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Bioaccumulation and Translocation of Heavy Metals in Paddy (*Oryza sativa* L.) and Soil in Different Land Use Practices

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Abstract: Rice tends to accumulate heavy metals present in soil that have been introduced by human activities and pass them up the food chain. The present study aimed to evaluate the accumulation of selected trace elements (Cu, Zn, and Pb) in paddy and soil and the transfer of these metals from soil to rice by analysing the bioconcentration factor (BCF), bioaccumulation factor (BAF), and translocation factor (TF) of heavy metals in paddy (*Oryza sativa* L.) and soil. Samples of matured paddy and the substrates were collected from three different areas located near a rural point (RP), a transportation point (TP), and an industrial point (IP). Heavy metal concentrations present in the soil and various parts of the plants were ascertained using an atomic absorption spectrophotometer (AAS). Cu, Zn, and Pb accumulation in the soil were detected in increasing orders of RP > TP > IP, IP > TP > RP, and IP > RP > TP, respectively. The BCF_{shoot}, BAF, and transfer factor of both Zn and Pb from soil to rice were detected in the order of TP > IP > RP, which was different from Cu, where BCF_{shoot} and TF showed the order of RP > IP > TP but the BAF indicated IP > RP > TP. TF > 1 was discovered for Zn and Pb at the TP, and for Cu at the RP, which could be attributed to the TP's strongly acidic soil and Cu's abundance in the RP's soil. Paddy height and yield traits were the most significant at the IP site, showing the highest number of fertile spikelets, the average weight of a 1000-paddy spikelet, and the harvest index (0.56). These findings can be related to the normal range of Zn and Pb found in rice plants that support growth. Thus, the findings of this study demonstrated that soil properties and metal abundance in soil from certain land use practices can partially influence the mobility and transfer of metals through soil–plant pathways.

Keywords: rice plant; bioaccumulation; translocation; soil geochemistry; heavy metals; rice growth

1. Introduction

Rice holds a paramount position in Asia, as it is both the primary producer and consumer of this crucial staple crop [1]. However, the escalating contamination of heavy metals in soil poses a serious threat to the environment and human health. One of the major concerns regarding heavy metal pollution is the potential for bioaccumulation in crops. Paddy is especially prone to heavy metal contamination due to being cultivated in submerged conditions with extensive root systems. Heavy metals in soil, such as lead (Pb), copper (Cu), and zinc (Zn) are a broad collection of metallic elements that are typically considered environmental contaminants due to their high persistence and partial biodegradation [2]. The accelerated pace of modern industry, urbanisation, and infrastructural development has considerably contributed to the anthropogenic release of massive amounts of heavy metals into soil. Understanding the mechanisms and extent of heavy metal bioaccumulation in a paddy is thus crucial for devising effective long-term solutions that prioritise food safety. Hence, the current study evaluated heavy metal accumulation and translocation in soil and paddy (*Oryza sativa* L.) under various land use practices, thereby addressing rising concerns about food safety. This study also intended to provide relevant information on the influence of heavy metal accumulation on rice yields to promote safe rice cultivation.

Copper, lead, and zinc were the most common heavy metals found in paddy fields in China, with elevated levels associated with industrial activities and influenced by a variety of factors including soil chemistry, plant physiology, and environmental conditions [3,4]. Concentrations of Pb, Cu, and Zn in road dust also increased with traffic density, primarily originating from vehicle emissions, diesel fuel, tire dust, brake dust, body rust, and tire wear [5,6]. Meanwhile, railway operations would consistently discharge zinc, copper, chromium, and lead during transportation through wheel–rail friction [7]. The accumulation of heavy metals may alter the soil pH and change it to alkaline or acidic, and further increases the uptake of heavy metals by rice plants through root accumulation and translocation [8]. Certain metal ions can be adsorbed in the roots, where a small fraction is transported to the aerial parts of the plants. Then, a portion of the heavy metals could be converted into plant-accessible forms through various soil processes, and influenced by soil properties and plant species [9]. Ultimately, this would reduce the concentration levels in the edible parts of the plant, as well as the toxicity of the metals.

Plants may also respond to a variety of phytoremediation mechanisms when exposed to heavy metals. Phytoextraction is a built-in process in plants that allows some species to efficiently remove heavy metals from contaminated areas through metal uptake and deposits in their tissues at a high growth rate and subsequent harvesting at a low cost [10]. Hyperaccumulator plants can even be improved using chelate agents, microbial aid and genetic recombination [11]. Certain metal-tolerant plant species can immobilise heavy metals belowground through root surface adsorption, organic acid complexation, and precipitation, thus reducing their bioavailability and preventing metals from migrating into the wider environment and entering the food chain [12]. A field experiment in China looked further at the efficacy of using plant residue pyrolysis biochar as liquid fertiliser to recycle the high biomass of polluted plant residues [13]. A recent study on a synergistic microalgae–bacteria consortium demonstrated multiple biosorption mechanisms such as electrostatic attraction, ion exchange, redox and enzymatic mechanisms, surface complexation, cell transformation, and cellular absorption to remove, reduce, or oxidise metal ions in wastewater, which are much faster and reversible [2]. These findings indicated that heavy metal contamination is a complex issue that requires multifaceted solutions, including effective heavy metal mitigation and treatment measures.

Hence, the present study focused on identifying the accumulation and transport patterns of toxic elements in paddy and soil obtained from multiple land use categories in Perlis, Malaysia. The primary objectives of this study were as follows: (i) to measure the concentrations of heavy metals (Cu, Zn, and Pb) in soil substrates and various paddy parts (belowground and aboveground), as well as soil physicochemical parameters (pH and organic matter content); (ii) to determine the bioconcentration factor (BCF), bioaccumulation factor (BAF), and translocation factor (TF) of plant components; and (iii) to analyse several important rice growth and yield traits. These findings were then utilised to assess the potential risks and implications for paddy productivity. Consequently, the findings of this study are critical knowledge for society since they can help environmental engineers and technologists to better protect human health by measuring the hazards of heavy metal exposure through major food supplies. Furthermore, the knowledge gained will pave the way for more business optimisation in crop outputs by identifying appropriate and feasible land use practices to ensure that paddy may be cultivated sustainably.

2. Material and Methods

2.1. Study Area

This study was conducted in Kangar, the capital of Perlis, Malaysia, which has an area of 2619.4 hectares and a population of 48,898. Situated at the northernmost point of Peninsular Malaysia, Kangar is the smallest town in the country, with most of its inhabitants being farmers and government workers. This study selected three sites in Perlis, namely rural, transportation, and industrial areas, as shown in Figure 1, to investigate the distribution of heavy metals in soil and rice plants around different types of land use, including agricultural area (AG), residential area (RE), commercial area (CM), institutions and community facilities (INT), industrial area (IND), infrastructure and utilities (INF), transportation area (TP), mixed development area (MD), paddy field (PF), and others (OT).

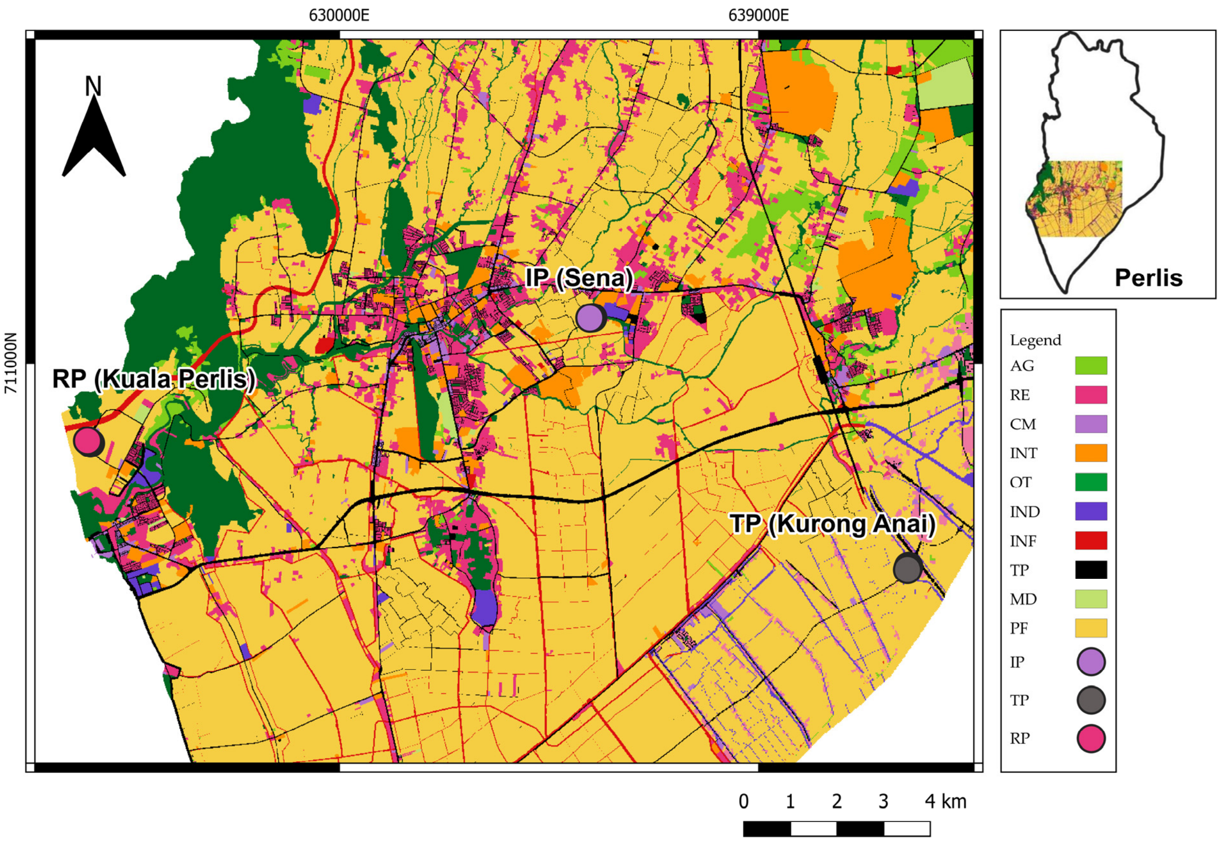

Figure 1. Location of study areas in Perlis.

Description of the study areas:

(a) Kuala Perlis as the rural point (RP)

The selected sampling site is located in Kampung Bukit Puteh, Kuala Perlis. This site is located in a rural area, which is 13.6 km from the capital city, Kangar (Figure 1). The site was selected to determine the effects of agrochemicals on rice plants.

(b) Kurong Anai as the Transportation Point (TP)

The study site in Kurong Anai is located approximately 150 m from the railway and close to the Arau station (Figure 1). The railway system, which was initially built in 1910 during the British colonial era to carry tin, now annually transports more than 13,000 people and millions of tonnes of goods. To investigate the impacts of railway transport on soil and rice plants, a sampling area of roughly 500 m perpendicular to the railway was chosen. This location was particularly chosen as a transportation hub. Water supply for paddy cultivation is provided by the Muda Agricultural Development Authority (MADA).

(c) Sena as the industrial point (IP)

A sampling site in Sena, which is located in the Jejawi industrial zone, was chosen to investigate the potential impact of industrial activity on rice plants (Figure 1). Sena is home to a rubber processing plant owned by a Japanese company that manufactures and sells high-grade industrial gloves. This sampling site was chosen as an industrial point, and it is located approximately 100 m from the paddy field. MADA also provides water for paddy cultivation in this area.

2.2. Sampling of Soil, Rice Plants, and Grain, as Well as Sample Preparation

Soil samples were collected from each sampling site at six different points (RP1-6; TP1-6; IP1-6) of the paddy fields. To avoid heavy metal contamination, soil samples were collected from the surface layer (0–10 cm) using a stainless steel spade. The soil samples were then air-dried in the laboratory at room temperature, disaggregated with a pestle and mortar, and sieved through a 2 mm sieve mesh. Fresh rice plants were carefully washed under running tap water to remove soil particles and dust, followed by three rinses with distilled water to eliminate any surface contaminants or impurities that could affect the accuracy of subsequent analysis. The potential for contamination of the plants by agrochemical residues was taken into account during this process. The plant samples were subsequently oven-dried at 80 °C for 24 h to prevent fungus infection, and later stored in plastic zip-lock bags prior to further analysis.

2.3. Sample Analysis

The pH of the collected soil samples was determined using the 1:2.5 soil-to-water ratio method, while the percentage of organic matter was calculated using the loss on ignition method [14]. Heavy metal analysis was conducted by digesting 1 g of air-dried soil sample and 0.5 g of oven-dried plant sample in 20 mL of concentrated nitric acid (HNO_3). Concentrated HNO_3 was used as the oxidising agent due to its high efficiency in digesting carbonates, phosphates, and other constituents to determine the total heavy metal content in a sample [15]. Before undergoing acid digestion, oven-dried plant tissues, such as roots and shoots, were cut into smaller pieces, and the rice grains were deshelled. During the acid wet digestion, the sample was heated in the fume hood under a hot plate until the brown fume had completely dissipated. After acid digestion, the sample was allowed to cool before being added with 50 mL of distilled water and later filtered using the standard filter paper. This solution was diluted by a factor of 10, and the heavy metal concentration of Pb, Cu, and Zn was determined using atomic absorption spectroscopy (AAS). For final calculations, the AAS value of each metal's blank was subtracted from the sample value. All analyses were performed in triplicate.

2.4. Accumulation of Heavy Metals in Plant

Soil-to-plant transfer factor is an important component of human exposure to metals through the food chain, and it may reveal heavy metal bioavailability in studied soils [16]. The bioconcentration factor (BCF), bioaccumulation factor (BAF), and translocation factor were used in this study to calculate metal uptake and distribution in various plant parts (TF). BCF represents the ratio of metal concentration in plant tissues to that in the soil, while BAF is the ratio of metal concentration in plant tissues to that in the roots. TF is the ratio of metal concentration in the aboveground plant parts to that in the roots. These factors are useful for assessing the ability of plants to take up and accumulate metals from contaminated soils, and to translocate metals to the edible parts of the plants. Analysing these factors can lead to a better understanding of the risks associated with consuming crops grown on contaminated soils.

2.4.1. Bioconcentration Factor (BCF)

A plant's ability to accumulate metals from the soil can be estimated using the BCF. The BCF was calculated using the following equation [17]:

$$BCF = C_p/C_s$$

where Cp is the metal concentration present in plant part and Cs is the concentration of metal in soil. The BCF value is a ratio of the metal content in different parts of the plant to that in the soil. A BCF value of greater than 1.00 suggests that the plant can accumulate metals, whereas a BCF value of $\leq$1.00 implies that the plant can only absorb heavy metals [18]. In other words, a BCF value of greater than 1.00 means that the plant can take up more metals than what is available in the soil, indicating that it has the ability to hyperaccumulate heavy metals. However, a BCF value of $\leq$1.00 indicates that the plant cannot hyper accumulate metals, but only absorb them in proportion to their availability in the soil.

2.4.2. Bioaccumulation Factor (BAF)

The BAF is an important index for understanding the potential of plants to accumulate heavy metals from soil into their edible parts. This index reflects the extent of metal bioaccumulation in plants relative to their concentration in soil, which can be useful for assessing the safeness of consuming food crops grown in contaminated soil. A high BAF value indicates that the plant has a strong capacity for metal accumulation, which may pose risks to human health when consumed [19]. Conversely, a low BAF value implies that the plant is less efficient in accumulating metals, indicating a lower risk of metal contamination in food crops. Therefore, the BAF can be used as an important index for evaluating the safety and quality of agricultural products in terms of heavy metal contamination. The BAF value was calculated as follows:

$$BAF = C_{rice\ grain/}C_{soil}$$

where heavy metal concentrations in the edible parts of the plants and the soils are represented by $C_{rice\ grain}$ and C_{soil}, respectively.

2.4.3. Translocation Factor (TF)

The TF is known as the plant's ability to transfer metals from roots to the aerial parts. TF was calculated based on the ratio of the metal concentration of plant shoots to roots using the following equation [19]:

$$TF = C_{shoot}/C_{root}$$

This index provides a quantitative interpretation of the relative differences in the biological availability of metals to plants. A TF value of less than 1 indicates that the plant has the potential for phytostabilisation, where the metal is immobilised in the roots, preventing it from being translocated to the shoots. In contrast, a TF value of greater than 1 indicates that the metals are effectively translocated from the roots to the shoots [20]. Therefore, TF can provide valuable insights into the potential risks associated with metal contamination in soil–plant systems and is an important parameter for assessing the environmental impact of heavy metals.

2.5. Rice Growth and Yield Traits

At harvest time, various yield components were assessed to determine the productivity of rice plants. These components include plant height (cm), culm height (cm), panicle length (cm), panicle number, number of tillers, number of grains per panicle (grain yield), 1000-grain weight (g), dry weight of plant biomass (g), number of fertile spikelets, and harvest index [21,22]. The panicle length was measured using a digital calliper and recorded after being averaged. The weight of the panicle was recorded after oven-drying. The filled and unfilled grains were separated from the spikelet, counted, and weighed after being oven-dried. The dry weight of all aboveground plant parts, including shoots, panicles, and grains, was recorded to calculate the plant biomass. To evaluate rice grain yield, the total dry weight and dry grain yield were measured, and the ratio of these two values was computed to determine the harvest index (HI) as follows:

$$\text{Harvest index (HI)} = (E_{\text{yield}})/(B_{\text{yield}}) = (\text{Dry grain yield})/(\text{Total dry weight})$$

where B_{yield} refers to the biological yield of the plant, which includes all of the aboveground plant parts, including shoots, panicles, and grains. E_{yield}, on the other hand, refers to the economic yield of the plant, which is the amount of grain harvested after the removal of chaff, husks, and other nongrain components [22]. The HI is a useful indicator of a plant's ability to convert total dry weight into economic yield. A higher HI indicates that the plant is more efficient at producing grain, whereas a lower HI suggests that the plant allocates more resources to nongrain components.

2.6. Statistical Analysis

To investigate the relationship between study sites and heavy metal types and concentrations, an ANOVA analysis was conducted. Meanwhile, Pearson correlation analysis was used to determine the associations among various factors, such as soil properties, concentrations of copper (Cu), zinc (Zn), and lead (Pb) in soil, as well as the concentration of Cu, Zn, and Pb in different parts of the plant (roots, shoots, and grains), and paddy yield. To perform the statistical analyses, SigmaPlot statistical software (14th version) was utilised.

3. Results and Discussion

3.1. Heavy Metal Concentrations in Soil

The concentrations of Cu, Zn, and Pb in the air-dried paddy soil from three different agricultural areas were determined. The findings are presented in Table 1, which displays the concentration of different heavy metals in the paddy soil of the selected areas in Perlis. The mean Cu concentration was found to be higher at the RP (100.55 mg/kg) compared to at the TP (85.24 mg/kg) and the IP (77.43 mg/kg). Cu concentrations at all sites were within the range of critical concentration in soil (60–125 mg/kg). Cu concentrations detected at all sampling areas were relatively higher than those reported in a previous study in India [23]. These results suggested that agricultural lands were directly influenced by anthropogenic sources, such as industrial activities, transportation, and agricultural practices, as the mean values of heavy metals were higher than the background values (30 mg/kg).

Table 1. Mean concentrations of heavy metals along with the standard deviation in the soil at each sampling site.

Heavy Metal	RP (mg/kg)	TP (mg/kg)	IP (mg/kg)
Cu	100.55 ± 15.41	85.24 ± 11.41	77.43 ± 11.05
Zn	76.59 ± 35.69	84.39 ± 40.90	123.78 ± 21.18
Pb	21.46 ± 19.34	7.95 ± 12.71	32.48 ± 31.78

Interestingly, the RP, which is situated quite far from the capital city, has a higher Cu concentration despite being less likely to be affected by transportation and industrial activities. This high concentration of Cu at the RP could most likely be due to agricultural practices and the application of agrochemicals. Previous studies have shown that long-term use of Cu-based chemicals in agriculture can increase the concentration of Cu in soil [24]. Different copper-based materials such as frit, chelate, phosphate, sulphate, and oxide have been suggested for use as fertilisers. A significant amount of copper-based fertiliser is widely used in most of the European Union, evidenced by high Cu accumulation in these countries [25].

The origin of Cu concentration at the TP could likely be caused by railway traffic, as Cu is often obtained from head-over traction cable abrasion [7]. When trains run on the tracks, the wheels create friction with the rails, which can cause tiny particles of Cu to be released into the air. These particles can then settle onto nearby soil and become incorporated into the soil. However, it was observed that Cu concentration began to decrease with increasing distance from the railway, indicating that the contribution of Cu to the soil was less further away from the railway. As shown in Tables 2 and 3, Cu concentration detected at the TP is lower than at the RP, with soil pH of 4.92 and 5.08, respectively. It is known that the availability of Cu increases with lower pH values, which aligned with the strongly acidic soil pH found at both the RP and the TP sites. This observation can explain the fact that Cu ions in soil can form very stable complexes over a broad range of pH [26]. Intriguingly, the average Cu concentration (240.59 mg/kg) found in Perlis in 2014 [27] was much lower at all three sampling sites, suggesting that there were consistent expansions of urban sprawl, industries, and vehicles up to the current time. However, the percentages of soil organic matter (OM) were not substantially different between sites; hence, no major dependent variation in terms of OM can be elaborated.

Table 2. Means of soil physicochemical characterisation at different study sites.

	RP	TP	IP
pH	5.08 ± 0.58	4.92 ± 0.11	6.50 ± 0.70
OM %	5.20 ± 1.09	5.24 ± 0.39	6.46 ± 1.08

Meanwhile, the mean concentration of Zn at the RP and the TP was 76.59 and 84.39 mg/kg, respectively, which did not exceed the average Zn concentration (90 mg/kg) in soil. The maximum values of Zn found in these areas (the RP and the TP) was high at 114.23 and 149.47 mg/kg, respectively, which indicated potential sources of contamination. One potential source of high Zn concentration is automobile traffic, which includes the wearing of brake lining and tires, and losses of oil and cooling liquid [28]. However, the mean Zn concentration at the IP exceeded the natural level, with a high value of 123.78 mg/kg, suggesting that industrialisation may be a major source of Zn contamination in this area. This hypothesis is supported by the fact that the IP is located near a rubber processing plant, which may have contaminated the surrounding paddy fields through wastewater effluent containing Zn. A previous study found that large quantities of Zn in the wastewater of industrial plants contaminated the water bodies and nearby soil in Bangladesh [29]. Another study also discovered that Zn was more available in soils with higher organic matter content [30], which could explain the higher Zn concentration at the IP.

The mean concentrations of Pb at the three research sites are in the following increasing order of IP > RP > TP at 32.48, 21.46, and 7.95 mg/kg, respectively, as indicated in Table 3. The Pb values obtained from the samples at the IP and the RP were marginally higher than the average Pb concentration in soil (14 mg/kg). The higher availability of Pb at the IP region is well supported by the findings of another study published in 2021. They stated that the closer to industries, the higher the level of Pb due to the high solid waste disposal, air emissions, wastewater discharges, and dry deposition transferred into the topsoil [31]. Pb concentrations in rural soil have also exceeded the normal range in soil and earth crust due to free emissions from automobile exhaust and continuous deposition near highways and roads. Pb accumulated on road surfaces because of soil accumulation, as well as ongoing contributions from urban traffic. A previous study demonstrated that high organic matter in soils can also cause Pb to be bound to organic exchange sites, eventually reducing its mobility and availability for uptake by plant roots, and resulting in lower concentrations of the metal found in plants [32]. Additionally, Pb from industrial sources is often present in the form of a more bioavailable soluble or exchangeable Pb, compared to Pb found geochemically in soil, which tends to be more tightly bound to soil particles [33]. Another study suggested that industrial activities, such as mining, smelting, and manufacturing, often produce acidic waste materials that can acidify the surrounding soil and increase Pb availability for plant uptake [34].

Table 3. Results of the present study compared with normal and critical metal concentrations in soil.

Heavy Metal	The Normal Range in Soils * (mg/kg)	Earth Crust Concentrations * (mg/kg)	Critical Soil Total Concentrations (mg/kg)	Present Study		
				RP (mg/kg)	TP (mg/kg)	IP (mg/kg)
Cu	2–250	50	60–125	100.55	85.24	77.43
Zn	1–900	75	70–400	76.59	84.39	123.78
Pb	2–300	14	100–400	21.46	7.95	32.48

Note. * Data mainly from [35,36].

In the overall results, the TP site has the lowest heavy metal concentrations (Cu, Zn, and Pb) compared to the other sites, even though the sampling points were located only 7 m from the rural track and 150 m away from the railway track. The low levels of Pb in the soil may be attributed to the low volume of vehicles passing through the area. This finding is well supported by a previous study [37], which found that samples taken near rural roads have lower Pb concentrations compared to areas with heavy traffic. Pb typically accumulates in the surface horizons of contaminated soils due to its strong chemical affinity for soil organic matter [32]. This study also found a link between soil organic matter (p0.05) and pH (p0.01), as well as Pb concentrations in the soil at the TP. A study that was conducted 1 km away from major roads found that Pb concentration in the soil ranged between 20 and 30 mg/kg. The majority of the metal was deposited within 30 to 50 m of the road, resulting in a significant reduction in Pb concentration in roadside soils [38].

The one-way ANOVA results revealed a significant difference in Cu concentrations among the study areas (F = 5.03, Ms = 664.02, p = 0.02). There were minor statistical differences in Zn concentrations across the study areas (F = 3.39, Ms = 3840.61, p = 0.06), while no significant difference in Pb concentration was detected (F = 1.76, Ms = 905.32, p = 0.21). In addition, Cu accumulation in soil can be ranked in the following decreasing order: IP < TP < RP. This behaviour can be explained by the fact that transportation sites may have higher levels of metal deposition from vehicular traffic, which can increase metal contents in soil and plants. According to a previous research, traffic-related metal deposition is greater near highways than in rural areas, and metal content in soil and vegetation is positively correlated with traffic density [39]. The presence of other contaminants may reduce the number of metals deposited in soil, which may possibly alter the physical and chemical properties of soil, making it less conducive to metal deposition. A published study discovered that the presence of PAHs might lower the number of heavy metals deposited in soil, which would most likely be due to metal adsorption and sequestration onto the PAHs [40].

The significant difference observed between Cu and Zn concentrations among the study sites could be attributed to the differences in the sources of Cu and Zn contamination. For instance, one study area might have more industries that release Cu and Zn into the environment than the other areas. Additionally, differences in soil pH, organic matter content, and other soil properties could also affect the availability and mobility of both metals in soil, leading to variations in their concentration among the study sites. The statistically significant differences in Pb concentrations across the study areas suggested that the sources of Pb contamination may be similar throughout the study sites. The primary sources of anthropogenic inputs, such as industrial emissions (leaded petrol, wastewater, and waste), agricultural outputs (herbicides, pesticides, fertilisers, and livestock dung), and urbanisation activities (traffic, building dust, and home waste) have all contributed to Pb contamination in soils [41]. Climate aspects such as wind speed and direction, as well as soil environment components such as pH and soil organic matter, have all influenced Pb deposition and migration [31]. Overall, the correlation between metal values and site type can be complicated, which can be influenced by several factors such as pollution levels, soil composition, and metal deposition.

3.2. Heavy Metals in Different Parts of the Plant

The current study discovered that the heavy metals under investigation were present in various plant parts. This finding is consistent with a previous research that detected heavy metals, including Pb, Cu, and Zn in various parts of the rice plant (*Oryza sativa* L.) [42]. Cu and Zn are essential micronutrients for plant growth and development. They play critical roles in photosynthesis, respiration, and other metabolic processes, including enzyme activation and gene regulation [30]. Hence, it is not surprising that these metals were present in all parts of the sampled rice plants, including the roots, stems, leaves, and grains. However, the presence of Pb in plant parts was of primary concern because this metal serves no purpose in plants and can become toxic to the plant during bioaccumulation. Pb can enter the plant through the roots and accumulate in the leaves, stems, and grains. Once inside the plant, Pb can interfere with various physiological and biochemical processes, including photosynthesis, respiration, enzyme activity, and protein synthesis. Pb toxicity can lead to stunted growth, chlorosis, necrosis, and even death of the plant [32].

Table 4 shows the mean concentrations of Cu in different parts of the rice plants at the RP, TP, and IP. Cu accumulated more in the shoots than in the roots and grains of paddy at the RP, with values of 183.42, 155.64, and 150.69 mg/kg, respectively. However, for samples collected at the TP, Cu accumulation was in the ranking order of root > grain > shoot, with concentrations of 105.89, 104.20, and 101.39 mg/kg, respectively. Meanwhile, Cu accumulated almost at the same level in the roots and grains, with values of 130.77 and 130.70 mg/kg, respectively, and a slightly lower level in the shoots (128.73 mg/kg) of samples collected at the IP. These results are consistent with the findings of earlier studies [42,43]. Copper is an essential nutrient for various metabolic processes [30]. It is known that Cu plays a vital role in root metabolism, which is why it is often concentrated in the roots of plants compared to in other tissues [44]. Furthermore, Cu has a strong affinity for the outer root membranes and displaces other ions from root exchange sites [44]. This can explain why roots have higher Cu concentrations based on samples collected from the TP and the IP compared to other plant parts. However, the concentration of Cu in soil did not show any significant relationship with rice grains among all study sites ($p < 0.05$), which is consistent with another study's findings [45].

Table 4. Mean concentrations of Cu in different parts of the plants.

Point	Root (mg/kg)	Shoot (mg/kg)	Grain (mg/kg)
RP	155.64	183.42	150.69
TP	105.89	101.39	104.2
IP	130.77	128.73	130.7

As per Table 5, the average concentrations of Zn in paddy parts collected at the RP reveal that Zn has accumulated more in the roots than in shoots and grains, with values of 87.28, 24.95, and 51.23 mg/kg, respectively. For samples collected at the TP, the shoots accumulated the most Zn, followed by the roots and grains, with values of 181.61, 152.50, and 125.95 mg/kg, respectively. Zn accumulation in the aboveground parts of rice plants, particularly the shoots, was mediated by the activity of specific Zn transporters, which were more abundant in the shoot than in other parts of the plants [46].

Table 5. Mean concentrations of Zn in different parts of the plants.

Point	Root (mg/kg)	Shoot (mg/kg)	Grain (mg/kg)
RP	87.28	24.95	51.23
TP	152.5	181.61	125.95
IP	249.11	180.37	133.14

Normally roots contain more Zn than the shoots, but Zn may be translocated from the roots and accumulated in the shoots, as similarly reported in a previous study [42]. Zn in grains is caused by continued root uptake of Zn during grain filling under Zn-sufficient conditions [47]. Moreover, results of samples at the IP showed that Zn accumulated more in the roots than in other plant parts, shoots, and grains, with values of 249.11, 180.37, and 133.14 mg/kg, respectively. In conclusion, there were no significant ($p < 0.05$) correlations between Zn bound to organic matter and soil properties at all sampling sites, which displayed results similar to past results [48].

Based on Table 6, samples collected at the RP show that Pb can be found only in the roots of the paddy. This may be because clay and organic matter contents in soil play a dominant role in the sorption of Pb in soil [30]. Samples collected at the TP showed that Pb has accumulated in the shoots, with the highest value of 42.54 mg/kg compared to in the shoots and grains, with values of 9.80 and 9.28 mg/kg, respectively. As the sampling sites were near the road, the leaves or stems may have absorbed heavy metals from atmospheric particles [33]. However, the results of samples at the IP showed that Pb has accumulated more in the roots than in other plant parts, namely shoots and grains, with values of 24.40, 17.63, and 6.54 mg/kg, respectively. This study found that lead levels in different parts of the rice plants were increased in the following order: root > shoot > grain. Thus, the majority of Pb content was taken up by the plant's roots, following Zn [42]. Furthermore, Pb level in the roots collected at the TP was higher than in the roots collected at the IP, which can be explained by the fact that the soil pH at the TP was more acidic (4.92) than at the IP (pH 6.50). The high pH of soil decreased Pb uptake, including root uptake of the rice plants, which is consistent with the results reported in a previous study [49]. Another study also reported a positive correlation between Pb concentration in soil and its concentration in plant tissue [50]. This finding is consistent with the findings of the current study at the TP, which also revealed a strong correlation between Pb concentrations in plant roots and shoots and soil Pb levels. The Pearson's correlation coefficient values at $p < 0.05$ for the roots and shoots were 0.81 and 0.90, respectively. These results showed that soil Pb concentration was increased with increasing Pb level in both the roots and shoots. However, these findings contradict the findings of a previous research that claimed that metals in aboveground tissues have a weaker positive correlation with metals in the substratum than with metals in underground tissues [51].

Table 6. Mean concentrations of Pb in different parts of the plants.

Point	Root (mg/kg)	Shoot (mg/kg)	Grain (mg/kg)
RP	0.16	0	0
TP	9.8	42.54	9.28
IP	24.4	17.63	6.54

Finally, heavy metal concentrations in rice plants at the RP show a decreasing order of Cu > Zn > Pb (Figure 2; Table 7). Cu concentration was the highest in all parts of the rice plants. In the case of samples from the TP, heavy metal concentrations in rice plants decreased in the following order: Zn > Cu > Pb. Zn concentration was the highest in all parts of the rice plants. Heavy metal concentrations in rice plants at the IP were in the decreasing order of Zn > Cu > Pb. Zn concentration was also the highest in all parts of the rice plants. The high Zn concentration was most likely the result of treated wastewater discharge from the nearby industry. Although the industrial wastewater was treated, heavy metals can still be found in it [52]. When comparing the three sites, the RP has the lowest Pb concentration compared to the other two sites; this could be due to the paddy field's distance from vehicle emission sources, which contributed significantly to the presence of Pb in soil.

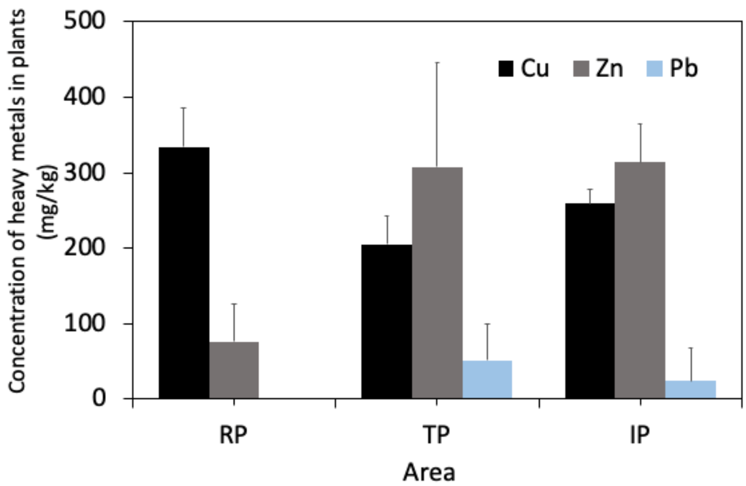

Figure 2. Concentration of heavy metals in plants.

Table 7. Results of the present study compared with normal and critical concentration of metals in plants.

Heavy Metal	Normal Range to Plants (mg/kg)	Critical Concentration in Plants (mg/kg)		The Present Study (mg/kg)		
		Upper	Lower	RP	TP	IP
Cu	5–20	20–100	5–64	334.11	205.59	259.43
Zn	1–400	100–900	100–400	76.18	307.55	313.51
Pb	0.2–20	30–300	-	0.16	51.82	24.17

3.3. Heavy Metal Bioaccumulation in Plants

As per Table 8, the BCF value of Cu at the RP for various parts of paddy was 1.55 (roots) and 1.82 (shoots), while the BAF value was 1.50 (grains). These BCF and BAF values of greater than 1 indicated that Cu can accumulate in all parts of the rice plants, including the roots, shoots, and grains. As a result, Cu bioavailability was extremely high in the collected soil and paddy samples. More of the Cu in the shoots collected at the RP and IP were translocated and partitioned in the grains, as evidenced by the higher BAF results of 1.50 and 1.69, respectively. The same trend was observed at the TP and IP, whereby the BCF values of Cu were 1.24 (roots) and 1.19 (shoots) for the former, and 1.69 (roots) and 1.66 (shoots) for the latter. The BAF values recorded at the TP and IP were 1.22 and 1.69 (grains), respectively. This means that Cu bioavailability was very high in the soil and paddy at both sites. This means that Cu in the soil was readily taken up and transported within the rice plants, leading to the potential toxic effects on the plant's growth and development. The TF values of < 1 at both the TP and IP, on the other hand, highlighted the fact that paddy roots have internal restrictions, resulting in low Cu translocation from the roots to the shoots. This may be due to the plant's natural defence mechanisms that prevented the uptake of excess metals, or due to the limited mobility of Cu in the soil. The mobility of Cu in soil was limited due to strong adsorption and precipitation [53], whereas organic complexes, such as phytochelatins and metallothionein that were formed in root cells, can increase Cu retention at the soil–root interface [54].

Table 8. Bioaccumulation of Cu in plant samples.

Area	BCF (Root)	BCF (Shoot)	BAF	TF
RP	1.55	1.82	1.50	1.18
TP	1.24	1.19	1.22	0.96
IP	1.69	1.66	1.69	0.98

Table 9 shows the BCF values for Zn in various parts of the paddy. At the RP, the BCF for Zn was higher in the roots (1.14) than in the shoots (0.33), while the BAF value was 0.67 (grains), which meant that paddy can only accumulate Zn in the root zone. Since the BCF and BAF values for shoots and grains were less than 1.00, Zn can be absorbed from the soil, but did not accumulate in these parts. The TF at the RP was extremely low at 0.29 (TF < 1), implying that Zn was only weakly transported from the roots to the shoots. Meanwhile, at the TP site, even though the BCF value of Zn in the roots (1.81) was lower than in the shoots (2.15) and the BAF was 1.49 (grains), the analysed paddy can still accumulate Zn in all parts of the plant (BCF > 1) with exceptionally high bioavailability of Zn in the soil and paddy. The TF value of 1.19 (>1) confirmed earlier findings that *Oryza sativa* can hyperaccumulate zinc from roots to shoots [42]. However, in this case, Zn translocated to the nonedible part of the plants, i.e., the shoots, where the potential for human exposure may be lower. At the IP, the BCF value of Zn was much higher in the roots (2.01) compared to in the shoots (1.46), while the BCF was 1.08 (grains). Hence, Zn can be accumulated in all parts of the paddy (BCF > 1). The TF result was slightly low (TF = 0.73), indicating that Zn was less efficiently translocated from the roots to the shoots (TF < 1).

Table 9. Bioaccumulation of Zn in plant samples.

Area	BCF (Root)	BCF (Shoot)	BAF	TF
RP	1.14	0.33	0.67	0.29
TP	1.81	2.15	1.49	1.19
IP	2.01	1.46	1.08	0.73

Table 10 shows that the BCF and BAF values of Pb in various parts of the paddy are less than 1, except for at the TP. At the RP, the bioavailability of Pb was extremely low in the soil; thus, the paddy was not able to absorb Pb. The results showed that Pb was not transported from the roots into the shoots (TF < 1). However, at the TP, the BCF value of Pb was relatively higher in the shoots (5.35) than in the roots (1.23). Meanwhile, the BAF value (grain) was 1.17, showing that xylem vessels could have transported the adsorbed Pb ions before bounding them into the cell walls of the shoots. Pb was strongly transported from the roots into the shoots, as indicated by the TF of 4.34 (TF > 1). The greater biomass of shoots in paddy resulted in the higher uptake of heavy metals in the shoots compared to in the roots [55]. In an analysis conducted at the IP site, the BCF values of Pb were 0.75 in the roots and 0.54 in the shoots, while the BAF was 0.20 in the grains. These results indicated that Pb was weakly transported from the roots to the shoots, since the TF value was 0.72 (<1). These results are consistent with the results of a previous study that stated that rice plants are non-accumulators of Pb [56]. Pb uptake in plants occurs primarily through the roots, which can take up significant amounts of Pb, while restricting its translocation to aboveground parts [57]. Another research discovered that the endodermis in roots can act as a barrier to prevent Pb from being transferred from the roots to other organs [58]. Furthermore, the compartmentalisation of $Pb^{(2+)}$ ions within the roots and their reduced transference to the shoots indicated that the rice plants have a well-adapted response to Pb-toxic environments [32]. These results showed that the soil was not polluted by Pb. However, Pb concentrations in rice grains surpassed the threshold values. This observation could be attributed to heavy metal deposition from motor vehicles and the fertilisers used in the paddy fields. These finding indicated that Pb was highly immobile in the soil and has a high affinity for soil organic matter.

Table 10. Bioaccumulation of Pb in plant samples.

Area	BCF (Root)	BCF (Shoot)	BAF	TF
RP	0.01	0	0	0
TP	1.23	5.35	1.17	4.34
IP	0.75	0.54	0.2	0.72

3.4. Effects of Heavy Metals on Rice Growth

Plant height is key in determining plant biomass, productivity, and growth rate [59]. An element deficiency or toxicity can reduce plant height and stunt its growth [60]. In this regard, the effect of Zn and Cu concentrations on plant height has been extensively researched in a variety of crops, including rice. The mean paddy height recorded at the RP, TP, and IP was 82.50, 93.27, and 102.33 cm, respectively. This improvement in plant height at the IP site with abundant Zn could be attributed to Zn's role as a transition metal in promoting cell elongation, enlargement, and division, which ultimately resulted in taller paddy [61]. These results are in line with the reported results of a previous study, which found that the application of Zn and NPK fertilisers significantly increased the height of rice cultivars [62]. In contrast, high levels of Cu in the soil can have adverse effects on plant growth and development. The height of mature rice plants decreased by 4.2% to 6.6% when the soil Cu levels were at 100 and 200 mg/kg, respectively [63]. This negative effect of Cu on plant height could be due to a disturbance in the balance of essential nutrients, as Cu can compete with other essential elements, such as Zn, Fe, and Mn [63]. This observation could explain the shortest rice plants being found at the RP among all study sites.

Lodging is a major concern in rice production, as it may reduce grain quality and quantity, resulting in significant yield losses [64]. Culm height, which is defined as the length from the plant's base to the panicle neck node, is an important trait for examining lodging resistance in rice and plays a key role in the final plant architecture [65]. The mean culm length of the paddy at the RP, TP, and IP was recorded as 53.99, 55.48, and 60.36 cm, respectively (Table 11). Several recent studies on the association between culm height and lodging resistance in rice have been published, where they observed that culm length was positively correlated to lodging resistance, implying that taller plants with longer culms were more resistant to lodging due to their greater culm diameter and strength [66]. Meanwhile, the flexibility of the culm would be more significant than its rigidity for the rice plants' lodging resistance [67]. The obtained results also revealed a favourable proportion between culm height and plant height in paddy, where a plant hormone known as brassinosteroid is essential in the regulation of plant height [68]. The relationship between these two traits can be influenced by a variety of factors, such as genotype, environmental conditions, developmental stage, and agricultural practices [69].

Table 11. Characteristics of paddy growth.

	Paddy Height (cm)	Culm Height (cm)	Length of Panicle (cm)	No. of Panicles	No. of Tillers
RP	82.50	53.99	19.80	11	12.30
TP	93.27	55.48	28.28	5.5	10.78
IP	102.33	60.36	23.97	9.83	6.70

The number of panicles in paddy can be affected by the timing of tiller emergence and the proportion of productive tillers. More panicles in paddy can enhance the number of filled spikelets, and as a result, the grain output of the plant. The number of panicles is regarded as the most vital component of rice yield, as it accounts for a considerable part of its yield variance. The results in Table 12 show that higher panicle lengths and plant heights can indirectly enhance the grain yield of rice by enhancing the number of spikelets per panicle, which can be exerted by the strong genotype of rice, as reported by another study [70]. Spikelets are an important part of the panicle because they contain both filled and unfilled grains. Increasing the number of filled spikelets can improve the plant's harvest index and overall grain yield. This study found that the paddy at the RP has the highest average number of panicles at 11, followed by paddy at the IP and TP, with values of 9.83 and 5.50, respectively. A significant relationship ($p < 0.05$, R = 0.87; $p < 0.01$, R = 0.96) was also observed between the number of panicles and grain yield in the samples collected at the IP and TP, indicating that a greater number of panicles can lead to a higher grain yield. Additionally, the mean panicle length for samples at the RP, TP, and IP was 19.80, 28.28, and 23.97 cm. respectively.

Table 12. Components of paddy yield.

	Grain Yield	Harvest Index	Average Weight of 1000 Paddy Spikelets	The Dry Weight of Plant Biomass (g)	No. of Fertile Spikelets
RP	12.11	0.38	25.4	31.84	475.67
TP	15.05	0.55	25.62	27.14	586.67
IP	28.42	0.56	26.16	50.9	1071

The number of tillers in rice plants can be used to assess their growth status. A deficiency or toxicity can reduce the number of tillers, which would reduce the area of the photosynthetic leaf surface, and, ultimately, lower the rice yield. Among the three locations being studied, paddy at the RP produced the most tillers (11.17), followed by at the IP (9.83) and at the TP (5.50). Thus, this study concluded that the RP, which contained a high Cu level, was not significant, as this site has produced paddy with the most tillers and panicles compared to the other locations. These results could be related to the fact that Cu as a transition metal was not consistently distributed throughout the rice grains, where it would form a complex on the protein–ligand chain in the rice grain cortex (embryo) [71]. With these findings on rice growth, farmers and related authorities can optimise resource utilisation, minimise yield-limiting constraints, and create a favourable environment for maximising rice output by making the best use of available resources.

3.5. Effects of Heavy Metals on Yield Components

Table 12 shows that the highest average weight of 1000 paddy spikelets is 25.40, followed by 25.62 and 26.16 g for samples collected at the RP, TP, and IP, respectively. This table shows that the IP has the highest grain yield. In contrast, the RP has the lowest grain yield and plant biomass, which could be due to the high Cu concentration (100.55 mg/kg) at the site. These results are consistent with the results of another study, which reported that the high soil Cu level decreased rice grain yield by up to 10% and significantly affected paddy biomass [42]. Previous study found that exposure to Zn can increase rice grain yield, which is in agreement with the higher grain yield observed in the IP [72]. Total dry weight is a crucial growth factor that measures a rice plant's photosynthetic performance. The present results showed that the IP has the highest total dry matter, which can be linked to its greatest grain yield compared to the other study areas.

The harvest index (HI) quantifies the economically useful portion of biological yield. The HI of the yields at the RP, TP, and IP is 0.38, 0.55, and 0.56, respectively, following an increasing order of IP > TP > RP, as shown in Figure 3a. Based on the current findings, paddy production can increase significantly with higher plant biomass and HI. The higher Pb concentration at the IP clearly did not affect the HI. MADA's efficient management system at both the TP and IP clearly resulted in greater harvest indices than at the RP. A high HI signified that the rice plants were using their energy efficiently to produce more grains relative to other vegetative parts. On the other hand, the lowest HI reported at the

RP could be elucidated by water stress. It can limit nutrition delivery to the grains, resulting in significantly reduced grain weight and increased empty grains during the blooming and head development stages [73]. As shown in Figure 3b, paddy at the IP has the highest number of fertile spikelets (1071), as stimulated by its higher biomass production. Previous research demonstrated that rice yield can improve with an increase in paddy biomass [74], while high sterile spikelets reduced the grain yield. The lower numbers of fertile spikelets found at the RP (475.67) and the TP (586.67) can be attributed to elevated Pb levels in the soil, which has been shown to enhance paddy sterility by 111.95% [75]. In conclusion, the yield components, such as the HI and the number of fertile spikelets, are crucial factors in paddy yield. Their values can be influenced by various factors, such as water stress and heavy metal contamination. These findings could contribute significantly to the understanding of heavy metal dynamics in paddy fields under diverse land use practices.

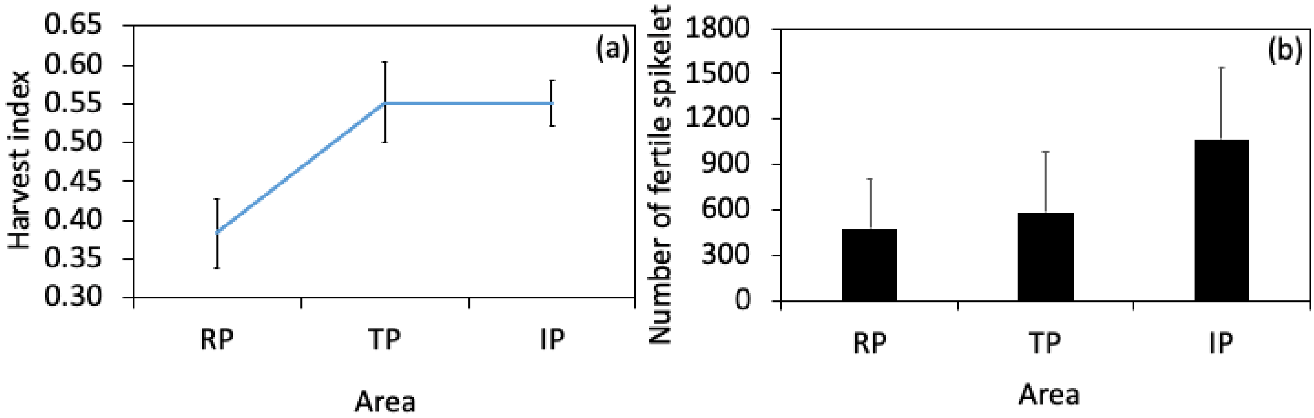

Figure 3. (**a**) Harvest Index of paddy at different study locations and (**b**) the number of fertile spikelets.

4. Conclusions

Heavy metal concentrations in soil can be affected by land use practises, with pH varying from 4.92 to 6.50 and organic matter content ranging from 5.20% to 6.45%. The highest Zn and Pb concentrations were found at the IP at 123.78 and 32.48 mg/kg, respectively, whereas the highest Cu concentration was found at the RP at 100.55 mg/kg. High Zn and Pb levels may be caused by industrialisation and urbanisation, whilst high Cu levels at the RP may be caused by agrochemicals. Metal concentrations were varied across all plant components gathered from these three study locations, with paddy collected at the RP having the greatest Cu level in the roots and grains, while paddy at the IP had the highest Zn concentration. Furthermore, the contamination analysis demonstrated that the paddy soils were slightly to moderately contaminated due to the presence of heavy metals. The bioaccumulation findings suggest that plant mechanisms connected to heavy metal translocation to the grains as an edible component can be further studied to prevent excessive metals from accumulating in grains under high root and shoot intakes. However, this study may have certain drawbacks, such as an insufficient sample size, data collection covering only a short period of time due to time constraints, and a lack of access to detailed information on land use practices, which may impede a thorough analysis.

Nonetheless, this study can be crucial for establishing the level of heavy metal pollution in paddy soils and its potential repercussions towards paddy growth. This means that although pollution does exist in these areas, it is not dangerously high. These indicators underline the importance of restoration and repair actions for preventing future soil quality decline and enhancing paddy yields. Thus, this study highlights the necessity of continual monitoring and regulating potential heavy metal contamination and sources in agricultural areas to assure the safety of agricultural products and preserve human health.

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