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Sewage sludge and NPK Application to enhance growth, yield and quality of kale and spinach crops

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Sewage sludge is considered as a valuable supplement because of its high organic matter, macro and micro nutrients. Its application has been studied in combination with recommended fertilizer dose (NPK is combination of N, P and K) for crops, viz. Kale and Spinach. The experiment comprised nine treatments (T_1=0%SS+0%NPK as Control, T_2=25%SS+0%NPK, T_3=50%SS+0%NPK, T_4=75%SS+0%NPK, T_5=100%SS+0%NPK, T_6=25%SS+75%NPK, T_7=50%SS+50%NPK, T_8=75%SS+25%NPK and T_9=0%SS+100%NPK) which were laid in a completely randomized manner with triplicates. The study revealed that morphological characters, viz. root length per plant, shoot length per plant, root fresh and dry biomass, leaf area index and weight of edible part were significantly higher in T_9 (0%SS+100%NPK) in both kale and spinach. Similarly, leaf area was significantly higher in T_8 (75%SS+25% NPK) treatment in both kale and spinach. The various parameters related to plant quality like ascorbic acid, carbohydrate content and protein content were significantly higher with application of 75% Sewage sludge+25%NPK and hence improve growth, yield and quality of kale and spinach.

Key words: Sewage sludge, NPK, growth, yield, quality, kale, spinach.

INTRODUCTION

The sewage sludge, also known as bio-solids, is an obligatory by-product of wastewater recovered after its proper treatment (Singh and Agrawal, 2008; Khaliq et al., 2017). Its scientific composting is being increasingly considered as an eco-friendly disposal strategy. The beneficial soil improvements in the form of sewage sludge and organic compost enhance most of the soil properties like soil pH, organic matter and other nutrients, particularly for the soils which are deficient in organic content. The effect of aerobically digested sewage sludge has proved to be one of the significant promoter of soil production, which was demonstrated through change in soil chemical properties and total above ground biomass of native plants (Singh and Agrawal, 2008; Khaliq et al., 2017; Bożym and Siemiątkowski, 2018; Kaur and Sharma, 2020).

There has been a routine for undue application of inorganic fertilizers to the vegetable crops that even with

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short growing season like kale and spinach for merely achieving effective yield (Usman et al., 2012; Khaliq et al., 2017; Mohamed et al., 2019). This trend is proving to be much problematic for both human health and environment. As an alternative to these inorganic fertilizers, it is worthwhile to switch for organic fertilizers. The most common organic fertilizer used is the farmyard manure, but the availability of farmyard manure in all required places and throughout the seasons is not possible. Therefore, sewage sludge, containing both inorganic and organic nutrients, is gaining importance for its use in agricultural management.

LITERATURE REVIEW

The domestic and industrial sewage sludge is increasingly being used for growth of vegetable crops. The application of biosolids is expected to increase structural nutrients (C, H, and O) and macro-nutrients (N, P, K, Ca, S, and Mg) coupled with balance of micro-nutrients (Sreesai et al., 2013). Most of the studies conducted globally have shown the benefits of administering the sewage sludge as cost-effective procedure to improve the crop productivity through soil fertility. Application of sewage sludge compost, reduces the environmental threats such as leaching of N and P and heavy metal accumulation in the soil (Singh and Agrawal, 2008). The microbial activity along with its biomass and organic carbon content has been observed to improve upon the supplementation of sewage sludge with low metal content. The significant feature which directs the heavy metal bioavailability in soil, is due to the organic content found in the sewage sludge (Usman et al., 2012; Alvarenga et al., 2015; Khaliq et al., 2017). The exchange sites for organic matter (soluble) from sewage sludge has got two groups, one binding to Ca, Mg, Zn, Ni, Co, Mn, Cd, Pb, Fe and the other binding at Cu, Pb, H (Bożym and Siemiątkowski, 2018; Hallouz et al., 2018; Traven et al., 2018; Kumar et al., 2019). The activity of free metals in soil solution is significantly reduced by organic ligands (Sreesai et al., 2013; Hallouz et al., 2018; Mohamed et al., 2019). Additionally, there are several advantages of supplementation of sewage sludge to crop cultivation soils in the form of range of nutrients being added to the soil (Alvarenga et al., 2015; Kumar et al., 2019), decreasing soil acidification (Khaliq et al., 2017), less need for pesticides and fertilizers, preventing soil erosion, increase in the number of beneficial soil organisms, physical, chemical and biological properties of soil get improved.

Study objectives

The objective of the present work was to investigate the effect of different ranges of sewage sludge and recommended fertilizer doses (NPK) on (1) Growth, (2) Yield and (3) Quality parameters of kale and spinach.

MATERIALS AND METHODS

Study area description

The present study was done at the experimental farm located in Wadura Campus of the Faculty of Agriculture and Regional Research Station, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir. Climatically, the experimental site is well known for its cold winters and hot summers at mid to high altitude. Experimental site was located in mid to high altitude temperate zone (Figure 1) characterized by hot summers and very cold winters. The mean monthly meteorological data collected during the growing season recorded at Meteorological Observatory Division of Agronomy, Shalimar. It is observed from the data that mean maximum temperature was 16.71 and 15.94°C and mean minimum temperature was 2.70 and 2.08°C during the cropping seasons of 2010 and 2011, respectively. Maximum relative humidity was 85.74 and 86.28% during 2010 and 2011, respectively, whereas total annual precipitation (rainfall) amounted to 383.70 and 428.10 mm during 2010 and 2011, respectively.

Characterization of sewage sludge

The experiment comprised of nine varying combinations (9 treatments), viz. T1 = Control, T2=25% Sewage Sludge +0% NPK, T_3=50% Sewage Sludge+0% NPK, T_4=75% Sewage Sludge+0% NPK, T_5=100%Sewage Sludge+0% NPK, T_6=25% Sewage Sludge+ 75% NPK, T_7=50% Sewage Sludge+50% NPK, T_8=75% Sewage Sludge+25% NPK and T_9=0% Sewage Sludge+100% NPK. The treatments were designed in a completely randomized manner with three replications. Two crops, viz. Kale (*Brassica oleracea* L. cv. 'GM Dari') and Spinach (*Spinacia oleracea* L. cv. 'Shalimar Green'), were used as an experimental material. These crops were considered for the study because of their short growing season (45 days) and high market value. The seeds of kale and Spinach were purchased from the production section of Agriculture Department (Government of Jammu and Kashmir).

The texture of soil used in the experimental beds was silty clay loam with neutral pH and moderate in nitrogen, phosphorus and potassium. Soil of selected field was brought to fine tilth by giving 3-4 ploughings with tractor followed by clod breaking and levelling. The samples were collected prior to treatments with sewage sludge and NPK and results of pre-test are shown in Table 1. The sewage sludge was air dried and then crushed properly to mix uniformly with soil and left for 10 days in the field. Furthermore, the experimental fields were divided into 54 plots of 1.5×1.5 m^2 plot size, by making 30 cm wide bunds between the plots as per the layout specifications. Proper and recommended protocols were adapted to grow the crops. Spinach seeds were sown directly to the experimental plots, while seeds of kale were sown separately raised in the nursery beds. The soil used to grow the plants was amended with sewage sludge at various ratios as per the treatments for both kale and Spinach. The nursery beds were watered prior to transplanting of the seedlings. Vigorous and healthy seedlings of almost uniform size were transplanted in well prepared plots. Plant spacing of 30×15 cm^2 was maintained for kale, while for spinach it was maintained at 30×10 cm^2, respectively. After transplanting, light irrigation was given and subsequent irrigations carried out whenever it was required. The protection of plants against various diseases was carried throughout the growing period of both crops in both years of

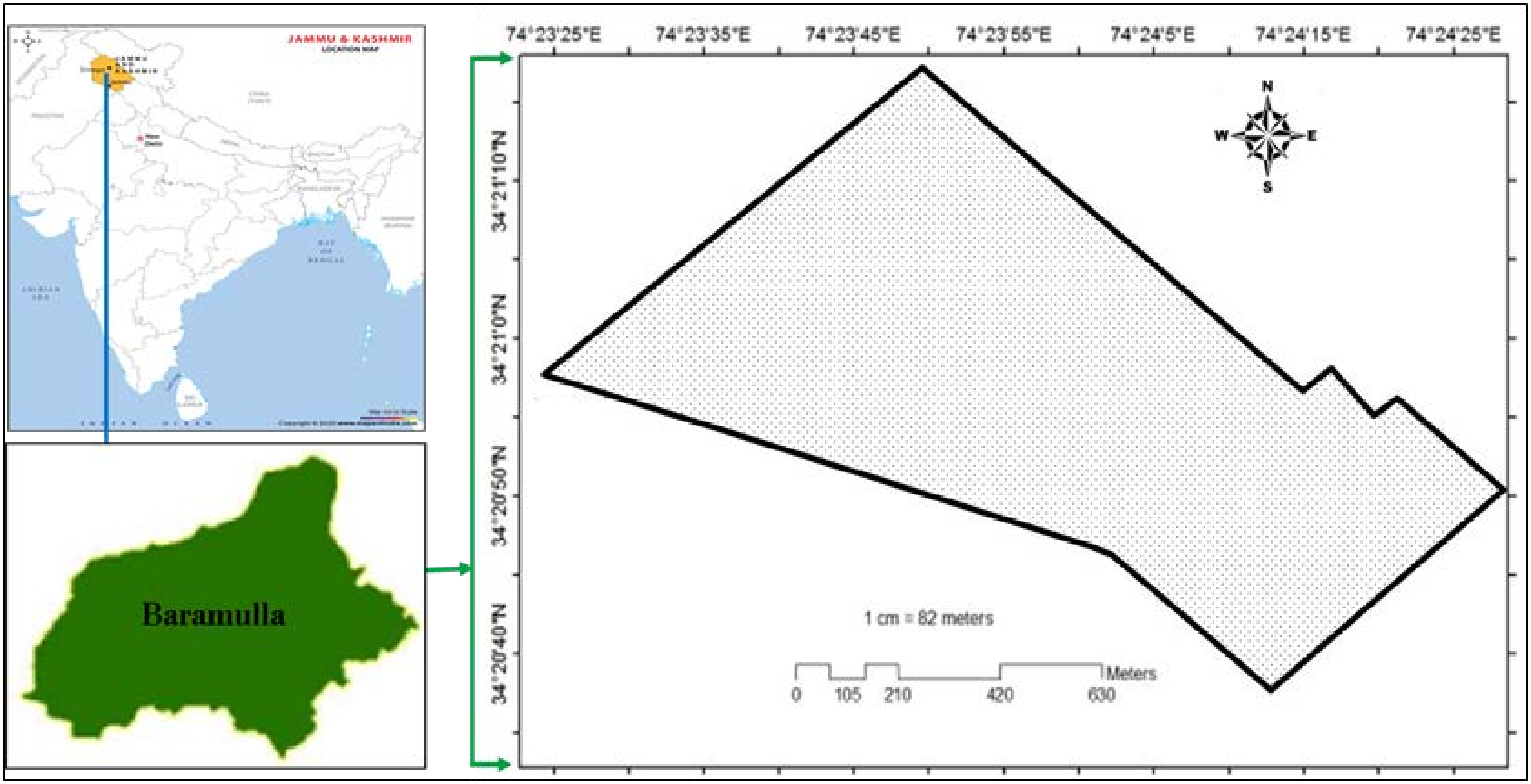

Figure 1. Location map of experimental farm located in Wadura Campus of Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K).

experimentation. The necessary cultural practices such as weeding and hoeing were timely done, so as to keep the experimental plots free from any weed.

Analysis - plants quality

The Laboratory analysis of plant material was carried out by the following methods.

Photosynthetic pigment content analysis

The pigment content levels like chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids were estimated as per the method given by Hiscox and Israelstam (1979) using DimethyleSulphoxide (DMSO). One hundred grams of fresh leaves were homogenised in 10 ml of DMSO and after keeping it in oven for 30 min at 45°C. The absorption of solution was noted at 480, 510, 645 and 663 nm on double beam UV spectrophotometer. The quantity of chlorophyll pigments were calculated by using the formula below (Maclachlan and Zalik, 1963; Duxbury and Yentsch, 1956).

$$\text{Chlorophyll-a mg/g tissue} = \frac{12.3\,(D\,663) - 0.86\,(D\,645)}{d \times 1000 \times W} \times V$$

Table 1. Physico-chemical properties of un-amended soil.

Parameter	Mean
Mechanical properties	
Fine sand (%)	9.30
Silt (%)	55.80
Clay (%)	34.90
Texture class	Silty clay loam
Chemical properties	
pH (1:2.5)	7.00
EC (dSm^{-1})	0.24
Organic Carbon (%)	0.54
Total nitrogen (%)	0.14
Available Nitrogen (mg/kg)	52.3
Available Phosphorus (mg/kg)	4.43
Available Potassium (mg/kg)	91.1
Available Calcium (mg/kg)	21.15
Available Magnesium (mg/kg)	72.23
Micro nutrients	
Zn (ppm)	0.77
Cu (ppm)	1.12
Fe (ppm)	21.29
Mn (ppm)	3.62
Heavy metals	
Cd (ppm)	0.004
Cr (ppm)	1.13
Pb (ppm)	0.32
Ni (ppm)	0.371

$$\text{Chlorophyll-b mg/g tissue} = \frac{19.3\,(D\,645) - 3.60\,(D\,663)}{d \times 1000 \times W} \times V$$

$$\text{Total Chlorophyll mg/g tissue} = \frac{20.2\,(D\,645) + 8.02\,(D\,663)}{d \times 1000 \times W} \times V$$

$$\text{Carotenoid content mg/g tissue} = \frac{7.6\,(D\,480) - 1.49\,(D\,510)}{d \times 1000 \times W} \times V$$

Where, D_{663}, D_{645}, D_{510}, D_{480} are the values of optical density at respective absorption spectra.
V= Final volume of chlorophyll extract in DMSO.
W= Fresh weight of sample.
d= Length of light path (always taken as 1)

Ascorbic acid

The estimation of Vitamin C was carried out by following the protocol of Ranganna (1986), where the dye used was 2, 6-dichlorophenol indophenol. In the procedure, the calculation of dye factor was estimated by titrating 5 ml (3%) metaphosphoric acid plus 5 ml standard ascorbic acid against 2, 6 dichlorophenol indophenol until the required colour was produced and the volume used was then noted;

$$\text{Dye factor} = \frac{0.5}{\text{Titre value}}$$

Estimated of Vitamin C in 10ml of sample was done, by using 3% metaphosphoric acid volume was raised upto 100 ml and filtered. After that, the 10 ml aliquot of the filtered solution was used for titration against 2, 6-dichlorophenol indophenol until the required colour (pink) appeared (persisting for at least 15 s). The calculation was done using the following formula (AOAC, 1975).

$$\text{Ascorbic acid (mg/100 g)} = \frac{\text{Titre value} \times \text{dye factor} \times \text{volume made up}}{\text{ml of filtrate taken for estimation} \times \text{weight of sample taken for estimation}} \times 100$$

Carbohydrate contents

The estimation of total carbohydrate content in the samples of the test crop was estimated by following the Anthrone method of Hedge and Hofreiter (1962). For the calculation of carbohydrate content present in the plant sample, the optical densities of the prepared plant samples were compared with the calibration cure prepared from the know concentration of glucose and the actual values were extracted. The formula for calculation of carbohydrate content present in samples was calculated as:

$$\text{Amount of carbohydrate present in sample (\% mg)} = \frac{\text{Sugar value from graph (mg)}}{\text{Aliquot sample used (0.5 or 1 ml)}} \times \frac{\text{Total volume of extract}}{\text{Wt. of sample (mg)}} \times 100$$

Protein contents

Determination of protein levels in plant samples were carried out by Micro- Kjeldahl's method (AOAC, 1975). By using the formula below to calculate protein percentage:

$$\text{Percentage} = \frac{\text{Sample liter (ml)} \times 0.014 \times \text{volume of digest} \times \text{normality of acid}}{\text{Aliquot taken (ml)} \times \text{weight of sample (g)}} \times 100$$

The data obtained in various observations during the study was pooled and "F" and "t" tests were statistically analyzed at 5% significance level. After significance of "F" test, the difference was determined. By using the Statistical Package for Social Science (SPSS for windows version 16.0, SPSS Inc, Chicago, IL), all the analyses were performed.

Data analysis

After carrying all the observations, the statistical analysis of collected data was carried out by using SPSS Version 18 (SPSS Inc., Chicago, IL, USA) software. The analyses of variance (ANOVA) were performed with the experimental results. The mean ± SE were subjected to Duncan's multiple range test at $p < 0.05$ level.

RESULTS AND DISCUSSION

Impacts of sewage sludge and recommended fertilizer dose (NPK) on growth parameters of kale

The present study revealed that root/shoot length, root fresh/dry biomass of the test crops, viz. kale and spinach, were significantly high with application of 100% NPK followed by application of 75% Sewage Sludge+25% NPK, whereas the values for theses parameters were significantly low in the plots which served as control (Tables 2 and 3). Significant improvement in fresh and dry root biomass is the reflection of increased root length due to application of 100% NPK. In fact, nitrogen being the essential constituent of plant tissue, favored rapid cell division and its enlargement and, thereby, improved both root and shoot length of kale and spinach (Borowski and Kucner, 2019). In a study, Wange et al. (1995) had also showed that the growth parameters of cabbage were increased significantly when nitrogen application was increased. Phosphorus is involved in electron transport and potassium is known to improve availability of nitrogen to the plants. Similar results were also reported by Alvarenga et al. (2015); Kirchmann et al. (2017) and Assimakopoulou et al. (2019). Kumar et al. (2019) also reported that applications of N, P and K fertilizer are closely related with the root and shoot enlargement of plants like *Brassica juncea*. Highest root growth in terms of percentage dry weight was observed in plants treated with NPK, which was further supported by its root sapogenin content (steroidal saponin glycosides). On the other hand the decrease in root length due to application of higher dosage of sewage sludge in combination with NPK could be attributed to increase in nutrient concentration that restricted the availability of nutrients to the plant. Alvarenga et al. (2015) reported that root elongation in crops (Chinese cabbage, *Brassica parachinesis* and Chinese radish) were retarded at higher concentrations of sludge extracts. Singh and Agrawal (2008) as well as Singh and Agrawal (2010) have also noticed reduced root length in *Beta vulgaris* due to increase in nutrient concentration under waste water irrigation.

5.2 Impacts of Sewage Sludge and Recommended Fertilizer Dose (NPK) on Growth Parameters of Spinach

The study also reveals significantly highest leaf area index in kale and spinach were recorded with application of 100% NPK followed by 75% sewage sludge+25% NPK treatment (Table 2 and 3). Leaf area index is a vital photosynthetic character in the plant and efficient availability of nitrogen may have lead to improved protoplasmic components and enhanced the function cell division and elongation, which resulted in luxuriant vegetative growth with increased leaf surface area exposed for harvesting more sunlight. It was shown that integrated application of waste (organic) and fertilizers (chemical) proved to improve leaf area significantly better results in leaf area (Kominko et al., 2017; Mohamed et al., 2019). Furthermore, Singh and Agrawal, (2010) also reported that leaf area of *Beta vulgaris* increased significantly when grown under various sewage sludge amendment. Besides, increment in the phosphorus doses might result in increased crop vigour as evident from improvement in root length and root biomass. The other reports that showed the reasons for increased leaf area index might be the addition of available P and exchangeable K content in the soil from inorganic fertilizers (Alvarenga et al., 2015; Khaliq et al., 2017). Similar type of results were also observed by (Assimakopoulou et al., 2019; Kumar et al., 2019; Borowski and Kucner, 2019; Kaur and Sharma, 2020).

Impacts of sewage sludge and recommended fertilizer dose (NPK) on weight of edible parts and yield of kale and spinach

It has been concluded from Table 3 that application of 100% NPK recorded the highest weight of edible parts of

Table 2. Effect of sewage sludge and recommended fertilizer dose (NPK) on growth parameters of kale.

Treatments	Root length plant^{-1} (cm)	Root fresh biomass (g)	Root dry biomass (g)	Shoot length plant^{-1} (cm)	Leaf area index	Leaf area (cm^2)
T_1 (Control)	10.01	21.99	7.06	33.67	0.401	181.85
T_2 (25:0)	11.80	24.32	8.57	36.42	0.521	197.76
T_3 (50:0)	12.92	26.97	8.20	37.99	0.558	214.10
T_4 (75:0)	14.13	30.08	9.13	38.49	0.597	231.98
T_5 (100:0)	14.65	30.91	9.39	39.46	0.612	238.77
T_6 (25:75)	15.50	31.22	9.47	40.71	0.710	247.22
T_7 (50:50)	15.68	32.61	9.90	41.12	0.833	260.76
T_8 (75:25)	15.77	33.24	10.09	41.75	0.952	259.65
T_9 (0:100)	16.23	33.89	10.29	42.21	0.960	264.32
SE(m) +	0.069	0.122	0.034	0.085	0.007	1.722
C.D ($p \leq 0.05$)	0.169	0.299	0.084	0.256	0.016	4.25

T indicates treatments; no fertilizer or sewage sludge as a control , (T_1); 25% Sewage Sludge + 0% Recommended fertilizer dose (RFD), (T_2); 50% Sewage Sludge + 0% RFD, (T_3); 75% Sewage Sludge + 0% RFD, (T_4); 100% Sewage Sludge + 0% RFD, (T_5); 25% Sewage Sludge + 75% RFD, (T_6); 50% Sewage Sludge + 50% RFD, (T_7); 75% Sewage Sludge + 25% RFD, (T_8); 0% Sewage Sludge + 100% RFD, (T_9).

Table 3. Effect of sewage sludge and recommended fertilizer dose (NPK) on growth parameters of spinach.

Treatments	Root length plant^{-1} (cm)	Root fresh biomass (g)	Root dry biomass (g)	Shoot length plant^{-1} (cm)	Leaf area index	Leaf area (cm^2)
T_1 (Control)	4.97	7.56	1.36	9.22	0.153	46.38
T_2 (25:0)	6.04	9.80	1.76	10.84	0.219	51.28
T_3 (50:0)	6.15	10.44	1.88	11.72	0.269	66.00
T_4 (75:0)	7.06	11.88	2.14	12.04	0.280	70.28
T_5 (100:0)	8.32	13.86	2.49	12.70	0.282	70.93
T_6 (25:75)	7.97	14.06	2.53	13.07	0.289	80.73
T_7 (50:50)	8.65	14.38	2.59	13.69	0.315	82.68
T_8 (75:25)	8.99	14.92	2.68	13.87	0.348	84.88
T_9 (0:100)	9.62	15.91	2.86	14.49	0.372	85.58
SE(m) +	0.069	0.254	0.046	0.088	0.003	0.825
C.D ($p \leq 0.05$)	0.209	0.769	0.138	0.216	NS	2.037

both kale (139.70 g) and spinach (91.18 g), while lowest weight of edible part of both the test crops were recorded in control treatment. This could be attributed to enhancement in leaf area in both vegetables. The study also revealed that plots treated with 75% sludge +25% NPK and 50%

Table 4. Effect of sewage sludge and recommended fertilizer dose (NPK) on weight of edible part and yield of kale and spinach.

Treatments	Kale		Spinach	
	Weight of edible part (g)	Yield (q ha^{-1})	Weight of edible part (g)	Yield (q ha^{-1})
T_1 (Control)	103.68	230.40	45.23	150.77
T_2 (25:0)	126.24	280.53	62.84	209.47
T_3 (50:0)	128.18	284.84	68.01	226.70
T_4 (75:0)	129.39	287.53	74.19	247.30
T_5 (100:0)	134.31	298.47	79.71	265.70
T_6 (25:75)	136.23	302.73	82.85	276.17
T_7 (50:50)	137.79	306.20	87.26	290.87
T_8 (75:25)	139.47	309.93	90.36	301.20
T_9 (0:100)	139.70	310.44	91.18	303.93
SE(m) +	2.066	2.765	2.151	2.809
C.D ($p \leq 0.05$)	5.104	6.83	5.314	6.94

sludge+50% NPK recorded equal yield to the plots treated with 100% NPK in kale, while in case of spinach T_8 (75% sludge+25% NPK) recorded equal yield to the plots treated with 100% NPK. Significantly lowest yield in both the crops were recorded in control treatment (Table 4). The improvement in yield with mixed application of sewage sludge and fertilizer (chemical) could be accredited for that sewage sludge releases nutrients slowly (Kaur and Sharma 2020). Amendment of soil by biosolids prevents loss of nitrogen through volatilization, leaching and denitrification by effective binding the nutrients to their transit substrates and releasing slowly with the passage of time thereby increasing nutrient use efficiency (Khaliq et al., 2017). The findings are in conformity with Usman et al. (2012), who found that the yield of brinjal was highest in plots treated with recommended dose of fertilizers. Similar type of results were also reported in other such studies (Borowski and Kucner, 2019; Kaur and Sharma, 2020).

Impacts of sewage sludge and recommended fertilizer dose (NPK) on quality parameters of kale

It has been inferred from the results obtained during the present study that application of 75% sludge+25% NPK significantly increased (Tables 5 and 6) foliar ascorbic acid contents in both kale (120.82 mg/100 g) and spinach (21.89 mg/100 g). The conversion of proline to hydroxyproline is due to ascorbic acid, which is involved in this enzymatic hydroxylation. This hydroxyproline is considered to be an essential constituent present in the cell walls (Lamport and Northcote, 1960). Its role has been found in cyclic redox reactions and also offers protection to plants during photosynthesis against the harmful side-effects of light. Gupta et al. (2009) reported that *Raphanus sativus* grown in sewage sludge showed higher production of ascorbic acid.

Effect of sewage sludge and recommended fertilizer dose (NPK) on quality parameters of spinach

It is the plant carbohydrates which form the main structural components and products of energy in two forms, viz. cellulose and hemicelluloses, which are polymers of glucose and 5-carbon sugars and other compounds respectively. The principal form is starch, a polymer of glucose, which is stored in cells in the form of granules when produced more than which is required. These starch granules are being broken down through enzymatic reactions for energy production in the times of metabolic need. The study shows that application of 75% sludge+25% NPK recorded significantly highest carbohydrate content in both kale (4.47%) and spinach (4.74%). Recently, Oloyede et al. (2012) reported that higher doses of NPK increased the carbohydrate content of pumpkin which is in harmony with the present findings. The enhanced levels of carbohydrates in sewage sludge supplemented plants are because of the occurrence of some important mineral ions in it e.g. the Mn and Cu elements present are known to stimulate the two photosystems. For PS II which is an O_2 evolving system, Mn^{+2} is required and in case of PS I, the interaction between copper and ferredoxin on the reducing site is also considered to be the important one (Marschner, 1986). The rate of electron transfer from water to NADP is also managed by Cu^{++} (Marschner, 1986). Zeid and Abou-El-Ghate (2007) reported that in plants irrigated with sewage water, the polysaccharide and total carbohydrate contents were higher compared to the controls plants. These carbohydrate levels decreased when sewage water was treated by precipitation, rice residue or EDTA before irrigation.

Table 5. Effect of sewage sludge and recommended fertilizer dose (NPK) on quality parameters of kale.

Treatments	Ascorbic acid (mg/100 g)	Carbohydrate content (%)	Protein content (%)	Chlorophyll-a (mg/100g)	Chlorophyll-b (mg/100 g)	Total chlorophyll (mg/100 g)	Carotenoids (mg/100 g)
T_1 (Control)	82.16	1.31	5.938	1.00	0.271	1.225	2.280
T_2 (25:0)	89.05	2.32	7.262	1.39	0.417	1.842	3.231
T_3 (50:0)	91.26	2.61	8.232	1.41	0.487	1.957	3.832
T_4 (75:0)	100.22	2.88	8.361	1.46	0.489	1.975	3.939
T_5 (100:0)	105.76	3.19	8.888	1.82	0.600	2.420	4.019
T_6 (25:75)	110.53	3.62	9.982	1.39	0.465	1.905	3.340
T_7 (50:50)	116.16	4.19	10.353	1.27	0.407	1.703	3.122
T_8 (75:25)	120.82	4.47	10.645	1.16	0.324	1.578	3.032
T_9 (0:100)	112.55	3.74	9.481	1.17	0.360	1.671	3.118
SE(m) +	1.771	0.050	0.068	0.007	0.007	0.069	0.042
C.D ($p \leq 0.05$)	4.371	0.143	0.167	0.017	0.016	0.169	0.102

Table 6. Effect of sewage sludge and recommended fertilizer dose (NPK) on quality parameters of spinach.

Treatments	Ascorbic acid (mg/100 g)	Carbohydrate content (%)	Protein content (%)	Chlorophyll-a (mg/100g)	Chlorophyll-b (mg/100g)	Total chlorophyll (mg/100g)	Carotenoids (mg/100 g)
T_1 (Control)	10.61	1.59	5.013	0.927	0.145	1.106	1.211
T_2 (25:0)	12.49	2.41	6.387	1.020	0.199	1.482	1.307
T_3 (50:0)	13.59	2.79	7.135	1.316	0.208	1.527	1.313
T_4 (75:0)	13.79	3.02	7.005	1.339	0.215	1.600	1.331
T_5 (100:0)	14.25	3.23	7.664	1.502	0.326	1.806	1.433
T_6 (25:75)	17.18	3.86	8.467	1.284	0.203	1.464	1.328
T_7 (50:50)	19.06	4.16	8.978	1.269	0.174	1.435	1.273
T_8 (75:25)	21.89	4.74	9.966	1.005	0.164	1.390	1.256
T_9 (0:100)	17.05	3.59	8.255	1.269	0.170	1.428	1.271
SE(m) +	0.705	0.132	0.191	0.005	0.0016	0.004	0.003
C.D ($p \leq 0.05$)	1.739	0.324	0.471	0.014	0.004	0.011	0.009

Importance and proteins formation

Since proteins play an important role in plant development, in a typical plant cell near about half of its dry matter constitutes protein. It is the protein, which forms the structural constituents, antibodies and most of the hormones in a plant cell. Almost 90% of the proteins are reported to function as enzymes, which are key players for many fundamental cellular reactions required for proper development in the plant system. It was found that the plots treated with 75% sludge+25%

Table 7. Effect of sewage sludge and recommended fertilizer dose (RFD) on micro nutrients of soils for kale and spinach.

T	Zn (ppm)		Cu (ppm)		Mn (ppm)		Fe (ppm)		Cr (ppm)		Ni (ppm)		Pb (ppm)		Cd (ppm)	
	K	S	K	S	K	S	K	S	K	S	K	S	K	S	K	S
T_1	12.04	25.19	2.45	1.65	3.91	3.58	75.34	86.64	1.311	0.756	1.339	0.998	1.636	0.955	0.292	0.341
T_2	14.57	28.19	3.26	2.21	5.13	4.91	77.78	90.06	1.402	0.855	1.415	1.249	1.848	1.329	0.349	0.454
T_3	16.21	37.01	4.86	2.59	5.58	4.92	78.05	90.33	1.441	0.936	1.545	1.686	2.021	1.999	0.389	0.489
T_4	17.01	44.01	4.97	2.86	5.74	5.41	78.30	90.58	1.485	0.950	1.611	1.902	1.955	2.057	0.421	0.528
T_5	18.81	46.01	6.98	3.61	6.13	5.77	78.71	90.99	1.526	0.967	1.677	1.966	2.087	2.123	0.441	0.568
T_6	20.01	49.00	6.90	4.00	6.33	5.97	79.90	91.67	1.541	1.477	1.811	2.011	2.353	2.191	0.461	0.593
T_7	20.77	49.80	9.08	4.49	6.44	6.09	79.90	92.18	1.565	1.522	1.943	2.110	2.681	2.257	0.492	0.626
T_8	21.37	58.01	9.83	4.98	6.99	6.64	80.36	92.64	1.754	1.542	2.273	2.309	2.221	2.841	0.501	0.644
T_9	16.91	44.60	6.15	3.18	5.53	5.18	78.46	90.74	1.560	1.062	1.679	2.078	2.087	2.116	0.445	0.551
PL	300-600		135-270		2000		50000		100		75-150		250-500		3-6	

NPK recorded highest protein (percent) both in kale and spinach (Tables 5 and 6). This could be due to the fact that higher rates of sludge may have increased the availability of nitrogen in the plant thereby improving protein content as these are the product of nitrogen concentration in the plant. These results are in conformity with the earlier reports (Usman et al., 2012; Kirchmann et al., 2017; Borowski and Kucner, 2019; Kumar et al., 2019; Singh and Agrawal, 2010). Some earlier reports have also shown that cultivation of some edible plants like *Raphanus, Corchorus, Lactuca, Eruca, Spinacia* and *Daucus*) on sewage sludge supplemented soil has resulted in higher content of total proteins (Mazen, 2003).

During the present study it was found that pigments like chlorophyll-a, chlorophyll-b, total chlorophyll along with carotenoids were significantly highest after application of 100% sewage sludge in both the test crops, while lowest readings for these parameters were observed under control conditions (Tables 5 and 6). The sewage sludge favoured both the synthesis and accumulation of chlorophyll, which may be accredited to the nitrogen availability provided by sewage sludge, which is essential in the structure of porphyrin, found in such metabolically important compounds as chlorophyll. Also, nitrogen is needed for the synthesis of the protein molecules to which chlorophyll is bound, or in which it is embedded (Usman et al., 2012). These results are in agreement with those of Singh and Agrawal (2008). It was reported that chlorophyll-a, -b and total chlorophyll in the leaves of developing cattail plants did not show any significant toxic effect due to the sewage sludge metals ions (Mohamed et al., 2018). However, reduction in the ratio of chlorophyll-a and b suggested some increase in chlorophyll hydrolysis due to metal accumulation. Mazen et al. (2010) observed that application of sewage sludge enhanced the levels of photosynthetic pigments. This enhancement slightly continued and was parallel with the application of sludge in the soil. Mazen et al. (2010) further reported that the plants which have been raised in sewage sludge treated soils did not show any symptoms of deficiency or toxicity (Tables 1 and 7). However, the performance of plants cultivated on sewage sludge supplemented desert soil was better, which showed higher accumulation of chlorophyll content. These results find support from earlier reports observed in plants like *Daucus*, *Spinacia Corchorus*, *Eruca*, *Lactuca*, and *Raphanus* (Mazen, 2003). So all the earlier results are in consistency with our present finding, which support the application of sewage sludge to various plant cultivating soils improve plant growth and development.

Neutral pH of soil after harvest of kale was recorded with 100% sewage sludge treatment, whereas significantly highest pH was noticed under the control treatment. In spinach significantly highest pH of soil after crop harvest was recorded under the treatment of 75% sludge + 25% NPK for two repeated years. Similarly, EC in both the test crops was highest in soil treated with 75% sludge + 25% NPK. Organic carbon, total N, available nitrogen and phosphorus, contents in the soil after final harvest of crop remained significantly higher under the treatment of 75% sludge + 25% NPK. Available potassium, calcium, magnesium and cadmium contents in soil after crop harvest did not differ significantly among the treatments during two years of study like (Mohamed et al., 2019). Manganese and cobalt contents in soil after final crop harvest were significantly higher under the treatment of 100% sewage sludge, whereas lowest values of these parameters were observed in control treatment. Similarly other heavy metals viz., Cd, Cr, Pb, Zn, Cu, Fe, Pb and Ni were within the permissible limit and their concentration did not vary significantly, the results are in line with studies like (Kumar et al., 2019; Kaur and Sharma 2020; Mohamed et al., 2019).

The results of the study showed that the concentration of all macro nutrients (N, P, K, Ca and Mg), micro nutrients (Mn, Cu, Zn and Fe) and heavy metals (Cd, Cr, Pb and Ni) in both kale and spinach were significantly highest in the plots treated with 75% sludge + 25% NPK, whereas, the values of these elements in control treatments were recorded to be the lowest. The nutrients were also in permissible limits for soils as shown in Table 7. Thus application of 75% sludge + 25% NPK has been recorded as a suitable practice to cultivate these two important crops. Thus same practices may be replicated for the cultivation of other crops.

Conclusion

The study concluded with the finding that in both kale and spinach, application of 100% NPK exhibited higher yield in edible part and other morphological parameters but was statistically on a par with 75% sludge + 25% NPK. The said treatment also significantly improved most of the quality parameters. In view of this, it is recommended that for obtaining higher quality yield in kale and spinach the crops should be fertilized with 75% sewage sludge + 25% NPK. However, further work needs to be carried out at different agro-climatic zones to arrive at final recommendations for the Valley.

Recommendations

(1) Treated sewage sludge is an end product of municipal wastewater treatment and contains many pollutants that are removed during treatment. Thus, its use as a soil conditioner improves soil properties in a manner similar to other organic-based soil amendments.

(2) Farming using sewage and sludge yields more nutritious and safe food and popularity of these foods increases because healthier and safer nature and ensures food safety from farm to plate. Thus, The National Programme for Organic Production (NPOP) provides an institutional mechanism for the implementation of NSOP for use of sewage sludge as fertilizer. The standards and procedures have been formulated in harmony with other International Standards regulating production, import and export of organic products.

(3) The financial incentive for farmers to use sewage sludge in crop production is debatable. Fertilizers account for a small percentage of total production costs and sewage sludge may be difficult to use as commercial fertilizer. Thus various government departments like Municipalities, civil works department, water distribution department and other agriculture allies must incentivize application of sludge for agriculture.

(4) Farmers must be educated about the schemes and grants provided by the government. Thus, An increasing number of NGOs encourage farmers to participate in agriculture based on 3R. One of the international NGOs working in the field is the International Union for the Conservation of Nature and Natural resources (IUCN) by focusing on providing information, training, capacity building and networking. Other NGOs in India have worked extensively with governments to help implementation of national strategies for agriculture and help raise the standards of farmers.

(5) The use of sewage and sludge as fertilizer is suited to all crops and specially when grown on loam soil. The use of sewage sludge if it has increased chemical and biological impurities, the soil has capacity to sequester the impurity through stabilisation. Thus future course of the study is to find the plants that have more capacity to grow on sewage sludge amended soils.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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