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Exploring the Impact of Different Irrigation and Fertilizer Applications on Zinc (Zn) Accumulation in Citrus Fruits in Semi-arid Environment

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Article Info

Abstract

This study aimed to assess the concentration of Zinc (Zn) in the edible parts of *Citrus sinensis, Citrus reticulata*, and *Citrus limetta* irrigated with sewage water and canal water along with different application of fertilizers. Plant, water, and soil samples were randomly chosen from two different locations for metal analysis. Water samples containing Zn contents vary significantly between sites, ranging from 0.501 to 0.737 mg/L. The amounts of Zn in fruit samples ranged from 0.208 to 1.607 mg/kg, where the concentrations of Zn in soil varied between 0.208 to 1.607 mg/kg. The Contamination Factor (CF) values was higher in sewage water as compared to canal water while Transfer Factor showcasing maximum values in *C. limetta and* minimum values in *C. reticulata*. hazard quotient value of Zn was ranged from 0.0215 to 0.1661 in Citrus fruits. Consuming citrus cultivated on soil contaminated with Zinc was dangerous. The Zn moves from water to soil, then to fruits, and finally is consumed by humans. Our findings indicated that the presence of Zinc in soil lowers the effectiveness of fertilizers and has health concerns for people. Ultimately, this research offers an extensive evaluation of the levels of Zn in fruit, soil, and water samples, emphasizing possible health hazards and ecological consequences. The results highlight the necessity of focused agricultural methods and water resource management in order to reduce citrus crop stress caused by Zn, guaranteeing environmentally friendly farming methods.

Keywords: Heavy Metal, Toxicity, Sewage Water, Health Risk

Introduction

Citrus, along with its associated goods and byproducts, may play a part in creating jobs that will boost the profitability of the Pakistani citrus industry. Because the output of citrus in Pakistan totals 2.89 million metric tons, exporting citrus is a significant source of foreign cash. Punjab province is a major contributor to more than 95% of this production from the citrus-growing regions of Pakistan. Citrus greening disease (CGD), despite Pakistan being one of the top 16 countries that produce citrus, has had an impact on its output (Sajid *et al*., 2022) [30].

The majority of heavy metals are rare elements that naturally occur in soils; they are mostly found in relatively high quantities in specific minerals. They primarily exist in forms that are difficult for living things to access. Some of these elements are needed by living cells in trace amounts for normal metabolism, although they may be sensitive to higher concentrations. Their abrupt release, which frequently takes the form of a biologically accessible substance, has the potential to threaten human health as well as harm natural and artificial ecosystems (Tyler, 2021) [34].

The capability of metal ions to move inside the plant's food source is known as the bio-concentration factor (CF). Trace metal pollution causes metal toxicity in humans and is a result of soil contamination as measured by the Enrichment Factor (EF) (Ghosh *et al*., 2021) [12].

Pakistan canal system is the largest in whole world. The Indus River's water is one of many that are kept and diverted for irrigation and hydropower. Water for irrigation is dispersed across broad plains in the Punjab and Sindh provinces by channels then distributaries that come since several dams. The results show that Pakistan is not water secure despite having access to such important resources when evaluating public, financial, and ecological welfares (Yu *et al*., 2022) [37].

Modern agriculture mainly relies on the use of inorganic fertilizers, which not only render the soil barren but also negatively affect the stability and sustainability of the ecosystem. The research trial was set up to determine whether the use of bio-fertilizers and both inorganic and organic nitrogen sources had a substantial impact on plant growth, production, quality, and nutrient content of both plants and fruits (Bakshi & Wali, 2019) [7].

The use of fertilizers not only boosts crop productivity but also changes the physicochemical and biological characteristics of the soil. However, regular use of chemical fertilizers is to blame for the loss in agricultural soil quality as well as the SOM (soil organic matter) content. (Pahalvi *et al*., 2021) [26].

Zn is a crucial metal that play role in cell development. Skin sores, sluggish development, dark issues, cuts, and injuries take longer to heal, but these symptoms heal more quickly after taking zinc supplements and eating meals enriched with zinc (Mason, 2016) [21]. Zn is more commonly applied to leaves than to soil. Zinc can significantly improve the fruiting and flowering characteristics of limes. Foliar application is unquestionably the best way to avoid the issues with nutrient availability. Because citrus trees are deeply rooted plants, adding micronutrients to the soil may not be of much use. The alternative method is to use foliar spray to deliver micronutrients (Lin *et al*., 2022) [19].

Micronutrients are crucial for plant and zinc (Zn) is one of them. It is a crucial cofactor for numerous enzymes involved in protein synthesis and nitrogen metabolism as well as major player other plant photosynthesis redox processes. Zn is advantageous in a small range of doses, much like other plant micronutrients and in soil its presence rises at less pH. Zn concentrations are typically below 125ppm in unpolluted soils (Song *et al*., 2019) [33].

Vital metal called zinc (Zn) is necessary for cell growth and division as well as the body's defense mechanisms. Cuts and injuries take longer to heal completely due to hypogonadism, having a weak appetite, having problems with taste and smell, delayed growth, dark pigmentation, skin sores, and other factors. These symptoms and signs immediately go away after ingesting meals or supplements high in zinc (Mason, 2016) [21]. Zn mostly travels from roots to fruit pulp. Although various health issues could result from a zinc deficit, fruit pulps contain a large amount of zinc that is good for human health (Rahimi *et al*., 2017) [28].

Materials and Methods
Area of Research
Punjab province of Pakistan is where Sargodha is found. Punjab's Sargodha is located at latitude of 32.08246 and a longitude of 72.66912.GPS coordinates for Sargodha in Pakistan is 32°4′56″N and 72°4′8″E. The Sargodha district spans 5,854 square kilometers. Northwest of Lahore City is the Sargodha District, which is located 187 kilometers away (116 miles). It is a commercial and agricultural trading hub with many different businesses. The third largest division in the Punjab province is Sargodha.

In Sargodha, the summers are oppressively hot, humid, and clear, while the short, dry, chilly winters are normally clear. Throughout the year, temperatures normally range from 42 to 105 degrees Fahrenheit, with only very unusual dips below 37 or ascents beyond 113 degrees. Most of Sargodha's blessings are attributed to agriculture. Sargodha is referred to as the "California of Pakistan" since it is the best citrus-producing region in the country.

Sample collection
We randomly selected the soil, water, and plant samples from two distinct locations. Samples were taken from Bhalwal (irrigated with fresh water and treated with three different types of fertilizers, CW-T1 (treated with organic fertilizer), CW-T2 (treated with inorganic fertilizer), and CW-T3 (treated with combined fertilizer)) and Ratto Kala. (Irrigated with sewage water and treated with three distinct types of fertilizers, designated as SW-T1 (treated with organic fertilizer), SW-T2 (treated with inorganic fertilizer), and SW-T3 (treated with combined fertilizer). From the chosen locations we collected the soil samples and kept in plastic bags. 500 mL plastic containers were used at each location to collect water samples. The water samples were then filtered and chilled before being tested for metals (Hassan & Basahi, 2013) [4]. Three copies of each sample are used. Each sample was selected at random, put the samples in envelope, labeled, and carried to the lab used for additional study.

Preparation of samples
We collected soil samples from various sites, allowed them to air dry, and then baked them for three days at 72 °C. After being removed from the oven and dried by air until no moisture was left, the samples were weighed with an electronic scale. The wet acid digestion technique was used to digest the materials. From two different locations, water samples were taken. The samples were packaged in bottles, and the acid digestion method was used to process them. A total of two separate locations yielded fruit samples that could be consumed. Firstly, placed them at 72 °C for 48 hours an electric oven to remove any remaining moisture content, air dried the samples. The samples were reduced to a powder, stored in desiccators at room temperature. The materials were broken down by wet acid digestion.

Digestion of samples by Wet Acid Digestion Method
For the metal analysis, only fruit parts that could be eaten were used. Fruit, water, and soil samples were all digested in

an acidic solution to make sure that they were completely solubilized. A 10 mL solution of concentrated HNO_3 (65%) was added to water sample of 1 mL and 1 g of the powdered samples of soil and fruit, and the mixture was kept undisturbed overnight. Once evaporation began, the mixture was heated on an electric burner for a further 70 °C (Abbasi et al., 2016) [23]. 5 mL of undiluted $HClO_4$ (70%) was added only after the liquid had reached room temperature. After that, the mixture was heated to 70 °C until the digestion was finished and thick, white vapors started to form. The samples were then filtered via Whatman paper # 42. According to Parveen et al. (2020) [27] the digested samples were placed in a 50 mL flask and filled with 0.1 HNO3 to the required level.

Standard Preparation
Atomic absorption spectrophotometer was used for Zinc analysis on water, soil, and fruit sample. Standard solutions were created in order to set specified curves.

Method of Standard Solution Preparation
Glassware, a funnel, and a flask were rinsed with distilled water. Analytical weight balance was employed to weigh the samples. Any solvent or water in modest quantities was used to dissolve the samples. Before moving on to the next step, it was ensured that the samples were completely dissolved. The samples were then moved to a heated plate to measure the rate of dissolution. Quantitatively adding the solution ensured that the volumetric flask received the dissolved samples.
In a volumetric flask that was below the 100 ml level, a few milliliters of water were added, followed by drop wise additions of deionized water. Hold the cover tightly while giving the flask several good shakes to ensure proper mixing. Verify that the solution has been thoroughly homogenized and that the absorption is uniform. In order to confirm that the solution's concentration is identical to its bottom and precisely on the 100 ml mark, check the meniscus once more, if necessary.

Analysis of Zinc
To analyze the zinc metal content of the samples atomic absorption spectrophotometer was used. In the atomic absorption principal, the wavelength Zn is 213.8 mm, while the slit width is 0.2 and lamp current is 2 (Chiroma et al., 2014) [9]. The permissible limits of zinc in water, soil and fruits are 5 mg L^{-1}, 250 $mgkg^{-1}$ and 100 $mgkg^{-1}$ respectively (Griffiths et al., 2012) [13].

Statistical Analysis of Data
A statistical analysis was done on the data collected from the water, fruits, and soil samples using the Atomic Absorption Spectrophotometer. SPSS 23 was utilized as the statistical analysis tool.

Contamination Factor (Cf)
The contaminant factor (Cf) is used to determine the degree of pollution in the sediment based on the concentrations of the pollutants in the sample and their previous concentration describes the amount of metal in the sediment (Haris et al., 2017) [14]. Metal Reference Values of soil in zinc is 44.19 $mgkg^{-1}$ (Hassan & Basahi, 2013) [4].
The C_f is calculated as follows:
"C_m sample' describe the amount of metal in the sediment"
"C_m background" is the metal content of a naturally occurring source.

$$CF = C_m \text{ Samples}/C_m \text{ Background}$$

Transfer Factor (TF)
The Transfer Factor (TF) indicates the number of channels that metals may reach soils through, including runoff from roads and industry (Gall et al., 2015) [10].

$$TF = C_{plant}/C_{soil}$$

Metals in the plant at their highest concentration (fresh weight)
Total Metal Concentration in Soil (Dry Weight)

Enrichment Factor (EF)
Barbieri et al. (2015) [8] introduced the soil EF as a metric to measure the number of heavy metals deposited in soil. Standard Concentration of zinc in soil and fruits are 44.9 and 60 respectively ($mgkg^{-1}$) (Singh et al., 2010) [32].

$$EF = \frac{(Metal\ Value\ in\ fruits/Metal\ value\ in\ soil)Sample}{(Metal\ Value\ in\ fruits/Metal\ value\ in\ soil)Standard}$$

Estimated Daily Intake (EDI)
A calculation of the highest daily intake of substance from food that doesn't pose major risks to health over the course of a period.
The EDI in ($mgkg^{-1}day^{-1}$) is expressed as the formula (**Amarh et al., 2023**) [5]

$$EDI = \frac{C \times DI \times C.F}{BW}$$

C stand for Metal conc. In mg/kg, DI stand for daily intake the value of DI is 31.5g/person/day, C.F stand for conversion factor the value of C.F is 0.085 (WHO, 2011) and BW stand for reference values of metal such as zinc the value of BW is 70 kg (Chiroma et al., 2014) [9].

Hazard Quotient (HQ)
The hazard quotient is the ratio between a substance's potential exposure and the concentration at which no negative effects are anticipated. Oral Reference Dose of zinc is 0.37 ($mgkg^{-1}days^{-1}$) (USPEA,2011).

$$HQ = \frac{EDI}{RfD}$$

"EDI" stands for Estimated Daily Intake of metals (mg $kg^{-1}day^{-1}$).
Metals' "RID" (mg/kg/day) reference dosage is used ($mgkg^{-1}days^{-1}$)

Result and Discussion
Concentration of Zn (mgL^{-1}) in water samples
The ANOVA showed significant effect (0.001>p) on Zn in water (Table:1). Our values show that Zn in water varied between 0.501 to0.737 mgL^{-1}. In this study, highest level of Zn (0.737 mgL^{-1}) in water samples was found at SW-T2 in C. sinensis and lowest (0.501 mgL^{-1}) concentration of Zn was detected at CW-T1 in C. reticulata (Tables:2) overall the higher values are present in SW rather than CW (Fig.1).

Concentration of Zn (mg/kg) in soil samples
The outcome from ANOVA exhibited that they were significantly affected (0.001>P) on Zn concentration in soil (Table:1). Findings exhibited that Zn concentrations were higher in SW soil than those determined in CW soil (Table:3). The concentration of Zn in soil was 0.516 to 0.976 mgkg^{-1} at CW. The absorption of Zn in soil samples were 0.604 to 2.767mgkg^{-1} at SW (Fig.2). the highest Zn concentration was present in *C. limetta* at SW-T2 site while the lowest value of Zn concentration was present in *C. sinensis* at CW-T1 site.

Concentration of Zn (mgkg^{-1}) in *C. limetta*, *C. sinensis* and *C. reticulata*
The findings from ANOVA revealed that were significantly affected (0.001>P), on Zn concentration in fruits (Table:1). Average value of Zn was noticed in range of (0.208 to 1.607 mgkg^{-1}) in fruits. The lowest value was found in *C. reticulata* at CW-T1. Highest concentration of Zn was observed in *C. limetta* at SW-T2 (Fig.3).

Table 1: Analysis of Variance Data for Zn in water, soil and fruit

Analysis of Variance		Water	Soil	Fruit
Source of Variation	Degree of freedom	Mean square	Mean square	Mean square
Sites	1	0.251604***	28.7547***	9.56091***
Treatments	2	0.029495***	2.0320***	0.77164***
Plants	2	0.001399***	0.1189***	0.12094***
Sites*Treatments	2	0.001931***	0.2741***	0.14032***
Sites*Plants	2	0.000710***	0.1028***	0.06563***
Treatments*Plants	4	0.000091***	0.0161***	0.00701***
Sites*Treatments*Plants	4	0.000182***	0.0179***	0.00556***
Error	36	0.000000	0.0000	0.0000
Total	53			

Table 2: Zn Concentration in water (Mean±SEM) in different sites

Plants	CW			SW		
	T1	T2	T3	T1	T2	T3
C. limetta	0.502±0.010	0.553±0.010	0.518±0.20	0.609±0.020	0.707±0.030	0.657±0.030
C. sinensis	0.503±0.011	0.562±0.011	0.526±0.030	0.634±0.020	0.737±0.030	0.672±0.030
C. reticulata	0.501±0.010	0.572±0.020	0.508±0.020	0.604±0.030	0.703±0.030	0.652±0.040
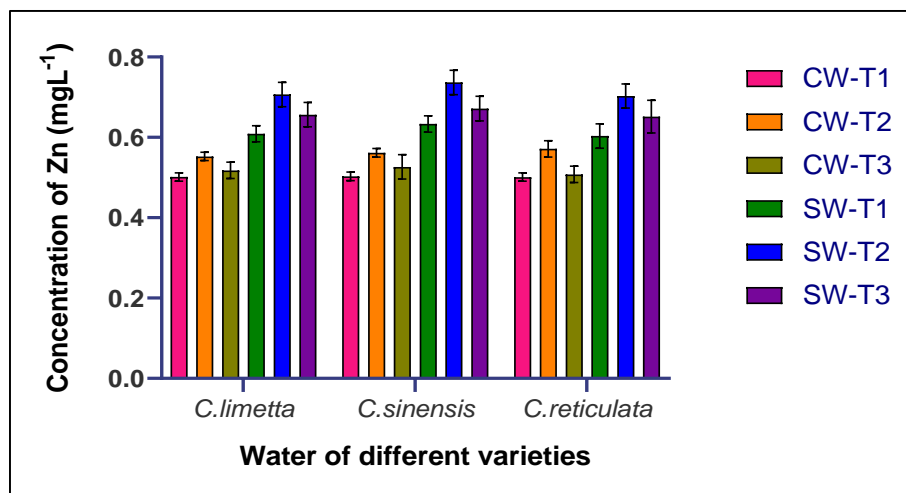
Fig 1: Variation in the levels of Zn (mg/L) in water samples at different sites

Table 3: Zn Concentration in Soil (Mean±SEM) in different sites of Sargodha

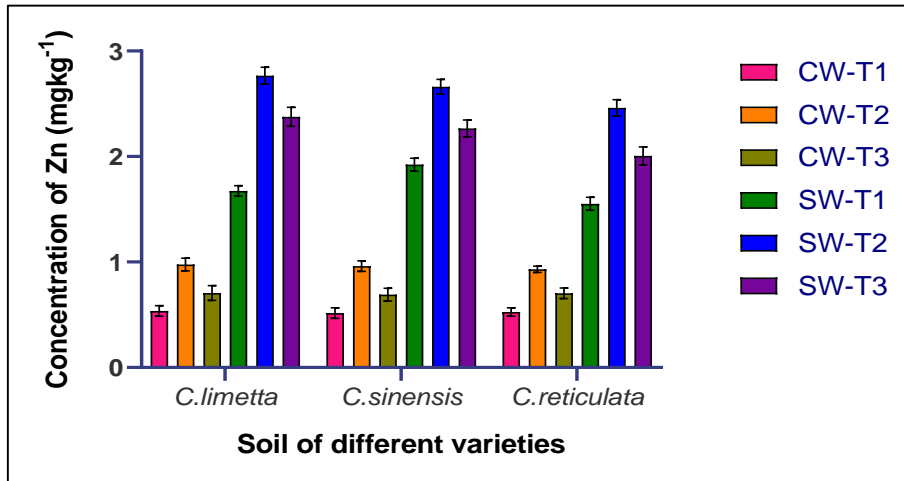| Plants	CW			SW		
	T1	T2	T3	T1	T2	T3
C. limetta	0.536±0.050	0.976±0.060	0.706±0.070	1.673±00.050	2.767±0.080	2.376±0.090
C. sinensis	0.516±0.050	0.961±0.050	0.691±0.060	1.922±00.060	2.661±0.070	2.266±0.080
C. reticulata	0.526±0.041	0.932±0.030	0.704±0.050	1.552±0.060	2.462±0.076	2.006±0.085

Fig 2: Variation in levels of Zn (mg/kg) present in soil samples at selected sites

Table 4: Zn Concentration in Fruits (Mean±SEM) in different sites of Sargodha

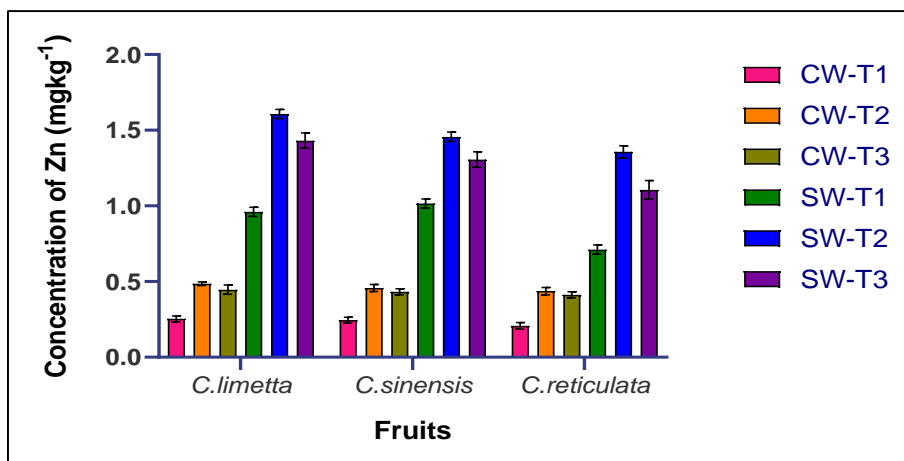| Plants	CW			SW		
	T1	T2	T3	T1	T2	T3
C. limetta	0.254±0.019	0.486±0.011	0.446±0.030	0.961±0.030	1.607±0.030	1.431±0.05
C. sinensis	0.246±0.019	0.458±0.023	0.432±0.020	1.017±0.030	1.456±0.030	1.306±.050
C. reticulata	0.208±0.021	0.437±0.023	0.412±0.020	0.712±0.030	1.357±0040	1.10±0.060

Fig 3: Variation in levels of Zn (mg/kg) in citrus fruits

Contamination Factor

Contamination factor of zinc concentration in samples collected from two separate CF content of Zn (0.0616) in *C. limetta* was higher at SW-T2 as compared to other samples. While, low concentration for Zn (0.0115) was determined in *C. sinensis* at CW-T1 (Fig. 4).
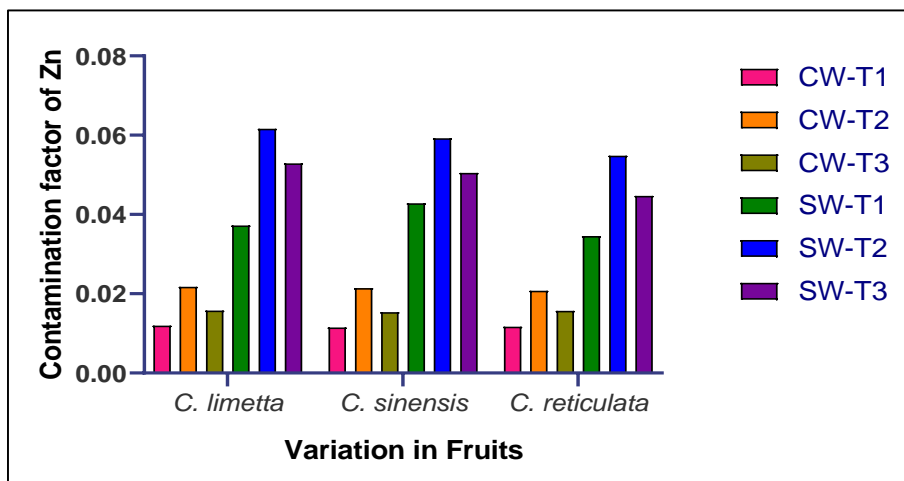

Fig 4: Variation in values of Contamination Factor for Zn

Estimated Daily Intake

Zn metal EDI showed an upper peak concentration in *C. limetta* (0.0615) at SW-T2 and lower values were observed in *C. reticulata* (0.0079) at CW-T1. The sequence of EDI in *C. limetta, C. sinensis* and *C. reticulata* is SW>CW (Fig. 5).
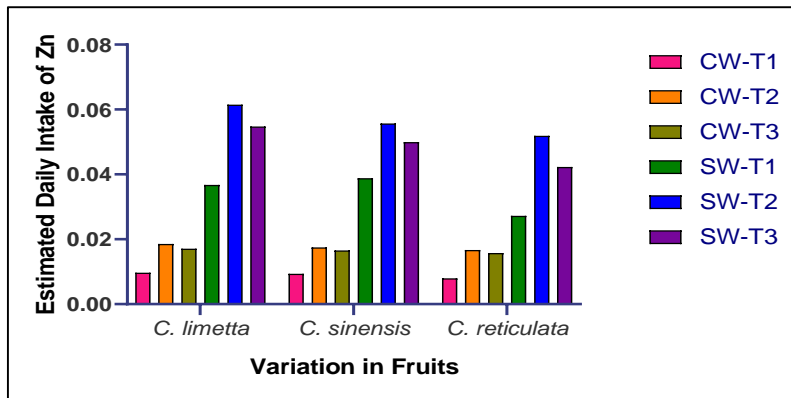

Fig 5: Variation in Estimated Daily intake for Zn

Hazard Quotient

Maximum values of HQ were found in *C. limetta* (0.1661) at SW-T2, while the lowest values were found in *C. reticulata* (0.0215) at CW-T1. *C. limetta, C. sinensis* and *C. reticulata* showed the order for HQ as SW>FW (Fig. 6).
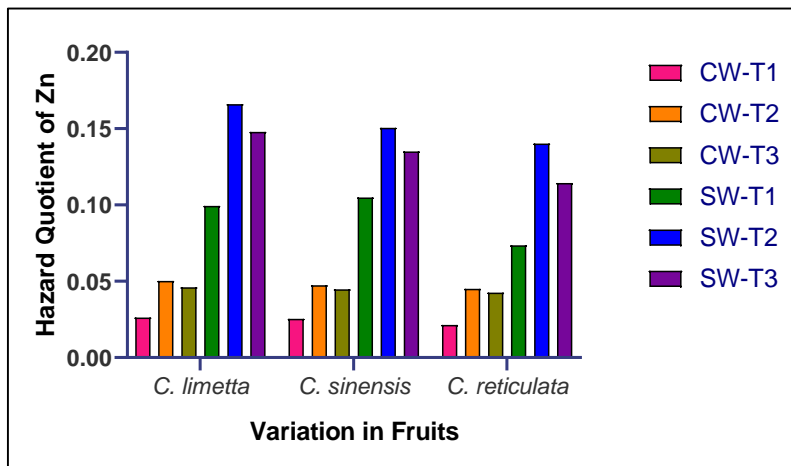

Fig 6: Variation in Hazard Quotient for Zn

Transfer Factor

The concentration of TF ranged from 0.3947 to 0.6319. The maximum TF of Zn metal was observed in *C. limetta* i.e., 0.6319 at CW-T3 site and TF of Zn was in *C. reticulata* at site CW-T1 i.e., 0.3947 (Fig. 7).
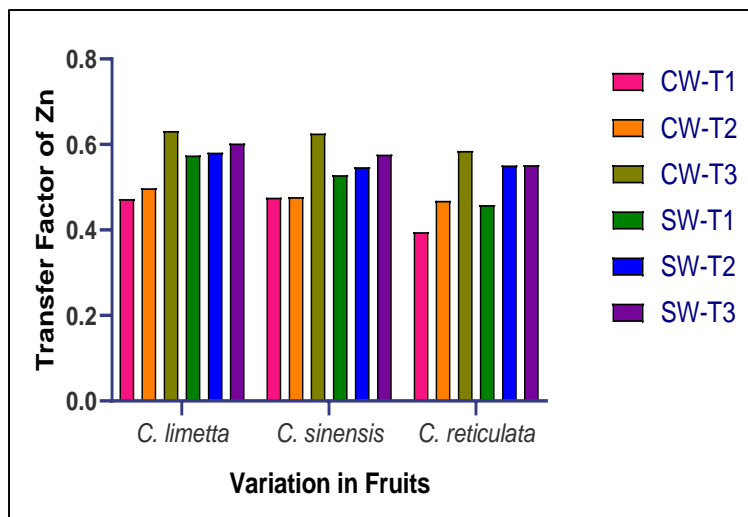

Fig 7: Variation in Transfer factor for Zn

Enrichment factor

The concentration of EF ranged from 0.2907 to 0.4654. A high EF level was found in *C. limetta* at SW-T1, while a lower EF level was observed in *C. reticulata* at Site CW-T1 (Fig. 8).
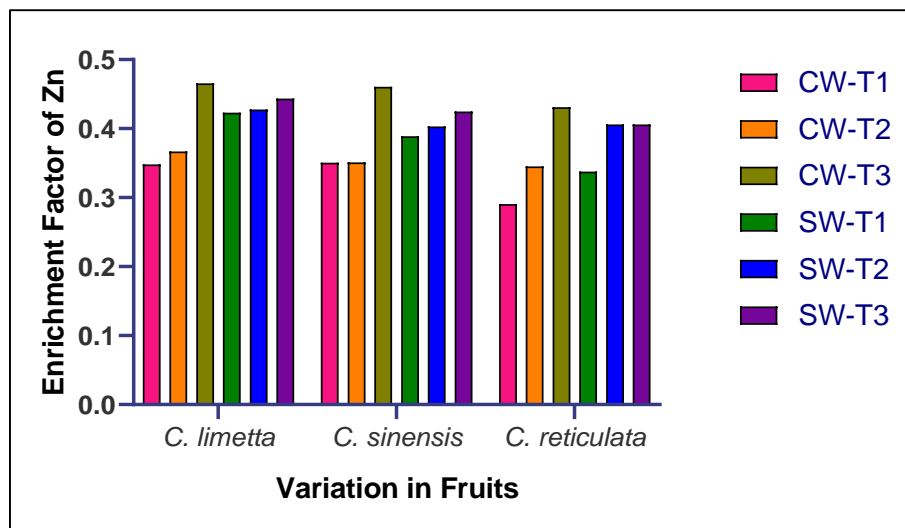

Fig 8: Variation in Enrichment factor for Zn

Discussion

Zn is a crucial metal that play role in cell development. Skin sores, sluggish development, dark issues, cuts, and injuries take longer to heal, but these symptoms heal more quickly after taking zinc supplements and eating meals enriched with zinc (Mason, 2016) [21]. Zn concentrations in the water ranged from 0.501-0.737mg/L, which was below than the recommended permissible level of 5 mg/L (USEPA, 2012). According to Almeelbi *et al*. (2014) [4], who stated that the micronutrient concentrations in potable water were 0.17 mg/L.

According to Ahmad *et al*. (2014), SW contain more nutrients than other types of fluids, with Zn concentrations in CW, and SW being 0.61, and 0.62 mg/l, respectively. Zn average content (mg/L) in water found between 0.55 and 0.761. When the current study was compared to other research, it was found that the Zn levels in the water sample were less than the quantities reported by Aurangzeb *et al*. (2014) [6], which was 6.12 mg/L. Mousavi & Shahsavari (2014) [24], found low levels of Zn (0.010 - 0.021 mg/L) in canal water, which they compared to the findings of the current work. According to Hassan & Basahi (2013) [4], the Zn level of water was 7.2 mg/L greater than the present values. (Kashif *et al*., 2009) [16] discovered high Zn concentrations in the Lahore Canal water. The maximum allowable level of 250 mg/kg, as indicated by Adagunodo *et al*. (2018) [1], was not reached in the current study's soil concentration, which ranged from 0.501-2.76 ppm. Our results fell short of the levels noted by Almeelbi *et al*. (2014) [4], the quantity of Zn irrigated with Sewage water treated with inorganic fertilizer was 371.24 and the amount in potable water was 45.87 g. Our findings exceeded those provided by Shah *et al*. (2013), whose mean Zn concentration in soil samples from citrus chards in the Swat Valley ranged between 0.24 and 10.10 and 0.38 and 5.63 ug/g, respectively. Zn concentrations in CW, and SW irrigated soil were 2315, and 3.307 mg/kg, respectively, according to Khan *et al*., (n.d.). According to Ahmad *et al*. (2016), there were 6.71 to 9.96 mg/kg of Zn ingested in soil with waste water. Mapanda *et al*. (2005), claimed maximum Zn concentrations of 228 mg/kg in wastewater-irrigated soil in Harare, Zimbabwe,

which were lower than the amounts in the current study. It is found the highest value for zinc, 83.3-58.8 mg/kg, when compared to recent research results.

Fruit types ranged in Zn content from 0.208 to 1.6907 g/kg. Zn levels in all fruit samples were significantly lower than the 100 mg/kg maximum tolerated limit reported by Chiroma *et al*. (2014) [9]. In conjunction with Shah *et al*. (2013), our findings revealed that the content of zinc in fruits values between 0.12 to 0.48 mg/100 g, citrus having 0.15 mg/100 g. Our findings did not agree with those of Rasool *et al*. (2020), who stated that Zn levels in *C. reticulata* was 1.80, 0.05 mg/kg in Sargodha. Zn concentrations were 0.321 in citrus fruit samples irrigated with Sewage water treated with inorganic fertilizers and 0.032 mg/L at the potable water site treated with organic fertilizers (Almeelbi *et al*., 2014) [4].

According to Ghani (2017), the Zn concentration varied between 26.463 and 31.726 mg/kg in various tehsils in the Sargodha district. The zinc concentration findings were constant with those described in (Mbong *et al*., 2014). Results showed that the urban and rural orchards contained 16.4 and 24.4 ppm of zinc, respectively. According to Gall *et al*. (2015) [10], the average Zn concentration in navel orange pulp was 6.82 mg/100 g at a sewage water irrigated site along with inorganic fertilizers application and 1.52 mg/100 g at a site that is irrigated with canal water along with combined fertilizers that used.

Despite the fact that Zn was present in high concentrations in the soil and water, only a small portion of it was found in the fruit. This was owing to the metals' slow translocation to the plants' fruits. The sewage water irrigated with the application of inorganic fertilizers location showed the highest concentration (1.60), and the Canal water irrigated site reported the lowest value (0.21).

The corresponding concentrations in CF, EF, and EDI was 0.0616, 0.4654, and 0.0615, respectively, as shown above. In this result, the less values of CF, EF and EDI was 0.0115, 0.2907, and 0.0079 respectively. TF value in the current study was less than the soil's reference value, which was given as 8.15 by Singh *et al*., (2010). Ahmad *et al*. (2016) found that Zn had a higher pollutant load level in soil (2.96-3.77).

Comparing the findings of the current experiment to those of Ahmad *et al*. (2014), it was found that Zn had a higher TF level (2.84 mg/kg).

Ahmad *et al*. (2016) reported CF values (0.778 -2.65) in CW and SW samples, and it was discovered that these values were just a little bit higher than those in the current experiment. Opaluwa *et al*. (2012) discovered a lower value for Zn CF than the one discovered in a recent study. An HQ grade, according to the USEPA, indicates that there isn't a significant risk to the health of consumers (Liang *et al*., 2015). HQ ranged from 0.1661 to 0.0215. According to the findings of a study Zn in citrus fruit samples therefore poses non-carcinogenic health concerns to consumers, it might be concluded.

Conclusion

In conclusion, this research elucidates that, the concentration of Zinc in various component of the agriculture such as water, soil and citrus fruits. The level of zinc in citrus fruits ranged from 0.208 to 1.607 mg/kg. The highest concentration was observed in *C. limetta* irrigated with sewage water with the application of inorganic fertilizer. In soil *C. limetta* also showed high concentration such as 2.767 mg/kg irrigated with sewage water with the application of inorganic fertilizer. The finding revels an interplay between Zn levels and their impact on human health and agricultural produce. While water and soil concentration generally fall within acceptance limits, the study underscores the need for vigilant monitoring and managements strategies. The contamination factor values ranged from 0.0616 in *C. limetta* was higher at sewage water with the treatment of inorganic fertilizer site While, low concentration for Zn (0.0115) was determined in *C. sinensis* at canal water with the application of organic fertilizer site. So, the study result showed that Zinc accumulation was higher as a result of sewage water with inorganic fertilizer application for all samples than the canal water irrigation with other fertilizer application. After this it is important to emphasize the need for controlled monitoring of Zn, and specially check the irrigation system which alter the soil properties. The variation in fruit Zn content across different types highlights the complexity of Zn translocation in plants. Ultimately, this research provides valuable insights for policymakers, environmentalists, and agricultural practitioners to ensure the sustainable managements of zinc in ecosystems, safeguarding both human well-being and crop productivity.

Authors contribution

IS carried out the investigation, original draft preparation, KA, ZIK, AA and MN supervised the study, data curation, AIB, SU, HM, IRN, IU and IS contributed to review and editing. FS, TA, TL and AI contributed to formal analysis.

Ethics Declarations
Ethical Responsibilities of Authors

All authors have read, understood, and have compiled as applicable with the statement on Ethical Responsibilities of Authors as found in the instructions for authors

Ethical Approval and Consent to Participate

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. In this study involving human participants, informed written consent to take part in the research have been obtained prior to the commencement of the study. The authors declare that manuscript has not been published previously.

Consent for publication

All subjects, gave their consent for the publication of details within the text ("Material") to be published in the above Journal and Article.

Competing Interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this research article.

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