Mitigation of Lead (Pb) Toxicity in Rice Cultivated with Ground Water and Waste Water by Application of Acidified Carbon

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Abstract

Toxicity induced by a higher concentration of lead (Pb) can significantly decrease plants growth, gas exchange and yield attributes. It also caused cancer in humans. Use of organic amendments especially biochar has the capability to alleviate Pb toxicity in different crops. Application of biochar can decrease the uptake Pb by roots in plants. However, high pH of thermo pyrolyzed biochar makes it unfit amendment for high pH soils. As Pb is an acute toxin and its uptake in rice is a major issue, current experiment was conducted with the aim to explore the efficacy of chemically produced acidified carbon (AC) for mitigation of Pb toxicity in rice. Leas has introduced artificially at the rate of (0, 15 and 30 mg kg⁻¹ soil) in combination with 0, 0.5 and 1 % AC underground water (GW) and waste water irrigation (WW) in rice. Results confirmed that addition of 1% AC significantly improved plant height, number of tillers, spike length fresh and dry weights of spikes and straw, 1000 grains weight, photosynthetic rate, transpiration rate and stomatal conductance over 0 % AC under GW and WW irrigations at 30 mg Pb kg⁻¹ soil (30Pb) toxicity. A significant decrease in electrolyte leakage and plant Pb concentration by application of 0.5 and 1 % AC validated their effectiveness for mitigation of 30Pb toxicity in rice over 0 % AC under GW and WW. In conclusion, 1 % AC is effective amendment in the alleviation of Pb toxicity in rice irrigated with GW and WW at 30Pb.

Introduction

Toxicity induced by heavy metals, is a serious concern of soil and environmental health. These heavy metals entered in food chain when plants are cultivated in heavy metals contaminated soils^{1,2}. Among different heavy metals, lead (Pb) is a potential pollutant and highly toxic for plants and humans. It has been observed that sulfide ores melting and burning of fossil fuels played a significant role in the emission of Pb in environment³. Higher uptake of Pb can cause mutagenesis and cancer in humans^{4,5}. It also adversely affect the growth attributes, macronutrients uptake and chlorophyll contents of plants⁵. Low photosynthetic rate, transpiration rate and stomatal conductance are also some of negative effects of Pb which played significant role in making poor plants growth⁶.

On the other hand, limited availability of good quality irrigation water is a big hurdle for the achievement of optimum crops yield. On an average, the consumption of good quality water for domestic purposes and industries has tremendously increased. Such conditions resulted in generation of significant quantity (962,334 M gallons) of untreated waste water⁷. Most of farmer applied 30% of this untreated waste water as irrigation in urban and semi-urban agricultural lands (22 M ha land) to overcome the problem of irrigation water scarcity^{7,8}. Readily available essential nutrients and low cost of waste water attracts the farming community for its utilization as irrigation⁹. However, they ignored the health hazards that are associated with the consumption of such untreated waste water irrigated crops¹⁰. It also induced detrimental effect on plants and soil¹¹. Persistent organic pollutants and enrichment of heavy metals in waste water coming from sewage and industries make it unfit. Microbes in soil remained unable to degrade the accumulated heavy metals which become the part of soil when waste water is irrigated¹².

To deal with heavy metals induced toxicity in plants scientists have suggested many technologies ¹³. However, need of time is to adopt such methodologies and techniques which are cost-effective and remained actively beneficial for long duration. That's why, bio-sorbent have suggested for mitigation of heavy metals toxicity in plants. Activated carbon biochar is one of such amendment that has potential to immobilize heavy metals in soil ¹⁴⁻¹⁶. Pyrolysis of organic waste compounds resulted in sequestration of carbon. It remained in soil for longer time period due to high resistant against decomposition ¹⁷. Many essential nutrients that are integral part of biochar structure is also released in soil which also facilitate in the improvement of plants vegetative growth ¹⁸. High porosity, surface charges stability and improved exchange sites played an imperative role in decreasing the uptake of these heavy metals in plants. When biochar is applied in soil, it not only contribute in better uptake of essential nutrients but also improve soil organic carbon pool which positively affect soil health ^{5,14,19-28}.

As a regular diet, > 50 million people consumed grains of rice ²⁹. According to the FAO survey report, analysis of rice nutritional status from different regions in the world, it contains various essential nutrient like N, P, K, Ca, Zn, Fe and Na. It is estimated that in 100 grams of rice contains 349–373 kcal energy, proteins (6.3–7.1 g), lipids (0.3–0.5 g), carbohydrates (77–78 g), fibers (0.2–0.5 g), riboflavin (0.02–0.06 mg), niacin (1.3–2.4 mg), thiamine (0.02–0.11 mg) and vitamin E (0.075–0.30 mg) are obtained ³⁰. In Bangladesh, Vietnam and Indonesia, 60% calories are taken from rice ³¹. That's why current experiment was conducted to check the influence of acidified carbon in alleviation of Pb toxicity in rice plant. To the best of our knowledge, this is the first kind of study where we use chemically produced acidified carbon in rice as an amendment for immobilization of Pb. We hypothesized that combined use of wastewater and chemically produced acidified carbon can be effective in minimizing Pb toxicity and enhancement growth, gas exchange attributes and yield of rice under Pb toxic conditions.

Material And Methodology

Experimental site and design

A pot study was done for assessing the effect of acidified carbon on rice growth and yield under Pb contaminated soil. The rice was irrigated with ground water and waste water. The study was conducted in research area of Department Soil Science, Bahauddin Zakariya University Multan, Pakistan. The research area is located in semi-arid region characterized with low average rainfall and high mean temperature (Figure 1).

Design of Experiments and Treatments

The study was designed in complete randomized design. Total eighteen treatments were applied with three replications. The treatments includes control+GW (no Pb+no AC), 15Pb+GW (15 mg Pb kg⁻¹ soil+noAC), 30Pb+GW (30 mg Pb kg⁻¹ soil+noAC), control+WW, 15Pb+WW, 30Pb+WW, control+GW+0.5AC (no Pb+0.5% acidified carbon), 15Pb+GW+0.5AC, 30Pb+GW+0.5AC, control+WW+0.5AC,

15Pb+WW+0.5AC, 30Pb+WW+0.5AC, control+GW+1AC (no Pb+1%AC), 15Pb+GW+1AC, 30Pb+GW+1AC, control+WW+1AC, 15Pb+WW+1 AC and 30Pb+WW+1AC.

Preparation of acidified carbon (AC)

Waste syrup of sugar was used to manufacture acidified carbon as described by Sultan et al. ³². In a designed reactor, H₂SO₄ (conc.) was added with sugar syrup at 1:2 (v/v) ratio. In an immediate and irreversible reaction, water vaporized and left behind the AC. Following reaction was occurred by mixing of H₂SO₄ and syrup:

Characterization of acidified carbon

The volatile matter, ash content and fixed carbon in AC were assessed according to McLaughlin et al. ³³ and Danish and Zafar-ul-Hye ³⁴. The pHs and ECe of AC were determined in filtered aliquots of biochar and distilled water at ratio of 1:10 ³⁵ by the help of pH and EC meter. Physiochemical characteristics of AC is provided in Table 1.

Table 1. Chemical properties of acidified carbon

Parameters	Units	Values
pHs	-	6.32
ECe	dS m ⁻¹	3.17
Ash	%	15
Fixed Carbon	%	65
Volatile Contents	%	20
Total Lead	µg g ⁻¹	1.34
Total Zn	µg g ⁻¹	0.45
Total B	µg g ⁻¹	0.10

Soil Characterization

Soil was collected from mango orchard of Bahauddin Zakariya University. Soil was sieved in to 2 mm mesh. Properties of experimental soil is given in Table 2. Recommended rate of NPK fertilizers were added at 136:89:62 kg ha⁻¹ for rice. Except of that, 14.8 kg/ha of ZnSO₄ (33 % Zn) and 7.41 kg ha⁻¹ boric acid (10.5 % Boron) was also added in soil and mixed uniformly. According to the study design, soil was

divided into three portions with respect to Pb concentration. Along with control soil, 15 and 30 mg kg⁻¹ of Pb was applied using lead sulfate (PbSO₄) source. After incubating the soil for 3 days with Pb, soil was properly mixed and 7 kg soil was filled in pots for further application of AC treatments in control and Pb contaminated soil. AC was incorporated well in soil at 0.5 % and 1 % rate.

Table 2. Characterization of experimental soil and irrigation water

Soil Parameter	Units	Value	Irrigation Parameter	Units	Value	
					WW	GW
pHs	-	8.63	pH	-	6.04	7.19
ECe	dSm ⁻¹	2.91	EC	dSm ⁻¹	3.53	0.34
Organic Matter	%	0.45	Carbonates	meq./L	0.34	0.00
Organic Nitrogen	%	0.23	Bicarbonates	meq./L	1.04	1.36
Available Phosphorus	mg kg ⁻¹	5.13	Chlorides	meq./L	0.50	0.20
Extractable Potassium	mg kg ⁻¹	124	Ca+Mg	meq./L	3.41	4.01
Total Lead	mg kg ⁻¹	4.63	Soluble Lead	mg kg ⁻¹	5.19	0.25
Extractable Zinc	mg kg ⁻¹	0.17	Soluble Zinc	mg kg ⁻¹	0.45	0.11
Extractable Boron	mg kg ⁻¹	0.11	Soluble Boron	mg kg ⁻¹	0.23	0.10

Seedlings Transplantation

About 8-10 kg of rice seedlings were transplanted from nursery on 31st May, 2019. Transplantation was done by hand manually.

Irrigation

Sewage water and ground water was used as treatment for irrigation purpose. Biochemical and chemical properties of waste water is described in Table 2.

Harvesting of crop

Crop was harvested manually after full maturity in November, 2019. The harvesting was done manually. Following agronomic parameters were noted in rice by taking the average value of each treatment's plants. Height of rice plant was recorded with meter scale from spike top to shoot bottom. Length of each spike was recorded with scale and the average value of all spike in each pot was determined. Number of tillers was recorded manually and means were taken. Fresh harvested whole plant (shoot and spikes)

were weighed on weighing balance. After separation of kernels from spikes, thousand grains were separated and weighed for determining the quality of seed.

Electrolyte leakage

For estimating the electrolyte leakage (EL) of plants, 0.2 g of fresh plant sample was taken and chopped in to small pieces and immersed in 10 ml of distilled water ³⁶. Incubation was done at 25 °C for 24 h. The electrical conductivity (EC1) was examined using pre-calibrated EC meter. The second EC (EC2) was measured by heating the test tubes in a water bath at 120 °C for 20 min. The final value of EL was calculated using the equation as follows;

$$\text{EL (\%)} = \text{EC1/EC2} \times 100$$

Chlorophyll contents

Fresh leaves were taken and cut in to small pieces and 0.5 g leaf samples were immersed in 10 ml acetone for 24 hours. Extract of chlorophyll was measured and color intensity was determined at 645 nm and 663 nm by the help of spectrophotometer ³⁷. From intensity values, chlorophyll contents were determined by following formula:

$$\begin{aligned}\text{Chlorophyll a (mg g}^{-1}\text{)} &= [12.7 \times \text{OD}_{663} - 2.69 \times \text{OD}_{645}] \times \frac{V}{1000} \times W \\ \text{Chlorophyll b (mg g}^{-1}\text{)} &= [22.9 \times \text{OD}_{645} - 4.68 \times \text{OD}_{663}] \times \frac{V}{1000} \times W \\ \text{Total chlorophyll (mg g}^{-1}\text{)} &= \text{Chlorophyll a} + \text{Chlorophyll b}\end{aligned}$$

where, OD = Optical density (wavelength), V = Final volume made, W = Fresh leaf made (g)

Photosynthetic rate and stomatal conductance of rice leaves were determined by using Infrared gas analyzer (CI-340 Photosynthesis system, CID, Inc. USA). On a sunny day, the readings were taken between 10:30 and 11:30 AM at saturating intensity of light ³⁸.

Plant Digestion

Plants shoot, leaves and grains samples were oven dried and grinded separately to form homogeneous powder. A mixture of di-acid was prepared by 10:1:4 HNO₃, H₂SO₄ and HClO₄. Plant material was mixed with conc. H₂SO₄ and kept overnight. Before heating, 10ml acid mixture was added in that suspension. Mixture was heated till clearing the solution and white fumes emerge ³⁹. Mixture was than cooled and stored for further nutrient analysis. For Pb determination, sample was filtered and directly ran on atomic spectrophotometer.

Acidified Carbon and Soil Total Cadmium

Cadmium can be quantified by the soil di-acid wet digestion. For this one-gram air dried soil, 3 ml HNO_3 was added and heated on hot plate at 145°C for an hour. After that, 4ml of per chloric acid (HClO_4) was added and hot plated temperature was raised to 240°C for one hour again ⁴⁰. After clearing the solution, flask was removed and kept for cooling. When solution temperature reached at room temperature, 50 ml DI water was added in it and then filtered that solution. Sample was run with deionized water as blank at atomic absorption spectrophotometer (graphite furnace; Agilent 200) for estimating the Pb concentration in soil.

Statistical analysis

Data computation was made on Microsoft Excel 2019 ® and statistical analysis one-way ANOVA was performed by Origin 2020b. Difference among treatment means was assessed by using the least square difference (LSD) at 0.5% probability level. Pearson correlation and principal component analysis was done ⁴¹.

Results

Results showed that application of different levels of acidified carbon (AC) significantly affect the plant height of rice cultivated under 0, 15 (15Pb) and 30 mg Pb kg^{-1} (30Pb) soil irrigated with waste water (WW) and ground water (GW) irrigations. No significant change was observed in plant height in 15Pb and 30Pb from control where WW was applied at 0%AAC. In 0%AAC, plant height was significantly higher in control + WW, 15Pb + WW and 30Pb + WW as compared to control + GW, 15Pb + GW and 30Pb + GW. Increasing level of Pb was non-significant for plant height over control (no Pb) under GW at 1%AAC. No significant change was also observed in plant height in 15Pb and 30Pb from control where WW was applied at 1%AAC. In 1%AAC, plant height was statistically alike in control + WW, 15Pb + WW and 30Pb + WW as compared to control + GW, 15Pb + GW and 30Pb + GW (Fig. 2A). Increasing level of Pb was non-significant for plant height over control (no Pb) under GW at 1%AAC. Significant change was noted in plant height in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, plant height was significantly high in control + WW and 15Pb + WW over control + GW and 15Pb + GW. Chord diagram confirmed that 1%ACC gave maximum increase in plant height as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better plant height as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 2B).

Addition of different levels of acidified carbon (AC) significantly changed spike length of rice cultivated with waste water (WW) and ground water (GW) irrigations under 0, 15 (15Pb) and 30 mg Pb kg^{-1} (30Pb) soil irrigated. In 0%AAC, spike length was significantly higher in control + WW, 15Pb + WW and 30Pb + WW as compared to control + GW, 15Pb + GW and 30Pb + GW. Increasing level of Pb was non-significant for spike length over control (no Pb) under GW at 1%AAC. No significant change was also observed in spike length in 15Pb and 30Pb from control where WW was applied at 1%AAC. In 1%AAC, spike length was statistically alike in control + WW, 15Pb + WW but differed significantly in 30Pb + WW as compared to control + GW, 15Pb + GW and 30Pb + GW respectively (Fig. 3A). Increasing level of Pb was non-significant

for spike length over control (no Pb) under GW at 1%AAC. Significant change was noted in spike length in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, spike length was significantly high in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW. Chord diagram showed that 1%ACC gave maximum increase in spike length as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better spike length as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 3B).

Number of spikes did not differ significantly in 15Pb + GW over control + GW. No significant change was observed in number of spikes in 15Pb and 30Pb from control where WW was applied at 0%AAC. In 0%AAC, number of spikes was significantly higher in control + WW, 15Pb + WW and 30Pb + WW as compared to control + GW, 15Pb + GW and 30Pb + GW. Increasing level of Pb was non-significant for number of spikes over control (no Pb) under GW at 1%AAC. No significant change was also observed in number of spikes in 15Pb and 30Pb from control where WW was applied at 1%AAC (Fig. 4A). In 1%AAC, number of spikes was significantly higher in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW respectively. Increasing level of Pb was non-significant for number of spikes over control (no Pb) under GW at 1%AAC. Significant change was noted in number of spikes in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, number of spikes was significantly high in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW. Results also confirmed that 1%ACC gave maximum increase in number of spikes as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better number of spikes as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 4B).

Number of tillers remained non-significant in 15Pb + GW over control + GW. Significant decrease was observed in number of tillers in 30Pb from control where WW was applied at 0%AAC. In 0%AAC, number of tillers was significantly higher in control + WW, 15Pb + WW and 30Pb + WW as compared to control + GW, 15Pb + GW and 30Pb + GW. Increasing level of Pb was non-significant for number of tillers over control (no Pb) under GW at 1%AAC. No significant change was also observed in number of tillers in 15Pb and 30Pb from control where WW was applied at 1%AAC. In 1%AAC, number of tillers was significantly higher in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW respectively. 15Pb was non-significant but 30Pb different significantly for number of tillers over control (no Pb) under GW at 1%AAC (Fig. 5A). Significant change was noted in number of tillers in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, number of tillers was significantly high in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW. Results also confirmed that 1%ACC gave maximum increase in number of tillers as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better number of tillers as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 5B).

In 0%AAC, fresh weight of straw was significantly higher in 15Pb + WW and 30Pb + WW as compared to 15Pb + GW and 30Pb + GW. Increasing level of Pb was non-significant for fresh weight of straw over control (no Pb) under GW and WW at 1%AAC. In 1%AAC, fresh weight of straw was significantly higher in control + WW and 15Pb + WW over control + GW and 15Pb + GW respectively. Treatments 15Pb and 30Pb

were non-significant for fresh weight of straw over control (no Pb) under GW at 1%AAC (Fig. 6A). Significant change was noted in fresh weight of straw in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, fresh weight of straw was significantly high in control + WW and 15Pb + WW over control + GW and 15Pb + GW. Results also confirmed that 1%ACC gave maximum increase in fresh weight of straw as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better fresh weight of straw as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 6B).

Effects of different application rates of acidified activated carbon and lead (Pb) levels under waste water (WW) and ground water (GW) irrigation was significant for fresh weight of spike. It was noted that 15Pb did not decrease fresh weight of spike over control (no Pb) under GW and WW at 0%AAC. However, 30Pb significantly decreased fresh weight of spike from control in GW at 0%AAC. In 0%AAC, fresh weight of spike was significantly higher in 15Pb + WW and 30Pb + WW as compared to 15Pb + GW and 30Pb + GW. Increasing level of Pb was non-significant for fresh weight of spike over control (no Pb) under GW and WW at 1%AAC. In 1%AAC, fresh weight of spike was non-significantly higher in control + WW and 15Pb + WW over control + GW and 15Pb + GW respectively (Fig. 7A). Treatments 30Pb were non-significant for fresh weight of spike over control (no Pb) under GW at 1%AAC. Significant change was noted in fresh weight of spike in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, fresh weight of spike was significantly high in control + WW and 15Pb + WW over control + GW and 15Pb + GW. Results also confirmed that 1%ACC gave maximum increase in fresh weight of spike as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better fresh weight of spike as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 7B).

No significant change in dry weight of straw was observed by increasing Pb levels in WW at 0%AAC. In 0%AAC, dry weight of straw was significantly higher in 15Pb + WW and 30Pb + WW as compared to 15Pb + GW and 30Pb + GW. Increasing level of Pb was non-significant for dry weight of straw over control (no Pb) under GW and WW at 1%AAC. In 1%AAC, dry weight of straw was statistically alike in control + WW and 15Pb + WW over control + GW and 15Pb + GW respectively. Treatments 30Pb was significant for dry weight of straw over control (no Pb) under GW at 1%AAC (Fig. 8A). Significant change was noted in dry weight of straw in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, dry weight of straw was significantly high in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW. Results also confirmed that 1%ACC gave maximum increase in dry weight of straw as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better dry weight of straw as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 8B).

In 0%AAC, dry weight of spike was significantly higher in 15Pb + WW and 30Pb + WW as compared to 15Pb + GW and 30Pb + GW. Increasing level of Pb was non-significant for dry weight of spike over control (no Pb) under GW and WW at 1%AAC. In 1%AAC, dry weight of spike was statistically alike in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW respectively. Treatments 30Pb was significant for dry weight of spike over control (no Pb) under GW at 1%AAC (Fig. 9A). Significant change was noted in dry weight of spike in control over 15Pb and 30Pb where WW was

applied at 1%AAC. In 1%AAC, dry weight of spike was significantly high in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + WW. Results also confirmed that 1%ACC gave maximum increase in dry weight of spike as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better dry weight of spike as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 9B).

Results showed that 0%AAC, 1000 grains weight was significantly higher in 15Pb + WW and 30Pb + WW as compared to 15Pb + GW and 30Pb + GW. Increasing level of Pb was non-significant for 1000 grains weight over control (no Pb) under GW and WW at 1%AAC. In 1%AAC, 1000 grains weight was statistically alike in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW respectively. Treatments 30Pb was significant for 1000 grains weight over control (no Pb) under GW at 1%AAC. Significant change was noted in 1000 grains weight in control over 15Pb and 30Pb where WW was applied at 1%AAC (Fig. 10A). In 1%AAC, 1000 grains weight was significantly high in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + WW. Results also confirmed that 1%ACC gave maximum increase in 1000 grains weight as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better 1000 grains weight as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 10B).

No significant change was observed in chlorophyll content where WW was applied under increasing level of Pb. In 0%AAC, chlorophyll content was significantly higher in 15Pb + WW and 30Pb + WW as compared to 15Pb + GW and 30Pb + GW. 30Pb significantly decreased chlorophyll content over control (no Pb) and 15Pb under GW and WW at 1%AAC. In 1%AAC, chlorophyll content was significantly higher in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW respectively. Treatments 30Pb was significant for chlorophyll content over control (no Pb) under GW at 1%AAC. Significant change was noted in chlorophyll content in control over 15Pb and 30Pb where WW was applied at 1%AAC (Fig. 11A). In 1%AAC, chlorophyll content was significantly high in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + WW. Results also confirmed that 1%ACC gave maximum increase in chlorophyll content as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better chlorophyll content as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 12B).

A significant decrease was observed in electrolyte leakage where WW was applied under increasing level of Pb. In 0%AAC, electrolyte leakage was significantly low in 15Pb + WW and 30Pb + WW as compared to 15Pb + GW and 30Pb + GW. 30Pb significantly increased electrolyte leakage over control (no Pb) and 15Pb under GW and WW at 1%AAC. In 1%AAC, electrolyte leakage was significantly low in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW respectively. Treatments 30Pb was significant for electrolyte leakage over control (no Pb) under GW at 1%AAC. Significant change was noted in electrolyte leakage in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, electrolyte leakage was significantly low in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + WW (Fig. 12A). Results also confirmed that 1%ACC gave maximum decrease in electrolyte leakage as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC

also gave significantly low electrolyte leakage as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 12B).

Effects of different application rates of acidified activated carbon and lead (Pb) levels under waste water (WW) and ground water (GW) irrigation was significant for photosynthetic rate. It was noted that 30Pb significantly decreased photosynthetic rate over control (no Pb) under GW at 0%AAC. A significant change was observed in photosynthetic rate where WW was applied under increasing level of Pb. In 0%AAC, photosynthetic rate was significantly higher in 15Pb + WW and 30Pb + WW as compared to 15Pb + GW and 30Pb + GW. 30Pb significantly decreased photosynthetic rate over control (no Pb) and 15Pb under GW and WW at 1%AAC. In 1%AAC, photosynthetic rate was significantly higher in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW respectively. Treatments 30Pb was significant for photosynthetic rate over control (no Pb) under GW at 1%AAC (Fig. 13A). Significant change was noted in photosynthetic rate in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, photosynthetic rate was significantly high in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + WW. Chord diagram confirmed that 1%ACC gave maximum increase in photosynthetic rate as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better photosynthetic rate as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 13B).

A significant change was observed in transpiration rate where WW was applied under increasing level of Pb. In 0%AAC, transpiration rate was significantly higher in 15Pb + WW and 30Pb + WW as compared to 15Pb + GW and 30Pb + GW. 30Pb significantly decreased transpiration rate over control (no Pb) and 15Pb under GW and WW at 1%AAC. In 1%AAC, transpiration rate was significantly higher in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW respectively. Treatments 30Pb was significant for transpiration rate over control (no Pb) under GW at 1%AAC. Significant change was noted in transpiration rate in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, transpiration rate was significantly high in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + WW (Fig. 14A). Results also confirmed that 1%ACC gave maximum increase in transpiration rate as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better transpiration rate as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 14B).

In 0%AAC, stomatal conductance was significantly higher in 15Pb + WW and 30Pb + WW as compared to 15Pb + GW and 30Pb + GW. 30Pb significantly decreased stomatal conductance over control (no Pb) and 15Pb under GW and WW at 1%AAC. In 1%AAC, stomatal conductance was significantly higher in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW respectively. Treatments 30Pb was significant for stomatal conductance over control (no Pb) under GW at 1%AAC (Fig. 15A). Significant change was noted in stomatal conductance in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, stomatal conductance was significantly high in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + WW. Results also confirmed that 1%ACC gave maximum increase in stomatal conductance as compared to 1%AAC and 0%AAC in GW and WW.

However, application of 1%AAC also gave significantly better stomatal conductance as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 15B).

A significant change was observed in Pb concentration of rice where WW was applied under increasing level of Pb. In 0%AAC, Pb concentration of rice was significantly higher in 15Pb + WW and 30Pb + WW as compared to 15Pb + GW and 30Pb + GW. 30Pb significantly decreased Pb concentration of rice over control (no Pb) and 15Pb under GW and WW at 1%AAC. In 1%AAC, Pb concentration of rice was significantly higher in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW respectively. Treatments 30Pb was significant for Pb concentration of rice over control (no Pb) under GW at 1%AAC. Significant change was noted in Pb concentration of rice in control over 15Pb and 30Pb where WW was applied at 1%AAC (Fig. 16A). In 1%AAC, Pb concentration of rice was significantly low in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + WW. Results also confirmed that 1%ACC gave maximum decreased in Pb concentration of rice as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly low Pb concentration of rice as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 16B).

In 0%AAC, soil Pb concentration was significantly low in 15Pb + WW and 30Pb + WW as compared to 15Pb + GW and 30Pb + GW. 30Pb significantly increased soil Pb concentration over control (no Pb) and 15Pb under GW and WW at 1%AAC. In 1%AAC, soil Pb concentration was significantly low in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + GW respectively. Treatments 30Pb was significant for soil Pb concentration over control (no Pb) under GW at 1%AAC. Significant change was noted in soil Pb concentration in control over 15Pb and 30Pb where WW was applied at 1%AAC (Fig. 17A). In 1%AAC, soil Pb concentration was significantly high in control + WW, 15Pb + WW and 30Pb + WW over control + GW, 15Pb + GW and 30Pb + WW. Results also confirmed that 1%ACC gave maximum increase in soil Pb concentration as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly high soil Pb concentration as compared to 0%AAC under different levels of Pb in GW and WW (Fig. 17B). Pearson correlation showed that electrolyte leakage and plant Pb concentration has significant negative correlation with all the growth, yield and gas exchange attributes. Significant positive correlation was noted between electrolyte leakage and plant Pb concentration (Fig. 18).

Discussion

A significant decrease in growth and yield attributes of rice by increasing Pb concentration was due to toxicity induced by Pb in plants. High exposure of Pb to plants significantly enhanced the production of reactive oxygen species (ROS). These ROS induced oxidative stress in plants that adversely affect the growth attributes⁴². Scientists have observed that ROS disturb the electron transport chain and metabolism of different nutrients elements in plants^{43,44}. Increase in uptake of Pb also restrict the activity of enzymes which regulate the mineral nutrients in plants. It also decrease the permeability of membrane and react with sulfhydryl groups at cellular level^{45,46}. As an abiotic stress, heavy metals also stimulate endogenous production of stress generating ethylene. This ethylene react with cell membrane

and activates chlase gene which played an imperative role in decreasing the chlorophyll contents in plants leaves ⁴⁷. Higher accumulation of stress ethylene in plant roots, play an imperative role in decreasing the root elongation. It promotes the thickness of plant roots via accumulation of dead cell in the root cortex. Such accumulation of death cells in root cortex results in the formation of lysigenous aerenchyma. In hypocotyls region, stress ethylene also decreases the cell division. Low cell division eventually results in poor elongation of roots and shoot in plants ⁴⁸.

Results of current study showed that application of waste water significantly increased the growth and 1000 grains weight of rice over ground water as irrigation under different levels of Pb (0, 15 and 30Pb). This increase was due to high N, P and K contents of waste water over ground water. Hamid *et al.* ⁴⁹ also reported similar kind of results. They argued that waste water is a rich source of nutrition for the plants. It not only provide irrigation water for crops cultivation but also has potential to decrease the application rates of inorganic macronutrients fertilizers ⁴⁹.

Furthermore, addition of 0.5 and 1% AAC performed significantly better over control (0%AAC) for the increase in growth and 1000 grains weight in rice under 15 and 30Pb. The improvement in growth, chlorophyll contents and 1000 grains weight were due to less uptake of Pb as compared to control. However, activated carbon ability to sorp nutrients also reduced the losses of N and improved its uptake in plants ^{50,51}. According to Chan *et al.* ⁵² the high surface area of activated carbon is basic reason for improved cation exchange sites in soil which resulted in better bioavailability of nutrients. In addition, geometry, size, distribution and number of microspores in activated carbon play an efficacious role in the sorption of nutrients and water. Application of activated carbon in soil also makes rapid cycling of nutrients. Higher retention of nutrients and diversity of rhizobacteria improve the fertility level of soil and nutrients availability to the plants ⁵³. It also releases a significant amount of nutrients in soil solution that become part of the activated carbon structure during pyrolysis. The concentration of nutrients present in activated carbon is dependent on the type of waste feedstock which is used to develop the activated carbon ⁵⁴.

Younis *et al.* ⁵⁰ regarding uptake of P by addition of cotton sticks activated carbon. According to Singh *et al.* ⁵⁵ it is the higher amount of K in activated carbon ash that contributes in the improvement of K concentration in plants. Better uptake of K might have maintained the turgor of cells and stomatal conductance by osmoregulation ^{56,57}. Polar and dispersive surface of biochar along with solid surface energy is directly associated with the retention of water molecules. The negative zeta potential of biochar in majority cases shows the presence of negative charges at biochar surface. Electrostatic force of attraction between negatively charged biochar surface and cations in soil solution facilitates the adsorption of nutrients at biochar surface ¹⁸. Progressive degradation of cellulose and lignin in waste feedstock make the amorphous surface of biochar. This amorphous surface of biochar has micropores. Emission of volatile compound during pyrolysis creates the spaces which plays a role in absorption of water when biochar is applied in soil as an amendment ⁵⁸.

Declarations

Author Contributions: Concept, S.D.; I.I.; method, S.D.; software, N.A.; A.E.; validation, M.A.A.; S.D.; N.A.; and A.E.; formal analysis, A.E.; investigation, A.E.; N.A.; A.E.; resources, S.T.; A.A.S.; R.D.; data curation, A.E.; writing—original draft preparation, S.D.; N.A.; A.E.; S.F.; R.D.; A.E.; writing—review and editing, S.F.; R.D.; I.I.; S.T.; A.A.S.; N.A.; A.E.; visualization, N.A.; A.E.; supervisor, N.A.;

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Figures

Figure 1

No significant change was also observed in number of spikes in 15Pb and 30Pb from control where WW was applied at 1%AAC (Figure 4A). In 1%AAC, number of spikes was significantly higher in control+WW, 15Pb+WW and 30Pb+WW over control+GW, 15Pb+GW and 30Pb+GW respectively. Increasing level of Pb was non-significant for number of spikes over control (no Pb) under GW at 1%AAC. Significant change was noted in number of spikes in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, number of spikes was significantly high in control+WW, 15Pb+WW and 30Pb+WW over control+GW, 15Pb+GW and 30Pb+GW. Results also confirmed that 1%ACC gave maximum increase in number of spikes as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better number of spikes as compared to 0%AAC under different levels of Pb in GW and WW (Figure 4B).

Figure 1

Treatments 30Pb was significant for dry weight of straw over control (no Pb) under GW at 1% AAC (Figure 8A). Significant change was noted in dry weight of straw in control over 15Pb and 30Pb where WW was applied at 1% AAC. In 1% AAC, dry weight of straw was significantly high in control+WW, 15Pb+WW and 30Pb+WW over control+GW, 15Pb+GW and 30Pb+GW. Results also confirmed that 1% AAC gave maximum increase in dry weight of straw as compared to 1% AAC and 0% AAC in GW and WW. However, application of 1% AAC also gave significantly better dry weight of straw as compared to 0% AAC under different levels of Pb in GW and WW (Figure 8B).

Figure 1

Significant change was noted in soil Pb concentration in control over 15Pb and 30Pb where WW was applied at 1%AAC (Figure 17A). In 1%AAC, soil Pb concentration was significantly high in control+WW, 15Pb+WW and 30Pb+WW over control+GW, 15Pb+GW and 30Pb+WW. Results also confirmed that 1%ACC gave maximum increase in soil Pb concentration as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly high soil Pb concentration as compared to 0%AAC under different levels of Pb in GW and WW (Figure 17B).

Figure 1

The daily minimum, maximum and average temperature and rainfall in Multan, Pakistan during the year 2019. The dotted lines are showing the duration of cluster bean crop.

Figure 1

Treatments 30Pb was significant for stomatal conductance over control (no Pb) under GW at 1% AAC (Figure 15A). Significant change was noted in stomatal conductance in control over 15Pb and 30Pb where WW was applied at 1% AAC. In 1% AAC, stomatal conductance was significantly high in control+WW, 15Pb+WW and 30Pb+WW over control+GW, 15Pb+GW and 30Pb+GW. Results also confirmed that 1% AAC gave maximum increase in stomatal conductance as compared to 1% AAC and 0% AAC in GW and WW. However, application of 1% AAC also gave significantly better stomatal conductance as compared to 0% AAC under different levels of Pb in GW and WW (Figure 15B).

Figure 1

Significant change was noted in Pb concentration of rice in control over 15Pb and 30Pb where WW was applied at 1%AAC (Figure 16A). In 1%AAC, Pb concentration of rice was significantly low in control+WW, 15Pb+WW and 30Pb+WW over control+GW, 15Pb+GW and 30Pb+WW. Results also confirmed that 1%ACC gave maximum decreased in Pb concentration of rice as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly low Pb concentration of rice as compared to 0%AAC under different levels of Pb in GW and WW (Figure 16B).

Figure 1

Treatments 15Pb and 30Pb were non-significant for fresh weight of straw over control (no Pb) under GW at 1% AAC (Figure 6A). Significant change was noted in fresh weight of straw in control over 15Pb and 30Pb where WW was applied at 1% AAC. In 1% AAC, fresh weight of straw was significantly high in control+WW and 15Pb+WW over control+GW and 15Pb+GW. Results also confirmed that 1% ACC gave maximum increase in fresh weight of straw as compared to 1% AAC and 0% AAC in GW and WW. However, application of 1% AAC also gave significantly better fresh weight of straw as compared to 0% AAC under different levels of Pb in GW and WW (Figure 6B).

Figure 1

15Pb was non-significant but 30Pb different significantly for number of tillers over control (no Pb) under GW at 1% AAC (Figure 5A). Significant change was noted in number of tillers in control over 15Pb and 30Pb where WW was applied at 1% AAC. In 1% AAC, number of tillers was significantly high in control+WW, 15Pb+WW and 30Pb+WW over control+GW, 15Pb+GW and 30Pb+GW. Results also confirmed that 1% ACC gave maximum increase in number of tillers as compared to 1% AAC and 0% AAC in GW and WW. However, application of 1% AAC also gave significantly better number of tillers as compared to 0% AAC under different levels of Pb in GW and WW (Figure 5B).

Figure 1

In 1% AAC, plant height was statistically alike in control+WW, 15Pb+WW and 30Pb+WW as compared to control+GW, 15Pb+GW and 30Pb+GW (Figure 2A). Increasing level of Pb was non-significant for plant height over control (no Pb) under GW at 1% AAC. Significant change was noted in plant height in control over 15Pb and 30Pb where WW was applied at 1% AAC. In 1% AAC, plant height was significantly high in control+WW and 15Pb+WW over control+GW and 15Pb+GW. Chord diagram confirmed that 1% AAC gave maximum increase in plant height as compared to 1% AAC and 0% AAC in GW and WW. However, application of 1% AAC also gave significantly better plant height as compared to 0% AAC under different levels of Pb in GW and WW (Figure 2B).

Figure 1

In 1%AAC, transpiration rate was significantly high in control+WW, 15Pb+WW and 30Pb+WW over control+GW, 15Pb+GW and 30Pb+WW (Figure 14A). Results also confirmed that 1%ACC gave maximum increase in transpiration rate as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better transpiration rate as compared to 0%AAC under different levels of Pb in GW and WW (Figure 14B).

Figure 1

Treatments 30Pb was significant for photosynthetic rate over control (no Pb) under GW at 1%AAC (Figure 13A). Significant change was noted in photosynthetic rate in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, photosynthetic rate was significantly high in control+WW, 15Pb+WW and 30Pb+WW over control+GW, 15Pb+GW and 30Pb+WW. Chord diagram confirmed that 1%ACC gave maximum increase in photosynthetic rate as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better photosynthetic rate as compared to 0%AAC under different levels of Pb in GW and WW (Figure 13B).

Figure 1

Significant change was noted in chlorophyll content in control over 15Pb and 30Pb where WW was applied at 1%AAC (Figure 11A). In 1%AAC, chlorophyll content was significantly high in control+WW, 15Pb+WW and 30Pb+WW over control+GW, 15Pb+GW and 30Pb+WW. Results also confirmed that 1%ACC gave maximum increase in chlorophyll content as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better chlorophyll content as compared to 0%AAC under different levels of Pb in GW and WW (Figure 12B).

Figure 1

Significant change was noted in 1000 grains weight in control over 15Pb and 30Pb where WW was applied at 1%AAC (Figure 10A). In 1%AAC, 1000 grains weight was significantly high in control+WW, 15Pb+WW and 30Pb+WW over control+GW, 15Pb+GW and 30Pb+WW. Results also confirmed that 1%ACC gave maximum increase in 1000 grains weight as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better 1000 grains weight as compared to 0%AAC under different levels of Pb in GW and WW (Figure 10B).

Figure 1

Treatments 30Pb was significant for dry weight of spike over control (no Pb) under GW at 1%AAC (Figure 9A). Significant change was noted in dry weight of spike in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, dry weight of spike was significantly high in control+WW, 15Pb+WW and 30Pb+WW over control+GW, 15Pb+GW and 30Pb+WW. Results also confirmed that 1%ACC gave maximum increase in dry weight of spike as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better dry weight of spike as compared to 0%AAC under different levels of Pb in GW and WW (Figure 9B).

Figure 1

In 1%AAC, electrolyte leakage was significantly low in control+WW, 15Pb+WW and 30Pb+WW over control+GW, 15Pb+GW and 30Pb+GW (Figure 12A). Results also confirmed that 1%ACC gave maximum decrease in electrolyte leakage as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly low electrolyte leakage as compared to 0%AAC under different levels of Pb in GW and WW (Figure 12B).

Figure 3

In 1%AAC, spike length was statistically alike in control+WW, 15Pb+WW but differed significantly in 30Pb+WW as compared to control+GW, 15Pb+GW and 30Pb+GW respectively (Figure 3A). Increasing level of Pb was non-significant for spike length over control (no Pb) under GW at 1%AAC. Significant change was noted in spike length in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, spike length was significantly high in control+WW, 15Pb+WW and 30Pb+WW over control+GW, 15Pb+GW and 30Pb+GW. Chord diagram showed that 1%ACC gave maximum increase in spike length as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better spike length as compared to 0%AAC under different levels of Pb in GW and WW (Figure 3B).

Figure 7

In 1%AAC, fresh weight of spike was non-significantly higher in control+WW and 15Pb+WW over control+GW and 15Pb+GW respectively (Figure 7A). Treatments 30Pb were non-significant for fresh weight of spike over control (no Pb) under GW at 1%AAC. Significant change was noted in fresh weight of spike in control over 15Pb and 30Pb where WW was applied at 1%AAC. In 1%AAC, fresh weight of spike was significantly high in control+WW and 15Pb+WW over control+GW and 15Pb+GW. Results also confirmed that 1%ACC gave maximum increase in fresh weight of spike as compared to 1%AAC and 0%AAC in GW and WW. However, application of 1%AAC also gave significantly better fresh weight of spike as compared to 0%AAC under different levels of Pb in GW and WW (Figure 7B).

Figure 18

Pearson correlation showed that electrolyte leakage and plant Pb concentration has significant negative correlation with all the growth, yield and gas exchange attributes. Significant positive correlation was noted between electrolyte leakage and plant Pb concentration (Figure 18).