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Accumulation of toxic metals in citrus plants due to heavy traffic pollution in industrial areas

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Abstract

Sargodha is famous for the production of different citrus varieties due to its quality throughout the country. Keeping in view the importance of citrus, this experiment was done to evaluate the effect of air contamination/pollution on growth of *Citrus limon* (Lemon) and *Citrus sinensis* (Kinu) and accumulation of heavy metals in their leaves in industrial areas of Tehsil Shahpur Sadar, Sargodha, Pakistan. Various biochemical and physiological parameters (chlorophyll *a* and *b*, relative water contents) were studied. Lessening of fresh and dry weight of lemon (23.38% and 2.19%) and kinu (12.16% and 1.49%) was noted. In lemon, values of 16.63%, 13.95% and 2.41% were noted for chlorophyll a, b and total while 37.07%, 3.48% and 6.24% for kinu. Copper (lemon = 0.175 & kinu = 0.789 mg L^{-1}) and nickel (lemon = 0.345 & kinu = 0.450 mg L^{-1}) concentrations were analyzed. Same trend for Ca (2.42 and 2.27 mg g^{-1} in lemon and kinu respectively), N (1.93 and 2.96 mg g^{-1} in lemon and kinu respectively) and P (0.064 and 0.58 mg g^{-1} in lemon and kinu respectively) contents were noted. It is concluded that contamination of polluted site significantly reduced the growth and physiological attributes of *Citrus limon* (Lemon) and *Citrus sinensis* (Kinu).

Keywords: Heavy metals, Industrial pollution, Kinu, Lemon, Mineral ions

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Introduction

Throughout the globe, different varieties of citrus are very popular among the people. These are very rich sources of phenolic compounds, folic acid, flavonoids (Samraj & Rajamurgugan, 2017), ascorbic acid, antioxidant, pectin and potassium (Czech et al., 2020). Citrus occupies a prominent position in the Pakistani fruit industry. During 2018, area cultivated with citrus in Pakistan was 200461 hectares. The total production was 2247956 tons and average yield of 112139 kg/hectare (Ahmad & Jamali, 2020).

Accumulation of heavy metals in the food chain becomes toxic for human beings as well as animals (Ali & Khan, 2018). Activities such as the extraction and refining of metallic minerals, manufacturing discharges and usage of pesticides and mineral manures have contributed to increased levels of toxic elements/heavy metals in the environment (Zhou et al., 2005). Heavy metals gather simply in significant human tissues and deteriorate human healthiness (Alloway, 2013). Finster et al. (2004) stated that lead (Pb) is a heavy metal with precise fear for municipal farming due to its risky harmfulness and its frequency in inner-city soils. Among biological methods, phytoremediation is a growing cost effective, ecological and plant-based knowhow that plays a vital part in rebuilding (Sheoran et al., 2008). The remedy of fight is usage of plants to excerpt or decompose pollutants (Sarwar et al., 2017). Plant extraction uses resistant accumulation plants to adsorb heavy metals and move them above the ground for storage (Lee et al., 2019).

Climatic air nourishes plants and living beings. Air quality determines the health of the population. Rapid urbanization has caused the spread of urban pollution in larger urban communities, especially in developing countries. Extensive pollutant measurements have been reported as a result of this urbanization and industrialization, where there are always many ecological complications, such as air, water and noise pollution or contamination and managing the waste materials (Yami et al., 2016). Relative water content (RWC) is a measure of water which may have when it is not equipped to absorb more water. More relative water content supports the drought resistance of the plants. At that time, the plant does not transport minerals from roots to leaves where biosynthesis happens and does not cool leaves. Chlorophyll measurement has an imperative part in metabolism, and reduced chlorophyll concentration links straight with development of plants (Liu & Ding, 2008). Therefore, objectives of the current study were to assess the effect of heavy metal pollution on growth of lemon and kinu and also to quantify the heavy metal's contents in the leaves of lemon and kinu.

Materials and Methods

Present experiment was performed during winter season, 2019-2020 to assess the pollution tolerance of plants cultivated in industrial areas of Tehsil Shahpur Sadar (32.2576º N, 72.4672º E and 210 m a.s.l.), Sargodha, Pakistan. In the study area, many industries were located that are strongly influencing the air quality. Treatments of the experiment were as under:

A: Citrus Varieties = 02 [Citrus lemon (Lemon) and Citrus sinensis (Kinu)]

B: Locations = 02 [Unpolluted/control (5km away from industrial area) and highly polluted (around 5 km of Arabia sugar mill limited)]

During the winter season of 2019-2020, plant materials were collected from Shahpur Sadar. Matured leaves from these plants were randomly collected in polythene bags from the sampling sites with similar light, water and soil condition and were shifted to laboratory immediately for analysis and were placed in the ice box to avoid the adverse effect of temperature as well as light. Fresh weight of the sample (leaf) was measured by weighing on balance very quickly after arrival in the laboratory. Samples were stored at freezing temperature in the refrigerator for further required analysis. Relative water contents of leaves of lemon and kinu were determined by using the methods which were proposed by Liu & Ding (2008).

RWC = (WF - WD) × 100 / (WT - WD)

Where

WF = Fresh weight of the leaf, WD = Dry weight of the leaf and WT = Turgid weight of fresh weigh of leaf

Chlorophyll, *a* and *b* contents were analysed using the method of Arnon, (1949). 0.1 g of leaf sample was chopped in 5 ml of 80% acetone and placed overnight at 4° and then centrifuged. A UV-spectrophotometer was used for it. Method, proposed by Barrs and Weatherly (1962) was used to record pH of leaf extract. A digital meter was used to note pH of leaf extract from supernatant. Samples were digested by “wet digestion” according to Vukadinovic (1988). Three samples of each material are digested. Atomic absorption spectrophotometer with a hollow cathode lamp for target elements was used for analysis.

To explore the performance and quality of plant produce in the term of metal accumulation, metal accumulation index is measured by using the following formula: MAI= $(1/N)\sum_{j=1}^{N} Ij$; Where N = total number metal analysed, I*j* = x / δx, a sub-index and x = mean value of metal calculated according the Liu et al. (2006).

All collected data were subjected to statistical analysis for Analysis of Variance (ANOVA) using R software (Steel et al., 1997).

Results

Fresh & dry weight of leaves

Analysis of variance (ANOVA) depicted that leaf fresh weight of lemon and kinu were significantly influenced by the sites. Polluted area significantly (*P*< 0.01) reduced leaf fresh & dry weight of lemon and kinu (Table 1). Lemon produced leaf fresh weight of 3.85 g and 2.95 g under normal and industrial area respectively. Lessening in fresh and dry weight of lemon was 23.38% and 2.19% respectively (Fig. 1). More reduction was observed in fresh weight as compared to dry weight of lemon. Polluted environment also significantly (*P*< 0.001) reduced the leaf fresh and dry weight in kinu plants. Kinu plants which were cultivated under a polluted environment produced less leaf fresh weight while those plants which were grown under normal environment produced maximum leaf fresh weight. Kinu produced leaf fresh weight of 2.44 g and 2.14 g under normal and industrial area respectively. Fresh and dry weights of kinu were reduced 12.16% and 1.49% respectively because of heavy metal’s pollution. Overall, the polluted site reduced the leaf biomass of both species as compared to normal site.

Table 1 ANOVA for leaf resh and dry weight of lemon and kinu

Source of Variance	DF	Leaf fresh weight	Leaf dry weight
Plants	1	0.433***	0.568***
Sites	1	2.376***	0.298***
Plants × Sites	1	0.811***	0.244***
Error	8	1e-4	1e-4

DF = Degree of freedom; SS = Sum of squares; MS = Mean sum of squares;
* = Significant at 0.05; ** = Significant at 0.01; *** = Significant at 0.001;
NS = Non-significant at 5% probability level.

Fig. 1 Leaf fresh and dry weight of lemon and kinu cultivated under normal and polluted environment

Chlorophyll contents

Statistical analysis of chlorophyll contents revealed that chlorophyll a, b and total chlorophyll in leaves of lemon and kinu were found statistically significant ($P< 0.001$) under normal and polluted environment (Table 2). Lemon plants synthesized 1.57 and 1.31 mg g^{-1} chlorophyll b under normal and 1.10 and 0.95 mg g^{-1} under polluted conditions respectively. In lemon, a reduction of 16.63%, 13.95% and 2.41% in chlorophyll a, b and total chlorophyll contents were observed (Fig. 2). In case of kinu plantation, a reduction of 37.07% was recorded in chlorophyll a content. Chlorophyll b and total chlorophyll reduced up to 3.48% and 6.24% respectively. Maximum chlorophyll a, b and total contents were 1.72, 1.08 and 2.16 mg g^{-1} respectively under normal conditions while in a polluted environment their values were 1.08, 1.04 and 2.02 mg g^{-1}.

Table 2 ANOVA for a, b and total chlorophyll contents of lemon and kinu

Source of Variance	DF	Chlorophyll a	Chlorophyll b	Total chlorophyll
Plants	1	7.5e-7ns	0.009***	0.008***
Sites	1	9.075e-5***	0.166***	0.170***
Plants × Sites	1	6.75e-6*	0.013***	0.011***
Error	8	1e-6	1e-6	3.158e-5

DF = degree of freedom; SS = Sum of squares; MS = Mean sum of squares; * = Significant at 0.05; ** = Significant at 0.01; *** = significant at 0.001; NS = Non-significant at 5% probability level.

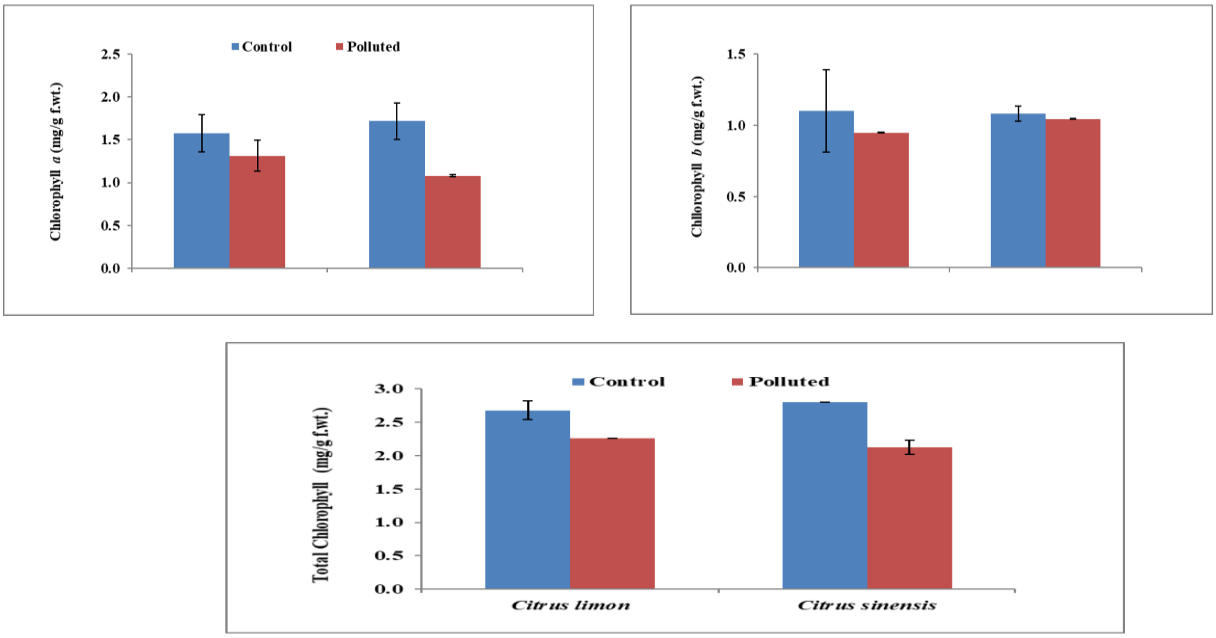

Fig. 2 Chlorophyll a, b and total chlorophyll contents of limon and kinu cultivated under normal and polluted environment

pH of leaf extracts

ANOVA (analysis of variance) showed that pH of leaf extracts of lemon and kinu were found statistically ($P < 0.001$) different (Table 3). pH was recorded higher in normal condition compared to the polluted area. Minute difference was noted in soil pH by cultivation of lemon and kinu plantation. A reduction of 6.9% and 15% in pH of leaves extract of lemon and kinu respectively (Fig. 3). Kinu plantation is more responsive to soil pH of normal and polluted areas as compared to lemon plantation. Polluted sites reduced the pH as compared to normal soil.

Table 3 ANOVA for plant leaves pH of lemon and kinu

Source of Variance	DF	pH of leaf
Plants	1	1.268***
Sites	1	0.188**
Plants × Sites	1	0.188**
Error	8	0.01

DF = degree of freedom; SS = Sum of squares; MS = Mean sum of squares; * = Significant at 0.05; ** = Significant at 0.01; *** = Significant at 0.001; NS = Non-significant at 5% probability level.

Relative water contents

Relative water contents (RWC) are presented in Table 4 and Fig. 4. Analysis of variance showed that RWC were significantly ($P < 0.001$) influenced among the plant species and growing conditions. Maximum RWC of 90.31% were observed in lemon plants which were growing under normal conditions. RWC were reduced up to 45.33% by the polluted environment in lemon plants. Polluted environment also significantly ($P < 0.001$) reduced the RWC in kinu plants up to 7.83% decrease. Kinu plants which were cultivated under a polluted environment produced 43.2% RWC while those plants which were grown under normal environment produced 46.87%. Overall, the polluted site reduced the production of RWC of both species as compared to normal sites.

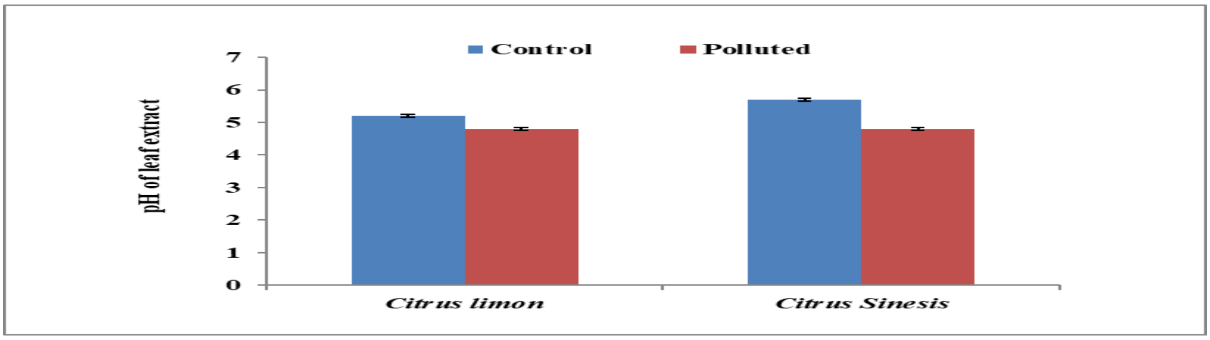

Fig. 3 pH of leaf extracts of lemon and kinu cultivated under cultivated normal and polluted environment

Table 4 ANOVA for relative water contents and carotenoid
of lemon and kinu

Source of Variance	DF	Relative water contents
Plants	1	1219***
Sites	1	1540***
Plants × Sites	1	1295***
Error	8	1^{-4}

DF = Degree of freedom; SS = Sum of squares; MS = Mean sum of squares; * = significant at 0.05; ** = significant at 0.01; *** = Significant at 0.001; NS = Non-significant at 5% probability level.

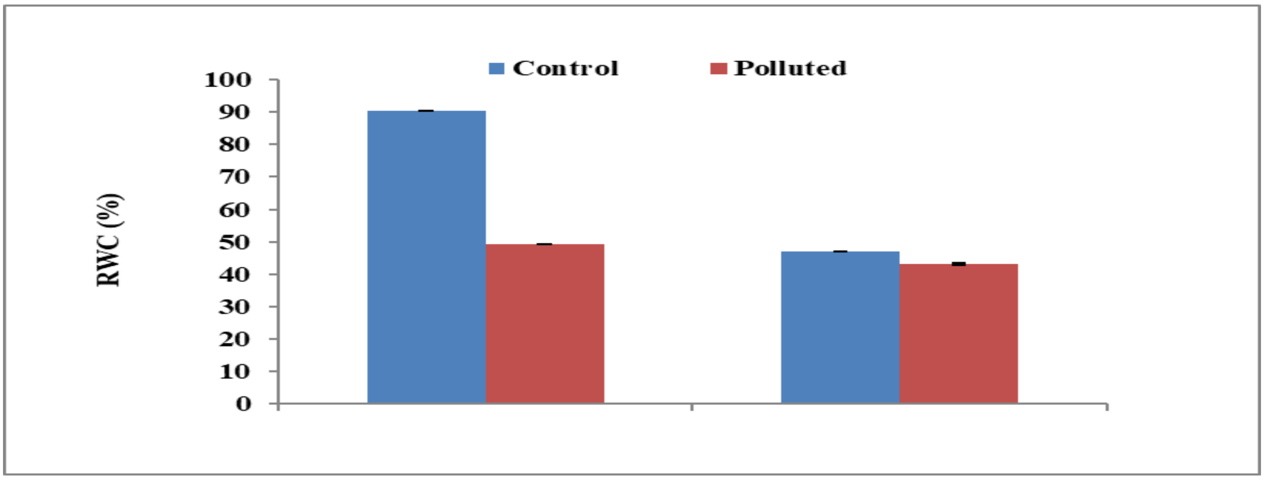

Fig. 4 Relative water contents of lemon and kinu under normal and polluted environment

Heavy metals contents in leaves

Significant (*P*< 0.001) increase was recorded in the absorption of heavy metals including Cu, Ni, Pb and Zn in the leaves of lemon and kinu (Table 5). Cu and Ni accumulations were significantly influenced by production sites and among the plant's species. Polluted environment is more responsible for the buildup of heavy metals as compared to a normal environment. Maximum Cu concentration of 0.175 and 0.789 mg L^{-1} was recorded in the leaves of lemon and kinu plants respectively cultivated in polluted sites. While minimum Cu contents (0.07 and 0.285 mg L^{-1}) were analyzed in lemon and kinu plants grown under normal conditions. An increase of 149.53% and 177.3% was recorded in lemon and kinu plants respectively (Fig. 5). Maximum concentration of Ni with 0.345 and 0.45 mg L-1 were found in lemon and kinu plantations respectively in polluted conditions. Ni concentration was increased with 474.8% and 30.88% in lemon and kinu respectively. Maximum increase of Pb of 182% and 4.73% was recorded in the leaves of lemon and kinu respectively. An increase of 94.55% and 4.74% in Zn concentration was observed in lemon and kinu plantations, respectively.

Table 5 ANOVA for heavy metals of leaves of lemon and kinu

Source of Variance	DF	Cu in leaf	Ni in leaf	Pb in leaf	Zn in leaf
Plants	1	0.217***	0.204***	1.995***	0.923***
Sites	1	0.809***	0.717***	0.489***	1.330***
Plants × Sites	1	0.166***	0.021***	1.735***	0.748***
Error	8	1e-10	1e-8	2.5005e-5	5.05e-7

DF = degree of freedom; SS = Sum of squares; MS= Mean sum of squares; * = Significant at 0.05; ** = Significant at 0.01; *** = Significant at 0.001; NS = Non-significant at 5% probability level.

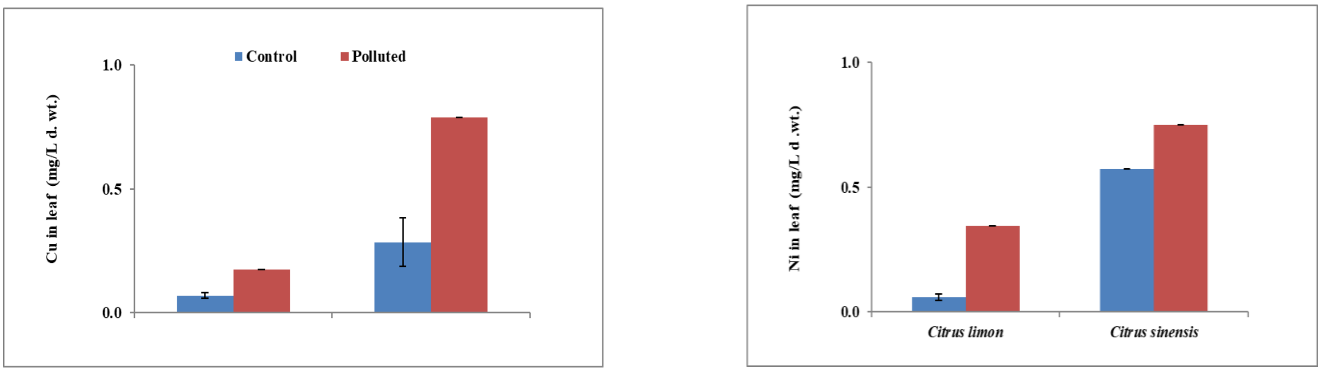

Fig. 5 Cu, Ni, Pb and Zn in leaves of lemon and kinu cultivated under normal and polluted environment

Mineral composition of leaves

Results indicated that mineral composition of lemon and kinu leaves was significantly ($P < 0.001$) inclined (Table 6). Extreme Ca content of 2.42 was 2.27 mg g^{-1} with a decrease of 10.6% and 9.95% in lemon and kinu respectively. A decline of 13.97% and 14.78% was noted in the content of K in lemon and kinu respectively. Extreme content of N was 1.93 and 2.96 mg g^{-1} while lowest was 1.62 and 2.57 mg g^{-1} in lemon and kinu respectively. Nitrogen content was reduced up to 16.06% and 13.18% in lemon and kinu respectively. Maximum content of P was 0.064 mg g^{-1} in lemon while lowermost was 0.012 mg g^{-1} in lemon. In kinu, P content was 0.58 mg g^{-1} as maximum and 0.01 mg^{-1} as lowest. A decrease of 82.25% and 98.22% was found in the content of P in lemon and kinu (Fig. 6).

Table 6 ANOVA for mineral elements of leaves of lemon and kinu

Source of Variance	DF	Ca in leaf	K in leaf	N in leaf	P in leaf
Plants	1	17.21***	0.097***	2.940***	0.007***
Sites	1	0.022***	0.120***	0.368***	4.41e-5 NS
Plants × Sites	1	6.75e-4*	3e-4 NS	0.005***	1.41e-5 NS
Error	8	1e-4	1e-4	1e-4	2.3333e-5

DF = degree of freedom; SS = Sum of squares; MS = Mean sum of squares; * = Significant at 0.05; ** = Significant at 0.01, *** = Significant at 0.001; NS = Non-significant at 5% probability level.

Heavy metals contents in soil

Fig. 7 depicted that Cu content in soil was significantly different. Maximum Cu concentration was recorded in polluted or industrial sites while less Cu content was noted in normal soil which was away from the industrial zone. Soil of non-industrial areas contained less amount of Cu. On the other hand, soil of polluted or industrial soil has more Cu content. Concentration of Ni in soil was significantly affected by the experimental sites. Pollution showed its impact on the content of Ni in soil. Normal site contained very minute content of Ni in the soil. Polluted area or industrial site contained large quantities of Ni in soil as compared to soil of normal site (Table 7). Polluted environment contained high content of Ni in the soil. Data depicted that Pb content in soil was significantly different. Maximum Pb content was recorded in polluted or industrial sites while less Pb content was noted in normal soil which was away from the industrial zone. Soil of non-industrial area contained less amount of Pb. On the other hand, soil of polluted or industrial soil has more Pb content. Concentration of Zn in the soil was significantly affected by the experimental sites. Pollution revealed its impact on the content of Zn in the soil. Normal site contained very minute content of Zn in the soil. Polluted area or industrial site contained large quantities of Zn in soil as compared to soil of normal site (Fig. 7). Polluted environment contained high content of Zn in soil.

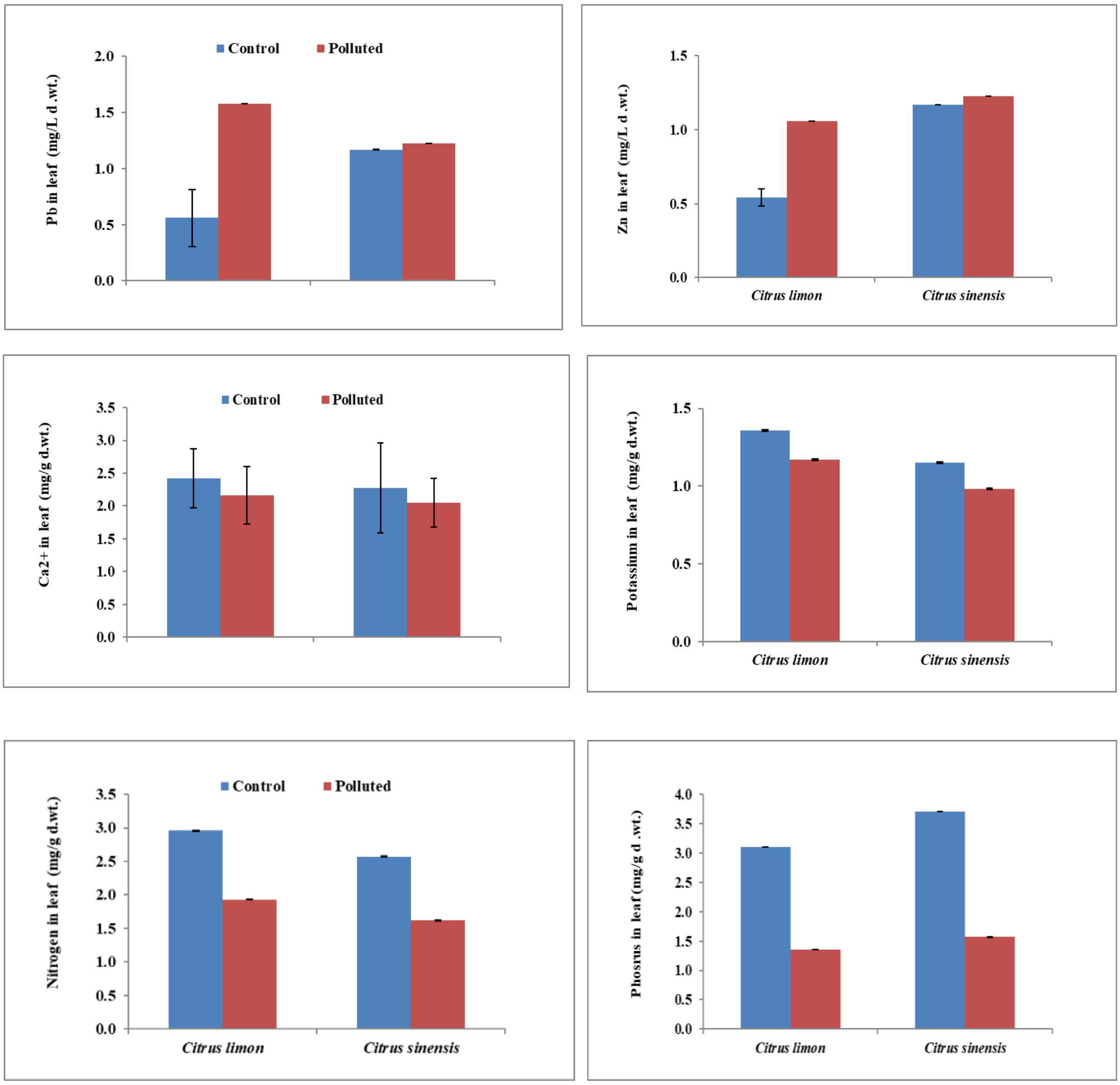

Fig. 6 Ca, K, N and P in leaves of lemon and kinu cultivated under normal and polluted environment

Table 7 ANOVA for heavy metals in soil

Source of Variance	DF	Cu in soil	Ni in soil	Pb in soil	Zn in soil
Plants	1	0.204***	1.995***	0.009***	0.008***
Sites	1	0.717***	0.489***	0.166***	0.170***
Plants × Sites	1	0.021***	1.735***	0.013***	0.011***
Error	8	1e-8	2.5005e-5	1e-6	3.158e-5

DF = Degree of freedom; SS = Sum of squares; MS = Mean sum of squares; * = Significant at 0.05; ** = Significant at 0.01; *** = Significant at 0.001; NS = non-significant at 5% probability level.

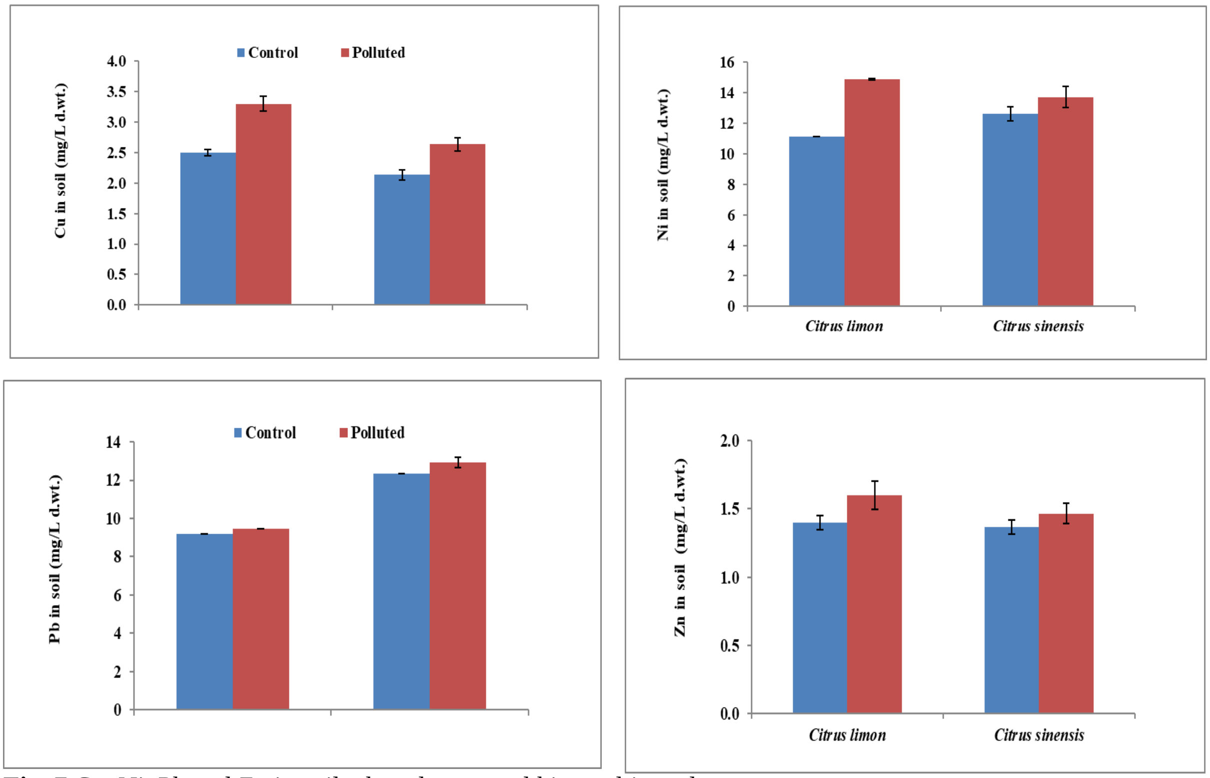

Fig. 7 Cu, Ni, Pb and Zn in soil where lemon and kinu cultivated

Discussion

Air pollution is one of the core issues which is affecting many biological processes. Effects of air pollution on human healthiness and crop cultivation are of major concern. Outcomes of present experimentation showed that polluted environments significantly inclined growth attributes of *Citrus limon* (Lemon) and *Citrus sinensis* (Kinu). Air pollution adversely affected the leaf fresh and dry weights and air pollution tolerance index. Plant leaves are considered primary photosynthetic organs. Biochemical and physiological parameters including chlorophyll *a* and *b* contents as well as relative water contents were also adversely impaired by polluted growing conditions. These findings are in line with the outcomes of AlObaidy & Rabee (2018). They reported that ascorbic acid, water contents and APTI were reduced when *Citrus aurantium* plants were cultivated along the road side and commercial places as compared to control sites. Chlorophyll contents are considered an index of plant productivity. In a present study, it was observed that leaf fresh and dry weights were reduced when plants were cultivated under a polluted environment. Reduction in biomass production is linked with reduction in synthesis of chlorophyll contents. Szabela et al. (2019) stated imposition of heavy metal trauma significantly reduced chlorophyll contents in leaves, net photosynthesis rate, intercellular CO_2 concentration, transpiration rate and stomatal conductance. Higher absorption of heavy metals in the growing atmosphere creates toxicity and ultimately reduces the growth and productivity of plants. Air pollution has appeared to be a complex problem as compared to any other environmental issues as nobody can create fresh air to respire in the biosphere (Kalagbor et al., 2014). Different species of plants can play a vital role to minimize the impact of pollution though their photosynthetic factories particularly leaves and other aerial parts from air by impaction and sedimentation processes reported that imposition of heavy metal stress reduced the dry weight of seedlings (Kaur et al., 2016).

Hippler et al. (2018) described imposition of heavy metal stress reduced dry weight of seedlings. In the current study, copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) concentrations were recorded maximum in leaves of lemon and kinu plants. It is also reported that mostly heavy metals are non-biodegradable that is why these cannot be removed from the atmosphere (Burakova et al., 2018; Afonne & Ifediba, 2020). Inhibited growth, chlorosis retarded root growth and ion leakage are some common symptoms of plants growing under Cu stress (Bouazizi et al., 2010). Nutrient deficiency, chlorosis, disturbed function of cell membrane and necrosis are the symptoms that are observed in case of Ni deficiency (Mazzafera et al., 2013). High concentration of Pb significantly reduced the plant growth. Pb stress also increased actions of antioxidant enzymes (Malar et al., 2014). Zn toxicity symptoms in plants include chlorosis, accumulation of other heavy metals (Mausi et al., 2014).

Dust load was increased under polluted environments in lemon and kinu plants under present study. Chaturvedi et al. (2013) reported that dust is the most widely distributed pollutant. Jaarsveld (2008) reported that dust deposition rate, chemical composition of dust, and its particulate size are main elements which determine toxicity on plants. Dust particles larger in size of stomatal opening commonly stack on opening and those particles which are smaller got entry in the leaves through stomatal opening and affect the biochemical and physiological processes including water retention, gaseous exchange and photosynthesis ultimately plant growth is affected (Rai et al., 2010).

Mineral elements of leaf including Ca, K, N and P were decreased in the leaves of lemon and kinu plants grown under polluted conditions as compared to normal conditions. Lemon plants performed better regarding mineral elements accumulation as compared to kinu. Maximum mineral contents were observed in lemon plants which were growing under normal conditions. Overall, the polluted site reduced the accumulation of mineral elements of both species as compared to normal site. These findings are in line with AlObaidy & Rabee (2018). They reported that mineral elements, especially nitrogen concentration was recorded higher in those citrus plants which were cultivated under polluted conditions as compared to normal environment. Such conclusions are in contrast to results of Chaturvadi et al. (2013). Alva et al. (2006) reported that nitrogen is the utmost imperative element for citrus growth and development. Soil pH decreased under polluted environment as compared to normal conditions. Heavy metals content in soil including Cu, Ni, Pb, Zn were increased. Heavy metals can migrate and accumulate in soil environment. Plants may absorb the metal pollutants present in the rhizosphere through vascular and root systems. Ashraf et al. (2011) reported that presence of heavy metals in high content in plants adversely affect synthesis of chlorophyll contents, weaken stomatal resistance and increase oxidative stress in plants. Artificial adding of heavy metal in soil may reduce plant growth (Street, 2012). Heavy metal contents were higher in plants present along roadside as compared to plants which were present at normal site (Sulaiman & Hamzah, 2018). It is also stated that heavy metal concentration is normally high in the upper layer of soils (Zhang et al., 2018).

Conclusion

It is determined that the polluted site significantly reduced growth and physiological attributes of *Citrus limon* (Lemon) and *Citrus sinensis* (Kinu). Biochemical and physiological parameters (chlorophyll *a* and *b* contents, and relative water contents) were also adversely affected by polluted growing conditions. Copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) concentrations were recorded maximum in leaves of lemon & kinu plants cultivated under polluted conditions. Mineral elements of leaf including Ca, K, N and P were decreased in leaves of lemon and kinu plants grown under polluted conditions as compared to normal conditions. Soil pH decreased under polluted environment as compared to normal conditions. Heavy metals concentration in soil including Cu, Ni, Pb, Zn were found higher under polluted environments.

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