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Compost and residues from biogas plant as potting substrates for salt-tolerant and salt-sensitive plants

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Dedication

This thesis is dedicated to my beloved family: my husband (**Trần Đăng Thuần**), my parents (**mẹ Mai, mẹ Nhung & bố Thược**), my brothers and my sister.

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LIST OF ABBREVIATIONS

CEC	Cation exchange capacity
Coco	Cocofiber
Comp	Unleached compost
Comp-S	Leached compost
CTC	Chlortetracyline
BioR	Unleached residues from biogas plant
BioR-S	Leached residues from biogas plant
DM	Dry matter
E_c	Salt content (mS/cm)
E. coli	*Escherichia coli*
FS	*Fecal streptococci*
HC soil	Soil with a high carbon content
Hy	Hygromull
LC soil	Soil with a low carbon content
Le	Lecaton
Per	Perlite
SMZ	Sulfamethazine
SPS	Standard soil substrate type ED 73 produced from 70% white peat and 30% clay
Sty	Styromull
TKS_0	Standard soil produced from 100% white peat
TKS_1	Standard soil produced from mainly white peat and partially black peat, lower salt content
TKS_2	Standard soil produced from mainly white peat and partially black peat, higher salt content
WHC	Water-holding capacity

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Summary

Compost and residues from biogas plant have been increasingly recognized as potting substrates in horticulture. To investigate the suitability of both materials to grow salt tolerant plants in 2010 a pot experiment was conducted in the greenhouse of INRES-Plant nutrition, University of Bonn. Ryegrass (*Lolium perenne* L.), rape (*Brassica napus*) and sunflower (*Helianthus annuus*) were chosen as experimental plants. To reduce the high salt content compost and residues from biogas plant were leached. To improve physical characteristics of raw materials, additives including Perlite, Styromull, Hygromull, Lecaton, Peat, Cocofiber were incorporated into compost or residues from biogas plant with the volumetric ratio of 4:1. Plant growth (DM) and nutrient uptake (N, P, K, Mg, Ca, Na and S) of the experimental plants grown in compost-based or residue-based substrates with and without additives and standard soil as a control were determined. Preliminary results reveal that origin compost and residues from biogas plant without leaching are suitable potting substrates for those plants. For compost leaching may not be recommended while for residues from biogas plant the effect of leaching was not distinct and needs further investigations. The incorporation of additives into the basic materials partially resulted in higher plant dry matter yield and nutrient uptake. However, differences between the additives on both parameters were mainly insignificant. Incorporation of Hygromull or Peat, especially into residues from biogas plant favored plant growth and enhanced total nutrient uptake.

In 2011, pot experiments were continued with the salt-sensitive ornamental plants, Pelargonium (*Pelargonium zonale Toro*) and Salvia (*Salvia splendens*). Two separate experiments were carried out for the mixtures of compost and additives (SPS standard soil type 73 based on Peat, Hygromull or Cocofiber) with different volumetric ratios (4:1, 1:1, 1:4) and the mixtures of Peat incorporated with small proportions of compost and additives (Styromull or Perlite). The results show that the large percentage (> 50% by volume) of compost in the substrate had negative effects on plant growth and nutrient uptake (N, P, K, Mg and Na) because of its high salt content in compost-based substrates. However, both yield formation and nutrient uptake of the studied plants when grown in peat-based substrates significantly increased comparing to those of compost-based substrates and almost gained the level of the control. Especially, the growth of Salvia was significantly improved. Consequently, compost-based media (with more than 50% of compost) may not be recommended for salt sensitive ornamental plants, while less than 25% volume of compost incorporated with Peat creates favorable peat-based substrates which reasonably enhanced growth of Pelargonium and Salvia.

Investigating antibiotic uptake by cabbage (*Brassica oleracea* var. capitata f. abba) from the manure-amended soils containing high and low carbon content spiked with the two antibiotics Chlortetracycline and Sulfamethazine was targeted as the third objective. The input concentrations of the studied antibiotics were 100, 200 and 400 µg/kg regarded to their present concentration range in Chinese top soils. The antibiotics in plant materials were analyzed by HPLC-MS after extraction. The results reveal that the presence of available high carbon content in the soil increased crop yield of cabbage. However antibiotics were not detected in the cabbage materials according to the antibiotic employment with the initial studied concentrations. It may be concluded that with the small amounts of antibiotics applied to the soils, there is no risk of uptake of antibiotics by plants.

Zusammenfassung (Summary in German)

Kompost und Reststoffe aus Biogasanlagen werden zunehmend recycelt, um schließlich als Kultursubstrate für gartenbauliche Zwecke zu dienen. Um die Eignung beider Materialien für den Anbau Salz-toleranter Pflanzen zu überprüfen, wurde im Jahr 2010 im Gewächshaus am INRES-Pflanzenernährung ein Gefäßversuch durchgeführt. Als Versuchspflanzen wurden Ausdauerndes Weidelgras (*Lolium perenne* L.), Raps (*Brassica napus*) und Sonnenblume (*Helianthus annuus*) angebaut. Um deren hohen Salzgehalt der Substrate abzusenken, wurden Kompost und Reststoffe aus einer Biogasanlage ausgewaschen. Zur Verbesserung physikalischer Eigenschaften beider Materialien wurden verschiedene Zuschlagstoffe (Perlit, Styromull, Hygromull, Lecaton®-Blähton, Torf oder Kokosfaser) in Kompost und Reststoffe aus einer Biogasanlage in einem Volumen-Verhältnis von 4:1 eingemischt. Der Trockenmasse-Ertrag und die Nährstoffaufnahme (N-, P-, K-, Mg-, Ca-, Na- und S-Gehalt) von Weidelgras, Raps und Sonnenblume wurden erfasst und mit der Kontrolle verglichen. Die Ergebnisse zeigen, dass Kompost und Reststoffe aus einer Biogasanlage auch ohne Salzextraktion als Pflanzsubstrate geeignet sind. Bei Kompost wird deshalb eine Salzextraktion nicht empfohlen, bei Reststoffen aus einer Biogasanlage war der Einfluss dagegen nicht eindeutig. Hierzu sind weitere Untersuchungen erforderlich. Die Einarbeitung der Zusätze in das Ausgangsmaterial erhöhte den Trockenmasse-Ertrag und die Nährstoffaufnahme der Pflanzen. Dabei war zwischen den verschiedenen Zusätzen meist kein signifikanter Unterschied festzustellen. Wurden Hygromull oder Torf als Zusatz verwendet, so begünstigten diese, besonders in Kombination mit Rückständen aus der Biogasanlage, das Pflanzenwachstum und die gesamte Nährstoffaufnahme.

Im Jahr 2011 wurden weitere Gefäßversuche mit den salzempfindlichen Zierpflanzen Geranie (*Pelargonium zonale)* und Feuersalbei (*Salvia splendens*) durchgeführt. Es handelte sich um zwei unabhängige Versuche, einer mit Kompost-Mischungen (SPS Bodensubstrat Typ 73, basiert auf Torf; Hygromull oder Kokosfaser) mit verschiedenen volumetrischen Verhältnissen (4:1, 1:1, 1:4) und der andere mit einem Gemisch aus Torf, etwas Kompost und Styromull oder Perlit. Die Ergebnisse zeigen, dass ein hoher Kompost-Anteil (> 50% des Volumens) aufgrund des relativ hohen Salz-Gehalts negative Auswirkungen auf das Pflanzenwachstum und die Nährstoffaufnahme (N, P, K, Mg und Na) hat. Auf Torf basierende Substrate jedoch erzielten sowohl einen signifikant höheren Ertrag als auch eine gesteigerte Nährstoffaufnahme im Vergleich zu auf Kompost basierenden Substraten und erreichten beinahe die Werte der Kontrolle. Besonders das Wachstum von Feuersalbei wurde signifikant verbessert. Folglich wird für Salz-sensitive Pflanzen empfohlen, nicht mehr als 50 Vol.-% Kompost in das Substrat zu mischen. Eine Substrat-Mischung mit Torf und weniger als 25 Vol.-% Kompost bildet eine gute Grundlage für ein gesundes Wachstum von Geranien und Salbei.

Drittes Ziel der Arbeit war zu überprüfen, ob Antibiotika aus einem mit Mist gedüngtem Boden mit hohem bzw. niedrigem Kohlenstoff-Gehalten von Weißkohl (*Brassica oleracea var. capitata f. abba*) aufgenommen werden. Dazu wurde der Boden mit den Antibiotika Chlortetracyclin und Sulfamethazin (100, 200 und 400 µg/kg Boden) versetzt, was die aktuelle Konzentrationsbreite chinesischer Oberböden widerspiegelt. Die Antibiotika im Pflanzenmaterial wurden nach Extraktion mittels HPLC-MS bestimmt. Die Ergebnisse zeigen, dass hohe verfügbare C-Gehalte im Boden den Ertrag bei Kohl erhöhen. Antibiotika wurden jedoch nicht im Kohl nachgewiesen. Es besteht also kein Risiko, dass Böden, die bis 400 µg/kg Antibiotika enthalten, komtaminierte Pflanzen hervorbringen.

Chapter 1

General Introduction

1.1. Organic waste recycling

1.1.1. Situation of organic waste recycling in China

Organic wastes have been increasingly released as a result of dramatically over-population development, urbanization, and human activities that are mostly occurring in Asian developing countries. These countries are facing with very serious environmental problems such as air, water, and soil pollution which are the result from shortage of organic solid waste management. Notably, China is one of the third largest countries with the highest population of over 1.3 billions in 2011 (Data source: World Bank 2011) contributing to a huge mass and volume of organic wastes in the world. Recently, China has become the largest waste generator in 2004 after surpassing United States (US) (Zhang et al. 2010). Burden of a huge population and fast industrialization in China, it has forced a pressure on Chinese food production meeting high demands of vegetables, meat, milk, eggs, etc. Therefore, the industry of slaughtering livestock (pig, cattle, buffalo, chicken, duck …) in China has been increasingly developed. In 2005 about 50% of pig meat and about 76% duck meat of the total world production were produced (Data source: Food and Agriculture Organization of the United Nations 2004). The intensive pig farms in China are mainly concentrated in five regions including Beijing, Tianjin, Zhejiang, Fujian and Hainan. Total output of animal wastes from livestock raising industry in China was estimated as 3.19 billion tons, which was accounted for 3.2 times the solid wastes generated by industrial sectors in 2004 (Data source: Chinese statistics of livestock production 2004). The amount of animal wastes is likely to reach 6 billion tons until 2015. The average animal waste load on farmland was about 24 tons/ha in 2008, especially high as 49 tons/ha in the Beijing area. With an extremely high contribution to the total waste amount, the waste source from livestock husbandry is determined as the main pollution source in China (Table 1).

Table 1: Total annual amounts of organic raw materials in China

Crop straws and stalks	400 million tons
	490 million tons
	673 million tons
	795 million tons
Animal excreta (feces and urine) (wet weight)	2.7 billion tons
	3.2 billion tons
	4.3 billion tons
Oliseed cake wastes	19.6 million tons
	25 million tons
Green manures	342 million tons
Human excreta	300-350 million tons
Slaughtered house waste	50-65 million tons
Sewage sludge (dry)	55 million tons
Municipal Solid Waste (MSW)	0.5-0.7 billion tons

Data source: Ju et al. (2005)

Parallel problems with the intensive livestock and crop production in Chinese agriculture are dealing with a huge mass of organic wastes from animals and plant residues. Consequently, new environmental pollutions have been formed, which are organic wastes such as remnants of vegetables and flowers, animal wastes, straw, etc. In connection to these agricultural activities, a series of environmentally hazardous pollutions in China are included: polluted aquifers due to direct discharge without pre-treatment, overloading of nutrients because of over application of animal manures to cropland, leaching of nitrate, soil pollution from feed additives including heavy metals, antibiotics, increased air pollution and greenhouse gas emission (CO_2) as a consequence of straw burning, etc. Furthermore, the sanitary of Chinese agriculture has been still very low, which causes negative effects on human health. The over application of animal manure to crop land may result in an enrichment of human pathogens and parasites such as *Salmonella, Escherichia coli, Campylobacter, Rotavirus Cryptosporidia*, Aujezsky's Disease (Pseudorabies) virus ….On the other hand, the soils in China are exhausted in nutrients due to intensive cropping and contain low soil organic matter content. Thus, Chinese farmers often choose a simple and conventional way to fertilize the soils by adding ash from burning straw, which unexpectedly caused other air pollution.

The producing scale in China is mainly on a household-based size. Therefore, the management and treatment of wastes have still a low level, especially in rural and suburban regions. Also, Chinese farmers have lack knowledge about the potential nutrition value in agricultural wastes and psychological resistance to waste materials as usefulness such as branches of plants, straw, plant stem and roots which are improper used and freely disposal. In fact, Chinese organic wastes from agricultural production are plentiful and abundant of organic matter and contain essential nutrients for plants (N, P, K) which can be recycled (Table 1). Bao et al. (2003) conducted some surveys in 18 Chinese provinces on total nutrient input derived from organic manure added to cropland. They found that about 35% of the total nutrient from manure and mineral fertilizers, containing 18% N, 28% P, 75% K, were applied to cropland (Bao et al. 2003). It was estimated that only 29.2% N, 43.5% P and 66.1% K in organic manure were averagely recycled. Therefore, at the present K mainly comes from organic manure, and N and P are mainly supplied as chemical fertilizers (Ju et al. 2005). Organic manure was partially distributed to different cropping systems, occupying some percentage of the total area such as grain crop (52.3%), vegetables (81.3%), fruit tree (77.0%), and remaining cash crop (29.2%), respectively (Ju et al. 2005). In 2007, China was ranked as the top country for cultivation of vegetable production, but the fertilizer application rate in China was high, reaching 51 million tons with a very high level of N up to 450 kg/ha in 2007 (Liu et al. 2009). The high content of N (Urea and N-NH_4^+) in the soil may be transformed into nitrate (NO_3^-) causing nitrate contamination of vegetables. However, there are still some horticulture areas where not enough fertilizers are applied, but also some areas like cash crops might be supplied excessive with mineral N fertilizers (Ju et al. 2004). Furthermore, livestock manure contaminated with antibiotics may be added to soils and then accumulated in plants and vegetables (Boxall et al. 2006). These substances may potentially become harmful to people’s health. Therefore, it is very important and necessary to develop a sustainable and safe food production in agriculture. Recycling nutrients in Chinese organic wastes are potential and abundant which can be applied to improve the quality and quantity of soil, crops and bring a lot of benefits to farmers and environment.

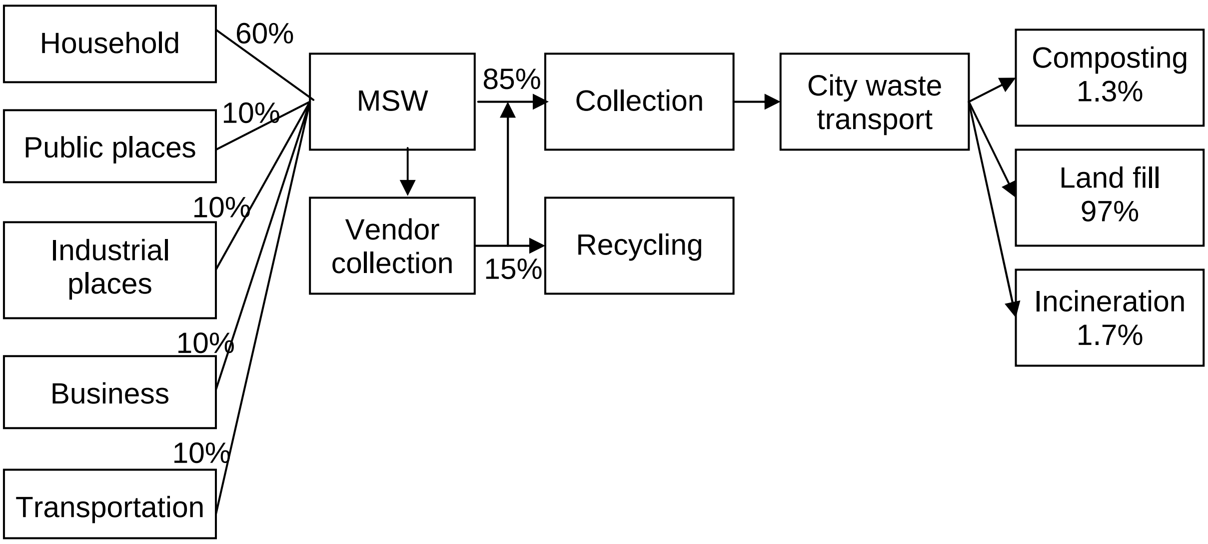

Figure 1. Municipal solid waste flow in Chinese cities (Idris et al. 2004)

So far landfill has still been a traditional dominant method of municipal solid wastes (MSW) treatment of Chinese cities. In a survey conducted for 138 Chinese cities in 2000, most of collected MSW was treated by 11 landfills (97%), followed by incineration (1.7%), only small percent occupied by composting (1.3%) (Figure 1) (Idris et al. 2004). This means there are a lot of environmental pollutions related to landfill sites such as groundwater pollution due to less or ignored leachate treatment of wastes, pollutions of surface water, land and air (Hui et al. 2005). Furthermore, landfill needs a lot of space, but land area is limited in cities (Hui et al. 2005). Moreover, the second popular method of MSW treatment in China was incineration requiring a costly installation, operation, and maintainance investment which were unaffordable for most Chinese cities (Idris et al. 2004). With high moisture content and low caloric value in solid wastes, the incineration systems in China are unable to avoid creating dioxins and fly ash pollution which becomes a very urgent problem and challenge (Hui et al. 2005). Therefore, the mentioned MSW methods above are not completely benificial and not sustainbale waste-treating methods in a long term. Nevertheless, composting occupies a very limited percent among MSW treatment methods in China. The composting scale of organic waste treatment is improper with the huge mass and volume of Chinese solid wastes and still much lower than in industrialized countries such as Germany, Sweden, Japan, USA, etc. (Zhang et al. 2010).

Organic wastes in China were accounted to have a large proportion in the whole MSW, however only 2.9 million tons were composted in 2006 (Chen et al. 2009). It is a waste of recylable abundant nutrient resources if wastes are dumped and burned without recycling and reusing. All residues or organic wastes from Chinese farms and households should be well-managed, reused and processed in a simple way by composting. Products from composting can then be used and applied widely as potting substrates in horticulture, which is very important for the new system of recycling organic residues in China.

Besides, Chinese government has recently invested and encouraged to develop a number of biogas plants (Liu et al. 2009). That is aimed at not only supplying more energy for the huge population country but also contributing to improving environmental quality. Residues from biogas plants were recognized to be beneficial fertilizers as well as potting substrates for planting. Yield, nutrition quality and stress resistance of vegetables were proved to be better when biogas manure was applied in horticulture. Referred to China National Plan on Rural Biogas Construction Projects in the period of 2006-2010, over 20% the amount of chemical fertilizers and pesticides, and less than 1% of pesticide residues were reduced by utilizing biogas manure (Liu et al. 2009).

It is clear that China has enough advantageous conditions to develop and extend the scale of organic waste treatments such as composting, biogas plants which are considered to overcome and solve all problems of the mentioned conventional methods of waste treatment. The application of products of compost or residues from biogas plant brings environmental, economical and nutritional values for safe crop production.

1.1.2. Brief introduction of Sino-German project

Regarding the sustainable agricultural production, the vegetable quality and the security of food, and the environment quality need to be improved in China, the project “Recycling of organic residues from agricultural and municipal origin in China” cooperates between Germany and China was born and ended during three years (September 1, 2008 - March 31, 2012). The project was divided into 9 sub-projects and this study belongs to the sub-project 7 which focuses on improving vegetable quality and increasing the security of food and environment by cycling organic wastes from agriculture in the ecosystem of greenhouse. The objectives of this sub-project are to optimize the use of residues from intensive livestock raising and further to develop substrates and raising for intensive ornamental and vegetable production. In detail, the objectives of sub-project 7 could be addressed as:

- To optimize the production of substrates and fertilizers derived from anaerobically and/or aerobically treated pig manure fractions for intensive horticultural production
- To optimize the substrate and fertilizer application with respect to yield and quality of vegetables and ornamentals in greenhouses

1.1.3. Research objectives

There are some focused objectives that the study targets:

1. Developing potting substrates derived from compost and residues from biogas plant as basic raw materials for salt-tolerant plants (pot experiments in 2010)
2. Developing potting substrates derived from compost as potting media component for salt-sensitive plants (pot experiments in 2011).
3. Investigating uptake of antibiotics by cabbage (pot experiment in 2011).

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Chapter 2

Compost and residues from biogas plant as basic raw materials for potting substrates of salt-tolerant plants

Abstract

Organic wastes should be recycled from an ecological as well as an economical point of view. For this reason compost and residues from biogas plant have been increasingly recognized as a viable management method for solid organic wastes aimed at recycling of its end-product as a potting substrate in horticulture. Plants including ryegrass (*Lolium perenne L.*), rape (*Brassica napus*) and sunflower (*Helianthus annuus*) were chosen to study in the first step because of their salt tolerance. For this first-stage plant experiment, to reduce high salt content and improve physical characteristics of the raw materials, available additives including Perlite (Per), Styromull (Sty), Hygromull (Hy), Lecaton (Le), Peat (Peat), Cocofiber (Coco) were incorporated into compost or residues from biogas plant with the volumetric ratio of 4:1. Besides, compost and residues from biogas plant were leached to produce raw materials having a much lower salt content. This chapter reports plant growth (dry matter yield) and nutrient uptake (N, P, K, Mg, Ca, Na and S) of plants grown in compost or anaerobically digested residues with and without additives and standard soil as a control. The results reveal that untreated compost and residues from biogas plant are suitable potting substrates, while leaching of raw materials is not recommended for compost. For residues from biogas plant it was not pronounced and needed further investigation. The incorporation of additives into the basic materials partially resulted in higher yield formation and nutrient uptake. However, the effect among additives on yield formation as well as nutrient uptake of the studied plants was mainly insignificant. Incorporation of Hygromull or Peat, especially into residues from biogas plant favored plant growth and enhanced the total uptake of nutrients, which was attributed to the fact that Hygromull or Peat stored nutrients and delivered them even at later stage during the growing period. Furthermore these additives with high water holding capacity reduced the salt concentration of the medium and thus favored growth of younger plants.

Key words: Compost, residues from biogas plant, additives, nutrient uptake, yield formation.

2.1. Introduction

Organic wastes increasingly released as a result of development of agriculture, industry, and other activities of humankind have been concerned as recyclable resources. These organic wastes could be recycled by applying aerobic and anaerobic digestion in composting and biogas production processes. Products of composting as well as biogas production are compost and residues from biogas plant which could be used as essential materials for soil improvement. By application of these two methods, waste volume could be largely reduced and essential nutrients were stabilized and reused in horticulture and agriculture.

Composting represents an effective way to recycle residues originated from agriculture, industry and other activities from an ecological as well from an economical point of view. For this reason, composting has been incrementally recognized as a viable management method for solid organic wastes. One of the advantages of composting is the possible recycling of its end-product, compost as a soil conditioner. There are plenty of compost benefits such as gradually releasing nutrients like N (Scherer et al. 1996) and P (Scherer 2004), supplying mineral or synthetic nutrient sources for plant growth, increasing buffer properties, cation exchange capacity (CEC), pH value, enhancing soil physical properties (reduction of soil density and soil loss as well as runoff of surface water, good drainage) (Amlinger et al. 2007) etc. Because of lots of benefits, the application of compost in agriculture has been recognized for decades. However, scientific researches have been recently published and illustrated its beneficial use of compost in crop production since the 20th century years (Jackson 2005, Sarwar et al. 2007). There were series of studies on compost used as potting substrates for ornamental plants derived from a lot of organic sources including biosolid (municipal) wastes (Wilson et al. 2004, Mami and Peyvast 2010), agroindustrial wastes (Garcia-Gomez et al. 2002), pruning wastes (Benito et al. 2005), poultry wastes (Das et al. 2002, Hachicha et al. 2006), yard trimmings (leaves and green wastes) (Cook et al. 1998, Lópeza et al. 2010), sewage sludge and horticultural wastes (Stabnikova et al. 2005), olive-mill wastes (Papafotiou et al. 2004), seagrass (Klock-Moore 2000), earthworm castings (vermicompost) (Suthar 2009, Theunissen et al. 2010), paper mill wastes (Hachicha et al. 2006) etc. Other directions of research on compost in agriculture and horticulture are still potential with other different materials. Recently, the incorporation of compost and other supplements has been received considerable concerns. This was aimed at improving physical soil conditions and increasing productivity and quality of crop production (Wilson et al. 2004, Mami and Peyvast 2010, Garcia-Gomez et al. 2002). This study also presents the suitability of using compost as one of target basic materials for crop production.

Residues from biogas plant are by-products of biogas production which remnants are partly undegraded and finally remained from the anaerobic digestion. The wastes for biogas production are mainly originated from plants and animal wastes, therefore the residues contain abundant amounts of micro and macro elements which are essential for crop production. Notably, residues from biogas plant are a sufficient nitrogen source for plant growth (Båth and Rämert 1999). The concept of the application of residues from biogas plant in agriculture is relatively new (Odlare 2005). These residues are not only an useful and rich nutrient source but also recycling indirectly contributes to reducing the greenhouse gas emission into the atmosphere as a result of its potential replacement of chemical fertilizers in the future (Arthurson 2009, Ahmad and Jabeen 2009). Governmental funding for biogas production has been becoming more important in Germany since the two last decades. Also in China, due to the demand of energy to supply for a huge population and dramatic industrial development, numbers of biogas plants supported by the Chinese government have been increasingly established (Liu et al. 2009). Liu et al. (2009) indicated that 18 million methane tanks were used by farmers at the end of 2005 in China rural areas, and 1500 large-scale plants of livestock production were run by enterprises. According to an estimation of the 11th five-year plan of Renewable Energy Development of China, there were about 40 million biogas-using farmers, 4700 biogas projects for large-scale plants of livestock production and 1600 biogas projects for treatment of organic wastes from industry China by 2010 (Liu et al. 2009). Thus, a large amount of residues from biogas plant has been annually produced that leads China to a new environmental and ecological problems such as eutrophication of surface water, enrichment of nitrate in the groundwater, soil salinization, space limitation, etc. Acknowledgement of these issues, China has been developing a win-win safe use of biogas manure as crop fertilizer to solve the environment issue and gain an economic effectiveness (Liu et al. 2009). Therefore, residues from biogas plant are available and increased incrementally in China. They have been studied to apply for crop production since two recent decades and applied for some vegetables such as lettuce, Chinese cabbage, rape, tomato, cucumber, garlic, green pepper, bitter gourd, etc., achieving a lot of benefits (Liu et al. 2009). Additionally, anaerobic digested residues are also applied to many other plants including ornamental plants for example sunflower to improve plant weight and replace chemical fertilizers in organic farming (Ahmad and Jabeen 2009), corn with higher crop production proportional to application rate of residues (Rivard et al. 1995), spinach and komatsuna having a high fresh yield and N uptake at early harvest (Furukawa and Hasegawa 2006). One important concerned issue on the hygienic security of the application of residues from biogas plant is that they do not contribute to a contamination of *E. coli*, FS, or *V. parahaemolyticus* in the soil or on plant leaves (Furukawa and Hasegawa 2006). However, scientific knowledge about the utilization of residues from biogas plant documented in agriculture is still limited. Thus, it is necessary to have more intensive research on the exploring application and effectiveness of anaerobically digested residues recycling in agriculture. Therefore, this is targeted as an important task mentioned in the previous research objectives.

Soilless culture

Due to many advantages, compost and residues from biogas plant have been widely applied in agriculture. However, these two raw materials were reported to have negative effects on plant growth when they are applied alone to use as potting substrates (Svensson et al. 2004). Many reports have recently documented that compost or residues from biogas plant were incorporated with various additives aimed to improve the quality of potting medium, resulting in higher crop yield and quality of plants (Benito et al. 2005, Garcia-Gomez et al. 2002, Jackson 2005, Mami and Peyvast 2010). These additives such as Perlite, Fine bark, Styromull, Hygromull, Peat, Cocofiber, Lecaton, etc. are mainly artificially produced. To choose a material as an additive incorporated into potting substrates, it must have optimal pore space, high CEC, good-heat capacity, and low heat-conductivity (Boodt and Verdonck 1971). That means a substrate value must be taken into account some aspects of water, air, plant nutrition, and heat economies. Moreover, based on plant species and characteristics of potting media, those additives were decided to be added into basic materials with optimal well-specified physical properties. Below, some characteristics of available additives being considered as standards for this study:

(i) Perlite: a light weight, white, expanded, closed volcanic Al-silicate mineral which increases the aeration because of its physical stability and ability to provide no capillary pore space in a mixture (Ingram et al. 1993). Perlite increases conductivity, but does not change pH and P and K concentration in the substrate (Gachukia and Evans 2008).

(ii) Styromull: a very light and closed-pore polystyrene foam containing 98% volume of air. Therefore, this substance can improve the substrate aeration and highly-withstanding natural compression and being important for greenhouse and outdoor horticulture. However, there is also a disadvantage of Styromull that is low bulk density determined as 25 kg/m^3 (Boodt and Verdonck 1971).

(iii) Hygromull: an open-pore hydrophilic polymer of urea and formaldehyde foam with 60-70% volume of open and 30-40% volume of closed pores; increases the water-holding capacity and the air economy (Boodt and Verdonck 1971). Similar to Styromull, Hygromull has a very low bulk density of 35-40 kg/m^3.

(iv) Peat: a type of soil originated from partially decomposed mosses or sedges accumulating in bogs ranging from over hundreds to thousands of years (Ingram et al. 1993). It has a high water-holding capacity, mostly sterile and an excellent structure for plant growth. For instance, Peat derived from sedges, reeds and grasses has the ability to bind certain soil-applied plant growth regulators, such as Cycocel, more than other types of Peat (Ingram et al. 1993). Peat moss is the most popular horticultural media (Ingram et al. 1993). Also, Sphagnum peat is often used as basic material (Olympios 1992).

(v) Lecaton: thermally lightweight expanded and burnt clay granules which are proven for over 20 years as the leading substrate in hydroponics and containers (Gartenversand Omega, Lauf, Germany). Lecaton is completely inert, pH neutral and reusable. It can be used alone or amended with other growing media to increase oxygen level and improve drainage of substrates, this helps to prevent root rot and reduce watering requirements. Additionally, Lecaton gives plants extra weights to resist collapse by strong winds or animals. On the other hand, it is able to absorb cations and may release Ca.

(vi) Cocofiber: made from fibers of coconut hull (mesocarp). Coconut fiber has a large content of lignin and low content of cellulose due to its malleability and high durability. Cocofiber has good physical and biological conditions for root development of plants (Treder 2008). Additionally, Cocofiber is strongly hygroscopic and readily absorbs moisture. These characteristics help to improve the aeration and being good at water-holding capacity.

This study was aimed to investigate the suitability of compost and residues from biogas plant as target basic materials alone or in combination with additives for potting substrates. To get a first insight ryegrass, rape and sunflower with medium salt tolerance were chosen as experimental plants.

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2.2. Materials and methods

2.2.1. Raw materials

Two basic raw materials were used including compost (Comp) originated from green wastes of Reterra compost plant in Erftstadt, Germany and residues from biogas plant (BioR) derived from pig manure and maize as main pig feeding of Julius-Kühn-Institut in Braunschweig, Germany (Figure 2).

Compost Residues from biogas plant

Figure 2. Raw materials

Compost and residues from biogas plant contain significantly higher salt content comparable to the standard soil (Table 2), which may have a potentially negative effect on plant growth, especially at the young growing or seeding stage (Rosen et al. 1993). It was suggested that compost and residues from biogas plant must probably pretreated by leaching before use (Rosen et al. 1993, Liu et al. 2009). Therefore, before setting up the pots in the greenhouse, compost and residues from biogas plant were leached to produce raw materials having much lower salt content. By this way, four types of materials including unleached compost (Comp), leached compost (Comp-S), unleached residues from biogas plant (BioR), leached residues from biogas plant (BioR-S) were created. The chemical characteristics of raw materials were determined in which the standard soil TKS_1 (Peat-based control) was included (Table 2). The salt content (E_c) was lowest in TKS_1, followed by unleached compost and residues from biogas plant. The same order was found for plant available P, K and Mg and total S, while total N was highest in compost. After leaching, the salt content in compost and anaerobically digested residues was remarkably reduced while the content of nutrients was almost not changed. These parameters were used as fundamental information to make a decision for setting up potting substrates in the greenhouse.

Table 2: Chemical characteristics of raw materials for pot experiment in 2010

Material	DM %	pH ($CaCl_2$)	E_c mS/cm	P_{Total} mg/kg	CAL-P mg/kg	K_{Total} mg/kg	CAL-K mg/kg	Mg_{Total} mg/kg	Mg-$CaCl_2$ mg/kg	Na_{Total} mg/kg	N_{Total} mg/kg	S_{Total} mg/kg	C_{Total} mg/kg
TKS_1	59.4	5.6	1.4	754	387	2187	255	1505	950	3850	9840	40	481080
Comp	63.7	8.0	2.8	3398	1417	14130	7308	6474	1045	1781	18770	9530	198630
Comp-S	43.7	8.0	1.3	2681	1396	10410	7450	5312	881	1057	17400	9560	202520
BioR	54.1	7.8	3.7	5361	3003	25830	12599	6040	1571	1292	17070	20140	448240
BioR-S	81.8	7.6	2.3	5632	2557	17690	8400	6767	1707	926	18150	20430	448900

Samples were analyzed with three replicates and average values are presented

2.2.2. Additives

The studied additives included Perlite (Per), Styromull (Sty), Hygromull (Hy), Lecaton (Le), Peat (Peat), Cocofiber (Coco) (Figure 3). Each additive was incorporated into compost or residues from biogas plant at the rate of 20% by volume.

Perlite Styromull Hygromull

Lecaton Peat Cocofiber

Figure 3. Additives added to basic materials

2.2.3. Experimental design

Experimental plants were ryegrass *(Lolium perenne* L.*),* rape (*Brassica napus*) and sunflower (*Helianthus annuus*) grown during the periods of April to September, April to June and June to July 2010, respectively, in the greenhouse of INRES-Plant nutrition, University of Bonn, Germany (Figure 4). The reasons to choose these plants are because of their moderate salt tolerance and ability of grass re-growing after cutting several times and the development roots of rape and sunflower which can take up a high amount of nutrients from substrates. Plants were seeded in 6-liter pots (25 cm diameter × 30 cm height). Ryegrass was sown in four rows and regularly cut for four times (each 30 day interval) at 2 cm above the surface of pot substrates. Rape (four seeds per pot) grown in the compost and residues from biogas plant was harvested after 51 and 58 days, respectively; then sunflower (five seeds per pot) was in turn seeded on the same substrates as rape and harvested after 40 days of cultivation. The plants were watered with distilled water every day (60-70% of the maximum water-holding capacity).

Figure 4. Pot experiments in the greenhouse with salt-tolerant plants

The experiment consisted of 30 treatments with four replications (Table 3) divided in two groups of unleached/leached compost and unleached/leached residues from biogas plant. After each harvest, fresh plant materials were dried at 60^{o}C in a thermal oven until constant weight, then finely ground into powder and stored in plastic bottles before analysis.

Table 3: Treatments of pot experiments in 2010

No.	Treatment	Potting substrate	No.	Treatment	Potting substrate
1	Control	Standard soil: TKS$_1$	16	Control	Standard soil: TKS$_1$
2	Comp	100% unleached compost	17	BioR	100% unleached residues from biogas plant
3	Comp Per	80% unleached compost + 20% Perlite	18	BioR Per	80% unleached residues from biogas plant + 20% Perlite
4	Comp Sty	80% unleached compost + 20% Styromull	19	BioR Sty	80% unleached residues from biogas plant + 20% Styromull
5	Comp Hy	80% unleached compost + 20% Hygromull	20	BioR Hy	80% unleached residues from biogas plant + 20% Hygromull
6	Comp Le	80% unleached compost + 20% Lecaton	21	BioR Le	80% unleached residues from biogas plant + 20% Lecaton
7	Comp Peat	80% unleached compost + 20% Peat	22	BioR Peat	80% unleached residues from biogas plant + 20% Peat
8	Comp Coco	80% unleached compost + 20% Cocofiber	23	BioR Coco	80% unleached residues from biogas plant + 20% Cocofiber
9	Comp-S	100% leached compost	24	BioR-S	100% leached residues from biogas plant
10	Comp-S Per	80% leached compost + 20% Perlite	25	BioR-S Per	80% leached residues from biogas plant + 20% Perlite
11	Comp-S Sty	80% leached compost + 20% Styromull	26	BioR-S Sty	80% leached residues from biogas plant + 20% Styromull
12	Comp-S Hy	80% leached compost + 20% Hygromull	27	BioR-S Hy	80% leached residues from biogas plant + 20% Hygromull
13	Comp-S Le	80% leached compost + 20% Lecaton	28	BioR-S Le	80% leached residues from biogas plant + 20% Lecaton
14	Comp-S Peat	80% leached compost + 20% Peat	29	BioR-S Peat	80% leached residues from biogas plant + 20% Peat
15	Comp-S Coco	80% leached compost + 20% Cocofiber	30	BioR-S Coco	80% leached residues from biogas plant + 20% Cocofiber

2.2.4. Analysis Methods

All raw materials, standard soil and plant materials were analyzed according to the standard analysis methods of VDLUFA (Hoffmann 1991, 1995, 1997).

pH-CaCl$_2$ was measured in $CaCl_2$ solution (0.01 M) (m/V ratio: 1/10) using a pH meter with glass combination electrode (MP 220) according to VDLUFA.

Salt content/Electric conductivity (E_c) was determined in water (m/V ratio: 1/10) by electric conductivity measurement (LP 340) according to VDLUFA.

Plant available Mg was determined by extracting 5 g air dried material with particle size less than 2 mm in 50 mL $CaCl_2$ solution (0.0125 M) for 2 hours following the VDLUFA method. The mixed solution was filtered through a 619 gL/4 paper. The filtrates were measured by Atomic absorption spectrophotometer at 285.2 nm (AAS).

Plant available P and exchangeable K were determined by extracting 5 g air dried material with particle size less than 2 mm in 100 mL Calcium-Acetat-Lactat (CAL) solution with a pH of 4.1 for 2 hours following the VDLUFA method. The solution was filtered through a 619 gL/4 paper. The filtrates were analyzed within the next 6 hours using a colorimeter at 430 nm measuring the blue colour intensity after the addition of ammonium monovanadate and ammonium heptamolybdate solution (for available P) and a flame photometer (for exchangeable K).

Dry ashing is suitable for total P, K, Mg, Ca and Na analysis. Using the procedure according to Chapmann and Pratt (1961) with some slight modifications, one gram of material (compost, residues from biogas plant, standard soil or plant materials) was annealed in a 50 mL porcelain crucible for 6-8 hours at 550^{o}C to obtained dried ash materials. After cooling down, saturated NH_4NO_3 (650 g/L) solution was added and dried in a thermal oven at 100^{o}C for 30 min. The dried ash was reannealed for further carbon oxidation in 2 hours. 5 mL HCl (6 N) was added to the oxidized ash and heated up until boiling. The mixture was transferred into 100 mL graduated flask and brought to volume with deionized water. The solution was filtered with filtration paper MN (640 m ø125 mm) into 100 mL flasks. These solutions were ready to dilute for analysis.

Total P, K, Ca and Mg: as previously mentioned, the filtered solutions after dry ashing were used for analysis of P, K, Na, Ca and Mg by the colorimeter (P), flame photometer (K, Na, Ca) and AAS (Mg), respectively.

Total N, S and C were measured by the Elemental Analysis Method. Briefly, 5 mg of samples in a silver cup added with a catalyst V_2O_5 were combusted at 1020°C and separated by gas chromatographic gauge (EuroEA 3000, CHNS-O Elemental vector analyzer). Based on the area of peaks in the detector, the results are determined. All element analyses above were followed strictly by VDLUFA.

Statistical data analysis

Data was processed by using the SPSS 18.0 (Chicago, IL, USA) software with multivariate analysis (ANOVA). Mean differences among treatments for dry matter, nutrient uptake of rape and sunflower harvests, or among different potting substrates for accumulated yield and nutrient uptake of ryegrass after 4 cutting periods were compared at a significance level of $p = 0.05$ by Tukey test.

2.3. Results and discussions

In the past many potting substrates were based on Peat. However, Peat is not a renewable resource and moreover, Peat is becoming difficult to obtain, because of new legislation for the conservation of non-renewable resources and environmental protection (Alexander et al. 2008). Therefore it is widely recognized that compost from solid wastes and residues from biogas plant are valuable sources for potting substrates. However, the high pH and electrical conductivity of both materials (Table 2) are assumed to restrict their use as potting substrates. To overcome these problems Peat and Cocofiber with a share of 20% volume were incorporated to each of the raw materials. In other treatments Perlite, Styromull, Hygromull and Lecaton were added because of the positive effects on the water-holding capacity, aeration or bulk density. In this section, results of parameters (dry matter-DM, uptake of nutrients including total N, P, K, Mg, Ca, Na and S) for the first phase potting experiments executed in 2010 were presented (Figure 5-20).

2.3.1. Grass

The influence of the different additives on plant growth (Figure 5) and uptake of nutrients was not clear cut (Figure 6-12). However, it should be pointed out that especially with unleached residues from biogas plant (BioR) the application of Hygromull resulted in a higher yield formation of ryegrass (Figure 5) and total nutrient uptake, which was significant in the case of P (Figure 7) and Mg (Figure 9). The higher total nutrient uptake is mainly caused by a higher uptake of the fourth cut. This is attributed to the fact that Hygromull with high water-holding capacity is able to store nutrients and deliver them even later in the growing season.

Furthermore based on the dilution effect the salt concentration of the medium is reduced, resulting in favored plant growth. Otherwise, anaerobically digested residues after leaching favored to mix with Peat which slightly increased total P, Mg, Ca uptake (Figure 7, 9, 10) because of the higher speeding rate of growth in the first cut.

2.3.1.1. Yield

Yield formation of ryegrass planted in the control medium (TKS_1) decreased according to the time of cuttings and gained the highest value of 17.4 g/pot in the 1st harvest and the lowest of 2.6 g/pot in the 4th cut (Figure 5). These results differed from all treatments with compost and residues from biogas plant as raw materials, both with and without additives. As compared to the control, DM yield of ryegrass grown in unleached compost and residues from biogas plant treatments was lower in the 1st cut while in the following three cuts the reverse hold true. The highest DM yield of grass planted in these unleached compost and anaerobically digested residue treatments was generally observed in the 2nd cut ranging from 13.9-19.4 and 11.4-15.5 g/pot, respectively. These peak results are related to the high salt and available nutrient content in the raw materials (Table 2), which is assumed to be limiting factors for plant growth in the first stage (Rivard et al. 1995), but soluble salts were strongly reduced later in the growing season (Papafotiou 2004). DM yield of the 1st cut between treatments of unleached compost with additives was comparatively as high as in the 3rd and 4th cuts and ranged between 4.3-11.6 g/pot. The grass kept growing and branching in the last cuts, but the growth rate started to slow down and the height was reduced (Ostos et al. 2008). For the treatments of unleached residues from biogas plant, the 1st cut was observed to have the lowest DM yield of 5.7-12.5 g/pot, lower than the 3rd and 4th cuts ranging from 10.0-14.5 g/pot (Figure 5). This is assumed to be caused by the higher nutrient delivery potential of origin anaerobically digested residues in the later growing period (Rivard et al. 1995). On the other hand, the incorporation of additives into unleached compost resulted in enhancement of DM yield formation of the 1st cut, while these additives added to residues from biogas plant slightly reduced ryegrass yield formation at the same cut (Figure 5). Nevertheless, the trend of ryegrass growth planted in leached compost or residues from biogas plant differed from those of the unprocessed materials in which DM of the 1st cut remarkably increased in the range of 7.4-11.7 g/pot or 11.5-18.3 g/pot, respectively (Figure 5). The significant improvement of yield formation resulted from leaching of raw materials that reduced the limitation of high salt content in potting substrates. However, DM yield of plants observed in the leached compost treatments in the later cuts (3rd and 4th cuts) was estimated as 4.6-8.2 g/pot, significantly lower than those in the unleached compost at the same cuts while yield of plants grown on the leached residues from biogas plant were still relatively high in the 2nd cut (10.9-15.6 g/pot), slightly lower in the last cut (7.3-8.5 g/pot) (Figure 5). This means leaching had positive effects on plant growth at the first stage of planting in both materials. The salt content of residues from biogas plant after leaching still remained high enough to have a positive influence on ryegrass growth later in the growing season, however reversible effect on the yield formation of the plants was observed in the leached compost.

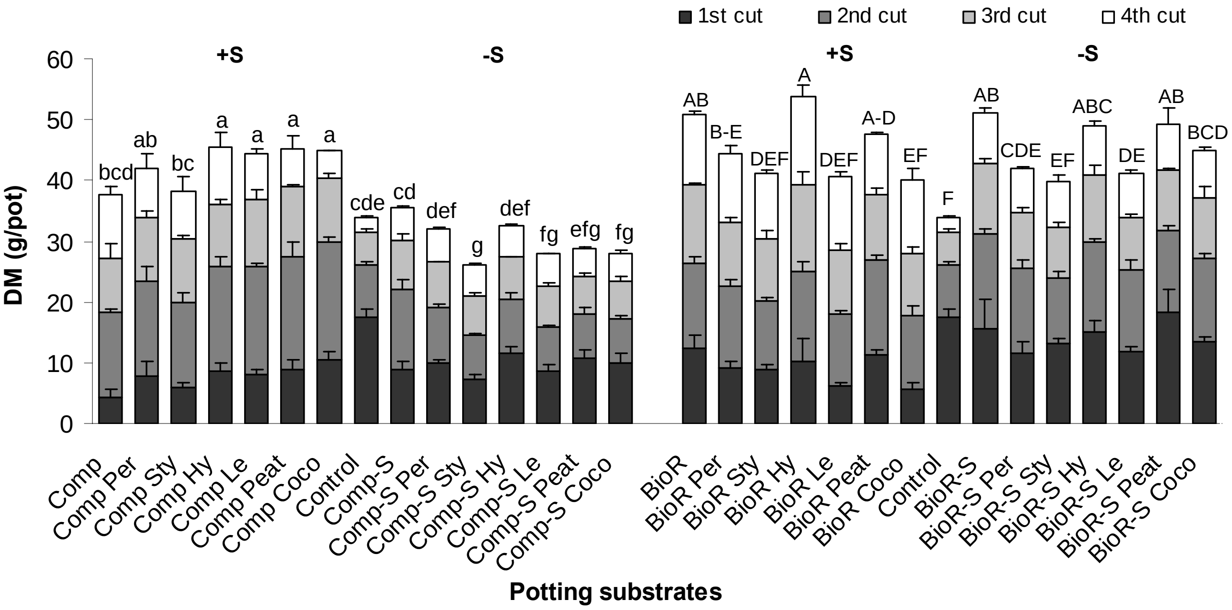

Figure 5. Dry matter yield of ryegrass planted in different potting substrates

Error bars represent the standard deviation of 4 replicates. Accumulative means of different treatments followed by the same letters are not significantly different ($p < 0.05$) by Tukey test.

(+S: unleached Comp/BioR; -S: leached Comp/BioR)

Accumulated DM yield (total of 4 cuts) of ryegrass grown in both pure unleached and leached compost and residues from biogas plant was as high as or remarkably greater than that in the control (Figure 5). Therefore both materials may be recommended for crop production (Svensson et al. 2004). Accumulated DM yield of ryegrass observed in leached compost-based substrates was reduced significantly while it was not distinctly observed in leached residues from biogas plant-based materials (Figure 5). It indicates that the reduction of the salt content after leaching had a negative influence on plant yield formation in compost but unaffected DM yield when planted in residues from biogas plant. Concerning yield production, leaching is not recommended for compost, however, it may be applied for residues from biogas plant using as raw material for young plants.

On the other hand, the impact of additives was not clear cut. The presence of some additives (Perlite, Hygromull, Lecaton, Peat, Cocofiber) favored yield formation, but the difference between the single additives was insignificant (Figure 5). The influence of Styromull on total DM yield was negligible (Figure 5). Moreover, in a study on some kinds of perennials such as Bolivian sunset (*Gloxinia sylvatica*), Brazilian plume (*Justicia carnea*), and Golden globe (*Lysimachia congestiflora*) planted in compost-based media, it was concluded that compost from biosolids and yard trimmings mixed with 75% of Vermiculite/Perlite improved the physical properties of media and gained marketable values of plant size, visual color, and quality of flowers as compared to sole compost (Wilson et al. 2004). In contrast, the incorporation of additives except Hygromull and Peat to the studied residues from biogas plant did not improve or even decreased the grass yield (Figure 5). Intensively, yield formation of ryegrass in the pure residues from biogas plant was remarkably higher than that in the pure compost (Figure 5). Similar results were documented by previous publications in which the authors carried out experiments with leek (Båth and Rämert 2000), oat and barley (Svensson 2004) planted in composted and anaerobically digested residues originating from organic household wastes. Additionally, Hygromull-mixed residues from biogas plant were shown to positively support ryegrass growth, while yield formation was negatively influenced by the other additives. Benito et al. (2005) also reported that the incorporation of an additive, for instance 10% or 25% volume of Peat to pruning waste compost significantly increased the germination index and yield of ryegrass as compared to pure compost. In the present study, the supplement of 20% additives (Perlite, Hygromull, Lecaton, Peat, Cocofiber) into compost is recommended, while only incorporation of Hygromull (20% by volume) into anaerobically digested residues might be recommended for enhancing the yield formation of ryegrass.

2.3.1.2. Uptake of nutrients

Nitrogen

Total N uptake (sum of 4 cuts) was observed at the lowest value in the control determined as 478.8 mg/pot and highest in the unleached compost-based materials estimated as 1360.4-1684.1 mg/pot, while in the residue-based treatments total N uptake ranged between 603.5-1092.6 mg/pot (Figure 6).

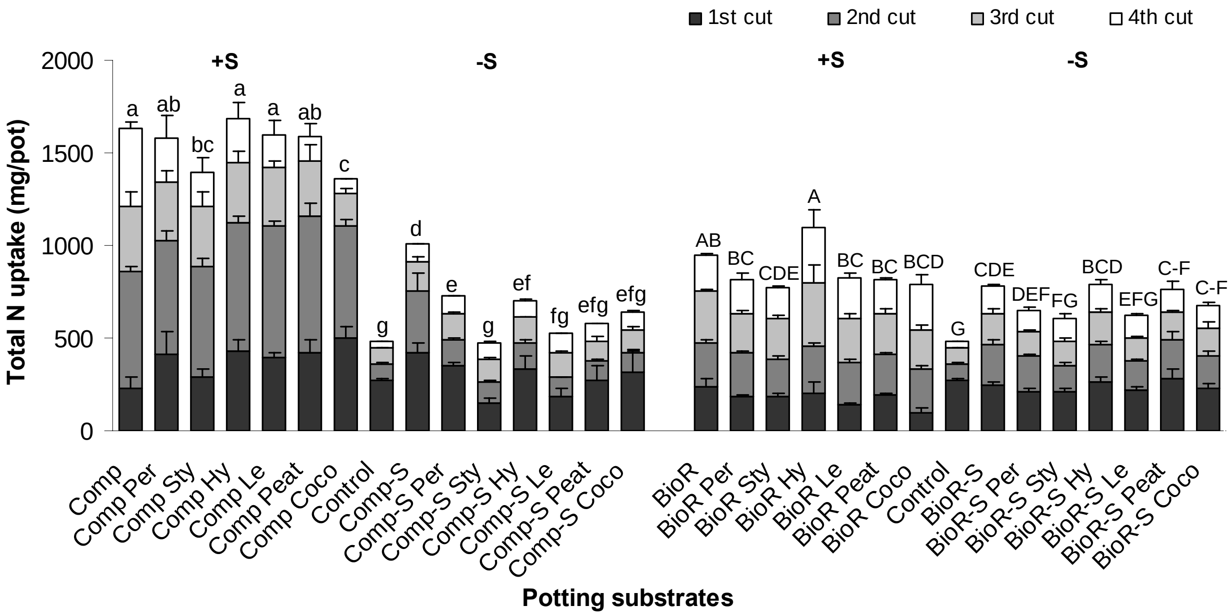

Figure 6. N uptake of ryegrass planted in different potting substrates

Notably, total N uptake by ryegrass was significantly reduced in leached compost-based substrates and it was slightly decreased in leached materials based on anaerobically digested residues. It seems that leaching had an obvious negative influence on N uptake of plants. Except Hygromull added to unleached residues from biogas plant favored total N uptake, remarkably in the 3rd and 4th cuts. However, the influence of the other additives incorporated into both materials was less pronounced. The addition of Styromull and Cocofiber to compost resulted in a significantly lower total N uptake compared to the pure compost. According to Verdonck et al. (1983) Cocofiber contains high carbon content (> 45%), therefore it promotes the fixation of plant available nitrogen. Moreover, the low total N uptake of ryegrass grown in the standard soil (control) may be caused by the lower amount of total available N as compared to compost and residues from biogas plant (Table 2). In addition, the high C:N ratio (49:1) of this control material must be also taken into the consideration. Although compost and residues from biogas plant contained almost the same amount of N (Table 2), total N uptake of ryegrass from the unleached compost treatments was relatively as twice as that in the unleached residues from biogas plant (Figure 6). This may be caused by the different C:N ratios, which is around 11:1 for compost and 26:1 for anaerobically digested residues (Table 2), resulting in a lower N delivery rate or even in N immobilization later in the growing season (Grigatti et al. 2011). Gunnarson et al. (2010) estimated that only about 12% of the organic N in residues from biogas plant was mineralized throughout a six-month experimental period because organic N compounds are relatively recalcitrant for biodegradation. In addition, Clemens et al. (2006) determined total NH_3 emission representing about 5-23% of the NH_4-N applied in anaerobic digested slurry. Furthermore, the digestion of highly degradable organic C in residues from biogas plant during cultivation causes a considerable N loss through NH_3 volatilization (Rivard et al. 1995, Arthurson 2009), resulting in a lower amount of plant available N and therefore a reduced N uptake of ryegrass from anaerobically digested residues. Moreover, Rubæk et al. (1996) observed N losses via denitrification after application of untreated cattle slurry and anaerobically digested slurry to the potting substrates before planting of ryegrass. Therefore, cumulated NH_3 emission after application of untreated and anaerobically digested slurry in horticulture should be taken into consideration in term of mitigation strategies for greenhouse gas emissions (Wulf et al. 2002). Additionally, Hafeez et al. (1988) agreed that total nitrogen concentration in pod grain and whole plant of *Vigna radiata* (L.) Wilczek significantly decreased as a result of an increased salinization.

Phosphorus

Accumulated P uptake of ryegrass grown in the control medium was figured as 147.9 mg/pot, which was in the same magnitude as that of ryegrass grown in all compost-based treatments. In contradiction, accumulated P uptake was significantly higher in anaerobically digested residue-based substrates (351.0-504.1 mg/pot) (Figure 7).

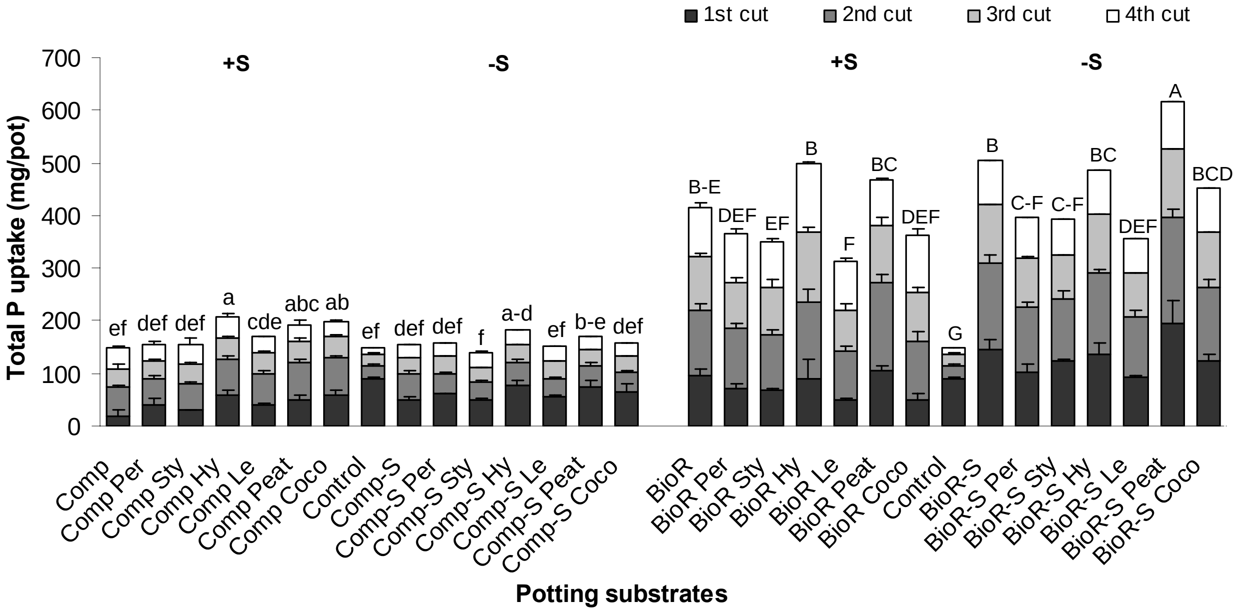

Figure 7. P uptake of ryegrass planted in different potting substrates

Leaching of both materials had less influence on P uptake of ryegrass. As compared to the control and compost treatments, P uptake of ryegrass grown in either unleached or leached residues from biogas plant was about two fold higher. The addition of Hygromull slightly favored P uptake by grass from all the compost and residue-based materials (Figure 7). Specifically, the incorporation of Hygromull and Peat into leached and unleached residues from biogas plant enhanced total P uptake obviously. The higher P uptake of ryegrass grown in residues from biogas plant may be caused by the higher content of plant available P of this material (Table 2). Furthermore according to Güngör and Karthikeyan (2008) organic P compounds partially mineralized during the anaerobic digestion in biogas plants. Therefore, Bachmann et al. (2011) assumed that P supplied with residues from biogas plant is a more effective P source than P from compost.

Potassium

As compared to the standard soil (control) total K uptake of ryegrass was significantly higher in the materials based on compost and anaerobically digested residues. Total K uptake of ryegrass grown in both media had the same high magnitude which was estimated as 2000.6-2966.5 mg/pot and 1438.0-2313.0 mg/pot for both the unleached and leached compost/residues from biogas plant, respectively (Figure 8).

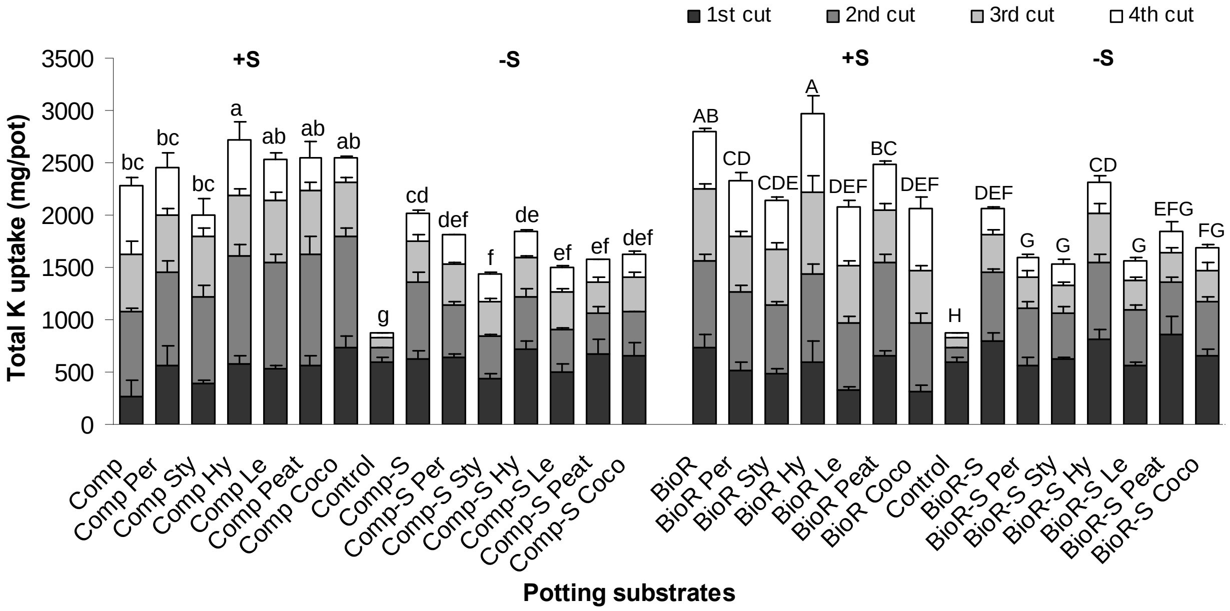

Figure 8. K uptake of ryegrass planted in different potting substrates

While K uptake of the first cut caused an exhaustion of available K in the control, K uptake of the latter cuts was significantly decreased. However, caused by the longer lasting source of K available in compost and residues from biogas plant K uptake of ryegrass was still relatively high as compared to the first cut (Soumare et al. 2003). Additionally, the high available K content in raw materials confirmed to show no depressive effects on K consumption by plants at the latter stages (Wen et al. 1997). For this reason compost appears to be as good as residues from biogas plant as a K supplier for ryegrass. Furthermore, leaching caused a notably decrease of K uptake by ryegrass from both compost and residues from biogas plant. Thus, in term of K uptake, leaching may not be proposed for both compost and residues from biogas plant. The impact of different additives incorporated into compost and residues from biogas plant on K uptake by ryegrass was generally not clear. Exceptionally, the supplement of additives excluding Styromull into unleached compost and Hygromull into either leached or unleached residues from biogas plant tended to enhance total K uptake of ryegrass. Therefore, Perlite, Hygromull, Lecaton, Peat and Cocofiber incorporated into unprocessed compost, while Hygromull mixed with either leached or unleached residues from biogas plant was pronounced to improve K uptake of ryegrass.

Magnesium

Leaching of residues from biogas plant considerably increased Mg uptake of ryegrass, notably at the 1st and 2nd cuts (Figure 9), while leaching of salt from compost caused a remarkable depression of Mg uptake. Generally, the potting substrates based on anaerobically digested residues were more favorable for ryegrass to take up Mg than compost-based materials and the standard soil. As compared to the control, Mg uptake of ryegrass from the unleached compost-based treatments were estimated to have the same magnitude as 81.4-95.4 mg/pot, which was lower than that of the unleached residues from biogas plant (98.2-187.9 mg/pot) and the leached residues from biogas plant (121.0-187.9 mg/pot). This may be due to the higher content of available Mg in anaerobically digested residues than compost (Table 2).

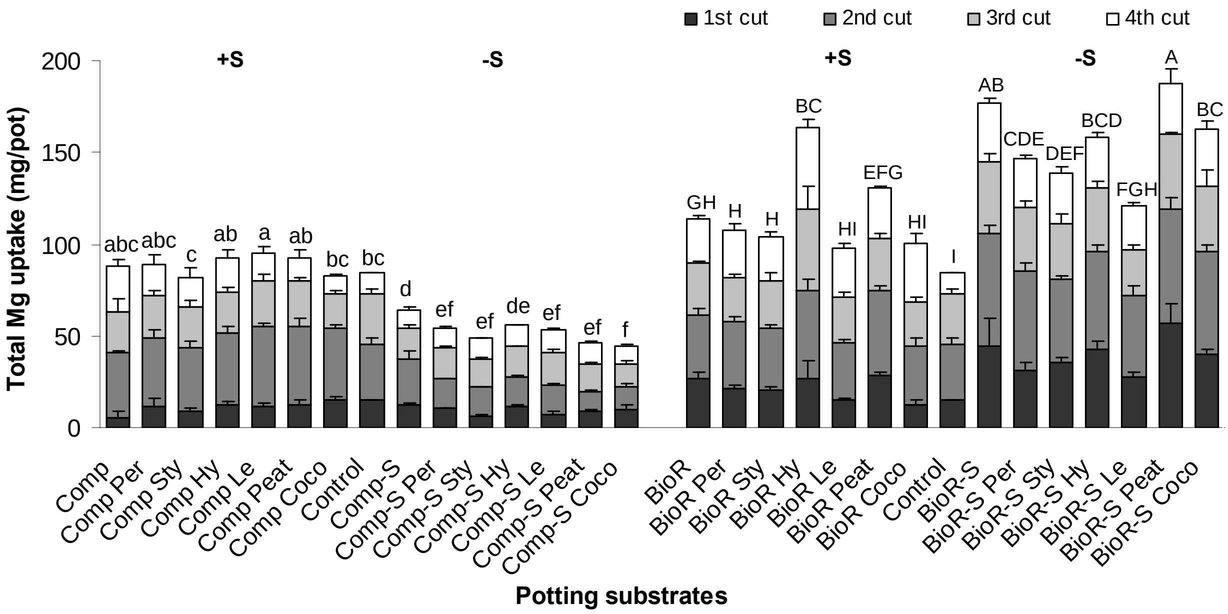

Figure 9. Mg uptake of ryegrass planted in different potting substrates

The effect of the incorporation of additives such as Perlite, Styromull, Hygromull, Lecaton, Peat, Cocofiber into compost was less pronounced, while Hygromull and Peat enhanced Mg uptake from both leached and unleached residues from biogas plant, respectively. Incorporation of both materials may therefore be recommended. However, it should be pointed out that this effect is not caused by the influence of Hygromull or Peat on the Mg delivery, but on the improvement of water-holding capacity which favors the growth of plants.

Calcium

Similarly to the trend of Mg uptake, leaching of residues from biogas plant slightly improved Ca uptake of ryegrass, while with compost leaching had little effect (Figure 10). Most values of Ca uptake determined in both the unprocessed and leached compost were statistically equal in the range of 86.4-135.4 mg/pot and those were slightly increased by 26.3-64.9 mg/pot in the leached biogas residue-based substrates.

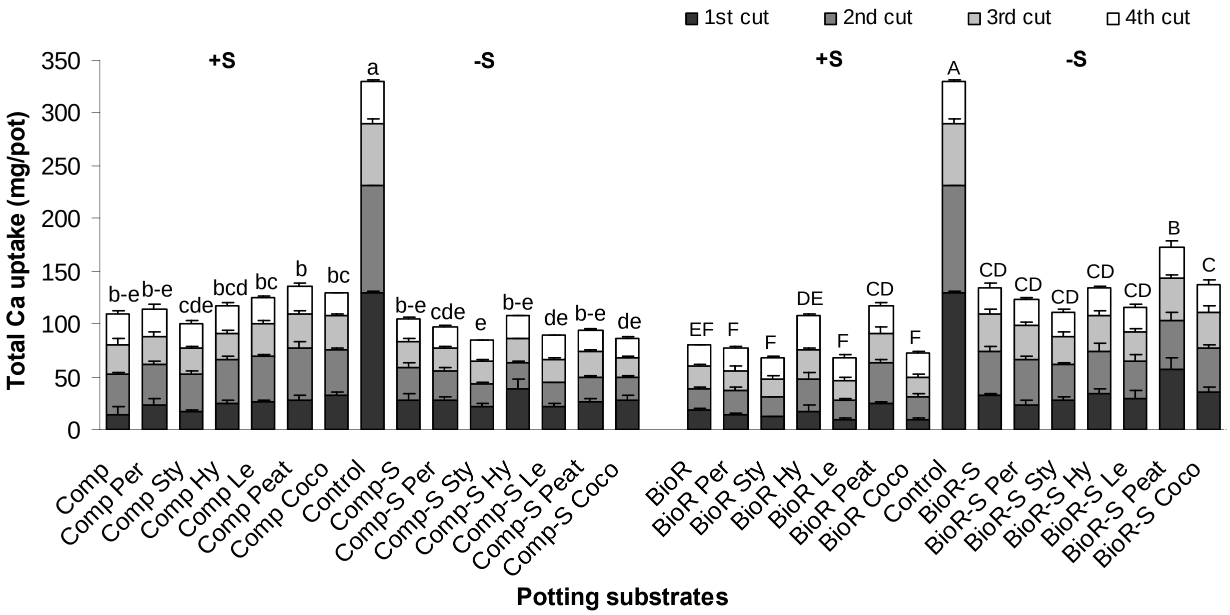

Figure 10. Ca uptake of ryegrass planted in different potting substrates

Surprisingly, cumulative uptake of Ca by ryegrass was estimated in the control as 329.6 mg/pot which was significantly higher than those in either compost or anaerobically digested residue treatments (Figure 10). The reason was reportedly caused by a depressive effect, since both compost and anaerobically digested residues contain high amounts of available nutrients existing in the cations including K^+, Mg^{2+}, NH_4^+ which induced a strong competition with Ca^{2+} during absorption and utilization by ryegrass, thus depressing Ca uptake (Bangerth 1979). Except the incorporation of Peat into the unleached and leached residues from biogas plant, the effect of other additives in both compost and residues from biogas plant on Ca uptake by ryegrass was not recognizable.

Sodium

Na uptake of ryegrass from compost was significantly higher as compared to residues from biogas plant and standard soil (control) (Figure 11). The values of Na uptake were evaluated as 26.4, 50.5-87.1, and 138.4-166.0 mg/pot in the control, the residues from biogas plant, and the compost-based materials, respectively (Figure 11).

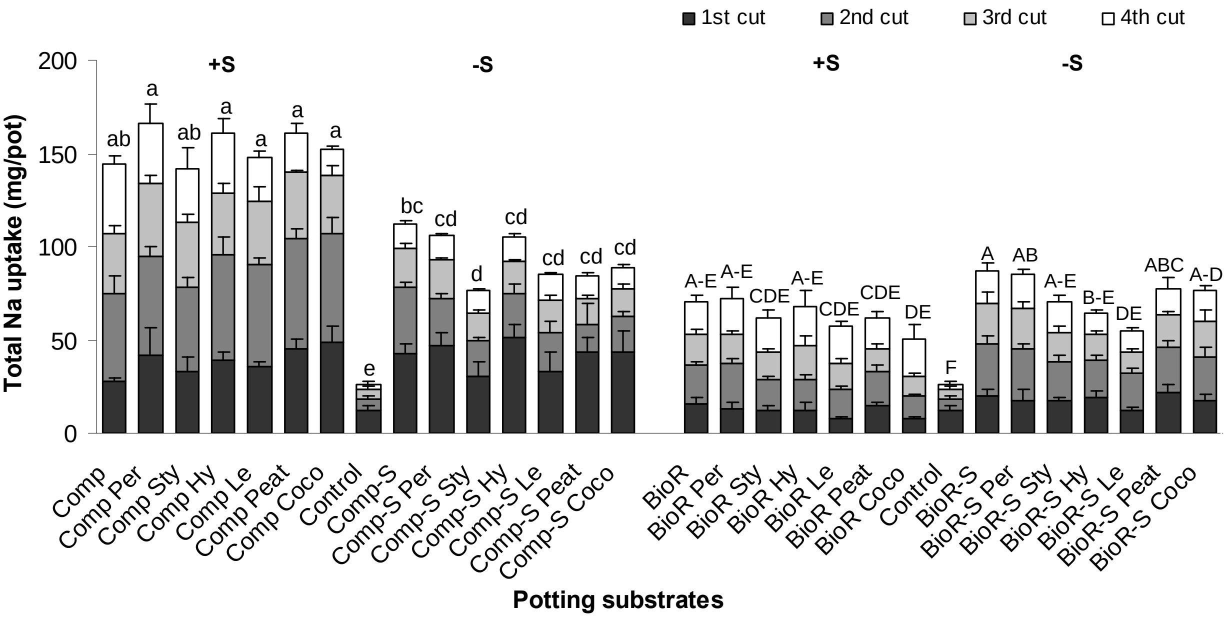

Figure 11. Na uptake of ryegrass planted in different potting substrates

Similar to N and K uptake, leaching of compost has caused a slightly reduction of Na uptake of ryegrass, while leaching salt from anaerobically digested residues had almost no effects on total Na uptake of ryegrass. One of the mechanisms related to salt stress is Na exclusion from the root or control of Na uptake (Zhang et al. 2010). For most plants, high Na concentrations are toxic which is closely linked and competitive to the cation K^+ (Mäser et al. 2002). K uptake of ryegrass from the anaerobically digested residue treatments was reported to be abundant (Figure 8), likely substituting Na during plant growth. Also, there was no significantly different influence among the presence of additives or without additives mixed into both materials, therefore the effects of additives on Na uptake of ryegrass can be ignored.

Sulfur

Original compost and standard soil (control) rather than pretreated compost and residues from biogas plant favored S uptake of ryegrass (Figure 12). Total S uptake of ryegrass grown in the unleached compost was as high as that in the control ranging from 99.8-116.5 mg/pot, while it was reduced significantly to 52.2-80.5 mg/pot in the leached compost. Total S uptake of ryegrass in the unpretreated and leached residues from biogas plant was estimated as 63.3-116.2 and 66.2-108.5 mg/pot, respectively (Figure 12).

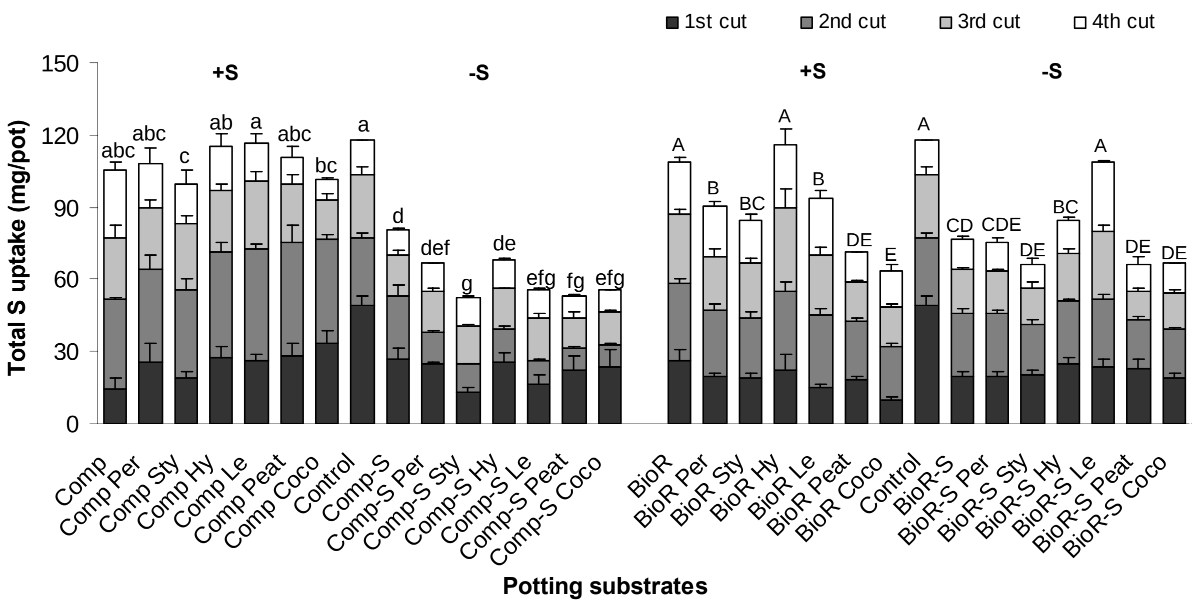

Figure 12. S uptake of ryegrass planted in different potting substrates

Regarding the effect of compost and additives incorporation, total S uptake was not recognizably influenced by the additives. However, with unleached residues from biogas plant S uptake was reduced except by the addition of Hygromull. Besides, Lecaton and Hygromull mixed into the leached residues from biogas plant resulted in higher S uptake of plants than the others. Intensively, the incorporation of Peat and Cocofiber into the original residues from biogas plant has caused a significant depression of S uptake of ryegrass (Figure 12). In addition, Peat and Cocofiber mainly contain cellulose and natural fibers having a great amount of hydrocarbon (Indayaningsih et al. 2011). Thus, the addition of both materials increased total carbon content in the substrates causing S immobilization (Chapman 1997). Therefore, ryegrass planted in the mixtures of residues from biogas plant and Peat or Cocofiber showed a lack of S uptake of the plants. In general, the reduction of S uptake in the treatments of residues from biogas plant is assumed to be the result of microbiological S immobilization. As Chowdhury et al. (2000) stated materials with wide C:S ratios normally immobilize inorganic S, because the development of the microbial biomass in the decomposing material needs more S than is provided by the substrate.

2.3.2. Rape and Sunflower

Rape with a higher salt tolerance was firstly planted in compost and residues from biogas plant and harvested after 51 and 58 days, respectively. Sunflower was planted in turn on the same used-substrates after harvesting rape. Sunflowers were harvested 40 days after sowing.

2.3.2.1. Yield

DM yield of rape planted in unpretreated compost treatments were in the range of 16.5-23.7 g/pot and almost reached the high level of the control (24.4 g/pot), but was several times higher than that in the treatments of unprocessed residues from biogas plant (5.6-12.0 g/pot) (Figure 13) even rape planted in compost was harvested one week earlier than in residues from biogas plant. This means the remarkably higher salt content in the residues from biogas plant is also the limiting factor for rape growth at the beginning. Leaching of compost resulted in a steep rape yield decrease, while the effect of leaching of residues from biogas plant was unclear and even resulted in a yield increase (Figure 13). These results were somehow consistent with those of ryegrass. Therefore, in term of yield production of rape leaching was not suggested for compost but it might be applied for residues from biogas plant. Regarding to the supplement of additives mixed with two basic materials, some additives such as Perlite, Styromull, Peat and Cocofiber mixed into the unleached compost or residues from biogas plant slightly increased the yield formation as compared to the singular raw materials. However, the different effect among additives on DM formation of rape was insignificant. The influence of additives incorporated into the leached materials was negligible except Hygromull mixed with the leached compost or residues from biogas plant in which DM yield peaked among other additives as 13.3 and 16.4 g/pot, respectively, and higher than in the unpretreated materials without the supplement of additives (Figure 13). As previously discussed, Hygromull with a relatively high amount of salt and available nutrients (P, K, Mg) can further supply nutrients for rape as a salt-favorable plant in the latter developmental stage.

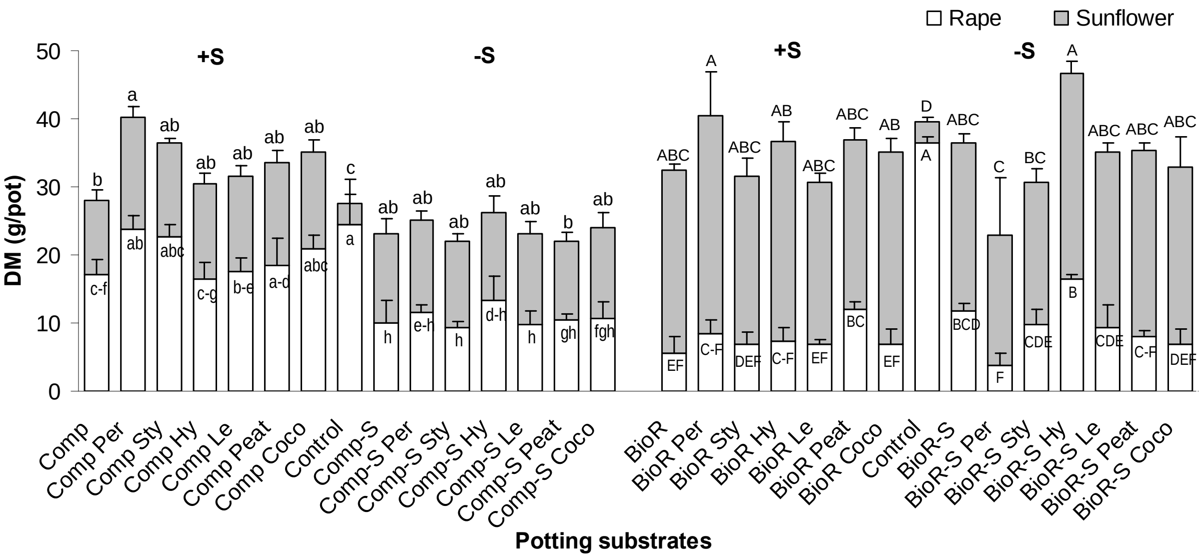

Figure 13. Dry matter yield of rape and sunflower planted in different potting substrates

Error bars represent the standard deviation of separated plants with 4 replicates. Means of different treatments followed by the same letters are not significantly different ($p < 0.05$) by Tukey test.

(+S: unleached Comp/BioR; -S: leached Comp/BioR)

However, yield formation of sunflower after harvesting rape planted in various potting substrates showed a different trend. DM yield of sunflower cultivated in the standard soil (TKS_1) was estimated as 3.1 g/pot, which was reduced significantly and remarkably lower than those in the treatments of compost and residues from biogas plant (Figure 13). This was because of available nutrients in the standard soil were mainly exhausted by rape. Nevertheless, nutrients were still abundant in compost and especially residues from biogas plant. Sunflower well adapted and strongly developed in the residues from biogas plant after the reduction of salt during the period of rape cultivation. The highest DM yield was observed in the residues from biogas plant, ranging between 19.1-31.9 g/pot and was two folds higher than that in compost, estimated as 10.9-16.5 g/pot. Leaching did not cause any different effects on yield formation of sunflower between the leached and unleached materials (Figure 13). Additionally, there were no significant differences in DM yield formation of sunflower between the pure materials and those mixed with additives. These results reveal that in the later-stage of cultivation the leaching was not necessary and the presence of additives had negligible influences on plant yield formation. Generally, the accumulated dry matter yield of both plants grown in compost and residues from biogas plant was slightly higher or reached the high levels as in the standard soil. This means the basic raw materials originated from compost and residues from biogas plant may be used as potting substrate, confirming results with ryegrass cultivated in the same potting materials.

2.3.2.2. Uptake of nutrients

Nitrogen

Original compost was observed to be more favorable for rape and sunflower to take up N (Figure 14). N uptake of rape planted in unprocessed compost was highest ranging from 722.4-947.5 mg/pot and lowest in the treatments with residues from biogas plant, estimated as 88.8-332.8 g/pot. After leaching, N uptake of rape grown in the compost treatments decreased significantly and was estimated as 146.0-232.8 mg/pot being as high as in the control media valued as 261.7 mg/pot. While in the leached residues from biogas plant it was statistically equal or slightly increased as compared to the unleached residues. The presence of additives mixed into the raw materials had little positive effects on N uptake of rape (Figure 14).

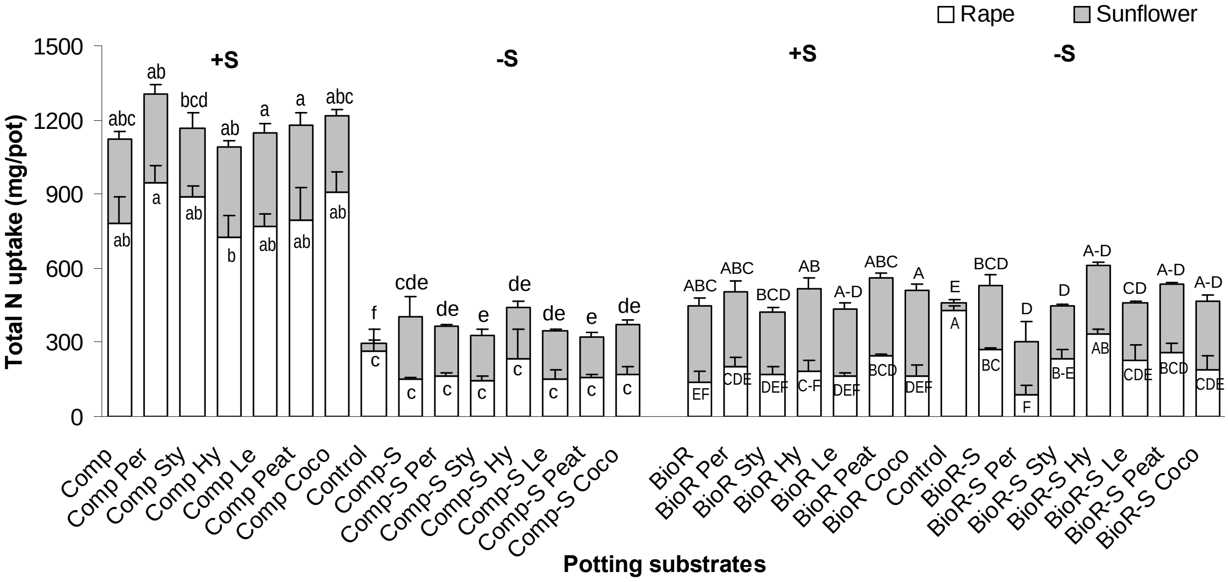

Figure 14. N uptake of rape and sunflower planted in different potting substrates

At the stage of sunflower cultivation, N taken up by sunflower from the unleached compost (278.6-388.2 mg/pot) was lower than that of rape but still remained higher as compared to the control, while in the leached residues from biogas plant N uptake of sunflower (273.6-347.3 mg/pot) was partially higher as compared to rape. While leaching of compost resulted in a lower N uptake of sunflower, an influence of leaching of residues from biogas plant could not be observed. The effect of additives on N uptake of sunflower was insignificant. The sum of N uptake of rape and sunflower was significantly highest in the unprocessed compost materials. The results prove the effectiveness of available N in compost.

Phosphorus

In contrast to N uptake, as compared to the control, the lowest P uptake of rape was observed in leached compost treatments (52.3-72.9 mg/pot), followed by unprocessed compost which was as high as the control (82.7-107.8 mg/pot) (Figure 15). The incorporation of Peat to unleached residues from biogas plant or Hygromull to leached residues resulted in the highest P uptake of rape valued as 220.9 or 322.8 mg/pot, respectively (Figure 15).

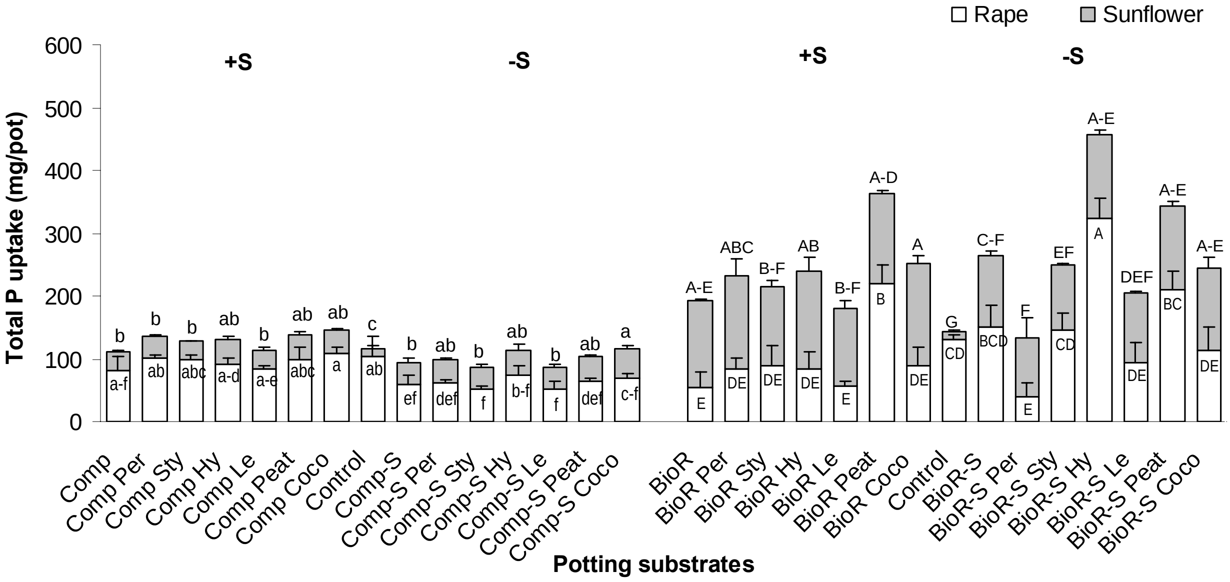

Figure 15. P uptake of rape and sunflower planted in different potting substrates

P uptake of sunflower from compost treatments was lower as compared to rape, which was in the range between 28.2-48.2 mg/pot (Figure 15). However, P uptake of sunflower in both unleached and leached residues from biogas plant was mainly higher as compared to rape. As compared to the compost and the standard soil, P uptake was significantly higher in the treatments with residues from biogas plant (determined as 91.9-164.0 mg/pot). The influence of leaching or the presence of additives in compost and residues from biogas plant could be excluded in the latter stage of planting. Due to the higher P uptake of sunflower as the later crop, total P uptake of rape and sunflower grown in the residues from biogas plant was remarkably higher than that of the compost treatments. This illustrates the higher P availability in residues from biogas plant, using pig manure and maize as input material.

Potassium

In the control K uptake of rape was in the range of the treatments with leached compost and unleached/leached residues from biogas plant, respectively. K uptake of rape was highest in the unpretreated compost (1362.2-1807.6 mg/pot), and lowest in the anaerobically digested residues (140.5-937.8 mg/pot). With sunflower K uptake was lowest in the control (114.2 mg/pot) and highest in the unprocessed residues from biogas plant (1215.7-1717.8 mg/pot) (Figure 16).

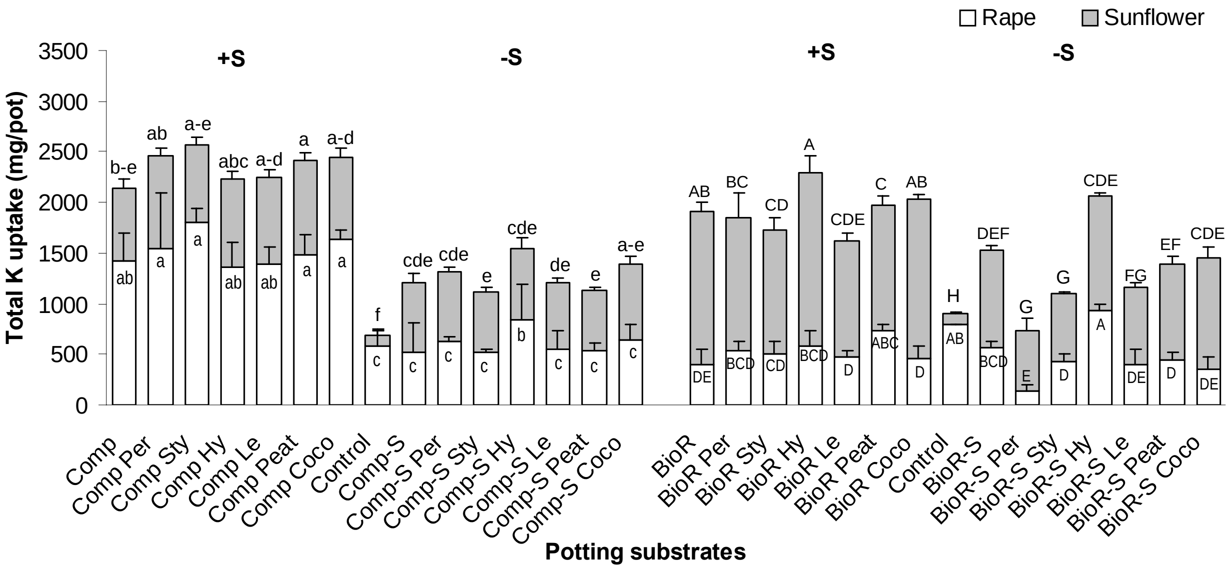

Figure 16. K uptake of rape and sunflower planted in different potting substrates

As compared to the unleached compost, leaching resulted in a significant decrease of K uptake of rape, while with sunflower K uptake of the treatments with leached compost were almost in the order of magnitude as the unleached treatments (Figure 16). With the residues from biogas plant the influence of leaching on K uptake of rape was not distinct, while with sunflower leaching resulted in a lower K uptake. In the leached residues from biogas plant the application of Hygromull favored K uptake of rape as well K uptake of sunflower in the unleached and leached residues (Figure 16). By summation of K uptake of rape and sunflower, the results reveal that leaching caused negative effects on K uptake of both plants during intensive cultivation. Therefore, leaching was not recommended for both raw materials in concerning K uptake.

Magnesium

As compared to the standard soil (control), Mg uptake of rape grown in unprocessed compost had the same levels of 25.3-32.5 mg/pot which were significantly higher than that in the leached compost (Figure 17). This means leaching of salt from compost had a negative effect on Mg uptake of rape at the beginning. In contrary, Mg uptake of rape from almost all treatments with residues from biogas plant (20.1-56.2 mg/pot) was significantly lower than that of the standard soil (Figure 17). Leaching resulted in a slight increase of Mg uptake of rape planted in anaerobically digested residues as compared to unleaching. Among additives mixed into compost, the effect on Mg uptake of rape was insignificantly different. Peat added to the unleached residues from biogas plant and Hygromull incorporated into the leached residues from biogas plant favored Mg uptake of rape.

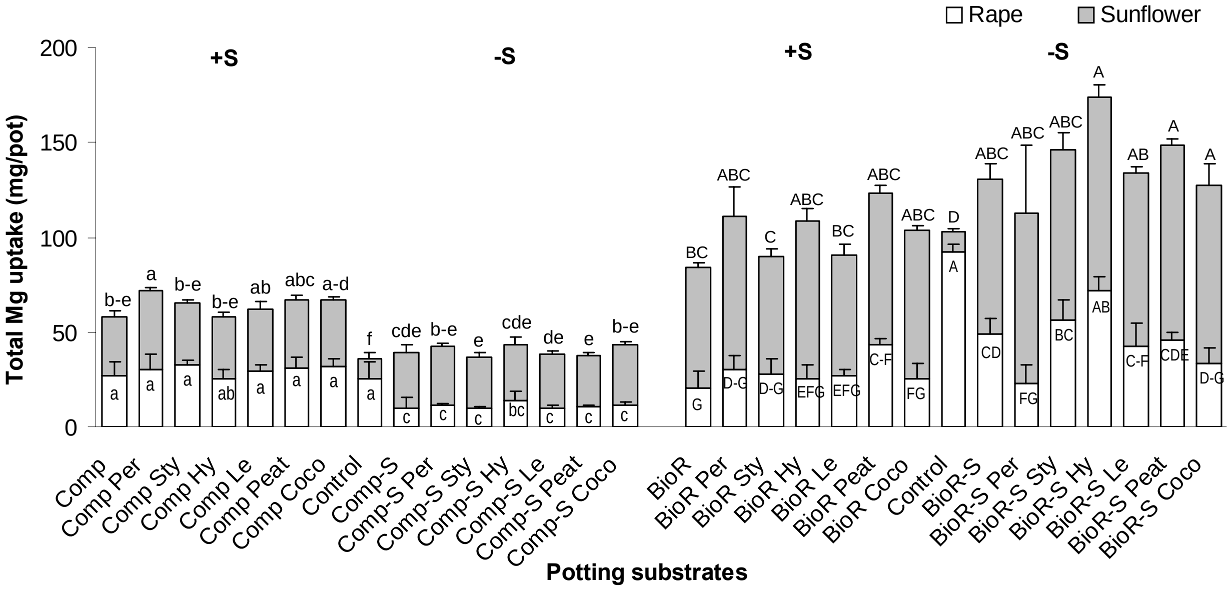

Figure 17. Mg uptake of rape and sunflower planted in different potting substrates

Mg uptake of sunflower planted in both materials was mostly significant higher as compared to rape. Notably, Mg uptake of plants grown in the residues from biogas plant was ranging from 61.8-102.8 mg/pot, several times higher than that grown in the compost treatments ranging between 27.1-40.9 mg/pot (Figure 17). With both raw materials Mg uptake of sunflower was not influenced by the application of additives. Caused by the high Mg uptake of sunflower grown in the residues from biogas plant, total Mg uptake of both plants was highest in these treatments.

Calcium

Similarly to Ca uptake of ryegrass, the values of Ca uptake by rape grown in both raw materials were significantly lower than that in the standard soil (Figure 18). Ca uptake of rape observed in unprocessed compost (148.1-208.1 mg/pot) was significantly higher than that in leached compost (67.5-104.2 mg/pot), while for leached residues from biogas plant Ca uptake of rape (40.1-92.4 mg/pot) was higher. Even rape planted in residues from biogas plant was harvested one week later than that planted in compost, Ca uptake of rape found in the original biogas residue-based treatments (21.6-57.0 mg/pot) was lowest (Figure 18).

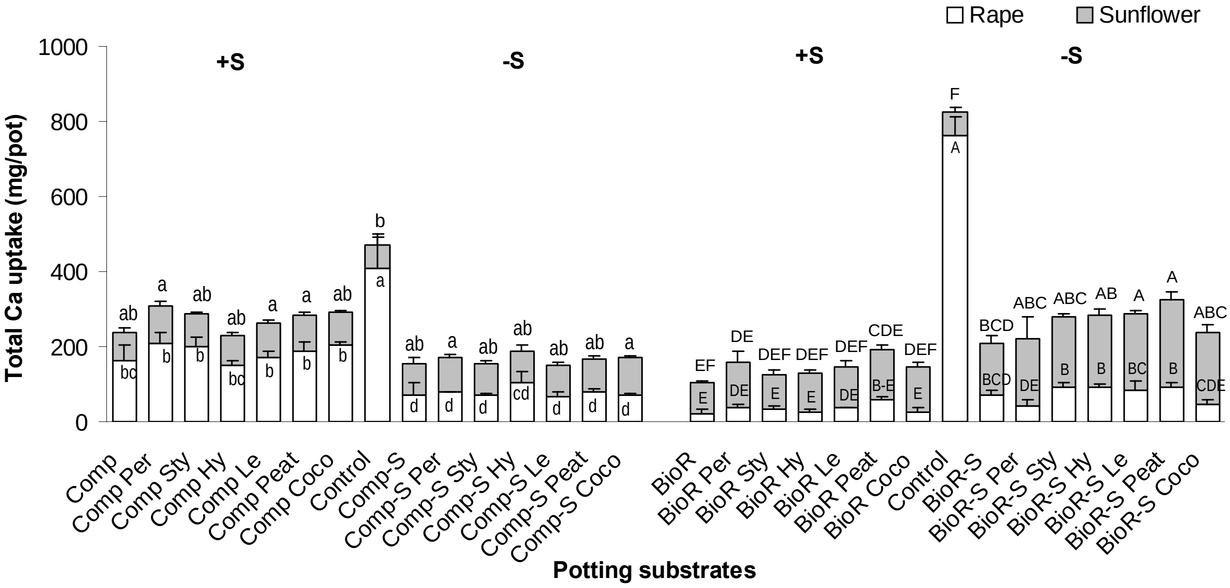

Figure 18. Ca uptake of rape and sunflower planted in different potting substrates

Additionally, Ca uptake of sunflower found in both unprocessed and leached compost (77.4-102.3 mg/pot) was statistically equal or slightly higher than that in the control media. The effect of leaching of compost on Ca uptake of sunflower was not pronounced. However, concerning total Ca uptake of rape and sunflower leaching of compost may not be recommended. Furthermore, Ca uptake of sunflower grown in the leached material with residues from biogas plant peaked at 138.9-236.2 mg/pot, followed by that in the unleached ones ranging from 82.2-135.4 mg/pot, the lowest observed in the standard soil as 63.4 mg/pot (Figure 18). Total Ca uptake of the two plants grown in the leached residues from biogas plant was slightly higher than in the unleached substrates. This indicates that leaching of anaerobically digested residues had a positive effect on Ca uptake. The impact of the presence of additives added into the raw materials on Ca uptake of the two plants was almost unclear. Total Ca uptake of both plants in the raw materials was generally significantly lower than the standard soil which was explained by the Ca depression phenomenon similar to the ryegrass results.

Sodium

Na uptake of rape grown in the unleached compost treatments peaked at 54.7-99.9 mg/pot, followed by that in the leached compost ranging 18.3-34.3 mg/pot, which was at the same level of the standard soil (control). Lowest Na uptake was found in the unleached and leached residues from biogas plant (1.9-8.9 mg/pot), which was significantly lower than in the control (Figure 19). This indicates that leaching of both raw materials especially with compost resulted in a strong reduction of Na uptake of rape at the early cultivation. It was noted that total Na uptake from the unprocessed compost was several folds higher than the residues from biogas plant. That is because of the remarkably higher content of Na available in the original compost (Table 2). Concerning total Na uptake of plants the influence of additives was negligible.

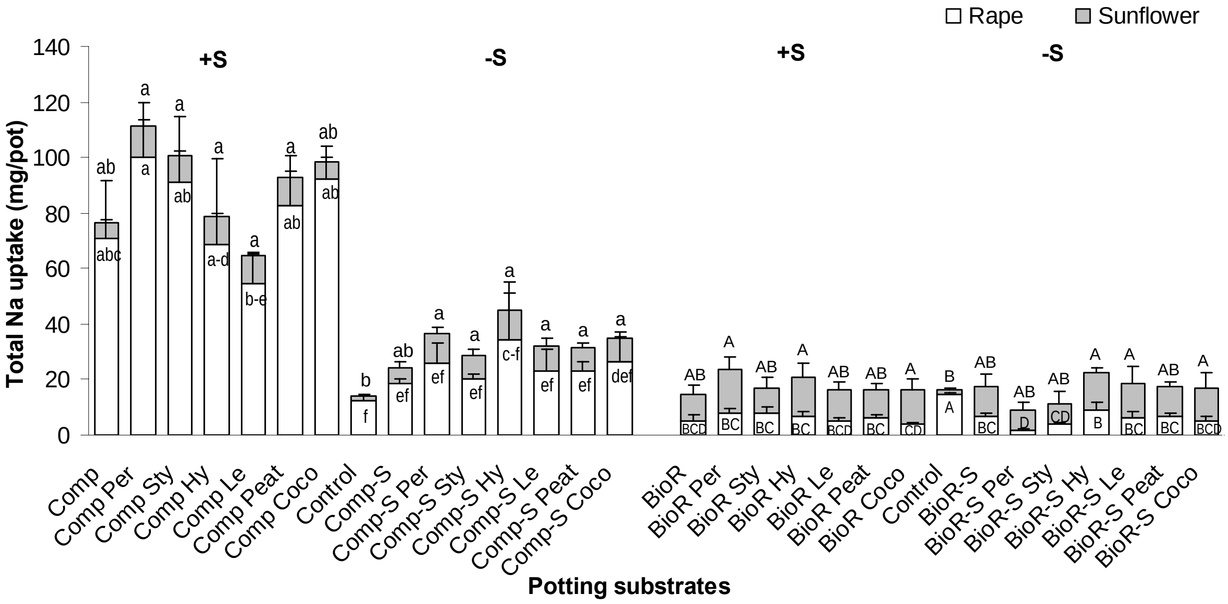

Figure 19. Na uptake of rape and sunflower planted in different potting substrates

Na uptake of sunflower from both groups of compost and residues from biogas plant was equally low ranging between 5.5-11.3 and 6.9-14.4 mg/pot, respectively, but still significantly higher than that of the control (Figure 19). Consistently, in the latter planting, the effect of leaching and addition of additives were negligible. Generally, the accumulated Na uptake of both plants grown in the unprocessed compost was highest due to the contribution of high Na uptake of rape and it was significantly lower found in the residues from biogas plant. When comparing to Na uptake of ryegrass, the result of rape and sunflower cultivated in the substrates based on anaerobically digested residues was significantly lower.

Sulfur

S uptake of rape grown in the standard soil (control) was in the same range as plants of the different treatments with unleached compost. In the unleached compost treatments S uptake of rape ranged between 180.6-231.2 mg/pot, and between 95.2-140.3 mg/pot in the leached compost treatments (Figure 20). In the unleached as well as leached residues from biogas plant S uptake was lowest, determined as 17.9-120.9 mg/pot (Figure 20). Therefore, leaching of compost negatively resulted in lower S uptake, but not recognized for residues from biogas plant. Additionally, compost was good at providing more abundant available S for rape than residues from biogas plant. The additive Lecaton added into the residues from biogas plant (both leached and unleached) favored S uptake of rape.

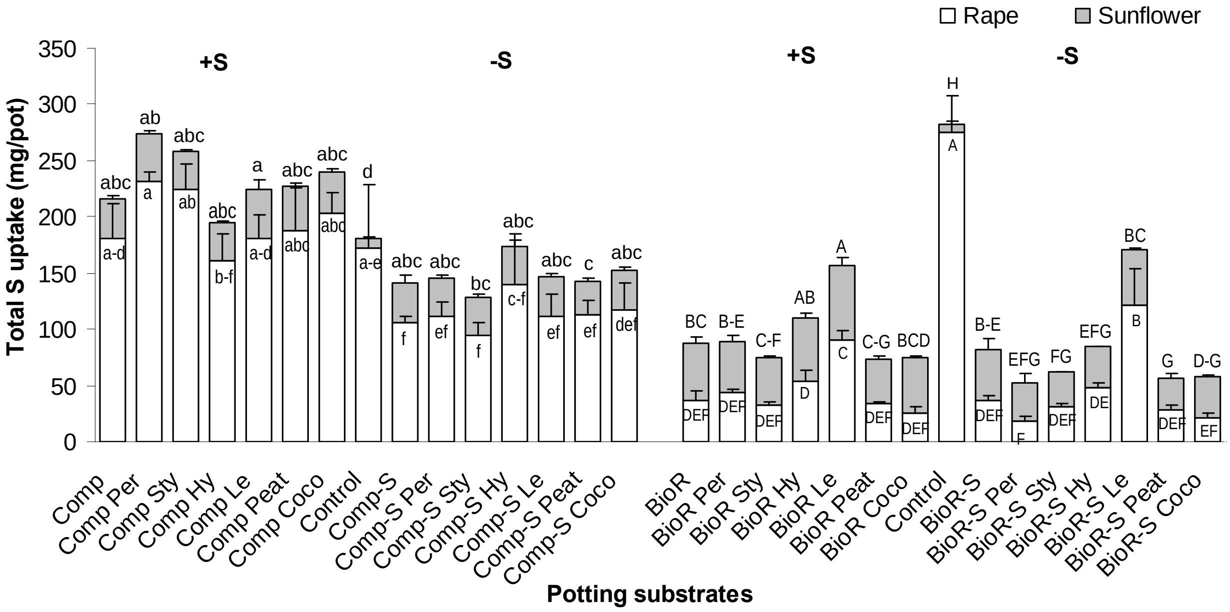

Figure 20. S uptake of rape and sunflower planted in different potting substrates

In all treatments S uptake of sunflower was significantly higher than that of the control (Figure 20). However, it is difficult to recognize the effect of the additives incorporated into raw materials and between unprocessed and leached materials. Regarding the accumulative S uptake of both plants, the highest S uptake was found in the unleached compost treatments. The distinct difference of S uptake among the material groups may be caused by the different ratio of available N to available S which was related to the ratio of total N to total S. In the literature, the optimum ratio of available N to available S in the soil for rapeseed production was estimated to be 7 to 1 (Janzen and Bettany 1982). It was estimated that the ratios of total N to total S for compost and residues from biogas plant in the present study were 1.8 and 0.8, respectively (Table 2) which were obviously lower than 7, thus inferring the inefficient utilization of the assimilated S (Janzen and Bettany 1982).

2.4. General discussion

Yield formation and nutrient uptake of rape and sunflower grown in residues from biogas plant were significantly lower than that grown in compost, even rape grown on the residues from biogas plant was harvested one week later as compared to plants grown in the compost. This means that compost was good for plants at the first stage of cropping. However, the accumulative results of DM yield and nutrient uptake of both plants grown in the anaerobically digested residues increased strongly and being as high as or even higher than those of the standard soil. Further total P and Mg uptake were significantly higher than those of plants grown in compost. This explains that residues from biogas plant continued to be biologically decomposed and released more available nutrients, which are necessary for plant growth. As previously mentioned in the Section 2.3.1, both compost and residues from biogas plants are good potting substrates, which supply abundant nutrients for plant development in term of intensive horticulture (Arthurson 2009). Moreover, plants grown in compost and residues from biogas plants were documented to increase not only yield formation but also nutrient uptake or crop quality (Liu 2009) and these materials were supposed to be potential to replace chemical fertilizers in horticulture (Arthurson 2009, Liu 2009). Practically, residues from biogas plant contain a high water content, however, they are highly porous and have a low water-holding capacity, thus quickly lost water and became dry on sunny days. Therefore, the residues from biogas plants daily needed a further water supply. In addition, daily watering may gradually cause leaking of nutrients. As a result, plant growth and nutrient uptake may be negatively affected. Residues from biogas plant might be added as crop fertilizer to provide more plant available nutrients for cultivation in a large scale field (Liu 2009). As matter of fact, further investigation for utilization of residues from biogas plant is necessarily needed. Nevertheless, it was further reported that compost is stable, has a good water-holding capacity and therefore reducing irrigation volume which is necessary for large-scale horticulture in a greenhouse (Cole et al. 2005). Intensively, compost has been recognized as an alternative commercial soilless media for traditional potting substrates like Peat in horticulture (Wilson 2004, Benito et al. 2005, Berecha 2011). Above mentioned short review demonstrates that compost has been employed with high rate application in potting media for growing medium salt-tolerant plants including ryegrass, rape and sunflower planted in either pure compost or mixtures of 80% compost and 20% additive in this study.

As discussed earlier in Section 2.3.1, the salt content in the raw materials (compost and residues from biogas plant) was relatively high. Therefore it was necessary to reduce or dilute the salt by leaching or mixing with additives. Leaching as discussed above was not recommended for compost but not pronounced for residues from biogas plant. Therefore further investigations are necessary. Confirming results of Olympios (1992) the supplement of additives for instance Hygromull and Peat are generally recognized to improve quality of the raw materials, resulting in improvement of crop productivity and quality. Hygromull incorporated into leached compost or anaerobically digested residues resulted in the highest yield as well as nutrient uptake. Hygromull has a relatively high content of available nutrients (P, K, Mg), therefore this additive further supplied nutrients for salt-favorable plants such as rape in the last lifetime of growing before harvested. Besides, Hygromull has the highest water-holding capacity among additives used in these experiments. Additionally, the presence of Peat in unprocessed residues from biogas plant increased the yield and nutrient uptake because Peat has a high water-holding capacity like Hygromull (Penningsfeld 1978). As discussed earlier with the results of ryegrass, the salt content in unpretreated residues from biogas plant was relatively high, it was necessary to reduce or dilute the salt by mixing additives having a high water holding-capacity. Therefore the incorporation of Peat into unleached residues from biogas plant archived the best among additives. Clearly, the incorporation of additives to compost or residues from biogas plant achieved benefits but it also had some disadvantages in economical and technical point of view for example requirements of capital, labor, skill and management and disease infections (Olympios 1992).

2.5. Conclusion

In the present study, compost derived from green wastes was a rich source for N and K supply while anaerobically digested residues derived from pig manure and maize were abundant in P and K supplement. Therefore, compost and residues from biogas plant are suitable raw materials to develop good potting substrates for plants.

Despite the high salt content in both raw materials, leaching of compost before using as a potting substrate was not recommended due to negative effects on DM yield and nutrient uptake of experimental salt-tolerant plants. Nevertheless, with residues from biogas plant the influence of leaching was not distinct. Yield formation of the studied plants grown in the residues was insignificantly affected after pretreated. However in concern of total nutrient uptake of plants for instance N, K and S, leaching of anaerobically digested residues is not strongly recommended for salt-tolerant plants.

The application of the studied additives (20% by volume) to compost had a positive effect on yield formation and nutrient uptake, therefore additives mixing were recommended. However, for a recommendation of an additive, further investigations are needed. The influence of the supplement of some studied additives (Perlite, Styromull, Cocofiber, Lecaton) to residues from biogas plant was unpronounced. With respect to yield formation and nutrient uptake, Hygromull or Peat is recommended as an additive to residues from biogas plant.

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Chapter 3

Compost as potting media component for salt-sensitive plants

Abstract

Composting has been considerably recognized as a viable management method for solid organic wastes as well as beneficial substitution for Peat, which is aimed at recycling of its end-product as a potting substrate for ornamental plants. In a pot experiment Pelargonium and Salvia as salt-sensitive plants were grown in the mixture of compost and additives with different volumetric ratios (4:1, 1:1, 1:4). Since plants may suffer from the high salt content, investigations with Peat incorporated into the mixture of compost in a further experiment were carried out.

This chapter reports plant growth by evaluation of dry matter yield and nutrient uptake (N, P, K, Mg and Na) of plants cultivated in compost with and without additives as potting substrates in comparison to standard soils. Both yield formation and nutrient uptake significantly increased and almost gained levels of those in the control in the second pot experiment when planted in the peat-based substrates. Especially, the growth of Salvia was significantly improved.

The obtained results indicated that compost-based media (> 50% compost) cannot be recommended for salt-sensitive ornamental plants, while a volume of compost lower than 25% incorporated into Peat creates peat-based substrates which reasonably enhanced growth of Pelargonium and Salvia.

Key words: compost, Peat, additives, potting substrates, nutrient uptake, yield formation.

3.1. Introduction

Peat is a natural product, similar to a type of soil, originated from partially decomposed mosses or sedges accumulating in bogs or mires ranging from over hundreds to thousands of years (Ingram et al. 1993). Over 400 million ha of peatland were estimated, discovered on the earth except places where the weather is too severe with extremely high or low temperature for plants to grow (Robertson 1993). Peat is well-known and popularly applied as growing media in horticulture and agriculture in many different countries all over the world including China, Ireland, UK, USA, Germany, Japan, Sweden, Finland, Holland, etc. (Robertson 1993, Fischer and Schmitz 1997). It is widely applied because of various advantages such as high water-holding capacity, good aeration, light weight (Berecha et al. 2011), low pH and nutrient content suitable for a lot of applications in horticulture, resistance to decay, free of weeds and animal pathogens, safety and reasonable price (Robertson 1993), and an excellent structure favored for plant growth, disease reduction, etc. (Mami and Peyvast 2010).

Peat as a traditional substrate was commonly used for ornamental plant cultivation. There were various kinds of substrates used for ornamental plants, namely pine mould, white peat, and frozen-out black peat (Boodt and Verdonck 1971). Black peat is found at a much thicker layer than white peat. Peat moss was regarded as the most popular horticultural media (Ingram et al. 1993). In greenhouse, Peat was considered as the best option to gain the optimum quality and yield for ornamental planting (Robertson 1993).

Peat is not an easily renewable resource, however, the demand of Peat exploitation in low land mires has increased dramatically for decades which has raised issues of supply security and mire conservation, ecological and environmental shift (Robertson 1993, Alexander et al. 2008). Activities of Peat extraction lead to lose animal habitats in peatland areas and alter water systems contributing to climatic changes. Besides, exploitation of Peat bogs as plentiful carbon storage source could release a huge volume of carbon dioxide which was estimated as 50 kg of CO_2 per m^3 of Peat extraction (Alexander et al. 2008). According to Lindsay (1993) concerning intensive resource exploitation, Peat extraction might lead to the complete destruction of all lowland mires by the end of the decade (Robertson 1993). Moreover, following the European legislation of biodiversity conservation, a campaign calling for important peatland conservation and protection and development of alternatives for Peat in horticulture was eagerly committed by the UK Royal Society for Nature Conservation since 1990 (Robertson 1993, Alexander et al. 2008). After this positive agreement, over 1000 ha of peatland area were conserved as a nature reserve in the UK which was then welcomed and advocated by the Government, its conservation agencies and other European developed countries such as Ireland and Finland (Bragg et al. 1993).

Although the high value and advantages of Peat in horticulture were undoubtedly confirmed, scientists have recognized the importance of finding available Peat substitute in growing media since the late 1990s (Alexander et al. 2008) for the purpose of conserving ecological nature resources, less Peat dependence and sustainable agricultural development. Considerable studies on alternatives for Peat as main based media have been encouraged and continued (Robertson 1993). An endless organic sources as an alternative for Peat as a conventional pot media may be coir (coconut fiber waste), nut husk (Meerow 1994, Hernández-Apaolaza et al. 2005, Treder 2008), bark or wood products, rockwool, straw, vegetable remains (Robertson 1993), composts generated from biosolids, pruning wastes, municipal solid wastes, green wastes, sewage sludge, etc. (Garcia-Gomez et al. 2002, Benito et al. 2005, Grigatti et al. 2007). Among substitutive substrates, although coir has a comparative bulk density, porosity and nutrient content as Peat, it has a rather low water storage capacity. Because nutrients are easily leached, thus more fertilizers may be required when using it as a main growing media (Robertson 1993). Wood fiber with open structure enhanced leaching of soluble nutrients that resulted in poor growth worse than coir and peat-based media (Bragg et al. 1993). Rockwool as an inert mineral alternative caused some disposability problems after tomato cropping (Robertson 1993). So far, compost originated from different organic wastes was considered as beneficial and excellent partial substitution for Peat in term of highly abundant nutrient recycle and supply, improving soil fertility and plant growth, and having environmental and economical values in horticulture (Amlinger et al. 2007, Grigatti et al. 2007, Oberpaur et al. 2010). However, composting is rather expensive due to its processing and technology investment (Robertson 1993). Robertson (1993) supposed that alternatives for Peat cannot be compared equivalent to peat-based substrates in term of quality, price and volume.

Compost has been used in agriculture and horticulture as crop substrate, fertilizer, Peat substitution as well as soil conditioner (Mami and Peyvast 2010, Berecha et al. 2011). The compost utilization contributed to reduce huge waste mass by 50% and volume by 80% (Cook et al. 1998) and provided abundant macro and micro nutrients (Amlinger et al. 2007). Therefore, compost has been used as a growing media component to meet the crop demands. However, the application rate of compost used in growing media is limited due to high salt and nutrient content, sodium, $CaCO_3$, and chloride (Fischer and Schmitz 1997, Fischer and Popp 1998), presence of unexpected contaminants such as trace elements, heavy metals, glass, and organic chemicals (Ostos et al. 2008). To optimize growing conditions, compost was not proposed to be used alone, but it was popularly added as additive or combined with Peat to minimize negative characteristics of a single material to obtain best plant growth (Ostos et al. 2008). Therefore, numerous studies on combination of Peat and compost sourced from multiple organic wastes mixed at different proportions have been experimentally conducted for various plant species. According to investigations of Fischer and Popp (1998) with *Deutzia scabra* grown in mixtures of Peat and various composts derived from bio-wastes and anaerobically treated refuses from dry fermentation, garden wastes or anaerobically treated refuses from wet fermentation applied at the rate of 20%, 40% or 60%, respectively, such mixtures are as good as Peat substrates. Fischer and Schmitz (1997) confirmed that mixtures of 75% sphagnum peat and 25% compost from residues of the KOMPOGAS-procedure (a one-step, thermophilic dry fermentation with 25-40% solid matter) was a favorable growing media with low pH and nutrient contents ideal for cultivation of sensitive plant spieces like *Euphorbia pulcherrima.* However, Mami and Peyvast (2010) recommended that compost from municipal solid wastes should not exceed 5% by volume added into Peat for cucumber (*Cucumis sativus* L., cv. Radian) transplant production, while coffee pulp compost applied at 15% or 50% rate was possible to replace 50% commercial growing media for tomato production (Berecha et al. 2011). Additionally, additives with low contents of nutrients, soluble salts and stable structure such as crushed brick, bark, wood chips (Fischer and Popp 1998), inert products like Polyurethane-ether, Perlite, Foam-plastics, Polystyrene and Vermiculite (Boodt and Verdonck 1971, Lamanna et al. 1991, Wilson et al. 2004) would be suitable to be added to compost and Peat mixtures to improve the physical characteristics of media and reduce the proportion of Peat. Boodt and Verdonck (1971) determined that white peat added to Perlite or Polyether (volumetric ratio = 3:1) increased the volume of air aeration from 8-26% or 8-45%, respectively, optimizing for root cuttings of *Begonia Tuberhyride Multiflora*. Ostos et al. (2008) studied mixtures of 40% compost mixed with Pine bark and Peat as potting susbtrates for the shrub *Pistacia lentiscus* L. In addition, 50% Perlite added to Peat and compost mixtures were studied as growing media for poinsettia production by Papafotiou et al. (2004). In a study of Smith et al. (2004), Perlite was also used as an additive substrate mixed into 70% Peat as potting media to cultivate Geranium (Pelargonium x hortorum). In another study of Wilson et al. (2004), a peat-based media consisting of 25-35% Polystyrene, 5-15% Vermiculite and 55-65% Peat was used as control materials for some tested subtropical perennial production.

As reviewed from various cited publications, authors proposed many different mixing ratios of compost and Peat or added some artificial additives to develop suitable potting media for wide range of crops including perennials, vegetables, fruits, shrubs, foliage plants, ornamental plants. The proportion rate of compost recommended to add into Peat is dependent on various plant species, sources of compost and growth stage of plants. For each purpose in horticulture, it is crucial to have more deep and special studies to find optimal growing conditions for individual plant production.

This study was conducted to investigate the suitability of compost-based and peat-based substrates used as potting media for ornamental plants in the greenhouse and develop optimal growing conditions for growth and quality of salt-sensitive plants. In order to address these objectives, two pot experiments were conducted in 2011. Experiment 1 was aimed at studying the effects of compost-based media on yield formation and nutrient uptake, while Experiment 2 was targeted to produce peat-based substrates as standard soils to optimize ornamental values for tested sensitive plants, Pelargonium and Salvia.

3.2. Materials and methods

3.2.1. Experiment 1: Compost-based susbtrates

3.2.1.1. Raw materials

In this experiment, the same source of compost from green wastes studied in Chapter 2 was used as the main basic raw material without leaching. Standard soils produced from mainly white peat and partially black peat (TKS_1 and TKS_2) as the control were included. Hygromull and Cocofiber chosen as additives containing a specific content of nutrient salts were mixed into compost. Besides, standard soil substrate type ED 73 produced from 70% white peat and 30% clay (SPS), an additive for medium salt sensitive plants was used as test additive to decrease the salt content in potting substrates significantly.

The chemical characteristics of compost, standard soils (TKS_1 and TKS_2), and additives for the first experiment are shown in Table 4. TKS_0 produced from 100% white peat which will be mentioned in the later experiment is also included in Table 4. The salt content and the nutrient content of compost were significantly higher than that of standard soils. The nutrient and salt content of standard soils are in the following range: $TKS_0 < TKS_1 < TKS_2$. The chemical indexes of TKS_2 were mostly two folds higher than TKS_1. Therefore, TKS_1 was used as control substrate for the moderately salt-sensitive Salvia (*Salvia splendens*) and TKS_2 for the less salt-sensitive Pelargonium (*Pelargonium zonale Toro*). SPS, standard soil substrate, has a low salt content and available P and K and was therefore chosen as an additive substrate for the first experiment. Additionally, plant available P and exchangeable K in Hygromull and Cocofiber were rather high and increased the available P and K of substrates in comparison with mixtures of compost and SPS.

Table 4: Chemical characteristics of compost, standard soils and additives

Parameters	**Comp**	**TKS_0**	**TKS_1**	**TKS_2**	**SPS**	**Coco**	**Hy**
DM (%)	47.0	50.1	54.7	59.3	49.5	83.4	0.0
pH ($CaCl_2$)	7.2	5.6	5.3	5.4	5.9	4.5	2.6
E_c (mS/cm)	3.09	0.36	0.92	2.94	0.20	0.38	0.69
P_{Total} (mg/kg)	3887	212	621	1060	449	376	10238
CAL-P (mg/kg)	1410	25	263	742	89	194	10574
K_{Total} (mg/kg)	12117	850	1273	2967	1500	10800	20833
CAL-K (mg/kg)	1773	77	163	419	205	11396	8180
Mg_{Total} (mg/kg)	4980	1300	1061	1525	1707	1094	165
Mg-$CaCl_2$ (mg/kg)	1010	941	860	1150	430	1686	95
Na_{Total} (mg/kg)	2776	216	317	427	176	2373	969
N_{Total} (mg/kg)	18910	7410	8400	7870	3965	3900	264913
S_{Total} (mg/kg)	2405	1760	1830	1855	460	830	1680
C_{Total} (g/kg)	210	461	491	482	191	459	296

Samples were analyzed with three replicates and average values are presented.

3.2.1.2. Experimental design

To compost as the main substrate some additives including SPS, Cocofiber and Hygromull were added. The experimental plants, Pelargonium and Salvia were transplanted in 6 liter pots (diameter: 25 cm, height: 30 cm).

Ten treatments with 4 replications were established (Table 5). The plants were watered with distilled water everyday (50% WHC). The increase of the additive proportion in growing media enhanced the maximum WHC of the substrates. The experiment was conducted from April to May 2011 in the greenhouse of INRES-Plant nutrition, University of Bonn, Germany (Figure 21). After 60 days cultivating whole fresh plant material including flowers and leaves was harvested and dried at 60°C in a thermal oven until the dry mass was constant. Dried plant material was finely ground into powder and stored in plastic bottles before analysis.

Table 5: Treatments of experiment 1 in 2011

No.	Treatment	Potting substrate	WHC (%)
1	Control	Standard soil: TKS_1 for Salvia	145.2
		Standard soil: TKS_2 for Pelargonium	160.7
2	Comp	100% Compost	32.3
3	Comp/SPS 4:1	80% Compost + 20% Standard soil type ED 73	37.3
4	Comp/SPS 1:1	50% Compost + 50% Standard soil type ED 73	60.3
5	Comp/SPS 1:4	20% Compost + 80% Standard soil type ED 73	67.3
6	Comp/Coco 4:1	80% Compost + 20% Cocofiber	24.1
7	Comp/Coco 1:1	50% Compost + 50% Cocofiber	29.5
8	Comp/Coco 1:4	20% Compost + 80% Cocofiber	43.2
9	Comp/Hy 4:1	80% Compost + 20% Hygromull	41.7
10	Comp/Hy 1:1	50% Compost + 50% Hygromull	71.7

Hygromull with its density of 35-40 kg/m^3 was impossible to set up the mixture of Compost and Hygromull (with Comp/Hy ratio = 1:4) as other mixtures, because the ideal bulk density for growing substrates should be about 150-500 kg/m^3 (Nappi and Barberis 1993).

Figure 21. Pelargonium and Salvia planted in compost-based substrates

3.2.2. Experiment 2: Peat-based substrates

3.2.2.1. Pretreatment of material to produce TKS_0

In contrast to experiment 1, TKS_0 produced from 100% Peat without supplement of nutrient salts was the main potting media in experiment 2. Styromull and Perlite containing no salt and nutrients were the additives used in this experiment. The aim of the use of TKS_0, compost and additives was to produce a suitable potting substrate with a low salt content but supplying enough nutrients for salt-sensitive ornamental plants.

TKS_0 was produced from 100% white peat. Slightly compacted Peat was moistened by a micronutrient solution (5.1g/L water) (volumetric ratio of moistening solution and peat = 1.47/100) of the complete fertilizer FERTY® 10 produced by Planta Düngemittel GmbH (Regenstauf, Germany) containing 10% MgO, 0.5% B, 2% Cu, 3.5% Fe, 0.5% Mn, 0.8% Mo and 0.3% Zn. Then 3.5 g/L of limestone ($CaCO_3$) was added to Peat and mixed evenly and the limed Peat was kept for 3 days, mixing once per day to get the lime uniformly distributed. This preparation method was the same as described by Olympios (1992). Water was also added to have a slightly wet substrate which helped to equilibrate the Ca saturation of Peat. After 3 days, pH ranged between 5.5-6.0. After these steps, TKS_0 was ready to set up the pot experiment.

3.2.2.2. Experimental design

The experiment included eight treatments with 4 replications (Table 6).

Similarly to the first experiment, Pelargonium and Salvia were chosen as experimental plants. They were transplanted into 6-liter pots and watered with distilled water everyday (50% of the maximum WHC). The procedure of planting in the greenhouse was the same as the previous experiment. After 2 months (from July to August 2011), whole fresh plant material of both plant species was harvested and dried at $60^{o}C$ in a thermal oven. Dried plant material was then finely ground into powder and stored in plastic bottles before analysis.

Table 6: Treatments of experiment 2 in 2011

No.	Treatment	Potting substrate	WHC (%)
1	Control	Standard soil: TKS_1 for Salvia	145.2
		Standard soil: TKS_2 for Pelargonium	160.7
2	Comp	100% Compost	32.3
3	TKS_0/Comp 3:1	75% Standard soil from 100% Peat + 25% Compost	105.7
4	TKS_0/Comp 6:1	86% Standard soil from 100% Peat + 14% Compost	123.3
5	TKS_0/Comp 6:1 + 5% Sty	81% Standard soil from 100% Peat, 14% Compost + 5% Styromull	118.0
6	TKS_0/Comp 6:1 + 10% Sty	76% Standard soil from 100% Peat, 14% Compost + 10% Styromull	120.0
7	TKS_0/Comp 6:1 + 5% Per	81% Standard soil from 100% Peat, 14% Compost + 5% Perlite	124.8
8	TKS_0/Comp 6:1 + 10% Per	76% Standard soil from 100% Peat, 14% Compost + 10% Perlite	125.3

3.2.3. Analysis methods

For compost and standard soils, parameters including DM, pH ($CaCl_2$), salt content, available P, K, Mg and total N, P, K, Mg, Na, C and S and for plant materials N, P, K, Mg and Na contents were determined according to the standard methods of VDLUFA (Hoffmann, 1991, 1995, 1997). For more details see the analysis methods in Section 2.2.4. Total nutrient uptake (mg/pot) is determined by nutrient content (mg/g DM) x dry matter (g/pot).

Multivariate analysis (ANOVA) was performed using the SPSS 18.0 software package (Chicago, IL, USA). Means of DM, content and total uptake of nutrients in plant materials were compared at significance level of $p = 0.05$ by Tukey test throughout the study.

3.3. Results and discussions

3.3.1. Experiment 1: Compost-based substrates

3.3.1.1. Plant growth

The first experiment set up for two low salt-tolerant flowering plants, Pelargonium (less salt-sensitive) and Salvia (more salt-sensitive) grown in compost-based substrates is shown in Figure 22.

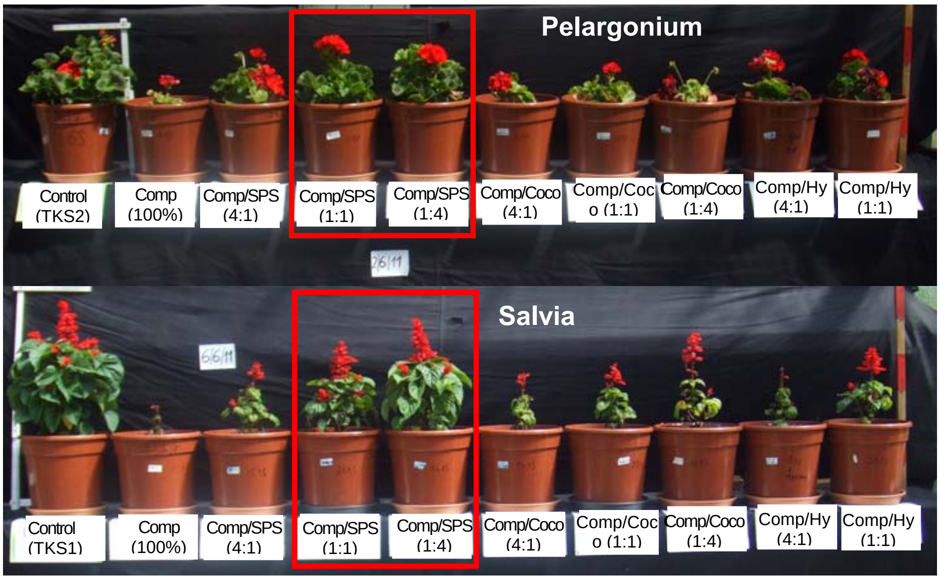

Figure 22. Pelargonium and Salvia grown in compost-based substrates 45 days after planting

As shown in Table 7, the salt content in pure compost and mixtures with a share of > 50% compost and additives at the start and at the end of the experiment, respectively, was notably higher than that of the standard soils. Caused by nutrient uptake of plants the salt content was reduced in all substrates. It is assumed that the high salt content in the compost-based substrates caused the poor plant growth of Pelargonium and Salvia (Figure 23).

Table 7: Salt content in growing media before and after harvest

Substrate	Initial E_c (mS/cm)	E_c after harvest (mS/cm)
Pelargonium		
Control-TKS_2	0.50	0.30
Comp 100%	1.52	1.35
Comp/SPS 4:1	1.24	1.10
Comp/SPS 1:1	1.11	0.60
Comp/SPS 1:4	0.42	0.30
Comp/Coco 4:1	1.27	1.08
Comp/Coco 1:1	1.18	0.83
Comp/Coco 1:4	0.64	0.53
Comp/Hy 4:1	2.03	1.13
Comp/Hy 1:1	1.51	1.00
Salvia		
Control-TKS_1	0.27	0.10
Comp 100%	1.52	1.28
Comp/SPS 4:1	1.24	0.93
Comp/SPS 1:1	1.11	0.58
Comp/SPS 1:4	0.42	0.25
Comp/Coco 4:1	1.27	1.08
Comp/Coco 1:1	1.18	0.75
Comp/Coco 1:4	0.64	0.48
Comp/Hy 4:1	2.03	1.18
Comp/Hy 1:1	1.51	0.90

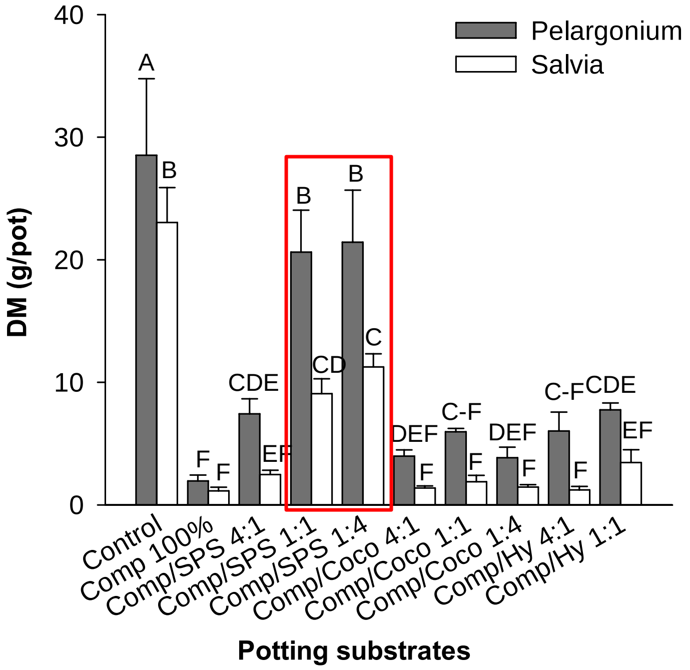

Figure 23. Dry matter yield of Pelargonium and Salvia planted in compost-based substrates

Error bars represent the standard deviation of 4 replicates. Means of different treatments followed by the same letters are not significantly different ($p < 0.05$) by Tukey test.

Dry matter yield of Pelargonium and Salvia was highest in the control treatments (standard soils TKS_2 and TKS_1, respectively) and lowest in the 100% compost (Figure 23). Although the yield of both plants was very low in the treatment with a share of 20% SPS, plant growth was favored by the small addition of SPS. DM yield of both plants increased significantly with an increasing share of SPS. However, the yield could not reach that of the control. While in the control treatments DM yield of Pelargonium amounted to 28.5 g/pot and of Salvia to 23.0 g/pot, 20.6 and 21.4 g/pot were reached for Pelargonium and 9.1 and 13.1 g/pot determined for Salvia with the ratios Comp/SPS 1:1 and 1:4, respectively (Figure 23). It should be pointed out that with both Comp/SPS ratios the improvement of yield formation was higher, demonstrating the higher salt tolerance of Pelargonium. While with Pelargonium the addition of Cocofiber and Hygromull partially favored yield formation as compared to 100% of compost, growth of Salvia was not favored (Figure 22). It is assumed that the high salt content in compost (Table 7) as the main substrate component negatively affects plant growth (Papafotiou et al. 2004). Furthermore, Cocofiber resulted in a highly porous, less water holding substrate which may favor partially leaching of nutrient from the potting material. In addition, Hygromull with a relatively high salt content (Table 7) negatively affected plant growth. Therefore, both additives are not recommended.

The results of the present investigation are in agreement with results of Fischer and Popp (1998) or Grigatti et al. (2007), who investigated the impact of a share of compost (25%) from anaerobically treated refuse with dry fermentation or green wastes and sewage sludge mixed with Peat on growth of *Deuzia scabra* 'Candidissima' or *Salvia splendens* 'maestro', respectively. In contrast, Klock-Moore (2000) found that compost from seaweed with a high initial salt content added at 0, 30, 60 and 100% by volume caused no depression on Salvia growth and even produced salable quality of Salvia flowers. In a report by Ostos et al. (2008), the compost-based substrates with the presence of up to 40% compost from sewage sludge strongly enhanced biomass weight of the shrub *Pistacia lentiscus* L. which was significantly higher than that grown in the commercial Peat as control. Wilson et al. (2001, 2002, 2003, 2004) conducted four experiments with peat-based soilless media amended with 25, 50, 75, 100% compost from biosolids and yard trimmings to examine the growth of 24 ornamental perennials including Salvia. Despite both positive and negative effects on plant growth were observed at high rates of compost addition (50-100%), flowering or visual quality of these tested perennial species were more or less affected, but still marketable in horticulture. The diverse results among studies on compost-based substrates were due to the differences of sources of compost and plant species having different salt tolerance (Grigatti et al. 2007).

3.3.1.2. Content and total uptake of nutrients (N, P, K, Mg and Na)

As compared to the standard soil TKS_2, N, P, Mg contents (mg/g DM) of Pelargonium planted on compost treatments were generally lower (Figure 24i, 25i, 27i), but K and Na contents were higher (Figure 26i, 28i). These results were similar for those of Salvia, except N content which reached the same level or was slightly higher than the standard soil TKS_1 (Figure 24i–28i). Furthermore, Na content of Salvia was generally higher than that of Pelargonium (Figure 24i–28i). The reduction of the share of compost incorporated into potting materials resulted in a proportional lower salt content. As a result, K and Na uptake contents were corresponding decreased (Figure 26i, 28i), while N, P, and Mg uptake contents were orderly increased (Figure 24i, 25i, 27i).

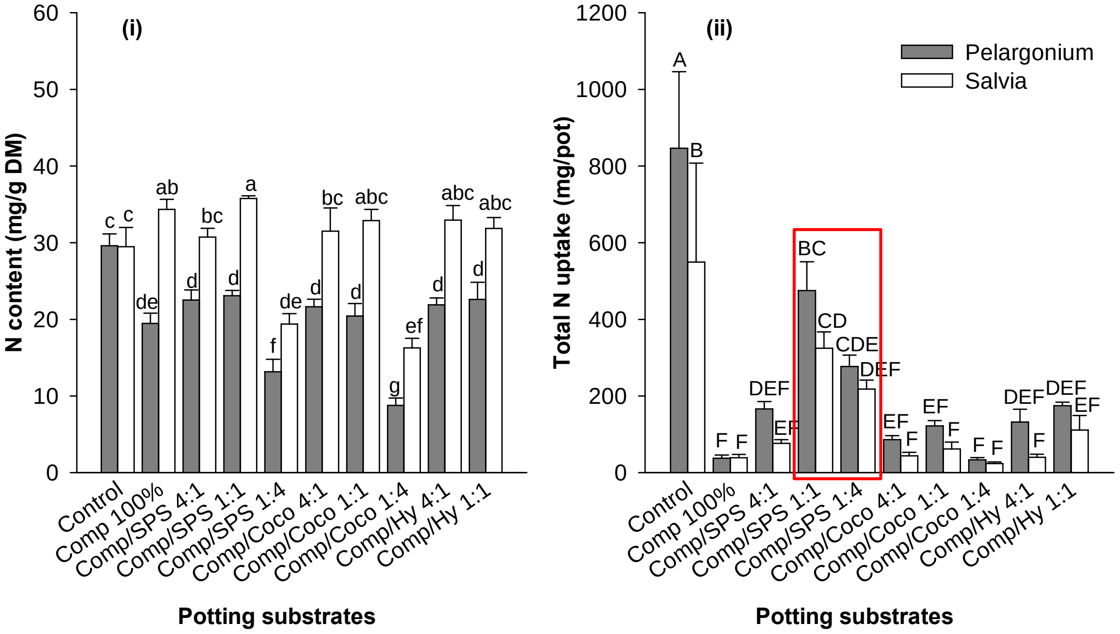

Figure 24. N content (i) and total N uptake (ii) of Pelargonium and Salvia planted in compost-based substrates

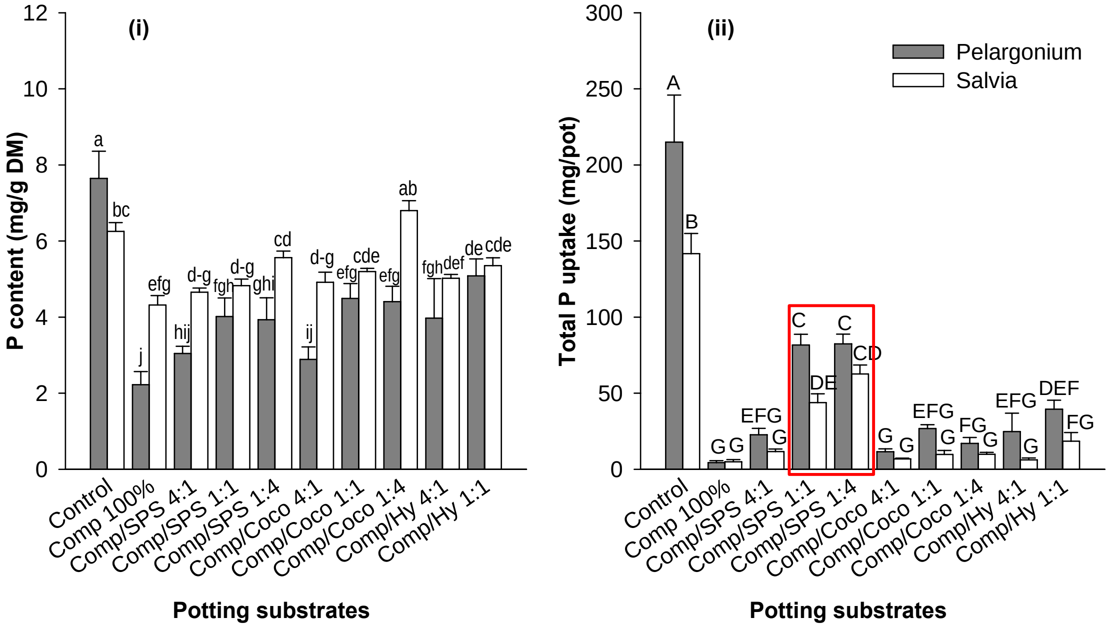

Figure 25. P content (i) and total P uptake (ii) of Pelargonium and Salvia planted in compost-based substrates

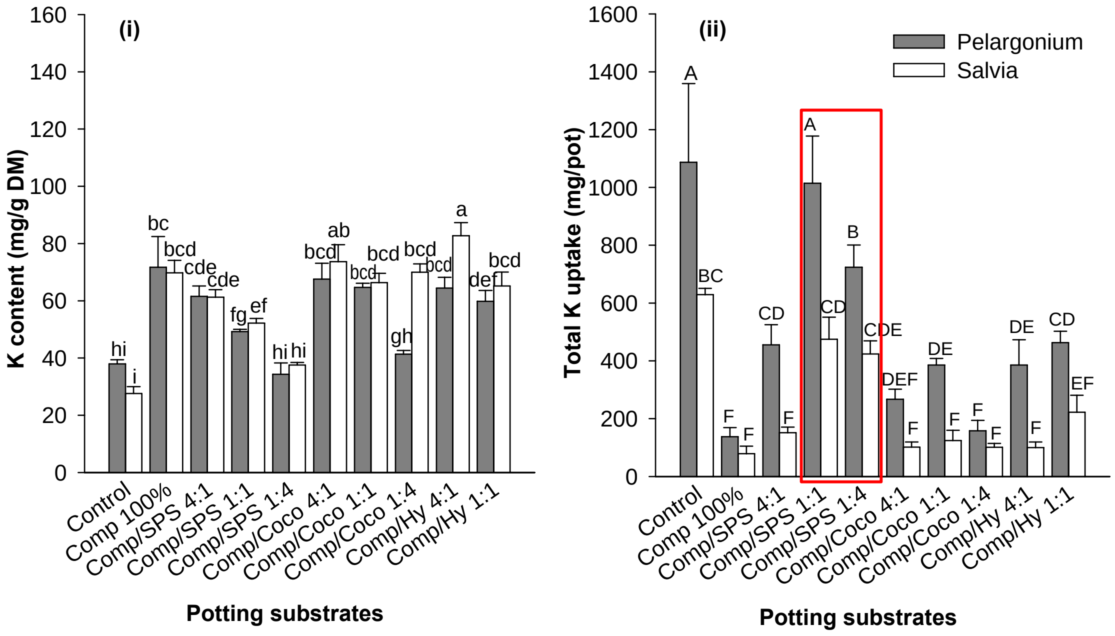

Figure 26. K content (i) and total K uptake (ii) of Pelargonium and Salvia planted in compost-based substrates

Total nutrient uptake of both plants from almost all treatments including the pure compost and the additive-mixed composts, except the mixtures of compost and SPS (with the volumetric ratios 1:1 and 1:4), were statistically not different and had the lowest levels (N: 33.3-174.5; P: 4.3-39.5; K: 138.0-463.3; Mg: 3.8-18.6 and Na: 11.0-29.8 mg/pot for Pelargonium and N: 23.7-111.0; P: 4.9-18.4; K: 79.5-222.2; Mg: 2.5-7.0 and Na: 24.4-54.8 mg/pot for Salvia, respectively) in comparison to the control treatments (N: 846.1; P: 215.0; K: 1087.2; Mg: 90.1 and Na: 34.2 mg/pot for Pelargonium and N: 549.6; P: 141.7; K: 628.9; Mg: 73.7 and Na: 135.3 mg/pot for Salvia, respectively) (Figure 24ii–28ii). Among these compost treatments, the highest total nutrient uptake of both plants was observed in the mixtures of 20% compost, followed by 50% compost and SPS, reaching close the level of the control. Notably, total Na uptake of Salvia from the Comp/SPS mixtures with volumetric ratios of 1:1 and 1:4 was estimated as 142.2 and 127.1 mg/pot (Figure 28ii), respectively. It significantly increased and being as high as the control treatment which is the result of the high yield formation of these plants. In contrast, total Na uptake of the 100% compost treatment was extremely low caused by the low yield of this treatment.

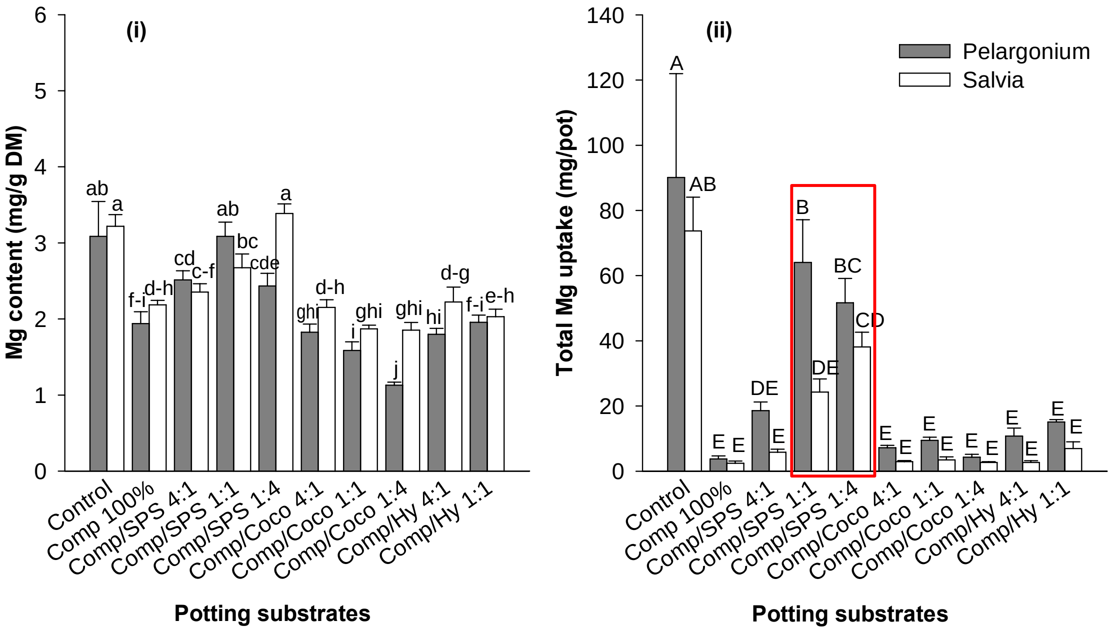

Figure 27. Mg content (i) and total Mg uptake (ii) of Pelargonium and Salvia planted in compost-based substrates

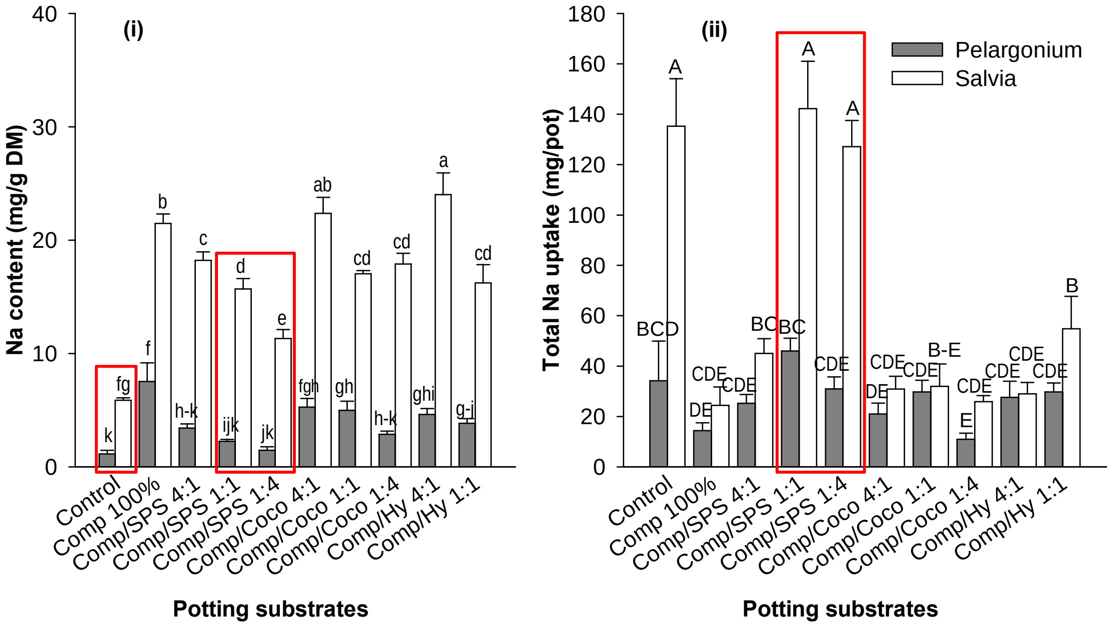

Figure 28. Na content (i) and total Na uptake (ii) of Pelargonium and Salvia planted in compost-based substrates

In the literature, the mixture of 40% compost originated from pine bark and moss was found as potential and adequate growing materials for lettuce (*Lactuca sativa* var. *capitata*) (Oberpaur et al. 2010), or compost-based susbtrates with 40% compost from sewage sludge or municipal solid wastes significantly increased nutrient concentrations and total nutrient uptake of a shrub (*Pistacia lentiscus* L.) (Ostos et al. 2008). However, the authors only mentioned the positive results when mixing 40% compost as main component and Peat substitution, other applied percentage of compost in compost-based substrates probably resulted in unknown effects on plant growth and nutrient uptake. In this study, the large proportion of compost incroporated into potting materials caused some damages which was phytotoxic for the development and quality of the salt sensitive plants, Salvia and Pelargonium. The same negative effects were recorded for Poinsettia production when replacing over 37.5% volumetric Peat by olive-mill waste compost (Papafotiou et al. 2004).

On the other hand, the presence of Hygromull or Cocofiber with different proportions in the growing media, despite partially salt content dilution and water-holding capacity enhancement (Table 6), seemed to depress the growth of Salvia and Pelargonium. Thus total nutrient uptake was not obviously improved as when the plants were grown in SPS-incorporated compost. Therefore, based on the results of this experiment the addition of Hygromull or Cocofiber to compost may not be recommended. Moreover, total nutrient uptake except Na by Pelargonium from all compost-based materials was larger than that of Salvia (Figure 24ii–28ii). This may be explained by the better adaptation and salt tolerance of Pelargonium as compared to Salvia when growing in media having high salt content. Clearly, the content and total uptake of nutrients into plant tissues among and within species varied remarkably as compost fraction increased in the substrate (Grigatti et al. 2007).

In summary, both Pelargonium and Salvia grown in the compost-based media with a high share of compost more than 50% suffered from the high salt content which negatively affected plant growth and nutrient uptake. Yield formation and total nutrient uptake of plants were observed to be highest in the control, followed by the potting substrates created by compost and SPS with the mixing ratios of 1:1 and 1:4. The results demonstrated that the high rate application of mainly peat-containing substrates like SPS favored plant growth. However, the incorporation of Cocofiber and Hygromull into compost negatively influenced the growth of the experimental plants. The negative results on DM weight and low nutrient uptake of Pelargonium and Salvia cultivated on compost-based substrates in this experiment were a basic foundation to understand the low salt-tolerant plant behavior and address the changes of salt content in materials to improve plant growth by mixing more additives into substrates and reducing the proportion of compost application in potting substrates. Therefore, a second experiment with peat-based substrates was conducted.

3.3.2. Experiment 2: Peat-based substrates

3.3.2.1. Plant growth

The second experiment was conducted with the same experimental plants as in the previous experiment. TKS_0 originated from 100% white peat was used as basic material and compost was added as partial Peat substitution to provide sufficient nutrients and an ideal salt content of the potting substrates.

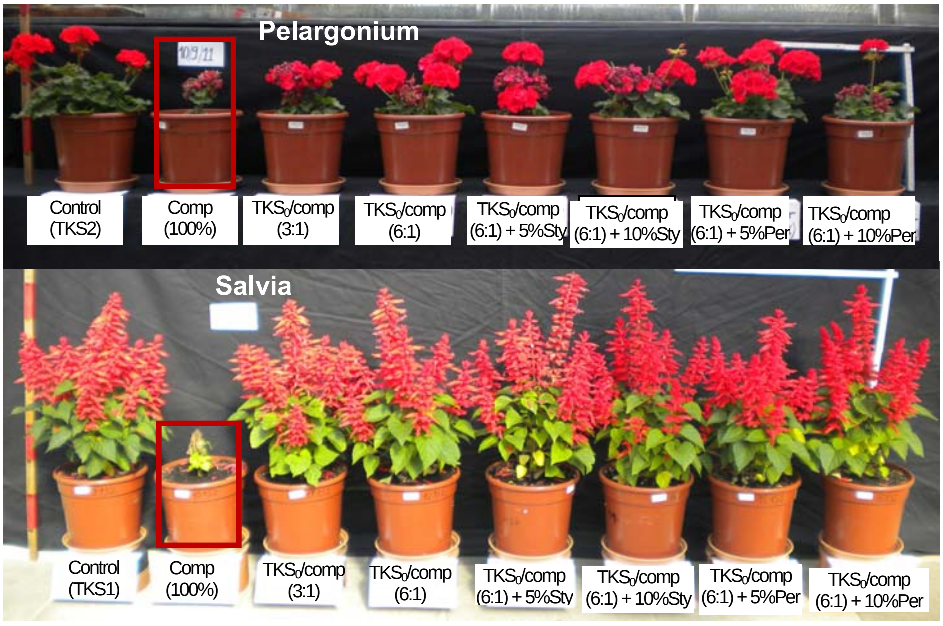

Figure 29. Pelargonium and Salvia planted in peat-based substrates 55 days after planting

Table 8: Estimation of salt content in TKS_0-based substrates before and after harvest

Substrate	**Initial E_c (mS/cm)**	**E_c after harvest (mS/cm)**
Pelargonium		
Control-TKS_2	0.50	0.28
Comp 100%	1.52	1.02
TKS_0/Comp 3:1	0.65	0.44
TKS_0/Comp 6:1	0.53	0.30
TKS_0/Comp 6:1 + 5%Sty	0.51	0.29
TKS_0/Comp 6:1 + 10%Sty	0.49	0.35
TKS_0/Comp 6:1 + 5%Per	0.51	0.31
TKS_0/Comp 6:1 + 10%Per	0.49	0.27
Salvia		
Control-TKS_1	0.27	0.05
Comp 100%	1.52	1.09
TKS_0/Comp 3:1	0.65	0.29
TKS_0/Comp 6:1	0.53	0.28
TKS_0/Comp 6:1 + 5%Sty	0.51	0.25
TKS_0/Comp 6:1 + 10%Sty	0.49	0.26
TKS_0/Comp 6:1 + 5%Per	0.51	0.24
TKS_0/Comp 6:1 + 10%Per	0.49	0.22

As shown in Table 8, except the extremely high salt content of the pure compost, the salt contents of other potting materials were close to the level of the standard soils (TKS_1 and TKS_2). Additionally, the salt content of all TKS_0-based treatments or peat-based treatments were decreased remarkably after harvest.

Table 9. The influence of growing media on plant height, diameter, number of leaves and flower buds of Pelargonium 31, 46 and 62 days after planting

Parameter	Number of days	Control	Comp	TKS_0/Comp (3:1)	TKS_0/Comp (6:1)	TKS_0/Comp (6:1) + 5%Sty	TKS_0/Comp (6:1) + 10%Sty	TKS_0/Comp (6:1) + 5%Per	TKS_0/Comp (6:1) + 10%Per
Height (cm/plant)	**0**	8.5	8.0	8.5	6.5	8.0	7.0	7.0	7.5
	31	13.3	12.8	13.5	14.3	14.5	15.5	11.8	14.5
	46	22.5	13.3	19.5	18.0	19.5	18.0	19.0	20.0
	62	23.0	13.3	19.5	18.5	20.5	22.0	21.5	24.0
Diameter (cm/plant)	**0**	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
	31	19.0	11.3	15.0	16.5	14.0	16.5	16.0	17.5
	46	24.5	11.5	19.5	23.5	18.0	22.5	25.0	25.0
	62	33.5	12.5	25.0	26.5	22.5	28.0	27.0	27.0
Leaves per plant	**0**	10.0	12.0	7.5	10.0	8.0	9.5	8.5	7.0
	31	28.0	13.0	18.0	23.5	17.0	26.5	26.5	20.5
	46	52.5	12.5	22.0	30.5	23.5	36.5	34.5	30.0
	62	59.0	18.0	29.0	34.0	27.0	39.5	40.0	40.0
Flower buds per plant	**0**	1.0	0.5	1.0	1.0	1.0	1.0	0.0	1.0
	31	3.0	1.5	2.0	3.0	3.0	2.5	3.0	1.5
	46	6.5	2.0	2.0	3.0	3.0	4.0	5.5	4.0
	62	7.5	1.0	2.5	2.0	2.0	5.0	3.5	4.5

Table 10. The influence of growing media on plant height, diameter, number of leaves and flower buds of Salvia 31, 46 and 62 days after planting

Parameter	**Number of days**	**Control**	**Comp**	**TKS$_0$/Comp (3:1)**	**TKS$_0$/Comp (6:1)**	**TKS$_0$/Comp (6:1) + 5%Sty**	**TKS$_0$/Comp (6:1) + 10%Sty**	**TKS$_0$/Comp (6:1) + 5%Per**	**TKS$_0$/Comp (6:1) + 10%Per**
Height (cm/plant)	**0**	8.0	11.0	9.3	8.5	10.0	9.3	9.5	8.8
	31	30.0	14.5	27.0	21.0	35.0	28.5	26.0	24.0
	46	45.5	14.5	43.0	38.5	47.5	43.0	41.0	41.5
	62	50.0	13.5	47.0	46.5	49.0	46.8	47.0	49.5
Diameter (cm/plant)	**0**	5-7	5-7	5-7	5-7	5-7	5-7	5-7	5-7
	31	23.5	8.8	20.0	18.5	23.5	21.0	20.5	21.5
	46	37.5	7.0	31.5	30.5	30.5	35.0	33.0	30.5
	62	42.5	died	37.0	37.5	38.0	38.0	38.0	40.0
Leaves per plant	**0**	8.5	9.5	8.0	8.0	10.0	9.0	10.0	9.0
	31	101.5	38.5	89.5	81.0	94.5	99.5	105.0	93.0
	46	175.5	30.5	151.0	125.5	127.3	137.5	143.5	145.0
	62	218.5	died	169.5	133.0	133.0	146.5	158.0	160.0
Flower buds per plant	**0**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	31	6.5	1.0	8.5	4.5	9.0	8.5	7.5	5.0
	46	7.0	1.0	8.5	11.5	12.5	13.0	13.0	12.0
	62	13.0	died	13.5	13.5	12.0	15.5	14.5	13.5

The results of plant development were partially shown in Table 9 and Table 10. Pelargonium and Salvia massively grew during the period between the day 31th and 46th after planting, and afterwards gradually slowed down their growth rhythm until harvest. The same observation was reported by Papafotiou et al. (2004) for *E. pulcherrima* cv. ‘Peterstar white’ when planted in 12.5-25% olive-mill waste compost and Peat mixtures. Pure compost media was shown to have worsened effects on growth of Pelargonium and Salvia. There was mostly no increase in the height and diameter. Plants withered, leaves were burned and felt, and even Salvia started to have dead symptoms after the first month of planting and died at the harvest time (Figure 29). In contrast, plants grown in the standard soils were observed to have much more improvements than those in the pure compost (Table 9). Although Pelargonium and Salvia planted in the peat-based treatments were lower in height, diameter, and number of leaves than plants grown in the standard soils, they were still marketable ornamental plants. Besides, the number of flower buds of Salvia planted in almost peat-based substrates had the same quantity as the control. This means the peat-based media seem to be good potting substrates for salt-sensitive flowering plants like Pelargonium and Salvia in term of marketable ornamental production. Similarity, Grigatti et al. (2007) found that DM and number flowers peaked for *Begonia semperflorens* when they were cultivated in a mixture of white peat and 25% compost which was originated from biosolids. However, *Mimulus* and *Salvia splendens* grew best in the media incoporated with 25-50% compost (Grigatti et al. 2007).

Similar to results of the previous experiment, DM weight of both plants obtained in the sole compost was negligible (Figure 30). The yield formation of Pelargonium or Salvia planted in TKS_0-based substrates supplied with 25% or 14% compost, respectively, was equally lower than the standard soils. However, in comparison with the results of the previous experiment, dry matter yield (DM) of both plants grown in the peat-based treatments ranged from 8.0-10 g/pot for Pelargonium and 13.8-15.9 g/pot for Salvia which was close to the DM values of the control determined as 14.0 g/pot for Pelargonium and 25.1 g/pot for Salvia, respectively. Although the yield formation obtained from peat-based treatments was lower than that of the control, it was at the same high magnitude and had still ornamentally valuable, especially Salvia (Figure 30). Additionally, there was an insignificant difference of DM of the plants among peat-based treatments. This indicates that the presence of additives Styromull or Perlite in these mixtures had slight effects on plant growth when cultivated in mainly peat-derived potting media.

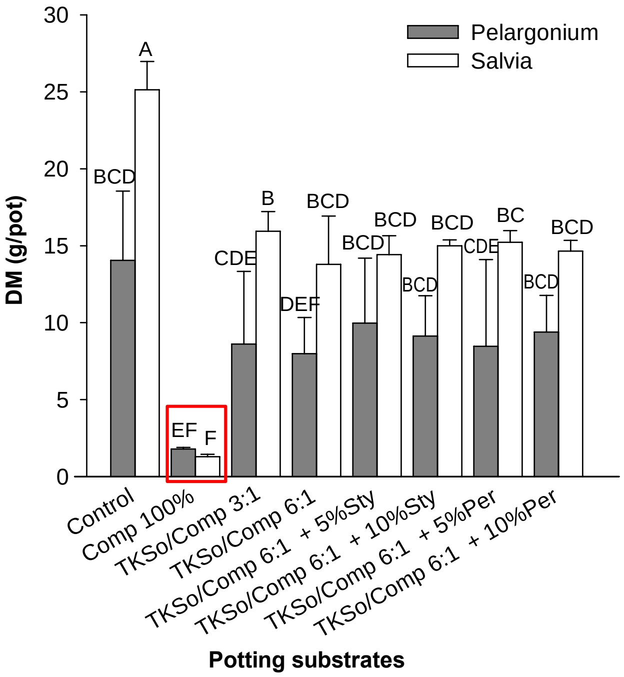

Figure 30. Dry matter yield of Pelargonium and Salvia planted in peat-based substrates

Error bars represent the standard deviation of 4 replicates. Means of different treatments followed by the same letters are not significantly different ($p < 0.05$) by Tukey test.

In this study, compost replaced 14-25% volume of Peat in the growing media notably improved the biomass production and achieved marketable ornamental values. The relatively low proportion of compost incorporated into Peat tended to be suitable for sensitive ornamental plants. Papafotiou et al. (2004) pointed out that the mixtures containing 12.5% olive mill waste compost for poinsettia cultivation were ready for the market. Bragg et al. (1993) found that in peat-based media up to 30% of compost from sewage sludge, based on volume, had no depressive effects on the growth of plant species like *Geraniums Fl hybrid*, *Petunia grandiflora* and *Impatiens super elfin*. Furthermore, the most convenient substrate for studied ornamental plants for instance *C. sempervirens* and *C. arizonica* conducted by Hernández-Apaolaza et al. (2005) was figured out as the mixing of 30% of compost from sewage sludge or biosolid and other additives like Pine bark or Coconut fiber, while Grigatti et al. (2007) reported that a greatly wider range of 25-50% compost as a substitutive in growing media produced significantly higher plant weight and ornamental values comparable to the commercial Peat medium.

3.3.2.2. Content and total uptake of nutrients (N, P, K, Mg and Na)

Nutrient uptake (N, P, K, Mg and Na) of Pelargonium and Salvia grown in all peat-based substrates excluding the 100% compost treatment was mostly as high as that of the control, ignoring the different rate of compost addition (14% or 25% by volume) and additives like Perlite and Styromull (5% or 10% by volume) (Figure 31i–35i). In the pure compost, N, K and Na content of Salvia were notably higher than that of Pelargonium (Figure 31i, 33i, 35i). Furthermore, the contents of N, K and Na of Salvia grown in the sole compost were relatively high as compared to that of the other potting materials (Figure 31i, 32i, 35i), while both Pelargonium and Salvia in the 100% compost were evaluated to have lower P and Mg contents than that of the plants cultivated in the control as well as peat-based treatments (Figure 32i, 34i). Besides, Na content of Salvia was six times higher than Pelargonium (Figure 35i). However, the contents of nutrients (N, P, K, Mg and Na) within these two plant species cultivated in the peat-based substrates were estimated in the ranges of N: 17.4-22.6, P: 2.6-7.6, K: 41.4-44.5, Mg: 1.6-2.5 and Na: 1.9-3.0 mg/g DM for Pelargonium and N: 20.4-31.6, P: 4.0-6.4, K: 39.3-45.6, Mg: 2.0-3.1 and Na:11.4-26.8 mg/g DM for Salvia, respectively, which had the same magnitude as the data recorded in compost-based materials of the first experiment. The results have shown that improvement of nutrient contents (mg/g DM) within ornamental plants grown in peat-based treatments was insignificantly as compared to that in compost-based substrates.

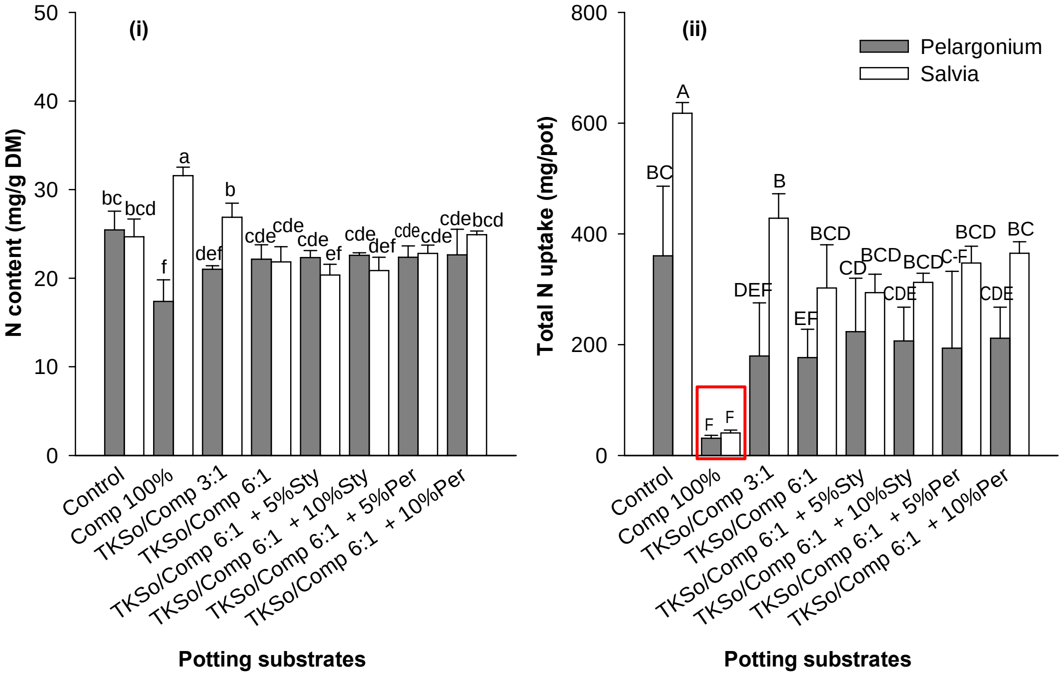

Figure 31. N content (i) and total N uptake (ii) of Pelargonium and Salvia planted in peat-based substrates

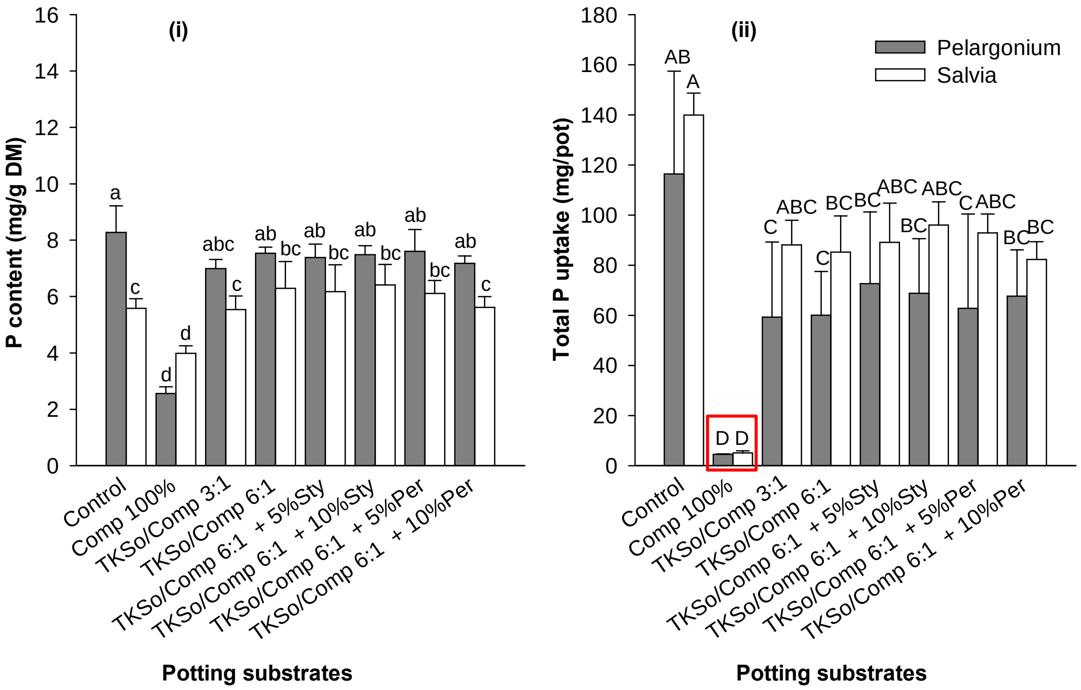

Figure 32. P content (i) and total P uptake (ii) of Pelargonium and Salvia planted in peat-based substrates

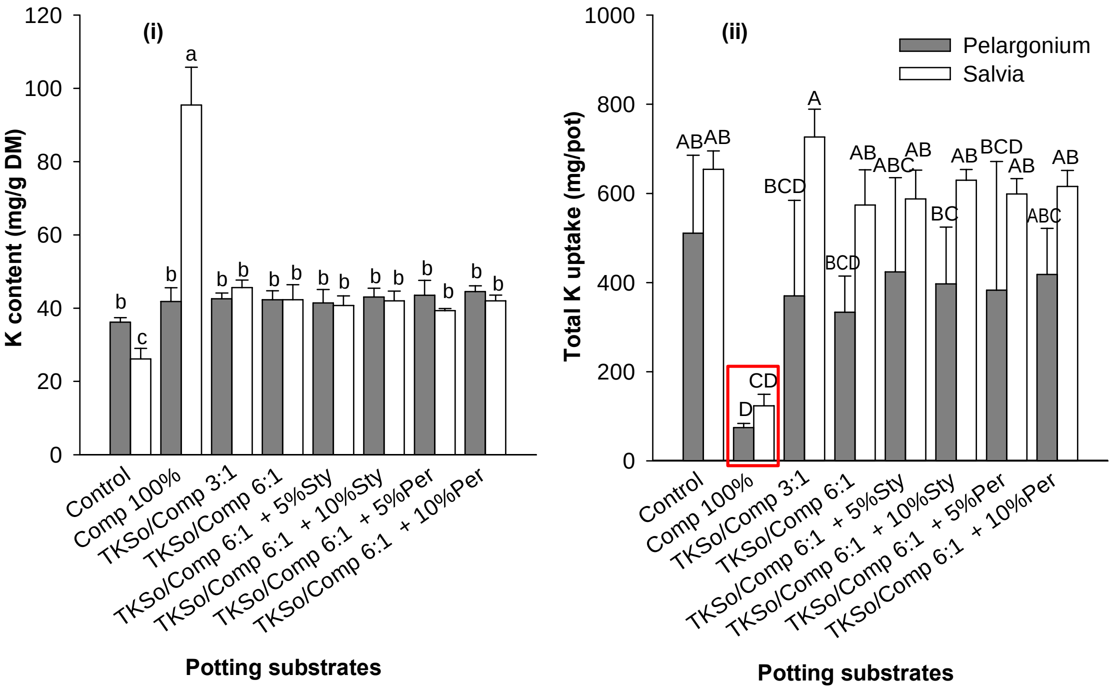

Figure 33. K content (i) and total K uptake (ii) of Pelargonium and Salvia planted in peat-based substrates

Total nutrient uptake by two plants cultivated in 100% compost was negligible (Figure. 31ii-35ii), which is due to poor growth of the plants (Figure 30). The incorporation of 14% or 25% compost into peat-based substrates resulted in remarkable enhancement of total nutrient uptake of both experimental plants compared to the pure compost. Among these treatments, the standard soil (control) was the best potting material in which total nutrient uptake of both plants was highest: total N (360.4), P (116.4), K (510.7) and Mg (33.7) (mg/pot) for Pelargonium and total N (617.8), P (140.0), K (653.9) and Mg (79.5) (mg/pot) for Salvia, respectively (Figure 31ii-34ii), while total Na uptake of Pelargonium and Salvia from the control was figured out to have the same magnitude with that on peat-based treatments. Despite lower of total nutrient uptake observed in the TKS_0-based treatments as compared to those of the control, total N (176.6-223.4), P (59.3-72.6), K (333.2-424.1), and Mg (19.4-24.2) uptake (mg/pot) for Pelargonium and total N (293.9-428.2), P (82.3-96.0), Mg (39.9-48.9) uptake (mg/pot) for Salvia, respectively, achieved marketable values in term of ornamental production (Figure 31ii-35ii). Intensively, total K and Na uptake of Salvia from different TKS_0-based treatments were at the same magnitude of 573.8-726.6 and 157.5-199.2 mg/pot, respectively, which were equal or slightly higher than that of the standard soil (TKS_1) (Figure 33ii, 35ii). Total Na uptake of Salvia increased significantly as compared to the result of the first experiment. This reflects the better adaptation and yield formation of plants when cultivated in peat-based substrates as compared to compost-based ones. Otherwise, total Na uptake of Pelargonium from all treatments including control (TKS_2), pure compost and TKS_0-based substrates was significantly low in the range of 3.9-19.8 mg/pot (Figure 35ii). That is similar to the Na content of Pelargonium as reported in the first experiment. This proves a less influence of the salt level of the growing medium on the growth of Pelargonium. The results of either nutrient content or total nutrient uptake of Salvia from peat-based treatments enhanced remarkably as compared those from compost-based potting materials and being significantly higher than Pelargonium.

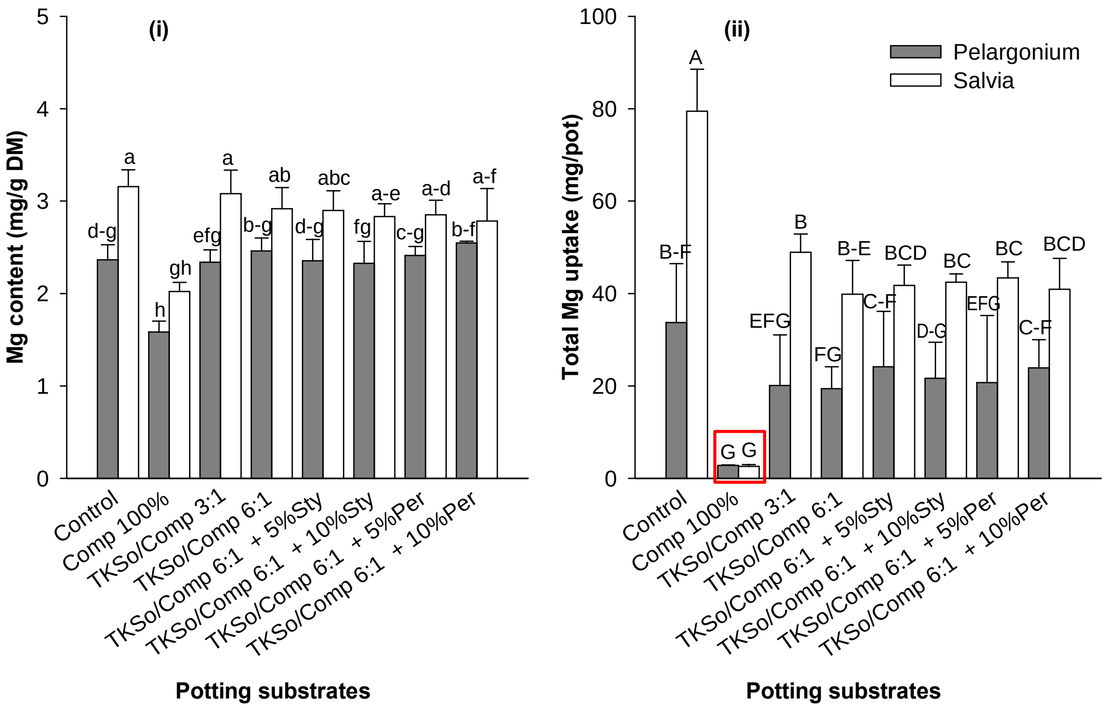

Figure 34. Mg content (i) and total Mg uptake (ii) of Pelargonium and Salvia planted in peat-based substrates

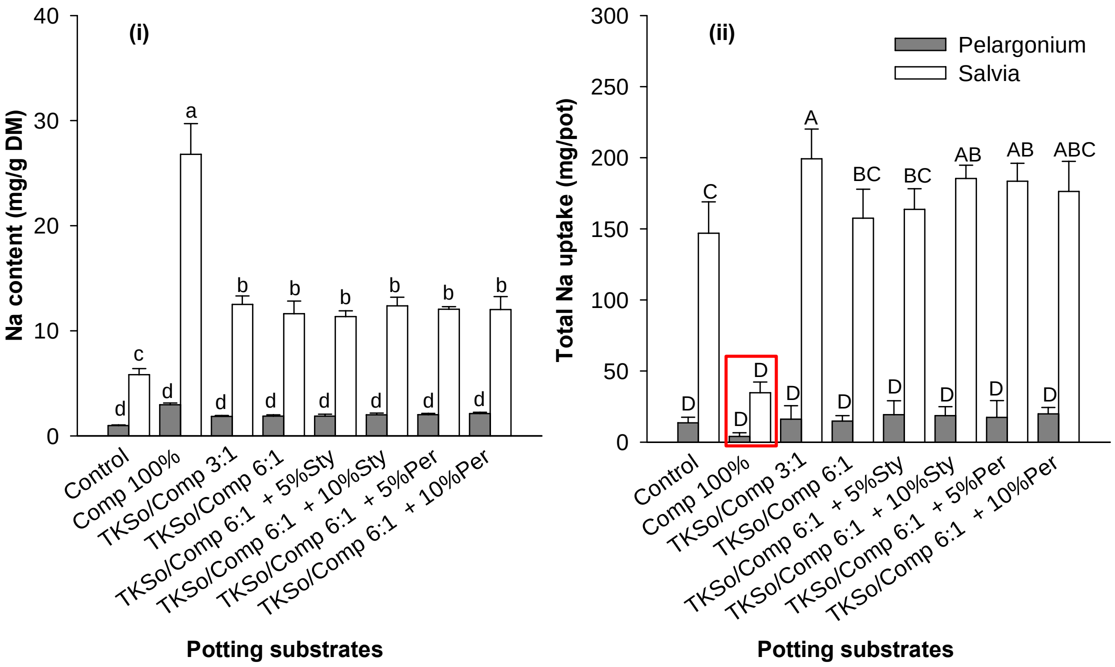

Figure 35. Na content (i) and total Na uptake (ii) of Pelargonium and Salvia planted in peat-based substrates

On the other hand, there was no significant difference of the total nutrient uptake among peat-based treatments without or with the presence of some available additives such as Styromull or Perlite. These results were similar to the results of experiment 1 and confirmed that the supplement of artificial studied additives to compost or peat-based potting substrates practically had unclear effects on plant growth and nutrient uptake. Therefore, these additives were also not recommended to be added to peat-based substrates but they might be suggested in concern of Peat substitution and reducing the dependence on Peat. In this experiment, compost had more or less a role as fertilizer while TKS_0 was used as the main substrate diluting salt content and balancing available nutrients close to the levels of the standard soil, which enhanced total nutrient uptake of plants significantly. Additionally, the addition of 14-25% volume of compost to these peat-based media obviously favored plant growth, especially of more-salt sensitive plants like Salvia. This was further reported by Lamanna et al. (1991) for most of the ten studied ornmental plants grown in all mixtures of compost and Peat as well as reducing Peat volume to 25% compost by volume which was considered as a standard substrate having better plant quality than singular Peat or compost.

It was concluded that salt-tolerant plants such as Pelargonium and Salvia were suitable to grow in the potting substrates based on Peat. Compost should be incorporated at a lower rate than 25% into substrates to supply abundant available nutrients for plant growth and decrease the dependence on Peat. By cultivation in peat-based potting materials, yield formation and total nutrient uptake by Pelargonium and Salvia were enhanced significantly in term of marketable ornamental plant production. However, the supplement of additives Styromull or Perlite had little effects on plant growth and nutrient uptake, thus they might be not recommended for incorporation into peat and compost.

3.4. Conclusion

Pure compost is obviously unsuitable to cultivate Pelargonium and Salvia. The replacement of more than 50% compost by SPS would reduce the high available content of salt in the sole compost and make it more favorable for the growth of these two salt-sensitive plants and significantly improve their yield formation and nutrient uptake as compared to pure compost, or compost- (> 50% by volume) based treatments. Otherwise, the best growth of Pelargonium and Salvia was reported in the control (TKS_2 and TKS_1, respectively) as standard soils.

The incorporation of less than 25% volume of compost into Peat was recommended for making reasonable peat-based potting media which favored the growth of Pelargonium and

Salvia and significantly enhanced their yield formation and nutrient uptake in term of marketable ornamental plant production. The incorporation was recognized to create abundant available nutrients and decrease Peat volume as well as reduce the dependence on Peat during production of potting materials for plants, especially, salt-sensitive plants (Pelargonium and Salvia).

However, the presence of typical artificial additives (Hygromull, Cocofiber, Styromull or Perlite) created unclear effects on both plant growth and nutrient uptake, thus they were not recommended for the partial supplement into Peat and compost.

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Chapter 4

Investigation on antibiotic uptake from manure-amended soil

Abstract

For food security, it is necessary to evaluate the hygienic and safety of potting substrates for vegetable cultivation before applying as growing media for plants. Especially, food security should be taken into account for the potting substrates as media for food or vegetable plant cultivation. As a consequence of intensive livestock raising some popularly used antibiotics mixed into feeding food and excreted into livestock manure might be detected in the growing media when adding livestock manure as fertilizer. In this experiment, the uptake of two typical kinds of antibiotics (Chlortetracycline, Sulfamethazine) existing in Chinese top soils were studied. The initially experimental concentrations of these antibiotics were 100, 200 and 400 µg/kg regarded to their present concentration range in Chinese top soils. Chinese cabbage was chosen as a target plant to investigate the uptake of these antibiotics from the pig manure-amended soils having high and low carbon content, respectively. Cabbage was cultivated from May to August, 2011 in the open greenhouse of INRES-Plant Nutrition, University of Bonn, Germany. Antibiotics were extracted from plant materials after harvest and analyzed by HPLC-MS. The results showed that the presence of available high carbon content in the soil increased crop yield of cabbage. However, antibiotics were not detected in the cabbage materials when the initial studied concentrations of the antibiotics in the soil were employed. It was revealed that with the small concentrations of the applied antibiotics, the uptake of these antibiotics by cabbage might be negligible.

Keywords: Chlortetracyline, Sulfamethazine, antibiotic uptake, cabbage, soil, manure.

4.1. Introduction

Antibiotics have been widely used to improve livestock growth in food for animals or to reduce disease outbreak in husbandry and poultry all over the world. The over use of antibiotics as feeding additives for cattle, pig, cow, chicken, etc. may resulted in an incomplete digestion, leading to a large amount of antibiotics (up to 90%) excreted with urine or feces, which are used as fertilizers (Heuer et al. 2011). For example Chlortetracyline was reported to be excreted over 70% with urine and feces (Kumar et al. 2005a). Therefore, antibiotic-containing manure applied to supply nutrients and improve crops has unexpectedly increased the amount of antibiotics accumulated in the soil.

The amount of antibiotics has been used differently among countries. In Europe, it was estimated that about 5000 tons of antibiotics were used in the 1990s (Zhao et al. 2010). In China, the use of antibiotics in livestock industries has been developed in the last three decades and estimated approximately 6000 tons of average veterinary antibiotics annually (Zhao et al. 2010). A large amount of antibiotics have been found in hectares of agricultural lands and contributing to change the soil microbial ecosystem (Kumar et al. 2005a). The presence of antibiotics, especially less mobile antibiotics, poses a potential of being toxic to plants or soil microorganism (Kumar et al. 2005a), or negatively develops antibiotic resistance genes and increases selections of resistant native bacteria in soil (Heuer et al. 2011). These impacts could directly or indirectly affect soil flora and fauna as well as nutrient availability because of changing soil microorganisms (Kumar et al. 2005a, Liu et al. 2009). Besides, a significant amount of antibiotics and its resistant bacteria originated from animal feces exist in soils, they may be leached and percolated into surface and ground water (Boxall et al. 2002, Christian et al. 2003, Stone et al. 2010). This is potential to cause hazardous health problems to human and animals via exposure with antimicrobial-contaminated water or food consumption (Heuer et al. 2011).

The concentration of antibiotics in manure was administrated in the range of trace levels of 200 mg/kg or L, with typical ranges from 1-10 mg/kg or L (Kumar et al. 2005b). Many antibiotics such as Chlortetracycline (Jiang et al. 2010, Stone et al. 2010), Tylosin (Stone et al. 2010), Tetracycline (Zhao et al. 2010), Sulfonamides (Zhao et al. 2010), Fluouroquinolones (Zhao et al. 2010), Sulfamethazine (Jiang et al. 2010), etc. were typically documented at a high using frequency as feed additives and detected in agricultural regions. The concentrations of Tetracycline and Chlortetracycline were respectively reported of 4.0 and 0.1 mg/kg in manure and released up to 86.2-198.7 and 4.6-7.3 µg/kg into the soil in Germany (Hamscher et al. 2002). Among collected reports, there were several studies recording various antibiotics popularly used in agriculture and food industries in China. According to a survey implemented in eight provinces in China from the North to the South (Shandong, Jiangsu, Shanghai, Zhejiang, Jiangxi, Hubei, Hunan and Guangxi), Chlortetracycline has been found in high concentrations in cow, chicken and pig manure, ranging between 21.1 and 27.6 mg/kg (Zhao et al. 2010). Additionally, thirteen antibiotics residues in manure (5 Sulfonamides, 4 Tetracyclines, 2 Quinolones, Furazolidone, and Chloramphenicol) sampled from 4 livestock husbandries in Tianjin (China) were detected at the concentration range of 0.3-173.2 mg/kg (Hu et al. 2008). Hu et al. (2010) further reported on detection of antibiotics in four typical farms of Tianjin in China and came to an important conclusion that there was no obvious geographical difference in antibiotic distribution between soil and manure.

In this study, two antibiotics Chlortetracycline and Sulfamethazine setting at the initial concentration range of 100, 200 and 400 µg/kg were incorporated into pig manure-amended soils to investigate growth and uptake of these antibiotics by Chinese cabbage.

4.2. Materials and methods

4.2.1. Chemicals

Based on the results of sub-project 6 of accumulation and leaching of P, heavy metals, and antibiotics following extended organic fertilization in light soils, Chlortetracyline (CTC) and Sulfamethazine (SMZ) were popularly present in the monitoring top soils of China. Therefore, these two antibiotics were selected to study at different concentrations of 100, 200 and 400 µg/kg.

The standards of the investigated substances CTC (in form of hydrochloride), and SMZ were purchased from Sigma-Aldrich (Schnelldorf, Germany), 4-epi Chlortetracycline (also in form of hydrochloride) was obtained from Fisher Scientific (Schwerte, Germany). $^{13}C_6$-Sulfamethazine, (Cambridge Isotope Laboratories, Saarbrücken, Germany) served as internal standard. All substances had a declared purity of > 95%. Internal standard solution was prepared in acetonitril at a concentration of 1 ng/mL and stored in the dark at -20°C. During extraction epimerization of Chlortetracycline was likely to take place, thus when CTC is mentioned in the following this refers to the sum of the original substance and the associated epimer.

4.2.2. Soil, manure and plant

Table 11: Texture of soil

Soil texture	Sand (%)	Silt (%)	Clay (%)
Low C soil	49.9	33.2	16.9
High C soil	56.1	30.1	13.8

(Source: INRES-Soil Science, University of Bonn, 2011)

The soils used as potting media for this experiment (0-20 cm depth) were collected from the experimental field of INRES-Soil Science, University of Bonn, Germany. The experimental soils differ in the carbon content (high carbon content (HC soil), low carbon content (LC soil)). However, the texture of both these soils was very similar (Table 11).

Table 12: Quality index of soil and manure

Material	DM %	pH ($CaCl_2$)	E_c mS/cm	P_{Total} mg/kg	CAL-P mg/kg	K_{Total} mg/kg	CAL-K mg/kg	Mg_{Total} mg/kg	Mg-$CaCl_2$ mg/kg	Na_{Total} mg/kg	N_{Total} mg/kg	S_{Total} mg/kg	C_{Total} mg/kg
HC soil	2.7	6.8	20.1	838	207	1650	451	1528	633	194	1260	150	12060
LC soil	4.4	6.5	10.0	545	62	1200	181	1417	385	164	880	90	8170
Pig manure	74.9	7.2	91.8	15748	9060	19003	20440	12881	13982	1964	31150	3510	366360

Samples were analyzed with three replicates and average values are presented

The pig slurry was incorporated into the soil aimed to produce experimental soil conditions similar to the studied Chinese top soil at the application rate of 30 tons/ha (3.10^{-4} kg/cm^2). Table 12 shows the quality index of soil and manure. All parameters of the high carbon-containing soil including pH, salt content, available nutrients, and contents of total macro nutrients were significantly higher than the low carbon-containing soil. Additionally, the nutrient salts in the pig manure were abundant.

White cabbage (*Brassica oleracea* var.capitata f.abba), a typical Chinese vegetable, was chosen as target plant for this experiment.

4.2.3. Experimental design

Cabbage, cultivated during three months from May to August, 2011, was transplanted into pots (diameter: 22 cm, height: 23 cm), containing 9 kg of the experimental soil. The plants were watered with distilled water every day (70-80% of the maximum WHC).

Chinese cabbage was grown in the greenhouse of INRES-Plant nutrition, University of Bonn, Germany. There were totally 10 treatments including one control treatment of the soil with high or low carbon content without antibiotics, three treatments of the soil with high or low carbon content spiked both with antibiotics at three initial concentrations (100, 200 and 400 µg/kg), one treatment of the soil with high or low carbon content spiked sole Chlortetracycline or Sulfamethazine at the highest concentration of 400 µg/kg. The pot replication of each treatment was four (Figure 36).

Figure 36. Cabbage planted in antibiotics spiked-soil

Nutritient solutions (NH_4NO_3, K_2HPO_4 and $MgSO_4.7H_2O$) containing 0.5 g N, 0.8 g K, 0.32 g P and 0.25 g Mg per pot were added to the soils three times equally (at the beginning, after 2 weeks and 8 weeks after planting) to supply macro nutritional elements for cabbage. After three months, cabbage bulbs were harvested. Whole fresh plant materials were freeze dried at -30^{o}C and 0.630 mSav by Christ Gamma 1-16 LSC freezer (SciQuip Ltd., Shropshire, UK) for one week and then finely ground into powder by a Siebtechnik mill (TS 250, Labexchange, Burladingen, Germany) and stored dry in plastic bottles and kept in a freezer until extraction.

4.2.4. Analysis methods

4.2.4.1 Extraction method of antibiotics from plant materials

2.5 grams of cabbage DM sample was suspended in 20 mL of buffered extraction solution (0.1M Citric acid monohydrate; 0.2M $NaH_2PO_4 \cdot H_2O$.; Titriplex 37.2g/L) (pH = 4.1) and manually shaken and ultrasound for 5 min. After ultrasound, the samples were centrifuged for 20 min at 2500 x g, and the supernatant was collected. To the remaining solid plant material, 20 mL of buffered extraction solution was added again and repeated the same steps before to collect all supernatant in glasses. The supernatant liquid was then centrifuged for 40 min at 2500 x g. The liquid was passed through an OASIS HLB 200 mg (Waters Corporation, Milford, MA) cartridge (preconditioned with 6 mL of methanol and 6 mL of nanopure water). After all liquid had passed through the cartridge, it was dried by a vacuum pump for 30 min preparing for solid phase extraction. Then the sample was eluted with 6 mL methanol. The eluted liquid was condensed under nitrogen flow at 200 ± 35 mbar and 40^{o}C. Finally, the residue liquid sample was diluted by adding about 1 mL of Millip/MeOH (4/1) buffered with 0.5% formic acid for HPLC-MS analysis (Grote et al. 2007). After extraction CTC was analyzed on the same day. SMZ was stored in the freezer until all samples were ready to be analyzed.

4.2.4.2. HPLC and MS/MS conditions

The antibiotics were analyzed by liquid chromatography coupled with mass spectrometry (Quantum Ultra, Thermo Electron Corporation, Dreieich, Germany), equipped with electro spray ionisation source (ESI) for CTC, atmospheric pressure chemical ionisation source (APCI) for SMZ both in the positive ion mode and an injection volume of 10 µl.

For SMZ a Nucleodur Sphinx RP column (3 µm, Macherey & Nagel; Düren, Germany) at a temperature of 15°C, with the mobile phases A (Millipore:MeOH – 1:20 (v:v) with 0,5% formic acid), B (MeOH:Millipore – 1:20 (v:v) with 0,5% formic acid) and C (ACN with 0.1% formic acid) was used. The HPLC gradient was run with a flow of 300 µl min^{-1} from 100% B to 95% A and 5% C in the first 22 min, then back to 100% B within 4 min, which was kept constant for 4 min. For CTC the same column conditions and mobile phases were used, the gradient run from 100% B to 95% A and 5% C within 16 min and then back to 100% B in 10 min which stayed constant for 4 min. For SMZ the APCI and MS system was run under the following conditions: the spray voltage was set to 4000 V, sheath and aux gas pressures were 25 and 20 arbitrary units, respectively. The vaporiser temperature was set to 355°C and the capillary temperature to 200°C. Argon served as collision gas with a pressure of 1.9 mTorr. For CTC the spray voltage was 3500 V, the vaporiser and capillary were heated to 300°C, a sheath gas of 30 arbitrary units was applied and the collision pressure was 1.6 mTorr.

SMZ was quantified by the response ratio of the study compound and the internal standards, using an external standard sequence (1–100 ng ml^{-1}). CTC was quantified by the method of single point standard addition according to the following equation:

$$y_0 = X * PA_{(sample)} / (PA_{(standard)} - PA_{(sample)}),$$

where:

y_0 is the unknown concentration of the sample

X is the amount of standard added (25 ng)

$PA_{(sample)}$ is the peak area of the sample

$PA_{(standard)}$ is the peak area of the sample with standard addition.

4.3. Results and discussions

Dry matter yield formation of cabbage planted in the same soil treatments containing high or low carbon without antibiotics or with the presence of antibiotics was insignificantly different (Figure 37) indicating that the presence of antibiotics (CTC and SMZ) in the soils had unclear effects on cabbage growth. This result agrees with the study of Boxall et al. (2006) who carried out research with carrot roots (tubers) and lettuce grown in a light loamy sand soil with a low organic carbon content spiked with veterinary medicines such as Tylosin, Diazinon, Florfenicol, Sulfadiazine, Phenylbutazone, Oxytetracycline, Levamisole, Amoxicillin, Trimethoprim and Enrofloxacin. However, in the present investigations DM yield of cabbage planted in the high C soil treatments was higher as compared to that in the low C soil treatments because of higher available nutrients in the high C soil than in the low C soil.

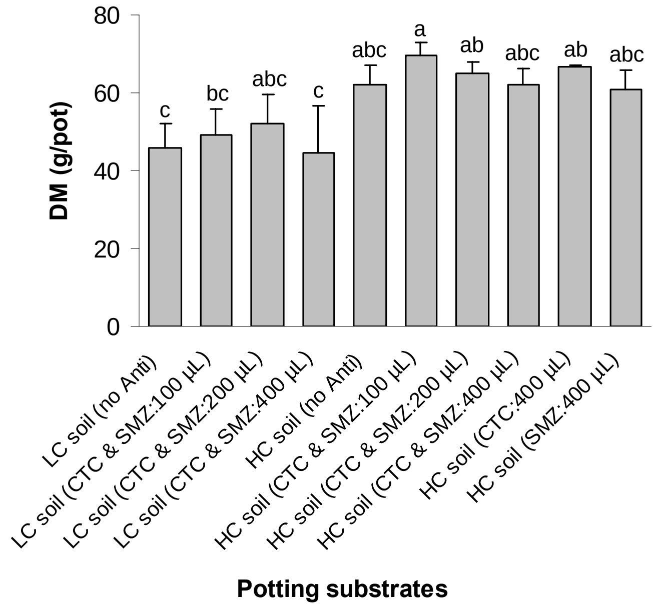

Figure 37. Dry matter yield of cabbage planted in antibiotic-spiked soils

Error bars represent the standard deviation of 4 replicates. Means of different treatments followed by the same letters are not significantly different ($p < 0.05$) by Tukey test.

Regarding the antibiotic uptake from the manure-spiked soil by the plants there were no antibiotics detected in cabbage samples. The lack of uptake may be due to the high limits of detection of the column of HPLC-MS or strong degradation of these two antibiotics during the period of experiment on sunny summer days (from May to August 2011). In addition, the initial studied concentrations of the antibiotics in soil were quite low (microgram/kg) while in other investigations applying milligram/kg soil less than 0.1% of Sufamethazine could be detected in corn (*Zea mays* L.), lettuce (*Lactuca sativa* L.), and potato (*Solanum tuberosum* L.) (Dolliver et al. 2007). Boxall et al. (2006) reported that none of the investigated compounds (Amoxicillin, Sulfadiazine and Tylosin) were found in lettuce leaves, whole carrot roots or carrot root peeling materials. Therefore they assumed that over 90% dissipated in the soil. Similarly, Chlortetracyline can be decomposed during the summer days (56% biodegradation at 30^{o}C, 12% biodegradation at 20^{o}C, 0% at 4^{o}C in 30 days) (Kumar et al. 2005a). Kumar et al. (2005b) also could find no Tylosin in the plant tissues of corn (*Zea mays* L.), green onion (*Allium cepa* L.), and cabbage (*Brassica oleracea* L. Capitata group). The uptake of antibiotics is very complicated to explain, because the uptake depends on many affecting factors such as chemical characteristics of antibiotics, plant growth stage, plant species, plant organs, experimental conditions, studied antibiotic concentration, etc. Therefore, the results of antibiotic uptake were documented differently among reports (Dolliver et al. 2007).

4.4. Conclusion

In the present investigations an uptake of antibiotics into cabbage was undetectable. This is positive from the view point of health effect, because an accumulation of antibiotics by plants would result in uncertainness of people.

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Chapter 5

General conclusions and recommendations

Compost and residues from biogas plant as raw materials are good potting substrates for salt-tolerant plants (grass, rape and sunflower). Compost derived from garden wastes supplied abundant available N and K content while anaerobic digested residues originated from pig manure and maize offered rich available P and K for plants.

As negative results of leaching of compost, the yield and total nutrient uptake of salt-tolerant plants were decreased. Therefore the leaching of compost before using as a potting substrate is not recommended.

The influence of leaching of residues from biogas plant was not distinct. While yield formation of grass, rape and sunflower was not significantly influenced, the uptake of K, N and S was slightly reduced. Therefore leaching of residues from biogas plant is not recommended in term of nutrient uptake by salt-tolerant plants. To make a final decision further investigations are necessary.

Regarding the additives supplemented into the raw materials (20% by volume), the incorporation of additives such as Perlite, Hygromull, Lecaton, Peat, Cocofiber into compost materials had positive effects on yield formation and nutrient uptake of salt-tolerant plants, especially of younger plants at the first stage of cultivation. However, to give a specific suggestion of an additive to compost, further investigations are necessary.

Different from compost, the supplement of additives to residues from biogas plant resulted in an indistinct influence. Only Hygromull or Peat may be recommended as an additive to anaerobically digested residues because of their favor for plant growth and nutrient uptake.

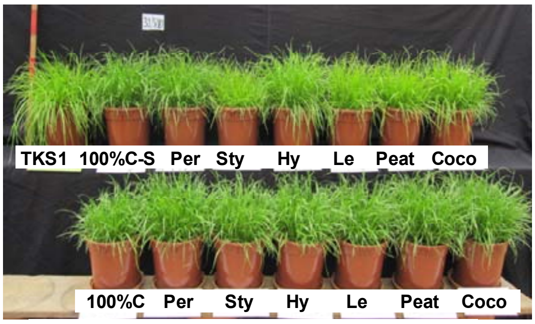
Because of the high salt content, 100% compost is improper to grow salt-sensitive plants. Even less compost with the application rate of 80% or 50% by volume and combination with an additive (Hygromull or Cocofiber) resulted in a low yield formation and total nutrient uptake of Pelargonium and Salvia. The replacement of more than 50% compost by SPS is favorable for salt-sensitive plants and resulted in the highest yield and total nutrient uptake among compost mixtures. Consequently, compost-based substrates with more than 50% by volume are not recommended as potting substrates for salt-sensitive plants.

Changes of the compost proportion (25%-14%) added into peat-based substrates remarkably increased yield formation and nutrient uptake of Pelargonium and Salvia as compared to the results of compost-based substrates and reached marketable values in term of ornamental production. Therefore, the incorporation of less than 25% volume of compost into Peat was recommended to create reasonable peat-based potting media for salt-sensitive plants like Pelargonium and Salvia.

The presence of typical artificial additives such as Hygromull, Cocofiber, Styromull or Perlite had insignificant effects on both plant growth and nutrient uptake, thus they were not recommended for the partial supplement into Peat and compost mixtures. However, in concerns of Peat substitution these additives might be suggested.

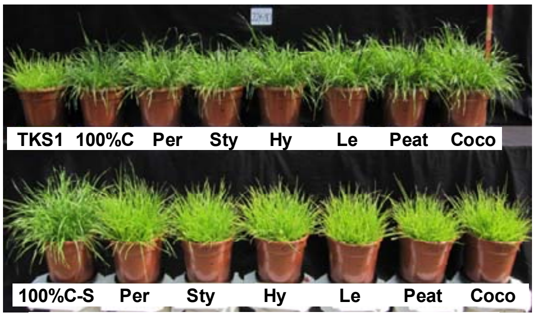
Regarding the results with antibiotics an uptake of Chlortetracyline and Sulfomethazine by cabbage could not be detected even with the highest application rate.

Appendices

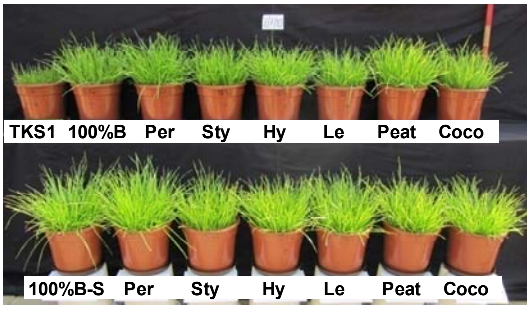


1st Harvest_Comp & Comp-S

1st Harvest_BioR & BioR-S

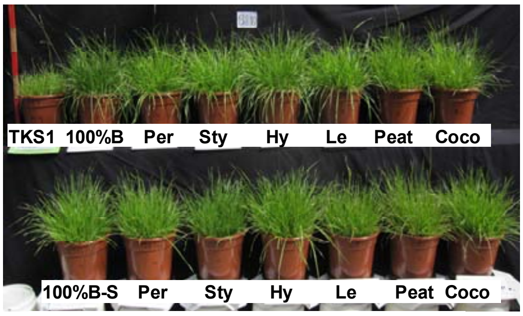


2nd Harvest_Comp & Comp-S

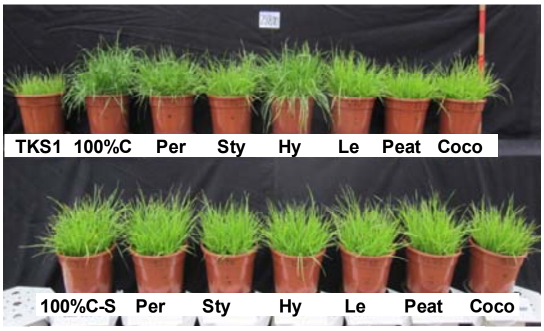


2nd Harvest_BioR & BioR-S

3rd Harvest_Comp & Comp-S



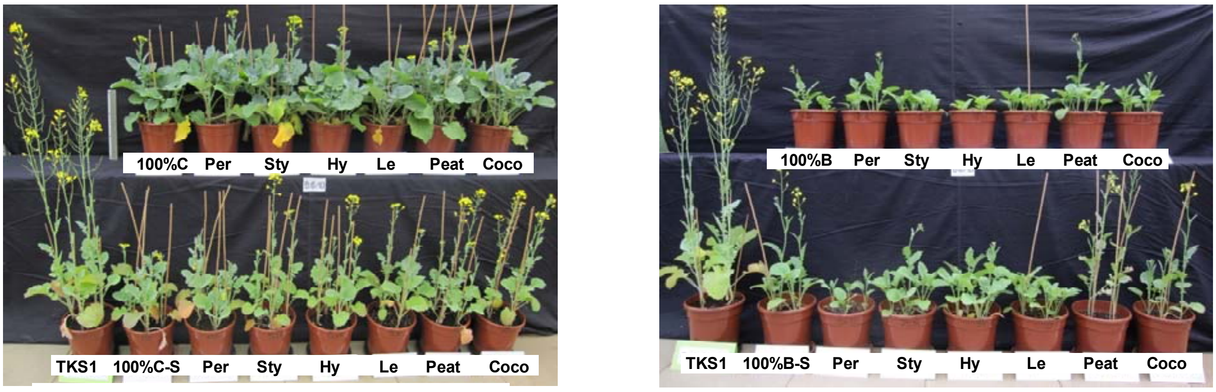
3rd Harvest_BioR & BioR-S



4th Harvest_Comp & Comp-S

4th Harvest_BioR & BioR-S

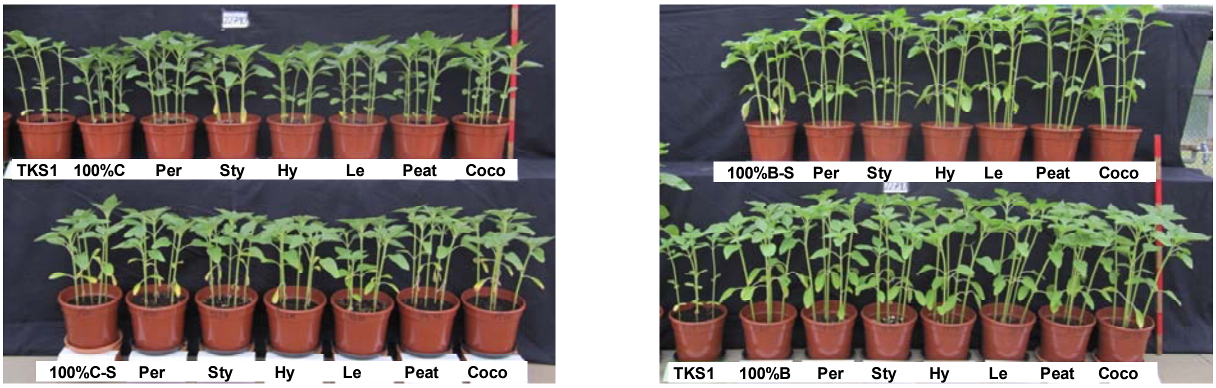
Appendix 1. Ryegrass planted in the substrates based on compost and residues from biogas plant before harvest



Comp & Comp-S_after 51 days

BioR & BioR-S_after 58 days

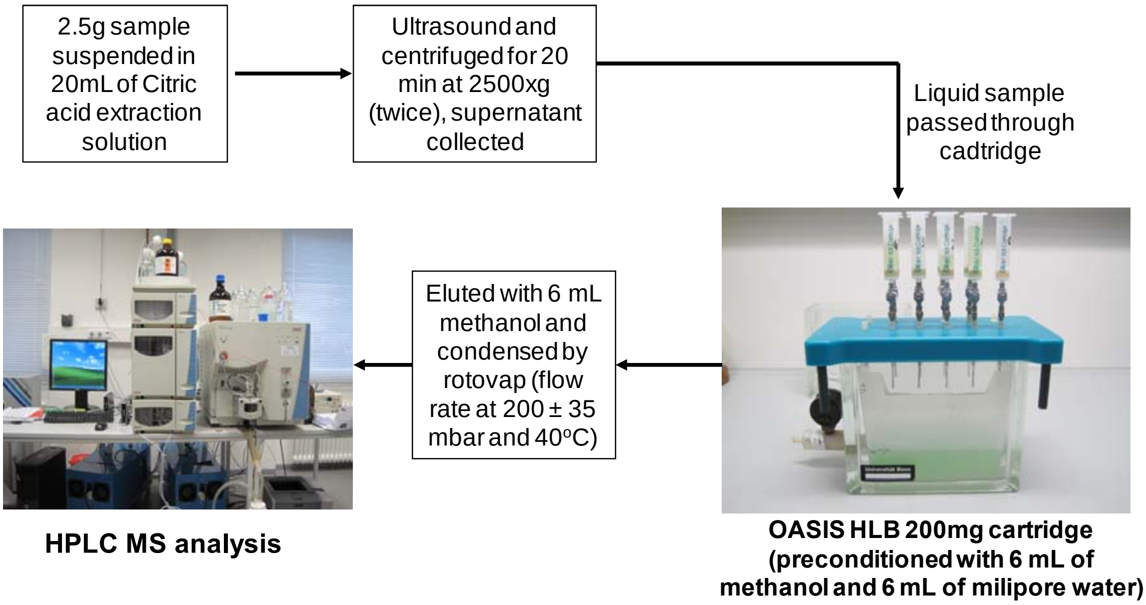
Appendix 2. Rape planted in the substrates based on compost and residues from biogas plant before harvest



Comp & Comp-S_after 40 days

BioR & BioR-S_after 40 days

Appendix 3. Sunflower planted in the substrates based on compost and residues from biogas plant before harvest



2.5g sample suspended in 20mL of Citric acid extraction solution

Ultrasound and centrifuged for 20 min at 2500xg (twice), supernatant collected

Liquid sample passed through cadtridge

OASIS HLB 200mg cartridge (preconditioned with 6 mL of methanol and 6 mL of milipore water)

Eluted with 6 mL methanol and condensed by rotovap (flow rate at 200 ± 35 mbar and 40°C)

HPLC MS analysis

Appendix 4. Extraction procedure of antibiotics from plant material