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Research Article

An experimental study on the effects copper and lead on the seedlings of some economically important vegetable species

Feyza CANDAN[1], Munir OZTURK[2]*, Volkan ALTAY[3],
Ibrahim Ertugrul YALCIN[4]

[1]*Manisa Celal Bayar University, Faculty of Science and Arts, Biology Department, Manisa, Turkiye; feyzacandan2002@yahoo.com*
[2]*Ege University, Botany Department & Centre for Environmental Studies, Izmir,*
*Turkiye; munirozturk@gmail.com (*corresponding author)*
[3]*Hatay Mustafa Kemal University, Faculty of Science & Arts, Biology Department, Hatay, Turkiye; volkanaltay34@gmail.com*
[4]*Bahcesehir University, Faculty of Engineering and Natural Sciences, Department of Civil Engineering, Besiktas, Istanbul,*
Turkiye; ibrahimertugrul.yalcin@eng.bau.edu.tr

Abstract

Bioaccumulation of toxic heavy metals in vegetables is closely related to the problems of safety concerns as they negatively affect plants in particular those consumed by the humans. Among the food systems the vegetables are the most noticeable foods affected by environmental pollution. Vegetables can take up the metals like copper and lead and store them in excessive levels. Keeping this in view this investigation was undertaken to study the effect of copper and lead concentrations (20, 40, 80, 160, 240, 320, 640, and 1280 μM) and assess their toxic affects on germination and seedling growth at early stages of eight vegetable cultivars; kidney bean, peas, black-eyed bean, artichoke, kale, lettuce, rocket and radish. The results were evaluated by multivariate analysis of variance and Pearson correlation statistical analysis. Our results indicate that the seeds of the vegetables studied by us are generally tolerant to both copper as well lead, except higher concentration exposures which showed no improvement when applied to artichoke (for Cu 1280 μM) and lettuce seeds (Cu 1280 μM; Pb 1280 μM). An application of copper and lead ended up with a decrease in barium, calcium, iron, magnesium, manganese, sodium and zinc content in all seedlings studied. In all vegetables exposed to copper and lead a promotion in copper and lead accumulation was recorded. There was a decrease in nutrient element intake which interrupted the mineral element uptake in the seedlings.

Keywords: copper; heavy metals; lead; vegetables; seedlings; uptake

Introduction

Heavy metals (HM) may not prove toxic all the time but some of these even in trace quantities such as cobalt, zinc, nickel, molybdenum, copper, iron and manganese may lead to adverse effects on plants used as vegetables and ultimately humans consuming these. Unlike nutrients, HMs like lead, cadmium, arsenic, silver, mercury and chromium are toxic and enter our environments through different sources (Ozturk, 1989; Ghori *et al.*, 2019; Khan *et al.*, 2019). The involvement of anthropogenic activities is highly responsible for the heavy

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metal content build-up in soils. These involvements include crop irrigation with sewage water, synthetic fertilizers used for growing vegetables, metal industries and vehicular traffic (Khan *et al*., 2019).

An accumulation of HMs in crops and vegetables up to toxic levels leads to safety concerns, negatively affecting the health of plants, humans and our environment (Clemens and Ma, 2016; Khan *et al*., 2019). Their bioaccumulation in edible plants end up with dangerous carcinogenic affects (Khan *et al*., 2019). Globally, their concentration constitutes essential aspect of food quality, food safety rules only permit only low concentrations in the food items consumed by living beings (Khan *et al*., 2019).

For growth of plants copper is a vital micronutrient. In general, 5-30 mg kg^{-1} are considered satisfactory for plants (Kumar *et al*., 2021). In uncontaminated soils normal values vary between 2-109 mg kg^{-1} (Kumar *et al*., 2021). The soil amendments, fertilizers, waste incineration, mining, fossil fuel combustion, traffic together with few other sources are the key anthropogenic additions (Kumar *et al*., 2021). The copper ore mining and industrial activities are responsible for increasing the copper levels in our surroundings (Kumar *et al*., 2021). The safe value suggested for food crops is 30 mg kg^{-1} (Kumar *et al*., 2021). Additionally, copper in excess in the plants triggers oxidative stress by generating reactive oxygen species (ROS), detrimental to the plants (Huang *et al*., 2020).

Another dangerous pollutant toxic to living organisms is lead included among the nonessential trace elements, mainly accumulating in agricultural soils because of anthropogenic activities, generally showing an uneven distribution in the earth's crust, mainly present in a divalent form (Gottesfeld *et al*., 2018; Rizwan *et al*., 2018; Ghori *et al*., 2019). Lately, in agricultural areas its concentration has dramatically gone up because of increased anthropogenic emissions following mining, use of polluted water- sewage sludge for crop irrigation, pesticides, lead-acid batteries, smelting pigment additives as well as overuse of leaded benzene (Ozturk, 1989; Ashraf *et al*., 2015; Ozturk *et al*., 2015; Aziz *et al*., 2016; Sardar *et al*., 2018; Khan *et al*., 2019; Ozturk *et al*., 2019). The acceptable limits of lead vary in different countries but in general the range is 50-300 mg Pb kg^{-1} of soil (Rizwan *et al*., 2018). The soil microbial activity and plant growth are highly affected following excessive lead in the soil (Kushwaha *et al*., 2018; Rizwan *et al*., 2018). The increasing levels lead in the soils lead to increase in its concentration in plants. This ultimately leads towards the risk of increase in the lead toxicity through food crops (Gul *et al*., 2018; Lai *et al*., 2018; Rizwan *et al*., 2018). The critical phases of plant growth/development are immediately affected by the stress originating from lead, in particular the germination and early plant growth stages are critical ones which play a crucial role in the subsequent plant growth (Seneviratne *et al*., 2017; Rizwan *et al*., 2018). The cultivars, dose and soil type are highly affective in lead toxicity related to the serious effects on seed germination (Rizwan *et al*., 2018). The urban as well as industrial solid/liquid waste deposition, agricultural practices, and bedrock are some of the natural -man-made activities, highly involved in spoiling the land by adding heavy metals like copper and lead (Kumar *et al*., 2021). Their deposition on the land in high doses ends up with soil contamination, this affects the food crops cultivated in such area. These heavy metals reach living organism through a consumption of the vegetables growing in such areas. Due to aerial load, the vegetables are the most important food items in the human food chain are vegetables and following their heavy aerial load in our environment causes severe pollution, also proving effective in them (Lokeshappa *et al*., 2012; Kumar *et al*., 2021). The uptake of heavy metals like copper and lead is very important because if these accumulate in excessive amounts they can lead to serious health problems (Maleki and Zarasvand, 2008; Kumar *et al*., 2021).

We planned this investigation to find out the heavy metal content and mineral nutrient status following application of different doses of copper and lead and follow their stress effects using certified seeds of 8 different varieties of vegetables known as global economic crops.

Materials and Methods

Experimental details

As a test of global economic crops, 8 different cultivar plant seeds (Kale: *Brassica oleracea* L. 'Yalova01'; Artichoke: *Cynara scolymus* L. 'Bayrampaşa'; Rocket: *Eruca vesicaria* (L.) Cav. 'Roka Izmir'; Lettuce: *Lactuca sativa* L. 'Yedikule 5701'; Kidney bean: *Phaseolus vulgaris* L. 'Sırık'; Peas: *Pisum sativum* L. 'Biorillo'; Radish: *Raphanus sativus* L. 'Fındık'; and Black-eyed bean: *Vigna sinensis* L. 'Karnıkara') were used. The certified seeds of these crops were obtained from the Manisa Provincial Directorate of Agriculture and Forestry in Turkiye.

85 cells of the seedling trays were filled with sterilized peat up to the surface. Uniformly sterilized and seeds of 8 different cultivated vegetables were planted in each cell of the tray. The seedling trays were divided into 17 groups, each group included control and 8 different concentrations of each of the lead and copper solutions. All trays were irrigated with 15 mL of each 20, 40, 80, 160, 240, 320, 640, 1280 µM concentrations of Pb $(CH_3COO)2•3\ H_2O$ solutions, and 15 mL for each 20, 40, 80, 160, 240, 320, 640, 1280 µM concentrations of $CuCl_2•2\ H_2O$ copper solutions. These processes were applied 7 times in total, every two days. Each treatment had three replicates. At the end of 14 days of treatment, dry weight of the seedlings sown were measured (Candan and Batır, 2015; Batır *et al.*, 2016).

Determination of element content and statistical analysis

The seedlings were isolated in sterile bags and dried in glass Petri dishes at 80 °C for 24 hours. The samples were ground in a porcelain mortar and weighed. The samples weighing 0.150-0.200 g were placed in Teflon containers. 8 ml of 65 percent Merck-HNO_3 solution was added to these containers. These samples were digested with a Berghof-MSW2 microwave device and transferred into sterile 50 mL falcon tubes with ultrapure water (Human-Zeener Power I). These were then filtered with a Whatman blue filter.

The concentrations of elements boron (B), calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), lead (Pb) and zinc (Zn) (mg kg^{-1} dw) were determined in the seedlings of vegetables by inductively coupled plasma optical emission spectroscopy (ICP-OES / PerkinElmer - Optima 7000DV) (Ozturk *et al.*, 2017, 2019; Yalcin *et al.*, 2020).

All calculations were based on the parameters of seedlings. Pearson correlation and the one-way analysis of variance (ANOVA) were applied to the concentration values by using IBM SPSS Statistics 25 software. Statistical significance was expressed as $*p<0.05$ and $**p<0.01$ level (Aldrich, 2018; Ozturk *et al.*, 2019).

Quality control and quality assurance

B, Ca, Cu, Fe, K, Mg, Mn, Na, Pb, and Zn concentrations of seedling samples were measured in triplicate and average used for linearity. All elemental concentrations were determined with relative standard deviation of ±0.85-1.45% using standard solutions for calibration (Table 1). Seedling samples were digested according to the EPA 3051A Analytical Method for ICP-OES. Stock solutions of multi-element standards (Merck) were used to prepare calibration solutions by dilution (1,000 mg L^{-1}). To create the calibration lines, 8 different calibration solutions were used with appropriate concentrations to determine the concentration of each element with high precision ($R^2>0.999$) (Table 1).

Limit of Detection (LoD) and Limit of Quantification (LoQ) values were calculated by analysing blank solutions and using the mathematical expressions given below.

LoD= 〖Ø〗_n×SD (1)

LoQ= 〖Ø〗_q×SD (2)

In equations 1 and 2, SD refers for the standard deviation of 10 replicates of the blank solution, while the values of 〖Ø〗_n and 〖Ø〗_q explains multiplying factors of 3 and 10, respectively (Cao *et al.*, 2016; Goncalves *et al.*, 2019).

The spectral lines were chosen from relevant literatures (Barin *et al.*, 2012; Kim *et al.*, 2017; Goncalves *et al.*, 2019) for the determination of element concentrations in our samples and plasma torch positions were also tabulated in Table 1.

Table 1. ICP-OES parameters of the analytical method

Elements	Spectral Lines (nm)	Plasma Torch Position	LoD (mg kg^{-1})	LoQ (mg kg^{-1})	RSD (%)	R^2
B	249.677	Axial	0.022	0.073	1.03	.999923
Ca	317.933	Radial	0.415	1.383	1.15	.999815
Cu	327.393	Axial	0.074	0.247	1.06	.999863
Fe	238.204	Axial	0.236	0.787	0.85	.999871
K	766.490	Radial	0.305	1.017	1.23	.999902
Mg	285.213	Radial	0.258	0.860	1.06	.999845
Mn	257.610	Axial	0.111	0.370	1.25	.999937
Na	589.592	Radial	0.086	0.287	1.45	.999862
Pb	220.353	Axial	0.015	0.050	0.93	.999849
Zn	213.857	Axial	0.093	0.310	1.19	.999909

LoD: limit of detection; LoQ: limit of quantification;

RSD: relative standard deviation; R^2: determination coefficient.

Results

In all vegetable seedlings studied in this study, the concentration values applied to the seeds exposed to copper (Cu) stress for 14-days-old seedlings increased. An increase in Cu accumulation has been observed in the seedlings. Similar situation has been observed in the seeds exposed to lead (Pb) stress, with an increase in Pb accumulation (Tables 2-9).

The Cu exposure at 1280 µM showed no improvement when applied to artichoke (*Cynara scolymus* 'Bayrampaşa') seeds. Similarly, lettuce (*Lactuca sativa* 'Yedikule 5701') seeds also showed no improvement at Cu exposure 1280 µM and Pb exposure 1280 µM when applied separately. In all seedlings exposed to Cu stress, the concentration values of B, Ca, Fe, Mg, Mn, Na, Pb and Zn decreased; while in all seedlings exposed to Pb stress, the values of B, Ca, Cu, Fe, Mg, Mn, Na and Zn decreased (Tables 2-9).

An evaluation of the concentrations of mineral elements in the seedlings exposed to different concentrations of Cu and Pb stresses is given below. B, Ca, Fe, Mg, Mn, Na, Pb and Zn concentrations gradually decrease as the amount of Cu exposure increases in all vegetable seedlings. Same situation has been noted as the amount of Pb concentration increased, the concentrations of B, Ca, Cu, Fe, Mg, Mn, Na and Zn began to decrease. However, only potassium (K) concentration values of seedlings exposed to both Cu and Pb stresses differed according to the variety of vegetables. In kidney bean (*Phaseolus vulgaris* 'Sırık') seedlings, the potassium (K) concentration in seedlings tends to decrease gradually as the Cu exposure increases up to 80 µM. However, as compared to the control group, K is still high at exposed level of Cu 160 µM (Table 2). However, the K concentration in the seedlings decreases after Pb exposure increases up to 40 µM compared to the control group (Table 2).

Table 2. Heavy metals and mineral nutrient content (mg kg^{-1}) in the seedlings of kidney beans (*Phaseolus vulgaris* 'Sırık') exposed to different concentrations of Pb and Cu stresses

	B	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Pb**	**Zn**
Control	28.462±0.358	21075.924±735.519	8.461±0.29	222.881±1.386	24156.811±317.277	6457.117±180.349	195.041±2.878	153.735±4.675	3.104±0.108	43.628±1.178
Cu 20 µM	22.803±0.552	16860.829±85.118	43.128±1.186	178.411±5.497	26325.514±585.191	5165.705±155.316	156.081±0.793	123.025±0.435	2.564±0.072	34.964±0.906
Cu 40 µM	19.934±0.704	14753.168±536.871	60.994±0.052	156.057±2.846	27909.844±172.134	4519.991±111.217	136.56±3.486	107.721±1.451	2.211±0.087	30.542±0.441
Cu 80 µM	17.133±0.611	12645.634±314.492	72.108±1.919	133.821±1.846	29494.189±728.963	3874.33±23.995	117.095±0.445	92.284±2.014	1.954±0.019	26.182±0.158
Cu 160 µM	14.239±0.295	10538.034±98.65	110.934±2.567	111.469±1.05	25078.443±350.292	3228.594±90.605	97.602±0.996	76.966±0.014	1.617±0.033	21.825±0.552
Cu 320 µM	11.468±0.394	8430.412±76.652	168.522±4.032	89.2±1.67	19662.811±661.013	2582.928±27.665	78.052±2.141	61.568±0.486	1.285±0.029	17.559±0.281
Cu 640 µM	10.403±0.426	8022.414±102.363	211.008±0.816	79.259±1.047	18423.81±309.552	2183.867±14.947	66.046±0.989	52.597±0.331	1.051±0.027	14.468±0.459
Cu 1280 µM	8.55±0.271	6322.84±59.753	246.935±2.904	66.928±0.727	17247.112±354.937	1937.17±51.307	58.583±1.279	46.151±0.68	0.98±0.021	13.14±0.525
Pb 20 µM	22.811±0.377	16425.917±316.518	6.416±0.189	180.114±2.183	25466.338±461.984	5726.105±59.486	164.118±1.471	123.016±0.495	16.337±0.246	31.059±0.166
Pb 40 µM	19.974±0.339	15026.724±490.116	5.825±0.027	160.044±2.967	26105.447±168.472	5285.461±73.459	140.066±4.038	107.618±3.207	23.007±0.501	27.442±0.36
Pb 80 µM	17.112±0.675	13818.792±491.453	4.339±0.182	152.997±1.906	23411.935±536.687	4915.107±80.361	125.305±2.32	92.256±0.535	36.994±0.301	23.461±0.486
Pb 160 µM	14.256±0.471	12825.427±195.226	4.007±0.162	136.714±2.255	20118.136±445.318	4315.128±103.696	126.808±4.598	76.933±2.615	65.176±1.513	19.007±0.58
Pb 320 µM	11.415±0.347	10846.388±236.446	2.514±0.03	92.155±2.913	15042.733±271.247	4246.558±130.251	91.135±1.268	61.553±1.83	95.337±0.164	18.162±0.111
Pb 640 µM	9.413±0.114	9886.411±244.088	2.105±0.074	81.006±0.496	13765.422±167.616	4152.451±86.646	85.198±2.295	52.568±1.12	126.715±2.214	15.125±0.197
Pb 1280 µM	8.64±0.163	8465.132±174.612	1.834±0.031	71.135±0.54	10258.614±250.347	3814.107±49.108	72.224±2.559	46.2±1.828	175.366±1.218	13.881±0.173

In peas (*Pisum sativum* 'Biorillo') seedlings, potassium (K) concentration in seedlings tends to decrease gradually after the Cu exposure increases up to 40 µM. However, when compared to the control group, K is still high at exposed Cu 160 µM (Table 3); the K concentration in the seedlings starts to decrease after Pb exposure increases up to 40 µM compared to the control group (Table 3).

Table 3. Heavy metal and mineral nutrient content (mg kg^{-1}) in the seedlings of peas (*Pisum sativum* 'Biorillo') exposed to different concentrations of Pb and Cu stresses

	B	Ca	Cu	Fe	K	Mg	Mn	Na	Pb	Zn
Control	10.093± 0.139	2764.109± 99.786	3.864± 0.042	82.007± 1.316	12764.911± 286.162	1725.008± 53.346	25.073± 0.126	196.814± 4.289	1.205± 0.012	33.934± 0.433
Cu 20 µM	8.147± 0.154	2211.363± 65.329	9.374± 0.332	65.69± 0.66	13211.956± 421.841	1380.114± 34.046	20.096± 0.347	157.543± 4.057	0.998± 0.047	27.234± 0.276
Cu 40 µM	7.079± 0.194	1934.947± 17.701	15.073± 0.216	57.459± 1.405	18935.439± 158.226	1207.525± 13.23	17.566± 0.491	137.774± 3.417	0.881± 0.03	23.764± 0.549
Cu 80 µM	6.128± 0.079	1658.562± 27.15	21.683± 0.269	49.273± 0.081	17658.951± 334.449	1035.097± 12.632	15.139± 0.292	118.174± 0.568	0.762± 0.031	20.424± 0.459
Cu 160 µM	5.128± 0.118	1382.086± 7.253	35.072± 1.091	41.111± 0.013	16382.492± 210.266	862.508± 11.933	12.62± 0.289	98.415± 2.841	0.647± 0.011	16.997± 0.361
Cu 320 µM	4.085± 0.013	1105.754± 25.213	57.497± 1.054	32.867± 1.195	10106.03± 303.113	690.013± 13.777	10.078± 0.212	79.752± 3.059	0.489± 0.023	13.629± 0.059
Cu 640 µM	3.702± 0.047	987.708± 29.661	70.968± 2.491	28.879± 0.604	9319.07± 141.934	567.023± 12.201	8.05± 0.128	68.836± 0.539	0.461± 0.011	11.669± 0.21
Cu 1280 µM	3.104± 0.025	829.324± 31.315	94.785± 0.614	24.701± 0.426	8829.491± 29.182	517.526± 8.927	7.539± 0.13	59.076± 1.273	0.425± 0.015	10.25± 0.118
Pb 20 µM	7.898±\ 0.06	2071.643± 18.756	2.706± 0.073	69.922± 1.09	14661.157± 312.471	1596.357± 7.362	22.218± 0.293	180.75± 1.428	5.62± 0.077	18.64± 0.436
Pb 40 µM	6.4± 0.01	1762.587± 18.948	2.411± 0.104	51.288± 1.144	13328.613± 366.487	1446.889± 43.672	18.64± 0.398	152.967± 4.247	13.297± 0.249	16.327± 0.264
Pb 80 µM	5.894± 0.037	1653.682± 15.734	2.079± 0.067	42.453± 0.508	10995.954± 348.362	1097.204± 19.476	16.224± 0.525	133.49± 5.22	19.704± 0.507	14.052± 0.327
Pb 160 µM	5.601± 0.019	1344.786± 12.286	1.784± 0.071	38.764± 0.96	9663.442± 252.738	847.837± 18.726	15.748± 0.055	117.924± 2.643	25.869± 0.153	11.847± 0.27
Pb 320 µM	3.977± 0.048	1135.769± 34.229	1.457± 0.052	30.105± 0.379	8330.72± 216.281	798.243± 29.441	13.157± 0.109	100.334± 1.615	41.9± 0.919	9.487± 0.258
Pb 640 µM	3.479± 0.05	1035.758± 24.068	1.024± 0.04	25.082± 0.138	7652.788± 68.456	678.271± 11.669	11.101± 0.073	93.379± 0.896	63.92± 1.109	8.064± 0.086
Pb 1280 µM	2.556± 0.039	926.839± 7.754	0.986± 0.029	21.278± 0.326	5998.067± 24.242	548.65± 12.613	9.724± 0.015	89.756± 1.142	77.084± 2.038	7.141± 0.207

In black-eyed bean (*Vigna sinensis* 'Karnıkara') seedlings, the potassium (K) concentration in seedlings tends to decrease gradually after the Cu exposure increases up to 40 µM. However, when compared to the control group, K is still high at exposed Cu 80 µM concentration (Table 4). However, K concentration in the seedlings started to decrease after Pb exposure increased up to 40 µM compared to the control group (Table 4).

Table 4. Heavy metal and mineral nutrient content (mg kg^{-1}) in the seedlings of black-eyed bean (*Vigna sinensis* 'Karnıkara') exposed to different concentrations of Pb and Cu stresses

	B	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Pb**	**Zn**
Control	42.927± 1.219	12455.898± 150.942	16.308± 0.269	231.066 ±5.087	16452.288± 306.184	4315.108 ±0.818	142.033 ±4.394	1204.399 ±32.342	2.084± 0.03	28.614± 0.368
Cu 20 µM	34.439± 0.991	9964.749± 325.186	39.247± 0.62	184.874 ±6.351	19161.834± 253.342	3452.16± 62.076	113.666 ±0.187	963.593± 35.98	1.749± 0.016	22.988± 0.217
Cu 40 µM	30.134± 1.177	8719.23± 183.442	42.443± 1.012	161.815 ±3.786	21516.64± 608.843	3020.592 ±56.355	99.5± 2.624	843.169± 28.286	1.558± 0.039	20.091± 0.148
Cu 80 µM	25.798± 0.348	7473.591± 222.301	49.024± 0.463	138.666 ±2.763	19871.478± 339.356	2589.148 ±18.235	85.238± 2.71	722.7± 27.825	1.33± 0.011	17.228± 0.19
Cu 160 µM	21.478± 0.171	6228.034± 18.044	63.614± 1.144	115.54± 1.576	15226.208± 536.067	2157.604 ±30.016	71.069± 0.562	602.243± 19.937	1.095± 0.021	14.391± 0.12
Cu 320 µM	17.212± 0.365	4982.382± 59.67	93.515± 3.595	92.535± 0.746	13580.973± 360.657	1726.138 ±62.199	56.838± 0.674	481.775± 3.049	0.861± 0.013	11.54± 0.301
Cu 640 µM	14.172± 0.286	4661.414± 55.867	118.303 ±0.725	80.504± 1.358	11380.732± 347.131	1555.1± 48.702	50.91± 0.964	404.837± 3.277	0.706± 0.02	9.474± 0.039
Cu 1280 µM	12.937± 0.157	3736.815± 99.636	134.426 ±3.924	69.347± 0.404	10935.787± 274.645	1294.577 ±44.284	42.673± 0.593	361.379± 6.784	0.682± 0.036	8.677± 0.245
Pb 20 µM	25.347± 0.547	7274.285±1 24.224	9.671± 0.36	135.11± 3.135	17608.271± 149.894	2520.12± 92.593	83.188± 1.556	703.479± 16.367	6.64± 0.033	16.801± 0.295
Pb 40 µM	22.149± 0.307	6365.079± 246.461	8.616± 0.277	118.291 ±2.405	18407.201± 151.644	2205.197 ±35.685	72.697± 2.025	615.658± 12.741	10.561± 0.169	14.884± 0.204
Pb 80 µM	18.879± 0.235	5455.726± 46.508	7.402± 0.047	101.253 ±3.445	15206.187± 127.974	1890.192 ±64.855	62.306± 1.657	527.715± 10.293	19.793± 0.379	12.775± 0.139
Pb 160 µM	15.748± 0.137	4546.693± 37.649	6.101± 0.231	84.487± 1.965	14005.327± 209.758	1575.198 ±22.65	52.019± 0.784	439.728± 12.811	35.196± 1.141	10.522± 0.243
Pb 320 µM	12.712± 0.067	3637.209± 51.586	5.034± 0.134	67.666± 2.532	12804.247± 370.809	1260.268 ±39.503	41.7± 0.179	351.773± 8.564	44.445± 0.25	8.587± 0.264
Pb 640 µM	10.714± 0.047	3035.39± 41.535	4.296± 0.099	55.756± 0.954	11119.387± 128.215	1047.125 ±17.504	34.612± 0.51	311.824± 5.104	69.023± 0.921	7.468± 0.123
Pb 1280 µM	9.501± 0.039	2728.056± 19.855	3.633± 0.131	50.706± 1.394	10603.234± 99.763	945.044± 8.53	31.346± 1.058	263.982± 7.933	78.413± 2.317	6.378± 0.175

In artichoke (*Cynara scolymus* 'Bayrampaşa') seedlings, K concentration in the seedlings decreases after Cu exposure increases up to 80 µM compared to the control group (Table 5). However, K concentration in the seedlings tends to decrease gradually after the Pb exposure increases up to 40 µM. When compared to the studied control group K is still high at exposed Pb 320 µM (Table 5).

Table 5. Heavy metal and mineral nutrient content (mg kg^{-1}) in the seedlings of artichoke (*Cynara scolymus* 'Bayrampaşa') exposed to different concentrations of Pb and Cu stresses

	B	Ca	Cu	Fe	K	Mg	Mn	Na	Pb	Zn
Control	9.107±0.218	2617.008±73.865	2.071±0.036	62.083±1.589	12563.719±175.017	751.139±3.892	7.551±0.208	954.108±0.138	0.867±0.041	20.185±0.35
Cu 20 µM	7.328±0.108	2093.693±52.609	12.053±0.111	49.754±1.335	13051.003±105.913	600.941±11.442	6.06±0.208	763.342±20.444	0.699±0.027	16.151±0.234
Cu 40 µM	6.436±0.219	1831.991±46.473	31.425±1.155	43.512±0.649	15794.604±190.968	525.897±16.224	5.352±0.04	667.938±22.124	0.709±0.038	14.221±0.501
Cu 80 µM	5.526±0.114	1570.244±49.53	46.292±0.818	37.275±0.835	16538.308±430.077	450.742±7.025	4.629±0.157	572.47±11.96	0.558±0.014	12.192±0.013
Cu 160 µM	4.603±0.036	1308.593±17.542	78.08±0.961	31.051±0.593	12281.907±363.049	375.66±1.513	3.871±0.074	477.066±6.797	0.466±0.025	10.144±0.202
Cu 320 µM	3.644±0.049	1046.811±14.995	99.346±2.941	24.886±0.819	10025.556±335.296	300.552±1.642	3.074±0.101	381.74±7.234	0.421±0.015	8.149±0.096
Cu 640 µM	3.148±0.105	876.83±4.469	111.297±3.894	19.858±0.271	9725.556±144.521	281.563±8.579	2.818±0.106	351.682±4.332	0.35±0.014	7.805±0.232
Pb 20 µM	6.323±0.118	1800.663±44.236	1.649±0.035	42.856±0.789	14644.007±133.026	516.92±15.641	5.459±0.053	656.482±11.154	3.289±0.057	13.957±0.105
Pb 40 µM	5.719±0.101	1575.687±12.234	1.398±0.037	37.608±0.274	17563.414±386.322	452.321±11.255	4.761±0.181	574.585±12.804	14.338±0.059	12.225±0.253
Pb 80 µM	4.909±0.067	1350.667±37.18	1.262±0.028	32.264±0.536	16482.896±302.856	387.857±10.077	4.001±0.114	492.503±7.62	25.788±0.139	10.491±0.306
Pb 160 µM	3.974±0.015	1125.431±32.713	1.048±0.01	26.779±0.079	15402.443±155.664	323.159±2.013	3.437±0.051	410.45±5.101	47.498±0.366	8.903±0.172
Pb 320 µM	3.192±0.083	900.488±16.907	0.884±0.021	21.581±0.118	13322.076±360.797	258.661±5.437	2.725±0.098	328.275±0.759	62.041±0.859	7.064±0.044
Pb 640 µM	2.695±0.063	810.269±14.404	0.833±0.03	18.494±0.17	11102.054±282.941	228.447±3.786	2.447±0.086	301.413±0.946	74.288±1.014	6.216±0.182
Pb 1280 µM	2.293±0.085	675.421±15.218	0.765±0.04	16.061±0.211	10441.561±53.26	194.037±6.529	2.243±0.044	246.356±1.508	98.925±3.059	5.344±0.051

In kale (*Brassica oleracea* 'Yalova01') seedlings, K concentration in the seedlings tends to decrease gradually after the Cu exposure increases up to 80 µM. However, compared to the control group, K is still high at exposed Cu 640 µM (Table 6). The K concentration in the seedlings tends to decrease gradually after the Pb exposure increases up to 40 µM, but compared to the studied control group, K is still high at exposed Pb 320 µM (Table 6).

Table 6. Heavy metal and mineral nutrient content (mg kg^{-1}) in the seedlings of kale (*Brassica oleracea* ‘Yalova01’) exposed to different concentrations of Pb and Cu stresses

	B	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Pb**	**Zn**
Control	8.558±0.07	4964.138±80.594	20.007±0.325	516.764±18.901	3978.511±9.04	456.127±6.478	125.209±0.553	318.722±0.886	4.047±0.145	64.138±2.121
Cu 20 µM	6.893±0.147	3971.361±40.522	48.096±1.409	413.466±10.907	4182.903±27.154	364.959±13.387	100.185±1.159	255.064±5.072	3.315±0.036	51.339±2.034
Cu 40 µM	6.076±0.035	3474.935±15.013	52.039±1.544	361.781±6.961	4785.035±113.902	319.395±10.636	87.723±2.644	223.149±1.517	2.923±0.049	44.988±1.531
Cu 80 µM	5.189±0.072	2978.524±14.339	60.026±2.355	310.061±7.908	5387.146±4.042	273.728±7.895	75.152±2.996	191.304±0.115	2.537±0.032	38.56±0.317
Cu 160 µM	4.356±0.124	2482.177±44.872	78.123±2.514	258.472±8.415	4989.275±182.517	228.085±1.356	62.652±0.223	159.409±4.433	2.035±0.024	32.153±0.692
Cu 320 µM	3.452±0.105	1985.75±55.099	90.146±0.554	206.779±3.685	4591.474±4.926	182.523±1.008	50.14±1.392	127.544±4.936	1.684±0.074	25.67±0.421
Cu 640 µM	3.106±0.115	1725.665±4.464	108.3±1.938	186.728±0.356	4021.418±34.074	161.469±2.115	44.086±1.294	107.572±4.142	1.329±0.061	21.662±0.63
Cu 1280 µM	2.64±0.073	1489.308±16.327	128.129±3.498	155.06±1.312	3893.583±2.657	136.91±1.888	37.648±1.144	95.665±2.223	1.234±0.052	19.315±0.757
Pb 20 µM	4.831±0.091	2700.727±29.893	11.036±0.272	281.279±6.916	4364.466±168.108	248.345±1.814	68.133±0.883	173.586±3.649	11.677±0.364	35.082±0.023
Pb 40 µM	4.227±0.152	2363.127±21.745	9.632±0.246	246.097±2.794	4893.914±6.366	217.372±4.31	59.869±1.854	151.878±0.651	25.24±0.491	30.739±0.29
Pb 80 µM	3.59±0.022	2025.473±59.147	8.374±0.303	210.989±4.808	4623.363±21.244	186.273±2.738	51.336±1.753	130.221±0.161	40.992±1.509	26.243±0.266
Pb 160 µM	3.204±0.046	1687.869±51.264	6.901±0.059	175.821±0.734	4352.816±121.509	155.221±1.907	42.813±1.093	108.534±3.505	77.128±0.183	21.909±0.458
Pb 320 µM	2.439±0.068	1350.413±21.442	5.493±0.14	140.759±3.14	4082.396±52.209	124.344±4.102	34.307±1.338	86.755±0.052	93.926±2.847	17.677±0.012
Pb 640 µM	2.037±0.022	1250.495±22.269	5.061±0.054	122.699±3.966	3772.221±112.394	104.18±2.884	30.182±0.722	73.848±0.798	112.507±2.932	15.32±0.232
Pb 1280 µM	1.825±0.08	1012.943±19.31	4.313±0.117	105.569±0.253	3511.852±113.939	93.105±1.079	25.627±0.586	65.293±1.901	139.604±0.381	13.294±0.513

In lettuce (*Lactuca sativa* ‘Yedikule 5701’) seedlings, K concentration in the seedlings decreases gradually after Cu exposure increases up to 40 µM. As compared to the studied control group, K is still high at Cu 80 µM (Table 7) level exposure. The K concentration in the seedlings tends to decrease gradually after the Pb exposure increases up to 40 µM and compared to the studied control group, K is still high at exposed Pb 80 µM (Table 7).

Table 7. Heavy metal and mineral nutrient content (mg kg^{-1}) in the seedlings of lettuce (*Lactuca sativa* 'Yedikule 5701') exposed to different concentrations of Pb and Cu stresses

	B	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Pb**	**Zn**
Control	8.114± 0.066	1603.824 ±8.003	18.3± 0.085	140.088± 2.453	3425.946 ±73.779	312.482± 2.41	25.208± 0.873	91.462± 2.652	5.007± 0.201	51.743± 1.609
Cu 20 μM	6.561± 0.234	1301.096 ±0.936	43.952± 0.614	112.146± 3.823	3740.853 ±94.77	250.015± 0.09	20.23± 0.305	73.179± 1.065	4.094± 0.16	41.461± 0.924
Cu 40 μM	5.753± 0.187	1124.741 ±10.378	77.645± 2.206	98.091± 1.496	4398.26± 111.742	218.841± 7.377	17.755± 0.415	64.106± 1.133	3.545± 0.094	36.299± 0.384
Cu 80 μM	4.96± 0.074	978.295± 16.795	94.942± 1.127	84.138± 1.153	4055.654 ±93.338	187.593± 3.343	15.193± 0.126	54.921± 1.056	3.101± 0.118	31.09± 0.595
Cu 160 μM	4.073± 0.081	881.963± 4.653	131.48± 2.494	70.14± 0.216	3313.071 ±74.52	156.303± 0.933	12.678± 0.404	45.841± 0.97	2.614± 0.019	25.874± 0.138
Cu 320 μM	3.31± 0.127	765.544± 11.938	182.482± 4.098	56.06± 0.706	2970.439 ±14.829	125.01± 0.448	10.101± 0.342	36.667± 0.949	2.027± 0.057	21.704± 0.629
Cu 640 μM	3.189± 0.096	652.554± 22.901	219.053± 5.146	44.066± 0.604	2570.459 ±81.254	106.079± 1.852	8.114± 0.324	27.601± 0.124	1.705± 0.049	16.766± 0.26
Pb 20 μM	4.758± 0.165	1294.575 ±35.665	10.605± 0.121	80.847± 1.101	3973.434 ±152.703	180.116± 1.988	14.711± 0.569	52.744± 1.429	15.198± 0.607	29.864± 0.552
Pb 40 μM	4.243± 0.054	1082.843 ±1.597	9.443± 0.095	72.784± 0.016	4126.776 ±11.245	157.607± 0.102	12.826± 0.031	46.235± 0.657	23.033± 0.837	26.327± 0.196
Pb 80 μM	3.615± 0.035	870.958± 12.33	7.97± 0.305	63.632± 1.489	3780.244 ±66.948	135.055± 3.677	11.122± 0.095	39.675± 1.038	37.429± 0.408	22.45± 0.074
Pb 160 μM	3.145± 0.05	759.139± 28.109	6.606± 0.246	51.574± 1.584	3233.389 ±92.301	112.714± 0.527	9.303± 0.222	32.983± 1.224	55.379± 1.743	18.698± 0.627
Pb 320 μM	2.348± 0.026	717.436± 20.835	5.31± 0.025	40.575± 0.292	2986.827 ±19.304	90.208± 0.273	7.306± 0.094	26.559± 0.111	77.545± 0.778	12.034± 0.32
Pb 640 μM	2.088± 0.074	641.332± 24.193	4.513± 0.066	33.407± 1.036	2786.894 ±96.857	82.092± 1.088	6.403± 0.081	21.607± 0.364	98.731± 3.21	10.999± 0.302

In rocket (*Eruca vesicaria* 'Roka Izmir') seedlings, K concentration in the seedlings tends to decrease gradually after the Cu exposure increases up to 80 μM. But compared to the control group, K is still high at exposed Cu 320 μM (Table 8). The K concentration in the seedlings tends to decrease gradually after the Pb exposure increases up to 40 μM; but compared to the studied control group, K is still high at exposed Pb 160 μM (Table 8).

Table 8. Heavy metal and mineral nutrient content (mg kg^{-1}) in the seedlings of rocket (*Eruca vesicaria* 'Roka Izmir') exposed to different concentrations of Pb and Cu stresses

	B	Ca	Cu	Fe	K	Mg	Mn	Na	Pb	Zn
Control	31.934 ±0.316	5384.174± 190.333	25.811± 0.211	315.839± 2.031	12006.418± 303.395	486.117± 6.727	27.016± 0.656	416.339± 1.953	8.137± 0.127	361.104± 11.364
Cu 20 µM	25.621 ±0.705	4307.423± 90.181	61.987± 2.106	252.687± 9.459	14605.208± 160.959	388.915± 4.562	21.713± 0.115	333.161± 5.219	6.617± 0.271	288.895± 6.026
Cu 40 µM	22.391 ±0.821	3768.959± 10.164	67.216± 1.496	221.157± 5.673	17004.561± 597.261	340.376± 13.082	18.936± 0.513	291.499± 6.709	5.728± 0.124	252.774± 3.369
Cu 80 µM	19.208 ±0.205	3230.53± 61.999	77.446± 2.965	189.578± 4.132	17230.902± 234.292	291.686± 2.691	16.273± 0.486	249.834± 9.898	4.958± 0.049	216.688± 6.933
Cu 160 µM	16.011 ±0.396	2692.141± 18.477	100.762± 0.923	157.949± 6.054	16043.275± 504.485	243.098± 5.953	13.618± 0.51	208.221± 7.533	4.163± 0.045	180.572± 5.676
Cu 320 µM	12.829 ±0.037	2153.713± 52.849	116.331± 3.857	126.431± 2.088	12302.614± 407.157	194.459± 4.535	10.907± 0.389	166.553± 6.447	3.26± 0.023	144.455± 1.907
Cu 640 µM	10.797 ±0.18	1853.731± 71.275	139.642± 5.568	106.444± 3.491	11504.627± 292.522	170.463± 3.881	8.905± 0.347	142.588± 4.503	3.003± 0.114	114.449± 0.635
Cu 1280 M	8.62± 0.318	1615.253± 10.436	165.209± 6.025	94.837± 0.141	10411.991± 272.624	145.852± 0.994	7.161± 0.033	124.929± 3.667	2.541± 0.025	108.351± 4.102
Pb 20 µM	18.158 ±0.628	3015.29± 29.149	14.624± 0.205	176.997± 3.774	13723.721± 186.221	272.37± 9.537	15.293± 0.112	233.37± 2.749	24.064± 0.728	202.335± 0.146
Pb 40 µM	15.704 ±0.225	2638.514± 51.001	12.764± 0.142	154.83± 5.278	15883.238± 431.842	238.387± 1.135	13.429± 0.416	204.15± 7.223	31.363± 0.164	177.186± 6.993
Pb 80 µM	13.488 ±0.433	2261.434± 46.319	10.915± 0.24	132.81± 0.147	14042.856± 207.206	204.361± 3.43	11.486± 0.354	175.059± 6.423	43.365± 1.627	151.743± 3.409
Pb 160 µM	11.351 ±0.06	1884.707± 47.357	9.295± 0.36	110.652± 1.182	12202.252± 120.398	170.205± 4.962	9.65± 0.279	145.919± 2.196	55.994± 0.799	126.656± 4.896
Pb 320 µM	9.207± 0.211	1507.776± 23.335	7.421± 0.196	88.649± 1.883	10361.959± 318.203	136.322± 3.385	7.826± 0.147	116.811± 1.806	90.669± 2.591	101.224± 1.464
Pb 640 µM	8.189± 0.013	1337.868± 40.27	7.028± 0.083	75.689± 1.607	8861.885± 103.089	116.198± 1.092	7.166± 0.198	95.817± 0.526	108.33± 1.494	91.328± 2.258
Pb 1280µM	6.855± 0.074	1130.757± 13.525	5.676± 0.202	66.406± 0.905	7521.517± 74.392	102.135± 0.791	5.706± 0.129	87.659± 2.774	153.735± 5.258	75.936± 0.012

In radish (*Raphanus sativus* 'Fındık') seedlings, K concentration in the seedlings tends to decrease gradually after the Cu exposure increases up to 20 µM. When compared to the control group, K is still high at exposed Cu 40 µM (Table 9). K concentration in the seedlings tends to decrease gradually after the Pb exposure increases up to 40 µM (Table 9).

Table 9. Heavy metal and mineral nutrient content (mg kg^{-1}) in the seedlings of radish (*Raphanus sativus* 'Fındık') exposed to different concentrations of Pb and Cu stresses

	B	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Pb**	**Zn**
Control	2.143±0.028	1045.388±38.839	3.629±0.021	196.304±4.424	2341.096±34.962	615.399±16.725	62.388±0.78	75.31±1.913	1.086±0.042	97.324±2.472
Cu 20 µM	1.781±0.038	896.313±10.712	28.779±0.055	157.095±2.23	2872.973±89.696	492.372±6.541	50.01±0.509	60.34±1.887	0.928±0.025	77.889±1.843
Cu 40 µM	1.579±0.03	821.813±25.868	49.505±0.733	137.505±1.619	2638.864±78.866	430.802±7.89	43.765±1.148	52.828±1.292	0.77±0.025	68.127±1.805
Cu 80 µM	1.3±0.055	747.257±15.033	70.996±0.487	117.884±2.501	2104.747±59.732	369.299±12.513	37.493±0.574	45.241±1.711	0.746±0.025	58.429±0.025
Cu 160 µM	1.148±0.053	572.71±3.626	104.215±1.363	98.251±0.954	1970.614±19.185	307.762±7.795	31.227±0.796	37.723±0.837	0.565±0.019	49.668±1.476
Cu 320 µM	0.862±0.025	498.196±18.39	126.372±0.81	78.607±0.752	1836.462±46.829	246.177±3.144	24.996±0.254	30.157±1.076	0.493±0.016	39.012±1.279
Cu 640 µM	0.701±0.037	358.194±1.966	149.695±3.169	66.557±2.644	1656.465±9.027	216.195±1.253	20.064±0.227	26.192±0.174	0.404±0.011	33.028±0.119
Cu 1280 µM	0.647±0.033	323.63±1.863	173.297±4.986	58.968±0.153	1502.358±14.574	184.652±6.015	18.755±0.548	22.627±0.653	0.384±0.025	29.225±0.597
Pb 20 µM	1.502±0.014	777.268±4.325	2.415±0.08	125.761±2.774	2498.406±67.808	394.002±4.396	40.054±1.393	48.365±1.702	13.857±0.028	62.402±1.323
Pb 40 µM	1.401±0.063	617.592±13.289	2.212±0.042	109.975±4.102	2511.236±15.729	344.702±0.72	35.191±1.373	42.294±0.81	34.81±0.488	54.572±0.816
Pb 80 µM	1.307±0.046	557.995±7.566	1.901±0.086	94.419±2.65	2123.876±7.672	295.573±0.175	30.036±0.235	36.275±0.053	46.809±0.277	46.925±0.016
Pb 160 µM	1.088±0.047	398.282±10.159	1.565±0.064	78.618±3.139	1936.583±45.024	246.258±5.765	25.069±0.347	30.148±1.099	68.676±2.319	39.069±0.451
Pb 320 µM	0.743±0.031	338.576±9.705	1.381±0.038	62.879±1.331	1749.355±44.095	197.125±7.568	20.116±0.306	24.198±0.722	83.919±3.046	31.252±0.494
Pb 640 µM	0.612±0.034	216.586±5.875	1.105±0.03	53.96±0.523	1509.396±37.374	176.992±3.573	17.028±0.124	19.212±0.272	116.754±1.919	26.32±0.195
Pb 1280 µM	0.545±0.012	178.996±4.239	1.031±0.018	47.426±1.456	1461.971±4.781	147.979±1.413	15.244±0.095	15.172±0.184	141.955±5.215	23.514±0.072

The correlation coefficients of the values of elements in all vegetable seedlings analysed within the scope of this study are presented in Table 10. The table shows that a positive correlation exists between B and the availability of Ca, K, Mg, Mn as well as Na (>0.60, >0.74). High positive correlations are found between Ca and the availability of K, Mg and Mn elements (>0.75, >0.96); between Fe and the availability of Mn (>0.58); between K and the availability of Mg (>0.74); and finally, between Mg and the availability of Mn (>0.84) (Table 10).

Table 10. Correlation coefficients between the elements determined in the samples exposed to both Cu and Pb stresses

Correlation Matrix (R)									
Pearson Correlation	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Pb**	**Zn**
B	.735**	-.062	.379**	.684**	.655**	.597**	.593**	-.251**	.366**
Ca		-.045	.333**	.747**	.956**	.899**	.216*	-.118	-.015
Cu			-.051	-.036	-.101	-.062	-.160	-.450**	.022
Fe				-.017	.133	.575**	.113	-.253**	.435**
K					.743**	.485**	.384**	-.220*	.039
Mg						.842**	.198*	-.067	-.185*
Mn							.160	-.159	-.107
Na								-.276**	-.015
Pb									-.118

Note: **. Correlation is significant at the 0.01 level (2-tailed);
*. Correlation is significant at the 0.05 level (2-tailed).

Discussion

The correlation coefficients data on different elements in the seedlings exposed to Cu and Pb stress groups of the vegetable seedlings analysed within the scope of this study, are shown in Table 11. The correlation coefficient relationship in vegetable seedlings exposed to Cu stress is as follows; the table shows that a positive correlation exists between B and the availability of Ca, K, Mg, Mn as well as Na (>0.57, >0.74). High positive correlations are found between Ca and the availability of K, Mg and Mn (>0.76, >0.95); between Fe and the availability of Mn and Pb (>0.56, >0.60); between K and the availability of Mg (>0.77); between Mg and the availability of Mn (>0.82); and finally, between P and the availability of Zn (>0.81) (Table 11).

Looking at the correlation coefficient relationship in vegetable seedlings exposed to Pb stress we note that a positive correlation exists between B and the availability of Ca, Cu, K, Mg, Mn as well as Na (>0.57, >0.74); high positive correlations are found between Ca and the availability of K, Mg and Mn elements (>0.74, >0.96); between Cu and the availability of Fe and Zn (>0.75, >0.66); between Fe and the availability of Mn (>0.59); between K and the availability of Mg and Mn (>0.74, >0.52); and finally between Mg and the availability of Mn (>0.87) (Table 11).

One-way analysis of variance (ANOVA) with Tukey's post-hoc HSD test regarding the results of element values in the vegetable seedlings exposed to Cu stress and Pb stress are presented in Table 12. The table presents the results related to the examined 8 stress groups, including control (0 µM) group. Based on the results obtained, there are 4 different significant groups for Cu stress and 6 different significant groups for Pb stress. In the case of Cu stress; control-20-40-80 µM groups, 20-40-80-160 µM groups, 160-320-640 µM groups, and finally 320-640-1280 µM groups differ from others by showing significantly similar changes. For Pb stress; the control-20-40 µM groups, 20-40-80 µM groups, 80-160 µM groups, 160-320 µM groups, 320-640 µM groups, and finally 640-1280 µM groups differ from others by showing significantly similar changes.

Table 11. Separate correlation coefficients between the elements determined for the samples exposed to Cu and Pb (written in bold) stresses

Correlation Matrix (R)										
Pearson Correlation	**B**	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Pb**	**Zn**
B	1	.738**	-.278*	.329**	.663**	.679**	.567**	.608**	.449**	.360**
Ca	.738**	1	-.159	.292*	.756**	.954**	.878**	.252*	.168	-.028
Cu	.569**	.203	1	-.319*	-.176	-.195	-.221	-.406**	-.167	-.117
Fe	.425**	.377**	.753**	1	-.082	.080	.562**	.099	.600**	.417**
K	.706**	.743**	.040	.042	1	.768**	.450**	.332**	.051	.031
Mg	.650**	.962**	.001	.193	.739**	1	.823**	.268*	-.059	-.211
Mn	.629**	.918**	.258*	.590**	.523**	.866**	1	.186	.099	-.140
Na	.567**	.174	.212	.110	.442**	.136	.123	1	-.022	-.029
Pb	-.390**	-.175	-.376**	-.361**	-.326**	-.125	-.227	-.445**	1	.814**
Zn	.363**	-.008	.658**	.446**	.033	-.162	-.080	-.011	-.164	1

Note: **. Correlation is significant at the 0.01 level (2-tailed);
*. Correlation is significant at the 0.05 level (2-tailed).

Table 12. The one-way analysis of variance (ANOVA) with Tukey's post-hoc HSD were performed and means for group in homogeneous subsets were displayed. Tukey's post-hoc test were for stress group

Stress Type	Stress Group	Cu	Stress Type	Stress Group	Pb
	Control	12.306^{*a}		**Control**	3.192^{*a}
Cu	**20 µM**	35.827^{*ab}	**Pb**	**20 µM**	12.085^{*ab}
	40 µM	49.543^{**ab}		**40 µM**	21.956^{*ab}
	80 µM	61.564^{**ab}		**80 µM**	33.859^{**bc}
	160 µM	87.785^{**bc}		**160 µM**	53.865^{**cd}
	320 µM	116.776^{**cd}		**320 µM**	73.723^{**de}
	640 µM	141.033^{**cd}		**640 µM**	96.284^{**ef}
	1280 µM	157.130^{**d}		**1280 µM**	123.583^{**f}

Note: The levels of statistical significance were expressed as 0.01 (**) and 0.05 (*) levels

The most critical stages in the plant growth are germination and seedling development, as these are important early stages of growth and these initial phases determine the yield and successful future growth of plants (Moreira *et al.*, 2020). One of the most sensitive physiological processes is germination in plants, as this phase is more susceptible to heavy metal contamination, in particular if they reach embryo due to lack of defensive strategies (Moreira *et al.*, 2020). The first step in the life of a plant is germination and if it takes place in an adverse environment the seeds will hardly germinate (Ozdener and Kutbay, 2009; Moreira *et al.*, 2020). Its inhibition is the first defense mechanism which a seed possesses against unfavourable environmental conditions (Moreira *et al.*, 2020). In the plants germination and seedling growth are the critical stages, playing crucial role in future plant growth (Rizwan *et al.*, 2018).

The absorption and nutrient uptake is inhibited by the heavy metals which produce an affect on the overall composition of seedlings inducing phytotoxicity by impairing the plant nutrient metabolism (Kumar *et al.*, 2021).

A well-known fact is that copper is a necessary co-factor of various proteins (Cambrolle *et al.*, 2015) however, its optimal quantity is needed to ensure cellular roles, whereas excessive amounts lead to harmful affects on primary production and plant survival (Printz *et al.*, 2016; Kumar *et al.*, 2021). As a vital metal it takes part in many plants' metabolic activities, but its over uptake by roots can rigorously compromise plant growth and productivity. It then ends up in changes in the design of root system, oxidative stress, nutritional inequities and accretion of ROS (Kumar *et al*., 2021).

Ambrosini *et al*. (2018) have reported that, as the copper levels increase, a gradual decline in the quantity of K follows, stressing that copper in excess results in the nutrient uptake impairment as observed in our study as well. A prominent sign of copper toxicity is lesser nutrient uptake, and membrane permeability of membrane and the role of transporters are influenced adversely (Ambrosini *et al*., 2018; Kumar *et al*., 2021). The copper levels in the rhizosphere, treatment time and growth in such surroundings affects the plants (Adrees *et al*., 2015). Also, under copper stress complex dynamics of nutrient uptake and transport may be because of the competition between copper and nutrients for transporters, alterations in gene expression which participate in the uptake of nutrients at the transcriptional or post-transcriptional level and variations in plasma membrane permeability (Kumar *et al*., 2021). The varying nutrient levels in plants under copper stress are due to diverse tolerance approaches in different plants (Yruela, 2009).

Some studies by different researchers reported observed a decline in some nutrient uptake (especially K, Ca, Mg and Fe) in young grapevines under excessive Cu stress (de Oliveira *et al*., 2015; Toselli *et al*., 2009). In *Lactuca sativa* mineral nutrient accumulation under copper stress has been investigated by Shams *et al*. (2019). Their results show that under the highest 400 mM treatment of $CuSO_4$ the B, Mg, Na and Zn accumulation declined significantly in seedlings. Li *et al*. (2019) also reported that B, Ca, Fe, K and Mg contents declined with Cu stress in *Citrus* seedlings.

The nutrient uptake is affected by copper toxicity followed by allocation within plant tissues due to disruption of water homeostasis, ionic allocation and cell permeability barriers (Jung *et al*., 2003). Both antagonistic or synergistic interaction takes place during nutrient uptake, which may affect plant metabolism and growth (Chrysargyris *et al*., 2019). We can mention that excessive copper leads to nutrient uptake impairment (Kumar *et al*., 2021).

Lead is not essential for plant growth, but can bio-accumulate in different plant parts, especially inside the roots (Khan *et al*., 2019). In the case of lead uptake in plants, factors involved primarily include physiochemical characteristics of soil, concentration of lead and type of the plant (Clemens and Ma, 2016; Khan *et al*., 2019). The lead is primarily adsorbed onto root hairs; the reason being interaction with root epidermal cells polysaccharides (Seregin and Ivanov, 2001). Inside the plant roots these interfere in its passive uptake with the water stream (Pourrut *et al*., 2011). Almost 95 percent of lead is accumulated by plant taxa but retained in roots (Pourrut *et al*., 2011). After entering plants, growth and biochemical functioning are interfered at multiple levels including root growth, photosynthetic activity, nutrient uptake, seed germination as well as establishment and reduction in the plant biomass (Khan *et al*., 2019). Lead also interferes in the cellular metabolism, potentially leading towards an initiation and overproduction of ROS followed by the generation of an oxidative stress and affecting growth of plants, damaging vital biomolecules and may trigger cell death (Bidar *et al*., 2007; Khan *et al*., 2019). The high ROS production within sub-cellular compartments has been attributed to lead induced inhibition of antioxidant enzymes, as these detoxify excessive ROS (Shahid *et al*., 2014). The antioxidant activities of these enzymes vary among different plant taxa or even different plant parts; these are mainly correlated with tissue lead concentrations (Khan *et al*., 2019).

Depending upon cultivars and doses; lead toxicity results in destructive effects on seed germination. Its inhibitory effects on seed germination in cereals are well known; including rice, wheat and maize (Hussain *et al*., 2013; Ahmad *et al*., 2011; Lamhamdi *et al*., 2011). In all plants generally mineral nutrition is part and parcel of the proper growth and development. A negative correlation has been reported in the case of plant mineral nutrient content and lead stress in many plants (Lamhamdi *et al*., 2013; Rizwan *et al*., 2018). Similar behaviour has been reported in maize cultivars with a decrease in the concentrations of calcium, copper, magnesium, sodium and potassium under higher levels of lead contamination in a dose-additive manner (Sing *et al*., 2015).

In wheat 5- to 30-days old seedlings exposed to lead (0, 1.5, 3, and 15 mM) there is a reduction in the concentration of calcium, copper, iron, magnesium, sodium and zinc in the plants (Lamhamdi *et al*., 2013).

Conclusions

The data published by different workers shows that copper and lead accumulation result in a decrease in the elemental movement as a result promoting their accumulation in all vegetable seedlings growing in the area. The uptake of mineral elements and their distribution in the tissues is inhibited by their toxicity which creates deficiencies or imbalances in mineral elements, thus impairing plant growth (Feng *et al*., 2018; Zeng *et al*., 2020).

Authors' Contributions

Conceptualization: FC, MO, VA and IEY; Investigation: MO, VA and IEY; Methodology: FC, MO, VA and IEY; Formal analysis: IEY and VA; Writing-original draft: MO and VA; and Writing-review and editing: MO and VA. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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