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EFFECT OF *RHIZOBIUM LEGUMINOSARUM* ON TOMATO PLANTS GROWING IN HEAVY METAL CONTAMINATED SOIL

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Abstract- An experiment was conducted to study the effect of *R. leguminosarum* biovar TF17 inoculation on tomato (*Lycopersicon esculentum*) plants treated with four different heavy metal salts. A pot experiment used complete randomized block design pattern with three replicates. Prior to pot study the rhizosphere of fenugreek (*Trigonella foenum-graecum* L.) plant was screened for efficient *Rhizobium leguminosarum* biovars. From the 152 bacterial isolates screened, 11 biovars of *R. leguminosarum* were identified and screened for PGPR traits. The isolated *R. leguminosarum* biovar TF17 with multi-plant growth promoting traits was selected for pot study. Effect of heavy metals on tomato seed germination was also studied. Treatment of $CoSO_4$, $CuSO_4$, $HgSO_4$ and $ZnSO_4$ with or without *R. leguminosarum* TF17 inoculum was provided to tomato seeds at the dose of 20 mg Kg^{-1} soil. The final observation of 14 weeks showed highest (96.41 cm) and the lowest (50.47 cm) plant length (shoot and root length) from $ZnSO_4$ and $CuSO_4$ treatments, respectively. Similarly, the highest (0.947 g) and the lowest (0.401 g) plant dry weights were recorded with $ZnSO_4$ and $CuSO_4$ treatments, respectively. Application of *R. leguminosarum* TF17 along with heavy metal salts mitigated the plant growth retarding effect to some extent. In a separate experiment spectrophotometric analysis showed negative relation between heavy metal concentration and *R. leguminosarum* TF17 growth. The lowest bacterial growth, in the form of optical density (OD) drop, was observed with 40 mg L^{-1} $CoSO_4$ treatment, followed by $CuSO_4$ of same concentration.

Keywords- Bioinoculant, Heavy metals, *Rhizobium leguminosarum*, Tomato

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Introduction

Those elements with metallic properties and atomic number > 20 have a rare natural abundance and are not used by living system; neither did this system evolve mechanisms to cope with them. Such metals are called heavy metals. When man started extracting these elements from natural abundance and used them as a consequence, also released them into the environment. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb and Zn.

Many farmlands in India rely on wastewater for irrigation due to scarcity of water, which causes heavy metal accumulation in soil [1,2] leading to reduced agricultural yield. Heavy metal accumulating plants commonly suffer from low growth and biomass. Some heavy metals at lower concentrations are considered harmless and are essential plant micronutrients; but at higher concentrations, they may cause plant growth retardation by inducing metabolic disorders [3]. Evidence shows that there is a linear correlation between the heavy metal content of plants with soil [4]. The higher concentrations of heavy metal ions in the soil affect physiology of plants by altering the functionality of enzymes and biological compounds, thus hindering the supply of some vital compounds thought the synthesis of unwanted metabolites [5]. Heavy metals are also known to cause oxidative stress in plants either by causing free radical production or by hindering the activity of enzyme and non-enzyme based antioxidants [6].

The plant-rhizobial interaction should be the prime focus for soil remediation study due to its wide distribution in soil, ability to reduce organic and non-organic pollutants and stimulation of other PGPRs [7]. The nitrogen-fixing rhizobia are integral part of soil and root ecosystems and are known to increase legume growth by facilitating nitrogen supply. In agricultural soil rhizobial strains are exposed to non-leguminous plants during legume-cereal rotations and mixed intercropping. The rhizobial exposure may sometimes lead to the infection of non-legumes [8-10]. This association can improve plant growth due to rhizobial ability of growth hormone production [10], siderophore production [11], phosphate solubilisation [12,13], HCN production[10], biocontrol from plant pathogens [14] and plant bioremediation from pollutants [7,15-17].

Although the energy demanding treatments like removal of contaminated soil, chemical extraction and application of chemical reagents are the most efficient methods known for rapid heavy metal removal from contaminated sites [16], the energy efficient method, called bioremediation, finds its potential in the energy hungry world. Since the reports of rhizobial effect on plants exposed to heavy metal soil are few and are of environmental interest, a study was designed to examine the effect of four common heavy metal salts on rhizobial inoculated tomato plants.

Materials and Methods

Study area

A study was conducted at Balawala region of Dehradun, India (30° 25'49" N, 78°11'26" E) in SBSPGI campus polyhouse from March to mid-June, during which the region received an average rainfall of 38.16 mm and relative humidity of 47.15% with an average temperature of 25.85°C (annual average rainfall 170 mm, relative humidity 66% and temperature 21.67ºC, respectively).

Bacterial isolation and identification

The root nodules of *Trigonella foenum graecum* plant were randomly collected from various regions of Balawala, Dehradun. Healthy root nodules were washed with tap water, surface sterilized with 0.7% (w/v) NaOCl for 4-5 min and rinsed

with plenty of sterilized distilled water. The nodules were crushed in a sterile culture tube using a sterile glass rod; 5 mL sterile water was added to it and mixed well. A 0.1 mL of aliquot from culture tube was pipetted and spread onto the surface of yeast extract mannitol agar plates (YEMA, HiMedia). Plates were incubated at 28° C for 48 h and after incubation, well separated single colonies on the plates were re-streaked on freshly prepared YEMA plates in order to obtain pure cultures. A total of 152 bacterial isolates were used in rhizobial screening. The bacterial isolates were morphologically and physiologically characterized and identified as per Bergy's manual of determinative bacteriology [18]. The isolated *R. leguminosarum* biovars were maintained on yeast extract mannitol agar medium and repeatedly purified. The identified biovars of *R. leguminosarum* were *in vitro* screened for growth promoting traits *viz.* cyanogen production, IAA production and siderophore production and P solubilization, respectively.

In vitro* effect of heavy metal treatment on *Rhizobium leguminosarum

The nutrient broth (HiMedia) was prepared, filled in 10 mL tubes, charged with metal salts at the concentrations of 10 mg, 20 mg, 30 mg and 40 mg L^{-1}, respectively and autoclaved. The tubes were inoculated with 0.1 mL overnight grown *R. leguminosarum* TF17 broth culture and incubated for 24 h at 27°C. The Optical Density of solution was measured at 620 nm wavelength using UV-Visible spectrophotometer.

Seed germination

Tomato seeds (Pusa Hyb - 4) were surface sterilized with 0.7% (w/v) NaOCl for 4-5 min and rinsed several times with distilled water. The seeds were placed in 20 mL capacity sterilized culture tubes containing water agar medium (20 g L^{-1} agar) and charged with 20 mg L^{-1} dose of respective heavy metals ($CoSO_4$, $CuSO_4$, $HgSO_4$ and $ZnSO_4$). The same experiment was performed with *R. leguminosarum* TF17 treated seeds, which were pre-soaked in overnight grown rhizobial culture contained in a conical flask. For germination study, the treated slants were kept under natural photoperiodic conditions in the laboratory and observed for 21 days.

Experimental design and setup

A study was laid out in completely randomized design with three replications for each treatment. The soil was collected from the nearby forest of Balawala, Dehradun and mixed with acid washed and neutralized sand. The ratio of sand and soil (3:2) was made by their volumes. The soil mixture was tested as per the available standard methods [19]. The soil was autoclaved at 20 psi for twenty minutes for two subsequent days. Each pot was uniformly filled with 8 kg soil and treated with heavy metal salts @ 20 mg kg^{-1} soil. Half of the total pots were sown with uninoculated tomato seeds and the rest half with *R. leguminosarum* TF17 inoculated seeds. The bacterial inoculum was prepared by inoculating nutrient broth contained flask and incubating it at 30ºC for 24 h to obtain a concentration of 1 x 10^9 cfu mL^{-1}. The seeds were bacterized by soaking in nutrient broth flask containing *R. leguminosarum* TF17 for 2-3 h. Non-inoculated seeds were kept in a flask containing sterilized distilled water for the same duration. Irrigation was done as and when necessary.

Plant harvest and analysis

The plants were carefully harvested from pots after 14 weeks of growth. The adhering soil particles sticking to the plants were removed by gently washing with tap water. The plants were blotted dry, measured for root-shoot length, oven dried at 70˚C for 72 h and weighted.

Statistical analysis

The data of the experiment was analyzed using Analysis of Variance (ANOVA) followed by post hoc test using SPSS®. All the observations were the mean of three replicates at $p \leq 0.05$ level of significance.

Results and Discussion

Bacterial isolation, identification and *in vitro* screening of plant growth promoting traits

In the present study, the bacterial screening was performed to select the effective rhizobia with plant growth promoting traits. Of the 152 bacterial isolates used in screening, 11 biovars of *R. leguminosarum* were identified using biochemical tests [Table-1]. These biovars were *in vitro* screened for plant growth promoting traits. Among them, two were cyanogen producers, three IAA producers, four siderophore producers and four P solubilizes, respectively [Table-2]. The *R. leguminosarum* biovar TF17 demonstrated maximum (three) growth promoting traits and hence selected for the further study.

Table-2 *R. leguminosarum biovars showing PGPR traits in vitro*

Biovar	Cyanogen producers	IAA producers	Siderophore producers	Phosphate solubilizers
TF2	-	-	+	+
TF5	-	+	+	-
TF17	-	+	++	+
TF22	-	-	-	-
TF27	+	-	-	-
TF31	-	-	-	-
TF32	-	-	-	+
TF49	-	-	-	-
TF52	+	-	+	-
TF58	-	-	-	+
TF61	-	+	-	-

-: negative; +: positive; ++: strongly positive

Effect of heavy metals on *in vitro* growth of *R. leguminosarum* TF17

The spectrophotometric observation reveals that heavy metal concentration affect *in vitro R. leguminosarum* TF17 growth. The OD of nutrient broth decreased with increase in heavy metal concentration. The lowest OD of *R. leguminosarum* TF17 growth was obtained with 40 mg L^{-1} $CoSO_4$ followed by $CuSO_4$ with similar concentration [Fig-1]. Similar observations have also been reported from previous study with *Rhizobium* sp. [20].

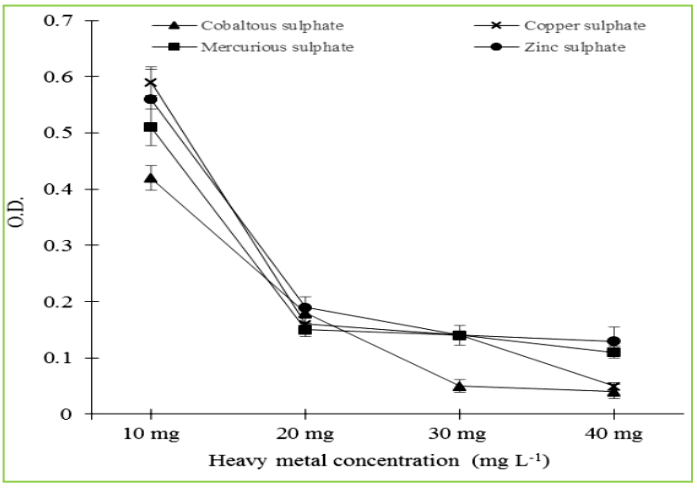

Fig-1 Effect of heavy metal concentrations on *in vitro* growth of *R. leguminosarum* TF17. OD of control = 0.67; Vertical bars represent standard error

Effect of heavy metals on tomato seed germination

The reports show that heavy metal effect on seed germination vary from metal to metal and from concentration to concentration [21]. In the present investigation heavy metal treatment considerably reduced tomato seed germination.

After 21 days of sowing the lowest seed germination percentage (81 %) was recorded from un-inoculated $CuSO_4$ treatment and highest with inoculated control (97.33 %). In comparison to control the tomato seed germination percentage was lower in all the four heavy metal treatments. The heavy metal induced reduction in seed germination has earlier

been documented on *Arabidopsis thaliana* [22], *Medicago sativa* [23], *Pisum sativum* [24] and *Sinapis alba* [25]. Overall, a moderate improvement in tomato seed germination was observed due to *R. leguminosarum* TF17 inoculation in heavy metal rich agar medium [Table-3]. The germination improvement was consistent during early observations of second and fourth week which diminished later. The prominent germination improvement due to rhizobial inoculation was obtained against $CuSO_4$ treatment which remained consistent throughout the observation. Rhizobial cells extracellularly release nodulation signals like lipo-chito-oligosaccharides (LCOs) which are known to stimulate seed germination in a wide range of plant species by unknown mechanism [26].

Table-1 *Morphological and biochemical characteristics of the R. leguminosarum biovars*

Isolate number	TF2	TF5	TF17	TF22	TF27	TF31	TF32	TF49	TF52	TF58	TF61
Morphology	rods	rods	rods	rods	rods	rods	rods	rods	rods	rods	rods
Gram's reaction	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+
Acid production	++	++	+	++	+	+	+	+	++	++	++
Catalase test	+	+	+	+	+	+	+	+	+	+	+
H_2S production	-	-	-	-	-	-	-	-	-	-	-
Urea hydrolysis	-	-	-	-	-	-	-	-	-	-	-
Carbon source											
Citrate	-	-	-	-	-	-	-	-	-	-	-
Gluconate	+	++	++	++	+	+	++	+	++	++	+
Glucose	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Glycerol	++	+++	+++	++	++	+++	+++	++	+++	+++	++
Inositol	++	++	++	++	++	++	+++	++	++	+++	++
L-Arabinose	++	+++	++	++	+++	++	+++	+++	+++	+++	+++
Maltose	+++	+++	++	+++	+++	++	+++	++	+++	+++	+++
Mannitol	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Sorbose	-	-	-	-	-	-	-	-	-	-	-
Succinate	++	+	+	+	++	++	++	++	+	++	++
Sucrose	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Nitrogen source											
Yeast extract	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Peptone	+	+/-	+	+	+	+	++	+	+	+	+/-
Glycine	++	++	++	++	+++	++	++	++	++	++	++
NH_4Cl	++	++	++	++	++	++	++	++	++	++	++
KNO_3	+	++	++	+	+	+	+	+	+	++	++
Growth factor required											
Biotin	+	+	+	+/-	+/-	+/-	+	+	+	+	+
Thiamine	+	+	+	+/-	+	+/-	+	+/-	+	+/-	+
Nodulation test											
Mean No. of nodules/plant	81	83	94	71	73	79	97	112	69	70	83

-: negative; +: positive; ++: strongly positive; +++: intensely positive

Table-3 *Percent germination of tomato seeds treated with heavy metals salts and inoculated with R. leguminosarum TF17*

	Treatment	Germination % of seeds					
		6 days	9 days	12 days	15 days	18 days	21 days
	Control	24.00a	76.67a	80.67a	89.33a	92.33a	96.67a
Non-inoculated	$CoSO_4$	20.33ab	46.00c	71.67b	75.67c	83.33b	85.33bc
	$CuSO_4$	15.00b	40.00d	54.33c	70.67d	72.67d	81.00c
	$HgSO_4$	15.33b	45.00cd	62.00c	76.33bc	78.67c	86.67b
	$ZnSO_4$	16.00b	53.67b	72.67ab	81.00b	88.00b	89.00b
	LSD ($P \leq 0.05$)	4.36	5.85	8.31	4.70	3.96	4.60
	Control	26.00a	77.00a	85.00a	88.00a	92.67a	97.33a
Inoculated	$CoSO_4$	21.00b	50.33c	64.67c	74.67c	86.00b	86.00c
	$CuSO_4$	16.00c	41.67d	55.67d	73.00c	76.67c	81.67d
	$HgSO_4$	15.00c	47.33c	60.67c	76.00c	79.67c	82.33cd
	$ZnSO_4$	16.33c	58.33b	75.33b	81.67b	86.33b	91.33b
	LSD ($P \leq 0.05$)	4.10	5.36	4.97	4.99	5.29	4.25

Means with the same letter in each column are not significantly different ($P \leq 0.05$)

Effect of heavy metals on tomato plant growth

In pot study, the sown tomato seeds treated with the four different heavy metal salts (20 mg Kg^{-1} soil) were observed for 14 weeks. The soil mixture used in the study had 3.02% organic C, 0.62% N, 0.005% P and 0.007% K, respectively [Table-4]. Compared to control the biweekly observations displayed notable plant length retardation with three out of four treatments. Overall, the plant length enhancement due to $ZnSO_4$ treatment and significant retardation due to $CuSO_4$ treatment was observed.

Table-4 *Physico-chemical properties of experimental soil*

Parameter	Value
Soil-Sand ratio	2:3
Organic carbon (%)	3.02
N (%)	0.62
P (%)	0.005
K (%)	0.007
pH	7.55
Sand (%)	63
Silt (%)	19
Clay (%)	18
Texture	Loamy

In un-inoculated pots the final data of 14th week showed maximum shoot-root length (73.18 cm and 23.23 cm, respectively) against $ZnSO_4$ treatment and minimum (36.46 cm and 14.01 cm, respectively) with $CuSO_4$ [Table-5]. Similarly maximum shoot-root dry weight (0.466 g and 0.480 g) was observed with $ZnSO_4$ treatment and minimum (0.188 g and 0.202 g, respectively) with $CuSO_4$ [Table-6]. Zn has been reported beneficial to plant up to 50 and 100 mg kg^{-1} concentration. Beyond 150 mg kg^{-1} level, Zn displays detrimental effect on tomato plants [27]. In current study this explains the reason for higher tomato plant growth treated with Zn at the concentration of 20 mg Kg^{-1} soil. The Cu requirement for normal growth in most plants is generally 5-20 mg Kg^{-1}. Decrease in tomato biomass due to Cu exposure has already been documented [28]. The amount in excess of 20 mg kg^{-1} soil is considered toxic [29] and is known to reduce the soil microflora. But in our study Cu treatment at 20 mg Kg^{-1} caused notable height and dry weight retardation of tomato plant. The Cu exposure retards plant growth by repressing photosynthetic O_2 evolution [30], free radical formation, oxidative breakdown of polyunsaturated lipids [31] and by lipid peroxidation [29]. The $HgSO_4$ application also retarded tomato plant length and dry weight in the study. The Hg concentration as low as 10 µM in the root zone has been reported toxic to tomato [32]. The phytotoxic effects of Hg are due to enhanced production of active oxygen species (mainly H_2O_2) and subsequent lipid peroxidation. It has been reported that Hg predominantly accumulate in tomato roots rather than in the shoot [32]. In our study considerable tomato growth retardation was observed with Co exposure. The application of Co has been reported to significantly reduce tomato plant biomass with the concentration as low as 0.05 mM [33].

Table-5 *The shoot-root length (cm) of non-inoculated tomato plants growing on heavy metal treated soil*

	Treatment	Weeks after sowing						
		2	4	6	8	10	12	14
Shoot length	Control	5.66ab	12.57a	14.44b	21.97a	27.37b	39.2b	57.64b
	$CoSO_4$	5.56ab	11.86a	13.18b	18.65b	23.57c	28.65d	44.93c
	$CuSO_4$	5.18b	9.01b	10.78c	17.86b	18.91d	23.68e	36.46d
	$HgSO_4$	5.60ab	11.66a	14.06b	20.99a	24.97bc	32.92c	48.50c
	$ZnSO_4$	6.02a	12.90a	16.38a	23.17a	33.35a	49.89a	73.18a
	LSD (P ≤ 0.05)	0.50	1.82	1.79	2.32	3.11	2.95	4.10
Root length	Control	2.85ab	3.79bc	4.70c	8.27ab	11.45a	12.35a	20.80ab
	$CoSO_4$	2.64b	3.13c	4.58c	7.63cb	8.67b	9.62bc	14.27c
	$CuSO_4$	2.18c	3.56bc	4.55c	6.78c	7.73b	7.92c	14.01c
	$HgSO_4$	2.65b	3.96ab	6.92a	7.83b	9.24b	10.41b	18.54b
	$ZnSO_4$	2.94a	4.68a	5.82b	9.17a	13.09a	13.43a	23.23a
	LSD (P ≤ 0.05)	0.25	0.75	1.05	7.72	1.74	1.75	2.55

Means with the same letter in each column are not significantly different (P ≤ 0.05)

The inoculated treatments provided higher growth values than non-inoculated counterparts. The final data of 14th week shows that inoculation of *R. leguminosarum* TF17 enhanced plant growth by yielding maximum shoot-root length (76.36 cm and 24.52) with $ZnSO_4$ treatment and minimum with $CuSO_4$ (41.86 and 14.73, respectively) [Table-7]. The maximum plant shoot-root dry weight (0.484 g and 0.509 g, respectively) was obtained with $ZnSO_4$ treatment and minimum (0.197 g and 0.213 g, respectively) with $CuSO_4$ [Table-8].

A consistent plant growth mitigation due to *R. leguminosarum* TF17 was observed against $CoSO_4$ and $CuSO_4$ treatment. The rhizobial mitigation effect was consistent against $CuSO_4$ treatment in the form of tomato shoot length with 14.81 percent enhancement of 14 weeks old plants. Maximum rhizobial based mitigation in the form of root length enhancement was observed with $CoSO_4$ treatment. Best mitigation effect among all the treatments was observed in the form of increased shoot dry wt of 2 weeks old $CoSO_4$ treated tomato plants (33.33 % increase) followed by increased root dry wt with $HgSO_4$ treatment (25 % increase) for the same period. However the mitigation effect temporally decreased within the treatment.

This plant growth mitigation effect could be either due to heavy metal assimilation by rhizobial cells or due to rhizobial counteraction of the growth retarding effect of heavy metals by providing additional nutrients to plant. Various strains of *Rhizobium* and *Bradyrhizobium* have already been reported to show plant growth promotion [34]. Most rhizobial strains attract towards amino acids, organic acids and sugars as a result of chemotactic response [35]. The rhizobial trait of attraction and attachment to root surface of non-nitrogen fixing plant is strain specific and thus cause difference in the rate of plant growth promotion.

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Conclusion

The overall analysis of a study concludes that out of four heavy metal salts used in the study three were detrimental to tomato plant growth at 20 mg Kg^{-1} soil concentration. The inoculation of *R. leguminosarum* TF17 mitigated the growth

retarding effect of heavy metals on tomato plant to some extent. The mitigation effect was visible in the form of increased plant height and dry weight of inoculated treatments. The study reveals the potential of *R. leguminosarum* as bioremediant and plant growth promoting rhizobacteria (PGPR) for non-legume plants.

Table-6 *The shoot-root length (cm) of R. leguminosarum TF17 inoculated tomato plants growing on heavy metal treated soil*

	Treatment	Weeks after sowing						
		2	4	6	8	10	12	14
	Control	6.01a	13.78a	16.94a	23.12ab	28.22b	41.05b	61.11b
Shoot length	$CoSO_4$	5.63a	12.64ab	13.53b	19.61c	25.27b	31.19c	46.27cd
	$CuSO_4$	5.32b	9.47c	11.81c	19.27c	20.30c	26.32d	41.86d
	$HgSO_4$	5.67ab	11.94b	14.36b	21.92b	26.04b	34.98c	50.61c
	$ZnSO_4$	6.14a	13.75a	17.67a	24.37a	35.16a	53.69a	76.36a
	LSD (P≤ 0.05)	0.52	1.67	1.69	1.92	3.37	3.96	5.22
	Control	3.04a	4.07ab	5.98ab	8.81ab	12.01b	13.15ab	21.54b
Root length	$CoSO_4$	2.78ab	3.24c	4.84bc	7.95bc	9.36c	10.17cd	15.70c
	$CuSO_4$	2.33c	3.81bc	4.85c	7.17c	8.34c	8.38d	14.73c
	$HgSO_4$	2.73b	4.32b	6.53ab	8.36b	9.58c	11.18bc	19.65b
	$ZnSO_4$	3.05a	5.04a	6.98a	9.80a	13.90a	14.58a	24.52a
	LSD (P≤ 0.05)	0.28	0.81	0.91	1.19	1.57	2.23	2.59

Means with the same letter in each column are not significantly different (P ≤ 0.05)

Table-7 *The shoot-root dry weight (mg) of non-inoculated tomato plants growing on heavy metal treated soil*

	Treatment	Weeks after sowing						
		2	4	6	8	10	12	14
	Control	5.67a	9.5a	18.47b	42.83ab	95.07ab	146.27b	410.11b
Shoot dry wt.	$CoSO_4$	3.43b	7.68a	13.20c	22.40c	57.03b	135.90b	198.91d
	$CuSO_4$	3.00b	12.63b	12.63c	21.40c	51.63b	92.77c	188.45d
	$HgSO_4$	3.60b	7.93a	22.13ab	39.13b	62.17b	130.30b	299.60c
	$ZnSO_4$	5.60a	9.03a	24.73a	50.37a	128.53a	208.43a	466.37a
	LSD (P ≤ 0.05)	1.77	2.22	5.14	10.18	39.52	18.14	38.13
	Control	5.10ab	11.37a	22.30a	444.43a	93.73b	146.27b	412.99b
Root dry wt.	$CoSO_4$	3.93bc	7.30b	11.83c	23.80b	60.37c	134.90bc	223.87d
	$CuSO_4$	3.00c	7.07b	10.40c	25.33b	53.30c	107.43c	202.85d
	$HgSO_4$	4.43bc	7.77b	16.63b	31.33b	63.50c	124.97b	320.26c
	$ZnSO_4$	6.67a	10.60a	20.40a	52.77a	129.87a	223.40a	480.77a
	LSD (P ≤ 0.05)	1.74	2.49	2.56	10.37	15.40	30.74	29.88

Means with the same letter in each column are not significantly different (P ≤ 0.05)

Table-8 *The shoot-root dry weight (mg) of R. leguminosarum TF17 inoculated tomato plants growing on heavy metal treated soil*

	Treatment	Weeks after sowing						
		2	4	6	8	10	12	14
	Control	6.02a	10.15a	21.85a	45.11ab	98.26ab	158.82b	430.95b
Shoot dry wt	$CoSO_4$	3.57b	8.21a	13.98b	22.83c	59.74bc	145.61bc	207.97d
	$CuSO_4$	3.11b	5.50b	13.33b	23.84c	55.04c	99.75d	197.12d
	$HgSO_4$	3.84b	8.34a	22.50a	41.56b	66.45bc	136.47c	316.20c
	$ZnSO_4$	5.90a	9.60a	26.87a	54.88a	139.60a	219.45a	484.20a
	LSD (P ≤ 0.05)	1.91	2.27	5.76	10.00	41.49	17.73	39.27
	Control	5.56ab	12.28a	23.01a	46.10a	97.65b	151.98b	435.34b
Root dry wt	$CoSO_4$	4.43bc	8.27c	12.38c	24.74b	63.45c	146.47bc	234.43d
	$CuSO_4$	3.31c	7.59c	11.19c	26.58b	56.54c	114.11c	212.87d
	$HgSO_4$	4.54bc	9.23bc	17.48b	32.53a	67.44c	135.77bc	337.45c
	$ZnSO_4$	6.98a	11.23ab	21.57a	55.57a	134.82a	232.92a	509.51a
	LSD (P ≤ 0.05)	1.73	2.65	2.71	10.72	14.93	35.95	34.97

Means with the same letter in each column are not significantly different (P ≤ 0.05)

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