Evaluation of macroalgal biomass and extracts for soil improvement and plant growth stimulation of *Sorghum bicolor*

By

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ABSTRACT

Marine macroalgae are plant-like organisms regarded as a valuable natural bioresource in a variety of agricultural applications. Applications in agriculture improved the physical and chemical qualities of soil. The agricultural value of marine macroalgae is further enhanced by their ability to produce a diverse range of biologically active biocidal compounds effective in boosting protection against plant-infecting diseases.

The literature review, which is a component of the presented research, covers characteristics of prospective macroalgal agricultural applications. Commercial production and utilization of certain marine macroalgal chemicals with interesting biotechnological value, such as biofertilizers, bio stimulators, and soil conditioners, are emphasized and addressed in detail.

In the research component of this project, *Undaria pinnatifida*, a brown alga was used as a bio-fertilizer to investigate its effect on the growth of *Sorghum bicolor*. A greenhouse growth trial with *Sorghum bicolor* seeds was carried out with different biofertilizer treatments, such as Seasol fertiliser treatment, Algardis (derived from post-fucoidan extracted biomass of the brown seaweed *Fucus vesiculosus*), *Undaria pinnatifida* biomass, microwave-assisted extracts of *Undaria pinnatifida* biomass at 40, 60, and 80^{o}C, compost (positive control), and unfertilized nutrient-poor topsoil (negative control). N, P, K, and C contents of pre-seeded (except for the Seasol treatment) and end of growth trial soils were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) and elemental analysis, respectively.

A total of 24 pots (25 cm diameter) were set up in a randomized design in a greenhouse in triplicate * eight treatment combinations with daily rotation. The light intensity was between 500-600 µmol m^{-2} s^{-1} set to a photo period of 16:8-hour light/dark cycle at a set temperature of 28^{o}C during the day and 18^{o}C at night. To only account for the contribution of C to plant growth, soils, except for 3 pots of topsoil that served as a negative control, were fertilised with the respective fertiliser with N, P, and K levels brought to the same levels as found in compost soil. Pots were only fertilised once prior to seeding except for compost soil (positive control) and unfertilised topsoil (negative control).

Highest growth of *Sorghum bicolor* was achieved in compost soil, but water holding capacity (WHC) was highest in topsoil fertilised with *Undaria pinnatifida* biomass, 40, 60, and 80^{o}C extracts, and Seasol treatment. In comparison between unfertilised topsoil and compost soil the

unfertilised topsoil (negative control) had the highest C (41.5 g/kg) content on day 0, and compost soil (positive control) had the highest N (1.3 g/kg), P (1.32 g/kg), and K (3.14 g/kg) content on day 0. The C content was much higher in *U. pinnatifida* biomass in compare to all other treatments at before seeding day-0. After 56 days (post-harvest), an increased soil elemental nutritive value was noticed. The concentrations of N (4.4 g/kg), P (1.455 g/kg), K (3.377 g/kg), and C (56 g/kg) of compost soil were increased, while N (1.6 g/kg), C (24.86 g/kg), and P (0.37 g/kg) contents in unfertilised topsoil (negative control) decreased, as did the N (1.566 g/kg), C (25.33 g/kg), P (0.57 g/kg), and K (3.22 g/kg) contents of the Algardis treatments. Post-harvest K (2.883 g/kg) and P (0.779 g/kg) contents were higher compared to starting conditions for treatments fertilised with *Undaria* biomass and K (3.345 g/kg), C (25.733 g/kg), and N (1.633 g/kg) contents for *Undaria* biomass MAE 40, 60, and 80˚C, N (1.266 g/kg), and C (22.9 g/kg) concentrations were lower for the latter treatments. Postharvest C (1.633 g/kg) and N (25.733 g/kg) concentrations were higher for topsoil fertilised with MAE 40˚C extracts compared to MAE obtained at 60 and 80˚C.

The plants were grown in compost soil (positive control) Algardis, Seasol, *Undaria* biomass, and *Undaria* MAE 40, 60, and 80℃ treatments. Seeds of *Sorghum bicolor* sown in the unfertilised topsoil (negative control) did not germinate. Best growth was observed for seeds germinated in compost soil, followed by Algardis, Seasol, and *Undaria* biomass treatments, while *Undaria* MAE 40, 60, and 80℃ treatments did not support growth to the same extent.

Plants grown in compost soil had higher above and below ground biomass, followed by Algardis, Seasol, and *Undaria* biomass treatments. There was no increase in the development of above and below-ground biomass between the fertilisation regimes using the MAE extracts.

In conclusion, fertilisation with Seasol with compost soil shows the best result, and the treatment with *Undaria pinnatifida* biomass and the Algardis liquid extract of the brown macroalga *Fucus vesicolosus* (Marinova) treatments supported growth to the same extent were the best alternative options to Seasol and can be used for the cultivation of *Sorghum bicolor*. Whilst in theory, *Undaria pinnatifida* biomass and Algardis can be cost-effective, eco-friendly, and easily available, techno-economic and life cycle analyses are required to demonstrate cost-effectivity and environmental sustainability.

Keywords; Marine macroalgae; bio-stimulants; *Undaria pinnatifida*, sustainable agriculture, Algardis, *Sorghum bicolor*, *Fucus vesiculosus*, Water holding capacity (WHC).

DECLARATION

I certify that the thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Takarir Faatema Sheliya

2022

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LIST OF ABBREVIATIONS

C	Degree Celsius
Cm	Centimeter
Kg	Kilogram
G	Gram
ICP-OES	Inductive coupled plasma optical emission spectroscopy
C, N, P, K	Carbon, Nitrogen, Phosphorus, Potassium
L	Liter
MAE	Microwave-assisted extract
Nm	Nanometer
mL	Milliliter
MQ water	Milli-Q water
Mg	Milgram
HNO_3	Nitric acid
Ppb	parts per billion
ppm	parts per million
%	Percentage
W	Watt
dS/m	Deci Siemens per meter
meq/100 g	milliequivalent per 100 g
cmolc/kg	centimole positive charge per kg

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Chapter 1

INTRODUCTION

1.1. REVIEW OF LITERATURE

Australia has some of the oldest land surfaces on the planet, its soils and seas are among the most nutrient-poor and unproductive in the world. Land clearing, water extraction, and low soil conservation are all contributing factors to a decrease in soil quality in Australia due to intense weathering. Although there are still areas of highly fertile Australian soils, acidification, salinization, erosion, nutrient imbalance, structural deterioration, loss of organic matter (and thus carbon), and other types of degradation affect a large proportion of cropland. Such degraded Australian soils are characterized by low carbon, nitrogen and soil moisture contents, and water holding - and cation exchange capacity (Mishra & Dash 2014).

Agriculture is our country's backbone. Farmers apply fertiliser to the soil to increase crop yield and plant development. Fertilizers might be chemical or biological. When compared to bio-fertilisers, synthetic fertilisers are now utilized more frequently in agriculture. Prolonged use reduces soil fertility, causes soil erosion because fertilisers kill vital soil microbes and plant remains into nutrient-rich organic materials. Synthetic fertilisers based on nitrogen and phosphate drain into groundwater, posing a significant water contamination risk. Aquatic ecosystems are harmed when fertilisers seep into streams, rivers, lakes, and other bodies of water (Mishra & Dash 2014).

Although Australian native plants are adapted to these poor soil conditions, introduced crops and pasture grasses are not, hence nitrogen fertilisers must be applied to the soil to achieve efficient yields according to (Erulan et al. 2009).

As commodity prices increase, farmers are encouraged to plant more profitable crops. As a result, the market prognosis for the agricultural economy had greatly improved, and markets for corn, soybeans, and wheat in 2021 were predicted to expand rather than shrink or remain static when compared to 2020 (Chnitkey et al. 2021).

Not fossil fuel derived as compared to synthetic nitrogen fertiliser, lessening the impact on atmospheric carbon dioxide levels and associated climate change predictions. If it is produced here or not import required, no shortages to be expected when supplies are produced at scale. Prices will not sky-rocket due to shortages in import.

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Figure 1.1. Increase in chemical fertiliser price (Chnitkey et al. 2021).

Sorghum (*Sorghum bicolor*) is used in this study as a surrogate for wheat because it is a low-cost alternative to maize due to its tolerance of dry growth conditions, requiring less water (450 – 650 mm) than maize throughout the entire growing season to generate comparable yields. Sorghum has an exceptional ability to take water from deeper soil layers. Sorghum is naturally heat tolerant and as carbon dioxide (CO_2) levels rise due to climate change, this crop is more sustainable than other C4 (a specific form of photosynthesis) species. Climate change has been shown to reduce yields of commodity crops such as wheat, cocoa, and corn around the world. Other research has indicated that when cultivated at higher levels of CO_2, barley, wheat, potatoes, and rice have 6 to 15% lower protein concentrations; however, the protein content of sorghum did not decrease considerably (Hao et al. 2021).

Macroalgae stimulate strong root growth and are beneficial for the growth of soil micro-organisms, promote vigorous flowering, fruiting, general plant health, and assist to improve plant environmental stress tolerance against heat, drought, and frost (Sathya et al.2010). Macroalgal soil treatment has also been shown to improve seed germination, reduce transplant shock, and improved root development increasing nutrient uptake. Macroalgal fertilisers improve carbon, nitrogen, and moisture contents of soils, water holding and cation exchange capacity (Erulan et al. 2009).

The study presented in this thesis aimed to investigate the usefulness of fertilisation with macroalgae and their microwave-assisted extracts (MAEs) to improve growth of *Sorghum*

bicolor compared to an Industry standard control (commercial algal extract containing garden fertiliser) It is hypothesised that fertilisation with macroalgae and/or their MAEs will improve root development, promoting healthy growth through improved provision and utilization of soil nutrients (nitrogen (N), phosphorous (P), potassium (K), and carbon (C) and soil water holding capacity.

1.2. Chemical fertiliser versus biofertiliser

In agriculture, nutrient depletion of soils is a common problem, hence large amounts of NPK chemical fertiliser are applied to maintain optimal nutrient levels. Chemical fertilisers tend to release nutrients fast, resulting in increased top growth which is not met by equal root development (Feigin & Halevy 1989). This type of growth often results in weaker, disease-prone plants with lower yields. When chemical fertilisers are used over an extended period, the soil may become chemically "over-loaded," ultimately poisoning the soil profile to the point that plants cannot develop (Khan et al. 2009).

Even fertiliser applications in ideal conditions only yield 50% of the usage of the applied nitrogen, 2-20% volatilises, 15-25% reacts with organic compounds in the soil and the remaining 2-10% pollute surface and groundwater (Feigin & Halevy 1989).

Fertiliser run-off can cause harm by leaching into subsurface aquifers and larger catchments, ultimately flowing into rivers, lakes, and the ocean. Environmental damage can be serious, and one example is damage to the Great Barrier Reef caused by nutrient run-off from coastal agriculture (Khan et al. 2009).

Though chemical fertilisers boost crop yields, their overuse has hardened the soil, reduced fertility, possibility of more insecticides, polluted air and water, and emitted glasshouse gases like CO_2, CH_4 and N_2O, and these emissions are responsible for severe global climate change and air pollution, posing risks to human health and the environment (Sathya et al. 2020). The continued use of these chemical fertilisers depletes vital soil nutrients and minerals, characteristic of fertile soils. Because nitrogen lowers the pH of the soil, artificial fertilisers can cause the topsoil to become acidic. The optimal pH range for best plant growth and agricultural productivity is 5.5 to 8. Crop output will be reduced if the soil is excessively acidic (pH less than 5.5) (Sharma & Singhvi 2017).

Food crops grown with chemical fertilisers, at the very least, may be less nutritious than they should be. This is due to the fact that chemical fertilisers trade quick growth for plant health,

resulting in produce with lower nutritional value. Plants will grow on little more than NPK, but they will be deficient in key elements like calcium, zinc, and iron. This may have a minor but cumulative impact on the health of those who consume them (Sharma & Singhvi 2017).

In the worst-case scenario, chemical fertilisers may raise the risk of acquiring cancer in adults and children while also negatively impacting foetal brain development. Scientists are aware of this. According to a 1994 study conducted by the University of Wisconsin, average quantities of nitrate (a common fertiliser) and a pesticide in groundwater may endanger the neurological, endocrine, and immunological systems of young children and foetuses of pregnant women. A 1973 study linked high levels of sodium nitrate in groundwater to the prevalence of stomach cancer, and a 1996 study linked it to the prevalence of testicular cancer (Sharma & Singhvi 2017).

Seaweeds, in addition to stimulating plant growth, influence the physical, chemical, and biological properties of soil, which in turn influences plant growth. Seaweeds and seaweed extracts improve soil quality by improving moisture-holding capacity and by encouraging the development of beneficial soil microbes (Khan et al. 2009).

The application of seaweeds and seaweed extracts stimulates the development of beneficial soil microbes and rhizosphere microbes through the secretion of soil conditioning substances. Alginates influence soil properties and promote the growth of beneficial fungi. Alginate oligosaccharides, produced by enzymatic degradation of alginic acid a main cell wall component of brown macroalgae which is responsible for plant growth (Khan et al. 2009).

The addition of macroalgal extracts is thought to improve soil carbon and nitrogen contents of structurally poor and degraded soils and has been shown to improve soil moisture content, and water holding and cation exchange capacity due to improved soil structure (Sathya et al. 2010).

1.3. Macroalgae and their application as a plant growth stimulator

Biofertilisers serve an important role in preserving soil fertility and are critical components required for organic farming. Seaweed extracts are natural organic fertilisers and bio-stimulants, promoting faster seed germination and a balanced nutrient supply to growing seedlings and the plants (Khan et al. 2009). Seaweed extracts can be utilized as fertilisers in sustainable agriculture to help solve soil fertility and nutrient problems and boost the efficiency of plant production (Selvam & Sivakumar 2014).

The presence of plant hormones in seaweed extracts infers bio-stimulant activity. Auxis, cytokines, gibberellins, abscisic acid, and ethylene are among the phytohormones found in seaweed extracts. In comparison to other marine algae, Phaeophyceae (brown algae) outperform Chlorophyceae (green algae) and Rhodophyceae (red algae) (Sathya et al. (2010). In addition to containing phytohormones, seaweeds contain essential macro- and micronutrients that stimulate faster seed germination and improve yields (Fig. 1.2). In coastal regions, brown seaweeds are the second most prevalent algae. Seaweed Liquid Fertiliser are generally extracts obtained from seaweeds (Hurtado et al. 2009).

Like the seaweeds themselves, seaweed extracts contain growth regulators, carbohydrates, plant growth hormones like auxins and gibberellins, and vitamins, aiding in maintaining soil fertility (Selvam & Sivakumar 2014). Application of seaweeds or seaweed extracts is both financially effective and environmentally friendly for long-term agriculture (Hong, Hien & Son 2007). Seaweed extract fertilisers are biodegradable, non-polluting, non-toxic, and non-hazardous to humans, animals, and birds (Hong, Hien & Son 2007). Seaweed extracts are widely used as a soil addition for insect control and plant disease management (Selvam & Sivakumar 2014).

Current research primarily focuses on the application of seaweed extracts as foliar sprays and for seed and soil treatments (Selvam & Sivakumar 2014).

Treatment with seaweed extracts improved plant nutrient uptake and resulted in improved resistant to environmental stress. The most promising and beneficial properties of seaweed-derived fertilisers in agriculture are increased growth rate, nutrient uptake, shoot and root development, and plant resistance to climate stress and pests (Fig. 1.2.). Maxicorp (Seaborn), Sea spray, Goemar GA 14, Algifert (Marinure), Seasol, Sea crop 16, Cylex, and SM3 are commercially marketed seaweed fertilisers (Hao et al. 202; Quitain et al. 2013).

Any of the following ways can be used to apply Seaweed Liquid Fertiliser. Seed treatment (dipping seeds in seaweed liquid manure before cultivation), soil treatment (treating soil with seaweed liquid manure), or foliar spray application are all options for using macroalgal extracts (spraying seaweed liquid manure to crops) (Sasikala et al. 2016).

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Figure 1.2 Schematic representation of physiological effects of seaweed extracts (Sasikala et al. 2016).

1.3.1. Undaria pinnatifida

Diverse types of marine algae contain useful organic chemicals. *Undaria pinnatifida* (Fig. 1.3), a brown macroalga known as "Wakame" in Japan, is one of these. *Undaria pinnatifida* is high in nutrients and minerals like calcium, iron, iodine, as well as protein, vitamins, and beta-carotene. *Undaria pinnatifida* is endemic to the northern hemisphere, including Japan, Korea, and China, where it is largely farmed for human use. It can be found in miso soup and is occasionally used as a seasoning in tofu salads or as a salted snack. Japan used to be the leading producer of seaweed, but now China is the leading producer, with French farmers joining the ranks as demand grows throughout the world (Quitain et al. 2013).

Undaria pinnatifida has been accidentally introduced into the French Mediterranean, Argentina, Italy, Australia, the European Atlantic, the United States of America, and New Zealand over the last few decades (Hurtado et al. 2009).

Harvest and economic use were forbidden, as it was classified as an unwanted organism. Ministry of Agriculture and Forestry (MAF) Biosecurity has just permitted the harvest of *U. pinnatifida* from man-made structures such as mussel farms because it is here to stay and has a high commercial value (Quitain et al. 2013).

Undaria pinnatifida contains a high concentration of bioactive components, including the polysaccharide fucoidan and the pigment fucoxanthin. Fucoidan is a sulphated polysaccharide that contributes to the slippery texture of the seaweed. It is found in the cell walls of numerous different varieties of brown seaweed and protects them from harsh environmental conditions

(Quitain et al. 2013). Fucoidan has recently been found to have a variety of bioactivities, including anti-oxidant and antiviral properties, weight-loss effects, and blood-thinning qualities (Quitain et al. 2013).

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Figure 1.3 Structure of Undaria pinnatifida

1.3.2. Algardis liquid extract and Seasol

Algardis liquid extract is organic liquid seaweed extract rich in nutrients produced by the sustainable harvest of seaweed by Marinova Pty. Ltd. (Hobart, Tasmania). It has been developed as a growth promoting biofertiliser and crop protector for commercial growers with broadacre applications and is suitable for application through a wide range of agricultural systems. Algardis liquid extract contains significant quantities of potassium, sulphate, carbohydrates, and essential trace elements (Table 1.1). The Algardis fertiliser used in the presented research was made from *Fucus vesiculosus* biomass residues after fucoidan extraction.

Fucus vesiculosus can be used as a fertiliser to supply microelements to plants. Essential microelements can be biofortified in edible plants using *Fucus* products (Michalak et al. 2021).

The brown algae *Fucus vesiculosus* is a rich source of polysaccharides (fucoidans, laminarin, and fucoxanthin), as well as important compounds like mannitol sugar alcohol and sugar polymers. These compounds have beneficial effects on plants and animals, as well as their oligosaccharide derivatives' electoral activity (Michalak et al. 2021).

Because of their high amount of betaines, an organic osmolytic substance that can potentially play a significant role in successful protection against extreme environmental conditions, methanolic extracts of *Fucus serratus* have been used for large-scale manufacturing of biofertilizers. Furthermore, spraying aqueous extracts from *Fucus spiralis* on tomato plants reduced crown gall infections caused by the bacterial pathogen *Agrobacterium tumefaciens* in a glasshouse experiment. The most researched *Fucus vesiculosus* extracts with antifungal activity are those produced using polar solvent extraction (Michalak et al. 2021).

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Table 1.1. Algardis liquid extract composition

Seasol fertiliser (containing macroalgal extracts and compost, which is a hobby gardener product) which is too expensive in compared to macroalga extract. Seasol plus Nutrients All Purpose including Natives is a pelletised plant and soil treatment boosted with a fast-acting fertiliser to revitalise soil health and promote vigorous growth in all plants. Boosted with a rich source of natural compost and organic matter that enriches the soil, it helps to improve the soil structure and worm and microbial activity. It also helps to improve soil moisture retention and nutrient uptake. It delivers a special blend of nitrogen(N), phosphorous(P) and potassium(K) and trace elements to help promote vigorous growth. Typical Analysis: (W/W): Nitrogen (N)10.7%, Phosphorus (P) 0.6%, Potassium 9.6%, Carbon (14) %, plus trace elements.

1.4. Sorghum as surrogate for wheat

A growing global population, along with climate change causing more frequent natural disasters, puts unprecedented pressure and challenges on the world's food security. The world population is predicted to reach 9.7 billion by 2050, necessitating the production of around 70% more food to meet demand (Hao et al. 2021).

Sorghum (*Sorghum bicolor*), the fifth most important cereal crop in terms of production and planting area, has received a lot of attention in recent years as a possible "star" crop for addressing global food security concerns. To begin with, farmed sorghum is remarkable in that it has a wide range of end uses, including food, feed, forage, fuel, and beverage. Four major varieties of sorghum are grown worldwide, including grain sorghum, sweet sorghum, forage sorghum, and broom sorghum, and the use of different forms of sorghum varies greatly between regions (Fig. 1.4) (Hao et al. 2021).

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Figure 1.4. A diagram exhibiting sorghum's unique property as a multipurpose crop (Hao et al. 2021).

Not only can it be used for forage, food, bioenergy, and brooms, but it can also be utilized for bioremediation of contaminated crops by using new technology where green plants are used in phytoremediation to dewater, remove inorganic contaminants such as heavy metals and

radionuclides, and decompose organic toxins as plants absorb nutrients through their roots. To lower the volume of aqueous waste, evapo- transpiration is used. Phytoremediation, or the use of plants to remove or degrade contaminants from soils and surface waters, has been presented as a low-cost, long-term, effective, and environmentally friendly alternative to traditional remediation procedures (Zhuang et al. 2009).

Sorghum is used in a variety of ways in different parts of the world. In general, it is utilized mostly for food in developing countries and as feed in developed countries (Hao et al. 2021).

Sorghum has better water use efficiency natural heat tolerance, drought resistant because sorghum's DNA makeup contributes to favourable properties such as drought and heat tolerance, water retention, deep, and fibrous root structure of sorghum boosts its ability to mine water. (Fig. 1.5. (Hao et al. 2021).

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Figure 1.5. Sorghum is a sustainable grain (Hao et al. 2021).

Grain sorghum is a staple diet for millions of people in arid and semi-arid regions around the world, mainly in Africa. It is the primary source of animal feed and industrial usage in developed countries such as the United States, Canada, and Australia. Global sorghum production was around 59 million tons in 2018/19, with 64 percent used for food-seed-industrial (FSI) and 36 percent used for feed and residuals consumption (Mace et al. 2021). Furthermore, sorghum is gaining popularity as a good source of physiologically active

chemicals that can help avoid chronic diseases and boost human health. As a result, sorghum is becoming increasingly important in the global food supply and agro business (Hao et al. 2021; Mace et al. 2021)

Sorghum goes through ten formal stages of growth and development, beginning with emergence (Stage 0) and ending with physiological maturity of the grain (Stage 9) (Fig. 1.6). The number of leaves on a sorghum plant during its vegetative stage is frequently used to define the plant's stage. Stage 0: Plant Emergence occurs when the first leaf, known as the coleoptile leaf, breaks through the soil surface. The time it takes for seedlings to emerge can range from three to fourteen days, depending on soil temperature, moisture, planting depth, and seedling vigour (Hao et al. 2021).

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Figure 1.6. The growth stages of sorghum (Hao et al. 2021).

Stage 1: Three-Leaf - The third leaf's collar is visible. Depending on the weather, this stage occurs 10 to 15 days following emergence. Stage 2: Five-Leaf- This stage occurs approximately 20 to 25 days after emergence when the plant is 7 to 9 inches tall. Stage 3: Growing Points Differentiation (GPD). When the plant is 12 to 15 inches tall, this stage usually occurs 30 to 40 days following emergence. Stage 4: Visible Flag Leaf - The flag leaf is the last leaf to emerge before heading. Typically, the plant grows from the flag leaf to the boot stage in 5 to 7 days (Hao et al. 2021).

1.4.1. Sorghum cultivation in Australia

Sorghum is Australia's third-largest grain crop, supplying vital animal feed, but it is also increasingly utilized in cereals and other meals for human use (Thaxton et al. 2020). For thousands of years, sorghum has been an important nutritional source of starch in Africa, but

it is increasingly regarded in Western diets as a low-GI, gluten-free, and nutritious grain. Larger grains are easier to digest for both humans and animals, and they boost processing efficiency. Sorghum is a popular crop among Australian farmers, notably in Queensland and New South Wales, and previous research has assisted breeders in expanding the crop's potential (Thaxton et al. 2020).

Queensland is Australia's leading producer of sorghum with 313,206 tonnes in 2020, accounting for 78.8% of total Australian sorghum production followed by New South Wales, Victoria, Western Australia, and South Australia for the remaining 21.2%. In 2020, Australia's total sorghum production was expected to be 397,485 tonnes. Other than being temperature and water-stress resilient, outperforming maize on low potassium (K) soils, renders Sorghum a viable cultivation alternative in areas where other major grain crops are not suitable (Thaxton et al. 2020).

1.4.2. Growth requirements for Sorghum

Sorghum requires a warm summer growing season of 4–5 months, with planting typically taking place between September and January. Whilst drought-tolerant, the crop responds strongly to rainfall, particularly during the head-forming and grainfill stages. From sowing to harvest, the sorghum growing season lasts between 115 and 140 days, depending on location, planting time, and hybrid. Temperature and moisture, as well as soil fertility and insect and disease damage, all have a strong influence on growth rates (Thaxton et al. 2020).

1.5. Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) is known as the green method of extraction of biologically active compounds from algal biomass, used to produce plant growth stimulants and biofertiliser. This method is an alternative to traditional liquid extraction, which has several limitations such as the use of large volumes of solvent and multiple extraction cycles. MAE can be run at high temperatures, boosting analyte diffusion rates from a solid sample into a solvent. Microwave power, time, algal type, and temperature are the essential parameters in microwave-assisted extraction. The organic and inorganic makeup of the obtained extract will define its future applicability in plant science (fertilisers, bio-stimulants) (Michalak, Tuhy & Chojnacka 2015).

1.6. Biotechnology Significance

Because of their high concentrations of mineral elements, amino acids, vitamins, and plant growth regulators such as auxins, cytokinin, and gibberellic acid, marine macroalgae are regarded as excellent resources for plant development. Brown algal extracts and algae are both commonly used in agriculture. They have been found to boost the productivity of a range of agricultural plants. The use of marine macroalgae in plant biotechnology has produced healthy plants, as well as a considerable increase in the number and weight of fruits. They also provide a non-toxic alternative method of disease management (Hamed et al. 2018).

Trace minerals found in marine macroalgal extracts play crucial roles in plant nutrition and physiology. A bio-stimulant is an organic compound that, when treated in small amounts, improves plant growth and development in ways that typical plant nutrients cannot. Macroalgal extracts have been used as agricultural bio-stimulants (Hamed et al. 2018). Extracts of macroalgae that are given to plants or soils to improve the physiological processes of the crop, making it more efficient. As a result, these substances can improve nutrient availability and water-holding capacity (Hamed et al. 2018).

1.7. Research gaps

A rise in food supply demand by the exponentially growing global population has led to significant growth of agricultural businesses in recent decades. Rising food demand, along with current agricultural practises, necessitates the inclusion of areas with sub-optimal soil quality for cultivation, as well as the replacement of chemical fertilisers (Mazepa et al.2021).

Organic agriculture approaches are also becoming more popular, owing to a growing global awareness of the need to build sustainable production systems. In 2018, the agricultural area certified organic or in the process of being converted to organic reached 71.1 million hectares, up from 35.9 million ha in 2013 (Mazepa et al.2021). The goal to improve health and food quality, eliminate negative environmental impacts, and ensure sustainability drives interest in organic agriculture (Mazepa et al.2021).

It is required to create approaches that promote organic production, such as the use of biofertiliser or natural bio stimulants. One of the possible levers to promote this transformation is the usage of bioproducts that are less harmful to human health and more environmentally friendly. Blue biotechnology, and more specifically seaweed and microalgae, are gaining popularity in the scientific community year by year (Mazepa et al.2021).

A previous study evaluated at the utilisation of the seaweed *Undaria* as a soil amendment for organic-matter-depleted soil. *Undaria* addition to a substrate comprising vermiculite: organic soil mix (95:5) stimulated tomato growth as evidenced by increases in aerial and root biomass and redox status (Salcedo et al. 2020). Use of chemical and functional assays validated the presence of plant nutrients, minerals, vitamins, antioxidant capacity, and phytohormone-like activities in *Undaria* extracts. Hence, *Undaria* extracts should be able to boost growth and contribute to redox homoeostasis in early plant developmental phases, which are crucial for tomato productivity (Salcedo et al. 2020). Recommendations arising from the research were that *Undaria* supplementation should be used to supply nutritional and growth promoter components to tomato crops grown in poor soils (Salcedo et al. 2020).

There is dearth of research that examines the effect of macro and microalgae: (1) Seaweeds' carbohydrate polymers as plant growth promoters (Pacheco et al. 2021). (2) Proteins extracted from seaweed *Undaria pinnatifida* and their potential uses as foods and nutraceuticals (Nadeeshani et al. 2021). (3) Diverse applications of marine macroalgal example for human food, livestock and agriculture, cosmetics, pharmaceutics (Leandro et al. 2020). (4) Cultivation and utilization of *Undaria pinnatifida* (wakame) as food (Yamanaka & Akiyama 1993).

Fucus vesiculosus was used as a raw material to make bio-products for sustainable agriculture. Biosorption was used to create microelement fertiliser additions. Cu(II) and Zn(II) ions were added to *F. vesiculosus* (Michalak et al. 2021). Plant growth bio stimulants were obtained by extracting seaweed with potassium hydroxide. In a germination test on garden cress (*Lepidium sativum*), several doses of enriched *F. vesiculosus* (1, 2, 4, 6 mg/per Petri dish) and concentrations of seaweed extract (2.5, 5, and 10%) were tested (Michalak et al. 2021). The results showed that biomass enrichment had no effect on plant length or RGB metrics (red, green and blue) parameters in their leaves. Taking these two parameters into account, the group treated with natural *F. vesiculosus* produced the best outcomes. There was a significant effect on plant length and RGB parameters. This biomass was recommended for the biofortification of plants with a specific micro element. The zinc content in the group treated with Zn-*F. vesiculosus* (6 mg) was about 6 times greater than in the group treated with natural *F. vesiculosus* and 8 times higher than in the control group, and for Cu- *F. vesiculosus* (6 mg) - 220 and 340 times higher, respectively (Michalak et al. 2021).

The natural, unenriched *Fucus vesiculosus* had much greater results in terms of plant length, chlorophyll content, and biofortification in all micro- and macro elements.

The *Fucus vesiculosus* extract improved plant length, chlorophyll content, and multielement composition in garden cress. For a 5 percent extract, the longest length and RGB parameters, as well as plant biofortification in Cu, Fe, Mn, Zn, Ca, S, P, and K, were determined. The length of plants treated with 5% extract was 20% greater than the length of plants treated with native *F. vesiculosus* powder (Michalak et al. 2021). However, the overall content of micro- and macro elements in garden cress was higher after application of raw *F. vesiculosus* rather than algal extract. In the absence of hazardous metals in the biomass, they proposed that *Fucus* can be used directly in agriculture. Nonetheless, *Fucus* extract is recommended for future research. Further research into the use of the acquired post-extraction residue in agriculture is also required. Further investigation into seaweed extract as a bio stimulant of plant growth was suggested (Michalak et al. 2021).

This study aims to fill this research gap by investigating the effects of macroalgal extract produced using the green extraction technology (microwave assisted extraction (MAE)). MAE is being developed using contemporary technologies that use fewer or no organic solvents to minimise environmental and health implications and increases the yield. Microwaves are electromagnetic waves with wavelengths ranging from 1 mm to 1 m and frequency ranging from 300 MHz (1 m) to 300 GHz (1 mm). Because microwave ovens or customised equipment are easily available at low cost, the method is gaining popularity (Michalak, Tuhy & Chojnacka 2015).

Considering this, the present study examined the suitability of *Undaria pinnatifida* biomass, its MAEs and the Algardis liquid extract (*Fucus vesiculosus*) to determine the potential to enhance growth of *Sorghum bicolor* over the commercially available gardening fertiliser Seasol (which contains (W/W): Nitrogen (N) 10.7%, Phosphorus (P), 0.6%, Potassium 9.6%, Carbon (C) 14% plus trace elements) or compost applications.

1.8. Aims and objective of the study

The study aimed to investigate if macroalgae extracts (Algardis), *Undaria pinnatifida* biomass or its MAEs would improve the growth *Sorghum bicolor* over the commercially available gardening fertiliser Seasol, and carbon-rich compost soil.

Specific objectives

1. Determination of soil mineral content (C, N and P, K) using elemental analysis and inductively coupled plasma optical emission spectrometry (ICP-OES), respectively.
2. Determination of effects on plant growth via assessment of
    a) Above-ground biomass
    b) below ground biomass
    c) Plant leaf numbers and dimensions
    d) Plant height

1.9. Project overview and expected outcomes

This project is based on the positive effect obtained in previous research using seaweed extracts to improve the growth of tomatoes, soil fertility, and productivity (Hussain, Kasinadhuni, & Arioli 2021). Contents of N, P, K, and C will be analysed for the positive control (compost soil), negative control (nutrient poor topsoil), Seasol, Marinova Algardis (Liquid extract), *Undaria pinnatifida* biomass and microwave-assisted extracts obtained at 40, 60, and 80°C.

Prior to applying fertilisation regimes, determination of soil mineral content (P, K) using inductively coupled plasma optical emission spectrometry (ICP-OES), and elemental analysis for C and N was performed on the carbon-rich compost soil and the nutrient poor topsoil used for the fertilisation treatments, the Algardis extract, *Undaria pinnatifida* biomass and the MAEs obtained from the different temperature extractions. Treatments amounts for N, P, K for the algal treatments were adjusted to the levels found in the compost soil. The same mineral analysis of all controls and treatment soils was performed after harvest. A greenhouse pot trial was carried out with three replicates per treatment. Plant growth measurements were carried out at harvest by assessing number of leaves and size, dry and wet weight of below ground and above ground biomass.

The expected outcome of the study

To assess the suitability of *Undaria pinnatifida* biomass or its MAEs for enhancing growth of *Sorghum bicolor* over the commercially available gardening fertiliser Seasol or compost applications. In addition, a macroalgal liquid extract produced by Marinova (Algardis) was tested for its suitability along with the *Undaria* treatments.

Chapter 2

MATERIALS AND METHODS

MATERIAL AND METHOD

2.1. Materials

2.1.1. Macroalgae, Algardis liquid extract, Soils, and Seasol

Macroalgae and Algardis liquid extract- 20 kg of *U. pinnatifida* powder and 25 L of Algardis liquid seaweed extract were provided by Marinova Pty Ltd Tasmania, Australia. Algardis seaweed liquid extract was made from *Fucus vesiculosus*, as a by-product after fucoidan extraction.

Seasol fertiliser- Seasol plus nutrients all purpose (which contains (W/W): Nitrogen (N) 10.7%, Phosphorus (P) 0.6%, Potassium 9.6%, plus trace elements, C 14%) was purchased online from Bunnings.com.au model number (10758) 1 bag.

Soils- Compost and nutrient-poor topsoils were purchased from SA composter Pty Ltd.

2.1.2. Determination of minerals in macroalgae, and Algardis liquid extract using ICP-OES and elemental analysis

Inductively coupled plasma optical emission spectrometry and elemental analysis for analysis of P and K by (ICP-OES) and elemental analysis C and N was done by Flinders Analytical Center. Perkin Elmer ICP-OES Optima 8000, DigiPREP block digestion system, ICP tubes, and Elemental Vario Isotope cube were used.

2.1.3 Greenhouse experiment

A greenhouse experiment was carried out in the Biology greenhouse 4 (southern compound) Bay 4 Flinders University. *Sorghum bicolor* (Broom corn ornamental) was purchased online via Eden Seeds in seven packets which contained a total of 380 seeds. Six kilogram of soil was used per pot (n = 3) with 21 pots receiving topsoil and 3 pots receiving compost soil. Pots were purchased online from Bunnings.com.au. Eight different treatments were applied, each being replicated three times (a total of 24 pots). A soil moisture meter was purchased online from Bunnings.com.au. Soil moisture meter-p2961033; model number 64737.

2.1.4. Chemicals

HNO_3, Na_2HPO_4 and KCl, were obtained from the chemical storage room on level 5 of the Health Sciences Building at Flinders University, Adelaide, Australia.

2.1.5. Equipments

Microwave-assisted extractor (Milestone Innovations), freeze dryer (Virtis, NSW), rotary evaporator (Buchi Flawil, Switzerland), hot air oven (Scientific equipment manufacturer SEM), centrifuge, Sartorius microbalance, -80°C freezers, Perkin Elmer ICP-OES Optima 8000, DigiPREP block digestion system, ICP tubes, Elemental Vario Isotope cube were available in the College of Medicine and Public Health and Flinders Analytical at Flinders University, Adelaide, Australia**.**

2.2 Extraction

2.2.1 Microwave-assisted extraction (MAE)

The process was carried out in a StartSYNTH-microwave synthesis lab station, equipped with an industrial magnetron that can deliver up to 1200 Watt. A microwave diffuser which is located above the microwave chamber evenly distributes the microwaves throughout the cavity, preventing localized hot and cold spots. It is operated using a compact terminal, with a bright, high resolution, touch screen display in which the parameters such as irradiation power, temperature, and time are entered.

Exactly 300 g of *Undaria pinnatifida* powder was weighed and placed in a 2,000 mL round bottom flask with a quick-fit wide neck, fitting into the condenser tube inside the chamber.

All microwave extractions were performed under set microwave irradiation as detailed in (Table 2.1) for 30 min at 40, 60, and 80°C. Due to the large quantity of extraction volumes required two extractions were carried out to obtain the MAE extracts. In the first extraction, 200 g of *Undaria pinnatifida* powder was weighed and placed in a 2,000 mL round bottom flask with 1,400 mL of water whilst the remaining 100 g of *Undaria pinnatifida* powder was mixed with 700 mL of water. Following extraction, the extracts were centrifuged at 2,040 rcf for 5 min to separate the supernatant from the residual solids (Table 2.1).

Table 2.1. Extraction condition for microwave-assisted extraction of *Undaria pinnatifida*

Extraction	Biomass / Algal powder	Solvent Water	Temperature	Energy
1	300 g	2100 mL	40°C	1000 W
2	300 g	2100 mL	60°C	1100 W
3	300 g	2100 mL	80°C	1200 W

2.2.2 Determination of minerals content in compost soil, topsoil, Undaria pinnatifida biomass, and Algardis liquid extract using ICP-OES and elemental analysis

Analysis of P and K by (ICP-OES) and elemental analysis of C and N in compost soil, topsoil, *Undaria* powder, Algardis liquid extract, and MAE extracts of *Undaria* obtained at three different temperatures was done before planting (day 0). Soil elemental analysis was also performed after harvest on day 56.

Analysis of P and K was done by (ICP-OES) in solid and liquid samples and elemental analysis of C and N in solid samples. These analyses were conducted by Chemical Analysis Service, Flinders Analytical Centre (Flinders University, Adelaide, Australia).

2.2.3 ICP-OES method for P and K analysis of Undaria pinnatifida extracts

For P and K analysis [(APPENDIX A 1.1.) samples provided for ICP-OES], samples were diluted 1,000 times in 0.5% HNO_3in two steps. First 0.1 mL of the sample was diluted with 9.9 mL MQ water and 1 mL of that solution was diluted with 9 mL 0.5% HNO_3, giving a total dilution of 1,000. The calibration was made in 0.5% HNO_3 between 50 ppb and 10 ppm for K and from 50 ppb to 2 ppm for P. Two wavelengths were used for each elements P 177.434 (nm), P 178.221 (nm), and K 766.490 (nm), K 76.896 (nm).

The 40°C extract was analysed using only water and water plus 0.5% HNO_3 to see if the results were similar which they were. So, the MQ plus 0.5% HNO_3 was used in line with conditions used for calibration. Samples and standards were analysed on a Perkin Elmer ICP-OES Optima 8000.

2.2.4 ICP-OES for P and K analysis of Undaria pinnatifida biomass and the freeze-dried extracts

For P and K analysis [(APPENDIX A1.1.) samples provided for ICP-OES], samples were acid-digested in a DigiPREP block digestion system in the following way: Between 50 and 100 mg of the samples was weighed into 50 mL digest tubes.
5 mL concentrated high purity HNO_3 was carefully added to each tube, making sure that no violent reaction occurred. The samples were allowed to be pre-digested under ambient

conditions overnight before placing them into the block digestor. The following digest method was used: ramping up to 80°C for approx. 20 min, held at 80°C for 30 min, before ramping to 120°C and holding at 120°C for 2 h. Samples were allowed to cool to room temperature. After cooling, samples were diluted with MQ water to 50 mL which gives an HNO_3 concentration of 10%. A 5 mL aliquot of each sample was transferred to 15 mL ICP tubes and diluted to 10 mL with MQ water giving an HNO_3 conc of 5%. A calibration ranging from 50 ppb up to 10 ppm was made in 5% HNO_3 to match the acid matrix of the samples. Two wavelengths were used for each element P 177.434 (nm), P 178.221 (nm), and K 766.490 (nm), K 76.896 (nm). Samples and standards were analysed on a Perkin Elmer ICP-OES Optima 8000.

2.2.5 Elemental analysis of C and N in freeze-dried Undaria pinnatifida extracts

The samples [(APPENDIX A1.1.) samples provided for elemental analysis] were run using the Elemental Vario Isotope cube with the combustion tube set at 950°C and the reduction tube at 600°C. 15 mL of *Undaria pinnatifida* extracts obtained at MAE 40°C, MAE 60°C, and MAE 80°C were dried for 5 days at -80°C, followed by freeze-drying for 5 days. In addition, 10 g of *Undaria pinnatifida* biomass**,** and 20 g of compost and topsoil were analysed by elemental analysis**.**

2.3 Greenhouse pot trial
2.3.1 Introduction

A greenhouse experiment was conducted in the Flinders University greenhouse area. A total of 24 pots (25 cm diameter) were used to set up the experiment with each condition set up in triplicate * 8 treatments. Pots were set up in a randomized design with daily rotation to avoid any impacts of possible uneven lighting. Soil moisture levels were determined daily. The light intensity was between 500-600 µmol m^{-2} s^{-1} and the temperature was set to 28ºC during the day, 18ºC at night.

Soil moisture levels were maintained by measuring soil moisture daily using a soil moisture metre and supplying make up watering to the starting water level conditions. Each pot was filled with 6 kg soil with which topsoil in 21 pots and compost soil in 3 pots. Nine seeds of sorghum are sown at equal distance in each pot at 2cm depth by hand. An equal amount of

water viz. 1000 mL was added to each pot. The preparation details for each treatment are given in APPENDIX A.1.2 and Fig.2.1 outlines the experimental design and endpoint measurements taken. Plant height measured from at 30 days. In which pots were potted from the 2nd of November till the 2nd of December 2021, and for the growth of 56-day pots were potted from the 2nd of November till the 28th of December 2021 plant height was determined by measuring from the ground level to the apex of the growing point using a measuring tape.

The leaf size was determined by using a ruler from the pointy part at end of the leaf to the point where the leaf joins the stalk. To determine above and below-ground biomass dry weight, plant materials were washed thoroughly with running tap water and padded dry gently with a paper towel then kept for drying for 3 days at 37°C hot room (Level 3 room no. 318 Biological science building). For dry weight above and below-ground biomass was oven dry for 3 days at 37°C.

2.3.2 Methods

Treatment regime of potted plant experiment

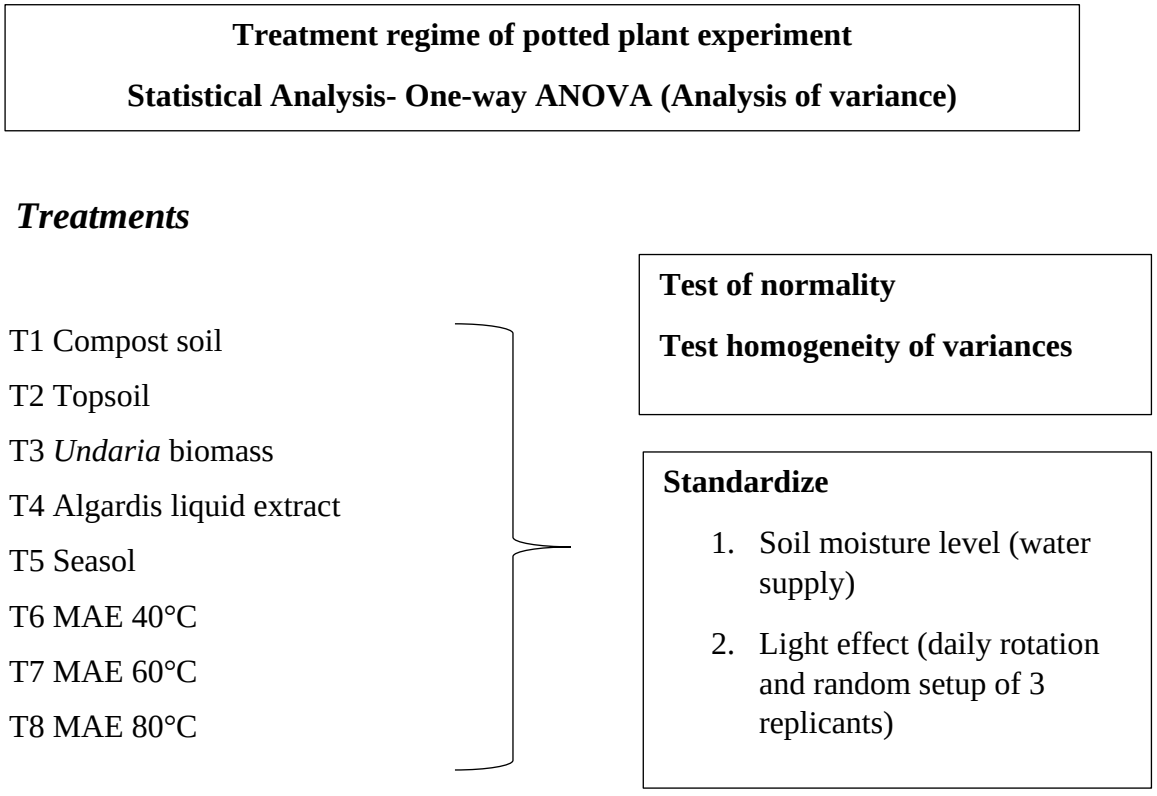


Figure 2.1. Overview of experimental set up of soil fertilisation experiments and end-point measurements

2.3.3 Daily measurements

a. Measure soil moisture

Soil moisture was measured before adding water to identify the need to water for plant growth The pot weight of each pot was recorded before adding water. Water was then added to pots. After adding water weight of each pot was recorded. The soil moisture was measured with soil moisture meter daily

b. Daily rotation

Pots were rotated daily for equal distribution of light conditions and for maintaining growth equally.

c. pH

pH was measured with a pH meter daily

2.3.4. Water Holding Capacity

The percentage of water that a specific soil can hold without dripping after being wet is referred to as its holding capacity (Atkinson et al. 2018). Sand has a low holding capacity because water drains quickly from it. This is since when particle size grows, particle surface area density drops, and hence water tension lowers (Atkinson et al. 2018). Clay soils, on the other hand, have a higher holding capacity since the particle size is so minute and the water adheres to the particles (Atkinson et al. 2018).

The water holding capacity of each pot was measured from day 1 to day 55 for the three replicates for each of the treatment. The dry weight (before adding water), and wet weight (after adding water) was determined for *Undaria* MAE extracts, Algardis, Seasol, and *Undaria* biomass treatments and the positive and negative controls. The water was added according to its need. The water holding capacity was calculated according to the formula below (Atkinson et al. 2018).

Mw= Mt-Ms,

where Mw is the water mass (g).

Mt is the total mass (g) of the container and moist soil.

Ms is the total mass (g) of the container and dry soil.

Water Holding Capacity: (VWC percent) = Vw/Vt*100;

Where Vt indicates the saturated soil's total volume (eq. 2.1).

Water Holding Capacity (VWC %) =

$$\frac{\text{The total mass of container with wet soil} - \text{Total mass of container with dry soil} \times 100}{\text{The total volume of the soil}}$$

Eq. 2.1 Calculated water holding capacity

2.3.5. Water content in above and below-ground biomass

Water content in roots and leaves was calculated in % where fresh weight, and dry weight of leaves were taken.

$$Water\ content\ (\%) = \frac{Wf - Wd}{Wf} * 100$$

Eq. 2.2 Calculated water content in leaves and root

2.4 Statistical data analysis

All statistical analysis were made by IBM SPSS Statistics, Version 28.0. Differences were deemed statistically significant at a set p-value of $p < 0.05$. Assumptions of normality and homogeneity of variance was carried out using the Kolmogorov Smirnov test. Assumptions of normality and homogeneity of variance were done for the N, P, K, and C content, leaf dimension, plant height, above and below-ground biomass fresh and dry weight, water holding capacity. One-way analysis of variance (ANOVA) was applied to data that met the underpinning assumptions of normal distribution and homogeneity of variances to determine significant differences of the mean concentration of mineral content in soils. A Tukey post hoc

test was used to determine which treatment led to the significance of differences between the groups.

Chapter 3

RESULTS

3. Results

3.1 Determination of mineral content in samples before seeding (Day-0)

Contents of N, C, P, and K (g/kg) of *Undaria* biomass, Algardis, and *Undaria* MAE-biomass extracts obtained at 40, 60, and 80°C at the time of seeding are shown in (Table 3.1.) In freeze-dried reconstituted *Undaria* biomass had a higher content of N (9.6 g/kg), and C (240.1 g/kg) compared to all other fertilisers. Algardis had a higher content of P (5.3532 g/kg) and K (125.922 g/kg) compared to all other fertilisers. MAE 40° and 80°C had almost similar contents of K (1.689 – 1.721 g/kg) and P (61.151 – 69.471 g/kg) while the MAE 40°C extract had a higher C (190.2 g/kg) content. *Undaria* biomass MAE extracts 40, 60, and 80°C had all most similar P and K content in raw extracts.

Soil contents of N, C, P, and K (g/kg) for compost soil (positive control), and unfertilised topsoil (negative control) at the time of seeding are shown in (Table 3.1). Topsoil (nutrient-poor negative control) had a higher content of N (3.3 g/kg), and C (41.5 g/kg). While the compost soil had the lowest amounts of C (21.7 g/kg), and N (1.3 g/kg).

Table 3.1. P, K, C, and N content of MAE 40, 60, and 80°C, *Undaria* biomass, Algardis, Seasol, Topsoil, and Compost soil on (day -0)

Treatments	Raw extract P [g/kg]	Raw extract K [g/kg]	Reconstituted Freeze-dried Extracts P [g/kg]	Reconstituted Freeze-dried Extracts K [g/kg]	N [g/kg]	C [g/kg]
MAE 40°C	0.449657	18.2127	1.682944	61.15172547	2.6	190.2
MAE 60°C	0.468732	18.74445	1.473332	57.73311073	2.5	167.7
MAE 80°C	0.451424	19.056975	1.72169	69.4718473	2.3	168.1
***Undaria* biomass**			4.008827	97.0098522	9.6	240.1
Algardis	0.312619	7.690996871	5.353257	125.9229256	4.6	112.89
Seasol			0.12	1.92	2.14	2.8
Topsoil			0.443476	2.867103	3.3	41.5
Compost soil			1.328965	3.145154	1.3	21.7

3.2 Determination of soil mineral content (post-fertilisation) Day-56

After 56 days there was an increased in soil elemental nutritive value after application of fertilisation) (Table 3.2) (Figure 3.1 B). The concentration of N, P, and K, content of compost soil had increased but decreased in unfertilised soil (topsoil) (C 24.867 g/kg) (Figure 3.1 B) in comparison to C (41.5 g/kg) before seeding day-0 (Figure 3.1 A).

N (1.567 g/kg), P (0.5701 g/kg), K (3.228 g/kg, and C (25.33 g/kg) concentrations decreased for the Algardis extracts after day 56 (compare Figure 3.1 B with Figure 3.1 A: N (4.6 g/kg), P (5.36 g/kg), K (125.92 g/kg), and C (112.89 g/kg)). K (2.883 g/kg), P (0.779 g/kg), N (1.266 g/kg), and C (22.9 g/kg) also decreased in *Undaria* biomass treatments to K (97.009 g/kg), P (4.008 g/kg), N (9.6 g/kg), and C (240.1 g/kg) (Table 3.2) (compare Figure 3.1 B with figure 3.1 A).

In contrast, on day 56 P, C, and N concentrations had increased in treatments with *Undaria* biomass MAE 40˚, 60˚, and 80˚C (compare Figure 3.1 B with Figure 3.1 A). C content was higher in MAE 40˚C (C (25.73 g/kg), P (1.000 g/kg), and N (1.633 g/kg)) compared to MAE 60 and MAE 80˚C (Figure 3.1 B).

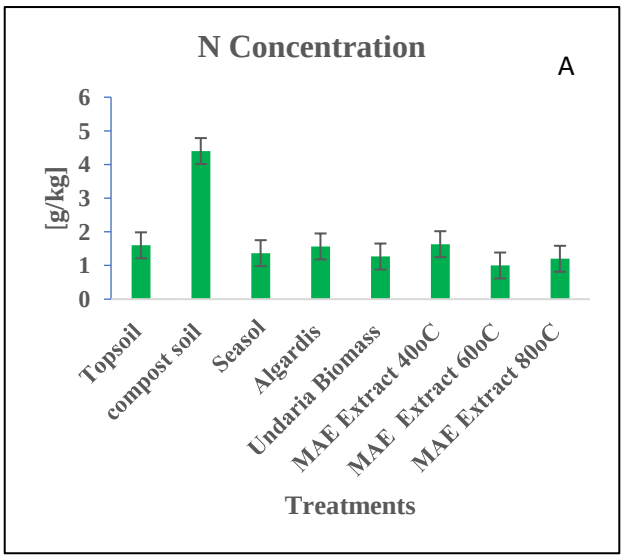
Table 3.2. P, K, C, and N content of MAE 40, 60, and 80°C, *Undaria* biomass, Algardis, Seasol, Topsoil, and Compost soil on (day -56)

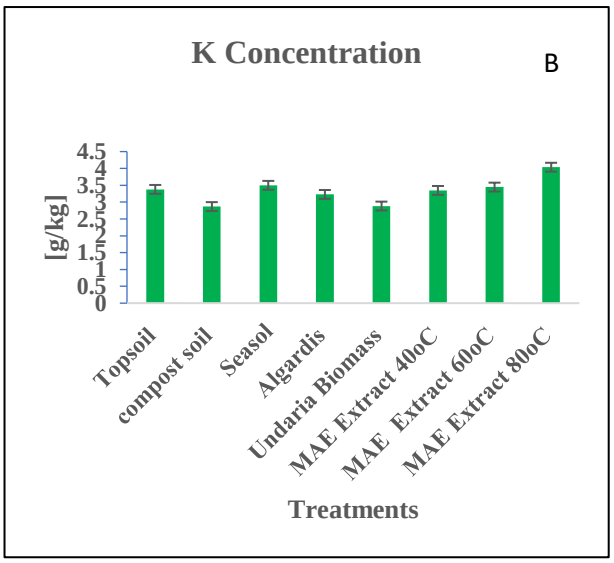
Treatments	K [g/kg]	P [g/kg]	N [g/kg]	C [g/kg]
Topsoil	3.37787	0.37325	1.6	24.8667
Compost soil	2.86803	1.45588	4.4	56
Seasol	3.498	0.46816	1.36667	19.2
Algardis	3.22865	0.57013	1.56667	25.3333
***Undaria* Biomass**	2.8832	0.77961	1.26667	22.9
MAE 40°C	3.34676	1.00015	1.63333	25.7333
MAE 60°C	3.44597	0.65635	1	14.7667
MAE 80°C	4.03591	0.6682	1.2	18.6

There was a statistically significant difference between groups as demonstrated by one-way ANOVA for post-harvest (day-56) Potassium (K) content between groups, and within groups F= 4.319, (p = .007) for Phosphorus (P) content between groups and within groups F= 2.919,

(p = .036), C between groups and within groups F= 6.082, (p = .001). for Nitrogen (N) content between groups and within groups F= 6.883, (p = <0.01) A Tukey post hoc test showed that in the P, N, K, and C group there was statistically significant difference between the treatments (p= < 0.05).

A multiple comparisons analysis test (APPENDIX A 1.3) showed a statistically significant difference in soil content for N, C, P, and K between the group (treatments) p < 0.05. A Tukey's HSD test for multiple comparisons established Potassium content K contents of compost soil were different to fertilization treatments in which compost soil has significant differences between the group (MAE extract 80°C) P= .005. There was a significant difference of dependent variable Potassium content (K) *Undaria biomass* with (MAE extract 80°C) P= .006 (APPENDIX A 1.8).





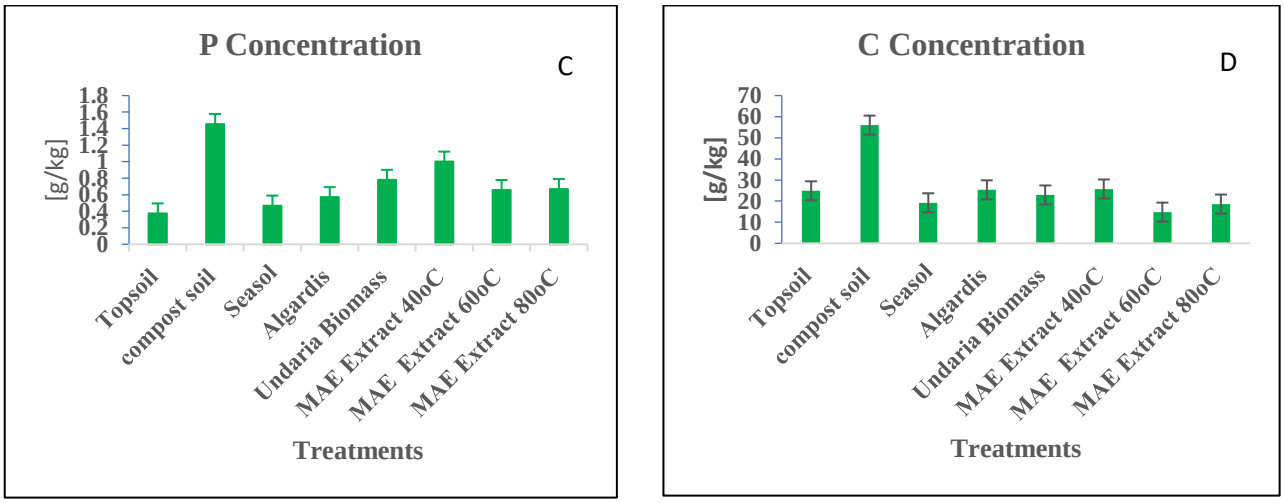


Figure 3.1. Soil N, P, K, and C contents of the different fertiliser treatments post-harvesting (day-56 (B)).

After 56 days, there soil elemental nutritive value after application of fertilisation increased. Whiel the concentration of N, P, and K content of compost soil increased; the C content of topsoil decreased in comparison of day-0 concentrations.

N, P, K and C concentrations decreased in Algardis and *Undaria* biomass treatments compared to day-0 concentrations.

In contrast, day 56 P, C, and N concentrations increased in treatments with *Undaria* MAE 40˚, 60˚, and 80˚C compared to day-0. C content was higher in MAE 40˚C compared to MAE 60 and MAE 80˚C.

A one-way ANOVA for post-harvest (day-56) showed a significant difference for Potassium (K) Phosphors (P), nitrogen (N) and Carbon (C) contents between and within groups, ($p = < 0.05$). A Tukey post hoc test showed that in the P, N, K, and C group there was statistically significant difference between the treatments ($p= < 0.05$).

3.3 Nutrient uptake

In the plant physiologists there are three mechanisms by which nutrients reach the surface of the root hairs. These are: 1) root interception, 2) mass flow, and 3) diffusion. The heights nutrient uptake of N content was in *Undaria* biomass (8.33 g/kg), C content in *Undaria* biomass MAE extract 60℃ (152.93 g/kg), K content in *Undaria* biomass (94.12 g/kg), and P content in Algardis (4.78 g/kg). The low nutrient uptake was observed of K content in Compost soil

(0.277 g/kg), Topsoil (0.007 g/kg), N content in Seasol (0.77 g/kg), and C content in Unfertilised topsoil (16.63 g/kg).

3.3 Nutrient uptake

In the plant physiologists there are three mechanisms by which nutrients reach the surface of the root hairs. These are: 1) root interception, 2) mass flow, and 3) diffusion. The heights nutrient uptake of N content was in *Undaria* biomass (8.33 g/kg), C content in *Undaria* biomass MAE extract 60℃ (152.93 g/kg), K content in *Undaria* biomass (4.78 g/kg).

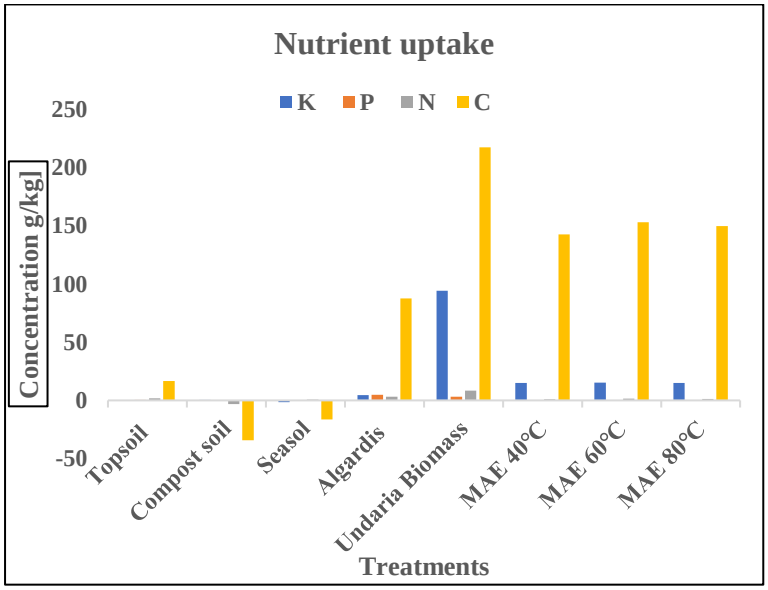


Figure 3.2. Nutrient uptake of concentration N, P, K, and C contents of the different fertiliser treatments

3.4 Growth of Sorghum bicolor

3.4.1 Day-30 growth response of Sorghum bicolor to fertilisation regimes

Growth was measured on 30th day after planting from day 0 to 30th day. Compost soil seeding resulted in fastest growth, followed by Algardis, and *Undaria* biomass, while fertilisation with *Undaria* MAE extracts resulted in much poorer growth and seed sowed into topsoil did not germinate (Figure 3.3).

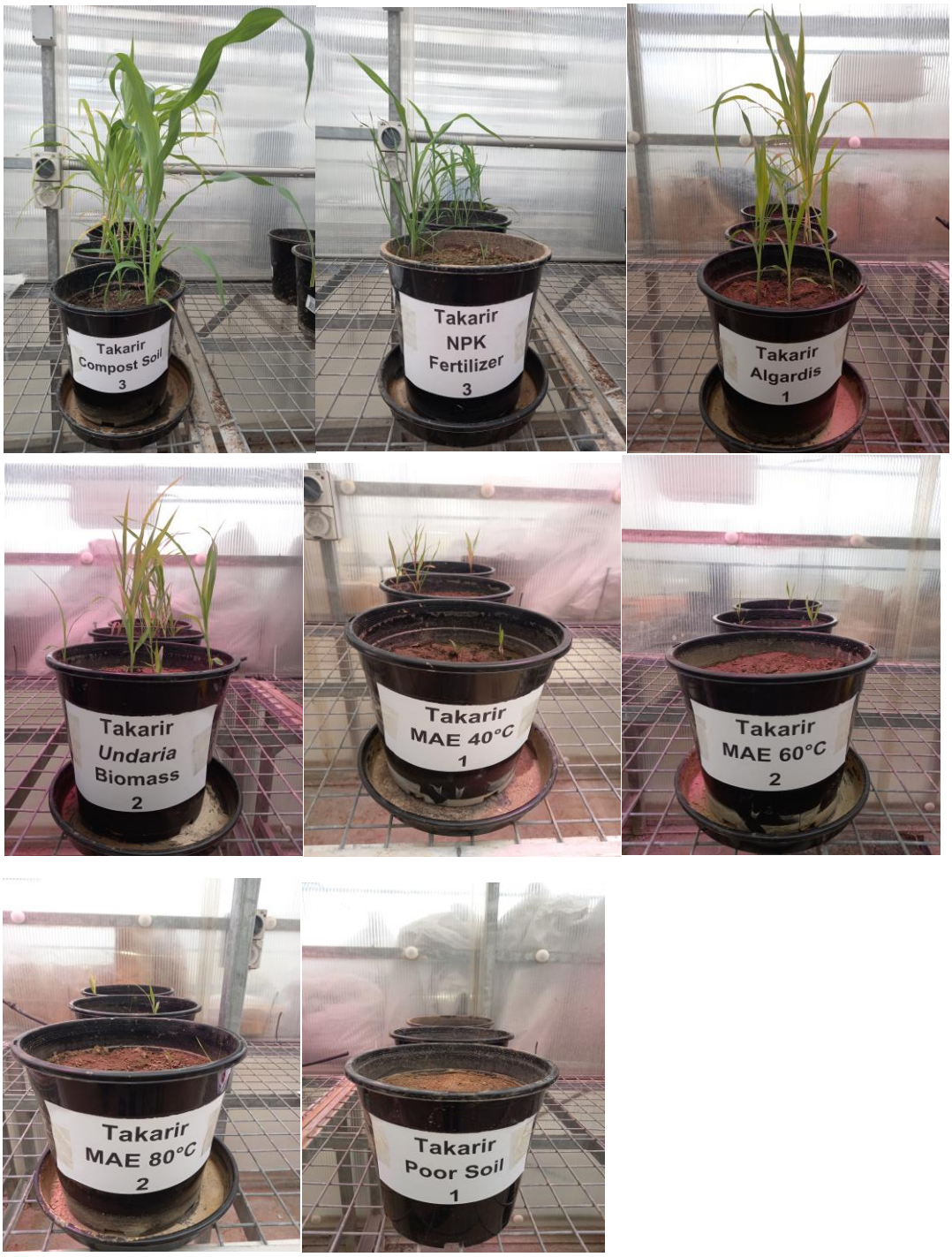


Figure 3.3. Plant growth response of *Sorghum bicolor* on day 30 to unfertilised topsoil (negative control), compost soil (positive control), Seasol, *Undaria* biomass, and *Undaria* MAE extracts at 40, 60, and 80°C

3.4.2 Growth on day 56

Growth was measured on the 56th day after planting (Figure 3.4). Compost soil seeding (positive control) resulted in fastest growth, followed by Algardis, *Undaria* biomass, and Seasol while fertilisation with MAE extracts resulted in poor growth and seeds did not germinate in unfertilised topsoil (negative control). Some plants showed yellowing leaf tips (Fig. 3.4).

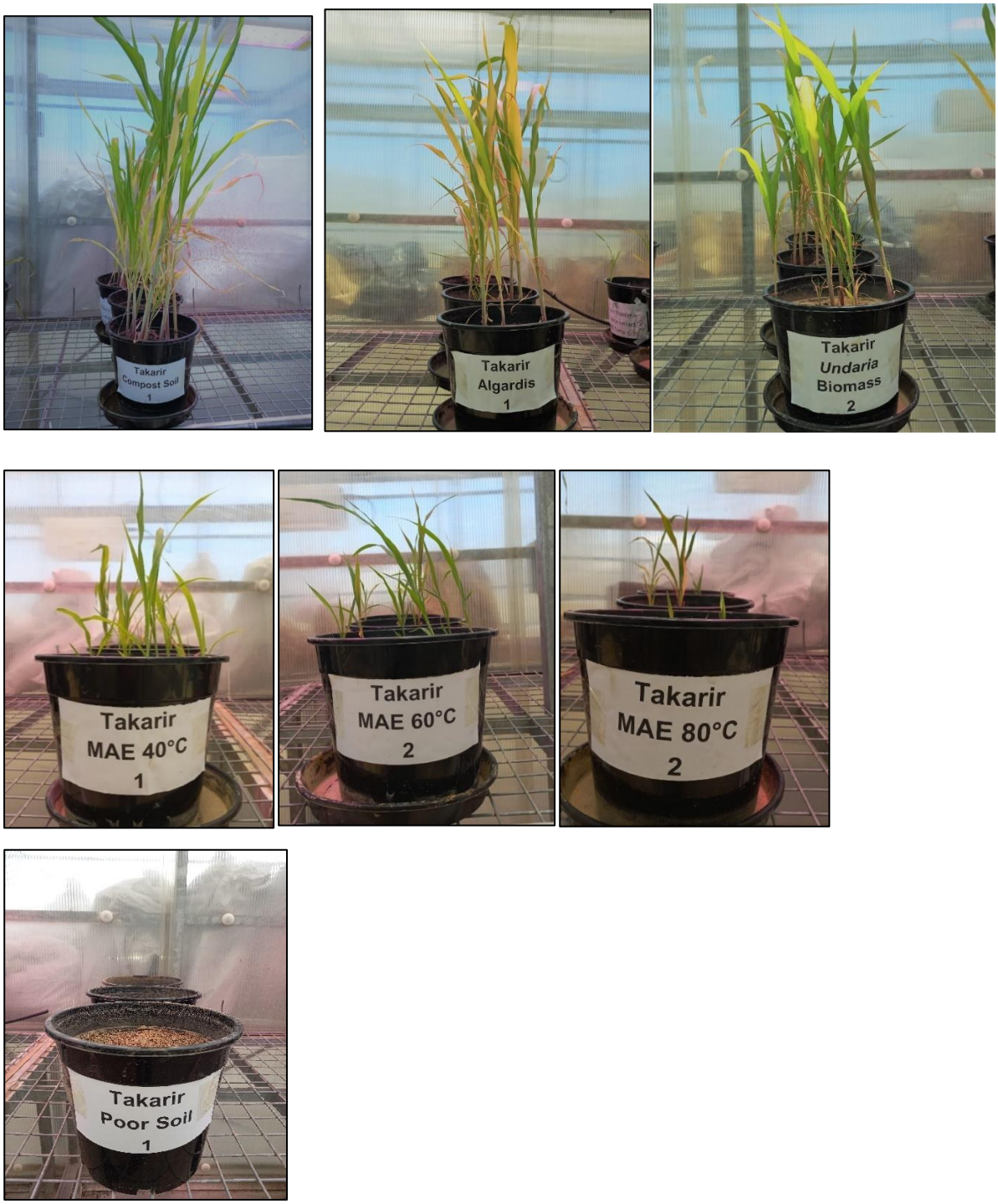


Figure 3.4. Plant growth response of *Sorghum bicolor* on day 56 to unfertilised topsoil (negative control), compost soil (positive control), Seasol, *Undaria* biomass, and *Undaria* MAE extracts at 40, 60, and 80°C

3.4.3 Plant height on day 30 and day 56 after sowing

In one-way ANOVA the Levene's tests of homogeneity of variances for day-30 plant height was significant ($p < 0.01$), identifying that data did not meet this assumption of the ANOVA. There was a significant difference based on median p= <0.01 (Fig. 3.5 A) (APPENDIX A 1.4). A one-way ANOVA determined a significant effect of fertilisation regime on plant height on 30^{th} day, F= 10.079, ($p = 0.001$). A multiple comparison (APPENDIX A 1.4) determined a significant effect of compost soil and fertilisation with Seasol, *U. pinnatifida* biomass, and MAE 40, 60, and 80°C extracts $p < 0.001$. There was no statistically significant difference between plant growth in compost soil and topsoil fertilised with Algardis $p = 0.471$ (Fig. 3.5 A).

A Tukey's HSD test for Seasol to all other fertilization treatments identified a significant difference to outcomes in compost soil ($p = 0.011$). There was a significant difference for *U pinntifida* biomass and compost soil ($p < 0.001$), MAE 40°C, MAE 60°C, and MAE 80°C with compost soil ($p < 0.001$) and Algardis (Fig. 3.5 A).

A multiple comparison analysis one-way ANOVA determined a significant difference of all fertilisation treatments on plant height on day 56 ($p < 0.001$ and F= 12.391, ($p = 0.001$), respectively). (Fig. 3.5 B). A Tukey's HSD test showed that Seasol treatments were significantly different to fertilisation outcomes with Algardis, compost soil, MAE 60°C, and MAE 80°C, while there was no significant difference between Seasol, *U. pinnatifida* biomass and the MAE 40°C.

There was a significant difference of dependent variable *Undaria* biomass with (compost soil, MAE 60°C, and MAE 80°C) P= <0.001. There is no significant difference in *Undaria* biomass with MAE 40°C, algardis, and industry standard control. There is a statistically significant difference in algardis with MAE 40C, MAE 60°C, and MAE 80°C (figure 3.5 B) (APPENDIX A 1.5).

Tests of Homogeneity of Variances for 30th-day plant height cm Levene Statistic based on mean is (8.013) p= <0.01. There was a significant difference based on median p= <0.01(figure 3.5 B) (APPENDIX A 1.4).

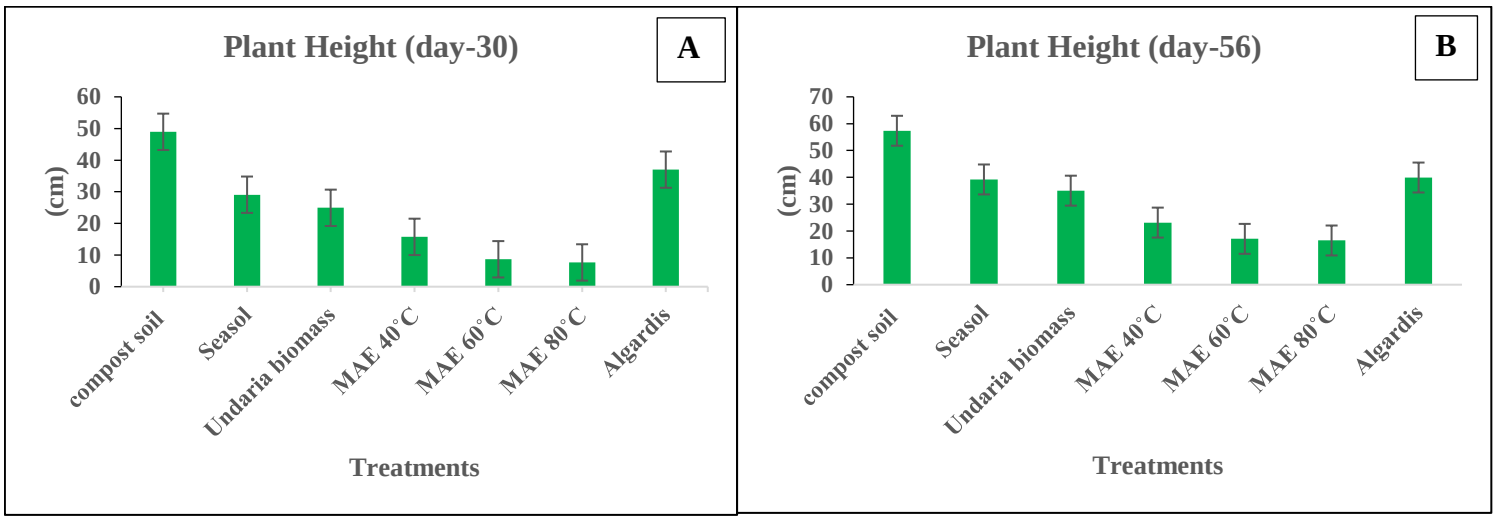


Figure 3.5. Effect of fertiliser treatment on *Sorghum bicolor* plant height on day 30 (A) and day 56 (B). Standard deviation is shown; n=3

Statistical analysis determined a significant effect of fertilisation regime on plant height on 30 day and 56-day, P= < 0.001. Tests of homogeneity of variances for day-30 and 56 plant height was significant (P < 0.01), For day 30 Seasol treatment to all other fertilization treatments identified a significant difference. There was a significant difference for *U pinntifida* biomass with compost soil (P < 0.001). MAE 40°C, 60°C, and 80°C with compost soil (P < 0.001) and Algardis

But in day 56 analysis showed that Seasol treatments were significantly different to fertilisation outcomes with Algardis, compost soil, MAE 60°C, and MAE 80°C, while there was no significant difference between Seasol, *U. pinnatifida* biomass. There was a significant difference of dependent variable *Undaria* biomass with (compost soil, MAE 60°C, and MAE 80°C) P= < 0.001.

3.4.4 Leaf size on day 30 and day 56 from sowing

The leaf size (mean 25 ± SD 11.44 cm) was greatest in the *Sorghum bicolor* grown in compost soil, followed by plants grown with Algardis, and *U. pinnatifida* biomass. Fertilisation regime significantly affected day-30 leaf size (one-way ANOVA F= 24.058, $p < 0.001$) and multiple

comparisons (APPENDIX A 1.6) showed that compost soil was different to all other treatments $p < 0.001$.

In one-way ANOVA a Tukey's HSD test identified that Seasol grown Sorghum bicolor leaf sizes differed from those grown with Algardis, compost soil, MAE 60°C, and MAE 80°C, but there was no significant difference between Seasol and *U. pinnatifida* biomass and the MAE 40°C (Fig. 3.5 A). There was a significant difference of *U. pinnatifida* biomass with compost soil and Algardis ($p < 0.001$), *U. pinnatifida* biomass no significant with MAE 40°C, MAE 60°C, MAE 80°C, and Seasol. For Algardis treatments, differences were significant compared to MAE 40°C, MAE 60°C, MAE 80°C, Seasol, and *U. pinnatifida* biomass, no significant of Algardis treatments with compost soil ($p = 0.071$) (Fig. 3.6 A). The MAE 40°C, MAE 60°C, MAE 80°C treatment outcomes were significantly different to compost soil and Algardis ($p = <0.001$) (Fig. 3.6 A) (APPENDIX A 1.6).

A one-way ANOVA for effect of fertilisation regime on leaf size of Sorghum bicolor on day 56 determined a significant effect, F= 32.085, ($p = 0.001$) (Fig.3.6 B) (APPENDIX A 1.7). A multiple comparison analysis (APPENDIX A 1.7) showed that a significant difference between compost soil day-56 leaf size and all other treatments ($p < 0.001$).

A Tukey's HSD test determined that day-56 leaf sizes of plants fertilised with Seasol were significantly different to those of Algardis, compost soil, MAE 60°C, and MAE 80°C, but not to *U. pinnatifida* biomass and in MAE 40C (Fig. 3.6 B) (APPENDIX A 1.7).

Likewise, leaf sizes of *U. pinnatifida* biomass fertilised plants differed to those of compost soil, MAE 60°C, MAE 80°C ($p < 0.001$), while differences were not significant with MAE 40°C, Algardis, and Seasol. Similarly, Algardis was significantly different to MAE 40°C, MAE 60°C, MAE 80°C, and compost (Fig. 3.6 B). Differences were not significant for MAE treatments (Fig. 3.6 B) (APPENDIX A 1.7).

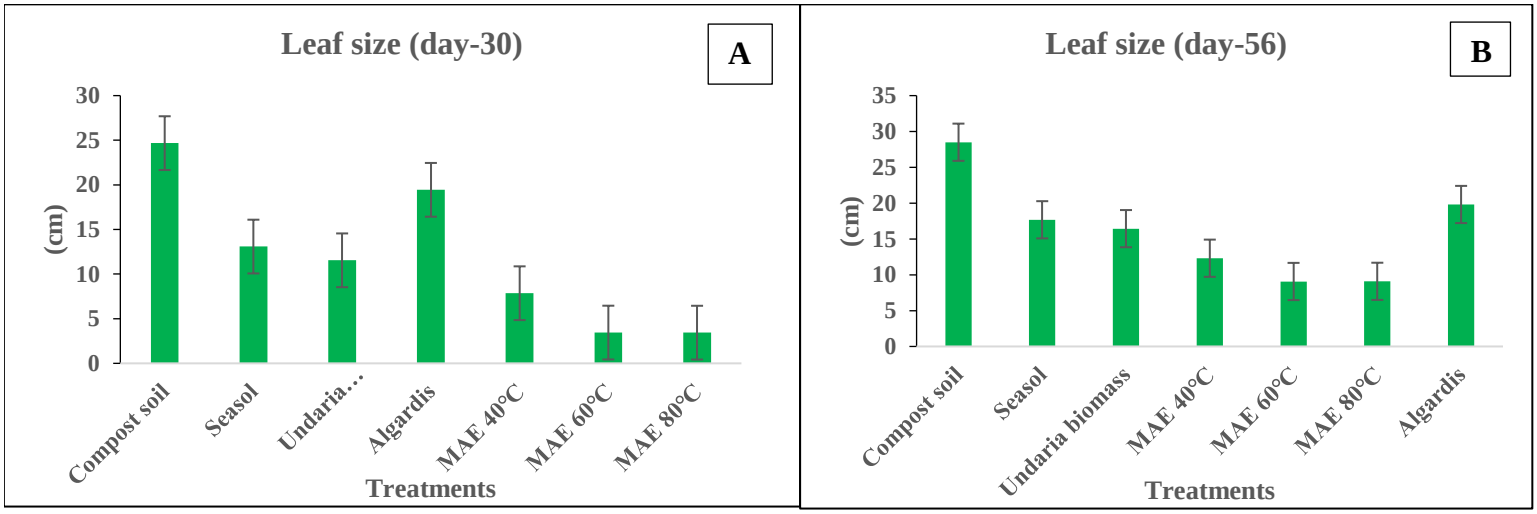


Figure 3.6 Effect of fertiliser treatments on *Sorghum bicolor* leaf size on day 30 (A) and day 56 (B). Standard deviation is shown; n=3

The leaf size was greatest in the *Sorghum bicolor* grown in compost soil, followed by plants grown with Algardis, and *U. pinnatifida* biomass. A one-way ANOVA for effect of fertilisation regime on leaf size of Sorghum bicolor on day 30 and day 56 determined a significant effect ($p = < 0.001$) and for day 30 and 56, a Tukey's HSD test identified that Seasol-grown *Sorghum bicolor* leaf sizes differed from those grown with Algardis, compost soil, MAE 60°C, and MAE 80°C, but there was no significant difference between Seasol with *U. pinnatifida* biomass

There was a significant difference of *U. pinnatifida* biomass with compost soil and Algardis ($p < 0.001$) on day 30, but no significant effect of *U. pinnatifida* MAE 40°C, MAE 60°C, MAE 80°C, and Seasol. and day 30 leaf sizes in treatments fertilised with *U. pinnatifida* biomass differed to those of compost soil, MAE 60°C, MAE 80°C on day 56 ($p < 0.001$)

On day 30, Algardis-treatments were significantly different compared to MAE 40°C, 60°C, and 80°C, Seasol, and *U. pinnatifida* biomass, while there was no significant difference to compost soil ($p = 0.071$). On day 56, however, Algardis-treated plants were significantly different to MAE 40°C, 60°C, and 80°C, and compost. The MAE 40°C, 60°C, and 80°C treatment outcomes were significantly different to compost soil and Algardis on days 30 and 56 ($p = < 0.001$).

3.4.5 Wet and dry weight of above and below ground biomass

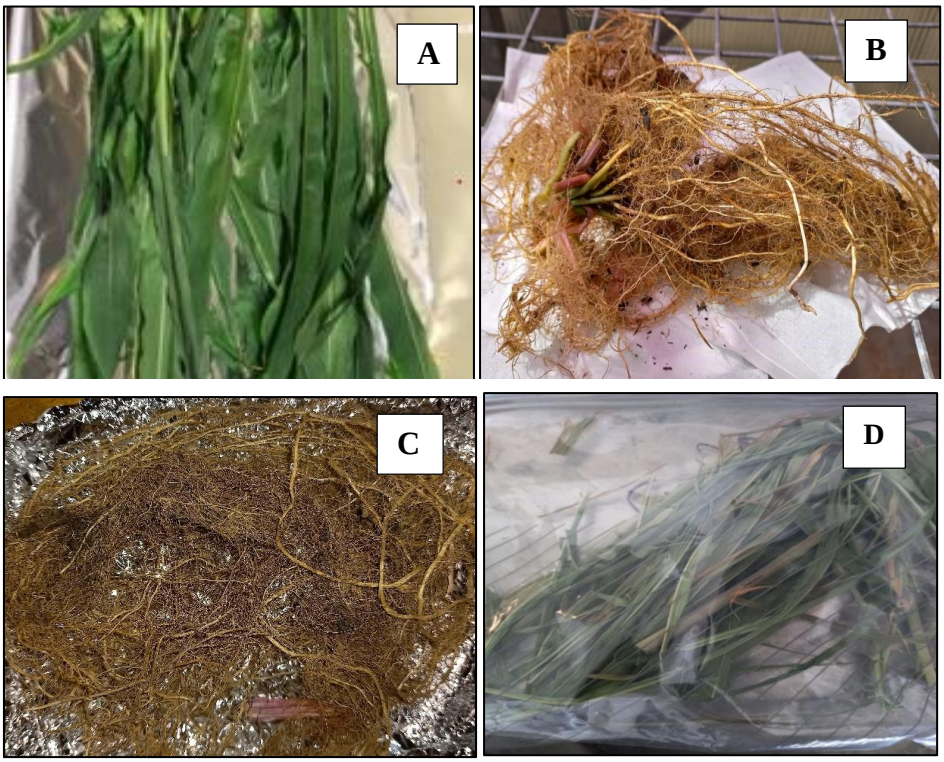


Figure 3.7. Fresh and dry biomass of *Sorghum bicolor*; were A. Fresh leaf, B. Fresh roots, C. Dry roots, and D. Dry leaf

Fresh weight of above and below-ground biomass of *undaria* biomass, and algardis are almost similar in weight. *Undaria* biomass leaf weight (54.12 g), root weight (52.94 g) algardis leaf weight (42.54 g), root weight (41.14 g) (figure 3.9). The highest leaf fresh weight was in compost soil treatments (102.63 g). And the highest root fresh weight was in compost soil and *undaria* biomass treatments (52 g).

In the leaves highest water contain was observed in MAE 60 (75.633%), and 80℃ (75.638%) respectively. In roots highest water contain was observed in *Undaria* biomass (85.379%) (Fig. 3.8).

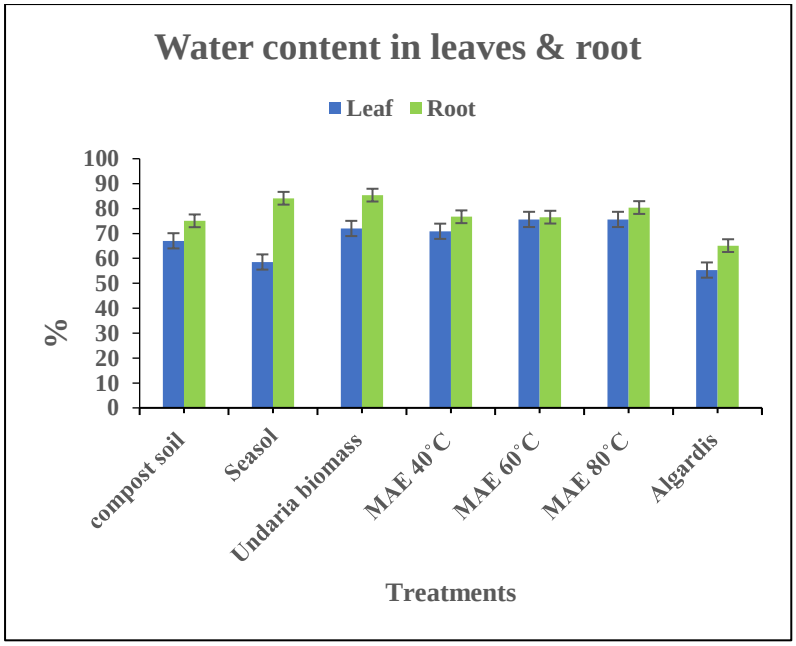


Figure 3.8 Water content in leaves and root of *Sorghum bicolor* on (day-56). Standard deviation is shown; n=3

One-way ANOVA was performed for fresh and dry weight leaf (above and below groun biomass) of the leaves and roots were also measured for all the treatments. One-way ANOVA was performed to determine whether there was a significant difference in fresh leaf weight, and dry leaf weight between the treatments.

Fresh leaf and root weights for all the treatments were normally distributed by using Shapiro Wilk test: $p > 0.05$ and the homogeneity assumption were also met, $F= 0.16$, $p = 0.62$. The one-way ANOVA showed that there were no significant differences in fresh leaf and root weights between the treatments (Fig. 3-9A).

The root and leaf dry weight were also analysed to determine whether there was a significant difference between any two treatments. Root and leaf dry weights were normally distributed for all the treatments (Shapiro-Wilks test: $p > 0.05$) and homogeneity of variance was satisfied for both the leaf dry weight, and root dry weight. The one-way ANOVA results showed that there was no significant mean difference in leaf dry weight, and root dry weight $p > 0.05$ (Fig. 3-9 B).

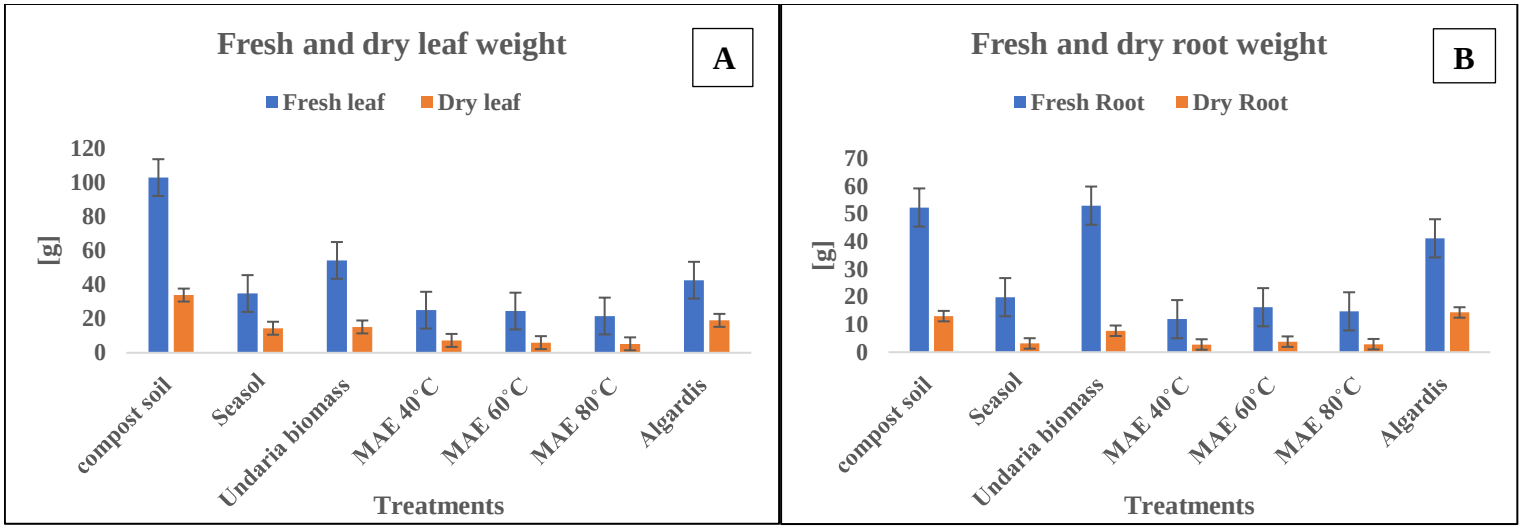


Figure 3.9. Effect of fertiliser treatment on the fresh and dry weight of leaf (A) and root biomass fresh and dry weight (B) (above-ground biomass and below-ground biomass) of *Sorghum bicolor* grown in compost soil, topsoil, and fertilised with Seasol, *U, pinnatifida* biomass, and *Undaria* MAE extracts obtained at 40, 60, and 80°C. Standard deviation is shown; n = 3.

3.5 Water holding capacity

A one-way ANOVA determined that there was significant effect of fertilisation on water holding capacity from day 0 to 56 (excluding the negative control) F= 2.045, (p = 0.050). The homogeneity of variance assumption was also met, F (2.045), p = 0.473. The one-way ANOVA results showed that there was a significant difference in water holding capacity between the treatments. Nonetheless, water holding capacity was highest in treatments fertilised with Compost soil, and more similar extent with MAE 40 and 60°C (Fig. 3.10) (APPENDIX A 1.8).

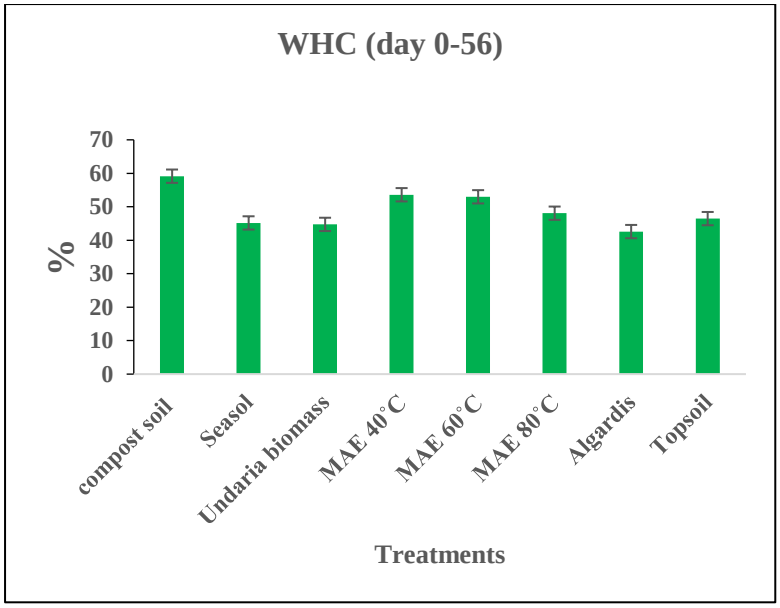


Figure 3.10. Effect of fertiliser treatment on water holding capacity of soil on day 56 for Compost soil, Topsoil, and Seasol, *U. pinnatifida* biomass, MAE 40, 60, and 80°C extracts, and Algardis. Mean ± standard deviation shown. n = 3.

Chapter 4

DISCUSSION

4.1 GENERAL DISCUSSION

Soil is an important source of nutrients for plant growth. Nitrogen (N), phosphorus (P), and potassium (K) are the three major nutrients. They form the trio known as NPK. Calcium, magnesium, and sulfur are also vital nutrients (Fernandes et al. 2017). Plants need only light, water, and up to 20 elements to meet all their biochemical requirements (Fernandes et al. 2017).

Hormones are classified into five types in plants. 1. Auxins- Auxins increase stem elongation and restrict lateral bud growth (maintains apical dominance). They form in the stem, buds, and root tips. Indole Acetic Acid is an example (IA). Auxin is a plant hormone that promotes cell elongation and is produced in the stem tip. Gibberellins- these hormones stimulate stem elongation. They are not made at the stem tip. Gibberellic acid was the first hormone of this type to be found (Wong et al. 2015).

Cell division is promoted by cytokinins. They form in growing places, such as meristems at the tip of the shoot. By suppressing cell development, Abscisic Acid promotes seed dormancy. Ethylene is a gas that mature fruits release. Ethylene is used to ripen crops simultaneously. (Wong et al. 2015).

The primary functions of N and P are that they are components of proteins and nucleic acids, both of which are essential components of plant tissue. K is the only nutrient that is not found in organic plant components but is essential for the regulation of plant processes such as osmosis and enzyme activity (Tang et al. 2018). In general, K has a significant impact on the quality of harvested plant products. Nutrients are available in the soil in several forms that differ in their availability to plants. For example, the majority of nitrogen in the soil is present in organic form as part of organic matter, whereas it can only be taken up in mineral forms (ammonium and nitrate) (Tang et al. 2018).

Before plant roots can absorb organic nitrogen, it must be mineralized into mineral forms. Phosphorus is also found in organic materials in the soil; however, it is usually in chemical forms that vary in solubility and plant availability (Tang et al. 2018). Potassium is primarily present in soil solution and is adsorbed to soil particles such as clay and organic matter, from which it can be desorb relatively quickly due to changes in equilibrium between the surface of soil particles and the soil solution. Plant roots absorb available nutrients from the soil's top layer (Tang et al. 2018).

This study investigated the efficacy of macroalgal fertilisation regime for germination and growth of *Sorghum bicolor*. Fertilisation regimes with *Undaria pinnatifida* biomass, *Undaria* MAEs obtained at 40, 60, and 80°C, and Algardis, a Marinova product derived in this case from *Fucus vesicolosus* from biomass residues following fucoidan extraction, were compared to effects of the commercially available gardening macroalgae containing fertiliser Seasol, compost soil (positive control) and nutrient-poor topsoil. The rationale for testing *Undaria* was it being a good source of iodine, calcium, iron, vitamins A, C, E, D, and K vitamin B2, folate, and omega 3 (Fernandes et al. 2017).

Compost and topsoil N, P, K, and C contents and contents in Algardis, *U. pinnatifida* biomass and the *Undaria* MAEs were determined before seeding day-0 to determine that macroalgal fertiliser amounts were adjusted to the N, P and K content found in compost soil. This left carbon as the only variable between the treatments, excluding the negative control. Increasing soil carbon can help reduce glasshouse gas concentrations in the atmosphere. It also enhances soil quality in a variety of ways, including providing soil structure, storing water and minerals required by plants, and feeding critical soil organisms (Tang et al. 2018).

In contrast, Seasol was used unadjusted as per package instructions 20 g/replicate P (0.12 g/kg), K (1.92 g/kg), N (2.14 g/kg), and C (2.8 g/kg) content. The C content was much higher in *U. pinnatifida* biomass in compare to all other treatments at before seeding day-0. Whereas after 56 days (post-harvest) here was an increased in soil elemental nutritive value after fertilization. The concentration of N, P, K, of compost soil is increased there was a decrease in C content (24.867 g/kg) in unfertilised topsoil on the day 56 (post-harvest) in comparison to C content (41.5 g/kg) before seeding day-0.

There was a decrease in Algardis concentration in N (1.567 g/kg), P (0.5701 g/kg), K (3.228 g/kg), and C (25.33 g/kg) at (post-harvest) in comparison to before seeding day-0 N (4.6 g/kg), P (5.36 g/kg), K (125.92 g/kg), and C (112.89 g/kg). The treatment with *Undaria* biomass has an increase in K and P content on the day 56 (post-harvest) in comparison to before seeding day-0 and a decrease in the concentration of N and C content on the day 56 (post-harvest).

The findings also revealed that the carbon contents also enhanced the growth of sorghum treated with *Undaria* biomass, Algardis and Seasol. There was increase in the carbon soil contents of the Compost soil, Algardis, and Seasol, contrary to what was expected at post-harvest (day 56). The increase in carbon concentration could be due to the initial topsoil and

compost soil samples collected having over surplus carbon contents and could also be due to soil samples obtained having undisturbed humus (Prescott et al. 2010). There was increase in the nitrogen content in soil of the Compost soil at post-harvest (day 56).

The heights nutrient uptake of N content was in *Undaria* biomass (8.33 g/kg), C content in *Undaria* biomass MAE extract 60℃ (152.93 g/kg), K content in *Undaria* biomass (94.12 g/kg), and P content in Algardis (4.78 g/kg). The low nutrient uptake was observed of K content in Compost soil (0.277 g/kg), Topsoil (0.007 g/kg), N content in Seasol (0.77 g/kg), and C content in Unfertilised topsoil (16.63 g/kg). The low nutrient uptake could be due to Environmental factors, Solution concentration, Chelates, Surfactants.

The soil phosphorous and potassium content was also determined after post-harvest (day 56) for all treatments. The findings showed that almost all the phosphorous content was taken up by the sorghum plants treated with Compost soil, *Undaria* biomass, Algardis, and Seasol. This implies that phosphorous contents are necessary for plants growth In line with this study (Malhotra et al. 2018). So, from the growth of compost soil, *Undaria* biomass, Algardis, and Seasol outcome of the study support this (Malhotra et al. 2018).

Plant growth parameter was measured by plant heigh, and leaf size of the sorghum plants. The highest growth was observed with the compost soil, next with Algardis, Seasol, and *Undaria* biomass in sorghum plants. On the other hand, its treated with *Undaria* MAE extracts 40C, 60, and 80℃ were found not in support of sorghum growth to the same extent. It could be due to microwave treatment which transforms natural product composition resulting in change of the quality and activity of the natural product (Hu et al. 2021). So, because of change of the quality and activity of the natural product which directly affected to treatments with *Undaria* MAE extracts 40C, 60, and 80℃.

Furthermore, observed an increase in the development of above and below-ground biomass with the Compost soil, following Algardis, Seasol, and *Undaria* biomass treatments pot. There was found no increase in the development of above -ground biomass when the extract was treated with microwave-assisted extracts. There was increase in below ground biomass development in compared to above -ground biomass.

Yellowing leaves on plants could be a sign of insufficient or excessive water (moisture stress) or nutrients, e.g. magnesium deficiency or oversupply of nitrogn, which can impair plant performance. Magnesium defiance can be another reason for yellow patches between leaf veins

on older leaves. Yellowing caused by nitrogen deficit begins with older leaves and then with new leaves (Fernandes et al. 2017).

4.2 Conclusion

Current information on the possible roles of marine macroalgae in plant growth and improvement is available. In general, marine macroalgae are distinguished by the presence of certain biotechnological components of importance in combine, such as microbicides, biofertiliser, and bio stimulators.

The study aimed to find an alternative for expensive fertiliser use. Based on measured growth parameters, compost soil was best suited to support growth of *Sorghum bicolor*, followed by Algardis, Seasol, and *Undaria* biomass, whilst *Undaria* MAEs inhibited growth.

Fertilisation with compost soil showed the best result, and treatment with *Undaria pinnatifida* biomass and the Algardis liquid extract of the brown macroalga *Fucus vesicolosus* (Marinova) treatments supported growth to the same extent. Seeds of *Sorghum bicolor* sown in the unfertilised topsoil (negative control) did not germinate.

Highest growth of *Sorghum bicolor* was achieved in compost soil, but water holding capacity (WHC) was highest in topsoil fertilised with *Undaria pinnatifida* biomass, 40, 60, and 80^{o}C extracts, and Seasol treatment. The heights nutrient uptake of N content was in *Undaria* biomass (8.33 g/kg), C content in *Undaria* biomass MAE extract 60°C (152.93 g/kg), K content in *Undaria* biomass (94.12 g/kg), and P content in Algardis (4.78 g/kg). The low nutrient uptake was observed of K content in Compost soil (0.277 g/kg), Topsoil (0.007 g/kg), N content in Seasol (0.77 g/kg), and C content in Unfertilised topsoil (16.63 g/kg).

Undaria pinnatifida biomass and Algardis can be best alternative options for too expensive Seasol fertilizer for the cultivation of *Sorghum bicolor*. Whilst in theory, *Undaria pinnatifida* biomass and Algardis can be cost-effective, eco-friendly, and easily available, techno-economic and life cycle analyses are required to demonstrate cost-effectivity and environmental sustainability.

4.3 Future direction

This study contributes to investigate do macroalgae extracts (Algardis), *Undaria pinnatifida* biomass or its MAEs improve the growth *Sorghum bicolor* over the commercially available gardening fertiliser Seasol, and carbon-rich compost soil.

Future research could include NPK fertiliser with macroalgae extracts (Algardis), *Undaria pinnatifida* biomass, Compost soil, to study the effect of fertilisation on the growth of different crop (including water holding capacity, above and below ground biomass fresh and dry weight)

Study needs to conduct soil analysis using pre and post fertilised soil test to clarify whether these N, P, K and C content significantly enhance plant growth

Future study needs to observe effect of fertilisation treatments conducted at same temperature, water level, light intensity, and photo period of light/dark cycle as per present study.

REFERENCES

Atkinson, C 2018, 'How good is the evidence that soil-applied biochar improves water-holding capacity?', *Soil Use and Management*, vol. 34, no. 2, pp. 177-86.

Erulan, V, Soundarapandian, P, Thirumaran, G & Ananthan, G 2009, 'Studies on the effect of Sargassum polycystum (C. Agardh, 1824) extract on the growth and biochemical composition of Cajanus cajan (L.) Mill sp', *American-Eurasian Journal of Agricultural and Environmental Science*, vol. 6, no. 4, pp. 392-9.

Feigin, A & Halevy, J 1989, 'Irrigation-fertilisation-cropping management for maximum economic return and minimum pollution of ground water', in *Research report, Inst. Soil Water, ARO, The Volcani Center, Bet Dagan*.

Fernandes, AM, Gazola, B, Nunes, JGdS, Garcia, EL & Leonel, M 2017, 'Yield and nutritional requirements of cassava in response to potassium fertilizer in the second cycle', *Journal of Plant Nutrition*, vol. 40, no. 20, pp. 2785-96.
Gary Schnitkey, NP, and Krista Swanson 2021, *2021 Fertilizer Price Increases in Perspective, with Implications for 2022 Costs."*, August 3,, farmdoc daily (11):114,.

Hamed, SM, Abd El-Rhman, AA, Abdel-Raouf, N & Ibraheem, IB 2018, 'Role of marine macroalgae in plant protection & improvement for sustainable agriculture technology', *Beni-Suef University Journal of Basic and Applied Sciences*, vol. 7, no. 1, pp. 104-10.

Hao, H, Li, Z, Leng, C, Lu, C, Luo, H, Liu, Y, Wu, X, Liu, Z, Shang, L & Jing, H-C 2021, 'Sorghum breeding in the genomic era: opportunities and challenges', *Theoretical and Applied Genetics*, pp. 1-26.

Hong, DD, Hien, HM & Son, PN 2007, 'Seaweeds from Vietnam used for functional food, medicine and biofertilizer', *Journal of Applied Phycology*, vol. 19, no. 6, pp. 817-26.

Hu, Q, He, Y, Wang, F, Wu, J, Ci, Z, Chen, L, Xu, R, Yang, M, Lin, J & Han, L 2021, 'Microwave technology: a novel approach to the transformation of natural metabolites', *Chinese Medicine*, vol. 16, no. 1, pp. 1-22.

Hurtado, AQ, Yunque, DA, Tibubos, K & Critchley, AT 2009, 'Use of Acadian marine plant extract powder from Ascophyllum nodosum in tissue culture of Kappaphycus varieties', *Journal of Applied Phycology*, vol. 21, no. 6, pp. 633-9.

Jeannin, I, Lescure, J-C & Morot-Gaudry, J-F 2021, 'The effects of aqueous seaweed sprays on the growth of maize', in *Volume 34, 1991*, De Gruyter, pp. 469-74.

Khan, W, Rayirath, UP, Subramanian, S, Jithesh, MN, Rayorath, P, Hodges, DM, Critchley, AT, Craigie, JS, Norrie, J & Prithiviraj, B 2009, 'Seaweed extracts as biostimulants of plant growth and development', *Journal of Plant Growth Regulation*, vol. 28, no. 4, pp. 386-99.

Leandro, A, Pereira, L & Gonçalves, AM 2020, 'Diverse applications of marine macroalgae', *Marine drugs*, vol. 18, no. 1, p. 17.

Mace, ES, Cruickshank, AW, Tao, Y, Hunt, CH & Jordan, DR 2021, 'A global resource for exploring and exploiting genetic variation in sorghum crop wild relatives', *Crop Science*, vol. 61, no. 1, pp. 150-62.

Mazepa, E, Malburg, BV, Mógor, G, de Oliveira, AC, Amatussi, JO, Corrêa, DO, Lemos, JS, Ducatti, DR, Duarte, MER & Mógor, ÁF 2021, 'Plant growth biostimulant activity of the green microalga Desmodesmus subspicatus', *Algal Research*, vol. 59, p. 102434.

Michalak, I & Baśladyńska, S 2021, 'Effect of Fucus extract and biomass enriched with Cu (II) and Zn (II) ions on the growth of garden cress (Lepidium sativum) under laboratory conditions', *Italian Journal of Agronomy*, vol. 16, no. 2.

Michalak, I, Tuhy, Ł & Chojnacka, K 2015, 'Seaweed extract by microwave assisted extraction as plant growth biostimulant', *Open Chemistry*, vol. 13, no. 1.

Mishra, P & Dash, D 2014, 'Rejuvenation of biofertilizer for sustainable agriculture and economic development', *Consilience*, no. 11, pp. 41-61.

Nadeeshani, H, Hassouna, A & Lu, J 2021, 'Proteins extracted from seaweed Undaria pinnatifida and their potential uses as foods and nutraceuticals', *Critical Reviews in Food Science and Nutrition*, pp. 1-17.

Pacheco, D, Cotas, J, Rocha, CP, Araújo, GS, Figueirinha, A, Gonçalves, AM, Bahcevandziev, K & Pereira, L 2021, 'Seaweeds' carbohydrate polymers as plant growth promoters', *Carbohydrate Polymer Technologies and Applications*, vol. 2, p. 100097.

Panja, P 2018, 'Green extraction methods of food polyphenols from vegetable materials', *Current Opinion in Food Science*, vol. 23, pp. 173-82.

Quitain, AT, Kai, T, Sasaki, M & Goto, M 2013, 'Microwave–hydrothermal extraction and degradation of fucoidan from supercritical carbon dioxide deoiled Undaria pinnatifida', *Industrial & engineering chemistry research*, vol. 52, no. 23, pp. 7940-6.

Reganold, JP & Harsh, JB 1985, 'Expressing cation exchange capacity in milliequivalents per 100 grams and in SI units', *Journal of Agronomic Education*, vol. 14, no. 2, pp. 84-90.

Salcedo, MF, Colman, SL, Mansilla, AY, Martínez, MA, Fiol, DF, Alvarez, VA & Casalongué, CA 2020, 'Amelioration of tomato plants cultivated in organic-matter impoverished soil by supplementation with Undaria pinnatifida', *Algal Research*, vol. 46, p. 101785.

Sasikala, M, Indumathi, E, Radhika, S & Sasireka, R 2016, 'Effect of seaweed extract (Sargassum tenerrimum) on seed germination and growth of tomato plant', *International Journal of ChemTech Research*, vol. 9, no. 09, pp. 285-93.

Sathya, B, Indu, H, Seenivasan, R & Geetha, S 2010, 'Influence of seaweed liquid fertilizer on the growth and biochemical composition of legume crop, Cajanus cajan (L.) Mill sp', *Journal of Phytology*, vol. 2, no. 5.

Selvam, GG & Sivakumar, K 2014, 'Influence of seaweed extract as an organic fertilizer on the growth and yield of Arachis hypogea L. and their elemental composition using SEM–Energy Dispersive Spectroscopic analysis', *Asian Pacific Journal of Reproduction*, vol. 3, no. 1, pp. 18-22.

Sharma, N & Singhvi, R 2017, 'Effects of chemical fertilizers and pesticides on human health and environment: a review', *International journal of agriculture, environment and biotechnology*, vol. 10, no. 6, pp. 675-80.

Tang, Z, Xu, W, Zhou, G, Bai, Y, Li, J, Tang, X, Chen, D, Liu, Q, Ma, W & Xiong, G 2018, 'Patterns of plant carbon, nitrogen, and phosphorus concentration in relation to productivity in China's terrestrial ecosystems', *Proceedings of the National Academy of Sciences*, vol. 115, no. 16, pp. 4033-8.

Thaxton, RA 2020, 'THREE. The Peasant Saltmakers' Struggle in Puyang', in *Salt of the Earth*, University of California Press, pp. 82-108.

Vijayanand, N, Ramya, SS & Rathinavel, S 2014, 'Potential of liquid extracts of Sargassum wightii on growth, biochemical and yield parameters of cluster bean plant', *Asian Pacific Journal of Reproduction*, vol. 3, no. 2, pp. 150-5.

Wong, W, Tan, S, Ge, L, Chen, X & Yong, J 2015, 'The importance of phytohormones and microbes in biofertilizers', in *Bacterial metabolites in sustainable agroecosystem*, Springer, pp. 105-58.

Yamanaka, R & Akiyama, K 1993, 'Cultivation and utilization of Undaria pinnatifida (wakame) as food', *Journal of Applied Phycology*, vol. 5, no. 2, pp. 249-53.

Zhuang, P, Wensheng, S, Zhian, L, Bin, L, Jintian, L & Jingsong, S 2009, 'Removal of metals by sorghum plants from contaminated land', *Journal of Environmental Sciences*, vol. 21, no. 10, pp. 1432-7.

APPENDIX A

Appendix A 1.1. Samples provided for quantification of minerals content

No	Sample name	Liquid Sample [mL]	Solid Sample [g]
1	Compost soil	-	20
2	Top soil	-	20
3	*Undaria* MAE extract 40°C	50	4.0761
4	*Undaria* MAE extract 60°C	50	4.1793
5	*Undaria* MAE extract 80°C	50	3.4188
6	*Undaria* biomass	-	10
7	Algardis liquid extract	15	1.673

Appendix A 1.2. Treatments as fertilization for greenhouse pot trial

	Dry Weight of 300g biomass extract [g]	[N] requirement [g]/pot	Factor [g]	MAE extract need to add mL/Pot	Water mL/pot	P undersupply requirement mL/pot	K undersupply mL/pot	NPK [g]/pot	Total volume/pot (MAE extract mL + P + K + water mL)	Seeds/pot	Algardis
40˚C	4.0761	31.70192308	0.128576	388.9	591.1	10	10	No	1000	9	No
60˚C	4.1793	32.97	0.126761	394.44	585.56	10	10	No	1000	9	No
80˚C	3.4185	35.83695652	0.09539	524.16	455.84	10	10	No	1000	9	No
Algardis	1.673	17.91	0.093412	No	518.27	No	No	No	1000	9	481.73
Positive control (compost soil)	0.02	No	No	No	1000	No	No	No	1000	9	No
Negative control (Topsoil)	0.02	No	No	No	1000	No	No	No	1000	9	No
Seasol	No	No	No	No	1000	No	No	20	1000	9	No
***Undaria* biomass**	8.6	No	No	No	980	10	10	No	1000	9	No

Appendix A 1.3.

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
K	Based on Mean	.837	7	16	.573
	Based on Median	.144	7	16	.993
	Based on Median and with adjusted df	.144	7	10.243	.991
	Based on trimmed mean	.754	7	16	.632
P	Based on Mean	6.259	7	16	.001
	Based on Median	1.044	7	16	.440
	Based on Median and with adjusted df	1.044	7	5.361	.494
	Based on trimmed mean	5.556	7	16	.002
N	Based on Mean	3.497	7	16	.018
	Based on Median	2.889	7	16	.037
	Based on Median and with adjusted df	2.889	7	2.486	.240
	Based on trimmed mean	3.463	7	16	.019
C	Based on Mean	4.426	7	16	.007
	Based on Median	1.984	7	16	.121
	Based on Median and with adjusted df	1.984	7	3.037	.306
	Based on trimmed mean	4.233	7	16	.008

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
K	Between Groups	2.897	7	.414	4.319	.007
	Within Groups	1.533	16	.096		
	Total	4.430	23			
P	Between Groups	2.492	7	.356	2.919	.036
	Within Groups	1.952	16	.122		
	Total	4.444	23			
N	Between Groups	25.013	7	3.573	6.883	<.001
	Within Groups	8.307	16	.519		
	Total	33.320	23			
C	Between Groups	3415.658	7	487.951	6.082	.001
	Within Groups	1283.667	16	80.229		
	Total	4699.325	23			

ANOVA Effect Sizes[a,b]

		95% Confidence Interval
		Lower
K	Eta-squared	.096
	Epsilon-squared	-.299
	Omega-squared Fixed-effect	-.283
	Omega-squared Random-effect	-.033
P	Eta-squared	.000
	Epsilon-squared	-.437
	Omega-squared Fixed-effect	-.412

	Omega-squared Random-effect	-.043
N	Eta-squared	.279
	Epsilon-squared	-.036
	Omega-squared Fixed-effect	-.035
	Omega-squared Random-effect	-.005
C	Eta-squared	.229
	Epsilon-squared	-.109
	Omega-squared Fixed-effect	-.104
	Omega-squared Random-effect	-.014

a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model.

b. Negative but less biased estimates are retained, not rounded to zero.

K

Treatments	N	Subset for alpha = 0.05

			1	2
Tukey HSD[a]	Compost Soil	3	2.868026471189023	
	Undaria Biomass	3	2.883195874029786	
	Algardis	3	3.228650666242922	3.228650666242922
	MAE Extract 40C	3	3.346761239146503	3.346761239146503
	Top Soil	3	3.377868823292981	3.377868823292981
	MAE Extract 60C	3	3.445967089478796	3.445967089478796
	Seasol	3	3.498000552952016	3.498000552952016
	MAE Extract 80C	3		4.035906049096089
	Sig.		.265	.082

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

P

			Subset for alpha = 0.05	
	Treatments	N	1	2
Tukey HSD[a]	Top Soil	3	.373253625907659	
	Seasol	3	.468160633221336	
	Algardis	3	.570130004675929	.57013000467592 9
	MAE Extract 60C	3	.656345065157756	.65634506515775 6
	MAE Extract 80C	3	.668195456532466	.66819545653246 6
	Undaria Biomass	3	.779605601700966	.77960560170096 6
	MAE Extract 40C	3	1.000150533475528	1.000150533475528
	Compost Soil	3		1.455880116982458
	Sig.		.401	.096

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

N

	Treatments	N	Subset for alpha = 0.05 1	2
Tukey HSD[a]	MAE Extract 60C	3	1.000000000000000	
	MAE Extract 80C	3	1.200000000000000	
	Undaria Biomass	3	1.266666666666667	
	Seasol	3	1.366666666666667	
	Algardis	3	1.566666666666667	
	Top Soil	3	1.600000000000000	
	MAE Extract 40C	3	1.633333333333334	
	Compost Soil	3		4.400000000000000
	Sig.		.953	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

C

	Treatments	N	Subset for alpha = 0.05 1	2
Tukey HSD[a]	MAE Extract 60C	3	14.766666666666666	
	MAE Extract 80C	3	18.599999999999994	
	Seasol	3	19.200000000000000	
	Undaria Biomass	3	22.900000000000006	
	TopSoil	3	24.866666666666664	

	Algardis	3	25.333333333333332	
	MAE Extract 40C	3	25.733333333333334	
	Compost Soil	3		56.000000000000000
	Sig.		.797	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Appendix A 1.4

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Plant height 30 day (cm)	Based on Mean	8.090	6	78	<.001
	Based on Median	5.032	6	78	<.001
	Based on Median and with adjusted df	5.032	6	39.363	<.001
	Based on trimmed mean	7.562	6	78	<.001

ANOVA

Plant height 30 day (cm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15546.624	6	2591.104	10.079	<.001
Within Groups	20053.188	78	257.092		
Total	35599.812	84			

ANOVA Effect Sizes[a]

		Point Estimate	95% Confidence Interval Lower	Upper
Plant height 30 day (cm)	Eta-squared	.437	.230	.530
	Epsilon-squared	.393	.170	.494
	Omega-squared Fixed-effect	.391	.169	.491
	Omega-squared Random-effect	.096	.033	.138

a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model.

Plant height (cm)

	Treatments	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a,b]	MAE Extract 80	6	7.667		
	MAE Extract 60C	6	8.667		
	MAE Extract 40C	8	15.750	15.750	
	Undaria biomass	21	24.929	24.929	
	Seasol	14	29.071	29.071	29.071
	Algardis	10		37.000	37.000
	Compost soil	20			48.950
	Sig.		.064	.068	.107

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.624.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix A 1.5

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Plant Height 56 day (cm)	Based on Mean	8.013	6	109	<.001
	Based on Median	7.123	6	109	<.001
	Based on Median and with adjusted df	7.123	6	66.599	<.001
	Based on trimmed mean	7.992	6	109	<.001

ANOVA

Plant Height 56 day (cm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21554.602	6	3592.434	12.391	<.001
Within Groups	31602.363	109	289.930		
Total	53156.966	115			

ANOVA Effect Sizes[a]

		95% Confidence Interval	
	Point Estimate	Lower	Upper
Eta-squared	.405	.235	.493

Plant Height 56 day (cm)	Epsilon-squared	.373	.193	.465
	Omega-squared Fixed-effect	.371	.192	.463
	Omega-squared Random-effect	.089	.038	.126

a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model.

Plant Height

			Subset for alpha = 0.05			
	Treatments	N	1	2	3	4
Tukey HSD[a,b]	MAE Extract 80C	11	15.82			
	MAE Extract 60C	12	17.08	17.08		
	MAE Extract 40C	14	23.14	23.14	23.14	
	Undaria biomass	23		35.04	35.04	
	Seasol	18			39.22	39.22
	Algardis	16			39.94	39.94
	Compost Soil	22				57.36
	Sig.		.894	.060	.098	.056

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 15.464.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix A 1.6

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Leaf size30 day (cm)	Based on Mean	29.041	6	388	<.001
	Based on Median	21.287	6	388	<.001
	Based on Median and with adjusted df	21.287	6	252.733	<.001
	Based on trimmed mean	27.508	6	388	<.001

ANOVA

Leaf size (cm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21315.271	6	3552.545	24.058	<.001
Within Groups	57293.428	388	147.663		
Total	78608.699	394			

ANOVA Effect Sizes[a]

		Point Estimate	95% Confidence Interval Lower	Upper
Leaf size 30 day (cm)	Eta-squared	.271	.190	.330
	Epsilon-squared	.260	.178	.320
	Omega-squared Fixed-effect	.259	.177	.319
	Omega-squared Random-effect	.055	.035	.072

a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model.

Leaf size 30 day (cm)

			Subset for alpha = 0.05			
	Treatments	N	1	2	3	4
Tukey HSD[a,b]	MAE Extract 80C	17	3.735			
	MAE Extract 60C	20	3.975			
	MAE Extract 40C	25	8.780	8.780		
	Undaria Biomass	97	12.273	12.273	12.273	
	Seasol	70		13.136	13.136	
	Algardis	57			20.640	20.640
	Compost Soil	109				26.275
	Sig.		.054	.745	.063	.456

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 34.976.
b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix A 1.7

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Leaf size 56 day	Based on Mean	29.404	6	665	<.001
	Based on Median	22.388	6	665	<.001
	Based on Median and with adjusted df	22.388	6	463.965	<.001
	Based on trimmed mean	27.094	6	665	<.001

ANOVA

Leaf size 56 day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29349.938	6	4891.656	32.085	<.001
Within Groups	101384.365	665	152.458		
Total	130734.303	671			

ANOVA Effect Sizes[a]

		Point Estimate	95% Confidence Interval	
			Lower	Upper
Leaf size 56 day	Eta-squared	.225	.166	.271
	Epsilon-squared	.218	.159	.264
	Omega-squared Fixed-effect	.217	.158	.264
	Omega-squared Random-effect	.044	.030	.056

a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model.

Leaf size 56 day

	Treatments	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a,b]	MAE Extract 60C	53	9.066			
	MAE Extract 80C	51	9.088			
	MAE Extract 40C	66	12.311	12.311		
	Undaria Biomass	127		16.437	16.437	
	Seasol	111		17.676	17.676	
	Algardis	97			19.809	
	Compost Soil	167				28.533
	Sig.		.637	.086	.593	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 80.638.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix A 1.8

Tests of Homogeneity of Variances

			Levene Statistic	df1	df2	Sig.
WHC 0-56 DAY		Based on Mean	.937	7	256	.478
		Based on Median	.944	7	256	.473
		Based on Median and with adjusted df	.944	7	215.845	.474
		Based on trimmed mean	.948	7	256	.470

ANOVA

WHC 0-56 DAY

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.729	7	.104	2.045	.050
Within Groups	13.041	256	.051		
Total	13.770	263			

ANOVA Effect Sizes[a,b]

			Point Estimate	95% Confidence Interval Lower	Upper
WHC 0-56 DAY		Eta-squared	.053	.000	.089
		Epsilon-squared	.027	-.027	.064
		Omega-squared Fixed-effect	.027	-.027	.064
		Omega-squared Random-effect	.004	-.004	.010

a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model.

WHC 0-56 DAY

	Treatments	N	Subset for alpha = 0.05 1
Tukey HSD[a]	Algardis	33	.425851851851852
	Undaria biomass	33	.447393939393939
	Seasol	33	.451727272727273
	TopSoil	33	.464649831649831
	MAE Extract 80 C	33	.480774410774411
	MAE Extract 60 C	33	.529840067340067
	MAE Extract 40C	33	.535936026936027
	Compost soil	33	.591405723905724
	Sig.		.062

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 33.000.