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Research article

Effectiveness of cattle dung biogas digestate on spinach growth and nutrient uptake

Bridget Tshikalange [a,*], Olusola Ololade [a], Chipa Jonas [b], Zaid A. Bello [c,**]

[a] Centre for Environmental Management, University of the Free State, Bloemfontein, South Africa
[b] Limpopo Department of Agriculture and Rural Development, Limpopo, South Africa
[c] Agricultural Research Council - Grain Crops, Potchefstroom, South Africa

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ABSTRACT

New farming techniques should be introduced to improve yield quality and quantity while taking preservation of the environment into consideration. This study investigated effectiveness of cattle dung biogas digestate on spinach growth and nutrient uptake. Spinach was grown with cattle dung biogas digestate (BD), inorganic fertiliser (IF) and unfertilised control (CO) treatments under complete randomised design field conditions. Spinach planted under BD showed significantly higher growth in terms of plant height and number of leaves compared to spinach under CO and IF. A linear relationship between leaf numbers and leaf area index (LAI) (R^2 0.691, $p < 0.0001$) was established for the study. Cattle dung biogas digestate (BD) plants produced significantly the longest roots followed by IF plants. The IF plants produced more biomass per rooting depth (0.85 g cm^{-1}) than BD (0.61 g cm^{-1}) and CO (0.35 g cm^{-1}). Regarding macronutrient content of the spinach leaves, significant differences were only observed for potassium (K) in the order of IF (8.6 g kg^{-1}), BD (6.8 g kg^{-1}), and CO (6.7 g kg^{-1}). Significantly higher amounts of zinc (Zn^{2+}) accumulated in spinach leaves under BD compared to IF and CO. Fertilising spinach with BD improves growth and development just as much as IF. Additional benefits include improving nutrient content of the spinach, assisting with environmental preservation and decreasing production cost.

1. Introduction

Modern crop production focuses on improving yield quality and quantity while taking preservation of the environment into consideration. To ensure that increased yield and environmental preservation takes place, new farming techniques need to be introduced. These techniques should ensure an increase in food production in a sustainable way for the ever-rising population (Tiang et al., 2016). There are many factors that contribute towards the sustainability of food production and among these factors is maintenance of soil fertility (Ebrahimi et al., 2019). Inorganic fertilisers have played an important role in increasing agricultural production and improving world food security (Goulding et al., 2008; Bayu, 2020). However, intensive use of inorganic fertilisers in crop production systems have negative agricultural and environmental consequences (Nkoa, 2014). Over time, increased use of inorganic fertiliser under crop production systems result in soil degradation in the form of soil acidification, and heavy metal accumulation, which leads to a decrease in crop production (Khan et al., 2018). Environmental problems such as nutrient pollution of both ground and surface water and increase in greenhouse gas emissions, which is one cause of climate change, are also accelerated (Kusin et al., 2015).

Balancing human needs with environmental sustainability is possible. Shifting towards organic production ensures that agricultural land is preserved for current and future generations (McIsaac, 2003; Giller et al., 2021). Therefore, farming making use of organic wastes has gained attention as an environmentally friendly production means. Nutritional and soil amendment benefits of organic wastes have been investigated (Odlare et al., 2011; Walsh et al., 2012; Renaud et al., 2017). Various types of soil organic amendments such as farm and municipal waste are used for organic production (Scotti et al., 2015). Organically grown foods are perceived to be of better quality, healthier and more nutritious than conventionally grown food (Warman and Harvard, 1998; Meemken and Qaim, 2018). Several studies have shown that organically grown fruits or vegetables contain more minerals and vitamins than those grown conventionally (Worthington, 2001; Citak and Sonmez, 2010; Kyriacou and Rouphael, 2018). Organic fertiliser is a low-cost input and readily

* Corresponding author.
** Corresponding author.
E-mail addresses: 2007108208@ufs4life.ac.za (B. Tshikalange), Belloz@arc.agric.za (Z.A. Bello).

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available, but comes in different forms. One source of soil organic amendments is biogas digestate. Biogas digestate is the product of substrates fed into a digester during the process of biogas production. Up to 80–90% of the quantity used to feed a biogas digester is retained after biogas production. Biogas digestate has proven to have fertilisation and soil amending benefits compared to inorganic fertilisers, which only offers a fertilisation benefit (Barbosa et al., 2014).

Food security does not only refer to availability, but also the quality of food. Nutritious food should provide carbohydrates, protein and vitamins in adequate quantities. Leafy vegetables are a source of different vitamins, which helps in providing a healthy living. High fruit and vegetable intake is known to have a positive impact on human health and is linked to decreased risk of many of the degenerative diseases associated with ageing, such as cardiovascular disease (Christen, 2000; Arts and Hollman, 2005; Kyriacou and Rouphael, 2018), Alzheimer's disease (Commenges et al., 2000), cataracts (Brown et al., 1999), and several forms of cancer (Williamson, 1996; Gandini et al., 2000; Joshipura et al., 2001; Kang et al., 2005). However, the nutritional quality in vegetables can be affected by pre- and post-harvest factors (Kader, 2008; Arah et al., 2015; Taghavi et al., 2019). Spinach (*Spinacia oleracea L.*) is an annual leafy vegetable that is ready for harvesting between 60 and 70 days after transplant. Apart from food and nutrition, spinach also possesses medicinal attributes. As a food, spinach can be used raw as a salad or cooked as a potherb. Nutritionally, the leaves contain low calories, vitamins A, B1 and B2 and minerals (Toledo et al., 2003). Spinach is an important source of total phenolic content and has an antioxidant capacity, which is beneficial for human health (Ismail et al., 2004; Murcia et al., 2020). It can also be used in treating urinary calculi and lung inflammation due to its hypoglycaemic effect (Miri and Roughani, 2019). These attributes have influenced worldwide consumption of spinach on a daily basis (Lisiewska et al., 2011; Murcia et al., 2020). Spinach has caught the attention of farmers in South Africa and is one of the most common leafy vegetables consumed in the country (Van Rensburg et al., 2007).

Spinach is mainly cultivated in the country under controlled environments such as greenhouses, hydroponics and shade nets and not many farmers use field cultivation. Many smallholder farmers in the country grow spinach. One of the challenges they face is the high input cost of spinach production. Greenhouses, hydroponics and shade nets are not cheap, and the cost of inorganic fertiliser is ever increasing. Hence, a better production technique that is cost effective and sustainable is needed. Fertilisation is one of the most practical and effective agronomic practices that helps improve the yield and nutritional quality of crops for human consumption (Arts and Hollman, 2005; Bindraban et al., 2015; Röös et al., 2018). However, there is need to investigate whether organic fertiliser, in the form of biogas digestate, will contribute to spinach growth better than inorganic fertiliser. This will elucidate whether there are nutritional benefits associated with using soil amendments in spinach production. Therefore, this study investigated the effectiveness of using

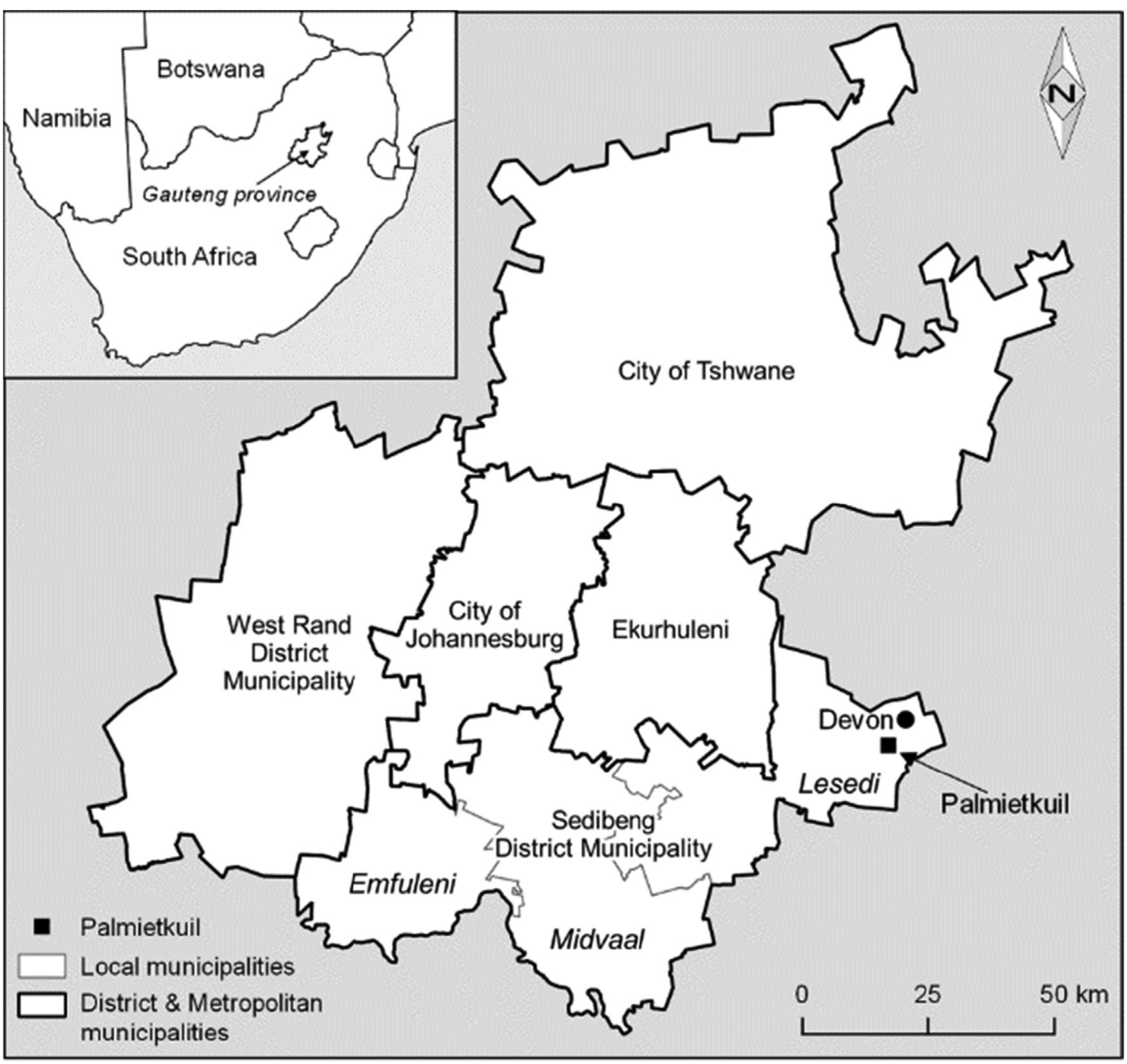

Figure 1. Location of the experimental site (Palmietkuil) in the Gauteng Province of South Africa.

cattle dung BD as soil amendment for spinach growth, nutrient uptake and production. Organic farming is a sustainable means of crop production and considered a viable option in developing countries. Therefore, results from this study will serve as a step towards better understanding and application of this system.

2. Material and methods

2.1. Experimental site

The study was conducted in an animal production farm located at Palmietkuil, Devon area in the Gauteng Province (South Africa) (Figure 1) between 8 September and 28 October 2017. The farm is located on 26°24′04′′.9 S latitude and 28°44′49′′.4 E longitude, covering a total area of 285 Ha. Devon area receives an average of 525 mm rainfall, mostly during the summer season (December, January and February). Mean daily maximum temperatures in the area range from 18 °C to 27 °C with mean daily minimum temperatures from 2 °C to 14 °C. Weather conditions for the area were obtained from the South African Weather Service (SAWS) station in the Delmas area, nearest to the experimental site.

2.2. Treatments and layout

Three treatments, namely cattle dung biogas digestate (BD), inorganic fertiliser (IF) and control (CO) were compared in the experiment. Treatment plots were replicated three times and laid out in complete randomised design. Each treatment was allocated a 3 m × 3 m plot with a 0.5 m space to make provision for border effects between plots. To ensure that spinach plants' nutritional demands were met, a basic spinach fertilisation of 125 kg N ha^{-1}, 20–25 kg P ha^{-1} and 125 kg K ha^{-1} of 2:3:4 (30) was applied to the soil (Ndololwana, 2015). Due to mass and combination difficulty in calculating the required amount of phosphorous and potassium needed at planting, recommended nitrogen content of the inorganic fertiliser was used as the reference for calculating the amount of biogas digestate needed. As a result, a total mass of 1.48 kg BD was applied. Both IF and BD were incorporated into the soil using a rake. Transplanting spinach took place on the 16^{th} of September 2017. Seedlings were transplanted at a spacing of 0.25 m × 0.30 m. At four weeks after transplanting, a recommended actual rate of 175 kg ha^{-1} limestone ammonium nitrogen (LAN) containing 49 kg N ha^{-1} was applied as top dressing. Calculated equivalent amounts of 0.6 kg in BD per plot was applied. Irrigation was carried out following ARC-VOPI (2007) guidelines of 25 mm–35 mm of water per hectare a week.

2.3. Biogas digestate characteristics

Biogas digestate originated from cattle dung substrate containing no other organic material. It was collected for characterisation at the farm biogas digestate tank making use of a clean 500 ml container rinsed with distilled water. The collected biogas digestate was analysed for pH using the procedures of Van Reeuwijk (2002) for both aliquot and dry samples. Total carbon and nitrogen was determined using an Erba NA 1500 at 1280 °C heating according to Jimeneze and Ladha (1993). Phosphorous and potassium quantities were determined using Inductively Couple Plasma- Optical Emission Spectrometric (ICP-OES) analytical procedure by Zasoski and Burau (1977). In order to justify plant uptake, food quality assurance and environmental application suitability, BD was analysed for heavy metal content. The material had a pH of 8.36, which had the capability of increasing soil pH. The BD contained heavy metals that were within acceptable ranges of organic fertilising material limits stipulated under the Fertiliser, Farm Feeds Agricultural Remedies and Stock Remedies Act, Act 36 of 1947 (Department of Agriculture, Forestry and Fisheries (DAFF), South Africa), and the Environmental Conservation Act, Act 73 of 1989 (Department of Environmental Affairs (DEA), South Africa). The characteristics of the BD nutrients and heavy metal content are given in Table 1.

2.4. Soil properties

Up to 1.2 m depth of soil was extracted with an auger for soil type identification. The soil type of the experimental site was clay textured and belonged to the Arcadia soil form (Soil Classification Working Group, 1991). The soil's texture distribution was 48% clay, 36% sand and 16% silt (Table 2). At the beginning of the experiment, soil was taken from each treatment plot at the first 30 cm layer and then mixed to obtain a homogeneous composition for soil chemical properties analyses. The soil samples were dried at 40 °C and pH determined using Van Reeuwijk's (2002) procedure for both aliquot and dry samples. Nitrogen (N), phosphorous (P), potassium (K^{+}), calcium (Ca^{2+}), magnesium (Mg^{2+}) and sulphur (S) content of the soil were determined using the ICP-OES analytical procedure by Zasoski and Barau (1977). The soil contained a pH of 6.67 with 2340 mg kg^{-1} N, 102.1 mg kg^{-1} P, 1250 mg kg^{-1} K, 4310 mg kg^{-1} Ca and 10.8 mg kg^{-1} Na (Table 3). The high clay content and the nature of the soil form indicated that the soil needed care before planting, but had a high plant nutrient sorption and water holding capacity beneficial for spinach growth (Tahir and Marschner, 2017). Therefore, prior to planting, the field was ploughed and rotovated. Plots were marked according to plot sizes and labelled. Weeds from the plots were controlled manually.

2.5. Agronomic measurements

Plant height, leaf area and number of leaves were recorded on a weekly basis throughout the experimental period. Two plants in each replicate plot of each treatment were marked for weekly observations of plant height and number of leaves. Plant height was measured from the crop base to the top of the last two leaves. Leaf area was measured with the aid of a leaf area meter (LI3000; LI-COR, Lincoln, NE, USA). Leaf area index was calculated using Eq. (1). At the end of the experimental cycle, which was 49 days after planting, leaves from the three treatments were collected for nutrient accumulation analysis. One leaf per replicate was

Table 1. Macronutrients, micronutrients and heavy metal content of biogas digestate.

Digestate	Element	Amount*
Macronutrients (g kg^{-1})	N	12.2
	P	2.43
	K	8.96
Micronutrients (g kg^{-1})	Ca	6.1
	Mg	1.77
	Fe	3.41
	S	2.5
	Al	2.72
	Na	5.53
	Cu	0.12
	Mn	2.15
	Zn	0.54
	B	0.09
Heavy metals (g kg^{-1})	Cd	0.012 (2)
	Cr	2.036 (175)
	Co	3.255 (10)
	Cu	1.302 (75)
	Hg	0.001 (1)
	Mo	0.036 (2.5)
	Ni	1.095 (20)
	Pb	0.262 (40)
	Zn	9.89 (275)
	As	0.049 (1.5)

* Maximum heavy metal concentration limits (Department of Agriculture, Forestry and Fisheries, 2012) in brackets.

Table 2. Soil texture distribution of the experimental site.

Texture class	Particle %
Sand	36
Clay	48
Silt	16

Table 3. Soil chemical properties of the experimental site.

Parameter	Values
pH	6.67
N (mg kg^{-1})	2340
P (mg kg^{-1})	102.1
K (mg kg^{-1})	1250
Ca (mg kg^{-1})	4310
Na (mg kg^{-1})	10.8

harvested and analysed individually. The collected leaves were digested and analysed for nitrogen (N), phosphorous (P), sulphur (S), potassium (K^{+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), copper (Cu^{2+}), aluminium (Al^{3+}), manganese (Mn^{+}), iron (Fe^{2+}), boron (B) and sodium (Na^{+}) making use of an ICP-OES. For the above ground biomass, three stands of spinach per replication were harvested and manually dried in the sun until the leaves reached constant mass (Hassan et al., 2007). Spinach plant roots were washed, measured and noted for comparative effect between the three treatments.

$$\text{Leaf area index (LAI)} = \text{leaf area per plant} \times \text{plant population} \qquad \text{Equation 1}$$

2.6. Statistical analysis

Prior to analysis of variance, data were tested for normal distribution using Shapiro-Wilks normality test, which is one of the preferred tests for normality (Stevens, 2002; Gomez and Gomez, 1984). Since there was no significant departure from normality, analysis of variance (ANOVA) using Statistical Analysis System (SAS) Program 9.4 package for Windows V8 (Statistical Analysis System Institute Inc., Cary, NC, 1999–2010) was carried out. Means were compared using the least significant difference (LSD), tested at a 95% confidence level. Data consisting of the number of leaves and LAI for all the treatments were pooled, linear trendlines constructed and tested for significance of relationship using Microsoft Excel.

3. Results and discussion

3.1. Weather conditions

The experimental area's weather conditions are given in Figures 2 and 3. During the experimental period, the station recorded average minimum temperatures ranging from 1 °C to 12 °C and average maximum temperatures ranging from 23 °C to 31 °C. Highest rainfall of 35 mm was recorded during the second week after transplanting date. The experiment started after frost season had lapsed, resulting in temperatures not dropping below 0 °C throughout the experimental period. An evaporative demand between 21 and 39 mm, which was higher than average rainfall received, was experienced during the study. This was part of the reason why supplementary irrigation was needed for crop growth.

3.2. Spinach growth

3.2.1. Plant height

Effect of cattle dung BD, IF and CO on spinach growth in terms of plant height is given in Figure 4. There were significant differences in the plant height as affected by different nutrient sources (d.f. = 2, P = 0.0004, LSD = 3.23). Spinach plant height varied from 8.2 ± 0.26 to 9.4 ± 0.29 cm during the first seven days after transplanting and 9.4 ± 0.29 to 20.4 ± 0.64 cm at the completion of the experiment. During the first twenty days after transplanting, spinach from BD and IF treatments plots were not significantly different in plant height compared to plants from CO plots, which showed lower plant height throughout the planting period. This was an indication that irrespective of the sources of the nutrients, application of soil amendments increased spinach plant height.

3.2.2. Number of leaves

Figure 5 shows the effect of BD, IF and CO on the number of spinach leaves. The results indicated that there was significant effect of treatments on number of spinach leaves (d.f. = 2, P < 0.0001, LSD = 0.97). Biogas digestate showed a steady increase in number of leaves throughout the experimental period. However, 14 days after transplanting, all the treatments contained same number of leaves. During the first 21 days after transplanting, number of BD leaves were significantly different from those of IF and CO, which were not significantly different from each other. Since the experimental site was a cattle production farm, comparable number of leaves on IF and CO may have been due to cattle dung droppings, which had since decomposed and mineralised to be available for plant nutrients. Both IF and BD showed a sharp increase in number of leaves from day 28–35.

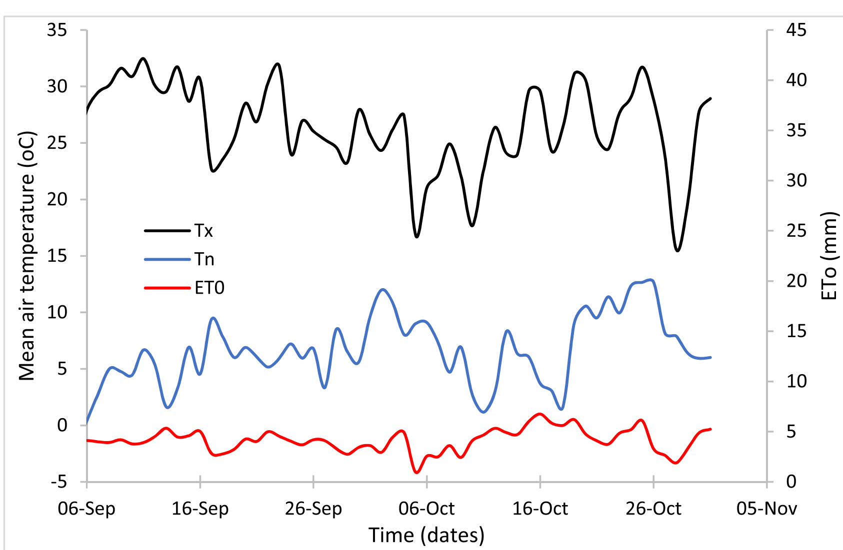

Figure 2. Average maximum (Tx), minimum (Tn) temperatures and reference evapotranspiration (ETo) experienced in Devon area during the experimental period.

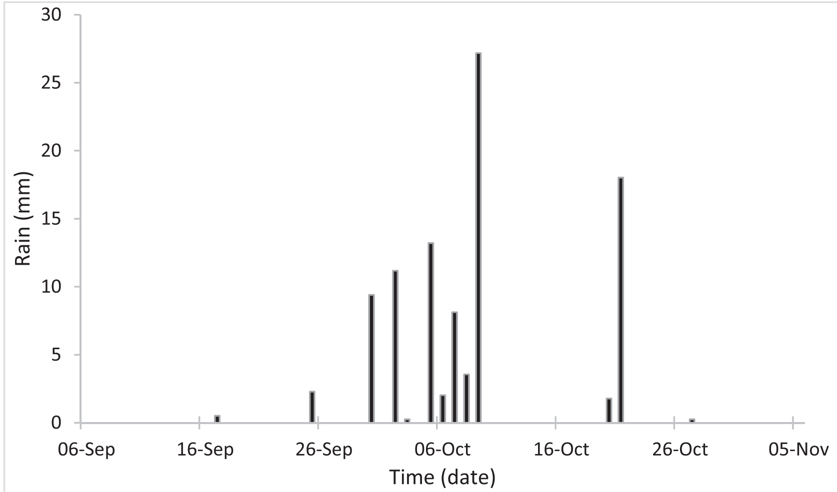

Figure 3. Rainfall experienced in Devon area during the experimental period (2017 growing season).

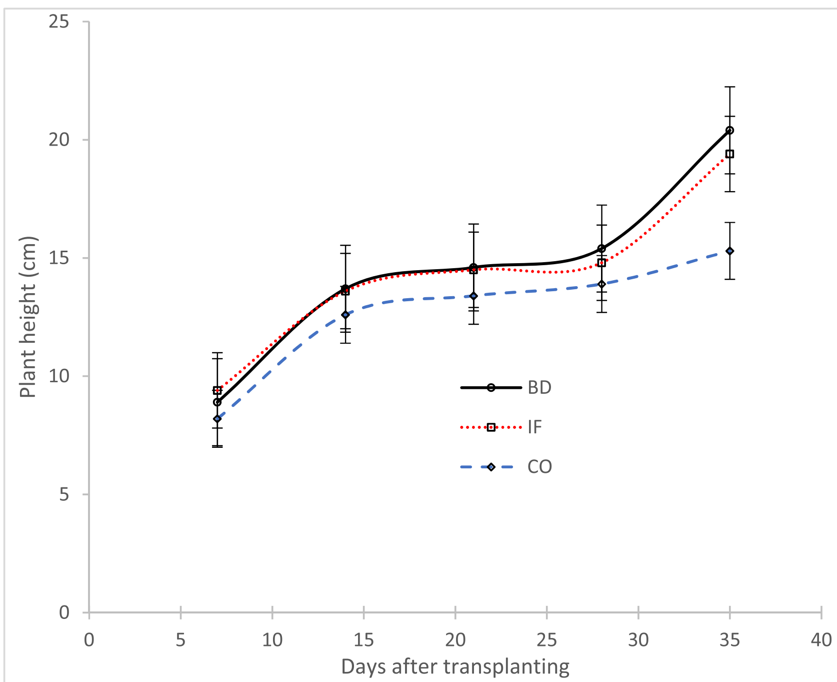

Figure 4. The effect of biogas digestate (BD), inorganic fertiliser (IF) and unfertilised control (CO) on spinach plant height (intervals represent standard errors).

3.2.3. Leaf area index (LAI) and relationship with number of leaves

The effect of BD, IF and CO on spinach LAI is illustrated in Figure 6. The LAI was significantly affected by different nutrient sources (d.f. = 2, P < 0.0001, LSD = 0.53). At 28 days after transplanting, LAI of plants from IF plots increased from 2.57 ± 0.08 to 6.11 ± 0.19. At 28 days after transplanting, LAI of BD and IF were not significantly different. Higher LAI of plants from IF treatment plots could be due to the readily available nitrogen from those plots. As presented in Figure 7, there was a significant positive linear relationship between the leaf numbers and LAI at P < 0.0001 and correlation (R^2) of 0.69. The higher the number of leaves, the larger the leaf areas. This showed that number of leaves contributed to the leaf area of spinach.

3.2.4. Biomass

An illustration of spinach dry and wet mass under BD, IF and CO treatments is given in Figure 8. Dry mass (d.f. = 2, P = 0.0002, LSD = 67.5), wet mass (d.f. = 2, P = 0.0005, LSD = 23.41) and root length (d.f. = 2, P = 0.00001, LSD = 46.98) of spinach were significantly different between the treatments. Economic yield of spinach was measured in fresh leaf and total mass. Therefore, the yield of spinach for this study was the total aboveground fresh mass at the end of 35 days after transplanting. At the end of the study, 10.7 ± 0.13 g plant^{-1} more fresh mass spinach was produced under BD than IF treatment, but there was no significant difference between the two treatments. The CO plants produced significantly the least fresh and dry mass of 201 ± 4.32 and 78 ± 2.98 g plant^{-1} respectively.

3.2.5. Root length and biomass: root length (BM/RL) ratio

The roots of the plants from the BD plots were longer (333 ± 20.14 cm) than the roots of plants from the IF (232 ± 17.23 cm) and CO (220 ± 11.05 cm) plots (Figure 8). However, root length of the plants from IF and CO were not significantly different. In Figure 8,IF showed higher biomass to root length (0.85 g/cm) compared to BD (0.61 g cm^{-1}) and CO (0.35 g cm^{-1}) treatments. The BD, however, showed some improvement from the CO.

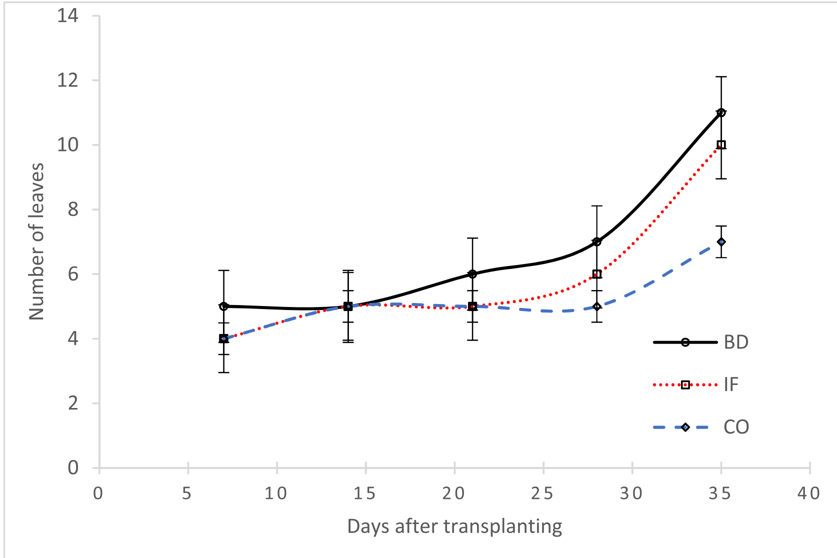

Figure 5. The effect of biogas digestate (BD), inorganic fertiliser (IF) and unfertilised control (CO) on number of spinach leaves (intervals represent standard errors).

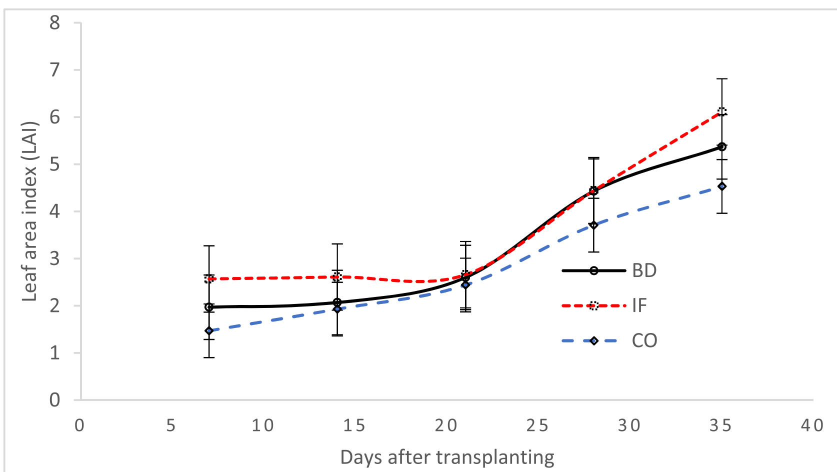

Figure 6. The effect of biogas digestate (BD), inorganic fertiliser (IF) and unfertilised control (CO) on leaf area index (LAI) of spinach (intervals represent standard errors).

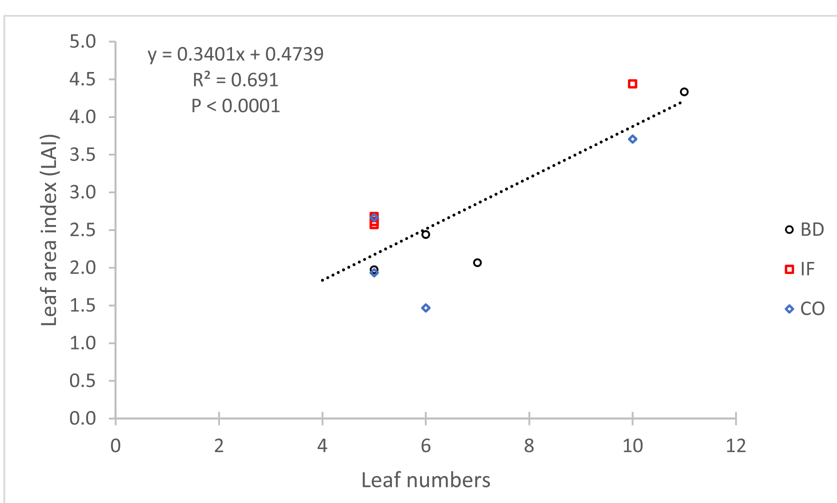

Figure 7. Relationship between leaf numbers and leaf area index of spinach as affected by biogas digestate (BD), inorganic fertiliser (IF) and unfertilised control (CO).

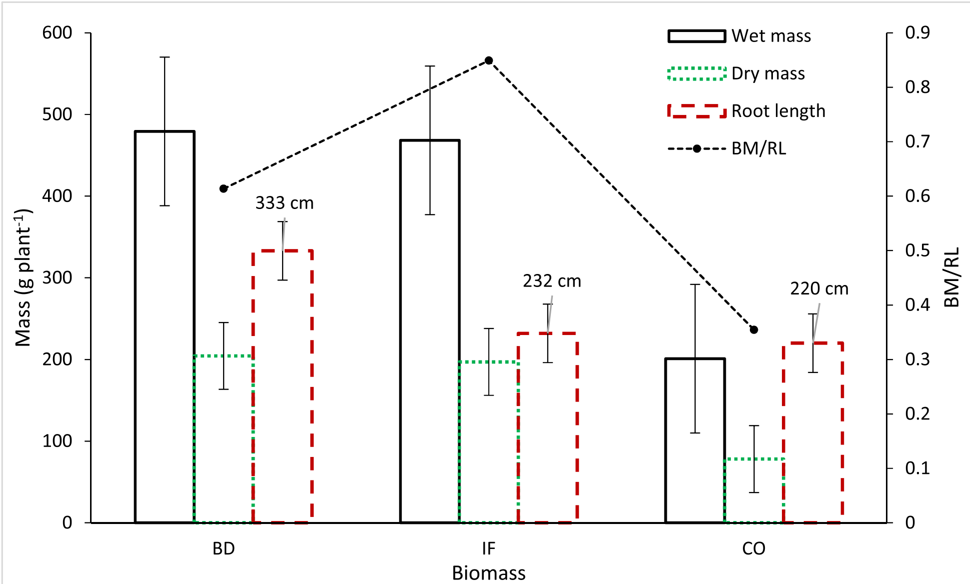

Figure 8. Biomass (wet and dry mass – g plant^{-1}), root length (cm) and biomass/root length (BM/RL – g cm^{-1}) of spinach plants under biogas digestate (BD), inorganic fertiliser (IF) and unfertilised control (CO) treatments after seven weeks (intervals represent standard errors).

3.3. Spinach nutrient uptake

3.3.1. Macronutrients

The macronutrients (N, P, K) content that accumulated in spinach leaves under BD, IF and CO are illustrated in Figure 9. The results showed that the treatments only affected K accumulation significantly, compared to N and P macronutrients (N: d.f. = 2, P = 0.104, LSD = 0.9; P: d.f. = 2, P = 0.433, LSD = 0.6; K: d.f. = 2, P = 0.0374, LSD = 1.4). The amount of N accumulated under both BD and IF treatments were not significantly different compared to CO, which accumulated less N. Although not significantly different, P accumulation in spinach leaves under CO was slightly higher compared to BD by 0.4% and IF by 0.3%. Significantly, higher amount of K accumulated in spinach leaves under IF compared to BD and CO.

3.3.2. Micronutrients

A summary of the micronutrient concentrations that accumulated in spinach leaves under BD, IF and CO is given in Table 4. No significant differences were observed in the accumulation of Mg^{2+} (d.f. = 2, P = 0.421, LSD = 8221.2), Fe^{2+} (d.f. = 2, P = 0.314, LSD = 134.9), Mn^{+} (d.f. = 2, P = 0.546, LSD = 32.3), B (d.f. = 2, P = 0.434, LSD = 10.7), Na^{+} (d.f. = 2, P = 0.122, LSD = 7862.7) and Al^{3+} (d.f. = 2, P = 0.075, LSD = 136.8) content in spinach leaves under the three different treatments. Significantly higher (d.f. = 2, $P < 0.0001$, LSD = 11.3) amounts of Zn^{2+} accumulated in spinach leaves under BD compared to IF and CO treatments. Accumulation of Cu^{2+} varied significantly (d.f. = 2, P = 0.0055, LSD = 4.3) with treatments in the order of BD (13.5 ± 1.18 mg kg^{-1}) > CO (11.9 ± 1.62 mg kg^{-1}) > IF (8.8 ± 0.4 mg kg^{-1}). Significantly higher Ca^{2+} content (d.f. = 2, $P < 0.0001$, LSD = 768.2) accumulated in spinach leaves under CO compared to BD and IF.

4. Discussion

Plant production practices that consider good environmental management contribute to sustainable food production (Ibrahim and

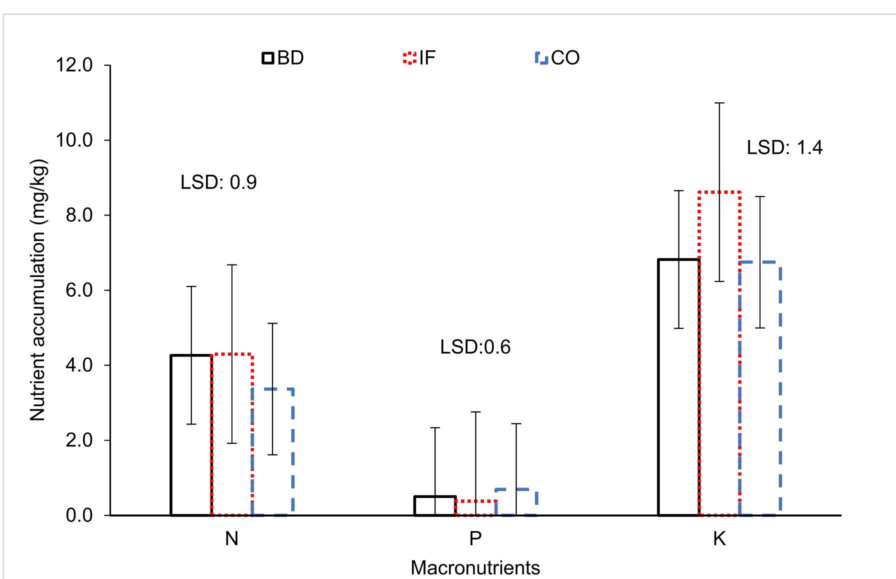

Figure 9. Macronutrients nitrogen (N), phosphorus (P), and potassium (K) accumulation in spinach leaves under biogas digestate (BD), inorganic fertiliser (IF) and unfertilised control (CO) treatments (intervals represent standard errors).

El-kader, 2015). Plant yield, height, number of leaves, biomass and the ability to accumulate plant nutrients through absorption is a measure of soil amendments' fertilisation capability. Biogas digestate is an environmentally friendly organic soil amendment that contains considerable amounts of nutrients necessary for plant growth (Głowacka et al., 2020). The current study showed plant height elongated with BD application, which concur with findings of Nasir et al. (2012) where cabbage plant height increased with cattle dung BD soil fertilisation. The CO became competitive with IF and BD in terms of number of leaves during the first growing weeks. This was attributed to plant nutrients from cow droppings and dead matter of grass, which mineralised before the experiment was conducted. According to Purbajanti et al. (2019), organic materials release plant nutrients slowly while storing them for a long time. The results were different from that of Islam et al. (2014), in which no significant difference was obtained on the number of leaves of maize crop after BD application. This indicated that the impact of organic material might not have been the same for all crops.

Leaf area is one of the spinach harvest economic aspects. The larger the leaf area, the more marketable and profitable the spinach (Pandey and Singh, 2011). Type and nature of the fertiliser influences the plant's LAI. Inorganic fertiliser contains readily available soluble plant nutrients and as result, an early increase in leaf area of IF spinach compared to BD was observed. Spinach biomass under BD fertilisation was higher by 10.7 g per plant compared to IF and CO. Similarly, Šimon et al. (2015), reported more biomass under BD compared to other soil amendments. It is common knowledge that roots serve as an anchor and are the means by which plants take up water and nutrients necessary for survival and growth. In this study, root length increased with BD soil application compared to IF and CO. Similarly, Mupambwa et al. (2019), obtained elongated root length on tomato plants fertilised with BD. Comparing root length to biomass ratio of the three treatments, IF showed higher root length to biomass ratio, followed by BD and CO. There is not enough research on the effect of BD on the ratio of root to biomass. However, Blaha (2019) states that an increase in plant root length to biomass indicates that there is more absorption of nutrients and plants are able to tolerate various stresses. The development and state of roots are important, because they act as a bridge between plant and soil (physical, chemical and biological properties). Therefore, any form of soil amendments such as organic fertilisers, which promote adequate growth and development of root systems will help crops adjust to abiotic stress such as water stress, during the plant development phases (Kul et al., 2020; Seo et al., 2020). This will promote good growth and distribution of biomass in crops.

Macro and micronutrients that accumulate in plant leaves play a role in maintaining good health and preventing disease in humans (Njeme et al., 2014). N accumulation in spinach leaves treated with BD did not differ from those treated with IF. This showed that consumption of spinach under BD did not disadvantage nitrogen benefits during human consumption. An increment of 0.4% in P accumulation under BD compared to IF benefits physiological functions during human consumption. Low potassium content in vegetables has an impact on conditions such as high blood pressure, renal and heart failure (He and MacGregor, 2018). Macronutrient (NPK) leaf accumulation results from the current study differed from Barbosa et al. (2014), where BD significantly increased the accumulation of all the macronutrients in maize plants. Biogas digestate used by Barbosa et al. (2014), contained high concentrations of K (37.5 g kg^{-1}) compared to 6.80 g kg^{-1} K contained in BD in the current study. While Ca^{2+} and Zn^{2+}accumulation increased with BD application, other micronutrients such as Mg^{2+}, Fe^{+}, Mn^{+}, B, Na^{+} and Al^{+} showed similar accumulation with inorganic fertiliser treatment. Alkalinity increases precipitation of Ca^{2+} as hydroxide (Hooda, 2010), which might have been the reason for increase in Ca^{2+} accumulation in the spinach leaves since BD used in this study was alkaline. Contrary to the present study, Cole et al. (2016) found that Zn^{2+} accumulation increased linearly with a controlled release of IF application than manure fertiliser. However, accumulation of more Zn^{2+}, as found in this study, is beneficial for human consumption. Zinc interacts with body enzymes and proteins to perform critical structural, functional and regulatory roles that boost the immune system to fight various diseases and disorders (Cakmak and Kutman, 2018).

Table 4. Micronutrients accumulation (mg kg^{-1}) in spinach leaves planted under biogas digestate (BD), inorganic fertiliser (IF) and unfertilised control (CO) treatments.

Elements	BD	IF	CO	LSD (5%)
Calcium	5876.3 ± 333.68[b]	5461.7 ± 110.67[b]	7629.3 ± 294.56[a]	768.2
Magnesium	11575.7 ± 2315.87[a]	14187.7 ± 1540.85[a]	12963.3 ± 1376.82[a]	8221.2
Iron	202.6 ± 19.75[a]	209.7 ± 45.76[a]	217.3 ± 11.86[a]	134.9
Manganese	56.1 ± 0.78[a]	96.7 ± 8.63[a]	75.4 ± 6.08[a]	32.3
Zinc	66.23 ± 2.11[a]	47.7 ± 4.49[b]	49.2 ± 4.49[b]	11.3
Boron	40.5 ± 0.7[a]	33.1 ± 2.90[a]	37.6 ± 3.62[a]	10.7
Sodium	13538.0 ± 882.76[a]	10856.3 ± 3032.12[a]	11332 ± 1938.83[a]	7862.7
Aluminium	213.3 ± 13.86[a]	219.7 ± 49.84[a]	232.3 ± 17.84[a]	136.8
Copper	13.5 ± 1.18[a]	8.8 ± 0.48[b]	11.9 ± 1.62[ab]	4.3

LSD: least significant difference.
*Means with different superscripts are significantly different.

5. Conclusion

The study showed various spinach morphological growth responses to biogas digestate (BD), inorganic fertiliser (IF) and unfertilised control (CO) treatments. An increase in biomass, number of leaves and higher plant stands in BD compared to IF show effective BD recycling in agricultural soil. Increased leaf area in IF compared to BD show low leaf area contribution on BD soil recycling. The fact that the number of leaves increased with top dressing showed the importance of nutritional supplements at vegetative stages regardless of the type of the soil amendment used. Fertilising crops with BD can be advantageous to both crop growth and environmental preservation. Biogas digestate fertilisation of leafy vegetables with similar nutritional requirements as spinach can effect a positive response in biomass and absorption of some plant nutrients. A reduction in leaf area can, however, disadvantage market value. Fertilising with BD will improve spinach as source of Zn, which is an important element in human diet. From this study, apart from sustainable production of spinach, BD will provide financial benefit if used as nutrient source for spinach production.

Declarations

Author contribution statement

Bridget Tshikalange: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Zaid A. Bello: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Olusola Ololade: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Chipa Jonas: Performed the experiments; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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