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Assessment of Human Health Risk of Zinc and Lead by Consuming Food Crops Supplied with Excessive Fertilizers

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ASSESSMENT OF HUMAN HEALTH RISK OF ZINC AND LEAD BY CONSUMING FOOD CROPS SUPPLIED WITH EXCESSIVE FERTILIZERS

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ABSTRACT

For the study of heavy metals impact on human beings, areas in Sargodha city that were supplied with various types of fertilizers were chosen. The three industrial areas; (Bhalwal, Sillanwali, and Sahiwal) of this city were explored for research reasons. The researchers wanted to know how much heavy metal was in the soil, food crops, and human. Excess fertilizer use contributes to global pollution. Farmyard manure, urea, and potassium chloride were used on Site 1; urea phosphate, manure, and ammonium sulphate were used on Site 2; and super phosphate, ammonium phosphate, and nitrate phosphate were used on Site 3. Samples of commonly used food crops, their respective soils and blood of residents who ingested the food crops of the studied area were collected. The zinc and lead levels in soil (8.30-16.80 and 1.80-12.71 mg/kg) and food crops (0.26-2.02 and 2.26-4.70 mg/kg) were far lower than WHO permitted limits. Blood mean concentration of both Zn (2.30-4.30 mg/L) and Ni (0.24-0.70 mg/L) were found maximum in residents of Site 3. The values of pollution load index, bioconcentration factor, enrichment factor for both zinc and lead were (0.18-0.37 and 0.220-0.948), (0.027-0.138 and 0.316-1.705), (0.020-0.144 and 0.515-2.780), respectively. Daily intake of metal (0.004-0.008 and 0.001-0.002 mg/kg/day) and health risk index (0.0001-0.016 and 0.005-0.115) values were observed to be lower in individuals for Zn and Pb, respectively. In present work values of all pollution indices were <1 indicated that there was no elevated accumulation of zinc and lead in soil and food crops. So, there would be no human health hazard.

Keywords: Ammonium phosphate, fertilizers, food crops, pollution load index.

INTRODUCTION

The word food crops refer to the word's major supply of food that is derived from plants. Grains, legumes, nuts, vegetables, fruits and beverages plants all are included in food crops. Wheat, rice, cotton and sugarcane are the major crops of Pakistan. Agriculture is a very essential part of Pakistan's economy and it is thought that 26 percent of gross domestic product (GDP). The major important agricultural food crops include wheat, rice, cotton, sugarcane fruits and vegetables (Rosa et al., 2019). To increase the yield of crops various types of fertilizers are used extensively. The extensive use of fertilizers contaminates the food crops.

Organic fertilizers are derived from plants and animal residues that are rich in nutrients. Plant based fertilizers include the compost, cottonseed meal, alfalfa and soybean meal, seaweed. The fertilizers that are based on animal include the bone meal, blood meal, animal manure, fish meal, fish mixture/emulsion and shellfish. Mineral fertilizers include the green sand and rock phosphate. There are the several advantages and disadvantages of the application of organic and chemical fertilizers. Although the chemical fertilizers are rapidly available to the plants and also rich in nutrients but the excessive application of fertilizers can cause the loss of essential elements and nutrients, acidification, basification reduction of the useful microbes and the contamination of surface and ground water. Organic matter is slowly decomposed and has the low nutrient content. But as compared to the chemical fertilizers organic manure has the number of benefits like balanced nutrient availability, increased the soil microbial activity, improvement of soil structure and enhances availability of soil water (Han et al., 2016).

Heavy metals from different sources such as from industrial effluents, mining, smelting, textiles, leather industry, electroplating and different agricultural practices (fertilizers and pesticides) contaminate the agricultural soil. These heavy metals pose a risk to the soil microbes as well as the human beings (Gall et al., 2015; Akhtar et al., 2022). The bioaccumulation of zinc (Zn) in human body due to the heavy metals contaminated food could affect the immune system and can also change the high- density lipoprotein (Zhou et al., 2016; Hussain et al., 2022). In the previous work of Zhou et al. (2016) it was observed that due to the ingestion of the food having zinc (Zn) in excess amount can lead to the liver damage and gastrointestinal diseases. Heavy metal-polluted soil and vegetables, including as Ni, Cu, Zn, and manganese, have had a significant impact on regional eco-safety due to their ability to stay for prolonged time and long- term persistency in soil (Khan et al., 2010; Akhtar et al., 2022; Chen et al., 2022).

The study's goal was to check out the consequence of contaminated food crops and propose future guidance to manage the feeding requirements. The concentrations of heavy metal in agricultural soil and food crops treated with many types of fertilizers and their impact on human beings.

MATERIALS AND METHODS

Study Area

Three different areas of Sargodha (Bhalwal, Sillanwali, and Sahiwal) of Punjab, Pakistan were selected to evaluate the effect of different fertilizers on various food crops. Sargodha is the 11th largest city of Pakistan. It is known as city of Eagle. Weather in Sargodha is influenced by subtropical dry semiarid steppe climate. The main food crops of Sargodha include wheat, sugarcane, maize and citrus fruits.

Sampling of Soil and Food Crops

The research locations were dug up thirteen to fifteen centimeter with a drill and one kilogram of soil was taken, including all levels. A total of 45 samples from five different food crops were taken. For each location, five duplicate specimen selections were performed. Each sampling site gathered samples of barley (*Hordeum vulgare*), maize (*Zea mays*), spinach (*Spinacia oleracea*), wheat (*Triticum aestivum*), and garlic (*Allium sativum*) alongside a road. All samples of food crops and soil were air dried before being baked for 45 hours at 75 degrees Celsius. All of the oven dried samples were then put into polythene bags and incubated at 71 degrees Celsius for four days. All of the samples had labeled**.**

Blood Sampling

Round about 45 human blood samples were collected from three distinct places in order to determine the adverse effects of various heavy metals ingested through contaminated food crops.

Soil and Crops Sample Digestion

Wet digestion was utilized to break down all of the natural components in the samples of food crops and soil, and the weight of each crucible was recorded using a weight balance before the dry digestion process. Food crops (5g) were added to each crucible, and the combined weight of the food crops and the crucible was recorded once more. It was heated until the entire material had gone to ash, then dried and stored for 24 hours. The ash was weighed again before dissolving it in 50 ml of water and storing it for heavy metal analysis. In the digesting chamber, 5g of soil and 2 ml of sulfuric acid were added.

Blood Digestion

According to Memon et al., (2007) the traditional wet acid procedure was used to digest the blood samples. In a Pyrex flask, precisely 0.5 ml of blood was taken. Then, for 10 minutes, a 3 ml mixture of HNO_3 and H_2O_2 (2:1V/V) was added and digested for one to two hours at 60 to 70^{0}C. The digests were then treated with 2 ml of nitric acid and a few drops of H_2O_2 while still being boiled on a hot plate at about 80 ^{0}C in order to produce a clear digested solution. The surplus acid mixture was evaporated down to a semi-dry mass, cooled, and then diluted with 0.1 ml nitric acid. These were put into a 100 ml volumetric flask and diluted with distilled water to the mark.

Statistical Analysis

The mean heavy metal level of each duplicate was discovered after analyzing the results from food crops, soil, and blood samples. The correlation and variance were calculated using SPSS software and ANOVA. Indices for pollution exposure evaluation were investigated.

Pollution Load Index:

It is used to assess the total pollution in the soil samples. If pollution load index is equal to one or greater than one it means soil is not suitable to grow the food crops. Pollution load index can be determined by using the following formula.

$$\text{PLI} = \frac{\text{Concentration of metal (mg/kg) in known soil}}{\text{Reference value of metal in soil}}$$

Metal reference value for zinc and lead are 44.9 and 8.15, respectively (Singh et al., 2010).

Bioconcentration Factor

Bioconcentration factor was determined to assess the level of zinc in food crops in accordance with the soil. If its value is greater than one it means food crops accumulate the dangerous level of heavy metal in it. BCF can be calculated by using the following formula

$$\text{BCF} = \frac{\text{Metals value in food crops}}{\text{Metals value in soil}}$$

Enrichment Factor

To assess the impact of anthropogenic activities on the heavy metals indices was used to calculate EF following formula is used

$$\text{EF} = \frac{\frac{\text{Concentration of metal value in plant}}{\text{Metal value in soil}}}{\left(\frac{\text{Metal value in plant}}{\text{Metal value in soil}}\right)\text{standard}}$$

Standard concentration of Zn and Pb in food crops were 60 and 8.15 mg/kg, whereas the standard value for zinc and lead in soil were 44.19 and 5 mg/kg (FAO/WHO, 2007).

Daily Intake of Metal

The harmful effects of heavy metals on the health of human beings can be determined by assessing the daily intake of heavy metal contaminated food.

DIM is obtained by following formula

$$\text{DIM} = \frac{\text{C metal} \times \text{F conversion factor} \times \text{D food intake}}{\text{B average weight of sheep}}$$

The average dietary intake and weight of human were 0.345 mg/kg and 55.9 mg/kg, respectively (Ratul et al., 2018).

Health Risk Index

The HRI (Health Risk Index) is a tool for determining the health risks associated withconsuming polluted feed

$$\text{HRI} = \frac{\text{Daily intake of metal}}{\text{Rf D}}$$

Oral reference dose values for Zn and Pb were 60 and 0.214 mg/kg, respectively (FAO/WHO, 2007).

RESULTS

Metal Analysis in Soil

Variance analysis indicated that the zinc had significant effect on Site and site varieties (Table 1). Concentration of Zn ranged from 8.30 to 16.80 mg/kg. The maximum concentration was shown by the plants of site-3 treated with super phosphate, ammonium phosphate and nitrate phosphate while the minimum concentration was represented by the food crops of site-1 supplied with Urea, farmyard manure and potassium chloride (Table 2 and Figure 1).

Metal Analysis in Food Crops

The results showed significant variations for Zn and Pb (Table 3). The values were in the range of 0.26 to 2.02 mg/kg. The food crops of Site-2 that was treated with with urea phosphate, manure, ammonium sulphate fertilizers had the mean content in between the site-1 and 2. The following (Table 4 and Figure 2) showed the results of mean content of heavy metal in food crops treated with different fertilizers at three different sites.

Metal Analysis in Human Blood

The table 5 showed the mean content of zinc in the blood of human beings that used the food crops of three different sites treated with different types of fertilizers. The concentration of Zn was varied from 2.30 to 4.30 mg/L (Table 5 and Figure 3). The residents of site-1 had the low level of Zn concentration in their body. The inhabitants of Site- 3 showed the high concentration of Zn in their blood.

Correlation

Negative significant correlation was observed at site-1, positive significant correlation was found at site-2 and at site-3 negative non-significant correlation for Zn between the soil and food crops was observed. At site-1 and 3 there was a negative non-significant correlation while at site-2 positive non-significant correlation was observed between the food crops and blood (Table 6).

Pollution Load Index (PLI)

The maximum PLI values was observed in *S. oleracea* that was grown at the site-3 treated with super phosphate, ammonium phosphate and nitrate phosphate fertilizers and the minimum concentration was found in *Z. mays* treated with urea, farmyard manure and potassium chloride (Table 7).

Bio Concentration Factor (BCF)

The values of BCF were varied from 0.027 to 0.138 mg/kg. The site-2 supplied with urea phosphate, manure, ammonium sulphate fertilizers showed the highest value of BCF (Table 8). The plant *T.aestivum* treated with urea, farmyard manure and potassium chloride had the minimum BCF value.

Enrichment Factor (EF)

According to the treatments of three different sites, the order of EF values was as follows; Site treated with super phosphate, ammonium phosphate and nitrate phosphate fertilizers > site supplied with urea phosphate, manure, ammonium sulphate fertilizers > site treated with urea, farmyard manure and potassium chloride (Table 9).

Daily Intake of Metal (DIM)

The inhabitants of site-1 ingested the contaminated food crops treated with various fertilizers showed the low level of zinc in their blood while the residents of site-3 took the food crops as diet.

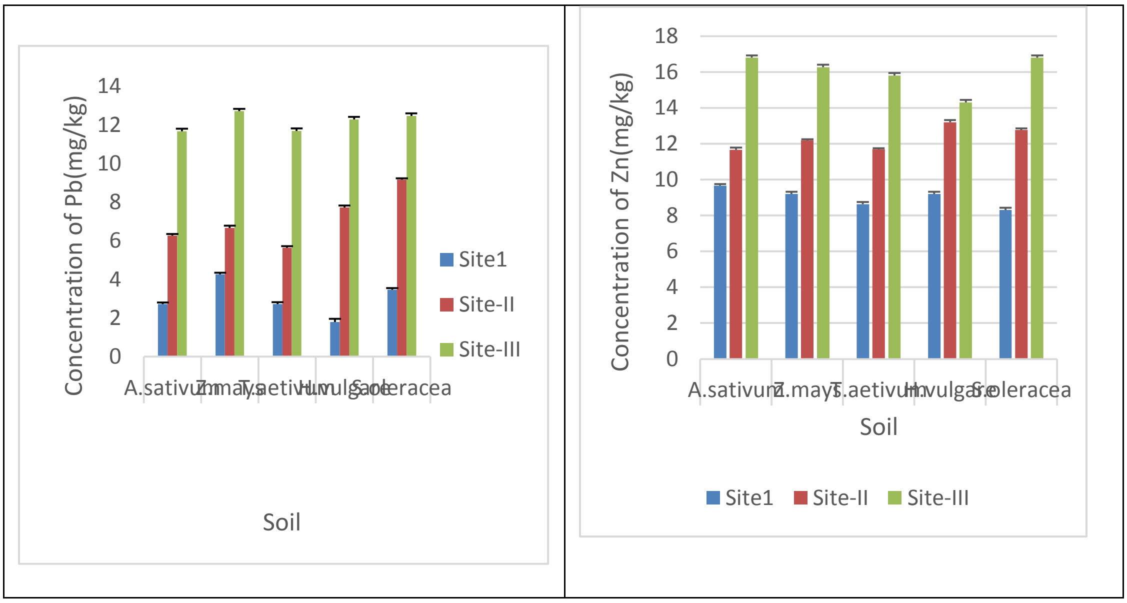

Figure 1: The fluctuating level of Zn and Pb in soil samples treated with different fertilizers

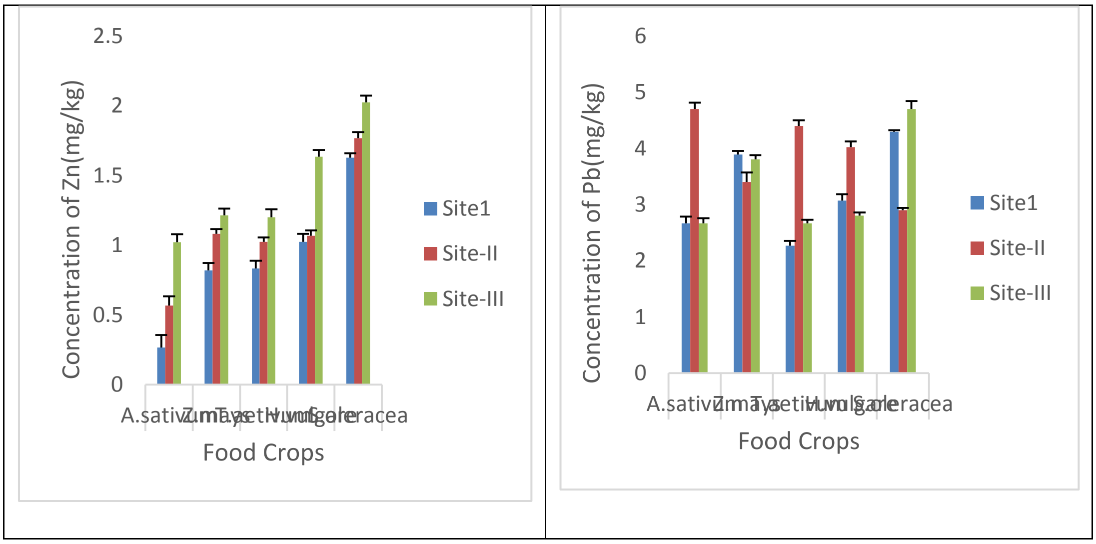

Figure 2: The fluctuating level of Zn and Pb in food crops samples treated with different fertilizers

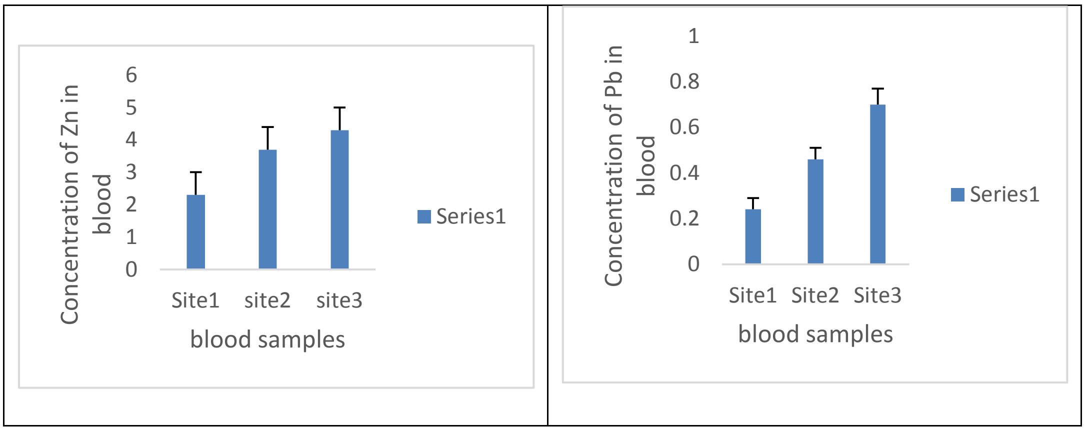

Figure 3: The fluctuating level of Zn and Pb in blood samples treated with different fertilizers

Table 1: Analysis of data variance for zinc and lead in the soil treated with different fertilizers.

Variables	Degree offreedom	Mean square of Zn	Mean square of Pb
Site	2	183.581***	2.865***
Varieties	4	0.718***	13.268***
Site * Varieties	8	2.352***	0.589***
Error	30	0.016	0.028

***=Significant (P < 0.001)

Table 2: Concentration of zinc and lead (mg/kg) in soil samples

Sites	Site1	Site2	Site3	Site1	Site2	Site3
T.aestivum	9.66±0.088	11.66±0.120	16.80±0.058	2.73±0.067	6.26±0.088	11.67±0.009
A.sativum	9.200±0.057	12.20±0.57	16.26±0.088	4.26±0.088	6.66±0.120	12.71±0.058
Z.mays	8.63±0.088	11.70±0.058	15.80±0.058	2.73±0.089	5.63±0.088	11.68±0.057
H.vulgare	9.20±0.058	13.20±0.058	14.30±0.058	1.80±0.058	7.73±0.089	12.27±0.058
S.oleracea	8.30±0.058	12.76±0.088	16.80±0.057	3.46±0.088	9.16±0.067	12.46±0.088

Table 3: Variance analysis of data for zinc and lead in the food crops treated with different fertilizers

Variables	Degree offreedom	Mean square ofZn	Mean square ofPb
Site	2	0.974***	1.842***
Varieties	4	1.690***	1.060***
Site * Varieties	8	0.037***	2.566***
Error	30	0.008	0.057

***=Significant (P < 0.001)

Table 4: Concentration of zinc and lead in food crops treated with various fertilizers

Sites	Concentration of Zn in food crops samples			Concentration of Pb in food crops samples		
	Site1	Site2	Site3	Site1	Site2	Site3
T.aestivum	0.26±0.088	0.56±0.057	1.02±0.006	2.66±0.120	4.70±0.115	2.66±0.088
A.sativum	0.82±0.052	1.08±0.006	1.21±0.009	3.89±0.065	3.40±0.173	3.80±0.077
Z.mays	0.83±0.055	1.02±0.007	1.20±0.067	2.26±0.088	4.40±0.100	2.66±0.064
H.vulgare	1.02±0.008	1.06±0.12	1.63±0.089	3.07±0.016	4.02±0.006	2.80±0.058
S.oleracea	1.62±0.009	1.76±0.107	2.02±0.009	4.29±0.033	2.90±0.404	4.70±0.140

Table 5: Concentration of zinc and lead in human blood

Sites	Site1	Site2	Site3	Site1	Site2	Site3
Mean concentration of Zn and Lead	2.30±0.7	3.70±0.70	4.30±0.7	0.24±0.05	0.46±0.05	0.70±0.07
Mean square	5.26^{ns}			0.265^{**}		

Table 6: Correlation values for zinc and lead

Sites	Soil-food crops	Food crops-blood
Correlation for Zn		
Site-1	-0.812***	-0.126^{ns}
Site-2	0.567**	0.370^{ns}
Site-3	-0.128^{ns}	-0.53^{ns}
Correlation for Pb		
Site-1	0.610^{ns}	-0.464^{ns}
Site-2	-0.704***	-0.461^{ns}
Site-3	0.750^{ns}	0.083^{ns}

Table 7: Pollution load index for zinc and lead

Sites	Pollution load index for Zn			Pollution load index for Pb		
	Site1	Site2	Site3	Site1	Site2	Site3
T.aestivum	0.215293	0.259837	0.374165	0.335378	0.768916	0.819087
A.sativum	0.2049	0.271715	0.362287	0.523517	0.817996	0.946149
Z.mays	0.192279	0.260579	0.351893	0.335378	0.691207	0.820723
H.vulgare	0.2049	0.293987	0.318486	0.220859	0.948875	0.892979
S.oleracea	0.184855	0.284336	0.374165	0.425358	1.124744	1.038855

Table 8: Bio Concentration factor for zinc and lead

Sites	Bio Concentration Factor for Zn			Bio Concentration Factor for Pb		
	Site1	Site2	Site3	Site1	Site2	Site3
T.aestivum	0.027586	0.048571	0.060714	0.97561	0.75	0.399467
A.sativum	0.08913	0.088525	0.07459	0.911719	0.51	0.493228
Z.mays	0.096525	0.087464	0.075949	0.829269	0.781065	0.398173
H.vulgare	0.111232	0.080808	0.114219	1.705556	0.519828	0.384733
S.oleracea	0.115984	0.138381	0.120436	1.238461	0.316364	0.555118

Table 9: Enrichment factor of zinc and lead

Sites	Enrichment Factor for Zn			Enrichment Factor for Pb		
	Site1	Site2	Site3	Site1	Site2	Site3
T.aestivum	0.02031727	0.035773	0.044716	1.5902443	1.2225	0.651132
A.sativum	0.06564457	0.065198	0.054936	1.48610145	0.8313	0.803961
Z.mays	0.07109071	0.064418	0.055937	1.35170768	1.273136	0.649022
H.vulgare	0.08192226	0.059515	0.084122	2.78005556	0.847319	0.627114
S.oleracea	0.1443422	0.101918	0.088701	2.01869196	0.515673	0.904842

Table 10: Daily intake of zinc and lead by human beings via contaminated food ingestion

Sites	Daily Intake of Zn			Daily Intake of Pb		
	Site1	Site2	Site3	Site1	Site2	Site3
T.aestivum	0.005071	0.00612	0.008813	0.001399	0.002466	0.001399
A.sativum	0.004826	0.0064	0.008533	0.002041	0.001784	0.001995
Z.mays	0.004529	0.006138	0.008289	0.001189	0.002308	0.001397
H.vulgare	0.004826	0.006925	0.007502	0.001611	0.002109	0.001469
S.oleracea	0.004354	0.006697	0.008813	0.002252	0.001521	0.002466

Table 11: Health risk index for human beings

Sites	Health Risk Index for Zn			Health Risk Index for Pb		
	Site1	Site2	Site3	Site1	Site2	Site3
T.aestivum	0.00112	0.000102	0.000147	0.006537383	0.011523	0.006537
A.sativum	0.00164	0.000107	0.000142	0.009537383	0.008336	0.009322
Z.mays	0.001138	0.000102	0.000138	0.005556075	0.010785	0.006528
H.vulgare	0.001925	0.000115	0.000125	0.007528037	0.009855	0.006864
S.oleracea	0.016697	0.000112	0.000147	0.010523364	0.007107	0.011523

These were grown in the soil treated with excessive amount of fertilizers had the high zinc concentration in their blood (Table 10). The values were ranged from 0.0043 to 0.008 mg/kg/day.

Health Risk Index (HRI)

HRI values (mg/kg/day) varied from 0.000102-0.016697 for Zn and 0.0055-0.0115 for Pb, respectively. The highest values of HRI for Zn were observed in *S.oleracea* at site-1, while *T.aestivum* at site-2 and *S.oleracea* at site-3 for Pb, respectively. The lowest values of HRI for Pb were examined in *T.aestivum* and *Z .mays* at site-2, while for Pb in *Z.mays* at site-1, respectively (Table 11). However, HRI of both metals (Zn and Pb) were below than 1 at all the sites and showed no danger to human health.

DISCUSSION

Current value of Zn in soil treated with different fertilizers was varied form of 8.30 to 16.80 mg/kg which was lower than Nadeem et al., (2020), who determined the level of Zn in soil ranged from 1.3752-18.84 mg/kg. The current observed value was lower than mean concentration of Zn in soil (137.6 mg/kg) noted by Ejaz et al., (2022). Present level of Zn was less than (65.89 to 76.54 mg/kg), recorded by Leogrande et al., (2019), but higher than (Batool et al., 2023), whose Zn concentration in soil was 0.18-1.21 mg/kg. Essien et al., (2019) described the Zn level varied from 35.9–47.5 mg/kg in soil that was greater than present concentrations. Present concentration of Zn in soil (8.30 to 16.80 mg/kg) was also lower than Ahmad et al. (2022), their value was 27.76 mg kg^{-1} to 37.25 mg kg^{-1}**.** The Zn concentration in the soil that was recorded by Ahmad et al., (2016) ranged from 2.35 to 3.54 mg/kg was found to be lower than current value of Zn in soil.

The concentration of zinc in fodder was ranged from 0.26 to 2.02 mg/kg which was lower than Ahmad et al., (2022), whose value was 8.60-16.49 mg kg^{-1} but higher than (0.20–0.32 mg/kg), as the value reported by Ugulu et al,. (2022). Maqsood et al., (2022) reported the value of Zn in forage (31.98–44.47 mg/kg), which was higher than present concentrations. Latif et al., (2018) reported the Zn concentration in the food crops varied between 19.5 to 41 mg/kg that was higher than the results of current study. Present value of Zn in forage was also lower than 30.83 to 35.04 mg/kg and 4.53 to 17.63 mg/kg as the values described by Ahmad et al., (2016) and Hussain et al., (2022), respectively.

According to Sharma et al., (2018), when BCF $\leq$ 1 indicated that food crops could not accumulate metal although metal is absorbed, while BCF > 1 indicated that metal could be readily and potentially accumulated by plants. In present work BCF value (0.027 to 0.138 mg/kg) of all food crops were less than Chen et al., (2022), whom reported value was 0.840 to 2.01 mg/kg. Current value of PLI (0.1848-0.3741) was lesser than 2.45, as the value reported by Shirani et al., (2020). In present study PLI was less than 1 at all sites showed no contamination of metal EF > 1 it indicates that distribution as well as availability of metals is enhanced in polluted site and their uptake by food crops will increases. Our present value of EF for Zn (0.0203-0.1443) was lower than (0.1776–0.3007), value reported by Khan et al., (2021). According to USEPA (2000), HRI values less than 1 showed no health risk while greater than 1 value indicated health risk for humans. In the Current investigated study all the HRI values were less than one so there would be no human health hazard.

In the present study lead mean content in soil samples collected from three different sites were varied from 1.80 to 12.71 mg/kg that was lesser than Pb concentration in soil, 19.77 to 22.48 mg/kg reported by Leogrande et al., (2019). Hamid et al., (2017) reported the higher concentration of Pb in collected soil samples ranged from 7 to 82 mg/kg than the current study. The Pb concentration was less than the concentration investigated by Liu et al., (2016) ranged from 13.18 to 36.48 mg/kg in soil, but higher than value reported by Chen et al., (2021), their value was 0.279 to 1.58 mg/kg. In the work of Ahmad et al., (2016) the concentration of lead in soil was ranged from 24.13 to 30.74 mg/kg that was elevated level than the present study results in the soil samples. The mean content of lead in the soil of current study was also less than the permissible limit of lead in soil 30 mg/kg given by FAO/WHO (1996). The Pb concentration reported in the investigated soil samples (8.65 to 22.5 mg/kg), studied by Salman et al., (2019) was higher than the present work results. The Pb level in the investigated food crops were in the range of 2.26 to 4.70 mg/kg that was lower than the Pb level reported by Ahmad et al., (2016), varied from 4.79 to 5.21 mg/kg. Chen et al., (2021) reported the value of Pb in forages was 0.048 to 2.002 mg/kg which was lower than present value. The present Pb concentration in forages was also lower than 2.2152-13.0352 mg/kg, value investigated by Ahmad et al., (2020). The Lead concentration in the collected vegetables in the work of (Kacholi and Sahu, 2018) varied from 0.23 to 2.92 mg/kg that was lower than the results of current study.

Chen et al., (2021) reported the PLI value (0.03-9.19), which was higher than current PLI value (0.220859-1.124744), but the observed value was higher than the PLI by Ahmad et al., (2020), there was 0.0235-0.1555. In the current investigated study all the values of HRI were lower lower than one it means Pb did not accumulate in the human body by eating the food crops but in the previous work of (Kacholi & Sahu, 2018), the HRI values were greater than one that could pose the public health risk. By doing different studies it was observed that the HRI values (0.020 to 0.057), reported by Nag and Cummins (2022) was also higher than present study (0.0055-0.0115), and could not threaten to human health. Akhtar et al., (2020) conducted value of BCF for Pb (0.06-0.14), which was lower than present studied value (0.3163-1.7055).

CONCLUSION AND RECOMMENDATIONS

According to the current study over usage of various fertilizers resulted in the accumulation of heavy metals in soil and food crops. Lead (Pb) levels in food crops were within acceptable EU and USEPA guidelines. Food crops are the primary source of nutrition for humans. However, farmers are being forced to use excessive amounts of fertilizers to boost crop productivity for agricultural reasons due to a scarcity of food as a result of rising population. These fertilizers contain a huge amount of contaminants in addition to essential nutrients. Humans that eat polluted food crops accumulate a wide range of hazardous metals in their organs and blood. It is now imperative to enhance public awareness about the health risks associated with eating polluted goods.

AUTHORS CONTRIBUTION

All authors have equal contribution in this article.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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