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Suitability of biosolids from university sewage ponds as a substrate for crop production

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Currently, sewage waste management is a serious environmental problem and one of the major growing concerns for urban areas all over the world. Utilization of biosolids (BS) for crop production may be a sustainable waste management strategy. The present study evaluated the physico-chemical and biological characteristics of biosolids from sewage ponds at Egerton University, Kenya. This was to determine its suitability for crop production. Biosolids were evaluated separately then as mixture with forest soil at rates of 0, 10, 20, 30, 40, 50 and 60% and compared with tea compost (TC) and coco peat (CP) in a completely randomized design experiment with four replications. Data collected included: macro-elements, micro-elements, heavy metals, pH, electrical conductivity (EC), bulk density (BD), water holding capacity and biological properties. Results showed that total organic carbon (0.03%), total organic nitrogen (2.0%) and Molybdenum (22 mg kg^{-1}), in biosolids were significantly ($p < 0.05$) higher compared with forest soil, but not significantly different from tea compost. For heavy metals, Hg (0.33 mg kg^{-1}), As (5.9 mg kg^{-1}), Cr (31.1mg kg^{-1}), Cd (0.38 mg kg^{-1}), Ni (16.3 mg kg^{-1}) and Zn (127 mg kg^{-1}) were significantly ($p < 0.05$) higher in biosolids but within the allowable limits according to Environmental Protection Agency (EPA) standards. bulk density (1.2 to 1.5) g cm-3 and pH (5.4 to 5.8) units, but high organic matter (195 to 230) g kg-1, water holding capacity (35 to 42 %) and EC (2.6 to 5.4) µSm-1). For microbial load, total viable count (TVC) and colony forming units (CFU) registered 5 ×10-7 and 6.5×10-7 respectively. However, Escherichia coli, Salmonella sp. and Staphylococcus sp. were not detectable in the fully composted biosolids. Similar trend of these results were subsequently observed in the substrates formed in the mixture of biosolids and forest soil and this provide insight on the potential of biosolids as substrate for crop production and a reliable alternative to soil alone.

Key words: Biosolids, forest soil, organic amendment, substrate.

INTRODUCTION

Application of mineral fertilizer has been the norm of maintaining soil fertility because of its uniformity and ease of application. Hence, fertilizers have reduced the use of organic nutrient sources (Shaheen and Tsadilas, 2013). This massive use of such mineral fertilizers and other inappropriate cultivation practices, including stubble burning, has greatly reduced soil organic-matter content, subsequently, influenced the physical, chemical, and

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biological properties leading to soil degradation risk. Such agronomic practices could lead to the mineralization and desertification (Tejada et al., 2001).

The generation of sewage wastewater has been increasing with rapid world population increase and urbanization. Application of treated wastewater in landfill is generally considered the most economical and beneficial way of disposing biosolids (Haynes et al., 2009). According to Al-Gheethi et al. (2018), biosolids is a sewage sludge that has been treated by advanced processes including aerobic and anaerobic, heat or lime treatment, which has met standards required for beneficial use. The organic and inorganic contents of biosolids are essential for soil and plants (Nowak, 2007). So they are nutrient-rich with organic matter content of up to 50% (Qin et al., 2012). They are also rich in nitrogen, phosphorus, and other trace elements and present a good source of nutrients for plant growth (Sukkariyah et al., 2005). Application of biosolids has been observed to improve the physico-chemical and biological properties of soils, which in turn facilitates better growth of plants (Mtshali et al., 2014). Besides acting as a food source for microorganisms, organic matter is the major binding agent for soil aggregate formation and stabilization (Tisdall and Oades, 1982). The soil structure formed, in turn, improves many other important soil physical and chemical properties such as bulk density, porosity, water holding capacity, cation exchange capacity, aeration and drainage, microbial communities and soil fauna, thus contributing to disease suppression and reduced soil erosion. However, the use of biosolids depends on a number of factors such as food habits, culture, socioeconomic and climatic conditions (Abur et al., 2014). It varies not only from city to city but also within the same city (Gakungu et al., 2012). Therefore, before using them for crop production, it is always necessary to characterize biosolids.

For crop production, biosolid waste generally contains significant concentrations of organic matter, nitrogen, phosphorus and potassium and to a lesser extent, calcium (Ca), sulphur (S) and magnesium (Mg). According to Kirchmann et al. (2016), about 10% of the total nitrogen (N) in biosolid waste is present as ammonium nitrogen, which is plant accessible, while 90% is present in organically bound forms that need to be mineralized to become plant available. Biosolids also insure against unforeseen nutrient shortages by supplying essential plant nutrients such as sulphur (S), manganese (Mn), zinc (Zn), copper (Cu), iron (Fe), molybdenum (Mo), and boron (B) that are seldom purchased by farmers because crop responses to their application are unpredictable (Sukkariyah et al., 2005). They can be applied on micronutrient deficient soils like alkaline soils (Moral et al., 2002) and sandy soil (Ozores-Hampton et al., 2011). Nutrient values of biosolids vary with sources of wastewater and wastewater treatment processes. Processes such as digestion or composting result in the loss of organic matter through decomposition increase concentrations of phosphorous and reduce trace elements (Mtshali et al., 2014). It also leads to a decrease in ammonium nitrogen by volatilization and a decrease in potassium by leaching. However, nutrient composition of biosolids is significantly altered by stabilization processes and mineralization.

Trace elements and heavy metals are of particular concern in regard to their effects on human and animal health (Qin et al., 2012). The USEPA (1995) analysed their risks to humans, animals, plants, and soil organisms from exposure to pollutants in biosolids via different pathways for land-applied biosolids. Nine trace elements: Arsenic (As), Cadmium (Cd), Copper (Cu), Lead (Pb), Mercury (Hg), Molybdenum (Mo), Nickel (Ni), Selenium (Se) and Zinc (Zn), were deemed to be of sufficient risk to regulate. Land application of biosolids must meet the ceiling concentrations and cumulative loading rates for these nine trace elements, above which, the biosolids cannot be applied in agricultural land. There are also concerns about the pathogen contaminations in biosolids (Qin et al., 2012). Biosolids applied to the land for crop production, both for human or as fodder, should not show any unacceptable microbial level or have adverse impact on human health (NRMMC, 2004).The objective of the current study was to determine the suitability of biosolids from Egerton University sewage ponds as potting substrate in terms of its physico-chemical and biological properties.

MATERIALS AND METHODS

Experimental site

The work was done in Njoro sub-county in Nakuru, Kenya from January to February 2018. The site is located on latitude 0° 23' S and longitude 35° 35' E in the Lower Highland III (LH3) agro-ecological zone at an altitude of 2238 m above sea level (Jaetzold et al., 2012). The analyses of the samples were done in Soils and Food Science laboratories of Egerton University (Figure 1).

Biosolids and forest soil samples collection

Naturally dried biosolids samples were collected from the seventh pond of the Egerton University wastewater treatment plant (Figure 2). The wastewater treatment plant is made of seven open aerated ponds. Wastewater undergoes aerobic digestion, in an oxygen-rich environment lagoon aerated naturally. Every year, the dry spell occurs in the months of December to March, during which the sixth and seventh pond normally dry up, leaving dry biosolids ready for disposal (Figure 2). The biosolid samples were collected (Plate 1) and solarized for two month sunder clear polythene paper gauge 200 mm thick. After solarization process, the biosolids were further stored in a plastic greenhouse for 10 months and then comprehensively analyzed in the laboratory.

Forest soil (FS) on the other hand was collected from an indigenous forest surrounding Egerton University botanic garden. This was an area, which has not been subjected to any farming activity for the last 20 years (Plate 2). After collection, the soil was solarized two months and then taken to the laboratory for comprehensive physico-chemical analysis along with the biosolids, tea compost (TC) and coco peat (CP).

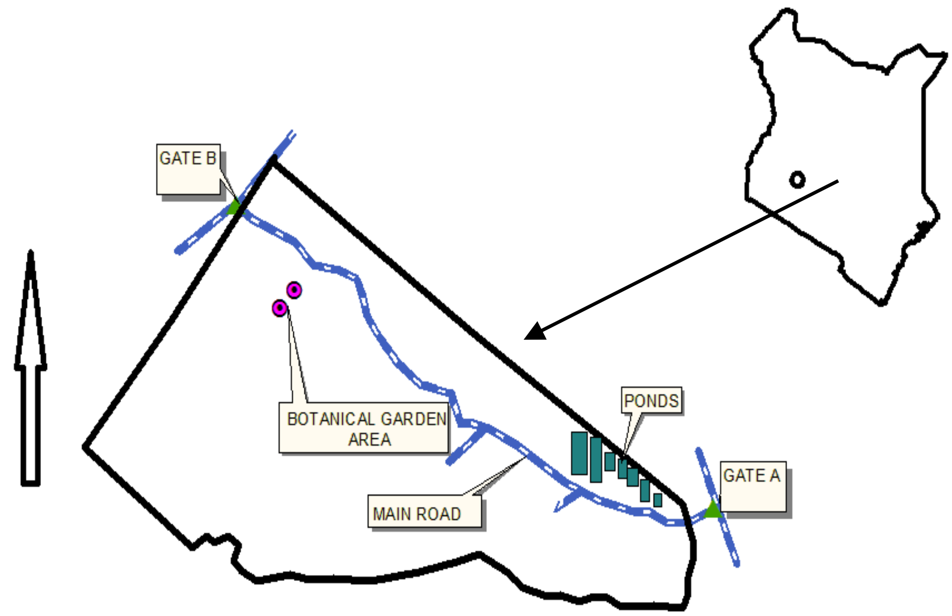

Figure 1. Map of the site of sampling forest soil and biosolids. Source: Agricultural Engineering Department, Egerton University (2019).

Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6	Pond 7
Effluent Water	Water	Water	Water	Water	Dewatered Biosolids	Dewatered Biosolids

Figure 2. The arrangement of lagoons at Egerton University wastewater treatment plant.

Preparation of biosolids as a substrate

Forest soil and biosolids were mixed at rates of 10, 20, 30, 40, 50 and 60% (v/v) and taken to the laboratory for comprehensive analysis of their physico-chemical characteristics. The physico-chemical characteristics of Tea compost (TC) and coco peat (CP) were also analyzed as reference commercial substrates. For microbial analysis, both water and dried biosolids portions were collected separately from different ponds (Figure 1) for presence or absence of the specified pathogens-*Salmonella* sp., *Escherichia coli* and *Staphylococcus* sp.

Determination of physico-chemical properties of the substrates

Electrical conductivity

Salinity was determined using conductivity meter with a conductivity bridge (Model CM-1 Mark V) for each growing medium. A ratio of 1:1 (substrate: water) suspension was prepared and filtered using a Buchner funnel with filter size 14. Each growing medium was filtered through Buchner funnel. After the filtrate was clear, it was transferred into a 50-ml bottle and the conductivity cell was immersed in the solution to take reading according to Okalebo et al. (2002).

Measuring pH of the substrates

The pH was measured using pH-meter (digital ion analyzer). Sample of 50 g of air-dried growing medium was taken into a 100-ml glass beaker; thereafter 50 ml distilled water was added using a graduated cylinder, mixed well and allowed to stand for 30 min. The suspension was stirred after every 10 min and pH determined according to the procedure described by Okalebo et al. (2002).

Organic matter content and organic carbon

One gram of air-dried growing medium was placed into a 500-ml beaker. Ten millilitres of 1 N potassium dichromate solution and 20 ml concentrated sulphuric acid was added in a beaker and swirled to mix the suspension. After 30 min, 20 ml of distilled water was added along with 10 ml concentrated orthophosphoric acid and the mixture was allowed to cool. Ten drops of diphenylamine indicator was added. The solution was titrated with 0.50 M ferrous ammonium sulphate solution and upon colour change from violet blue to

Plate 1. Harvesting of biosolids in the sixth (semi solid) and seventh (dry) lagoons**.**

Plate 2. Harvesting of forest soil

green, the reading was recorded, and organic matter content determined. Organic carbon was determined by method of Walkey and Black (1934).

Bulk density

A core ring of 5 cm diameter with known weight (W_1) and volume (V) was inserted 5 cm in the substrate to scoop the amount of substrate material of the same volume. It was then removed from the substrate and samples around the core was wiped and trimmed at the bottom and top using a knife. They were then placed in an oven at 105°C for two days after which they were allowed to cool and weighed (W_2), according to Okalebo et al. (2002).

Bulk density (g cm^{-3}) = (W_2(g) –W_1(g)/V (cm^3).

Where, W_1 was the fresh weight of the sample with the core and W_2 was the weight of the dried sample in the core and V was the volume of the core.

Water holding capacity

This is the maximum amount of water that freely drained soil can hold, estimated after saturated soil has been drained without allowing its moisture stores to be depleted by evaporation. The substrate was filled with water and free water allowed to drain off, then covered with plastic containers for 2 days. Moisture content was thereafter determined based on the initial and final weights and using the formula below:

Field capacity of substrate (%) =(W_2 –W_3)/(W_3-W_1)) x 100

Where, W_1 was the wet substrate in moisture container with known weight, W_2 was the total weight and W_3, was weight of the dry soil in moisture container (Okalebo et al., 2002).

Determination of nutrient and heavy metal elements

Total nitrogen (Kjeldahl method)

Substrate sample weighing 0.3 g was digested in digestion tubes using a digestion mixture comprising of N/140 HCl, HNO_3, Se and $CuSO_4$. The temperatures in the heating block was maintained at 360°C for two hours after which the samples were allowed to cool, transferred to 50-ml volumetric flasks and the volume made to the mark. The sample was then allowed to settle and 5 ml of the aliquot was put into the distillation bottle where 10 ml of 1% NaOH was added. It was then steam distilled into 5 ml 1% boric acid containing 4 drops of mixed indicator for 2 min, from the time the indicator turned green. Distillate was titrated using HCl and the end point was reached when the indicator turned green through grey to definite pink (Okalebo et al., 2002), modified by Juma et al., (2018). A blank experiment was prepared using the same procedure, according to the method described by Kirk (1950).

Total phosphorous

Total phosphorus in the substrate samples was determined by the method described by Juma et al. (2018). A substrate sample of 0.3 g was digested in digestion tubes using a digestion mixture comprising of HCl, HNO_3, Se and $CuSO_4$. The temperatures in the heating block were maintained at 360°C for two hours after which the samples were left to cool, transferred to 50-ml volumetric flasks and volume made to the mark. Five ml of the aliquot was transferred into the sample bottles with 1 ml of developing colour solution (Ammonium Vanadate and Ammonium Molybdate in the ratio of 1:1). The samples were left to stand for 30 min after which they were transferred to cuvettes. Readings (absorbance) were taken using a spectrophotometer at λ_{max}= 430 nm. Calibration curve was done using laboratory certified standards containing 0, 0.2, 0.4, 0.6, 0.8 1.0 and 1.2 ppm P respectively.

Potassium analysis

A substrate sample weighing 0.3 g was digested in digestion tubes using a digestion mixture comprising HCl, HNO_3, HF and $H_3 BO_3$. The temperatures in the block was maintained at 360°C for two hours, thereafter samples were cooled, transferred to 50-ml volumetric flasks and volume made to the mark. Calibration was done for each element using certified standards. Samples were analysed using Atomic Absorption Spectrophotometer (AAS), Varian spectra AA10 AAS machine.

Determination of Na, Ca, Mg, Fe, Zn, Cu, Mn, Pb, Cu and Cd

The determination of these elements in the substrate was done using double acid method of extraction. AAS was used for estimation of these available elements in the tested substrate. This followed the procedure of Okalebo et al. (2002).

Determination of biological properties of the substrates

This work was done to determine the presence or absence of faecal contaminants, specifically *Salmonella* sp., *Escherichia coli* and *Staphylococcus* sp., as microbial organisms of health concern on in dry biosolids ready for disposal.

Isolation of microbial cultures

Fifty grams of compost were added to 950 ml of normal saline and homogenized for 30 min. Ten-fold serial dilution (10^{-1} to 10^{-6}) was made from the homogenate. The homogenate was used for enumeration of bacteria of medical importance in the biosolids. For enumeration of bacteria, 1 ml of homogenate was aseptically transferred onto plate count nutrient agar (Oxoid, England) in triplicates. The plates were incubated at 37°C for 24 h under aerobic atmosphere. After incubation and isolation, the number of colonies was counted with a colony counter, recorded as colony-forming unit (CFU).g^{-1} and Total Viable Count (TVC).g^{-1} of the growing medium. The evaluation of cellular concentration in a substrate samples were determined by plate counting of serials dilutions. The presence or absence of microbial organisms of health concern such as pathogenic *Salmonella* sp., *E. coli* and *Staphylococcus* sp., was determined in each sample in triplicates.

Data analysis

Data for each variable measured were analyzed using the statistical model for completely randomized design with five treatments and four replications. Data analysis was carried out using Statistical Analysis System software statistical package version 9.1 (SAS Institute, Cary Inc., 2001). Shapiro Wilk test was used to check for normality of the data before analysis. Numerical data were subjected to analysis of variance (ANOVA) at $p \leq 0.05$ and means for significant treatments separated using Tukey's Honestly Significant Difference (HSD) test at $p \leq 0.05$.

RESULTS

The results on quantity of macro-, micro- elements and heavy metals determined in the biosolids (BS), forest soil (FS), tea compost (TC) and coco peat (CP) substrates are shown in Tables 1 and 2.

Macro- and micro-elements and heavy metals

Forest soils (FS) was used as reference and TC and CP as commercial substrates to compare the suitability and potential of BS in crop production (Table 1). In the four substrates, total organic carbon (ToC) content was detected in the range of 0.02 to mg g-1), which was not significantly ($p < 0.05$) different among them. Biosolids (BS) was significantly ($p < 0.05$) higher in molybdenum (22 mg kg^{-1}) and total organic nitrogen (2.0%), which was not significantly ($p < 0.05$) different from that of tea compost (TC). In comparison to the four substrates, FS as reference material was significantly ($p < 0.05$) higher in Fe (7.2%), Mn (0.6% mg kg^{-1}) and B (81 mg kg^{-1}).

Heavy metals

Forest soil (FS) was higher than the rest of the

Table 1. Chemical characteristics of biosolids from Egerton University waste water treatment plant.

Substrates[a]	ToN % (0.01)*	ToP % (0.007)	K % (0.07)	Mg % (0.07)	Ca % (0.1)	S %	Fe %	Mn % (0.002)	Mo (mg kg^{-1})	B (mg kg^{-1})
FS	0.26±0.02[c]	0.13±0.01[c]	0.52±0.01[b]	0.14±0.01[b]	0.56±0.01[c]	1.72±0.01[b]	7.15±0.20[a]	0.61±0.02[a]	9.35±1.30[b]	81.25±2.30[a]
BS	2.00±0.02[a]	0.25±0.01[b]	0.50±0.02[b]	0.16±0.01[b]	0.49±0.02[c]	0.64±0.01[c]	4.26±1.40[b]	0.26±0.01[c]	22.47±4.00[a]	61.73±2.80[b]
CP	1.01±0.09[b]	0.16±0.02[c]	0.83±0.02[a]	0.27±0.02[a]	1.02±0.02[b]	ND	0.68±0.01[c]	0.19±0.01[c]	4.84±0.90[c]	52.98±1.50[c]
TC	2.10±0.20[a]	0.87±0.01[a]	0.01±0.00[c]	0.25±0.02[a]	4.27±0.60[a]	55.56±5.70[a]	0.47±0.01[c]	0.44±0.01[b]	5.04±1.20[c]	88.77±7.60[a]

Means ± standard deviation followed by the same letter within a column are not significantly different according to Tukey's HSD test at $p \leq 0.05$. *Maximum recommended values of plant nutrient in the soil/ substrate for tomatoes production (Sainju et al., 2003) modified. [a] N = 4 in the substrate analysis. TC = Tea compost, CP= Coco Peat, FS =Forest soil ND= Not detected.

Table 2. Heavy metal characteristics of biosolids from Egerton University waste water treatment plant.

Substrates[a]	Pb (mg kg^{-1}) (150) *	Hg (mg kg^{-1}) (1)	As (mg kg^{-1}) (20)	Se (mg kg^{-1}) (100)	Cr (mg kg^{-1}) (100)	Cu (mg kg^{-1}) (100)	Cd (mg kg^{-1}) (1)	Ni (mg kg^{-1}) (60)	Zn (mg kg^{-1}) (200)
FS	46.15±2.73[a]	0.04±0.00[b]	5.73±0.35[a]	16.12±1.23[a]	22.52±2.75[b]	9.24±0.97[c]	0.39±0.04[a]	12.01±0.47[a]	66.41±4.21[b]
BS	21.82±1.99[b]	0.33±0.01[a]	5.85±0.55[a]	1.07±0.07[b]	31.12±2.15[a]	34.43±1.24[b]	0.38±0.01[a]	16.73±0.49[a]	127.02±4.97[a]
CP	3.27±0.03[c]	0.03±0.00[b]	0.87±0.01[b]	0.79±0.03[b]	35.63±1.25[a]	63.22±3.57[a]	0.08±0.00[b]	7.05±0.05[b]	127.05±1.56[a]
TC	3.38±0.05[c]	0.07±0.00[b]	0.78±0.01[b]	2.31±0.02[b]	36.77±2.53[a]	64.75±2.97[a]	0.06±0.00[b]	7.26±0.06[b]	28.88±2.03[c]

Means ± standard error followed by the same letter within a column are not significantly different according to Tukey's HSD test at $p \leq 0.05$.*Maximum ceiling values of heavy metals for agricultural land application according to New South Wales EPA, (2000). [a] N = 4 in the substrate analysis. [a] N = 4 in the substrate analysis. Key: TC = Tea compost, CP= Coco Peat, FS =Forest soil, BS= solarized for 2 months and stored for 10 months.

substrates in Pb (45 mg kg^{-1}), As (5.7 mg kg^{-1}), Se (16 mg kg^{-1}), Cd (0.4 mg kg^{-1}) and Ni (12 mg kg^{-1}) which was nevertheless not significantly ($p < 0.05$) different from that of biosolids (16.3 mg kg^{-1}). Biosolids registered significantly ($p < 0.05$) higher Hg (0.33mg kg^{-1}), As (5.9mg kg^{-1}), Cr (31.1mg kg^{-1}), than the other substrates. On the other hand TC and CP recorded significantly ($p < 0.05$) higher contents of Cr (36mg kg-1) and Cu (64mg kg-1) respectively (Table 2).

Substrate analysis

Forest soil was mixed with BS at different rates (V/V) and compared with FS, TC and CP as controls, to get the best BS combination with significantly higher macro- and micro-nutrient composition (Tables 3 and 4). Forest soil was significantly higher in Mg (130 mg kg^{-1}) and C:N ratio of 21. Biosolids at 30% was significantly higher in most of the elements analyzed: ToN (13 mg g^{-1}), ToP (101 mg kg^{-1}), K (428 mg kg^{-1}), Mg (119 mg kg^{-1}) and ToC (114 mg g^{-1}). Both BS at 10 and 20% were higher in K, Mg and C, but not ToN and ToP. Biosolids at 40% was significantly higher in K (422 mg kg^{-1}), ToC (122 mg g^{-1}). On the other hand BS at 50 and 60% were significantly higher in Na 350 mg kg^{-1} and 376 mg kg^{-1}, respectively. In comparison, TC (commercial substrate) was significantly higher in ToN (16 mg g^{-1}) and ToP (116 mg kg^{-1}) but was not significantly different from BS at 30%. Both commercial substrates TC and CP were significantly ($p < 0.05$) higher in Ca (44 mg kg^{-1}) and (39 mg kg^{-1}) and Mg (127 mg kg^{-1}) and (115 mg kg^{-1}). Mg content in both TC and CP was not significantly different from those of FS and BS at 10 to 30%. Total organic carbon was significantly higher in TC but not different from that of BS at 30%. Potassium and Mg were significantly higher in BS at 10 to 30%. Mg content significantly reduced in BS at 40 to 60%, as Na content became significantly ($p < 0.05$) higher in the substrate. Manganese level was significantly ($p <$

Table 3. Macro-element characteristics of the substrate's mixtures (BS: FS).

Substrates (v/v)	ToN (mg g^{-1})	ToP (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	C (mg g^{-1})	C:N
Recommended ^{x}	**0.1^x**	**70^x**	**700^x**	**1000^x**	**700^x**	**-**	**-**	**-**
FS	4.3±0.8^e	69.1±8.8^e	132.5±3.3^e	21.9±1.5^b	131.1±9.7^a	62.9±5.6^f	91.7±9.4^c	21.3±1.6^a
BS 10%	5.9±0.9^{de}	83.0±9.7^{bcde}	412.3±1.9^{ab}	24.0±2.9^b	126.1±3.9^{ab}	254.8±9.1^c	115.0±3.6^{ab}	19.7±2.7^{ab}
BS 20%	7.4±0.3^{cde}	90.3±6.9^{bcd}	419.9±14.0^{ab}	22.8±1.8^b	117.7±7.7^{ab}	242.1±16.1^c	114.4±5.5^{ab}	15.4±1.2^{bc}
BS 30%	12.9±1.4^{ab}	101.0±2.8^{ab}	427.8±6.2^a	29.5±1.7^b	119.1±3.5^{ab}	252.8±8.2^c	122.1±6.4^a	9.6±1.4^{dc}
BS 40%	9.6±0.6^{bcd}	95.9±1.7^{abc}	422.4±5.7^{ab}	27.0±5.7^b	113.8±7.3^b	343.3±13.2^b	122.0±3.5^a	12.7±0.6^{cde}
BS 50%	8.9±1.5^{bcd}	79.3±6.8^{cde}	403.7±7.8^b	28.5±1.6^b	47.7±3.0^c	349.8±7.7^{ab}	127.9±5.6^a	14.7±2.^{8bcd}
(BS) 60%	10.5±1.8^{bc}	70.3±4.4^{de}	403.5±15.5^b	27.5±2.0^b	37.2±2.8^c	376.3±12.1^a	129.6±5.7^a	12.5±1.7^{cde}
Tea C (TC)	16.3±3.0^a	116.1±11.5^a	369.6±7.3^c	43.5±5.0^a	126.6±5.8^{ab}	114.8±3.4^e	120.5±7.9^a	7.6±1.4^e
C P	9.2±0.9^{bcd}	33.8±2.0^f	344.1±2.5^d	38.5±5.9^a	114.6±7.9^{ab}	164.4±7.0^d	99.6±5.8^{bc}	10.8±1.1^{cde}

Means ± standard error in a column followed by letter are not significantly different according to Tukey's HSD test ($p<0.05$).^{X} Recommended rates for plant nutrient content in tomato.
Source: Sainju et al. (2003).

Table 4. Micro-element characteristics of the substrates.

Substrates (v/v)	Mn (mg kg^{-1}) (20)^y	Fe(mg kg^{-1})	Zn(mg kg^{-1})
Forest soil (control)	69.6±8.0^c	27.0±1.9^f	4.7±0.3^d
Biosolid 10%	530.4±13.9^a	2490.0±29.3^a	47.4±1.2^a
Biosolid 20%	524.8±7.4^a	2473.9±8.8^a	44.0±2.3^a
Biosolid 30%	539.4±31.9^a	2479.1±18.6^a	44.0±1.1^a
Biosolid 40%	553.9±24.7^a	2471.5±34.6^a	45.9±0.9^a
Biosolid 50%	551.9±11.3^a	1184.1±4.9^b	24.4±2.1^b
Biosolid 60%	544.8±18.3^a	852.5±6.5^c	25.4±0.9^b
Tea compost	167.0±18.5^b	207.4±6.7^d	21.9±2.6^b
Coco peat	29.8±1.2^c	114.1±9.9^e	16.4±2.2^c

Means ± standard error in a column followed by letter are not significantly different according to Tukey's HSD test ($p<0.05$). ^{y} Recommended value of nutrient in substrate for tomato production.
Source: Sainju et al. (2003).

Zn content were significantly ($p < 0.05$) higher in BS from 10 to 40% (Table 4).

Heavy metals

Copper (Cu) content varied from 4.4 to 14 mg kg^{-1} and was significantly ($p < 0.05$) higher in all the substrates except FS and CP (Table 5). Likewise, Cd content varied from 0.0023 to 0.0128 mg kg^{-1} and was significantly ($p < 0.05$) higher in all the substrates except FS. Lead (Pb) content varied from 2.1 to 109.6 mg kg^{-1} but was significantly ($p < 0.05$) higher in FS and lower in the rest of the substrates.

Physico-chemical properties of the substrates

Physical properties of the substrates showed

Table 5. Heavy metal characteristics of the substrates**.**

Substrates (v/v)	Cu (mg kg^{-1}) (100)^z	Cd (mg kg^{-1}) (1)	Pb (mg kg^{-1}) (150)
Forest soil (control)	4.4±0.5^b	0.0023±0.1^b	109.6±9.0^a
Biosolid 10%	12.2±0.6^a	0.0128±0.8^a	2.8±0.9^c
Biosolid 20%	12.7±1.0^a	0.0115±0.4^a	2.1±0.4^c
Biosolid 30%	10.3±0.9^a	0.0127±0.6^a	5.1±0.4^c
Biosolid 40%	12.7±0.9^a	0.0122±0.7^a	3.1±0.5^c
Biosolid 50%	13.1±0.9^a	0.0122±0.4^a	6.0±0.8^c
Biosolid 60%	13.3±0.9^a	0.0122±0.7^a	2.5±0.5^c
Tea compost (Control)	14.0±0.2^a	0.0122±0.7^a	20.1±4.1^b
Coco peat (Control)	6.5±0.3^b	0.0121±1.6^a	4.3±1.0^c

Means ± standard error in a column followed by the same letter are not significantly different according to Tukey's HSD test *($p<0.05$).* *Maximum ceiling values of heavy metals for agricultural land application.
Source: NSW EPA (2000).

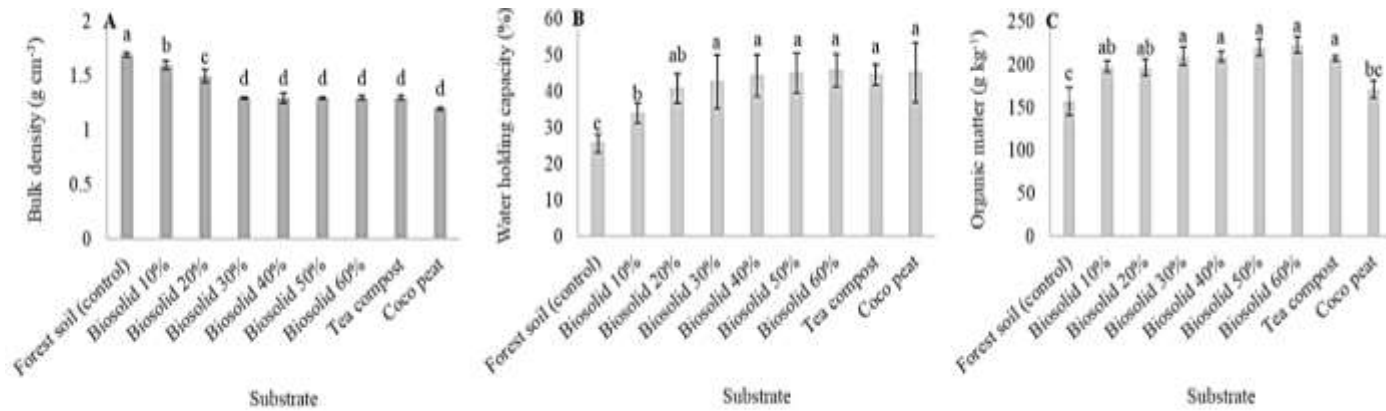

Figure 3. A- Bulk density, B - Water holding capacity and C- Organic matter characteristics of different growing media. Means ± standard deviation followed by the same letter are not significantly different according to Tukey's HSD test *($p<0.05$).*

significant differences on bulk density (BD), water holding capacity and organic matter contents (Figure 3). Bulk density varied from 1.2 to 1.7 g cm^{-3} and was significantly ($p < 0.05$) higher in FS, followed by BS at 10% and lower in the rest of the substrate. Water holding capacity varied from 26 to 46% but was significantly ($p < 0.05$) higher in the all substrates except forest FS and BS at 10%. Organic matter content was higher in the all the media except FC and CP. Electrical conductivity varied from 2.6 to 5.4 µSm^{-1} but registered higher in BS 40 to 60%. Forest soil registered a higher pH but was not significantly ($p < 0.05$) different from the two commercial substrates TV and CP. However, BS recorded significantly ($p < 0.05$) lower pH than the other substrates with a reducing trend from BS at 20 to 60% (Figure 4).

Biological properties of biosolids

Selected bacteria of economic importance: Salmonella sp., Escherichia coli and Staphylococcus were observed in the substrates (Table 6). Total viable counts (TVC) showed a rising trend from BS at 10 to 20% followed by a downward trend up to BS at 100%. The presence of microbes was evident in the substrates at different dilution levels. Forest soil recorded a TVC of 42×10-4; tea compost had moderate growth while coco peat had no growth (NG). For the colony forming units (CFU), FS and BS at 10 to 40% had too numerous to Count (TNTC); however above BS 40%, it was possible to numerate them at dilution level 10^{-7}. Regardless of the rates of BS tested, the targeted microbes *Salmonella* sp*.*, *E. coli* and *Staphylococcus* were absent on the dry BS substrates tested. Further confirmation test done on the pond for the presence or absence of targeted microbes of the seven ponds indicated the presence of *Salmonella* sp*.* and *Staphylococcus* sp*.,* at various stages except in pond 6 and 7, which had dry BS sample (Table 7). However, *E. coli* persisted on pond 6 and 7.

DISCUSSION

The utilization of biosolids in agriculture has gained popularity as a source of waste disposal. Analysis of biosolids alone for the macro- and micro-elements in

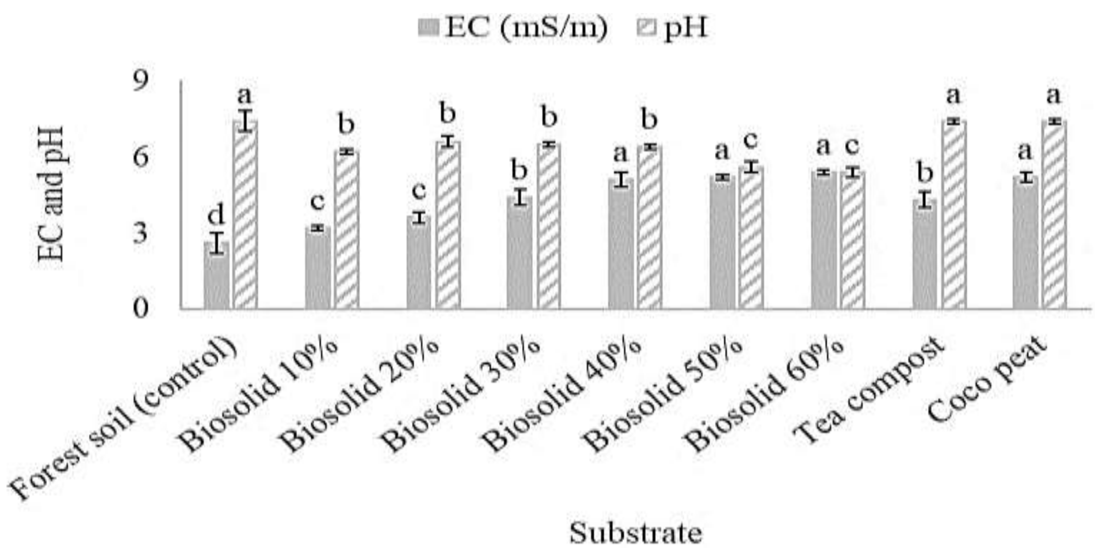

Figure 4. Electrical conductivity and pH on different type of substrates. Means ± standard deviation followed by the same letter are not significantly different according to Tukey's HSD test *($p<0.05$).*

Table 6. Numeration of *Salmonella* sp., *Escherichia coli* and *Staphylococcus* sp.in the substrates.

Substrates	TVC (PCA)	CFU (MAC)	*E. coli* (EMB)	*Salmonella sp.* (BPA)	*Staphylococcus sp.* (BPA)
Forest soil (FS)	42×10^{-4}	TNTC	-ve	-ve	-ve
Biosolids 10%	50×10^{-7}	TNTC	-ve	-ve	-ve
Biosolids 20%	96×10^{-7}	TNTC	-ve	-ve	-ve
Biosolids 30%	72×10^{-7}	TNTC	-ve	-ve	-ve
Biosolids 40%	52×10^{-7}	TNTC	-ve	-ve	-ve
Biosolids 50%	22×10^{-7}	13×10^{-7}	-ve	-ve	-ve
Biosolids 60%	11×10^{-7}	6.5×10^{-7}	-ve	-ve	-ve
Biosolids 100%	5×10^{-7}	6.5×10^{-7}	-ve	-ve	-ve
Tea compost	58×10^{-7}	6.5×10^{-7}	-ve	-ve	-ve
Coco peat	N/G	N/G	-ve	-ve	-ve

TVC- Total Viable Count, CFU- Colony-Forming Units, PCA- Plate Count Agar, EMB- Eosin Methylene Blue (*E. coli*), MacConkey Agar, BPA- Bairvd Parker Agar (*Staphylococcus sp.*), BPA- Bairvd Parker Agar (*Salmonella sp.*). TNTC- Too Numerous to Count, VFC- Very Few Colonies, N/G- No Growth, -ve= absent, +ve = Present, ++ve =highly present, +++ve - Very highly present.

Table 7. Numeration of bacterial total viable counts (TVC) and colony forming units (CFU) in Egerton University ponds.

Pond type	TVC (PCA)	CFU (MAC)	*E. coli (EMB)*	*Salmonella* sp.	*Staphylococcus* sp.
POND 1	TNTC	TNTC	+++ve	++++ve	+++ve
POND 2	TNTC	TNTC	+++ve	+++ve	+++ve
POND 3	TNTC	TNTC	+++ve	+++ve	+++ve
POND 4	TNTC	TNTC	+++ve	+++ve	+++ve
POND 5	TNTC	TNTC	+++ve	+++ve	+ve
POND 6	TNTC	TNTC	++ve	.ve	-ve
POND 7	TNTC	VFC	+ve	-ve	-ve

POND 1- Effluent, POND 2 -Second pond (water), POND 3-Third pond (water), POND 4- Fourth pond (water), POND 5- Fifth pond (water), POND 6 -Sixth pond (water), POND 7-Seventh pond (dewatered biosolid). TVC- Total Viable Count, CFU- Colony Forming Units, PCA- Plate Count Agar, EMB- Eosin Methylene Blue (*E. coli*), MacConkey Agar (MAC), BPA- Bairvd Parker Agar (*Staphylococcus sp.*), BPA- Bairvd Parker Agar (*Salmonella sp.*). TNTC- Too Numerous to Count, VFC- Very Few Colonies, N/G- No Growth, -ve= absent, +ve = Present, ++ve =highly present, +++ ve – Very highly present.

comparison to other substrates revealed the significant presence of organic nitrogen (N), molybdenum (Mo) and to lesser extent, phosphorus (P), potassium (K) calcium (Ca), sulphur (S), magnesium (Mg), iron (Fe), manganese (Mn) and boron (B). On the other hand, forest soil (FS) as a natural substrate significantly had higher in Fe, Mn and B. This study suggests that dewatered biosolids (BS) would be a better source for slow release nitrogen and molybdenum. However, if BS is combined with FS their ability as substrate would possibly be enhanced to provide five elements fore-mention. Total organic carbon was significantly higher and has a big role in plants, fostering healthier and more productive growth of the plants through photosynthesis process. Amending soil with organic carbon not only facilitates healthier plant life, but also helps the substrate to drain well with enhanced bulk density, prevents water pollution and besides, it is beneficial to useful microbes, insects and eliminates the need for using synthetic fertilizers. Additionally, availability of C and N in organic form, in BS has benefits to crop since the plant nutrients are released over entire period of crop production. The organic nitrogen present in BS is an essential macro nutrient for the synthesis of amino acids, the building blocks of proteins, and also a major part of the chlorophyll molecule necessary for photosynthesis. Even though BS did not show significance in ToP, K and Mn compared to other substrates tested, they were within maximum recommended values of plant nutrient in the substrate according to Sainju et al. (2003). Among the macro-elements of importance in plant nutrition, K however, was critically lower in the BS. This was probably leached out in the process of BS formation since it is in ionic form and therefore deficient. Pakhnenkoa et al. (2009) earlier reported the same on the availability of K in biosolids used in agriculture. These results are in agreement with report by Kirchmann et al. (2016) who also reported on organic form of N and P with only 10% of each that may be available. In another study, Paz-Ferreiro et al. (2018) reported similar findings, that N availability is partially controlled by type of treatment process sludge undergo and that agricultural value of biosolids will also depend on the mineralization rate of the organic N pool. In terms of its functions in plant, significant level of molybdenum (Mo) in BS observed in this study is indicative of the potential of the substrate in providing this trace element for crop development. Molybdenum is a necessary component of two major enzymes in plants, nitrate reductase and nitrogenase, which are required for normal assimilation of N in plant function (Silva and Uchida, 2000). The result also showed forest soil (FS) as a better source of Iron, Manganese and Boron. These are important trace elements essential in plant nutrition. When compared to maximum recommended values of plant nutrient in the soil or substrate suggested by Sainju et al. (2003), these elements are found to be sufficient for crop productivity in the three substrates tested: FS, BS and TC (Commercial substrate). Considering the significant presence of trace elements, Fe, Mn and B in FS indicated the potential of the substrate in combination with BS. This combination would possibly contain Fe, Mn, B and Mo which are equally essential in plant functions and crop production. Even though these elements are very important in plant physiology and development, they may not be found at instant in synthetic fertilizer (Zamann et al., 2002), unless supplemented by other means to crops. Boron was significantly available in FS. Its role in the synthesis of one of the bases for RNA formation particularly in cellular activities has been reported by Silva and Uchida (2000).

Combination of BS with FS to form substrates at different rates in the range of 0 to 60% revealed a new trend in plant nutrient availability, showing complex variations of important plant nutrients in the substrates. Significantly higher Mg observed in FS in a reducing trend to BS 60% was an indication of FS being the source and the donor in the substrate complex mixes. This study shows significant presence of Mg in FS and BS rates from 10 to 30% of the substrate. The reducing trend of Mg in the substrate mixes with the declining of the pH of the substrate observed indicated that the element is pH dependent. Studies have shown that Mg as reported by Sainju et al. (2003). This leaves a deficit of 580 mg kg^{-1} to be supplemented or the liming of the substrate to pH can make the element available. Magnesium tends to become unavailable as pH decreases. Calcium followed similar trend reducing in the substrate with increasing BS rates and this may allude to the pH as a factor in the substrate. Similarly, increase of EC with Na in the substrate probably was also a factor influencing the presence of macro element like Mg. Magnesium is normally available within a pH range of 6.0 to 8.5. As the rate of BS increased from 40% and above, the pH of the substrate was reducing and this was a limiting factor that probably caused significant reduction of Mg and Ca. The results of this study is consistent with Sullivan et al. (2015) who reported that depending on the process of producing BS, the pH may be acidic to alkaline, and therefore adjusting the pH by liming the substrate would be necessary to get the right substrate with biosolids. This is also in agreement with Ingram et al. (2016) on the effect of soil pH and nutrient availability. In this work, decrease in Mg with increased EC observed is associated with higher Na and subsequently higher salinity in BS at 50 and 60%. This is in line with observation made by Mtshali et al. (2014) that high Na concentration is associated with elevated EC of soils amended with sludge, with or without lime. The deficiency of K^+ which may necessitate the supplement of the elements in the substrate to support plant growth has also been reported by Paz-Ferreiro et al. (2018).

The C:N ratio is normally used as a growing media index to represent its stability, as it has been known that a C:N ratio of 15 permits plants N uptake without leaching as nitrate, and any C:N ratios above 15 represent values within which nitrogen is immobilized according to Dresboll and Magid (2006). The results indicates that FS without BS had significantly higher C:N ration of above 15 at rates of BS 10 and 20% and this was a sign of better chemical property of the substrate for crop production. From BS 30% and below, the C:N ration was within require range for nutrients availability. The Influence of BS in the substrate reduced the C:N ratio, which was a positive effect on nutritional quality. This concurred with Rawat et al. (2013) observation on BS waste as a source of high mineral elements, important in crop production.

The significance in BS combination with FS (BS 10 to 60%) observed was indicative of a better substrate that provides the three Mn, Fe and Zn. Plants use the element in Mn^{2+} or Mn^{3+} form and primarily functions as part of the plant enzyme system, activating several metabolic functions (Silva and Uchida, 2000). Like Manganese trace element, Fe is essential in the heme enzyme system in plant metabolism (photosynthesis and respiration). Iron is also part of protein ferredoxin and is required in nitrate and sulfate reductions, essential in the synthesis and maintenance of chlorophyll in plants and strongly associated with protein metabolism. All these physiological function may contribute to the crop growth and development, hence higher yield. The results on substrate analysis also revealed metals (Zn, Cu, Mo, and Ni), which are essential for plant growth and crop development available in both FS and BS combined, especially at BS 30%. On the elements essential for plant growth, Copper was significantly high in BS 30% and TC (commercial substrate), indicating the potential of substrate BS in plant growth at the same rate. In particular, Cu is essential in several plant enzyme systems involved in photosynthesis and is part of the chloroplast protein plastocyanin, which forms part of the electron transport chain and also plays a role in the synthesis stability of chlorophyll and other plant pigments (Sainju et al., 2003).

Heavy metal concentration indicated that, even though Cu and Cd were high in BS waste, they were found to be below the permissible limits of Standards (NSW EPA, (2000). Thus, the BS in this study at rate of BS 30% and below were observed to be suitable for use in agricultural purposes. This is in line with Miezah et al. (2015) who reported that availability of heavy metals is less in a more compost form of sludge as it has more humic acid, thus binds more metals and decreases their availability. Similarly, this work is in agreement with recommendation by Naveen et al. (2017) on quality of good biosolids; fully decomposed, and low in heavy metal and salt contents. The present study revealed the concentration of heavy metals below EPA 2000 standards. Further, these results are also in agreement with the work of Mohammad et al. (2011), where heavy metal concentration did not exceed the permissible level, on wastewater (which forms biosolids) for tomato production. However, the concentration of heavy metals in BS normally depends on the source and processes involved on production as reported by Paz-Ferreiro et al. (2018), and since BS are produced from different source, they are unique and comprehensive test is necessary before use in crop production systems.

Trend in EC level with increase in BS rates was observed and this was an indicator of higher salt concentration in the substrate, where Na was higher in BS 40 to 60%. This also confirms a report by Mtshali et al. (2014) that BS normally have higher Na concentration. EC is an important parameter to determine the substrate quality as high salt concentration can inhibit the plant growth (Fathi et al., 2014). Higher EC would result in reverse osmosis which would be detrimental to crop physiology and development leading to salt stress. On pH, a study has shown that sewage sludge may vary between slightly acidic to neutral and alkaline ranges depending on the degree of treatment and application of sludge conditioners (Yilmaz, and Temizgul, 2012). Moreno et al. (1997) earlier reported, confirms our result that application of BS may reduce the pH of soils due to humic acid release and may also increase the EC of soils or substrate. In line with our study, Youssef and Eissa (2017) reported that soil pH was decreased due to the production of organic acids during the mineralization of organic manure. However, acidic soils (Forest) have been observed to increase in pH following sludge amendment as earlier reported by Parkpain et al. (2000) and Wong et al. (2001). In addition, pH of soils or substrate may increase due to the exchangeable Ca and other cations present in sewage sludge such as Na (Tsadilas et al., 1995).

The physical properties of the substrate are the most important parameters related to plant performance. In this work, combination of FS and BS at 20 to 60% played a vital part in the structure adjustment, decrease in the BD, increased water holding capacity and soil organic matter. Bulk density (BD) was enhanced by FS as indicated in these results, while water holding capacity and organic matter contents were influenced by BS presence at any level, indicating that the essence of organic matter in the BS. Reduction observed in BD did not only make the substrate lighter, but also creates large pores that play an important role in roots growth, gas and water to penetrate into the substrate, and in any growing media, aeration is positively related to air-filled porosity (Wallah, 2007). As have been reported by other authors in various studies, BS has higher level organic matter of which 60 - 90% are biodegradable (Mami and Peyvast, 2010). This study has demonstrated that water holding capacity is an important parameter in a substrate because it dictates how frequent crops can be irrigated in a given period. Similarly, the increasing trend of water holding capacity of the substrate with increased organic matter indicates the important property of organic matter in water retention for plant use in favour of biosolids application. In line with this study, Pascual et al. (2018) reported that easily available water in a substrate should range from 20 to 30%. These results were found to be with the ideal water holding capacity of 40–65% that corresponds with water retention of 25–30% (Abad et al., 2001). These results also concur with Naveen et al. (2017) that BS increases the buffering capacity of soil and also improve water holding capacity of soil.

On the biological properties of the substrate, main focus was on the presence and or absence of selected bacteria of economic importance; *Salmonella* sp., *E. coli* and *Staphylococcus* sp. The presence of microbes in the soil or substrate depends on the physic-chemical condition of the substrates. These results confirm that pH may dictate the presence and absence of microbes. There are microbes that may survive in acidic soils while others are neutral. The other factor that determines the population, survival and mobilization would be moisture and EC of the media. In this study, these factors are proposed to be determinants of the presence and absence of *Salmonella* sp., *E. coli* and *Staphylococcus* sp. The results of this work can be explained from the soluble salt concentration in the BS substrates rates. At BS 50% and above, the EC was at its highest in the substrate, which was also reflected by higher Na. This was an indication of increased osmotic pressure (salt stress), that would possibly make the substrate draw water out of the cells of microorganism, hence death in the media or reduced chance of survival. This observation is in agreement with Andronov et al. (2012), that salt stress can reduce microbial activities, biomass and community structures in soil or substrate as in this case. Results are also in agreement with the observation of Yan et al. (2015) on the reduction of soil microbes with the influence of soluble salts in the substrate. Overall, making a better substrate for crop production requires balancing of the biosolids amount applied in soil mixes.

On enumeration of specific bacteria, *E. coli* was present and persisted even in the dewatered samples in 6^{th} and 7^{th} pond. This observation was in agreement with Arthurson (2008) findings on persistence of *E. coli*, which was the most resistant pathogen in whole process of sludge treatment. The reduction of *Salmonella* sp., *E. coli* and *Staphylococcus* sp. may indicate that in the process of solarisation, storage and further decomposition was a possible elimination process for the targeted pathogens including the stubborn *E. coli* which persisted in the 7^{th} pond. Our general observation in this study was also in line with an earlier report by De´portes et al. (1998), that there was a decrease in faecal contamination indicators and disappearance of faecal pathogens when BS were stored before use over a period of one year.

Conclusion

The present study evaluated the physico-chemical and biological characteristics of biosolids to determine its suitability for crop production as a substrate. The results indicated that application of biosolids increases organic matter and may possibly reduce mineral fertilizers with addition of elements such as potassium, magnesium and calcium. However, addition of biosolids beyond 30% may increase soil EC and decrease pH. Biosolids contain efficient amount of organic matter, ToN, which can improve soil physico-chemical and biological properties of the substrate for crop production, while heavy metals were within the allowable range. This study recommends use of BS as a substrate mixture with soil (forest soil), to the limit of 30% (v/v) and below for crop production. Liming may be a good option for adjusting the pH of biosolids substrate. It is thus recommended that more studies be conducted on various test crops as potted substrate and comprehensive analysis be carried out on plant response as well as biosafety of these agricultural food products.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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