Effective Amendments on Cadmium, Arsenic, Chromium and Lead Contaminated Paddy Soil for Rice Safety

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Abstract: Contamination of paddy soils with heavy metals and metalloids poses a risk to human health through the food chain. For safe agricultural production in contaminated paddy soils, “in situ” remediation through the unavailability of heavy metals from contaminated soils was investigated in order to develop cost-effective and eco-friendly approaches for soils contaminated with complexed heavy metals (HMs) and metalloids. In the present study, the effectiveness of different soil amendments, including sulfur-containing materials (hair or cysteine), manganese compounds as an antagonist and an oxidant, zeolite and iron oxide as adsorbents, and their combinations through coating or modification, was investigated by a pot experiment with rice plants and chemical analysis. Two levels of each treatment were made, termed X1 and X2, which were a single and double dose of each treatment respectively, while CaCO3 was used as a basal and pH enhancement amendment in all treatments to identify the best combination of the above treatments in the in situ remediation of heavy metals and metalloids. The rice plants were kept under the flooded condition. Results indicated that the bioavailability of As, Cd, Cr, and Pb in soil was significantly decreased with level two (X2) of iron oxide coated with modified hairs (T7 × 2) followed by level two (X2) of zeolite coated with KMnO4. The iron oxide coated with modified hairs (X2) decreased the concentrations of heavy metals and metalloids in rice plants in the order Pb > As > Cr > Cd by 81%, 80%, 79% and 46%, respectively, followed by zeolite coated with KMnO4 (X2) in the order Pb > As > Cr > Cd by 78%, 76%, 71%, and 31%, respectively, to control. The available content of these elements in soil was decreased in the order As>Cr>Pb>Cd by 67%, 66%, 64% and 60%, respectively, through iron oxide coated with modified hairs, followed by zeolite coated with KMnO4 in the order Pb > Cr > Cd > As by 57%, 53%, 50%, and 46%, respectively, to control, which can explain the decreased bioavailability by these amendments. In addition, the maximum shoot dry and leaf area were noticed in the pots treated with iron oxide coated with modified hairs and zeolite coated with KMnO4. There is potential to use modified hairs (MHs) with iron oxide and KMnO4-coated zeolite as soil amendments to develop a cost-effective and efficient “in situ” remediation technology for As, Cd, Pb, and Cr-contaminated rice paddy soils, especially for the soils with complex contamination by Cd and As.

Keywords: amendments; toxic elements; paddy soil; eco-friendly; cost-effective and economical

1. Introduction

Worldwide, soil and food crops are contaminated by uncontrolled industrialization, unsustainable urbanization, and intensive agricultural practices. These problems have resulted in a
global environmental problem of contamination with heavy metals and metalloids. Together these heavy metals and metalloids are known as toxic elements (TEs) that are a threat to human health and soil quality. The TEs released through different natural and anthropogenic processes finally reach the soil, as soil is the ultimate source of waste disposal. The TEs present in the discarded waste material enter our water resources, and end up in our food chain. The use of environmentally friendly and cost-effective amendments are therefore more necessary for the remediation of moderate to high TE contaminated soil. A large amount of organic waste is produced every day, which can be used with inorganic materials for plants’ nutrient supply. Organic material alone and in combination with different inorganic materials has the capability of heavy metal remediation [1]. Other commonly used amendments include lime, phosphates, zeolites, iron oxides, manganese oxides and organic amendments like biochar, keratinous material, microbes, and bio-molecules [1–6]. Keratinous materials such as wool and hairs are inexpensive and abundantly produced materials that can remove HMs due to the presence of amino, carboxyl, hydroxyl, and sulfur-containing functional groups [7]. Cysteine also contains groups like thiol, amine, and carboxylic acid that can interact with, bind and detoxify TEs. Cysteine has the capability of forming bonds with metals because it contains a thiol group that forms metal or hydrogen bonds through lone pairs of an electron between a local dipole moment [8,9]. Zeolite also has been reported to remove HMs from sewage [10], water [11], and soil [12]. The negative charge of zeolite, and an increase in soil pH and cation exchange capacity (CEC), are mainly responsible for stabilization of HMs in soil [13]. The HMs in the soil can be (i) dissolved (in soil solution), (ii) exchangeable (in organic and inorganic components), (iii) structural components of the lattices in soils, and (iv) insolubly precipitated with other soil components [14]. The uptake of TE is mainly dependent upon the mobility and availability of these elements, so in this context, the first two are very important in their absorption and utilization by plants. The overall mobility and availability of HMs in the soil are controlled by adsorption and desorption characteristics [15]. Antoniadis [16] reported that soil properties, such as organic matter content, pH, soil redox potential (Eh), CEC, clay minerals, calcium carbonate, and Fe and Mn oxides, control the adsorption and desorption of TEs. It is well known that the organic amendments can immobilize HMs in the soil through adsorption reactions or forming stable complexes [17]. Due to the decomposition of organic matter, the effect of organic amendments varies with the bioavailability of elements over time.

Exposure to toxic elements, like Ni, Cd, Cr, and Hg, can cause bone fractures, hypertension, kidney dysfunction and even cancer in humans [18]. Additionally the most phytotoxic HMs are considered to be Cd and Pb as they constrain plant growth when cultivated on contaminated soil [19]. The co-exposure of crops to HMs may exhibit more toxicity than individual HMs [20,21]. The soil of Liuyang city, Hunan province, which has been reported contaminated with HMs, was used in these experiments [22]. The soil of several regions in China has been reported to be contaminated with HMs [23,24].

In the present study, the effectiveness of different soil amendments, including sulfur containing materials (hair or cysteine), manganese compounds as an antagonist and an oxidant, zeolite and iron oxide as adsorbents, and their combinations through coating or modification, were investigated by a pot experiment with rice plants and chemical analysis. New soil amendments were created for remediation of HMs through the use of these materials alone and in different combinations. The effect of surface coating on different materials should be tested to increase the surface area for complexation and the negative charge for adsorption. These amendments, which are practical, eco-friendly, and cost-effective in-field applications, are recommended after the comparison between all treatments.

2. Materials and Methods

2.1. Soil Sampling and Characteristics of the Experimental Location

The paddy soil from the rice paddy field was collected at a depth of 20 cm from Liuyang city of Hunan province, which is a subtropical red soil region of southern China. Liuyang city lies at 28°09′49″ N and 113°38′36″ E with a subtropical monsoon climate zone, 17.3 °C annual average
temperature, and 1562 mm annual precipitation. The soil of Liuyang city is mainly derived from the Quaternary Red Clay and recent alluvial deposits and is traditionally classified into red soil and paddy soil [25].

A pot experiment was conducted at the Graduate School Chinese Academy of Agriculture Sciences, 100081, Beijing, China. The experiment was conducted in a greenhouse with fluctuating temperatures between 35 and 20 °C day and night, 24 h light (roughly 13 h day/11 h night, 500–1100 µmol m−1 s−1), and 70% relative humidity was maintained throughout the growth period.

2.2. Soil Sampling

The paddy soils of Liuyang city have been reported to be contaminated with heavy metals and metalloids, especially cadmium, lead, chromium, and arsenic [26]. About 350 kg of soil from Hunan province was air-dried in shade at about 20 °C to 25 °C. The dried soil samples were passed through a 2-mm sieve. Physical and chemical properties of the soil were analyzed before the application of fertilizers and amendments. The soil had the following properties: pH (10:1 distilled water:soil) 4.92, organic matter 34.18 ± 1.67 g kg−1 [27], total N 2.31 ± 0.73 g kg−1 [28], extractable P 16.46 ± 1.10 mg kg−1 [29], total P 1.14 ± 0.51 g kg−1 [30], extractable K 100 ± 4.31 mg kg−1 [29], total K 19.69 ± 1.52 g kg−1 [31], total Cd 0.95 mg ± 0.21 kg−1, total Pb 494 mg ± 15.67 kg−1, total Cr 70 ± 5.01 mg kg−1 and total As 98 ± 4.82 mg kg−1 [32]. The Na2-EDTA and NH4H2PO4 extractable content of HMs was As 34.3 ± 3.84 mg kg−1, Cr 4.00 ± 0.37 mg kg−1, Pb 7.9 ± 0.61 mg kg−1, and Cd 0.17 ± 0.03 mg kg−1 [33].

2.3. Pot Experiment

A pot experiment in the greenhouse was conducted at the Chinese Academy of Agriculture Sciences, Beijing, China during August and October 2018. The rice (Oryza sativa L.) variety Huang Huazhan was used. Seeds of rice were surface sterilized with 18% H2O2 for 5 min and then germinated in moist perlite. The rice nursery was kept under a flooded condition and under continuous observation. All treatments were applied one month before transplanting the seedlings and were kept under flooded conditions during the whole month. After 3 weeks, uniform seedlings were selected and transplanted to PVC pots containing the contaminated soil. The pots had 20 cm diameter and 26 cm height, while each pot contained 5 kg of soil. There were nine treatments in total with two different levels of X1 and X2, which made a total of 51 pots. X2 was double that of X1 in treatment and lime application rate only (Table 1). The details of the experimental design are given below in Table 1. All pots were treated equally with surface standing water of 2–3 cm throughout the growth period. Each pot except T1 received the fertilizer as a basal dose at the rate of one gram of urea (N content 46%) and 1 g of K2HPO4·3H2O as nutrient source. Only urea was applied in split doses to fulfill the nitrogen requirements in a timely manner. The temperature and light described above were maintained until the harvesting of the crop. The above ground portion of each rice plant was harvested at the final tillering stage (60 days after transplantation).

| Treatments | Rate for Level 1 (X1) (g/pot) | Rate for Level 2 (X2) (g/pot) |
|------------|-------------------------------|-------------------------------|
| T1         | 0                             | 0                             |
| T2         | 5 g CaCO3                     | 10 g CaCO3                    |
| T3         | 0.25 g modified hairs + 5 g CaCO3 | 0.5 g modified hairs + 10 g CaCO3 |
| T4         | 0.25 g cysteine + 5 g CaCO3   | 0.5 g cysteine + 10 g CaCO3   |
| T5         | 0.5 g MnSO4 + 5 g CaCO3       | 1 g MnSO4 + 10 g CaCO3        |
| T6         | 0.5 g KMnO4 + 5 g CaCO3       | 1 g KMnO4 + 10 g CaCO3        |
| T7         | 0.5 g modified hairs-FeO3 + 5 g CaCO3 | 1 g modified hairs-FeO3 + 10 g CaCO3 |
| T8         | 0.5 g zeolite-modified hairs-FeO3 + 5 g CaCO3 | 1 g zeolite-modified hairs-FeO3 + 10 g CaCO3 |
| T9         | 0.5 g zeolite-KMnO4 + 5 g CaCO3 | 1 g zeolite-KMnO4 + 10 g CaCO3 |
2.4. Sample Preparation and Analysis Methods

After harvest, the above-ground portion of each rice plant was weighed. The leaf area was calculated [34] and washed thoroughly with tap water and deionized water several times. The plants were oven-dried at 70 °C to constant weight and were ground in a stainless steel grinder (FW-100, Tianjin Taishite Instrument Ltd., Tianjin, China) to pass through a 100-mesh sieve.

2.5. Preparation of the Amendment Materials

Human hair was collected from different salons (it was collected randomly in Beijing, China, and contained both male and female hair of different ages), washed with distilled water, and dried at room temperature. The hair was cut in small pieces of 1 mm using an electrical cutter and immersed into (0.1 mol/L) NaOH for 5 h. The solution was filtered, washed several times with distilled water, and allowed to dry at room temperature.

The surface coating of iron oxide with MHs was prepared according to the procedure described by Kataby et al. [35]. Ten grams of MH powder was dissolved in 250 mL ethanol and then 10 g of iron oxide was added into it. The mixture was shaken for 22 h, centrifuged, and washed repeatedly in ethanol (8–10 times) in a glove-box, and dried in vacuum for 2 h.

The zeolite was surface coated with iron and hairs through the procedure described by Zhang et al. [36]. About ten grams of MHs, iron oxide and zeolite were added to toluene. The mixture was refluxed for 24 h at 110 °C. Then the resulted product was filtered, washed with toluene followed by ethanol, and finally dried overnight at room temperature.

The procedure of Mohsenibandpei et al. [37] was followed for the surface coating of zeolite with KMnO₄. One gram of KMnO₄ was dissolved in 250 mL water and then nine grams of zeolite was added into it. The mixture was boiled at 100 °C for 10 min. The resulting product was washed with distilled water and dried at room temperature.

The weight of all the amendments were taken according to the experimental design and were added to the dry soil and mixed thoroughly with the soil using a small spade. All this happened one month before the transplantation and all the pots with and without amendments were kept under flooded conditions.

2.6. Analysis of Total Elements in Plant and Soil Samples

Five grams of air dried and grinded soil samples were taken into 100 mL Teflon tubes (Jiangsu Yihao Fluorine Plastic Co., Ltd, Yancheng, China) for analysis of As, Pb, Cr, and Cd. About 9 mL of concentrated and highly pure HNO₃ was added to the Teflon tubes (followed by 4 mL of H₂O₂ for plant samples or 4 mL HF for soil samples) and were left standing overnight. The samples were digested in a microwave digestion oven (Mars 5, CEM Corporation, Matthews, NC, USA) at 180 °C for 30 min. The quality control of the digestion and analysis process was carried out through standard reference material tea (GBW07605). Teflon tubes were uncapped carefully, allowed to cool, filtered and diluted with ultra-pure water up to 50 mL. The concentration of elements in the digestion solution were analyzed by ICP-MS (Agilent-7500, Agilent Technologies Co. Ltd., Palo Alto, CA, USA).

2.7. Analysis of Extratcable Elements in Soil Samples

The procedure described by Burridge and Hewitt [33] was followed for extraction of Cd, Pb, and Cr. Soil (5 ± 0.01 g) was taken in a 50 mL conical centrifuge tube and 25 mL of 0.05 mol/L Na₂-EDTA solution was added to the tubes. The pH of the solution was adjusted to 7.0 using NaOH. The solution was shaken for 2 h in the oscillating instrument and centrifuged at 2000 g for 20 min in a centrifuge. A medium-speed filter paper was used to filter the supernatant. The concentration of elements was determined using ICP-MS (Agilent-7500, Agilent Technologies Co. Ltd., Palo Alto, CA, USA) [38].

The procedure described by Wenzel et al. [39] was followed for extraction of As. NH₄H₂PO₄ was weighted accurately at 5.7515 g (relative molecular mass 115.03 g/mol) in a 1 L beaker. Ultrapure water (400 mL) was added to the beaker. When the NH₄H₂PO₄ was completely dissolved, the solution was transferred into a 1 L volumetric flask. The soil of one gram was taken in a 50 mL conical
centrifuge tube and 25.0 mL of extractant was added to the tube. The solution was shaken at 60 rpm for 16 h in a constant temperature shaker. The solution was centrifuged at 2200 rpm for 15 min and was filtered with a 0.45 µm filter paper. An atomic fluorescence spectrometer (AFS) (AF-620, Beijing Rayleigh Analytical Instrument Company, Beijing, China) was used to determine the amount of arsenic in the soil.

2.8. Soil pH and Eh

The soil pH and Eh were noted through a soil pH and Eh meter (Combo pH & Eh. HANNA instruments Woonsocket, RI USA).

2.9. Statistical Analysis

One way ANOVA was conducted to compare different treatment means using SPSS 26.0. All the results are presented as an average of three replicates and standard error were estimated using Microsoft Excel 2013. The principal component analysis (PCA) and correlation analysis were conducted through R software (R version 3.6.1, R Core Team, Vienna, Austria) and used for graphical presentation.

3. Results

Statistical analysis of the data revealed that the application of iron oxide, modified hair, zeolite, and manganese base complexation agents to the soil significantly reduced \( (p < 0.05) \) the extractable content of HMs in the soil and their concentration in the plant tissues. The plant available content of HMs was reduced due to the transformation or change in their speciation in the amended soils. According to Figure 1 Figure 2 Figure 3 and Table 2 Table 3 Table 4, among all the amendments in the pots, those that received both levels of T7 and T9 were highly effective in reducing the concentration of HMs in rice plants. T7 \( \times \) 2 was the most effective in reducing the concentration of HMs followed by T9 \( \times \) 2. Results on the base of the effectiveness of treatments are given below.

3.1. Principal Component Analysis and Interpretation

The principal component analysis (PCA) clustering techniques are usually performed to identify the difference among the treatments and to show a correlation among the variables. PCA interprets data more meaningfully than other tools of its kind and is a most useful tool for exploratory data analysis and for making predicative modes. This process is generalized by main component analysis and allows you to perform it on a data set with many different dimensions. The analysis creates a new set of axes, rotated in such a way to align maximally with variance, thus distilling high-dimensional data into a few linear combinations. The PCA in Figure 1 shows the treatment effect on the concentration of different elements in rice plants. The total variability of the first two axes of PC-1 and PC2 revealed about 91.7%, as shown in Figure 1. Variability was observed in all elements as described in the PCA analysis. The HMs such as As, Cr, Cd, and Pb were significant components between two treatment levels. This fluctuation of different treatments poses a major impact on these metal’s complexation in plant concentrations. The variation in the first principal component PC1 was mainly attributed to As, Cr, Fe, Mn, Pb, and Cd, for which the eigenvectors are 4.51, 0.98, 0.26, 0.14, 0.05 and 0.03, respectively. The variation in the PC2 was attributed to As and Pb with eigenvectors of 2.5 and 0.48, respectively. Heavy metals in the plants showed positive associations with each other and negative associations with Fe and Mn. The right axis of the PCA graph showed that As, Pb, Cr, and Cd showed a positive correlation with each other, while Mn and Fe showed a negative relationship. An increase in Fe concentration of T7 caused a decrease in Cd, As, Mn, and Pb. The same pattern was noted with Mn in T9, T5, and T6. The high concentrations of Cd, As, Pb, and Cr were noted in both levels of T1, T2, and T3.

The results of the total concentrations of the above-mentioned elements in the soil is presented in Figure 2, which reveals that the first two components (PC1 and PC2) accounted for 90.4% of the total variation. In both biplots, the first component (PC1), which accounted for 68.8% of the total
variation, illustrated that the highest contribution to total variation was from Cd content (21.30%), followed by Pb (20.23%), Cr (19.90%), As (18.45%), and Fe (10.64%), whereas Mn (9.45%) made the smallest contribution. The main attribute in variation were As, Cr, Fe, Mn, Pb and Cd and their eigenvectors are 4.12, 1.29, 0.24, 0.17, 0.08 and 0.07, respectively. The graph at the right axis of PCA shows a positive correlation among Mn, Cr, Pb, As and Fe. Mn and Cr show a positive correlation with both levels of T9 and Fe–As with T7. The concentration of Mn and Fe increased the concentration of TEs.

Heavy metals were scattered in biplots by origin, as shown in Figure 3. The first two axes (i.e., PC1 and PC2) accounted for 91.6% of the total variability. The variation in the first principal component PC1 70.4% was mainly attributed to As, Cr, Fe, Mn, Pb, and Cd, for which the eigenvectors are 4.22, 1.27, 0.29, 0.11, 0.05 and 0.03, respectively. The variation in the PC2 component was attributed to As, Cr and Pb, with eigenvectors of 1.29, 0.41 and 0.29, respectively. In soil solution, the heavy metals showed positive associations with one another, while Fe and Mn showed a negative association. This result revealed that As, Cr, Pb, and Cd showed a positive correlation with each other and a negative correlation with Fe and Mn. The high concentrations of As, Cr, Pb, and Cd were noted in T1, T2, and T3. Fe and Mn are at the left axis and have a negative relationship with the other elements.

3.2. The Content of Toxic Elements in Plants and Soil Affected by Modified Hairs-Fe2O3 (X2)

The mean value along with the standard error of total Cd concentration in the soil was 0.72 mg kg\(^{-1}\). The total concentration of As, Cr, and Pb was 67 mg kg\(^{-1}\), 43 mg kg\(^{-1}\), and 378 mg kg\(^{-1}\), respectively. The order of the decrease of toxic elements in plant tissues was Pb > As > Cr > Cd, which decreased by 81%, 80%, 79%, and 46%, respectively. The order of extractable toxic elements decrease in soil was As > Cr > Pb > Cd, which decreased by 67%, 66%, 64%, and 60%. The highest iron content was also recorded in these pots. The mean concentration of iron in the plant tissues was 16 mg kg\(^{-1}\), while 1.5 mg kg\(^{-1}\) was recorded in the control plants. Extractable Fe in these was 32 mg kg\(^{-1}\), while 6 mg kg\(^{-1}\) was recorded in control pots. The total concentration of Fe in these pots was 71 mg kg\(^{-1}\), while 15 mg kg\(^{-1}\) was recorded in control pots. A maximum Eh of 297 mV, pH of 7.6, leaf area of 3.2 cm\(^2\), wet weight of 37 g and dry weight of 8.5 g were noted in pots with treatment modified hairs-Fe2O3 (X2). The highest iron content was also recorded in these pots. The details of the Eh and pH of soil and leaf area, dry, and wet weight of rice plants is given in Table 5.

![Figure 1](image-url). Principal component analysis of total element’s concentration in rice plants. PC: Principal Components.
3.3. The Content of Toxic Elements in Plants and Soil Affected by Zeolite-KMnO₄ (X2)

The mean value along with the standard error of total Pb concentration in the soil was 375 mg kg⁻¹. The total concentration of As, Cr, and Cd was 58 mg kg⁻¹, 41 mg kg⁻¹, and 0.70 mg kg⁻¹, respectively. The order of the decrease in toxic elements in plant tissues was Pb > As > Cr > Cd, which decreased by 78%, 76%, 71%, and 31%, respectively. The order of the decrease in extractable toxic elements in the soil was Pb > Cr > Cd > As, which decreased by 57%, 53%, 50%, and 46%, respectively.
An Eh of 270 mV, pH of 8.01, leaf area of 3 cm², wet weight of 33 g and dry weight of 7.5 g were noted in pots with treatment T9 × 2. High manganese content was also recorded in these pots. The mean concentration of Mn in plant tissues was 12 mg kg⁻¹, while 4 mg kg⁻¹ was recorded in control plants. The mean value of soil extractable Mn in these pots was 23 mg kg⁻¹ and 10 mg kg⁻¹ was recorded in control pots. The total concentration of Mn in the soil of these pots was 294 mg kg⁻¹ and 209 mg kg⁻¹ was noted in control pots.

3.4. The Content of Toxic Elements in Plants and Soil Affected by Modified Hairs-Fe₂O₃ (X1)

The mean value along with the standard error of total As concentration in soil was 61 mg kg⁻¹. The total concentration of Pb, Cr, and Cd was 362 mg kg⁻¹, 41 mg kg⁻¹, and 0.7 mg kg⁻¹, respectively. The order of the decrease in toxic elements in plant tissues was Pb > As > Cr > Cd, which decreased by 71%, 65%, 56%, and 36% respectively. The order of the decrease in extractable toxic elements in the soil was As > Cr > Pb > Cd, which decreased by 66%, 56%, 53%, and 52%, respectively. A soil Eh of 267 mV, pH of 7.87, leaf area of 2.86 cm², wet weight of 26.5 g and dry weight of 6.1 g were noted in pots with the above treatment. The mean concentration of iron in plant tissues was 10 mg kg⁻¹, while 1.5 mg kg⁻¹ was recorded in control plants. The mean value of soil extractable Fe was 25 mg kg⁻¹ and 6 mg kg⁻¹ was recorded in control pots. The total concentration of Fe was 49 mg kg⁻¹ and 15 mg kg⁻¹ in pots containing soil without any amendments.

| X1   | As (mg kg⁻¹) | Cr (mg kg⁻¹) | Fe (mg kg⁻¹) | Mn (mg kg⁻¹) | Pb (mg kg⁻¹) | Cd (mg kg⁻¹) |
|------|--------------|--------------|--------------|--------------|--------------|--------------|
| T1   | 43 ± 2       | 34 ± 3       | 15 ± 1       | 210 ± 17     | 308 ± 10     | 0.51 ± 0.02  |
| T2   | 44 ± 1       | 34 ± 3       | 16 ± 1       | 218 ± 18     | 309 ± 15     | 0.53 ± 0.02  |
| T3   | 54 ± 3       | 37 ± 3       | 23 ± 2       | 231 ± 16     | 341 ± 16     | 0.56 ± 0.01  |
| T4   | 52 ± 2       | 36 ± 2       | 29 ± 2       | 233 ± 18     | 326 ± 18     | 0.59 ± 0.01  |
| T5   | 51 ± 2       | 39 ± 2       | 14 ± 1       | 276 ± 17     | 235 ± 10     | 0.59 ± 0.01  |
| T6   | 49 ± 1       | 39 ± 2       | 15 ± 1       | 286 ± 18     | 354 ± 10     | 0.59 ± 0.01  |
| T7   | 61 ± 3       | 41 ± 3       | 48 ± 3       | 251 ± 10     | 362 ± 14     | 0.69 ± 0.01  |
| T8   | 53 ± 2       | 36 ± 3       | 39 ± 3       | 221 ± 11     | 352 ± 11     | 0.59 ± 0.01  |
| T9   | 55 ± 2       | 39 ± 3       | 23 ± 2       | 282 ± 10     | 361 ± 16     | 0.68 ± 0.01  |

| X2   | As (mg kg⁻¹) | Cr (mg kg⁻¹) | Fe (mg kg⁻¹) | Mn (mg kg⁻¹) | Pb (mg kg⁻¹) | Cd (mg kg⁻¹) |
|------|--------------|--------------|--------------|--------------|--------------|--------------|
| T1   | 43 ± 1       | 34 ± 2       | 15 ± 1       | 210 ± 11     | 308 ± 10     | 0.51 ± 0.02  |
| T2   | 51 ± 1       | 35 ± 2       | 17 ± 1       | 225 ± 14     | 310 ± 11     | 0.56 ± 0.01  |
| T3   | 58 ± 2       | 39 ± 3       | 25 ± 2       | 234 ± 14     | 350 ± 9      | 0.55 ± 0.01  |
| T4   | 53 ± 3       | 37 ± 3       | 25 ± 1       | 273 ± 13     | 340 ± 7      | 0.59 ± 0.01  |
| T5   | 55 ± 2       | 42 ± 4       | 16 ± 1       | 294 ± 15     | 329 ± 10     | 0.60 ± 0.01  |
| T6   | 47 ± 2       | 40 ± 3       | 16 ± 1       | 307 ± 14     | 366 ± 11     | 0.61 ± 0.01  |
| T7   | 67 ± 2       | 42 ± 3       | 71 ± 1       | 267 ± 13     | 378 ± 7      | 0.71 ± 0.01  |
| T8   | 57 ± 3       | 37 ± 2       | 45 ± 2       | 223 ± 13     | 351 ± 7      | 0.59 ± 0.01  |
| T9   | 58 ± 2       | 41 ± 3.12    | 26 ± 1.38    | 294 ± 12     | 375 ± 13     | 0.70 ± 0.01  |

3.5. The Content of Toxic Elements in Plants and Soil Affected by Zeolite-KMnO₄ (X1)

The mean value along with the standard error of total Cr concentration in soil was 39 mg kg⁻¹. The total concentration of Pb, As, and Cd was 361 mg kg⁻¹, 55 mg kg⁻¹, and 0.7 mg kg⁻¹, respectively. The order of the decrease in toxic elements in the plant tissues was Pb > As > Cr > Cd, which decreased by 74%, 67%, 65%, and 27%, respectively. The order of the decrease in extractable toxic elements in the soil was Cr > Pb > Cd > As, which decreased by 46%, 44%, 43%, and 33%, respectively. An Eh of 272 mV, pH of 7.89, leaf area of 2.58 cm², wet weight of 23 g, and dry weight of 4.7 g were noted in the pots treated with the above treatment. The mean concentration of Mn in plant tissues was 11 mg kg⁻¹, while 4 mg kg⁻¹ was recorded in control plants. Extractable Mn was 21 mg kg⁻¹ and 10 mg kg⁻¹ was recorded in control pots soil. The total concentration of Mn was 282 mg kg⁻¹ and 209 mg kg⁻¹ in pots containing soil without any amendments.
Table 3. Mean value and standard error of total elements (mg kg\(^{-1}\)) in rice plants.

| X1 | As (mg kg\(^{-1}\)) | Cr (mg kg\(^{-1}\)) | Fe (mg kg\(^{-1}\)) | Mn (mg kg\(^{-1}\)) | Pb (mg kg\(^{-1}\)) | Cd (mg kg\(^{-1}\)) |
|----|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| T1 | 3.7 ± 0.2           | 6.6 ± 0.3           | 1.5 ± 0.3           | 4.1 ± 0.3           | 1.1 ± 0.2           | 0.33 ± 0.02         |
| T2 | 3.4 ± 0.3           | 6.3 ± 0.2           | 1.4 ± 0.2           | 3.4 ± 0.3           | 0.8 ± 0.2           | 0.30 ± 0.01         |
| T3 | 2 ± 0.3             | 4.6 ± 0.1           | 2.2 ± 0.2           | 4.8 ± 0.3           | 0.3 ± 0.1           | 0.26 ± 0.01         |
| T4 | 2.6 ± 0.3           | 5.3 ± 0.2           | 2.2 ± 0.1           | 5.8 ± 0.3           | 0.4 ± 0.2           | 0.25 ± 0.02         |
| T5 | 1.3 ± 0.2           | 3.6 ± 0.4           | 2 ± 0.3             | 11 ± 0.3            | 0.4 ± 0.1           | 0.23 ± 0.01         |
| T6 | 1.6 ± 0.2           | 3.6 ± 0.4           | 2.2 ± 0.3           | 12.3 ± 0.3          | 0.4 ± 0.1           | 0.23 ± 0.01         |
| T7 | 1.3 ± 0.3           | 2.6 ± 0.4           | 10 ± 0.6            | 7.8 ± 0.3           | 0.3 ± 0.10          | 0.201 ± 0.02        |
| T8 | 2.6 ± 0.3           | 3.7 ± 0.5           | 4.4 ± 0.1           | 4.5 ± 0.2           | 0.4 ± 0.05          | 0.273 ± 0.02        |
| T9 | 1.2 ± 0.4           | 2.3 ± 0.4           | 3.5 ± 0.1           | 11.3 ± 0.3          | 0.2 ± 0.07          | 0.239 ± 0.01        |

3.6. The Content of Toxic Elements in Plants and Soil Affected by MnSO₄ (X2)

The T5 × 2 and T6 × 2 treatments, mainly applied as Mn sources, produced good results in reducing toxic elements significantly (p < 0.05). T5 × 2 reduced the toxic element concentration in the plants in the order: As > Pb > Cr > Cd by 72%, 66%, 53%, and 30%, respectively. The extractable content of elements was reduced in the order: Cd > As > Pb > Cr by 49%, 42%, 36%, and 30%, respectively. The mean value, along with the standard error, of total Pb concentration in the soil was 328 mg kg\(^{-1}\). The total concentration of As, Cr, and Cd was 56 mg kg\(^{-1}\), 42 mg kg\(^{-1}\), and 0.6 mg kg\(^{-1}\), respectively. A mean soil pH of 7.89, Eh of 283 mV, leaf area of 2.63 cm\(^2\), wet weight of 21 g and dry weight of 5.2 g were noted in the pots treated with the above amendment on the day of harvest. The mean concentration of Mn in plant tissues was 12 mg kg\(^{-1}\), while 4 mg kg\(^{-1}\) was recorded in control plants. The mean value of extractable Mn in the soil was 22 mg kg\(^{-1}\) and 10 mg kg\(^{-1}\) was recorded in control pots. The total concentration of Mn was 294 mg kg\(^{-1}\) and 209 mg kg\(^{-1}\) in pots containing soil without any amendments.

Table 4. Mean value and standard error of extractable elements (mg kg\(^{-1}\)) in the soil.

| X1 | As (mg kg\(^{-1}\)) | Cr (mg kg\(^{-1}\)) | Fe (mg kg\(^{-1}\)) | Mn (mg kg\(^{-1}\)) | Pb (mg kg\(^{-1}\)) | Cd (mg kg\(^{-1}\)) |
|----|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| T1 | 33 ± 0.5            | 3.6 ± 0.4           | 6 ± 0.39            | 10 ± 0.5            | 6.2 ± 0.3           | 0.10 ± 0.01         |
| T2 | 31 ± 0.5            | 3.6 ± 0.6           | 6 ± 0.46            | 9 ± 0.3             | 6.3 ± 0.2           | 0.09 ± 0.01         |
| T3 | 22 ± 0.8            | 2.9 ± 0.5           | 9 ± 0.49            | 11 ± 0.2            | 3.0 ± 0.2           | 0.07 ± 0.01         |
| T4 | 26 ± 0.6            | 3.0 ± 0.5           | 9 ± 0.56            | 12 ± 0.3            | 4.2 ± 0.3           | 0.07 ± 0.01         |
| T5 | 24 ± 3.3            | 2.7 ± 0.5           | 7 ± 0.42            | 20 ± 0.4            | 4.5 ± 0.1           | 0.05 ± 0.01         |
| T6 | 25 ± 0.6            | 2.7 ± 0.7           | 7 ± 0.42            | 22 ± 0.3            | 4.6 ± 0.1           | 0.05 ± 0.01         |
| T6 | 15 ± 1.2            | 1.6 ± 0.2           | 24 ± 1.25           | 10 ± 0.2            | 2.9 ± 0.4           | 0.04 ± 0.01         |
| T8 | 27 ± 1.0            | 3.4 ± 0.5           | 13 ± 0.94           | 9 ± 0.3             | 4.1 ± 0.3           | 0.09 ± 0.01         |
| T9 | 22 ± 2.3            | 1.9 ± 0.3           | 11 ± 0.50           | 21 ± 0.3            | 3.4 ± 0.4           | 0.05 ± 0.01         |

| X2 | As (mg kg\(^{-1}\)) | Cr (mg kg\(^{-1}\)) | Fe (mg kg\(^{-1}\)) | Mn (mg kg\(^{-1}\)) | Pb (mg kg\(^{-1}\)) | Cd (mg kg\(^{-1}\)) |
|----|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| T1 | 33 ± 0.5            | 3.6 ± 0.3           | 6 ± 0.3             | 10 ± 0.5            | 6.2 ± 0.1           | 0.10 ± 0.01         |
| T2 | 28 ± 0.6            | 3.2 ± 0.3           | 5 ± 0.3             | 7 ± 0.2             | 5.8 ± 0.4           | 0.08 ± 0.01         |
| T3 | 20 ± 0.6            | 2.5 ± 0.2           | 11 ± 0.3            | 12 ± 0.4            | 2.4 ± 0.2           | 0.07 ± 0.01         |
| T4 | 23 ± 0.7            | 2.9 ± 0.2           | 10 ± 0.3            | 11 ± 0.1            | 3.3 ± 0.1           | 0.06 ± 0.01         |
| T5 | 19 ± 1.1            | 2.5 ± 0.2           | 7 ± 0.3             | 22 ± 0.3            | 3.9 ± 0.2           | 0.05 ± 0.01         |
| T6 | 21 ± 1.4            | 2.3 ± 0.2           | 7 ± 0.4             | 24 ± 0.3            | 2.7 ± 0.2           | 0.05 ± 0.01         |
| T7 | 10 ± 1.1            | 1.2 ± 0.1           | 32 ± 0.8            | 13 ± 0.2            | 2.2 ± 0.2           | 0.04 ± 0.01         |
3.7. The Content of Toxic Elements in Plants and Soil Affected by KMnO4 (X2)

The mean value, along with standard error, of total Cd concentration in the soil was 0.6 mg kg\(^{-1}\). The total concentration of As, Cr, and Pb was 47 mg kg\(^{-1}\), 40 mg kg\(^{-1}\), and 366 mg kg\(^{-1}\), respectively. T6 × 2 reduced the toxic element concentration in the plants in the order: Pb > As > Cr > Cd by 79%, 60%, 50%, and 25%, respectively. The extractable content of elements was reduced in the order: Pb > Cd > Cr > As by 55%, 52%, 36%, and 34%, respectively. A mean soil pH of 7.77, Eh of 248 mV, leaf area of 2.7 cm\(^2\), wet weight of 25 g and dry weight of 6.2 g were noted in pots treated with the above treatment on the day of harvest. The mean concentration of Mn in plant tissues was 13 mg kg\(^{-1}\), while 4 mg kg\(^{-1}\) was recorded in control plants. Extractable Mn was 24 mg kg\(^{-1}\) and 10 mg kg\(^{-1}\) was recorded in control pot soil. The total concentration of Mn was 307 mg kg\(^{-1}\) and 209 mg kg\(^{-1}\) in control pots.

Table 5. Mean values of plant leaf area, dry weight, wet weight, soil pH, and Eh.

|     | X1 Leaf Area (cm\(^2\)) | Dry Weight (g) | Wet Weight (g) | pH | Eh (mV) |
|-----|-------------------------|----------------|----------------|----|---------|
| T1  | 1.74                    | 1.31           | 8.64           | 6.6| -20     |
| T2  | 1.83                    | 1.82           | 11.70          | 7.9| 37      |
| T3  | 2.12                    | 4.91           | 18.01          | 7.8| 160     |
| T4  | 2.11                    | 4.14           | 16.94          | 8.0| 150     |
| T5  | 2.39                    | 4.01           | 14.03          | 7.9| 208     |
| T6  | 2.38                    | 3.92           | 17.91          | 8.0| 207     |
| T7  | 2.84                    | 6.14           | 26.54          | 7.8| 267     |
| T8  | 2.53                    | 2.93           | 15.83          | 7.9| 250     |
| T9  | 2.58                    | 4.71           | 23.31          | 7.8| 272     |

|     | X2 Leaf Area (cm\(^2\)) | Dry Weight (g) | Wet Weight (g) | pH | Eh (mV) |
|-----|-------------------------|----------------|----------------|----|---------|
| T1  | 1.74                    | 1.31           | 8.64           | 6.6| -20     |
| T2  | 1.98                    | 3.64           | 17.83          | 8.3| 35      |
| T3  | 2.70                    | 5.71           | 22.54          | 8.1| 165     |
| T4  | 2.40                    | 4.43           | 22.81          | 8.1| 152     |
| T5  | 2.63                    | 5.22           | 21.12          | 7.5| 283     |
| T6  | 2.76                    | 6.24           | 25.10          | 7.7| 248     |
| T7  | 3.20                    | 8.44           | 36.71          | 7.6| 297     |
| T8  | 2.86                    | 4.41           | 22.91          | 8.0| 265     |
| T9  | 3.00                    | 7.53           | 33.12          | 8.0| 270     |

3.8. Correlation between Different Elements in the Rice Plant

Correlation of different elements in the plants was performed to evaluate the relationship between the effect of the elements on each other. The correlation matrix of the plant samples is presented in Figure 4. The correlation is significant at the 0.01 level (2-tailed) of plant X1 and X2. The correlations between Cr-Cd, Cr-Pb, and Cr-As in X1 were strongly correlated with each other. Among all the samples, Pb, As, Cd, Cr showed a negative correlation with Fe. Fe-As and Mn-As also showed a negative relationship. All metal pairs revealed significant strong positive correlations (\(p = 0.01\)) between plant Cr-As, plant Cd-As and plant Pb-As.

The correlation was significant at the 0.05 level for plant X2. The correlations between Cr-Cd, Cr-Pb, and Cr-As were strongly correlated with each other. Among all the samples, Pb, As, Cd, and Cr showed a negative correlation with Fe. Fe-As and Mn-As also showed a negative relationship. All element pairs revealed significant strong positive correlations (\(p = 0.01\)) between plant Cr-As, plant Cd-As and plant Pb-As.

The correlation matrix of available concentration in a soil sample is presented in Figure 5. The correlation is significant at the 0.01 level soil at X1. A strong negative correlation was noted in Fe-Mn. Most of the elements showed a strong to low positive correlation with each other. The correlation
matrix of Mn-Cd showed a positive correlation, and a positive correlation was found between other elements including Pb-Cd, Pb-As, Cd-As and Mn-As.

The correlation of extractable element concentrations in X2 was achieved to calculate the relationship between the effect of elements on each other. The correlation is significant at the 0.01 level of soil X2. A negative correlation was noted between Fe-Mn. The correlation matrix of Mn-Cd showed a strong positive correlation. Pb-Cd, Pb-Cr, Cd-As, and Cd-Cr also showed a positive correlation.

The correlation matrix of the total concentration of different elements affected through different treatments is presented in Figure 6. The correlation was significant at the 0.01 level (2-tailed) X1 and X2. The correlation analysis of X1 revealed that all HMs showed a negative correlation with Fe and Mn. A negative correlation was noted in Fe-Cd, Fe-As, Fe-Cr, Fe-Pb. A negative correlation was found between Mn and the other elements, including Mn-Cd, Mn-As, Mn-Cr, and Mn-Pb.

At the X2 level, all HMs showed a negative correlation with Mn and Fe, while a positive correlation was observed among TEs. A strong negative correlation was noted in Fe-Cd, Fe-As, Fe-Cr, and Fe-Pb. A negative correlation was found between Mn and the other elements, including Mn-Cd, Mn-As, Mn-Cr, and Mn-Pb.

![Figure 4. Correlation between total concentrations of different elements in plants.](image)

![Figure 5. Correlation between extractable content of different element concentrations in soil.](image)
4. Discussion

All of our treatments except the control contained two levels of lime (CaCO₃) as a basal dose, which significantly increased the soil pH as compared to control. The bioavailability of heavy metals (HMs) and metalloids are largely dependent upon the pH. The mobility of HMs were significantly decreased due to the application of lime that caused alkalinity in the soil; as a result, the precipitation and adsorption of HMs were enhanced. This adsorption and precipitation increased the stability of HMs and metalloids in soil [40,41]. Lee et al. [42] used granulated lime (Ca(OH)₂) and calcium carbonate (CaCO₃) for removal of heavy metals from contaminated water and about 98% of As and 96% Cd were removed from the contaminated water through sweep precipitation. Krebs et al. [43] noted that lime used as alkaline treatment reduced Pb concentration, compared to control, which was attributed to a pH increase, and consequently enhanced metal precipitation. Another important phenomenon governing the availability of HMs is redox potential. The redox potential of all the pots was increased except the control. The pH and Eh together have a significant effect on the availability of HMs and metalloids. The availability of Cd, As, Pb and Cr decreases as the Eh and pH of the soil solution increase [32,40,44–51].

The results of this study indicate that minimum concentration of Cd, As, Pb and Cr were observed in treatments that contain iron oxide, modified hairs, zeolite, and Mn. In the literature, many scientists have reported the efficiency of the iron oxide, modified hairs, zeolite, and Mn in reducing heavy metal toxicity [6,52–54]. The treatments that contained MHs and iron oxide were statistically highly significant in reducing the available and total HMs and metalloids in plants and soil followed by the treatments that contained zeolite and KMnO₄. Iron oxide and human hairs have been reported to be effective in the in situ remediation of HMs. The smaller size of iron oxide and hairs provided more surface area to HMs and increased the adsorption sites that resulted in an increase in the sorption of metals and metalloids. The iron oxide has been reported to enhance the formation of iron plaque, which acts as a barrier to reduce the permeability of roots towards HMs [55]. The competition for adsorption sites through Fe and H ions, the formation of soluble complexes, high surface area, high CEC, and specific retention may have played a significant role in decreasing the availability of Cd, Pb, Cr, and As. The binding capacity of iron oxide towards heavy metals, including Cd, As, Cr, Pb, Cu, Co, and Ni, is well known and might be another factor that supports our results [45,48,56,57]. We know that Eh is also a very important factor in reducing the availability of metals and metalloids to rice plants. Maximum Eh was observed in these treatments, which was
due to the application of iron oxide that resulted in reducing the available fraction of HMs in soil and eventually in the plants too.

Hairs, as keratin-based adsorbents, are reported to reduce the concentration of HMs in both soil and water [7,52,58–62]. There are two main mechanisms called physiosorption and chemisorption through which keratin material can remove HMs from air and solution. The amino acids like cysteine, aspartic acid, glutamic acid, lysine, tyrosine, glycine, and histidine present in the hair protein increase the detoxification and adsorption of HMs [63,64]. In this study, the human hairs were modified with the application of NaOH to enhance their efficiency by creating high resistance and strong cation resin that resulted in decreasing the availability of HMs. We found that the alkaline environment of the solution and NaOH modified hairs increased the adsorption of metals to soil and keratin fibers. Our findings are similar to [52,60–62].

The treatments that contained KMnO4 coated on zeolite also showed encouraging results in reducing the availability of HMs to rice plants. High amounts of Cd, As, Pb, and Cr were adsorbed into the soil and reduced the available fraction of the above HMs in soil solution. KMnO4 has been reported to reduce the availability of Cr, Pb, Cd, and As through adsorption when it is coated on the surface of different materials [65–69], while zeolite has the capability to adsorb Pb, Cd, As, and Cr [53,70–74]. We coated KMnO4 on the surface of zeolite so that it can increase the surface area and produce a more negative charge on the surface of the coated zeolite. Similar results were found by [75] when MnO2 was coated on the surface of diatomite. [67] used KMnO4 and FeSO4.7H2O solution and found a 36% increase in surface area of diatomite, while the pore structure of diatomite was not disturbed. Surface-coated zeolite with KMnO4 increased the sorption of HMs mainly due to two reasons: (I) an increase in surface area, and (II) an increase in negative charge on the surface of our treatment.

Leaf area, and wet and dry weight of rice plants were also studied as they represent maximum production. Most of our treatments positively affected the growth parameters of our crop. A balanced fertilizer is also important for the growth of any plant and we used the most effective and moderate level of fertilizer according to [76]. Furthermore, fertilizers, iron fertilizer and keratin material have been reported to be effective in enhancing the growth of plants [77–80]. Iron oxide promoted the growth of rice through regulating the antioxidant enzyme activity and phytohormone contents. In addition, Fe also participates in respiration, photosynthesis, electron transfer during redox reactions and in the biosynthesis of chlorophyll and phytomolecules. Waste hairs applied to soil can act as a good fertilizer source as hair protein contains a lot of amino acids, which, on decomposition, release essential plant nutrients. Human hair contains 51% carbon, 21% oxygen, 17% nitrogen, 6% hydrogen, and 5% sulphur, which makes waste human hair a good source of organic fertilizer [79]. Zeolite increases crop yield and biomass through improving soil physical and chemical properties, which include improving water holding capacity, facilitating root growth, decreasing the availability of HMs, and increasing N use efficiency through minimizing N lose and maximizing N recovery for the high CEC of zeolite, especially for ammonium (NH4+) [81–83].

Results from the principal correlation analysis revealed that Fe and Mn had a high negative correlation with Cd, As, Cr, and Pb in both plants and soil, while there was a strong positive correlation within HMs and metalloids. Our results were similar with [41,84–86]. This indicates that Fe and Mn reduce the metal toxicity both in plants and soil. Furthermore, the availability and mobility of HMs in soil is dependent on organic sources, clay minerals, soil pH and Fe/Al oxides. Organic amendment provides a novel environmentally friendly option for reducing trace metal accumulation in edible parts of plants. Adsorption, complexation and/or precipitation are the possible mechanisms involved in immobilization of metals by organic amendments [87]. The role of natural clay minerals (sepiolite, zeolite) has already been conferred for remediation of HM-contaminated sites due to their easy availability and low cost [88].

5. Conclusions

In summary, amendments that contained iron oxide, human hair, manganese, and zeolite were most significant in reducing the concentration of HMs in rice plants. The correlation effect of these
elements indicated that Fe and Mn addition decreased the HM concentration in rice plants. These treatments positively affected the biomass production of rice plants. The pH and redox potential were also enhanced due to these amendments, which resulted in high detoxification of TEs. T7 was the most significant among all the treatments with maximum plant biomass production and iron concentration in rice plant and a high redox potential, pH, and iron concentration in soil. T7 decreased the availability of As, Cd, Pb, and Cr in the soil as a minimum concentration was noted in pots that contained T7. T9 was second after T7 in decreasing HM concentration in soil and plants. There was a positive effect on Eh, pH, and plant biomass production in pots that received T9. The maximum contraction of Mn was found in T9. We recommend the use of iron oxide, human hair waste, zeolite, CaCO3, MnO2 and potassium permanganate as effective amendments for remediation of TEs. Furthermore, it is recommended that different levels and combinations of the above-mentioned materials should be tested to improve the complexation and adsorption power. These materials also improved the biological growth of rice plants. Maximum biomass production was noted in pots that received the above-mentioned material.

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