Incidence of Heavy Metals in the Application of Fertilizers to Crops (Wheat and Rice), a Fish (Common carp) Pond and a Human Health Risk Assessment

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Abstract: Though manure has been used for centuries to enhance soil fertility and final agricultural yield, the overuse of fertilizers on the land can cause serious pollution issues, such as heavy metals and eutrophication, that can further lead to health problems. The current study aimed to assess the heavy metals in the most consumed crops (wheat and rice) and fish (common carp) and their risk to human health. Results revealed that there was an irregular trend of heavy metals in different sites of the study area; however, the general trend that was observed during the current research work was Pb>Cr>Cd>Mn in soil samples and crops, while in the case of fish muscle, the trend was Cr>Pb>Cd>Mn. Furthermore, after health index analysis, it was revealed that in some of the sites, the hazard index (HI) was above 1. Principal component analysis suggests a strong positive correlation of the heavy metals from the soil and water to the crops and fish muscles, respectively. Cd was highly bioaccumulated in the crops and fish muscles from different sites. There was an unusual increase in the metal concentration in the samples from the Sargodha. This result suggests a rapid increase in the metal concentration within the agricultural land and its products that can put human health at risk of developing multiple diseases related to the heavy metals, as indicated by the HI values. It is highly recommended that fertilizers should be used carefully so that human populations and animals can safely consume heavy-metal-free food.

Keywords: heavy metals; fertilizers; Cyprinus carpio; Triticum aestivum; Oryza sativa; risk assessment

1. Introduction

The term ‘fertilizer’ refers to any natural or synthetic substance applied to soil or plant tissues to provide nutrients for plants [1]. Fertilizers may be distinguished from liming materials or other soil amendments that do not contain nutrients [2]. They can be obtained from a variety of sources, both natural and industrial. Generally, fertilization is based on three main macronutrients in modern agriculture: nitrogen, potassium (K) and phosphorus (P), with the rare addition of micronutrient supplements such as rock dust [3]. There are several ways in which farmers apply these fertilizers: dry, liquid and pelletized, using machinery and hand-tool applications [4]. Using fertilizers can also enhance the natural food supply for cultured fish in ponds [5]. Fertilizing and liming fishponds will dramatically affect the growth of the system, much as it does in agricultural situations [6]. Applying fertilizers can also help reduce aquatic weeds by shading the bottom of the pond,
depriving aquatic weeds of an opportunity to grow [7]. Fertilizers can significantly increase crop yields but can also damage the soil nutrient balance and can cause environmental pollution [8,9]. In streams and water bodies with excessive fertilizer runoff, eutrophication can occur, which creates an overabundance of nutrients, resulting in algae and other microorganisms which can develop uncontrollably [10]. Eutrophication may negatively affect the ecology of reservoirs as well as their natural stability [11]. In addition, excessive fertilizer use can contaminate the groundwater with inorganic chemicals [12]. During the production of fertilizers, varying amounts of different heavy metals are transferred to fertilizers, and these are later applied to the soil, which then enters the food chain [13]. Globally, pollution from heavy metals in water, air, soil, plants and aquatic species is a significant environmental problem because they enter the human food chain [14]. The presence of heavy metals in the environment poses a potential health risk because they are mutagenic, carcinogenic and reproductive toxicants. Due to this, heavy metals in aquatic environments and their accumulation in aquatic animals are potentially harmful to human health [15]. Several studies have been conducted on heavy metal pollution in crops, fish species and habitats, including water and soil [13,14,16]. Organic fertilizers may also contain a different concentration of heavy metals applied to fishponds and soil. Assimilation of metals by fish occurs through the ingestion of particulates suspended in the water [17]. In addition, crops and vegetables absorb heavy metals from soil contaminated with metals, which can lead to health issues in humans and animals who use them as food sources [18]. Good-quality, heavy-metal-free feed enhances the fish flesh quality, and the effective management and conservation of aquatic ecosystems can also be achieved through the monitoring of water quality [13,19].

The Sargodha district of Punjab, Pakistan, has a sub-tropical, semi-arid, continental climate. The summers are long-lasting, with hot, dry air and dust storms. Most of the land in the Sargodha district is used for the cultivation of major crops including wheat, rice, sugarcane, groundnut, jowar, bajra, guar seed, sunflower, moong, maash, masoor, gram, rapeseed and mustard, barley, sesamum, sugar beet, linseed, sunn hemp and canola. To balance soil fertility, farmers use different kinds of organic and inorganic fertilizers [20]. Other studies have reported the accumulation of heavy metals in plants, such as Khan et al. [21], who analyzed the heavy metals in wheat grain irrigated with wastewater, and reported Cd was above the permissible limits for children; they also reported a significant association between the metals in the soil and grains. Recently, Akhter et al. [22] reported bioaccumulation in tomatoes from the use of different fertilizers in the Sargodha district. Several other studies also reported different heavy metals from the Sargodha district [23–25].

Globally, wheat and rice are the most cultivated and economically important crops for human consumption. They are a nutritionally essential and rich source of carbohydrates. The fish selected for the current study have a high market demand worldwide and are a rich source of protein. The present study aimed to assess the heavy metals (Mn, Cd, Cr and Pb) in wheat (Triticum aestivum), rice (Oryza sativa) and fish (Cyprinus carpio) and their risk to human health. The study demonstrates the need for the quantitative determination of heavy metals from fertilizers in wheat, rice and fish to ensure their suitability for human consumption.

2. Materials and Methods

2.1. Study Area Specification

A study was conducted in the district of Sargodha (Figure 1), which covers an area of 5864 km² and has the geographical coordinates of 32°05′02″N and 72°40′18″E. In Pakistan, Sargodha is ranked as the 11th most populous city and is known as the City of Eagles. The region is also called the California of Pakistan due to its prolific production of citrus fruits, particularly “Kinu”. The Jehlum River flows to the northwest of Sargodha and the Chenab River lies to the east. According to the 2017 Census, the total population of the Sargodha district was 3,703,588. The summer season in Sargodha is humid, sweltering and mostly
clear, whereas winter is dry, cool and short. As a result, summer temperatures can reach 50 °C while winter temperatures can drop below freezing. It is estimated that the Sargodha district receives 413.0 mm of rainfall annually. The main crops of the district are wheat, rice, cotton and sugarcane. For agricultural crops, the water source is mainly tube well or river water. In addition, Sargodha is an essential place for warm-water fish, mainly carp, tilapia and catfish species which are mostly cultured in earthen pond systems.

Figure 1. Satellite map of Sargodha district (adapted from google map).

2.2. Soil and Fish Sampling

Before collecting soil samples, detailed investigations were made regarding the applied fertilizer quantities, the frequency of application and application times. It was found that the fertilizer consumption rate for land crops (wheat and rice) was 380, 200, 100 and 20 kg/ha per year for poultry wastes and cow dungs; nitrogen, phosphorus and potassium fertilizers; inorganic fertilizers and poultry wastes; and poultry wastes and wastewater from fishponds, respectively. On the other hand, in fishponds, it was found that 250, 200 and 200 kg/ha for inorganic, cow and poultry wastes and cow dungs, respectively, was applied. Farmers use a lot of fertilizer without the evaluation of land requirements by experts. Details of the fertilizer grades used are given in Table 1.

Table 1. Fertilizer grade details used by farmers.

| Type of Fertilizer                        | Nitrogen (N) | Phosphorus (P) | Potassium (K) |
|------------------------------------------|--------------|----------------|---------------|
| Poultry wastes and cow dungs             | 46           | 0              | 0             |
| Nitrogen, Phosphorus, and Potassium fertilizer | 12           | 12             | 12            |
| Poultry wastes                           | 40           | 1              | 1             |
| Poultry wastes and wastewater from fishpond | 45           | 0              | 1             |
| Inorganic                                | 25           | 5              | 14            |
| Cow and poultry waste                    | 36           | 2              | 8             |
| Cow dungs                                | 49           | 8              | 17            |
Soil samples from three different depths, such as topsoil, 1.5–3 inch and 4–6.5 inch, were collected from three separate regions of the selected study sites. The soil samples were randomly selected but at appropriate distances from each other.

The selected fish for the current study was *Cyprinus carpio*, which is mainly cultured and has a high rate of consumption. Fish samples were collected from three different cultured ponds in the Sargodha district. During collection, fish were captured with the help of a cast net. The average fish weight in grams was 450.43 ± 13.41, with no clinical sign of infection.

### 2.3. Heavy Metal Analysis in Soil and Crops

A wet digestion method was used to determine the total heavy metal content of the soil samples. A solution of 0.1M HNO3 was used to extract heavy metals from the soil. With the use of an inductively coupled plasma atomic emission spectrometer “ICP-AES, PerkinElmer, Optima 5300DV”, the total and available concentrations of Cr, Pb and Mn were determined, whereas the concentration of Cd in the soil samples was analyzed using inductively coupled plasma mass spectrometry “ICP-MS, PerkinElmer SCIEX, Elan 9000”. The properties of the soil, including soil organic matter (SOM), pH, Clay, EC (electrical conductivity) and CEC (cation exchange capacity), were analyzed following the procedure of Li et al. [26]. The details of soil properties are given in Table 2. In this study, powdered wheat and rice grains were digested by HClO4 and HNO3, and heavy metal concentrations were determined using ICP-AES. In order to prepare the crucibles for digestion, all crucibles were dipped in nitric acid and rinsed with deionized water. Standard reference materials, such as GBW07405 for soil samples and GBW07602 for crop samples, were used for quality control. The reagent blanks and all soil samples and crop samples were analyzed in triplicates.

### Table 2. Soil properties and the texture of different study sites.

| Sites  | Crops | SOM (%) | pH (−log H⁺) | Clay (%) | EC (us/cm) | CEC (cmol/kg) | Soil Texture     |
|--------|-------|---------|--------------|----------|------------|---------------|-----------------|
| Control site | Wheat | 3.68    | 6.35         | 22.75    | 1437.54    | 15.98         | Loam            |
|        | Rice  | 3.34    | 6.21         | 17.84    | 1543.92    | 16.21         | Silty clay loam |
| Site 1 | Wheat | 4.12    | 6.43         | 29.42    | 1784.43    | 16.43         | Loam            |
|        | Rice  | 4.03    | 6.73         | 20.34    | 1832.78    | 16.23         | clay            |
| Site 2 | Wheat | 4.65    | 6.54         | 25.43    | 1843.19    | 18.34         | Loam            |
|        | Rice  | 4.32    | 6.47         | 26.32    | 1821.34    | 19.68         | Silty clay loam |
| Site 3 | Wheat | 4.31    | 6.66         | 27.75    | 1923.32    | 19.46         | Loam            |
|        | Rice  | 5.02    | 6.42         | 26.98    | 1845.85    | 20.43         | Silt clay       |
| Site 4 | Wheat | 4.64    | 6.43         | 27.54    | 1954.23    | 18.43         | Loam            |
|        | Rice  | 4.53    | 6.34         | 26.43    | 1943.95    | 19.43         | Silt clay       |

### 2.4. Heavy Metal Analysis in Water and Fish Muscle

Each 80 mL of water sample was filtered to remove unwanted macroscopic substances, and digested with aqua regia, HCl: HNO3 (3:1), following the method of APHA [27]. Acid-mixed samples were subjected to thermostatically controlled hot-plate digestion up to 60 °C for 15 min, allowed to cool, filtered through Whatman filter paper grade 42 and analyzed using ICP-MS. The physicochemical parameters of the pond water samples were analyzed with the help of different instruments. Dissolved oxygen (DO) was determined in situ using a DO meter, the pH using a pH meter, and total dissolved solids (TDS) with a TDS meter. The API Freshwater Master Kit was used for ammonium ion, nitrate, nitrite and alkalinity determination. The physicochemical parameters of fishpond water from different sites are depicted in Table 3.
Table 3. Water quality parameters of different fishpond sites.

| Water Quality Parameters | Control Site | Site 1       | Site 2       | Site 3       |
|--------------------------|--------------|--------------|--------------|--------------|
| pH (−log $H^+$)          | 7.8 ± 0.13   | 7.3 ± 0.12   | 7.2 ± 0.13   | 7.1 ± 0.21   |
| TDS (ppm)                | 148.4 ± 9.64 | 163.4 ± 6.63 | 166.5 ± 14.23 | 164.1 ± 6.35 |
| Temperature (°C)         | 27.3 ± 0.21  | 28.0 ± 1.21  | 27.8 ± 0.21  | 27.8 ± 0.19  |
| Ammonia (ppm)            | 0.01 ± 0.006 | 0.03 ± 0.007 | 0.03 ± 0.002 | 0.02 ± 0.005 |
| DO (mg/L)                | 5.9 ± 0.32   | 5.7 ± 0.43   | 5.8 ± 0.42   | 5.8 ± 0.24   |
| Nitrite (ppm)            | 0.004 ± 0.003 | 0.02 ± 0.00  | 0.03 ± 0.005 | 0.03 ± 0.003 |
| Nitrate (ppm)            | 0.01 ± 0.009 | 0.03 ± 0.003 | 0.01 ± 0.003 | 0.02 ± 0.003 |
| Alkalinity (mg/L)        | 31.8 ± 0.84  | 32.9 ± 1.32  | 30.9 ± 1.62  | 33.9 ± 1.44  |

Dried fish muscle tissue was digested in concentrated HNO$_3$ and H$_2$O$_2$ in a 1:1 ratio in a microwave digestion system at 130 °C, diluted with Milli-Q water and filtered with Whatman filter paper grade 42. The samples were further diluted with Milli-Q water for analysis.

2.5. Bioaccumulation Factor

The bioaccumulation factor (BAF) was established with the help of the following equation:

$$BAF = \frac{C_{substance \ in \ the \ organism}}{C_{substance \ in \ the \ matrix}}$$

In the case of crops, the organism is either wheat or rice and the matrix is soil; in the case of the pond fish, the organism is $C. \ carpio$ and the matrix is pond water.

2.6. Estimated Daily Intake

Fish muscle is a major source of food for 50% of the human population. Therefore, fish muscles are used for calculating the human health risk through an estimated daily intake (EDI) of metals. The estimated daily intake (EDI) is calculated using the following formula [28].

$$EDI \left( \frac{mg}{kg \ body \ weight \ of \ consumer \ day} \right) = \frac{(Concentration \ of \ metal \times \ Weight \ of \ fish \ consumed \ per \ day)}{Body \ weight \ of \ consumer}$$

where the concentration of metals in the fish muscle were converted into a dry weight by dividing with a concentration factor of 4.8 as per [29]. The average weight of fish consumed per day was 70 g and wheat and rice consumption was 2.73 kg, while the average consumer body weight was taken to be 60 kg for Pakistani men [30–32].

2.7. Human Health Risk Assessment

Heavy metal accumulation in a food chain is one of the risk factors for human health. Some metals adversely affect the nervous system [33], and many are reported to be carcinogenic [34]. This study will be a pioneer for the neurotoxic risk of heavy metals through the consumption of common fishes and crops. In order to assess the risk due to the consumption of metal-loaded fish, we calculated the estimated daily intake (EDI) [28], target hazard quotient (THQ) and hazard index (HI), which gives an account of the potential risk to health due to heavy-metal consumption through contaminated food [35,36], and is calculated as:

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

where EDI is calculated using the formula. RfD is the standard dose intake of a particular metal in a day (mg/kg body weight/day) that is within a tolerable and healthy range [37]. A THQ of more than 1 indicates deleterious health effects due to contaminated food exposure in the population.
HI indicates risk due to multiple metals present in contaminated food and is calculated as:

\[
HI = \sum \text{THQ}
\]

2.8. Statistical Analysis

The statistical difference among heavy metals of sampling sites and samples was estimated with the help of one-way ANOVA using MS excel 2016. Further Principal Component Analysis (PCA) was carried out to determine the relationship between the biotic and abiotic metal concentration.

3. Results

On the basis of the soil layer, it was found that the topsoil of the studied sites contained a greater concentration of heavy metals as compared to the other layers. Moreover, the least concentration was recorded in the last (deepest layer of the soil). The highest concentration of Mn, Pb and Cr was recorded for site 1, where poultry wastes and cow dungs were used as fertilizer. Cd was recorded as highest for site 4, where poultry waste and wastewater from the fishpond were used. The details are depicted in Table 4.

Table 4. Metal concentration (mg/kg) on the basis of soil layers from the cultivating lands.

| Sites             | Type of Fertilizer | Soil Layers | Mn     | Cd     | Cr     | Pb     | p-Value |
|-------------------|--------------------|-------------|--------|--------|--------|--------|---------|
| Control site      | No fertilizer      | Layer 1     | 0.58   | 0.843  | 6.1    | 47.16  | < 0.001 |
|                   |                    | Layer 2     | 0.04   | 0.033  | 4.58   | 40.04  | < 0.001 |
|                   |                    | Layer 3     | 0.001  | 0.0045 | 2.12   | 18.81  | < 0.001 |
| Site 1            | Poultry wastes and cow dungs | Layer 1 | 2.29   | 3.43   | 60.37  | 255.51 | < 0.001 |
|                   |                    | Layer 2     | 1.99   | 2.98   | 52.02  | 150.66 | < 0.001 |
|                   |                    | Layer 3     | 1.33   | 1.34   | 28.67  | 139.65 | < 0.001 |
| Site 2            | Nitrogen, Phosphorus, and Potassium fertilizer | Layer 1 | 1.53   | 2.753  | 50.61  | 188.19 | < 0.001 |
|                   |                    | Layer 2     | 1.1    | 1.993  | 40.37  | 110.62 | < 0.001 |
|                   |                    | Layer 3     | 0.73   | 1.023  | 20.53  | 75.583 | < 0.001 |
| Site 3            | Inorganic fertilizers and poultry wastes | Layer 1 | 1.92   | 3.90   | 36.82  | 155.74 | < 0.001 |
|                   |                    | Layer 2     | 1.16   | 3.07   | 29.82  | 80.62  | < 0.001 |
|                   |                    | Layer 3     | 0.54   | 1.58   | 16.71  | 34.26  | < 0.001 |
| Site 4            | Poultry wastes and wastewater from fishpond | Layer 1 | 2.12   | 4.63   | 35.6   | 167.66 | < 0.001 |
|                   |                    | Layer 2     | 1.97   | 4.01   | 41.05  | 100.25 | < 0.001 |
|                   |                    | Layer 3     | 0.87   | 2.18   | 20.26  | 45.87  | < 0.001 |

The results of the studied crops (wheat and rice) from the current study revealed that Pb concentration was higher in samples from all sites (wheat and rice). Besides Pb, it was found that Cr was significantly higher in concentration in rice found in all sites except for site 4, where it was recorded as significantly high in wheat. Mn was found to be at a significantly lower concentration in all sites. The details of the results can be seen in Table 5.
Table 5. Heavy metals concentration analysis in most consumed crops.

| Sites                  | Type of Fertilizer                        | Crops   | Mn (µg/L) | p-Value | Cd (µg/L) | p-Value | Cr (µg/L) | p-Value | Pb (µg/L) | p-Value |
|------------------------|------------------------------------------|---------|-----------|---------|-----------|---------|-----------|---------|-----------|---------|
| Control site           | No fertilizer                            | Wheat   | 0.0012    | 0.17    | 0.040     | 0.0129  | 0.41      | 0.034   | 0.53      | 0.002   |
|                        |                                          | Rice    | 0.000167  | 0.001   | 0.001     | 0.028   | 0.118     | 0.028   | 11.88     |         |
| Site 1                 | Poultry wastes and cow dungs              | Wheat   | 0.24      | 0.000  | 0.81      | p < 0.001| 1.923     | p < 0.001| 5.293     | p < 0.001|
|                        |                                          | Rice    | 0.08      | 0.42   | 2.643     |         | 5.66      |         |           |         |
| Site 2                 | Nitrogen, phosphorus, and potassium fertilizers | Wheat   | 0.043    | 0.030  | 1.056     | p < 0.001| 2.65      | p < 0.001| 6.39      | p < 0.001|
|                        |                                          | Rice    | 0.356    | 0.273  | 3.23      |         | 5.34      |         |           |         |
| Site 3                 | Inorganic fertilizers and poultry wastes  | Wheat   | 0.021    | p < 0.001| 1.33     | p < 0.001| 1.27      | p < 0.001| 4.393     | p < 0.001|
|                        |                                          | Rice    | 0.476    | 1.16   | 3.22      |         | 6.293     |         |           |         |
| Site 4                 | Poultry wastes and wastewater from fishpond | Wheat   | 0.543    | p < 0.001| 1.16     | p < 0.001| 6.283     | p < 0.001| 3.306     | p < 0.001|
|                        |                                          | Rice    | 0.018    | 1.263  | 4.21      |         | 5.36      |         |           |         |

Furthermore, in the case of fish muscle and water of the pond analysis, it was found that Cr concentration was significantly greater in the water and fish muscle from all ponds, followed by Pb. The description is given in Table 6.

Table 6. Concentration of heavy metals on the basis of water of the pond.

| Pond Sites     | Type of Fertilizer                        | Mn      | Cd      | Cr      | Pb       |
|----------------|------------------------------------------|---------|---------|---------|----------|
| Control        | No fertilizer                            | Pond Water 0.02 | Fish Muscle 0.47 | Pond Water 0.11 | Fish Muscle 0.81 | Pond Water 0.21 | Fish Muscle 0.11 |
| Site 1         | Inorganic                               | 2.13    | 1.23    | 1.92    | 2.46     | 4.9      | 3.80     | 4.81      | 3.88     |
| Site 2         | Cow and poultry waste                   | 1.71    | 0.73    | 2.64    | 2.16     | 5.11     | 3.33     | 2.84      | 2.2      |
| Site 3         | Cow dungs                               | 1.92    | 0.70    | 2.63    | 2.03     | 4.08     | 4.83     | 3.32      | 3.23     |

After applying PCA, it was found that heavy metals from the soil and water were positively correlated to the heavy metals found in the crops and fish muscles. PCA analysis can be seen in Figures 2 and 3.
Figure 2. PCA analysis between the heavy metals found in soil and crops.
The bioaccumulation analysis showed that Cd was highly bioaccumulated in wheat and rice from site 3 compared to the other sites and metals. Complete details are depicted in Table 7. In the case of fish muscles, it was found that Cd was bio-accumulated in the fish muscles from site 1 (Table 8).
Table 7. Bioaccumulation of heavy metals in wheat and rice crops from different sites.

| Groups       | Wheat |     |     |     |     |     |     |     |
|--------------|-------|-----|-----|-----|-----|-----|-----|-----|
|              | AS    | Cd  | Ni  | Pb  | AS  | Cd  | Ni  | Pb  |
| Control group | 0.000269 | 0.047619 | 0.067213 | 0.011238 | 0.000288 | 0.00119 | 0.00459 | 0.251908 |
| Site 1       | 0.104803 | 0.236152 | 0.031854 | 0.020715 | 0.034934 | 0.122449 | 0.04378 | 0.022152 |
| Site 2       | 0.028105 | 0.384 | 0.052361 | 0.033955 | 0.23268 | 0.099273 | 0.063821 | 0.028376 |
| Site 3       | 0.010938 | 0.341026 | 0.034492 | 0.028207 | 0.247917 | 0.297436 | 0.087452 | 0.040407 |
| Site 4       | 0.256132 | 0.25054 | 0.113004 | 0.019718 | 0.008491 | 0.272786 | 0.075719 | 0.031969 |

Table 8. Bioaccumulation of heavy metals in the fish muscles from different sites.

| Groups | AS | Cd | Ni | Pb |
|--------|----|----|----|----|
| Control group | 0 | 1 | 7.36 | 0.52 |
| Site 1 | 0.57 | 1.28 | 0.77 | 0.80 |
| Site 2 | 0.42 | 0.81 | 0.65 | 0.77 |
| Site 3 | 0.36 | 0.77 | 1.18 | 0.97 |

The findings of the human health risk analysis showed that in site 2 (wheat), where nitrogen, phosphorous and potassium fertilizers were used, HI was recorded above 1, which means it is in health probability risk. However, in the case of rice in site 3 and site 4, where ‘inorganic fertilizers and poultry waste’ and ‘poultry waste and wastewater from fishpond’, respectively, were used, HI was recorded above 1 for probability risk. Besides this, the HI index for others was near 1, such as in sites 1, 3 and 4 of the wheat crop. In the case of rice, site 1 was near to 1 HI. However, in the muscle of C. carpio, it was found that site 3 had a HI above 1. The details are depicted in Table 9.

Table 9. Health risk analysis of the heavy metals.

| Crops | Sites                     | Type of Fertilizer | Metals | RfD mg kg⁻¹ day⁻¹ | EDI mg kg⁻¹ day⁻¹ | THQ | HI |
|-------|---------------------------|--------------------|--------|-------------------|-------------------|-----|----|
|       | Control                   | No fertilizer      | Mn     | 0.140             | 0.000             | 0   | 0.62|
|       |                            |                     | Cd     | 0.001             | 0.001             | 0.3 | 0.52|
|       |                            |                     | Cr     | 1.500             | 0.016             | 0.001| 0.84|
|       |                            |                     | Pb     | 0.004             | 0.021             | 0.001| 0.63|
|       | Site 1                    | Poultry wastes and cow dungs | Mn | 0.140 | 0.009 | 0.0064 | 0.84|
|       |                            |                     | Cd     | 0.001 | 0.031 | 0.31  | 0.41|
|       |                            |                     | Cr     | 1.500 | 0.079 | 0.005 | 0.41|
|       |                            |                     | Pb     | 0.004 | 0.209 | 0.52  | 0.41|
|       | Site 2                    | Nitrogen, phosphorus, and potassium fertilizer | Mn | 0.140 | 0.001 | 0.0007 | 1.04|
|       |                            |                     | Cd     | 0.001 | 0.041 | 0.41  | 0.80|
|       |                            |                     | Cr     | 1.500 | 0.104 | 0.0069| 0.80|
|       |                            |                     | Pb     | 0.004 | 0.252 | 0.63  | 0.80|
|       | Site 3                    | Inorganic fertilizers and poultry wastes | Mn | 0.140 | 0.000 | 0.000  | 0.95|
|       |                            |                     | Cd     | 0.001 | 0.052 | 0.52  | 0.95|
|       |                            |                     | Cr     | 1.500 | 0.050 | 0.0033| 0.95|
|       |                            |                     | Pb     | 0.004 | 0.173 | 0.43  | 0.95|
|       | Site 4                    | Poultry wastes and wastewater from fishpond | Mn | 0.140 | 0.021 | 0.015  | 0.80|
|       |                            |                     | Cd     | 0.001 | 0.045 | 0.45  | 0.80|
|       |                            |                     | Cr     | 1.500 | 0.248 | 0.016 | 0.80|
|       |                            |                     | Pb     | 0.004 | 0.130 | 0.32  | 0.80|
Table 9. Cont.

| Crops | Sites | Type of Fertilizer | Metals | RfD mg kg\(^{-1}\) day\(^{-1}\) | EDI mg kg\(^{-1}\) day\(^{-1}\) | THQ | HI |
|-------|-------|-------------------|--------|------------------|------------------|-----|----|
| Rice  | Control | No Manure | Mn  | 0.140 | 0.000 | 0 | 0.11 |
|       | Site 1 | Poultry wastes and cow dungs | Cd  | 0.001 | 0.000 | 0 | 0.002 |
|       |       |                 | Cr  | 1.500 | 0.001 | 0.000006 | 0.117 |
|       | Site 2 | Nitrogen, phosphorus, and potassium fertilizer | Pb  | 0.004 | 0.469 | 0.16 |
|       | Site 3 | Inorganic fertilizers and poultry wastes | Mn  | 0.140 | 0.003 | 0.011 |
|       | Site 4 | Poultry wastes and wastewater from fishpond | Cd  | 0.001 | 0.016 | 0.010 |
|       |       |                 | Cr  | 1.500 | 0.104 | 0.0006 |
|       |       |                 | Pb  | 0.004 | 0.223 | 0.55 |
|       |       |                 | Mn  | 0.140 | 0.014 | 0.01 |
|       |       |                 | Cd  | 0.001 | 0.010 | 0.10 |
| Fish  | Site 1 | Inorganic | Cr  | 1.500 | 0.127 | 0.0084 |
|       | Site 2 | Cow and poultry wastes | Pb  | 0.004 | 0.210 | 0.52 |
|       |       |                 | Mn  | 0.140 | 0.018 | 0.012 |
|       |       |                 | Cd  | 0.001 | 0.006 | 0.10 |
|       |       |                 | Cr  | 1.500 | 0.127 | 0.0084 |
|       |       |                 | Pb  | 0.004 | 0.248 | 0.62 |
|       |       |                 | Mn  | 0.140 | 0.000 | 0 |
|       |       |                 | Cd  | 0.001 | 0.049 | 0.49 |
|       |       |                 | Cr  | 1.500 | 0.166 | 0.0011 |
|       |       |                 | Pb  | 0.004 | 0.211 | 0.52 |
|       |       |                 | Mn  | 0.140 | 1.435 | 0.10 |
|       |       |                 | Cd  | 0.001 | 2.87 | 0.287 |
|       |       |                 | Cr  | 1.500 | 4.433 | 0.295 |
|       |       |                 | Pb  | 0.004 | 4.526 | 0.113 |
|       |       |                 | Mn  | 0.140 | 0.851 | 0.607 |
|       |       |                 | Cd  | 0.001 | 2.52 | 0.252 |
|       |       |                 | Cr  | 1.500 | 3.885 | 0.259 |
|       |       |                 | Pb  | 0.004 | 2.566 | 0.641 |

4. Discussion

Fertilizer has been used for centuries to enhance soil fertility and final agriculture yield [38], but overuse of fertilizers on the land can cause serious pollution issues such as eutrophication that can then lead to other problems [39]. Heavy metal pollution is one of the most significant issues related to fertilizers that can enter the food chain and cause severe risks to human health [40]. The World Health Organization reported that more than 25% of the world’s diseases are due to long exposure to environmental contamination, including heavy metals [41]. The current study evaluated the heavy metals concentration in the most consumed crops (wheat and rice) and fish (common carp) and their risk to human health. The trend obtained in the current study was Pb>Cr>Cd>Mn in soil samples and crops, while in the case of fish muscles, the trend was Cr>Pb>Cd>Mn. Overall, there was an irregular trend among the sampling sites and samples. Further results revealed that the topsoil of the studied sites contained a high concentration of all heavy metals compared to any other soil layer. Further, Pb was recorded in high concentrations in all studied sites and crops.

Researchers reported that if food contaminated with Pb metal can cause serious health issues, then even if a person is exposed for a short period and minimum quantity, it can still cause some symptoms such as abdominal pain, constipation, tiredness, headache, pain or tingling in the hands and feet and even memory loss [14,42]. Within our body, Pb is absorbed from food and deposited in blood, tissue and bones, from where it becomes a continual source of internal lead exposure [43]. In older age, when our bone demineralizes and internal exposure may increase due to a large quantity release of lead from the bone tissue [44], a high level of lead exposure can lead to weakness, anemia, brain and kidney
damage and even death [45]. An unborn child can be directly affected by lead if food contaminated with lead is ingested by pregnant women because it can cross the placental barrier very easily. In children, lead can damage the developing nervous system, negatively affecting barriers and intelligence. In adults, it can cause stillbirths, miscarriage and infertility [46,47]. A study by Ahmad et al. [48] suggested that the concentration of Pb was lower than the concentration in the current study. Later, in 2021, Akhter et al. [22] reported a Pb concentration of 0.0529 to 0.17, which is lower than the values recorded in the current study. However, the current study reported that crops contain lead concentrations within the limits permitted [49] (Table 10). However, the lead concentration is far greater than the permissible limits within fish muscles. The permissible limits of fish muscles can be seen in Table 8. The current study contrasts Khan et al. [49], who reported the least quantity of Pb (0.65–1.38 mg kg⁻¹) in a soil sample from the district of Sargodha. Another study conducted by Ahmad et al. [50] also reported values lower than ours, such as 0.3 to 0.41 mg/kg from the Sargodha district, but consistent with Habib et al. [14]. They reported that fish muscle contained lead concentrations above the permissible limits. No other studies related to heavy metal analysis in Sargodha district were found. Further, it was observed that the concentration of Pb was increasing gradually.

After Pb, Cr had the next highest concentration in all studied sites such as water and soil, crops and fish muscle. Although Cr is considered one of the most important metals that plays a major role in the immune system, enhancing the lipid profile, helping in insulin function, weight gain and treating polycystic ovary syndrome (PCOS), overconsumption of Cr can lead to serious health hazards. Although the absorption of Cr is very poor, it can be highly absorbed through the lungs or gastrointestinal tract [51]. Once absorbed in a high amount above the permissible limits, it can lead to cancer and metabolic syndrome and damage the liver and kidney [52]. Ugulu et al. [53] and Khan et al. [49] reported the value of Cr at 0.493 to 1.154 mg/kg and 0.11–1.9 mg/kg, respectively, which is lower than the values of the wheat grain in the current study. There is very limited literature available on Cr analysis in rice grain in Sargodha district. In the present study, the Cr level in the water and soil is only in the permissible range in the control group, while in the fish body, Cr concentration is far above the allowable limits.

In the current research, Cd concentration was above the permissible limit in the soil in the topsoil layer of almost every site, grain of rice and wheat, and fishpond water and muscle. Cd is predominantly lethal to the kidney [49], particularly to the proximal tubular cells; this portion of the kidney is the main site of accumulation. Besides this, Cd can also cause the demineralization of bone, either through direct bone damage or indirectly due to renal dysfunction [36]. Mn was reported in the permissible range in soil, most of the grains (wheat and rice) from different sites and fish muscle. However, fishpond water contained values above the permissible limits. Ahmad et al. [50] reported a Cd value lower than our study in grain and soil in the permissible range. Tariq et al. [54] studied the high level of Cd in basmati rice from the Sargodha district and found consistent results with our study.

| Metals | Water (FAO) [55] | Fish (FAO) [55] | Soil (FAO) [56] | Grain (US-EPA) [57] |
|--------|----------------|----------------|----------------|-------------------|
| Pb     | 15 µg/L        | 0.2 mg/kg      | 8.15 mg/kg     | 0.3 µg/L          |
| Mn     | 0.05 mg/L      | 980 µg/kg      | 25.5 mg/kg     | 55 µg/L           |
| Cr     | 0.1 mg/L       | 0.05 µg/g      | 64.27 mg/kg    | 50 µg/L           |
| Cd     | 0.005 mg/L     | 0.02 µg/g      | 2.8 mg/kg      | 0.10 µg/L         |

5. Conclusions

The current study found that topsoil contained a high concentration of heavy metals compared to the lower layers. Most of the values recorded in the present study were above the permissible limits. The trend obtained in the current study was Pb>Cr>Cd>Mn in soil samples and crops, while in the case of fish muscle, the trend was Cr>Pb>Cd>Mn. Overall,
there was an irregular trend among the sampling sites and samples. We have noticed an unusual increase in the metal concentration in all studied samples (soil, water, grains and fish muscles). This result suggests a rapid increase in the metal concentration within the agricultural land and its products that can put human health at risk of developing multiple diseases related to heavy metals, as indicated by the HI values obtained for some sites. Mn in pond water was recorded above the tolerance range but was lower in fish muscle. This may create a serious human health risk in the future. Overuse of fertilizer without examination can also decrease the final yield. It is highly recommended to use metal-free fertilizers, so that heavy-metal-free and safe food can be available for the general population.

Based on the current study results, we recommend that:

- In the future, further research should be conducted to ensure the suitability of edible crops and fish for human consumption.
- Fertilizer manufacturers should take appropriate measures to ensure their quality and prevent heavy metals pollution.
- Awareness and proper training should be given to farmers about using fertilizers.

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**References**

1. Du Jardin, P. Plant biostimulants: Definition, concept, main categories and regulation. *Sci. Hortic.* 2015, 196, 3–14. [CrossRef]

2. Ye, L.; Camps-Arbestain, M.; Shen, Q.; Lehmann, J.; Singh, B.; Sabir, M. Biochar effects on crop yields with and without fertilizer: A meta-analysis of field studies using separate controls. *Soil Use Manag.* 2020, 36, 2–18. [CrossRef]

3. Sarkadi, L.S. Effects of Fertilizer on Food Supply. In *Chemistry’s Role in Food Production and Sustainability: Past and Present*; American Chemical Society: Washington, DC, USA, 2019; pp. 129–145.

4. Savari, M.; Gharechaee, H. Application of the extended theory of planned behavior to predict Iranian farmers’ intention for safe use of chemical fertilizers. *J. Clean. Prod.* 2020, 263, 121512. [CrossRef]

5. Boyd, C.E. Aquaculture pond fertilization. *CABI Res.* 2018, 2018, 1–12. [CrossRef]

6. Hussain, N.; Abbasi, T.; Abbasi, S.A. Generation of highly potent organic fertilizer from pernicious aquatic weed *Salvinia molesta*. *Environ. Sci. Pollut. Res.* 2018, 25, 4989–5002. [CrossRef]

7. Cai, A.; Xu, M.; Wang, B.; Zhang, W.; Liang, G.; Hou, E.; Luo, Y. Manure acts as a better fertilizer for increasing crop yields than synthetic fertilizer does by improving soil fertility. *Soil Tillage Res.* 2019, 189, 168–175. [CrossRef]

8. Kumar, R.; Kumar, R.; Frakash, O. Chapter-5 the Impact of Chemical Fertilizers on Our Environment and Ecosystem. *Chief Ed* 2019, 35, 69.

9. Nadarajan, S.; Sukumaran, S. Chemistry and toxicology behind chemical fertilizers. In *Controlled Release Fertilizers for Sustainable Agriculture*; Academic Press: London, UK, 2021; pp. 195–229.

10. Al prol, A.E.; Heneash, A.M.; Soliman, A.M.; Ashour, M.; Alsanie, W.F.; Gaber, A.; Mansour, A.T. Assessment of water quality, eutrophication, and zooplankton community in Lake Burullus, Egypt. *Diversity* 2021, 13, 268. [CrossRef]
38. Zhang, X.; Fang, Q.; Zhang, T.; Ma, W.; Velthof, G.L.; Hou, Y.; Oenema, O.; Zhang, F. Benefits and trade-offs of replacing synthetic fertilizers by animal manures in crop production in China: A meta-analysis. *Glob. Chang. Biol.* 2020, 26, 888–900. [CrossRef] [PubMed]

39. Babaei, H.; Nazari-Sharabian, M.; Karakouzian, M.; Ahmad, S. Identification of critical source areas (CSAs) and evaluation of best management practices (BMPs) in controlling eutrophication in the Dez River Basin. *Environments* 2019, 6, 20. [CrossRef]

40. Qaswar, M.; Yiren, L.; Jing, H.; Kaillou, L.; Mudasar, M.; Zhenzhen, L.; Hongqian, H.; Jianhua, J.; Ahmed, W.; et al. Soil nutrients and heavy metal availability under long-term combined application of swine manure and synthetic fertilizers in acidic paddy soil. *J. Soils Sediments* 2020, 20, 2093–2106. [CrossRef]

41. Han, R.; Zhou, B.; Huang, Y.; Lu, X.; Li, S.; Li, N. Bibliometric overview of research trends on heavy metal health risks and impacts in 1989–2018. *J. Clean. Prod.* 2020, 276, 123249. [CrossRef]

42. Godfray, H.C.J.; Aveyard, P.; Garnett, T.; Hall, J.W.; Key, T.J.; Lorimer, J.; Pierrehumbert, R.T.; Scarborough, P.; Springmann, M.; Jepp, S.A. Meat consumption, health, and the environment. *Science* 2018, 361, eaam5324. [CrossRef] [PubMed]

43. Dignam, T.; Kaufmann, R.B.; LeStourgeon, L.; Brown, M.J. Control of lead sources in the United States, 1970-2017: Public health progress and current challenges to eliminating lead exposure. *J. Public Health Manag. Pract.* 2019, 25 (Suppl. S1), S13. [CrossRef]

44. Rodríguez, J.; Mandalunis, P.M. A review of metal exposure and its effects on bone health. *J. Toxicol.* 2018, 2018, 4854152. [CrossRef]

45. Andjelkovic, M.; Buha Djordjevic, A.; Antonijevic, E.; Antonijevic, B.; Stanic, M.; Kotur-Stevuljevic, J.; Spasojevic-Kalimanovska, V.; Jovanovic, M.; Boricic, N.; Wallace, D.; et al. Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney. *Int. J. Environ. Res. Public Health.* 2019, 16, 274. [CrossRef]

46. Dórea, J.G. Environmental exposure to low-level lead (Pb) co-occurring with other neurotoxicants in early life and neurodevelopment of children. *Environ. Res.* 2019, 177, 108641. [CrossRef]

47. Green, R.E.; Pain, D.J. Risks to human health from ammunition-derived lead in Europe. *Ambio* 2019, 48, 954–968. [CrossRef]

48. Ahmad, K.; Ashfaq, A.; Khan, Z.I.; Ashraf, M.; Akram, N.A.; Yasmin, S.; Batool, A.I.; Sher, M.; Shad, H.A.; Khan, A.; et al. Health risk assessment of heavy metals and metalloids via dietary intake of a potential vegetable (*Coriandrum sativum* L.) grown in contaminated water irrigated agricultural sites of Sargodha, Pakistan. *Hum. Ecol. Risk Assess Int. J.* 2016, 22, 597–610. [CrossRef]

49. Khan, Z.I.; Ahmad, K.; Rehman, S.; Siddique, S.; Bashir, H.; Zafar, A.; Sohail, M.; Ali, S.A.; Cazzato, E.; De Mastro, G. Health risk assessment of heavy metals in wheat using different water qualities: Implication for human health. *Environ. Sci. Pollut. Res.* 2017, 24, 947–955. [CrossRef] [PubMed]

50. Ahmad, K.; Kokab, R.; Khan, Z.I.; Ashfaq, A.; Bashir, H.; Munir, M.; SHER, M.; WAJID, K.; Memona, H.; Sana, M.; et al. Assessment of heavy metals in wheat variety “Chagi-2” under short-term wastewater irrigation. *Biologia* 2018, 64, 15–25. [CrossRef]

51. Yang, D.; Liu, J.; Wang, Q.; Hong, H.; Zhao, W.; Chen, S.; Yan, C.; Lu, H. Geochemical and probabilistic human health risk of chromium in mangrove sediments: A case study in Fujian, China. *Chemosphere* 2019, 233, 503–511. [CrossRef] [PubMed]

52. Maret, W. Chromium supplementation in human health, metabolic syndrome, and diabetes. *Met. Ions Life Sci.* 2019, 19, 231–251. [CrossRef]

53. Ugulu, I.; Ahmad, K.; Khan, Z.I.; Munir, M.; Wajid, K.; Bashir, H. Effects of organic and chemical fertilizers on the growth, heavy metal/metalloid accumulation, and human health risk of wheat (*Triticum aestivum* L.). *Environ. Sci. Pollut. Res.* 2021, 28, 12533–12545. [CrossRef]

54. Tariq, F.; Wang, X.; Saleem, M.H.; Khan, Z.I.; Ahmad, K.; Saleem Malik, I.; Munir, M.; Mahpara, S.; Mehmoood, N.; Ahmad, T.; et al. Risk assessment of heavy metals in basmati rice: Implications for public health. *Sustainability* 2021, 13, 8513. [CrossRef]

55. FAO/WHO. *Codex Alimentarius Commission, Food Additives and Contaminants*; Joint FAO/WHO Food Standards Program, FAO/WHO: Geneva, Switzerland, 2001.

56. WHO. *Heavy Metals Environmental Aspects*; Technical Report, Environmental Health Criteria No. 85; WHO: Geneva, Switzerland, 1995.

57. Samuel, Y.G.; Ochube, E.O. Evaluation of the levels of selected heavy metals in leafy vegetables from irrigation farming sites in Jos, Plateau, Nigeria. *J. Toxicol. Environ. Health Sci.* 2021, 13, 28–36. [CrossRef]