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Bio solids influence on spinach leaf quality grown in Peri Urban areas

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Abstract
In order to offset the conflicting information regarding biosolids on the public domain the present study has been carried out at College of Agriculture farm, PJTSAU, Rajendranagar. The pot culture study entitled "Biosolids Influence on Spinach Leaf Quality Grown in Peri Urban Areas "was conducted during *kharif*, 2014 at College of Agriculture, PJTSAU, Rajendranagar, Hyderabad. The experiment was laid out in Completely Randomized Design (CRD) with four replications. There were five treatments of biosolids application rate comprising T1 (3 t ha^{-1}), T2 (4t ha^{-1}), T3 (5 t ha^{-1}) T4-Control (Spinach) and T5-Control (Fenugreek). Applicaion of 5 t ha^{-1} of biosolids to Spinach increased the leaf yield by per cent as compared with 4 t ha-^{1} and 3 t ha $^{-1}$ due to more availability of NPK and Zinc. Similarly, maximum chlorophyll content in leaf was recorded in treatment with 5 tonns/ha of biosolids to spinach (49.57 SPAD units). Uptake of major nutrients (NPK) and micronutrient (Zn) was found to be highest in 5 t ha^{-1} of biosolids. Among heavy metals, though uptake of Cd, was more followed by Ni and Co, found to be with in the maximum permissible limits as per WHO standards (2007) [21]. The transfer factor values for Cd were found to be more as compared with Ni and Co indicating that spinach has higher affinity towards Cd absorption.

Keywords: Biosolids, spinach, NPK and heavy metals uptake, transfer factor

Introduction
Biosolids contains nutrients and valuable trace elements essential to plants and animals. It is an efficient source of phosphate and slow-release nitrogen, besides high organic matter content. Thus, improves soil physical, chemical and biological properties and enhances crop yields and quality. Moreover, waste management has become a major environmental challenge, and land application of biosolids, treated sewage sludge, is generally considered the best option instead of its disposal either as landfill or incineration because it offers the possibility of recycling plant nutrients, provides organic material, improves chemical along with physical properties of soil and in turn enhances crop yields and quality of produce (Epstein, 2003; Lu *et al*., 2012) [6, 9].

The study addresses the potential agronomic value of biosolids as an alternate organic source to fertilizers to cultivate vegetables in peri-urban areas. The study also demonstrates techno – economic viability of land application of biosolids for production of horticulture crops more so leafy vegetables in peri-urban areas for two reasons. The first reason being raising vegetables is the main occupation of peri urban farmers and second being proximity to sewage treatment plants.

On the flip side, the confliction information about biosolids is hindering its marketability unlike inorganic fertilizers. Hence, to offset this reserved opinion and also to gain firsthand information the research study has been executed.

Material and Methods
The pot culture study entitled "Biosolids Influence on Spinach Leaf Quality Grown in Peri Urban Areas "was conducted during *kharif*, 2014 at College of Agriculture, PJTSAU, Rajendranagar, Hyderabad. The experiment was laid out in Completely Randomized Design (CRD) with four replications. There were five treatments of biosolids application rate comprising T1 (3 t ha^{-1}), T2 (4t ha^{-1}), T3 (5 t ha^{-1}) T4-Control (without biosolids to Spinach) and T5-Control (without biosolids to Fenugreek). The reason to include fenugreek as another control in the study is its ability to withstand to heavy metal contamination by the quality of less phytoextraction as compared with spinach. Thus, conclusive evidence can be arrived about leaf quality of spinach. Spinach (*Spinacea oleracea*) variety Allgreen and Fenugreek (*Trigonella foenumgraecum*) local variety were selected for the study. Data on nutrients uptake, yield, quality, heavy metal content were recorded.

Source of sewage sludge

Sewage sludge used in the experiments was collected from Noor Mohammad Kunta-Sewage Treatment Plant (NMK-STP). Plant Capacity is 4.0 MLD. It is situated 2.5 km away from College of Agriculture Rajendranagar, Hyderabad.

Biosolids used in present study was analyzed in the laboratory following standard procedures before studying its effect on quality of leafy vegetables. The results revealed that the biosolids was moderately acidic in reaction with a pH of 5.66, EC of 5.52 dS m^{-1}, total organic carbon content of 24.02 per cent and with a sludge volume index (SVI) of 438.61 mL g^{-1}. Total N, P and K contents of biosolids were 2.84, 1.45 and 2.76 per cent, respectively. The triacid extractable zinc in biosolids was 26.58 mg kg^{-1} while, the diacid extractable heavy metals *viz*., Cd, Co and Ni were 0.84, 0.42, 1.82 mg kg^{-1}, respectively and were within the permissible limits as per the standards of USEPA.

In all the treatments after adding biosolids as per the treatment schedule on air dry weight basis, rest of the pot volume was filled with air dried red soil. Necessary care was taken to protect the crops from pests and diseases. Plant growth parameters like plant height, number of leaves, chlorophyll content, leaf area and dry matter production were recorded at different growth stages of both spinach. The plants samples were analyzed for N, P, K, Zn, Cd, Co and Ni uptake. Soil samples were analyzed for pH, EC, OC, N, P, K, Zn, Cd, Co and Ni. However, leaf yield, quality and nutrient uptake were only mentioned as rest of the parameters are relatively beyond the scope of article.

Physiological observations

Chlorophyll content of spinach and fenugreek

Chlorophyll content of the intact leaves at 15 DAS, 30 DAS, 45 DAS and harvest stage (60 DAS) was recorded with a SPAD meter and the readings were expressed in SPAD units.

Plant chemical analysis

Plant samples collected for recording dry matter production at 60 days age were dried, powdered and utilized for chemical analysis of Nitrogen uptake using Kjelplus, Phosphorus uptake by Vanado molybdo phosphoric yellow colour method (Piper, 1966) [13] and Potassium uptake by flame photometer (Piper, 1966) [13] and expressed as:

$$\text{Nutrient uptake (g plant-1)} = \frac{\text{Nutrient content (\%) x Dry matter production (g plant-1)}}{100}$$

Zinc and Heavy metals uptake

Zinc or heavy metal content (µg g^{-1}) in the filtrate was determined using Atomic Absorption Spectrophotometer (AAS) and uptake was expressed in mg $plant^{-1}$.

$$\text{Zn or heavy metal uptake (mg plant}^{-1}\text{)} = \frac{\text{Zn or heavy metal content (µg g}^{-1}\text{) x Dry matter production (g plant-1)}}{1000}$$

Transfer factor (TF)

It is also called as Bio-concentration factor (BCF). It is an index demonstrating the potential of whole plant or its tissue for phytoextraction.

$$\text{TF (mg kg}^{-1}\text{)} = \frac{\text{Heavy metal content in plant}}{\text{Heavy metal content in soil}}$$

Results and Discussion

Chlorophyll content (SPAD units)

Chlorophyll content was measured with SPAD meter on intact leaves at 15DAS, 30 DAS, 45 DAS and 60 DAS, which is presented in Table 1. The SPAD units increased as crop age advanced. The highest chlorophyll content of 49.57 SPAD units was recorded at 60 DAS in 5 t ha^{-1} of biosolids treatment (T3) followed by 4 t ha^{-1} (48.50) and 3 t ha^{-1} of biosolids application (48.00) at same interval of time. The SPAD units recorded in 5 t ha^{-1} (49.57) and 4 t ha^{-1} of biosolids application (48.50) were on par with each other. The results also further revealed that spinach recorded increase in chlorophyll content with incremental dose of biosolids application rate which resulted in higher nitrogen availability and uptake (Table) Similarly, Kanbi and Bhatnagar (2005) [7] reported that chlorophyll content (47.6 SPAD units) in potato was highest with application of 30 t ha-1 farm yard manure as compared with rest of lower rates of FYM application.

Table 1: Effect of Biosolids on Chlorophyll content (SPAD units) of Spinach

Treatments		Chlorophyll content (SPAD units)			
		15 DAS	30 DAS	45 DAS	60DAS Harvesting stage)
T_1	3tha^{-1} of biosloids	38.45	45.85	50.21	48.00
T_2	4tha^{-1} of biosloids	40.21	45.92	51.02	48.50
T_3	5tha^{-1} of biosloids	42.37	46.62	52.32	49.57
T_4	Control (Spinach)	37.23	45.67	48.24	46.37
T_5	Control Fenugreek)	20.25	23.90	30.67	32.57
	CD	0.97	1.19	2.24	2.22
	SE (d)	0.47	0.57	1.05	1.04
	SE (m)	0.33	0.40	0.74	0.73
	CV (%)	2.21	2.29	3.06	3.03

Leaf yield (g plant-1)

The leaf yield (g $plant^{-1}$) data was recorded at 15, 30 and 45 DAS was used for comparison. But, leaf yield recorded at harvesting stage (60 DAS) is considered as final leaf yield data and is presented in Table 2. Significantly highest leaf yield (3.45 g $plant^{-1}$, Table.2) at 15 DAS was observed in 5 t ha^{-1} of biosolids to Spinach (T3) followed by 4 t ha^{-1} (3.02 g $plant^{-1}$, T2) and 3 t ha^{-1} of biosolids application (2.61 g $plant^{-1}$, T1). The lowest leaf yield of 2.12 g $plant^{1}$ was observed in control of spinach (T4) and Fenugreek (T5). Observation of highest leaf yield at harvesting stage was noticed in 5 t ha^{-1} of biosolids (T3) was due to more plant height, number of branches and number of leaves. As biosolids is rich in macro and micro nutrients, various growth parameters showed positive response. Sewage sludge amendment at higher dose (20 t ha^{-1}) showed enhancement in shoot length, root length, number of leaves and leaf area in maize as stated by Qasim *et al*. (2001) [14]. Sinha *et al*. (2008) [18] also reported significant increments in growth parameters of two varieties of *Vigna radiata* at higher rate (30 t ha^{-1}) of sludge amendment. Mazen *et al* (2010.) [10] reported that the fresh weight of wheat plants was increased by increasing the sewage sludge levels in soil.

Table 2: Effect of Biosloids on leaf yield (g $plant^{-1}$) of Spinach

Treatments		Chlorophyll content (SPAD units)			
		15 DAS	30 DAS	45 DAS	60 DAS (Harvesting stage)
T_1	3 t ha^{-1} of biosloids	2.61	8.31	18.55	37.97
T_2	4 t ha^{-1} of biosloids	3.02	9.14	21.55	48.47
T_3	5 t ha^{-1} of biosloids	3.45	10.25	23.12	51.42
T_4	Control (Spinach)	2.12	5.10	16.77	35.10
T_5	Control (Fenugreek)	1.24	3.25	6.65	14.26
	CD	0.08	0.22	0.52	1.16
	SE (d)	0.03	0.10	0.24	0.54
	SE (m)	0.02	0.07	0.17	0.38
	CV (%)	2.10	2.17	2.22	2.19

Nutrient content (%) and uptake (mg plant-1)
Nitrogen

The nitrogen content (%) and uptake at harvesting stage (60 DAS) was estimated and is presented in Table 3. Significantly highest nitrogen content (2.31%) and uptake (0.076 g $plant^{-1}$) uptake was recorded at harvesting stage in 5 t ha^{-1} followed by 4 t ha^{-1} (1.8%, 0.046 g $plant^{-1}$)) and 3 t ha^{-1} of biosolids (1.4%,0.032 g $plant^{-1}$). The lowest nitrogen content (1.12%) was observed in control of both spinach and fenugreek (T4 and T5). The nitrogen content and uptake was more by 27.76% 39.47%, respectively in 5 t ha^{-1} of biosolids (T3) than 4 t ha^{-1} of biosolids application (T2) was due to more availability of nitrogen (Table.) Significantly higher N content in seeds of lentil grown with sewage sludge @ 1 to 6 kg m^{-2} was also reported by Yamur *et al*. (2005) [22]. Another similar finding was reported by Singh and Agrawal (2010) [17] that, nutrients concentration of N, P, K, Ca, Na and Mg in seeds of *Vigna radiata* linearly increased at all application rates of sewage sludge (3, 9 and 12 kg m^{-2}) which suggests more availability of nitrogen due to more sewage sludge amendment application. Khankhane and Yadav (2003) [8] reported that NPK uptake was highest by sewage sludge application @ 15 t ha^{-1} which induced 1.40 and 0.59 g pot^{-1} of nitrogen uptake by brinjal and tomato plants, respectively.

Table 3: Effect of Biosloids on Nutrient content and uptake of Spinach

Treatments		N Content (%)	N Uptake (kg ha^{-1})	P Content (%)	P Uptake (kg ha^{-1})	K Content (%)	K Uptake (kg ha^{-1})	Zn Content (%)	Zn Uptake (kg ha^{-1})
T_1	3 t ha^{-1} of biosloids	1.40	0.032	1.60	0.037	0.51	0.012	19.03	0.438
T_2	4 t ha^{-1} of biosloids	1.80	0.046	2.40	0062	0.68	0.018	21.97	0.567
T_3	5 t ha^{-1} of biosloids	2.31	0.076	3.30	0.108	0.90	0.029	24.84	0.812
T_4	Control (Spinach)	1.12	0.024	0.38	0.008	0.17	0.004	17.66	0.371
T_5	Control (Fenugreek)	0.49	0.010	0.23	0002	0.12	0.003	14.27	0.144
	CD	0.04	0.002	0.05	0.002	0.04	0.002	1.64	0.016
	SE (d)	0.02	0.002	0..02	0.001	0.02	0.001	0.79	0.008
	SE (m)	0.01	0.001	0.01	0.001	0.01	0.001	0.56	0.005
	CV (%)	2.98	2.362	3.61	2.973	2.52	4.125	5.64	2.215

Phosphorus

The phosphorus content (%) at harvesting stage (60 DAS) was estimated and is presented in Table 3. Significantly highest phosphorus content (3.3%) and uptake (0.108g $plant^{-1}$) were observed in 5 t ha^{-1} of biosolids to spinach (T3) followed by 4 t ha^{-1} (2.40%) and 3t ha^{-1} of biosolids (1.6%). Similar to trend observed in case of Nitrogen. The lowest phosphorus content (0.38%) was recorded in control (T 4 and T5). The increase in phosphorus content was more by 37.49% and uptake by 42.59% in 5 t ha^{-1} of biosolids (T3) than 4 t ha^{-1} biosolids to spinach (T2). More availability of phosphorus (Table 4.), higher organic carbon (Table 4.) in the soil and favorable soil pH caused more phosphorus availability in 5 tha^{-1} of biosolids as compared with rest of the treatments. Increase in P content of corn (Bozkurt *et al*., 2000) [4] and barley seeds (Naggar and Ghamry, 2001) [11] grown in sewage sludge amended soil as compared to un amended soil was also reported. Higher uptake and translocation of P to pods in *Vigna radiata* might have led to higher P content in seeds at increasing sewage sludge application rates from 3 to 12 kg m^{-2} (Singh and Agrawal, 2010) [17]. Significantly higher P content was recorded in control of spinach when compared to P content in fenugreek.

Potassium The trend in case of potassium is similar to Nitrogen and Phosphorus (Table 3). Similarly, increase in K content with increased dose of sewage sludge was reported by Topcuoglu *et al*. (2013) [20]. Stark and Clapp (1980) [19] revealed that, crop yield and K uptake increased in potato with application of sewage sludge as compared with other treatments that did not receive sludge. Similar opinion was expressed by Singh and Agrawal (2010) [17] which was already mentioned in previous pages.

Table 4: Effect of Biosloids on uptake of heavy metals at harvesting stage in Spinach

Treatments		Cd uptake (mg $plant^{-1}$)	Co uptake (mg $plant^{-1}$)	Ni uptake (mg $plant^{-1}$)
T_1	3 t ha^{-1} of biosloids	0.013	0.012	0.017
T_2	4 t ha^{-1} of biosloids	0.018	0.015	0.025
T_3	5 t ha^{-1} of biosloids	0.027	0.024	0.036
T_4	Control (Spinach)	0.008	0.007	0.012
T_5	Control (Fenugreek)	0.003	0.003	0.004

	CD	0.002	0.001	0.001
	SE (d)	0.001	0.000	0.000
	SE (m)	0.001	0.000	0.000
	CV (%)	3.266	3.198	2.452

Zinc

Significantly highest Zinc uptake in 5 t ha^{-1} (T3) was more by 30.17%, 46.05% and 54.31%, respectively as compared with 4 t ha^{-1}, 3 t ha^{-1} of biosolids and control (T4 and T5; Table 3). This finding can be supported by the observations made by Paulraj and Sreeramulu (1994) [12], who observed that increasing levels on sewage sludge application caused corresponding increase in the uptake of nutrients like Fe, Zn and Pb in the tomato crop. This finding can be supported by the observations made by Badawy and CEI-Moutaium (2000) [2] reported that the concentrations of Zn in tomato leaves increased with the increase in sewage sludge application rate. Concentrations of Zn in the above ground lettuce tissue and to lesser extent significantly increased by sewage sludge application was found by Sloan *et al*., (1997). Highest zinc concentration (22.07 mg kg^{-1}) was recorded in mung bean seeds at higher dose (12 kg m^{-2}) of sewage sludge application (Singh and Agrawal, 2010) [17].

Heavy metals content (%) and uptake (mg plant^{-1})

Cadmium, Cobalt, Nickel

Maximum cadmium (Cd) content and uptake (0.027 mg plant^{-1}) though, was noticed in 5 t ha^{-1} of biosolids (Table. 4) as compared with rest of the treatments, was within the permissible limits of WHO (2007) [21] limits. This finding can be corroborated by the observation made by Akdeniz *et al*. (2006) [1] that, the concentration of Cd had increased linearly in sorghum leaves (0.56, 0.49, 0.49 mg kg^{-1}) and seeds (0.44, 0.46 and 0.45 mg kg^{-1}) when sewage sludge (@ 7, 14, 21 Mg ha^{-1}, respectively) application rate increased linearly. The higher content of cobalt (Co) and uptake (0.024 mg plant^{-1}) and nickel its respective uptake in 5 t ha^{-1} biosolid application treatment as compared with rest of the treatments can be attributed to more concentration and total availability in 5 t ha^{-1} of biosolids. The explanation given by Singh and Agrawal (2010) [17] and Begum (2011) [3] regarding other heavy metal like nickel and cobalt also stand good here. Similar result was also expressed by Saruhan *et al*. (2010) [16] in *Lotus corniculatus* that, the concentration of cobalt (Co) in plant increased (0.42, 0.56 and 0.63 mg kg^{-1} respectively) with the increase in sewage sludge application rates (@ 3, 6 and 9 t ha^{-1}). Similar results were given by Paulraj and Sreeramulu (1994) [12], who observed that increasing levels of sewage sludge application caused corresponding increase in the uptake of nutrients like Cu, Zn and Cd in the tomato.

Transfer factor analysis of Heavy metals (mg kg^{-1})

Transfer factor (TF) is an index demonstrating the potential of whole plant or its issues to accumulate metal from soil. High TF value indicates suitability of the plant or its tissue for phyto-extraction. Current study revealed that the TF values varied significantly among all the heavy metals studied (Table.5). The range of Co varied from 0.51 to 1.02 mg kg^{-1}, Cd from 1.71 to 3.31 mg kg^{-1} and Ni from 0.86 to 1.83 mg kg^{-1}. Among the three metals, TF values of Cadmium were found to be more in all the treatments followed by Ni and Co.

Significantly highest transfer factor value of Cadmium, Nickel and Cobalt were observed in 5 t ha^{-1} (T3) followed by 4 t ha^{-1} and 3 t ha^{-1} of biosolids similar to major and micro nutrient (Zn) The lowest transfer factor value (1.03 mg kg^{-1}, Table.5) of heavy metals was observed in Contro l of fenugreek followed by control of spinach.

Rangnekar. *et al.* (2013) [15] also reported that TF values of Cd were more in spinach as compared to other two leafy vegetables *viz*, fenugreek and amaranthus which they studied at Maharastra in India. Demi rezen and Aksoy (2006) [5] also found a similar pattern while working with leafy vegetables of Turkey.

Table 5: Influence of Biosolids on Transfer Factor Analysis of Heavy Metals (mg kg^{-1}) in Spinach

Treatments		Transfer Factor Analysis of Heavy Metals (mg kg^{-1})		
		Ni	Co	Cd
T_1	3 t ha^{-1} of biosloids	1.18	0.90	2.19
T_2	4 t ha^{-1} of biosloids	1.23	0.93	2.21
T_3	5 t ha^{-1} of biosloids	1.83	1.02	3.31
T_4	Control (Spinach)	1.03	0.73	1.90
T_5	Control (Fenugreek)	0.86	0.51	1.71
	CD	0.03	0.079	0.094
	SE (d)	0.001	0.026	0.031
	SE (m)	0.001	0.037	0.044
	CV (%)	0.188	2.037	4.686

Future scope of work

Biosolids generated from Noor Mohammed Kunta Sewage Treatment Plant (NMKSTP) located near College of Agriculture, PJTSAU, Hyderabad, was found to be a good source of nutrients to cultivate leafy vegetables in peri-urban areas of Hyderabad. However, the trial needs further study to confirm the results before its extensive and intensive use in peri urban areas in Rangareddy district of Telangana state.

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