



**Full Length Article**

## Wastewater Induced Manganese Toxicity Affects Growth and Bioavailability in Spinach (*Spinacia oleracea*)

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### Abstract

The different types of sewage sludge generated from different urban sources are commonly used for irrigation in agriculture and contain heavy metals (as manganese, cadmium, lead and zinc). This sewage water is mostly used for vegetable production in peri-urban areas and is a source of heavy metals. Therefore, this study was conducted to evaluate the effects of wastewater with different toxicity levels of manganese (Mn) on growth and bio-availability of Mn in spinach. The sewage water with two levels of Mn was applied exogenously as i) 150 mg L<sup>-1</sup> sewage water (moderately toxic), ii) 350 mg L<sup>-1</sup> sewage water (toxic level) and no Mn as control; (only sewage water). Two cuttings of spinach were taken after 45 and 90 days of germination. There was significant accumulation of Mn in leaves. The maximum concentration of Mn (11.13 and 12.67 mg L<sup>-1</sup>) was recorded with 350 mg L<sup>-1</sup> Mn in sewage water at both cuttings, respectively. Minimum number of leaves with decrease of 43 and 25% were recorded with Mn 350 mg L<sup>-1</sup> (toxic) and 150 mg L<sup>-1</sup> (moderate) respectively. Plant biomass and dry weight showed negative behavior in second cutting as compared to first. The results showed that long-term application of Mn contaminated wastewater decreases the growth rate and yield of spinach by increasing its accumulation in leaves up to toxic levels. In conclusion, the application of sewage water to vegetables (especially spinach) should be avoided to prevent human life from health disorders. © 2019 Friends Science Publishers

**Keyword:** Wastewater; Heavy metals; Accumulation; Bioavailability; Human health

### Introduction

Water is an important component of living entities. As per Quran “We made every living thing from water. Will they not then believe?” (The Nobel Quran, 21: 30). Sewage and industrial wastewater is used commonly for irrigation source by farmers because it is available easily. Heavy metals like Cd, Zn, Cr, Mn, Hg and Pb are present in wastewater (Sato *et al.*, 2014; Afifa *et al.*, 2017) and its application has some disadvantages when enter the food chain causing brain and nervous disorders (Siddique *et al.*, 2010).

A variety of industries could produce wastewater with Mn contamination, most remarkably by dying and textiles manufacturing industries, approximately 95% of total Mn used by them (Levy and Nassetta, 2003; Nasir *et al.*, 2017). It is much difficult to remove Mn from aqueous media, due to its high solubility under both alkaline and acidic conditions (Silva *et al.*, 2012). The waste produced containing excess of Mn, textile industries, steel manufacturer and dying industries lead to environmental pollution (Noureen and Javed, 2017). While the Mn is essential nutrient for human life, but if its level exceeding

0.1 mg L<sup>-1</sup> in water supplies may causes hazardous effect (WHO, 2007). Excessive Mn in wastewater may lead to its bio-accumulation in soil and plants (Patnaik, 2002).

Excessive Mn concentration in plant tissues may cause the alteration in various processes, including enzymatic activity, nutrient uptake behavior and their redistribution and causing antagonistic effects on the use of other major nutrients (Ca, Fe, Mg, N) (Lavres Junior *et al.*, 2010). Early research linking environmental effects to Mn stress in crop plants coupled with recently improved understanding support the phototoxicity of Mn in plants (Fernando and Lynch, 2015). Re-evaluation is warranted based on new findings showing that excessively accumulated Mn can become phytotoxic upon interaction with environmental triggers such as solar radiation and atmospheric ozone (González and Lynch, 1999; Clair *et al.*, 2005). Excessive Mn can affect physiological stress mechanisms including photobleaching under solar radiation and oxidative stress due to Mn antagonism against metal co-factors integral to stress-mitigating enzyme activities (Clair and Lynch, 2005; Fernando and Lynch, 2015).

The metal toxicities in plants altered the nucleus functions, reduce cell division and cause poor leaf development (Shu *et al.*, 2012; Zancheta *et al.*, 2015). Moreover, the toxicity of Mn restricts the translocation of other essential nutrients (calcium, magnesium) in plants (Huang *et al.*, 2016). Under Mn toxicity the availability of oxygen is reduced which lower the efficiency of electron transport chain and cause reduction in energy production and CO<sub>2</sub> fixation rate (Foyer and Shigeoka, 2011; Gururani *et al.*, 2015). According to Weng *et al.* (2013), the Mn toxicity reduced photosynthesis rate in plants. In hyper-accumulator species (*Phytolacca acinosa*) of Mn, its toxic effect degraded the photosynthetic apparatus of chloroplast (Rojas-Lillo *et al.*, 2014).

Vegetables are mostly used for cooking purposes; the leafy vegetables are good source of the quality and high value nutrition (Sobukola and Dairo, 2007). These are one of the major foods help in prevention and curing of different diseases (D'Mello, 2003). Spinach is important selling vegetables in many areas and it is hyper-accumulator of heavy metals and toxic ions (Teerakun and Reungsang, 2005).

The present study was conducted to estimate the availability and accumulation of Mn in spinach by applying sewage water with different levels of Mn concentration. The objective of this experiment was to monitor the Mn effects on growth and bioavailability in spinach by wastewater irrigation under toxic level of Mn.

## Materials and Methods

### Sewage Water Sampling Sites

The samples were collected from three different wastewater drains of Faisalabad (31°25'45" N 73°4'44" E). Selected drains were Bawachak Drain, Industrial Eve, Sarghodha Road (31°28'41.0" N 73°04'36.3" E) (Fig. 1), Ganda Nala, Mustafabad Jail Road (31°26'41.4" N 73°04'51.1" E), and Chokera Drain, Chokera village (31°27'19.4" N 73°00'20.8" E). Sampling was done at randomly from different location of these drains with three depths (0–6, 6–12, 12–18 cm). Nine samples with three replicates were collected from each drain.

### Pre-analysis of Wastewater

Wastewater samples were analyzed for the physical and chemical parameters. Physical parameters determined were pH, color, turbidity, total dissolved solids (TDS), and chemical parameters were electrical conductivity (EC), sodium adsorption ratio (SAR) and residual sodium carbonate (RSC). All these parameters were measured following the method described in U.S. Salinity Lab. Staff (1954). Five heavy metals zinc (Zn), manganese (Mn), ferrous (Fe), lead (Pb) and cadmium (Cd) were analyzed

from samples by using atomic absorption spectrophotometer (Model Varian Spectra AA 250 plus) (Table 1).

## Experimental Site and Treatment

The pot experiment was conducted by using completely randomized design at wirehouse Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Two level of Mn toxicity were developed in wastewater using MnCl<sub>2</sub>. Three treatments were designed in experiment i) toxic level (350 mg L<sup>-1</sup> Mn in wastewater), ii) moderately toxic level (150 mg L<sup>-1</sup> Mn in wastewater) (Balkhair and Ashraf, 2016) and iii) sewage water (control) with four replications. Plants were harvested two times at the interval of 45 and 90 days from the date of sowing.

## Observations, Measurements and Analysis

The plants were examined for the growth parameter like number of leaves per plant, plant biomass, plant dry weight and leaf surface area of plant after each harvest. After recording the growth parameters, samples were dried in an oven (Memmert, 854 Schwabach, Germany) for 10 days at 60°C and then grinded for further analysis.

## Metal Ion Analysis

The 0.25 g of ground sample was digested using wet digestion method as described by U.S. Salinity Lab. Staff (1954). Di-acid mixture of HNO<sub>3</sub> and HClO<sub>4</sub> with 2: 1 ratio was added in each sample and leave overnight after which sample was digested on hot plate till the whitish color appears. After digestion, the digested volume of the solution was made to 25 mL and filtered the solution. Then, it was used to estimate the Mn concentration accumulated in plants. Atomic absorption spectrophotometer (Model Varian Spectra AA 250 plus) was used for Mn analysis.

## Results

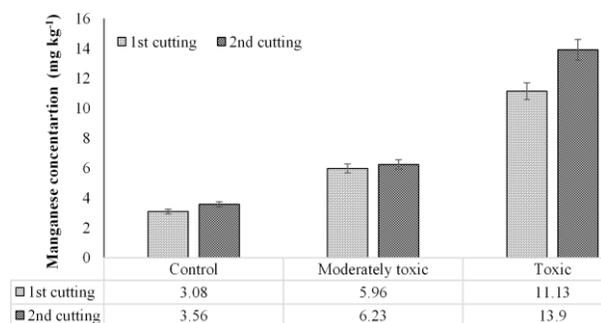
### Irrigation Water Quality

Water used for the irrigation purpose was collected from Bawachack drain, Faisalabad, Pakistan. The wastewater of this drain derived mostly from textile and dyeing industries with considerable quantity of household and sewage waste. The water contained high concentration soluble salts with EC 5.4 dS m<sup>-1</sup> and total dissolved solids (TDS) 3388 mg L<sup>-1</sup> which was much higher from the permissible limit 1.5 dS m<sup>-1</sup> and 1200 mg L<sup>-1</sup> respectively (U.S. Salinity Lab. Staff., 1954). The sodium adsorption ratio (SAR) was 18.5 *i.e.*, 85% more than its critical limit. Critical limits used as were given by US Salinity Lab. Staff (1954). Heavy metals founded in water were Zn, Mn and Fe with an average concentration 0.3, 0.4 and 0.3 mg L<sup>-1</sup> respectively while Pb and Cd not founded.

**Table 1:** Results of Physical and Chemical parameters of wastewater analyzed

Site	Parameters	Range	Mean	Permissible limit	
Ganda Nala	Color	Yellowish green	-	-	
	Turbidity	Yes	-	-	
	EC (dS m <sup>-1</sup> )	3.7-4.0	3.85	1.5*	
	SAR (mmol L <sup>-1</sup> ) <sup>1/2</sup>	9.8-11.5	10.7	10*	
	RSC (me L <sup>-1</sup> )	17-21	19.4	2*	
	pH	8.1-8.5	8.2	-	
	TDS (me L <sup>-1</sup> )	2400-2550	2464	1200*	
	Zn (mg L <sup>-1</sup> )	0.1-0.4	0.3	2.0**	
	Mn (mg L <sup>-1</sup> )	0.2-0.6	0.3	0.2**	
	Fe (mg L <sup>-1</sup> )	0.4-0.6	0.3	5.0**	
	Pb (mg L <sup>-1</sup> )	Nd	-	-	
	Cd (mg L <sup>-1</sup> )	Nd	-	-	
	Bawachak Drain	Color	Blackish grey	-	-
		Turbidity	Yes	-	-
EC (dS m <sup>-1</sup> )		4.7-5.9	5.4	1.5*	
SAR (mmol L <sup>-1</sup> ) <sup>1/2</sup>		15-21.5	18.5	10*	
RSC (me L <sup>-1</sup> )		24-29.5	27.5	2*	
pH		8.1-8.6	8.3	-	
TDS (me L <sup>-1</sup> )		3040-3616	3388	1200*	
Zn (mg L <sup>-1</sup> )		0.2-0.6	0.3	2.0**	
Mn (mg L <sup>-1</sup> )		0.2-0.6	0.4	0.2**	
Fe (mg L <sup>-1</sup> )		0.3-0.6	0.3	5.0**	
Pb (mg L <sup>-1</sup> )		Nd	-	-	
Cd (mg L <sup>-1</sup> )		Nd	-	-	
Achakhera Drain		Color	Blackish grey	-	-
		Turbidity	Yes	-	-
	EC (dS m <sup>-1</sup> )	4.7-5.1	4.8	1.5*	
	SAR (mmol L <sup>-1</sup> ) <sup>1/2</sup>	14.4-15.9	15.4	10*	
	RSC (me L <sup>-1</sup> )	19.5-27.3	24.6	2*	
	pH	6-8.7	7.5	-	
	TDS (me L <sup>-1</sup> )	3015-3213	3100	1200*	
	Zn (mg L <sup>-1</sup> )	0.1-0.3	0.1	2.0**	
	Mn (mg L <sup>-1</sup> )	0.1-0.5	0.3	0.2**	
	Fe (mg L <sup>-1</sup> )	0.2-0.6	0.4	5.0**	
	Pb (mg L <sup>-1</sup> )	Nd	-	-	
	Cd (mg L <sup>-1</sup> )	Nd	-	-	

\* U.S. Salinity Lab. Staff. (1954); \*\* Ayers and Westcot (1985)



**Fig. 1:** The uptake of manganese concentration (ppm) by spinach at two different cutting with different levels of toxicity (1<sup>st</sup> cutting after 45 days, 2<sup>nd</sup> cutting after 90 days, moderately toxic (150 mg L<sup>-1</sup> Mn), and toxic (350 mg L<sup>-1</sup> Mn)

### Growth Parameters

The results showed that number of leaves per plant reduced with increasing Mn toxicity in wastewater. Maximum numbers of leaves (7 and 8) per plant were recorded in control (sewage water) with 1<sup>st</sup> and 2<sup>nd</sup> cutting respectively.

Minimum numbers of leaves (4 and 6) were recorded with 350 mg L<sup>-1</sup> Mn (toxic level) in 1<sup>st</sup> and 2<sup>nd</sup> cutting respectively. However, plant biomass, dry weight and leaf surface area seems significantly affected at 2<sup>nd</sup> cutting as compared to 1<sup>st</sup> cutting. Under toxic level of Mn, at 1<sup>st</sup> cutting plant biomass, dry weight and leaf surface area showed 107, 121 and 83% increase from control while these figures reduced to 49, 58 and 28%, respectively in 2<sup>nd</sup> cutting which showed its toxicity (Table 2).

### Manganese uptake by Spinach

Due to increasing Mn toxicity in wastewater used for irrigation the accumulation of Mn in spinach increased. Maximum concentration accumulated with 350 mg L<sup>-1</sup> Mn (toxic level) in wastewater was 11.13 and 13.9 mg L<sup>-1</sup> at 1<sup>st</sup> and 2<sup>nd</sup> harvesting respectively. The minimum concentration was found in control (wastewater) treatment. On the other hand, in 1<sup>st</sup> and 2<sup>nd</sup> cutting it showed 261% and 290% respectively increase in Mn uptake in spinach with respect to control (Fig. 1).

### Pearson Correlation Coefficient

The Pearson correlation chart for the Mn uptake and growth parameters was developed which showed that Mn concentration uptake by plant showed highly negative correlation on number of leaves per plant ( $r=0.98-1.00$ ). The Mn concentration showed positive correlation on plant biomass with coefficient value of 0.92 which reduced with increasing the time to 90 days ( $r=0.81$ ). The Mn concentration showed positive correlation on plant dry weight ( $r=0.95$ ) which reduced with increasing time span to 90 days ( $r=0.86$ ).

### Discussion

The excessive amount of Mn application with sewage water badly affected the spinach growth and development (Table 2 and 3). The excessive Mn affects physiological and metabolic processes involved in plant growth and development (Hauck *et al.*, 2003; Shi *et al.*, 2006; Shu *et al.*, 2012; Zancheta *et al.*, 2015). Shoot growth of spinach was reduced due to Mn toxicity (Table 2) as excessive Mn inhibits chlorophyll biosynthesis, photosynthetic rate (Subrahmanyam and Rathore, 2000; Shi *et al.*, 2006), CO<sub>2</sub> assimilation rate and stomatal conductance (Santos *et al.*, 2017).

The toxic level of Mn (350 mg L<sup>-1</sup>) in sewage water significantly reduced the leaves number per plant, plant biomass and leaf surface area (Table 2) as excessive concentration of Mn in plant tissue changes the enzymatic activity, uptake, redistribution and use of other nutrients as P, N, Ca, Mg and Fe which are essential for normal plant growth (Lavres Junior *et al.*, 2010; Huang *et al.*, 2016) and cause oxidative damage (Santos *et al.*, 2017). In the present study, wastewater collected from Bawachak

**Table 2:** Growth parameters status of two cuttings of crop

Treatment	Parameters	Cuttings	
		1 <sup>st</sup> (after 45 days)	2 <sup>nd</sup> (after 90 days)
Control	Number of leaves per plant	7.0 ± 0.289	8.0 ± 0.85
	Plant biomass (g/plant)	2.01 ± 0.16	9.03 ± 0.45
	Dry weight (g/plant)	0.14 ± 0.013	0.55 ± 0.054
	Leaf surface area (cm <sup>2</sup> )	39.35 ± 1.42	154 ± 1.47
150 mg L <sup>-1</sup> Mn (moderately toxic)	Number of leaves per plant	6.0 ± 0.25 (-14%)	7.0 ± 0.25 (-13%)
	Plant biomass (g/plant)	3.55 ± 0.25 (+77%)	12.28 ± 1.28 (+36%)
	Dry weight (g/plant)	0.25 ± 0.022 (+78%)	0.61 ± 0.021 (+11%)
	Leaf surface area (cm <sup>2</sup> )	67.43 ± 1.20 (+71%)	175 ± 1.08 (+14%)
350 mg L <sup>-1</sup> Mn (toxic level)	Number of leaves per plant	4.0 ± 0.25 (-43%)	6.0 ± 0.25 (-25%)
	Plant biomass (g/plant)	4.16 ± 0.16 (+107%)	13.44 ± 0.44 (+49%)
	Dry weight (g/plant)	0.31 ± 0.012 (+121%)	0.87 ± 0.036 (+58%)
	Leaf surface area (cm <sup>2</sup> )	72.8 ± 1.45 (+85%)	191 ± 1.55 (+24%)

Each value is Mean of 4 replications ± SE; Value in ( ) are percentage increase or decrease to its respective control, \*+ increase in value, - decrease in value

**Table 3:** Pearson correlation coefficients for effects of Mn concentration and growth of spinach

	MC (45 days)	MC (90 days)	BM (45 days)	BM (90 days)	NoL (45 days)	NoL (90 days)	DW (45 days)
MC (90 days)	0.98						
BM (45 days)	0.92	0.83					
BM (90 days)	0.91	0.82	0.99				
NoL (45 days)	-0.99	-0.98	-0.91	-0.89			
NoL (90 days)	-0.98	-0.93	-0.97	-0.96	0.98		
DW (45 days)	0.95	0.86	0.99	0.99	-0.93	-0.98	
DW (90 days)	0.98	1.00	0.83	0.82	-0.98	-0.94	0.87

MC = Manganese concentration BM = Biomass NoL= Number of leaves DW = Dry weight 45 days = first cutting 90 days = second cutting

drain Faisalabad, Pakistan contain Mn concentration above the permissible limit > 0.2 mg kg<sup>-1</sup> (Table 1) and was applied to spinach to check its bioavailability.

There was high accumulation of Mn in spinach leaves (Fig. 1) when applied at toxic level may be owed to its more availability. The high Mn concentration negatively affects the carbon fixation process (Rojas-Lillo *et al.*, 2014; Santos *et al.*, 2017), due to reduced availability of NADP<sup>+</sup> for carbon fixation and reduced stability of photosystem II (Gururani *et al.*, 2015). Manganese toxicity negatively affects the reactive oxygen species (ROS) accumulation in chloroplasts and inhibits renewal of D1 protein required for Photosystem II repairment (Nishiyama *et al.*, 2011) (Table 3). Moreover, the suppression of ROS further degrades the chloroplast enzymes (Kato and Sakamoto, 2014) and promotes breakdown of thylakoid membrane (Gratão *et al.*, 2009).

Spinach is one of the hyper-accumulative crops show high resistance to high EC and metal ion concentration with application of irrigation water and did not affect the yield (Singh *et al.*, 2004). In this study, the growth parameters of the crop were not significantly affected because industrial wastewater was also a rich source of nutrients which enhanced the growth and yield (Pathak *et al.*, 1999). The Mn concentration accumulated in vegetative part of spinach was above the permissible limit (WHO, 2007). Hence, the spinach grown with wastewater for irrigation was not suitable for human consumption because due to the high concentration of accumulated Mn in vegetative part (Ronaq *et al.*, 2005; Opaluwa and Aremu, 2012; Khan *et al.*, 2013).

## Conclusion

In conclusion, the growth of spinach is not much affected by the application of wastewater alone. However, the toxic level of Mn in sewage water increased its bioavailability in spinach leaves as spinach is the most commonly used vegetable in Pakistan.

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