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COMPARISON OF THREE DIFFERENT WOODY PLANT SPECIES FOR THEIR ABILITY TO UTILIZE NUTRITIONAL ELEMENTS SUPPLIED IN THE FORM OF BIOSOLIDS AND MINERAL FERTILIZERS

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United Arab Emirates University

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College of Agriculture and Veterinary Medicine

Department of Integrative Agriculture

COMPARISON OF THREE DIFFERENT WOODY PLANT SPECIES FOR THEIR ABILITY TO UTILIZE NUTRITIONAL ELEMENTS SUPPLIED IN THE FORM OF BIOSOLIDS AND MINERAL FERTILIZERS

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November 2021

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Fatima El-Telib El-Tayeb Hassan

This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Horticulture

Under the Supervision of Dr. Elke Neumann

November 2021

Declaration of Original Work

I, Fatima El-Telib El-Tayeb Hassan, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled “*Comparison of Three Different Woody Species for Their Ability to Utilize Nutritional Elements Supplied in the Form of Biosolids and Mineral Fertilizers*”, hereby, solemnly declare that this thesis is my original research work that has been done and prepared by me under the supervision of Dr. Elke Neumann, in the College of Agriculture and Veterinary Medicine at the UAEU. This work has not previously formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

Student’s Signature Fatima Date: 04.01.2023

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Abstract

Crop production systems of the future will need to focus on recycling rather than flow-through of mineral nutrients. Much of the nutrients that leave agricultural systems with crop products eventually end up in household waste and sewage. Returning nutrient-rich products from wastewater treatment to agricultural soils must be done in an environmentally sound and culturally acceptable manner. More than 300,000 hectares of forest have been planted in the United Arab Emirates (UAE), consisting mainly of native species, such as *Prosopis cineraria* (Ghāf) and *Vachellia tortilis* (Samr). It has been proposed that sewage sludge, the dry residue from wastewater treatment, is returned to crop production via land application to these forests, as they are not directly used in food production for human consumption nor are they in close proximity to human settlements. However, little is known about how native desert trees would respond to such an additional fertilizer supply. In the present study, the ability of Ghāf and Samr trees to utilize nutrients provided either in the form of sewage sludge as biofertilizers or mineral fertilizer salts was compared. Wild jasmine shrubs (*Clerodendrum inerme*) were included as a third species in this experiment because they are an exotic and faster growing woody plant. The young jasmine shrubs were grown in the greenhouse in pots filled with sandy dune soil to which nutrients were added at three different levels. The nutrients were supplied either in the form of sewage sludge or mineral fertilizers. The soil prepared for fertilization with sewage sludge was mixed with 3.2, 6.4 and 12.8 g of dry sewage sludge per kg of dry soil, respectively. This was equivalent to 60, 120 and 240 mg N per kg dry soil. Plants were harvested nine months after the start of the experiment, and their dry weight and shoot nutrient uptake were evaluated. Compared to the desert tree species, wild jasmine shrubs had significantly higher dry weights at the end of the experiment. Nevertheless, none of the species showed a positive growth response to the increase in the supply of nutritional elements. Wild jasmine shrubs showed increased uptake of macronutrients with increasing fertilizer supply and were equally capable of utilizing nutrients from sewage sludge and mineral fertilizers. No increase in elemental uptake in response to increasing fertilizer supply was observed in the indigenous trees. The growth of Ghāf trees responded negatively to a high supply of sewage sludge but not to a high supply of mineral fertilizer. The results of our study suggest that the ability to absorb and utilize nutrients supplied in the form of sewage sludge may be limited in desert trees such as Ghāf and Samr. The potential to utilize sewage sludge could be increased by planting exotic species which have higher growth and element uptake potential compared to indigenous trees.

Keywords: Biosolids, Sewage Sludge, Biofertilizers, Mineral Fertilizers, Nutritional Elements, Soil Amendment, Native Desert Plants, Ghāf, Samr, United Arab Emirates.

Title and Abstract (in Arabic)

مقارنة بين ثلاثة أنواع مختلفة من النباتات الخشبية لقدرتها على الاستفادة من العناصر الغذائية المتوفرة في شكل سماد حيوي وأسمدة معدنية

الملخص

ستحتاج أنظمة إنتاج المحاصيل في المستقبل إلى التركيز على إعادة التدوير بدلاً من تدفق المغذيات المعدنية. ينتهي المطاف بالكثير من العناصر الغذائية التي تترك الأنظمة الزراعية مع منتجات المحاصيل في النفايات المنزلية ومياه الصرف الصحي. يجب أن تتم إعادة المنتجات الغنية بالمغذيات من معالجة مياه الصرف إلى التربة الزراعية بطريقة سليمة بيئيًا ومقبولة ثقافيًا. تمت زراعة أكثر من 300000 هكتار من الغابات في دولة الإمارات العربية المتحدة، وتتألف بشكل رئيسي من الأنواع المحلية، مثل الغاف (*Prosopis cineraria*) والسمّار (*Vachellia tortilis*). تم اقتراح إعادة المخلفات الجافة الناتجة عن معالجة مياه الصرف الصحي، إلى إنتاج المحاصيل عن طريق تطبيق الأراضي على هذه الغابات، حيث لا يتم استخدامها بشكل مباشر في إنتاج الغذاء للاستهلاك البشري كما أنها ليست قريبة من المستوطنات البشرية. تم تضمين شجيرات الياسمين البرية (*Clerodendrum inerme*) كنوع ثالث في هذه التجربة لأنها تنمو بشكل أسرع من النباتات الخشبية. نمت شجيرات الياسمين الصغيرة في مشاتل واقية وفي أواني مملوءة بتربة رملية التي أضيفت إليها المغذيات على ثلاثة مستويات مختلفة. تم توفير المغذيات إما في شكل أسمدة حيوية (من الصرف الصحي) أو الأسمدة المعدنية. تم خلط التربة المعدة للتسميد بحمأة الصرف الصحي مع 3.2 و6.4 و12.8 جم من المواد العضوية الجافة المستخلصة من الصرف الصحي لكل كجم من التربة الجافة على التوالي. كان هذا يعادل 60 و120 و240 ملليغرام لكل كيلوجرام من التربة الجافة. تم حصاد النباتات بعد تسعة أشهر من بدء التجربة، وتم تقييم وزنها الجاف وامتصاص المغذيات الجذعية. بالمقارنة مع أنواع الأشجار الصحراوية، كان لشجيرات الياسمين البرية أوزان جافة أعلى بشكل ملحوظ في نهاية التجربة. ومع ذلك، لم يظهر أي من الأنواع استجابة نمو إيجابية لزيادة الكمية من العناصر الغذائية. أظهرت شجيرات الياسمين البرية زيادة في امتصاص المغذيات الكبيرة مع زيادة الإمداد بالأسمدة، كما كانت قادرة على الاستفادة من المغذيات من عناصر الصرف الصحي العضوية والأسمدة المعدنية. لم يلاحظ أي زيادة في امتصاص العناصر كاستجابة لزيادة الإمداد بالأسمدة في الأشجار محلية النشأة. نجد استجاب نمو أشجار الغاف سلبًا للإمداد العالي من مواد الصرف الصحي العضوية، ولكن لم يستجب لارتفاع الإمداد بالأسمدة المعدنية.

تشير نتائج الدراسة إلى أن القدرة على امتصاص واستخدام العناصر الغذائية العضوية المتوفرة في الصرف الصحي قد تكون محدودة في الأشجار الصحراوية مثل الغاف وسمار. يمكن زيادة إمكانية استخدام مياه الصرف الصحي عن طريق زراعة الأنواع غير محلية النشأة والتي تتمتع بإمكانية نمو أعلى وامتصاص عنصر أعلى مقارنة بالأشجار محلية النشأة.

مفاهيم البحث الرئيسية: المواد الصلبة الحيوية، الصرف الصحي، والأسمدة الحيوية، الأسمدة المعدنية، العناصر الغذائية، تسميد التربة، النباتات الصحراوية الأصلية، الغاف، سمر، الإمارات العربية المتحدة.

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Dedication

To my beloved parents and family

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List of Abbreviations

Al	Aluminium
AM	Arbuscular Mycorrhizal
AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis of Variance
BOD	Biochemical Oxygen Demand
C	Carbon
Ca	Calcium
Cd	Cadmium
CFR	Code of Federal Regulations
Cr	Chromium
Cu	Copper
DW	Dry Weight
EEC	European Economic Commission
EPA	Environmental Protection Agency
EU	European Union
F	Fluoride
FAO	Food and Agriculture Organization
Fe	Iron
ICP-OES	Inductively Coupled Plasma - Optical Emission Spectrometry
Hg	Mercury
K	Potassium
KOH	Potassium Hydroxide
Mg	Magnesium
Mo	Molybdenum
N	Nitrogen
Na	Sodium
Ni	Nickel
P	Phosphorus
Pb	Lead
PFM	Prairie Forest Management
pH	Potential of hydrogen

PTE	Potentially Toxic Elements
SS	Sewage Sludge
Se	Selenium
TSE	Treated Sewage Effluent
UAE	United Arab Emirates
US	United Stats
WHO	World Health Organization
WTP	Wastewater Treatment Plant
Zn	Zinc

Chapter 1: Introduction

1.1 Overview

Biosolids generated as a waste from Sewage Treatment Plants (STP) can cause pollution problems when dumped into landfills in large amounts or used inappropriately for agricultural purposes. Biosolids are primarily the nutrient-rich organic solid material produced by the municipal sewage treatment process, previously referred to as sewage sludge. Wastewater solids become biosolids when they are stabilized by digestion or other physical, chemical, and/or biological treatments like composting, alkalization, or heating. Stabilized biosolids are a potentially beneficial liming agent or fertilizer to be used in agriculture.

Organic matter for the application to soil is scarce in hyper-arid environments like the UAE, and farmers often find it difficult to raise the organic matter content of their sandy soils. The use of mineral fertilizers as the only source of nutrient supply can be associated with high leaching and volatilization losses of nutritional elements. Farmers thus often seek alternatives to conventional fertilizers in form of an organic soil amendment available at a competitive price and with a low C: N ratio. Biosolids of adequate quality offer landholders such an organic material. Land application of biosolids has been practiced for decades and continues to be the most common strategy for their valorisation. Biosolids, have been shown to be beneficial to plant growth through nutrient supply and soil property improvement. Since this material would otherwise be disposed to waste, farmers and municipalities are interested in this potential sustainable recycling path (Mok et al., 2013).

In many cases, the agricultural use of biosolids is less expensive than disposal into landfills (Council et al., 2002). Biosolids composting might add costs in first place, but the resulting compost then has a wide variety of uses, and with it a commercial value gain. Furthermore, composting eases storage and application as it reduces weight, odors, and organic contaminants. Land application of biosolids can enhance carbon (C) sequestration by soils (Torri & Cabrera, 2017), provide nutrients to crops (Magesan & Wang, 2003), and improve soil fertility (Scharenbroch et al., 2013).

The material can also reduce soil erosion as the organic matter in biosolids aggregates soil particles, thereby retaining nutrients and improving water retention characteristics. Nutrients (e.g., nitrogen and phosphorus), micronutrients including essential trace metals (e.g., copper, zinc, molybdenum, boron, calcium, iron, magnesium, and manganese), and organic matter in the biosolids are beneficial for gardening, forestry, turf growth and landscaping. However, there are also hesitations and restrictions pertaining to the application of biosolids to soils serving in the production of fresh food or recreational purposes.

Biosolids can contain harmful elements such as heavy metals or aluminium. Though the microbial load can be minimized through composting or other heat treatment, they might still contain antimicrobial residues and other organic residues of the pharmaceutical and chemical industry. For this reason, the application of biosolids to plant production systems that do not serve in food production and are not in proximity to residential areas has often been preferred. For example, biosolids can increase tree growth and subsequent economic returns of forest plantations serving timber, fibre, or biofuel production. When biosolids derive mainly from household effluents and contain little industrial waste, their use can also involve their application to various types of land, including agricultural fields, forests, reclamation sites, parks, and golf courses.

Composted and treated biosolids can be used by landscapers and nurseries and by homeowners for lawns and home gardens. Application of biosolids to forests, currently involving a relatively small percentage of biosolids, can help shorten pulp wood and lumber production cycles by accelerating tree growth (EPA, 1994). At reclamation sites, biosolids help revegetate barren land and control soil erosion. Relatively large amounts of biosolids are used to achieve reclamation of disturbed sites, such as former mines or waste depositions. Before biosolids can be safely used for soil improvement and nutrient supply to plants, their impact on the agroecosystem needs to be carefully studied. While many studies have been conducted on the use of biosolids in temperate and hamate climates, little is known about the impact that biosolids application has on soils and plants of the hyper-arid environments of the Gulf Region.

A proper environmental impact assessment would require the study of plant responses, as well as impacts on other organisms living in the soil. Desert soils are naturally poor in organic matter and nutritional elements, and roots of indigenous plants often associate with beneficial soil microorganisms to facilitate element and water uptake. It is important that soil fertilization or amendment practices support rather than reduce such beneficial microbes in the soil, e.g., nitrogen fixing bacteria and arbuscular mycorrhizal fungi. The latter are widespread root symbionts in the soils throughout the world. Mycorrhizal symbioses enhance plant growth through their contribution to nutrient uptake via external hyphae that can grow more than 10 cm away from the root. External mycorrhizal mycelium is important for exploration of soil pores and interaction with organic matter in the soil.

The hyphal network is also important for establishing soil aggregates, which is important to maintain a stable soil structure. Mycorrhiza fungi can contribute 80% to total plant uptake of P, 25% to total N,10% to total plant K, 25% to total Zn, and 60 % to Cu uptake (Ying-Ning & Wu, 2011). Moreover, plants depend heavily on mycorrhizal fungi in low P soil for adequate P and N uptake. In contrast, high available P and N amounts lead to suppress the relationship between the fungus and host plant.

1.2 Statement of the Research Problem

The growing population of residents and tourists in the UAE is increasing pressure on wastewater treatment facilities. In 2015 the residential population of the UAE exceeded 9 million ,while population forecasts for 2030 are between 10.5 and 11.5 million. Biosolids are treated and stabilized solids resulting from wastewater treatment. On average, the biosolids production per capita is 0.05 kg/day. Therefore, it can be estimated that 457 tons/day of biosolids were produced in the UAE in 2015. Currently, in the UAE, biosolids are mainly disposed in landfills (Ospina & Hassan, 2017). In addition, the impact of erosion in plant biodiversity has created environmental and socioeconomic problems which subsequently triggered the need of conservation of plant resources.

In some other countries, a growing market is the use of biosolids in manufactured soils and soil amendments, which can be used for erosion control, roadway construction, and parks (EPA, 1998). Composted and heat dried or pelletized biosolids for use on public lands, lawns, and home gardens are not yet available in the UAE, though such materials can be of these forms' excellent quality with incredibly low levels of metals and pathogens below detection levels. However, cultural hesitations and uncertainty about the response of desert soils and plants to biosolids application currently restrict their use in the UAE. Recycling paths might, however, involve plant cultivation systems that are neither close to human settlements, nor serve in the production of fresh food, such as forests.

The UAE is lightly forested, with man-made tree plantations covering 2 – 3 percent of the land surface. These forests comprise mainly of native trees, such as *Prosopis cineraria* (Ghāf) and *Vachellia tortilis* (Samr) species. They serve in the maintenance of biodiversity, prevention of erosion, and as a cultural heritage. Recently, opportunities for the conversion of some of these forests into production systems for animal feed have been discussed. This would need to involve an intensification of the system, possibly achieved through the input of additional irrigation water and fertilizers. Such intensified forests might be an ideal system for the valorisation of biosolids. However, to date truly little is known about the ability of native trees to utilize additional nutritional elements provided in form of biosolids or mineral fertilizers for growth. Native desert species are highly stress tolerant and might have a limited ability to translate additional input into growth.

The native tree species *Prosopis cineraria* (Ghāf), and *Vachellia tortilis* (Samr) are most widely grown in manmade forests of the UAE. These species tolerate to dry and saline conditions and can survive in extremely poor soils. Fast growing plant genotypes used for biomass production, erosion control or urban greenery in the UAE are largely non-native, such as, e.g., *Clerodendrum inerme* (wild jasmine) species (Sakamoto et al., 2012).

1.3 Research Objectives

The overall goal of this research was to assess the ability Ghāf and Samr trees to utilize nutritional elements supplied in form of biosolids or mineral fertilizers for growth. The non-native wild jasmine shrub (*Clerodendrum inerme*) served as a control. The specific objectives were (i) to find out whether tree element uptake would differ depending on the form of fertilizer supplied (mineral vs. biosolids), and (ii) to which extent trees native to desert environments would be able to acquire nutrients from fertilizers supplied at different levels (low, medium, and high), and (iii) the extent the root colonization of the arbuscular mycorrhiza fungi (AMF) was assessed in the desert tree species in response to the different fertilization treatments.

1.4 Research Hypotheses

Nutritional elements within biosolids will be largely available to plants during a growth period. There will not be a significant difference in growth and element uptake between plants supplied with biosolids and nutritional elements in mineral form. However, it is hypothesized that the desert tree species will have a lower ability to utilize additional nutrients for growth, compared with wild jasmine shrubs. The application of biosolids might thus need to be limited to relatively low application rates. It is expected that the results of the present study will broaden the current understanding of the effects that biosolids application might have on desert tree stands. Such knowledge would be important to develop environmentally sustainable paths for biosolids valorisation through man-made forests of the UAE.

Chapter 2: Literature Review

2.1 Applications of Biosolids

Despite considerable environmental risks associated with the application of poorly treated and/or uncontrolled application of sewage sludge to agricultural soils, the use of such waste for agriculture also has many advantages, and may ultimately be key to closing element cycles in world's food supply systems (Kumar et al., 2017). The application of biosolids can increase soil microbial biomass and activities of some soil enzymes, such as urease, alkaline phosphatase and β-glucosidase linked to C, N, P and S soil cycles.

Chen et al. (2019) also reported that the incorporation of organic amendments to soil stimulates dehydrogenase activity because the added material may contain intra- and extracellular enzymes that encourage microbial activity in the soil. It was found that compost or sewage sludge were effective in the remediation of the saline soil. It was reported that the application of municipal solid waste compost (13.3 g kg^{-1}) and sewage sludge (26.6 g kg^{-1}) to soil significantly improved its physical and chemical properties, especially carbon and nitrogen contents (Lakhdar et al., 2010).

Plant nutritional element concentrations in biosolids vary with sources of wastewater and wastewater treatment processes. The total amounts of nutrients in biosolids, and their availability to plants is significantly altered by stabilization processes. Similarly, the rate of nutrient release (or mineralization) is also affected by the processes. Mineralization of N from aerobically digested biosolids was reported to be significantly higher than that from anaerobically digested material throughout a 26 weeks incubation study (Baballari, 2019).

Soil type, temperature, soil moisture content, aeration, and species and number of soil microorganisms play a role in organic matter mineralization in biosolids. Mineralization rate is also closely related to the C: N ratio. The higher the C: N ratio in soil, the lower the N mineralization rate. In some cases, the mineralization process was more influenced by soil type than by rate and type of sludge applied (Lu et al., 2012). The use of biosolids in agriculture is strictly regulated in most developed countries, but safe paths for their use are increasingly identified and deployed. But can be encouraged like in Michigan’s biosolids and septage programs. This is driven by the intention of closing nutrient loops to ensure that nutrients are returned to agricultural land to improve soil fertility.

Returning biosolids to food production systems can also reduce the needs for mineral fertilizer inputs and related resource depletion and environmental impact. In the past decades, a lot of research on the use of biosolids for agricultural purposes has focused on assessing risks arising from their possible contamination with heavy metals, pathogens, and other pollutants. Based on such studies, strict guidelines for the use of such material for agricultural purposes have been put in place, especially in developed countries. Comparatively little research has been conducted on assessing the plant availability of nutritional elements that biosolids contain. Guidelines that would make sure that applied rates of biosolids to the soil correspond to the plant demand are important to prevent nutrient leaching or nutritional imbalances.

Efficient biosolids management strategies need to have a main focus on economic, technological, and societal constraints. At the same time, the assessment of the overall sustainability of biosolids application as a long-term recycling strategy needs to be carefully evaluated by scientists, researchers, and policy makers to prepare for appropriate decision-making for sustainable development in the future. Therefore, more scientific research is required on the different aspects of biosolids or sewage sludge to make it a feasible component of sustainable development.

2.2 Legislations

In many countries, the increasing production of biosolids and sewage sludge, and costs associated with their appropriate disposal, are a matter of concern (Lamastra et al., 2018). Regarding worldwide institutions, FAO and WHO provide their own guidelines. In the FAO's document regarding wastewater treatment and its use in agriculture, in Section 6 (Agricultural use of Sewage Sludge), point 6.2 (Sludge Application), the authors present maximum permissible concentrations of potentially toxic elements (PTE) in the soil after the application of sewage sludge (Pescod, 1992). This document also states maximum annual rates of addition. Among well-known contaminants such as zinc and mercury, the document also provides guidelines related to maximum addition of molybdenum (Mo), selenium (Se), fluoride (F), and arsenic (As) (Nunes et al., 2021). Additionally, is possible to find examples of effective sludge treatment processes and further information about sewage sludge and crops.

The UAE imports more than 90% of its food, and considerable amounts of plant nutritional elements that this food contains. A significant portion of this ultimately ends up in the sewage system and is released in form of biosolids and treated sewage effluent (TSE). While TSE is widely used for the irrigation of public urban greenery in the UAE, recycling of biosolids occurs only to a minor extent, possibly due to cultural hesitations and practical difficulties. It is likely that in the future, these can be increasingly overcome, such as they did for the use of TSE. In preparation of this, the

UAE has put forward comprehensive regulatory frameworks to ensure that the use of waste materials occurs in a safe manner.

Of the different Emirates, Abu Dhabi has most aggressively taken to the subject of sewage treatment and use over the past few years. In order to protect citizens from the potential health hazards of uncontrolled release of sewage effluents, the government gave top priority to the improvement of the sewerage/drainage systems as well as to sewage treatment. In 2005, the Abu Dhabi Sewage Services Company (ADSSC) took over the management of all sewage treatment plants (STP) under the regulatory control of the Abu Dhabi Regulation and Supervision Bureau (ADRSB).

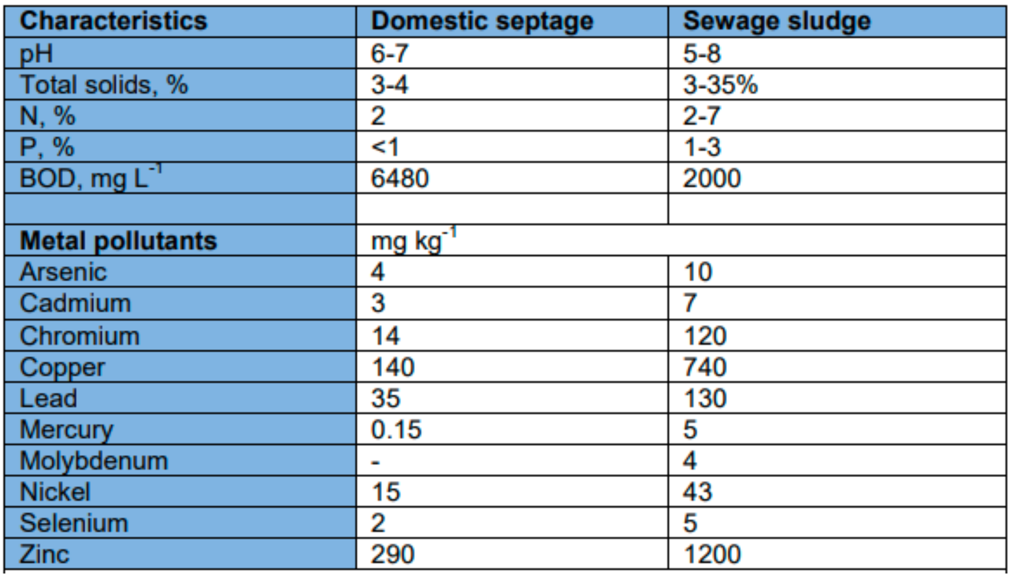
The Regulation and Supervision Bureau (RSB) introduced two key regulations on June 1st, 2010; these were termed as the “Recycled Water and Biosolids Regulations 2010”. These regulations established a legal framework for the safe and economic management of *Recycled Water and Biosolids by Sewerage Services Licensees* in the Abu Dhabi Emirate. They define the minimum microbiological, physical and chemical requirements for recycled water and biosolids (Alshankiti et al., 2014).

Dubai abides by the ‘Technical Guideline for the Environmental Regulations for the Reuse of Treated Wastewater for Irrigation and Thermal Treated Sludge for Agricultural Purpose’, issued by Dubai Municipality in June 2011. Fujairah Municipality follows the ‘*Federal Ministry of Environment Standards for Sludge Disposal’*, as well as rules and regulations of Dubai Municipality. In Ajman Municipality, there are regulations in place for the hazardous materials in the sewage system at source of effluents, by imposing pre-treatment system in facilities that are expected to produce pollutants. Ras Al-Khaimah has adopted the same standards for sludge disposal as Dubai and Abu Dhabi emirates do.

2.3 Characterizations of Sewage Sludge and Biosolids for Land Applications

In present day society, the everyday activities of human's result in the discharge of many substances to the wastewater stream. Untreated wastewater sludge may contain pathogens, inorganic and organic pollutants. As expected, the data shows that the concentration of different metals increased by several times following the processing of the domestic septage to sewage sludge (SS). The data presented in this table does show the addition of heavy metals which occurs on the way between home toilets and the treatment plant. Interestingly, the biochemical oxygen demand (BOD) decreased to about one third of its former value during this period, while the metal pollutants increased by 133-3233%, with the increase being exceptionally high for Mercury (Alshankiti et al., 2014). Table 1 provides a comparison of the pollutants in domestic septage versus those in sludge.

Table 1: Chemical characteristics and metal pollutants in domestic septage to SS (Iverson et al., 2022)



Characteristics	**Domestic septage**	**Sewage sludge**
pH	6-7	5-8
Total solids, %	3-4	3-35%
N, %	2	2-7
P, %	<1	1-3
BOD, mg L^{-1}	6480	2000
Metal pollutants	mg kg^{-1}	
Arsenic	4	10
Cadmium	3	7
Chromium	14	120
Copper	140	740
Lead	35	130
Mercury	0.15	5
Molybdenum	-	4
Nickel	15	43
Selenium	2	5
Zinc	290	1200

2.4 Comparison between Biosolids and Mineral Fertilisers

The study, Comparing the Benefits and Risks of Agricultural Amendments and Fertilizers (99-PUM-1), examines how to reconcile agricultural needs with several controversial biosolids land application issues. The authors suggest that, despite risks of metal accumulation, plant uptake of harmful compounds, contamination of groundwater, and potential prevalence of pathogens and viruses, the use of biosolids for agricultural purposes may not impose a greater risk that the use of manure Generally, manures and biosolids contain similar amounts of macronutrients, such as, P and K. Study data indicate that nitrogen comprises 1 to 10.8% (dry weight) of amendments and that phosphate makes up another 0.7% to 7.5% of the total. However, the forms in which the nutritional elements are present in the organic materials may vary, and with their availability to plants. For example, depending on the sewage treatment process, biosolids may contain significant amounts of P in form of phytate, which is sparingly available to plants and microorganisms.

Chemical fertilizers have the advantage that amounts of nutritional elements added to the soil can be very well adjusted and tailored to the requirements of crops. This is not always possible for nutrients supplied in organic form, where plant available N:P:K ratios can differ depending on the material used, as well as mineralization rates. In addition, organic fertilization often involves that greater amounts of fertilizer need to be incorporated into the soil, requiring special machinery. Though the same amount of nutrients can be delivered either in mineral or organic form, the amount of work involved can be greater for biosolids or manure compared with mineral fertilizers.

As long as chemical fertilizers are available at relatively low prices, the incentive for the use of biosolids may be relatively low. However, an increasing need for reduction of CO_2 emissions, future scarcity of fossil energy, and pollution problems arising from poor recirculation of nutritional elements within the food supply cycle, may lower the feasibility of mineral fertilizers in the future. The Haber Bosch Process used for fixation of atmospheric nitrogen into mineral fertilizers is one of the most energy consuming industrial processes worldwide, contributing a major portion to the CO_2 footprint of food supply systems (Daelman et al., 2019). So far, global food supply chains are through-flow rather than recirculation systems.

Mineral fertilizers are used by farmers to replace nutritional elements lost with the harvest products that are sold and never returned. On the other side of the system, nutritional elements are released into the natural environment, causing eutrophication, groundwater pollution and global warming. Nitrogen flow through food supply systems into the environment has dramatically increased over the last 50 years, along with the demand of the world population for protein-rich food based on animal production systems (Martinez et al., 2019). Recirculation of nutritional elements contained in biosolids, food waste and crop residues are not only an opportunity for municipalities to reduce waste management costs, but rather a necessity to achieve sustainability and food security.

2.5 Indigenous Plant Species in the UAE

Ghāf (غاف) is the local Arabic name for *Prosopis cineraria* (Family: Leguminosae / Mimosoideae). A fully grown Ghāf tree has a straight unbranched trunk, and a weeping light-textured crown. There is a considerable genetic diversity within the Ghāf species, and several different morph types are known. Some have smaller leaves and rather hard, thorny branches, while others have less thorns and larger leaves. Ghāf is an indigenous species of the Arabian Desert and known as the National tree of the UAE.

It is a drought-tolerant, evergreen, leguminous tree, able to withstand the harsh climate of the desert environment (Figure 1). Despite being evergreen under conditions of the UAE, Ghāf trees undergo cycles of leaf fall and renewal (Yamani et al., 2017).

Figure 1: Ghāf tree (*Prosopis cineraria*)(Yamani et al., 2017)

The Ghāf tree can withstand high salinity levels up to 4,500 ppm, as reported by Yamani et al. (2017). The Ghāf trees can survive even when irrigated with high levels of salinity. When growing naturally in the Abu Dhabi desert, they often occur alone or in small clusters that constitute an important refuge and source of food for indigenous animals like gazelles and camels Ghāf trees are also grown in large numbers in man-made forests where they are supplied with irrigation water. The latter is often a mixture of low-quality groundwater and TSE Naturally occurring Al Ghāf trees depend on the availability of groundwater, and form symbiosis with arbuscular mycorrhizal fungi and rhizobia to acquire nutritional elements. Their roots can grow as deep as 30 meters to access groundwater. The soil fertility around desert trees often increases over time, forming ‘islands of fertility’ in a harsh environment, and important repositories of indigenous biodiversity (Ruiz et al., 2008).

As overgrazing and urban expansion have reduced the number of such natural tree stands over the last decades, the man-made forests strive to support wildlife, and combat erosion. Samr (سمّر) is the local Arabic name for *Vachellia tortilis* or *Acacia tortilis* (Family: Fabaceae), which is common and widespread in the eastern part of the UAE. It occurs as either a large shrub or a small tree up to approximately 6 m height (Figure 2). Its crown has a characteristic triangular shape with a flat top, and often grazed by gazelles, goats, and camels (Yamani et al., 2017).

Figure 2: Samr tree (*Vachellia tortilis*) (Yamani et al., 2017)

Similar with Ghāf, it is a leguminous tree, well-adapted to the Arabian desert. Both tree species have T-shaped root systems with shallow roots foraging for nutritional elements and scarce surface water, and deep roots connected to underground water pools. Samr trees tend to have a wider network of shallow roots compared with Ghāf, allowing them to grow where hardpans are present, or on alluvial fans (Yamani et al., 2017). It is also planted in forestry blocks in the eastern parts of Abu Dhabi emirate.

Wild jasmine (*Clerodendrum inerme*), known as 'Chou-Wu-Tong' in China, is an ornamental plant found in wild areas with a temperate climate in China, Japan, Korea, and the Philippines. As a widespread broad-leaved understory shrub, *C. inerme*, is distributed primarily in thickets near hillsides, riversides, and roadsides below an elevation of 2400 m (Siripuram et al., 2018). It is pollinated nocturnally and diurnally by animals, including hawkmoths, bees, and swallowtails (Figure 3).

Figure 3: Wild Jasmine (*Clerodendrum inerme*)(Siripuram et al., 2018)

In addition, *C. trichotomous* has been reported as a tree species with a strong capability to adapt to environmental challenges such as drought, barren, and salt resistance, and therefore, it could be used for ecological restoration in mining areas and for afforestation in saline-alkali land (Sakamoto et al., 2012).

2.6 Mycorrhiza

Mycorrhizae are major components of all terrestrial ecosystems, and essential for the survival of many plant species. They also act as indicators of plant health and soil fertility. Plants associate with other life forms (animals, bacteria, or fungi) to complete their life cycle, fight against pathogens, or to thrive in adverse environments. The plant root, soil under the influence of the root, and microorganisms associated with it, are together called 'rhizosphere'. There are several types of mycorrhizal symbioses between plant roots and soil fungi, of which the endomycorrhizal symbiosis between plants and members of the *Glomeromycotan* are the most widespread. Members of the *Fabaceae* family, like Ghāf and Samr, are known to form endomycorrhizal associations in nature, as do members of the *Lamiaceae*, such as *Clerodendrum inerme*.

Endomycorrhizal fungi are obligate biotrophs that colonize the cortex of their host plant, forming an intraradical mycelium. The extraradical mycelium extends into the soil and can increases the nutrient absorbing surface of the root system by 100-fold. Finely branched extraradical mycorrhizal hyphae take up P and other nutritional elements and transfer and transport these into the host root where they are delivered to the plant. In return, the symbiotic fungus is supplied with sugar in form of hexose by the plant. The endomycorrhizal fungi commonly form haustorium-like structures called ‘arbuscules’ within the plant cortical tissues. For this reason, they have been termed arbuscular mycorrhizal fungi (AMF).

Though endomycorrhizal associations are primarily a strategy to acquire sparingly available nutritional elements such as P, associations can also protect plants from excessive uptake of harmful elements. Endomycorrhizal fungi have been shown to protect trees from high concentration of toxic heavy metals like copper, zinc, iron, manganese, cadmium, nickel, etc., by accumulating and immobilizing them in their mycelium. Mycorrhiza is one of the best examples of symbiotic associations between plants and fungi. The extraradical mycelium of mycorrhizal fungi can also enhance the soil structure and its porosity, thus facilitating soil water retention, especially during the dry season. The rhizosphere is the site where microorganisms interact with both plant roots and soil constituents (Pathan et al., 2020).

Excessive application of fertilizers and pesticides can reduce the abundance of mycorrhizal fungi, as plants no longer rely on their symbiotic partner for nutrient uptake. This has led to a decline in the abundance of mycorrhiza fungal propagules and species diversity in intensively managed agricultural soils. Especially in plantations serving biodiversity conservation, fertilization practices should not lead to such biodiversity losses, and a careful monitoring of the impact of fertilizer application on the extent of mycorrhiza root colonization and sporulation is necessary.

Techniques to detect and quantify AMF in roots are thus essential tools in mycorrhizal research. These methods are primarily used to identify mycorrhizal associations and measure the degree of root colonization. A range of light microscopy-based techniques can be used to detect and quantify AMF in roots including in vivo observations of fungal structures in living roots, non-vital staining methods and vital root staining methods (Vierheilig et al., 1998). Microscopical methods, which allow details of associations to be clearly seen, are essential to all work with endomycorrhizal associations, as these are defined by morphological criteria and must be identified by the presence of key features, especially arbuscules (Vierheilig et al., 1998).

Chapter 3: Materials and Method

3.1 Set-Up of the Experiment

To investigate element uptake from biosolids and mineral fertilizers by young Ghāf, Samr trees and *Clerodendrum* shrubs, a pot experiment was conducted at the UAEU research farm in Al Foah (24°21'26.88"N; 55°48'3.90"E). The Ghāf (*Prosopis cineraria*) and Samr (*Vachellia tortilis*) trees had been obtained from the Barari Nursery in Salamat and were approximately 8 months old by the time the experiment was set up. The plants had been cultivated in a mixture of sand and compost in plastic bags in an open nursery bed prior to being planted. The wild jasmine (*Clerodendrum inerme*) shrubs were obtained from a commercial plant nursery in Dubai. They had been propagated from stem cuttings and were approximately two months old at the time of planting. The plants had been grown in a commercial potting mix. The shoots of the Ghāf and Samr plants were between 30 and 40 cm tall at the time of planting, with a fresh weight of 7 – 10 g. The *Clerodendrum* shoots were slightly smaller. Trees of homogeneous size were selected for the experiment.

The experiment was a two-factorial trial, involving 90 planting pots. Each pot was planted with one plant and represented one experimental unit. The planting pots were set up completely randomized in a polycarbonate Quonset greenhouse. at the UAEU farm. Each planting pot was filled with 8.8 kg dry soil at a bulk density of 1.6 g DW cm^{-3}. The soil had been taken from the slope of a sand dune in Al-Foah area, Al Ain city (Arenosol, Lat.: 24°21’12.16” N; Long.: 55°47’54”81E). The site from where the substrate was taken had never been cultivated and was devoid of actively growing higher plants. Prior to being used in the experiment, the soil substrate was passed through a sieve with a mesh width of 1 mm to remove stones and other debris.

Analysis of representative subsamples of the substrate revealed that it had a pH of 7.83, and an EC value of 0.96 dS m^{-1}, measured in a 1:1 soil: water (w/vol.) extract. Subsamples of approximately 0.5 g dry weight were microwave digested in presence of 10 ml of concentrated (70% in H_2O) HNO_3, 2 ml conc. (37% in H_2O) HCl, and 1 ml conc. (30% in H_2O) H_2O_2. After digestion, the cooled liquid was brought to a volume of 50 ml with deionized water and passed through blue ribbon filter paper (Grade 589/3; Whatman, UK), before being analysed for element concentrations by Inductively coupled plasma - optical emission spectrometry (ICP-OES, Varian 710-ES; Varian, USA). The measurements revealed that the soil substrate contained (mg kg dry $soil^{-1}$) 48.64 Ca, 30.19 Mg, 0.12 Na, 1.10 K, 0.08 P, 0.01 Cu, 8.65 Fe, 0.17 Mn, 0.26 Ni, 0.01 Zn.

3.2 Set-Up of the Fertilisation Treatments

The biosolids were obtained from the Al Saad Wastewater treatment plant in Al Ain in October 2019. The material was dried for two days in a drying oven at a temperature of 65°C. Representative subsamples of the dry material were analysed for mineral element concentrations as described in Section 3.1. The experimental soil was brought to a water content of 10% w/w to avoid dust evolution and was then thoroughly mixed with biosolids at a rate of 3.2 (low), 6.4 (medium) or 12.8 (high) g per kg dry soil. The soil was then filled into round plastic planting pots with a volume of 7 L.

The soil for the mineral fertilization treatments was supplied with nutritional elements in form of solutions added to the filled pots. Planting pots filled with biosolids amended soil received a K_2SO_4 solution to add 97.24 mg (Low), 194.48 mg (Medium) or 388.97 mg (High) K per kg dry soil. This was meant to compensate for a low K concentration in the biosolids, and equalized K supply between the biosolids and mineral fertilization treatments. The latter were supplied with P and K in form of KH_2PO_4. The soil in all pots was further supplied with micronutrients in form of a water soluble, chelated compound fertilizer (Microcare Fort Complex 2, Abu Dhabi Fertilizer Industries, LLC, Abu Dhabi, UAE) at an equal rate. Table 2 shows concentrations of nutritional elements in the biosolids, and amounts applied to the soil prepared for the low, medium, and high treatment.

Table 2: Nutrients concentration in the biosolids applied to soil at various levels

Element	**Element concentration in the biosolids (mg per kg DW)**	**Applied amounts of elements to the soil in (mg per kg DS).**			**Applied form to Mineral fertilization treatments**
		Low	Medium	High	
N	18.81	60.00	120.00	240.00	$Ca(NO_3)_2*4H_2O$
P	24.17	77.10	154.19	308.39	KH_2PO_4
K	2.55	8.13	16.27	32.54	
K	Added as K_2SO_4	97.24	194.48	388.97	
Ca	57.14	182.27	364.53	729.06	
Mg	14.52	46.32	92.63	185.26	$MgSO_4*7H_2O$
Fe	12.63	40.28	80.56	161.12	
Fe	Added as chelate	10	10	10	Fe-EDTA
Cu	0.28	0.90	1.80	3.60	
Cu	added as chelate	2.5	2.5	2.5	Cu-EDTA
Zn	0.75	2.39	4.78	9.56	
Zn	added as chelate	10	10	10	Zn-EDTA
Mn	0.27	0.87	1.74	3.48	
Mn	Added as chelate	7.5	7.5	7.5	Mn-EDTA
Na	1.19	3.79	7.39	15.18	

The Biosolids treatments received 3.2, 6.4 and 12.8 g of biosolids per kg dry soil. In addition, K was supplied to the biosolids treatments in form of potassium sulphate

(K_2SO_4). Amounts of K supplied to the biosolids in organic and mineral form corresponded to amounts added to the mineral fertilization treatments with the KH_2PO_4. The soil for all treatments was supplied with a micronutrient compound fertilizer providing Fe, Cu, Zn and Mn in chelated form. The amount of additional micronutrient supply did not differ depending on the treatment.

The experimental Ghāf and Samr plants were removed from the plastic planting bags in which they had been cultivated before being used in the experiment, and the soil surrounding their roots was gently shaken off until the roots were bare. They were then planted into the experimental pots (one plant per pot), and the soil moisture regime was brought to a volume of 20% w/w immediately afterwards. The root system of the *Clerodendrum* plants could not be separated from the peatmoss substrate in which the plants had been cultivated. For this reason, the plants were transferred with the entire root bale, including approximately 200 ml of peatmoss substrate. The weight of every planting pot involved in the experiment was recorded after planting and establishment a soil moisture (20% w/w) that was close to field capacity.

The planting pots were set up completely randomized in the greenhouse in December 2019. Between December and February, the temperature around the trees averaged 25°C during the day, and 19°C during the night. Between March and September, the average temperature around the plants was 33°C during the day, and 27°C during the night. The greenhouse was cooled with an evaporative cooling pad, and the trees were set up on a table near the cooling system. The trees were irrigated every second day. The daily water loss from the planting pots was estimated gravimetrically once every 10 to 15 days, and irrigation water supply was based on these estimations. Five replicates were prepared of each treatment, as shown in Table 3.

Table 3: Nutrient treatments of the two-factorial experiment conducted for three species

Tree species	*Cleodendrum inerme*						*Prosopis cineraria*						*Acacia tortilis*					
Factor 1: Amount of nutrients supplied	Low		Medium		High		Low		Medium		High		Low		Medium		High	
Factor 2: Form of nutrients supply	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M

Key: B = Biosolids; M = Minerals

3.3 Harvest of Experimental and Element Analysis of Plant Materials

The trees were harvested ten months after planting. At the time of harvest, the shoots of the trees were cut off above the ground, chopped into pieces and dried in a drying oven at 65°C. The planting containers were emptied into aluminium trays, and their contents left to dry in the greenhouse. The roots were then separated from the soil by passing the dry material gently through a sieve with a mesh of 1 mm. The root systems were washed with tap water and dried at 65°C. The weight of all dry plant material was then estimated. Representative subsamples of the chopped shoots were then ground into a fine powder using a hammer mill.

Samples of approximately 300 mg of the ground plant material were then microwave digested in presence of a 1:2 mixtures of concentrated per chloric acid and nitric acid. The digested samples were transferred to graduated bottles and brought to a volume of 30 ml. They were filtered through a blue-ribbon filter paper prior to being analysed for concentrations of P, K, Ca, Mg, Na, Fe, Zn, Mn, Cu, Pb, Ni, Cd, and Co using

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The nitrogen concentrations in the shoot material were estimated according to Kjeldahl, 1883, using a semi-automated procedure.

3.4 Estimation of AMF Colonised Root Length

A representative subsample of approximately 0.5 g dry weight was taken from each Ghāf or *Clerodendrum inerme* root system. These samples consisted of root fragments of 1-3 cm length. The samples were soaked in water for around 30 minutes prior to being stained according to (Vierheilig et al., 1998). In a first step, the roots were submerged in 95°C hot 10% KOH solution for 15 minutes for clearing and making the cell wall permeable to the dye. The samples were then thoroughly rinsed with tap water and acidified in white vinegar. They were then stained for 3 minutes in boiling 5% ink in white vinegar solution. Blue fountain pen ink was used for staining (Flamingo Stationery Trading, UAE). The roots were detained in a 50:50 vinegar/water mixture overnight before the samples were spread out on graduated (1 cm) petri plates for estimation of the colonized.

3.5 Statistical Analysis

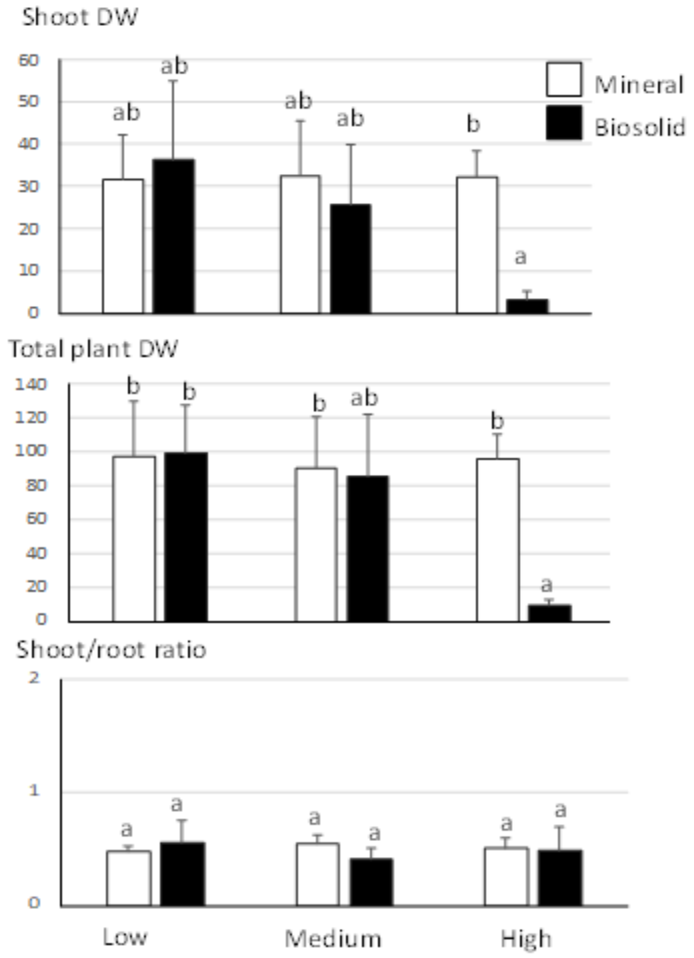
The results were presented as mean values and standard deviations. Data were tested using statistical analysis in IBM SPSS statistics 26 software. Means were compared using One Way ANOVAs at significance level of $P < 0.05$. Differences between means of the treatments were compared by the Tukey's Multiple Comparison. A Two-Way ANOVA was performed to assess whether the type of fertilizer (biosolids vs. mineral, factor 1), or the fertilization level (low, medium, or high, factor 2) had a significant ($P < 0.05$) effect on the obtained results, and whether variation within one factor significantly depended on the level of the respective other factor.

Chapter 4: Data Analysis and Results Interpretation

4.1 Plant Dry Weights at Harvest Period

Under a high supply, however, the Ghāf trees receiving biosolids remained much smaller than those under mineral fertilization. The total plant DW of Ghāf trees growing in soil supplied with high amounts of biosolids was more than 80% lower than that of all other treatments. Ghāf plants receiving mineral fertilizer did not differ in their DW depending on the amount of fertilizer supplied. When the soil was amended with biosolids, the DW of Ghāf trees of the low and medium supply level did not differ. Ghāf trees of all treatments had a shoot/root ratio of around 0.5, indicating that the DW of the plant root systems was nearly twice as high as that of the corresponding shoots. The soil fertilization treatment had no impact on the shoot/root ratio.

At the time of harvest, the total DW of Samr trees was less than half of that achieved by Ghāf trees of the same experiment. The growth of the Samr trees differed greatly between the replicates of each treatment, resulting in high standard deviations around mean values. Total DW of Samr tree was highest growth under a low fertilization regime, irrespective of the fertilizer type. There was no difference in plant DW depending on whether biosolids or mineral fertilizer was applied. The Samr plants had higher shoot/root ratios than the Ghāf trees, with shoots being two to three times heavier than the roots. However, there was no difference in the shoot/root ratio depending on the fertilization treatment. The shoot dry weight (DW), as well as the DW of the entire tree at the time of harvest did not differ depending on the fertilizer type. Among all woody species involve in this experiment, the *Clerodendrum inerme* shrubs achieved the highest total DW and shoot DW in, as shown in Figures 4 - 6.

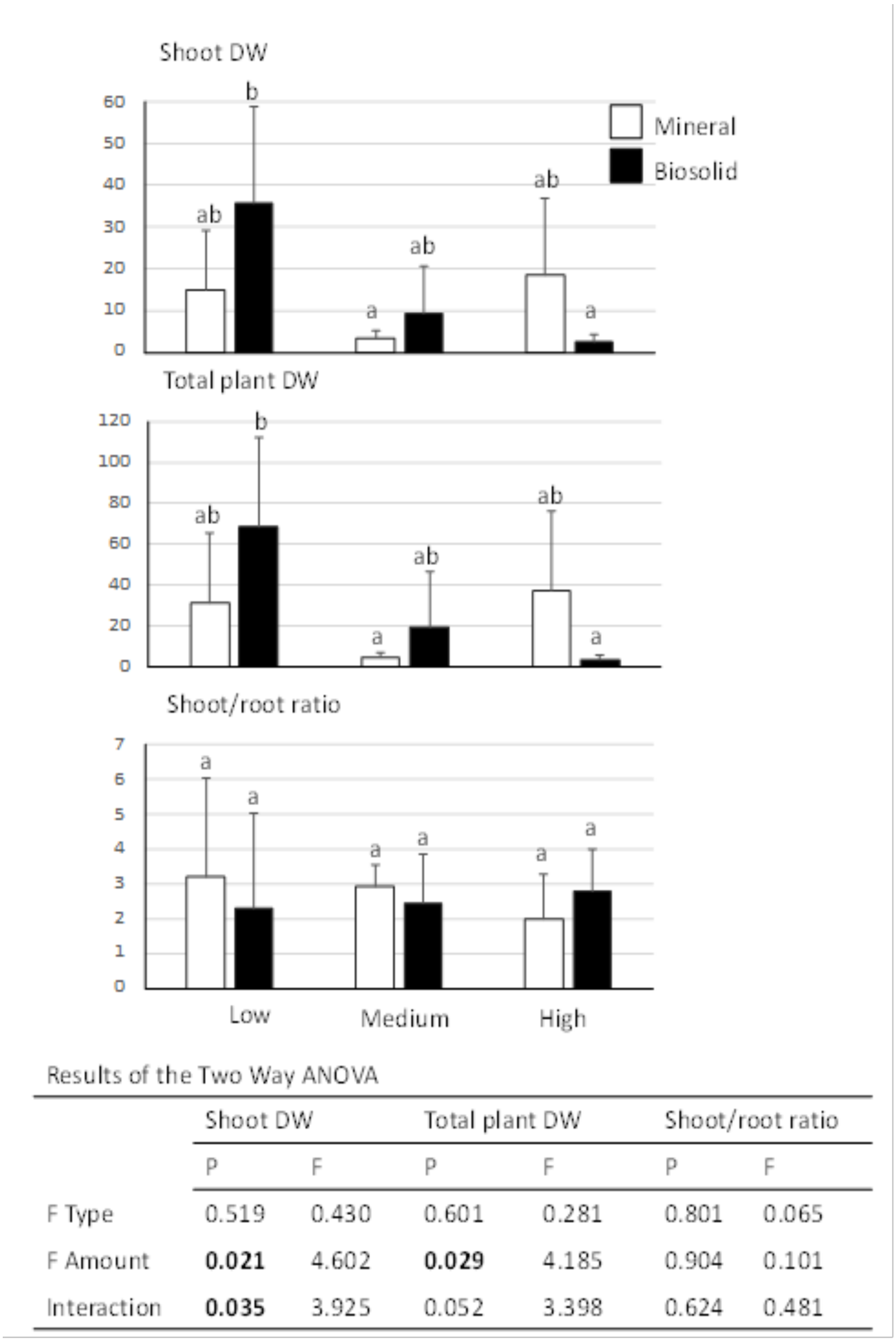


Results of the Two Way ANOVA

	Shoot DW		Total plant DW		Shoot/root ratio	
	P	F	P	F	P	F
F Type	0.057	4.138	0.078	3.463	0.499	0.475
F Amount	**0.046**	3.662	**0.049**	3.539	0.863	0.148
Interaction	**0.035**	4.057	0.055	3.382	0.315	1.228

Figure 4: Dry weight of Ghāf plants at the time of harvest in g per plant

Note: Mean values ± standard deviation for the shoot DW (top), the total plant dry weight (middle) and the shoot/root ratio (bottom). Mean values followed by the same letter are not significantly different (One Way ANOVA; P <0.05). The table below the figures shows the results of the Two-Way ANOVA. P values indicating a significant (<0.05) effect of the fertilizer type (F Type), the fertilization level (F Amount), or an interaction between both factors, are printed in bold.

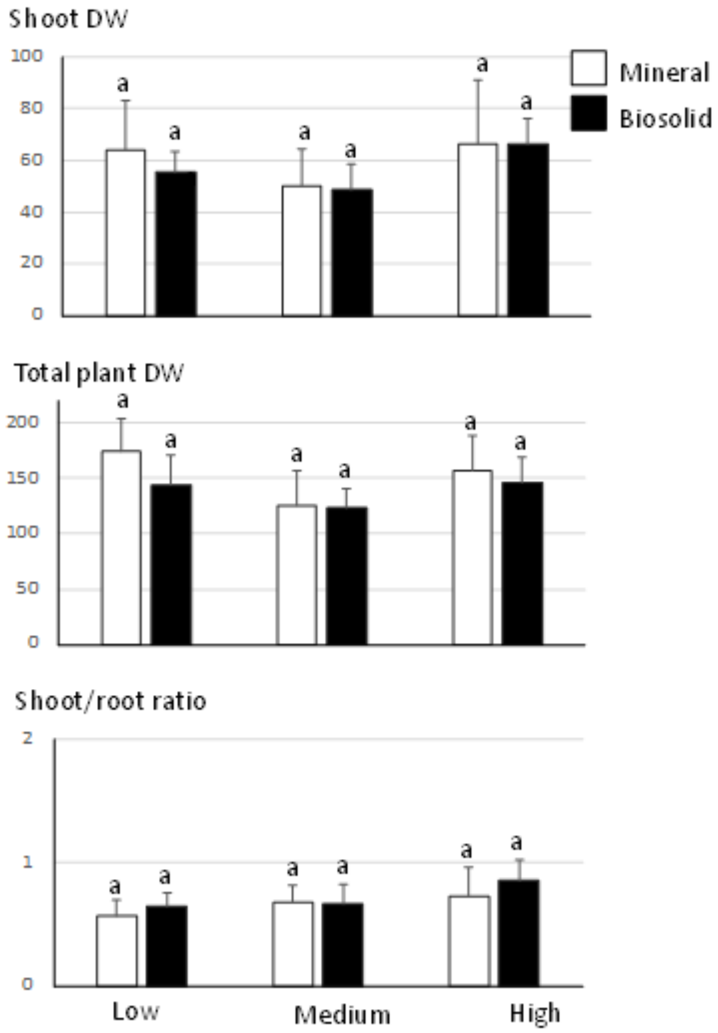


Results of the Two Way ANOVA

	Shoot DW		Total plant DW		Shoot/root ratio	
	P	F	P	F	P	F
F Type	0.519	0.430	0.601	0.281	0.801	0.065
F Amount	**0.021**	4.602	**0.029**	4.185	0.904	0.101
Interaction	**0.035**	3.925	0.052	3.398	0.624	0.481

Figure 5: Dry weight of Samr plants at the time of harvest in g per plant

Note: Mean values ± standard deviation for the shoot DW (top), the total plant dry weight (middle) and the shoot/root ratio (bottom).



Results of the Two Way ANOVA

	Shoot DW		Total plant DW		Shoot/root ratio	
	P	F	P	F	P	F
F Type	0.573	0.326	0.156	2.145	0.246	1.415
F Amount	0.067	3.036	0.019	4.670	0.072	2.947
Interaction	0.834	0.183	0.486	0.744	0.553	0.608

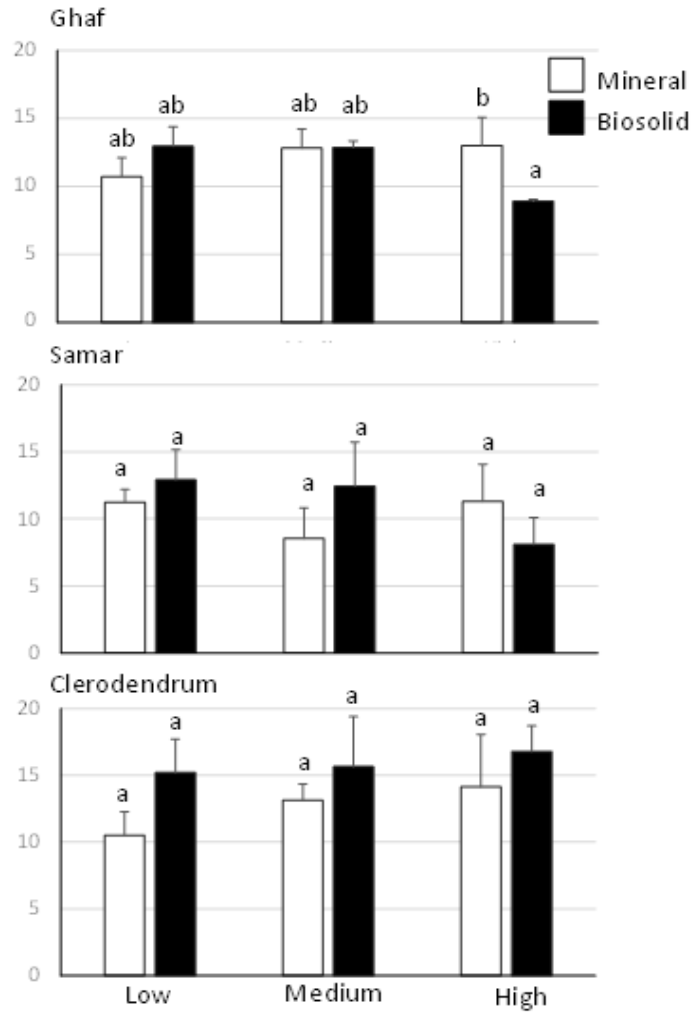
Figure 6: Dry weight of *Clerodendrum* shrub at the time of harvest in g per plant

Note: Mean values ± standard deviation for the shoot DW (top), the total plant dry weight (middle) and the shoot/root ratio (bottom).

4.2 Nitrogen and Phosphorus Concentrations in Shoots

Under the high nutrient supply level, Ghāf trees receiving biosolids had a lower N concentration compared with those receiving mineral fertilizer supply. All other Ghāf trees did not differ in their N supply status, depending on the fertilizer type or amount. The shoot N concentrations did also not differ much between Ghāf and Samr trees. Both species had N levels of 10 - 15 mg per g DW in their shoots. In Samr trees there was no difference in the shoot N status between the treatments, while *Clerodendrum* shrubs growing in biosolids amended soil had higher N concentrations in their shoots compared with plants receiving mineral fertilizers.

In this woody species, the shoot P concentrations also increased with increasing fertilizer supply level. The Ghāf trees supplied with high amounts of biosolids had higher shoot P concentrations than all other treatments, but no further differences in shoot P status were observed, depending on the fertilization treatment. In the Samr trees, shoot P concentrations did not differ amount the treatments, but were in a much higher range compared with those of wild jasmine shrubs or Ghāf trees. In wild jasmine shrubs shoots, not only the concentrations of N, but also those of P were higher for plants supplied with biosolids rather than mineral fertilizers, as detailed in Figure 7 and Figure 8, respectively.

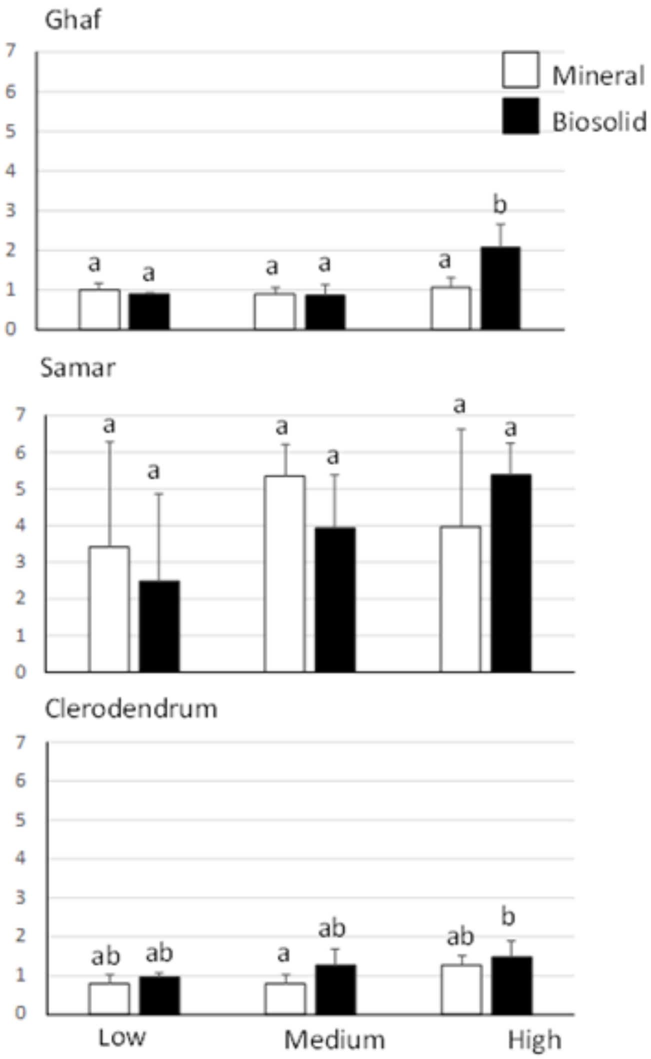


Results of the Two Way ANOVA

	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	0.465	0.576	0.312	1.123	**0.023**	6.767
F Amount	0.146	2.347	0.684	0.393	0.312	1.286
Interaction	**0.015**	6.516	0.316	1.280	0.751	0.293

Figure 7: Nitrogen concentrations in shoots of Ghāf, Samr trees and Wild Jasmine shrubs

Note: Mean values ± standard deviation. Mean values followed by the same letter are not significantly different (Tukey's multiple comparison, $P < 0.05$). The table below the figures shows the results of the Two-Way ANOVA. P values indicative of a significant ($P < 0.05$) effect of the fertilizer type, the amount or an interaction between both factors are printed in bold.



Results of the Two Way ANOVA

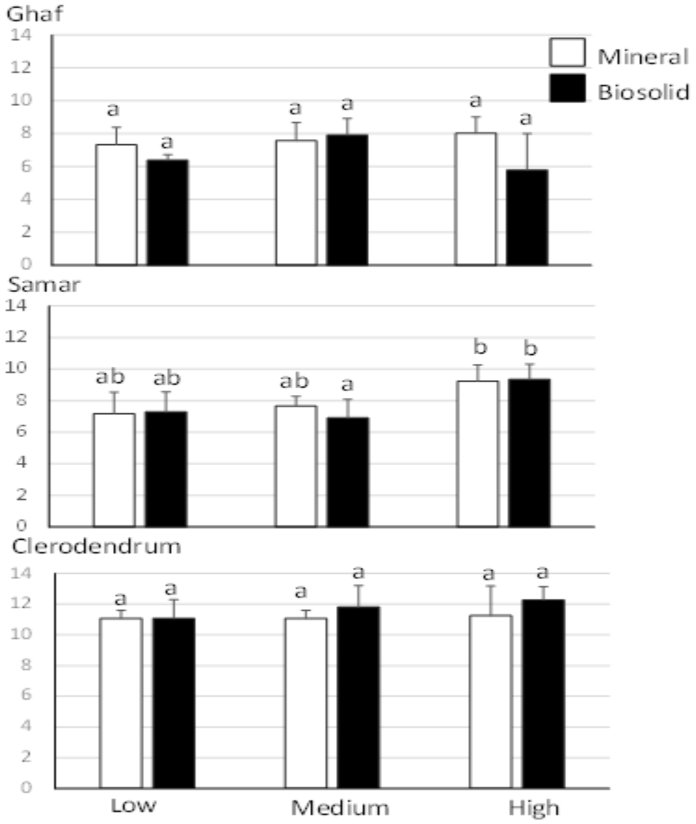
	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	0.055	4.196	0.696	0.157	**0.041**	4.680
F Amount	**0.001**	10.798	0.135	2.198	**0.007**	6.077
Interaction	**0.007**	6.622	0.312	1.230	0.221	1.607

Figure 8: Phosphorus concentrations in shoots of Ghāf, Samār trees and Wild Jasmine shrubs

Note: Mean values ± standard deviation (also see Figure 4)

4.3 Basic Cation Concentrations in Shoots

The supply status of Ghāf trees with K, Ca, Mg and Na showed no difference depending on the type of fertilizer or the fertilization level. Ca concentrations were above K levels, ranging from 10 – 13 mg per g DW, and Mg concentrations were lower (3 – 4 mg per g DW). Sodium concentrations were 4 to five times lower than concentrations of K. The K concentrations in shoot tissues were between 6 – 8 mg per g DW across all treatments, as detailed in Figure 9.



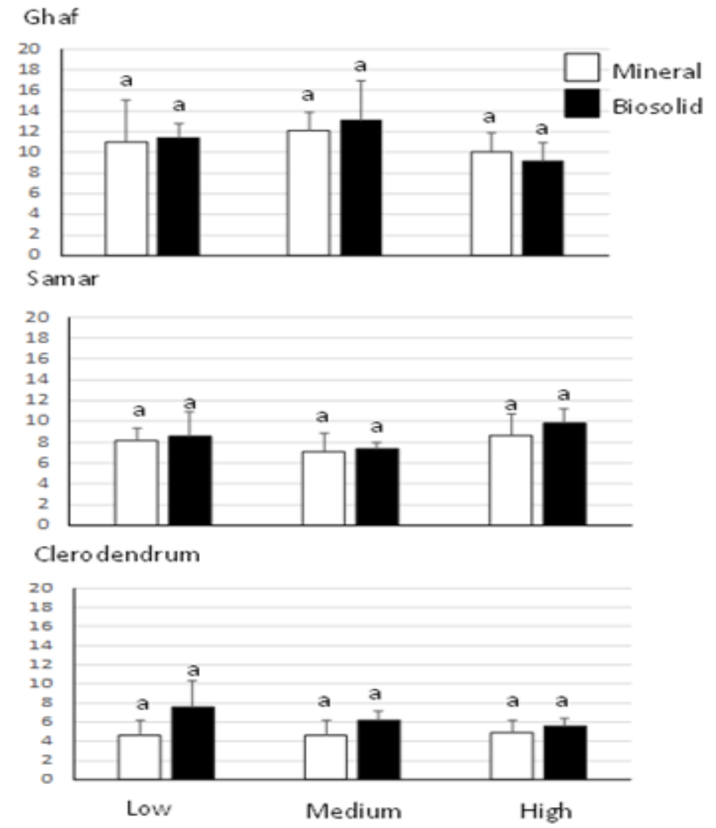
Results of the Two Way ANOVA

	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	0.093	3.119	0.683	0.171	**0.040**	4.716
F Amount	0.363	1.069	**<0.001**	10.126	0.083	2.760
Interaction	0.169	1.957	0.635	0.463	0.945	0.057

Figure 9: Potassium concentrations in shoots of Ghāf, Samār trees and Wild Jasmine shrubs

Note: Mean values ± standard deviation (also see Figure 4)

Irrespective of the type of fertilizer, shoots of Samār trees of the high supply treatment had higher K concentrations compared with trees of the medium and low supply levels. There was no difference in shoot K concentration depending on whether Samār trees received low or medium amounts of organic or mineral fertilizer. The fertilizer type had no effect on the basic cation concentrations in Samār shoots. Compared with the Ghāf trees, the basic cation concentrations in shoots of Samār trees were in the same range as those in Ghāf trees, as detailed in Figure 10.



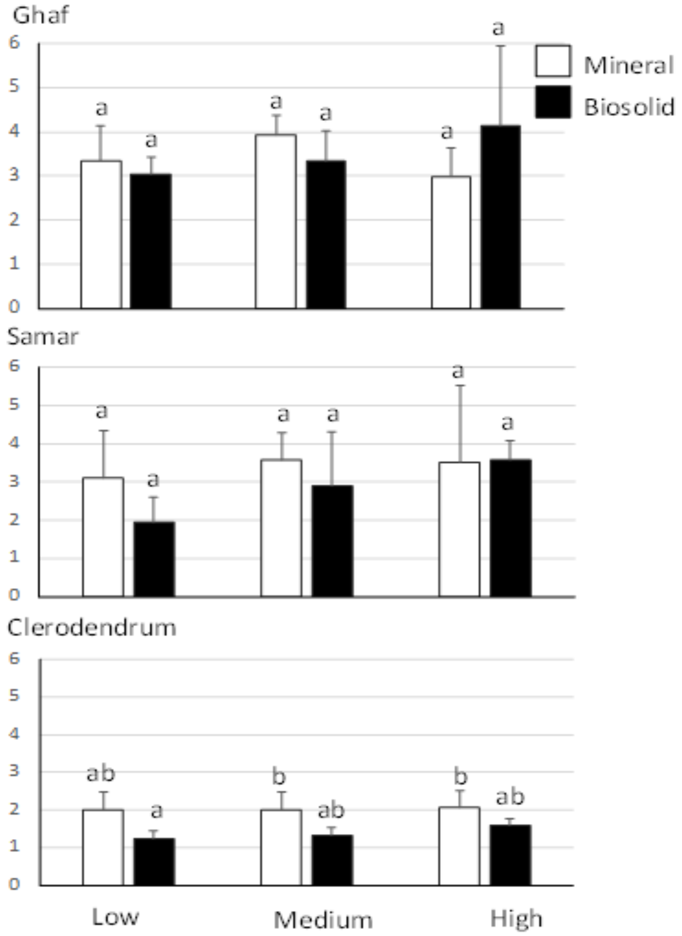
Results of the Two Way ANOVA

	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	0.869	**0.028**	0.306	1.101	0.106	2.820
F Amount	0.074	2.994	0.059	3.237	0.037	3.804
Interaction	0.735	0.313	0.852	0.161	0.850	0.164

Figure 10: Calcium concentrations in shoots of Ghāf, Samār trees and Wild Jasmine shrubs

Note: Mean values ± standard deviation (also see Figure 4)

The wild jasmine shrubs had higher shoot K concentrations compared with Ghāf and Samār trees, while their Ca and Mg concentrations were lower. There were no differences in wild jasmine shrubs shoot cation concentrations depending on the fertilization treatment. Whereas the shoot Na concentrations were in a similar range across all woody species and treatments involved in the experiment, as detailed in Figure 11 and Figure 12, respectively.

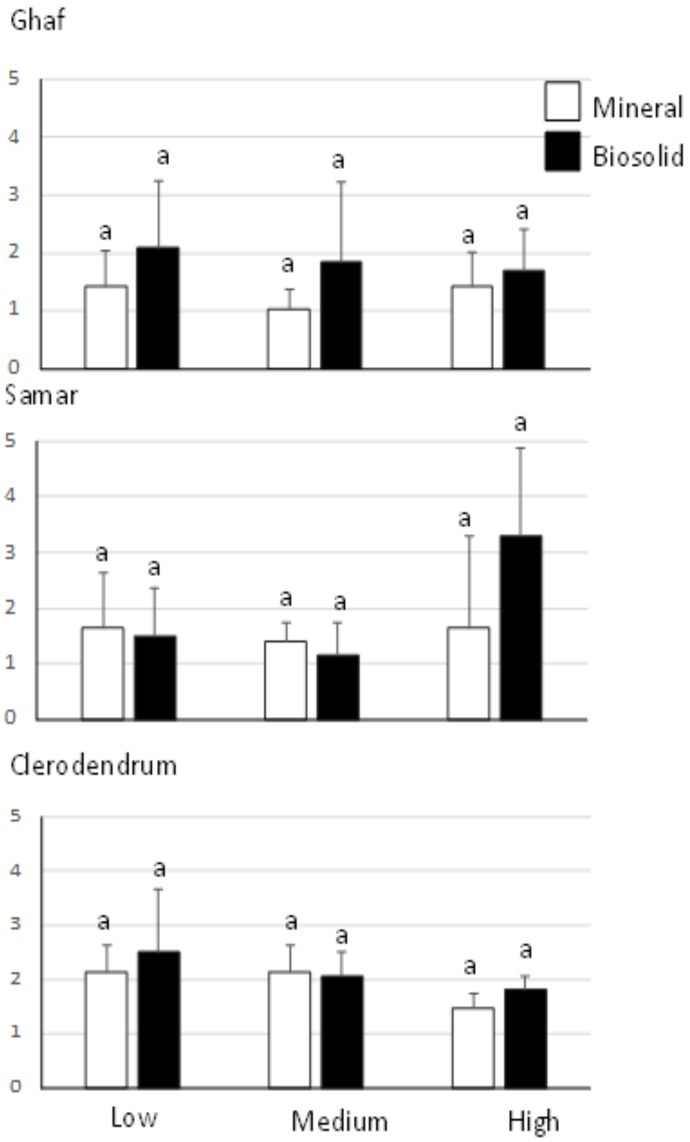


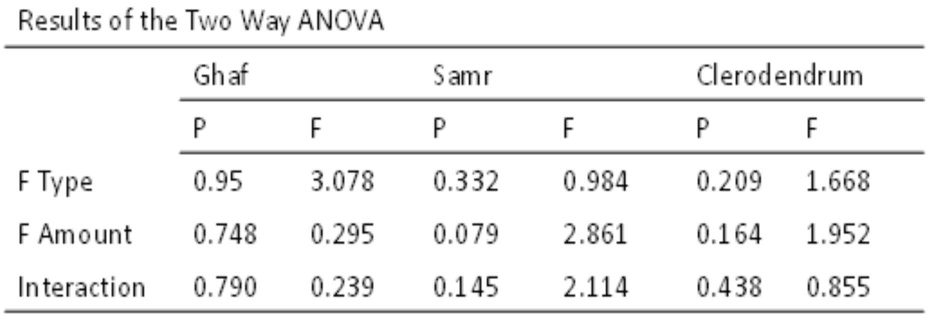
Results of the Two Way ANOVA

	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	0.826	**0.049**	0.193	1.807	**0.002**	12.598
F Amount	0.625	0.481	0.176	1.885	**0.040**	3.684
Interaction	0.166	1.973	0.528	0.657	0.550	0.612

Figure 11: Magnesium concentrations in shoots of Ghāf, Samār trees and Wild Jasmine shrubs

Note: Mean values ± standard deviation (also see Figure 4)





Results of the Two Way ANOVA

	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	0.95	3.078	0.332	0.984	0.209	1.668
F Amount	0.748	0.295	0.079	2.861	0.164	1.952
Interaction	0.790	0.239	0.145	2.114	0.438	0.855

Figure 12: Sodium concentrations in shoots of Ghāf, Samār trees and Wild Jasmine shrubs

Note: Mean values ± standard deviation (also see Figure 4)

4.4 Macronutrient Contents in Plant Shoots

Among the plant species involved in the experiment, wild jasmine shrubs showed the highest overall shoot macronutrient contents, followed by Ghāf and Samār trees. In Ghāf trees grown in mineral fertilized soil, shoot element uptake did not differ depending on the fertilization level (Table 4). When biosolids were supplied, plants of the high supply treatment took up much smaller amounts of nutritional elements compared with all other treatments involved in this trial. Similar with the Ghāf trees, Samār trees were negatively affected in their shoot element uptake by a high level of biosolids application (Table 5). Under a low and medium supply level, however, the biosolids treatments tended to have higher shoot contents of K, Ca, Mg and P compared with corresponding mineral fertilized plants. Neither in Ghāf nor in Samār trees did an increase in the fertilization level lead to an increase in shoot macronutrient contents.

The uptake of other macronutrients remained unaffected by an increasing element supply. Across all fertilization levels, wild jasmine shrubs supplied with mineral fertilizer had higher shoot contents of Mg compared with corresponding biosolids treatments. Though the soil amended with biosolids contained higher amounts of Na compared with the minerally fertilized soil, Na uptake was generally not different depending on the fertilization level, or the type of fertilizer used. In wild jasmine shrubs, shoot uptake of K and P increased with increasing fertilization level, irrespective of the type of fertilizer as detailed in Table 4.

Table 4: Macronutrients and Na concentrations in Ghāf, Samr trees and Wild Jasmine shrubs

F Amount	Low		Medium		High		Two Way ANOVA		
F Type	Mineral	Biosolid	Mineral	Biosolid	Mineral	Biosolid	F Amount	F Type	Amount x Type
N (mg / plant)	311.5 a ± 128.8	471.9 a ± 219.4	427.2 a ± 166.44	351.1 a ± 268.8	369.3 a ± 17.18	35.7 a ± 5.47	0.333	0.153	0.086
K (mg / plant)	227.7 ab ± 69.2	250.3 ab ± 117.5	254.8 ab ± 34.0	230 ab ± 108.2	209.4 b ± 129.2	15.4 a ± 7.5	**0.038**	**0.012**	**0.013**
Ca (mg / plant)	363.5 ab ± 205.4	415.1 b ±213.5	382.0 b ± 120.6	368.1 ab ± 306.7	318.2 ab ± 68.1	30.5 a ± 20.8	**0.021**	0.228	0.093
Mg (mg / plant)	106.3 b ± 47.0	105.1 b ±38.3	125.9 b ± 45.4	91.6 ab ± 68.3	93.9 b ± 11.7	15.4 a ±12.1	**0.012**	**0.024**	0.125
Na (mg / plant)	43.2 ab ±22.5	64.3 b ± 24.2	32.7 ab ± 12.8	60.1 ab ± 65.5	43.9 ab ± 16.8	6.0 a ± 5.7	0.083	0.747	**0.035**
P (mg / plant)	30.9 ab ± 10.5	32.7 ab ± 18.7	29.9 ab ± 14.3	24.1 ab ± 18.5	34.2 b ± 8.1	6.2 a ± 4.3	0.172	**0.050**	0.052

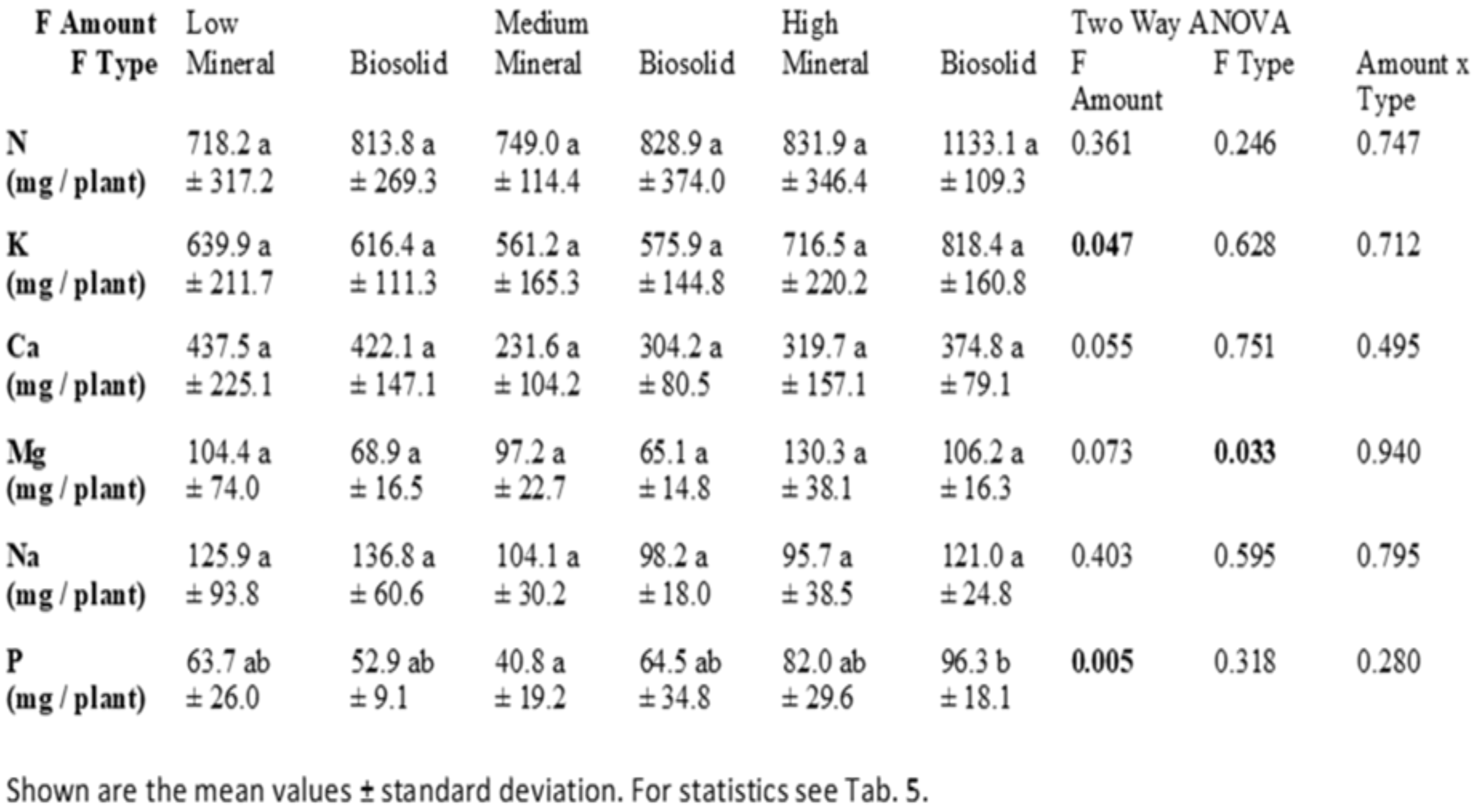
Shown are the mean values ± standard deviation. Mean values followed by the same letter are not significantly different (Tukey's multiple comparison, $P < 0.05$). The right side of the table shows the results of the Two Way ANOVA. P values indicative of a significant ($P < 0.05$) effect of the fertilizer type, the supply level (amount) or an interaction between both factors are printed in bold.

Table 5: Macronutrient and sodium contents of Samār tree shoot in mg per plant

F Amount	Low		Medium		High		Two Way ANOVA		
F Type	Mineral	Biosolid	Mineral	Biosolid	Mineral	Biosolid	F Amount	F Type	Amount x Type
N (mg / plant)	156.0 a ± 157.3	489.9 a ± 168.2	33.6 a ± 15.9	174.7 a ± 182.0	300.2 a ± 249.8	9.4 a ± 6.7	0.119	0.320	**0.019**
K (mg / plant)	93.0 ab ± 78.4	243.5 b ± 148.5	26.5 ab ± 11.8	56.3 a ± 58.9	158.4 ab ± 152.6	22.2 a ± 14.6	**0.026**	0.688	**0.012**
Ca (mg / plant)	115.8 ab ± 109.5	317.1 b ± 236.7	23.8 a ± 10.2	71.4 ab ± 88.8	130.9 ab ± 121.5	24.6 a ±17.2	**0.018**	0.331	**0.048**
Mg (mg / plant)	33.1 ab ± 22.2	61.6 b ± 40.5	12.7 a ± 6.9	17.6 ab ± 11.9	37.1 ab ± 27.0	9.1 a ± 6.6	**0.015**	0.834	**0.045**
Na (mg / plant)	23.1 ab ± 29.1	38.2 b ± 20.1	5.1 a ± 3.0	5.7 a ± 2.3	8.7 ab ± 1.9	6.2 ab ± 2.9	**0.002**	0.458	0.420
P (mg / plant)	22.1 ab ± 2.9	50.7 a ± 23.8	18.1 ab ± 7.5	26.3 ab ± 21.8	37.5 ab ± 28.7	13.3 a ±9.0	0.217	0.542	**0.015**

Shown are the mean values ± standard deviation. For statistics see Tab. 5.

Table 6: Macronutrient and sodium contents of Wild Jasmine shoot in mg per plant

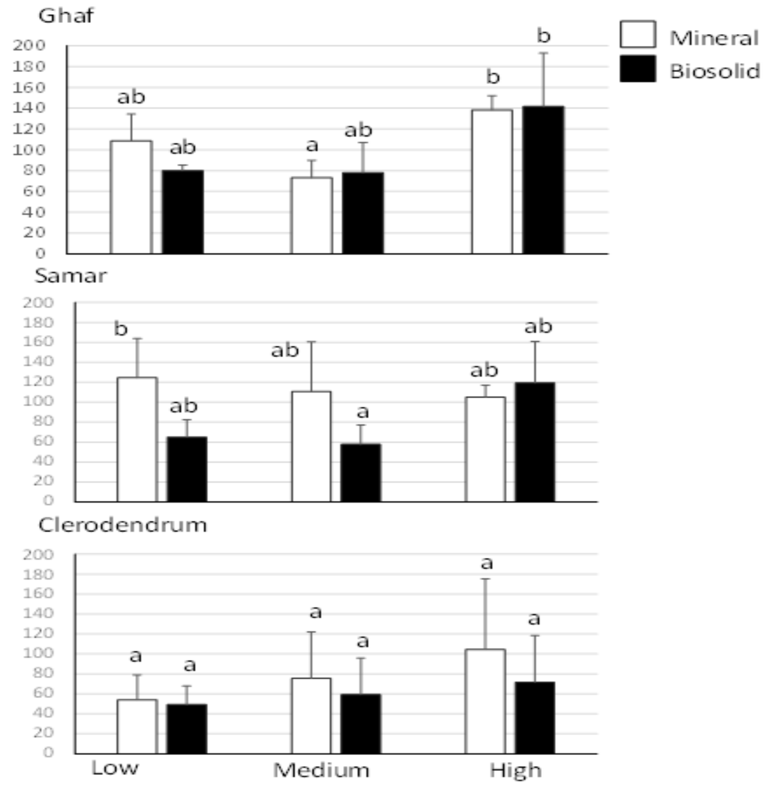


F Amount	Low		Medium		High		Two Way ANOVA		
F Type	Mineral	Biosolid	Mineral	Biosolid	Mineral	Biosolid	F Amount	F Type	Amount x Type
N (mg / plant)	718.2 a ± 317.2	813.8 a ± 269.3	749.0 a ± 114.4	828.9 a ± 374.0	831.9 a ± 346.4	1133.1 a ± 109.3	0.361	0.246	0.747
K (mg / plant)	639.9 a ± 211.7	616.4 a ± 111.3	561.2 a ± 165.3	575.9 a ± 144.8	716.5 a ± 220.2	818.4 a ± 160.8	**0.047**	0.628	0.712
Ca (mg / plant)	437.5 a ± 225.1	422.1 a ± 147.1	231.6 a ± 104.2	304.2 a ± 80.5	319.7 a ± 157.1	374.8 a ± 79.1	0.055	0.751	0.495
Mg (mg / plant)	104.4 a ± 74.0	68.9 a ± 16.5	97.2 a ± 22.7	65.1 a ± 14.8	130.3 a ± 38.1	106.2 a ± 16.3	0.073	**0.033**	0.940
Na (mg / plant)	125.9 a ± 93.8	136.8 a ± 60.6	104.1 a ± 30.2	98.2 a ± 18.0	95.7 a ± 38.5	121.0 a ± 24.8	0.403	0.595	0.795
P (mg / plant)	63.7 ab ± 26.0	52.9 ab ± 9.1	40.8 a ± 19.2	64.5 ab ± 34.8	82.0 ab ± 29.6	96.3 b ± 18.1	**0.005**	0.318	0.280

Shown are the mean values ± standard deviation. For statistics see Tab. 5.

4.5 Micronutrient Concentrations in Shoots

While the Fe nutritional status of Ghāf shoots did not differ depending on the type of fertilizer used, Samr trees of the low and medium supply level had higher shoot Fe concentrations when fertilizers had been supplied in mineral form. The overall shoot Fe concentrations were in a similar range for Ghāf and Samr trees whether biosolids or mineral fertilizers were supplied, while the Fe levels in wild jasmine shrubs shoots were lower than those of the native trees. Figure 13 shows Iron concentrations in shoots of Ghāf trees.



Results of the Two Way ANOVA

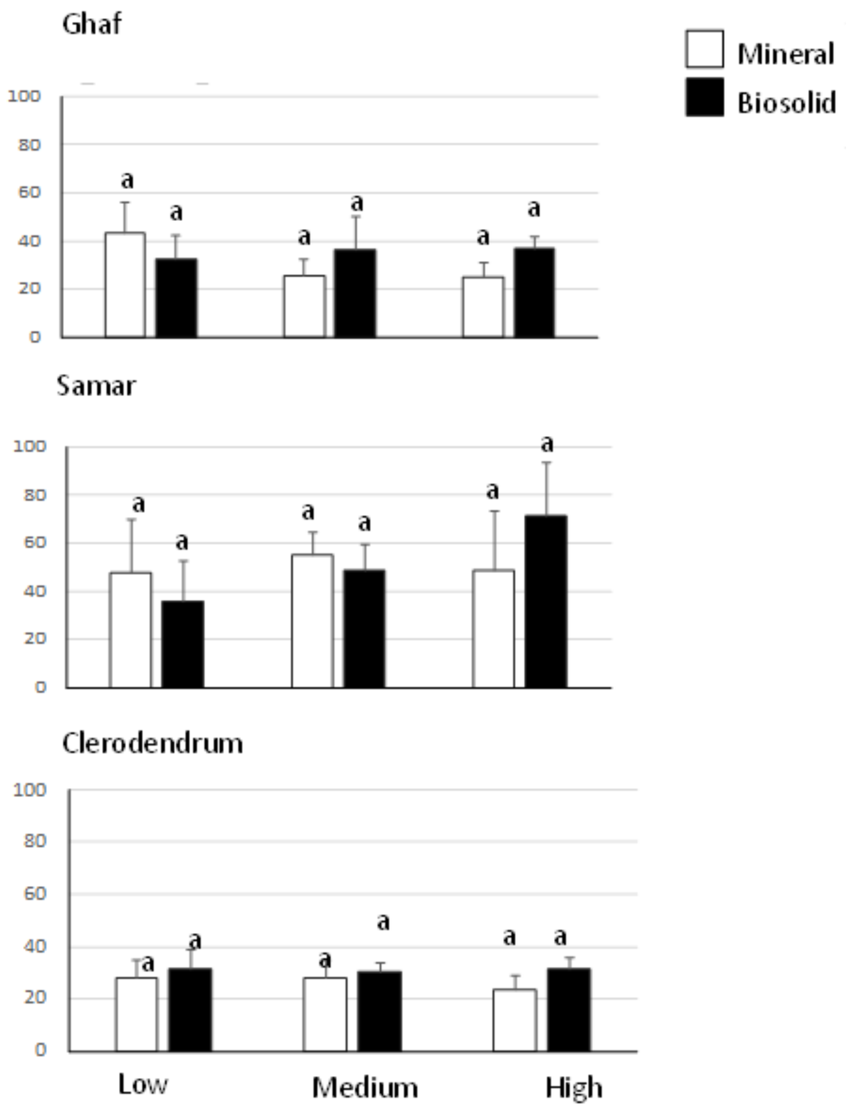
	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	0.564	0.345	**0.015**	6.924	0.274	1.253
F Amount	**<0.001**	11.308	0.217	1.639	0.200	1.721
Interaction	0.438	0.862	**0.047**	3.529	0.777	0.255

Figure 13: Iron concentrations in shoots of Ghāf, Samār trees and wild jasmine shrubs

Note: Mean values ± standard deviation (also see Figure 4)

Similar with Fe, the Zn and Cu concentrations in shoots were higher for Ghāf and Samār trees compared with wild jasmine shrubs. However, Zn concentrations did generally not differ depending on the fertilization level or the type of fertilizer used. The Samār trees showed a big variation in the shoot Cu concentrations within the same treatment, and no clear trend regarding the effect of the fertilizer type or supply level could be observed. Samār trees of the high supply level had higher Cu concentrations when fertilizers had been supplied in form of biosolids. Similar with Fe and Zn, wild jasmine shrubs did not differ in their shoot Cu concentrations depending on the fertilization treatment. In Ghāf tree shoots, the Cu concentrations were higher for plants growing in soil amended with biosolids compared with the corresponding mineral treatments.

The Co concentrations in shoots were in a similar range across all woody species and treatments of the present experiment. While the Cu concentrations tended to be higher for Ghāf and Samār trees growing in biosolids compared with mineral fertilized soil, an opposite effect was observed in shoot Mn levels. The latter were higher for mineral fertilized Ghāf trees and decreased with increasing fertilizer supply level. In Samār trees the fertilization level had no impact on the shoot Mn levels, but the Mn supply status tended to be slightly higher for mineral compared with biosolids fertilized plants. In wild jasmine shrubs there were no differences in shoot Mn concentrations depending on the fertilization level. The shoot Mn concentrations were in a similar range across all experimental woody species. Figures 14, 15, 16, and 17 showing concentrations of Zn, Cu, Co, and Mn, respectively.

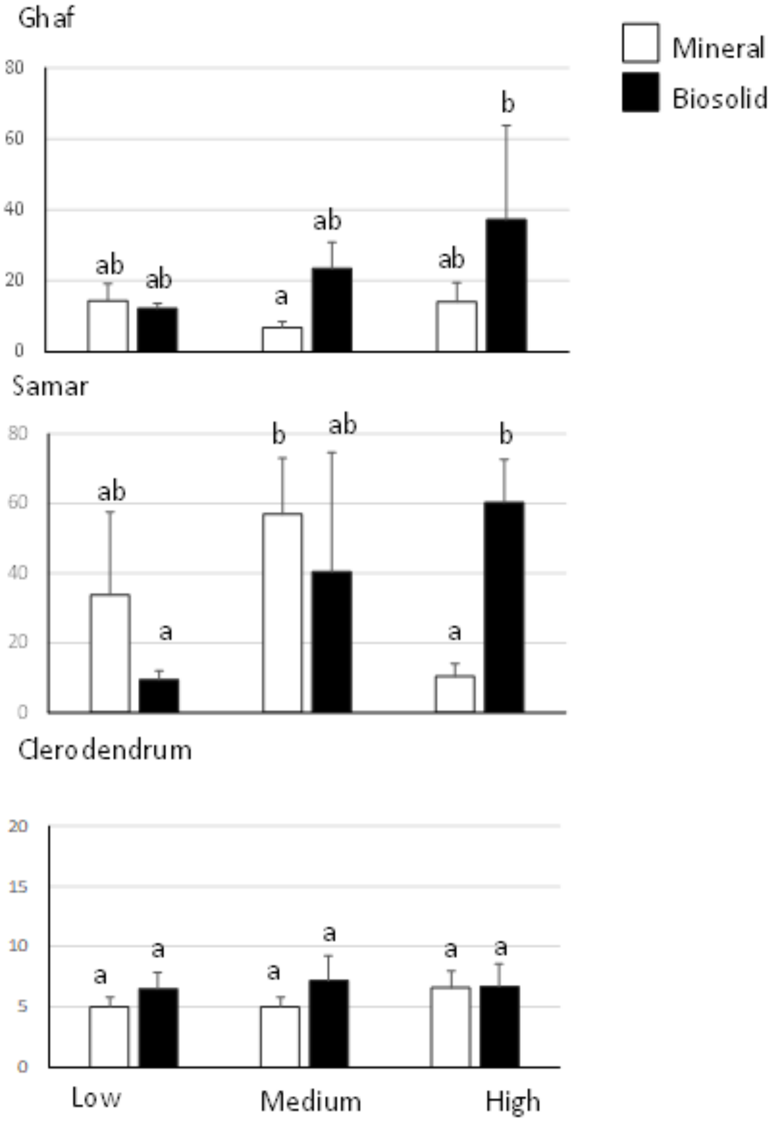


Results of the Two Way ANOVA

	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	0.304	1.115	0.804	0.063	0.206	1.687
F Amount	0.248	1.503	0.126	2.279	0.379	1.009
Interaction	**0.038**	3.901	0.128	2.263	0.378	1.014

Figure 14: Zinc concentrations in shoots of Ghāf, Samr trees and Wild Jasmine shrubs

Note: Mean values ± standard deviation (also see Figure 4)

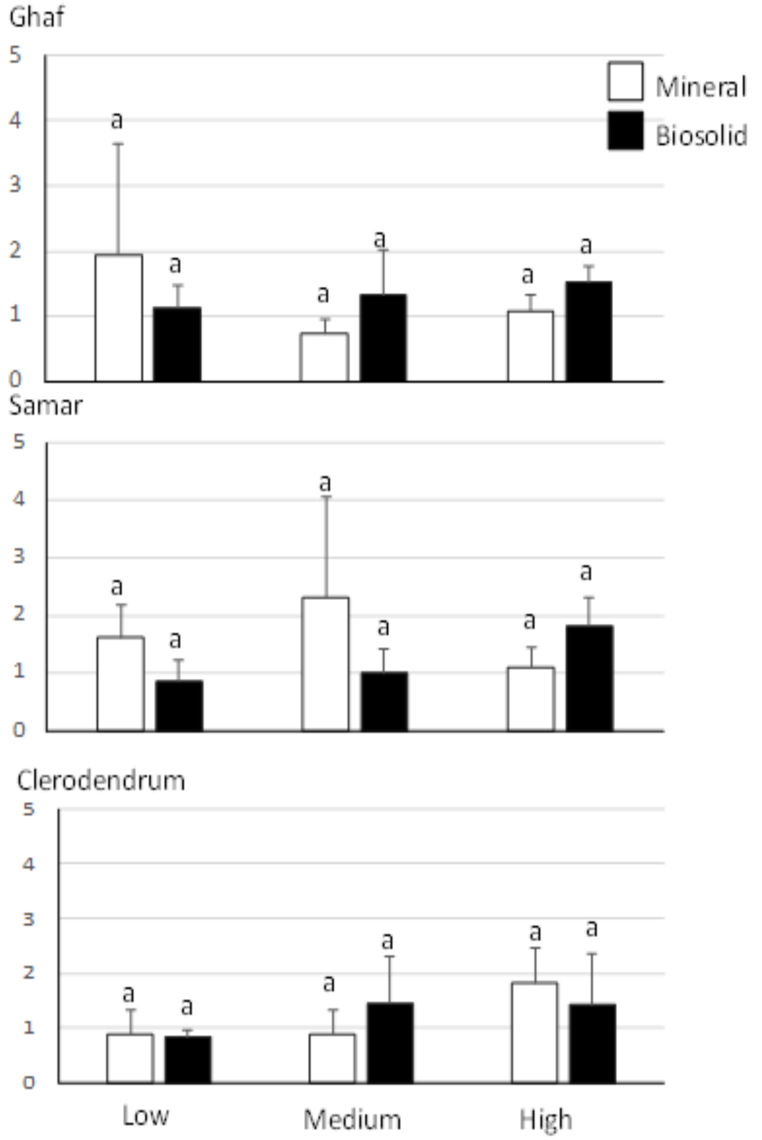


Results of the Two Way ANOVA

	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	**0.026**	5.836	0.688	0.166	0.478	0.520
F Amount	0.114	2.439	**0.022**	4.547	0.522	0.669
Interaction	0.133	3.343	**<0.001**	9.954	0.149	2.066

Figure 15: Copper concentrations in shoots of Ghāf, Samr trees and Wild Jasmine shrubs

Note: Mean values ± standard deviation (also see Figure 4)

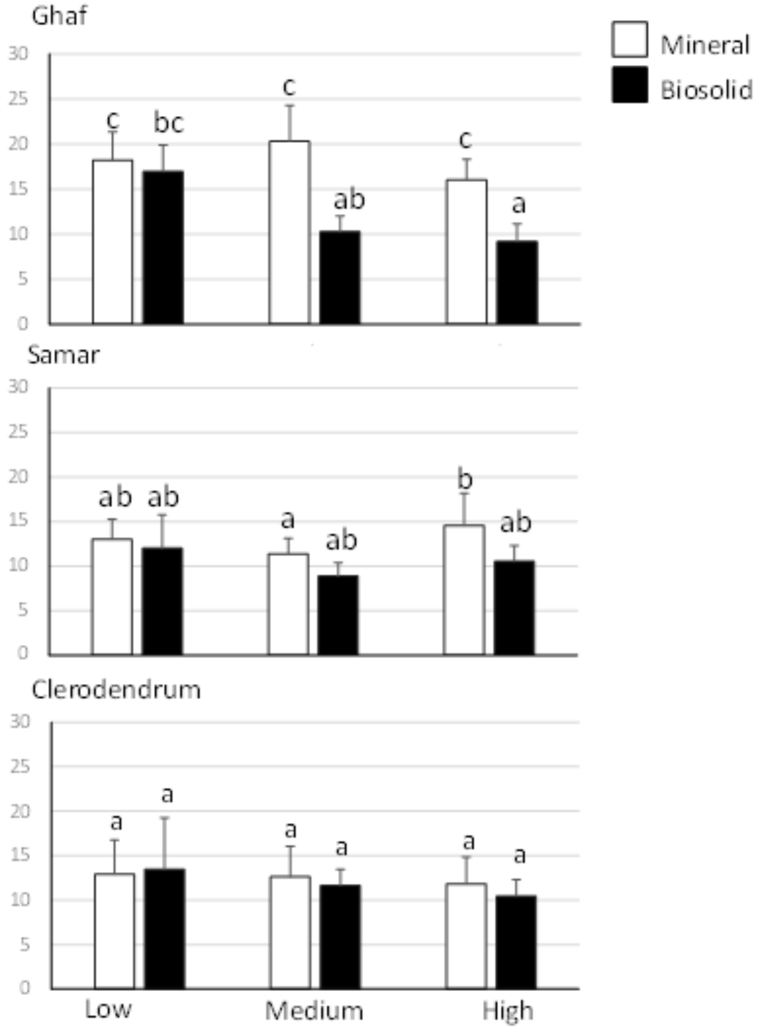


Results of the Two Way ANOVA

	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	0.896	0.018	0.153	2.191	0.817	0.055
F Amount	0.413	0.927	0.474	0.773	0.154	2.028
Interaction	0.141	2.177	0.030	4.129	0.230	1.565

Figure 16: Cobalt concentrations in shoots of Ghāf, Samār trees and Wild Jasmine shrubs

Note: Mean values ± standard deviation (also see Figure 4)



Results of the Two Way ANOVA

	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	**0.001**	28.745	**0.018**	6.502	0.645	0.218
F Amount	**0.005**	7.205	0.102	2.535	0.441	0.847
Interaction	**0.020**	4.829	0.464	0.794	0.807	0.217

Figure 17: Manganese concentrations in shoots of Ghāf, Samār trees and Wild Jasmine shrubs

Note: Mean values ± standard deviation (also see Figure 4)

4.6 Micronutrient Contents in Shoots

Ghāf and Samr trees grown in soil fertilized with high rates of biosolids not only had lower shoot macronutrient, but also micronutrient contents compared with all other woody species. The other fertilization treatments differed only little in their shoot micronutrient contents. In Ghāf trees, shoot Fe contents were slightly higher in mineral compared with biosolids fertilized plants, as shown in Table 7. In biosolids fertilized Samr trees, the shoot Zn and Mn contents decreased with increasing supply level, while no such effect was observed in mineral fertilized trees, as shown in Table 8. Wild jasmine shrubs did not differ in their shoot micronutrient contents depending on the fertilization treatment, as shown in Table 9. The shoot micronutrient contents of wild jasmine shrubs were generally higher compared with those of Ghāf and Samr trees.

Table 7: Micronutrient contents of Ghāf shoots in µg mg per plant

F Amount	Low		Medium		High		Two Way ANOVA		
F Type	Mineral	Biosolid	Mineral	Biosolid	Mineral	Biosolid	F Amount	F Type	Amount x Type
Fe **(mg / plant)**	3.61ab ± 1.89	2.96 ab ± 1.72	2.42 ab ± 1.31	2.23 ab ± 2.01	4.51 b ± 1.14	0.40 a ± 0.27	0.365	**0.010**	**0.018**
Zn **(mg / plant)**	1.36 b ± 0.67	1.14 ab ± 0.53	0.81 ab ± 0.29	1.06 ab ± 0.92	0.79 ab ± 0.20	0.11 a ± 0.06	**0.007**	0.279	0.164
Cu **(µg / plant)**	476.7 a ± 275.0	429.1 a ± 182.2	220.2 a ± 88.6	548.9 a ± 198.7	444.1 a ± 173.6	155.8 a ± 138.7	0.228	0.974	**0.010**
Co **(µg / plant)**	5.4 a ± 1.4	4.8 a ± 3.8	6.0 a ± 5.0	9.3 a ± 10.2	6.3 a ± 6.8	0.6 a ± 0.5	0.294	0.643	0.236
Mn **(µg / plant)**	589.6 b ± 271.8	591.5 b ± 234.4	669.2 b ± 299.2	264.8 b ± 144.9	518.5 b ± 137.2	30.2 a ± 23.1	**0.012**	**0.002**	**0.049**

Shown are the mean values ± standard deviation. For statistics see Tab. 5.

Table 8: Micronutrient contents of Samr shoots in µg per plant

F Amount	Low		Medium		High		Two Way ANOVA		
F Type	Mineral	Biosolid	Mineral	Biosolid	Mineral	Biosolid	F Amount	F Type	Amount x Type
Fe (mg / plant)	2.00 a *± 2.13*	2.23 a *± 1.73*	0.38 a *± 0.19*	0.37 a *± 0.26*	2.11 a *± 2.17*	0.25 a *± 0.13*	**0.048**	0.326	0.252
Zn (mg / plant)	0.47 ab *± 1478.7a*	1.02 b *± 538.7a*	0.19 a *± 453.8a*	0.36 ab *± 357.2a*	0.56 ab *± 483.8a*	0.16 a *± 574.2a*	**0.031**	**0.043**	0.480
Cu (µg / plant)	248.9 a *± 79.2*	323.9 a *± 216.3*	207.4 a *± 123.9*	203.4 a *± 108.4*	152.9 a *± 146.2*	142.55 a *± 92.9*	0.105	0.699	0.747
Co (µg / plant)	3.0 a *± 4.3*	4.7 a *± 5.2*	0.5 a *± 0.3*	2.6 a *± 3.1*	4.4 a *± 4.3*	0.8 a *± 0.7*	0.368	0.965	0.193
Mn (µg / plant)	208.0 ab *± 205.8*	478.8 b *± 331.8*	38.8 a *± 17.1*	89.9 a *± 125.3*	227.0 ab *± 212.4*	24.55 a *± 15.5*	**0.011**	0.591	**0.045**

Shown are the mean values ± standard deviation. For statistics see Tab. 5.

Table 9: Micronutrient contents of Wild Jasmine shoots in µg mg per plant

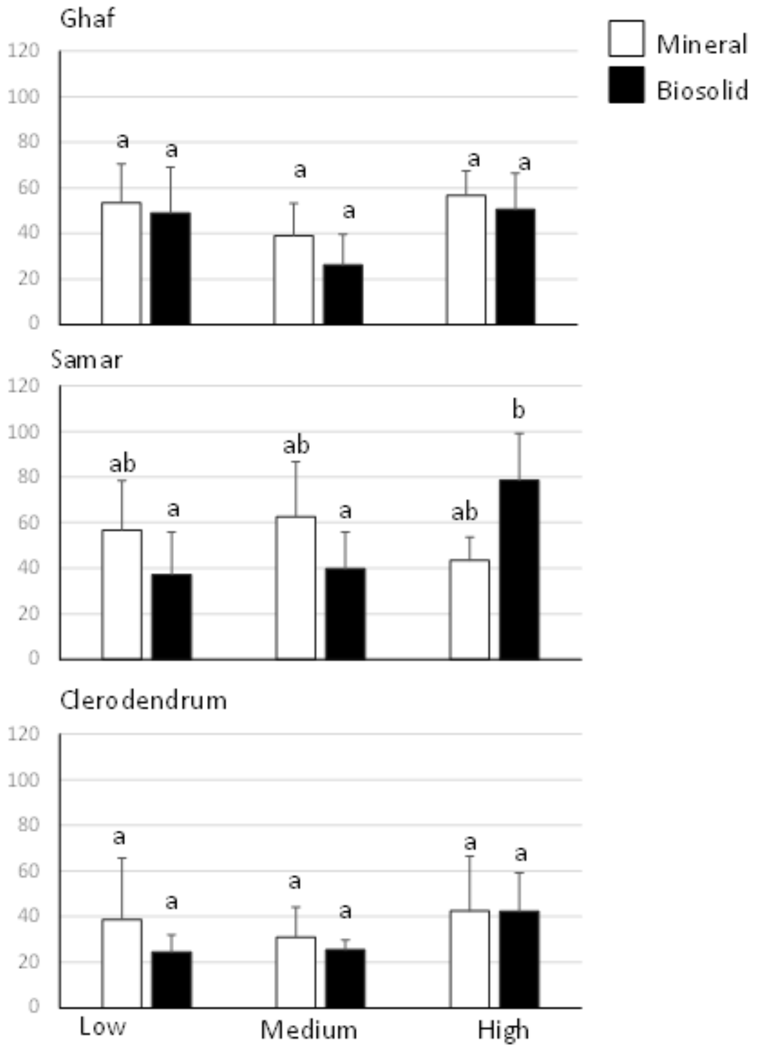
F Amount	Low		Medium		High		Two Way ANOVA		
F Type	Mineral	Biosolid	Mineral	Biosolid	Mineral	Biosolid	F Amount	F Type	Amount x Type
Fe (mg / plant)	3.17 a *± 1.02*	2.73 a *± 1.09*	3.62 a *± 2.57*	2.68 a *± 1.22*	5.92 a *± 3.48*	4.64 a *± 2.78*	0.054	0.291	0.915
Zn (mg / plant)	2.19 *± 1.48*	1.77 *± 0.54*	1.38 *± 0.45*	1.49 *± 0.36*	1.50 *± 0.48*	2.13 *± 0.57*	0.265	0.699	0.311
Cu (µg / plant)	471.4 *± 215.8a*	355.3 *± 54.5a*	256.7 *± 91.8a*	349.9 *± 121.4a*	410.9 *± 98.7a*	444.8 *± 124.2a*	0.078	0.939	0.190
Co (µg / plant)	29.9 a *± 27.9*	22.6 a *± 10.9*	26.4 a *± 17.6*	13.3 a *± 1.9*	39.7 a *± 21.8*	25.9 a *± 8.9*	0.260	0.080	0.898
Mn (µg / plant)	843.5 a *± 438.4*	745.3 a *± 352.3*	620.1 a *± 214.*	562.3 a *± 126.5*	774.2 a *± 331.3*	699.2 a *± 193.5*	0.304	0.483	0.988

Shown are the mean values ± standard deviation. For statistics see Tab. 5.

4.7 Concentrations of Potentially Harmful Elements in Shoots

The Al concentrations in shoots were higher for Ghāf and Samār trees compared with wild jasmine shrubs, as shown in Figure 18. Samr trees grown in soil amended with high amounts of biosolids had higher Al shoot concentrations compared with treatments that had received medium or low amounts of biosolids. Ghāf trees and wild jasmine shrubs did not show differences in shoot Al concentrations depending on the fertilization treatment. The shoot Cd and Ni concentrations were in a similar range across all fertilization treatments and experimental plants, as shown Figure 19 and Figure 20, respectively.

The shoot Ni concentrations were in a range of 2 – 3 µg per g DW, while Cd and Cr concentrations were in the range of 1 – 2 µg per g DW across all woody species involved in the experiment. In Ghāf trees, shoot concentrations of Cr tended to be higher for biosolids compared with mineral fertilized plants of the medium and high supply level, as shown in Figure 21. There were no differences in shoot Cr concentrations among the mineral fertilized plants.

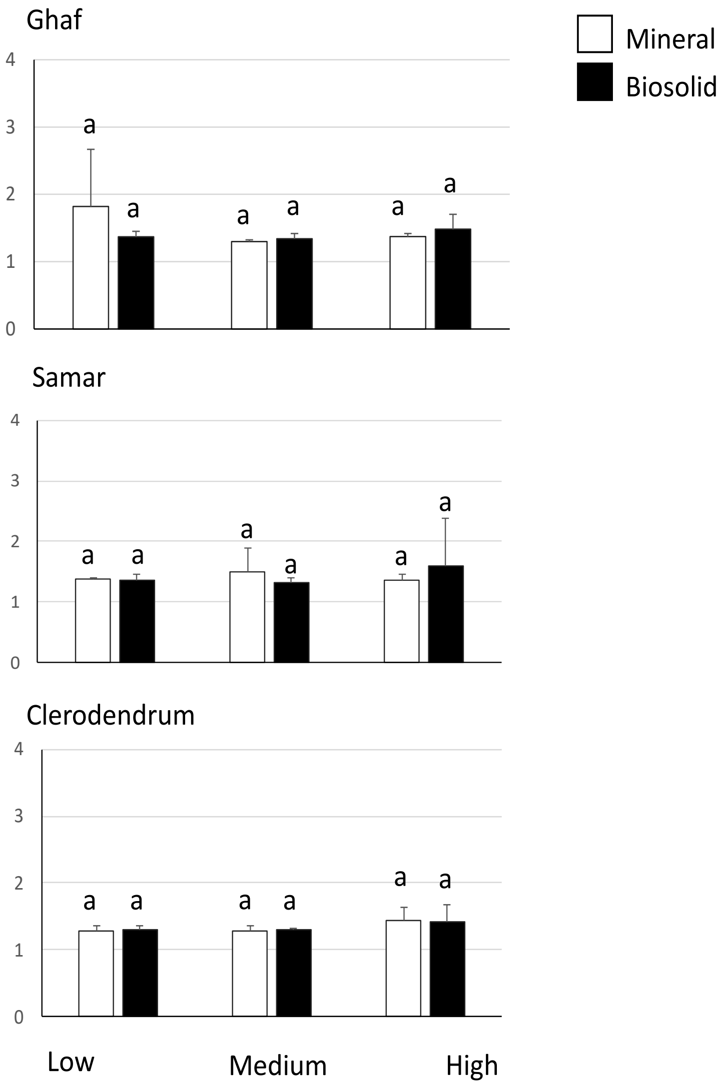


Results of the Two Way ANOVA

	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	0.228	1.551	0.745	0.109	0.305	1.098
F Amount	**0.031**	4.178	0.290	1.308	0.186	1.804
Interaction	0.150	0.862	**0.006**	6.469	0.668	0.411

Figure 18: Aluminium concentrations in shoots of Ghāf, Samr trees and Wild Jasmine shrubs

Note: Mean values ± standard deviation (also see Figure 4)

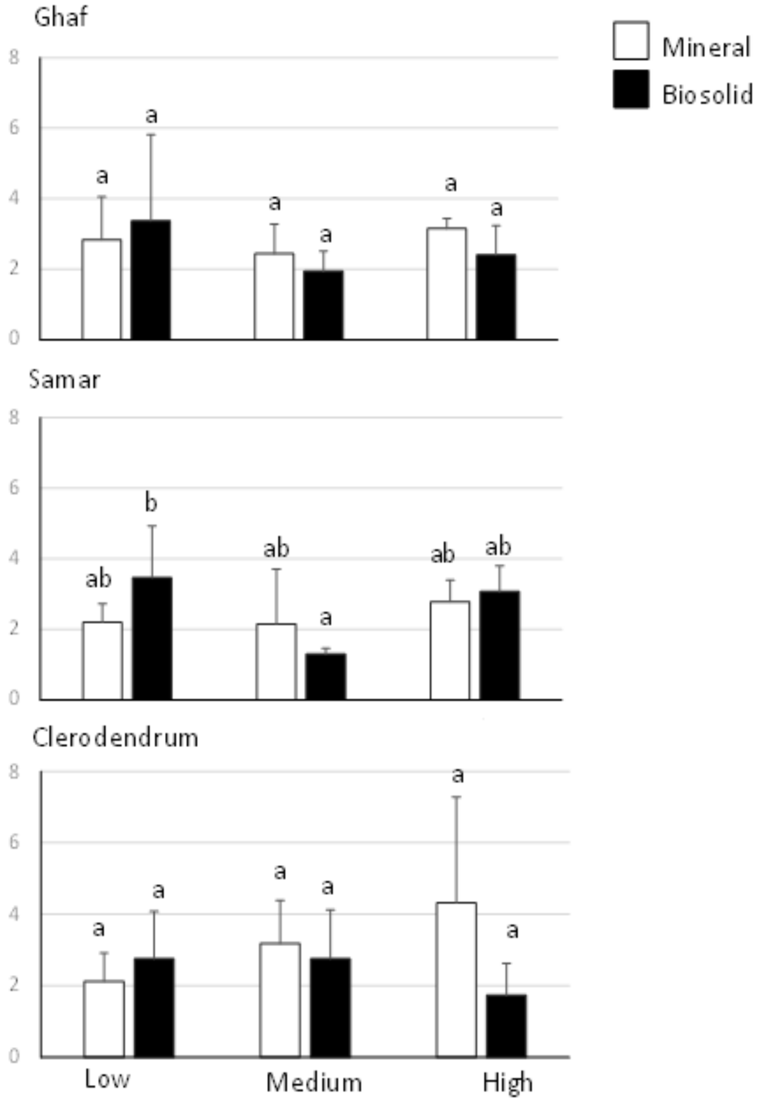


Results of the Two Way ANOVA

	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	0.497	0.479	0.963	0.002	0.642	0.222
F Amount	0.340	1.141	0.789	0.239	0.074	2.906
Interaction	0.244	1.519	0.454	0.818	0.830	0.188

Figure 19: Cadmium concentrations in shoots of Ghāf, Samār trees and Wild Jasmine shrubs

Note: Mean values ± standard deviation (also see Figure 4)

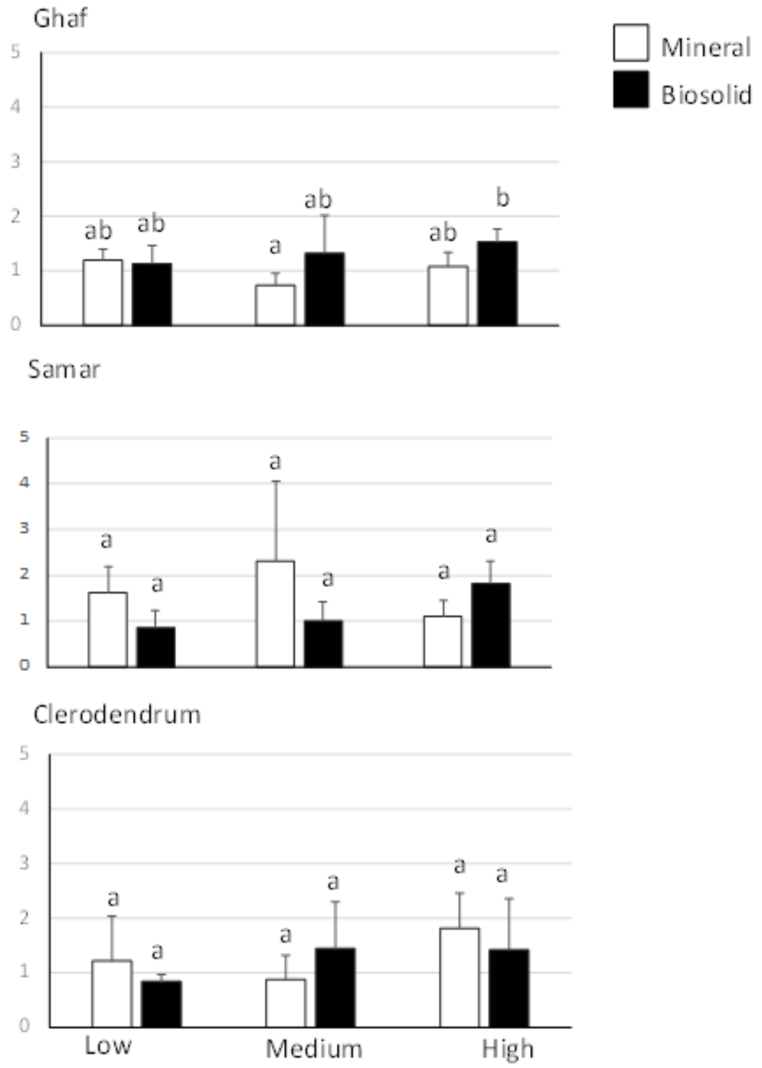


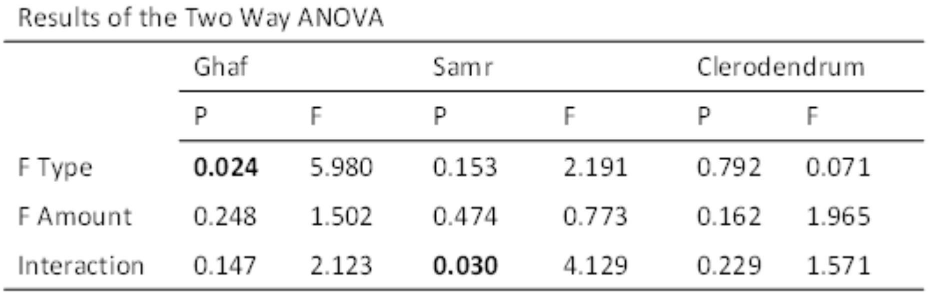
Results of the Two Way ANOVA

	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	0.643	0.223	0.523	0.421	0.188	1.835
F Amount	0.368	1.054	**0.025**	4.376	0.668	0.410
Interaction	0.529	0.659	0.078	2.865	0.093	2.626

Figure 20: Nickel concentrations in shoots of Ghāf, Samār trees and Wild Jasmine shrubs

Note: Mean values ± standard deviation (also see Figure 4)





Results of the Two Way ANOVA

	Ghaf		Samr		Clerodendrum	
	P	F	P	F	P	F
F Type	**0.024**	5.980	0.153	2.191	0.792	0.071
F Amount	0.248	1.502	0.474	0.773	0.162	1.965
Interaction	0.147	2.123	**0.030**	4.129	0.229	1.571

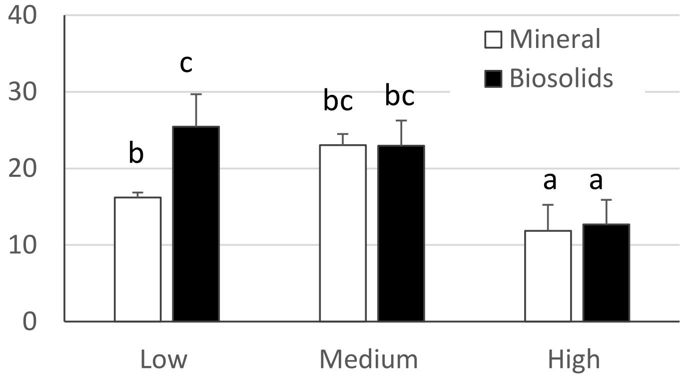
Figure 21: Chromium concentrations in shoots of Ghāf, Samār trees and Wild Jasmine shrubs

Note: Mean values ± standard deviation (also see Figure 4)

4.8 The Extent of Arbuscular Mycorrhiza Fungal Root Colonization in Ghāf and Wild Jasmine Plants

Amounts of roots in some of the Samr plants were too small to estimate the extent of AM fungal root colonization. For this reason, only the Ghāf and wild jasmine roots were stained and observed under the microscope. The wild jasmine roots did not show any sign of AMF colonization, and no extraradical fungal mycelium was observed around them. The Ghāf roots remained relatively dark despite extended KOH treatment and cortical tissues were not translucent in all places.

AMF hyphae, however, attached to the roots and some intraradical structures could be observed and were counted. It is possible that the extent of AMF colonization was slightly underestimated in roots of Ghāf plants due to this. Ghāf roots of the high and medium supply treatment did not differ in the extent AM fungal root colonization depending on whether fertilizer had been supplied in mineral form or biosolids, as shown in Figure 22. In the low supply treatment, however, AMF root colonization was higher for the biosolids treatment. Ghāf root colonization by AMF was generally lower for the high compared the medium supply treatment, while there was no difference among roots of the medium and low treatment.



Results of the Two Way ANOVA

	P	F
F Type	**0.031**	5.829
F Amount	**<0.001**	23.132
Interaction	**0.034**	4.421

Figure 22: The AMF colonized root length in percent of total root length

Note: Mean values ± standard deviation. Mean values followed by the same letter are not significantly different (One Way ANOVA, Tukey's Multiple Comparison). The table below the figure shows the results of the Two-Way ANOVA. P-values indicative of a significant effect of the fertilizer (F) type, the F amount, or an interaction between the two factors are printed in bold.

Chapter 5: Discussion

The primary objective of this study was to assess the ability of desert plants to acquire nutritional elements supplied in form of biosolids or mineral fertilizer and utilize these for growth. The extent of arbuscular mycorrhiza fungal root colonization was assessed in the desert tree species in response to the different fertilization treatments.

5.1 Quality of Biosolids Used in the Experiments

Biosolids can be utilized as soil amendments for agricultural crops and ornamental plants as they are rich in essential mineral nutrients. They should, however, only be used if toxicity from heavy metal accumulation in plant tissues can be avoided, and environmental risks remain low (Dad et al., 2019). The N and P concentrations in biosolids used in the present experiment were in an adequate range for organic fertilizers, and comparable to those found in animal manures (Szogi et al., 2015).

The basic cation concentrations in biosolids were within the range reported in studies of (Giusquiani et al., 1988; Sullivan, 2015). who reported concentrations of 3 – 8% N, 1.5 - 3.5% P, 0.1 - 0.6% K, 1 – 4% Ca and 0.4 - 0.8% Mg in biosolids. However, the K concentrations in the biosolids used in the present study were comparatively low, possibly due to the high solubility of K and related losses in the wet sewage treatment process. The optimum level of biosolids application have greatly dependent on the nature of biosolids, plant demands and pedo-climatic qualities to meet the plant requirements and reduce the environmental impact.

The Recycled Water and Biosolids Regulation of the UAE (2010) states threshold values for heavy metal concentrations in biosolids eligible for restricted or unrestricted application. The concentrations of Cu, Zn, Cd and Ni exceeded the stated permissible concentrations for unrestricted, but not for restricted use. Threshold values of other heavy metals were in an acceptable range, also when compared with European standards (Collivignarelli et al., 2019).

Amounts of biosolids applied to the plants of the present experiment were between 3.2 and 12.8 g per kg dry soil, corresponding to approximately 1.5 to 4.5 kg of biosolids per m^2 and 28.2 to 112.6 g of biosolids per plant. Despite relatively high concentrations of Cu, Mn, and Zn in the biosolids, it was expected that the amounts of micronutrients supplied to the soil in form of the biosolids were not sufficient to cover the plant demand, given that the pH of the soil was in an alkaline range and metal availability expected to be rather low. For this reason, additional micronutrients were supplied in chelated form.

5.2 Status of Plant Nutrients

According to Ericsson (1995), tree species differ only little in tissue element concentrations required for optimal growth. The author reported macronutrient concentrations in woody parts of various Eucalyptus and Poplar species to be in the range of 1.7 - 2.3 mg N, 0.3 – 0.9 mg P, 1.2 – 3.0 mg K, 0.4 – 0.6 mg Mg, and 4 – 8 mg Ca per per g DW. Foliar concentrations in the same plants were between 19 – 20 mg N, 0.9 – 1.5 mg P, 4 – 10 mg K, 1.3 – 3.9 mg of Mg and 5 – 9 mg Ca per g DW. Drumond (1988) reported macronutrient concentrations in leaves of young trees of various Prosopis species grown in semi-arid regions of Brazil to be in the range of 31 – 41 mg N, 1.5 – 2.4 mg P, 10.6 – 13.5 mg K, and 8.2 – 18.6 mg Ca per g DW. Rubanza et al. (2007) analysed various Acacia species for their leaf element concentrations and found that these were in the range of 23 – 37 mg N, 3.5 - 4.9 mg P, 14.6-31.5 mg Ca and 1.4-3.0 mg of Mg per g DW.

When compared with these findings, N concentrations in the shoot of the plants of the present experiment were in a rather low but most likely still sufficient range. The shoot K, Ca and Mg concentrations were in a likely optimal range across all treatments, while P levels in shoots of Ghāf trees and wild jasmine shrubs might have been rather low. Reasons why the trees of the present experiment did not benefit much from additional nutrient supply in terms of tissue element concentrations, total element uptake or growth remain speculative.

The wild jasmine shrubs showed higher shoot N and P concentrations with increasing fertilization level, but there was no difference in total uptake of these elements among the fertilization treatments. It cannot be excluded that shrubs of the lowest fertilizer supply level were already saturated with nutritional elements and largely unable to utilize higher amounts of fertilizer. Trees native to desert ecosystems are adapted to survive under a very limited supply with P and N (He et al., 2014). The response of forest trees native to N limited ecosystems to additional nutrient input can be very variable.

Previous studies have reported that beyond a state of nutrient saturation, where the availability of nutritional elements exceeds the demand of the trees, forest ecosystems decline in response to additional nitrogen intake (Aber et al., 1998). The reasons for this are not yet completely understood, but it has been suggested that element leaching, nutritional imbalances or susceptibility to diseases play a role (Emmett, 2007). Wallace et al. (2007) observed a ‘mosaic response’ of an oak forest to long-term additional experimental N input. While some trees were able to translate additional N into growth, others did not survive under such conditions.

The Samr trees of the present study also showed a considerable heterogeneity in growth and element uptake across all experimental treatments. Whether this was partially related to the relatively high nutrient input levels, however, remains speculative. Plants native to P impoverished Australian desert ecosystems have shown negative growth responses to increasing supply with P, mainly due to the inability to downregulate their P uptake systems and resulting P toxicity (Lambers et al., 2013). The P concentrations in the plants of the present study were not in a toxic range, but it is likely that they exceeded plant demand at higher input levels, and that the Ghāf and Samār trees were unable to utilize additional P for growth.

With the exception of Mg, the availability of macronutrients to plants of the present experiment did not seem to differ depending on whether mineral fertilizers or biosolids were used as a source of nutritional elements. This suggests that P, N and Ca supplied in form of biosolids were equally well available as those provided in mineral form. K concentrations were originally incredibly low in biosolids, so that the biosolids treatments had received additional K in form of a K_2SO_4 solution. Conclusions on plant availability of K from biosolids can thus not be made.

The total uptake of Mg by Ghāf and wild jasmine plants was lower when biosolids rather than mineral fertilizer had been applied. In sewage treatment plants $MgCO_3$ is sometimes used as a flocculating agent to remove colloids from water (Bratby, 2016). Under alkaline soil conditions, the solubility of $MgCO_3$ may be lower compared with that of $MgSO_4$ supplied to the minerally fertilized plants, resulting in a lower availability of Mg to plants. The availability of micronutrients to plants can be low on alkaline soils (Marschner, 2011) and micronutrient deficiency is relatively common in ornamental plants of the UAE. Though the concentrations of Cu and Zn in the biosolids used in the present experiment were relatively high, total amounts supplied to the soil were relatively low, due to the small amounts of biosolids that had been added. For this reason, the soil used in the present experiment was supplied with equal amounts of a micronutrient compound fertilizer base on EDTA chelates across all treatments.

Concentrations of micronutrients in leaves of different *Prosopis cineraria* species have been reported to range between 26.7 – 87.2 µg Cu, 59.3 – 251.9 µg Mn, 50.0 – 1653.5 µg Zn, 105.7 – 233.5 µg Fe and 0.05 – 0.65 µg Co per g DW (Drumond, 1988). Values provided by Rubanza et al. (2007) for Acacia species were in a similar range, from 4.5 – 23.8 µg Cu, 41.0 – 90.0 µg Mn, 10.9 – 22.2 µg Zn, and 146.2 – 432.0 µg Fe per g DW. Bergmann (1999) stated optimal values for micronutrient concentrations in leaves of some ornamental and fruit trees were in the range of 10 – 20 µg Cu, 30 – 100 µg Mn, 25 – 60 µg Zn, and 80 – 200 µg Fe per g DW. When compared with these values, Cu concentrations in the shoots of Ghāf and Samār trees were in a sufficient range, while those in wild jasmine shoots were in a rather low range.

The Zn, Fe and Co concentrations were in a sufficient range across all woody species and treatments, while Mn concentrations were generally in a range indicating deficiency. In Ghāf trees, trees supplied with biosolids had lower Mn shoot concentrations and contents compared with corresponding minerally fertilized treatments. The uptake of other micronutrients by the woody species involved in the experiment remained unaffected by the type of fertilizer applied. It is possible that Mn supplied to the soil remained poorly available to trees due to the alkaline nature of the experimental soil. In addition, Mn might have been immobilized by Al and Fe compounds that were present in the biosolids and might have remained from colloidal precipitation.

5.3 Potentially Harmful Elements in Plant Shoots

In general, domestic biosolids have lower heavy metal contents than industrial ones. Origin and treatment method of biosolids may markedly affect their characteristics. Application guidelines have been developed across all parts of the world to avoid the accumulation of toxic elements in agricultural soils, and/or their migration to groundwater bodies or adjacent ecosystems. However, there is still considerable debate about permissible limits and potential environmental impacts (Silveira et al., 2003).

Biosolids can contain considerable amounts of Al, mostly in precipitated and plant unavailable form. Despite high Al concentrations in biosolids of the present experiment, plants that grew in soil amended with biosolids did not show higher Al concentrations or contents in their shoots compared with plants that had grown in minerally fertilized soil. The Al concentrations in shoot tissues of the trees of the present study were in the range of 30 to 80 µg per g DW, which is below average concentrations of 830 µg per g DW reported for more than 500 terrestrial plants sampled across China (Zhang et al., 2011), and also far below concentrations considered toxic for plants (Brunner & Sperisen, 2013).

Heavy metal concentrations in the biosolids used as fertilizer in the present study were above values recommended for unrestricted use for Cd, Ni, Cu and Zn. However, total amounts of these elements applied to soil were relatively small in the present study, and below levels expected to cause elevated uptake or toxicity in plants. In accordance, there was no difference in shoot concentrations or contents of Ni, Cr, Cd, Zn or Cu, depending on whether the soil was supplied with biosolids or mineral fertilizers. Similar with Al, concentrations of Ni and Cr were in a range commonly observed in trees, and neither in a range toxic for plants (Kugonic & Grcman, 1999), nor in a range of concern with respect to human consumption (World Health Organization. Regional Office for Europe, 2000).

The Cd concentrations in shoots, however, were in an elevated rage across all woody species and irrespective of the fertilization treatment. Cadmium concentrations are usually in the range of 0.001 – 0.05 µg per g in fresh meat and fish, and up to 0.3 µg per g in cereals (World Health Organization. Regional Office for Europe, 2000). The FAO-WHO recommended that Cd concentrations in edible plant material should not exceed 0.3 µg per g dry weight. Reasons for elevated Cd in shoots of trees of the present experiment remain unknown. Elevated levels of Cd in vegetables grown on non-contaminated soils of Italy were reported by Baldantoni et al. (2016), suggesting that Cd acquisition and shoot transfer may strongly depend on the plant species.

5.4 Overall Plant Performance

It has been shown that biosolids are rich in nutritional elements, such as Nitrogen (N) and Phosphorus (P), making the material a suitable fertilizer for agricultural crops (Brisolara & Qi, 2015). In some studies, biosolids have been superior to other organic fertilizers in improving growth and yield of crop plants, possibly due to a relatively high bioavailability of the nutritional elements they contain (Rouch et al., 2011; Singh et al., 2008).

The results of the present study suggest that the availability of macronutrients from within biosolids is equal to that of mineral fertilizer. However, the ability of the woody trees used in the present study to utilize increasing supply levels of nutritional elements was limited, irrespective of the form in which the nutrients were supplied. While the desert trees might have been inherently unable to translate high supply of nutritional elements into growth, the reasons for the absence of growth responses in the wild jasmine shrubs remained unknown.

Some authors reported a decline in growth with increasing amounts of biosolids supplied, due to fact that the biosolids excessively increased the amount of NaCl in the substrate (Lefebvre et al., 2007). Bañuelos et al. (2007) reported that the application of biosolids to apricot orchards increased the soil salinity, along with total concentrations of most nutrients (except K) in the soil. The plants of the present experiment did not show any difference in their Na shoot concentrations or contents depending on the type of fertilizer supplied. This suggests that salt toxicity did not play a role in the present study.

Reasons for the poor growth of Ghāf and Samār trees supplied with high amounts of biofertilizers need to be further elucidated. An excessive availability of nutritional elements may not be the main reason, as corresponding minerally fertilized plants did not show such a response. The results of the present study suggest that Mn deficiency might have played a role in this context. It can also not be excluded that a high microbial activity in the soil substrate receiving high amounts of biosolids negatively affected the growth of the desert trees, which are adapted to soils particularly low in soil organic matter.

Chapter 6: The Conclusion

In conclusion, biosolids can be used to supply Ghāf (*Prosopis cineraria*) and Wild Jasmine (*Clerodendrum inerme*) with nutritional elements at a low overall fertilization regime. Higher levels of nutrient supply were not translated into additional element uptake or growth, suggesting that the ability of the tested plant species for biomass production may be rather low. Additional supply with micronutrients is recommended when biosolids are used a sole fertilizer for landscaping plants growing on alkaline soils of the UAE.

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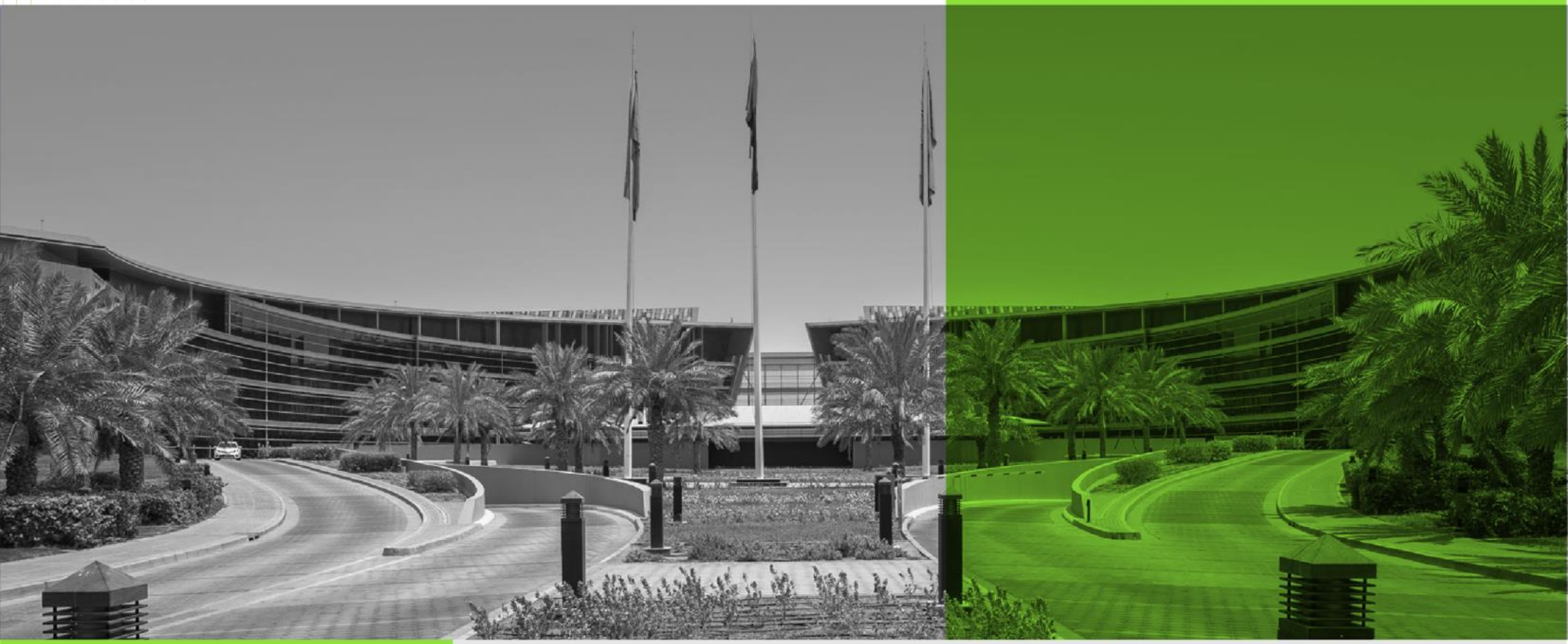

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In the present study, the ability of Ghāf and Samr trees to utilize nutrients provided in sewage sludge as biofertilizers or mineral fertilizer salts was compared. Wild jasmine shrubs (Clerodendrum inerme) were included as a third species in this experiment because they are an exotic and faster-growing woody plant. The potential to utilize sewage sludge could be increased by planting exotic species which have higher growth and element uptake potential compared to indigenous trees.

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