APPLICATION OF USING SLAUGHTERHOUSE WASTE FOR BOOSTING THE GROWTH OF SPINACH

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ABSTRACT

Slaughterhouse waste is capable of causing serious bacterial, viral and pathogenic diseases both in humans and animals. Slaughterhouse wastewater contains fat, proteins, suspended solids and diluted blood. Due to this, organic as well as nutrient profusion in wastewater is very high. These partially solubilized residues pose a real threat to human health if left out in riverbeds and other water bodies untreated. Processing of blood requires a high capital investment on heavy instruments for slaughtering and collection of blood. The hazard could be minimized by environmentally friendly and cost-effective disposal methods. This waste could be used for land application via composting, as it is a rich source of primary plant nutrients i.e. N, P and K as well as growth promoting microbes and organic substances. Keeping in view the above-mentioned facts, a pot trial was conducted in the wire house of the Institute of Soil and Environmental Sciences. We mixed the soil with slaughterhouse waste before sowing. Three seedlings of spinach were maintained after germination. Water was applied as needed by the plants. Recommended doses of N, P, and K fertilizers were applied. Data regarding physiological parameters, growth and yield was collected and analyzed at appropriate growth stages. After harvesting, plant tissues (root and leave) and soil samples was subjected to analysis for plant nutrients provision. Analysis of data was done following standard statistical procedure. Slaughterhouse waste improves the plant growth. Results showed that blood, intestine and dry matter significantly affect the plant growth parameter of spinach in natural soil. Plant height was increased up to 92 %, root length 194 %, shoot dry weight 60 % and root dry weight 169 % with blood at (120 ml), intestine (100 g) and dry matter (20 g) as compared to control. All the treatments significantly improved these parameters but blood, intestine and dry matter increased chlorophyll contents 84%, relative water contents 21 %, photosynthetic rate 91%, transpiration rate 95%, and stomatal conductance 58 %, and decreased electrolyte leakage up to 54% in spinach.

Keywords: soil health, growth, slaughterhouse waste, fertility.

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INTRODUCTION

Agricultural crop manufacturing in developing countries like Pakistan faces challenges due to weathered dry soils, ion toxicities, and nutrient deficiencies(Ahmed, Hasanuzzaman et al. 2020). Climate change, rising temperatures, and environmental changes also affect soil productivity and crop vitamins. Chemical fertilizers, high production costs, and decreasing uncooked materials pose threats to crop manufacturing(Singh and Reddy 2013). European regulations categorize animal by-products (ABP) into three types, with the most hazardous waste requiring pasteurization or sterilization before anaerobic digestion(Nag, Auer et al. 2019). Pre-treatments, such as chemical or high-pressure treatments, can improve waste bioavailability and energy balance in abattoir facilities(Harris 2017). Thermal pre-treatments can improve anaerobic digestion performance for various types of waste, but they can create recalcitrant, inhibitory, and toxic compounds due to Maillard reactions. Industrialization and urbanization contribute to environmental pollution, with developing countries focusing on industrialization and developed countries neglecting agriculture due to private sector dependence Industrialization and urbanization contribute to environmental pollution, with developing countries focusing on industrialization and developed countries neglecting agriculture due to private sector dependence(Long, Zou and Liu 200(Ullah, Ozturk et al. 2020). This pollution affects soil, air, water, and natural deposits, leading to heavy metals entering the food chain and toxic effects on humans, plants, and microbes.

Livestock production in Brazil has increased, creating over 900,000 tons of bovine rumen waste in 2012(Ezejiofor, Enebaku and Ogueke 2014). Organic fertilizers like poultry manure, biosolid waste, and pig slurry can enhance grain crops like soybeans and corn(Nunes, Menezes et al. 2015). Studies show that broiler litter and marketable fertilizer claim rates increase soybean grain yield, nitrogen, and phosphorus uptake. Poultry manure can also increase soil pH and P levels, and can be used as mineral fertilizer to supply P and K to soybean crops(Almaz, Halim et al. 2017). This study aims to estimate the agronomic effects of an organic compost made from slaughterhouse waste on soybean and off-season corn crops and soil fertility. The UASB technology is a promising anaerobic high-rate configuration for treating wastewater, offering a moderately high loading rate with a long SRT and short HRT due to sludge preservation(Stazi and Tomei 2018). The hybrid up flow anaerobic sludge blanket (HUASB) reactor configuration is an alternative for treating various types of wastewaters, combining the advantages of both UASB and UAF reactors(Dorji 2020). Methanogenic consortium granulation is crucial for the stable operation of anaerobic, high-rate biological systems. A study investigates the optimum and maximum loading rates of a hybrid UASB reactor for treating poultry slaughterhouse wastewater in high-rate anaerobic treatment processes(Musa, Idrus et al. 2018). The food industry aims to reduce waste and improve efficiency in recovering by-products from agricultural activities, including poultry by-products like offal, bone, viscera, feet, and feathers(Jayathilakan, Sultana et al. 2012). The poultry meat processing industry produces significant wastewater crucial for environmental preservation. Measuring organic matter content is essential for determining treatments and estimating costs. Meat processing effluents are high in nitrogen, phosphorus, solids, and BOD5 levels, potentially leading to eutrophication(Ng, Dalhatou et al. 2022). The industry may invest in recovering and collecting by-products to recover valuable commodities. Meat processing plants generate wastewater with high organic matter content, which must be treated before being sent to a municipal sewage system(Bustillo-Lecompte and Mehrvar 2015). Solid waste is typically transported to a composting plant or dump, but retaining it near processing plants can cause odor issues, spread diseases, and pollute groundwater(Ayilara, Olanrewaju et al. 2020). A study is being conducted to assess the risks of repetitive movements in poultry slaughterhouses, focusing on work-related musculoskeletal disorders (UL-WMSDs) caused by biomechanical factors like repetitiveness, high-frequency technical actions, excessive force, awkward postures, insufficient recovery time, tool use, and cold exposure(Caso, Ravaioli and Veneri 2007). The study aims to address the issue of waste management in poultry slaughterhouses, which has led to consumers ignoring the final fate of their waste and resenting financial penalties and legislative restrictions. Brazil, the largest exporter of chicken meat since 2004, has not improved working conditions in line with production growth(Hutz, Zanon and Brum Neto 2013). Research indicates that 67.2% of poultry slaughterhouse workers in Brazil and Iran experience discomfort, primarily on their shoulders and neck. The International Ergonomics Association has developed a checklist to address animal waste issues in developing countries(Ahasan 2002). Blood from slaughtered animals ends up as waste due to lack of facilities, high capital costs, and high water content(Nazifa, Saady et al. 2021). Alternative methods like field spreading, landfilling, and ensiling are not suitable for these countries. The EU is directing the use of animal waste due to BSE infection, while Hungary produces 100-120 million tons of waste, with 5% considered unsafe(Leoci 2014). Perilous waste can be treated with heat and fertilization to improve soil conditions and reduce stored waste and sewage slime(Ashokkumar, Flora et al. 2022). Bio-wastes like poultry manure, pig slurry, and sewage sludge have potential applications in crops like soybeans and corn. Composting is a cost-effective and environmentally friendly solution to large quantities of organic waste(Ravindran, Karmegam et al. 2021). The objective of this paper is to evaluate the overall effectiveness of slaughterhouse waste on the growth and yield of spinach and check the effect of slaughter house waste on soil nutritional status in order to manage slaughterhouse waste.

MATERIALS AND METHODS

The purpose of the current experiment was to assess the effects of a slaughterhouse on spinach crops in terms of the relative intake of NPK. This research was started in November 2017.

Experimental Site: During the winter season of 2017, the pot study was conducted in the wire house of the Institute of Soil and Environmental Science, University of Agriculture, Faisalabad.

Collection and Preparation of Soil: The bulk soil was obtained from the ISES farm at the University of Agriculture in Faisalabad. After air-drying, the soil was sieved through 2 mm mesh and well blended. A produced soil composite sample was tested for several physicochemical soil properties.

Soil Analysis before Planting: The US Salinity Lab Staff (1954) evaluated soil for physicochemical qualities and it was investigated that sandy loam with a pH of 8.4. There were no significant problems with plant growth, and the soil was productive. Saturated soil paste was prepared with distilled water and pH was measured with pH meter. After that extract was produced and the electrical conductivity of the saturated soil paste was noted by using digital (EC) meter (Jenway) in dSm^{-1}. This paste was dried in order to obtain the saturation percentage by using the formula:

Saturation % = $loss\ of\ weight\ on\ oven\ drying\ /weight\ of\ oven\ dried\ s$oil × 100

Textural analysis: The hydrometer method was used to evaluate the proportion of primary soil particles, including sand, silt, and clay. The soil was soaked with a sodium hexametaphosphate solution and then dispersed in a hydrometer jar. The hydrometer readings indicated the percentage of silt and clay in the soil. The suspension was mixed and left for four hours, resulting in the clay percentage. The textural class was then determined from the international triangle.

Soil analysis: The Institute of Soil and Environmental Sciences at the University of Alaska Fairbanks provided a soil sample for examination. The soil texture was determined using a hydrometer, and the pH of the saturated soil paste was measured using a Calomel glass electrode assembly. The soil paste extract had an electrical conductivity of 1.30 dS m-1 and an organic carbon content of 4.3 g/kg-1. The CEC in soil was calculated to be 14.3 cmol kg-1. The Kjeldhal technique yielded a soil total N value of 0.062%, and extractable K was 109 mg/kg1. Olsen P was measured to be7.31 mg kg, with a sodium bicarbonate solution. The quantity (%) of sand, silt, and clay was determined as follows (Clay+Silt) % = [(CHRsc) 100] /weight of soil, Clay % = [(CHRc) 100] / weight of soil Sand % = 100- [(Clay Silt) %], Silt% = % (Clay Silt) – Clay%.

At last, the value of different parameters of the soil were found by using different instruments. The value of sand, silt, clay, texture class, pH, ECe is 48.5, 32.2,27.3, sandy loam 8.41and 0.786 respectively.

Filling of pots and Different Treatments: 24 plastic pots filled with 4 kg of ready-made soil were stored in an open-air, rain-protected wire and Complete Randomized Design (CRD) layout was used for seven treatments. Data on organic fertilizer absorption and plant growth indices were collected. T_0 = Control (No Waste), T_1 = Intestine (100g), T_2 = Intestine + Dry Matter (100g + 20g), T_3 = Blood (120mL), T_4 = Blood + Dry Matter (120mL + 20g), T_5 = Blood + Dry Matter +Intestine (120mL + 20g + 100g), T_6= Blood + Intestine (120mL + 100g), T_7= Dry Matter (20g)

Seed material collection and Crop sowing Methodology: Spinach seeds were obtained from the Ayub Agriculture Research Institute in Faisalabad and primed with high-quality irrigation water. The crop was planted on December 30, 2017, with 10 seedlings initially planted and four remaining. Spinach was hand thinned 20 days after sowing to achieve the required plant population of four. Chemical management was modified to keep insect pests below a threshold level, and weeds were managed by hand weeding and hoeing. Irrigation was applied at appropriate intervals, and the crop was harvested on February 30, 2018. Plant shoots were collected, labeled, and sun dried.

Growth Yield Parameters: The study measured plant height and root length at 90 days after sowing (DAS). At 90 DAS, one plant was harvested and shoot fresh weight was recorded. Roots were divided into small portions and the fresh weight of roots was recorded. Shoot material was sun dried and oven dried for 48 hours. Samples of root were sun dried and then oven dried at 60°C. The dry weights of the samples were then recorded.

Plant Physiological analysis: The Biochemistry Lab at Agriculture University Faisalabad found that organic substrates significantly influenced photosynthesis and transpiration rates in plants. T1 showed the highest rate of photosynthesis, while T3 to T5 treatments showed similar trends. All organic substrate blending positively impacted transpiration rate, with T5, T4, and T2 plants showing a significant increase. Treatment combinations also increased stomatal conductance, with T4 and T3 showing similar effects but higher than the control. Wastes application did not significantly regulate carbon dioxide intake. The study measured the osmotic potential of flag leaf cell sap using an osmometer, and then collected, weighed, and cut leaf blades into segments. Initial electrical conductivity was measured, followed by rehydration and autoclaving. The total electrical conductivity was then measured, and electrolyte leakage was determined by using equations:

$$\text{Relative water content (\%)} = \frac{\text{Leaf fresh weight - Leaf dry weight}}{\text{Leaf turgid weight - Leaf dry weight}} \times 100$$

Electrical conductivity: $\frac{(ECf \times ECi)}{(ECt - ECi)} \times 100$

Plant chemical analysis: Plant samples were digested using Wolf's 1982 procedure, dried at 60°C for 48 hours, and ground into powder. The samples were then heated to

350°C, cooled, and distilled water was added. The extracted solution, containing 50ml of NPK, was then stored in plastic bottles to assess the concentration in the plant. The Kjeld Hal method was used to assess nitrogen concentration in plant samples. The N components were then assessed by following equation.

N% = (v2-v1) ×0.041×N×100/w

And:

V_1 = Volume of the H_2SO_4 which is required to titrate the blank solution

V_2 = Volume of H_2SO_4 which is required to titrate the sample solution

N = Normality of H_2SO_4 =0.01N

1 ml of 0.01N H_2SO_4 =0.0014g N

W = Weight of the sample

10 ml of digested sample solution × Weight of dry sample

Total volume of digested sample solution

The study involved determining the concentration of phosphorus in plant extract using a 50ml flask, adding a 10ml reagent, and recording readings using a spectrophotometer. A calibration curve was created to evaluate standards and samples. The potassium content in a plant was assessed using a flame photometer following Pratt and Chapman's 1961 procedure, and phosphorus concentration was measured in ppm and then converted into percentage by using formula:

Potassium = Parts per million (ppm) in graph × dilution × 100

10^6

Statistical analysis: To analyze the data statistically a software "Statistic 8.1" version was used. To compare results of the study a Duncan's multiple test (DMR) was used.

RESULTS

A study at the University of Agriculture, Faisalabad, evaluated the impact of slaughterhouse waste on spinach growth and soil health, using a pot trail.

Agronomic parameters: The study found that plant height was reduced in natural soil, but slaughterhouse waste significantly improved it. The maximum height was achieved when joint application of intestine, blood, and dry matter was used, which increased by 85% compared to the control. All treatments increased shoot length, with blood and intestine joint application increasing 60% plant height, intestine and dry matter increasing 66%, and dry matter increasing 70%. The treatments improved root length over control, with soil combinations of intestine, blood, and dry matter enhancing it by 170%, blood and dry matter increasing it up to 150%, and dry matter increasing 135%. Intestine and blood combined increased root length by 104% and 98%, respectively. The treatments improved root length over control, with soil combinations of intestine, blood, and dry matter enhancing it by 170%, blood and dry matter increasing it up to 150%, and dry matter increasing 135%. Intestine and blood combined increased root length by 104% and 98%, respectively. Slaughterhouse waste increased root dry weight, with the maximum weight observed being 1.5g with intestine, blood, and dry matter treatment, which was 80% higher than the control. All treatments improved root dry weight, with most being non-significant but significant over control. In soil, blood and dry matter combined increased root dry weight by 71%, while dry matter increased by 58%. Intestine and blood also increased by 40% and 27%, respectively. The study found that the application of intestine, blood, and dry matter significantly improved shoot fresh weight, with a maximum of 56g, 80% of the control. The soil application of blood and dry matter increased shoot fresh weight by 72%, while dry matter and intestine increased by 55% and 63%, respectively. The study found that the application of intestine, blood, and dry matter significantly improved root fresh weight, with a maximum of 70%. The soil application of blood and dry matter increased root fresh weight by 6g, while dry matter and intestine increased by 53% and 59%, respectively. Intestine and blood also increased by 39% and 31%, respectively.

Physiological parameters: Slaughterhouse waste increased chlorophyll content in spinach leaves, with maximum levels observed in intestine, blood, and dry matter treatments. All treatments improved chlorophyll content under organic amendments, with blood and dry matter increasing 83%, dry matter 70%, and intestine 39%. The combined application of intestine, blood, and dry matter increased relative water contents by 70% compared to the control. The treatments all improved photosynthetic rate over control. In a soil combination of intestine, blood, and dry matter, 110 % photosynthetic rate was enhanced. Blood and dry matter increased up to 95%, while dry matter increased 81%. Intestine and dry matter increased 87%, while blood and intestine combination increased 70%. Intestine and blood increased 61% and 55%, respectively. The study found that all treatments improved transpiration rate, with soil combinations of intestine, blood, and dry matter enhancing 70%, blood and dry matter increasing up to 63%, and dry matter increasing 49%. Intestine and dry matter increased 55%, blood and intestine combination increased 41%, and intestine and blood increased 35% and 27% respectively. Stomatal conductance was found to be affected by some treatments, but all treatments increased it significantly over control. The maximum conductance was observed at 225 mmol m-2S-1 in the treatment involving intestine, blood, and dry matter, which was 80% more than the control. In soil, blood and dry matter increased 71% stomatal conductance, while dry matter increased 58%. Intestine and dry matter increased 65%, blood and intestine increased 45%, and intestine and blood increased 30%. All treatments improved internal carbon, with soil combination of intestine, blood, and dry matter enhancing it. Intestine and blood significantly increased carbon compared to control. The study found that spinach leaves' osmotic potential was reduced in natural soil, but slaughterhouse waste significantly improved it. The maximum osmotic potential was achieved when intestine, blood, and dry matter were combined, with a 92% increase. All treatments significantly increased osmotic potential, with blood and dry matter increasing by 80%, dry matter by 65%, and slurry of intestine and dry matter by 73%. Intestine and blood increased by 45% and 36%, respectively. The study found that spinach's saturation percentage increased significantly, with the maximum increase observed in the combined application of blood, intestine, and dry matter. All treatments increased the saturation percentage over the control, with blood and dry matter in soil, dry matter in soil, and slurry in the intestine and blood significantly increasing over the control. The treatments improved soil pH over control, with a significant increase in soil pH and dry matter in the combination of intestine, blood, and dry matter compared to the control. Intestine and blood significantly improved soil pH. All the treatments improved organic matter over control. In soil combination of intestine, blood and dry matter enhanced organic matter over control. Blood and dry matter increased organic matter and dry matter significant as compared to control. Intestine and dry matter significant over control, while blood and intestine combination significant over control. Intestine increased organic matter and blood significant organic matter over control.

Chemical parameters: Phosphorus concentration in spinach increased significantly, with maximum concentration observed in treatments combining blood, intestine, and dry matter. All treatments increased phosphorus over control, with soil, blood, dry matter, slurry, and blood and intestine significantly increasing phosphorus. Intestine and blood increased by 45% and 36%, respectively, compared to the control. Potassium concentration in spinach significantly increased, with maximum concentration observed in treatments combining blood, intestine, and dry matter. All treatments increased potassium over control, with soil, blood, dry matter, slurry, and intestine significantly increasing Potassium. Intestine and blood increased by 45% and 36%, respectively, compared to the control.

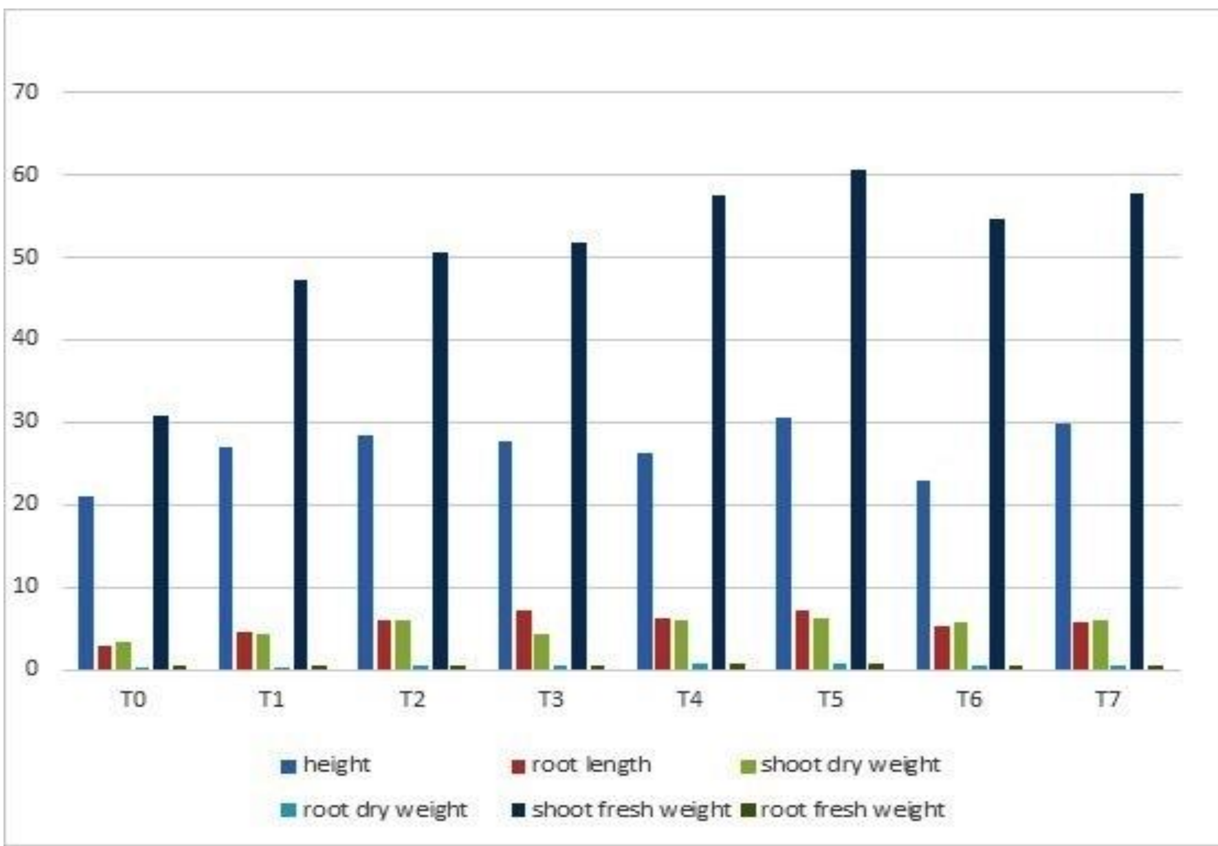

Fig (1): Effect of blood, intestine and dry matter on plant height (cm), root length (cm), shoot dry weight (cm), root dry weight (cm), shoot dry weight (cm), shoot fresh weight (cm), and root fresh weight of spinach in

soil. Column showed mean of three repeats and bars showed S.E of mean do not significant statistically at $p \leq 0.05$.

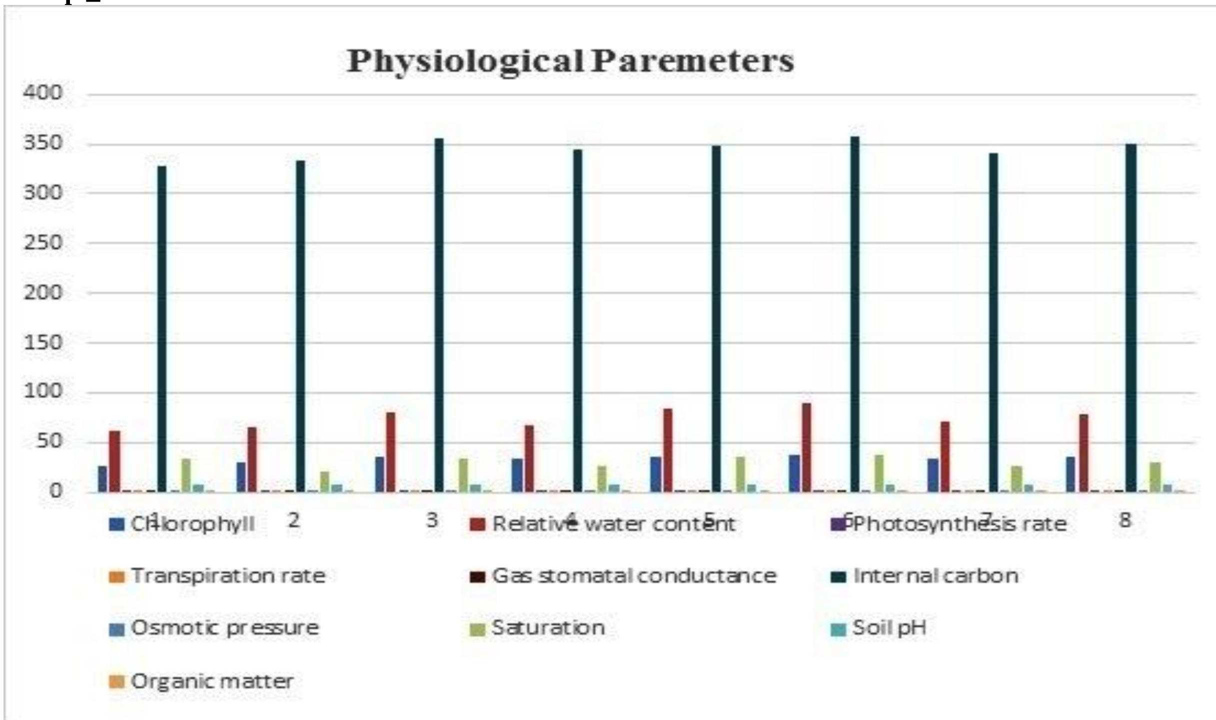

Fig 2: Effect of blood, intestine and dry matter on Chlorophyll content (SPAD), Relative water content (RWC), Photosynthesis rate (mmol m^{-2} S^{-1}), Transpiration rate (mmol m^{-2} S^{-1}), Gas stomatal conductance (mmol $m^{-2}S^{-1}$), Internal carbon (VPN), Osmotic potential, Saturation, Soil pH and organic matter. Column showed mean of three repeats and bars showed S.E of mean do not significant statistically at $p \leq 0.05$.

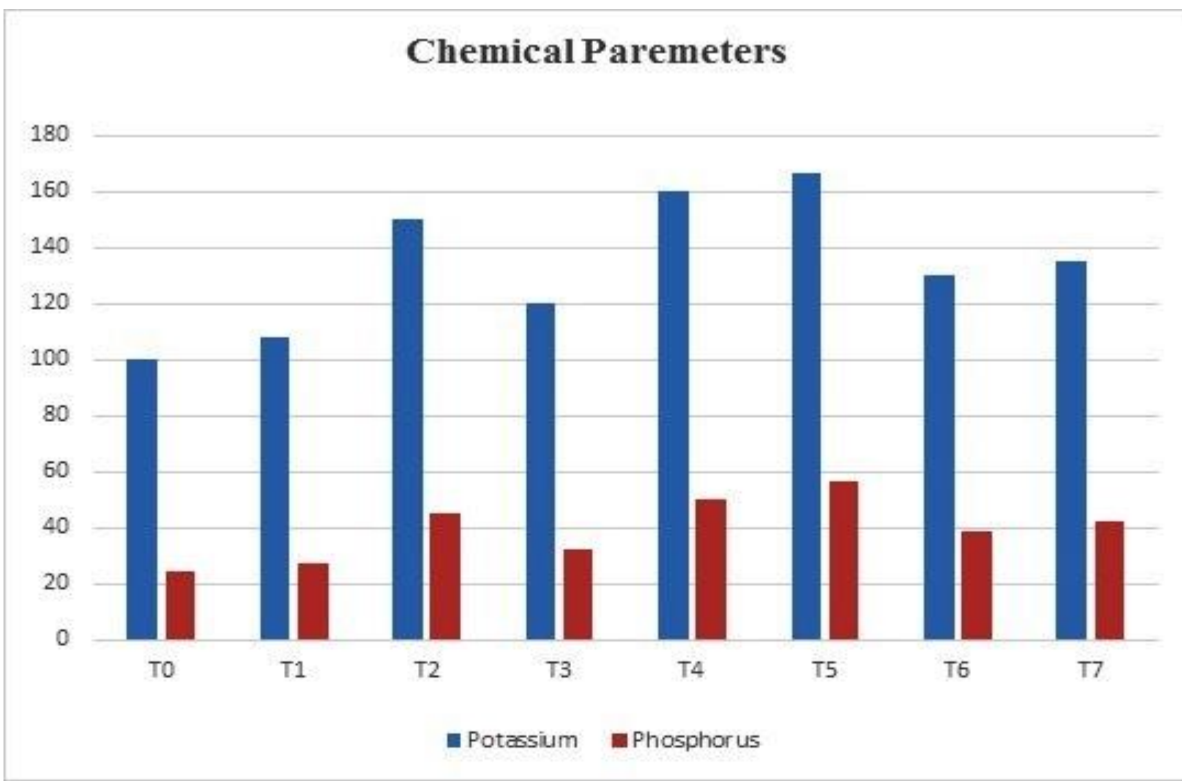

Fig 3: Effect of blood, intestine and dry matter on soil potassium and phosphorus. Column showed mean of three repeats and bars showed S.E of mean do not significant statistically at $p \leq 0.05$.

DISCUSSION

Heavy metal deposition in agricultural soil has been caused by industrialization and its effluents for decades, and their high concentration in soil environment may pose a substantial harm to the quality of natural waters, soils, plants, and human health. Due to a lack of fresh water, farmers were forced to use untreated waste water for agricultural production. These effluents are nutrient-rich, which can minimize fertilizer use for agricultural production, but they also contain heavy metals and other toxic compounds that are detrimental not just to terrestrial ecosystems but also to humans. Applying waste water repeatedly can cause phytotoxicity, which can contaminate food chains. Various approaches have been employed to mitigate these hazards that impact the development and biomass yield in heavily contaminated soils. The study's findings demonstrated that adding blood, intestine, and dry matter to soil greatly enhances spinach's physiological, chemical, and physiological characteristics. Several growth indices, including plant height, shoot and root dry weight, improved several times when blood, intestine, and dry matter were applied together. A reduction in root length greater than shoot length was noted; this could be because the intestine affects cell division and tears the root's cell wall. Under typical soil conditions, it saw a decrease in both total root biomass and root length. The decrease in root growth could be attributed to several factors, including the elongation of the cell cycle inhibition of root cell division, collapse of tissue, and incapacity for nutrients and water adsorption due to direct contact of roots with metal species. According to the results of the current investigation, all the agronomic parameters under naturally occurring soil were considerably elevated by slaughterhouse waste. Organic matter in the soil improved soil fertility because it offers all needed nutrients that led to higher vegetative development. The addition of waste to the soil boosted macro and micro nutrients (K, Ca, Zn, and Mn). The combination application of blood, intestine, and dry matter greatly enhanced the physiology of the spinach plant and had a considerable impact on the physiological characteristics of the plants. In our investigation, we found that while the control condition's chlorophyll contents were lower, the combined use of the intestine, blood, and dry matter increased the contents of chlorophyll. Because iron is the primary component of protoporphyrin, a precursor to chlorophyll synthesis, and because iron toxicity affects Fe availability, an adequate iron supply is essential for the production of chlorophyll pigment. The iron's ability to bond with protoporphyrin, the primary precursor of chlorophyll synthesis, may be the cause of this inhibition in chlorophyll content. Reactive oxygen species (ROS) were thought to harm the protein pigment complexes found in thylakoid membranes and prevent the formation of chlorophyll. There are a few possible explanations for the adsorption of heavy metals on the surface of biochar, including ion exchange, electrostatic interaction, coupling with the functional group of the biochar, and chemical precipitation. The surface of biochar has free or complexed carboxyl, phenolic hydroxyl, or alcoholic hydroxyl groups that are essential for the sorption of heavy metals through inner-sphere complex, electrostatic attraction, and precipitation.

Conclusion: Leather processing in eastern Pakistan is a significant industry, with over 800 tanneries operating in the region. The Kasur region has the highest number of tanneries, which discharge about nine million liters of highly contaminated wastewater daily. The waste generated from tanneries includes meat, fat tissue, hair, and sludge. The type of leather manufacturing process, skin resource, and techniques used affect the quantity of waste produced. The irrigation of waste water introduces heavy metals to the soil and aquatic system, causing negative environmental impacts due to their persistence. Heavy metal pollution is a major environmental problem, as metal ions persist in the environment due to their non-degradable nature. A pot experiment was conducted to test the effect of slaughterhouse waste on soil quality and the growth and physiology of spinach. Results showed that blood, intestine, and dry matter significantly affect plant growth parameters, with blood, intestine, and dry matter increasing chlorophyll contents, relative water contents, photosynthetic rate, transpiration rate, stomatal conductance, and electrolyte leakage.

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