Effects of Bacterial Consortium on Heavy Metal Accumulation and Growth Performance of *Zea mays* L. Plants

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Abstract

The present study aimed to assess the effects of a Cd-resistant bacterial consortium (ECO-M) on growth performances and heavy metal accumulation in Zea mays L. var DKC8205 grown on heavy metal contaminated soil. ECO-M inoculation has significantly increased the numbers of leaves, leaf area, height and biomass of Z. mays plants over the control in both ground (GWIS) and wastewater irrigated soil (WWIS) (P<0.05). The per cent increase in tested growth parameters of Z. mays plants due to ECO-M inoculation varied from 5% to 59% in the contaminated soils. The accumulation of heavy metals in roots, stems and leaves decreased significantly in ECO-M inoculated plants as compared to the non-inoculated plants (P<0.05). The maximum per cent reduction in Cu and Cd concentrations were found 43% in leaf, 54% in roots, 89% in stem, respectively in GWIS. Cr, Zn and Ni concentrations in leaf were decreased by 43%, 18% and 23%, respectively in WWIS. The per cent reductions of heavy metals in tested plants were higher in GWIS as compared to WWIS. From the results of present study, it can be concluded that the application of bacterial consortium ECO-M could be helpful in improving the growth of crops and prevent entry of heavy metals in food chain resulting from long-term use of contaminated irrigation water.

Index Terms: *Consortium, Heavy metals, Growth, Biomass, Bioaccumulation*

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INTRODUCTION

Heavy metal contamination of food chain and their associated human health risk is as one of the growing environmental concerns throughout the globe. Heavy metals pose adverse impacts on soils, plants, animals, and human beings (Singh *et al*., 2019; Lu et al., 2011). Long-term wastewater use for irrigation of agricultural crops elevated the concentrations of heavy metals in top soil and consequently in vegetable crops (Liu et al., 2005, Yang et al., 2008). In addition, other anthropogenic activities such as industrialization, unplanned urbanization, excess use of phosphatic fertilizers and pesticides in agricultural fields, etc., has also elevated heavy metals in soils (Allaire, M. *et al.,* 2028).

Conventional technologies are neither eco-friendly nor cost effective and generate secondary pollutants in the environment. Whereas phytoremediation. a green technology used to remediate the contaminated soil and is considered as one of the feasible, cost-effective and eco-friendly strategies for sustainable restoration of heavy metals contaminated soils (Rajkumar et al., 2017; Manoj et al., 2020). Plants such as *Zea mays, Brassica juncea,* etc., were selected for the efficient phyto-remediation due to their greater biomass and well established root system to accumulate contaminants (Ali *et al*., 2010). Heavy metals reduce the efficiency of phyto-remediation process by inhibiting plant's growth and their ability to remove/stabilize heavy metals present in the soil. Higher concentrations of heavy metals in the soil has adverse effects on plant growth and establishment by altering metabolic processes and sequentially reduces the efficiency of phytoremediation (Ling et al., 2017).

Microorganisms, in association with roots, form unique communities in the rhizosphere promote plant growth and detoxify hazardous compounds (Belimov et al., 2005; Rajkumar, and Freitas 2009). Metal-resistant microorganisms can affect the mobility of trace metal and their availability to the plants through release of chelating agents, organic acids, phosphate solubilization, and redox changes (Abou-Shanab et al., 2003; Idris et al., 2004). Therefore, improvement of the interactions between plants and beneficial rhizosphere microbes can enhance biomass production and tolerance of the plants to heavy metals and improve phytoremediation process. PGPRs can improve plant growth by direct or indirect methods (Dell'Amico et al., 2008; Tica et al., 2011). In recent years, studies about rhizobacteria and their interactions with hyper accumulating or accumulating plants have attracted the attention of several investigators (Barakat 2011; Barzanti et al., 2007; Dell'Amico et al., 2008; Idris et al., 2004; Sheng and Xia, 2006).

Recent studies have revealed that these PGPRs could promote plant growth and protect them against heavy metals toxicity in contaminated soils (Barakat 2011; Burd et al. 2000; Belimov et al. 2005; Dell'Amico et al. 2008; Idris et al. 2004). The present study aimed to assess the effect of Cd resistant bacterial consortium on heavy metal accumulation and consequence growth performance of *Z. mays* L. plants grown in soil contaminated with heavy metals.

Methodology

Consortium development

Cd resistant strains, BHUJ Cd-1, BHUJ Cd-3, BHUJ Cd-10, BHUJ Cd-15 and BHUJ Cd-20 were isolated from long-term wastewater irrigated soil of Varanasi region. A consortium, ECO-M was developed using these strains in 1:1:1:1:1 in ratio into an autoclaved nutrient broth medium amended with salt of Cd 50 µg ml^{-1} $CdCl_2H_2O$ (Tiwari *et al.,* 2012),. The developed consortium was stored at 4 ^{0}C in a refrigerator for further use.

Pot experiment

A pot experiment was conducted in the Botanical Garden of Department of Botany, Banaras Hindu University, Varanasi to assess the effects of consortium ECO-M on heavy metal accumulation and growth performance on maize (*Zea mays* L.) crops grown on soil under two irrigation regimes i.e. wastewater and ground water. The experimental soil (10 kg each) collected from a long-term wastewater and ground water irrigated area of Varanasi were amended with recommended doses of NPK (120:60:60 mg kg^{-1}) and farm yard manure (30 tons ha^{-1}) and were filled in 20 earthen pots. The physicochemical chemical and heavy metal concentrations in the experimental soils are in Table 1. Healthy seeds of *Z. mays* var. DKC8205 plants obtained from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi were sterilized with 2% $HgCl_2$ for five minutes and were treated with ECO-M aseptically amended with game cassia and put for 30 minutes. Five seeds were hand in sown at the depth of 2-cm in each pot (10 pots for each irrigation regimes). Ten pots under each irrigation regime were without ECO-M i.e. –ECOM. Others 10 pots were with ECO-M i.e. +ECO-M. These experiments were conducted in five replicates.

Equal volume of water was used for the irrigation. Manual weeding was also performed during the experimental period as and when required.

Soil analysis

The experimental soils (500g approx) were air dried followed by crushing with mortar and pastel and sieved with 0.2-mm sized sieve. Soil pH of the air-dried soil was measured in soil: water suspension (1: 5) using a pH electrode (Eutech Instruments, Singapore). The electrical conductivity of the soil samples was also measured by using a conductivity meter (Labman Scientific Instruments, India) in the same soil suspension. The organic carbon in soil was determined using Walkley and Black's Rapid Titration method (Allison et al., 1986). For the analysis of heavy metals, 0.25 g of dried soil was digested with 15 ml of HNO_3 and $HClO_4$ in 9:4 ratios at 80 °C until a transparent solution was obtained (Allen *et a*l., 1986). Then, the digested soil samples were filtered through a Whatman filter paper 42 and the final volume of filtrates was maintained to 25 ml using double distilled water. Concentrations of heavy metals Cu, Zn, Cd, Cr and Ni in the filtrates were determined using an atomic absorption spectrophotometer (Perkin- Elmer, 2130, USA) fitted with a specific lamp of particular metal and appropriate drift blank.

Plant sampling and analysis

The per cent seed germination was calculated by dividing the number of seeds germinated at seventh days after the sowing with total seed sown in the pot and the obtained values were multiplied by hundred. Three 50-days old *Z. mays* plants from the respective replicate of a treatment were collected for the growth and biomass analysis. Each plant was considered as a replicate. The plant samples were then brought back to the laboratory and washed under the running tap water to remove the adhered soil particles and dried using the blotting papers. The numbers of leaves were counted manually and the leaf area was measured using a graph paper. The lengths of roots and shoots were measured using measuring scale and both were summed up to find out the total length of plants. The roots, stems and leaves were separated and oven dried at 80 ^{0}C in hot air oven till constant weight was obtained. Dry weights of roots, stem and leaves were taken separately and then combined to obtain the total biomass using a pan balance. Root shoot ratio was also computed by dividing the dry weights of root with that of shoots and expressed as g g^{-1}.

For the determination of heavy metals such Cd, Cr, Ni, Cu and Zn in roots, stems and leaves of *Z. mays* plants , 0.1 g of ground material was digested by method as described above (Allen *et al*.,1986). Metal pollution index (MPI) in roots, stems and leaves of Z. mays plants was computed using the following equation (Usero et al., 1997).

$$MPI = (Cf_1 \times Cf_2 \times Cf_3 \times \ldots\ldots \times Cf_n)^{1/n}$$

Where, Cf is the concentration of n heavy metals in plant samples.

Statistical analysis

All the measurements were carried out in triplicates and results were expressed as a mean ± standard error. Paired-sample T-test technique was applied to separate the treatment means and considered statistically significant at $p<0.05$. All the statistical analyses were performed using SPSS software (SPSS, Inc., Version 16).

Table 1: Physicochemical properties of experimental soil

Parameters		**Ground water-Soil**	**Wastewater-Soil**
pH		7.18 ± 0.18	7.2 ± 0.20
EC	µs/cm	169 ± 9.23	815.66 ± 20.66
OC	%	0.72 ± 0.05	4.84 ± 0.03
Total nitrogen	kg ha^{-1}	547.45 ± 10.26	-
Total phosphorous	kg ha^{-1}	35.56 ± 2.21	85.00 ± 2.90
Total potassium	kg ha^{-1}	257.04 ± 1.28	-
Elemental content			
Cd	mg kg^{-1}	1.02 ± 0.04	8.43 ± 0.35
Cr	mg kg^{-1}	0.67 ± 0.05	129.5 ± 4.11
Ni	mg kg^{-1}	3.41± 0.40	47.73 ± 0.69
Cu	mg kg^{-1}	4.25 ± 0.35	124.67 ± 1.45
Zn	mg kg^{-1}	24.42 ± 0.76	152.17 ± 0.09
Ca	mg kg^{-1}	440 ± 45.01	-
Mg	mg kg^{-1}	1788 ± 72.45	-

Values are mean ± SE of three replicates.

Results

Physico-chemical properties of experimental soil

The physico-chemical properties of soil collected from the ground and wastewater irrigated areas of Varanasi were analysed and results are shown in Table 1. The results showed that pH, EC, P and organic carbon content in soil were generally higher in WWIS as compared to the GWIS (Table. 1). The concentrations of metals such as Cd, Cr, Ni, Cu, Zn, Ca, and Mg concentration were many times high in WWIS than the GWIS (Table 1). The concentration of Cu, Cr and Cd in WWIS has exceeded the European Union Standards (100, 100 and 3 mg/kg dw, respectively). Whereas, Cd exceeded the Indian standard (3-6 mg/kg dw) set for the soil.

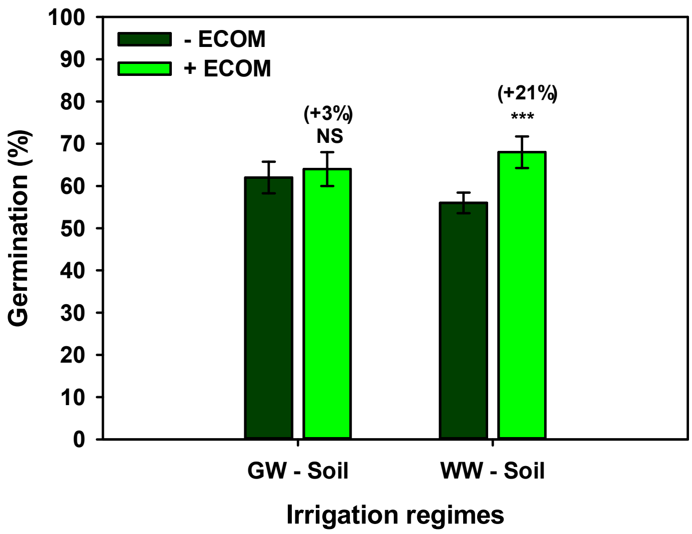

Figure 1: Effects of Cd resistant bacterial consortium application on seed germination of *Z. mays* plants grown on soil under different irrigation regimes. Bars are mean ± SE of three replicates. Level of significance: *** p<0.001; NS = not significant (p>0.05). Values in parenthesis are percent change.

Table 2: Effects of Cd resistant bacterial consortium application on growth performance and biomass accumulation in *Z. mays* plants grown on soil under different irrigation regimes

Growth performance	Ground water-Soil		Wastewater-Soil	
	-ECOM	+ECOM	-ECOM	+ECOM
No of leaves (Plant^{-1})	7.80 ± 0.37	9.60 ± 0.24**	8.60 ± 0.24	11.60 ± 0.40**
Leaf area (cm^{2} plant^{-1})	1360 ± 18.66	1426 ± 14.44^{ns}	1501 ± 43.36	1702 ± 17.74*
Plant length (cm plant^{-1})				
Root	14.80 ± 0.20	19.20 ± 0.86*	17 ± 0.89	22.40 ± 1.17**
Shoot	95.60 ± 1.89	122.32 ± 5.25**	135 ± 1.90	142.40 ± 3.16^{ns}
Total plant	110.40 ± 1.94	141.52 ± 6.02**	152 ± 2.02	164.80 ± 3.69*
Biomass (g plant^{-1})				
Root	0.84 ± 0.04	1.19 ± 0.03**	0.64 ± 0.03	2.48 ± 0.15***
Stem	3.71 ± 0.13	5.88 ± 0.19**	5.61 ± 0.24	8.90 ± 0.17**
Leaf	3.61 ± 0.25	4.81 ± 0.18*	5.27 ± 0.20	7.20 ± 0.37*
Total plant	8.70 ± 0.51	12.78 ± 0.52**	11.53 ± 0.41	18.58 ± 0.54**

Values are mean ± SE of three replicates.
Level of significance: *** p<0.001; ** p<0.01; * p<0.05; NS = not significant (p>0.05).

Effect of consortium on growth performance of *Zea mays* plants

The results of present study showed that seed germination, growth, biomass and root shoot ratio (RSR) of the *Z. meays* plants were improved due to the application of consortium ECO-M in both WWIS and GWIS as compared to the control i.e. without consortium (Tables 2 and 3, Figure 1). ECO-M application increased the seed germination of tested plants significantly by 21% over the control in WWIS ($p<0.001$). Application of ECO-M did not significantly affect the seed germination in GWIS (Figure 1). Application of ECO-M in GWIS has increased the tested parameters significantly, except leaf area ($p<0.01$). However, application of ECO-M enhanced the growth performance of *Z. mays* significantly over the control in WWIS (Table 2).

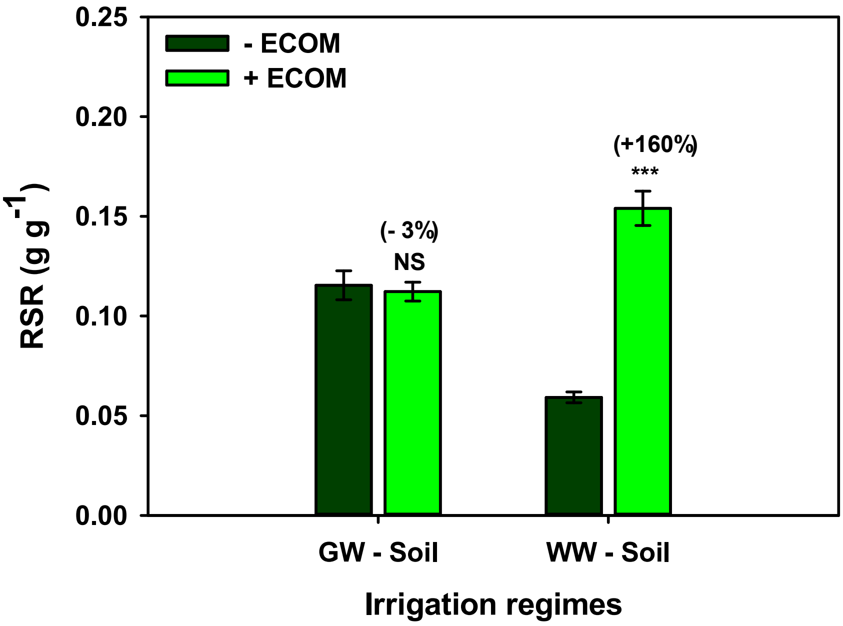

Figure 2: Effects of Cd resistant bacterial consortium application on root shoot ration of *Z. mays* plants grown on soil under different irrigation regimes. Bars are mean ± SE of three replicates. Level of significance: *** $p<0.001$; NS = not significant ($p>0.05$). Values in parenthesis are percent change.

The minimum i.e. 5% increase in leaf areas and shoot length of tested plants due to ECO-M application were found in GWIS and WWIS, respectively. The maximum i.e. 59% increase in stem biomass due to ECO-M application was found in tested plants in both types of soil (Table 2). Results further revealed that RSR significantly increased in WWIS with an insignificant decrease in GWIS due to inoculation of of *Zea mays* plant with ECO-M (Figure 2).

Table 3: Effects of Cd resistant bacterial consortium application on heavy metal **(µg g⁻¹ dw)** accumulation in different parts of *Z. mays* plants grown on soil under different irrigation regimes

Plant's parts/ Heavy metals		Ground water-Soil		Wastewater-Soil	
		-ECOM	+ECOM	-ECOM	+ECOM
Root	Cu	11.17 ± 0.51	7.97 ± 0.27^{ns}	80.03 ± 1.81	79.67 ± 1.02^{ns}
	Cd	0.42 ± 0.08	0.19 ± 0.03**	16.42 ± 0.65	10.58 ± 0.22**
	Cr	54.67 ± 0.22	50.53 ± 0.55*	111.83 ± 0.79	75.58 ± 1.10*
	Zn	104.08 ± 3.20	80.03 ± 1.81*	275.83 ± 1.67	233.58 ± 8.82*
	Ni	12.75±0.58	10.07 ± 0.28*	23.08 ± 1.47	16.58 ± 1.54*
Stem	Cu	9.75 ± 0.14	6.67 ± 0.09**	11.25 ± 0.58	8.00 ± 0.25*
	Cd	2.75 ± 0.14	2.20 ± 0.15*	7.25 ± 0.14	5.33 ± 0.22*
	Cr	51.50 ± 0.52	43.07 ± 1.32*	287.50 ± 1.44	244.92 ± 5.07*
	Zn	69.42±0.33	8.00 ± 0.25*	145.17 ± 2.42	133.88 ± 5.08^{ns}
	Ni	14.17 ± 0.22	11.40 ± 0.55*	22.00 ± 1.01	18.08 ± 0.44^{ns}
Leaf	Cu	20.58 ± 1.29	11.70 ± 0.70*	19.33 ± 2.96	15.58 ± 0.22^{ns}
	Cd	3.33 ± 0.22	2.87 ± 0.19^{ns}	10.25 ± 0.14	10.25 ± 0.43^{ns}
	Cr	55.58 ± 1.80	45.70 ± 1.57^{ns}	145.75 ± 3.50	102.08±1.67**
	Zn	56.92±0.55	15.58 ± 0.22***	146.92 ± 1.67	135.82 ± 0.59*
	Ni	17.58 ± 0.73	13.67 ± 0.49*	32.58 ± 0.36	27.08 ± 0.44**

Values are mean ± SE of three replicates.
Level of significance: *** p<0.001; ** p<0.01; * p<0.05; NS = not significant (p>0.05).

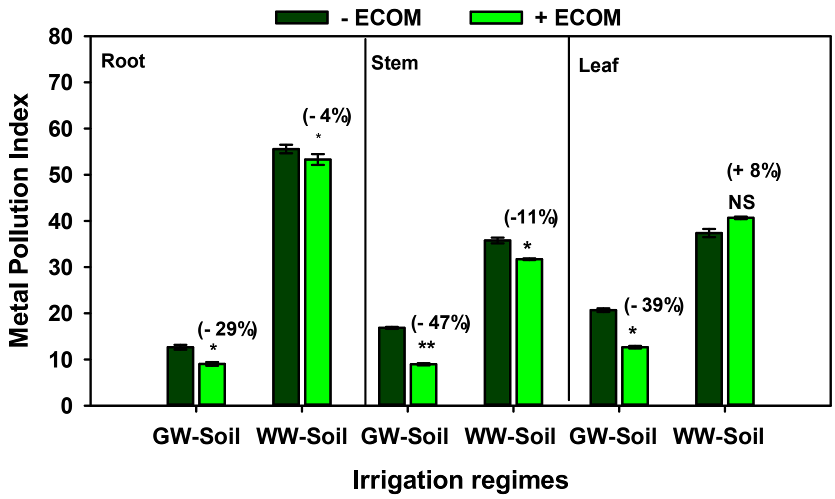

Figure 3: Effects of Cd resistant bacterial consortium application on metal pollution load in root, stem and leaf of *Z. mays* plants grown on soil under different irrigation regimes. Bars are mean ± SE of three replicates. Level of significance: *** p<0.001; ** p<0.01; * p<0.05; NS = not significant (p>0.05). Values in parenthesis are percent change.

Effect of Cd resistant bacterial consortium on heavy metal accumulation

The results of the effects of a Cd resistant plant growth promoting rhizobacterial consortium, ECO-M on heavy metal accumulation in different parts of *Z. mays* plants grown in GWIS and WWIS are given in Table 3. The results showed that Cu, Cd, Cr, Zn, and Ni accumulation were significantly reduced by 29%, 54%, 8%, 23% and 21 % in root tissues, 32%, 20%, 17%, 89%, and 20% in stem tissues and 43%, 14%, 18%, 73% and 23% in leaf tissues due to the application of consortium in *Z. mays* plants grown in GWIS. However, ECO-M application in WWIS reduced 0.5%, 36%, 48%, 18% and 28% in root tissues, 29%, 26%, 17%, 8% and 18% in stem tissues and 0%, 43%, 8% and 20% in leaf tissues, respectively. The results indicated that the application of ECO-M potentially reduces the heavy metal accumulation in Z. mays plants grown on both the soils significantly (Table 3).

DISCUSSION

Soil pollution with heavy metals is one of sever environmental problem worldwide. Further researches are required to minimize the soil pollution. Thus present study aimed to remediate heavy metals contaminated soil by using a Cd tolerance plant growth promoting rhizbacterial consortium. In the present study, results showed that ground water irrigated soil have lower pH, EC, OC, total nitrogen, phosphorous, potassium compared to wastewater irrigated soil, and was higher in wastewater compared to ground water. Pescod (1992) also reported that wastewaters have slightly higher (range: 2.05 to 3.05 dS/m) EC above the safe limit (0.7-3 dS/m). Due to long-term use of wastewater in agricultural field increases the EC of wastewater irrigated soil. Previous studies were also agreed with results of the present study, wastewater have higher organic matter compared to ground water (Kunhikrishnan et al. 2012; Shahid et al. 2014). Wastewater had organic matter and nutrients carrier therefore increases the organic carbon in waste water irrigated soil compared to ground water. Results showed waste water irrigated soil had higher phosphorous due to amendments' of carrier of nutrient. Wastewater irrigated soil have higher heavy metals such as Cd, Cr, Ni, Cu, Zn, Ca and Mg as compared to soil irrigated with ground water (well water). Singh *et al*. (2010) reported that wastewater have higher heavy metal concentrations in wastewater irrigated soil as compared to clean water irrigated soil. According to Singh *et al*. (2010) the concentrations of heavy metals such as Cd, Cu, Pb, Zn and Cr were increased by 109%, 151%, 162%, 32% and 161%, respectively of Dinapur area where treated wastewater is used for the irrigation of agricultural field as compared to the site irrigated by clean water.

In the present study, heavy metal resistant plant growth promoting rhizobacterial consortium was inoculated in *Z. mays* plants and grown in the ground water and wastewater irrigated soils. Consortium increased per cent germination, numbers of leaf, leaf area, plant heights, biomass and root shoot ratio as compared to without inoculated consortium in both the soils. Ntirry *et al.* (2018) have reported that metal tolerant plant growth promoting bacteria such as cellulosic *Microbium* sp. isolated from a multi contaminated site have ability to increase plant growth under metal contaminated soil. Some previous studies have also reported that PGPR application has improved plant growth and plant fitness in contaminated environment (Ma et al., 2011, Nadeem et al., 2014).

Metal resistant plant growth promoting rhizobacteria were also capable of decreasing heavy metal toxicity and consequently improve the plant's health in contaminated soil. PGPR increases the nutrient availability in soil to the plants through mobilizing essential nutrient elements e.g., P, Mg, K, Ca, N, S, Fe, Zn, Mn, etc., and making them available to plant's roots, thus playing an important role in plant resistance to metal stress and in increase of plant biomass (Ma et al., 2016, 2011; Rajkumar *et al*., 2010b; Saravanan *et al*., 2011; Sharma and Archana, 2016; Khatri et al., 2020). Cu accumulation was more in leaf tissue followed by root and least in stem in ground water and more in root followed by leaf and least in stem in contaminated soil but application of consortium reduces the accumulation of heavy metal in *Z. mays* plants grown in both the soils. The same trends were also observed for Cu accumulation in *Z. mays* plants by Rizvi and Khan (2018). In the present study, Cd accumulation was reduced in both soils by inoculation of consortium. The results of the present study were agreed with Silva *et al*. (2021).

Cd accumulation in roots and shoots of *Z. mays* plants by inoculation *Pseumonas*. *aeruginosa* was reduced by 16.43% and 13.78% (Khanna *et al*., 2019). In addition, Cd accumulation in roots and shoots of Cd exposed seedlings of *Lycopersicon esculentum* L. was found lowered by 21.83% and 7.73% by application of *Burkholderia gladioli*. The present study showed that Zn accumulation in *Z. mays* plants was highest in roots followed by leaf and least in stem in both the soil regimes but application of consortium has increased Zn accumulation in wastewater and reduces in grounded water irrigated soil. The same trend was also found for Cr accumulation. Silva *et al.* (2021) have reported that inoculation of IPA403 increased the shoot Cr as compared to without inoculated soil. Metal pollution index were reduced in roots, shoots and leaf in both the soil by inoculation of consortium. *Z. mays* plants is more consumable crops and application of consortium can reduces the Cu, Cd, Cr, Zn and Ni in ground and wastewater irrigated soil which ultimately reduced the MPI in different parts of this plants. The reduction in MPI in different parts of *Z. mays* plants may be ascribed to reduction of heavy metal accumulation due to consortium application.

CONCLUSION

The increasing concentration of heavy metals in food chain can cause many health hazards in human beings. Therefore the development of an easy and eco-friendly approach to mitigate the heavy metal toxicity is needed. The results of present study showed that the application of ECO-M has improved the germination, plant growth, biomass accumulation and root shoot ratio of *Z. mays* plants grown in ground water and wastewater irrigated soil. Bioaccumulation of heavy metals such as Cd, Cr, Ni, Cu and Zn accumulation and metal pollution index in *Z. mays* plants were also reduced due to inoculation ECO-M in both soils. Inoculation of ECO-M in soil increases the soil microbial community composition which may influence the availability of heavy metals and nutrients in the soil and consequently their uptake and accumulation in *Z. mays* plants. From the present study, It can be concluded that *Z. mays* L. plants assisted with ECO-M can reduce the heavy metal accumulation in plant tissues and enhance the plant growth and thus prevent the entry of heavy metal in food chain. Further studies are required at molecular level in soil, ECO-M and plant systems to understand the actual set of beneficial effects on plants and metal reduction.

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