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Evaluating the Human Health Risks of *Oryza sativa* Contaminated With Heavy Metals in Diversely Irrigated Fields of Sahiwal, Pakistan

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Authors' Contribution
ZIK and KA supervised the study. MA conducted the experiment. AA, FZ and IRN were responsible for the data analysis. HM and NR were responsible for analyzing and interpreting the data. RSA critically reviewed the manuscript.

Key words
Basmati, Copper, Food chain, Pollution, Public, Zinc

ABSTRACT

Heavy metals exposure in *Oryza sativa* varieties imposes great health risks to human health. The current study was conducted to investigate the heavy metal content in different varieties of rice grown in adversely irrigated fields of Sahiwal, Sargodha. This study evaluates the Cu and Zn content in paddy soil, in edible part of the rice as well as serum of local residents. Various pollution indices were applied to analyze the current status of these metals. Results demonstrated the metal trend in rice varieties as Zn > Cu. Observed Zn (11.43-23.487mg/kg) and Cu (10.524-20.50mg/kg) range in soil was within WHO limits. Analyzed Zn (0.967-2.87 mg/kg) and Cu (0.575-3.066 mg/kg) concentration in all rice grains lie within WHO/FAO limits. Basmati and Irri 6 varieties presented the highest Zn and Cu level in their grains. Positive correlation from soil to grains was observed for both metals. Transfer factor showed the minimum uptake of both metals in Kainat variety while metal accumulation in rice tissues was observed in Irri 6 variety. Zn (0.559-0.932mg/kg) and Cu (1.428 to 1.995mg/kg) range showed the minimal enrichment in soil. Target hazard quotient values for Zn (0.0021-0.0207) and Cu (0.00006-0.00078) were below than 1 for all age groups. In conclusion, there is no significant health risk observed for humans on consuming rice varieties from this area.

INTRODUCTION

Agriculture sector has prime importance in Pakistan economy. It supports 21% to total GDP and constitute about 60% of total exports. Pakistan is a country where both Rabi and Kharif crops are cultivated (Syed *et al*., 2022). Major kahrif crop of Pakistan is rice which is cultivated on 3.034 million hectares of land and is the second most consumed crop after wheat. It is a good source of mineral, vitamins and proteins (Abbas and Dastgeer, 2021).

In Pakistan, rice production is decreased and affected badly because of heavy metal contaminated soil and water being used for its irrigation. Plant's biological systems are affected by heavy metals accumulation *i.e.* uptake of oxygen, water and synthesis pathways for chlorophyll, breakdown of mitochondria, damage of cell wall thickness and nuclear envelop are being caused by heavy metals toxicity. Water potential of the plants are decreased by increasing osmolarity and disturbing osmo-regulatory process during development as a result of which transpiration level of leaf area is affected and reactive oxygen species (ROS) are produced due to bursting of lipid membrane DNA, and proteins (Akhtar *et al*., 2021). Hazardous heavy metals exposure imposes great health risks to human health. A study was carried out in Tamale Aboabo Market in the Northern Region of Ghana. Randomly selected 21 samples from the market containing cereal crop and legumes were taken for analysis of heavy metal contamination using an atomic absorption

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Spectrophotometer. As concentration was found to be 0.017mg/kg and the concentration of Cu ranged from 0.019 to 0.042mg/kg, and that of Zn concentrations were low. There was no cancer risk by consumption of cereals in Tamale market cereal samples. The overhead concentrations of As in the cereals can be preferable to the usage of arsenic containing pesticides, fertilizer and runoff from other cultivation fields (Adam *et al*., 2022). The maximum Pb, As, Hg and Cr concentrations were 1.455, 1.1, 2.8, and 1.05 times of their risk thresholds, respectively (Wei *et al*., 2023). The average Cu and Zn concentrations in commercial rice were 2.31 and 15.429 mg kg^{-1}, respectively, indicating a good nutritional status (Wei *et al*., 2023).

Zn and Cu are important micro-nutrient for growth, metabolism and protein synthesis in rice plant. Both metals also play their role in photosynthetic and respiratory machinery of plants (Schmidt *et al*., 2023). Enzymes for nitrogen metabolism uses Zn as co-factor so when there is Zn deficiency the enzymes functions are disturbed and capacity to cope with anaerobic conditions of soil is badly affected in rice seedlings. Flooding of water causing Zn deficiency which is observed shortly after transplanting that results in loss of productivity. Moreover, salinity destroys the physiology of rice plant by decreasing vegetative growth, flowering and vigor of rice seedlings (Nadeem *et al*., 2020). Cu is used as antifungal agent in agriculture sector. In humans, essential metals are also important like Cu is necessary for haemoglobin formation and metabolism of carbohydrates and some are important as co-factor of enzymes. However, when these essential metals present in excessive amount they cause cell damage. The accumulation of metals in food crops is dangerous as it transfers to animals and humans thus damaging their health badly. It is dire need to control the metals content in food. The aim of this research work is to investigate the heavy metal content in different varieties of rice grown in adversely irrigated fields of Sahiwal, Sargodha. The research objectives were: to evaluate the Cu and Zn content in paddy soil, in edible part of the rice as well as serum of local residents. Various pollution indices were applied to analyze the current status of these metals in diverse fields.

MATERIALS AND METHODS

Study area

Sahiwal is the one of the Tehsil of Sargodha. It is located at 38 km from Sargodha city which is the 12th major urban city of Pakistan (Fig. 1). Summer temperature observed is 50 °C while winters minimum temperature is 12 °C in this area. The samples were obtained from those areas which were irrigated with tube well water and mixed water (fresh and wastewater) in Tehsil Sahiwal of district Sargodha.

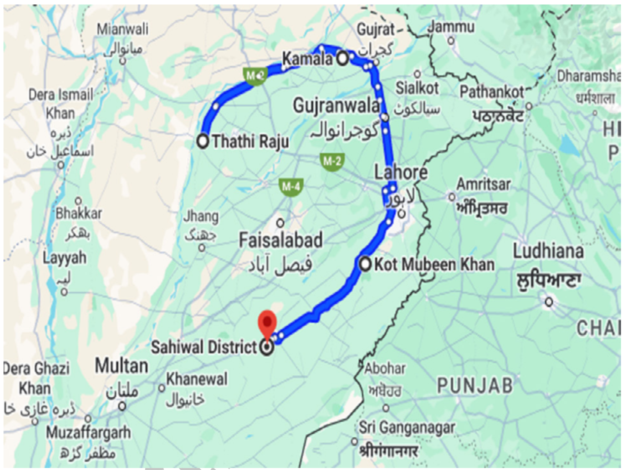

Fig. 1. Map of study area.

Sample collection and preparation

A total of 80 pairs of soil samples were taken from depth of 0-15 cm and their corresponding shoot and grain samples were also taken during harvesting season of rice. Each sampling point was based according to the type of irrigation water. All debris and stones were removed from soil samples and was stored in sampling bags. Soil samples were dried in air for almost 3-5 days and after that in oven for 72 hours at 80°C. At the end these samples were saved for additional digestion methods. All metallic contaminants on rice grains were removed by washing them twice. All rice and soil samples were dried at 65 ºC for 48h in a furnace to maintain constant weight for further analysis. After drying the samples were ground completely into a fine powder and then protected 2 g samples for digestion.

Individuals having age between 5-55 years were selected for blood sampling. All the individuals ages were divided into groups: Group1: 5-15 years (Y), Group 2: 16-25 Y, Group 3: 26-35 Y, Group 4: 36-45 Y and Group 5: 46-55 Y. G All the participants were local residents of study area. Five replicates for each group were taken. About 2g sample was acid-digested with 10 mL of HNO3-H2O2 (2:1) at 140–160°C. The digested solution was filtered by passing through syringe filters (0.45 µm) and the final volume (10 ml) was made by addition of Milli-Q water. The level of heavy metals was measured using CE™ 3400 AAS Atomic Absorption Spectrometer.

Following formulae were used to determine contamination factor (CF), transfer factor (TF), enrichment coefficient (EC) and target hazard quotient (THQ).

$$CF = \frac{\text{metal concentration in sample}}{\text{Background concentration of metal}}$$

$$TF = \frac{\text{Metal concentration in plant mg/kg}}{\text{Metal concentration in soil mg/kg}}$$

$$EF = \frac{\text{Conc. of metal in edible part / Conc. of metal in soil}}{\text{Standard conc. of metal in edible part/ Standard conc. of metals in soil}}$$

EF is defined as retention of metals in soils. Zn and Cu standard concentration was 2.8mg/kg and 9.07 mg/kg in soil (Singh *et al*. 2010) while 20mg/kg and 50mg/kg in rice grains (Satpathy *et al*., 2014).

THQ was used for assessing the non-carcinogenic risks for each individual metal through rice consumption. It is an estimated risk level of pollutant exposure.

$$THQ = \frac{EF \times ED \times FIR \times CM}{BW \times AT \times RfD} \times 10^{-3}$$

EF stands for exposure frequency (365 days per year), ED is for exposure duration (70 years in the study for non-cancer risk). FIR stands for food ingestion rate (g/person/day), and CM for rice's heavy metal content. RfD is for oral reference dose (mg/kg-d) which was 0.300mg/kg for Zn and 0.040mg/kg for Cu (Harmanescu *et al*., 2011); BW stands for mean body weight of consumers; and AT stands for the average time for non-carcinogenic effects (70 years).

Statistical analysis

ANOVA and correlation analysis was applied on the data by using SPSS (Statistical Package for Social Sciences) software, Version 16.0 and graphs are made through prism software 10.0.

RESULTS AND DISCUSSION

Zn and Cu in paddy soil samples

Statistical data showed significant effect of treatments, samples and treatments*samples for Zn and Cu content in the soil samples (Table I). Zinc metal was evaluated in various varieties of rice grown in different treatments like tube well water and mixed water. The mean concentration of Zn metal in paddy soil ranged from 11.43-23.487 mg/kg. The minimum concentration of zinc was detected for kainat (11.43 mg/kg) at tube well water and maximum concentration was detected for super kernel (23.48 mg/kg) at mixed water. The observed Zn order in paddy soil was: kainat<basmati<irri6<super kernel at tubewell water and kainat < irri6< basmati < super kernel at mixed water treatment (Fig. 2). According to Du *et al*. (2018), the Zn content in the soil was reported as 177 mg kg^{-1} which is much greater than in the recent study. The level of Zn in soil suggested by Qin *et al*. (2021) was 16.43 mg/kg which is lower than that of the recent study. Zheng *et al*. (2020) indicated the Zn level was 46.89 mg kg-1 in the paddy soil which is higher than that of present studies. The results of Jiang *et al*. (2020) the concentration of Zn was found to be 86.8 mg kg^{-1} which was higher than recent investigations. Present concentration of Zinc in paddy soil ranged from 15.87-23.4 mg kg^{-1} that was found to be within WHO/FAO (2007) limits. Deficient Zn concentration was a serious problem in paddy soil because it leads to the Zn deficiency in human body (Hussain *et al*. 2022). Chen *et al*. (2022) stated that soil type, dispersal of nitrogen and organic content in soil directly influence the Zn uptake in paddy soil.

Table I: ANOVA analysis in soil, rice and serum samples.

Source of Variance	Treatments	Samples	Treatments* Samples
	1	3	3
Soil			
Zn	418.267***	19.618***	7.379 ***
Cu	213.953***	14.171***	7.912***
Shoot			
Zn	384.101***	8.049***	23.665***
Cu	124.570***	15.607***	6.984***
Grain			
Zn	384.101***	8.049***	23.665***
Cu	21.2678***	1.0782***	0.7180***
Serum			
Zn	0.005926***	0.010723***	0.006648***
Cu	0.000295***	0.003536***	0.006455***

The concentration of copper in soil ranged from 10.524- 20.50 mg/kg. The lowest level of Cu was observed for Kainat (10.52 mg/kg) and its highest level was observed for Basmati (20.50 mg/kg). At Tube well water treatment, the order of concentration was Kainat < Basmati < Irri6< Super kernel. At mixed water treatment, the order of concentration was Kainat < Super kernel < Irri6< Basmati (Figure 3). Concentration of Cu reported by Ahmad *et al*. (2021) in soil was above the permissible limit that was opposite to present findings. Giri and Singh (2017) indicated the level of Cu in soil was 41.4 mg kg-1 which is much greater than current observation. Present Cu in paddy soil ranged from 10.524- 20.50 mg kg^{-1} that was found to be within WHO/FAO (2007) limits.

According to Kaninga (2022), pH factor negatively affect the Cu availability in soil. As the soil pH increased Cu distribution capacity in soil is decreased. Cu also form bonding with available organic matter in soil which also hinders its distribution on soil medium.

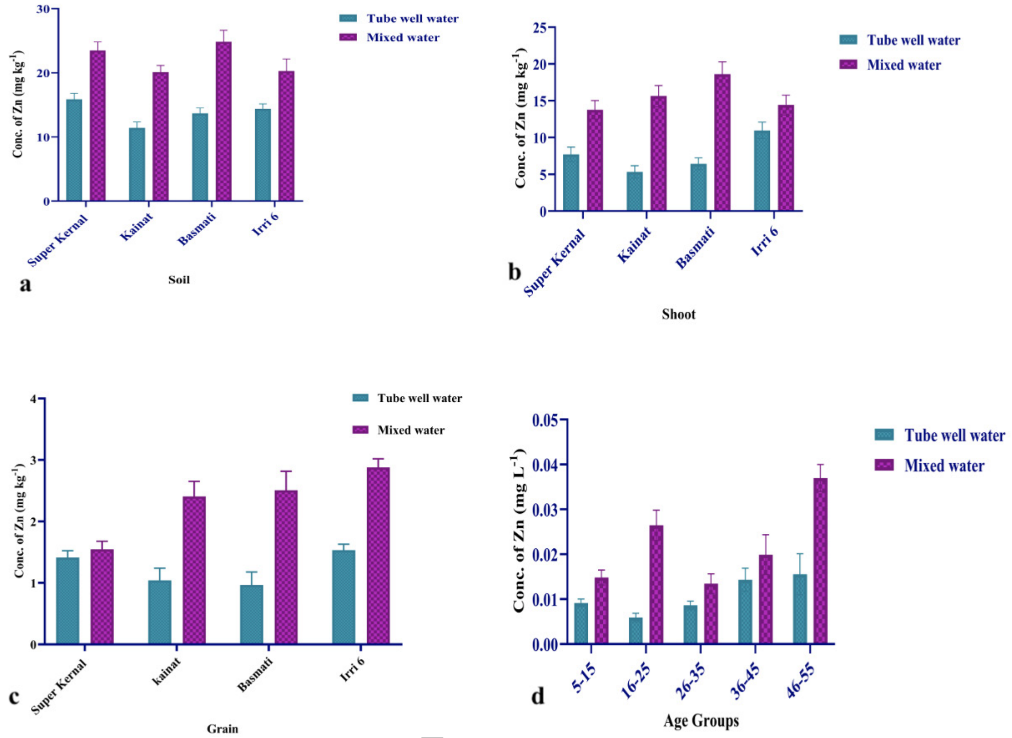

Fig. 2. Zn concentration in soil (a); rice shoot (b); rice grain (c) and human serum samples (d).

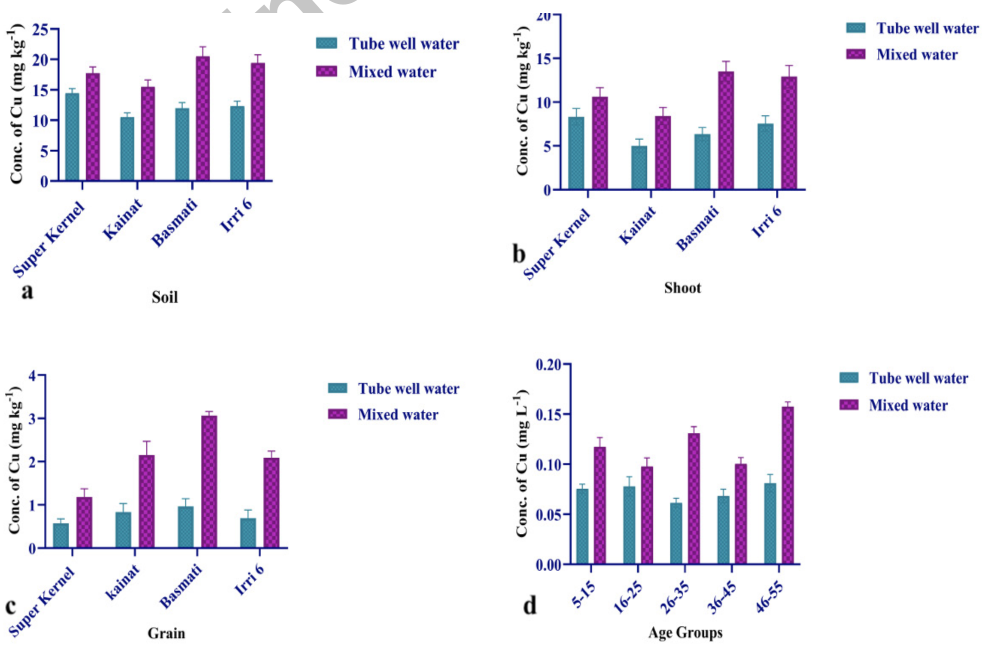

Fig. 3. Cu concentration in soil (a); rice shoot (b); rice grain (c) and human serum samples (d).

Zn and Cu in rice samples

The statistical analysis of data showed significant variation at the level of (p<0.001) along treatments, varieties and treatments*varieties for zinc content in the rice samples (Table I). The concentration of zinc ranged from 5.337-18.62 mg/kg in shoot. Zinc level in shoot was found lowest at T1 for kainat (5.33 mg/kg) and highest values were observed for basmati (18.62mg/kg). The order of Zn concentration in shoot was kainat <basmati < super kernal < irri 6 at tube well water. The order of Zn concentration was super kernal< irri 6< kainat< basmati at mixed water treatment. The concentration of Zn in the grains of different rice varieties was recorded as 0.967-2.87 mg/kg. The minimum values were recorded for basmati (0.967 mg/kg) and maximum were observed at T2 for irri 6 (2.87 m/kg). The order of Zn level in grains was basmati < kainat < super kernal< irri 6 at tube well water. The order followed by different varieties for Zn level at T2 was super kernal < kainat < basmati < irri 6 (Figure 2). Kong *et al*. (2018) recorded the value of Zn was 48 mg/kg which is much higher than recent observations. The previous studies by Zhao *et al*. (2010) indicated the Zn level in rice grain was 2.24 mg/kg which is in accordance with our study. Mamat *et al*. (2020) showed the Zn level in rice grain was 0.345 mg/kg which is lower than current observations. According to Tang *et al*. (2019) the concentration of Zn in rice grain was 1.189 mg/kg which is equal to current results. The previous studies by Abdul Sattar (2021) indicated the level of Zn was 11.23 mg/kg which is much greater than current observations. Present Zn concentration in rice grains was lowered than acceptable Zn limit suggested by WHO/FAO (2007). Nearly 2 billion people were facing Zn deficiency across the world. Hussain *et al*. (2022) stated that Zn fertilization and irrigation management are the main aspect that influence the Zn concentration in rice grains.

The statistical analysis of data showed significant variation at the level of (p<0.001) along treatments, varieties and treatments*varieties for copper content in the shoot (Table 1). Copper level in shoot ranged from 5.014-12.91 mg/kg. The lowest concentration of Cu was found in shoot of kainat (5.014 mg/kg) and highest values were observed for basmati (13.516 mg/kg). The level of Cu in grains samples obtained from various sites was ranged from 0.575 mg/kg to 3.066 mg/kg. At tube well water, the values ranged from 0.575 mg/kg to 0.965 mg/kg in grains of rice. The minimum values were observed for super kernal (0.575mg/kg) and maximum values were observed for variety basmati (0.965 mg/kg). At mixed water, the values ranged from 1.183 to 3.0660 mg/L. The minimum observed in super kernal and maximum value is found in basmati. The order of concentration of copper was super kernal <irri 6 < kainat < basmati at tubewell water the order of concentration observed at mixed water was super kernal < irri 6 < kainat < basmati (Fig. 3). Various shoot samples of different rice varieties using tube well water and mixed water as source of irrigation were determined for Cu level in them. The mean level of Cu varied from 5.014mg/kg- 13.516 mg/kg. Du *et al*. (2018) suggested the Cu content in Shoot of rice was 3.69 mg kg-1 which is lower than current investigation. This concentration of Cu was according to the results of Mahfooz *et al*. (2020) whose reported 1.19-2.29 mg/kg of Cu content. Low Cu content in the rice grains was observed by Xiao *et al*. (2017). Compared with the reports by Sharma *et al*. (2018) these observations were low. The level of Cu in the rice grain observed by Abualhaija (2023) was 2.49 which are comparable with the recent results. Level of Cu indicated by the Guadie *et al*. (2021) was found to be in accordance with current results. The level of Cu observed by Adewumi and Lawal (2022) was 0.25 mg/kg which is lower than current observation. Observed Cu range was lower than WHO/FAO (2007) limits that is 40 mg/kg. Results presented that there is low availability of copper metal in soil as well as low uptake by rice varieties.

Heavy metals in serum samples

The statistical analysis of data showed significant variation at the level of (p<0.001) along treatments, serum and treatments*serum for zinc content in serum (Table 1). The concentration of zinc in human serum ranged from 0.015-0.036 mg/L. At tube well water, minimum zinc concentration in serum was found in age group of 16-25 (0.0058 mg/L) and maximum value for age group of 46-55 (0.015 mg/L). At mixed water, the minimum values were observed for 5-15 (0.016 mg/L) and maximum values for age group between 46-55. The order of zinc level was 16-25 < 26-35 < 5-15 < 36-45 <46-55 at tube well water. The order of zinc level was 26-35 < 5-15 < 36-45 < 16-25 <46-55 at mixed water treatment (Fig. 2). Begoña-Zubero *et al*. (2023) evaluated the Zn content in the serum of Spanish women which were much greater than present observations. Our observed Zn range was much lower than results reported by Mashhadi *et al*. (2017). Relatively, higher Zn content in serum ((0.43-1.39mg/L) was observed by Markiewicz-Żukowska *et al*. (2015). Zn deficiency negatively affect the human immune system. Age, sexual category and area location directly influence the Zn level in human body. It also stated that low Zn level damage the mental health (Khan *et al*. 2023).

The statistical analysis of data showed significant variation at the level (p<0.001) along treatments, serum and treatments*serum for copper content in serum (Table 1). At tube well water treatment, maximum values were observed as 0.08106 mg/L in 46-55 age group and lowest value at the same treatment site was 0.06149 mg/L for age group of 26-35 year. The Cu order in blood was 26-35<36-45 < 5-15 <16-25 <46-55 at this site. At mixed water treatment, the maximum values were observed as 0.1575 mg/L for age group of 46-55 and minimum 0.097 mg/L for age group of 16-25. The order of level of Cu in serum was 16-25 <36-45 < 5-15 <26-35 <46-55 at mixed water site (Fig. 3). Our Cu range in human serum was lower than findings of Al-Ansari *et al*. (2020) in Iraq. Milde *et al*. (2001) observed the mean Cu level in human serum as 0.95 that was much higher than present results. Present results show consistency with Toro-Román *et al*. (2021). To observe the intake of Cu via rice consumption in the study area, the serum of human blood of various age groups was analyzed for Cu level. Cu mainly accumulated in the soft tissues of body, and its deficiency lead to muscle fatigue, stress as well as impaired central nervous system. It also helps in supply of Ca ion to bones (An *et al*. 2022).

Correlations between soil-shoot, shoot-grain, grain-serum samples

All correlations between soil-shoot, shoot-grain, grain-serum in tube well water and mixed water were non significant. Positive correlation was observed at both sites between soil and shoot. for shoot and grain a positive and non significant correlation was observed at tube well water and positive correlation was found at mixed water. For grain and serum, positive correlation was observed at both sites. all correlations between soil-shoot, shoot-grain, grain-serum in tube well water and mixed water were non significant. Positive correlation was observed between soil and shoot for both sites. For shoot and grain a negative correlation was observed of about at tube well water and positive correlation was found at mixed water. For grain and serum negative correlation for tube well and positive non significant correlation was observed for mixed water treatment (Table II). Khan *et al*. (2021) resulted the positive correlation between soil, shoot and grain for both Zn and Cu, which showed consistency with this study, but their significant variation was opposite to this study. Similar results to this study were also reported by Xie *et al*. (2011). In contrast, a weak negative correlation between Cu and Zn was observed by Maares *et al*. (2023). Noor *et al*. (2024) also found positive correlation for Zn in soil to grains. It also stated that limited Zn concentration in rhizosphere also produces a thrust for Zn uptake in plants that's why they modified their root system to efficiently absorb the nutrients during stress conditions.

Table II: Zn and Cu correlations among soil-shoot, shoot-grain, grain-serum for different treatments.

Treatment	Soil-shoot	Shoot-grain	Grain- Serum
Zn			
Tube well water	0.574^{ns}	0.876^{ns}	0.790^{ns}
Mixed water	0.519^{ns}	0.364^{ns}	0.339^{ns}
Cu			
Tube well water	0.942^{ns}	-0.754^{ns}	-0.555^{ns}
Mixed water	0.994**	0.481^{ns}	0.356^{ns}

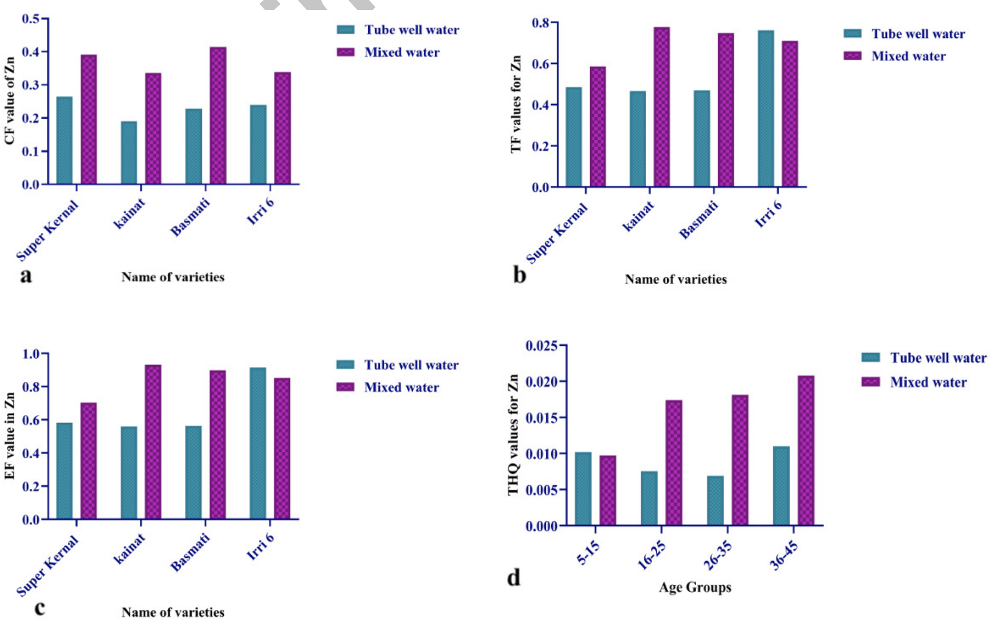

Fig. 4. Contamination factor (a); transfer factor (b); enrichment factor (c); target hazard quotient (d) of Zn in four varieties of rice irrigated with tube well water and mixed water.

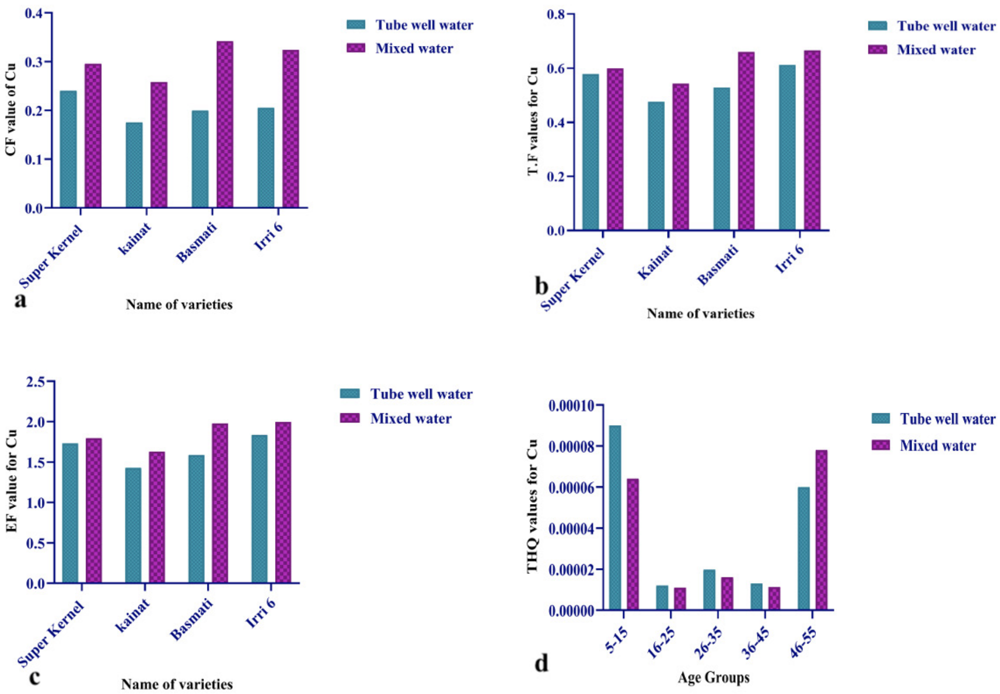

Fig. 5. Contamination factor (a); transfer factor (b); enrichment factor (c); target hazard quotient (d) of Cu in four varieties of rice irrigated with tube well water and mixed water.

Contamination factor

Contamination factor (CF) of the Zn ranged from 0.190-0.414 mg/kg. After irrigation with tube well water, minimum zinc contamination was found in kainat and maximum value in super kernal. After mixed water irrigation, the minimum values were observed in basmati and maximum values in kainat. The order of zinc level was: kainat < basmati < irri 6 < super kernal at tube well water. The order of zinc level was basmati < super kernal < irri 6 < kainat (Fig. 4). Mohammadi *et al*. (2019) reported CF 3.679 which is higher than current observations. Mahfooz *et al*. (2020) evaluated the CF 0.467 which is in accordance with recent studies. Sukri *et al*. (2018) evaluated the Zn level 0.19 which suggest the equality with current indications. Zhiyuan *et al*. (2014) observed the CF 1.23 which is higher than current study results. According to Kaur and Garg (2017) the CF of Zn was 3.45 which is greater than present outcomes. In both water sources, the CF values remained lower than 1 for all rice varieties, suggesting that the rice had lower contaminant concentrations compared to the respective water sources.

CF showed the Cu content between 0.175-0.345 mg/kg for various rice varieties. After tube well water irrigation, the values ranged from 0.175 mg/kg (kainat) to 0.2403 mg/kg (super kernal). The order of C.F values observed was kainat < basmati < irri 6 < super kernal after tube well water irrigation,. At mixed water treatment, highest C.F was observed in Basmati and minimum value was found in kainat. The order of C.F values were; kainat < irri 6 < super kernal < basmati at mixed water treatment (Fig. 5). CF of soil showed the Cu content of 0.175-0.345 for various rice varieties. The results given by Oubane *et al*. (2021) for Cu metal indicate the higher contamination compared with recent outcomes. Comparatively high CF was observed by Gemeda *et al*. (2021). Mahmood *et al*. (2020) noted that CF was recorded as 0.094 which was lower than current observations. Xiao *et al*. (2017) suggested that CF of current study was 0.321 which is comparable to the present study. Relatively low CF of 0.04 was observed by Ye *et al*. (2014). The results of present study were comparable to the investigations by Zeng *et al*. (2021). In both water sources, the Cu CF values remained lower than 1 representing the safer Cu level in soil.

Transfer factor

Zn TF ranged from 0.466-0.777 mg/kg. After tube well water irrigation, minimum zinc contamination was found in kainat and maximum value for irri 6. At mixed water, the minimum values were observed for super kernal and maximum values for kainat. The order of zinc level was: kainat < basmati < super kernal < irri 6 at tube well water (Fig. 4). The order of zinc level was super kernal

< basmati < irri 6 < kainat. Kang *et al*. (2020) recorded the TF of Zn was 1.19 which is higher than results of this study. According to Fan *et al*. (2017) the TF was observed as 0.067 which is lower than current findings. Li *et al*. (2012) indicated that the TF was almost equal to the present study results. The transfer factors indicate the extent of contaminant movement from soil medium to rice grains. The results demonstrated that all rice varieties are safe for human consumption.

Cu transfer from soil to grain was observed as 0.476-0.668mg/kg. In tube well water irrigation, the highest TF of copper was found to be in irri 6 and lowest of values were observed in kainat. The order of level of TF was: kainat < basmati < super kernal < irri 6 at tube well water. For mixed water, the highest values were observed for irri 6 and lowest was found in kainat. The order of level of T.F for copper was kainat < super kernal < basmati < irri 6 (Fig. 5). The level of risk reported by Abdulwahid (2023) was much higher than current investigations. According to the study by Liu *et al*. (2013) TF level was 1.33 for Cu which is higher than current study results. TF observed by Rezapour *et al*. (2019) was 0.278 which is lower than current observations. All Cu values were lower than 1 indicating that all rice varieties are not Cu accumulator.

Enrichment factor (EF)

Cu enrichment range was observed as 0.559-0.932mg/kg. In tube well water irrigation, the highest EF of copper was found to be in Irri 6 and lowest of values were observed in kainat. The order of level of Cu was; irri 6 < super kernal < basmati < kainat. for mixed water, the highest values were observed for kainat and lowest was found in super kernal. The EF order of level for copper was super kernal < irri 6 < basmati < kainat (Fig. 4). EF values noted by Afonne and Ifediba (2020) was indicated as 0.245 which is lower than current results. According to Wei *et al*. (2019) the level of EF was observed as 1.34 which is slightly higher than current findings. Previous studies by Karizaki (2016) showed the EF of 0.34 which shows the consistency of results with current observation. In terms of pollutant concentration, these enrichment variables shed light on how different rice cultivars interacted with metal enrichment in soil.

EF was evaluated as 1.428 to 1.995 mg/kg for Cu metal. For tube well water, the highest values were observed for irri 6 and lowest values were observed in kainat. The order of level of EF was observed as kainat< basmati< super kernal < irri 6 at tube well water. For mixed water, the highest values were observed in basmati and lowest values were observed for kainat. The order of level of E.F was observed as kainat< super kernal< basmati< irri 6 at mixed water treatment (Fig. 5). These values showed the variation from the results given by Ahmed at al. (2021). The values of EF recorded by Chandran *et al*. (2012) were 0.675 which is comparatively less than current findings. Novotná *et al*. (2015) investigated the EF for Cu was 4.098 which is higher than recent study results. According to Abdul Sattar (2021), if EF values lies between 1-2, then there will be minor metal enrichment. Present results suggested minor enrichment of Cu in studied area.

Target hazard quotient (THQ)

THQ range for Zn was 0.0021-0.0207 at both sites. At tube well water, minimum Zn value was observed in 26-35 age while maximum value found in 36-45. At mixed water treatment, highest THQ observed for 36-45 age group while lowest THQ is in 46-55 age group (Fig. 4). The THQ level of the Zn was recorded as 0.021 by Zheng *et al*. (2017) which greater than current observations. THQ level investigated by El-Kady and Abdel-Wahhab (2018) is consistent with current observations. Abualhaija (2023) recorded the THQ level of 0.12 for Zn in various age groups which is much higher than recent findings. THQ was calculated to evaluate the potential health risk. The calculated THQ of this study remained consistently below than 1 for all age groups representing the no potential health risks for Zn toxicity in human on rice consumption.

THQ was investigated to evaluate the health risk of rice consumption. THQ range for Cu was 0.00006-0.00078 at both sites. At tube well water, the minimum value was observed for age group 16-25 and maximum values were observed for age group of 46-55. The THQ values observed at mixed water were found to be in maximum at age of 46-55 and minimum at 5-15 age group. The order of level of Copper at T1 was followed as 16-25<46-55<36-45<5-15<26-35. The order followed by T2 was 5-15<16-25<<36-45<26-35<46-55 (Fig. 5). The study by Navaretnam *et al*. (2023) showed a value of 0.279 which is much greater than present findings. THQ level investigated by Ajah *et al*. (2022) is consistent with current observations. Hamid *et al*. (2020) recorded the THQ level for Cu in rice varieties as 0.66-0.89 which are much higher than recent findings. The results indicated the values recorded below 1 which indicates less potential risk for public.

CONCLUSION

Present results concluded that Zn and Cu concentrations were significantly increased in waste water treatment as compared to tubewell water. Basmati and irri 6 varieties presented the highest Zn and Cu level in their grains. Analyzed Zn (0.967-2.87 mg/kg) and Cu (0.575-3.066 mg/kg) concentration in all rice grains lie within

WHO/FAO limits. CF>1 showed higher Zn contamination in the study area as compared to Cu metal. Zn (0.559-0.932) and Cu (1.428 to 1.995) range showed the minimal enrichment in soil. As all THQ values were lower than 1 in all age groups, so there was no potential carcinogenic risk to local consumers. In conclusion, there is no significant health risk observed for humans on consuming rice varieties from this area. Government should organize strategies to regularly monitored the metal level in rice grains to ensure the metal level at harmless level.

DECLARATIONS

Ethical approval

All authors declared that manuscript has not been published previously.

Ethical statement

The institutional Human Ethics Committee of University of Sargodha (Approval No.25-A18 IEC UOS) has allowed all the protocols used in this experiment. All the experimental methods of this study have followed all the appropriate guidance and regulations including NRC standards.

Availability of data and materials

This published article contain all the generated data of this study.

Conflict of interest

All authors declared that they have no conflict of interest.

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