December, 2022, a total of four distinct sample locations were chosen for the sampling process. The purpose of this study was to ascertain the presence of the heavy metal arsenic in the soil. At each of these four locations, soil samples were collected once a month throughout the year, comprising all the four seasons. To ensure accurate results, the surface pollution was eliminated before taking the soil samples. Specifically, 0.5 kg of the soil samples was collected from the outer surface or from the depth of 5-15 cm, in polythene bags to prevent any contamination during transport and storage Mounia *et al.* (2013). Sample preparation procedures for spectrochemical determination of total recoverable elements followed the guidelines set forth by U.S Environmental Protection Agency (EPA) in 2002. This comprehensive approach allowed for insights into potential seasonal variations and trends in heavy metal contamination.

Four sample locations were used for the sampling, which took place from January to December, 2022. In order to detect the presence of heavy metal arsenic, soil samples (n=4) were taken from each location once a month during the four distinct seasons. After eliminating surface pollution, 0.5 kg of soil samples were taken from the outer surface, or 5-15 cm in depth, in polythene bags Mounia *et al.* (2013). Sample preparation procedures for spectrochemical determination of total recoverable elements were carried out as per U.S Environmental Protection Agency (2002).

Sample preparation

The sample was uniformly homogenized using motor and pestle, a portion of it was transferred to a tared weighing dish and readings were recorded to calculate the total recoverable analyses in the solid samples. Once the sample dries, it was sieved through a 5 mesh propylene sieve to further enhance homogeneity and remove any large particles or clumps. When feasible, high-purity reagents were employed. All of the acids utilized in this approach were ultra-purity grade and Rankem Compounds provided the chemicals for measurement. Thermo Scientific's ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) was used in conjunction with Iteva software to evaluate the samples.

A sample was completely mixed, a portion of it was transferred to a tared weighing dish, the sample was weighed and the weight was recorded in order to determine the total recoverable analyses in solid samples. The dry material was mashed in a mortar and pestle and sieved using a 5-mesh propylene sieve to obtain homogeneity. When feasible, high-purity reagents were employed. All of the acids utilized in this approach were ultra-purity grade and Rankem Compounds provided the chemicals for measurement. Thermo Scientific's ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) was used in conjunction with Iteva software to evaluate the samples.

Sample digestion and analysis

The sample were subjected to digestion using the method outlined by Goldberg *et al.* (1983). The digestion process involves the use of two regents: 32% HCl and 70% HNO_3 acids (Ramkem chemicals- Pure analytical grade). Top of FormConcentrated HNO_3 and HCl acids were combined in a 1:3 ratio to create aqua-regia. In a 250 mL conical glass flask, one gram of each reference material or dry powdered soil was combined with 28 mL of aqua-regia. The flask was gently swirled to mix the reactants and heated to a temperature of 120°C for 5 hours on a hot plate. The dissolved samples were filtered into 100 mL HDPE (High-Density Polyethylene) bottles after cooling down using filter paper that had been washed through with 3% HNO_3 acid for ICP-OES analysis. To ensure clean glassware for samples preparation, all the glassware was cleaned by immersing it in an acid solution containing 10 percent v/v HNO_3 for 24 hours, followed by a rinsing with deionized distilled water.

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 metals 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.

Using ICP-AES, pond water samples from the affected area and the control samples were examined for the presence of heavy metals and a rise in their concentration between April and October, 2022 Chakraborti *et al.,* (2013).

Sample preparation-Muscles

Adult and healthy male zebrafish 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 2010. The fish were then euthanized and dissected to extract muscle and gill tissues along with the brain for the estimation of selected antioxidant enzymes.

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

Each specimen was the subject of a 1g sample with three replications. All samples were dried for two hours in an oven set to 70°C. After the drying process was finished, the samples were moved to a muffle furnace where they were heated to between 450 and 500°C for four hours, with the temperature of the furnace being raised by 50°C at a time so that all of the samples could be turned into ash.

The digestion step was initiated when all the samples had been converted to ash. To ensure thorough digestion, one gram of powdered material was collected in tubes and digested using HCl and HNO_3 1:3 v/v 2 ml HCl and 6 ml HNO_3 before being heated for an hour in a water bath at 70°C. Each tube was then allowed to cool to room temperature before filtering with filter paper and collecting the filtrate in polyethylene tubes. Finally, each filtrate was then diluted with de-ionized water to make a total volume of 25 ml.

Statistical analysis

The statistical analysis was conducted using SPSS 23.0 and one-way analysis of variance (ANOVA) at the $P<0.05$ level.

RESULTS AND DISCUSSION

Iron (Fe)

According to WHO (1993), the maximum permissible limit of iron that can be present in soil is 21000 mg/kg. Table 1 shows the soil sample collected from site A had the highest concentration of Iron with 44680 mg/kg, which is 2 times the upper limit set by the WHO for safe limits. The average iron content in the soil samples taken from Oorgaum during season 4 was 41915.17 mg/kg, followed by 35979.25 mg/kg at site D, 24572.08 mg/kg at site C and 24280.67 mg/kg at site B. The average concentrations of iron in all four sampling sites were 30656.6 mg/kg, 32319.7 mg/kg, 32116 mg/kg and 31653 mg/kg in seasons 1, 2, 3 and 4 respectively. Season 2 shows the highest concentration followed by seasons 3, 4 and 1.

Sites A, B, C and D have Iron levels in their soil that are well above the legal limit. The Iron concentrations in the soil samples are rising from season 2 to season 3 in sites A and B. Initial concentrations tend to be lowest during season 1, with subsequent concentrations gradually rising throughout the year.

Table 2 shows a substantial and positive link between soil iron pollution during seasons 1 (January through March) and 2 (April through June) ($r= 0.993$, $p<0.05$). For Seasons 1 and 3, the correlation coefficients for iron contamination in soil were ($r= 0.993$, p 0.05) and ($r= 0.995$, $p<0.05$), respectively.

Table 3, displays the results of a statistical analysis comparing iron concentrations in the soil across different seasons. The results show that there is a marginally statistically significant difference between the iron concentrations in the soil between seasons 1 (January-March) and 2 (April-June) with a p-value of 0.049. We cannot statistically report a difference in iron levels between seasons 1 (January-March) and 3 (July-September) and seasons 1 (January-March) and 4 (October-December) since the p-value is greater than 0.05.

To assess the bioaccumulation of iron present in the pond water in the muscles of zebrafish.

The pond water samples from sampling sites A, B, C and D were collected to compare against the pond water located 55 km away from the KGF mining region as the control site

Table 1: Concentration of Iron in the soil (mg/kg) in sampling sites during January-December, 2022.

Sampling site	Maximum permissble limit	Highest	Lowest	Average
Oorgaum (A)	21000	44680	38000	41915.17
Tenants (B)		28765	22765	24280.67
Champion (C)		26754	23485	24572.08
Balghat (D)		43980	26540	35979.25

during the month of April and October 2022 to assess the entry of heavy metals from soil into the water bodies due to the contrasting amount of rainfall received, based on Indian Meteorological Department' data of past 100 years. Pond water samples from the affected region and the control samples were analyzed for the presence of heavy metal during April and October, 2022 using ICP-AES (Chakraborti *et al.,* 2013).

Concentration of Iron in pond water during April and October 2022

The maximum tolerable limit of iron in water is 1 mg/L as per WHO (1993) and USEPA (2002).

Table 4 shows the average iron concentration in April across all sample sites was 2.24 mg/L during April, which is 2.2 times higher than the legal limits. Sample site B had the highest iron concentration, at 3.41 mg/L, while site C had the lowest, at 1.48 mg/L, following the dry season. In contrast, the average concentration of iron in all four locations increased and the average concentration is 4.47 mg/L in October, after the southwest monsoon season, which resulted in HM being transferred to the pond water from tailings. Site B has the highest concentration of iron, at 5.56 mg/L, followed by D, A and C.

Displays that iron levels in pond water are excessive in the locations labeled B, D, A and C. An exponential rise in the concentrations of iron is observed between the month of April and October, 2021, against the maximum permissible limits of 1 mg/L. All control values were seen below permissible limits.

According to Table 5, there is a positive and significant correlation between iron concentration in pond water during April and October (r= 0.987, $p<0.05$).

Table 6, displays the results of a statistical analysis comparing iron concentrations in the pond water during April and October. There is a statistical significance in the amount of iron in the pond water during April and October since the p-value<0.05.

The concentration of Iron in the muscles of zebrafish in mg/Kg in during April and October, 2022

The pond water from four sampling sites A, B, C, D and one control sample was brought to the lab and zebrafish were raised in 5 different tanks labeled A, B, C and D, along with the control tank which had pond water located 55 Km away from KGF mining sites.

Table 7 shows the maximum permissible limit of iron in various parts of the fish is 100 mg/Kg as per WHO (1993). The average concentration of iron on day 1 of April was

Table 2: Correlation of Iron concentration in soil in various seasons during January-December, 2022.

Seasons during 2022	Correlation	Sig.
1 and 2	.993	.007
1 and 3	.993	.007
1 and 4	.995	.005

Table 3: Comparison of Iron concentration in soil in various seasons.

Seasons	Paired differences					t	df	P-value
	Mean	Std. deviation	Std. error mean	95% confidence interval of the difference				
				Lower	Upper			
1-2	-1662.750	1032.664	516.332	-3305.94959	-19.55041	-3.220	3	.049
1-3	-1460.000	1240.156	620.0781	-3433.36555	513.36555	-2.355	3	.100
1-4	-996.750	1158.28	579.1445	-2839.84655	846.34655	-1.721	3	.184

Table 4: Concentration of Iron in pond water in mg/L in sampling sites during April and October, 2022.

Sampling site	April	October
Control	0.52	0.98
Oorgaum (A)	1.541	3.89
Tenants (B)	3.41	5.56
Champion (C)	1.48	3.54
Balghat (D)	2.53	4.89
Max permissible limit	1	1
Average	2.24025	4.47

Table 5: Correlation of iron concentration in pond water in April and October, 2022.

During 2022	Correlation	Sig.
1 April and October	.987	.013

Table 6: Comparison of iron concentration in pond water in April and October, 2022.

Treatment during April and October	Paired differences					t	df	P-value
	Mean	Std. Deviation	Std. error Mean	95% Confidence Interval of the difference				
				Lower	Upper			
April-October 2022	-2.229750	.148729	.074364	-2.466411	-1.993089	-29.98	3	.000

Table 7: Concentration of iron in muscles of zebrafish in mg/Kg in various sampling sites during April and October, 2022.

	Apr day 1	Apr day 15	Apr day 30	Oct day 1	Oct day 15	Oct day 30
Control	830	850	947	850	874	874
Oorgaum	2345	5874	7419	5478	7847	9875
Tenants	6547	6547	8219	8974	8974	10457
Champion	5897	5897	6917.3	7845	7845	9875
Balghat	4978	4978	5872	6987	6987	8425
Max permissible limit	100	100	100	100	100	100
Average	4941.75	5824	7106.825	7321	7913.25	9658

Table 8: Correlation of iron concentration in muscles of zebrafish during April and October, 2022.

During assessment period	Correlation	Sig.
Apr day 1 and oct day 1	.947	.014
Apr day 15 and oct day 15	.999	.000
Apr day 30 and oct day 30	.991	.001

Table 9: Comparison of iron concentration in muscles of zebrafish in pond water in April and October, 2022.

Treatment during April and October	Paired differences					t	df	P-value
	Mean	Std. Deviation	Std. error Mean	95% Confidence interval of the difference				
				Lower	Upper			
Day 1- 1	-1907.400	1156.106	517.0265	-3342.89585	-471.90415	-3.689	4	.021
Day 15-15	-1676.2000	944.21751	422.266	-2848.60088	-503.79912	-3.970	4	.017
Day 30 -30	-2026.340	1202.231	537.654	-3519.10705	-533.57295	-3.769	4	.020

observed as 4941.75 mg/ kg. The highest concentration of iron was seen at site B with 6547 mg/Kg within 24 hours of treatment subsequently increasing on day 30 which is much higher than day 1 in April during dry season. Post South-West monsoon season, the amount of iron in muscles was observed to be 8974 mg/Kg on day 1 of October and 10457 mg/Kg on day 30 in site B, which is almost 104 times higher than the maximum permissible limit (Table 7). The average concentration of iron in the muscles of zebrafish in all 4 sampling sites was 7321, 7913.2 and 9658 mg/Kg on days 1, 15 and 30 of October 2022.

Displays that iron levels in the muscles of zebrafish are above permissible levels in all sampling sites except in the zebrafish raised in the pond water collected from the control site during April and October, 2022.

According to Table 8, there is a positive and significant correlation between iron concentration in the muscles of zebrafish between April day 1 and October day 1($r = 0.947$, $p<0.05$). There is also evidence of a positive and statistical significance observed in the muscles of zebrafish during in April day 30 and October day 30 assessment ($r = 0.991$, $p<0.05$) Apr day 15 and Oct day 15 ($r = 0.999$, $p<0.05$).

Table 9, shows the results of a statistical analysis comparing iron concentration in the muscles of zebrafish in April and October. There is a significant difference in iron levels in the muscles of zebrafish between Apr day 1 and Oct day 1, Apr day 15 and Oct day 15 and Apr day 30 and Oct day 30 since the p-value<0.05.

CONCLUSION

Analysis of heavy metals in soil samples indicated alarming levels of heavy metal Iron was found to be beyond the permissible limits set by WHO (1993) and USEPA (2002). Iron (Fe) was found more than the permissible limit in site A and 125% more in sites B, C, and D. Statistical analysis using One-way ANOVA was used followed by SPSS software showed an insignificant correlation between various seasons of the year to that concentration of heavy metals, thereby we infer that these metals are not dependent on seasons and are present throughout the year in soil due to the past gold mining activities. The present study accomplishes that further study is required to minimize the levels of contamination caused due to gold mining activities and the measures to be conceived to curb the entry of HM into both abiotic and biotic components of the study area, thereby reducing the effects caused by HM on higher order plants, animals and humans. This study also emphasizes measures to be taken to attain reclamation and remediation of the immediate environment of Kolar Gold Fields.

ACKNOWLEDGEMENT

We wholeheartedly, thank the management of Kristu Jayanti College, Autonomous, Bengaluru, for providing the infrastructure and facilities to conduct the research.

Conflict of interest: None.

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