Metal Toxicity to Certain Vegetables and Bioremediation of Metal-Polluted Soils

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Abstract

The production of quality vegetables is a crucial issue worldwide due to consistently deteriorating soil health. Plants including vegetables absorb a number of metals from soil, some of which have no biological function, but some are toxic at low concentrations, while others are required at low concentration but are toxic at higher concentrations. As vegetables constitute a major source of nutrition and are an important dietary constituent, the heavy metal uptake and bioaccumulation in vegetables is important since it disrupts production and quality of vegetables and consequently affects human health via food chain. Considering the serious threat of metals to vegetables, an attempt in this chapter is made to highlight the effects of certain metals on vegetables grown in different agroclimatic regions of the world. Also, the bioremediation strategies adopted to clean up the metal-contaminated soil is discussed. The results of different studies conducted across the globe on metal toxicity and bioremediation strategies presented in this chapter are likely to help vegetable growers to produce fresh and contaminant-free vegetables.

8.1 Introduction

Vegetables play an important role in humans' diet by providing and assisting the body with a variety of important constituents such as minerals, vitamins, complex carbohydrate, high dietary fibre, low levels of fat and high amount of water. Due to

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these, the consumption of vegetables is encouraged in human dietary systems. Consequently, there is an increasing demand of fresh and healthy vegetables which, however, may be contaminated by pathogens, heavy metals and/or toxins (Mello 2003). Mostly, growers engaged in vegetable production in horticulture practices worldwide often use poor-quality irrigation water due to unavailability of good-quality water (Drechsel and Keraita 2014). Apart from poor-quality waters, soil, human handling, organic fertilizers and wastewater are the major factors in contaminating the fresh vegetables. Among these factors, organic fertilizers and wastewater are considered the major source of vegetable contamination (Grant 2011). Depending on the source of contamination, the industrial wastewater contributes significant amounts of metals, metalloids and volatile or semi-volatile compounds, while domestic wastewater is most harmful due to its pathogenic load (Fiona et al. 2003). International Water Management Institute (IWMI 2006) has reported that at least 3.5 million ha is irrigated globally with untreated, partly treated, diluted or treated wastewater. Such poor-quality water containing toxic materials after uptake by plants may cause severe toxicity to vegetables. The consumption of such contaminated vegetables in turn affects the human health. And, hence, due to increasing concern of food safety, proper practices and methods of production have to be developed, and the hazards and the risks associated with toxic elements like heavy metals have to be fully understood before they pose any serious and consequential threat to consumer health. The heavy metals cannot be degraded by any biological, physical or chemical processes (Naz et al. 2015) and, hence, persist in the environment. However, numerous traditional physicochemical processes are available for remediations of polluted sites which are expensive and quite often inefficient as they do not permanently eradicate the pollutants. Also, the byproducts generated in the process become hazardous to human health (Singh et al. 2011). On the other hand, biological methods are more acceptable as they do not pose such problems, are easy to operate and do not produce secondary pollution. The biological approach generally called as bioremediation is considered a safe and inexpensive technique since they are based on the use of living organisms, microorganisms and plants (Karigar and Rao 2011) For instance, microorganisms adopt several mechanisms such as biotransformation (Xiong et al. 2010) and have varied ability of interacting with heavy metals. Another heavy metal removal strategy involves the use of plants, called as phytoremediation, wherein plants partially or completely remediate selected contaminants from soil, sludge, sediments, wastewater and groundwater. It is a cost-effective, efficient and eco-friendly in situ remediation technology driven by solar energy. There are however, certain drawbacks associated with this technology such as the pollutants or their metabolites accumulate within plant tissues, which in turn shorten plant life and releases contaminants into the atmosphere via volatilization.

8.2 Heavy Metals: A Brief Account

A heavy metal is defined as a member of a loosely defined subset of elements that exhibits metallic properties and mainly includes the transition metals, some metalloids, lanthanides and actinides. However, based on density, atomic number or atomic weight and chemical properties or toxicity, heavy metals have been defined variously (John 2002). For instance, any metallic chemical element that has a relatively higher density and is toxic or poisonous even at low concentration is defined as heavy metal (Alloway 1990). On the other hand, the elements such as cadmium, copper, nickel, mercury and lead which are commonly associated with pollution and exhibiting significant toxicity are considered heavy metals by Fiona et al. (2003). However, based on their importance as a nutrient, metals have been classified as (1) essential (e.g. Zn, Cu, Fe, Mn and Se), (2) probably essential (e.g. V and Co) and (3) potentially toxic (As, Cd, Pb, Hg and Ni) (Ebdon 2001). Besides this, all metals, in general, have toxic effects when there is excessive exposure (Woimant and Trocello 2014).

Heavy metals are a serious concern throughout the globe due to their toxic, mutagenic and teratogenic effects even at very low concentrations (Oluwole et al. 2013). While growing in metal-polluted soils, the plant can absorb metals through roots, or they can be deposited on foliar surfaces (Jassir et al. 2005). Heavy metal enters the human body mainly through inhalation of dust, direct ingestion of soil, consumption of food plants grown in metal-contaminated soil and drinking contaminated water. Due to non-destructive nature, heavy metals consequently accumulate in human vital organs and cause varying degrees of illnesses (Lenntech 2006). Elimination of heavy metals deposited on the surface, however, can often be accomplished simply by washing prior to consumption, whereas bio-accumulated metals are difficult to remove and are, therefore, of major concern (Michio 2005).

8.3 Sources of Vegetable Contamination by Heavy Metals

Because of soil contamination, heavy metal stress is becoming a major challenge to crop plants, particularly to vegetable crops. The heavy metals are derived from city/industrial effluent (Cai et al. 2012; Wang et al. 2013), mining and smelting (Zhao et al. 2012), fertilizers and pesticides (Nacke et al. 2013; Yu et al. 2013), electronic waste recycling/dismantling activities (Liu et al. 2013) and auto mobile depositions (Turer and Maynard 2003). Additionally, wastewater/sewage water can be another major source of heavy metals in areas where raw sewage water is used for irrigation (Li et al. 2013; Wang et al. 2013). Vegetable growing areas which are often situated in or near sources of atmospheric deposits have an elevated risk of potential contamination. Ingestion of vegetables that have been produced with contaminated water poses a serious risk to human health including various chronic diseases, particularly after prolonged dietary intakes (Sharma et al. 2009). Different vegetable species, however, tend to accumulate different metals based on environmental conditions, metal species and plant available forms of heavy metals (Lokeshwari and Chandrappa 2006). Uptake through roots depends on many factors such as soil pH, plant growth stages, the soluble content of heavy metals in soil, as well as type of crops, fertilizers and soil (Sharma et al. 2006). Most common heavy metals often

Table 8.1 Guidelines for safe limits of heavy metals

Sample/ source	Standards	Cd	Cu	Pb	Zn	Mn	Ni	Cr
Soil (μg/g)	Indian Standard (Awashthi 1999)	03–06	135–270	250–500	300–600	–	75–150	–
	European Union Standards (EU 2002)	3.0	140	300	300	–	75	150
Water (μg/ml)	Indian Standard (Awashthi 2000)	0.01	0.05	0.1	5.0	0.1	–	0.05
	FAO (1985)	0.01	0.2	5.0	2.0	0.2	0.2	0.1
Plant (μg/g)	Indian Standard (Awashthi 1999)	1.5	30	2.5	50	–	1.5	20
	WHO/FAO (2007)	0.2	40	5	60	–	–	–
	European Union Standards (EU 2006)	0.2	–	0.3	–	–	–	–

Adapted from Singh et al. (2010)

found in vegetables include Cd, Cu, As, Cr, Pb, Zn, Co and Ni. When present in trace quantities, some of them act as micronutrients. Comparing the accumulated concentrations of metals with permissible limits of the Indian Standard (Awashthi 1999) and safe limits given by WHO/FAO (WHO/FAO 2007), several studies have found that metal concentrations were higher in vegetables grown in metal-polluted soil as compared to the safe limits given by commission regulation (EU 2006) (Table 8.1).

Other than safety concerns, excessive heavy metals significantly deteriorate the fertility of soil and consequently affect the growth and quality of crops (Muchuweti et al. 2006). Several studies have indicated that vegetables, particularly leafy vegetables grown in heavy metal-contaminated soils, have higher concentrations of heavy metals as compared to those grown in non-polluted soil. The symptoms of phytotoxicity of heavy metals, however, vary from metal to metal (Table 8.2). Routine monitoring of heavy metal concentrations in soils and also in crops is, therefore, essential to know the pollution levels and to devise strategies to minimize contamination, in order to reduce the risks to human health.

8.4 Bioaccumulation of Heavy Metals: A Serious Concern

Contamination and subsequent accumulation of heavy metals in leafy (Table 8.3) and non-leafy (Table 8.4) vegetables from different sources have been widely reported. However, the concentration of heavy metals in vegetables varies from below the detection limit to above the safe limit depending upon the source of contamination. Among heavy metals, Cd, a relatively rare element (WHO 1992), is

Table 8.2 Some examples of phytotoxicity symptoms of heavy metals in plants

Metal	Symptoms	Reference
Cadmium	Brown margin in leaves, chlorosis, necrosis, curled leaves, stunted roots, reddish veins and reduction in growth, purple coloration	Singh (2006)
Lead	Dark green leaves, stunted foliage, increased amounts of shoots	
Zinc	Chlorosis, stunted growth, reduction of root elongation	
Copper	Chlorosis, yellow coloration, purple coloration of the lower side of the midrib, less branched roots, inhibition of root growth, brown, stunted, coralloid roots, inhibition of plant growth	
Iron	Dark green foliage, stunted top and root growth, thickening of roots, brown spots on leaves starting from the tip of lower leaves, dark brown and purple leaves	
Chromium	Reduction in root growth, leaf chlorosis, necrosis, inhibition of seed germination and depressed biomass, disturb water balance	Ghani (2011), Pederno et al. (1997)
Arsenic	Wilting leaves, violet coloration (due to increased anthocyanin levels), root discoloration, inhibition of root growth, cell plasmolysis and plant death	Kabata-Pendias and Pendias (2001), Quaghebeur and Rengel (2003), Liu et al. (2005), Barrachina et al. (1995)

used in electroplating and galvanization processes, in batteries, in the production of pigments, as chemical reagent and in miscellaneous industrial processes such as smelting (ATSDR 1989). Cadmium compounds have varying degrees of solubility ranging from highly soluble to nearly insoluble which affects their absorption and toxicity (ATSDR 1989). Cadmium among metals is the most toxic heavy metal because it bioaccumulates, has a long half-life (about 30 years) and may cause health disorders even at low doses (Lenntech 2006). The increase in Cd uptake by plant tissues occurs due to the use of contaminated water for irrigation, fertilizers, sewage and composts. The absorption of Cd by plants, however, depends on genotypes and physical and chemical properties of plants (Jing and Logan 1992). Several workers have reported that the concentration of Cd was high and not suitable for human consumption in vegetables such as lettuce, spinach, radish, etc. (Prabu 2009), brinjal (Jamali et al. 2007), carrot and potato (Ding et al. 2014) and cucumber, tomato, green pepper, parsley, onion, bean, eggplant, pepper mint, pumpkin and okra (Demirezen and Aksoy 2006). In a study, Jassir et al. (2005) reported that the levels of Cd in the garden rocket vegetable species were high in both washed and unwashed samples which could possibly be due to the relatively easy uptake of Cd by food crops, especially by leafy vegetables. Also it may be due to the foliar absorption of atmospheric deposits on plant leaves (Midio and Satake 2003). In a similar investigation, significant variation in Cd concentration within different

Table 8.3 Heavy metal concentrations (mg/kg) in edible portion of leafy vegetables

Vegetables	Source of heavy metals	Heavy metals									References
		Cu	Zn	Cd	Pb	Ni	Cr	Mn	As	Hg	
Amaranthus blitum (*Amaranthus*)	Agricultural activities	42.82	–	0.16	1.91	–	1.85	–	0.67	0.27	Lui et al. (2006)
	Wastewater irrigation	1.981	54.65	2.918	2.361	–	–	–	1.780	–	Zhou et al. (2016)
	Contaminated riverside	15.60	78.45	0.15	2.54	2.46	2.28	–	0.19	–	Islam and Hoque (2014)
	Tannery effluents	–	–	0.08	2.06	–	9.08		0.14	0.04	Islam et al. (2014)
Beta vulgaris var. all green (palak)	Wastewater irrigation	28.58	41.51	4.36	15.74	7.57	27.83	117.94	–	–	Sharma et al. (2007)
	Sewage sludge	25.30	79.0	23.70	1.90	5.65	2.90	56.0	–	–	Singh and Agrawal (2007)
Apium graveolens (celery)	Sewage sludge	0.91	–	0.020	0.42	–	–	–	–	–	Dogheim et al. (2004)
	Agricultural activities	109.89	–	0.10	1.76	–	0.08	–	0.49	0.31	Lui et al. (2006)
	Atmospheric deposition	8.21	49.70	0.23	2.98	8.50	1.21	75.82	–	–	Stalikas et al. (1997)
Coriandrum sativum (coriander)	Sewage sludge	1.62	–	0.020	0.35	–	–	–	–	–	Dogheim et al. (2004)
	Atmospheric deposition	25.42	41.05	0.495	0.143	–	–	–	–	–	Jassir et al. (2005)
	Wastewater irrigation	22.24	186.40	–	–	–	83.06	65.64	–	–	Sinha et al. (2006)

Lactuca sativa (lettuce)	Industrial effluents, vehicular pollution	59.93	39.50	0.34	9.70	6.30	–	–	–	–	Demirezen and Aksoy (2006)
	Urban and industrial activities	0.92	–	0.01	0.07	–	–	–	–	–	Dogheim et al. (2004)
	Compost amendment	13.5	1171.0	–	4.90	–	–	1246.6	–	–	Intawongse and Dean (2006)
	Atmospheric deposition	–	–	0.07	0.58	–	–	–	–	–	Radwan and Salama (2006)
Mentha piperita (mint)	Sewage sludge	2.15	–	0.010	0.59	–	–	–	–	–	Dogheim et al. (2004)
	Atmospheric deposition	–	–	1.89	43.00	–	–	–	–	–	Kachenko and Singh (2006)
	Wastewater irrigation	17.34	192.00	–	–	–	–	–	–	–	Sinha et al. (2006)
Nasturtium officinale (watercress)	Atmospheric deposition	17.19	46.45	0.495	0.106	–	–	–	–	–	Jassir et al. (2005)
	Urban and industrial activities	1.96	105.20	1.22	14.37	43.62	–	18.77	–	–	Mohamed et al. (2003)
	Sewage sludge	1.11	–	0.080	0.29	–	–	–	–	–	Dogheim et al. (2004)

(continued)

Table 8.3 (continued)

Vegetables	Source of heavy metals	Heavy metals									References
		Cu	Zn	Cd	Pb	Ni	Cr	Mn	As	Hg	
Petroselinum crispum (parsley, lettuce)	Atmospheric deposition	24.89	43.54	0.062	0.099	–	–	–	–	–	Jassir et al. (2005)
	Sewage sludge	1.82	–	0.010	0.43	–	–	–	–	–	Dogheim et al. (2004)
	Industrial effluents, vehicular pollution	53.12	259.20	0.84	9.90	3.47	–	–	–	–	Demirezen and Aksoy (2006)
	Urban and industrial activities	3.34	21.00	–	3.29	0.60	–	13.09	–	–	Mohamed et al. (2003)
	Atmospheric deposition	–	–	0.067	0.34	–	–	–	–	–	Kachenko and Singh (2006)
		–	–	–	3.29	–	–	–	–	–	
Spinacia oleracea (spinach)	Industrial effluents, vehicular pollution	50.0	282	9.2	9.00	–	–	–	–	–	Singh and Kumar (2006)
	Atmospheric deposition	4.48	20.9	0.11	0.34	–	–	–	–	–	Radwan and Salama (2006)
	Compost amendment	32.3	632	–	5.20	–	–	6631	–	–	Intawongse and Dean (2006)
	Urban and industrial activities	1.18	–	0.03	0.56	–	–	–	–	–	Dogheim et al. (2004)
	Wastewater irrigation	23.0	85.08	–	–	–	8.55	155.6	–	–	Sinha et al. (2005)
	Agricultural activities	37.62	–	0.076	1.0	–	0.66	–	0.81	0.21	Lui et al. (2006)

Modified from Agrawal et al. (2007)

Table 8.4 Heavy metal concentrations (mg/kg) in edible portion of non-leafy vegetables

Vegetables	Source of heavy metals	Cu	Zn	Cd	Pb	Ni	Cr	Mn	As	Hg	References
Abelmoschus esculentus L (lady’s finger)	Wastewater irrigation	11.30	116.26	–	–	–	6.00	29.22	–	–	Sinha et al. (2005)
	Industrial effluents, vehicular pollution	37.54	15.56	0.58	10.70	2.70	–	–	–	–	Demirezen and Aksoy (2006)
	Wastewater irrigation	5.10	132.70	6.60	28.0	10.60	12.80	–	–	–	Sharma et al. (2007)
Allium cepa (onion)	Industrial effluents, vehicular pollution	53.83	21.34	0.97	8.70	4.60	–	–	–	–	Demirezen and Aksoy (2006)
	Urban and industrial activities	2.81	17.60	0.76	10.29	18.37	–	3.26	–	–	Mohamed et al. (2003)
	Atmospheric deposition	1.49	11.40	0.02	0.14	–	–	–	–	–	Radwan and Salama (2006)
Brassica oleracea (cauliflower)	Industrial effluents, vehicular pollution	1.70	21.5	–	–	–	–	–	–	–	Singh and Kumar (2006)
	Wastewater irrigation	12.08	173.21	–	–	–	40.30	28.40	–	–	Sinha et al. (2005)

(continued)

Table 8.4 (continued)

Vegetables	Source of heavy metals	Cu	Zn	Cd	Pb	Ni	Cr	Mn	As	Hg	References
Daucus carota (carrot)	Atmospheric deposition	1.51	8.03	0.18	0.01	–	–	–	–	–	Radwan and Salama (2006)
	Compost amendment	37.60	149.50	27.40	8.50	–	–	758.90	–	–	Intawongse and Dean (2006)
	Agricultural activities	27.12	–	0.085	0.92	–	0.38	–	0.15	0.24	Lui et al. (2006)
	Urban and industrial activities	0.98	9.60	0.81	7.94	17.54	–	6.14	–	–	Mohamed et al. (2003)
	Wastewater irrigation	–	59.58	6.32	–	–	–	–	–	–	Sharma and Agrawal (2006)
Lycopersicon esculentum (tomato)	Agricultural activities	201.8	–	0.11	5.23	–	0.34	–	0.46	0.13	Lui et al. (2006)
	Atmospheric deposition	1.83	7.69	0.26	0.01	–	–	–	–	–	Radwan and Salama (2006)
	Wastewater irrigation	8.70	42.45	7.20	29.00	–	–	–	–	–	Sharma et al. (2006)
	Industrial effluents, vehicular pollution	32.60	3.56	0.41	9.70	3.10	–	–	–	–	Demirezen and Aksoy (2006)
	Urban and industrial activities	4.47	14.40	0.77	2.59	14.64	–	7.39	–	–	Mohamed et al. (2003)
Raphanus sativus (radish)	Agricultural activities	8.65	–	0.083	0.47	–	0.38	–	0.22	0.21	Lui et al. (2006)
	Compost amendment	26.90	500.30	68.20	11.80	–	–	271.0	–	–	Intawongse and Dean (2006)
	Tannery effluent irrigation	–	–	0.55	1.05	–	3.44	–	0.06	0.05	Islam et al. (2014)

Solanum melongena (brinjal)	Urban and industrial activities	2.93	50.70	0.69	4.57	11.87	–	21.66	–	–	Mohamed et al. (2003)
	Industrial effluents, vehicular pollution	37.38	9.35	0.43	7.20	4.6	–	–	–	–	Demirezen and Aksoy (2006)
	Atmospheric deposition	1.41	11.50	0.02	0.21	–	–	–	–	–	Radwan and Salama (2006)
	Agricultural activities	41.37	–	0.16	1.30	–	1.15	–	0.98	0.26	Lui et al. (2006)
	Wastewater irrigation	20.89	53.92	0.47	27.76	–	–	–	–	–	Parashar and Prasad (2013)
	Contaminated riverside	17.04	18.68	0.24	0.07	4.52	1.02	–	0.04	–	Islam and Hoque (2014)
Solanum tuberosum (potato)	Atmospheric deposition	0.83	7.16	0.02	0.01	–	–	–	–	–	Radwan and Salama (2006)
	Urban and industrial activities	0.88	4.50	0.84	2.81	10.74	–	5.67	–	–	Mohamed et al. (2003)
Cucurbita maxima (pumpkin)	Contaminated riverside	11.44	46.10	0.01	0.25	5.82	1.45	–	0.02	–	Islam and Hoque (2014)
	Sewage irrigation	–	–	0.365	1.79	–	12.4	53.5	–	–	Ashfaq et al. (2015)
Cucumis sativus (cucumber)	Sewage irrigation	20.38	54.84	0.5	26.27	–	–	–	–	–	Parashar and Prasad (2013)

Modified from Agrawal et al. (2007)

tomato genotypes was found (Hussain et al. 2015). The heavy metals that accumulated within tissues of various metal-tolerant tomato genotypes followed the order, shoot > fruit > leaf > root, while in susceptible genotypes, the order was fruit, shoot, leaf and root. The genotypic variation of a crop species makes it possible to select either Cd-accumulating cultivars to remediate contaminated soils or Cd-excluding cultivars to avoid Cd excessive uptake.

Chromium is yet another important metal which may enter through air, drinking water or eating food containing Cr or even through skin contact (Dinis and Fiúza 2011). However, for human and animals, it is considered as an essential metal for carbohydrate and lipid metabolism, and the recommended dietary intake of Cr is 50–200 μg/day for adults. However, exceeding normal concentrations (50–200 ug/day) lead to accumulation and toxicity that can result in hepatitis, gastritis, ulcers and lung cancer (Garcia et al. 2001). Several studies have demonstrated that some vegetables like cabbage and lettuce accumulate higher levels of Cr and could contribute to dietary problems (Biego et al. 1998; Castro 1998). Chromium has a low mobility and moves very slowly from roots to above-ground parts of plants (Skeffington et al. 1996). And, hence, the concentration of Cr is low in the upper organs of plants. Several studies have shown that the Cr concentration was higher than the maximum permitted metal concentration in lettuce, cabbage (Itanna 2002), spinach (Banerjee et al. 2011), luffa, brinjal, ladyfinger, cucumber and gourd (Kumar et al. 2016). In contrast, the Cr contents in different vegetables grown in the lands irrigated with wastewater were found within the safe limits (Sharma et al. 2006).

Mankind is exposed to the highest levels of lead (Pb) that occurs naturally as a sulphide compound and is a soft bluish-white, silvery grey metal that melts at 327.5° C (Budavari 2001). There are different sources of environmental pollution with Pb as Pb alkyl additives in petrol and manufacturing processes. This can bring Pb into the human food chain through uptake of food (about 65%), water (up to 20%) and from air and dust (about 15%) (IPCS 1992). Like other heavy metals, Pb can bioaccumulate over time and remain in the body for long periods. It therefore becomes important to detect such metals even at very low concentrations. In Sudan, for example, Dafelseed (2007) determined the level of Pb in selected fresh vegetables and reported 0.35, 0.86, 0.60 and 0.48 mg/kg in carrot, sweet pepper, garden rock and tomato, respectively. On the contrary, the FAO/WHO (2001) has reported that the maximum permissible level of Pb in vegetables is 0.3 mg/kg.

Human carcinogen, arsenic, is a well-known toxic element, widely distributed in the environment in both inorganic and organic forms (Hughes et al. 2011). It is well-recognized that inorganic arsenic is probably the most dangerous form of arsenic in food, being As (III) more toxic than As (V) (Pizarro et al. 2016). There are many routes by which As can enter the human body (1) via food chain (ingestion by water and food sources) and (2) occupational exposure (Rahman et al. 2009). There have been several reports of arsenic speciation in vegetables growing in natural or contaminated soils (Pell et al. 2013). Broccoli, lettuce, potato, carrots, etc., for example, can accumulate arsenic when such crops are grown in soil irrigated with As (V) containing water. In most of the vegetables, arsenic is taken up by plant roots via macronutrient transporters (Zhao et al. 2010; Wu et al. 2011). In a study, it was observed that the calculated accessible doses of As expressed as microgram arsenic per year are about 470 for carrots, 550 for beets and 180 for quinoa considering the maximum intake of 2.5 kg per year of quinoa and of 6 kg per year of carrots and beets. Therefore, quinoa seems to be the vegetable with the lower toxicological risk (Pizarro et al. 2016). When taken up by plants, significant changes have been observed in the growth, yield and accumulation characteristics of okra spiked with 20 and 50 mg/kg of As(III), As(V) and dimethylarsinic acid (DMA) (Chandra et al. 2016). The arsenic concentration in the aerial part followed the order As(V) > As(III) > DMA while it was As(III) > As(V) > DMA in the roots. Thus, the plant has the capacity to tolerate As stress and can be considered as a resistive variety. Similarly, arsenic accumulation has also been reported in different vegetables beyond the permissible limit. For instance, Santra et al. 2013 found that tuberous vegetables accumulated higher amount of arsenic than leafy vegetables which was followed by fruity vegetable. This is supported by the fact that the accumulation of As in plants occurs primarily through the root system and, hence, the highest As concentrations have been reported in plant roots and tubers (Marin et al. 1993). In this study, the highest arsenic accumulation was observed in potato, brinjal, arum, amaranth, radish, lady's finger and cauliflower, whereas lower level of arsenic accumulation was observed in beans, green chilli, tomato, bitter guard, lemon and turmeric. Rehman et al. (2016) in contrast reported that the As concentration in edible portions of vegetable ranged from 0.03 to 1.38 mg/kg. Similarly, the trend of As bioaccumulation in vegetables irrigated with arsenic contaminated water was spinach > tomato > radish > carrot, and this distribution of As in vegetable tissues was species dependent; As was mainly found in the roots of tomato and spinach, but accumulated in the leaves and skin of root crops as reported by Bhatti et al. (2013).

8.4.1 Vegetable Toxicity by Multiple Metals

The interactions between different metals in soil may lead to increased uptake of one or the other metal by plants which in effect may cause toxicity to animals and humans via food chain. In a study carried out in Slovakia, Musilova et al. (2016) reported the accumulation of Cd, Pb and Zn in potato tubers in a concentration-dependent manner. The correlation between heavy metal content in soil and its content in potato tubers followed the order cv. Laura-Spissky Stvrtok (Cd), cv. Red Anna-Odorin (Pb) and Marabel and Red Anna-Odorin, cv. Marabel-Belusa and cv. Volumia-Imel (Zn). Also, heavy metals have been found several folds higher than the safe limit in other vegetables like *Colocasia*, *Amaranthus*, cauliflower, etc. (Saha et al. 2015). Of the various metals detected, the concentrations of Pb, Cd and Ni were above the permissible limit in all vegetables, while *Colocasia* and *Amaranthus* accumulated highest metal contents. The highest mean transfer coefficients (TCs) values recorded for Zn, Cu, Pb, Cd and Ni were 0.59 (*cauliflower*), 0.67 (*Colocasia*), 0.93 (*Amaranthus*), 1.02 (*Colocasia*) and 1.09 (*Amaranthus*), respectively. The results further revealed that the maximum single element pollution index (SEPI) value was found for Cd which ranged from 2.93 to 6.03 with a mean of 5.32. In yet another investigation, Tiwari et al.(2011) assessed the edible parts of five vegetables such as spinach, radish, tomato, chilli and cabbage growing in field receiving mixed industrial effluent and reported a high level of toxic metals (As, Cd, Cr, Pb and Ni). It was concluded from this study that the cultivation of such vegetables is not safe under heavy metal-stressed soil. Similarly, parsley, followed by spinach, contained the highest concentration of heavy metals besides onion that contained high levels of toxic heavy metals. The content of Cu in parsley and spinach and Pb in onion exceeded the Codex limits (Osaili et al. 2016). In the western region of Saudi Arabia, the human health problems were found associated with the consumption of metal-contaminated okra (Balkhair and Ashraf 2016). The levels of Ni, Pb, Cd and Cr in the edible parts were 90, 28, 83 and 63%, respectively, above the safe limit. The uptake and accumulation of heavy metals by the edible portions followed the order Cr > Zn > Ni > Cd > Mn > Pb > Cu > Fe. Moreover, the health risk index (HRI) was >1 which indicated a significant health risk, and hence, okra among vegetables was not safe for human consumption.

Antonious et al. (2012) reported that regardless of soil amendments, the overall bioaccumulation factor (BAF) of seven heavy metals in cabbage leaves and broccoli heads were poor. For leafy vegetables collected in 15 ha of squatted land belonging to the international airport of Cotonou, total concentrations of metal (loids) measured in consumed parts of *Lactuca sativa* L. and *Brassica oleracea* were 52.6–78.9, 0.02–0.3, 0.08–0.22, 12.7–20.3, 1.8–7.9 and 44.1–107.8 mg/kg for Pb, Cd, As, Sb, Cu and Zn, respectively (Uzu et al. 2014). Ferri et al. (2015) reported that 60 and 10% of spinach samples exceeded maximum Pb and Cd European standards and recommended that washing before consuming vegetables can reduce toxicity risk to humans. Moreover, crude or untreated wastewater, the treated wastewater and groundwater used for irrigating vegetables also contribute significantly to bioaccumulation of heavy metals. As an example, Ghosh et al. (2012) in a study found that radish, turnip and spinach, irrigated with sewage water, were grouped as hyperaccumulator of heavy metals, whereas brinjal and cauliflower accumulated less heavy metal though the metal concentrations did not exceed the permissible limit and, hence, were considered safe for consumption.

Industrial activities also add heavy metals to the environment that pose risk to both human and plant health. Also, the atmospheric deposition through the particulate matter released from transport creates heavy metal pollution among vegetables grown along roadside or during marketing. The health risk assessment methods of United States Environmental Protection Agency (USEPA) are used to establish the potential health hazards of heavy metals in soils growing vegetables in different regions of the world. In a study conducted in three economically developed areas of Zhejiang Province, China suffering from increasing heavy metal damages from various pollution sources including agriculture, traffic, mining and Chinese typical local private family-sized industry, 268 vegetable samples which included celery, cabbages, carrots, asparagus lettuces, cowpeas, tomatoes and cayenne pepper and their corresponding soils were collected. Metal concentrations were measured in soil, settled atmospheric particulate matter (PM) and vegetables at two different sites near a waste incinerator and a highway. A risk assessment was performed using both total- and bio-accessible metal concentrations in vegetables. At both sites, total Cr, Cd and Pb concentrations in vegetables were found above or just under the maximum limit levels for foodstuffs according to Chinese and European Commission regulations. High metal bio-accessibility in the vegetables (60–79%, with maximum value for Cd) was observed (Xiong et al. 2016). In another study, leaves of mature cabbage and spinach were exposed to manufactured mono-metallic oxide particles (CdO, Sb_2O_3 and ZnO) or to complex process. Particulate matter was mainly enriched with lead, and it was found that high quantities of Cd, Sb, Zn and Pb were taken up by the plant leaves. The levels of metals depend on both the plant species and nature of the PM. A maximum of 2% of the leaf surfaces were covered up to 12% of stomatal openings. Metal (loid) bio-accessibility was significantly higher for vegetables due to chemical speciation changes (Xiong et al. 2014).

8.4.2 Effect of Metals on Physiological Processes of Vegetables Grown in Metal-Stressed Soil

Heavy metals have strong influence on nutritional quality of vegetables when grown in metal-contaminated soil. Therefore, the consumption of such vegetables may lead to nutritional deficiency in developing countries which are already facing malnutrition problems. In a study, effects of four different levels of Cd, Pb and mixture of Cd and Pb on different nutrients of three vegetables, potato, tomato and lettuce, grown in pots containing soil contaminated with Cd, Pb and Cd-Pb mixture were evaluated. The edible portions of each plant were analysed for Cd, Pb and different macro- and micronutrients including protein, vitamin C, N, P, K, Fe, Mn, Ca and Mg. Results revealed a significant variation in elemental concentrations in all the three vegetables. The projected daily dietary intake values of selected metals were significant for Fe, Mn, Ca and Mg but it was not significant for protein, vitamin C, N and P. The elemental contribution to recommended dietary allowance (RDA) was significant for Mn. Similarly, RDA for Fe and Mg was higher while Ca, N, P, K, protein and vitamin C showed the minimal contribution for different age groups. This study suggests that there can be substantial negative effects on nutritional composition when such vegetables cultivated in soil poisoned with Cd and Pb are consumed (Khan et al. 2015a). However, dosage of Cd higher than critical level (≥25 mg/kg soil in treatments) drastically alters plant growth (stunted), reduced yield as well as dietary contents (sugar and vitamin C) of these important vegetables especially its antioxidant content, and the hazardous effect was more visible at higher bioaccumulation of heavy metals during vegetative growth stage (Mani et al. 2012). Heavy metals also adversely affect the mineral uptake and metabolic processes in plants when present in excess. In a recent study, the accumulation of Cr in various plant tissues and its relation to the antioxidant activity and root exudation was evaluated (Uddin et al. 2015). The results revealed that 1 mM of Cr enhanced the weight of shoots and roots of *Solanum nigrum*, whereas weight of shoots and roots of *Parthenium hysterophorus* decreased when compared with lower levels of

Cr (0.5 mM) or control plants. In both plants, the concentrations of Cr and Cl were increased while Ca, Mg and K contents in root, shoot and root exudates were declined with increasing levels of Cr. The higher levels of Cr augmented SOD and POD activities and proline content in foliage of *S. nigrum*, while Cr at lower levels had stimulatory effects in *P. hysterophorus*. Citric acid concentration in root exudates increased with increasing rates of Cr by 35% and 44% in *S. nigrum*, while it was 20 and 76% in *P. hysterophorus*. Generally, *P. hysterophorus* exuded maximum amounts of organic acids. Moreover, the increasing amounts of Cd showed a differential impact on the content and translocation of micro- and macronutrients in tomato (Bertoli et al. 2012). Among different organs, the aerial part had 2.25 g/kg, 2.80 g/kg, 18.93 mg/kg and 14.15 mg/kg of K, Ca, Mn and Zn, respectively, compared to the control.

Apart from these effects, heavy metals in some studies have also been found to adversely affect the water and iron content in some vegetables. For example, 100 and 400 μM Cr had an obvious effect on iron metabolism and water relations of spinach (Gopal et al. 2009). Visual symptoms and increased accumulation of Cr were observed in roots than in leaves, when spinach was exposed to Cr. Moreover, the concentration of chlorophylls and the activities of heme enzymes, catalase and peroxidase decreased following exposure to excess Cr suggesting the intervention of Cr in iron metabolism of plants. These changes coupled with reduction in Fe concentration in Cr-exposed plants further indicate that by declining Fe absorption, Cr disrupts the chlorophyll-forming process and heme biosynthesis. Additionally, the transpiration rate along with proline accumulation was found to decrease in the leaves of Cr-treated plants which indicated water stress. In contrast, heavy metal has also been found to improve growth of celery more than lettuce and spinach, when irrigated with sludge containing heavy metals (Haghighi 2011). The stimulatory effect of sludge on growth rate of all three vegetables occurred via photosynthesis. It was, therefore, concluded from this study that the increasing element uptake induces photosynthesis and concurrently enhances the growth of leafy vegetable. In yet similar experiment, the impact of mixing native soil with municipal sewage sludge (MSS) or yard waste (YW) mixed with MSS (YW + MSS) was assayed to determine (1) yield and quality of sweet potato; (2) concentration of Cd, Cr, Mo, Cu, Zn, Pb and Ni in different organs of sweet potato (edible roots, leaves, stem and feeder roots); and (3) concentrations of ascorbic acid, total phenols, free sugars and β-carotene in edible roots at harvest (Antonious et al. 2011). The results revealed that even though the total concentrations of Pb, Ni and Cr were greater in plants grown with MSS and YW, applied together, compared to control plants, the mixture of MSS and YW increased yield, ascorbic acid, soluble sugars and phenols in edible roots of sweet potato by 53, 28, 27 and 48%, respectively, compared to plants grown in native soil. β-Carotene was greater (157.5 μg/g fresh weight) in the roots of plants grown in MSS compared to roots of plants grown in MSS + YW treatments (99.9 μg/g fresh weight). In a follow-up study, the concentrations of capsaicin, dihydro-capsaicin, β-carotene, ascorbic acid, phenols and soluble sugars in the fruits of *Capsicum annuum* L. (cv. Xcatic) grown under four soil management practices including YW, SS, chicken manure (CM) and no-much (NM) bare soil were determined. The total marketable pepper yield was increased by 34% and 15%, when it was grown in SS- and CM-treated soil, respectively, compared to NM bare soil. However, the number of culls (fruits that fail to meet the requirements of foregoing grades) was lower in YW-treated soils compared to SS- and CM-treated soils (Antonious 2012).

Elevated levels of heavy metals also affects the plants at the cellular and at the whole-plant level (Burzynski and Klobus 2004; Shaw et al. 2004). For instance, Cd and Cu have been reported to modify plasma membrane H^+-ATPase activity (Janicka-Russak et al. 2012). Also, an increased level of heat-shock proteins (hsp) in the tissues was observed as an adaptive process to survive under adverse conditions, and increased PM H^+-ATPase activity could further enhance the repair processes in heavy metal-stressed plants. In other investigations, metal ions have been found to inhibit root elongation, photosynthesis and enzyme activity and cause oxidative damage to membranes (Hernandez and Cooke 1997; Shaw et al. 2004; Sheoran et al. 1990). In a similar study, the inhibitory impact of metals on physiological, biochemical and morphological characteristics of spinach grown at 20 and 40% sewage sludge-amended soil is reported (Singh and Agrawal 2007). At 40% sewage sludge application, a substantial decrease in length of root and shoot and leaf area of spinach was observed. Among the biochemical parameters, photosynthetic rate was reduced by 23.6 and 28.8% in palak at 20 and 40% sewage sludge amendment, respectively. As compared to untreated soil, foliar thiol content decreased at 20 and 40% sewage sludge amendment. There was an increase in lipid peroxidation at different concentrations of sewage sludge used, and this is attributed to the formation of reactive oxygen species (ROS) and free radicals induced by Cd, Ni and Pb leading to disorganization of membrane structures of cells. In addition to these, chlorophyll content, fluorescence ratio (Fv/Fm) and protein content were also decreased, but peroxidase activity increased with increasing sewage sludge amendment ratio. These destructive effects in turn make plants more susceptible to additional stresses such as drought which reduces water uptake capacity and water use efficiency of the smaller root system and possibly blocks aquaporins (Yang et al. 2004; Ionenko et al. 2006 and Ryser and Emerson 2007). Heavy metal toxicity and drought stresses are likely to occur simultaneously, as metal-contaminated soils tend to have poor water-holding capacity (Derome and Nieminen 1998), and evaporation rates are high due to sparse vegetation cover (Johnson et al. 1994).

8.5 Bioremediation Strategies Adopted for Heavy Metal Removal

8.5.1 Phytoremediation

Remediation of metal-contaminated soils is indeed a major challenge before the scientists working in different countries. The conventional technologies employed to remove heavy metals from soils often involve stringent physicochemical agents (Neilson et al. 2003), which can destruct soil fertility and also negatively affect the agroecosystem.

Despite these, numerous methods including chlorination, use of chelating agents and acid treatments have been proposed to remove metals from contaminated sites. However, such methods are considered ineffective due to operational difficulties, high cost and low metal leaching efficiency. Due to these problems, there is an urgent need to find some viable alternative. In this regard, bioremediation which is the process of cleaning up of hazardous wastes involving the use of microorganisms or plants is considered the safest method of clearing polluted soil (Dixit et al. 2015). Among various bioremediation strategies, phytoremediation, also called as botanoremediation, green remediation and agro remediation, has been found inexpensive and more practicable method for minimizing/clearing metals from soil and water (Lasat 2000). Also, during phytoremediation practices, no hazardous product is generated. Broadly, this remediation system is plant based which is a solar-driven biological system (Santiago and Bolan 2010). Plants involved in phytoremediation have been categorized as:

1. Excluders: plants that survive through restriction mechanisms and are sensitive to metals over a wide range of soil. Members of the grass family, for example, sudan grass, bromegrass, fescue, etc., belong to this group.
2. Indicators: plants that show poor control over metal uptake and transport processes and correspondingly respond to metal concentrations in soils. This group includes the grain and cereal crops like corn, soybean, wheat, oats, etc.
3. Accumulators: plants which do not prevent metals from entering the roots, but they have evolved specific mechanisms for detoxifying high metal levels that accumulated in the cells (Baker 1981). Tobacco, mustard and Compositae families (e.g. lettuce, spinach, etc.) fall within this category.

Apart from these three categories, extreme accumulators, often called hyperaccumulators, form a fourth category which includes plants with exceptional metal-accumulating capacity. This property of accumulating excessive metal concentration, allows plants to survive and even thrive in heavily contaminated soils (or near ore deposits). To date, about 400 plant species (Table 8.5) have been identified as metal hyperaccumulators, representing <0.2% of all angiosperms (Brooks 1998).

Table 8.5 Numbers of known hyperaccumulating plants and their families

Heavy metals	Total number of plants	Families
Cadmium	1	Brassicaceae
Cobalt	26	Lamiaceae, Scrophulariaceae
Copper	24	Lamiaceae, Cyperaceae, Poaceae, Scrophulariaceae
Manganese	11	Apocynaceae, Cunoniaceae, Proteaceae
Nickel	290	Brassicaceae, Cunoniaceae, Euphorbiaceae, Flacourtiaceae, Violaceae
Selenium	19	Fabaceae
Thallium	1	Brassicaceae
Zinc	16	Brassicaceae, Violaceae

Adapted from Brooks (1998)

Phytoremediation involves many steps and techniques to clean up the contaminants from the polluted sites (Santiago and Bolan 2010). Some of the most commonly practised phytoremediation strategies are:

1. *Phytoextraction*: contaminants are taken up by roots and translocated within the plants and are removed by harvesting the plants. In this process, toxic metals from contaminated soils, sediment and sludge are absorbed, concentrated and precipitated into the above-ground biomass such as shoots, leaves, etc. (Singh et al. 2012).
2. *Phytodegradation*: involves the breakdown of organics, taken up by the plant to simpler molecules that are incorporated into the plant tissues (Dermentzis 2009).
3. *Rhizofiltration*: is primarily used to remediate extracted groundwater, surface water and wastewater with low contaminant concentrations. Rhizofiltration can be used for Pb, Cd, Cu, Ni, Zn and Cr, which are primarily retained within the roots.
4. *Phytostabilization*: primarily used for the remediation of soil, sediment and sludges. It involves the use of plant roots to limit contaminant mobility and bioavailability in the soil. Phytostabilization can occur through the sorption, precipitation, complexation or metal valence reduction. It is useful for the removal of Pb and As, Cd, Cr, Cu and Zn (Flora et al. 2008).
5. *Phytovolatilization*: involves the use of plants to take up contaminants from the soil, transforming them into volatile forms and releasing them into the atmosphere. Phytovolatilization occurs as growing trees and other plants take up water and the organic and inorganic contaminants. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentrations. Phytovolatilization has been primarily used for the removal of mercury (Durnibe et al. 2007).
6. *Phytostimulation*: using plants to stimulate bacteria and fungi to mineralize pollutant using exudates and root sloughing. Some plants can release as much as 10–20% of their photosynthates in the form of root sloughing and exudates (Pilon-Smits 2005).

Considering the importance of phytoremediation technology in metal clean up from the contaminated soils, several vegetables have also been explored for their phytoremediation ability in order to detoxify or reduce the heavy metal contamination in vegetable growing fields. For example, the growth response, metal tolerance and phytoaccumulation properties of water spinach and okra were assessed under different contaminated spiked metals by Ng et al. (2016) using control, 50 mg Pb/kg soil, 50 mg Zn/kg soil and 50 mg Cu/kg soil. Of the two vegetables, okra accumulated highest concentrations of Pb (80.20 mg/kg) in its root followed by Zn in roots (35.70 mg/kg) and shoots (34.80 mg/kg) of water spinach, respectively. Moreover, the accumulation of Pb, Zn and Cu in both water spinach and okra differed considerably. Though the accumulation of Pb in the shoots of water spinach and okra exceeded the maximum permissible limits of the National Malaysian Food Act 1983 and Food Regulations 1985 and the International Codex

Alimentarius Commission, both crops were found as good Pb and Zn phytoremediators. Generally, leafy vegetables have a higher tendency for uptake and accumulation of heavy metals; these can be used as indicator and also for removal of toxic heavy metals from polluted agricultural field. In yet other study, *Ipomoea aquatica* Forsk., an aquatic macrophyte, was assessed for its ability to accumulate Pb by exposing it to graded concentrations of this metal. Accumulation of Pb was the highest in root followed by stem and leaf. Furthermore, Pb at 20 mg/l induced colour changes in the basal portion of stem which had significantly higher Pb concentration than in the unaffected apical part. This resulted in sequestration of excess metal in affected stem tissue, which could take up Pb by the process of caulofiltration or shoot filtration and served as a secondary reservoir of Pb in addition to the root. The ability of the plant to store Pb in its root and lower part of stem coupled with its ability to propagate by fragmentation through production of adventitious roots and lateral branches from nodes raises the possibility of utilizing *I. aquatica* for Pb phytoremediation (Chanu and Gupta 2016). Even among different varieties of vegetables, difference in the bioaccumulation property that can be exploited for remediation of polluted soils is reported. The high accumulator genotypes may be useful for phytoremediation, while the low accumulator varieties might be appropriate selections for growing on metal-contaminated soils to prevent potential human exposure to heavy metals and health hazards through the food chain. To categorize the pepper accessions of *Capsicum chinense* Jacq, collected from eight different countries, grown in a silty-loam soil under field conditions as low or high heavy metal accumulators, Antonious et al. (2010) collected mature fruits and analyse Cd, Cr, Ni, Pb, Zn, Cu and Mo. Fruits accumulated significant concentrations of Cd (0.47 μg/g dry fruit), Pb (2.12 μg/g dry fruit) and Ni (17.2 μg/g).

8.5.2 Microbe-Assisted Remediation

Numerous microbial communities belonging to different genera have evolved certain mechanisms to tolerate and detoxify metals from contaminated environment (Mosa et al. 2016). Interestingly, the high surface to volume ratio of microorganisms and their ability to circumvent metal toxicity makes such organisms a viable and inexpensive alternative to chemical methods of metal remediation (Kapoor et al. 1999; Magyarosy et al. 2002). Biological mechanisms involved in microbial survival under metal-stressed environment include complexation, biosorption to cell wall and pigments, extracellular precipitation and crystallization, transformation of metals, decreased transport or impermeability, efflux, intracellular compartmentation and sequestration (Kang et al. 2016). One or many of these strategies may be adopted by microbiota to overcome metal problems. For example, synthesis of metallothioneins or γ-glutamyl peptides is a mechanism of Cu resistance in *Saccharomyces cerevisiae*, but Cu binding or precipitation around the cell wall and intracellular transport are also components of the total cellular response (Gadd and White 1989).

Considering the importance of microbes in metal detoxification/removal, identification of metal-tolerant microorganisms has become important to remediate the metal-polluted soils so that larger area can be used for vegetable cultivation. In this regard, the effect of two strains of *Trichoderma* (*T. harzianum* strain T22 and *T. atroviride* strain P1) on the growth of lettuce plants irrigated with As-contaminated water was assessed (Caporale et al. 2014). The results revealed the accumulation of this element mainly into the root system which subsequently reduced both biomass development and net photosynthesis rate (while altering the plant P status). However, both species of plant growth-promoting fungi (PGPF) *Trichoderma* alleviated, at least in part, the phytotoxicity of and eventually decreased As accumulation in tissues and concurrently enhanced plant growth, P status and net photosynthesis rate (Caporale et al. 2014). In a similar experiment, heavy metal-resistant strain J62 of *Burkholderia* sp. has been reported to promote the growth of tomato and maize (Jiang et al. 2008). In a follow-up study, the biological properties such as dry weights of fruit, roots, stem, leaf and whole tomato plants were increased by single or combined remediation of ryegrass and arbuscular mycorrhiza, while MDA contents and antioxidant enzyme activities of foliage and roots were declined in two varieties of tomato when exposed to Cd (20 mg/kg). Cadmium accumulation in tomato followed the order leaf > stem > fruit > root. However, the Cd concentrations in leaf, stem, root and fruit of both varieties were decreased by single or combined application of ryegrass and AM-fungi (Jiang et al. 2014).

Adequate nutrients are required for proper growth and development of a plant (Anil et al. 2003). Also, it is essentially required for maintaining normal metabolic reactions of plants. In contrast, the metal-contaminated soil is generally deficient in plant nutrients, and the plants that remain under constant stress fail to take up sufficient amounts of nutrients from soil. To overcome these problems, several metal-tolerant microbes possessing one or many plant growth-promoting activities such as ability to solubilize insoluble phosphate (Kim et al. 2013), phytohormone production (Franco-Hernández et al. 2010) or by some indirect mechanisms such as biocontrol activity (Khan and Bano 2016) involving siderophore production (Rajkumar et al. 2010) have been used. Besides these, microbes also aide in the phytoremediation process (Ullah et al. 2015), and as a result, the plants grow better in metal-stressed soils. As an example, two Cd- and Ni-resistant plant growth-promoting bacteria, *Pseudomonas* sp. ASSP 5 and ASSP 29, were isolated from fly ash-contaminated sites, and their plant growth promotion ability was tested by inoculating *Lycopersicon esculentum* plants grown in fly ash-amended soil (Kumar and Patra 2013). In most cases, strain ASSP 29 of *Pseudomonas* sp. produced more pronounced effect on biological (plant height and wet and dry weights) and chemical (protein and chlorophyll content in leaves) characteristics of plants and accumulation of metals in root and shoot of plants. Both the strains ASSP 5 and ASSP 29 showed a remarkable ability to protect the plants against the inhibitory effect of Ni and Cd besides promoting the growth of plants through production of IAA and siderophore and solubilization of P. Similarly, Dourado et al. (2014) evaluated Cd–*Burkholderia*–tomato interaction studies by inoculating a Cd-tolerant *Burkholderia* strain SCMS54 that exhibited a higher metabolic diversity and plasticity. Inoculated tomato plants in the presence of Cd grew well compared to non-inoculated plants indicating that the strain SCMS54 abated the toxicity of Cd and consequently enhanced tomato production grown under Cd stress. Based on this study, it was suggested that the bacterial strain isolated from Cd-contaminated soil could be used for tomato cultivation in soils even contaminated with Cd.

An endophytic bacterium *Serratia* sp. RSC-14 isolated from the roots of *S. nigrum* RSC-14 was used as an inoculant against *S. nigrum* plants grown in metal-stressed soils. In this study, the toxic effect of Cd-induced stress was relieved, and there were some significant improvements in root/shoot growth, biomass production and chlorophyll content, while MDA and electrolyte contents were found to decrease considerably. Besides the ability to tolerate Cd concentration up to 4 mM, the strain RSC-14 exhibited P solubilizing activity and secreted plant growth-promoting phytohormones such as IAA (54 μg/ml). The regulation of metal-induced oxidative stress enzymes such as catalase, peroxidase and polyphenol peroxidase had ameliorative effects on host growth. Activities of these enzymes were significantly reduced in RSC-14-inoculated plants as compared to control plants under Cd treatments. The current findings thus supported the hypothesis that *Serratia* sp. RSC-14 endowed with improved phytoextraction abilities could be used as metal-tolerant microbial inoculants to enhance the overall performance of *S. nigrum* plants when grown intentionally or inadvertently in Cd-contaminated soil (Khan et al. 2015b). Similarly, Luo et al. (2011) isolated endophytic bacterium *Serratia* sp. LRE07 from Cd-hyperaccumulator *S. nigrum* plants which, besides expressing the ability to promote plant growth, had high metal removal efficiencies also. Cadmium tolerant endophytic fungal community associated with *S. nigrum* has also been isolated and characterized for host plant growth modulation under Cd contamination (Khan et al. 2016). Owing to the levels of Cd tolerance detected, in order to simulate a tripartite plant–microbe–metal interaction, *S. nigrum* plants were inoculated with *Glomerella truncata* PDL-1 and *Phomopsis fukushii* PDL-10 under Cd spiking of 0, 5, 15, and 25 mg/kg. The results indicated that PDL-10-inoculated plants had significantly higher Cd content in shoots and in roots than those observed in the PDL-1-inoculated plants. Additionally, irrespective of Cd stress, PDL-1 and PDL-10 inoculation significantly improved plant growth attributes. He et al.(2009) reported that two Cd-resistant strains *Pseudomonas* sp. RJ10 and *Bacillus* sp. RJ16 increased plant growth through Cd and lead (Pb) solubilization and by secreting IAA, siderophore and 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase) besides enhancing Cd and Pb uptake ability of a Cd-hyperaccumulator tomato. Significant increase in Cd and Pb contents of above-ground tissues varied from 92 to 113% and from 73 to 79% respectively in inoculated plants grown in heavy metal-contaminated soil compared to the uninoculated control. These results show that the bacteria could be exploited for bacteria enhanced-phytoextraction of Cd- and Pb-polluted soils. Also, the effects of metal-resistant microorganisms and metal chelators on the ability of plants to accumulate heavy metals have been investigated. Though the application of Cd- or Pb-resistant microorganisms improved the ability of *S. nigrum* to accumulate heavy metals and increased plant yield, but the effects of microorganisms on phytoextraction were smaller than the effects of citric acid (CA).

When plants were grown in the presence of Cd contamination, the co-application of CA and metal-resistant strains enhanced biomass by 30–50% and increased Cd accumulation by 25–35%. In the presence of CA and the metal-resistant microorganisms, the plants were able to acquire 15–25% more Cd and 10–15% more Pb than control plants. It was therefore suggested from this study that the synergistic combination of plants, microorganisms and chelators can enhance phytoremediation efficiency in the presence of multiple metal contaminants (Gao et al. 2012).

Conclusion

Heavy metals are one of the major toxic pollutants whose removal from contaminated areas is urgently required in order to reduce their impacts on various food chains and ultimately human health. Among food commodities, vegetables are one of the main components of human dietary system because they provide essential micro and macronutrients, proteins, antioxidants and vitamins to the human body. All vegetables are often grown in suburban areas experiencing high concentrations of heavy metals both through aerial deposition and contamination accumulators through soil and irrigation water. Among vegetables, leafy vegetables have more potential to accumulate heavy metals from a contaminated environment due to their higher capacity of absorption both from contaminated soil and aerial deposits. The advantage of high biomass production and easy disposal also makes vegetables useful to remediate heavy metals from a contaminated environment, but the excessive intake and consequent accumulation in human beings through long-term consumption of contaminated food may result in negative effect on human health. Remediation and safe consumption of vegetables are, therefore, the two opposite concerns of heavy metal impact on the environment. Stringent enforcement of standards should therefore be followed for maximum allowable intake of heavy metals to avoid risk to human health. Heavy metals besides contaminating food also reduce the nutritional value of vegetables affecting other biochemical and physiological processes reducing the yield and quality of the crops. Thus regular monitoring of heavy metal contamination in the vegetables grown at wastewater irrigated area is necessary, and consumption of contaminated vegetables should be avoided in order to reduce the health risk caused by taking the contaminate vegetables. Furthermore, a safe and inexpensive metal-removing strategy like the use of plants and microbes both in isolation and association should be promoted to grow fresh and contaminant-free vegetables.

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