Sewage sludge from Taxco de Alarcón wastewater treatment plant as substrate to cultivate *Panicum maximum*

Erik Yoel Carreto-Morales[1,a], Jazmin Alaide López-Díaz[1,a], Mariana Martínez-Castrejón[2], Oscar Talavera-Mendoza[1], Roberto Carlos Almazán-Núñez[3], and Giovanni Hernández-Flores[4,*]

[1] Escuela Superior de Ciencias de la Tierra, Universidad Autónoma de Guerrero, Ex hacienda San Juan Bautista s/n,
Taxco el Viejo, Guerrero, C.P. 40323, Mexico.
[2] Centro de Ciencias de Desarrollo Regional, Universidad Autónoma de Guerrero, Privada de Laurel No. 13,
Col. El Roble, Acapulco, Guerrero. C.P. 39640, Mexico.
[3] Laboratorio Integral de Fauna Silvestre, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero,
Av. Lázaro Cárdenas s/n, Chilpancingo, Guerrero, C.P. 39000, Mexico.
[4] CONACYT - Escuela Superior de Ciencias de la Tierra, Universidad Autónoma de Guerrero,
Ex hacienda San Juan Bautista s/n, Taxco el Viejo, Guerrero, C.P. 40323, Mexico.
[a] Facultad de Ecología Marina, Universidad Autónoma de Guerrero,
Av. Gran Vía Tropical No. 20 Fracc. Las Playas, Acapulco, Guerrero, C.P. 39390, Mexico.
** ghernandez@conacyt.mx; ghernandez@uagro.mx*

ABSTRACT

The management and disposal of the sewage sludge (*SS*) generated by a wastewater treatment plant (*WWTP*) as part of the municipal wastewater (*MWW*) treatment process is one of the main socio-environmental issues faced by this type of system. Taxco de Alarcón, Guerrero, in southern Mexico has had a *WWTP* operating since 2016, and the *SS* disposal is a task that must be addressed by the *WWTP*. Thus, the aim of this work was to evaluate the growth capacity of *Panicum maximum*, also known as mombaza grass (*MG*), by using *SS* generated within the "Taxco de Alarcón wastewater treatment plant" as substrate. To do so, 4 g of *MG* seeds were scattered over 5 kg (dry basis) of *SS*. As a control, a commercial compost soil was used, hereafter called pattern soil (*PS*). The experiment was carried out in triplicates for three months and drinking water (water used for human consumption) was used for crop irrigation. Each month a *MG* harvest was carried out. The response variables analyzed for *MG* were germination time (one month after plant emergence), height (H_{MG}), growth rate Gr_{MG}, and yield (Y_{MG}), whereas in the *SS* and *PS* the content of organic matter was analyzed. Furthermore, the chemical composition was analyzed using scanning electron microscopy and X-ray energy dispersion spectroscopy (*SEM-EDS*) on the *MG*, *SS*, and *PS*. The results showed that *MG* germinated faster on *PS* (5 days) than germination on *SS* (7 days). However, the *MG* grown on *SS* reached a considerably higher height (45 cm) compared to the height reached on *PS* (17 cm). Furthermore, the maximum Gr_{MG} over *SS* was also higher than the maximum Gr_{MG} observed on the *PS*, 3.64 and 1.40 cm·day^{-1}, respectively. In terms of Y_{MG}, it was observed that on *SS* it reached an average monthly Y_{MG} of 416 g·m^{-2}, whereas in *PS* it reached a Y_{MG} of 72 g·m^{-2}. The chemical analysis detected P, K, Ca, Mg, and S, considered macronutrients in both substrates. Besides, some micronutrients identified in *SS* were Cu, Fe, Mn, and Zn, whereas in *PS* it was also possible to detect micronutrients except Mn and Zn. All the macronutrients detected in the substrates were observed in the harvested *MG*. However, in the *MG* harvested in *PS*, Mn and Zn were not detected. Hence, a feasible disposal strategy for the *SS* generated by the Taxco de Alarcón *WWTP* is as a substrate for grass forage *MG* by its high organic matter content, the significant presence of macro and micronutrients, and the performance shown by *MG* cultivated in *SS*. Furthermore, the *SS* characteristics provide added value and can be considered as organic amendments of agricultural soils.

Key words: biofertilizer; mombaza grass; *Panicum maximum*; sewage sludge; wastewater treatment plant; Mexico.

RESUMEN

El manejo y disposición de lodos residuales (LR) generados por una planta de tratamiento de agua residual (PTAR) como parte del proceso de depuración del agua residual municipal (ARM), es uno de los principales problemas socioambientales que enfrentan este tipo de sistemas. Taxco de Alarcón, Guerrero, ubicado al sur de México posee una PTAR que comenzó a funcionar en el año 2016. La generación y disposición de los LR es una tarea que se debe atender por parte de la PTAR. El objetivo de este trabajo fue evaluar la capacidad de crecimiento de Panicum maximum empleando LR generados por la "Planta de tratamiento de agua residual Taxco de Alarcón" como soporte de crecimiento. Panicum maximum mejor conocido como pasto mombaza (PM) fue la especie seleccionada para sembrar sobre los LR. Una masa de 4 g de semilla fue esparcida sobre de 5 kg (base seca) de LR. Como control se ocupó un suelo composta comercial al que se le denominó suelo modelo (SM). El experimento se realizó por triplicado durante tres meses y para el riego del cultivo se empleó agua purificada de calidad de consumo humano. Cada mes se realizó una cosecha del PM. Las variables de respuesta analizadas para el PM fueron tiempo de germinación (primer mes), la velocidad de crecimiento (v_{PM}*), altura y rendimiento(*r_{PM}*), mientras que en los LR y SM se analizó el contenido de materia orgánica. Además, en el PM, LR y SM se analizó la composición química mediante microscopía*

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electrónica de barrido y espectroscopía de dispersión de rayos X. Los resultados mostraron que el PM germinó en un tiempo menor en el SM (5 días) comparado con la germinación en los LR (7 días). Sin embargo, el PM cultivado sobre LR alcanzó una altura considerablemente superior (45 cm) comparado con la altura alcanzada en SM (17 cm). Además, la v_{PM} *máxima sobre LR también fue mayor comparado con la* v_{PM} *máxima observada en el SM, 3.64 y 1.40* $cm \cdot día^{-1}$*, respectivamente. En términos* r_{PM} *se observó que en LR alcanzó un* r_{PM} *promedio mensual de 416* $g \cdot m^{-2}$*, mientras que en SM alcanzó un* r_{PM} *de 72* $g \cdot m^{-2}$*. El análisis químico logró detectar la presencia de P, K, Ca, Mg y S, considerados como macronutrientes en ambos soportes de crecimiento. Además, algunos micronutrientes identificados en LR fueron Cu, Fe, Mn y Zn, mientras que en el SM también se lograron detectar micronutrientes con excepción de Mn y Zn. Todos los macronutrientes detectados en los soportes de crecimiento fueron detectados en el PM cosechado. Sin embargo, en el PM cosechado en SM no se logró detectar la presencia de Mn y Zn. En función de su elevado contenido de materia orgánica, presencia de macro y micronutrientes y los resultados observados en este trabajo, una estrategia de disposición para los LR generados por la PTAR Taxco de Alarcón que podría ser considerada es como soporte de crecimiento para el pasto forrajero PM. Además, las características de los LR otorgan un valor agregado y también podrían ser considerados para emplearlos como mejoradores de los suelos agrícolas de la región.*

Palabras clave: biofertilizante; lodos residuales; Panicum maximum; pasto mombaza; planta de tratamiento de agua residual; México.

INTRODUCTION

Wastewater treatment plants (*WWTP*) are systems that integrate a series of physical, biological, and chemical processes systematically established to carry out the removal of polluting elements found in municipal wastewater (*MWW*) (Noyola *et al.,* 2013). The *WWTP*s arose from a water stress situation and from the need to increase the water use efficiency. In Mexico, the distribution of operating *WWTP* has reached a figure of 2642 and 4698 *WWTP* for *MWW* and industrial wastewater, respectively (CONAGUA, 2019). A volume of ~141479 *MWW* $L \cdot s^{-1}$ is treated by different processes in the *WWTP*s, and *ca.* 30 % bases its operation on the activated sludge method. However, the flow rate treated using this method represents *ca.* 50 % of the *MWW* treated in Mexico (CONAGUA, 2019). The choice of *MWW* treatment using aerobic biological processes (specifically activated sludge) is based on methods efficiency and treatment rate (Noyola *et al.,* 2013).

Unfortunately, one of the main wastes generated by this type of process is the so-called sewage sludge (*SS*). Tchobanoglous *et al.* (2003) reported that *ca.* 0.94 kg of *SS* (dry basis) are produced when 3.78 m^3 of *MWW* are treated, *i.e.*, 1000 L of *MWW* treated produce *ca.* 250 g of *SS* and the greatest *SS* generation occurs during primary and secondary treatment within a *WWTP*. Considering the treated *MWW* and the relationship established by Tchobanoglous *et al.* (2003), around 3056 *SS* $Mg \cdot day^{-1}$ are generated in Mexico, *i.e.,* 1.12 million *SS* $Mg \cdot year^{-1}$ (dry basis). Moreover, the addition of chemicals (*e.g.,* lime) for conditioning and stabilization processes during *SS* production considerably increases the final mass of this waste (Oropeza García, 2006).

Currently, the *SS* generation and disposal resulting from the *MWW* treatment carried out by the *WWTP*s have become one of the major socio-environmental problems within urban areas. Hence, a standard was drawn up to control and regulate sludge and biosolids use and final disposal, NOM-004-SEMARNAT-2002, which contemplates two types of solid waste: sludge and biosolids (SEMARNAT, 2003). The standard defines biosolids as *"sludge that has been subjected to stabilization processes and that due to their content of organic matter, nutrients and characteristics acquired after stabilization, may be susceptible to use"*. Besides, it defines sludge as *"solids with a variable moisture content, originating from urban or municipal sewage systems unclogging, from water treatment plants and from wastewater treatment plants, which have not been subjected to stabilization processes"*. In Taxco de Alarcón, Guerrero, Mexico, recently the *WWTP* "Taxco de Alarcón" started operations in 2016. The maximum installed capacity is to treat a *MWW* flow of 100 $L \cdot s^{-1}$, *i.e.,* 8640 $m^3 \cdot day^{-1}$ (CONAGUA, 2017). From this, the estimated amount of *SS* produced is *ca.* 2.16 Mg of *SS* day^{-1} (dry basis) taking as a calculation basis the value reported by Tchobanoglous *et al.* (2003). These wastes, as in most *WWTP*s constitute a final disposal problem. Thus, a strategy is sought for the final disposal of the *SS* and to prevent them from becoming a severe problem.

Nowadays, agricultural soils have suffered fertility decline due to intensive management production, as well as the scarce or absent conservation agriculture (Vega-Carreño and Febles-González, 2005). Consequently, it has generated an increase in the global demand for fertilizers (Patel *et al.,* 2020). Nevertheless, one of the main disadvantages of applying synthetic fertilizers to increase crop production is their high cost (Gallardo *et al.,* 2009). An attractive characteristic of the *SS* is the high amount of nutrients that they present, *e.g.,* total nitrogen, organic nitrogen, total phosphorus, available phosphorus, calcium, magnesium, sodium, and potassium in an average percentage of 4.46, 4.31, 1.59, 0.11, 1.18, 0.24, 0.40, and 0.19 %, respectively (Ortiz-Hernández *et al.*, 1995). Besides, they have a high moisture content and organic matter (Ortiz-Hernández *et al.,* 1995). Therefore, in regions with eroded soils or with low organic matter content, the use of *SS* as a soil conditioner is better than the application of synthetic fertilizers and depicts one interesting and acceptable ways of biosolids disposal (Salcedo Pérez *et al.,* 2007; Vásquez Aleman and Vargas Martínez, 2018). Also, to reduce the application of synthetic chemical fertilizers and their associated effects, some developing countries have decided to adopt dispersing the *SS* in agricultural soils (Ottaviani *et al.,* 1991). The use of *SS* in nutritionally worn or eroded soils improves their physicochemical and microbiological characteristics. Its use decreases the soil bulk density, favors aggregate formation and stability, increases the organic matter content, moisture retention and pore size, microbial activity, and nitrogen and phosphorus content. All these characteristics mentioned are part of a fertile soil (Tester, 1990; Canet *et al.,* 1996; Salcedo Pérez *et al.*, 2007).

Campos Medina *et al.* (2009) evaluated the potential of using *SS* as quality enhancers of nutritionally depleted soil. The *SS* used received an alkalinization pretreatment to stabilize them. Then, *SS* composition was subject to analysis. The analysis showed that the *SS* used had a high content of nutrients feasible to incorporate into eroded soils. On the other hand, Vásquez Aleman and Vargas Martínez (2018) directly used *SS* to evaluate the growth of edible crops, such as *Lactuca sativa* and *Daucus carota*, lettuce, and carrot, respectively, during 120 days. However, using *SS* for direct consumption crops represents a health risk to human population. Thus, there are alternatives crops with a commercial interest that could be cultivated directly on *SS* without direct health risk, *e.g.,* *Panicum maximum* better known as mombaza grass (*MG*). It is a forage grass used as a food source for cattle in tropical grasslands. Cattle production is a principal economic activity in Mexico, especially in northern states (Moral Barrera and Murillo Villanueva, 2015). *MG* constitutes an important source of low-cost nutrients compared to other food sources (Minson, 1990; Mendoza Martínez and Ricalde Velasco, 2016). Therefore, this work aimed to evaluate the growth capacity of *Panicum maximum,* also known as *MG* using *SS* generated within the "Taxco de Alarcón wastewater treatment plant" as substrate.

MATERIALS AND METHODS

Study site

Taxco de Alarcón wastewater treatment plant is located in the northern zone of Guerrero state, in the Municipality of Taxco de Alarcón between coordinates 437964 m E and 2050086 m N (UTM coordinate system), within the INEGI topographic chart Taxco scale 1:50000 (E14-A68). It is located within the hydrological region No. 18 (HR 18) Balsas Region, Balsas-Mexcala Basin. The *WWTP* treated effluent is reincorporated to the Taxco River, and at a distance of ~ 21 km, it joins to Cacalotenango River (Figure 1).

Experimental design

Aluminum trays with dimensions of 45×34×6 cm were used as a seedbed for the *MG*. Each tray was washed three times using drinking water. Afterwards, 5 kg of *SS* previously weighed on an analytical balance were placed inside it, and 4 g of weighed *MG* seed were dispersed on the surface. An analytical balance (OHAUS brand, explorer model) was used. This seeding procedure was carried out by triplicate. As a control, a pattern soil (*PS*) was used on which *MG* was seeded and subjected under the same experimental conditions. The dehydrated *SS* were sampled from the "Taxco de Alarcón wastewater treatment plant" in a site destined for their internal disposal. The *PS* was purchased at a departmental store. It is an ecological, commercial fertilizer brand. The *PS* specifications indicate that it is a composted soil obtained from natural materials and organic waste from fruits and vegetables. It has a dark color and compacted appearance, unlike the *SS*, which have a grayish and porous appearance (Figure 2).

The seedbeds were placed in a greenhouse. In the beginning, the *SS* and *PS* substrates were fully hydrated using 4 and 1 L of drinking water, respectively. The difference in water volume used was due to the initial moisture content of every substrate. After this, each seedbed was hydrated daily with 1.5 L of the same water quality. The drinking water used for irrigation was purchased from a drinking water purifier located in Iguala, Guerrero. The water quality was evaluated previously and met with the NOM-127-SSA1-1994 (Ramírez Mata and Flores Real, 2008). The treatment method used for the drinking water purifier has remained the same.

Three different harvests of *MG* were carried out. Each one was at 30 days cutting approximately 1 cm from the base of the *MG* growth.

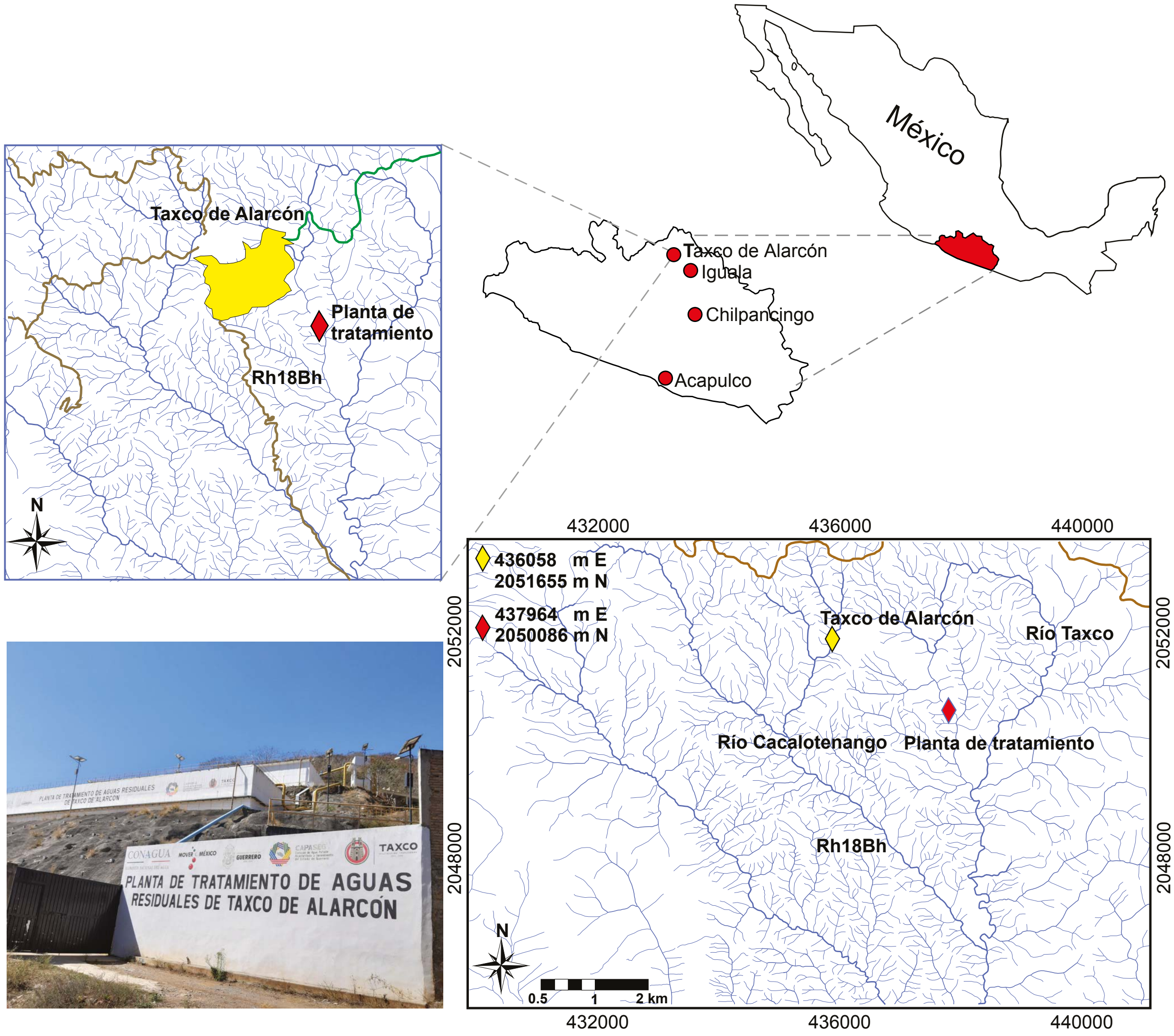

Figure 1. Taxco de Alarcón wastewater treatment plant location.

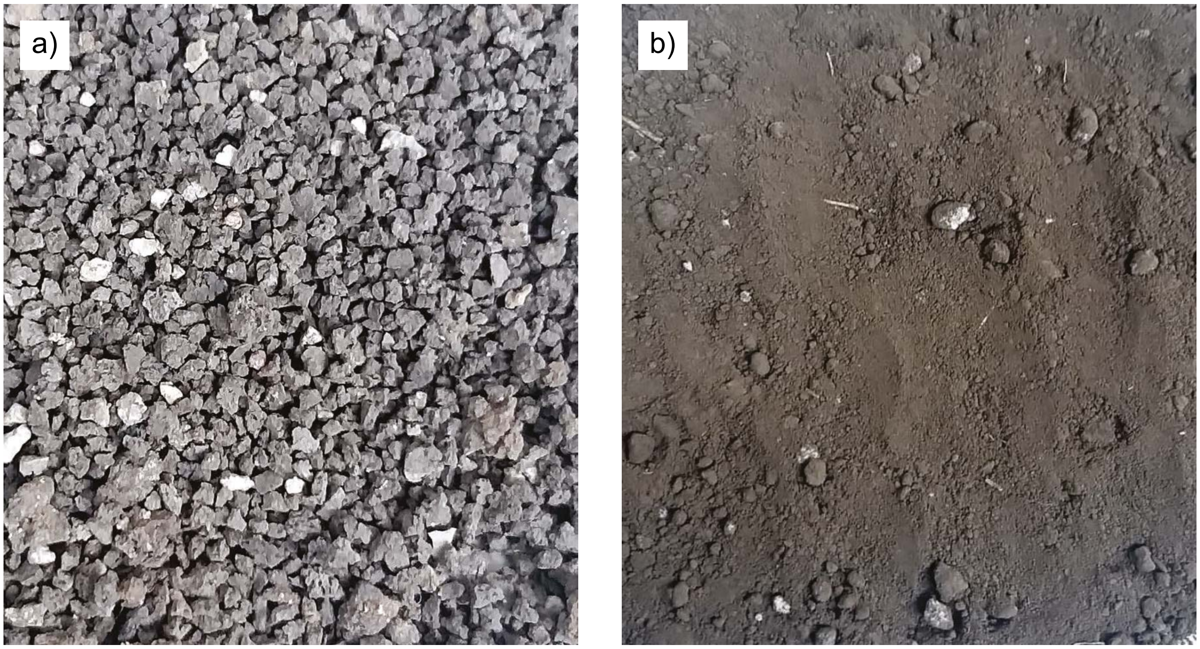

Figure 2. General view of the substrates used for the cultivation of Mombaza grass: a) dehydrated sewage sludge and b) pattern soil.

Each seedbed content of *SS* and *PS* was taken for analysis after each harvest. For *MG*, the response variables analyzed were germination time, growth rate (Gr_{MG}), height (H_{MG}), and yield (Y_{MG}), whereas for *SS* and *PS*, the organic matter content was analyzed. Additionally, a chemical composition analysis of *MG*, *SS*, and *PS* was carried out through scanning electron microscopy and X-ray energy dispersion spectroscopy (*SEM-EDS*).

In this study, the experimental design was completely randomized. The experimental model comprises a single factor, that is, the influence that the two substrates *SS* and *PS* have on Gr_{MG}, H_{MG}, and Y_{MG}.

Mombaza grass, sewage sludge, and pattern soil sampling

The sampling was carried out every 30 days. The *MG* was carefully harvested from each sown bed using a razor to avoid detaching the grass from the roots. The harvested *MG* was placed in Ziploc® bags and hermetically sealed. Afterward, the *MG* was dehydrated by direct exposure to sun heat for 6 hours. This process was executed during the three hottest months in Iguala de la Independencia: March, April, and May, where the temperature recorded were 36.5, 37.9, and 37.3 °C, respectively (https://es.climate-data.org/america-del-norte/mexico/guerrero/iguala-3422/?amp=true). Then, the *MG* samples were stored for analysis; meanwhile, each sampling, the total substrate (*SS* and *PS*) contained in one seedbed was recovered as a sample and deposited in hermetically sealed plastic bags. They were dried under the same conditions established for *MG* and later were analyzed.

Analysis

Moisture content and porosity determination

The initial moisture content determination of the substrates was achieved by gravimetry. A mass of 20 g of *SS* and *PS* previously weighed using an OHAUS brand analytical balance (navigator model) were placed into porcelain capsules of known mass. Subsequently, the capsules were placed in an oven at a temperature of 105 °C. Dehydration was performed until the weight of the samples remained stable. The moisture content (*Hu*) percentage was determined by using equation 1.

$$Hu = \left(\frac{m_i - m_f}{m_i}\right) \times 100 \tag{1}$$

where m_i depicts the *SS* or *PS* initial mass (g) and m_f the *SS* or *PS* final mass (g). The substrates initial moisture content was determined by duplicate.

The porosity for each substrate was calculated by eq. 2 using the bulk density and particle density (Sánchez-Vera *et al*., 2003).

$$Porosity = \left(\frac{Bulk\ density}{Particle\ density}\right) \times 100 \tag{2}$$

where the porosity is expressed in %, bulk density, and particle density in g·cm^{-3}. The bulk density was determined using equation 3, whereas the particle density was calculated using equation 4. Both values were determined by triplicate.

$$Bulk\ density = \left(\frac{Substrate\ dry\ mass}{Substrate\ volume}\right) \tag{3}$$

where bulk density is expressed in g·cm^{-3}, substrate dry mass in g, and substrate volume in cm^3.

$$Particle\ density = \left(\frac{Substrate\ mass}{Substrate\ volume}\right) \tag{4}$$

where particle density is expressed in g·cm^{-3}, substrate mass in g, and substrate volume in cm^3.

Mombaza grass germination time, height, growth rate, and yield calculation

The germination time was determined by measuring from the sowing of the *MG* seed until the appearance of the first germination shoots in each substrate. On the other hand, the H_{MG} was measured weekly using a scaler. From this data, it was possible to determine the Gr_{MG}, using equation 5.

$$Gr_{MG} = \frac{H_{MG}}{t} \tag{5}$$

where Gr_{MG} depicts the *MG* growth rate (cm·day^{-1}), H_{MG} the *MG* height (cm), and *t* the time to reach the corresponding height (days). On the other hand, the Y_{MG} was obtained from the monthly harvested mass of *MG* for each substrate per unit area (equation 6).

$$Y_{MG} = \frac{m_{MG}}{A} \tag{6}$$

where Y_{MG} represents the *MG* yield per month (*MG* g·m^{-2}), m_{MG} the *MG* dry mass (g) harvested every 30 days, and *A* the sowing area (m^2).

Prior to statistical analysis, normality and homogeneity of variances were evaluated for H_{MG}, Gr_{MG}, and Y_{MG} using a Shapiro-Wilk test ($p > 0.05$). After this, a paired *t*-test was used to compare the means for the variables before mentioned among *PS* and *SS* during the experiment. All the differences were statistically significant when $p \leq 0.05$.

Organic matter and removal quantification

Organic matter (*OM*) analysis was performed in duplicate for *SS* and *PS*. A mass (*W*) of ~5 g was brought to 105 °C for 2 hours to remove moisture and obtain weight 1 (W_1). Once W_1 was determined, the samples were heated at 450 °C for 2 hours in the muffle and the weight 2 (W_2) was recorded (Handbook, 1992). The *OM* content was determined by equation 7.

$$OM = \frac{W_1 - W_2}{W} \times 100 \tag{7}$$

where *OM* represents the organic matter content (%), *W* is the initial mass of the sample, W_1 the dehydrated mass (g) of the sample, and W_2 the calcined final mass (g).

From the *OM* content, the organic matter removal (η_{OM}) was estimated at the end of each cultivation period using equation 8.

$$\eta_{OM} = \frac{OM_1 - OM_2}{OM_1} \times 100 \tag{8}$$

where η_{OM} represents the organic matter removal (%), OM_1 the initial organic matter content (g), and OM_2 the final organic matter content (g).

Chemical composition analysis of mombaza grass, sewage sludge, and pattern soil

From previously dehydrated samples, a mass of ~5 g of *MG*, *SS*, and *PS* was pulverized using a mortar with a pestle until obtaining a thin and homogeneous powder, <0.063 mm. Afterwards, the *MG*, *PS*, and *SS* chemical composition determination was carried out. The procedure was performed using a mass of ~10 mg of each sample. It was placed in an aluminum sample holder with double-sided graphite tape. The sample was covered with graphite using the thermal evaporation of carbon. The device utilized for this purpose was a Denton Vaccum brand coater, Desk Carbon Accessory model. The analyzes were carried out using a *SEM* JEOL IT300-LV equipment, with an *EDS* Bruker, Quantax: Xflash 6/30.

The *SEM-EDS* working conditions were escape angle at 35°, 20 keV, and WD = 10 ± 0.5 mm. The chemical mapping was carried out for 60 min in an surface area of ~3.5 mm^2.

The *SEM-EDS* analysis were carried out at Laboratory of Scanning Electron Microscopy and Microanalysis of the Autonomous University of Guerrero.

RESULTS AND DISCUSSION

Moisture content in pattern soil and sewage sludge

Table 1 shows the initial average moisture present in the substrates used to germinate *MG*. It is observed that the *PS* presented a higher moisture content, *ca*. 15 % more than *SS*.

Table 1. Moisture content of pattern soil and sewage sludge used for the cultivation of Mombaza grass.

Substrates	**Average moisture** (%)
Pattern soil	19.93±0.01
Sewage sludge	5.45±0.04

Data are presented as mean ± standard error (n=2).

In economic terms, the moisture content in the *SS* is not a problem. The *SS* constitutes a waste generated by the *WWTPs*, therefore, its acquisition has not cost. In this sense, the production costs of the *MG* would not increase with the use of this nutritional source. However, in the case of using *PS* or another nutritional additive, it is necessary to consider its monetary value in the total production costs of the *MG*. Additionally, the variation of the moisture content in the *PS* affects the purchase price. In this work, 1 kg of *PS* was purchased at $6.00 MXN.

Mombaza grass germination time, height, and growth rate

The *MG* germination time in the two substrates used was notably dissimilar. The first germination shoots were observed in the seedbeds using *PS* as substrate. That happened five days after the *MG* seeds had sown. On the other hand, using *SS* as a substrate, the first germination shoots were observed until day 7. These values indicate that the germination of *MG* using *PS* requires less time, *i.e.*, the difference among *MG* germination in the substrates was 2 days. On the other hand, Figure 3 shows the height reached by the *MG* during the first month of growth. During the first two weeks, it was observed that the H_{MG} grown in *PS* was slightly higher than the height reached by the *MG* grown in *SS*. However, from the third and fourth weeks, the H_{MG} showed a favorable change for the *SS*. The H_{MG} grown in *SS* exceeded considerably the height reached in the *PS*. At the end of the first month, the maximum *MG* heights reached were 20 and 7 cm in *SS* and *PS*, respectively, *i.e.*, the maximum H_{MG} reached in *SS* was 2.85 times higher than the H_{MG} reached in *PS*. These differences among both treatments were significant only for weeks 3 and 4 according to the paired *t*-test (third week: $t = -6.348$, $p = 0.002$; fourth week: $t = -3.243$, $p = 0.017$).

Unlike the first month (Figure 3), in the second and third months (Figure 4), the initial H_{MG} reached during the first week of every month by the *MG* grown in the *SS* as substrate was higher than the H_{MG} reached in *PS*. This behavior is attributed to the fact that the germination factor

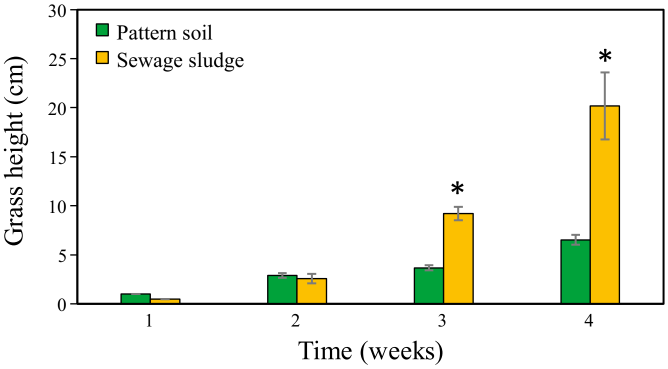

Figure 3. Average height of Mombaza grass plants cultivated on pattern soil and sewage sludge during the first month of experimentation. The asterisk (*) means significant difference (paired *t*-test, p <0.05) and vertical bars indicate the standard error.

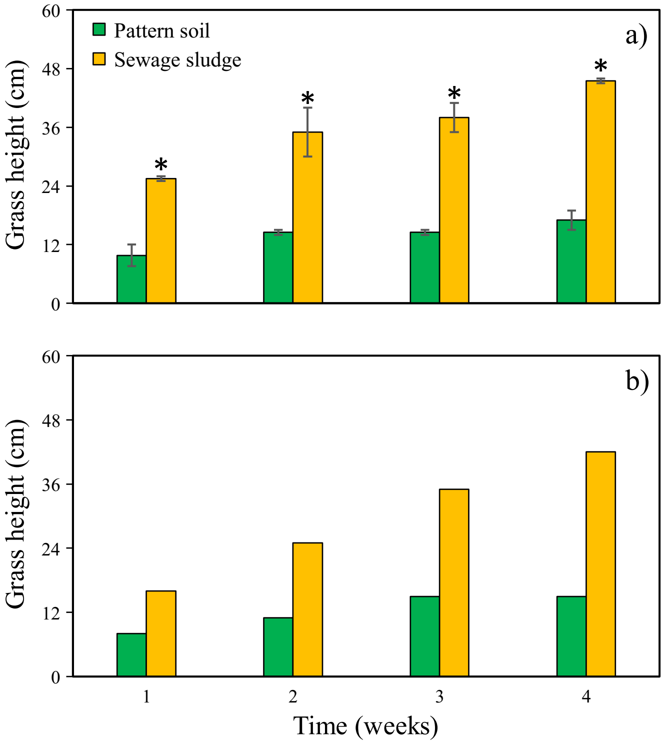

Figure 4. Average height of Mombaza grass plants cultivated on pattern soil and sewage sludge during the a) second and b) third month of cultivation. The asterisk (*) means significant difference (paired *t*-test, $p < 0.05$) and vertical bars indicate the standard error.

Figure 5. General view of the gleaning of Mombaza grass cultivated on pattern soil observed from seventh week of cultivation (second month).

was eliminated in these months. That is, the monitoring of the height in these months was from previously germinated *MG* crops.

In the second month, a linear tendency to increase the height was observed over time in the case of *MG* grown on *SS* (Figure 4a). The equation that describes this behavior is $y = 6.3x + 20.25$ ($R^2 = 0.9657$). However, in the case of *MG* grown in *PS*, the tendency to grow was lower, *i.e.*, the slope of the growth curve was lower. This can be observed from the equation that describes this behavior $y = 2.16x + 8.55$ with $R^2 = 0.8599$.

This behavior is attributed to the fact that the *MG* cultivated on *PS* began gleaning from the third week of culture, affecting its growth (Figure 5). The H_{MG} of the four weeks belonging to second month was significantly higher in *SS* compared to *PS* (paired *t*-test: $p \leq 0.05$).

Soil quality is an important variable in the constitution of plant growth. However, climatic conditions (photoperiod, temperature, and humidity), annual seasons, and even management practices are also responsible for altering plants' flowering and fruiting periods (Velasco *et al.,* 2018). One work carried out to evaluate grass production, dependent on defoliation frequency and height, showed that they also alter flowering (Febles *et al.,* 2009; Márquez, 2014; Velasco *et al.*, 2018). Once the grass is cut or harvested, a metabolic readjustment is generated that promotes a new leaf area formation. The purpose is to reestablish the plant's photosynthetic capacity; this, in turn, could promote flowering if the shoots are large enough or old (Febles *et al*., 2009). In this work, both crops were carried out under the same climatic and management conditions, therefore, the quality of the *PS* might be the main factor that caused the growth of *MG* to stagnate, reaching a *plateau* at a maximum height of 17 cm. On the other hand, in this second period, the maximum H_{MG} reached in *SS* was 45 cm (Figure 4a). The H_{MG} cultivated in *SS* was 2.64 times higher than the recorded in *PS*.

Finally, in the third month of cultivation, the H_{MG} showed a similar behavior to that of the second month (Figure 4b). The growth curves were $y = 8.8x + 7.5$ with $R^2 = 0.9954$ and $y = 2.5x + 6$ with $R^2 = 0.8993$ for *MG* grown on *SS* and *PS*, respectively. In the third month, the maximum height observed was 42 and 15 cm for *MG* grown in *SS* and *PS*, respectively, *i.e.*, the H_{MG} grown in *SS* was 2.80 times higher than the height recorded in *PS*. Additionally, the monthly H_{MG} reached in each substrate is shown in Figure 6. The *MG* heights among *PS* and *SS* were significantly different, both in the second (paired *t*-test: $t = -8.216$, $p = 0.003$) and third month ($t = -4.238$, $p = 0.024$).

The Gr_{MG} was another important factor that was analyzed. The Figure 7 shows the Gr_{MG} determined weekly during the *MG* cultivation.

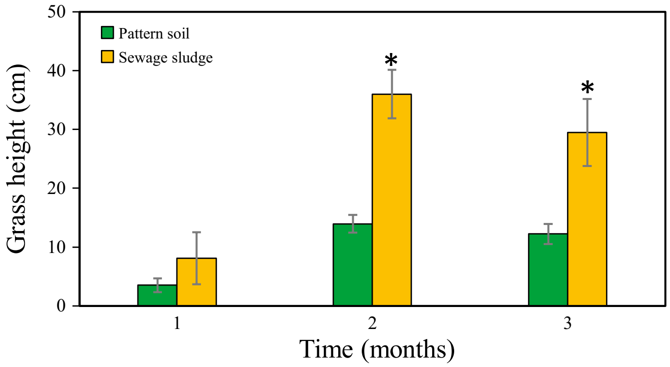

Figure 6. Monthly average height of Mombaza grass plants cultivated on pattern soil and sewage sludge. The asterisk (*) means significant difference (paired *t*-test, $p < 0.05$) and vertical bars indicate the standard error.

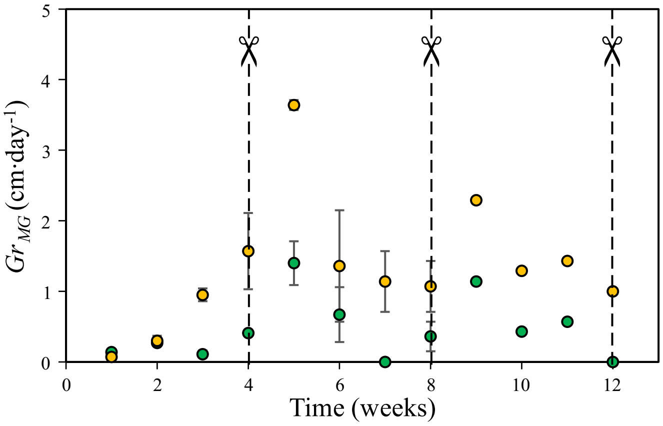

Figure 7. Average growth rate of Mombaza grass plants (Gr_{MG}) cultivated on pattern soil (presented with green circles) and sewage sludge (presented with yellow circles). The scissors indicate Mombaza grass plants harvest. Vertical bars indicate the standard error.

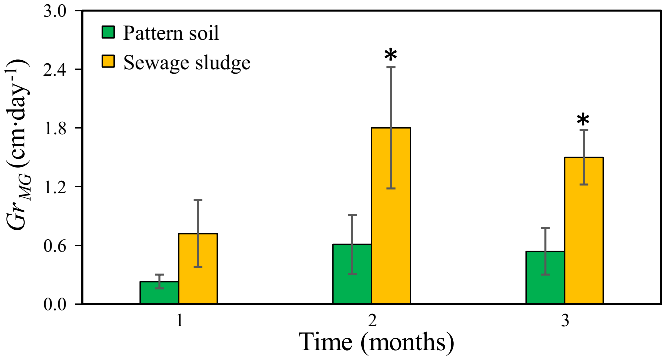

Figure 8. Monthly average growth rate of Mombaza grass plants (Gr_{MG}) cultivated on pattern soil and sewage sludge. The asterisk (*) means significant difference (paired *t*-test, $p < 0.05$) and vertical bars indicate the standard error.

In the first week of the first month, Gr_{MG} was higher in *PS*. This was because of the *MG* in *PS* presented a shorter germination time compared to *SS*. However, from the second week on, the Gr_{MG} observed in *SS* began to grow linearly with time. In the fourth week, the first harvest or cut of the *MG* grown in both substrates was carried out. During the first week of the second month, the *MG* reached its maximum Gr_{MG} in each substrate. In case of the *MG* grown on *SS*, the maximum Gr_{MG} reached was 3.64 cm·day^{-1} whereas in *PS* it was 1.40 cm·day^{-1}. In the following three weeks, Gr_{MG} showed a tendency to decrease in both crops. It was observed that as the H_{MG} increased, the Gr_{MG} decreased (Figure 4 and 7). Finally, in the third month of cultivation, the Gr_{MG} showed a similar behavior to that of the second month. The Figure 8 shows the monthly Gr_{MG}. A paired *t*-test showed that the monthly Gr_{MG} among *PS* and *SS* were statistical different in the second (t = -3.287, p = 0.044) and third month (t = -13.98, p = 0.000).

The environmental temperature and those of the substrates was monitored during *MG* cultivation (Figure 9). In general, the environmental temperature was higher, in a range of 34 – 42 °C during the twelve weeks of experimentation. This recorded temperature is within the range established for the optimal growth temperature for *MG*: tropical climate. This climate characterizes by a monthly average temperature of ~24 °C and no frosts. Also, in the arid tropical climate, temperatures of 40 °C can be recorded, similar to those reached in this experiment (Papadakis, 1980).

On the other hand, in the first week of cultivation, it was observed that the temperature in both substrates was higher than that recorded in the environment. This is attributed to the fact that there was not a considerable H_{MG} that prevented the supports from absorbing heat.

The environmental temperature was higher than the temperature measured directly on the supports from the second week on. It can be because each substrate already presented a significant *MG* growth density. Another aspect to highlight is that, in general, the *PS* temperature was always higher than the temperature determined in *SS*. The heat capacity of the substrates, related to the amount of water that each one retains can be the main reason because of this difference in temperatures was observed. The *PS* has a noteworthy effect absorbing water, whereas in *SS* the water percolates to the bottom of the seedbed. The *PS* has a silty structure, and the water retention phenomenon is larger than *SS*. A 60 % of the particles size of *PS* were <0.125 mm. On the other hand, the *SS* has a sandy structure (84 % of particles have a size >1 mm and 14.5 % have a size between 0.5 and 1

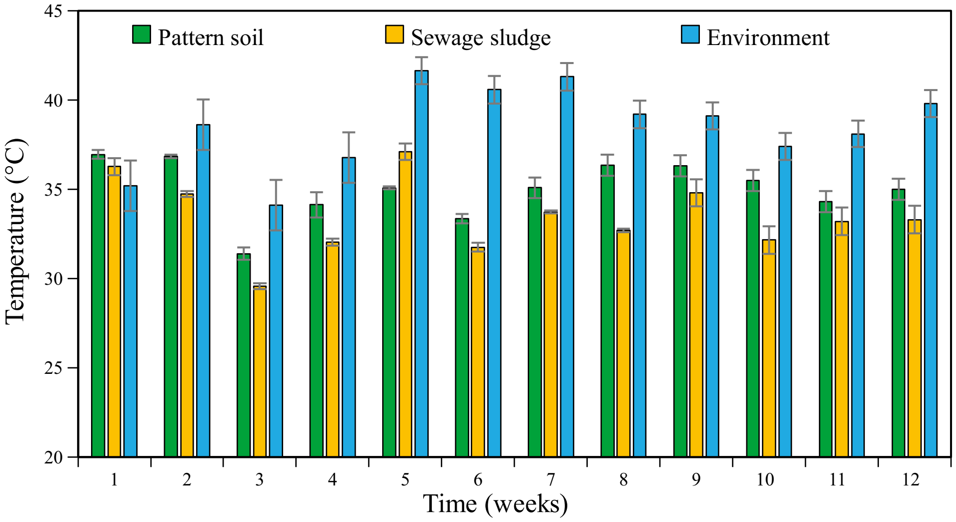

Figure 9. Mean temperature of pattern soil, sewage sludge, and environment throughout the three-month period of Mombaza grass cultivation. Vertical bars indicate the standard error; n=2.

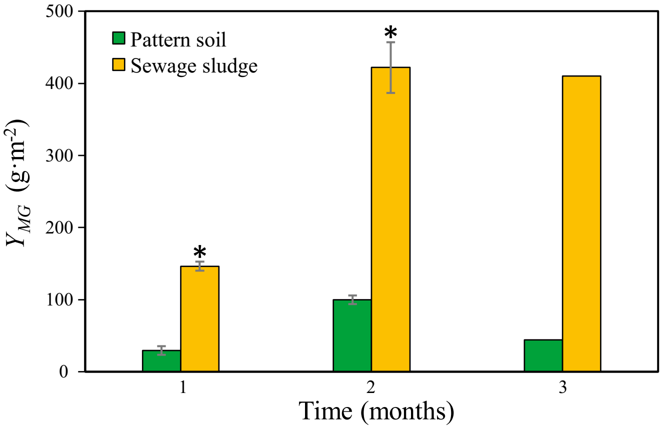

Figure 10. Monthly average yield of Mombaza grass plants (Y_{MG}). The asterisk (*) means significant difference (paired *t*-test, $p < 0.05$) and vertical bars indicate the standard error.

mm) and the porosity is bigger than the porosity observed by *PS*, 60 and 38 %, respectively. Thus, the defined structure present in *PS* leads to retaining more water in the porous space whereas the *SS* does not have a defined structure (Figure 2). Also, the porous structure and composition of *SS* promoted the evapotranspiration process. In *PS* the evapotranspiration phenomenon was noticeable lower than *SS* (Figure 2). In other words, the presence of water in the substrates affects their ability to absorb and/or release heat. Unlike other materials, water has a high heat capacity value. The heat capacity of water is 4187 $kJ \cdot m^{-3}$ $°C^{-1}$, and its specific heat 1000 cal $(kg\ °C)^{-1}$. Specific heat represents the amount of heat that water requires to raise its temperature by one degree (AQS, 2005).

Mombaza grass yield

The Y_{MG} was calculated monthly (Figure 10, Table 2). The first month showed a maximum Y_{MG} of 30 and 146 *MG* $g \cdot m^{-2}$ in *PS* and *SS*, respectively. In the first month, both crops needed about a week to germinate. For this reason, the Y_{MG} was lower compared to subsequent months. From the second month on, the *MG* on *SS* showed a Y_{MG} significantly higher than that observed in *PS*. It reached an average Y_{MG} of 416 *MG* $g \cdot m^{-2}$ whereas *PS* reached an average Y_{MG} 72 *MG* $g \cdot m^{-2}$. In the case of the *MG* cultivation on *PS*, in the second month the grass began to glean, this phenomenon affected the H_{MG} and Gr_{MG} and consequently the Y_{MG}. The Y_{MG} values obtained from months 1 and 2 were analyzed using a paired *t*-test, which showed a statistically significant difference among *SS* and *PS* ($p < 0.05$). The third month was not possible to analyze because of the absence of replicates. However, in the third month, the H_{MG} and Gr_{MG} values for *MG* cultivated in *SS* showed significant differences with the values obtained for the *MG* cultivated in *PS* (Figure 6 and 8). Moreover, the Y_{MG} depends on variables such as the H_{MG} and Gr_{MG}. Therefore, the Y_{MG} value for third month showed by *MG* cultivated in *SS* would represent a difference statistically significant.

Table 2. Monthly average and cumulative yields of Mombaza grass cultivated on pattern soil and sewage sludge for a 3-month period.

Time (month)	**Mombaza grass yield** ($g \cdot m^{-2}$)	
	Pattern soil	Sewage sludge
1	29.63±6.05	146.41±6.18
2	100.00±5.88	421.90±34.97
3	44.44	410.46
Cumulative total	174.07	978.77

Data are presented as mean ± standard error; n=3 and 2, for month 1 and 2, respectively.

On the other hand, the Y_{MG} accumulated throughout the three months of cultivation was calculated (Table 2). The results showed a cumulative Y_{MG} *ca.* 1.0 *MG* $kg \cdot m^{-2}$ using *SS* as substrate whereas in *PS* the Y_{MG} was 174 *MG* $g \cdot m^{-2}$.

Organic matter removal

Throughout the *MG* cultivation time the *OM* content was monitored. It was determined that the initial *OM* content in the evaluated substrates were 36 and 15 % for *SS* and *PS*, respectively. This difference in *OM* content is one of the factors related to the quality of the substrate that could have affected the growth of *MG* in the *PS*. In terms of η_{OM} determined for *PS*, it was practically not observed. This value is consistent with the observed Y_{MG} (Table 2). On the other hand, a significant η_{OM} was observed in *SS* (Table 3). Once again, this value was closely related to the estimated monthly and quarterly Y_{MG} (Table 2). In general, the cultivation of *MG* on *SS* contributes with the η_{OM} reached in *SS*, *ca.* 12 %. Nevertheless, it is important to consider the *OM* natural degradation. It is another factor that contributes with the η_{OM} in soils. The substrates used showed a bulk density of 0.4217±0.0218 and 0.5313±0.0150 $g \cdot cm^{-3}$ for *PS* and *SS*, respectively. The *SS* depicts a high and major porosity than *PS*, 60 and 38 %, respectively. The sandy soils such as *SS* substrate have a better aeration than *PS*. Hence, the rate of organic matter decomposition influences by chemical and microbial processes in *SS* is bigger than *PS* (Clapp *et al.*, 1986). Thus, the major η_{OM} reached by *SS* was expected (Terry *et al.*, 1979).

The removal value shown by *SS* (*ca.* 12 %) indicates that there is still enough *OM* that can be used to cultivate *MG* and that the crop can continue to be growing and obtain similar Y_{MG} to the values obtained if the physical space for the root growth was not limited.

It has been reported, the *OM* from *SS* exert significant influence on the physical, chemical, and biological properties of soils. The *OM* improves the soil's productive capacity, and its structure in terms of porosity and bulk density. Also, the water, air, and heat transmission are impacted. Finally, the soil strength is modified and translated into desirable physical conditions (Clapp *et al.*, 1986). Nevertheless, a detailed chemical analysis must be carried out to know the *SS* composition prior to agricultural soils application. This type of analysis will prevent the dispersion and accumulation of potentially hazardous compounds such as trace heavy metals and toxic organic compounds (Clapp *et al.*, 1986; González-Flores *et al.*, 2011).

In addition to the previously described important aspects, another difference was observed in the *MG* cultures: the coloration grass. The *MG* grown on *PS* showed a solid green color. In contrast, the *MG* grown on *SS* showed a yellow coloration. The yellow color emergence in the leaves of *MG* is known as chlorosis (Durán Quiroz *et al.*, 1998). This phenomenon originates in the leaf tissue due to the lack of chlorophyll. There are several causes associated with this color deficiency. Some of

Table 3. Percentage of organic matter removal in pattern soil and sewage sludge throughout the cultivation period of Mombaza grass.

Time (month)	**Organic matter removal** (%)	
	Pattern soil	Sewage sludge
1	0	11
2	10	4
3	2	3

them are insufficient drainage, damaged roots, compacted roots, high alkalinity, and nutritional deficiencies of the plant. Nutritional deficiencies can occur because the soil is not rich in nutrients or nutrients are not available. Nutrients such as Ca, Mg, Co, Cu, Fe, Mn, Mo, and Zn can be present, although associated to five fractions according to a classification established by Tessier *et al.* (1979) for particulate trace metals. The particulate trace metals associated with fraction 1 (exchangeable) can be released by a simple change in water ionic composition, whereas the fraction 2 (bound to carbonates) is a pH function. A slightly acid rain phenomenon can lead the trace metals releasing (Krauskopf and Bird, 2003). The particulate trace metals associated with fraction 2 can be released when the environmental conditions reach a pH value between 6.5–7.5. In fraction 3, the particulate trace metals are bound to iron and manganese oxides and under anoxic conditions they are thermodynamically unstable (low Eh value). On the other hand, the particulate trace metals bounded with organic matter (faction 4) requires oxidizing conditions to release soluble nutrients through the organic matter degradation. The *SS* usually can contain large quantities of trace metals associated to organic matter and the organic matter oxidation control their release (Clapp *et al.*, 1986). Finally, the particulate trace metals associated with fraction 5 (residual) are not expected to be released in solution. The releasing of particulate trace metals from fraction 5, needs conditions normally not encountered in a natural environment. In other words, the nutrients solubility and geodisponibility such as particulate trace metals are in function of environmental conditions (Tessier *et al.*, 1979; González-Flores *et al.*, 2011). Fe, Zn, or Mn inadequacy can cause the chlorosis phenomenon (Oliveira Prendes *et al.,* 2006). Alvira Serrano (2019) carried out a chemical-mineralogical characterization of *SS* of the Taxco de Alarcón *WWTP*. In his research results, he reported a pH of 7.1 for the *SS* and total concentrations of 19.38, 3.13, and 1.83 g of Fe, Zn, and Mn per kg of *SS*, respectively. However, in fraction 4, concentrations of 6.25, 0.70, and 0.20 g per kg of *SS* were found for Fe, Zn, and Mn, respectively. Also, in fraction 3, important concentrations were reported: 0.13, 0.70, and 0.50 g per kg of *SS* for Fe, Zn, and Mn, respectively. Finally, in fractions with major geodisponibility (fractions 1 and 2), the sum reached the following values: 4.33, 1.72, and 1.14 g per kg of *SS* for Fe, Zn, and Mn, respectively. Hence, pH and availability of nutrients could not be part of the reasons that cause chlorosis in the *MG* grown in *SS*.

In the case of *PS*, the growth of *MG* kept a relationship with the quantity and size of the roots, *i.e.,* the growth of the roots was abundant, fibrous, or fasciculate and they developed near the surface (Figure 11a). In the case of *MG* grown in *SS*, its roots were thicker and longer. Root mass per unit area was lower in *SS*. The roots dispersed in all directions within the *SS*. The length of these roots extended to the seedbeds depth where a drainage system was lacking (Figure 11b). In this area, due to the texture of the *SS* (sandy structure) and porosity (60 %) when the water irrigation was carried out, the water infiltrated and accumulated mainly at the bottom. From this, the *MG* cultivated in *SS* developed roots longer than in *PS*. Thus, the main cause of chlorosis observed in *MG* grown in *SS* can be related to the drainage lack in seedbeds.

Pattern soil and sewage sludge chemical analysis

The chemical analysis by *SEM-EDS* on *PS* showed the presence of the following elements considered as macronutrients for plants P, K, Ca, Mg, and S (Figure 12a). Also, other elements such as Cu, Cl, Na, Al, Ti, Fe, Si, and O were identified, of which Cu, Cl, Al, and Fe are part of the micronutrients of plants (Figure 12b, c). The combination of essential elements, macro, and micronutrients with sunlight, are the requirements for the correct metabolic development of plants (Kathpalia and Bhatla, 2018). On the other hand, Figure 13a shows all the macronutrients detected in *SS* (P, K, Ca, Mg and S). Additionally, in this substrate Cu, Fe, Ti, Si, Al, Na, Mn, and Zn were detected. From them Cu, Fe, Al, Mn, and Zn are part of the group of micronutrients for plants (Figure 13b, 13c). In nutritional terms, it was observed that *SS* and *PS* contain the same macronutrients. However, in terms of micronutrients, Mn and Zn were detected only in the *SS*, elements that were not detected in the *PS*. On the other hand, in *SS* the presence of Cl was not detected, whereas in *PS* it was detected.

Mombaza grass chemical analysis harvested on pattern soil

The same macronutrients detected in the *PS* (P, K, Ca, Mg, and S) were identified in each *MG* harvest on the corresponding substrate (Figure 14). Furthermore, some micronutrients detected in all the

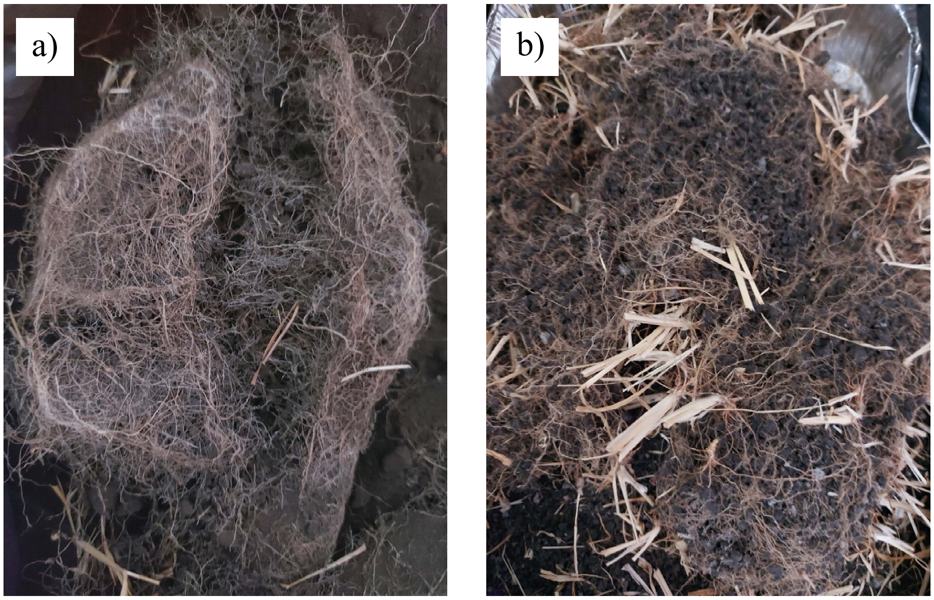

Figure 11. General view of Mombaza grass root system in a) pattern soil and b) sewage sludge.

harvests were Cu, Cl, and Al, whereas Fe was only found in the second harvest. Other elements detected in the *MG* harvested on the *PS* were Na detected during the first and third months, whereas Si was detected in the three crops forming aggregates within the *MG* structure. Finally, Ti element appears in the *PS*, however this element was not detected in *MG* crops, it indicates that *MG* does not incorporate it into its structure.

Mombaza grass chemical analysis harvested on sewage sludge

In case of the *MG* harvested on *SS*, the presence of the macronutrients observed in the substrate P, K, Ca, Mg, and S was observed in each crop (Figure 15). On the other hand, the micronutrients detected in the *MG* structure were Al, Cu, Mn, Fe, Zn, and Cl. The only microelements found in the three harvests were Al, Cu, and Mn, while Zn was detected in the first, and second harvest, Fe in the first, and Cl in the first, and third harvest. Other identified elements were Na and Si found in all crops. In the case of Si, it was again observed as part of aggregates, a form similar to that observed in the *MG* harvested in *PS*. Finally, in the case of Ti detected in the *SS*, this element was not detected in *MG* crops. Some micronutrients such as Cu, Zn, Mn, Fe, Al, and Mo are also heavy metals, and at high concentrations are toxic to plants. Other elements such as Cd, Pb, Cr, Ni, Hg, and As are heavy metals and all of them are toxic for living organisms and are often considered as pollutants (He *et al.,* 2005; Sánchez-Montoya *et al.,* 2019; Vélez-Pérez *et al.*, 2020).

The macronutrients detected in the *MG* of all the crops analyzed from *PS* and *SS* were the same, *i.e.*, there was no difference in terms of macronutrients in the *MG* harvested. However, in terms of micronutrients, Mn and Zn were detected in *MG* harvested from *SS*, whereas in *MG* harvested from *PS*, none of these elements were identified even though they are part of the nutrients that plants need for their proper development (He *et al.,* 2005). These results indicate that the concentration of these elements in *PS* and *MG* harvested from this substrate could be below detection limit to be detected by this technique. In the case of Cl, this element was not found in *SS*, however, it was detected in *MG* harvested from this substrate. This means that the concentration of this element in the *SS* was not enough to be detected by the technique directly in them or Cl was added through irrigation water from dissolved salts (Kathpalia and Bhatla, 2018). Therefore, the presence of Mn and Zn in *SS* was one of the main causes for which better results were observed from the *MG* harvested on this substrate.

The *SEM-EDS* is a semi-quantitative technique, it is used as a tool to detect the presence or absence of a component. It is difficult through this technique to know the real concentration, nevertheless, it is useful to identify chemical elements in a sample as in the case of the analyzed substrates (Carles-Melgarejo, 2010).

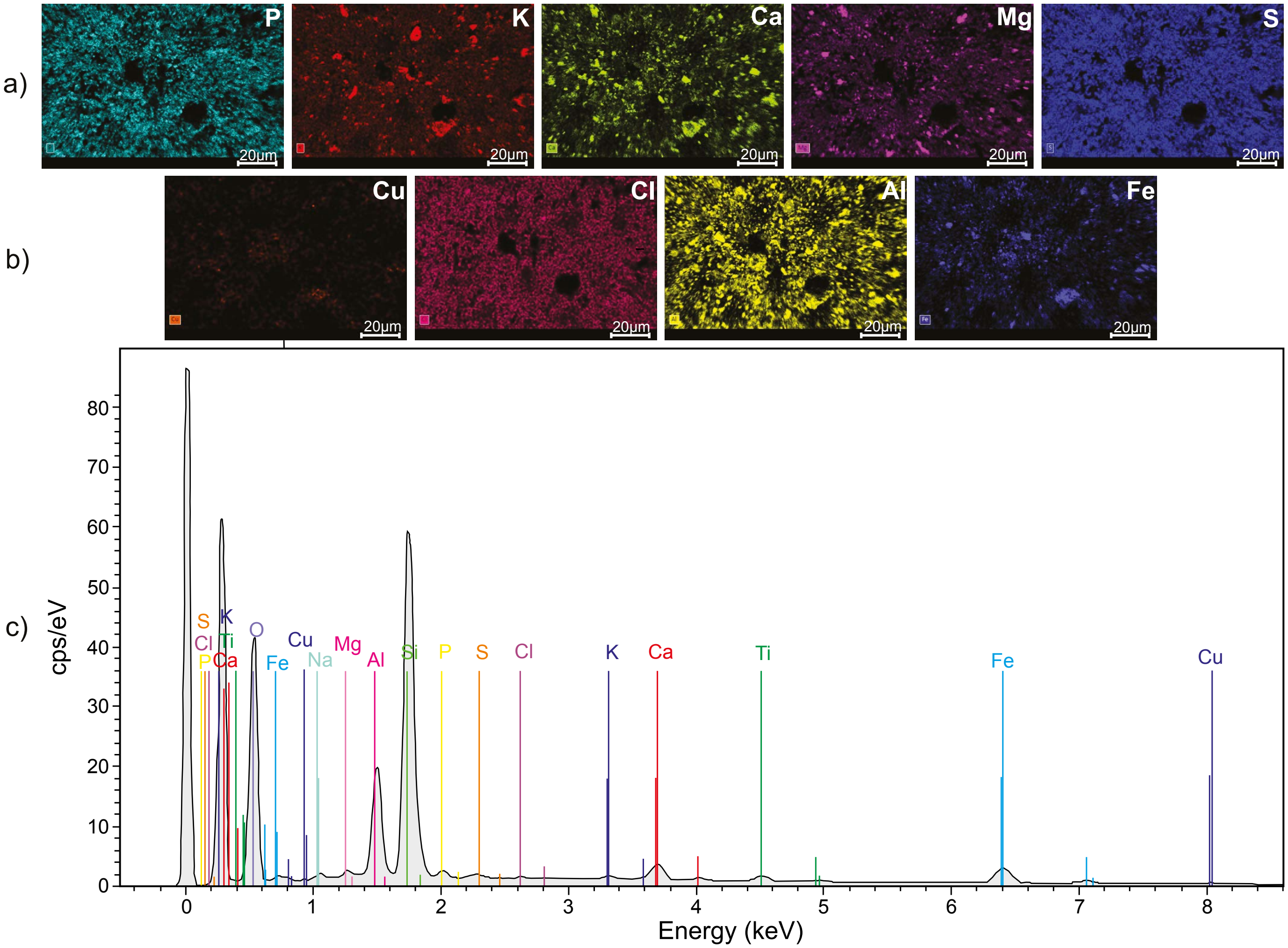

Figure 12. Chemical composition of the pattern soil used for the cultivation of Mombaza grass determined by scanning electron microscopy and X-ray energy dispersion spectroscopy, where the presence of a) macronutrients; b) micronutrients; and c) the full spectrum of all identified elements can be appreciated.

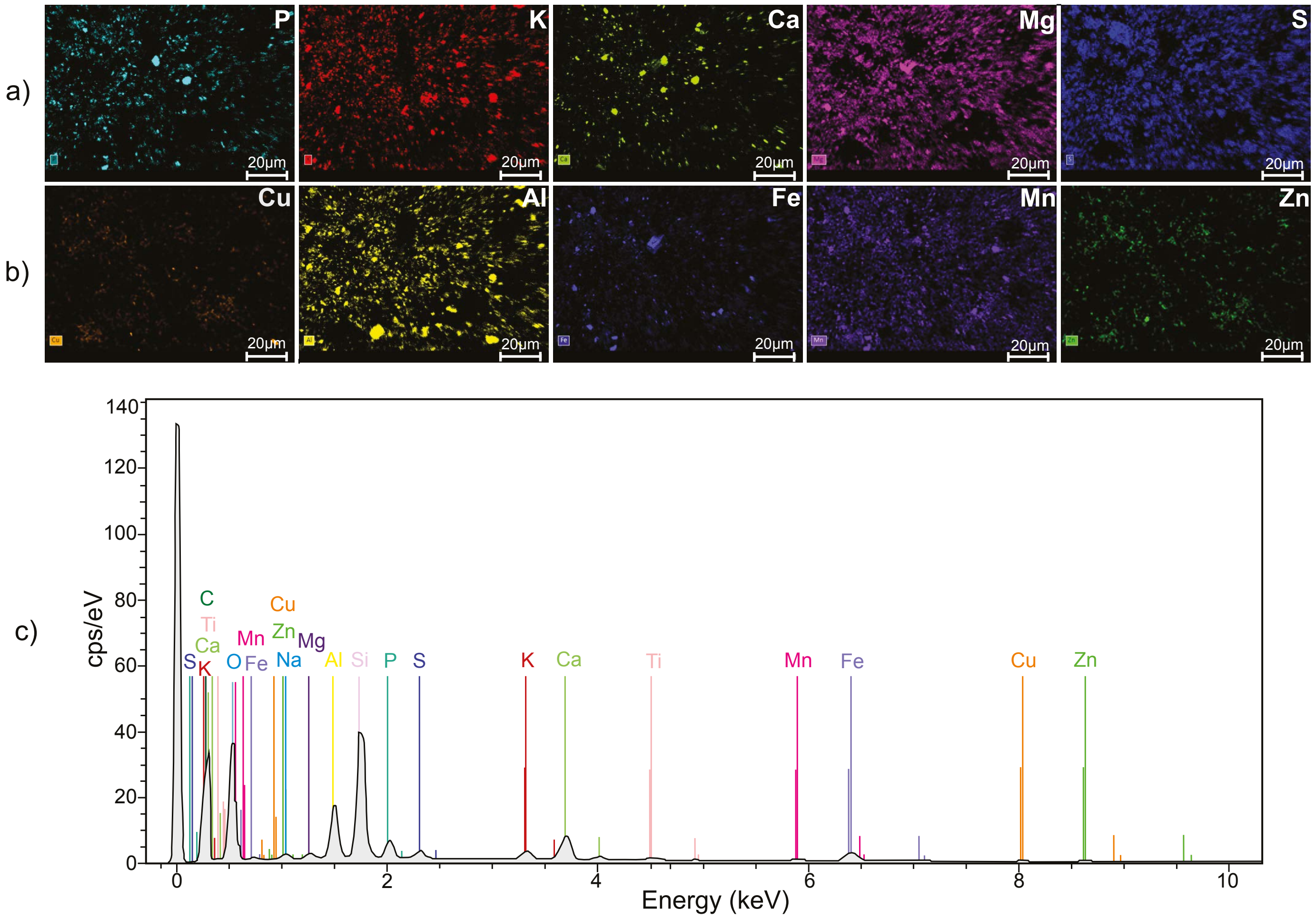

Figure 13. Chemical composition of sewage sludge used for the cultivation of Mombaza grass determined by scanning electron microscopy and X-ray energy dispersion spectroscopy, where the presence of a) macronutrients; b) micronutrients; and c) the full spectrum of all identified elements can be appreciated.

CONCLUSION

The commercial soil used as substrate (*PS*) contains 3.7 times more moisture than *SS*. In terms of germination rate, the *MG* germination on *PS* was faster than *MG* germination on *SS*. In *PS* it was within the first 5 days, whereas in *SS* it was observed until day 7. However, the *PS* was better than the *SS* only in this parameter. In terms of H_{MG}, the maximum height reached of the *MG* grown on *SS* was 2.80 times higher than the observed on *PS*. On the other hand, after the first harvest (first month), the highest Gr_{MG} (regardless of the substrate) was recorded during the first cultivation week. The Gr_{MG} in *SS* was *ca.* 2.5 times higher than Gr_{MG} in *PS*. Furthermore, it was observed that after the first growth week of the second month, as the H_{MG} increases, the Gr_{MG} decreases.

In terms of Y_{MG} arvested in *SS* was 562 % higher than the mass harvested in the *PS*, *i.e.*, the use of *SS* as a sowing substrate translates into a Y_{MG} of *ca*. 5.62 times better compared to *PS* as substrate. Another aspect to highlight is the *OM* content, which was 2.4 times higher in *SS* than the *OM* content in *PS*. The *OM* analysis throughout the three months *MG* cultivation on *SS* showed a decrease in *OM*, *ca*. 12 %, *i.e.*, during this time a considerable decrease in *OM* content was not observed. On the other hand, each substrate evaluated presented the same macronutrients (P, K, Ca, Mg, and S); however, the *SS* showed Mn and Zn, two micronutrients of importance that were not detec-ted in *PS*.

Based on the analysis of Gr_{MG}, η_{OM}, Y_{MG}, and chemical composition in *SS*, there is potential to continue cultivating *MG* for a lapse greater than three months in areas where do not exist physical limitation for the root's expansion.

The *SS* showed better nutritional characteristics than the *PS*. This was reflected in a major performance as a substrate for *MG*. The nutritional characteristics of the *SS* generated by Taxco de Alarcón *WWTP* and its performance as a substrate provide added value to this type of waste and allow it to be proposed as a substrate for this forage grass. Additionally, *SS* can be proposed as a physicochemical and nutritional characteristics improver for eroded soils in the region when fully chemically characterized.

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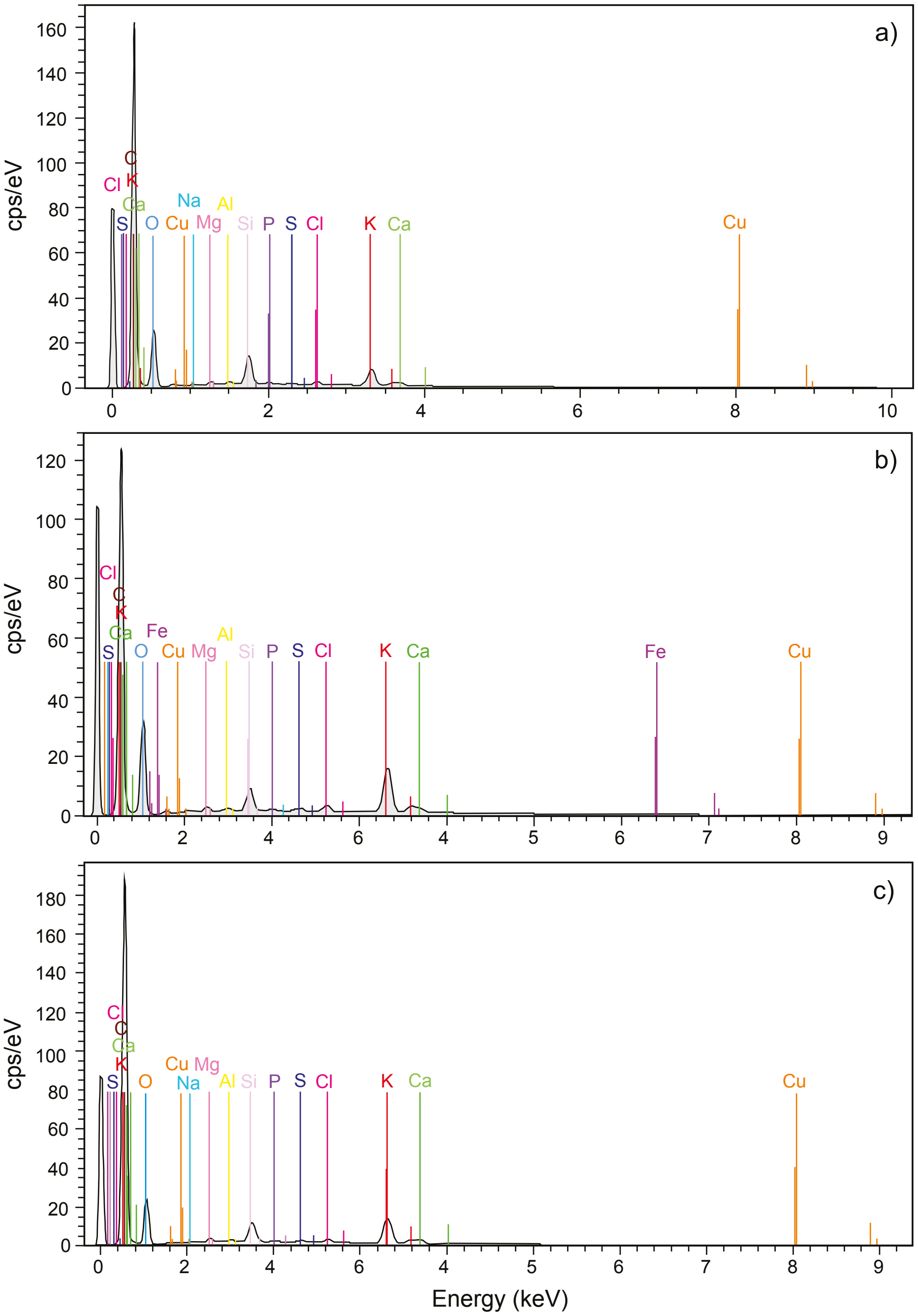

Figure 14. Chemical composition of Mombaza grass plants determined by scanning electron microscopy and X-ray energy dispersion spectroscopy, which were harvested on pattern soil during the a) first month; b) second month; and c) third month of cultivation.

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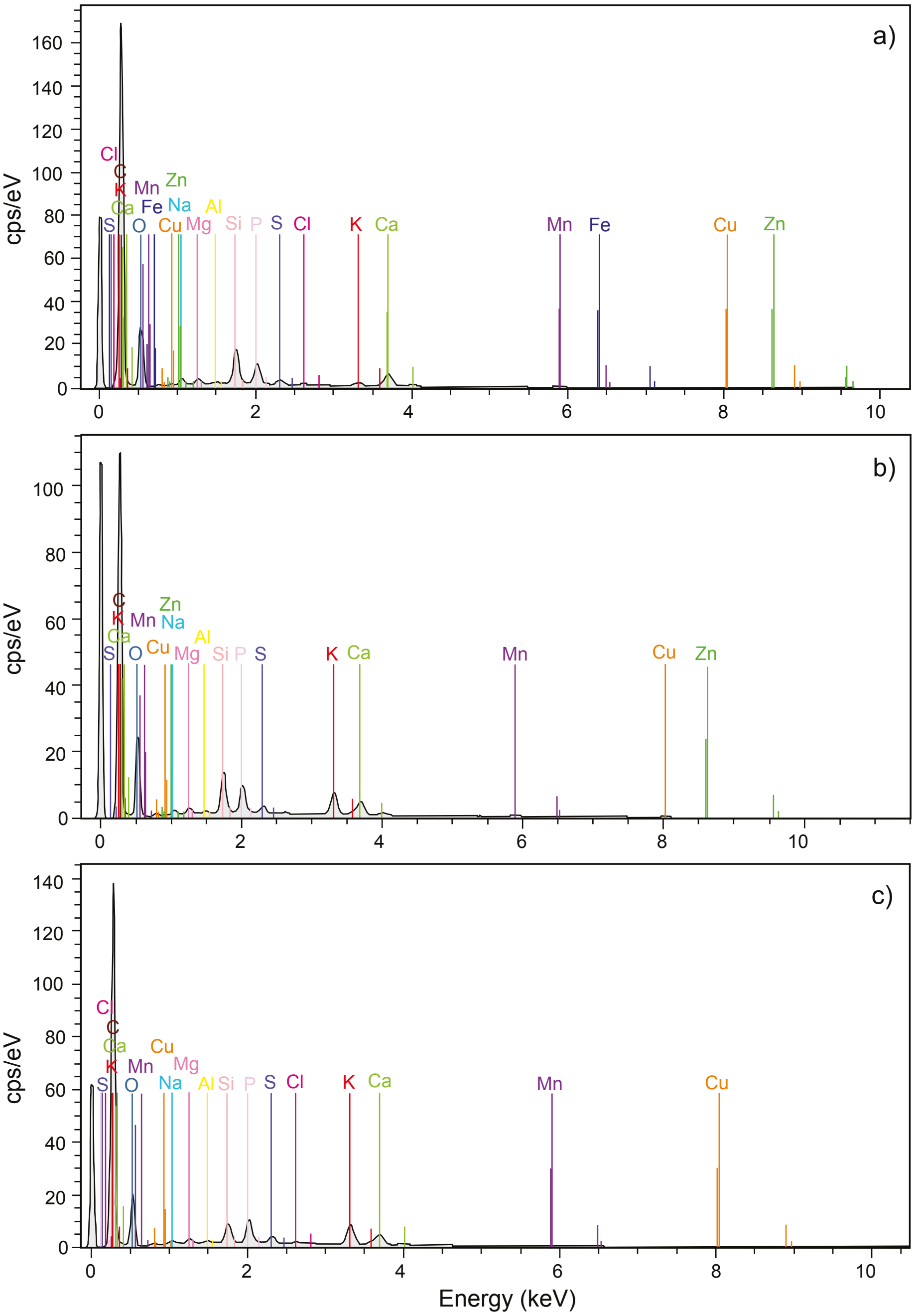

Figure 15. Chemical composition of Mombaza grass plants determined by scanning electron microscopy and X-ray energy dispersion spectroscopy, which were harvested on sewage sludge during the a) first month; b) second month; and c) third month of cultivation.

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