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ARTICLE

Poultry Manure as an Organic Fertilizer with or without Biochar Amendment: Influence on Growth and Heavy Metal Accumulation in Lettuce and Spinach and Soil Nutrients

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ABSTRACT

This pot-based study investigated the influence of poultry manure and 1:1 mixture of poultry manure + biochar (produced from farmyard manure [FYM] or wood), on the biomass production and concentration of heavy metals in leaves of lettuce and spinach. The concentration of mineral nitrogen (N) and soluble inorganic phosphorus (P) of soils cultivated with these vegetables was also investigated. The application of poultry manure or FYM biochar in soil as 10% (equivalent to 60 t ha^{-1}, an estimated 1726.8 kg ha^{-1} N in poultry manure and 1353.9 kg ha^{-1} N in FYM) and 15% amendment (equivalent to 90 t ha^{-1}, an estimated 2590.2 kg ha^{-1} N in poultry manure and 2030.8 kg ha^{-1} N in FYM) significantly decreased biomass production of lettuce as compared to control (no fertilizer added) treatment. However, mixture of poultry manure with wood-derived biochar at both application rates (i.e., 10% and 15%) and with FYM biochar at lower application rate (i.e., 10%) caused 2–3-fold increase in aboveground plant biomass and 2–14-fold increase in root biomass ($p < 0.05$). Furthermore, as compared to control treatment, a significant ~2–3-fold increase in aboveground plant biomass was also observed in response to mixture of poultry manure with wood-derived and FYM derived biochars at 10% amendment rates. As compared to control treatment, concentration of mineral N and soluble inorganic P were higher in soils of all other treatments. In spinach, amendment of poultry manure or its co-amendment with biochar of FYM significantly increased aboveground plant biomass at 7% (equivalent to 42 t ha^{-1}) as compared to 3% and 5% amendment rates (equivalent to 18 and 30 t ha^{-1} respectively). The concentration of soil mineral N and soil soluble mineral P was not different between treatments. In lettuce, wood-derived biochar did not reduce concentration of heavy metals (i.e., manganese (Mn), copper (Cu), iron, (Fe), cadmium (Cd), lead (Pb), nickel (Ni) and cobalt (Co) than FYM-derived biochar while in spinach, as compared to poultry manure, co-amendment of poultry manure with wood-derived biochar reduced concentration of heavy metals, indicating differential responses of crops to organic amendments.

KEYWORDS

Biochar; poultry manure; lettuce; spinach

1 Introduction

Poultry industry started in Pakistan in 1962, this industry turned into an important sector of livestock in Pakistan, providing employment to 1.5 million people and contributed in 1.4% gross domestic production (GDP) of the country in 2017–2018 [1]. To date, twenty-five thousand poultry farms are reported in Pakistan [2]. The production of cow meat is also increasing in Pakistan [1]. The growing industry of poultry and livestock production in Pakistan is also generating production of manure with the same rate. The utilization of manure produced from poultry and livestock as organic fertilizer in soil can be a means of its proper disposal and its prevention to be wasted, besides of its high potential to be utilized in biogas production for energy generation [2]. Poultry manure as a fertilizer was a part of organic crop production from centuries and it has been considered as the most suitable among the natural fertilizers because it has high nitrogen content in it [3].

Biochar is pyrogenous biomass that is made from burning biomass (e.g., stover, manure, bark, nutshells, algae, animal bones, etc.) in oxygen-free or oxygen-deficient conditions [4]. Biochar is hygroscopic can retain and attract water, because of its high surface area and porous structure [5]. It prevents the leaching of nutrients from the soil [6]. When biochar is mixed with compost or manure, it absorbs nutrients and become a slow-release fertilizer [7]. Many beneficial effects of biochar depend upon its different properties [8]. The utility of these manures can be maximized with the help of their co-amendment with biochar, which is reported to be more beneficial for soil quality and crop production than when these organic substances (manures and biochar) are amended alone [7,9–11]. Biochar-manure mixture can be more economical when it is applied in the root zone of crops as it will be used in many times less amount than when it is applied thoroughly in soil [12].

Lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*) are two important vegetable crops of Pakistan [13]. There is wealth of reports that demonstrate positive influence of manures and biochar-manure mixture on yield of these two important crops [14–17]. However, because in developing countries, there is no proper management of utilization of manures, they can contribute significantly in environmental pollution especially emission of greenhouse gases [18,19]. For this reason, there is need to assess if high application rates of these manures in soil with combination of biochar can have a positive influence on the yield of vegetable crops. Biochar due to its highly porous nature, captures nutrients and act as slow-release fertilizer [10] and therefore, if mixed with manures can attenuate high application rate-related negative influence of manure fertilizers. Such a study will help farmers to utilize these two important biowastes, which will in return help prevent environmental hazards related to their waste in environment. Objectives of the present study were to evaluate the influence of poultry manure and farmyard manure (FYM) as organic fertilizers, amended in soil alone or as a mixture with biochar, thoroughly mixed in soil or applied in the root zone of lettuce, on growth, water use efficiency (WUE) and concentration of heavy metals, i.e., manganese (Mn), copper (Cu), iron, (Fe), cadmium (Cd), lead (Pb), nickel (Ni) and cobalt (Co) in lettuce. Two types of biochars were used; biochar produced from wood and biochar produced from cow manure. We also tested poultry manure as organic amendment in soil alone or mixed with wood-derived biochar or biochar produced from FYM at lower rates than the rates applied to lettuce (as in lettuce high application rates of manure amendment reduced growth) on growth, WUE and concentration of heavy metals (as mentioned above) in spinach. Soil quality parameters such as organic matter, pH, electrical conductivity and concentration of mineral nitrogen (N) and soluble mineral phosphorus (P) of soils after harvest of crops were also tested. Our main hypotheses are mixture of poultry manure with biochar increases 1) plant growth performance and 2) reduces concentration of heavy metals in soil. Dikinya et al. [20] observed higher yield of spinach in Luvic Calcisol at 10% amendment rate of poultry manure as compared to 5% amendment rate. Yang et al. [21] used 20% amendment rate of poultry manure in their research that was related to evaluate abundance of endopytic bacteria in Pakchoi and antibiotic resistant bacteria in soil planted with Pakchoi. Hao et al. [22] observed approximately 15 times greater fresh biomass production of Pakchoi in response to amendment of poultry manure at 8% amendment rate. Therefore, we used 10% and 15% amendment rates for poultry manure for spinach. The main reason of using these high application rates was to provide an insight to local farmers to maximize the use of this manure for agriculture purpose, which can in return help substantially in reducing environmental pollution caused by its waste.

2 Materials and Methods

2.1 Biochar Production and Experimental Setup

In this study slow pyrolyzed wood-based biochar was purchased from the local timber market of Quetta, Balochistan, Pakistan. This biochar was produced in kilns, where the temperature is between 300°C and 500°C [23,24]. The biochar produced from cow manure (FYM in text) was prepared by the burning of dry cow manure under the oxygen-deficient conditions in kilns. The poultry manure in this project was taken from poultry farm in Quetta, Pakistan. Fresh poultry manure was kept under sunlight to properly dry it. The biochar-manure mixture was prepared as 1:1 w/w biochar:manure ratio on dry bases. Properties of biochars and poultry manure are provided in Tab. 1.

Table 1: Chemical properties of FYM biochar, wood-derived biochar and poultry manure on dry biomass bases

Properties	Poultry manure	FYM biochar	Wood-derived biochar
pH	5.42	5.51	5.29
EC (μs cm^{-1})	260	422	146.6
Organic matter (%)	63	55	75
Manganese (Mn) (mg g^{-1})	35.07	196.17	9.88
Copper (Cu) (mg g^{-1})	190.22	192.66	182.19
Iron (Fe) (mg g^{-1})	5.46	7.45	3.92
Cadmium (Cd) (mg g^{-1})	0.84	0.015	0.76
Nickel (Ni) (mg g^{-1})	6.44	12.02	3.37
Lead (Pb) (mg g^{-1})	8.51	10.69	10.44
Cobalt (Co) (mg g^{-1})	0.91	0.61	1.09

Sandy loam soil was selected for this study. The soil was air-dried completely and sieved through 2 mm mesh, 600 g soil was used for each plastic pot of lettuce plant. Commercially available plastic pots of 08 cm diameter and 15 cm height were filled with soil. For lettuce crop, experiment was designed as factorial with three factors; 1) fertilizer type 2) amendment rate and 3) fertilizer placement (thoroughly mixed or placed as root-zone fertilizer). For lettuce, five treatments except control treatment were selected for thoroughly mix type as 1) control (no organic amendment), 2) wood-based biochar, 3) poultry manure, 4) mixture of wood-based biochar-poultry manure as 1:1 ratio and 5) mixture of FYM biochar-poultry manure as 1:1 ratio. Each organic fertilizer was applied at two amendment rates10% (equivalent to 60 t ha^{-1}) and 15% (equivalent to 90 t ha^{-1}). Based on published reports regarding concentration of total nitrogen in poultry and FYM presented in Supporting Information Tab. S1, these amendment rates may contain respectively an estimated 1726.8 kg ha^{-1} N and 2590.2 kg ha^{-1} N input for poultry manure and respectively an estimated 1353.9 kg ha^{-1} N and 2030.85 kg ha^{-1} N input for FYM [25–29]. Previous reports suggest that the yield of lettuce was higher at higher application rate of synthetic fertilizer as 200 kg ha^{-1} [30] and 271 kg ha^{-1} [31]. Three treatments were selected for root zone fertilizer trial; 1) poultry manure, 2) mixture of wood-based biochar-chicken manure (mixed at 1:1 ratio) and 3) mixture of FYM biochar-chicken manure (mixed at 1:1 ratio). Each fertilizer was applied at three amendment rates, i.e., 5% (equivalent to 30 t ha^{-1}), 10%, and 15%). The pots were filled with soil, then an amount of fertilizer equivalent to 5%, 10% or 15% rate were surface applied and the fertilizers were then covered with soil. Each treatment had three replications.

For spinach, only fertilizer as thoroughly-mixed treatments were used and the amendment rates were kept lower than the ones for lettuce. The experimental design was factorial with factors; 1) fertilizer type and 2) amendment rate. This was because for lettuce, root-zone fertilizer and manures at higher rates (10% and 15%) without biochar mixture had a negative influence on crop growth. For lettuce following treatments were made; 1) poultry manure, 2) mixture of FYM biochar-poultry manure (mixed at 1:1 ratio) and the mixture of wood-based biochar—poultry manure (mixed at 1:1 ratio). Each fertilizer was amended at 3% (~18 t ha^{-1}), 5% (~30 t ha^{-1}) and 7% (~42 t ha^{-1}) amendment rate in soil. These amendment rates are approximately equal to 290 kg ha^{-1}, 483 kg ha^{-1} and 676 kg ha^{-1} of total N input respectively from poultry manure and 406 kg ha^{-1}, 677 kg ha^{-1} and 948 kg ha^{-1} of total N input from FYM manure according to the Supporting Information Tab. S1. Previous studies showed a positive linear growth rate of spinach in response to the amendment rate of synthetic fertilizer and the maximum yield was reported at highest application rate of 200 kg ha^{-1} [32,33]. All treatments had three replications. Treatments and abbreviations of each treatment are given in Tab. 2.

Table 2: Experimental design and abbreviations of treatments

Plant name	Treatments with amendment rates	Abbreviations of treatments
Lettuce (thoroughly mixed treatment)	Control	Cont
	Poultry manure 10%	P10
	Poultry manure 15%	P15
	Wood Biochar 10%	W10
	Wood Biochar 15%	W15
	FYM Biochar 10%	FYM10
	FYM Biochar 15%	FYM15
	Wood Biochar + poultry manure 10%	WB + P10
	Wood Biochar + poultry manure 15%	WB + P15
	FYM Biochar + poultrymanure 10%	FYM + P10
	FYMbiochar + poultrymanure 15%	FYM + P15
Lettuce (root zone fertilizer treatment)	Poultry manure 5%	P5r
	Poultry manure 10%	P10r
	Poultry manure 15%	P15r
	Wood biochar + poultry manure 5%	WB + P5r
	Wood biochar + poultry manure 10%	WB + P10r
	Wood biochar + poultry manure 15%	WB + P15r
	FYMbiochar + poultry manure 5%	FYM + P5r
	FYM biochar + poultry manure 10%	FYM + P10r
	FYM biochar + poultryanure 15%	FYM + P15r

(Continued)

Table 2 (continued).

Plant name	Treatments with amendment rates	Abbreviations of treatments
Spinach (thoroughly mixed treatment)	Poultry manure 3%	P3
	Poultry manure 5%	P5
	Poultry manure 7%	P7
	FYM biochar + poultry manure 3%	FYM + P3
	FYM biochar + poultry manure 5%	FYM + P5
	FYM biochar + poultry manure 7%	FYM + P7
	Wood biochar + poultry manure 3%	WB + P3
	Wood biochar + poultry manure 5%	WB + P5
	Wood biochar + poultry manure 7%	WB + P7

2.2 Cultivation and Harvesting

Lettuce seeds were purchased from the local market of Quetta city and broadcasted in the third week of November over all the pots. After two weeks of seed germination, the seedlings were thinned to six seedlings per pot [14]. The water use efficiency was carried out after two weeks of germination, till the time of harvesting the pots were irrigated with an equal amount of water at alternate days and once in a week were adjusted to ~60% water filled pore space (WFPS) as described in Gul et al. [34].

Spinach seeds for this research were purchased from the local market of Quetta city and were broadcasted in last week of November over all the pots [15]. After two weeks of seed germination, the seedlings were thinned to 10 seedlings per pot. The water use efficiency was carried out after two weeks of germination, till the time of harvesting the pots were irrigated with an equal amount of water at alternate days and once in a week were adjusted to approximately 60% WFPS as described in Gul et al. [34]. Water use efficiency was calculated by using the following formula [35]:

Water Use Efficiency = Plant biomass/Amount of water (L) provided.

2.3 Assessment of Growth Performance of Plants

At the time of harvesting (~7 weeks after germination), the above-ground biomass of plant and its roots were separated by cutting each pot from two sides with the help of a sharp scissor. The soil root system present in that was removed very carefully without damaging the root system. Roots were carefully washed. The above-ground biomass and roots were air-dried for 48 hours, afterword kept in an oven at 60°C for 48 hours then dry biomass and root weight was measured [15,36].

2.4 Assessment of Heavy Metals

Heavy metals assessment was carried out by burning 1 g sample of above-ground biomass of both lettuce and spinach plant tissues, wood biochar, cow manure biochar and chicken manure at 500°C in furnace till ash formation. Ash was then transferred in bottles, containing 30 ml distilled water and 1.5 ml of concentrated hydrochloric acid as described in Ghori et al. [15]. Heavy metals concentration of plant tissues, cow manure biochar, wood biochar, and chicken manure samples were analyzed by using flame atomic absorption spectrophotometer (AA 7000 Shimadzu). Protocol and instrumental conditions for heavy metal analysis are carried out following protocol of Khan et al. [37].

2.5 Assessment of pH, E.C, mineral Nitrogen and soluble Phosphorus in soil

The assessment of Nitrogen and soluble Phosphorus was carried out by extracting the soil samples with 2M KCl solution as it was described in Gul et al. [34]. Total mineral nitrogen as ammonium (NH_4^+) and nitrate (NO_3^-) was assessed by following the protocol of Sims et al. [42]. Soluble inorganic Phosphorus was analyzed by following the process as described in D'Angelo et al. [43]. The extracts of soil samples were analyzed on UV-visible spectrophotometer (Shimadzu UV-700) for nitrogen and soluble phosphorus. The pH and electrical conductivity of soil were analyzed by mixing soil samples in distilled water by 1:2 w/v ratio as described in Dupuis et al. [44].

2.6 Statistical Analysis

All the data sets were assessed for normal distribution followed by one-way analysis of variance (ANOVA) least significance difference (LSD) test. Statistical evaluation of all results was carried out by using the most advanced computerized statistical CoStat software (version 6.400) and Microsoft Excel.

3 Results

3.1 Plant Growth Performance and Water Use Efficiency

In lettuce plant growth performance and water use efficiency (WUE) was higher in organic amendments that were thoroughly mixed as compared to organic amendments in the root zone of plants. Organic amendments tend to increase the shoot biomass, root biomass and water use efficiency (WUE) of lettuce ($p < 0.05$). Amendment of poultry manure and FYM biochar significantly reduced biomass production than control; however, when mixed with wood-based biochar at both application rates and FYM biochar at lower rates caused 2–3-fold increase in aboveground plant biomass and 2–14-fold increase in root biomass (Figs. 1 and 2; $p < 0.05$; raw data in Supporting Information Tab. S2). Furthermore, mixture of poultry manure with wood-derived biochar and FYM-derived biochar at 10% amendment rate significantly increased aboveground plant biomass ($p < 0.05$; Fig. 2). Application of poultry manure and FYM biochar at the root zone of plants significantly reduced growth pf plants as compared to the treatments where these fertilizers were thoroughly mixed in soil, while amendment of wood-based biochar did not ameliorate their negative effect (Figs. 1 and 2; $p < 0.05$). The WUE of plants were significantly higher as compared to control in response to the co-amendment of wood-based and FYM biochar with poultry manure (Fig. 2; $p < 0.05$; raw data in Supporting Information Tab. S3). For spinach, aboveground plant biomass was significantly lower in response to the amendment of poultry manure at lower application rate (3%) than other treatments (Figs. 1 and 2; $p < 0.05$; raw data in Tab. S4). Root biomass did not show the same trend as for aboveground plant biomass (Fig. 2; $p < 0.05$).The WUE of spinach showed positive relation to the aboveground plant biomass (Fig. 2; $p < 0.05$; raw data in Tab. S5).

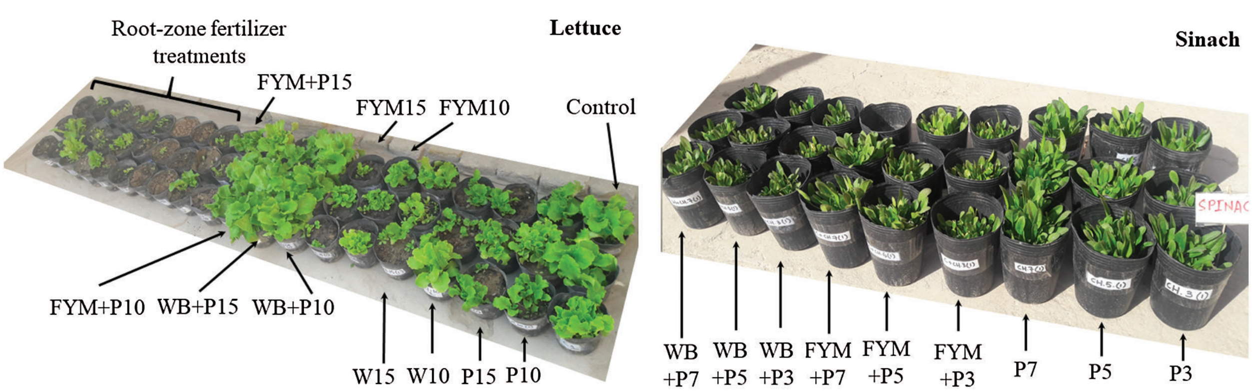

Figure 1: Lettice and spinach plants placed in sequential lines according to their treatments

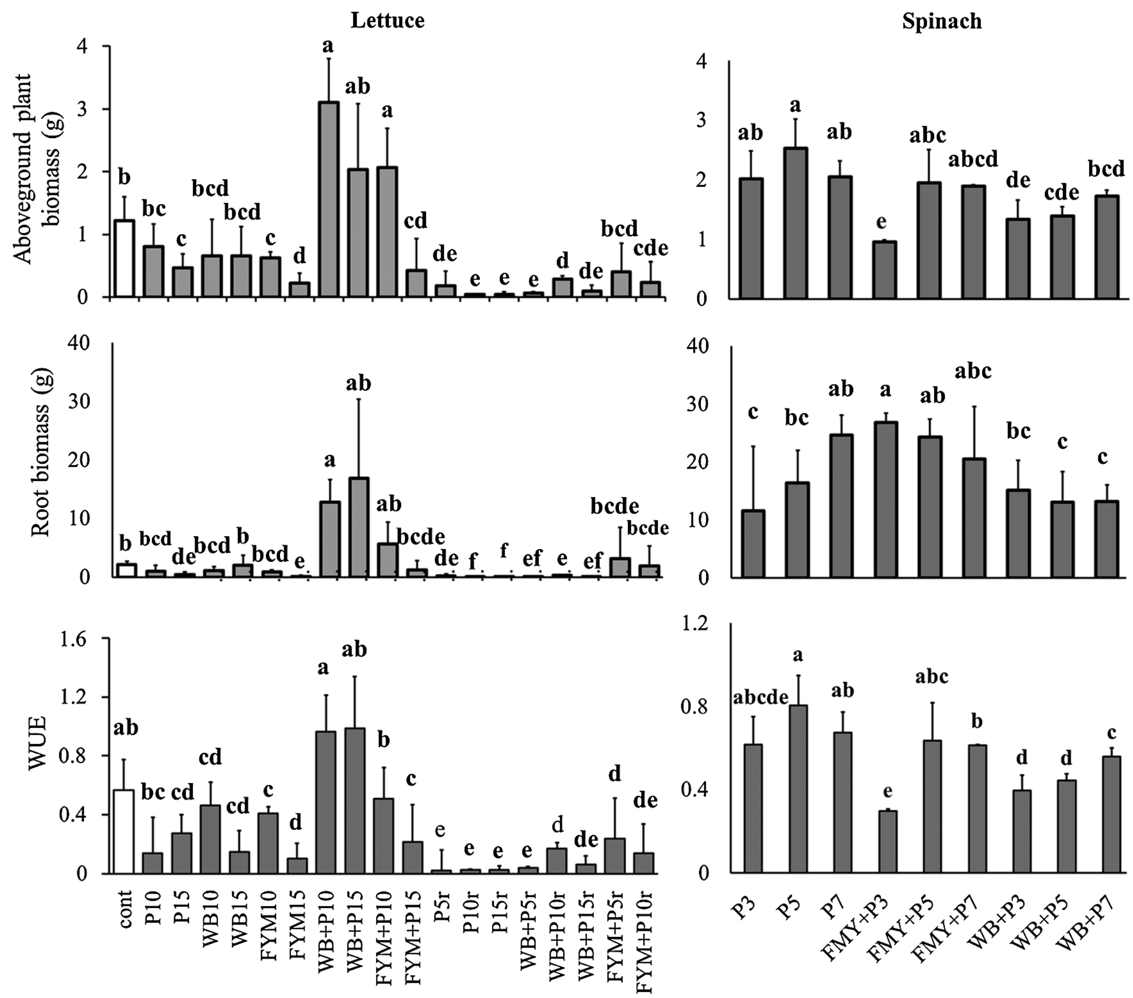

Figure 2: Average (±SD) values of aboveground plant biomass (g), root biomass (g) and water use efficiency (WUE) of lettuce and spinach in response to different treatments. Bars with different lowercase letters indicate significant difference ($p < 0.05$)

3.2 Soil Properties

In lettuce-grown soil, as compared to control, concentration of mineral N and soluble mineral P was significantly higher in response to organic amendments (Fig. 3; $p < 0.05$; raw data in Supporting Information Tabs. S6 and S7).

In spinach-grown soil, concentration of mineral N was significantly higher in response to the amendment of poultry manure and mixture of FYM + poultry manure at 7% rate as compared to the treatments of 3% amendment rates for poultry manure and mixture of FYM + poultry manure (Fig. 3; $p < 0.05$; raw data in Supporting Information Tab. S8). The concentration of P was significantly higher in response to the amendment of poultry manure at 7% rate and mixture of wood-based biochar and poultry manure at 5% amendment rate than the soil amended with mixture of wood-based biochar with poultry manure at 5% and 7% rates (Fig. 3; $p < 0.05$; raw data in Supporting Information Tab. S9).

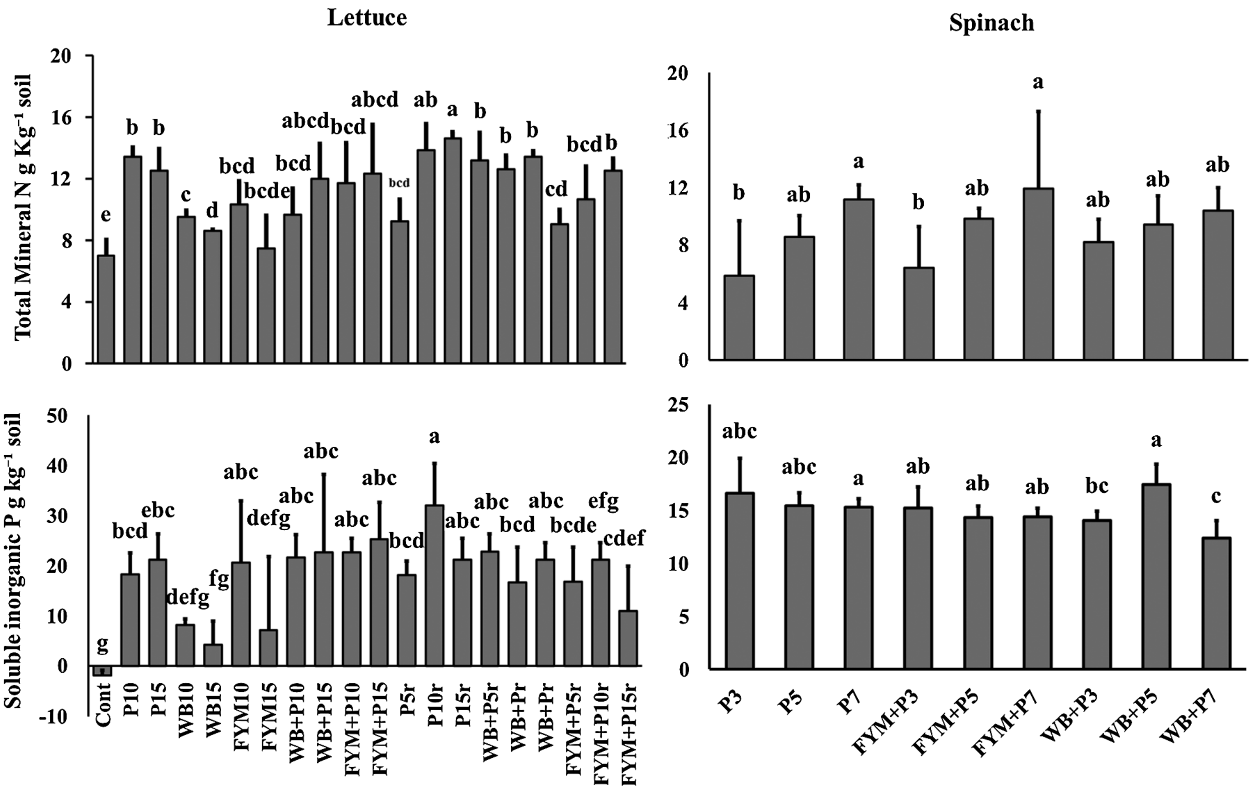

Figure 3: Average (±SD) values of mineral N and soluble inorganic P of soils grown with lettuce and spinach under different treatments. Bars with different lowercase letters indicate significant difference ($p < 0.05$)

3.3 Concentration of Heavy Metals in Aboveground Plant Tissues

The concentration of heavy metals (manganese [Mn], copper [Cu], iron [Fe], cadmium [Cd], lead [Pb], nickle [Ni] and cobalt [Co] was higher in tissues of lettuce plants grown in soils amended with organic fertilizers as compared to control (Tab. 3). However, the nutrient use efficiency (NUE) of plants for heavy metals tend to be higher in response to organic amendments as compared to control (Tab. 3). In spinach, increasing the amendment rate of poultry manure increased the concentration of heavy metals; however, amendment of mixture of wood-based biochar with poultry manure reduced concentration of heavy metals (Tab. 3). The NUE of plants for heavy metals had positive relation with plant biomass.

4 Discussion

4.1 Plant Growth Performance and Water Use Efficiency

Our findings show that amendment of poultry manure and the biochar of FYM had negative influence on the growth of lettuce but poultry manure when mixed with biochar of wood at both application rates (10% and 15%) and with biochar of FYM at low application rate (10%) caused 2–3-fold increase in aboveground plant biomass and 2–14-fold increase in root biomass. Interestingly, as compared to control treatment, mixture of poultry manure with wood-derived biochar and with FYM-derived biochar significantly increased aboveground plant biomass by ~3 and ~2 fold respectively at 10% amendment rate. Our results are consistent with the finding of Hameeda et al. [36] who also observed enhanced yield of tomato in response to the co-amendment of wood-derived biochar and FYM. Findings of Sun et al. [38–40] also suggested that positive influence of organic fertilizers are enhanced when they are amended in soil with combination of biochars. The soil amended with FYM biochar and with mixture of FYM biochar and poultry manure at higher application rates had significantly higher EC (Supporting Information Tab. S10), which might be the reason for reduced growth of plants possibly because of high concentration of nutrients. Nutrients in high concentration cause osmotic stress in roots and thus affect negatively plant growth [34]. Wood-based biochar have lower concentration of nutrients and act as nutrient capture when applied to soil in mixture with manure [34]. The co-amendment of poultry manure with wood-based biochar might ameliorated the toxic effect of high concentration of nutrients in manure and thus caused a significant increase in biomass of lettuce in our study.

Table 3: Concentration of heavy metals (mg g^{-1}) manganese (Mn), copper (Cu), cadmium (Cd), lead (Pb), Nickle (Ni) and cobalt (Co) in leaves of lettuce and spinach and the nutrient use efficiency of plants for these heavy metals as nutrient efficiency ratio, which was calculated as plant biomass/concentration of heavy metal in plant biomass ([45])

Crop	Treatment	Mn	NUE for Mn	Cu	NUE for Cu	Fe	NUE for Fe	Cd	NUE for Cd	Pb	NUE for Pb	Ni	NUE for Ni	Co	NUE for Co
Lettuce	Cont	93.05	0.078	116.48	0.062	1486.6	0.004	1.14	6.429	8.12	0.902	8.08	0.907	0.20	36.65
	WB + P10	106.25	1.47	141.61	1.104	1402.6	0.111	1.40	111.1	8.25	18.86	2.62	59.38	0.43	361.8
	WB + P15	91.06	1.58	179.51	0.802	1412.3	0.102	1.31	119.3	8.18	19.11	6.85	22.83	0.66	236.9
	FYM + P10	96.97	0.41	130.85	0.304	1488.5	0.026	1.11	129.8	8.43	17.09	8.04	17.92	0.19	758.4
	FYM + P15	85.04	0.46	184.33	0.215	1248.6	0.031	1.32	30.15	8.00	4.97	61.39	0.648	0.58	68.62
Spinach	P3	138.74	0.014	187.62	0.010	1628.7	0.001	1.39	1.45	8.34	0.24	9.00	0.22	1.17	1.72
	P5	187.84	0.013	182.88	0.013	16.16	0.156	1.42	1.78	6.74	0.37	10.20	0.24	0.90	2.8
	P7	154.32	0.013	150.52	0.013	11.47	0.179	1.55	1.32	8.45	0.24	5.61	0.36	0.81	2.53
	WB + P7	116.11	0.014	168.58	0.010	7.31	0.237	0.71	2.44	9.60	0.18	4.87	0.35	0.97	1.78

Values are based on pool samples of n = 3
High NUE of plant for a given heavy metal indicates "less" uptake of that heavy metal per unit (g) plant biomass production.

We also tested the influence of poultry manure and the mixture of poultry manure with FYM biochar or wood-based biochar on the growth of another important vegetable crop of Pakistan the spinach. As in lettuce, high amendment rates of poultry manure and biochar of FYM significantly reduced growth, we applied these fertilizers in lower rates, i.e., 3%, 5% and 7%. These amendment rates did not show any response in spinach growth while the mixture of poultry manure with wood-based biochar significantly reduced the growth of spinach as compared to when only poultry manure was applied. This indicates that these amendment rates are not enough to have a positive influence on growth of spinach and the addition of wood-based biochar might have caused nutrient reduction in plants, which caused a reduction in the growth of this crop. The WUE for both crops showed a positive relationship with the aboveground plant biomass. The WUE of lettuce was significantly higher for the treatment of mixture of wood-derived biochar with poultry manure and FYM-derived biochar with the poultry manure as compared to when only poultry manure or wood-derived biochar or FYM-derived biochar were applied to the soil. Such a trend, i.e., biochar + manure mixture positive influence on plant WUE, was however not observed for spinach. This non-consistent trend indicates that these fertilizers have differential influence on the growth performance of these vegetable crops. Crops exhibit variable interaction with their microbiome in response to fertilizer and its application rate, which influence crops growth performance [41]. It is worth further investigation to assess biochar-based organic fertilizer-plant rhizobium interaction in controlling plant growth performance in various crops. Such a study will help understand the crop-specific requirement of biochar-based organic fertilizers regarding their types and application rates.

Poultry manure and FYM in Balochistan province of Pakistan are many-times less expensive than inorganic NPK fertilizer as NPK fertilizer costs approximately 1 US dollar for one kg while FYM costs around 30–45 US dollars per "tone." Poultry manure if required in small amount (few kg) is even free of cost. These fertilizers when not utilized appropriately, they will be wasted as air and water pollutants [46] with no exception in Balochistan (personal observation). Similarly, major source of wood-derived biochar for Bar B-Q purpose is wood from pruned trees of orchards. The crushed small pieces as leftover of this biochar costs approximately 0.5 US dollars for 1 kg. Agriculture in this arid region can be promoted by utilizing biochar-manure mixture as organic fertilizer. The use of such fertilizer treatments can be proved as cost-efficient and environmental-friendly practice that can potentially also promote agriculture in this arid region of Pakistan.

4.2 Concentration of Heavy Metals (Mn, Cu, Fe, Cd, Pb, Ni, Co)

The NUE of heavy metals in this study was calculated as nutrient efficiency ratio of Baligar et al. [45], which was calculated as plant biomass/concentration of heavy metal in plant biomass [45]. Therefore, high NUE of plant for a given heavy metal (e.g., in our study Mn, Cu, Cd, Pb, Ni or Co) indicates "less" uptake of that heavy metal per unit plant biomass production. As observed the high concentration of heavy metals in poultry manure as compared to wood-based biochar, amendment of these fertilizers showed generally higher concentration of heavy metals in leaves of lettuce as compared to control. However, NUE of lettuce for heavy metals was higher when grown in the soil amended with mixture of poultry manure with wood and FYM-derived biochars. These are the amendments that increased significantly biomass production and WUE of lettuce. Due to limiting funding we could not analyze heavy metals in lettuce for all treatments; however, our observation is in agreement of our previous finding that biochar-based organic fertilizer, which improve biomass production and WUE of plants, also improve NUE of plants for heavy metal absorption [15,36]. For spinach, amendment of mixture of wood-derived biochar with manure reduced concentration of heavy metals. Our results are in agreement to previous findings that wood-derived biochar reduces concentration of heavy metals in plants grown in soil contaminated with high concentration of heavy metals [36]. Wood-derived biochar has low concentration of nutrients. When it is mixed with organic fertilizer that is nutrient-rich it absorbs nutrients from that fertilizer and supply nutrients to plants by acting as slow-release fertilizer [10] and therefore also reduces uptake of heavy metals in plants [47].

4.3 Soil Properties

In lettuce, concentration of total mineral N and soluble mineral P of soil were significantly and many-fold higher in response to organic amendments as compared to control, indicating that poultry manure, FYM biochar, wood-based biochar and their co-amendments with poultry manure increased concentration of these nutrients. In spinach, concentration of mineral N was higher in soil in response to the amendment of poultry manure and mixture of poultry manure with FYM biochar amended at higher rate (7%) as compared to when mixture of poultry manure and wood-derived biochar was amended at 7% application rate.

4.4 Conclusion

Our results indicate that high application rates (10% and 15% or 60 t ha^{-1} and 90 t ha^{-1}) of poultry manure or FYM biochar, if amended alone reduced significantly the growth of lettuce; however, when these nutrient-rich organic wastes were co-amended (1:1 w/w ratio on air-dry weight bases) with wood-derived biochar, significantly improved biomass production. At low application rates (3%, 5% and 7% or 18 t ha^{-1}, 30 t ha^{-1} and 42 t ha^{-1}), co-amendment of wood-derived biochar with poultry manure significantly reduced aboveground biomass of lettuce; whereas, co-amendment of FYM biochar with poultry manure at high application rates had no influence as compared to when only poultry manure was applied to soil. This indicates that at low application rates, mixing of poultry manure with wood-derived biochar has no positive influence on this crop, which may be due to at low application rates, these fertilizers do not increase soil nutrients. The mixing of manure-based fertilizers with wood-derived biochar reduced concentration of heavy metals per unit biomass (increased NUE) in both crops. Our findings indicate that higher application rates of poultry manure and FYM biochar need to be done with their mixture with wood-derived biochar. Poultry manure and FYM are many times less expensive than synthetic fertilizer while leftover of wood-derived biochar is available in timber markets of this province (~0.5 US$ per kg). Proper use of biochar-based these organic fertilizers can be cost-efficient for farmers and best waste-management practice.

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Supplementary Files

Table S1: Concentration of nitrogen, phosphorus and potassium in poultry and cattle manures

Nitrogen (g kg^{-1})		Phosphorus (g kg^{-1})		Potassium (g kg^{-1})		Country	Reference
Poultry	Cattle	Poultry	Cattle	Poultry	Cattle		
37.1	17.0	14.7	4.2	17.9	16.8	South Africa	[1]
14.5	5.46	8.1	2.25	3.6	6.13	Pakistan	[2]
24.3	—	20.1	—	27.6	—	Pakistan	[3]
51.9	48.2	41.2	47.1	36.5	40.7	Pakistan	[4]
16.1	19.6	3.37	9.68	11.21	22.54	South Africa	[5]

—indicates no data

Table S2: Dry biomass of aboveground plant parts and roots (g) of lettuce

Treatment	Dry biomass of shoot (g)	Dry biomass of Root (g)
cont	1.04	1.65
cont	1.65	2.78
cont	0.95	2.003
P10	0.39	0.443
P10	1.08	2.145
P10	0.94	0.679
P15	0.46	0.286
P15	0.69	1.006
P15	0.24	0.169
WB10	1.32	1.909
WB10	0.27	0.880
WB10	0.38	0.742
WB15	0.24	1.269
WB15	0.56	1.026
WB15	1.17	4.047
FYM10	0.73	1.022
FYM10	0.57	0.486
FYM10	0.58	1.160
FYM15	0.16	0.113
FYM15	0.40	0.403
FYM15	0.10	0.066
WB + P10	2.78	8.804

(Continued)

Table S2 (continued).

Treatment	Dry biomass of shoot (g)	Dry biomass of Root (g)
WB + P10	2.60	16.680
WB + P10	3.90	12.835
WB + P15	2.86	15.985
WB + P15	2.39	30.840
WB + P15	0.84	3.800
FYM + P10	1.38	2.950
FYM + P10	2.21	3.995
FYM + P10	2.61	9.988
FYM + P15	0.07	0.053
FYM + P15	0.78	2.41
P5r	0.453	0.614
P5r	0.032	0.010
P5r	0.058	0.089
P10r	0.034	0.014
P10r	0.054	0.023
P15r	0.010	0.003
P15r	0.078	0.028
WB + P5r	0.079	0.039
WB + P5r	0.071	0.027
WB + P5r	0.046	0.127
WB + P10r	0.258	0.387
WB + P10r	0.349	0.532
WB + P10r	0.254	0.346
WB + P15r	0.206	0.285
WB + P15r	0.053	0.108
WB + P15r	0.041	0.032
FYM + P5r	0.925	9.307
FYM + P5r	0.169	0.277
FYM + P5r	0.113	0.081
FYM + P10r	0.014	0.004
FYM + P10r	0.615	5.892
FYM + P10r	0.078	0.048

Table S3: Water use efficiency of lettuce

Treatment	WUE
cont	0.566
cont	0.934
cont	0.583
P10	0.140
P10	0.586
P10	0.525
P15	0.272
P15	0.398
P15	0.139
WB10	0.464
WB10	0.163
WB10	0.250
WB15	0.147
WB15	0.342
WB15	0.427
FYM10	0.411
FYM10	0.328
FYM10	0.351
FYM15	0.105
FYM15	0.252
FYM15	0.062
WB + P10	0.965
WB + P10	0.918
WB + P10	1.366
WB + P15	0.987
WB + P15	0.872
WB + P15	0.329
FYM + P10	0.507
FYM + P10	0.824
FYM + P10	0.909
FYM + P15	0.035
FYM + P16	0.392
P5r	0.274
P5r	0.019
P5r	0.034

(Continued)

Table S3 (continued).

Treatment	WUE
P10r	0.023
P10r	0.030
P15r	0.005
P15r	0.045
WB + P5r	0.046
WB + P5r	0.041
WB + P5r	0.027
WB + P10r	0.147
WB + P10r	0.214
WB + P10r	0.153
WB + P15r	0.128
WB + P15r	0.030
WB + P15r	0.024
FYM + P5r	0.556
FYM + P5r	0.094
FYM + P5r	0.064
FYM + P10r	0.008
FYM + P10r	0.365
FYM + P10r	0.048

Table S4: Dry biomass of aboveground plant parts and roots (g) of spinach

Treatment	Dry biomass of shoot (g)	Dry biomass of Root (g)
P3	2.23	2.55
P3	2.34	24.04
P3	1.47	8.22
P5	2.92	11.52
P5	1.97	15.45
P5	2.69	22.37
P7	2.12	21.10
P7	2.27	24.84
P7	1.76	27.88
FYM + P3	0.91	27.98
FYM + P3	0.96	25.06
FYM + P3	0.98	27.43

(Continued)

Table S4 (continued).

Treatment	Dry biomass of shoot (g)	Dry biomass of Root (g)
FYM + P5	1.53	21.57
FYM + P5	2.58	23.65
FYM + P5	1.72	27.66
FYM + P7	1.90	26.87
FYM + P7	1.88	14.09
WB + P3	1.10	9.118
WB + P3	1.18	18.48
WB + P3	1.71	17.70
WB + P5	1.57	18.96
WB + P5	1.32	11.28
WB + P5	1.27	8.924
WB + P7	1.62	12.49
WB + P7	1.81	10.83
WB + P7	1.75	16.35

Table S5: Water use efficiency of spinach

Treatment	WUE
P3	0.666
P3	0.718
P3	0.467
P5	0.933
P5	0.651
P5	0.831
P7	0.695
P7	0.762
P7	0.567
FYM + P3	0.292
FYM + P3	0.308
FYM + P3	0.293
FYM + P5	0.511
FYM + P5	0.844
FYM + P5	0.554
FYM + P7	0.616
FYM + P7	0.612

(Continued)

Table S5 (continued).

Treatment	WUE
WB + P3	0.357
WB + P3	0.348
WB + P3	0.481
WB + P5	0.480
WB + P5	0.429
WB + P5	0.423
WB + P7	0.523
WB + P7	0.602
WB + P7	0.556

Table S6: Concentration of ammonium (NH_4^+N g kg^{-1} soil), nitrate (NO_3^-N g kg^{-1} soil) and total mineral nitrogen (cumulative of ammonium and nitrate as g kg^{-1} soil)

Lettuce			
Treatment	NH_4^+N g kg^{-1} soil	NO_3^-N g kg^{-1} soil	Total mineral nitrogen g kg^{-1} soil
Cont	2.268	5.631	7.900
Cont	2.862	4.390	7.252
cont	2.635	3.224	5.860
P10	5.406	7.631	13.03
P10	5.094	8.002	13.09
P10	5.394	8.754	14.14
P15	5.398	8.256	13.65
P15	5.094	5.866	10.96
P15	5.108	7.903	13.01
WB10	3.618	5.513	9.131
WB10	3.295	6.554	9.849
WB10	3.302	6.364	9.667
WB15	3.387	5.275	8.663
WB15	4.067	4.443	8.511
WB15	2.925	5.702	8.628
FYM10	4.367	6.126	10.49
FYM10	4.131	7.631	11.76
FYM10	3.254	5.558	8.813
FYM15	3.853	5.702	9.556

(Continued)

Table S6 (continued).

Lettuce			
FYM15	3.466	4.053	7.520
FYM15	2.237	3.108	5.346
WB + P10	4.131	7.405	11.53
WB + P10	3.213	4.986	8.200
WB + P10	4.131	5.173	9.305
WB + P15	4.466	7.405	11.87
WB + P15	4.367	5.388	9.756
WB + P15	4.717	9.606	14.32
FYM + P10	4.722	8.754	13.47
FYM + P10	4.724	8.256	12.98
FYM + P10	3.439	5.312	8.751
FYM + P15	4.466	8.754	13.22
FYM + P15	5.394	9.606	15.00
FYM + P15	2.186	6.661	8.848
P5r	4.881	5.928	10.81
P5r	3.585	5.170	8.756
P5r	3.901	4.240	8.141
P10r	5.396	6.554	11.95
P10r	5.094	9.606	14.70
P10r	5.392	9.606	14.99
P15r	5.404	8.754	14.15
P15r	5.094	9.597	14.69
P15r	5.394	9.603	14.9
WB + P5r	4.881	8.754	13.63
WB + P5r	4.340	6.910	11.25
WB + P5r	5.145	9.606	14.75
WB + P10r	5.133	8.469	13.60
WB + P10r	4.580	7.215	11.79
WB + P10r	3.652	8.754	12.40
WB + P15r	5.099	8.754	13.85
WB + P15r	4.882	8.256	13.13
WB + P15r	5.094	8.256	13.35
FYM + P5r	4.067	4.596	8.663
FYM + P5r	3.439	4.904	8.344
FYM + P5r	1.826	8.256	10.08

(Continued)

Table S6 (continued).

Lettuce			
FYM + P10r	4.466	8.248	12.71
FYM + P10r	3.618	4.904	8.523
FYM + P10r	2.065	8.754	10.82
FYM + P15r	3.195	8.754	11.94
FYM + P15r	2.625	9.606	12.23
FYM + P15r	3.809	9.609	13.41

Table S7: concentration of soluble inorganic phosphorus (g kg^{-1} soil) of soil after harvest of lettuce plants

Treatment	Phosphorus g kg^{-1} soil
cont	−2.806
cont	−1.936
cont	−0.726
P10	18.021
P10	14.263
P10	22.670
P15	23.668
P15	24.602
P15	15.239
WB10	7.4267
WB10	7.5966
WB10	9.5074
WB15	6.4925
WB15	7.3842
WB15	−1.214
FYM10	34.199
FYM10	17.341
FYM10	10.186
FYM15	18.530
FYM15	−9.515
FYM15	12.39
WB + P10	19.231
WB + P10	18.743
WB + P10	26.959

(Continued)

Table S7 (continued).

Treatment	Phosphorus g kg^{-1} soil
WB + P15	11.460
WB + P15	40.335
WB + P15	16.131
FYM + P10	24.411
FYM + P10	24.114
FYM + P10	19.507
FYM + P15	17.405
FYM + P16	31.800
FYM + P17	26.747
P5r	21.290
P5r	16.067
P5r	17.087
P10r	27.787
P10r	41.715
P10r	26.471
P15r	17.065
P15r	25.558
P15r	21.184
WB + P5r	26.959
WB + P5r	21.078
WB + P5r	20.611
WB + P10r	15.579
WB + P10r	10.399
WB + P10r	24.199
WB + P15r	21.184
WB + P15r	24.581
WB + P15r	18.106
FYM + P5r	19.210
FYM + P5r	16.025
FYM + P5r	15.218
FYM + P10r	13.350
FYM + P10r	-1.0445
FYM + P10r	3.7961
FYM + P15r	1.0573
FYM + P15r	17.830
FYM + P15r	14.305

Table S8: Concentration of ammonium (NH_4^+N g kg^{-1} soil), nitrate (NO_3^-N g kg^{-1} soil) and total mineral nitrogen (cumulative of ammonium and nitrate as g kg^{-1} soil) in soil grown with spinach

Treatment	NH_4^+N g kg^{-1} soil	NO_3^-N g kg^{-1} soil	Total mineral nitrogen g kg^{-1} soil
P3	2.251	0.057	2.309
P3	2.586	2.700	5.287
P3	3.428	6.512	9.940
P5	2.338	4.625	6.964
P5	2.311	7.606	9.918
P5	3.933	4.924	8.857
P7	2.644	9.435	12.08
P7	2.798	7.279	10.07
P7	3.777	7.571	11.34
FYM + P3	0.224	2.999	3.223
FYM + P3	4.051	4.664	8.715
FYM + P3	2.340	5.026	7.367
FYM + P5	3.777	6.008	9.786
FYM + P5	3.929	6.666	10.59
FYM + p5	3.057	6.069	9.127
FYM + P7	3.374	4.224	7.599
FYM + P7	3.446	6.907	10.35
FYM + p7	6.004	11.89	17.90
WB + P3	3.801	4.385	8.186
WB + P3	2.358	4.301	6.660
WB + P3	3.777	6.037	9.815
WB + P5	4	3.246	7.568
WB + P5	6.362	5.148	11.51
WB + P5	3.875	5.437	9.313
WB + P7	5.140	4.988	10.12
WB + P7	4.156	7.975	12.13
WB + P7	4.195	4.770	8.966

Table S9: Concentration of soluble inorganic phosphorus (g kg^{-1} soil) of soil after harvest of lettuce plants

Treatment	Soluble inorganic P g kg^{-1} soil
P3	17.019
P3	13.247
P3	19.710
P5	16.306
P5	14.052
P5	15.984
P7	16.237
P7	14.857
P7	14.811
FYM + P3	16.260
FYM + P3	16.444
FYM + P3	12.925
FYM + P5	13.684
FYM + P5	15.547
FYM + P5	13.707
FYM + P7	14.006
FYM + P7	13.937
FYM + P7	15.294
WB + P3	13.109
WB + P3	14.604
WB + P3	14.558
WB + P5	17.617
WB + P5	15.409
WB + P5	19.273
WB + P7	14.259
WB + P7	11.844
WB + P7	11.177

Table S10: The pH and electrical conductivity of soils of various treatments

Plant Name	Treatments	pH	EC (μs cm^{-1})
Lettuce	Cont	9.1 ±0.26^{bcdef}	54.13 ± 2.343^{cd}
	P10	8.8 ± 0.20^{efg}	67.13 ± 26.07^{cd}
	P15	8.7 ± 0.20^{fg}	83.83 ± 51.78^{bcd}
	WB10	9.2 ± 0.26^{abcde}	62.96 ± 19.22^{cd}
	WB15	9.2 ± 0.25^{abcd}	54.63 ± 11.03^{cd}
	FYM10	9.4 ± 0.23^{ab}	73.20 ± 39.30^{cd}
	FYM15	9.4 ± 0.20^{ab}	133.4 ± 51.69^{ab}
	WB + P10	9.2 ± 0.15^{abcd}	70.66 ± 15.75^{cd}
	WB + P15	8.9 ± 0.35^{defg}	102.8 ± 45.79^{bcd}
	FYM + P10	9.3 ± 0.25^{abc}	81.40 ± 33.25^{bcd}
	FYM + P15	8.9 ± 0.10^{efg}	173.5 ± 18.37^{a*}
	P5r	9.1 ± 0.10^{bcdef}	58 ± 15.06^{cd}
	P10r	8.9 ± 0.05^{def}	69.33 ± 28.94^{cd}
	P15r	8.6 ± 0.17^{g}	49.86 ± 31.25^{d}
	WB + P5r	9.0 ± 0.17^{cdef}	62.33 ± 20.26^{cd}
	WB + P10r	9.1 ± 0.26^{bcdef}	60.69 ± 16.05^{cd}
	WB + P15r	9.0 ± 0.20^{cdef}	67.93 ± 34.84^{cd}
	FYM + P5r	9.4 ± 0.15^{ab}	51.40 ± 12.91^{d}
	FYM + P10r	9.5 ± 0.40^{a*}	105.3 ± 53.84^{bc}
	FYM + P15r	9.3 ± 0.05^{ab}	162.2 ± 45.01^{a*}
Spinach	P3	9.1 ± 0.26^{b}	36.21 ± 17.82^{b}
	P5	9.1 ± 0.11^{b}	33.76 ± 12.20^{b}
	P7	9.1 ± 0.05^{b}	32.84 ± 21.07^{b}
	FYM + P3	9.3 ± 0.10^{ab}	36.13 ±13.01^{b}
	FYM + P5	9.3 ± 0.05^{ab}	45.40 ± 3.34^{ab}
	FYM + P7	9.4 ± 0.11^{a*}	67.53 ± 37.12^{a*}
	WB + P3	9.2 ± 0.10^{ab}	44.46 ± 7.86^{ab}
	WB + P5	9.3 ± 0.17^{ab}	36.23 ± 10.68^{b}
	WB + P7	9.1 ± 0.11^{b}	42.46 ± 13.26^{ab}

Within column, values followed by different letters are significantly different at $p < 0.05$.

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