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PHYTOREMEDIATION POTENTIALITY OF BOTTLE GOURD PLANT (*Lagenaria siceraria*)

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Abstract

A pot experiment of bottle gourd plant with Pb, Zn, NPK and Vermicompost (VC) was carried out at the Department of Soil, Water and Environment, University of Dhaka. The aim of the study was to introduce a phytoremedial technique. The bottle gourd (*Lagenaria siceraria)* plant was used in the trial for evaluating its potentiality to accumulate Pb and Zn from soil and translocate these elements to different parts of the plant. Twelve treatments (T) were utilized in the experiment including control (T_1). The remaining treatment groups were composed of different ratios of VC and recommended dose of NPK- with and without lead and Zinc. Tolerance to metal stress by the bottle gourd plant was evident in terms of higher biomass and yield production with the Pb and Zn. The maximum total biomass (leaf + stem + root) of fresh (67.49 g) and dry yield (8.41g) was achieved with Pb addition to $T_{11.}$ Bioconcentration factor (BCF) and Translocation Factor (TF) greater than one (BCF>1 and TF>1) were considered indicators of heavy metal accumulators and translocators, respectively. Among the Pb treatments, highest BCF achieved in leaf (1.67) with T_{12}, in stem (1.82) with T_8, in root (4.67) with T_{11} and maximum TF (2.87) found with $T_{11.}$ The Zn treatments exerted the highest BCF value in leaf (1.80), and in stem (1.64) with T_{10}, in root (2.70) with T_{12}, lowest value of BCF and TF were displayed in control (T_1). Research exposed that bottle gourd plants had transferred both the heavy metals from soil significantly. The highest Pb accumulation in leaf was found to be 0.0023% (dry weight) in T_{10} and the lowest (0%) in T_1. The highest translocation factor was found in T5 for Pb and in T_{11} for Zn. Moreover, bioconcentration factor (Pb) in shoots exhibited a significant, strong, and negative correlation (r = -0.804, p = 0.002) with the soil organic carbon content. There was also a strong, significantly negative correlation between total Pb content in soil and BCF (leaf) (r = -0.717, p = 0.009), as well as BCF (stem) (r = -0.692, p = 0.013). Similar relationship was found in case of Zn. The study revealed that bottle gourd plants possess phytoremedial potentiality which will be beneficial for the pollution context of Bangladesh. Further investigation is needed for greater research interest.

Key words: Phytoremediation; Bottle gourd; Accumulation; Translocation; Pb; Zn.

INTRODUCTION

The unwanted abundance of heavy metals, such as lead, cadmium, chromium, nickel, zinc *etc*. has become a global concern with no long-term solution in sight. These substances are naturally occurring, however their presence at an excessive concentration in agricultural fields, and groundwater is thought to be mainly due to anthropogenic activities, such as mining, vehicle emissions, burning fossil fuel, industrial wastes, irrigation, application of fertilizers, and pesticides, *etc*. (Mensah *et al*. 2009, Huang *et al*. 2019). The upper limit value as a factor of their texture in unpolluted soils for Pb was set at 50 µg g^{-1} (sandy and loam soil) and for Zn at 100 µg g^{-1} (sandy soil) and 150 µg g^{-1} (loam soil) by De Temmerman *et al*. (1984). Zinc concentration was reported to be as high as 1955.5 µg g^{-1} in the soil of a region near Hame Kasi mine (Nouri *et al*. 2009). Metal pollution from anthropogenic source is evident, because soil in the surrounding area of Mahad AD'Dahab Mine in Saudi Arabia was found to contain

355 mg kg^{-1} of Pb and 1481 mg kg^{-1} of Zn (Al-Farraj *et al*. 2009). These trends also exist in the soil of Bangladesh. Mizan *et al*. (2023) reported that heavy metals, such as Cr. Pb, Cu, Zn, Ni and Cd were found increasingly in the soil adjacent to the industrial area, road site soil, tannery, textile industries including crop lands in Bangladesh.

Some of these metals are essential in trace amounts, yet toxic to living beings in slightly higher amounts. For example, zinc is essential for immunomodulatory functions as it is one of the cofactors of major proteins, however Zn toxicity can cause respiratory and cardiac issues in animals and imbalanced mineral nutrition, reduced photosynthesis rate in plants (Kaur and Garg 2021, Hussain *et al*. 2022). The recommended daily allowance (RDA) for Zn given by Institute of Medicine (US) Panel on micronutrients (2001) is 11 mgd^{-1} (adult male) and 8 mgd^{-1} (adult female) (Trumbo *et al*. 2001). On the other hand, lead having no benefit for either plants or animals, is detrimental even at trace level. The localized concentration of Pb in roots causes morphological disturbances in plants (Sharma and Dubey 2005) and is a known carcinogenic to humans with potential to other diseases like cardiovascular diseases, kidney damage, and high blood pressure (Collin *et al*. 2022).

Plants growing in the soil contaminated with heavy metals are the main source of human and animal exposure to these toxic elements because plants uptake and accumulate the metals into their tissue, which is the pathway of the metals to get into the food chain. The nature and extent of accumulation vary with species to species and do not necessarily correlate with the total metal concentration in the growing site (Dietz *et al*. 1999), which can be an opportunity or a threat to the environment because while low uptake rate of heavy metals ensures safe consumption of a crop, high accumulation means its potential to be used in clean-up technology (Gerhardt *et al.* 2017). When the conventional remediation technology fails to promise decontamination without altering the natural healthy state of soils at an economically feasible cost, phytoremediation shows real possibility (Baker *et al.* 1994). However, in order to implement successful phytoextraction scheme, suitable plant species needs to be discovered for each climatic region. For the successful extraction of Pb and Zn from contaminated mine area *T. latifolia* and *P. australis* are worth mentioning (Ye *et al*. 1997).

Bottle gourd (*Lagenaria siceraria*; Family-Cucurbitaceae) plant parts, the leaf and stem as a whole also known as ‘Lau shaak’, are one of the most widely consumed leafy vegetables in Bangladesh, and this species is well-adapted to the local climatic condition. Bottle gourd is well-known for its nutritional value and medicinal purposes, and thus has become an important part of the diet in many countries of the world. The edible portion is a rich source of carbohydrate, protein, fat, and minerals including phosphorus and calcium (Sithole *et al.* 2015). As evidence suggests that many species of Cucurbitaceae family including bottle gourd can accumulate heavy metals (Seshabala *et al*. 2007). As lead is a highly immobile element, unlike zinc, it tends to be accumulated mainly in plant roots rather than getting transported to the aboveground shoots. However, all metals get bound to the cell walls to some extent while moving from roots to plant shoots, which is a major limitation in successful phytoextraction (Dietz *et al.* 1999). Alam *et al.* (2003) showed evidence that the leaves of bottle gourd plant accumulates more lead (0.987 ppm) than many vegetables grown in that area. Islam *et al.* (2000) found average Pb and Zn contents in some parts of Bangladesh to be 28 mg kg^{-1} and 64.3 mg kg^{-1}, respectively. However, for being included as a suitable plant for phytoextraction, it has to withstand metal stress by not showing any significant decrease in morphological characters, plant growth, yield and nutrient status (Laghlimi *et al.* 2015), which were recorded under twelve different treatments including control in this study. Besides, large biomass production under spiked metal containing condition is an indicator of plants being suitable extractor even if they are not hyperaccumulators (Ebbs *et al*. 1997). For instance, Indian mustard (*Brassica juncea)* is an effective species for remediation of lead despite having about 5% of total uptake accumulated in roots (Begonia *et al.* 1998). As the long and branched vine of bottle gourd plant allows it to produce large biomass, this plant is surely worth studying. Moreover, ideal phytoextractors will have a high translocation factor meaning there will be more metal accumulated in the aboveground biomass than in the roots (Sekara *et al.* 2005, Sarwar *et al.* 2017). The results obtained in this study will allow determining its potential to be used as a phytoextractor.

Therefore, a study is urgent to overcome the toxicity of heavy metal contaminated soil of Bangladesh. This is to our knowledge that it is the first study done in Bangladesh regarding the accumulation ability of bottle gourd plant and its potentiality for phytoextraction. The aim of this study was to assess the Pb and Zn metal accumulation ability of bottle gourd plant species.

MATERIAL AND METHODS

Soil sample collection, preparation, and analysis

The bulk soil sample (0-15 cm depth) was collected from an agricultural farm in Gonokbari, Savar upazila, Bangladesh. Collected soil was air-dried for three days, and visible roots or debris were removed by hand. For the preparation of soil sample for the analysis of physical and chemical properties, a portion was separated and crushed gently with a wooden hammer to break down any large clods before passing through a 2 mm sieve, then the sieved soil was stored in a labeled plastic bottle. The rest of the collected soil was preserved for pot experiment.

All the analyses were carried out at the Department of Soil, Water and Environment, University of Dhaka. The texture was silt loam, which was deduced from the USDA soil texture triangle using the proportion percentages (Bouyoucos 1962). The pH and Electrical Conductivity (EC) of the soil samples were analyzed by taking soil and distilled water in ratios 1:2.5 and 1:5, in order (Jackson 1958) using HANNA Instrument H12211ph (Orp Meter) and EC Eutech instruments CON 700, respectively. The soil was found to have a pH of 5.51 and EC of 54.9 μS/cm. Organic carbon content was determined following the Walkley and Black's (1934) wet oxidation method, and was multiplied by Van Bemmelen factor of 1.72 (Huq and Alam 2005) for calculating organic matter content. The organic carbon and organic matter of the soil were 0.312 and 0.537%, respectively, which is considered very low in organic matter classes.

For the analyses of total P, S, Pb and Zn in soil, the 0.5 g sample was digested using HNO_3-H_2O_2-$HClO_4$ (2:1:1) following the method described by Chowdhury *et al.* (2017). Total P and S in the digest were measured with the aid of a T-60 OV-visible spectrophotometer following Vanadomolybdophosphoric yellow color method in nitric acid system (Jackson 1958) and turbidimetric method (using $BaCl_2$ and Tween-80 as suspending agents) (Huq and Alam 2005), respectively. Total Pb and total Zn were determined with an atomic absorption spectrophotometer (VARIAN AA240). The determination of total soil N was done by digesting soil sample separately using concentrated H_2SO_4 and digestion mixture and later distilling the digest following the Kjeldahl method (Jackson 1958). For the determination of available nitrogen (NH_4 + NO_3 and NO_3 separately), a procedure given by Jackson (1958) was followed. The soils were leached with 1N KCl solution (10:100 w/v, soil to extractant) to replace ammonium (NH_4^+) and nitrate (NO_3^-) ions from the colloidal surfaces. The soil leachates were then distilled with and without Devarda's alloy in 10% NaOH solution for both NH_4^+ and NO_3^- ions and for only NH_4^+ ions, respectively. Available P was measured by modified Olsen method using $NaHCO_3$ extractant (pH adjusted to 8.5 by NaOH) at an extraction ratio of 1: 20 (Olsen 1954). For available sulfur, the soil samples were extracted with the Morgan's solution following the procedure provided by Lunt *et al.* (1951). The results of the analysis of initial soil are presented in Table 1.

Table 1. Physical and chemical properties of the soil used.

Soil properties	Values	Soil properties	Values (%)
pH	5.51	Total Sulfur	0.045
EC (µS/cm)	54.9	Available Nitrogen	0.0031
Textural class	Silt loam	Available Phosphorus	0.0003
% Organic Carbon	0.312	Available Phosphorus	0.0003
% Organic Matter	0.537	Available Sulfur	0.013
% Total Nitrogen	0.164	Total Lead	0.0006
% Total Phosphorus	0.117	Total Zinc	0.0053

Experimental design

A pot experiment consisting of 12 treatments (T) was carried out at the Department of Soil, Water and Environment, University of Dhaka. Seven kilograms of air-dried soil were taken in each plastic pot of height 23 cm and diameter 25.6 cm. All the pots were crannied at the bottom and filled with the soil. No fertilizer was used in the control (T_1) and the remaining treatments were composed of different proportions of N, P, K, Pb, Zn and VC as per design. The treatments were as follows: T_1: Control, T_2: VC 6 t ha^{-1}, T_3: $N_{30} P_{15} K_{23}$ kg ha^{-1}, T_4: $N_{30} P_{15} K_{23}$ kg ha^{-1} + VC 2 t ha^{-1}, T_5: $N_{22} P_{11} K_{17}$ kg ha^{-1} + VC 4 t ha^{-1}, T_6: $N_{15} P_7 K_{11}$ kg ha^{-1} + VC 6 t ha^{-1}, T_7: T_4 + Zn 2kg ha^{-1}, T_8: T_5 + Zn 2 kg ha^{-1}, T_9: T_6 + Zn 2 kg ha^{-1}, T_{10}: T_4 + Pb 2 kg ha^{-1}, T_{11}: T_5 + Pb 2 kg ha^{-1}, and T_{12}: T_6 + Pb 2 kg ha^{-1}.

Fig. 1. General view of the pot experiment with bottle gourd (*Lagenaria siceraria*).

The commercially available vermicompost was collected from local market and used in the experiment. The nutrients N, P, K, Pb and Zn were incorporated to the pots from the sources of urea, TSP, MoP, PbO_2 and ZnO, respectively. Each treatment had three replications, and the pots were laid out in a Completely Randomized Design (CRD).

The seeds of bottle gourd were collected from a seed shop at Siddique Bazaar, Dhaka. Three seeds were sown in each pot during Rabi season (December 2021). After germination, two healthy seedlings were allowed to grow in each pot. Light irrigation was practiced every other day, and weeding was done when necessary. For the assessment of plant growth morphological characteristics, such as plant height, leaf area, and stem girth were measured at continuous interval of 7 days after germination up to 42 days prior to harvest. Leaf area was calculated by multiplying leaf length × width with the Montgomery factor of 0.83 (model-7) (Yu *et al.* 2020).

Harvesting and sample preparation

After 42 days of germination, the plants were harvested carefully by uprooting, then were washed with tap water and finally with distilled water. Later these were dried by using a soft tissue paper. Immediately after harvest, fresh weights of individual plant parts (leaf, stem, and root) were taken. The plants were air-dried for two days before finally oven-drying at 65°C for 48 hours. The dried plant samples were weighed again, and ground finely using a mortar and stored in labeled plastic bottles for chemical analysis. Post-harvest soil was collected from each pot and prepared as was the initial soil.

Chemical analysis of plant and post-harvest soil sample

All the post-harvest soil samples were digested and analyzed following the same procedure mentioned for the analysis of initial soil. For the digestion of plant parts 0.2 g samples were taken and digested as was the soil samples. Plant samples were analyzed for total N, P, S, Pb and Zn using the same procedure followed for analyzing soil samples.

Statistical analysis

Analysis of Variance (ANOVA), Fisher's Least Significant Difference (LSD) test and Pearson's correlation analysis were carried out with the results obtained. Other statistical analyses were done using Minitab v21 and MS Excel 2016.

RESULTS AND DISCUSSION

Biomass production

In order to evaluate the capacity of bottle gourd plants to withstand metal stress by producing substantial amount of biomass, growth and yield under various treatments were measured.

Plant growth assessment

Plant growth was assessed in terms of plant height (Table 2), leaf area (Table 3), and stem girth (Table 4) recorded weekly up to 42 days after seed germination. The height of bottle gourd plants on the first two weeks and the last week varied significantly ($p \leq 0.05$) among the twelve treatments. The control group had the shorter height throughout the whole observation period and all the lead and zinc included treatments varied significantly ($p \leq 0.05$) from the lead and zinc excluded control group in terms of plant height. According to the Fisher's pairwise comparison, T_{12}: ($N_{15} P_7 K_{11}$ kg ha^{-1} + VC 6 ton ha^{-1} + Pb 2 kg ha^{-1}) did not vary significantly from other treatment groups with various doses of vermicompost and NPK fertilizers that were not treated with lead addition (Table 2).

Table 2. Effects of vermicompost, NPK, Pb, and Zn on the height (cm) of bottle gourd plants at 7 day intervals from seed germination to harvest.

Treatments	Plant height (cm) at different days after germination					
	7^d	14^d	21^d	28^d	35^d	42^d
T_1: Control (-VC, -NPK, -Pb, -Zn)	12.13^d	26.00^{cd}	49.43	80.70	94.90	94.30^e
T_2: VC 6 ton ha^{-1}	14.20^{cd}	28.23^{abc}	63.17	108.63	134.23	167.00^a
T_3: $N_{30} P_{15} K_{23}$ kg ha^{-1}	15.87^{bc}	28.17^{abc}	56.17	127.23	140.97	150.77^{ab}
T_4: $N_{30} P_{15} K_{23}$ kg ha^{-1} + VC 2 ton ha^{-1}	15.13^{bc}	30.40^{ab}	69.30	123.07	127.03	136.13^{abc}
T_5: $N_{22} P_{11} K_{17}$ kg ha^{-1} + VC 4 ton ha^{-1}	15.73^{bc}	29. 97^{ab}	53.67	114.73	130.17	135.20^{abc}
T_6: $N_{15} P_7 K_{11}$ kg ha^{-1} + VC 6 ton ha^{-1}	16.43^{ab}	29.13^{ab}	55.97	107.73	119.43	122.20^{bcde}
T_7: T_4 + Zn 2 kg ha^{-1}	14.00^{cd}	23.70^d	36.70	84.13	96.47	106.37^{cde}
T_8: T_5 + Zn 2 kg ha^{-1}	15.07^{bc}	27.63^{abc}	51.73	105.50	122.30	128.97^{bcd}
T_9: T_6 + Zn 2 kg ha^{-1}	12.73^d	27.43^{bc}	45.67	99.77	117.07	100.47^{de}
T_{10}: T_4 + Pb 2 kg ha^{-1}	15.47^{bc}	25.73^{cd}	50.17	119.17	121.13	128.37^{bcd}
T_{11}: T_5 + Pb 2 kg ha^{-1}	15.33^{bc}	27.77^{abc}	60.83	119.03	140.47	149.03^{ab}
T_{12}: T_6 + Pb 2 kg ha^{-1}	18.30^a	30.47^a	71.87	128.63	144.83	154.47^{ab}
LSD at 5%	**1.02**	**1.42**	**NS**	**NS**	**NS**	**16.08**

^{abcde} Data bearing different superscripts within the same column differ significantly ($p<0.05$).

The largest leaf area was recorded in T_{11}: ($N_{22} P_{11} K_{17}$ kg ha^{-1} + VC 4 ton ha^{-1} + Pb 2 Kg ha^{-1}). All the plants receiving lead treatments showed decrease in leaf area after four weeks of seed germination, which was also true for the control group unlike plants from T_2: (VC 6 ton ha^{-1}) and T_7: ($N_{30} P_{15} K_{23}$ kg ha^{-1} + Zn 2 Kg ha^{-1}) groups. However, all the treatment groups varied significantly ($p \leq 0.05$) throughout the six weeks of observation (Table 3).

Table 3. Effects of vermicompost, NPK, Pb, and Zn on the leaf area (cm^2) of bottle gourd plants at 7 day intervals from seed germination to harvest.

Treatments	Leaf aea (cm^2)					
	7d	14d	21d	28d	35d	42d
T_1: Control (-VC, -NPK, -Pb, -Zn)	15.48^{bcd}	63.19^d	103.38^{cde}	113.57^{cdef}	97.80^{bcd}	98.71^{bcd}
T_2: VC 6 ton ha^{-1}	17.86^{abc}	83.33^{ab}	106.20^{cde}	87.64^f	109.06^{bc}	114.05^{abcd}
T_3: $N_{30} P_{15} K_{23}$ kg ha^{-1}	20.22^{ab}	77.82^{abcd}	123.37^{ab}	151.64^a	149.11^a	135.66^a
T_4: $N_{30} P_{15} K_{23}$ kg ha^{-1} + VC 2 ton ha^{-1}	23.77^a	86.42^a	114.56^{abcde}	125.10^{abcde}	86.94^{cd}	115.27^{abcd}
T_5: $N_{22} P_{11} K_{17}$ kg ha^{-1} + VC 4 ton ha^{-1}	17.00^{abcd}	83.92^{ab}	118.45^{abc}	120.26^{bcde}	86.54^{cd}	93.25^{cd}
T_6: $N_{15} P_7 K_{11}$ kg ha^{-1} + VC 6 ton ha^{-1}	21.55^{ab}	67.63^{cd}	111.55^{abcde}	129.52^{abcd}	73.82^d	107.23^{abcd}
T_7: T_4 + Zn 2 kg ha^{-1}	11.85^{cd}	69.18^{bcd}	115.77^{abcd}	120.85^{bcde}	122.43^{ab}	128.51^{ab}
T_8: T_5 + Zn 2 kg ha^{-1}	21.82^{ab}	77.38^{abcd}	98.89^e	106.94^{def}	110.50^{bc}	107.07^{abcd}
T_9: T_6 + Zn 2 kg ha^{-1}	10.94^d	75.35^{abcd}	98.85^e	137.55^{abc}	95.16^{bcd}	86.55^d
T_{10}: T_4 + Pb 2 kg ha^{-1}	18.21^{abc}	69.45^{bcd}	101.58^{de}	148.19^{ab}	124.00^{ab}	122.16^{abc}
T_{11}: T_5 + Pb 2 kg ha^{-1}	18.34^{abc}	79.37^{abc}	127.04^a	142.32^{ab}	124.97^{ab}	140.58^a
T_{12}: T_6 + Pb 2 kg ha^{-1}	20.66^{ab}	90.28^a	109.66^{bcde}	99.23^{ef}	98.21^{bcd}	89.30^{cd}
LSD at 5%	**3.26**	**7.43**	**7.50**	**13.33**	**16.41**	**15.91**

^{abcdef} Data bearing different superscripts within the same column differ significantly ($p<0.05$).

The stem girth of bottle gourd plants varied significantly ($p \leq 0.05$) only in the first three weeks after germination. The largest stem girth was recorded in plants from the zinc treated pot (T_7: $N_{30} P_{15} K_{23}$ kg ha^{-1} + VC 2 ton ha^{-1} + Zn 2 kg ha^{-1}) until the 14th day, on the third week into observation plants belonging to T_{11}: ($N_{22} P_{11} K_{17}$ kg ha^{-1} + VC 4 ton ha^{-1}+ Pb 2 kg ha^{-1}) showed an average stem girth of 2.97 cm on the 21st day, which was higher than any other groups, and significantly ($p \leq 0.05$) different from the control group. It was evaluated that the maximum stem girth (3.13 cm) was obtained with this treatment (T_{11}) on the 42nd day after sowing (Table 4).

Table 4. Effects of vermicompost, NPK, Pb, and Zn on the stem girth (cm) of bottle gourd plants at 7 day intervals.

Treatments	Stem girth (cm)					
	7d	**14d**	**21d**	**28d**	**35d**	**42d**
T_1: Control (-VC, -NPK, -Pb, -Zn)	1.63^{e}	2.03^{cde}	2.23^{c}	2.53	2.30	2.80
T_2: VC 6 ton ha^{-1}	1.67^{de}	1.97^{def}	2.53^{bc}	2.47	2.73	2.87
T_3: $N_{30} P_{15} K_{23}$ kg ha^{-1}	2.13^{a}	2.07^{bcde}	2.33^{bc}	2.67	2.43	3.33
T_4: $N_{30} P_{15} K_{23}$ kg ha^{-1} + VC 2 ton ha^{-1}	1.63^{e}	2.10^{bcd}	2.50^{bc}	2.50	2.60	3.07
T_5: $N_{22} P_{11} K_{17}$ kg ha^{-1} + VC 4 ton ha^{-1}	1.70^{cde}	2.07^{bcde}	2.50^{bc}	2.53	2.57	2.93
T_6: $N_{15} P_7 K_{11}$ kg ha^{-1} + VC 6 ton ha^{-1}	2.00^{ab}	2.20^{ab}	2.37^{bc}	2.73	2.63	3.20
T_7: T_4 + Zn 2 kg ha^{-1}	2.17^{a}	2.33^{a}	2.63^{ab}	2.73	2.80	3.10
T_8: T_5 + Zn 2 kg ha^{-1}	1.83^{bcd}	2.00^{cdef}	2.50^{bc}	2.63	2.57	2.93
T_9: T_6 + Zn 2 kg ha^{-1}	1.87^{bc}	2.03^{cde}	2.43^{bc}	2.43	2.43	2.87
T_{10}: T_4 + Pb 2 kg ha^{-1}	1.77^{cde}	2.13^{bc}	2.67^{ab}	2.53	2.70	3.00
T_{11}: T_5 + Pb 2 kg ha^{-1}	1.77^{cde}	1.87^{f}	2.97^{a}	2.77	2.57	3.13
T_{12}: T_6 + Pb 2 kg ha^{-1}	1.77^{cde}	1.93^{ef}	2.23^{c}	2.93	2.50	3.00
LSD at 5%	**0.08**	**0.07**	**0.17**	**NS**	**NS**	**NS**

^{abcdef} Data bearing different superscripts within the same column differ significantly ($p<0.05$).

Yield assessment

Yield was determined in terms of fresh and dry weights of leaves, stems and roots separately, and of total plant (Table 5).

Table 5. Fresh and dry matter production of bottle gourd for different doses of vermicompost, NPK, Pb, and Zn.

Treatments	Fresh weight (g) per plant				Dry weight (g) per plant			
	Leaf	**Stem**	**Root**	**Total**	**Leaf**	**Stem**	**Root**	**Total**
T_1: Control (-VC, -NPK, -Pb, -Zn)	13.76^{c}	20.03^{c}	1.73^{cdef}	35.52	2.70^{abc}	2.20^{cd}	0.37^{b}	5.27
T_2: VC 6 ton ha^{-1}	23.18^{a}	23.32^{bc}	5.24^{a}	51.74	3.09^{ab}	2.83^{bcd}	0.76^{a}	6.68
T_3: $N_{30} P_{15} K_{23}$ kg ha^{-1}	13.31^{c}	29.63^{bc}	1.53^{def}	44.47	1.81^{bc}	2.77^{cd}	0.20^{c}	4.78
T_4: $N_{30} P_{15} K_{23}$ kg ha^{-1} + VC 2 ton ha^{-1}	15.34^{bc}	26.63^{bc}	2.48^{bcd}	44.45	2.66^{abc}	3.15^{abc}	0.36^{b}	6.17
T_5: $N_{22} P_{11} K_{17}$ kg ha^{-1} + VC 4 ton ha^{-1}	15.84^{bc}	27.91^{bc}	3.19^{b}	46.94	2.66^{abc}	2.81^{bcd}	0.43^{b}	5.90
T_6: $N_{15} P_7 K_{11}$ kg ha^{-1} + VC 6 ton ha^{-1}	5.84^{d}	20.65^{c}	1.24^{f}	27.73	1.64^{c}	2.13^{cd}	0.18^{c}	3.95
T_7: T_4 + Zn 2 kg ha^{-1}	13.49^{c}	21.67^{c}	2.03^{cdef}	37.20	2.21^{bc}	2.58^{cd}	0.40^{b}	5.19
T_8: T_5 + Zn 2 kg ha^{-1}	15.89^{bc}	26.81^{bc}	2.80^{bc}	45.50	3.02^{ab}	2.97^{abcd}	0.42^{b}	6.41
T_9: T_6 + Zn 2 kg ha^{-1}	12.19^{cd}	17.27^{c}	1.34^{ef}	30.80	1.60^{c}	1.72^{d}	0.17^{c}	3.49
T_{10}: T_4 + Pb 2 kg ha^{-1}	24.71^{a}	34.59^{ab}	2.74^{bc}	62.05	3.82^{a}	4.08^{ab}	0.36^{b}	8.26
T_{11}: T_5 + Pb 2 kg ha^{-1}	21.28^{ab}	42.96^{a}	3.25^{b}	67.49	3.81^{a}	4.13^{a}	0.47^{b}	8.41
T_{12}: T_6 + Pb 2 kg ha^{-1}	16.30^{bc}	29.50^{bc}	2.47^{bcde}	48.27	2.72^{abc}	2.78^{cd}	0.37^{b}	5.87
LSD at 5%	**3.11**	**5.90**	**0.54**		**0.65**	**0.61**	**0.06**	

^{abcdef} Data bearing different superscripts within the same column differ significantly ($p<0.05$).

The fresh and dry weights of bottle gourd plants were found significant difference in treatments ($p \leq 0.05$). The Pb (2 Kg ha^{-1}) receiving treatments yielded higher rate of fresh leaf, stem and roots. The highest total biomass (leaf + stem + root) was yielded with T_{11}: ($N_{22}\,P_{11}\,K_{17}$ kg ha^{-1} + Pb 2 Kg ha^{-1}). Results revealed that dry weight of leaf and stem with T_{10}: (T_4 + Pb 2 Kg ha^{-1}) and T_2: (sole application of vermicompost: 6 ton ha^{-1}) were statistically similar. The Zn included treatments (T_7, T_8 and T_9) produced relatively lower rate of fresh and dry weight compared to Pd and sole vermicompost application (T_2). Dry weights (root) of plants that received Pb (T_{10}, T_{11}, T_{12}) were higher than those receiving 100% of recommended dose of NPK fertilizer (T_3, T_4). Study also indicated that addition of Zn was poor performer in terms of total biomass production in comparison with Pb and VC included treatments (Table 5).

Pb and Zn accumulation

According to the standards of hyper accumulators for plant leaves set by Baker *et al.* (2000) bottle gourd leaves were compared in terms of Pb and Zn concentrations (% dry weight). The concentrations in other plant parts (stem and root) were also measured for the calculation of Bioconcentration Factor (BCF) in each morphological plant parts in order to assess the accumulation ability. Translocation Factor was also calculated to determine the translocation of Pb and Zn from plant roots to shoots. When BCF>1, that plant tissue is considered to be accumulators and TF>1 is considered to be significant capacity for translocation from plant roots to shoots (Khodijah *et al.* 2019). All these data regarding Pb and Zn accumulation are arranged in Table 6 and Table 7, respectively and the correlations found were displayed in Fig. 2 and 3. BCF and TF were calculated as follows (Ng *et al.* 2016).

$$Bioconcentration\ Factor\ (BCF) = \frac{Heavy\ metal\ content\ in\ dry\ weight\ of\ plant\ parts\ (\%)}{Heavy\ metal\ content\ in\ soil\ (\%)}$$

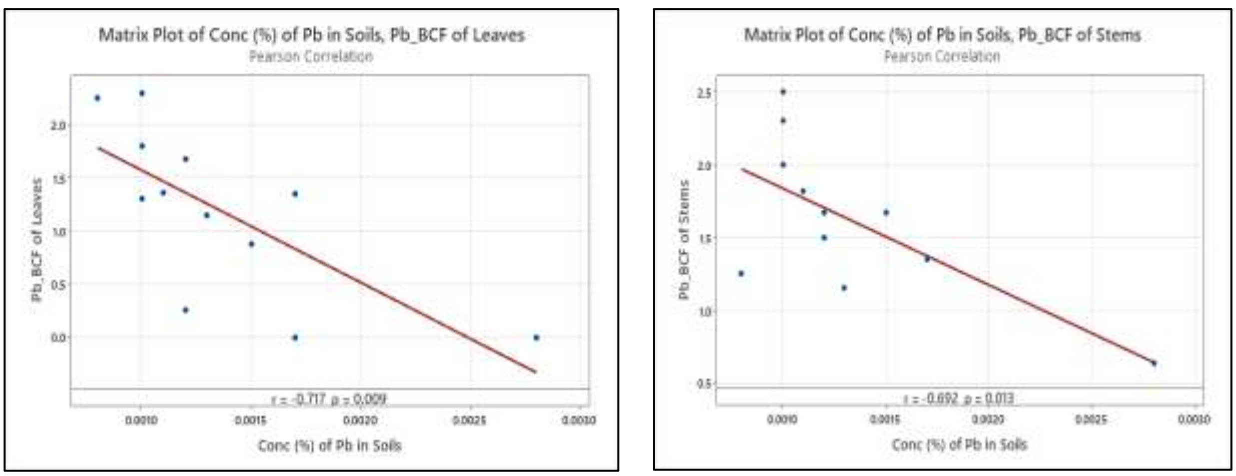
$$Translocation\ Factor\ (TF) = \frac{Heavy\ metal\ content\ in\ dry\ weight\ of\ plant\ shoot\ (\%)}{Heavy\ metal\ content\ in\ dry\ weight\ of\ plant\ root\ (\%)}$$

Table 6. Concentration (%) of Pb in different parts of bottle gourd plants and in corresponding soils.

Treatments	Soil	Leaf	Stem	Root	BCF (leaf)	BCF (stem)	BCF (soot)	TF
T_1: Control (-VC, -NPK, -Pb, -Zn)	0.0028^{a}	0^{c}	0.0018	0.0027^{cde}	0	0.64	0.96	0.67^{c}
T_2: VC 6 ton ha^{-1}	0.0017^{b}	0^{c}	0.0023	0.0027^{cde}	0	1.35	1.59	0.85^{bc}
T_3: $N_{30}\,P_{15}\,K_{23}$ kg ha^{-1}	0.0008^{c}	0.0018^{a}	0.001	0.0035^{cd}	2.25	1.25	4.38	0.8^{c}
T_4: $N_{30}\,P_{15}\,K_{23}$ kg ha^{-1} + VC 2 ton ha^{-1}	0.0012^{bc}	0.0003^{bc}	0.002	0.0023^{def}	0.25	1.67	1.92	1.0^{bc}
T_5: $N_{22}\,P_{11}\,K_{17}$ kg ha^{-1} + VC 4 ton ha^{-1}	0.001^{bc}	0.0023^{a}	0.002	0.0015^{ef}	2.3	2.0	1.5	0.54^{c}
T_6: $N_{15}\,P_7\,K_{11}$ kg ha^{-1} + VC 6 ton ha^{-1}	0.0013^{bc}	0.0015^{ab}	0.0015	0.0013^{f}	1.15	1.15	1.0	2.3^{b}
T_7: T_4 + Zn 2 kg ha^{-1}	0.001^{bc}	0.0013^{abc}	0.0023	0.0025^{def}	1.3	2.3	2.5	1.44^{bc}
T_8: T_5 + Zn 2 kg ha^{-1}	0.0011^{bc}	0.0015^{ab}	0.002	0.002^{ef}	1.36	1.82	1.82	1.75^{bc}
T_9: T_6 + Zn 2 kg ha^{-1}	0.001^{bc}	0.0018^{a}	0.0024	0.004^{bc}	1.8	2.5	4.0	1.08^{bc}
T_{10}: T_4 + Pb 2 kg ha^{-1}	0.0017^{b}	0.0023^{a}	0.0023	0.004^{bc}	1.35	1.35	2.35	1.15^{bc}
T_{11}: T_5 + Pb 2 kg ha^{-1}	0.0015^{bc}	0.0013^{abc}	0.0025	0.007^{a}	0.87	1.67	4.67	2.87^{a}
T_{12}: T_6 + Pb 2 kg ha^{-1}	0.0012^{bc}	0.002^{a}	0.0018	0.005^{b}	1.67	1.5	4.17	0.76^{bc}
LSD at 5%	**0.0004**	**0.0007**	**NS**	**0.0007**	**NS**	**NS**	**NS**	**0.624**

^{abcdef} Data bearing different superscripts within the same column differ significantly ($p<0.05$).

The Pb content in post-harvest soil reduced in all the treated pot than the control. Therefore, it is evident that Pb was transferred from soil to the biomass of bottle gourd plant with different fertilized treatments. The concentration of Pb (%) in leaves, and in roots varied significantly ($p \le 0.05$) among treatments, however, no plant parts showed any correlation with the total Pb content (%) in soil as suggested by Dietz *et al.* (1999). Both the control (T_1) and the group with sole application of vermicompost (T_2) had no Pb accumulation in leaf. The highest Pb accumulation in leaf was found to be 0.0023 % (dry weight) in T_{10}: $N_{30} P_{15} K_{23}$ kg ha^{-1} + Pb 2Kg ha^{-1}and the lowest (0%) with T_1. The highest Pb accumulation in stem obtained (0.0025%) was with T_{11}: ($N_{22}P_{11} K_{17}$ kg ha^{-1} + Pb 2 kg ha^{-1}) and the lowest (0.0015%) with T_6: ($N_{15} P_7 K_{11}$ kg ha^{-1} + VC 6 ton ha^{-1}). The highest Pb accumulation in the root was found (0.007%) in T_{11}: ($N_{22} P_{11} K_{17}$ kg ha^{-1} + Pb 2 kg ha^{-1}) and the lowest (0.0013%) with T_6: ($N_{15} P_7 K_{11}$ kg ha^{-1} + VC 6 ton ha^{-1}).

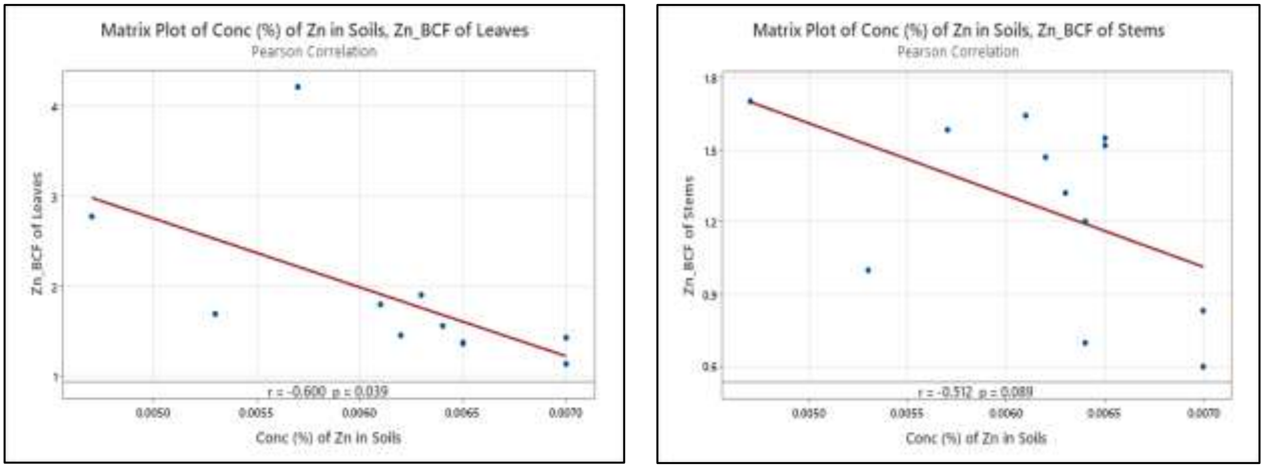


a **b**

Fig. 2. Relation of Pb content in soil with: **a**. BCF (leaf); and **b**. BCF (stem).

Similar uptake pattern was noticed in spinach leaves where lead content was found to be as high as 0.00725% (Syed *et al.* 2022). However, spinach leaves are the only edible part of the plant. On the other hand, bottle gourd fruits can be consumed if the rest of the aboveground biomass has to be eliminated from the diet due to possible health risks from intense metal accumulation. Therefore, further research is needed for evaluating safe consumption of bottle gourd fruits. In a study done by Sekara *et al*. (2005), the above ground biomass of field pumpkin was found to contain 7.55 mg kg^{-1} (dry weight) lead, and chicory leaves contained 12.25 mg kg^{-1} (dry weight) of lead. Total Pb in soil showed significant, strong, and negative correlation ($r = -0.717$, $p = 0.009$) with the BCF of leaves (Fig. 2a), which is comparable with the result obtained in a study by Alam *et al*. (2003), where the authors worked with 17 local vegetables including bottle gourd and found similar correlation for most heavy metals including Pb and Zn. Similar correlation was also found between Pb content in soils and BCF of plant stems ($r = -0.692$, $p = 0.013$) (Fig. 2b). However, correlation never proves causation. Translocation factor ranged from a maximum of 2.87 (T_5) to a minimum of 0.54 (T_{11}) with an average of 1.27±0.21. Sekara *et al.* (2005)

worked with nine vegetables and found translocation factor (Pb) to be as high as 4.38 and as low as 0.21. No significant correlation was seen between translocation factor and soil Pb content (Table 6).



a **b**

Fig. 3. Relation of Zn content in soil with: **a.** BCF (leaf); and **b**. BCF (stem).

It was observed from Table 7 that, Zn content in post-harvest soil was slightly higher in T_5, T_6, T_7, T_9, T_{11} and T_{12} than control (T_1). This might be the case of low Zn need of bottle gourd plant. But Zn transfer from soil to plant occurred in the leaf, stem and root somewhat lower as comparatively Pb treated soil. The highest Zn concentration (0.024%) was attained in leaf with T_2 (VC 6 ton ha^{-1}) and the lowest (0 .008%) with T_9: (T_6 + Zn 2 kg ha^{-1}). The concentration of Zn in stem was as high as 0.0101% in T_{11}: ($T_{4\,+}$ Pb 2 kg ha$^{-1)}$ and as low as 0.0042% with T_9: (T_6 + Zn 2 kg ha-^{1}).

Table 7. Concentrations (%) of total zinc in different parts of bottle gourd plants and in corresponding soils.

Treatments	Soil	Leaf	Stem	Root	BCF (Leaf)	BCF (Stem)	BCF (Root)	TF
T_1: Control (-VC, -NPK, -Pb, -Zn)	0.0062^{ab}	0.009^{b}	0.0091^{a}	0.007^{f}	1.45^{c}	1.47^{ab}	1.45^{d}	2.01^{ab}
T_2: VC 6 ton ha^{-1}	0.0057^{bc}	0.024^{a}	0.009^{a}	0.0144^{d}	4.21^{a}	1.58^{ab}	2.53^{bc}	2.29^{ab}
T_3: $N_{30}\,P_{15}\,K_{23}$ kg ha^{-1}	0.0053^{cd}	0.009^{b}	0.0053^{cd}	0.0132^{d}	1.7^{c}	1^{cde}	2.49^{bc}	1.08^{cd}
T_4: $N_{30}\,P_{15}\,K_{23}$ kg ha^{-1} + VC 2 ton ha^{-1}	0.0047^{d}	0.013^{b}	0.008^{ab}	0.0107^{e}	2.77^{b}	1.7^{a}	2.28^{c}	1.96^{b}
T_5: $N_{22}\,P_{11}\,K_{17}$ kg ha^{-1} + VC 4 ton ha^{-1}	0.0064^{ab}	0.01^{b}	0.0045^{d}	0.0179^{bc}	1.56^{c}	0.7^{e}	2.8^{b}	0.81^{cd}
T_6: $N_{15}\,P_7\,K_{11}$ kg ha^{-1} + VC 6 ton ha^{-1}	0.0063^{ab}	0.012^{b}	0.0083^{ab}	0.0171^{c}	1.9^{bc}	1.32^{abc}	2.71^{bc}	1.19^{c}
T_7: T_4 + Zn 2 kg ha^{-1}	0.007^{a}	0.01^{b}	0.0058^{bcd}	0.0192^{b}	1.43^{c}	0.83^{de}	2.74^{b}	0.82^{cd}
T_8: T_5 + Zn 2 kg ha^{-1}	0.0064^{ab}	0.01^{b}	0.0077^{abc}	0.0224^{a}	1.56^{c}	1.2^{bcd}	3.5^{a}	0.79^{cd}
T_9: T_6 + Zn 2 kg ha^{-1}	0.007^{a}	0.008^{b}	0.0042^{d}	0.0189^{b}	1.14^{c}	0.6^{e}	2.7^{bc}	0.65^{d}
T_{10}: T_4 + Pb 2 kg ha^{-1}	0.0061^{bc}	0.011^{b}	0.01^{a}	0.0079^{f}	1.8^{bc}	1.64^{ab}	1.3^{d}	2.66^{a}
T_{11}: T_5 + Pb 2 kg ha^{-1}	0.0065^{ab}	0.009^{b}	0.0101^{a}	0.0099^{e}	1.38^{c}	1.55^{ab}	1.52^{d}	1.93^{b}
T_{12}: T_6 + Pb 2 kg ha^{-1}	0.0065^{ab}	0.0089^{b}	0.0099^{a}	0.0176^{bc}	1.37^{c}	1.52^{ab}	2.7^{bc}	1.07^{cd}
LSD at 5%	**0.0004**	**0.0028**	**0.0012**	**0.0008**	**0.53**	**0.20**	**0.21**	**0.23**

^{abcdef} Data bearing different superscripts within the same column differ significantly (p<0.05).

Zinc concentration in leaves, stems, and roots had an average of 0.011% ± 0.0012, 0.0077% ± 0.00063, 0.0047% ± 0.0014, respectively which is quite low compared to the Zn concentration reported in a study by Al-Farraj *et al*. (2009) found in *Ochradenus baccatus* shoots (leaf + stem) and roots (0.043

and 0.017%). However, the average bioconcentration factor of bottle gourd roots in this study is 2.39, which is a lot higher than that reported in the study mentioned above. Translocation factor in bottle gourd plants ranged from a minimum of 0.65 to a maximum of 2.66. Nouri *et al.* (2009) has reported Translocation factor for zinc in *C. juncea* to be 1.36 and in *S. barbata* to be 1.04. Bioconcentration factor in different plant parts for Zn varied significantly ($p \le 0.05$), unlike for Pb (Table 7). There is a significant, strong, and negative correlation ($r = -0.600$, $p = 0.039$) between Zn concentration in soil and BCF of leaves, and a slightly moderate and less significant one ($r = -0.512$, $p = 0.089$) between Zn concentration in soil and BCF of stems (Fig. 3a and b). Similar to Pb, there were no correlation between soil Zn content and Zn uptake by any plant parts.

Chemical analysis of post-harvest soil

The soils with twelve different treatments (including control) were analyzed for organic carbon content as well as total and available concentration (%) of nitrogen, phosphorus and sulfur. Organic matter content (%) was derived from organic carbon content by multiplying with 1.72 (Bemmelen factor), and all the data are presented in Table 8. The purpose of this analysis is to correlate the accumulation ability of bottle gourd plants under various treatments with the properties of corresponding soil, although only significant results are mentioned in this paper.

Table 8. Concentration (%) of organic carbon, organic matter, total and available nitrogen, phosphorus, and sulphur in the post-harvest soil.

Treatments	Organic carbon	Organic matter	Total nitrogen	Total phosphorus	Total sulphur	Available nitrogen	Available phosphorus	Available sulphur
T_1	0.663	1.14	0.183^{e}	0.068^{b}	0.06^{b}	0.0048	0.0008^{de}	0.0087^{ef}
T_2	1.167	2.007	0.174^{fg}	0.073^{b}	0.083^{b}	0.0039	0.0001^{f}	0.013^{cd}
T_3	0.741	1.275	0.187^{de}	0.081a	0.092^{b}	0.0075	0.0012^{ab}	0.0063^{f}
T_4	1.162	1.999	0.171^{g}	0.084^{a}	0.059^{b}	0.0063	0.0011^{abc}	0.0137^{cd}
T_5	1.495	2.572	0.177^{f}	0.069^{b}	0.056^{b}	0.005	0.0006^{e}	0.0153^{bcd}
T_6	1.237	2.128	0.189^{d}	0.016^{de}	0.092^{b}	0.0042	0.0008^{de}	0.012^{de}
T_7	1.094	1.822	0.21^{a}	0.035^{c}	1.968^{a}	0.004	0.0014^{a}	0.0167^{bc}
T_8	1.162	1.999	0.175^{fg}	0.022^{d}	0.085^{b}	0.0043	0.0008^{cde}	0.012^{de}
T_9	1.459	2.509	0.174^{fg}	0.035^{c}	0.085^{b}	0.0044	0.0008^{cde}	0.0177^{b}
T_{10}	1.162	1.999	0.188^{de}	0.015^{de}	0.081^{b}	0.004	0.0006^{e}	0.0283^{a}
T_{11}	1.203	2.07	0.202^{b}	0.001^{f}	1.878^{a}	0.0033	0.001^{bcd}	0.0008^{g}
T_{12}	1.313	2.258	0.196^{c}	0.013^{e}	2.139^{a}	0.0035	0.0009^{bcde}	0.0123^{de}
LSD at 5%	**NS**	**NS**	**0.002**	**0.004**	**0.144**	**NS**	**0.0002**	**0.0018**

[abcdefg] Data bearing different superscripts within the same column differ significantly ($p<0.05$).

Total nitrogen, phosphorus, and sulphur in post-harvest soils varied significantly ($p \le 0.05$) while ranging from 0.171 to 0.21%, 0.001 to 0.084% and 0.056 to 2.13%, respectively. No correlation was found among the total and available concentrations of nutrients. The highest available phosphorus (0.0014%) was found in T_7: (N_{30} P_{15} K_{23} kg ha^{-1} + VC 2 ton ha^{-1}+ Zn 2 kg ha^{-1}), while T_{10}: (T_4 + Pb 3 Kg ha^{-1}) and T_3: (N_{30} P_{15} K_{23} kg ha^{-1}) had the highest available sulphur (0.0283%) and highest available nitrogen (0.0075%), respectively.

Although the organic carbon content (%) in soils (hence the organic matter content) did not show significant variance among treatment groups, it strongly correlated with lead accumulation in plant shoots (r = -0.804, p = 0.002) (Fig. 4). This negative correlation can be explained by the binding of metals to humic or fulvic acids in soil resulting in immobilization and consequently less availability of metals (Dietz *et al.* 1999).

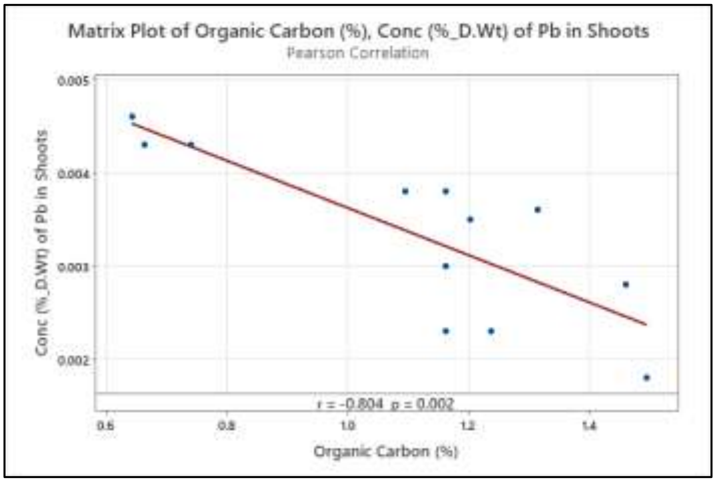


Fig. 4. Relationship between soil organic carbon content and Pb content in shoots.

Nutrient analysis of different plant parts

Total concentrations of three macronutrients- nitrogen, phosphorus and sulfur in different plant parts were analyzed to determine any possible correlation between the nutrient uptake and accumulation of Pb and Zn. Even though there was no significant correlation found, nutrient uptake significantly varied ($p \leq 0.05$) between treatment groups. The results are presented below in Table 9.

Table 9. Concentrations (%) of total nitrogen, phosphorus, and sulphur in different parts of bottle gourd plants.

Treatments	Nitrogen (N)			Phosphorus (P)			Sulphur (S)		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
T_1	2.531	1.803	2.179	0.051^{g}	0.386^{abcd}	0.127^{h}	1.450^{a}	0.569^{cd}	0.366^{b}
T_2	4.057	2.136	2.808	0.542^{b}	0.430^{ab}	0.397^{f}	1.232^{b}	0.813^{abcd}	0.205^{d}
T_3	1.56	2.247	2.843	0.519^{c}	0.482^{a}	0.560^{cd}	0.928^{cd}	0.543^{d}	0.111^{fg}
T_4	4.195	1.803	2.496	0.418^{d}	0.410^{abc}	0.538^{de}	1.183^{b}	0.998^{a}	0.138^{e}
T_5	3.696	2.025	2.08	0.218^{e}	0.436^{ab}	0.684^{a}	1.178^{b}	0.688^{bcd}	0.116^{ef}
T_6	4.057	2.469	2.843	0.099^{fg}	0.374^{bcd}	0.561^{cd}	1.089^{bc}	0.806^{abcd}	0.212^{d}
T_7	3.086	2.136	2.392	0.139^{ef}	0.312^{cde}	0.530^{e}	1.199^{b}	0.587^{cd}	0.257^{c}
T_8	2.808	2.136	2.774	0.230^{e}	0.387^{abcd}	0.586^{c}	1.211^{b}	0.832^{abc}	0.338^{b}
T_9	5.027	2.358	2.427	0.561^{c}	0.339^{bcde}	0.652^{b}	0.707^{e}	0.900^{ab}	0.587^{a}
T_{10}	2.947	2.247	2.184	0.094^{g}	0.292^{def}	0.084^{i}	0.927^{cd}	0.893^{ab}	0.062^{g}
T_{11}	3.64	1.775	1.976	0.843^{a}	0.242^{ef}	0.161^{g}	0.482^{f}	0.681^{bcd}	0.202^{d}
T_{12}	3.294	1.847	2.115	0.769^{ab}	0.200^{f}	0.094^{i}	0.868^{de}	0.833^{abc}	0.141^{e}
LSD 5%	**NS**	**NS**	**NS**	**0.05**	**0.05**	**0.01**	**0.08**	**0.13**	**0.02**

^{abcdefghi} Data bearing different superscripts within the same column differ significantly ($p<0.05$).

Treatment groups receiving Pb, showed lowest phosphorus contents in stems and roots, however the highest phosphorus accumulations (0.843 and 0769%) were recorded in T_{11} and T_{12} in order, both of which received 3 kg ha^{-1} of lead. In most cases, plants with lead treatments had lower sulfur content than those from other groups, even lower than the control group. However, plants treated with zinc showed intermediate effect.

From the data obtained in this study, it can be concluded that bottle gourd plant showed tolerance of metal stress or adverse pressure of Pb and Zn loaded soil in terms of biomass production, growth and yield. Besides, bottle gourd leaves and stems have high bioaccumulation capacity of Pb and Zn as well as high translocation factor, which makes them suitable phytoextractor. All these results indicate the possibility of bottle gourd plants being used in heavy metal clean-up technology and the need of further research. However, presence of organic carbon can reduce Pb uptake significantly ($p \leq 0.05$). In Bangladesh context the bottle gourd plant species was assessed as a biological tool for remediation and reduction of metal mobility.

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