



Bioaccumulation of zinc in rice (*Oryza sativa* L.) from River Swat, Panjkora, And Kabul Pakistan

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ABSTRACT

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Ecosystem contamination with heavy metals lead to the bioaccumulation of these elements in crops. Rice, a significant component of the human diet, can bioaccumulate heavy metals and is of public health concerns. Therefore, the current research aimed to investigate the bioaccumulation of the heavy metal zinc in several rice varieties from three rivers in Pakistan's Malakand Division. The Highest Zn concentration of 91.58 ± 6.25 mg kg⁻¹ dry weight was observed in rice roots of China Basmati variety grown across River Swat, while the highest Zn concentration of 60.06 ± 4.41 mg kg⁻¹ dry weight was observed in the stem of Mota Chawal on River Kabul. In a site-wise comparison of both River Swat and Kabul, the Zn concentrations in the stem of Sara Sela rice on River Panjkora were 55.5 ± 4.37 , showing significant differences among different sites on the rivers. Similarly, comparing metal concentrations in different rice varieties generally did not show significant differences between the varieties. Zn concentrations in rice were higher in the Swat River than in the rest in all cases. Bioaccumulation factor (BAF) values for Zn were in the order of soil > roots > stem > leaves > grains for River Swat and Kabul. In contrast, for River Panjkora Zn concentration, the order was soil > stem > root > grains > leaves, respectively. Zn absorption by rice in the form of ions or particles generally affects plant phenotypic, physiological, and molecular development; hence, it must be considered in present varieties for the future.

Contribution/Originality: The present study contributed to the safety of Rice field grown along the three most important river of Malakand Division. The safety of rice is of ought most importance as it's a major staple crop, utilized by the peoples of the area.

1. INTRODUCTION

In Pakistan, rice is the second-most significant food crop in terms of domestic consumption and exports. Agrochemical, industrial, and other human activities have caused major heavy metal contamination of agricultural soil all over the globe (Chibuikwe & Obiora, 2014). The most popular grain in Pakistan in rice (*Oryza sativa* L.). But compared to other grains, it collects more heavy metals (Huang et al., 2018). Both natural and artificial factors can potentially release heavy metals into the environment. Anthropogenic sources, notably mining activities, are the main emitters (Duruibe, Ogwuegbu, & Egwurugwu, 2007). Human activities such as agriculture, mining, building, and industrial operations are the major cause of heavy metal contamination (Upadhyay, Singh, & Singh, 2011).

It is essential to choose rice cultivars whose levels of zinc in edible sections are low enough to make them safe for ingestion by people even when grown in polluted soil. Zinc has greater bioavailability and soil-plant transfer rate than other heavy metals, making it a non-essential trace element for plant development. It is easily absorbed by plants, which enter the food chain and eventually the human body (Tang, Pang, Ji, Gao, & Nguyen, 2016; Touceda-González et al., 2015). Inorganic zinc is accumulated through the absorption of bioavailable zinc from the soil by rice's roots and leaves. Ninety percent of the exposure to zinc comes from diet, and plant-derived foods like grains are the main source (Chen et al., 2018). It is standard practice to utilize the species sensitivity distribution (SSD) to identify the pollutant concentration that protects a certain percentage of species (Ding et al., 2018).

The concentration and accumulation of heavy metals in plant tissue has been assessed using the bioaccumulation factor (BAF), which is the ratio of concentration of heavy metal in plants to that of soil (Chen et al., 2009; Wang et al., 2010). Similarly, the BAF for rice grains was evaluated following (Römkens et al., 2009; Ye, Li, Ma, Wu, & Sun, 2014) to assess the safety of rice crops that is of importance regarding human health. The BAF is directly related to soil important properties like pH, soil organic matter contents (SOM), cation exchange capacity (CEC), clay contents and redox potential (Eh). The most important element affecting the amount of Zn that accumulates in rice grains is the pH of the soil; raising the pH might immobilize heavy metals by increasing soil adsorption and converting freely accessible forms of the metal to immobile ones (Zhan et al., 2020). Additionally, when soil CEC grew, the capacity of the soil to store heavy metals increased (Zhang, Chen, Xu, Zhu, & Zhu, 2019).

Research to ascertain the contribution rate of various soil characteristics to the bioaccumulation of zinc in rice grains found that soil pH and organic carbon (OC) account for about 70% of the variation in the amount of zinc accumulated in the grains. Contrarily, the impact of soil Cd concentration was only mild, while soil CEC and clay content were minimal (Zhai et al., 2016). Key soil factors and plant traits related to heavy metal accumulation should be considered when investigating zinc bioaccumulation. In light of this, a soil-plant transfer model was developed for forecasting BCF values on a broader scale. This model includes soil characteristics (pH and OC) that are most likely to affect zinc bio-accumulation as well as the inherent sensitivity of rice (Liu et al., 2016). The addition of the model increases SSD accuracy and makes it possible to assess the bioaccumulation of heavy metals in rice grains under polluted soil.

Data on the bioaccumulation of zinc in various parts of rice in the study area were collected to:

1. Study the bioaccumulation of zinc in different parts of rice in the selected area.
2. Study the zinc contamination in paddy soil in the study area.
3. Assess the risk to human health from consuming zinc-contaminated rice.

2. MATERIAL AND METHODS

In the current investigation, random mature rice samples were taken from rice in Pakistan's Malakand division that had been irrigated with tainted water from the rivers Swat, Panchkora, and Kabul (Figure 1). Additionally, several images were shot using a digital camera, and the global positioning system was used in this experimental investigation to examine environmental factors. Geographically, it stretches from 71°13'8" to 72°22'13"E longitude and 34°39'30" to 35°47'17"N latitude, with a catchment area of 5905 km². These mountain ranges were the source of the Panjkora River basin, divided into the Swat and Kabul River basins by the western and eastern mountains. Due to the many glaciers in the valleys over 4000 meters above sea level, these mountains are blanketed with snow. The region under the current operation has a cold to warm summer with temperatures between 16 °C to 32 °C. From December through February, the temperature decreases below the freezing mark. The characteristic of the winter season is snowfall. The yearly rainfall data shows variations of more than 1000 mm with a range of 823 to 2149 mm (Mahmood & Ullah, 2016).

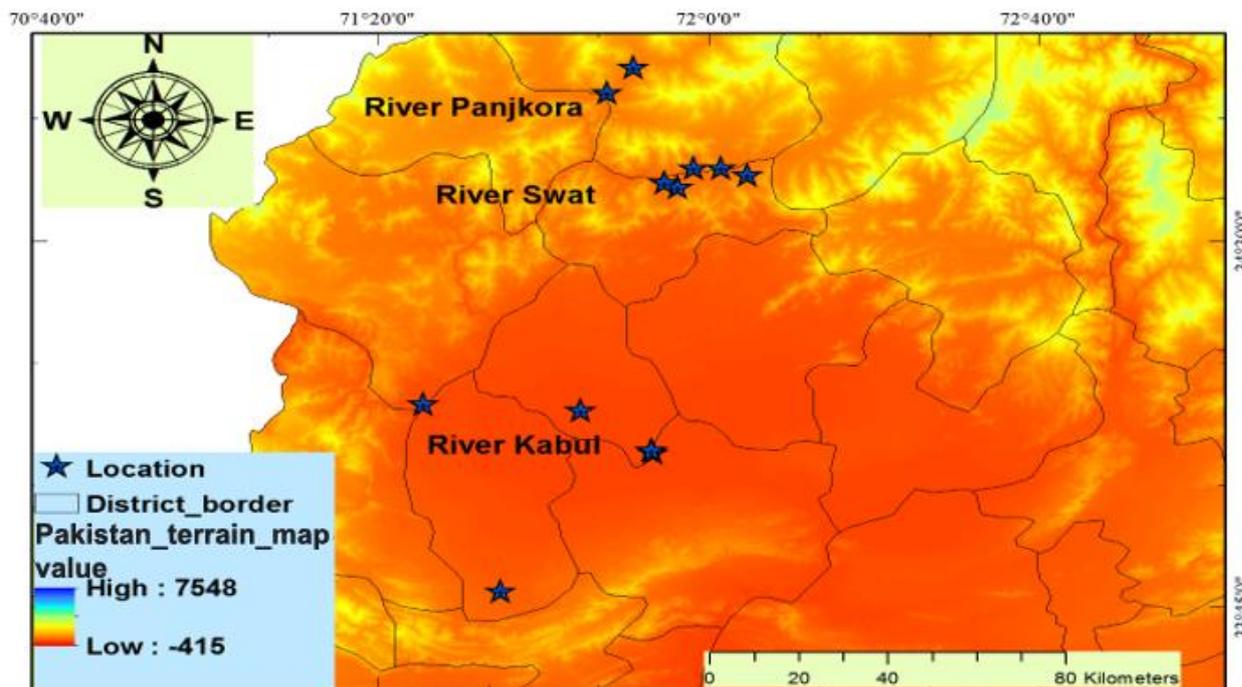


Figure 1. Digital elevation model showing the sampling point from where Rice plants were collected for analysis.

2.1. Sample Collection and Analysis

At a depth of 0 to 25 cm below the soil surface, a soil sample was taken from the paddy plants' surrounding roots for analysis at the Peshawar Agriculture Research Center. The soil and paddy plant clumps were selected as repeated samples from each site, placed into labeled plastic bags, and transferred to the laboratory, where they were preserved for 15 minutes in dry air at room temperature. Figure 1 provides detailed information about the sample collection locations. Each component was divided up into smaller parts in the lab. The white rice was separated from its husk by grinding the grains into a fine powder, which was then dried in a 105° oven for 24 hours. The soil sample was also carefully cleaned to remove any remaining roots, stones, twigs, and other pollutants so it could pass through a 1mm sieve.

2.2. Sample Digestion

O. sativa powder was heated on a hot plate to 70° for 30 minutes, at the point it was digested with a solution of HNO₃ in 7.5 ml and HClO₄ in 2.5 ml until a clear solution was generated and the odors stopped. The rice samples were cooled, then filtered with 45 m Whitman filter paper, and diluted with 50 ml of deionized water. Before performing the dry air heavy metal analysis, the filter samples were put into the polyethylene bottles and left at room temperature.

Like this, 0.5 g of soil samples were digested in 10 ml of a 3:1 aqua regia HCL and HNO₃ solution, then cooked on a hot plate for 40 minutes at 70 C. After cooling, the soil sample was filtered using a 2mm Whatman filter paper sieve. The soil samples were put into polypropylene bottles and stored in 2 dry air until the examination of the heavy metals.

2.3. Quantification

Utilizing the procedures outlined in GB/T 5009, 11/2003, 15/2003, 12/2010, and 17/2003, Chinese National Food Safety Standards for measuring arsenic, cadmium, lead, and mercury in foods, respectively (heavy metal contamination of China's rice fields), the zinc content in rice samples was evaluated. Duplicate samples, internal reference materials, reagent blanks, and certified foreign reference materials were all used as a quality control

procedure (Chen, Amarasiriwardena, & Christiani, 1999). The chemical analysis's accuracy and bias were both under 10%.

2.4. Data analysis

For the statistical analysis, we used the Microsoft Excel program and SPSS (Statistical package for social sciences) to calculate the selected data's mean and standard deviation.

3. RESULTS

Concentrations of Zinc (Zn) in soil and root stem and rice leaves (*Oryza sativa* L.) collected from different sites in River Panjkora are given in Table 1. The highest concentration of Zn was found in the root, followed by leaves, grains, and then in the stem. The concentration was found at the permissible level and is safe from a health point of view.

Table 1. Zn concentration in soil and rice (*Oryza sativa*) along with different sites of River Panchkura.

Site	Variety	Zn concentration (mg kg ⁻¹ dry weight)				
		Soil	Roots	Stem	Leaves	Grains
Haji Abad	China	42.16 ± 8.18	47.66 ± 3.81	18.63 ± 4.22	22.92 ± 3.30	21.8 ± 3.97
Khazana	Sara Sela	48.55 ± 1.44	33.22 ± 4.65	55.5 ± 4.37	17.95 ± 6.79	38.81 ± 23.33

Note: The table above shows the mean ± standard deviation (n = 3).

3.1. Bioaccumulation of Zinc

The accumulation of Zinc in the different parts of cultivated varieties of the plant *Oryza sativa* is shown in Figure 2. The bioaccumulation factor was higher in the various collected from Khazana.

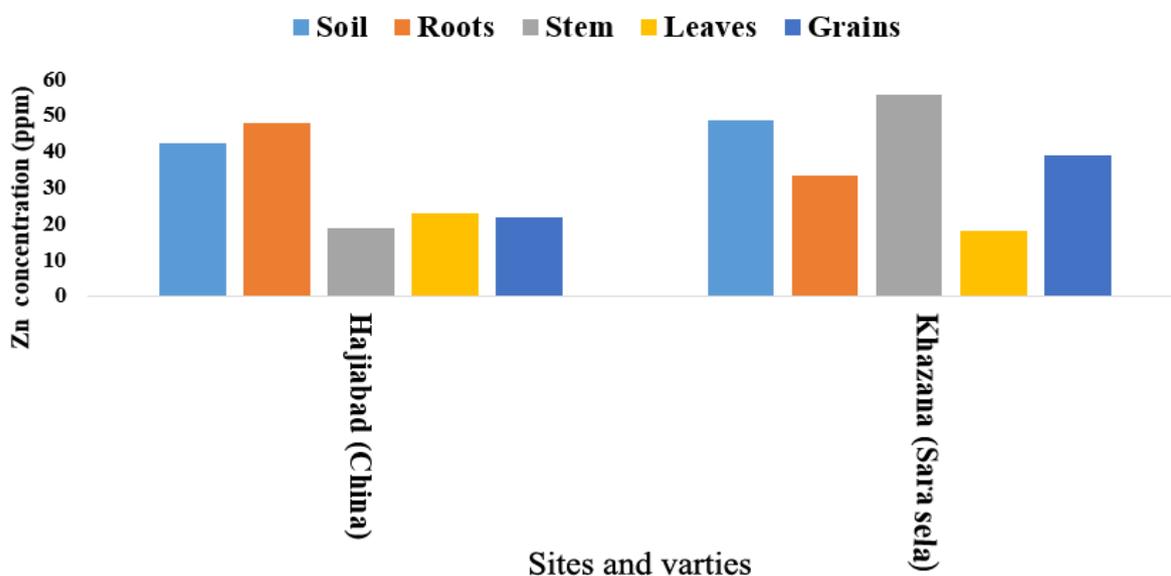


Figure 2. Zn concentrations (mg kg⁻¹ dry weight) in different parts of rice from different sites of River Panjkora.

3.2. The Concentration of Zn in Soil and Rice along with River Swat

The background concentration of the selected Heavy metals (Zn) found in the used Soil, Roots, Stem, Leaves, and Grains are given in Table 2.

Table 2. Zn concentration in soil and rice along with different sites of River Swat.

Site	Rice varieties	Zn concentration (mg kg ⁻¹ dw)				
		Soil	Roots	Stem	Leaves	Grains
Thana	Mardanai Sela	62.4 ± 7.27	39.16 ± 6.39	30.13 ± 1.94	29.93 ± 7.67	30.5 ± 1.21
	China Basmati	60.36 ± 2.76	44.03 ± 6.23	25.2 ± 2.48	25.26 ± 0.97	20.23 ± 2.13
Chakdara	China Begamai	44.1 ± 19.41	85.86 ± 21.02	35.93 ± 15.41	24.33 ± 5.96	41.6 ± 21.01
	Mardanai Sela	59.13 ± 4.04	30.8 ± 5.70	27.03 ± 2.95	23.06 ± 2.09	21.5 ± 3.68
	Garma Sela	47.73 ± 3.49	32.26 ± 2.30	28.26 ± 2.94	27.16 ± 2.83	20.76 ± 3.69
	Sara Sela	55.16 ± 4.15	43.73 ± 2.02	58.3 ± 7.1	29.36 ± 4.70	26.8 ± 6.20
Badwan	Garma Sela	57.36 ± 5.45	73.43 ± 18.36	91.33 ± 2.63	28.3 ± 3.14	22.4 ± 6.75
	China Basmati	70.53 ± 4.83	72.13 ± 7.07	58.16 ± 6.56	31.56 ± 1.52	22.93 ± 1.43
Amandara	Garma Sela	43.16 ± 4.25	31.0 ± 2.02	26.13 ± 7.36	26.43 ± 7.39	31.96 ± 13.17
	China Basmati	37.4 ± 0.96	56.91 ± 8.27	27.83 ± 8.20	22.86 ± 2.40	19.33 ± 0.65
Batkhela	China Basmati	32.4 ± 2.32	91.58 ± 61.25	44.53 ± 7.29	26.03 ± 6.50	23.46 ± 1.25

Note: Results are shown as mean ± standard deviation (n = 3).

3.3. Bioaccumulation of Zinc

The accumulation of Zinc in the different parts of cultivated varieties of the plant *Oryza sativa* is shown in Figure 3, respectively.

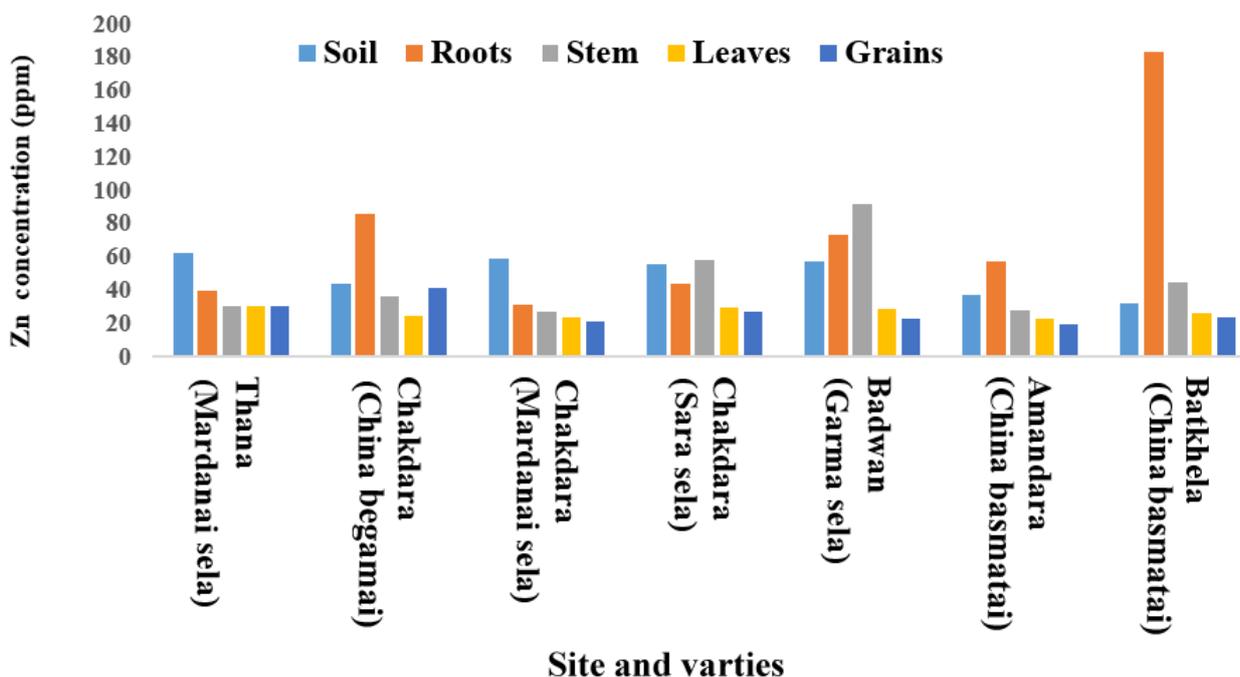


Figure 3. Show the uptake of Zn metal by the different parts of *Oryza sativa*.

Figure 4 show the geographical representation of Heavy metal (Zn) concentration in the crop plant *Oryza sativa*. The X-axis are shown the sites and varieties, and the Y-axis are shown the concentration of Zinc.

3.4. The Concentration of Zinc in Soil and Rice along with River Kabul

The background concentration of the selected Heavy Metals (Zn) found in the used Soil, Roots, Stem, Leaves, and Grains are given in the following Table 3.

Figure 4 shows the graphical result of the concentration of Zinc metal by the Y-axis and the varieties and sampling sites. The result shows Zinc uptake by the different parts of the *Oryza sativa* plant.

Table 3. Zn concentration in soil and rice along with different sites of River Kabul.

Sites	Rice varieties	Zn concentration (mg kg ⁻¹ dw)				
		Soil	Roots	Stem	Leaves	Grains
Patwar Payeen Warsak	Polo Wala	64.7 ± 2.26	43.06 ± 6.80	54.26 ± 7.18	25.13 ± 2.70	22.5 ± 2.29
Badin Kalay Adezai	Mota Chawal	63.06 ± 2.38	45.76 ± 3.07	60.06 ± 4.41	32.1 ± 3.48	24.1 ± 2.94
Rajar Charsada	Mota Chawal	57.7 ± 3.32	46.7 ± 7.40	26.53 ± 5.77	40.4 ± 18.96	19.06 ± 1.09
Babaji Kalay Nowshehra	Mota Chawal	46.6 ± 2.83	43.36 ± 7.07	18.9 ± 4.0	22.6 ± 2.04	17.63 ± 1.69
Godam Korona Nowshehra	Watanai Chawal	65.26 ± 2.17	35.23 ± 4.90	45.76 ± 14.92	29.4 ± 7.98	33.86 ± 9.01
	Kotado	59.86 ± 2.97	22.22 ± 3.70	26.23 ± 11.14	27.23 ± 1.50	22.3 ± 2.67

Note: Results are shown as mean ± standard deviation (n = 3).

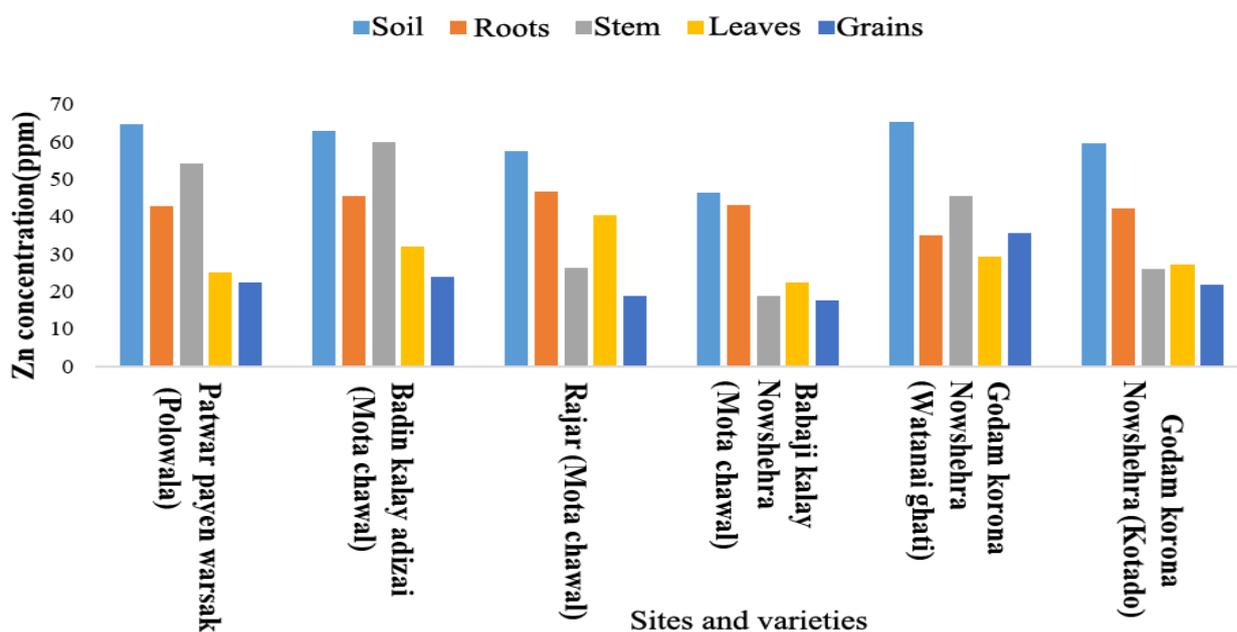


Figure 4. Shows Zn metal uptake by the different parts of *Oryza sativa*.

4. DISCUSSION

4.1. Bioaccumulation of Zinc (Zn) in rice at River Panchkora

Even while organisms need zinc to be healthy, too much of it may be dangerous (Noulas, Tziouvalekas, & Karyotis, 2018). For plants and animals, the free zinc ion in the solution may be very poisonous (Mudhoo, Garg, & Wang, 2012). In this research, the greatest soil Zn content was 48.55 mg kg⁻¹ dw, whereas Zn concentrations in rice of River Swat varied from 17.95 to 6.79 in leaves to 55.5 to 4.37 mg kg⁻¹ dw. The frequency of indica rice cultivars with grain concentrations surpassing the national threshold of 0.2 mg/kg was twice that of japonica, indicating that japonica is safer than indicated under Zn exposure. The difference in grain Zn concentration across rice cultivars was 7.6 times (Li, Cao, Ma, Su, & Li, 2019). This result was consistent with Zhang, Zhong, Liu, and Ouyang (2015) observation's that roots of rice treated with 10, 100, and 1000 mg/kg Zn had Zn levels that were 2.5, 2.7, and 3.9 times greater, respectively, plants subjected to Zn²⁺.

4.2. Bioaccumulation of Zn in Rice at River Swat

Similarly, Zn concentrations in rice varieties varied from 19.33 to 0.65 mg kg⁻¹ dw in grains to 91.58 to 61.25 mg kg⁻¹ dw in roots and from 32.04 to 2.32 to 70.53 to 4.83 mg kg⁻¹ dw in soil. According to Takahashi et al. (2011), the quantity of Zn accumulated in rice grains positively corresponds with the amount of Zn absorbed by roots. Similarly, it was also reported that the rice seedling that was not exposed to ZnO, have lower concentrations of

50.22 and 21.92 g/g dry weight, respectively in the roots. In the roots of rice seedlings, these values varied from 5702.55 to 8501.26 g/g dry weight, whereas in the shoots, they ranged from 1508.22 to 3065.65 g/g dry weight. Our reported Zn concentrations are lower than those described by Takahashi et al. (2011), described by Takahashi et al. (2011), which is due to the conduction of our study in field conditions.

4.3. Bioaccumulation of Zn in Rice at River Kabul

In addition, the Zn content in the River Kabul varied from 17.63-1.69 in the grains to 60.06-4.41 in the stem and from 46.60-2.83 to 65.26-2.17 in the soil, respectively. The BCF of 134 rice grains taken from various places was examined by Zhang, Liu, and Wang (2010), who discovered that the average value was 0.26. This value was comparable to the BCF of rice with moderate bio-accumulation (i.e., grade 3) grown in various soil conditions in the present research. Earlier research found that rice with low-Zn-accumulating features helped lower Zn content in hybrid grains and that following generations might inherit superior qualities from parents (Xiao et al., 2017).

5. CONCLUSIONS

This research was conducted on rice plants and paddy soil samples to determine the accumulation of zinc in plants and soil samples gathered during their harvesting time. The majority of the examined zinc was collected mostly in the rice paddy plant's soil, stem, and roots (*Oryza sativa*). Paddy plant and Soil samples were analyzed for Zinc contents from all three sites, River Panjkora, River Swat, and River Kabul, which indicated that in paddy plant and soil. Zinc concentration was in variable range these analyses showed that most of the Zinc absorbed from Soil by Roots of Rice plant. The most Zinc concentration was found in Soil, Stem, and Roots minute quantity in Grains and Leaves. However, it is not clear that the Zinc is transferred from pollutant water to paddy Soil and different parts (roots, Stem, Leaves, and Grains) of the Rice plant with a different distribution pattern.

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Authors' Contributions: Design the research, I.U.; write initial draft of the manuscript and field data collection, R.U.; data analysis and validation, H.B.; data visualization and interpretation, M.N.; metal analysis and accumulation, A.M.; proofreading and data interpretation, H.U. All authors have read and agreed to the published version of the manuscript.

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