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Evaluation of heavy metal element (Fe) in cereal food crops irrigated with diverse types of water and their transfer in blood of humans in a Semi-arid environmental gradient in Pakistan

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Abstract

In the present study the concentration of iron (Fe) was considered in Pakistan's Punjab, Sargodha, soil, water, and cereals. Samples were selected from three distinct Sargodha irrigation sites using three distinct water sources. Site 1 used municipal waste water for irrigation, Site 2 used canal water, and Site 3 used ground water. Significant amounts of heavy metals are existing in municipal waste water. In accumulation to being lower in soil and cereal crops, the amount of Iron (Fe) in water was also lower than the standard value. DIM had a high value, but EF, PLI, HRI, and BCF were below the acceptable range.

All results of every parameters have been discussed in the paper in detail.

Keywords: Municipal waste water, Standard limit, BCF, PLI, EF, DIM, HRI

Introduction

Heavy metal usually signifies to metallic element & metalloids getting densities more than five g cm cube. Substantial metallic element in the loams can be observed certainly or outcomes from human caused activities. Accepted resources encompass the atmospheric discharges since transport of continental eruptions, dust the delivery of mainland soils, and the enduring of metal-supplemented stalwarts (Ernst *et al*., 2004). The different important sources of contamination is the anthropogenic activities starting place, the utility of steel based pesticides the exploitation of different kind of mines & smelters and metal accompanied manure slush in husbandry; electronics (use, disposal and manufacture), the incineration of vestige petroleum, metallurgical productions & so on. (Alloway *et al*., 2007).

Heavy metals are reflected as risky chemical compounds in our environment. Non-essential heavy metals are highly toxic to animal's plant life, and human beings at also actual little meditations. Level the crucial weighty brass additionally purpose opposing strength outcomes at excessive focusses. Measures evolved for threat documentation of biochemical poisons within the water atmospheres recall 3 basic characteristic features: Determination, bioaccumulation & venomousness. Noxious ingredients which are together bio accumulative & obstinate are greater danger risk. (Mahboob *et al*., 2014) [18].

Further supply of loam effluence comprises the release of metallic element since bulky stream of transportation on highways which might also incorporate zinc, cadmium, and nickel and are determined in gasoline as antiknock. The testimony of automobile derivative steel and the repositioning of brass element dumped on through fare superficial by means of midair and excess sea partake directed to adulteration of mud .Road dust creating in all likelihood from the discharges of electrical semicircle and heater dirt (EAFD) is as defined to comprehend excessive amount meditations of different alloys The severe wear and rip of drains and brake pedal coatings can harvest excessive amount concentrations of Fe .The fly filth of char consumed electricity powers carries metals.

Collection of Sample

Sampling Sites

First collected a range of samples from four distinct locations throughout the District of Sargodha, including soil, human blood, and cereal crops, began work in April 2020 and finished in May 2021. Select three duplicates of each specimen from each site independently.

Samples of Water

Select the water which is given to the related cereals, took this water in 2ml bottle Then saved this for digestion.

Cereal Crops Samples

25 samples collected from five different cereal crops. For every site, chose five copies of each specimen. For every location, gathered Corn, Wheat, Flax (Alsi), Jodar, and Milllet from the edges of the roadways displayed the cereal samples to dry in the air, then stored for a week at 75°C in the oven. The samples are ground into a fine powder when they have completely dried. Additionally, kept a 2 gram sample safe for digestion.

Table 1: List of Cereals

Sr.	Scientific title	Common name
1	*Triticum aestivum*	Wheat
2	*Zea mays*	Corn
3	*Avena sativa*	Oat plant
4	*Linum usitatissimum*	Flex (Alsi)
5	*Pennisetum glaucum*	Millet

Soil Samples

Gathered forty-five soil samples from various Sargodha locations. Using a colorless steel auger, excavated at a depth of 12 to 15 cm in all of the chosen locations (Sanchez and Lacombe 1976). Soil samples weighing at least 2g were sampled and placed in elastic bags. Following nearly three days of air drying, soil samples were baked for seventy-two hours at 80°C. Ultimately, preserved these specimens in plastic bags for a subsequent digestive process.

Blood Serum

The initial step should be to select the many blood specimens from certain areas within the District of Sargodha. The study's methodology was clearly explained to all participants. The volunteers gave their permission to donate a 3-milliliter sample of blood. A certified technician from the approved laboratory BT Hyrocare pierced a vein to collect the sample, following established protocol for gathering of blood. Following, blood samples were shipped in 3-ml EDTA bottles to Thyro.

Metal Investigation

There was little thought put into how to measure the amount of metals.
1. Sifting
2. Distillation
3. Distillation
4. Atomic Absorption/Spectrophotometry

Tools and Chemicals

Gloves, two 100 mL digestion flasks, two 50% hydrogen peroxide (H2O2), a hot plate, two milliliters of sulfuric acid, a stirrer, and filter paper are needed for this experiment. A 50 mL measuring cylinder Tiny plastic bottles, tripod stands, and recently created distilled water.

Wet acid (digestion) method

Soil

One gram of sample of dried, powdered dirt were put to a digestion flask together with eight milliliters of H2SO4. After that, the acid and soil mixture cooked for around thirty minutes. Addition of 10 milliliters (mL) of H2SO4 over a flame until the deposits brightened. Subsequently, 4 (mL) of H2O2 was added and boiled again. Distilled water is retained until metal analysis and is used to dilute solutions up to a capacity of 50 milliliters (mL).

Cereals

1 gram cereal samples were digested at 250°C for at minimum three to four hours using a ratio of 4:1 H2SO4 to H2O2, resulting in an dense, colorless white vapors rising from the flask and an even, colorless solution. The distilled water used to wash the flask residues was filtered using Wattman filter paper. For the final analysis, a 50 (mL) diluted solution was created by adding distilled water.

Blood

The blood was heparinized and then centrifuged. After mixing 2 (mL) of blood plasma and 2 (mL) of H2SO4, the sample mixture was allowed to sit for the entire night. At 120°C, the sample combination was digested until every piece of organic matter had been dissolved. H2O2 has been supplied in an amount of 2 (mL) to expedite the digesting process' oxidation. Following digestion, the samples were cooled, and used ultrapure water to dilute them to 60 milliliters (mL) before placing them in glass tubes for examination.

Dilution & Filtration

Following digestion, each of the provided samples was thinned to a size of 50 milliliters using incredibly clear water. Following the observation, samples of dirt, forages, blood, and hair were filtered, and these samples were to be kept in plastic bottles for storage.

HMs analysis

Anon (1980) utilized an Atomic Absorption Spectrophotometer in a subsequent procedure toward the number of heavy metals present in the specimens submitted, In recent soil research, the following metals were examined: iron (Fe). Wavelength of Fe was 248.3, slit width was 0.2 and lamp current was 6.

Table 2: Critical limit of metal (mg/kg)

Metals	Soil	Cereals	Blood
Fe	150 a	40 h	2.5 p

Source: a* Rhue and kidder (1983), h, WHO (1996p*Kaneko (1980).

Arithmetical study (Statistical Analysis)

Average levels were determined of harmful metals found in soil, cereals, and blood samples that were taken in separate repeats. Next, determined variance and correlations using ANOVA and SPSS software.

Pollution Indices

Index of pollution load

$$PLI = \frac{\text{value of metal in studied soil}}{\text{Metals reference values in soil}}$$

Table 3: Metals and metalloids reference values in soil

Metal	Reference Values
Fe	56.9 (Dosumuet *et al*. (2005)

Bioconcentration factor

(Cui *et al*. 2004) was used this calculations for BCF

BCF soil to cereal = Metal content in grains/ Value of metals in soil

BCF cereal to plasma = Metal content of plasma / Metals value in cereals

Daily intake for metals

DIM = C metal * D food intake / A average weight

Table 4: Metal DIM

Humans	$D_{\text{food consumption}}$	$A_{\text{normal mass}}$	C.F
I	12 mg/kg (Johnsen &aaneby 2019)	600 kg (Johnsen &aaneby 2019)	0.085 Jan *et al* 2010)
II	12.5 mg/kg ((Briggs and Briggs 1980).	550 kg ((Briggs and Briggs 1980).	0.085 Jan *et al* 2010)
III	1.3 mg/kg	75 kg (Johnsen and aaneby.2019)	0.085

Health risk index

Approximation of HRI complete by this process

$$HRI = DIM / R_f D$$

Enrichment factor

$$\text{Enrichment factor (EF)} = \frac{\text{(Metal content in cereals/Conc. of metal in soil) sample}}{\text{(Value. of metal in crops/Conc. of metal in soil) standard}}$$

Table 5: Standard concentration of metals (mg/kg)

Metal	Soil	Cereals
Fe	56.9 d	425.4 d

a*FAO/WHO 2001 b* (singh *et al*., 2010) c* (Dutch standard 2000) d*(Dosssssumu *et al*.,2005)

Iron

Table 6: Examination of Iron Metal Water Variance Analysis

Site	S-1	S- 2	S- 3
Fe	1.6649±.16999	.7720±.0702	.0092±.000003
Average square	1.85^{ns}		

Results

Maximum mean concentration of Fe was found in site 1(1.6mg/liter). Minimum concentration was existing in site 3 (0.009mg/L).

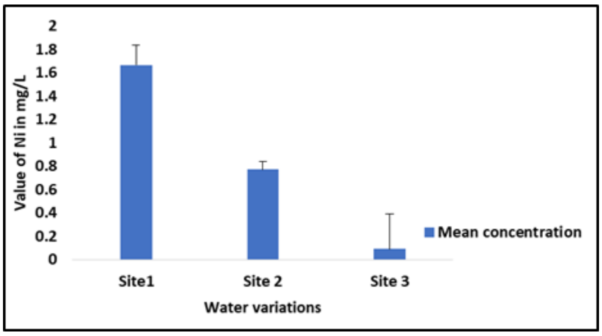

Fig 1: Fluctuations of Iron in waters samples

Soil Results

Table 7: Examining data variances for iron in soil treated by groundwater, canals, and municipal wastewater

Variables	Degree of freedom	Mean square
Site	2	20.839***
Plant	4	.184**
Site*Plant	8	.121***
Error	30	0.148

, *= significant at 0.01 and 0.001 level.

Result

At site 1 the extreme absorption of Fe was present in *L.usitatissimum* (3.22mg/kg).Minimum value was in *P.glaucum* (2.3mg/kg). At site 2 the supreme value was in *T.aestivum (71.95mg/kg).Minimum value was in P.glaucum* (1.65mg/kg). At site 3 extreme value was in *T aestivum* (0.5mg/kg). Least value was in *L.usitatissimum* (0.2mg/kg).

Table 9: Iron concentration in soil samples (Mean± S.E)

Cereal crops	I	II	III
T. aestivum	2.8563±4.870	1.9530±.773	0.5056±.601
L. usitatissimum	3.2230±.294	1.8476±.459	.2506±.132
Z. mays	2.6836±.673	1.9496±1.161	0.4106±0.463
Av. Sativa	2.5106±.692	1.6593±0.320	0.4110±0.331
P. glaucum	2.3603±1.157	1.6583±2.755	0.3623±0.577

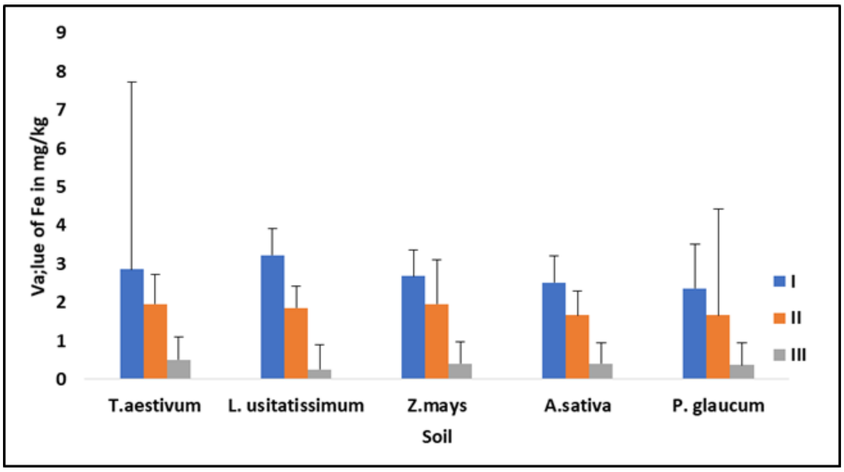

Fig 2: Iron levels in soil treated with groundwater, canal wastewater, and municipal wastewater fluctuate

Iron build up in Cereals

Table 10: Iron in forages treated with ground, canal, and sewer effluent was examined using analysis of variance

Variables	Degree of freedom	Mean square
Site	2	58.596***
Plant	4	3.981***
Site*Plant	8	1.098***
Error	30	.074

***= significant at 0.001 level.

Result

At place 1 the supreme concentration of Fe was present in *Z.mays* (7.6mg/kg). Least value was in *L.usitatissimum* (6.03mg/kg). At place 2 the maximum value was in *Z.mays* (6.4mg/kg).Minimum value was in *P.glaucum* (4.3mg/kg). At place 3 supreme value was in *T aestivum* (3.7mg/kg). Least value was in *A.sativa* (1.3mg/kg).

Table 11: Concentration of Iron in cereals samples

Cereal crops	1	2	3
T. aestivum	6.4420±.309	4.9300±.379	3.7773±.544
L. usitatissimum	6.0356±.615	4.4033±.560	2.5303±.100
Z. mays	7.6380±.421	6.4133±.481	3.4553±0.436
Av. Sativa	6.5620±.253	4.7300±0.288	1.3393±0.293
P. glaucum	7.4290±0.227	4.3900±0.482	3.6580±0.230

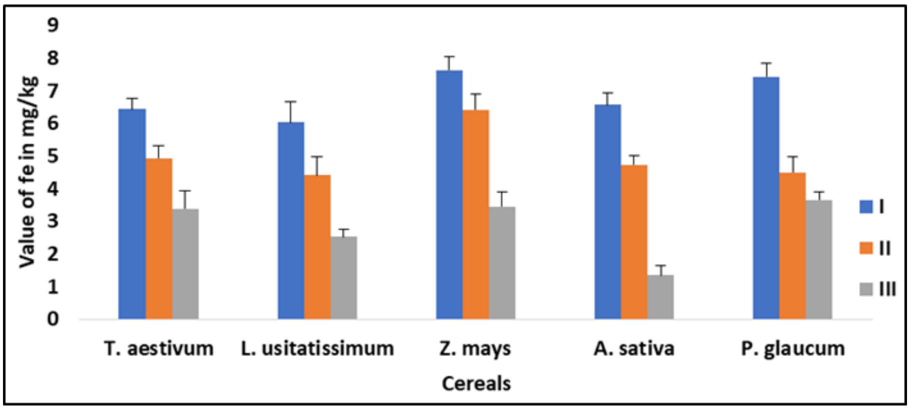

Fig 3: Fluctuation in level of Iron in Cereals ground, canal, and municipal wastewater treatment

Analysis of Iron in blood

Table 12: Variation analysis of iron in human blood

Site	S-1	S- 2	S -3
Fe	1.8628±.06310	1.4260±.07648	1.2324±.03629
Mean square	.521***		

*, **, ***= significant at 0.05, 0.01 and 0.001 level. ns = non-significant

Results

Maximum mean concentration of Fe was found in site 1(1.8mg/liter).Minimum concentration was present in site 3(1.2mg/l).

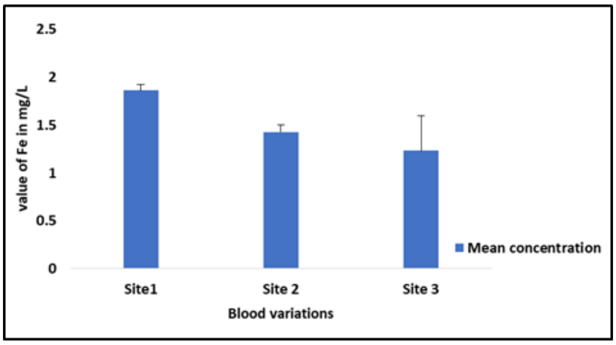

Fig 4: Variation in of Iron in human blood

Table 13: Correlation between soil, Cereals and blood of different sites

Site	Soil to Cereal	Cereal to Blood
S -1	-.242ns	-.779ns
S-2	-.086ns	-.464ns
S-3	-.328ns	.016ns

NS = non-significant

Pollution Load Index

Maximum PLI for soil was observed in *L.usitatissimum* (0.056mg/kg).Minimum concentration was found in *P.glaucum*(0.006mg/kg).at site 1 the order of PLI was *L.usitatissimum>T.aestivum>Z.mays>A.sativa>P.glaucum*. at site 2 the of PLI was *T.aestivum>A.sativa >Z.mays>L.usitatissimum>P.glaucum*.at site 3 the direction of PLI was *T.aestivum>L.usitatissimum>Z.mays.*

Table 14: Pollution load index of soil for Iron metal

Cereal crops	S-I	S- II	S- III
T.aestivum	.050199	.034323	.008887
L.usitatissimum	.056643	.032472	.007405
Z.mays	.047165	.034265	.007217
A.sativa	.044124	.029162	.007223
P.glaucum	.041482	.029145	.006368

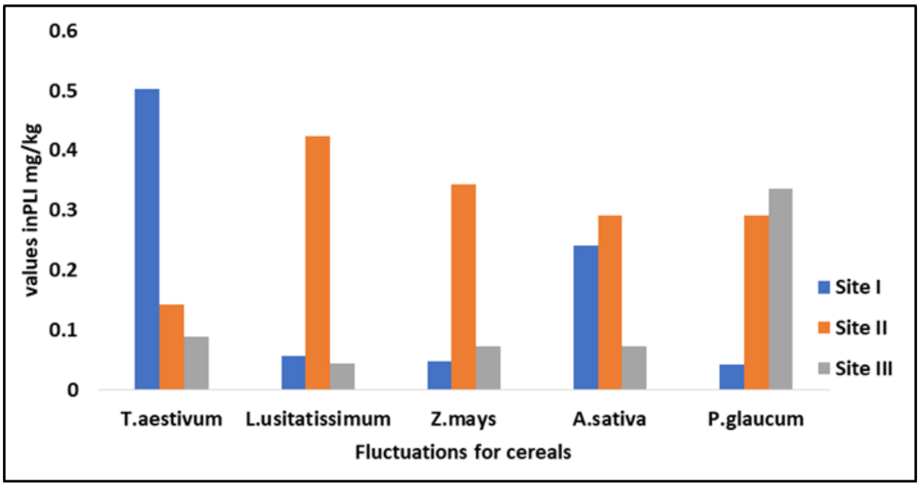

Fig 5

4.9 Fluctuations in PLI for Iron metal

Bioconcentration Factor

Maximum BCF for soil was observed in *P.glaucum* (10.09mg/kg).Minimum concentratin of BCF was found in *Z.mays*(1.87).at location 1 the order of BCF was *P.glaucum>L.usitatissimum>Z.mays>A.sativa>T.aestivum*. Site 2 has the direction of BCF was *Z.mays>A.sativa>P.glaucum>T.aestivum>L.usitatissimum*. at site 3 the order of BCF was *P.glaucum>Z.mays>L.usitaissimum>T.aestivum>A.sativa..*

Table 15: Study of Bioconcentration Factor of Iron metal

Cereal crops	S- I	S- II	S- III
T.aestivum	2.255339	2.524322	6.678901
L.usitatissimum	1.872665	2.383168	7.09427
Z.mays	2.846106	3.289434	8.413873
A.sativa	2.613648	2.850543	3.258637
P.glaucum	3.147437	2.707538	10.09569

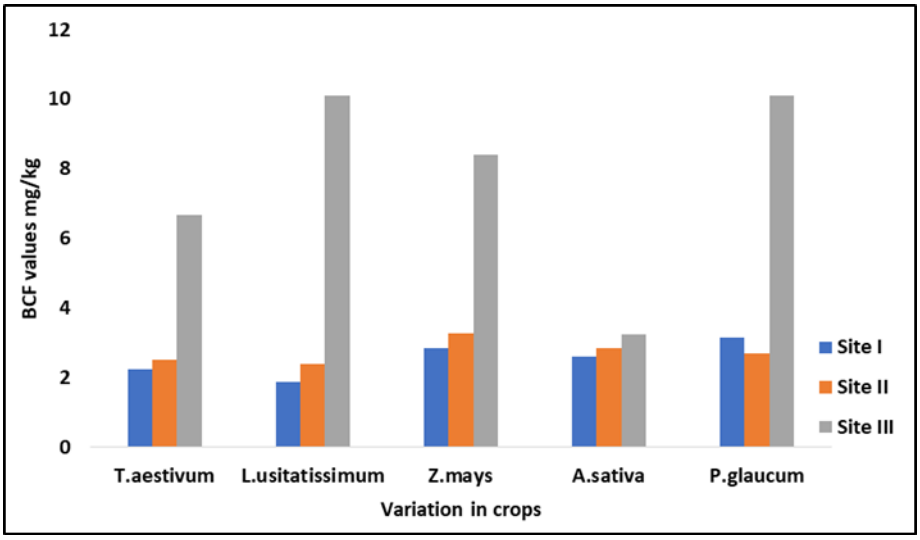

Fig 6: Fluctuations in level of iron in BCF

Enrichment Factor

Maximum EF for soil was observed in *P.glaucum* (1.35). Least concentration was found in *L.usitatissimum* (0.25). at location 1 the sequence of EF was *P.glaucum>Z.mays>A.sativa>T.aestivum>L.usitatissimum*. at site 2 EF was *Z.mays>A.sativa>P.glaucum>T.aestivum>L.usitatissimum*. at site 3 the directive of EF was *P.glaucum>L.usitatissimum>Z.mays>T.aestivum>A.sativa*.

Table 16: Analysis of Enrichment Factor in animals of Iron

Cereal crops	S -I	S- II	S- III
T.aestivum	.30192	.337928	.894097
L.usitatissimum	.250691	.319032	1.351308
Z.mays	.381005	.440353	1.126355
A.sativa	.349886	.381599	.43623ss
P.glaucum	0.421344	0.362455	1.351498

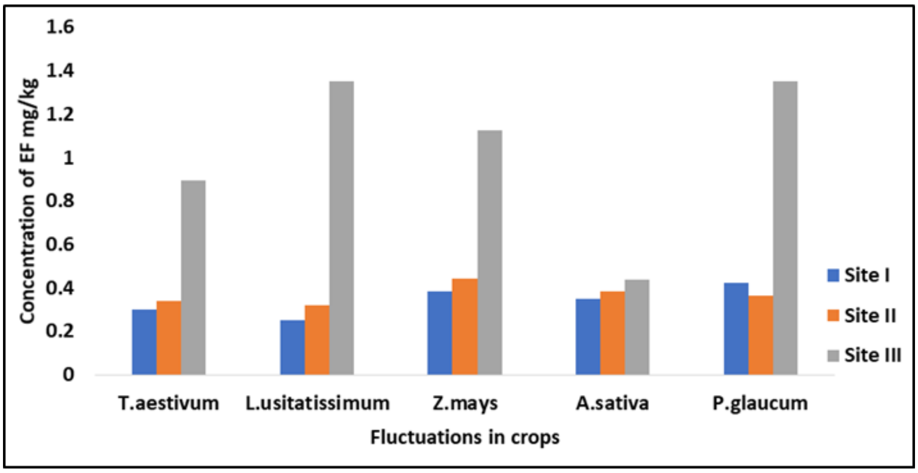

Fig 7: Fluctuations in EF of Iron level

Maximum DIM for soil was observed in *Z.mays* (0.0040mg/kg).Minimum concentration was found in *A.sativa*(0.0007mg/kg).

Table 17: Analysis for Daily intake of metal Iron

Cereal crops	S- I	S- II	S- III
T.aestivum	.003423	.00262	.001795
L.usitatissimum	.003207	.00234	.001344
Z.mays	.004058	.003408	.001836
A.sativa	.003487	.002513	.000712
P.glaucum	.003947	.002386	.001944

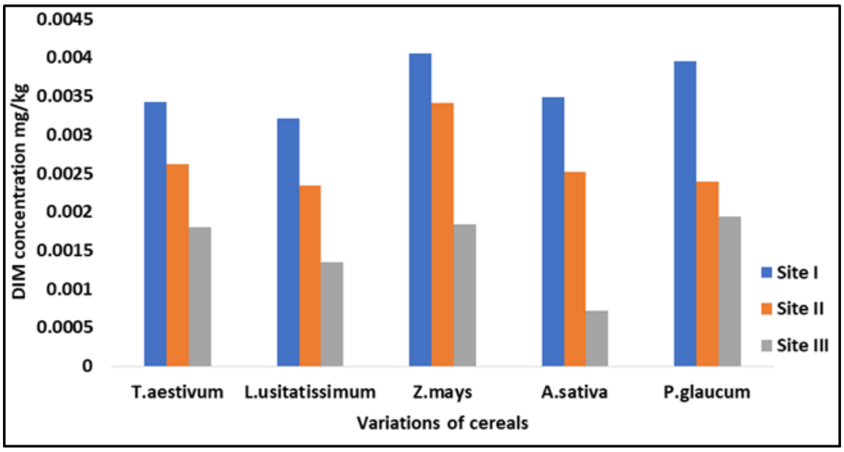

Fig 8: Fluctuation in level of Iron in DIM indices

Health Risk Index

Maximum HRI for soil was observed in *Z.mays* (0.0057mg/kg)Minimum concentration was found in *A.sativa* (0.0010).At location 1 the order of HRI was *Z.mays>P.glaucum>A.sativa>T.aestivum>L.usitatissimum*. at site 2 the order of HRI was *Z.mays>T.aestivum> L.usitatissimum >A.sativa>P.glaucum>*. At location 3 the order of *P.glaucum>Z.mays>T.aestivum>A.sativa>L.usitatissimum.*

Table 18: Analysis for Health Risk index for Iron

Cereal crops	S- I	S- II	S- III
T.aestivum	.00489	.003743	.002564
L.usitatissimum	.004581	.003343	.00192
Z.mays	.005797	.004869	.002623
A.sativa	.004981	.00359	.001017
P.glaucum	.005639	.003409	.002777

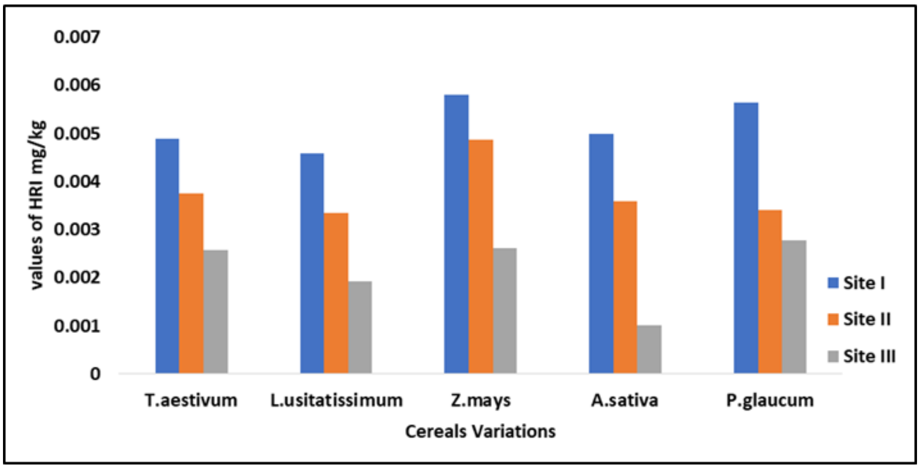

Fig 9: Fluctuation in level of Iron in HRI

Discussion

Maximum mean concentration of Fe was found in water in site 1(1.6mg/liter).Minimum concentration was present in site 3(0.009mg/liter).This value is less than that reported by (WHO, 2001).

The highest Fe absorption (3.22) was originate in *L.usitatissimum* at site 1.*P. glaucum* had the lowest value (2.3 mg/kg). *T.aestivum* had the highest result at location 2 (71.95 mg/kg).*P. glaucum* had the lowest value (1.65 mg/kg). The greatest value at site 3 was 0.5 mg/kg of *T aestivum*. The *L.usitatissimum* minimum value was 0.2 mg/kg.150 mg/kg of Fe was found in soil, according to (WHO, 2009). Our present concentration very less than this value. (Fuortes ***et al.***, 2000) stated the absorption of Fe in soil ranged from (235.5 to 341.90 $mgkg^{-1}$).this concentration is less than our current value.

At location 1 the extreme absorption of Fe was current in cereals in *Z.mays* (7.6mg/kg).Minimum value was in *L.usitatissimum* (6.03mg/kg). At site 2 the maximum value was in *Z.mays* (6.4mg/kg).Least worth was in *P.glaucum* (4.3mg/kg). At site 3 maximum value was in *T aestivum* (3.7mg/kg) (Silvanus *et al*., 2004).

Site 1 had the highest mean concentration of Fe (1.8 mg/liter) in human blood. The lowest concentration (1.2 mg/liter) was found at site 3.This figure is lower than what the FAO stated.

Maximum HRI for soil was observed in *Z.mays* (0.0057mg/kg)Lowest concentration was found in *A.sativa*(0.0010mg/kg).if HRI is greater than its dangerous, if it is less than 1 then it does not consist of metals.

Recommendations and Conclusions

Fe water concentration was below acceptable bounds. Fe concentrations in soil were already greater than recommended limits, at 235.5 mg/kg. Cereals' Fe value exceeded recommended limits as well. Human blood analysis reveals that the Fe concentration was likewise greater. PLI and HRI values for Fe were lower, whereas EF and BCF values were greater and DIM values were lower. The surrounding neighborhood may be at risk due to the increased level of hazardous metal contamination. This study suggested that before being used in contaminated areas, grains and remedial plants should be checked for heavy metals. Extreme Fe levels have the potential to seriously harm the nervous system, reproductive system, and all body functioning.

Ethical Approval for Research

The sampling protocols were approved by the Institutional Animal Ethics Committee, University of Sargodha (Approval No.25-A18 IEC UOS). All the experiments performed on animal complied with the rules of the National Research Council. 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 samples were taken from local farms by the consent of their owners by taking them into full confidence regarding the security of their animals as neglected nails can lead to discomfort or infection and it's a usual procedure as a part of animal care in farms. Keeping in view all Ethical aspects of Research whole work was done. In this Research no animal was sacrificed and only Blood was used. Approval of whole work was taken from the Ethical Committee of the University. Director ORIC has constituted an independent Research Ethics and Support Committee (hereinafter referred to as RESC) of the University to ensure compliance with ethical standards, legal aspects and professional standards in research process undertaken at University of Sargodha. At present, RESC comprises up to sixteen (16) members and is headed by Director, ORIC. RESC was formed by the recommendations of Director ORIC which included the Chairman, 05 Deans and 03 Directors and the same was approved by the worthy Vice Chancellor.

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