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Effect of Soil Contamination and Antibiotics Application on Growth and Some Physiological Traits of Beans in Greenhouse Condition

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Author
Ziba Najafzadeh Nobar, *Ph.D. Student.*[1*]
Ali Akbar Safari Sinegani, *Ph.D.*[2*]

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[1] Department of Soil Science, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran.
[2] Department of Soil Science, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran.

**** Correspondence***
Address: Department of Soil Science, Faculty of Agriculture, Bu-Ali Sina University, Shahid Mostafa Ahmadi Roshan Street, Hamedan
Cell: (+98) 914 100 6482
Tel: (+98) 813 4424366
Fax: (+98) 813 4424012
Postcode: 6516738695
Email: z_najafzadeh2002@yahoo.com

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ABSTRACT

Aims: The purpose was to specify the impacts of amoxicillin, cefixime, and metronidazole on sunray cultivar of green bean (*Phaseolus vulgaris* L.) growth and some of its metabolites, which were cultured in soils with various quantities of heavy metals.

Materials & Methods: For evaluation of the effects of the antibiotics (i.e., Amoxicillin, Cefixime, and Metronidazole) on plant growth, the Antibiotics were tested in 2 doses of 100 and 200 $mg.kg^{-1}$ soil, and zero for the control. After 42 days fresh and dry weights of green bean shoots and roots were analyzed, and before harvesting the quantity of Chlorophyll (Chl) a, Chl b, Carotenoid (Car), Flavonoids, Phenol, and Antioxidant activity in *P. vulgaris* leaves were measured.

Findings: Green bean treated with Amoxicillin (200 $mg.kg^{-1}$) produced the highest root dry weight in mine soil (42.91% increase compared to control) while it treated with cefixime (200 $mg.kg^{-1}$) produced the highest shoot dry weight at rangeland soil (19.21% increase compared to control). The quantity of Flavonoids (18.91%), Phenol (19.70%), Chl a (37.72%), and total Chl (37.40%) in the leaves of the plant with 200 $mg.kg^{-1}$ Metronidazole reduced in compared to their controls. The results showed that antioxidant activity in green bean tissues was enhanced in mine soil compared to agricultural and rangeland soils (37.76% and 18.43% respectively).

Conclusion: Though soil contamination with heavy metals and the usage of metronidazole had stressful results on green beans, these were not additive or synergetic. Residues of Antibiotics often enter agricultural soils through animal manure, so it is suggested to use at least decomposed manure to control their stressful effects and the arrival of Antibiotics into the food chain.

Keywords: Amoxicillin; Antioxidant; Cefixime; Metronidazole; Mine soil; *Phaseolus vulgaris*.

CITATION LINKS

[1] Prosser R.S., Sibley P.K. Human health ... [2] Liu F., Ying G.G., Tao R., Zhao J.L., ... [3] Farkas M.H., Berry J.O., Aga D.S. ... [4] Bewick M.W.M. ... [5] Kang D.H., Gupta S., Rosen C., Fritz V., Singh A., Chander Y. ... [6] Hu X., Gao Y., Fang Z. Integrating ... [7] El-Saadony M.T., Desoky E.S.M., Saad A.M., Eid R.S., ... [8] Desoky E.S.M., Elrys A.S., Rady M.M. ... [9] Yang L., Ren Q., Zheng K., Jiao Z., ... [10] Akhzari D., Shayganfar A. The Interaction of Artemisia ... [11] Ullah R., Hadi F., Ahmad S., Jan A.U., ... [12] Li M., Yang L., Yen H., Zhao F., Wang X., Zhou T., Chen L. ... [13] Farshad A. Soilscape ... [14] Helrich K. Official methods of analysis of ... [15] Srivastava V., Nerwal D.K., Kandan A., Akhtar J., ... [16] Arnon D.I. Copper enzymes ... [17] Mackinney G. Absorption of light by ... [18] Bedreag C.F.G., Trifan A., Bucur L.A., Arcus M., Tebrencu C., Miron A., Costache I.I. ... [19] Tezcan F., Gültekin-Özgüven M., Diken T., Özçelik B., Erim F.B. Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. Food Chem. 2009; 115(3): 873-877 ... [20] Yoo K.M., Lee C.H., Lee H., Moon B., Lee C.Y. ... [21] Brand-Williams W., Cuvelier M.E., Berset C.L.W.T. ... [22] Burda S., Oleszek W. Antioxidant and... [23] Guzel S., Terzi R. Exogenous hydrogen ... [24] El-Juhany L.I., Ali H.M., Basalah M.O., Shehatah A.M.S. ... [25] Sinegani A.A.S., Younessi N. Antibiotic ... [26] Xiong W., Wang M., Dai J., Sun Y., Zeng Z. Application of... [27] Yu X., Liu X., Liu H., Chen J., Sun Y. ... [28] De Filippis L.F., Ziegler H. Effect of ... [29] Elloumi N.B., Rhouma F., Ben A., Mezghani B.I., Boukhris M. ... [30] Van der Werf A., Nagel O.W. Carbon allocation ... [31] Koevoets I.T., Venema J.H., Elzenga J.T., Testerink C. Roots ... [32] Allito B.B., Ewusi-Mensah N., Logah V., Hunegnaw D.K. ... [33] Chandra R., Kang H. Mixed heavy metal ... [34] Kümmerer K. Antibiotics in ... [35] Opriş O., Soran M.L., Coman V., Copaciu F., Ristoiu D. ... [36] Khan K.Y., Ali B., Zhang S., Stoffella P.J., Yuan S., Xia Q. ... [37] Van Assche F., Clijsters H. ... [38] Rydzyński D., Piotrowicz-Cieślak A.I., Grajek H., Michalczyk D.J. Chlorophyll ... [39] Urwat U., Zargar S.M., Ahmad S.M., Ganai N.A. Insights into role of STP13 in sugar-driven signaling that leads to decrease in photosynthesis in dicot legume crop ... [40] Ilboudo O., Tapsoba I., Bonzi-Coulibaly Y.L., Gerbaux P. Targeting ... [41] Ampofo J.O., Ngadi M. Stimulation of the phenylpropanoid pathway and antioxidant capacities by biotic and abiotic elicitation strategies in common bean (Phaseolus vulgaris) sprouts. Process ... [42] Kosyk O.I., Khomenko I.M., Batsmanova L.M., Taran N.Y. ... [44] Hadi F., Ali N., Fuller M.P. Molybdenum (Mo) increases endogenous phenolics, proline...

Introduction

Antibiotics are used to control microbial diseases in humans, animals, or plants, and are sometimes excreted as bioactive metabolites after treatment. Unlike pesticides, Antibiotics are considered potential contaminants. Antibiotics enter the food chain through the soil-plant system [1]. Animal feces and sewage sludge may be the sources of Antibiotics in the environment. Antibiotic contamination may impair plant growth, photosynthetic, and Antioxidant activity. However, different plants have various vulnerabilities to antibiotics. Therefore, it is necessary to study the environmental effects (such as plant uptake, effects on soil organisms, Bacteria, and Antibiotic resistance) of Antibiotics related to the application of Animal manure and wastewater in agricultural soil [2].

The effect of Antibiotics on plants varies in soils and between plant species [3]. Antibiotic contamination in vegetables is important for food safety. Among the first research that examined the uptake of Antibiotics from fertilizers by plants, there was a research in which the fermented wastes of tylosin and terramycin (Oxytetracycline) were applied as fertilizer for tomatoes of the variety Eurocross B (*Solanum lycopersicum* L.) [4]. In the results obtained from this research, the presence of Antibiotics in plants grown in fermentation waste mixed with compost-containing peat was not seen [5]. Examining the metabolic changes of plants exposed to Antibiotics can show how plants respond to Antibiotic stress [6].

It is expected that in the future, due to the increasing population, legumes will be one of the suitable options for healthy nutrition. Furthermore, legume species are uniquely suited to enhance soil productivity and provide nutrient-enriched grains and vegetables for limited-resource farmers. It should also be said that *P. vulgaris* is one of the main legumes in human and livestock food. Beans are supposed to be the main legumes due to their high protein and carbohydrate content [7]. Green beans are used worldwide for their healthy nutritional properties. Beans are sometimes grown in soils contaminated with heavy metals, such as around factories or industrial areas. On the other hand, water and soil contaminated with heavy metals can have detrimental effects on the productivity of plant products. The lack of toxicity of minerals is one of the stresses that can affect the important functions of plant cells. The response to nutritional stress requires the regulation of systems involved in the absorption and transport and distribution of mineral ions [8].

The entrance of heavy metals into the food chain can threaten human and animal health, impair plant growth, and reduce the plant's accumulation capacity for metals. In a study, 43 pairs of soil and grape samples were assembled from China to determine the heavy metal (i.e., Pb, Cd, Cu, Zn, and Ni) pollution amount in soil, and the potential health risk by use of grapes. Studies have shown that people who regularly use grapes from this region are at risk of developing cancer [9]. Biotic and abiotic stresses have negative impacts on the plants' survival capacity [10]. In response to the toxicity of heavy metals, plants have a defense mechanism that includes enzymes and secondary metabolites such as Phenol, Proline, and Flavonoids. These secondary metabolites can act as free radical scavengers, plant protection, metal chelators, osmoprotectants, and stress tolerance [11].

Due to the annual consumption of Antibiotics (medical and veterinary) has reached about 100,000 tons worldwide, and nearly 30%-90% of Antibiotics will be returned either untransformed or as active metabolites into the environment [12] for the first time, the reaction of green beans (*P. vulgaris*) to the usage of Amoxicillin, Cefixime, and Metronidazole with heavy

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metals is investigated. The immune response of green beans is assessed by measuring Chl, Antioxidants, and Phenol. Therefore, the main goal of this research is to know the consequences of the application of Antibiotics on the responses of green beans in different amounts of heavy metals in soils.

Materials & Methods

Soil preparation

In this research, seeds of green bean (*P. vulgaris*) sunray cultivar was gotten from SPII (Seed and Plant Improvement Institution) Karaj, Iran.

Soil samples were taken from three various sites with different amounts of heavy metals (such as Cd, Cu, Fe, Mg, Mn, Pb, and Zn) in Hamadan province, with a semiarid climate. Agricultural soil, rangeland soil, and metal-contaminated mine soil were prepared for this research with various heavy metal contents. Agricultural soil samples were taken from the research farm of Bu-Ali Sina Faculty of Agriculture, Hamadan, Iran. This farm hadn't captured any Antibiotic-containing manure for at least five years. Most likely, this soil is in the Inceptisols category [13]. This land was fallow at the time of sampling. Two composite samples were obtained in triplicates by the top 30 cm layer (under the influence of plant roots) of tailing, rangeland, and contaminated mine soils in the Ahangaran site. Ahangaran mine is located 23 kilometers east of Malayer city. Its temperature range is from -5 to +23 degrees Celsius. It has medicinal plants, grasses, and mint types. The geographic peculiarities of the agricultural, Ahangaran mine, and rangeland soils are longitude 48°28'53" E, latitude 34°47'59" N; longitude 48°59'53" E, latitude 34°10'24" N, altitude 2029 (m), and longitude 48°58'53" E, latitude 34°9'33" N, altitude 1957 (m) respectively. Once air-dried, 2mm sieved soil samples were prepared and kept in special containers.

Table 1) Physicochemical characteristics of the researched soils (sig<0.001).

	Agricultural soil	Rangeland soil	Mine soil
pH (1:2)	6.74±0.03c	7.33±0.02a	7.16±0.01b
EC ($dS.m^{-1}$) (1:2)	0.44±0.00b	0.38±0.00c	0.50±0.00a
Cation exchangeable capacity ($cmol_c.kg^{-1}$)	19.75±0.00a	20.37±0.14a	4.12±0.27b
Sand%	58.96±0.62b	38.05±0.53c	65.90±0.30a
Silt%	26.73±1.12c	43.99±0.99a	31.01±0.30b
Clay%	14.30±0.82b	17.95±0.54a	3.07±0.30c
CaCO3%	1.43±0.22c	19.38±0.22a	11.35±0.36b
Organic carbon content (%)	0.90±0.02a	0.97±0.01a	0.32±0.02b
Organic matter content (%)	1.56±0.04a	1.68±0.02a	0.56±0.03b
Available K (mg. kg^{-1} dry soil)	161.20±1.35b	542.60±2.71a	3.96±1.33c
Available Na (mg.kg^{-1} dry soil)	161.75±5.00ns	175.98±2.00ns	164.74±10.46ns
Available P (mg. kg^{-1} dry soil)	21.66±0.45a	16.21±0.13b	6.94±0.28c
Soil texture	Sandy Loam	Loam	Sandy Loam

*Values are the mean± standard error. In each row, the values with similar letters are not significantly different according to Duncan's test (P<0.05), ns: non-significant.

Soil physical and chemical properties were measured according to standard methods (Table 1) [14]. Soil texture analysis was made using the hydrometer method. Soil pH and electrical conductivity were evaluated in extract a ratio of 1:2 (w/v) of soil to distilled water by a glass electrode. Organic matter was evaluated using dichromate oxidation, equivalent Calcium Carbonate was assessed by titration with acid, available K and Na were measured by the Ammonium acetate method, and Cation exchange capacity was also evaluated by the Ammonium acetate 1N method, and available P was measured in Sodium Bicarbonate extract. Soil heavy metal was analyzed with Varian SpectrAA 220FS PARTS (Table 2) [15].

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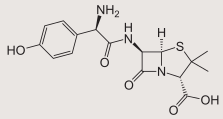
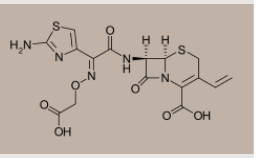
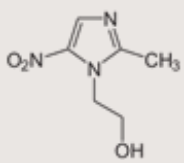
Table 2) Concentrations ($mg.kg^{-1}$) of heavy metals in soil samples.

Heavy metals	Agricultural soil	Rangeland soil	Mine soil
Cd	0.75±0.00b	1.97±0.01b	37.53±0.94a
Cu	16.45±0.11c	45.66±1.35b	89.58±2.31a
Fe	22691.66±557.16b	29835.00±584.07b	73110.00±8165.58a
Mg	6052.08±163.71ns	6365.62±43.73ns	4697.32±1269.40ns
Mn	387.50±7.21b	2200.00±37.52b	9816.66±1106.54a
Pb	33.20±1.13c	595.03±20.76b	9749.66±208.68a
Zn	58.33±4.10b	25.04±2.00b	3839.20±297.00a

*Values are the mean± standard error. In each row, the values with similar letters are not significantly different according to Duncan's test (P<0.05), ns: non-significant.

Mostly used Antibiotics in the universe including Amoxicillin (β-lactams), Cefixime (Cephalosporin), and Metronidazole (Nitroimidazole) were purchased from a pharmacy. Drugs were made by Daana Pharma Co. Tabriz-Iran (Amoxicillin and Cefixime) and Pars Darou Pharma Co.Tehran-Iran (Metronidazole) was used. Amoxicillin was offered in capsule form, and Cefixime and Metronidazole were presented in tablet form. Every Amoxicillin capsule was containing 500 mg (chemical formula; $C_{16}H_{19}N_3O_5S$), Cefixime tablet containing 400 mg ($C_{16}H_{15}N_5O_7S_2$), and Metronidazole 500 mg ($C_6H_9N_3O_3$) (Table3).

Table 3) Properties of the studied antibiotics.

Compound	Molecular weight ($g.mol^{-1}$)	Structure
Amoxicillin	365.40	
Cefixime	453.44	
Metronidazole	171.156	

All the other used chemicals in this research like those used in the measurement of some plant metabolites or soil heavy metal analysis were from Merck Co., Germany.

Greenhouse Experiments

To evaluate the effect of the Antibiotics on plant growth, the Antibiotic test was done using Amoxicillin, Cefixime, and Metronidazole in 2 doses of 100 and 200 $mg.kg^{-1}$ soil [16]. Aqueous solutions of Amoxicillin, Cefixime, and Metronidazole were provided by distilled water and added to the weight of 1.5 kg dry soil, and were thoroughly mixed. While mixing, water was added to the soil to the moisture value of near field capacity. For every Antibiotic treatment, two concentrations and a control treatment (without Antibiotic) in three replicates and a total of 63 experimental pots were designed. The experiments were operated in plastic pots (13 cm with a height of 10 cm). Next into each plastic pot, 5 seeds were implanted at a depth of 2.5 cm (one dry & 4 soaked to ensure the seeds germinate). The treated plastic pots were put in the greenhouse. Ten days after seed germination, the number of bean plants in each pot was reduced to two. Throughout the experiment time, the soil water moisture was held every day by adding a suitable quantity of water. The plants in the pots were harvested on the

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42nd day at the end of the flowering stage.

Growth and photosynthetic pigment

Fresh and dry weights of green bean shoots (SDW) and roots (RDW) were sampled and the ratio of SDW/RDW was estimated for all treatments.

Car and Chl a & b amount in *P. vulgaris* were measured according to the following procedure [17-19]. Fresh leaves (0.25 g) were rubbed in a mortar with liquid nitrogen then homogenized in 80% acetone (5 mL) and centrifuged (3,000 rpm) for 10 min. Supernatants were separated into test tubes. Absorbance was obtained at 645, 663, and 470 nm by spectrophotometer (model UV-2100).

Computation of Chl a, Chl b, and Car value:

$$Chla_{(mg.g^{-1})} = [(12.7_{\times}Abs_{663})-(2.69_{\times}Abs_{645})]_{\times} [V/(1000_{\times}0.25)] \quad \text{Eq.(1)}$$

$$Chlb_{(mg.g^{-1})} = [(22.9_{\times}Abs_{645})-(4.68_{\times}Abs_{663})]_{\times} [V/(1000_{\times}0.25)] \quad \text{Eq.(2)}$$

$$Car_{(\mu g.ml^{-1})} = [(1000_{\times}Abs_{470})-(1.82_{\times}Chl\ a)-(85.02_{\times}Chl\ b)]/198 \quad \text{Eq.(3)}$$

Where Abs is = absorbance measured with the sample analysis at 663, 645, and 470 nm and V is = extract volume.

Phenolic and Flavonoid measurement

The extract of plant leaves was obtained from 0.5 g of fresh leaves using liquid Nitrogen and 5 mL of 85% methanol and they were left at room temperature for 1h under persistent stirring, then the solution was centrifuged (3,500 rpm) for 15 min [20].

The total amounts of Phenolic phytochemicals were obtained utilizing the Folin–Ciocalteu procedure. 1.5 milliliter of 10% Folin–Ciocalteu phenol reagent was put into 300 microliters of the extracts and put in the dark at room temperature. After 5 min, 1.2 mL of 7.5% Na_2CO_3 solution was added to the mix. Then the samples were shaken for 90 min and the absorbance was read at 765 nm by a spectrophotometer. The total Phenolic amounts were represented in mg of Gallic Acid equivalents (GAE) per 1g of a fresh leaf [21].

Computation of phenol quantity:

$$TP_{(mgGAE.g^{-1}FW)} = [(Y/1000)_{\times}(V/FW)] \quad \text{Eq.(4)}$$

$$Y_{(\mu g.ml^{-1}GA)} = (51.813_{\times}Abs_{765})-2.399 \quad \text{Eq.(5)}$$

Where Y = Concentration earned from the standard curve and
V = Extract volume and
FW = Fresh weight and
Y/1000 = To turn micrograms into milligrams and
Abs = Absorbance earned with the sample analyzed at765 nm

The Flavonoid content was obtained using a colorimetric procedure. 275 microliter aliquot of the extracts was put into 0.3 mL of 5% (w/v) Sodium Nitrite. After 5 min, 0.6 ml of 10% (w/v) $AlCl_3$ was added, and, after 6 min 2 mL of 1M NaOH was also put into the mixture, accompanied by the addition of 1 mL distilled water. Absorbance was measured at 510 nm and the Flavonoid amount was represented as mg quercetin equivalents (QUE) per 1 g of a fresh leaf [22].

$$Y_{(\mu g.ml^{-1}QU)} = (166.67_{\times}Abs_{510})+4.5 \quad \text{Eq.(6)}$$

$$Total\ Flavonoids_{(mgQUE.g^{-1}FW)} = [(Y/1000)_{\times}(V/FW)] \quad \text{Eq.(7)}$$

Antioxidant activity determination

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a free radical used pro determining Antioxidant activity. Reduction of DPPH by an Antioxidant or by a radical species eventuates in a loss of absorbance at 515 nm. For determination of Antioxidant activity via the DPPH, a solution of DPPH (60 µM) was provided in 85% Methanol as 2.4 µg DPPH was added to 100 mL Methanol (This solution is prepared daily). 75 Microliter of the extracts was added to 2.925 mL of DPPH solution added to the flask in the dark at room temperature (~25°C) and vortex. After

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30min, absorbance at 515 nm was measured in a spectrophotometer [23]. The sample concentration with initial absorbance closest to that of the blank (DPPH+ solvent) was chosen for determination of antiradical activity (ARA), specified by Eq. (8) [24]:

$$ARA = 100\times[1-(Abs_{\text{of sample}}/Abs_{\text{of DPPH}})] \quad \text{Eq. (8)}$$

Blank DPPH = 0.783

Proline estimation

The dried milled leaves (0.25 g) were applied for proline extraction. Samples were thoroughly homogenized in 5 ml 3% Sulfosalicylic-Acid being beaten in a mortar, after shaking the analyze tube for 2 minutes, the liquid and solid phases were separated, then extracts were centrifuged at 3500 rpm for 10 min. The top part of the main liquid was separated and 1 ml of filtrated extract was mixed with 5 ml distilled water, then 1 ml was mixed with 1 ml of Acetic Acid and 1ml ninhydrin reagent (1.25 g ninhydrin, 30 ml of glacial Acetic Acid, 20 ml 6M H_3PO_4) and it had been placed in Bain Marie for 45 min at 100°C. The reaction was discontinued by putting the analyzed tubes into ice-cold water. The samples were severely blended with 3 ml toluene. After 30 min, the absorption of the toluene phase was determined at 520 nm by a spectrophotometer. The proline concentration was obtained utilizing a standard curve. Free proline quantity was represented as $\mu g.g^{-1}$ dry weight [25]. The concentration of proline was calculated according to Eq.(9)[26].

$$\text{Proline } \mu g.g^{-1}\text{dry weight} = [(\text{ml toluene})\times(\mu g\ \text{proline}.ml^{-1})]/(g\ \text{sample}\times 2/3) \quad \text{Eq.(9)}$$

Statistical analysis

Data were analyzed using a factorial experiment in a completely randomized design in which soil at three levels was at the first factor(agricultural, rangeland, and mine soil)and Antibiotics at seven levels (without Antibiotic, Amoxicillin 100 & 200 $mg.kg^{-1}$, Cefixime 100 & 200 $mg.kg^{-1}$ and Metronidazole 100 & 200 $mg.kg^{-1}$) was the 2nd factor. Control and treatment tests were performed in triplicates and data were statistically analyzed by SPSS (version 20) software. The normality of the residuals was determined with the Shapiro-Wilk test ($p>0.05$). Except for the data related to Chlorophyll a and Phenol, the rest of the data were standardized by the z-score normalization. The homogeneity of variance test was significant for Flavonoid data ($p>0.05$). By performing multivariate analysis of variance (MANOVA), the average values were compared by Tukey's multiple comparison test ($P\leq 0.05$). On the other hand, the average values of soil physicochemical properties and heavy metals were compared by Duncan's multiple comparison test ($P\leq 0.05$). Results were represented as mean± standard error of replicates.

Findings

Heavy metal content in the soils

The total concentration of heavy metals in soils is represented in Table 2. The presented amounts are the average of three values of researched soils. The concentrations of heavy metals in the soils were as the following: 0.75–39.16 $mg.kg^{-1}$ for Cd, 16.25–92.50 $mg.kg^{-1}$ for Cu, 22037.50–84140.00 $mg.kg^{-1}$ for Fe, 2177.25–6423.73 $mg.kg^{-1}$ for Mg, 375.00–11635.00 $mg.kg^{-1}$ for Mn, 31–10157.88 $mg.kg^{-1}$ for Pb and 21.06–3567.75 $mg.kg^{-1}$ for Zn.

Shoot and Root dry weight

Plants SDW and RDW were significantly influenced by the interaction between soil and Antibiotic treatments ($P<0.05$). Dry weight could reflect the growth and development of plants. Green beans treated with Cefixime 200 created the greatest SDW in rangeland soil (0.94±0.12 g) while plants treated with Metronidazole100 created the least SDW in mine soil (0.05±0.03 g) (Figure 1). The use of 200 mg of Metronidazole per kg of rangeland soil significantly ($p<0.05$)

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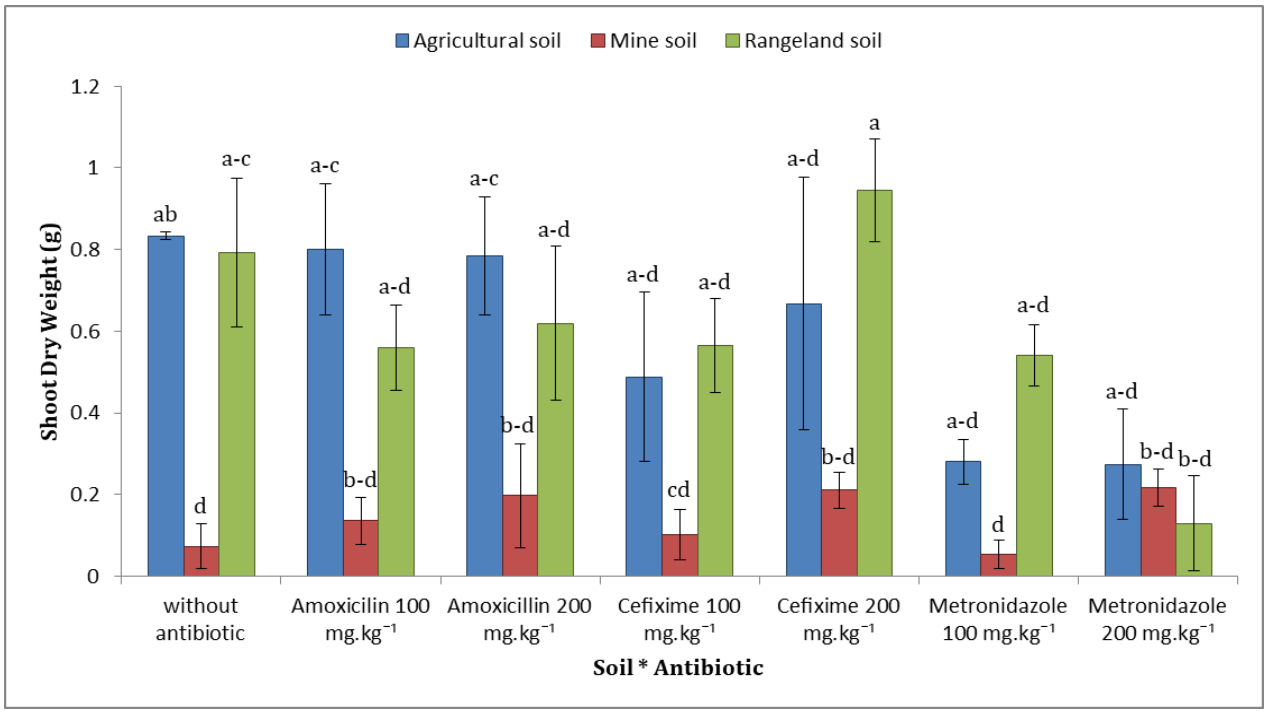

Figure 1) SDW of green bean in response to soil type and antibiotic treatments. Values are the mean± standard error. Bars with similar letters have no significant difference according to Tukey's test (P≤0.05).

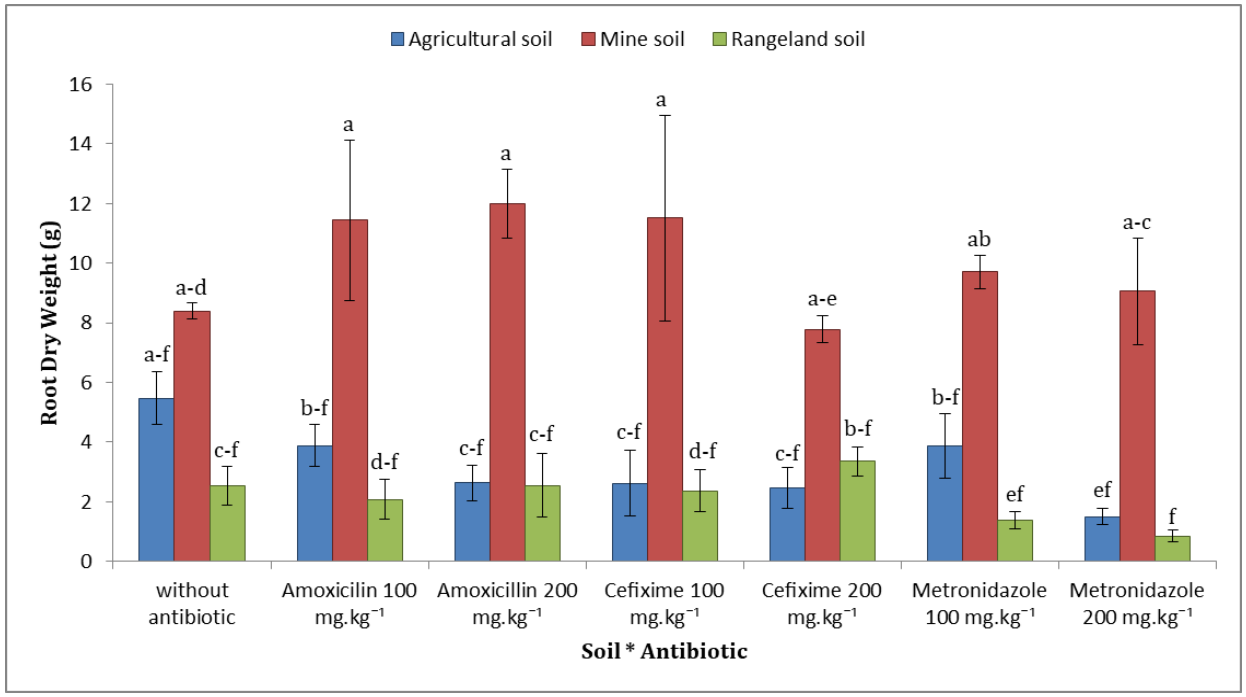

Figure 2) RDW of green beans in response to soil type and antibiotic treatments. Values are the mean± standard error. Bars with similar letters have no significant difference according to Tukey's test (P≤0.05).

reduced the SDW by 86.31% compared to the use of Cefixime (200 mg.kg^{-1}). The use of 200 mg of Cefixime per kg of mine soil afforded a significant ($p<0.05$) decrease of 77.76% of the SDW compared to the rangeland soil.

RDW was significantly altered by the interaction between soil and Antibiotic treatments ($P<0.05$). Green beans in rangeland soil and Metronidazole treatment had the lowest root growth. Nevertheless, the

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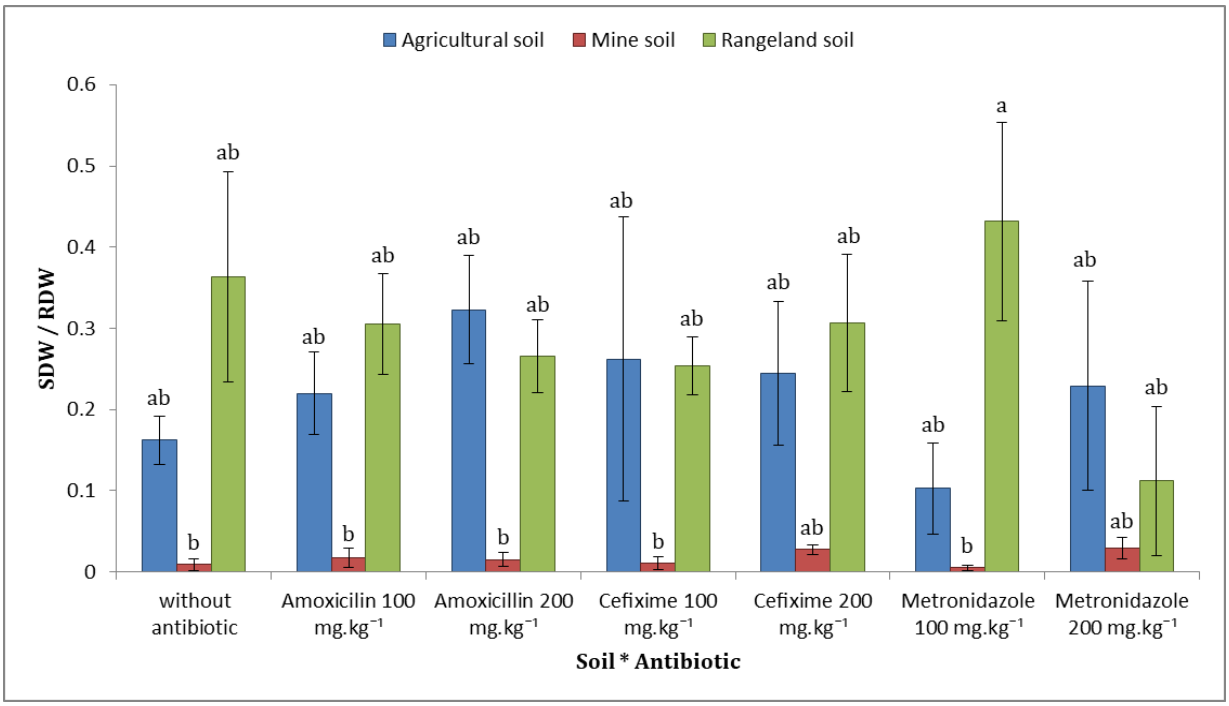

Figure 3) SDW/RDW ratio of green bean in response to soil type and Antibiotic treatments. Values are the mean± standard error. Bars with similar letters have no significant difference according to Tukey's test (P≤0.05).

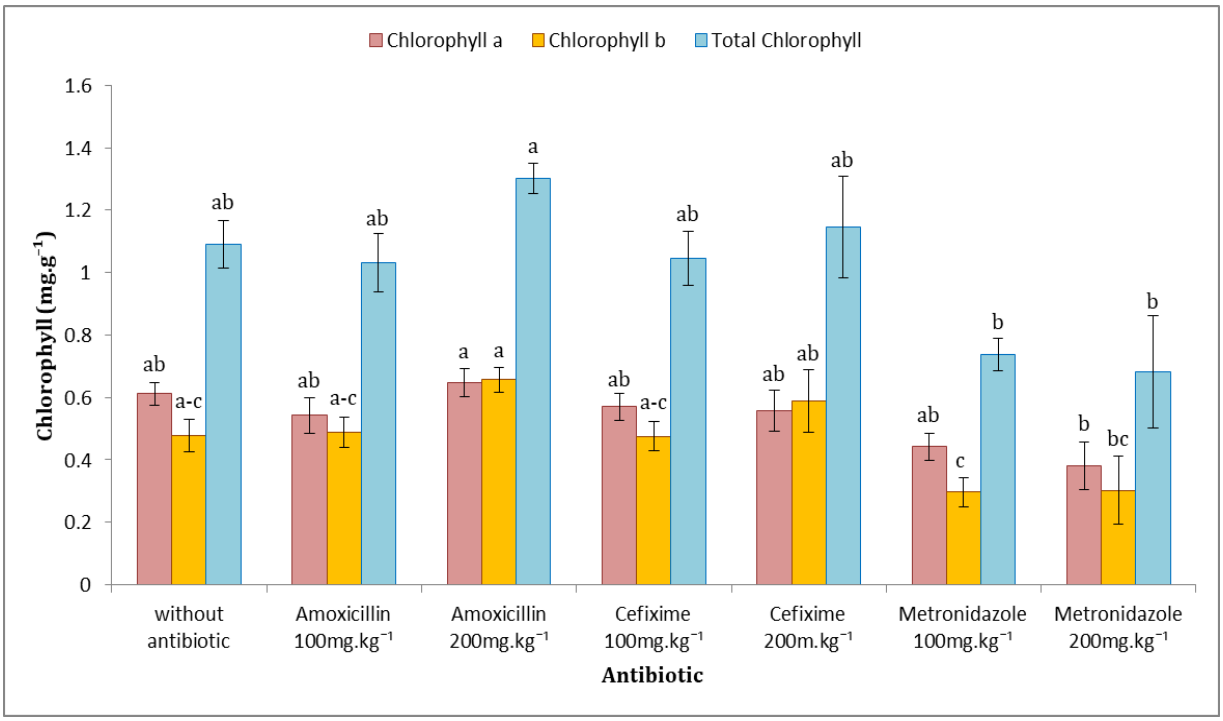

Figure 4) Chl a, Chl b, and total Chl content in response to antibiotic treatments. Values are the mean± standard error. In each data separately related to Chl a, Chl b, and total Chl, bars with similar letters have no significant difference according to Tukey's test (P≤0.05).

biomass of roots was relatively great in mine soil. Green beans treated with Amoxicillin 200 resulted in the maximum RDW in mine soil (11.98±1.14 g) while plants treated with Metronidazole 200 resulted in the lowest RDW in rangeland soil (0.85±0.18

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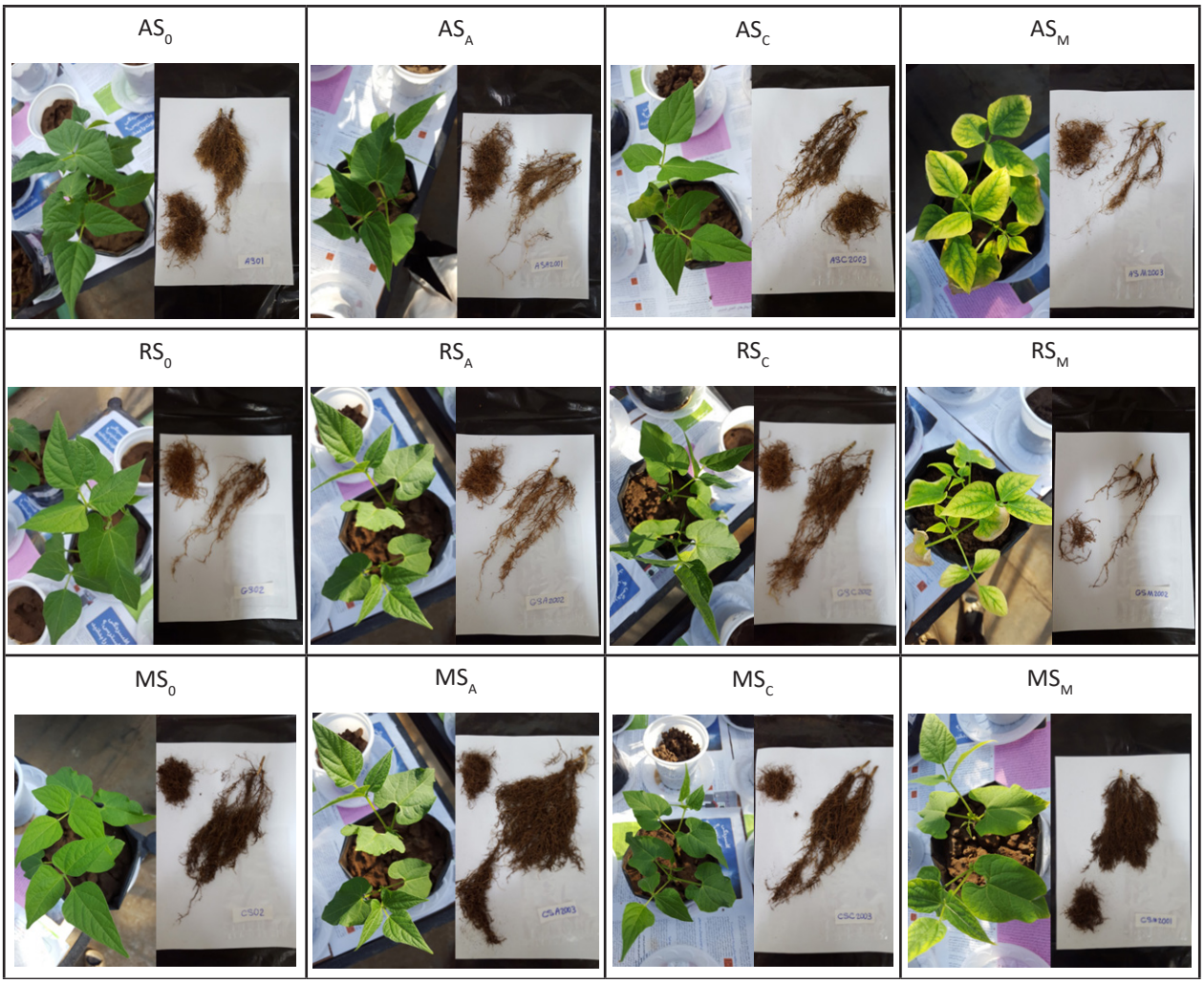

Figure 5) The effects of antibiotics on green bean shoot and root growth at AS: agricultural soil, RS: rangeland soil, MS: mine soil, $_0$:control, $_A$: amoxicillin (200 mg.kg^{-1}), $_C$: cefixime (200 mg.kg^{-1}) and $_M$: metronidazole (200 mg.kg^{-1}).

g) (Figure 2). Only in Amoxicillin (100 and 200 mg.kg^{-1}), Cefixime (100 mg.kg^{-1}), and Metronidazole (200 mg.kg^{-1}) treatments, there a significant ($p<0.05$) difference in RDW between mine soil and other two soils. In each soil separately, there was no significant difference between Antibiotic treatments.

In all the soil samples, it has been no significant difference in SDW/RDW ratio between Antibiotic treatments and control ($P<0.05$) (Figure 3). But this ratio was low in mine soil. The greatest and lowest amount for SDW/RDW ratio showed with the usage of Metronidazole (100 mg.kg^{-1}) in rangeland soil (0.43±0.12 g) and mine soil (0.005±0.003 g) respectively, so that this difference was a significant ($p<0.05$) reduction of 98.79% in mine soil compared to rangeland soil.

Photosynthetic pigments

After 4 weeks of growth, the Chl amount was evaluated in leaves of plants grown under diverse conditions (control, 100 and 200-Amoxicillin, 100 and 200-Cefixime, and 100 and 200-Metronidazole). In addition, Carotenoids were evaluated from the leaves of the plants grown under the conditions as was explained earlier. Multivariate analysis of variance indicated that Antibiotic usage had a significant effect on photosynthetic pigments ($p<0.01$) however soil type and its interaction with Antibiotic usage had no significant impact on photosynthetic pigments. The mean comparison of Chl a, Chl b, and total Chl in leaves under Antibiotic treatments are presented in Figure 4. In

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Metronidazole (100 & 200 mg.kg^{-1}) Chl a, Chl b, and total Chl quantity reduced compared to the control however, the reduction wasn't significant. On the other hand, in Metronidazole, Chl a, and total Chl amount were reduced by the enhancing Antibiotic concentration however, this decrease wasn't significant.

The use of 200 mg.kg^{-1} Metronidazole afforded a significant ($p<0.05$) reduction of 41.14% in the amount of chl a compared to Amoxicillin. Taking 100 and 200 mg.kg^{-1} of Metronidazole compared to 200 mg.kg^{-1} of Amoxicillin caused a significant ($p<0.05$) decrease in the amount of chl b (54.86% and 54.00% respectively). Also, the results

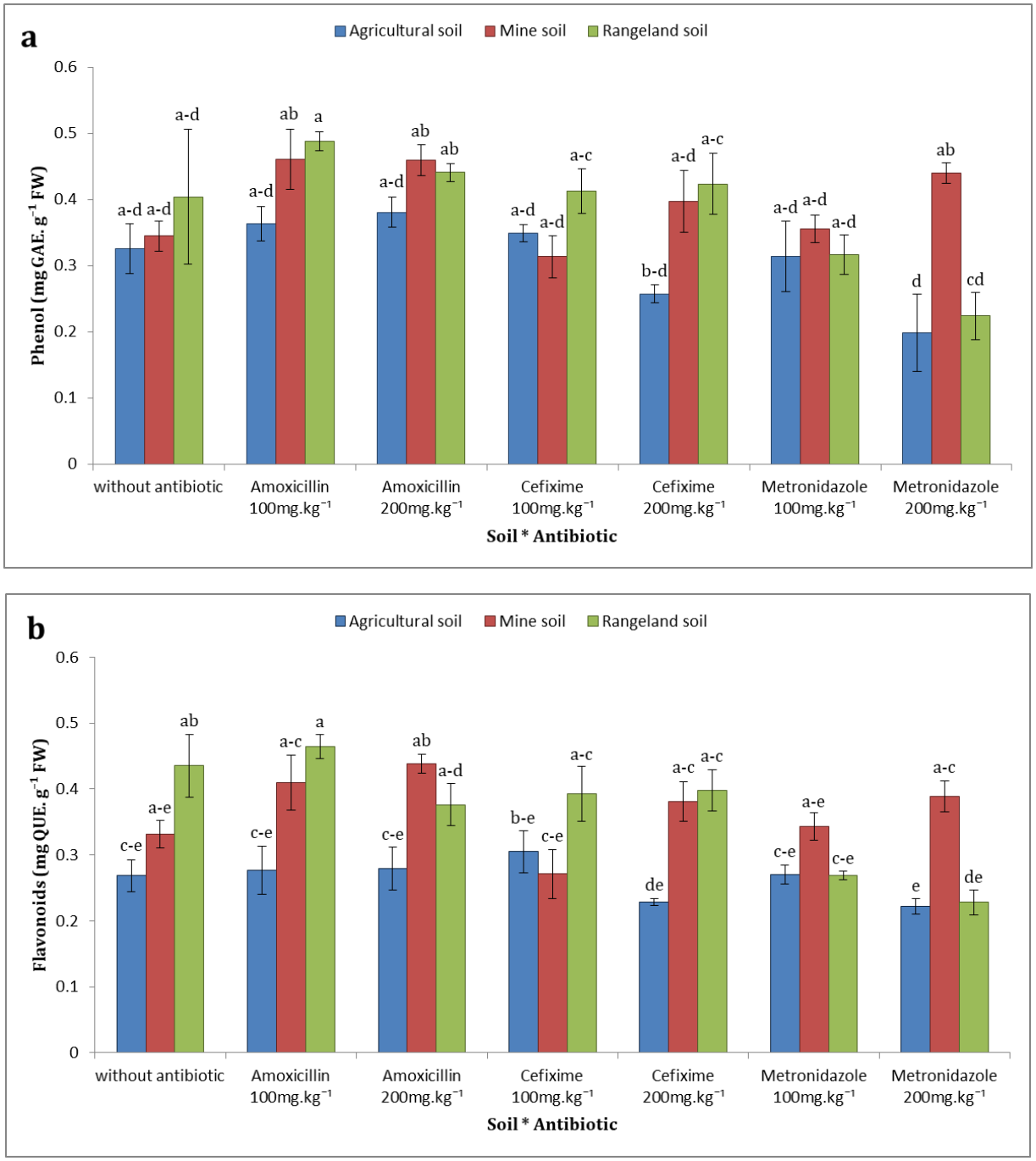

Figure 6) Phenol content (a) and flavonoid content (b) in response to soil type and antibiotic treatments. Values are the mean± standard error. Bars with similar letters have no significant difference according to Tukey's test (P≤0.05).

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showed that 100 mg.kg^{-1} Metronidazole compared to 200 mg.kg^{-1} Cefixime afforded a significant ($p<0.05$) reduction of 49.72% in chl b. Metronidazole 100 and 200 mg.kg^{-1} created a significant ($p<0.05$) decrease in total chl compared to Amoxicillin 200 mg.kg^{-1} (43.38% and 47.62% respectively). Generally, these answers are also supported in Figure 5 that the negative effects of metronidazole are more distinct in the agricultural and rangeland soils.

Phenolic and Flavonoid contents

The outcomes of soil type, Antibiotic usage, and their interaction with Phenolic and Flavonoid amounts were significant ($p<0.01$). Phenolic quantities in green beans were increased on exposure to Amoxicillin treatment (100 mg.kg^{-1}) in rangeland soil (Figure 6a). The current research represented that Metronidazole stress (100 & 200 mg.kg^{-1}) in rangeland soil significantly decreased Flavonoids synthesis in leaves of the green bean in comparison to the control (Figure 6b). Amoxicillin 100 and 200 mg.kg^{-1} rangeland soil significantly ($p<0.05$) increased the quantity of Phenol compared to Metronidazole 200 mg.kg^{-1} (117.84% and 96.66% respectively). The use of 200 mg of Metronidazole per kg of mine soil afforded a significant ($p<0.05$) enhancement in the amount of Phenol in two comparisons with agricultural and rangeland soils (121.53% and 96.34% respectively) as shown in figure 6a. In mine soil, the use of 200 mg of Amoxicillin per kg resulted in a significant ($p<0.05$) enhancement of 61.81% in the amount of flavonoids compared to the consumption of 100 mg.kg^{-1} of Cefixime. In rangeland soil, the use of 100 mg.kg^{-1} of Amoxicillin against 100 and 200 mg.kg^{-1} of Metronidazole created a significant ($p<0.05$) enhancement in the amount of Flavonoid (72.58% and 103.45% respectively) as shown in figure 6b. In mine soil, the use of 200 mg.kg^{-1} of all three Antibiotics; Amoxicillin, Cefixime, and Metronidazole separately, caused a significant ($p<0.05$) increase in the amount of Flavonoids evaluated in agricultural soil (56.97%, 66.53%, and 75.52% respectively). Rangeland soil represented maximum (0.48±0.01 mg gallic acid equivalents (GAE). g^{-1} fresh weight (FW) and 0.46±0.01 mg quercetin equivalents (QUE). g^{-1} FW) Phenol and Flavonoid amounts respectively in Amoxicillin (100 mg.kg^{-1}), and agricultural soil indicated the lowest Phenol and Flavonoid response (0.19±0.05 mg GAE. g^{-1} FW and 0.22±0.01 mg QUE. g^{-1} FW) at Metronidazole (200 mg.kg^{-1}) (Figure 6a, b).

Antioxidant activity and proline content

The multivariate analysis variance of Antioxidants by DPPH scavenging in various soils in all Antibiotic treatments determined that soil type and its interaction with Antibiotic usage had significant ($P<0.01$) results on inhibition%. Antioxidant activity in green bean tissues was increased in mine soil compared to agricultural and rangeland soil. Metronidazole (200 mg.kg^{-1}) in mine soil displayed the greatest answer of Antioxidant activity (90.76±1.18%) in comparison to different treatments, while rangeland soil indicated the lowest Antioxidant response (34.44±13.17%) at the similar treatment (Figure 7). The use of 200 mg of Metronidazole per kg of mine soil compared to Cefixime (100 mg.kg^{-1}) has resulted in a significant ($p<0.05$) enhancement of antioxidants (119.79%). The use of 200 mg of Cefixime per kg of rangeland soil has caused a significant ($p<0.05$) increase of 142.52% of Antioxidant compared to Metronidazole (200 mg.kg^{-1}). In treatment (Metronidazole 200 mg.kg^{-1}), the Antioxidant activity in mine soil was significantly ($p<0.05$) higher than in agricultural and rangeland soils (124.18% and 163.53% respectively).

In the current research, the Proline amount wasn't significantly ($P>0.05$) changed in leaves of *P. vulgaris* in answer to the tested doses of Antibiotics in a variety of soils.

Discussion

Heavy metal concentration

Except for Mg, the greatest amounts of the other metals were perceived in the soil

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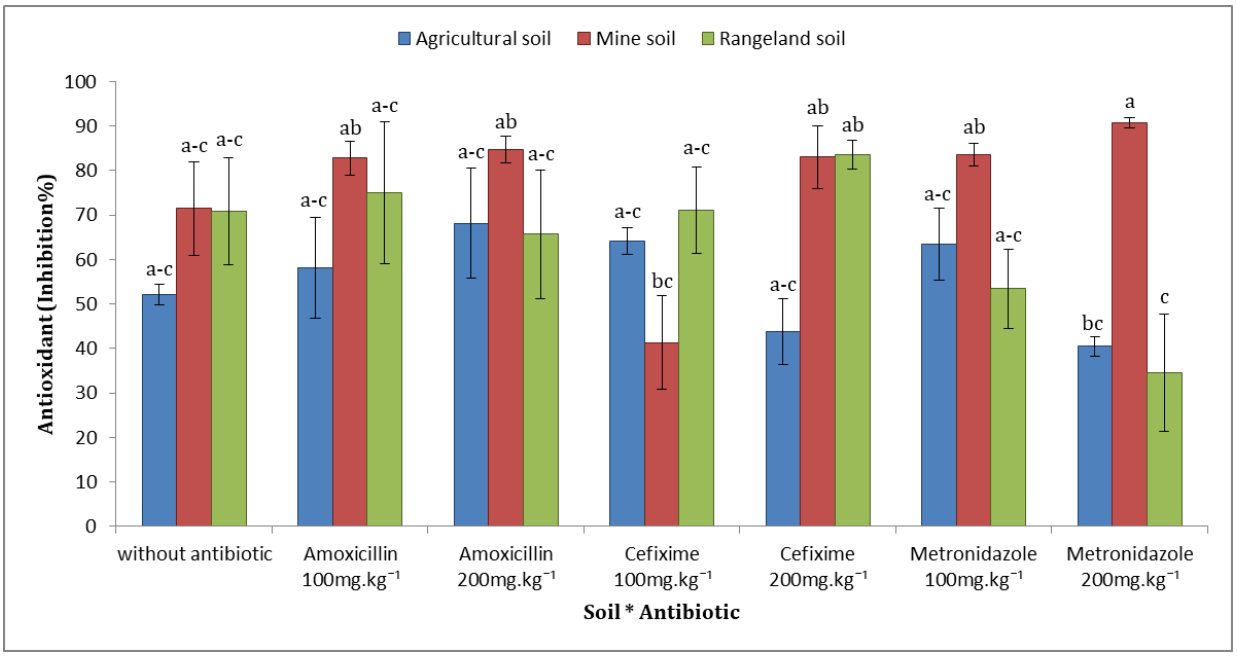

Figure 7) Antioxidant content (%) in response to soil type and antibiotic treatments. Values are the mean± standard error. Bars with similar letters have no significant difference according to Tukey's test (P≤0.05).

collected at the Ahangaran mine. The order of contamination was mine soil>rangeland soil> agricultural soil. Nevertheless, the levels of heavy metals were much high in the rangeland soil sampled near this mine too. Most concentrations of Mg were taken in the rangeland soil. The high quantities of heavy metals in the rangeland soil might be because of the strategic situation of the research area, which is set near the mining region. These findings are consistent with the results of Sinegani and Younessi [27].

Green beans shoot and root dry weight

Here usage of metronidazole had a significant and negative influence on SDW however Cefixime, especially in higher concentration (200 mg.kg^{-1}) had an optimistic result on green bean SDW. The result of Amoxicillin on SDW of green beans was positive in the mine soil. But this is not statistically significant.

Antibiotics altered soil microbial activity and thus affected plant growth [28]. Previous studies have shown that Antibiotics can reduce plant height and fresh weight [29]. IAA (Indole-3-Acetic Acid) produced by Rhizosphere bacteria increases the weight, size, number of branches, and root contact surface with soil. These changes increase the root potential for nutrient exchange and thus increase plant growth capacity [30]. According to the results of previous research, it was also seen in the present study, that the high dose of Cefixime significantly increased the SDW of green beans in the rangeland soil more than in the mine soil.

Photosynthetic Carbon reduction cycle enzymes are sensitive to Cadmium, which can reduce stem length and plant biomass by increasing heavy metals [31]. In comparing the effect of heavy metal on root growth and shoots, heavy metal has more negative effects on root growth [32]. According to these researches, in the present research as well, as the results showed, among the soils without Antibiotic treatment, SDW in mine soil was significantly lower than the other two soils. In nutrient stress, plant biomass is mainly allocated to the roots, which increases the absorbing organ. Besides, plants that have enough Nitrogen direct biomass to the shoot [33]. With inadequate nodulation and Nitrogen fixation, faba bean plants will need an extensive root system [34]. The results of these researches can also be seen in the

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present research, as in the mine soil, the RDW due to the application of all three Antibiotics (Amoxicillin 100 & 200, Cefixime 100, and Metronidazole 200 $mg.kg^{-1}$) was compared to the other two soils. By forming more nodules in the plant, more shoot and root dry matter is produced [35]. Rhizobium strains with higher nodulation increased SDW and shoot-to-root ratio in beans. Similar to these results were also seen in the present research so that the application of low concentration of Metronidazole increased the ratio of SDW/RDW in the rangeland soil against mine soil.

Photosynthetic pigments

The quantity of Photosynthetic pigments might avail as a significant bioindicator of stress in plants. As in the research by Chandra and Kang [36], in the recent research also there was no significant change in the Chl a/b ratio among all concentrations of Antibiotics.

There are many reports of the effect of Antibiotics on the Photosynthetic system of plants, which can be mentioned as follows: the effect of Tetracyclines, Fluoroquinolones, and Macrolides on the Synthesis of plant Chloroplastic Protein; the negative effect of Fluoroquinolones on Photosynthesis; reduction of Chl synthesis by streptomycin; Chl reduction of Photosynthesis and plant growth by Ciprofloxacin; effect on plastid division by β-Lactams; reduction of Photosynthetic pigments content, Chlorophylls, and Carotenoids in plants by Tetracyclines, Ciprofloxacin and Erythromycin; and effect on the Photosynthetic Electron transport rate by Penicillin's, Cephalosporins, and Tetracyclines [37]. Researchers investigated the effect of Antibiotics on Photosynthesis and concluded that Ciprofloxacin and Cephalosporins can reduce stomatal conductance and prevent net assimilation rate [38]. The most prominent effect of Antibiotics on Chloroplast and reduced plant growth is associated with plant metabolism [39]. In the present study, the high concentration of Metronidazole caused the greatest decrease in the amounts of Chl a, and total Chl and its low concentration caused the greatest decrease in the amount of Chl b.

Contrary to the research of Zhou et al. [40], the present results displayed that the high concentration of Amoxicillin used caused the greatest increase in the amounts of the Chl a, b, and total among the three studied Antibiotics. Research has shown that low doses of Antibiotics can affect the amount of Nucleic Acid and plant cell Protein by reducing Chlorophyllase activity and delaying Chl degradation, thereby increasing Chl biosynthesis [40]. The highest increase in total Chl at a concentration of 15 $mg.L^{-1}$ Zinc Oxide nanoparticles under lead stress [41]. Studies have shown that heavy metals affect Chl synthesis by directly inhibiting an enzymatic step or by inducing a deficiency of an essential nutrient [42].

Research has shown that soil contamination with Tetracycline and Cadmium can lead to Chl degradation. Tetracycline is more easily absorbed by plants than Cadmium. However, the Phytotoxic effect of Cadmium (effect on enzyme activity, dry mass, fresh mass, and leaf area) is stronger than Tetracycline. Cadmium also further reduces the amount of Chl. Oxidation of Photosynthetic pigments may be the cause of Chl depletion in metal-treated plants [43].

Reports suggest that mineral stress may reduce the expression of proteins involved in Photosynthesis. Among the minerals, Iron and Zinc are cofactors for many enzymes and are essential for plant growth. They are implicated in physiological responses and metabolic pathways, Chl synthesis, and photosynthesis. Iron and Zinc deficiency causes Chlorosis of plants and decreases photosynthetic activity. The deficiency of these micro-nutrients disrupts primary and secondary metabolism, Protein synthesis, and the distribution of Carbohydrates between tissues, ultimately reducing Photosynthesis [44].

In this research, the Car amount wasn't significantly varied ($P>0.05$) in the leaves of *P. vulgaris* in response to every tested dose of Antibiotics in contaminated and non-

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contaminated soils. The amount of Chl and Carotenoids decreased in all popular Hybrids except Hybrids that increased in concentrations of 200 and 500 ppm of heavy metals [36].

Phenolic, Flavonoid, and Antioxidant activity

Plants activate the Antioxidative defense system (Phenols and Flavonoids) against the stress of heavy metals. They protect plants as metal chelators and natural scavengers of reactive Oxygen species (ROS) [45]. Phenolic compounds are known as free, soluble bound, and insoluble bound forms. Free Phenols are in the form of Phenolic Acids and Flavonoids. Soluble Phenol is the same as esterified Phenolics. Insoluble Phenols are present in the cell wall along with Cellulose and Lignin. Beans are rich in Polyphenolic compounds that help in the poor absorption and digestibility of nutrients. One of the most important families of phytochemicals in beans is Phenolic compounds. Phenol in beans (*P. vulgaris*) varies depending on the type and growing conditions. The researchers concluded that the total Phenol content (TPC) is an indicator of Antioxidant activity. Salicylic acid (SA) is a water-soluble Phenolic compound that can regulate plant growth and play an important role in biotic and abiotic stresses. Mechanisms related to SA may include the inactivation of removing enzymes by this compound or the activation of the superoxide dismutase (SOD) enzyme [46].

Metal stress causes the making of reactive Oxygen species (ROS) such as $O_2^{\bullet -}$, H_2O_2, and $OH^{\bullet}$, which leads to the demolition of Proteins, DNA, and Chl. Relative to ROS characters, some are toxic and detoxified by several cellular enzymatic (catalase, superoxide dismutase, peroxidase, and glutathione reductase) and non-enzymatic (Carotenoids, Proline, Glutathione, and ascorbic acid) mechanisms. Plants have an antioxidant defense mechanism against metal toxicity, which includes enzymes and metabolites such as Phenolics, Proline, and Flavonoids. These enzymes and compounds preserve cell organelles against the oxidative damage produced by ROS. Proline, Phenols, and Flavonoids function as metal chelators, and free radical scavengers, protecting the plant and tolerating stress [8]. For example, an increase in Phenol content in *Euphorbia helioscopia* L. and *Parthenium hysterophorus* L. was shown in lead-contaminated soil. The Proline, total Phenolic was assessed under metals stress. The Phenolic and Proline quantities displayed a positive correlation with the increase of lead in the plants [11].

In the present study, the use of Antibiotics in soils with different levels of heavy metal had different effects on green bean growth and metabolites. The use of Antibiotics in mine soil caused a significant enhancement in the amount of Flavonoids in green bean leaves compared to the other two soils, which can be said to be an indication of the effect of heavy metals. The same results with a high concentration of Metronidazole were also seen in the mine soil for the amount of phenol compared to the other two soils. The effect of the type of Antibiotic in increasing or decreasing the amount of Phenol and Flavonoid in green bean leaves was confirmed in the results so that in rangeland soil, the use of Amoxicillin afforded a significant increase in the amount of both Phenol and Flavonoid compared to the use of Metronidazole.

The results of the present study showed that at a high concentration of Metronidazole, the Antioxidant (inhibition%) in mine soil was significantly higher than in the other two soils. Phenyl Ammonia-Lyase (PAL) is an enzyme that is used to protect the plant against abiotic and biotic stresses. This enzyme participates in the deamination of L-Phenylalanine to trans-cinnamic acid, which is the prerequisite required for the production of flavonoids and Phenols. Secondary metabolites, Phenols, and Flavonoids can scavenge the ROS produced under stress. Therefore, PAL plays an important role in plant protection to deal with heavy metal stress. For example, PAL activity in Lactuca *sativa* L. under Cadmium metal stress has been reported [47]. Phenolic compounds together with other compounds

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such as Proline and Jasmonic Acid stimulate the plant's defense system. When heavy metals reach the roots, the delivery of elements to the top parts is prevented or reduced to protect the plant [48].

Plants resist the stress of heavy metals by inducing the accumulation of secondary Metabolites and specific Antioxidant Enzymes. Under stress, a balance between the making of reactive Oxygen species (ROS) and its degradation is necessary to maintain metabolic function, otherwise, the cell is damaged. The amount of ROS is regulated by the Antioxidant system, which includes non-enzymatic Antioxidants (Proline, Phenolics, Organic Acids, Glutathione) and Antioxidant enzymes (catalase, Glutathione transferase, Peroxidase (POD), Superoxide Dismutase (SOD)). Free Proline and Phenolics can protect plants from Oxidative stress. Proline is one of the metabolic products of plants that accumulate to eliminate the toxic effects of metals. The Amino Acid Proline acts as a metal chelator, protects enzymes, and quencher of ROS [49].

Conclusion

This research revealed that metronidazole reduced green bean SDW and RDW and SDW/RDW ratio significantly but this negative effect wasn't perceived in Amoxicillin and Cefixime usage. Green beans cultured in agricultural soil and rangeland soil had higher shoot growth and biomass. But it had bigger root growth and biomass in mine soil. Thus, the lower shoot/root ratio was calculated for all cultured in mine soil. The usage of metronidazole highly reduced Chl a, total Chl, flavonoids, and phenol quantities in leaves of green beans in all the researched soils. Further, metronidazole treatment resulted in chlorosis in green bean leaves. This negative effect was more evident in non-contaminated agricultural soil. Application of Amoxicillin also enhanced Chl a, total Chl, Flavonoids, and Phenol quantities in leaves of green beans in all the tested soils. Soil heavy metal contamination had positive effects on Antioxidant activity in leaves of green beans in non-Antibiotic treated soils. This study proposed that heavy metals and Metronidazole can be stressful for the green bean. However, their negative effect wasn't synergetic.

This experimental hypothesis confirmed that the effect of Antibiotics on the growth and response of the plant depends on the type of Antibiotic and the characteristics of the soil so the negative effect of Metronidazole on the investigated parameters was more than the other two Antibiotics. On the other hand, according to the results, the high concentration of Amoxicillin used in this research caused the greatest increase in the quantities of Chlorophyll a, b, and total. While the high concentration of Metronidazole caused the greatest decrease in the amounts of Chlorophyll a and total. Therefore, it can be said that the effect of Antibiotics on plant parameters also depends on the type and characteristics of Antibiotics.

Since no response was observed in the symbiotic interactions of green bean roots with the application of the Antibiotic, it is suggested to investigate the Antibiotic resistance of symbiotic Microorganisms in the soil-plant system. It is also suggested to check the Antibiotic resistance of destructive and beneficial soil Microorganisms and reduce the bad effect of pathogens on the plant.

Since the residues of Antibiotics often enter agricultural soils through animal manure, it is suggested to use at least decomposed animal manure to control the stressful effect on plants and decrease the arrival of Antibiotics into the food chain.

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original work, which has not been previously published elsewhere.

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References

1. Prosser R.S., Sibley P.K. Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation. Environ Int. 2015; 75: 223-233.
2. Liu F., Ying G.G., Tao R., Zhao J.L., Yang J.F., Zhao L.Z. Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. Environ. Pollut. 2009; 157: 1636-1642.
3. Farkas M.H., Berry J.O., Aga D.S. Chlortetracycline detoxification in maize via induction of glutathione S-transferases after antibiotic exposure. Environ Sci Technol. 2007; 41(4): 1450-1456.
4. Bewick M.W.M. The use of antibiotic fermentation wastes as fertilizers for tomatoes. J Agr Sci. 1979; 92(3): 669-674.
5. Kang D.H., Gupta S., Rosen C., Fritz V., Singh A., Chander Y., et al. Antibiotic uptake by vegetable crops from manure-applied soils. J Agr Food Chem. 2013; 61(42): 9992-10001.
6. Hu X., Gao Y., Fang Z. Integrating metabolic analysis with biological endpoints provides insight into nanotoxicological mechanisms of graphene oxide: From effect onset to cessation. Carbon. 2016; 109: 65-73.
7. El-Saadony M.T., Desoky E.S.M., Saad A.M., Eid R.S., Selem E., Elrys A.S. Biological silicon nanoparticles improve *Phaseolus vulgaris* L. yield and minimize its contaminant contents on a heavy metals-contaminated saline soil. J Environ Sci. 2021; 106: 1-14.
8. Desoky E.S.M., Elrys A.S., Rady M.M. Integrative moringa and licorice extracts application improves Capsicum annuum fruit yield and declines its contaminant contents on a heavy metals-contaminated saline soil. Ecotox Environ Safe. 2019; 169: 50-60.
9. Yang L., Ren Q., Zheng K., Jiao Z., Ruan X., Wang Y. Migration of heavy metals in the soil-grape system and potential health risk assessment. Sci Total Envirov. 2022; 806: 150646.
10. Akhzari D., Shayganfar A. The Interaction of Artemisia persica Allelopathy, Drought and Arbuscular Mycorrhizal Fungi on Growth and Physiological Indices of Ferula haussknechtii H. Wolff ex Rech. f. ECOPERSIA 2019; 7(4): 203-210.
11. Ullah R., Hadi F., Ahmad S., Jan A.U., Rongliang Q. Phytoremediation of lead and chromium contaminated soil improves with the endogenous phenolics and proline production in Parthenium, Cannabis, Euphorbia, and Rumex species. Water Air Soil Poll. 2019; 230(2): 40.
12. Li M., Yang L., Yen H., Zhao F., Wang X., Zhou T., ... Chen L. Occurrence, spatial distribution and ecological risks of antibiotics in soil in urban agglomeration. J Environ Sci. 2023; 125: 678-690.
13. Farshad A. Soilscape Speaks Out; an Account of the Past, Present, and Future. ECOPERSIA 2015; 3(1): 881-899.
14. Rowell D.L. Soil science: Methods and applications. Longman Group UK Ltd., London. 1994.
15. Helrich K. Official methods of analysis of the Association of Official Analytical Chemists. AOAC. 1990.
16. Srivastava V., Nerwal D.K., Kandan A., Akhtar J., Sharma N., Kiran R., ... Agrawal A. Management of microbial contaminants in the In Vitro Gene Bank: a case study of taro [Colocasia esculenta (L.) Schott]. In Vitro Cell Dev-Pl. 2021; 57(1): 152-163.
17. Arnon D.I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant physiol. 1949; 24(1): 1.
18. Mackinney G. Absorption of light by chlorophyll solutions. J Biol Chem. 1941; 140(2): 315-322.
19. Lichtenthaler H.K., Buschmann C. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. Curr. Protoc. Food Anal. Chem. 2001; 1(1): F4-3.
20. Bedreag C.F.G., Trifan A., Bucur L.A., Arcus M., Tebrencu C., Miron A., Costache I.I. Chemical and antioxidant studies on Crataegus pentagyna leaves and flowers. Rom Biotech Lett. 2014; 19(6): 9859.
21. Tezcan F., Gültekin-Özgüven M., Diken T., Özçelik B., Erim F.B. Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. Food chem. 2009; 115(3): 873-877.
22. Yoo K.M., Lee C.H., Lee H., Moon B., Lee C.Y. Relative antioxidant and cytoprotective activities of common herbs. Food chem. 2008; 106(3): 929-936.
23. Brand-Williams W., Cuvelier M.E., Berset C.L.W.T. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol. 1995; 28 (1): 25-30.
24. Burda S., Oleszek W. Antioxidant and antiradical activities of flavonoids. J Agr Food Chem. 2001; 49(6): 2774-2779.
25. Guzel S., Terzi R. Exogenous hydrogen peroxide

[DOR: 20.1001.1.23222700.2022.10.4.2.1]

increases dry matter production, mineral content and level of osmotic solutes in young maize leaves and alleviates deleterious effects of copper stress. Bot Stud. 2013; 54 (1): 1-10.
26. El-Juhany L.I., Ali H.M., Basalah M.O., Shehatah A.M.S. Effects of water stress and salinity on the growth of Hibiscus tiliaceus trees. J Pure Appl Microbio. 2014; 8: 399-408.
27. Sinegani A.A.S., Younessi N. Antibiotic resistance of bacteria isolated from heavy metal-polluted soils with different land uses. J Glob Antimicrob Re. 2017; 10: 247-255.
28. Xiong W., Wang M., Dai J., Sun Y., Zeng Z. Application of manure containing tetracyclines slowed down the dissipation of tet resistance genes and caused changes in the composition of soil bacteria. Ecotox environ safe. 2018; 147: 455-460.
29. Yu X., Liu X., Liu H., Chen J., Sun Y. The accumulation and distribution of five antibiotics from soil in 12 cultivars of pak choi. Environ Pollut. 2019; 254: 113115.
30. Solano B.R., Maicas J.B., Mañero F.G. Physiological and molecular mechanisms of plant growth promoting rhizobacteria (PGPR). Plant-bacteria interactions-Strategies and techniques to promote plant growth. Weinheim, Wiley VCH. 2008; 41-54.
31. De Filippis L.F., Ziegler H. Effect of sublethal concentrations of zinc, cadmium and mercury on the photosynthetic carbon reduction cycle of Euglena. J Plant Physiol. 1993; 142(2): 167-172.
32. Elloumi N.B., Rhouma F., Ben A., Mezghani B.I., Boukhris M. Cadmium induced growth inhibition and alteration of biochemical parameters in almond seedlings grown in solution culture. Acta Physiol Plant. 2007; 29: 665-672.
33. Van der Werf A., Nagel O.W. Carbon allocation to shoots and roots in relation to nitrogen supply is mediated by cytokinins and sucrose: opinion. Plant Soil. 1996; 185(1): 21-32.
34. Koevoets I.T., Venema J.H., Elzenga J.T., Testerink C. Roots withstanding their environment: exploiting root system architecture responses to abiotic stress to improve crop tolerance. Front plant Sci. 2016; 7: 1335.
35. Allito B.B., Ewusi-Mensah N., Logah V., Hunegnaw D.K. Legume-rhizobium specificity effect on nodulation, biomass production and partitioning of faba bean (*Vicia faba* L.). Sci Rep-UK. 2021; 11(1): 1-13.
36. Chandra R., Kang H. Mixed heavy metal stress on photosynthesis, transpiration rate, and chlorophyll content in poplar hybrids. Forest Sci Technol. 2016; 12(2): 55-61.
37. Kümmerer K. Antibiotics in the aquatic environment–a review–part I. Chemosphere. 2009; 75(4): 417-434.
38. Opriş O., Soran M.L., Coman V., Copaciu F., Ristoiu D. Determination of some frequently used antibiotics in waste waters using solid phase extraction followed by high performance liquid chromatography with diode array and mass spectrometry detection. Cent Eur J Chem. 2013; 11(8): 1343-1351.
39. Khan K.Y., Ali B., Zhang S., Stoffella P.J., Yuan S., Xia Q., et al. Effects of antibiotics stress on growth variables, ultrastructure, and metabolite pattern of Brassica rapa ssp. chinensis. Sci Total Environ. 2021; 778: 146333.
40. Zhou W., Qiao X., Xiao H., Wang Y., Qu L. Effects of antibiotics on chlorophyll-a level in marine microalga. Mar. Environ. Sci. 2009; 28(3).
41. Hussain F., Hadi F., Rongliang Q. Effects of zinc oxide nanoparticles on antioxidants, chlorophyll contents, and proline in *Persicaria hydropiper* L. and its potential for Pb phytoremediation. Environ Sci Pollut R. 2021; 1-17.
42. Van Assche F., Clijsters H. Effects of metals on enzyme activity in plants. Plant Cell Environ. 1990; 13(3): 195-206.
43. Rydzyński D., Piotrowicz-Cieślak A.I., Grajek H., Michalczyk D.J. Chlorophyll degradation by tetracycline and cadmium in spinach (*Spinacia oleracea* L.) leaves. Int J Environ Sci Te. 2019; 16(10): 6301-6314.
44. Urwat U., Zargar S.M., Ahmad S.M., Ganai N.A. Insights into role of STP13 in sugar driven signaling that leads to decrease in photosynthesis in dicot legume crop model (*Phaseolus vulgaris* L.) under Fe and Zn stress. Mol Biol Rep. 2021; 48(3): 2527-2531.
45. Ilboudo O., Tapsoba I., Bonzi-Coulibaly Y.L., Gerbaux P. Targeting structural motifs of flavonoid diglycosides using collision-induced dissociation experiments on flavonoid/Pb2+ complexes. Eur J Mass Spectrom. 2012; 18 (5): 465-473.
46. Ampofo J.O., Ngadi M. Stimulation of the phenylpropanoid pathway and antioxidant capacities by biotic and abiotic elicitation strategies in common bean (Phaseolus vulgaris) sproutsProcess Biochem. 2021;100: 98-106.
47. Kosyk O.I., Khomenko I.M., Batsmanova L.M., Taran N.Y. Phenylalanine ammonia-lyase activity and anthocyanin content in different varieties of lettuce under the cadmium influence. Ukr. Biochem. J. 2017; 89(2): 85-91.
48. Karabourniotis G., Liakopoulos G., Nikolopoulos D., Bresta P. Protective and defensive roles of non-glandular trichomes against multiple stresses: Structure–function coordination. J Forestry Res. 2020; 31(1): 1-12.
49. Hadi F., Ali N., Fuller M.P. Molybdenum (Mo) increases endogenous phenolics, proline and photosynthetic pigments and the phytoremediation potential of the industrially important plant *Ricinus communis* L. for removal of cadmium from contaminated soil. Environ Sci Pollut R. 2016; 23 (20): 20408-20430.

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