Research Article

Role of hydroxyl benzoic acid foliar spray on amelioration of lead tolerance on *Triticum aestivum* L.

Naveen Dilawar¹, Fayaz Asad¹*, Sabrina Shahid¹, Samiullah² and Wisal¹

1. Department of Botany, Bacha Khan University Charsadda, KP-Pakistan
2. Department of Botany, University of Peshawar, KP-Pakistan

*Corresponding author’s email: fayaz.asad79@yahoo.com

Citation
Naveen Dilawar, Fayaz Asad, Sabrina Shahid, Samiullah and Wisal. Role of hydroxyl benzoic acid foliar spray on amelioration of lead tolerance on *Triticum aestivum* L. Pure and Applied Biology. Vol. 10, Issue 3, pp861-871. http://dx.doi.org/10.19045/bspab.2021.100088

Received: 01/08/2020 Revised: 17/11/2020 Accepted: 27/11/2020 Online First: 19/12/2020

Abstract
Heavy metal pollution of water and soil has become a global issue, which extensively effect the major cereal crops and human health in the world. Current study aimed to conduct the role of exogenously applied Hydroxyl Benzoic Acid (HBA) foliar spray on agronomic and physicochemical parameters of two varieties of *Triticum aestivum* L. i.e. Jhanbaz and Galaxy 2013 under the lead (heavy metal) stress. Leaves of the above plants were treated (after 7, 14, 21 days) with distilled water (control), 2ppm Pb²⁺ (T1), 2ppm Pb²⁺ +HBA (T2), 4 ppm Pb²⁺ (T3), and 2ppm Pb²⁺ +HBA (T4) and response of plants was observed at first, second and third week of the germination. Results of the lead (Pb²⁺) treatment of 4ppm and 2ppm showed high adverse effect on physiochemical and growth performance. Whereas HBA foliar spray revealed better performance by improving seedling vigorous index, agronomic parameters and percent field capacity under application of lead at 4ppm and 2ppm. This indicated that the toxic effects generated by heavy metal stress were relatively overcome by the application of HBA. However, results exhibited the better growth and physicochemical performance were recorded in Jhanbaz as compared to Galaxy 2013 and recommended variety under induced lead stress (lead).

Keywords: Growth performance; Heavy metal; HBA; Lead; *Triticum aestivum* L.

Introduction
Heavy metal pollution of water and soil supplies has become a major problem in the modern world. Due to a direct interaction between these sources and the food chain, the excessive concentration of heavy metals is a serious threat to human life [1]. More heavy metal presentation to ground water through polluted soil plays a vital role in health and environmental aspects. Pb²⁺ is one of the most toxic heavy metals without biological function, which is quickly absorbed by plants and it is toxic to living organisms even at low concentrations [2]. Inevitably, the use of industrial wastewater for agricultural land irrigation has skyrocketed due to water shortage crises and fertility benefits. Lead (Pb²⁺) is a heavy metal of anthropogenic origin [3]. Pb²⁺ is a pollutant that accumulates in soils, sediments and water and is extremely persistent in the environment [4]. Pb²⁺ has no biological function and is toxic to living organisms, even at low concentrations. Although Pb²⁺ is not an essential element, some plant species multiply in an area contaminated with Pb²⁺ and accumulate it...
in different parts. The roots are the first organ in contact with various components of the rhizosphere [5]. Lead is absorbed by the plants through roots and caused disturbance of ions with in plants [6]. It has been speculated that lead toxic drugs may cause physiological and biochemical changes in the Oryza sativa L. [7]. Lead is not essential for a relatively high reactions of plant cells but it will remove this metal if it is present in their environment, especially in rural area where it is polluted by automobiles exhausts and in farms, contaminated with fertilizers containing heavy metals as impurities [8]. Plants take Pb^{2+} from the solution in the soil at the roots, and then the largest amount of Pb^{2+} accumulates in the roots in insoluble form [9]. Accumulation of lead in plants increases with an increase in plant lead levels. Lead can cause a wide range of physical and biochemical dysfunctions in seed germination, plant growth, water conditions and nitrate uptake [10-12]. Lead is one of the most widely used heavy metals and is highly toxic to plants [13]. Throughout the plant, Pb^{2+} can affect photosynthesis at stoma levels, mesophyll cells, pigment content, and light and dark reactions. Heavy metal pollution of soil and water is a global environmental problem [14]. The objective of this study is to conduct the role of exogenously applied Hydroxyl Benzoic Acid (HBA) foliar spray on agronomic, and physicochemical parameters two varieties of Triticum aestivum L. such as Jhanbaz and Galaxy 2013 under the induced stress of heavy metal (lead).

**Materials and Methods**

Peshawar Industrial City Peshawar is a major producer of industrial wastewater in KPK Province. Due to their significant wastewater production, local farmers can use these alternative water resources for irrigation. Three samples of wastewater were taken from an industrial area of Peshawar city. The average concentration of Pb^{2+} in industrial wastewater was determined by a nuclear absorption spectrophotometer (AAS) and ranged from 2 ppm to 4 ppm. To perform this measurement, a lab specialist pumped 100 ml of dirty water directly into the laboratory [15]. However, the presence of metallurgical fluids in wastewater prevents plants from growing due to its complex biocides and chemicals [16]. Treating mineral fluids was not reasonable or cost-effective, so the wastewater was prepared with the infamous Pb^{2+} solution (100 mg / l) developed by Merck Millipour of Germany.

Two different concentrations of solution were prepared, 4ppm and 2ppm, medium and strong wastewater was used in this study, respectively. The experiment was conducted during the 2015 wheat growing season at the Department of Botany, Bacha Khan University, Charsadda (latitude, 34°43.080’N, longitude, 71°43’50.880’E) and altitude 282 m. Selected Local Blend Jhanbaz and Galaxy 2013 Triticum aestivum L. from Nowshera Cereal Crops Research Institute (CCRI). The seeds were sterilized in a solution of 10 ml of Clorox and 200 ml of water for 3 minutes and then washed with 50% ethanol for 3 minutes. After that, the seeds were rinsed with distilled water and planted in plastic pots (14 cm bottom inside diameter, 18.5 cm top inside diameter, 15.6 cm height and 0.5 cm depth). 'Thick) filled with air-dried soil and sand (3: 1) in triplicate pots. They were protected from the rain and 3 pots were submitted to each treatment. The total number of pots was 30. Plants were watered as needed. Sampling was performed 15 days after the start of treatment with heavy metals. For each treatment, 3 repetitions were taken. After the agronomic study, the samples were dried at room temperature for analysis of the heavy metal Pb^{2+}. (20) Grams of soil of a uniform depth were taken 6 inches from the surfaces of the pots. Dry weight was determined after drying the soil in an oven for 72 hours at 70 °C until constant weight.

**Soil moisture content**

The %age moisture content of soil is
calculated by following formula:

\[
\text{%age moisture content} = \frac{\text{Soil Fresh weight} - \text{Soil Dry weight} \times 100}{\text{Soil Fresh weight}}
\]

**Field capacity of rhizospheric soil**

The field capacity of rhizospheric soil was calculated following the method:

\[
\text{Field Capacity} \% = \frac{\text{Wet soil weight (g)} - \text{Dry soil weight (g)} \times 100}{\text{Dry soil weight (g)}}
\]

**Analysis of heavy metal lead for Rhizospheric soil and plant powder**

Distilled water of 1 mL and 9 mL of rhizospheric clay extracts was taken into a test tube and lead was analyzed on an atomic absorption spectrophotometer. Micronutrients, micronutrients and heavy metals rhizome soil were measured by the formula:

\[
\text{Nutrients (ppm)} = \left( \text{Extract in ppm - blank} \right) \times \frac{A}{W} \times \text{dilution factor}
\]

Whereas

\[A = \text{Total extract volume (ml)}\]
\[W = \text{Rhizospheric soil dry weight}\]

**Procedure**

Take the oven dried sample (0.25 g) in a 50 ml flask and add 6.5 ml of mixed acid solution, i.e. nitric acid, sulfuric acid, per chloric acid (5: 1: 0.1) and boil on a hot plate with extractor hood. Then a few drops of contaminated water were added and allowed to cool. The digested samples were then transferred to a 100 ml volumetric flask and the volume was increased to 100 ml by adding contaminated water. The extract was then filtered through whatman # 42 filter paper and stored in plastic bottles labeled filtrate. The concentration of these elements throughout the sample was determined by the Shamadzo AA-670 nuclear absorption spectrophotometer.

\[
\text{Plant Cation of Nutrient} = \left( \text{Extract in ppm - blank} \right) \times \frac{A}{W} \times \text{dilution factor}
\]

Whereas

\[A = \text{Total volume of extract (mL)}\]
\[W = \text{Dry Plant Weight}\]

**Results and Discussion**

**Germination percentage**

When applied Pb2⁺ stress *Triticum aestivum* L. showed reduction in germination but under HBA showed promotion. The maximum germination has been indicated after 7 day(d) and 14d treatment in T3 (V1) and T4 (V2), followed by T4 (V1) and T3 (V2), while the minimum was found in T1 (V1) and T5 (V2) while day after of 21 treatments the maximum germination has been found in T2 (V1) 5.618% and T4 (V2) with 16% (Table 1) while the lowest has been reported in T1 (V1) and T5 (V2) our result is also linked with [17, 18]. Who demonstrated that this metal exerts plant growth, seed germination, shoot growth, root growth, evaporation, chlorophyll production, lamellar organization in the cytoplasm and cell division. Sprouts are sensitive to environmental influences in the germination and germination stages [19]. Toxicity of Lead significantly affects the processes of biological in various plants like in maize, barley, wheat, Citrullus, cauliflower and in vegetables. lead
decreases the seedling growth, shoot length, root length; number of leaves [20].

**Shoot and root length**

Heavy metal stress directly affects the plant shoot and root length, the current study indicated that the maximum shoot length was documented in T2 (V1) and T1 (V2) whereas the lowest has been found in T1 (V1) and T5 (V2). After 14d treatment the maximum shoot length has been indicated in T5 (V1) with 28.6cm and T2 (V2), while the minimum has been found in T4 (V1) and T5 (V2). The maximum shoot length after 21d treatment was noted in T3 (V1) and T1 (V2), whereas the lowest has been reported in T4 (V1) and T2 (V2) (Table 2). While after 7d treatment the maximum root length has been found in T1 (V1, V2) followed by T3 (V1, V2), while the lowest has been noted in T5 (V1, V2). After 14d treatment the maximum root length has been reported in T2 (V1) with 17.25cm and T4 (V2) with 11.05cm, whereas the lowest has been indicated in T5 (V1) and T3 (V2). The maximum root length has been found after 21d treatment in T3 (V1) and T4 (V2), whereas the lowest has been noted in T1 (V1) and T5 (V2).

Soils contaminated with Pb²⁺ cause sharp decreases in crop productivity there by poisoning a serious problem for agriculture (21) (Table 3). Similarly, result was also found that Retarded or no root and shoot formation was observed in the *Triticum aestivum* L. plant when expose to higher concentration of Cr [21]. Enlarge in heavy metals uptake reduces growth of plant and development for the reason that they start interacting with the micronutrient and limits their availability to the plant. Lead adversely affects plant growth which depends upon its concentration and plant species. Reduction in plant tallness due to Pb²⁺ is reported in many plants, 11%, 22% and 41% decrease in height of oats [22], in *Lactuca sativa*, *Curcumas sativus* and *Panicum miliaceum* [23] and *Triticum aestivum* L. [24].

**Fresh and dry biomass**

The higher fresh shoot weight was found in T1 (V1,V2) after 7d treatment and was noted lowest in T4 (V1) with 0.207g and T5 (V2) with 0.721g. The shoot fresh weight after 14d treatment has been indicated in T2 (V1) and T3 (V2), while the lowest was indicated in T4 (V1) and T2 (V2). After 21d treatment the maximum shoot fresh weight has been indicated in T4(V1) and T3 (V2), whereas the lowest has been reported in T4 (V1) and T2 (V2) while After 7d treatment the highest shoot dry weight was found in T4(V1) and T2 (V2), whereas the lowest was noted in T3 (V1, V2). The upper limit shoot dry weight has been reported in T2 (V1) with 0.14g and T1 (V2) with 0.145g after 14d treatment, whereas the smallest has been reported in T4 (V1) and T2 (V2). The higher dry weight of shoot was noted after 21d treatment in T4 (V1) and T3 (V2), whereas the lowest was noted in T3 (V1) and T4 (V2) (Table 2) while root maximum fresh weight has been reported after 7d,14d.,and 21d in T2 (V1), and in T3 (V2), whereas the lowest has been reported after in T3 (V1) and T5 (V2), T4 (V1) and T2(V2), and T1 (V1) and T2 (V2) respectively whereas dry weight has been found maximum 7d and 14d treatment in T2, T4 (V1) and T3 (V2), but the lowest has been noticed after 7d in T5 (V1, V2), after 14d in T4 (V1), T2 (V2) and after 21d treatment in T1 (V1) and T2 (V2) (Table 3). Similarly, benzoic acid at 100-400 mg L⁻¹ increased seedling growth, biomass, flavonoid protein, carbohydrate and phenolic content of *physiolas valgaris* plant [25] and considerably increased totals insoluble and soluble carbohydrates in *Ammi visnaga* L. In previous studies it has also been reported that dry weight of root and their length of the *Caesalpinia pulcherrima* was repressed by 100ppm Pb²⁺ [26], total root weight and root length of *Triticum aestivum* L. was also affected by 20 mg Pb²⁺ (VI) kg⁻¹ [27].
Leaf numbers, area, fresh and dry biomass

Results of our study conform that Pb\(^{2+}\) stress is not only harmful for germination, shoot, root length and biomass but also harmful for leaf too. The maximum fresh leaf weight has been showed by T1 (V1, V2) after 7d treatment with 0.093g and 0.154g, while the lowest was found in T4 (V1) and T2 (V2). After 14d treatment the maximum leaf dry weight has been noted in T2 (V1) and T3 (V2) while the lowest has been reported in T4 (V1) and T2 (V2). After 21d treatment the maximum leaf dry weight has been found in T5 (V1) and T3 (V2), whereas the minimum has been showed by T4 (V1, V2) respectively (Table 4). The maximum leaf dry weight has been noticed after 7d treatment in T1 (V1) and T2 (V2), whereas the minimum has been reported in T3 (V1) and T2 (V2). After 17d treatment the maximum leaf dry weight has been indicated in T2 (V1) and T3 (V2), while the minimum was reported in T3 (V1) and T2 (V2). The maximum leaf dry weight has been found after 21d treatment in T5 (V1) with 0.022g and T3 (V2) with 0.038g, whereas the lowest has been reported in T2 (V1) and T4 (V2) (Table 4). No. of leaves has been found maximum in T1 (V1) and T3 (V2) after 7d treatment and the lowest has been noticed in T3 (V1) and T2 (V2). After 17d treatment the maximum No. of leaves has been reported by T5 (V1) and T4 (V2), while the minimum has been showed by T4 (V1) and T5 (V2). After 21d treatment the maximum No. of leaves has been indicated in T4 (V1) and T5 (V2) whereas the lowest has been reported in T2 (V1, V2) respectively (Table 4). Consequences of leaf area has been noticed maximum after 7d treatment in T2 (V1) and T4 (V2), followed by T5 (V1) with 5.35cm and T4 (V2) with 43.55cm, while the lowest was noticed in T4 (V1) and T5 (V2) respectively. After 14d treatment the maximum leaf area has been reported in T5 (V1) and T3 (V2), while the lowest was indicated by T4 (V1) and T2 (V2). After 21d treatment the maximum leaf area has been showed by T4 (V1) 5.61cm and T3 (V2) with 12.69cm, while the lowest has been found in T1 (V1) and T2 (V2) respectively (Table 5). Along with number of leaves in plant, heavy metals also affect the other aspects of leaves of different plants such as Pb\(^{2+}\) effect leaf biomass and area of Albizia lebbeck [28], decrease in leaf area of primary and trifoliate leaves of bush bean plant [29], dry leaf yield of bush bean plants was also decreased [30], decrease in leaf dry weight and leaf area of Oryza sativa, Acacia holosericea, Leucaena leucocephala [31], and P. vulgaris [32], reduced leaf size of spinach [33], pretentious young leaves in tomato plants [34].

Moisture contents, Field capacity, vigorous index

Table 1 showed that maximum % moisture content has been reported in T5 (untreated control) in V1 (Jhanbaz) and T1 (2ppm Pb\(^{2+}\)) in V2 (Galaxy 2013) after 7d treatment, followed by T3 (4ppm Pb\(^{2+}\)), whereas the lowest has been reported in T1 (2ppm Pb\(^{2+}\)) in V1 with 0.033% and T5 (untreated control) in V2. The maximum % moisture content after 14d treatment was noted in T1 (2ppm Pb\(^{2+}\)), followed by T3 (4ppm Pb\(^{2+}\)), while lowest was found in T4 (2ppm Pb\(^{2+}\)+HBA) in V1 and T5 (untreated control) in V2. After 21d treatment the maximum % moisture content was noted maximum in T5 (untreated control) in V1 with 5.925% and T2 (2ppm Pb\(^{2+}\)) in V1 and T3 (V2) whereas the lowest was found in T4 (2ppm Pb\(^{2+}\)+HBA) in V1 and T5 (untreated control) in V2. The maximum % moisture content after 14d treatment was noted in T1 (2ppm Pb\(^{2+}\)), followed by T3 (4ppm Pb\(^{2+}\)), while lowest was found in T4 (2ppm Pb\(^{2+}\)+HBA) in V1 and T5 (untreated control) in V2. After 21d treatment the maximum % moisture content was noted maximum in T5 (untreated control) in V1 with 5.925% and T2 (2ppm Pb\(^{2+}\)) in V1 and T3 (V2) whereas the lowest was found in T4 (2ppm Pb\(^{2+}\)+HBA) in V1 and T1 (2ppm Pb\(^{2+}\)) in V2 respectively. The maximum F.C (field capacity) has been indicated in T3 (V1) and T1 (V2), followed by T1 (V1) and T3 (V2), while the lowest was found in T1 (V1) and T5 (V2) after 7d treatment. After 14 days’ treatment the maximum F.C has been indicated in T1 (V1, V2) with 5.397-6.276%, followed by T3, whereas the lowest was noted in T4 (V1) and T5 (V2). The maximum F.C has been reported after 21d treatment in T5 (V1) and T3 (V2),
followed by T1 (V1) and T3 (V2), whereas the lowest was noted in T2 (V1) and T1 (V2). Maximum vigorous index has been reported in T3 (V1) and T5 (V2) after 7d treatment, while the minimum has been evaluated in T2 (V1, V2). After 17d treatment the maximum vigorous index has been found in T1 (V1) with 768.6 and T5 (V2) with 929.90, while the lowest has been reported by T4. The maximum vigorous index has been noticed in T3 (V1) and T5 (V2), while the minimum has been showed by T2 (V1) and T4 (V2) respectively. The maximum vigorous index has been reported in T1 (V1) and T2 (V2), while the lowest has been noticed in T1 (V1, V2). After 17d treatment the maximum vigorous index has been found in T1 (V1) with 768.6 and T5 (V2) with 929.90, while the lowest has been reported by T4. The maximum vigorous index has been noticed in T3 (V1) and T5 (V2), while the minimum has been showed by T2 (V1) and T4 (V2) respectively (Table 5). Similarly, result is also found by [35], the treatment T2 (2ppm+HBA) in both varieties showed the maximum values for the majority of agronomic characters among all the treatments. Treatment T2 (2ppm+HBA) showed the maximum vigor index in both varieties and was the best treatment of our research work. Maximum Vigor index was recorded in treatment T2 of Galaxy 2013 (2546.2 ± 992.778), followed by treatment T4 of the same variety [36] whereas, treatment T3 (4ppm) of both varieties was the worst treatment with the lowest vigor index in all samples.

### Rhizospheric soil and whole plant

Table 6 exhibited the maximum concentration of lead in rhizospheric soil has been found maximum after 7d treatment in T5 (V1) with 1.156 followed by T4 (V2), while the lowest concentration has been found in T1 (V1,V2). After 14d treatment the maximum concentration after 14d treatment has been recorded in T3 (V1,V2), whereas the lowest has been noticed in T1 (V1,V2). The maximum concentration of lead in rhizospheric soil has been showed after 21d treatment in T5 (V1) with 1.399 and T3 (V2) with 1.569, while the lowest has been reported in T1 (V1) and T5 (V2) respectively. The maximum concentration of lead in whole plant has been found maximum in T5 (V1) and T4 (V1) after 7d treatment, while the lowest has been noticed in T1 (V1) and T2 (V2). After 14d treatment the maximum concentration has been found in T5 (V1) with 1.484 and T3 (V2) with 1.429, whereas the minimum concentration has been reported in T1 (V1) and T2 (V2) respectively. The maximum concentration of lead in whole plant after 21d treatment has been found in T4 (V1) and T5 (V2), whereas the lowest has been reported in T1 (V1) and T4 (V2) respectively. Result is similar with the work of [37, 38] that showed Plants' responses to lead exposure are used as tools (biomarkers) in the context of environmental quality assessment. To develop tools for ecological study, understanding the mechanisms involved in plant absorption, transfer and toxicity is essential.
Table 1. Influence of foliar spray of Hydroxyl benzoic acid on percent field capacity, soil moisture, and germination percentage of *Triticum aestivum* L. under inoculation of Pb$^{2+}$ at 2ppm and 4ppm

| Treatment | % moisture | Field capacity | Germination |
|-----------|------------|----------------|-------------|
|           | 7d         | 14d            | 21d         | 7d         | 14d            | 21d         |
| T1        | 0.03±0.00  | 5.21±0.01      | 5.23±1.17   | 3.47±0.04  | 5.40±0.10      | 5.42±1.27   | 11.00±1.41 | 11.0±1.41  | 1.39±0.03 |
| T2        | 0.04±0.02  | 1.98±0.84      | 2.65±1.44   | 4.70±1.56  | 2.00±0.87      | 2.71±1.48   | 12.50±1.41 | 12.5±1.42  | 5.62±1.84 |
| T3        | 0.05±0.01  | 4.33±1.12      | 4.40±0.61   | 5.49±0.72  | 4.47±1.19      | 4.54±0.64   | 15.00±2.12 | 15.0±2.12  | 3.31±0.64 |
| T4        | 0.04±0.00  | 1.88±0.76      | 4.66±1.23   | 3.89±0.13  | 1.90±0.79      | 4.83±1.39   | 13.50±2.12 | 13.5±2.12  | 5.31±1.19 |
| T5        | 0.05±0.01  | 3.66±0.61      | 5.93±3.63   | 5.55±0.51  | 3.76±0.64      | 6.26±3.86   | 12.50±1.41 | 12.5±1.41  | 2.62±0.87 |

T1 stands for 2ppm + Pb$^{2+}$, T2=2ppm Pb$^{2+}$ +HBA, T3=4ppm Pb$^{2+}$, T4= 2ppm Pb$^{2+}$ +HBA, T5= Untreated/control

Table 2. Effect of foliar spray of Hydroxyl benzoic acid on shoot length, fresh and dry biomass of *Triticum aestivum* L. under inoculation of Pb$^{2+}$ at 2ppm and 4ppm

| Treatment | Shoot length | Jhanbaz | Shoot fresh weight | Shoot dry weight |
|-----------|--------------|---------|--------------------|------------------|
|           | 7d           | 14d     | 21d                | 7d               | 14d            | 21d               |
| T1        | 2.26±3.96    | 26.0±0.35 | 26.4±0.84         | 0.56±0.14        | 0.48±0.01      | 0.34±0.011         | 0.12±0.03 | 0.13±0.01 | 0.09±0.02 |
| T2        | 24.75±0.00   | 28.35±0.21 | 29.8±6.08         | 0.45±0.09        | 0.59±0.13      | 0.34±0.05          | 0.11±0.03 | 0.14±0.04 | 0.09±0.00 |
| T3        | 22.15±0.07   | 24.1±4.38  | 30.5±0.35         | 0.22±0.00        | 0.26±0.09      | 0.267±0.022        | 0.04±0.00 | 0.05±0.01 | 0.06±0.00 |
| T4        | 19.55±0.07   | 18.65±0.91 | 24.65±0.98        | 0.20±0.01        | 0.16±0.32      | 0.36±0.021         | 0.12±0.05 | 0.04±0.00 | 0.13±0.00 |
| T5        | 18.75±0.00   | 28.6±5.16  | 27.5±0.35         | 0.34±0.13        | 0.56±0.31      | 0.32±0.09          | 0.06±0.05 | 0.14±0.07 | 0.09±0.02 |

Galaxy 2013

| Treatment | Shoot length | Shoot fresh weight | Shoot dry weight |
|-----------|--------------|--------------------|------------------|
|           | 7d           | 14d                | 21d                |
| T1        | 9.85±2.05    | 0.08±0.03          | 43.45±7.99        |
| T2        | 7.7±0.77     | 0.09±0.04          | 24.85±10.53       | 0.49±0.29 | 0.24±0.20 | 0.37±0.37 | 0.09±0.04 | 0.06±0.03 | 0.09±0.08 |
### Table 3. Influence of foliar spray Hydroxyl benzoic acid on root length, dry and fresh biomass of *Triticum aestivum* L. under inoculation of Pb\(^{2+}\) at 2ppm and 4 ppm

| Treatment | 7d Root length | 14d Root length | 21d Root length | 7d Root fresh weight | 14d Root fresh weight | 21d Root fresh weight | 7d Root dry weight | 14d Root dry weight | 21d Root dry weight |
|-----------|----------------|----------------|----------------|---------------------|----------------------|----------------------|------------------|------------------|------------------|
| T1        | 11.25±0.21     | 10.5±0.00      | 12.0±2.13      | 0.103±0.00          | 0.13±0.06            | 0.04±0.01            | 0.06±0.00        | 0.09±0.03        | 0.04±0.01        |
| T2        | 10.88±4.56     | 17.25±5.30     | 13.75±1.41     | 0.33±0.11           | 0.28±0.15            | 0.11±0.32            | 0.21±0.10        | 0.18±0.10        | 0.08±0.02        |
| T3        | 10.3±0.49      | 10.45±1.62     | 14.5±1.41      | 0.09±0.13           | 0.12±0.02            | 0.05±0.01            | 0.06±0.01        | 0.07±0.00        | 0.04±0.01        |
| T4        | 10.8±4.66      | 14.6±7.70      | 14.0±1.41      | 0.17±0.08           | 0.09±0.01            | 0.13±0.02            | 0.05±0.02        | 0.07±0.00        | 0.12±0.02        |
| T5        | 0.83±1.20      | 0.55±0.42      | 1.24±0.56      | 0.11±0.05           | 0.12±0.02            | 0.06±0.01            | 0.03±0.02        | 0.05±0.00        | 0.04±0.01        |

### Table 4. Effect of foliar spray Hydroxyl benzoic acid on leaf numbers, leaf day and fresh biomass of *Triticum aestivum* L. under inoculation of Pb\(^{2+}\) at 2ppm and 4 ppm

| Treatment | 7d Leaf fresh weight | 14d Leaf fresh weight | 21d Leaf fresh weight | 7d Leaf dry weight | 14d Leaf dry weight | 21d Leaf dry weight | No of leaves |
|-----------|---------------------|-----------------------|-----------------------|------------------|------------------|------------------|--------------|
| T1        | 0.09±0.026          | 0.0815±0.001          | 0.052±0.011           | 0.025±0.007      | 0.0225±0.000     | 0.0175±0.004     | 11±0.707     |
| T2        | 0.091±0.008         | 0.094±0.013           | 0.057±0.012           | 0.022±0.002      | 0.024±0.004      | 0.017±0.004      | 8±0.707      |
| T3        | 0.057±0.000         | 0.058±0.012           | 0.061±0.004           | 0.013±0.000      | 0.02±0.007       | 0.019±0.000      | 6.5±0.707    |
| T4        | 0.037±0.007         | 0.0375±0.004          | 0.049±0.006           | 0.009±0.003      | 0.016±0.000      | 0.019±0.000      | 7.5±0.00     |
| T5        | 0.079±0.023         | 0.084±0.048           | 0.071±0.020           | 0.01±0.014       | 0.02±0.014       | 0.0225±0.002     | 8±0.707      |

**T1 stands for 2ppm-Pb\(^{2+}\), T2=2ppm Pb\(^{2+}\)+HBA, T3=4ppm Pb\(^{2+}\), T4= 2ppm Pb\(^{2+}\)+HBA, T5= Untreated/control**
T1 stands for 2ppm, T2=2ppm Pb²⁺+HBA, T3=4ppm Pb²⁺, T4= 2ppm Pb²⁺+HBA, T5= Untreated/control

Table 5. Effect of foliar spray Hydroxyl benzoic acid on vigorous index and leaf area of *Triticum aestivum* L. under inoculation of Pb²⁺

| Treatment | Leaf fresh weight | Leaf dry weight | No of leaves |
|-----------|------------------|----------------|--------------|
|           | 7d               | 14d            | 21d          | 7d             | 14d            | 21d          | 7d             | 14d            | 21d          |
| T1        | 0.154±0.037      | 0.1535±0.083   | 0.122±0.011  | 0.015±0.010   | 0.044±0.015     | 0.027±0.008   | 7±1.414       | 9.5±0.707      | 11.5±3.535   |
| T2        | 0.091±0.053      | 0.048±0.029    | 0.067±0.048  | 0.032±0.013   | 0.0185±0.004    | 0.023±0.011   | 6.00±0.00     | 8.00±0.707     | 9.5±2.828    |
| T3        | 0.072±0.039      | 0.162±0.076    | 0.122±0.048  | 0.021±0.006   | 0.0525±0.016    | 0.0385±0.013  | 7.5±0.00      | 9.5±1.144      | 11±1.144     |
| T4        | 0.105±0.016      | 0.104±0.009    | 0.065±0.141  | 0.021±0.005   | 0.035±0.009     | 0.0225±0.004  | 7.5±0.00      | 10.00±0.707    | 9.5±0.707    |
| T5        | 0.107±0.007      | 0.094±0.015    | 0.084±0.006  | 0.023±0.003   | 0.0245±0.002    | 0.024±0.002   | 7.5±0.00      | 9.00±0.00      | 12±2.121     |

Table 6. Influence of foliar spray Hydroxyl benzoic acid on rhizospheric soil and whole plant of *Triticum aestivum* L. under inoculation of Pb²⁺ 2ppm and 4ppm

| Treatment | Jhanbaz | Galaxy 2013 |
|-----------|---------|-------------|
|           | Leaf area | Vigorous index | Leaf area | Vigorous index |
|           | 7d       | 14d        | 21d       | 7d       | 14d        | 21d       |
| T1        | 5.72±1.89 | 7.32±0.035 | 4.47±0.99 | 645.57±5.39 | 1025.48±6.87 | 645.57±5.39 | 10.48±2.77 | 13.95±3.28 | 9.783±2.81 | 639.99±5.60 | 900.11±7.04 | 1053.0±5.66  |
| T2        | 6.08±0.05 | 8.08±1.30  | 6.12±1.61 | 509.76±7.29 | 1501.69±8.01 | 509.76±7.29 | 8.58±4.41 | 4.71±1.88 | 5.85±3.81 | 403.2±0.98 | 802.6±2.74  | 982.56±2.80  |
| T3        | 5.35±0.91 | 5.78±0.84  | 6.77±0.77  | 768.6±8.89  | 2072.7±7.32  | 768.6±8.89  | 43.55±5.64 | 18.66±8.73 | 12.69±4.62 | 885.51±4.36 | 1033.2±4.95 | 995.89±5.66  |
| T4        | 4.13±0.43 | 4.2±0.67   | 5.61±0.38  | 526.68±8.26 | 882.10±3.5  | 526.68±8.26 | 67.64±4.22 | 10.52±2.30 | 8.00±2.00 | 591.5±6.01 | 696.8±6.02  | 951.00±4.68  |
| T5        | 6.02±1.47 | 8.29±3.16  | 5.25±0.05  | 721.59±7.72 | 1055.68±9.86 | 721.59±7.72 | 7.5±0.91 | 10.01±0.79 | 9.26±1.03 | 929.90±5.40 | 1273.38±8.48 | 1682.36±5.49 |

T1 stands for 2ppm-Pb²⁺, T2=2ppm Pb²⁺+HBA, T3=4ppm Pb²⁺, T4= 2ppm Pb²⁺+HBA, T5= Untreated/control

| Treatment | Rhizospheric soil | Whole plant | Rhizospheric soil | Whole plant |
|-----------|------------------|-------------|------------------|-------------|
|           | Jhanbaz | Galaxy 2013 | 7d       | 14d    | 21d | 7d       | 14d    | 21d |
| T1        | 0.72±0.01 | 1.36±0.03   | 1.17±0.01 | 1.16±0.02 | 1.31±0.03 | 1.26±0.06 | 1.00±0.01 | 1.29±0.02 | 1.42±0.01 | 1.29±0.04 | 1.40±0.07 | 1053.0±5.66 |
| T2        | 1.04±0.04 | 1.19±0.04   | 1.26±0.03 | 1.24±0.02 | 1.33±0.02 | 1.31±0.10 | 1.07±0.02 | 1.34±0.03 | 1.50±0.03 | 1.29±0.06 | 1.28±0.00 | 982.56±2.80 |
| T3        | 1.04±0.04 | 1.38±0.01   | 1.30±0.02 | 1.24±0.05 | 1.40±0.06 | 1.37±0.07 | 1.05±0.01 | 1.54±0.05 | 1.56±0.07 | 1.31±0.02 | 1.42±0.09 | 995.89±5.66 |
| T4        | 1.07±0.03 | 1.26±0.05   | 1.35±0.07 | 1.28±0.03 | 1.37±0.03 | 1.55±0.08 | 1.13±0.07 | 1.37±0.12 | 1.52±0.02 | 1.35±0.06 | 1.34±0.15 | 951.00±4.68 |
| T5        | 1.15±0.05 | 1.31±0.02   | 1.39±0.03 | 1.39±0.04 | 1.48±0.04 | 1.42±0.09 | 1.31±0.05 | 1.32±0.03 | 1.38±0.01 | 1.30±0.01 | 1.36±0.07 | 1682.36±5.49 |

T1 stands for 2ppm-Pb²⁺, T2=2ppm Pb²⁺+HBA, T3=4ppm Pb²⁺, T4= 2ppm Pb²⁺+HBA, T5= Untreated/control
Conclusion
The effect of heavy metal (lead) research can be applied to so many current wheat problems including adverse effect on crop productivity. Keeping in views of these problems the present study was aimed to evaluate the effect of heavy metal (lead) on *Triticum aestivum* L. in response to hydro benzoic acid foliar spray. Results postulated that this lead had adverse effect on *Triticum aestivum* L. seedling agronomic characters. The effect of heavy metal (lead) resulted reduction in growth parameters in wheat has been improved by exogenous foliar application of hydro benzoic acid (HBA) thus illustrated that hydro benzoic acid plays a positive role in enhancing plant growth under heavy metal stress.

Authors’ contributions
Conceived and designed the experiments: N Dilawar, & F Asad, Performed the experiments: N Dilawar & Samiullah, Analyzed the data: N Dilawar & F Asad, Contributed materials/ analysis/ tools: N Dilawar Wisal & S Shahid. Wrote the paper: N Dilawar & F Asad.

References
1. Alexander PD, Alloway BJ & Dourado AM (2006). Genotypic variations in the accumulation of Cd, Cu, Pb and Zn exhibited by six commonly grown vegetables. *Environ Pollut* 144: 736–745.
2. Adriano DC (2001). Trace Elements in Terrestrial Environments; Biochemistry, Bioavailability and Risks of Metals. Springer-Verlag, New York, pp150–159.
3. Cheng C, Phipps D & Alkhaddar RM (2005). Treatment of spent metalworking fluids. *Wat Res* 39(17): 4051-4063.
4. Capuana M (2011). Heavy metals and woody plants-biotechnologies for phytoremediation. *J. Biogeogr Sci For* 4: 7-15.
5. Gao X, Flaten DF, Tenuta M, Grimmert MG, Gawalko EJ & Grant CA (2011). Soil solution dynamics and plant uptake of cadmium and zinc by durum wheat following phosphate fertilization. *Pla soi*, 338(1-2): 423-434.
6. Gupta D, Nicoloso F, Schetinger M, Rossato L, Pereira L, Castro G, Srivastava
7. S & Tripathi R (2009) Antioxidant defense mechanism in hydroponically grown *Zea mays* seedlings under moderate lead stress. *J Hazard Mater* 172(1): 479–484.
8. Johansson L & Pelliccari CE (1988). Lead induced changes in the stabilization of the mouse sperm chromatin. *Toxicology* 51: 11-24.
9. Kosobrukhov A, Knyazeva I & Mudrik V (2004). Plantago major plants responses to increase content of lead in soil: growth and photosynthesis. *Pla Gro Reg* 42: 145-151.
10. Lukaszek M & Taj P (1998), Development of photosynthetic apparatus and respiration in pea seedlings during greening as influenced by toxic concentration of lead. *Acta Phy Plant* 20: 35.
11. Lynch JM & Whipps JM (1990). Substrate flow in the rhizosphere. *Pla Soi* 129: 1–10.
12. Lamhamdi M, Bakrim A, Aarab A, Lafont R, Sayah F (2011). Effects of lead phytotoxicity on wheat (*Triticum aestivum* L.) seed germination and seedling growth. *Compt Rend biol* 334, 118–126.
13. Macpherson AN, Arellana JB, Fraser NJ, Cogdell RJ & Gilboro T (2001) *Biophys J* 80: 923-930.
14. Maestri E, Marmiroli M, Visioli G & Marmiroli N (2010). Metal tolerance and hyperaccumulation: costs and trade-offs between traits and environment. *Envi Exp Bot* 68(1): 1–13.
15. Robinson, Mills BH, Petit TM, Fung DLE, Green SR & Clothier BE (2000). Natural and induced cadmium-accumulation in poplar and willow: Implications for phytoremediation. *Pla soi* 227(1-2): 301-387.
16. Sharma, P. and R.S. Dubey. 2005. Lead toxicity in plants. *Braz J Plant Physiol.*, 17(1):35–52.
17. Shyama R, Weerakoon & Somaratne S (2009). Phytoextractive potential among mustard (*Brassica juncea*) genotypes in Serilanca. *Biol Sci* 38(2): 85-93.
18. Traunfeld JH & Clement DL (2001). Lead in Garden Soils. Home and Garden.College Park, MD: Maryland Cooperative Extension, University of Maryland.
19. Asad F, Begum HA, Hamayun M, Hameed R, Yaseen T & Khan A (2018). Efficacy of different solvent extracts from selected medicinal plants for the potential of...
antibacterial activity. Pure Appl Biol 7(2): 890-896.
19. Dey SK, Jena PP & Kundu S (2009). Antioxidative efficiency of *Triticum aestivum* L. exposed to chromium stress. *J Envi Biol* 30: 539-544.
20. Peralta JR, Gardea-Torresdey JL, Tiemann KJ, Gomez E & Arteaga S (2001). Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (*Medicago sativa* L.). *Bull Environ Contam Toxicol* 66: 727-734.
21. Mathur S, Kalaji HM & Jajoo A (2016). Investigation of deleterious effects of chromium phytotoxicity and photosynthesis in wheat plant. *Photosynthetica* 54: 185-192.
22. Del Real AP, Gonzalo PG, Rodriguez AG, Lobo MC & Sanz AP (2013). Effect of genotype, Cr (III) and Cr (VI) on plant growth and micronutrient status in Silene vulgaris (Moench). *Span J Agri Res* 11: 685-694.
23. Diwan H, Ahmad A & Iqbal M (2012). Characterization of Chromium Toxicity in Food Crops and their Role in Phytoremediation. *J Bior Biod 3*: 159.
24. Sharma DC & Mehrotra SC (1993). Chromium accumulation and its effects on wheat (*Triticum aestivum* L. cv. HD 2204) metabolism. *Pla Sci* 111: 145-151.
25. Sadak MSH, SR El-Lethy & MG Dawood (2013). Physiological role of benzoic acid and salicylic acid on growth, yield, some biochemical and antioxidant aspects of soybean plant. *Wol J Agric Sci* 9 (6): 435–442.
26. Barcelo J, Poschenrieder C & Gunse B (1986) Water relations of chromium VI treated bush bean plants (*Phaseolus vulgaris* L, cv Contender) under both normal and water stress conditions. *J Exp Bot* 37: 178-187.
27. Chen NC, Kanazawa S, Horiguchi T & Chen NC (2001). Effect of chromium on some enzyme activities in the wheat rhizosphere. *Soil Micro* 55: 3-10.
28. Barcelo J, Poschenrieder C, Ruano A & Gunse B (1985). Leaf water potential in Cr (VI) treated bean plants (*Phaseolus vulgaris* L.). *Pla Phy Supp* 77: 163-164.
29. Wallace, Soufi SM, Cha JW & Romney EM (1976). Some effects of chromium toxicity on bush bean plants grown in soil. *Pla Soi* 44: 471-473.
30. Karunyal S, Renuga G & Kailash P (1994). Effects of tannery effluent on seed germination, leaf area, biomass and mineral content of some plants. *Bior Tech* 47: 215-218.
31. Poschenrieder C, Gunse B & Barcelo J (1993). Chromium-induced inhibition of ethylene evolution in bean (*Phaseolus vulgaris*) leaves. *Phys Plant* 89: 404–408.
32. Singh AK (2001). Effect of trivalent and hexavalent chromium on spinach (*Spinacea oleracea* L.). *Envi Ecol* 19: 807-810.
33. Pedreno NJI, Gomez R, Moral G, Palacios J & Mataix J (1997). Heavy metals and plant nutrition and development. *Rec Res Dev Phytoe* 1: 173-179.
34. Kazmi A, Usman M & Muhammad W (2019). Effect of Hydroxybenzoic Acid Foliar Spray on Selected Wheat Varieties under Induced Heavy Metal Stress. *Glob J Res Rev* 6: 1-8.
35. Naeem MI, Bhatti I, Ahmad RH & Ashraf MY (2004). Effect of some growth hormones (GA3, IAA and kinetin) on the morphology and early or delayed initiation of bud of lentil (*Lens culinaris* Medik). *Pak J Bot* 36: 801-809.
36. Islam MK, Khanam S, Lee SY, Alam I & Huhl MR (2014). The interaction of arsenic (As) and chromium (Cr) influences growth and antioxidant status in tossa jute (*Corchorus olitorius*). *Pla Omics* 7: 499.
37. Wierzbicka MH, Przedpeska E, Ruzik R, Ouerdane L, Poeć-PawlakK, Jarosz M, Szpunar J & Szakiel A (2007). Comparison of the toxicity and distribution of cadmium and lead in plant cells. *Protoskima* 231, 99–111.
38. Begum HA, Asad F, Sadiq A, Mulk S & Ali K (2019). Antioxidant, antimicrobial activity and phytochemical analysis of the seeds extract of *Cucumis sativus* Linn. *Pure and Appl Biol* 8(1): 433-441.