Biogeochemical Behavior of Lead and Nickel as Influenced by Phosphatic Fertilizer Applied to Rice (Oryza sativa L.) Cultivars Grown under City Effluent Irrigation

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Abstract: The hydrology of arid to semi-arid regions is substantially different from that in humid regions due to scarce hydrological data distributions with dry climates and strong evapo-transpirative environments. In the perspective of global water shortage, food security for all of the living beings has become a matter of great concern. Efficient use of water resources both in urban and rural conditions. In the perspective of global water shortage, food security for all of the living beings has become a matter of great concern. Efficient use of water resources both in urban and rural environments and application of non-conventional water resources for irrigation are becoming increasingly important. In order to sustain crop production, the re-use of treated wastewater for irrigation of crops could be a good option. A pot experiment was set up to evaluate effects of different doses of di-ammonium phosphate (DAP) fertilizer on lead (Pb) and nickel (Ni) phyto-availability by two cultivars of rice irrigated with city effluent. Experiment was conducted in a completely randomized design (CRD) each with three replications. The results showed effective immobilization of Pb with applied phosphatic fertilizer. Among all of the tested treatments, the most effective treatment to reduce phyto-availability of Pb was T₄ (248 kg P ha⁻¹) due to antagonistic interaction. While Ni showed inconsistent behavior with both synergistic and antagonistic interaction (biphasic) to applied phosphorus (P) rates. Data regarding various growth parameters such as plant height, number of tillers, shoot and root dry weights, straw and grain yields, and physiological attributes such as total chlorophyll contents, photosynthetic and transpiration rates showed significant (p ≤ 0.05) responses to P application. An increasing trend was revealed in determined parameters with increased P application rates, with the exception of decreased plant height. The conclusion of this article is an open access article published maps and institutional affiliations.

Keywords: city effluent irrigation; remediation; lead; nickel; rice; phosphorus

1. Introduction

Pakistan lies in an arid and semi-arid region of the world and agricultural irrigation is prominently dependent upon Indus River system. Over 16 million hectares of land receive...
water from Indus Basin System and its tributaries having annual farm gate supplies up to 137.82 million acre foot [1,2], which is not enough to supply water to crops properly, as to maintain sustainable irrigated agriculture. One alternative can be use of wastewater for irrigation purposes. City wastewater often used for irrigation and farming vegetables in urban agricultural soils of Pakistan [3]. Farmers assume the city effluent as a good source of nutrients and irrigation, while city administrations consider it as a cheaper method for disposal [4]. Although sewage effluent streams are loaded in organic matter and plant nutrients, they also contain heavy metals such as Pb and Ni [5].

The concern of several toxic elements build-up in soils around the world is often due to industrial and urban activities, and the usage of raw and untreated sewage sludge has increased [6]. Their presence inflicts significant damage to the environment and puts human health at risk due to toxic elements mobility and solubility. Pb is found in relatively high concentration in effluents when compared to other heavy metals. The Pb content in soil is positively correlated with its concentration in plant tissue [7]. Discharge of Pb occurs through many anthropogenic activities including application of sewage sludge, fertilizers, and pesticides, which can contain surface soils and ground waters [8]. The Pb can form incredibly stable complexes such as \([\text{Pb}(\text{NO}_3)^+\), \([\text{Pb}(\text{OH})_3]^−\) and minutely soluble precipitates such as PbCO\(_3\), PbSO\(_4\), Pb(OH)\(_2\) and Pb\(_3\)(PO\(_4\))\(_2\) in soil [9]. Very small quantities of Ni are present in parent rock, and it mostly enters the soil and environment through various human actions. Average concentrations of Ni found in earth’s soil and crust is 40 and 75 mg kg\(^{-1}\), respectively. The Ni found in soils is released from copper (Cu) and Ni smelters, combustion Ni loaded diesel oil [10], urban effluents, bio-solids, impurities, and residues in fertilizers, wastes from agriculture sector and mining and smelting [11]. Despite being essential for plants [12], Ni has been found to be toxic to plants, animals, and humans at higher concentrations.

About 32,500 hectares of land is irrigated with the city effluent in Pakistan where vegetables, fodder, and cereal crops are grown [13] while 10 percent of crops are being irrigated with raw sewage on a global scale [14]. This is of great concern and demands attention, as untreated sewage is continuously being applied to for growing crops on urban lands [8]. The soil salinity troubles also enhanced owing to raw effluent irrigation containing toxic metals [15]. Due to high electrical conductivity (EC), sodium adsorption ratio (SAR), and residual sodium carbonate (RSC), the industrial and urban effluent drains of Faisalabad have been ascertained to be unfit for irrigational purposes [15,16]. Quantitative data statistics concerning the biogeochemical behavior of Pb and Ni in paddy soils and crop plants irrigated through industries utilized water are missing in Pakistan [17].

The urea and DAP are the major NP inputs in Pakistan for crop production. Apart from providing plants with nutrients and increasing the production of food, the application of fertilizers have an influence on soil’s pH, ionic strength, complex formation, surface charge, distribution, soil microbial activity, and rhizosphere composition [5,18]. A number of processes including phosphate induced metal sorption, cation exchange capacity (CEC), precipitation, and surface complex formation directly reacts with metal ions [8,19]. A few experiments indicated P induced immobilization of Pb in polluted water and soil by transforming soluble Pb into pyromorphite-like minerals [20,21]. Phosphorus often brings to a halt of Pb in highly polluted soil [22]. Kalavrouziotis et al. [23] found that Pb and Ni showed synergistic interaction in the leaves and antagonistic in the heads of broccoli when irrigated with treated municipal wastewater. Murtaza et al. [24] considered the application of DAP fertilizer can be an effective to reduce phyto-availability of three toxic metals (i.e., cadmium (Cd), copper (Cu) and zinc (Zn)). However, to-date no information is available on the biogeochemical behavior of Pb and Ni in coarse and fine/basmati rice cultivars, simultaneously, with varying rates of applied DAP.

Since, enough high-quality rice is grown in Pakistan to meet the domestic demands and holding good export potential in international market [1]. The proper amelioration of Pb and Ni contaminated paddy soils using sound scientific approaches on sustained basis would be a wise choice so as to minimize the issues and maximize the crop production.
Therefore, the present work aims at effective use of P fertilizer in the immobilization/remediation of Pb and Ni growing coarse and fine/basmati rice cultivars irrigated with city effluent. Phyto-availability of Pb and Ni and their possible interactions, accumulation, and distribution patterns in the different tissues of rice plants are also investigated.

2. Materials and Methods

2.1. Soil Sampling and Its Characterization

A pot experiment was set up in the wire house at the Institute of Soil and Environment Sciences (ISES), University of Agriculture Faisalabad (UAF), Pakistan. Bulk soil was collected from an experimental area of the institute and was air dried, finely grounded with a wooden roller to pass through a sieve (2 mm), homogenized and stored in plastic containers for further physical and chemical analysis following Iqbal et al. [25].

A hydrometer method [26] with sodium hexametaphosphate [(NaPO\(_3\))]\(_6\)] as the dispersion agent was employed for particle size analysis. Using UDSA textural triangle, the class of soil texture was revealed.

Total metals in soil were extracted by a method described by Amacher [27] using a flame atomic absorption spectrometer (FAAS; Model Thermo S-Series, Model Thermo S-Series, Thermo Electron Corporation, Cambridge, UK). A sample of 1 g of air-dried soil was taken in a 50 mL conical flask with the addition of 10 mL concentrated HNO\(_3\) and was placed overnight. Next morning, this mixture was heated at 200 °C and cooled. After addition of 1 mL of HNO\(_3\) and 4 mL of HClO\(_4\) sample was again heated at 200 °C until fumes if HClO\(_4\) appeared. Sample was cooled after fumes of HClO\(_4\) appeared and heated again to 70 °C for one hour after adding 5 mL of 1:10 HCl. The sample was cooled again and 1% HCl was added to make a final volume of 50 mL. The sample was filtered through a Whatman filter paper no. 42 to get the filtrate for heavy metal analysis. To avoid precipitation of salts during storage, sodium hexametaphosphate (0.1%) at the rate of one drop per 25 mL extract was added.

Analysis of electrical conductivity of saturation extract (EC\(_e\)) was carried out with conductivity meter (Jenway Model-4070, Loughborough, Leicestershire, UK) soil saturation percentage (SP), pH of saturated soil paste (pHs), CEC, sodium adsorption ratio (SAR) and soluble cations (i.e., Ca\(^{2+}\) + Mg\(^{2+}\), Na\(^+\)) and anions ions (i.e., CO\(_3^{2-}\), HCO\(_3^-\), Cl\(^-\)) were carried out in accordance with methods specified by U.S Salinity Lab Staff [28]. Soluble SO\(_4^{2-}\) ions were determined on the basis of difference method.

Soil organic matter and lime (calcium carbonate) were determined in according to the methods reported by Walkly-Black [29] and Calcimeter method [30], respectively.

The AB-DTPA extraction method [31] was followed to determine plant available Pb and Ni by using FAAS. The determined important initial physical and chemical characters of the soil are presented in Table 1.

Table 1. Physico-chemical properties of soil used for experiment.

| Characteristic | Value               |
|----------------|---------------------|
| Textural Class | Sandy clay loam     |
| Sand           | 45.14%              |
| Silt           | 24.30%              |
| Clay           | 30.56%              |
| pHs            | 7.71                |
| EC\(_e\)        | 1.71 dS m\(^{-1}\)  |
| CO\(_3^{2-}\)   | Nil                 |
| HCO\(_3^-\)    | 1.70 mmol\(_L\)\(^{-1}\) |
| Cl\(^-\)        | 5.70 mmol\(_L\)\(^{-1}\) |
### 2.2. Characteristics of Faisalabad City Effluent

Thirty samples of raw city effluent were collected from Nallah from Faisalabad metropolitan with the help of plastic bottle attached to a long wooden stick. Concentrated HCl (5 mL) was inserted to each water sample to maintain low pH to avoid any type of Pb and Ni precipitation. Afterwards, Pb and Ni were determined in the laboratory using FAAS [7,10]; the obtained data are presented in Table 2. The electrical conductivity of the city effluent was 2.9 to 6.5 dS m\(^{-1}\). Minimum and maximum concentrations of the Pb in city effluent were 0.015 and 1.137 mg L\(^{-1}\), respectively, averaging at 0.08 mg L\(^{-1}\) in collected city effluent. The Ni concentration was relatively low in city effluent as compared with Pb. Minimum and maximum concentrations of the Ni were 0.022 and 0.073 mg L\(^{-1}\), respectively, whereas average concentration of Ni was found 0.0437 mg L\(^{-1}\) in city effluent.

### Table 2. Pb and Ni concentrations (mg L\(^{-1}\)) in Faisalabad metropolitan city effluent.

| Sample No. | Ni (mg L\(^{-1}\)) | Pb (mg L\(^{-1}\)) | Sample No. | Ni (mg L\(^{-1}\)) | Pb (mg L\(^{-1}\)) |
|------------|-------------------|-------------------|------------|-------------------|-------------------|
| 1          | 0.047             | 0.015             | 16         | 0.049             | 0.054             |
| 2          | 0.050             | 0.019             | 17         | 0.037             | 0.038             |
| 3          | 0.027             | 0.023             | 18         | 0.043             | 0.049             |
| 4          | 0.052             | 0.032             | 19         | 0.054             | 0.061             |
| 5          | 0.034             | 0.022             | 20         | 0.051             | 0.079             |
| 6          | 0.046             | 0.031             | 21         | 0.032             | 0.073             |
| 7          | 0.030             | 0.028             | 22         | 0.038             | 0.055             |
| 8          | 0.043             | 0.065             | 23         | 0.046             | 0.051             |
| 9          | 0.073             | 0.071             | 24         | 0.029             | 0.044             |
| 10         | 0.025             | 0.034             | 25         | 0.022             | 0.049             |
| 11         | 0.042             | 1.137             | 26         | 0.039             | 0.039             |
| 12         | 0.041             | 0.033             | 27         | 0.051             | 0.054             |
| 13         | 0.053             | 0.034             | 28         | 0.041             | 0.056             |
| 14         | 0.061             | 0.045             | 29         | 0.046             | 0.046             |
| 15         | 0.071             | 0.029             | 30         | 0.039             | 0.035             |

### 2.3. Rice Nursery and Its Transplantation

Seeds of two cultivars of rice (Shaheen Basmati and KS-282) were taken from Rice Research Institute Kala Shah Kaku, Shiekhupura, Pakistan. Healthy seeds were chosen from both cultivars and were grown in polythene lined trays containing washed sand. Sand was washed with tap and distilled water, twice each and with 1% HCl solution. Nursery was irrigated with distilled water. Then, four weeks old rice seedlings were transplanted at three seedlings per hill and four hills were maintained per pot.

### 2.4. Experimental Setup and Design

Glazed pots were filled with 10 kg of soil. Five treatments viz. control (T\(_0\)) phosphorus at 62 kg ha\(^{-1}\) (T\(_1\)), phosphorus at 124 kg ha\(^{-1}\) (T\(_2\)), phosphorus at 186 kg ha\(^{-1}\) (T\(_3\)), and with phosphorus at 248 kg ha\(^{-1}\) (T\(_4\)) were set up and arranged in completely randomized
design (CRD). Three replicates were used for each treatment to increase experimental accuracy (Table 3). Rice crop was fertilized at 120: 62 kg ha\(^{-1}\) of N: K as Urea, and K\(_2\)SO\(_4\) (SOP), respectively. While P as DAP was applied at four different rates as mentioned in Table 3, whole of P\(_2\)O\(_5\) and K\(_2\)O was added at transplanting whereas urea was applied thrice in the same dose: 1/3rd at transplanting, 1/3rd after 25 days of transplanting and 1/3rd after 45 days of transplanting. Effluent samples were taken at each irrigation for analysis of Ni and Pb concentrations using FAAS.

Table 3. Treatments applied to rice in pot experiment.

| Treatment | N (kg ha\(^{-1}\)) | P (kg ha\(^{-1}\)) | K (kg ha\(^{-1}\)) |
|-----------|--------------------|--------------------|--------------------|
| T\(_0\)   | 0                  | 0                  | 0                  |
| T\(_1\)   | 170                | 62                 | 62                 |
| T\(_2\)   | 170                | 124                | 62                 |
| T\(_3\)   | 170                | 186                | 62                 |
| T\(_4\)   | 170                | 248                | 62                 |

2.5. Plant Sampling and Analysis

Randomly selected flag leaves in pots were used to measure transpiration rates, photosynthesis rates and total chlorophyll contents of both the rice cultivars. Leaf total chlorophyll content index in terms of Special Products Analysis Division (SPAD, a division of Minolta) values were determined, from leaf tip to leaf base via a handheld SPAD-502 meter (Minolta, Osaka, Japan). The photosynthetic and transpiration rates were measured using a portable infrared gas analyzer (IRGA, LCA-4, Analytical Development Company, Hoddesdon, England).

The rice crop was harvested at harvest maturity. Plants were dug out carefully from the pots. Roots were gently washed using tap water to remove attached soil particles and were then washed with distilled water. The uprooted plants were divided into roots, shoots, and paddy. Root and shoot samples were blotted in filter paper sheets, collected in separate paper bags, air dried and oven dried up to a constant weight at 65 °C. Oven-dried root and shoot were finely grinded with stainless steel Willey grinding machine and stored for analysis. Rice paddy were separated manually and weighed. Root and shoot dry weights of rice were also recorded. Soil samples from each pot after harvest were kept for further Pb and Ni analysis, and rice samples were also used for metal determination [32].

From this dried plant material, one gram was taken and digested by addition of 5 mL of concentrated H\(_2\)NO\(_3\) and 5 mL of HClO\(_4\) in a conical flask and was kept for overnight. The very next morning, 5 mL of concentrated H\(_2\)NO\(_3\) was added again for digestion a hot plate until the solution became clear. Following digestion, the resultant material was cooled before being diluted to 25 mL by adding distilled water and was stored in air-tight bottles and was kept for analysis of Pb and Ni via FAAS.

The Pb or Ni uptake (mg pot\(^{-1}\)) by root or shoot or paddy was computed by Pb or Ni concentration (mg kg\(^{-1}\)) in root or shoot or paddy \(\times\) root or shoot or paddy yield (g pot\(^{-1}\)) / 1000 [7,10].

2.6. Statistical Analysis

All the obtained data were subject to analysis of variance (ANOVA) at a significance level of \(p \leq 0.05\). The LSD test was used to differentiate between applied treatment effects [33] using M-STAT Version 1.10 computer based software package.

3. Results and Discussion

City effluent is a prosperous basis of plant nutrients and organic matter as well as contains heavy metals which when enter into food chain cause severe health problems. Phosphorus application may be one option for immobilizing heavy metals including Pb and Ni. In this section, under city effluent irrigation, rice growth, physiological responses
and metals uptake by different rice plant parts as affected by varying P application rates have been presented and discussed.

3.1. Rice Growth and Yield Responses

Addition of P along with city effluent irrigation brought a gradual increase in rice growth and yield attributes. Rice showed a positive response to increased P rates. Coarse rice cultivar (KS-282) produced more biomass as compared to fine cultivar (Shaheen Basmati).

3.1.1. Number of Tillers per Hill and Plant Height

Effects of P application were found to be significant ($p \leq 0.05$, Table 4) on the number of tillers per hill (Figure 1a) and plant height of rice (Figure 1b), irrigated with city effluent. The number of tillers increased significantly compared to the control. Maximum number of tillers was recorded with treatment T4 in both cultivars, but fine Shaheen basmati cultivar produced more tillers, and its plant height was higher as compared with number of tillers and plant height recorded in coarse KS-282 rice cultivar. But statistically, both cultivars remained non-significant in producing number of tillers at control treatment. The treatments T2, T3, and T4 differ significantly from each other and from control, but T1 remained at par with control. The treatment order for number of tillers per hill remained T4 ≥ T3 > T2 > T1 ≥ T0. Maximum total number of tillers of rice with T4 can be attributed to sufficient P concentration to support normal plant growth in the soil [34].

Table 4. F-values of two ways ANOVA for the effect of varying application rates of phosphatic fertilizer on growth, yield, physiological, and biogeochemical responses of coarse and fine rice (*Oryza sativa* L.) cultivars receiving city effluent irrigation.

| Parameter                              | Treatment (d.f. = 3) | Cultivar (d.f. = 1) | Treatment × Cultivar (d.f. = 3) |
|----------------------------------------|----------------------|---------------------|---------------------------------|
| Number of tillers per hill             | 22.11 *              | 2.78 ns             | 2.67 *                          |
| Plant height                           | 341.55 *             | 10.51 *             | 1.39 ns                         |
| Root dry weight                        | 196.42 *             | 89.62 *             | 1.47 ns                         |
| Shoot dry weight                       | 167.84 *             | 22.45 *             | 4.97 *                          |
| Paddy yield                            | 98.51 *              | 12.61 *             | 2.61 ns                         |
| 1000 grain weight                      | 89.79 *              | 3.98 *              | 2.75 *                          |
| Total chlorophyll contents             | 64.40 *              | 5.12 *              | 2.13 ns                         |
| Photosynthesis rate                    | 79.68 *              | 49.75 *             | 5.16 *                          |
| Transpiration rate                     | 25.36 *              | 0.01 ns             | 0.41 ns                         |
| Concentration of Pb in root            | 100.12 *             | 125.29 *            | 1.3 ns                          |
| Concentration of Pb in shoot           | 64.30 *              | 9.19 *              | 0.33 ns                         |
| Concentration of Pb in paddy          | 49.66 *              | 54.82 *             | 2.00 ns                         |
| Concentration of Ni in root            | 15.21 *              | 1.44 ns             | 3.87 *                          |
| Concentration of Ni in shoot           | 1.74 ns              | 7.52 *              | 4.00 *                          |
| Concentration of Ni in paddy           | 2.63 ns              | 0.28 ns             | 0.26 ns                         |
| Concentration of Pb in post rice soil  | 8.35 *               | 2.48 ns             | 1.15 ns                         |
| Concentration of Ni in post rice soil  | 1.19 ns              | 1.87 ns             | 1.21 ns                         |

* = Significant; ns = Non-significant.

Maximum plant height (169.6 cm) and (164.0 cm) was recorded in Shaheen basmati and KS-282, respectively, with T1 treatment while minimum with T0 treatment (control). Mean plant height of cultivars remained in the order of T1 > T2 > T3 > T4 > T0.
Effect of varying application rates of phosphatic fertilizer on growth and yield responses of rice (*Oryza sativa* L.) receiving city effluent irrigation. (Each value is a mean, $n = 3$ statistically significant at $p \leq 0.05$, T bars represents ± SE of means, i.e., SEMs). Whereas, $T_0 =$ Control, $T_1 = N_{170}P_{62}K_{62}$, $T_2 = N_{170}P_{124}K_{62}$, $T_3 = N_{170}P_{186}K_{62}$, and $T_4 = N_{170}P_{248}K_{62}$.

Crop yield is the product of yield components, of which total number of tillers is the most important component. A higher number of tillers reflects good crop stand [18]. Decrease in plant height with increase in P rates may be due to increased plant and soil metal owing to fertilizer application [35,36] which in turn decreased plant physiological processes. Brennan and Bolland [37] also observed increased Cd concentration of canola and wheat from P fertilization in a soil. In normal and salty Pb-anxious soils, Iqbal et al. [16] demonstrated that rice growth and yield was improved through phosphate amendments by forming Pb pyromorphite, thus reducing Pb bioavailability [38]. In another study, it was also evident that applied DAP increased the development of Pb pyromorphite, resultantly; growth was increased due to the abridged Pb availability to plants [39].
3.1.2. Rice Root and Shoot Dry Weight

The root dry weight (RDW, Figure 1c) and shoot dry weight (SDW, Figure 1d) increased significantly \( (p \leq 0.05, \text{Table 4}) \) with increasing P rates in both rice cultivars as affected by city effluent irrigation. The maximum RDW and SDW were obtained with treatment \( T_4 \) and minimum with control treatment. In fine Shaheen basmati cultivar, the highest SDW (94.4 g pot\(^{-1}\)) was observed in \( T_4 \) treatment which was 33.1% increased relative to the control treatment. Shoot dry weight increased by 4.8, 14.3, 27.9 and 33.1% with increasing P rates (\( T_1, T_2, T_3, T_4 \), respectively) compared to control. In coarse cultivar, the increase in SDW was 3.9, 11.5, 16.3 and 25% over control with \( T_1, T_2, T_3, T_4 \), respectively. Likewise, in case of KS-282, the increase in RDW was 6.1, 17.3, 30.7, and 41.4% while in Shaheen basmati it was 9.8, 21.8, 33.3, and 41% relative to the control treatment (\( T_0 \)). The two rice cultivars behaved differently for SDW and RDW; as fine Shaheen basmati cultivar showed more increase in SDW in comparison with coarse KS-282 cultivar. Mean SDW and RDW of both cultivars were found in the order of \( T_4 > T_3 > T_2 > T_1 > T_0 \). Coarse cultivar produced more SDW compared with the fine cultivar.

In agreement with the present study, comparable results were reported by Jamali et al. [40] that coarse cultivar produced more SDW as compared to fine cultivar irrigated with city effluent. Overall KS-282 produced significantly more relative to Shaheen basmati. Increase in RDW and SDW with increasing applied P rates owing to highly soluble phosphate resource was seen to enhance the potential for pyromorphite formation [41,42] thereby promoting root development and photosynthesis, and extensive root and shoots growth with increased P rates [43]. To explore the relative toxicity or sensitivity of plants to toxic metal, the growth of root was a greater intended feature [44,45].

In agreement with present study, Shaheen basmati produced the highest root and shoot weights followed by KS-282 under Cd [44] and Pb stress [46]. Iqbal et al. [16] established that increasing application rates Ca and P based amendments steadily increased plant height, biomass of straw and paddy yield in normal and salty Pb-anxious soils. The positive influence of P based amendments on growth and yield of plants can be documented either due to improved nutrition of P and additional energy, otherwise, the impact of P to lessen toxicity of metal; or amalgamation of both mechanisms [38].

3.1.3. Paddy Yield and 1000 Grain Weight

Effects of applied P treatments and cultivars on rice paddy yield (Figure 1e) and 1000 grain weight (Figure 1f) were found significant \( (p \leq 0.05, \text{Table 4}) \) under city effluent irrigation. Paddy yield and 1000 grain weight increased significantly with increasing P rates, being found maximum with treatment \( T_4 \). In KS-282, the increase in paddy yield was 3.7, 5.6, 11.1, and 22.2% compared to control. In fine cultivar the increase was 7.2, 11.6, 14.3, and 19.7% with its respective control. Total paddy yield increased gradually with increasing P rates and the highest paddy yield (32.8 g per pot) was recorded with treatment \( T_4 \).

Coarse KS-282 cultivar produced a little higher 1000 grain weight at 248 kg P ha\(^{-1}\) as compared with fine Shaheen basmati. Maximum 1000 grain weight was recorded with \( T_4 \) while minimum was with \( T_0 \) in both cultivars. In coarse cultivar the increase was 4.2, 14.2, 19.8, and 28.4% compared with that of control while in fine cultivar the increase was 5.5, 12.5, 15.2, and 21% as compared with the control. However, statistically both cultivars remained non-significant. The treatment’s effectiveness to produce mean paddy yield and 1000 grain weight of both cultivars was observed in decreasing order of \( T_4 > T_3 > T_2 > T_1 > T_0 \).

These results correlate with the findings of Jamali et al. [40] who found maximum wheat yield with the application of domestic sewage sludge at normal rates of nitrogen (N), P and potassium (K). City effluent contains organic matter, but increase or decrease in crop yield depends upon the nature of organic matter present in the effluent. If the organic material consists of easily decomposable fractions with low C: N ratio, it may have beneficial effects on crops yield, while organic matter with wider C: N ratio may immobilize N present in the soil or applied through fertilizer and accordingly may result in yield reduction [47]. Application of P enhances crop yield by promoting root development,
crop maturity, water use efficiency, photosynthesis, N fixation, sugar translocation and plant disease resistance [43]. Iqbal et al. [16] also found that the applied DAP increased the straw dry matter, plant height and paddy yield in normal and salty Pb-anxious soils.

The increase in grain yields can be due to the beneficial effect of applied P on the metabolism of plants, however, extent of increase depended on plant species and their cultivars [45]. Patra et al. [48] further illustrated that plant species and crop genotypes demonstrate disparity in tolerance or sensitivity to metal bearing on metacentric or diploid chromosomes number and total length of diploid complement. Iqbal et al. [46] also derived that Shaheen basmati had shown better growth, yield and physiological functions in comparison with KS-282 due to genetic differences under diverse levels of applied Pb in normal and salty anxious soils.

3.2. Rice Physiological Responses

In this study, under city effluent irrigation, physiological parameters such as total chlorophyll contents, photosynthesis rate and transpiration rate of both cultivars increased significantly ($p \leq 0.05$, Table 4) with increasing P rates. The response of both cultivars for total chlorophyll contents (Figure 2a) and photosynthetic rate (Figure 2b) statistically differed significantly from each other while their response for transpiration rate (Figure 2c) remained non-significant. Coarse and fine cultivars produced the highest total chlorophyll contents 36.8 and 36.4, respectively, with T4 treatment. Mean increase in total chlorophyll contents was found in the order of $T_0 > T_1 > T_2 > T_3 > T_4$.

The reason is the immobilization of heavy metals by P which in turn enhanced the chlorophyll contents. Similar results were reported in rice by Huang et al. [49]. They also reported that coarse rice cultivar produced more chlorophyll contents as compared to fine cultivar.

The increase in transpiration rate in KS-282 was 15.7, 34.1, 40.8, and 46.5% over its respective control treatment. However, in Shaheen Basmati the increase was 12.6, 22.6, 36.4 and 47% as compared with control. The reason may be that city effluent used for irrigation contains higher amount of plant nutrients and organic matter which increased plant growth that ultimately led to increased physiochemical processes of plants. The response of cultivars regarding transpiration rate remained non-significant. Mean increase in transpiration rate was remained in the order of $T_0 > T_1 > T_2 \geq T_3 \geq T_4$.

![Figure 2. Cont.](image-url)
Figure 2. Effect of varying application rates of phosphatic fertilizer on physiological responses of coarse and fine rice (*Oryza sativa* L.) receiving city effluent irrigation (Each value is a mean, *n* = 3 statistically significant at *p* ≤ 0.05, T bars represents ± SE of means i.e., SEMs). Whereas, T0 = Control, T1 = N170P62K62, T2 = N170P124K62, T3 = N170P186K62, T4 = N170P248K62.

Deviations in chlorophyll contents of crop plants due to abiotic or biotic disturbances are interrelated to illustrate symptoms of plant disorders and photosynthetic capability [45,50]. With escalating DAP rates, the lethal impacts of Pb on physiological and gas exchange rates were steadily diminish can be ascribed to lessen Pb concentration in tissues of rice [16]. The halt of Pb noxious was persuaded by P fertilizer via dropping Pb solubility owing to configuration of pyromorphite in soils [51], whereas augmented application rate of DAP gradually lessens the Pb accumulation in rice [16].

3.3. Concentration of Ni and Pb

3.3.1. Concentration of Pb in Root, Shoot and Paddy

The results showed Pb concentrations (mg kg⁻¹ DW) decreased significantly (*p* ≤ 0.05, Table 4) in root (Figure 3a), shoot (Figure 3b) and paddy (Figure 3c) at increasing P rates in both the rice cultivars under city effluent irrigation. Lead concentration was the highest with treatment T0 and the lowest with treatment T4 in both cultivars.

In roots, the decrease in Pb concentration in coarse cultivar was 32.3, 26.2, 20.1 and 10.9% as compared with control treatment. While in fine cultivar the decrease was 31.7, 28.4, 21.8, and 12.2% from the respective control.
Figure 3. Effect of varying application rates of phosphatic fertilizer on rice tissues concentration of Pb and Ni receiving city effluent irrigation. (Each value is a mean, \( n = 3 \) statistically significant at \( p \leq 0.05 \), T bars represents \( \pm \) SE of means i.e., SEMs). Whereas, \( T_0 = \) Control, \( T_1 = \) N\(_{170}\)P\(_{62}\)K\(_{62}\), \( T_2 = \) N\(_{170}\)P\(_{124}\)K\(_{62}\), \( T_3 = \) N\(_{170}\)P\(_{186}\)K\(_{62}\), \( T_4 = \) N\(_{170}\)P\(_{248}\)K\(_{62}\).

The highest Pb concentration in shoot of KS-282 was 3.9 mg kg\(^{-1}\) DW, while in Shaheen basmati it was 3.8 mg kg\(^{-1}\) DW with treatment \( T_0 \). Furthermore, its concentration reduced to 2.8 mg kg\(^{-1}\) DW in KS-282 and 2.6 mg kg\(^{-1}\) in Shaheen basmati with treatment \( T_4 \). In shoot, the decrease in Pb concentration in coarse cultivar was 27.4, 23.3, 15.6, and 9.7% as compared with its control treatment. While in fine cultivar the decrease was 30.4, 26.1, 15.4, and 7.4% with its respective control treatment.
Lead concentration in paddy was found the lowest with T₄ both in case of coarse (1.30 mg kg⁻¹ DW) and fine (1.03 mg kg⁻¹ DW) cultivars. The decrease in Pb concentration in paddy in KS-282 was 31.0, 26.1, 15.1, and 5.9% with respect to control. While in of Shaheen basmati the decrease was 42.6, 39.7, 32.7, and 17.3% in paddy Pb concentration from its respective control. Significantly more Pb concentration in paddy was accumulated in KS-282. The less Pb accumulation in Shaheen basmati might be due to the fact that it immobilized Pb more effectively.

The response of both cultivars regarding immobilization of Pb remained significantly different from each other and Shaheen basmati immobilized Pb more effectively as compared with coarse cultivar. Mean decrease in Pb concentration in roots, shoots, and paddy were found in the order of T₀ > T₁ > T₂ > T₃ ≥ T₄.

In present study, the low concentration of Pb in plant seems due to its low bioavailability, its retention by root surface and low mobility in soil owing to formation of Pb phosphate and other lead complexes in soil [52]. Zaragüeta et al. [53] also explored influence of long-term addition of sewage sludge to a calcareous soil on its total and bioavailable content of trace elements including Pb and Ni, and their transmissance to the tested barley and sunflower crops. Kartas et al. [54] assessed metal accretion in wheat irrigated by wastewater and reported that most of heavy metals (Cr, Mn, Pb, Zn, Ni and Cu) were retained in roots and their concentration was 4.2, 3.6, 42.8, 5.5, 1.7 and 40.1 mg kg⁻¹, respectively. Abbas et al. [55] also reported that sufficient quantities of trace elements (Zn, Cu, Fe, Mn) accumulated in rice straw and roots. Concentration of Pb in shoots and paddy was lower than that of Ni concentration. It was generally higher with the application of city effluent indicating antagonistic interaction of Ni with Pb absorption. It is reported that Ni had the enhancing effect on Pb in wheat straw [56]. The potential of other ions in also important on the stabilization of Pb (e.g., sulphate can reduce the solubility of Pb-phosphate) [57]. Reduced Pb uptake by Shaheen basmati can either be due to chelate secretion and/or its deposition of Pb in cell wall components [58].

Similar trends in results were also described by Iqbal et al. [59], Basta et al. [60], Cao et al. [61] and Bolan et al. [52] that Pb translocated to upper parts in very small amount as P immobilized Pb in soil due to phosphate induced Pb adsorption or precipitation with solution P as metal phosphates. Other reason for low Pb concentration in paddy could be the Pb accumulation mostly on cell walls of roots and permit restricted quantity of Pb for translocation to shoots, resultantly, less loading of grains with Pb [49]. Plants such as barley and rice did not absorb much Pb or transport Pb to grains [62].

### 3.3.2. Concentration of Ni in Root, Shoot and Paddy

The concentration of Ni in root (Figure 3d) of both cultivars remained non-significant (p ≤ 0.05, Table 4) as these accumulated statistically the same concentration of Ni (i.e., 23.9 and 23.3 mg kg⁻¹, respectively). Effects of treatments were found statistically significant. Mean Ni concentration was decreased in roots in the order of T₂ ≥ T₃ > T₀ ≥ T₄ > T₁. Treatment T₂ exhibited maximum decrease in Ni concentration. Among rice plant parts, Ni was mostly retained in roots.

The present results were in agreement with Wang et al. [63], who recognized significant variations for Ni accumulation among 72 rice varieties. The Ni concentration in rice shoot was found to be certainly associated with its translocation, however, not with Ni concentration in roots (due to diverse Ni-transport capabilities of rice varieties rather than the restricting Ni in roots). Andreeva et al. [64] reported that distribution of Ni in plants is dependent on the plant’s developmental stages and most of Ni accumulation was found in roots of oat (*Avena satia* L.) at booting and tillering stages.

In the present study, the response of both rice cultivars remained inconsistent for Ni in root and shoot against the applied P treatments. The Ni showed both synergistic and antagonistic interaction with P application. Nickel concentration in shoot (Figure 3e) of KS-282 increased with T₁ but it decreased with T₂, T₃, and T₄.
Statistically Shaheen basmati accumulated more Ni (14.7 mg kg\(^{-1}\) DW) as compared with KS-282 (14 mg kg\(^{-1}\) DW). The effect of treatments was statistically non-significant. The effectiveness of treatments for decreasing Ni concentration in shoot was in the order of \(T_0 > T_2 \geq T_3 > T_1 \geq T_4\). Overall, Ni accumulation was statistically more with control while the other treatments remained at par with each other.

Recognition of genetic variability among cultivars to take up essential and/or toxic substances in plant tissue is measured as satisfactory criterion [46,65]. A maximum permissible level of Pb in rice grain is 0.2 mg kg\(^{-1}\) [66]. Marschner [12] concluded that increasing P concentrations in growth environment can decrease the concentrations of Mn, Zn and Ni in typical soil. The present study results are in disparity with these reports since the maximum level of P we used in experiment was 248 kg ha\(^{-1}\) while some scientists have used P @ 2300 mg P kg\(^{-1}\) soil for immobilizing metals. Cao et al. [67] and Basta et al. [60] reported phosphate to be effectual for immobility of Pb than for Zn, Ni, Cu, and Cd. This may be the reason that Ni concentration in paddy, shoots and roots was more than Pb since Ni was not effectively immobilized due to its interactive effect with Pb under phosphate applications.

The Ni concentration in paddy (Figure 3f) of both coarse and fine cultivars had non-significant (\(p \leq 0.05\), Table 4) response to P application. Application of treatments i.e., \(T_0, T_1\) and \(T_2\) had similar response, whereas, treatments \(T_3\) and \(T_4\) were significantly different from the each other for Ni concentration in paddy. Statistically both cultivars remained non-significant as they accumulated almost similar concentration of Ni (2.7 mg kg\(^{-1}\) and 2.74 mg kg\(^{-1}\)). While the interactive effects of treatment and cultivar also remained non-significant.

Khoshgoftarmanesh and Kalbasi [68] established that with garbage leachate Ni < 1 mg L\(^{-1}\) applied at 600 t ha\(^{-1}\), the paddy Ni concentration is found not greater than 4 mg kg\(^{-1}\). Kashem and Singh [36] observed that solubility of Ni decreased by two to five times in P amended soils due to formation of metal complexes. Less concentration of Ni in grains than that in shoot indicates some physiological barrier which restricts the transport of Ni to grains [69].

### 3.3.3. Post Harvest Soil Pb and Ni Concentration

Lead was significantly (\(p \leq 0.05\), Table 4) immobilized in post rice soil with increasing P application rates (Figure 3g). Lead was effectively immobilized with \(T_3\) (36.3%) which was 3.52 mg Pb kg\(^{-1}\) soil and with \(T_4\) (39.3%) which was 3.3 mg kg\(^{-1}\) soil. Increasing P rates increased immobilization of Pb in soil under both cultivars, but both cultivars remained statistically non-significant. Mean increase in Pb immobilization in soil was in the order of \(T_0 > T_1 \geq T_2 \geq T_3 \geq T_4\). Maximum Pb was immobilized with \(T_4\) which was 39.4 % of the control.

Phosphorus treatments have been shown to exhibit effectiveness in chemical immobilization of Pb [60]. Application of DAP significantly reduced (92%) the bioavailability of Pb [39]. Lead in soil was immobilized mainly as Pb phosphate as reported by Bolan et al. [52], Cao et al. [61]. Other mechanism of immobilization includes direct metal absorption and phosphate anion induced metal absorption. Another reason can be that when superphosphate (SSP) are added in soil, monocalcium phosphate (MCP) dissolves and forms soluble dicalcium phosphate (DCP) and releases phosphoric acid in the vicinity of fertilizer granules. Phosphoric acid can dissociate into hydrogen ions (H\(^+\)) and phosphate. The protons can cause a reduction in pH around fertilizer granules down to very low rates and this lower pH leads to increased dynamics of Pb minerals and subsequent release of Pb [52].

The effect of treatments on Ni immobilization in post rice soil (Figure 3h) was found non-significant (Table 4). Statistically all of the applied P treatments had shown same trends in results. There was no immobilization of Ni with increasing P rates. Cultivars had non-significant effect with each other. The interactive effects of treatments and cultivars were also non-significant.

The present results can be due to the fact that P was effective in remediating Pb, Zn, Cu, and Cd (and to a very little extent Ni) [60]. Another reason might be the lower rates of P used in our experiment. Mcgowen et al. [70] evaluated the performance of DAP as chemical amendment and reported that among different concentrations applied, 2300 mg P kg\(^{-1}\) was
most valuable for immobilization Pb, Zn, and Cd. Iqbal et al. [16] established that increasing application rates of DAP gradually decreased AB-DTPA extractable Pb in post-rice soil in normal and salty noxious soil conditions. Equivalent tendency was noted for AB-DTPA Pb extractability in post-wheat soil under normal and salty soil environment [71].

3.4. Accumulation Pattern of Pb and Ni in Root, Shoot and Paddy

Similar behavior for Pb uptake by KS-282 and Shaheen basmati was observed, with applied P treatments receiving city effluent irrigation. The KS-282 showed maximum uptake (0.58 mg pot^{-1}) in roots (Figure 4a) with control treatment (T0) which decreased significantly with increasing P rates and the lowest uptake (0.43 mg pot^{-1}) was recorded with T4 treatment (248 kg P ha^{-1}). Similarly, maximum Pb uptake (0.35 mg pot^{-1}) in shoots was seen in T0 and decreased to the lowest value of (0.25 mg pot^{-1}) with T0 treatment. Furthermore, maximum uptake of Pb by paddy was 0.10 mg pot^{-1} with T0 decreasing to 0.04 mg pot^{-1} with T4.

![Figure 4. Effect of varying application rates of phosphatic fertilizer on Pb and Ni uptake by rice receiving city effluent irrigation. (Each value is a mean, n = 3 statistically significant at p ≤ 0.05, T bars represent ± SE of means i.e., SEMs). Whereas, T0 = Control, T1 = N_{170}P_{62}K_{62}, T2 = N_{170}P_{124}K_{62}, T3 = N_{170}P_{186}K_{62}, T4 = N_{170}P_{248}K_{62}.](image)

In Shaheen basmati, maximum uptake (0.39 mg pot^{-1}) was recorded by roots with T0 which decreased with increasing P rates to the lowest value of 0.26 mg pot^{-1}. Similarly, Pb uptake by shoots was maximum (0.27 mg pot^{-1}) with T0 and minimum (0.16 mg pot^{-1}) uptake was observed with T4 treatment. The lowest uptake of Pb (0.03 mg pot^{-1}) by paddy was recorded with treatment T4 (248 kg P ha^{-1}). Results showed the Pb concentration in different parts of Shaheen basmati and KS-282 was in the subsequent sequence: as roots > shoots > paddy.

In agreement with the present study, the Pb uptake by rice shoots and paddy of Pb grown under Pb-noxious soils were notably higher than normal and salty soils. Iqbal et al. [16] estab-
lished that the DAP was graded second effectual amendment among tested amendments and its application lessened Pb concentration in rice shoot and paddy under normal and salty Pb noxious soils.

The Ni uptake by plant root, shoot and paddy was also significantly affected by P treatment rates receiving city effluent irrigation. The maximum (1.30 mg pot\(^{-1}\)) recorded Ni uptake by shoots of rice cultivar KS-282 was with T\(_4\) (248 kg P ha\(^{-1}\)) treatment and its minimum uptake (1.12 mg pot\(^{-1}\)) was with T\(_0\) treatment (Figure 4b). Whereas, maximum (1.16 mg pot\(^{-1}\)) Ni uptake by root was with T\(_3\) and the lowest (0.75 mg pot\(^{-1}\)) was observed in case control (T\(_0\)). Moreover, maximum Ni uptake in paddy was (0.09 mg pot\(^{-1}\)) with T\(_4\) treatment in both cultivars. In Shaheen Basmati, maximum Ni uptake in shoots was 1.46 mg pot\(^{-1}\) with T\(_4\) (248 kg P ha\(^{-1}\)) and minimum (1.08 mg pot\(^{-1}\)) was recorded with T\(_0\) treatment. Likewise, maximum Ni uptake by roots was (1.09 mg pot\(^{-1}\)) with T\(_4\) and minimum uptake (0.69 mg pot\(^{-1}\)) was observed with control treatment. The both cultivars remained inconsistent to applied P rates and that most of Ni was accumulated in shoots followed by roots and paddy.

Aslam et al. [72] explained that based on heavy metals tolerance responses and mechanisms, the cereal crops can be categorized into three classes: (i) metal excluders, (ii) metal indicators, and (iii) metal accumulators. The metal ‘excluders’ use avoidance policy towards the accumulation and translocation of metal in their shoots. The metal ‘indicators’ accumulate toxic metals systematically in aerial parts or shoots. The metal ‘accumulators’ related to absorbance, translocation and ultimately these accumulate toxic ions, at levels higher than those recognized in soil medium, in diverse parts of their shoot tissues. In procession with present pot study, Murtaza et al. [24] revealed that N and P fertilization had significantly influenced the difference tissues concentration of Cd, Cu, and Zn in wheat and maize crops. Undue Ni in plants restrain photosynthetic system due to disturbed electron transport chain and assimilatory CO\(_2\) [73], openings and closings of stomata and conductance mechanisms [74]. Nevertheless, the accumulations of Ni in edible ingredients pollute the foodstuff succession, hence menacing health of human at risk [75]. Aziz et al. [76] investigated that the noxious impacts of Ni on rice growth, physiology, and Ni concentration in rice and illustrated the ameliorative functions of Ca on Ni toxicity to plants. Nickel is reported to induce the deficiency of Fe and Zn, and hampers the uptake of Cd, Co, Cr, and Pb [10].

4. Conclusions

In present study, the addition of P has been evaluated in soil irrigated by city effluent, and the bioavailability of Pb and Ni under their interactions in the presence of P in two cultivars of rice has been investigated. Lead was effectively immobilized in soil, showed antagonistic interaction with P and its translocation to upper plant parts was significantly decreased with applied P rates. The Ni showed both synergistic and antagonistic interaction (inconsistent behavior) to P applications. The Ni showed more bioavailability and most of it was accumulated in the shoots and roots of rice, while Pb was mostly retained in the roots. The increased Pb immobilization in soil was found in the order of T\(_0\) > T\(_1\) ≥ T\(_2\) ≥ T\(_3\) ≥ T\(_4\). The addition of P at higher rates also promoted rice growth parameters significantly with the exception of decreased plant height, while the physiological functions of both the rice cultivars were increased with increased P rates. Increasing rate of P along with steady level of NK appears to be an effective and viable option for immobilization of Pb in effluent irrigation. This remedial approach can considerably reduce the risks of growing rice with city effluent containing high Pb and Ni. There is need to carry out more detailed work to assess and establish a sound basis for the suitability and economic feasibility of P for the immobilization of Pb and Ni receiving city effluent for irrigation on farmer’s field and further need to be verified with other rice cultivars and crop species.
Author Contributions: Conceptualization, G.M., M.M.I. and T.N.; data curation, M.A.A.M. and M.M.I.; formal analysis, M.A.A.M., T.N. and M.I.Z.; funding acquisition, A.A., H.F. and I.N.; investigation, G.M., M.M.I. and M.I.Z.; methodology, M.M.I., G.M. and R.P.; resources, M.I.Z., H.F. and I.N.; software, H.F. and R.P.; supervision, G.M., T.N., M.M.I. and I.N.; visualization, H.F.; writing—original draft, M.A.A.M. and M.M.I.; writing—review and editing, T.N., M.I.Z. and R.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All obtained data is enclosed with this manuscript.

Acknowledgments: The researchers would like to thank the Deanship of Scientific Research, Qassim University for funding the publication of this project. The authors are also grateful to the ISES, University of Agriculture Faisalabad, Pakistan for their assistance in research work.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. GOP. Economic Survey of Pakistan 2020–2021; Ministry of Finance, Government of Pakistan: Islamabad, Pakistan, 2021.
2. Hashmi, A.; Bhatti, A.I.; Ahmed, S.; Ur Rehman Tariq, M.A.; Savitsky, A. Revisiting the Indus Basin Model for an energy sustainable Pakistan. Water 2022, 14, 702. [CrossRef]
3. Murtaza, G.; Shehzad, M.T.; Kanwal, S.; Farooqi, Z.; Ownes, G. Biomagnification of potentially toxic elements in animals consuming fodder irrigated with sewage water. Environ. Geochem. Health 2022. [CrossRef] [PubMed]
4. Zeeshan, N.; Nasir, A.A.; Haider, F.U.; Naveed, K.; Naseer, S.; Murtaza, G. Risk assessment of trace metals deposition and growth of Abelmoschus esculentus L. on industrially polluted soils of Faisalabad, Pakistan. Pak. J. Agric. Sci. 2021, 58, 881–889. [CrossRef]
5. Iqbal, M.M.; Naz, T.; Rehman, H.; Nawaz, S.; Qayyum, M.A.; Zafar, M.I.; Farooq, O.; Rehman, R.; Imitiaz, M.; Murtaza, G.; et al. Impact of farm manure application on maize growth and tissue Pb concentration grown on different textured saline-sodic Pb-toxic soils. Asian J. Agric. Biol. 2020, 8, 52–60. [CrossRef]
6. Iqbal, S.; Naz, T.; Sattar, M.A. Challenges and opportunities linked with waste management under global perspective: A mini review. J. Qual. Assur. Agric. Sci. 2021, 1, 9–13. [CrossRef]
7. Iqbal, M.M.; Murtaza, G.; Naz, T.; Niaz, A.; Mehdi, S.M.; Rehman, A. Genotypic differences in rice cultivars for lead (Pb) tolerance in salt affected soil. J. Agric. Res. 2017, 55, 627–637.
8. Iqbal, M.M.; Murtaza, G.; Naz, T.; Hussain, A.; Button, M.; Laing, G.D. Pb fractionation and redistribution as affected by applied inorganic amendments under different soil moisture regimes and incubation time in saline–sodic Pb-polluted paddy soil. Paddy Water Environ. 2018, 16, 875–885. [CrossRef]
9. Shahid, M. Biogeochemical Behavior of Heavy Metals in Soil-Plant System; Higher Education Commission: Islamabad, Pakistan, 2017; p. 577.14–dc23. ISBN 978-969-417-195-1. Available online: https://prr.hec.gov.pk/jspr/handle/123456789/49 (accessed on 8 February 2022).
10. Amjad, M.; Raza, H.; Murtaza, B.; Abbas, G.; Imam, M.; Shahid, M.; Naeem, M.A.; Zakir, A.; Iqbal, M.M. Nickel toxicity induced changes in nutrient dynamics and antioxidant profiling in two maize (Zea mays L.) hybrids. Plants 2020, 9, 5. [CrossRef]
11. Alloway, B.J. Heavy Metals in Soils: Trace Metals and Metalloids in Soils and Their Bioavailability, 3rd ed.; Springer: Dordrecht, The Netherlands, 2012; 614p, ISBN 978-94-007-4469-1. [CrossRef]
12. Marschner, H. Mineral Nutrition of Higher Plants, 3rd ed.; Academic Press: San Diego, CA, USA, 2012; ISBN 978-0-12-384905-2. [CrossRef]
13. Ensink, J.H.J.; Mahmood, T.; Van der Hoek, W.; Raschid-Sally, L.; Amerasinghe, F.P. A nation-wide assessment of wastewater use in Pakistan: An obscure activity or a vitally important one. Water Policy 2004, 6, 197–206. [CrossRef]
14. Baig, I.A.; Ashfaq, M.; Hassan, I.; Javed, M.I.; Khurshid, W.; Ali, A. Economic impacts of wastewater irrigation in Punjab, Pakistan. J. Agric. Res. 2011, 49, 5–14.
15. Murtaza, G.; Ghafoor, A.; Rehman, M.Z.; Sabir, M.; Naeem, A. Phytodiversity for metals in plants grown in urban agricultural lands irrigated with untreated city effluent. Commun. Soil Sci. Plant Anal. 2012, 43, 1181–1201. [CrossRef]
16. Iqbal, M.M.; Naz, T.; Zafar, M.I.; Imitiaz, M.; Farooq, O.; Rehman, A.; Ali, S.; Rizwan, M.; Hussain, S.; Javed, W.; et al. Green remediation of saline–sodic Pb-factored soil by growing salt-tolerant rice cultivar along with soil applied inorganic amendments. Paddy Water Environ. 2020, 18, 637–649. [CrossRef]
17. Mehdi, S.M.; Abbas, G.; Sarfraz, M.; Abbas, S.T.; Hussain, G. Effect of industrial effluents on mineral nutrition of rice and soil health. Pak. J. Agric. Sci. 2003, 3, 462–473. [CrossRef]
18. Iqbal, M.M.; Murtaza, G.; Mehdi, S.M.; Naz, T.; Rehman, A.; Farooq, O.; Ali, M.; Sabir, M.; Ashraf, M.; Sarwar, G.; et al. Evaluation of phosphorus and zinc interaction effects on wheat grown in saline-sodic soil. Pak. J. Agric. Sci. 2017, 54, 531–537. [CrossRef]
48. Patra, M.; Bhownik, N.; Bandopadhyay, B.; Sharma, A. Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. *Environ. Exp. Bot.* 2004, 52, 199–223. [CrossRef]

49. Huang, D.; Xi, L.; Yang, L.; Wang, Z.; Yang, J. Comparison of agronomic and physiological traits of rice genotypes differing in cadmium-tolerance. *Acta. Agron. Sin.* 2008, 34, 808–817.

50. Purnama, P.R.; Soedarti, T.; Purnobasuki, H. The effects of lead [Pb(NO$_3$)$_2$] on the growth and chlorophyll content of sea grass *Thalassia hemprichii* (ehrenb.) Aschers.] ex situ. *Vegetos* 2015, 28, 9–15. [CrossRef]

51. Wang, B.; Xie, Z.; Chen, J.; Jiang, J.; Su, Q. Effects of field application of phosphate fertilizers on the availability and uptake of lead, zinc and cadmium by cabbage (*Brassica chinensis* L.) in a mining tailing contaminated soil. *J. Environ. Sci.* 2008, 20, 1109–1117. [CrossRef]

52. Bolan, N.S.; Duraisamy, P.; Mani, S. Role of inorganic and organic soil amendments on immobilization and phytoavailability of heavy metals: A review involving specific case studies. *Aust. J. Soil Res.* 2003, 41, 1–23.

53. Zaragüeta, A.; Enrique, A.; Virto, I.; Antón, R.; Urmeneta, H.; Orcaray, L. Effect of the long-term application of sewage sludge to a calcareous soil on its total and bioavailable content in trace elements, and their transfer to the crop. *Minerals* 2021, 11, 356. [CrossRef]

54. Kartas, M.; Dursun, S.; Guler, E.; Ozedemir, C.; Argun, M.E. Heavy metal accumulation in wheat plants irrigated by wastewater. *J. Cellul. Chem. Technol.* 2006, 2006, 575–579.

55. Abbas, S.T.; Sarfraz, M.; Mehdi, S.M.; Hassan, G.; Rehman, U. Trace elements accumulation in soil and rice plants irrigated with the contaminated water. *Soil Tillage Res.* 2007, 94, 503–509. [CrossRef]

56. Nan, Z.; Zhao, C.; Li, J.; Chen, F.; Sun, W. Relations between soil properties and selected heavy metal concentrations in spring wheat (*Triticum aestivum* L.) grown in contaminated soils. *Water Air Soil Pollut.* 2002, 133, 205–213. [CrossRef]

57. Stanforth, R.; Qiu, J. Effect of phosphate treatment on the solubility of lead in contaminated soil. *Environ. Geol.* 2001, 41, 1–10. [CrossRef]

58. Meharg, A.A. Mechanisms of plant resistance to metal and metalloid ions and potential biotechnological applications. *Plant Soil* 2005, 274, 163–174. [CrossRef]

59. Isqbal, M.M.; Murtaza, A.; Naz, T.; Javed, W.; Hussain, S.; Ilyas, M.; Anjum, M.A.; Mehdi, S.M.; Ashraf, M.; Isqbal, Z. Uptake, translocation of Pb and chlorophyll contents of *Oryza sativa* as influenced by soil-applied amendments under normal and salt-affected Pb-spiked soil conditions. *Asian J. Agric. Biol.* 2017, 5, 15–25.

60. Basta, N.T.; McGowen, S.L. Evaluation of chemical immobilization treatments for reducing heavy metal transport in a smelter-contaminated soil. *J. Environ. Pollut.* 2004, 127, 73–82. [CrossRef]

61. Cao, R.X.; Ma, L.Q.; Chen, M.; Singh, S.P.; Harris, W.G. Phosphate-induced metal immobilization in a contaminated site. *Environ. Pollut.* 2003, 122, 19–28. [CrossRef]

62. Chandra, R.; Kumar, V.; Tripathi, S.; Sharma, P. Heavy metal phytoextraction potential of native weeds and grasses from endocrine disrupting chemicals rich complex distillery sludge and their histological observations during in-situ phytoremediation. *Ecol. Eng.* 2018, 111, 143–156. [CrossRef]

63. Wang, Y.; Shi, C.; Lv, K.; Li, Y.; Cheng, J.; Chen, X.; Fang, X.; Yu, X. Genotypic variation in nickel accumulation and translocation and its relationships with silicon, phosphorus, iron, and manganese among 72 major rice cultivars from Jiangsu Province, China. *Int. J. Environ. Res. Public Health* 2019, 16, 83281. [CrossRef] [PubMed]

64. Andreeva, I.V.; Matson, R.; Urmeneta, H.; Orcaray, L. Effect of the long-term application of sewage sludge on plant systems and the development of genetic tolerance. *Environ. Exp. Bot.* 2004, 52, 488–494. [CrossRef]

65. WHO. Joint FAO/WHO Expert Standards Program Codex Alimentarius Commission; World Health Organization: Geneva, Switzerland, 2007.

66. Cao, X.; Ma, L.Q.; Singh, S.P.; Chen, M.; Harris, W.G.; Kizza, P. Field Demonstration of Metal Immobilization in Contaminated Soils Using Phosphate Amendments; Final Report 32611-0290; Soil and Water Science Department, University of Florida: Gainesville, FL, USA, 2003.

67. Khoshgoftarmanesh, A.H.; Kalbasi, M. Effect of municipal waste leachate on soil properties and growth and yield of rice. *Commun. Soil Sci. Plant Anal.* 2002, 33, 2011–2020. [CrossRef]

68. Datta, S.P.; Biswas, D.R.; Saharan, N.; Ghosh, S.K.; Rattan, R.K. Effect of long-term application of sewage effluents on organic carbon, bioavailable phosphorus, potassium and heavy metals status of soils and uptake of heavy metals by crops. *J. Indian Soc. Soil Sci.* 2000, 48, 836–839.

69. Mcgowen, S.L.; Basta, N.T.; Brown, G.O. Use of diammonium phosphate to reduce heavy metal solubility and transport in smelter-contaminated soil. *J. Environ. Qual.* 2001, 30, 493–500. [CrossRef]

70. Ahmad, H.R.; Ghafoor, A.; Corwin, D.L.; Aziz, M.A.; Ullah, S.; Sabir, M. Organic and inorganic amendments affect soil concentration and accumulation of cadmium and lead in wheat in calcareous alkaline soils. *Commun. Soil Sci. Plant Anal.* 2011, 42, 111–122. [CrossRef]

71. Aslam, M.; Aslam, A.; Sheraz, M.; Ali, B.; Ulhassan, Z.; Najeeb, U.; Zhou, W.; Gill, R.A. Lead toxicity in cereals: Mechanistic insight into toxicity, mode of action, and management. *Front. Plant Sci.* 2021, 11, 587785. [CrossRef]

72. Sabir, M.; Hakeem, K.R.; Aziz, T.; Rehman, M.Z.; Rashid, I.; Ozturk, M. High Ni levels in soil can modify growth performance and mineral status of wheat cultivars. *Clean-Soil Air Water* 2014, 42, 1263–1271. [CrossRef]
74. Ouzounidou, G.; Moustakas, M.; Symeonidis, L.; Karataglis, S. Response of wheat seedlings to Ni stress: Effects of supplemental calcium. *Arch. Environ. Contam. Toxicol.* 2006, 50, 346–352. [CrossRef] [PubMed]

75. Wu, Y.; Hendershot, W.H. The effect of calcium and pH on nickel accumulation in and rhizotoxicity to pea (*Pisum sativum* L.) root--empirical relationships and modeling. *Environ. Pollut.* 2010, 158, 1850–1856. [CrossRef]

76. Aziz, H.; Sabir, M.; Ahmad, H.R.; Aziz, T.; Zia-ur-Rehman, M.; Hakeem, K.R.; Ozturk, M. Alleviating effect of calcium on nickel toxicity in rice. *Clean-Soil Air Water* 2015, 43, 901–909. [CrossRef]