

**CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE**

**Faculty of Tropical AgriSciences**



**Investigation of appropriate disposal methods of  
seagrass biomass with the focus on *Posidonia  
oceanica***

MASTER'S THESIS

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# Declaration

I hereby declare that I have done this thesis entitled “Investigation of appropriate disposal methods of seagrass biomass with the focus on *Posidonia oceanica*” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 22<sup>nd</sup> of April 2023

.....

Bc. Jan Faktor

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## Abstract

Seagrass meadows are crucial components of the Mediterranean's local ecosystems and greatly influence its productivity and functionality. They provide essential services to the ecosystem, such as oxygen production, fish shelter, and protection of the seafloor and sea banks from erosion. Unfortunately, a large amount of seagrass necromass accumulates on the shores of Mediterranean countries which are usually attractive tourist summer destinations. This seagrass residue can cause several problems if it is not managed in such touristic areas, potentially affecting the environment, social aspects, hygiene, and economy of the areas. Preventive beach management leads to high removal costs and improper disposal. Therefore, it is necessary to find appropriate disposal methods and provide solutions for seagrass biomass. As biomass is a popular source of alternative energy, it could be a feasible option to reduce disposal costs and at the same time benefit from energy production. The crucial question is how to effectively enhance the material's properties and characteristics to achieve better outcomes in solid biofuel production. Mainly as the initial untreated material has high ash content (27.30% d.b. for *Posidonia oceanica* (*Po*) and 33.60% d.b. in a mix of *Zostera noltii* and *Cymodocea nodosa* (*ZoCy*)) and low gross calorific value (12.86 MJ/kg d.b. in *Po* and 11.87 MJ/kg d.b. for the mixture). This research conducted a primary treatment test by sieving and water washing, followed by a major water treatment trial to evaluate the impacts on these key fuel-energy properties. Further trials focused on calorific value enhancements through the creation of biochar with torrefaction was also performed. The treatment trials were highly successful in improving the original properties of biomass by a significant amount, e.g., a reduction of ash content to 11.50 % d.b. for *Po* was achieved after 1 h of tap water treatment and furthermore, an increase of gross calorific of *Po* elevated to 22.32 MJ/kg d.b. by torrefaction at 350°C. Suggesting that *Posidonia oceanica* could be a suitable material for solid biofuel production.

**Key words:** *Posidonia oceanica* reuse, solid biofuels, torrefaction, sustainable waste management, water treatment, fuel-energy properties

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## List of the abbreviations used in the thesis

°C - Degrees Celsius	K - Potassium
AC - Ash content	kg/m <sup>3</sup> - Kilogram per cubic meter
AD - Anaerobic digestion	KOH - Potassium hydroxide
C: N - Carbon: Nitrogen Ratio	kW - Kilowatt
CeO <sub>2</sub> - Cerium dioxide	L - Litre
CHN - Carbon Hydrogen Nitrogen	LC - Least Concern
cm - Centimetre	LHV - Lower heating value
<i>Cy</i> - <i>Cymodocea nodosa</i>	MJ/kg - Megajoules per kilogram
d.b. - Dry basis	NCV - Net Calorific Value
DT - Deformation temperature	Nm <sup>3</sup> /kg - Normal cubic metre per kilogram
FT - Fluid temperature	NO <sub>x</sub> - Nitrogen oxides
g - Gram	pH - Potential of hydrogen
g/L - Gram per litre	<i>Po</i> - <i>Posidonia oceanica</i>
GCV - Gross calorific value	SB - Seagrass biomass
GDP - Gross domestic product	SO <sub>x</sub> - Sulphur oxides
H - Hydrogen	ST - Shrinkage starting temperature
H <sub>2</sub> O <sub>2</sub> - Hydrogen peroxide	TL13 - strain's polysaccharide-degrading enzymes
H <sub>2</sub> BO <sub>3</sub> - Boric Acid	VM - Volatile matter
H <sub>3</sub> PO <sub>4</sub> - Phosphoric acid	w.b. - Wet basis
HF - Hydrogen fluoride	wt% - Percentage by weight
HHV - Higher heating value	Zo - <i>Zostera Noltii</i>
HM - Heavy metal	<i>ZoCy</i> – Mixture of <i>Zostera noltii</i> and <i>Cymodocea nodosa</i>
HNO <sub>3</sub> - Nitric acid	
HT - Hemisphere temperature	
IUCN - The International Union for Conservation of Nature	

# 1. Introduction and Literature Review

Worldwide availability and distribution of algae and seaweed provides many ecosystem services to the world's oceans and brings many benefits to the local environments. Seagrasses, algae, and other aquatic species of flora are a vitally important part of the surrounding ecosystems. Supporting countless species of local fauna throughout their lifecycles, creating a safe, nutritious, and prosperous environment (Green & Short 2003; Boudouresque et al. 2012; IUCN 2016).

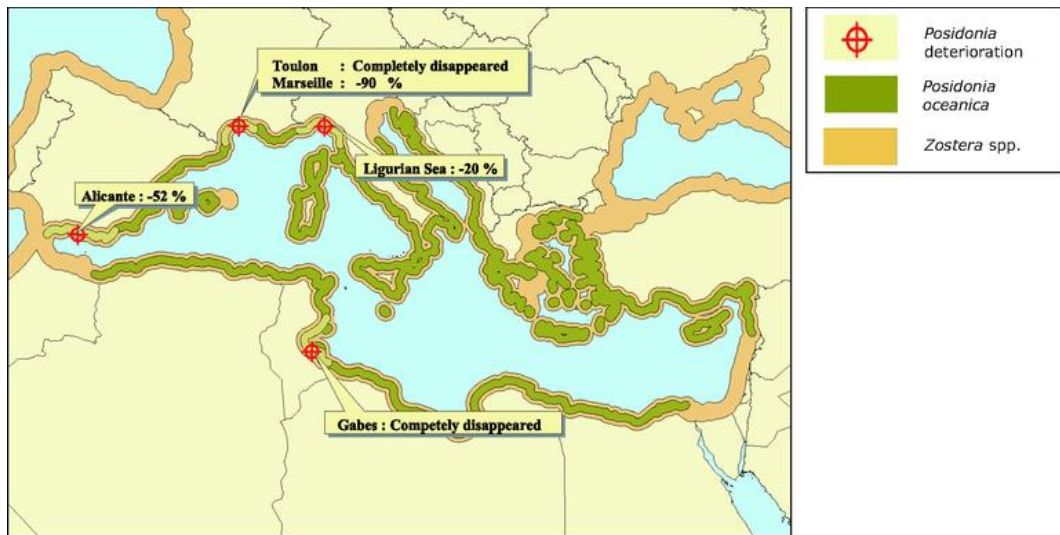
These benefits are numerous and some of the positive impacts on the local aquatic fauna and flora include the stabilisation of fine sediment, where leaves and roots help stabilise it, which can result in better water quality; Provision of shelter to various species of crustaceans, fish and many others, to whom it serves as spawning grounds and nurseries as well, protecting them from predators, rough weather and fisheries; it serves as feeding grounds for numerous species, providing nutrition for many levels from plankton to marine mammals, resulting in these areas becoming biodiversity hotspots which can be seen in Figure 1; Furthermore, these meadows are very important as they can photosynthesise and therefore serve as a carbon sink and produce oxygen (Green & Short 2003; Coccozza et al. 2011b; Boudouresque et al. 2012; Voca et al. 2019).



**Figure 1 Thriving *Posidonia* ecosystem**

Source: Author (2022)

*Posidonia oceanica* (*Po*), commonly called Neptune grass, is an endemic seagrass species found in the coastal waters of the Mediterranean Sea as can be seen in Figure 2, where even the presence of *Zostera* spp. is shown. In the figure it can be seen that the distribution is declining, and this can be caused by anthropogenic factors (Boudouresque et al. 2012).



**Figure 2** *Po* and *Zostera* spp. distribution and its deterioration

Source: European Environment Agency (2009)

Even though *Po* has been recorded within the Least Concern (LC) group in the last assessment from 2013 by the IUCN Red List, there are many national and multilateral (European Union) efforts to protect and safeguard this species including it in many laws as strictly protected species (Green & Short 2003; European Environment Agency 2009; Boudouresque et al. 2012; IUCN 2016). This fact could potentially create obstacles in the reuse of *Po* biomass.

Even though the algae and seagrasses are extremely important to its environments it is important to note that in certain cases these can cause numerous inconveniences, where high biomass production can influence the local ecosystems even in bad ways. These can be represented by high biomass output in specific months affecting the local coastline as most of it gets covered by large quantities of algae or seagrass residues of leaves, stems and other parts that are separated from the main body of the plant. Such biomass accumulation can result in affecting the whole communities, ranging from social, and ecological to economic impacts (Cocozza et al. 2011b; Voca et al. 2019).

As this seems to be an unsolved issue in many cases, which represents and will continue to represent significant challenges for the coastal communities. Yet it does not involve sustainable options for biomass use, meanwhile, tonnes of algae and seagrass covering coastlines are simply dumped and remain unused, losing a big potential in giving the material a second life and reusing the matter to improve the economic viability of the removal process. Solutions for the use, proper disposal and recycling of algae/seagrass biomass need to be developed to efficiently combat such cases when biomass accumulations affect the local coastline and imbalanced ecosystems (Cocozza et al. 2011a; Balata & Tola 2018; De Sanctis & Di Iaconi 2019; Voca et al. 2019).

Sustainable solutions might be the best way to follow in the future as these could present a viable pathway for proper disposal without losing the potential that the material has to offer. These methods can range from co-composting (Peruzzi et al. 2020), pyrolysis, co-pyrolysis (Conesa & Domene 2015), bio-oil production, bio-gas production (Dębowski et al. 2013; De Sanctis et al. 2019) various solid biofuels as well as classic liquid ones (Balata & Tola 2018) continuing to the possibility of the hydrogen production, which could be for the many the fuel of the future (Deniz et al. 2015).

## **1.1. *Posidonia oceanica* biomass**

As mentioned previously, this chapter will focus specifically on the possible uses of biomass from the most widely distributed Mediterranean seagrass, *Po oceanica*, which creates numerous issues along the coastline of many Mediterranean states such as Italy, Croatia, Greece, Cyprus, Turkey, France and Spain, which can be mainly felt in the cases of touristic destinations such as the one in Figure 3.



**Figure 3 Necromass-ridden coastline**

Source: Author (2021)

As can be observed in the Figure 3 above seagrass accumulation presents a significant challenge for local tourism, making many beaches not suitable for bathing (Voca et al. 2019). This is mainly caused by a bad odour that comes hand in hand with other unpleasant factors as physical pollution of the beaches due to the accumulation of dead leaves. The amounts of the leaves are season dependent and can vary throughout the months as well, which is mainly weather-dependent (Cocozza et al. 2011b; Voca et al. 2019). A one-kilometre-wide belt of *Po* meadows can annually produce approximately 125 kg of dry matter of biomass per meter of coastline each year (Voca et al. 2019). Additionally, Cocozza et al. (2011b) states that with an approximate calculation of removed material annually 900 metric tonnes are removed and taken to an authorised deposit. Furthermore, the costs can be estimated at 180,000 € annually.

One of the main issues of the problem itself is the inappropriate disposal of the matter, which causes further problems in the proximity of the beaches with higher accumulation levels resulting in the creation of dumping sites, where most of the biomass is transferred to clean the beach so it becomes more appealing again (Voca et al. 2019). To prevent these, mostly illegal, dumps from being created a strong need for the creation

of a proper framework and appropriate disposal and reuse methods are needed. This tendency in behaviour is visible in Figure 4.



**Figure 4** Illegal disposal place for *Po* and other waste

Source: Author (2021)

Furthermore, an explanation of why these methods is needed and how they could help in solving the situation is needed. The presence of accumulated *Po* biomass on the beaches creates countless problems for the communities that are focused on tourists, where most of the GDP is generated from tourism. Starting from the smell, and bad appearance as in some eyes it evokes the dirtiness of the water, however, opposite is true as *Po* is a water clarity bioindicator (Voca et al. 2019), which mainly affects the local touristic infrastructure as hotels in the proximity of affected beaches cannot compete with those that remain unaffected, therefore resulting even in economic impacts. In addition, those economic problems can develop into social issues. Another impact is hygienic, where the degradation process of the biomass creates unfavourable conditions for the beachgoers. A common goal would be to dispose of the material in such a manner that it would at the same time create revenue and therefore reduce the costs for biomass removal. This can be achieved through the reuse of the matter either through energy production or the production of other products (Cocozza et al. 2011b; Voca et al. 2019).



## **1.2. Energy use - biofuels**

### **1.2.1. Solid biofuels**

One of the possible uses for the *Po* biomass is pyrolysis, co-pyrolysis - in case another material is added, combustion and gasification. Voca et al. (2019) suggest that the biomass is highly available, cheap to obtain and has positive characteristics of high lignin contents, could be considered a favourable material due to low levels of sulphur and nitrogen and has higher gross calorific value (GCV) comparable to other lignocellulosic biomass (Chiodo et al. 2016; Voca et al. 2019), however, it has limitations as well for example high ash contents which are accompanied by high carbon content. These results can be further extended by the findings of Plis et al. (2014) who suggest that the use of *Po* as feedstock for biorefinery is recommended as no poisonous gases are created ( $\text{SO}_x$ ,  $\text{NO}_x$ ). Adding the highest rates of released gaseous compounds are observed within the range of 500-700 K and 900-1050 K.

Bio-oil and bio-char production from biomass has been compared with two other biomasses (white pine, and *Lacustrine alga*) using a fixed bed reactor and temperatures within 400-600 °C in the study of Chiodo et al. (2016). Suggesting that the yields of biochar and bio-oil produced by *Po* are comparable with those obtained by woody biomass. Furthermore, suggesting that bio-char products are very interesting for soil application due to their characteristics of stability (comparable to White-Pine) and alkalinity of pH = 10.75. On the other side, bio-oils resulted to have a low GCV (19.0–30.1 MJ/kg).

Furthermore, this subchapter will be mainly included in the discussion as it is the main focus of this work, minor results that we obtained during our research for my master's thesis, where it was confirmed that *Po* biomass pellets reach similar GCV values as other lignocellulosic materials. We have tested the biomass in the form of pellets and briquettes and the GCV ranged around 11.0–13.1 MJ/kg. In the Figure 5, found below, you can find the finished analytical samples, which have gone through processing, and raw material in the desiccation chamber to prevent a moisture build-up.



**Figure 5** Different *Po* samples

Source: Author (2023)

## **1.2.2. Liquid biofuels**

### **1.2.2.1. Bioethanol production**

A handful of articles focused on the potential of SB as a liquid biofuel in two main forms. Firstly, bioethanol production (Pilavtepe et al. 2013; Kyriakou et al. 2018; Souii et al. 2022) was considered and secondly, bio-oil was studied (Maisano et al. 2017).

Within the first study focusing on bioethanol production researchers aimed to find a solution to the problem with SB by exploring the potential of *Po* waste as a source of biofuel. They collected SB waste from the western coast of Turkey and used diluted sulfuric acid and cellulase to break it down using hydrolysis. The study found that *Po* waste has the potential to be a source of bioethanol, with a bioethanol yield of 62.3% based on sugar consumption and a productivity of 0.46 kg/m<sup>3</sup> h in flasks and 0.76 kg/m<sup>3</sup> h in a 2 L bioreactor, using consecutive enzymatic and acid hydrolysis (Pilavtepe et al. 2013).

The TL13 strain's polysaccharide-degrading enzymes were found to be highly effective in breaking down untreated *Po* waste into fermentable sugars, more so than commercial enzymes. This led to a bioethanol production that reached 33.35% of the theoretical maximum yield. These results suggest that *Microbacterium* biocatalysts have great potential as eco-friendly tools for seagrass-based biorefinery, based on both genomic and experimental findings. The degradation of plant cell walls by extremophilic bacteria represents an interesting biological process with potential applications in generating biofuels from agricultural wastes. However, a significant challenge in this process is the breakdown of the cell wall. In recent experiments, the effectiveness of this process was demonstrated in generating bioethanol from *Po* waste, with a yield of 33.35% of the theoretical maximum and a concentration of 2.28 g/L (5.7 g ethanol/100 g of waste). The results suggest that the utilization of extremophilic bacteria for plant cell wall degradation could hold significant promise in the production of biofuels from agricultural wastes (Souii et al. 2022).

The results of this study indicate that the physicochemical properties of biochar are heavily influenced by both the type of feedstock and the temperature used during pyrolysis. Specifically, a temperature of 500°C led to a higher specific surface area and porosity compared to 250°C. Preliminary fermentations revealed that biochar shows great promise as a support material to enhance bioethanol production using *Saccharomyces cerevisiae*. The study will further explore the use of different biochar samples derived from vineyard prunings and sea grass to ferment three yeast strains, while also examining the adsorption capacity of each biochar (Kyriakou et al. 2018).

### **1.2.2.2. Biooil production**

The study of Maisano et al. (2017) focused on bio-oil production through catalytic pyrolysis, which resulted in confirming that *Po* biomass might be suitable and promising material for fast catalytic pyrolysis to produce renewable fuels and chemical feedstocks. Showing that its high carbon content and low decomposition temperatures could be suitable. Reaching the highest yields at 500 °C when Ni/CeO<sub>2</sub> is added as a catalyst.

The research aimed to investigate the possibility of producing high-quality bio-oil from the Mediterranean Sea plant using a catalytic pyrolysis process. Experiments were conducted using *Po* at 500 °C in a fixed bed reactor to analyse the impact of different "home-made" Ni, Ce, and HZSM-5-based catalysts in terms of conversion yield, bio-oil

yield, and bio-oil oxygen and organic compounds content. The highest liquid yields were obtained at 500 °C, with CeO<sub>2</sub>, NiCe/HZSM-5, and Ni/ CeO<sub>2</sub> being the most effective catalysts. The oxygen level in bio-oil was decreased, and the hydrocarbon content was increased, with CeO<sub>2</sub>, Ni/CeO<sub>2</sub>, and NiCe/HZSM-5 catalysts, which promoted deoxygenation reactions, including dehydration, decarboxylation, and decarbonylation. The results indicated that catalytic pyrolysis of *Po* could produce high-quality bio-oil, particularly using zeolite and ceria-based catalysts (Maisano et al. 2017).

### **1.2.3. Gaseous biofuels**

#### **1.2.3.1. Anaerobic digestion**

Several studies focused on the use of algae as a potential substrate for biogas production through anaerobic digestion (Dębowski et al. 2013; Marquez et al. 2013; Ward et al. 2014; Misson et al. 2020), however, only a handful of articles mentioned or explored specifically the option of using *Po* as a substrate for biogas production (De Sanctis et al. 2019; De Sanctis & Di Iaconi 2019; Renzi et al. 2022). The current scientific findings were combined to assess marine biomass and create propositions.

Dębowski et al. (2013) suggest that algae biomass might be a suitable option for use in biogas production technologies. The impacts of the methane fermentation process should be directly impacted by anaerobic bacteria and cell wall degradation, hence experimental and implementation efforts should concentrate on technologies for pre-treating and conditioning algal biomass. Creating a suitable multipurpose feedstock that is suitable for numerous forms of biofuel production. Suggesting that biogas production might be the most viable option of them all to use its full potential. However, this might be dependent on the chosen technology used for methane production, therefore, this must be taken into consideration when choosing the best approach. One of the biggest challenges in methane production from algae will be finding an appropriate C: N ratio to make the reactor work. Interestingly a combination of algae production, wastewater treatment and AD could be potentially viable, however, this is not suitable for our research as our main focus is on seagrass necromass.

In addition to that, the study of Ward et al. (2014) suggests that a further understanding of algae biomasses for methane production will be needed as each species acts differently. Adding that algae-based wastewater treatment could be a possible

solution as well, however, biogas production must be the most focused topic as it has the highest potential. Furthermore, there are obstacles presented in the case of pre-treatment of the biomass because pure biomass would cause many technical difficulties ranging from low concentrations of biodegradable matter and cell wall thickness. Therefore, a better understanding of algae species is needed to improve the technologies applied.

Marquez et al. (2013) explored the potential of a combination of sea wrack biomass and various microbial seeds such as cow dung, marine sediment or microflora and found out that cow manure is the least suitable seed that has a very low methane yield, with the higher one achieved with marine sediment, however, it does not amount to the calculated potential, which might be significantly impacted by the salinity of the material.

Misson et al. (2020) & Renzi et al. (2022) suggest the reuse of SB instead of dumping it in landfills and one of the solutions mentioned in this article is anaerobic digestion. This has been further trialled by (De Sanctis et al. 2019; De Sanctis & Di Iaconi 2019) where it has been confirmed in both cases, that the energy retrieval process through AD in mesophilic conditions is inefficient, as the yield is very low, mainly because of the high lignin content in *Po*, however adding that chemical pretreatment could be a potential solution as it caused a steep increase in methane production (De Sanctis & Di Iaconi 2019).

Furthermore, a study focusing on efficiency improvement in AD of *Po* has been carried out and concluded that the process of thermal hydrolysis and AD to achieve elevated methane production from *Po* is not an efficient process, stating that the input of energy will always be 8-10 times higher than the amount that is produced. Therefore, it is not suggested to be used in the future. On the other hand, acidic thermal hydrolysis and AD could produce surplus energy of 22-35% (De Sanctis et al. 2019).

Lastly, Misson et al. (2020) studied a variety of different species of SB in the northern Adriatic Sea in Italy and their main findings concluded that washed seagrass had achieved significantly higher yields (by 94%) compared to the dry matter of the SB. Furthermore, it pointed out an important finding that the presence of HM in the digestate after AD is not considered harmful and therefore potential reuse in agriculture could be explored. The landfill costs of 280,000 €/year would be eliminated and 90,000 €/year could be generated through energy production.

### **1.2.3.2. Syngas**

In an article written by Maisano et al. (2019), the potential use of SB for the production of syngas through the use of a fluidized bed gasifier has been discussed. The characteristics of SB were explored through thermogravimetric analysis and compared to white pine and citrus peels, where it had significantly worse properties than the two other materials mainly because of its high ash and moisture content. The study concluded with interesting findings about the characteristics of the behaviour of *Po* biomass: air-steam gasification combined with *Po* decomposes the material earlier at decreased temperatures due to a higher AC of the material, which could potentially decrease the costs of the process as the temperature during the production phase can be lower; some deficiencies have been stated such as lower Cold Gas Efficiency, lower Nett Calorific Value and Carbon Conversion Efficiency; the production of bio-syngas could be suitable for the combination of steam gasification at moderate temperature (app. 1023 K) with SB; it has favourable C (46.1 wt%), H (6.82 wt%), and GCV (18.1 MJ/kg) values even compared to traditional lignocellulosic biomass as white pine; it showed a better performance than citrus peels; the best rates of the yield of *Po* gasification for syngas (2.64 Nm<sup>3</sup>/kg biomass) and hydrogen (0.65 Nm<sup>3</sup>/kg biomass) at Steam to Biomass equal to 1 wt/wt. The study does not mention any pre-treatment for SB, from our study, it can be seen that it plays a vital role in AC and GCV values and therefore it could be potentially interesting to reevaluate such action.

## **1.3. Other uses**

### **1.3.1. Animal Feed**

Numerous articles focused on a distinct way of reusing the material from banquettes, where in this case the focus was on animal feed. In these articles, it has been studied, whether *Po* could be a suitable and nutritionally sound substitute for commonly used feed for animals such as barley or straw or whether it would be a suitable mineral and fibre supplement in the diets of ruminants. Furthermore, scientists have studied the impacts of such feed on the production of these animals (Castillo et al. 2014, 2016; Van Eldik et al. 2017) and looked for solutions on how to improve the properties of the given material to be more suitable for feed (Abid et al. 2023).

In the article of van Eldik et al. (2017), it has been discussed that feeding Murciano-Granadina Goats for 25 days with *Po* instead of barley straw had insignificant sensorial and physiochemical impacts on the dairy products from the experimental group that consumed seagrass. Additionally, seagrass use could potentially improve the milk-clotting time performance. However, further analysis and trials would be needed to build strong evidence.

Spanish research group Castillo et al. (2014, 2016) focused on the influence of seagrass feed on goats and potentially other ruminants and its suitability regarding minerals and fibre. The main findings of the studies were that seagrass is potentially a suitable source of fibre for places with lower accessibility to grasslands. In one of the studies, it has been indicated that the intake of seagrass could have positive impacts on milk's fat content without detrimental impacts on other quality characteristics such as lactose, protein, and non-fatty solids and that the increase of milk fat has been higher in the group that had a lower intake of seagrass feed from the two experimental groups (Castillo et al. 2016). In Castillo et al. (2014) the presence of higher levels of Fe through mineral contents analysis has been identified and further linked with its interaction with Cu could be one of the possible hurdles in the implementation of such feed to prevent any harm to the animals. Further, it highlights low protein content compared to the more traditional cereal straw. Therefore, the article suggests additional studies focused on these interaction dynamics.

Additionally, the recently published study of (Abid et al. 2023) discusses the importance of modifying the seagrass-based feed to become more suitable for such applications as animal feed and it suggests that the pre-treatment with microwaves and exogenous fibrolytic enzymes from *Trichoderma longibrachiatum* are an auspicious solution for the conversion of dietary fibre into reducing sugars providing a larger supply of energy for lactation.

All these experiments show some potential in the use of *Po* as an animal feed that could become another positive solution for the disposal of the material. The suitability of the material should be proved by further research and additional solutions for improvements of the digestibility should be sought. Cabrita et al. (2016) suggest that the levels of essential elements and potentially toxic elements shall be monitored in new trials with seaweed and seagrass-based feed to rule out the possibility of toxicity.

### **1.3.2. Other uses of *Posidonia oceanica* fibres**

According to the articles fibres of *Po* may have interesting properties for use in materials production with a large variety ranging from a renewable source of biological adsorbent material for wastewaters (Ncibi et al. 2007; Coletti et al. 2013; Pennesi et al. 2013; Khiari & Belgacem 2017; Photiou et al. 2021; Rudovica et al. 2021), continuing with the use of such lignocellulosic material as a part of various natural or artificial composites (Khiari et al. 2011; Ntalos & Sideras 2014; Zannen et al. 2016; Khiari & Belgacem 2017; Benito-González et al. 2018; Rammou et al. 2021) to such interesting applications as enhancers of construction and building materials (Herráiz et al. 2016; Olacia et al. 2020) and bioactive compounds that can be used in food production (Benito-González et al. 2019).

#### **1.3.2.1. Adsorbents**

The solutions for water treatment varied throughout the studies, where Ncibi et al. (2007) focused on the use of prewashed *Po* leaves as an affordable and ecological adsorbent for red reactive textile dyes dissolved in water. Interestingly, it showed an increased efficiency by 80%, when the leaves were chemically pre-treated by  $H_3PO_4$  and  $HNO_3$ . Suggesting that it could be more suitable than some other studied natural adsorbents such as orange peels, apple pomace etc. Discussing the addition of some immobilized fungi or bacteria onto seagrass fibres could enhance the dye uptake capacity.

Moreover, few articles discussed the removal of various chemicals from synthetic and real wastewater samples (Pennesi et al. 2013; El Khames Saad et al. 2014; Photiou et al. 2021; Asimakopoulos et al. 2021). Where in the case of Pennesi et al. (2013) the study focused on the removal of trivalent vanadium and pentavalent molybdenum. *Po* has been considered a suitable material for such practice, mainly for the structure of its cell walls, the presence of favourable acids and its high metal-binding capacity (Ncibi et al. 2009). Not only that it confirmed positive adsorptive properties it has as well discussed the possibility of use for other chemicals as hexavalent chromium (Asimakopoulos et al. 2021) and suggests that the process should be configured for a potential trial at an industrial scale. Photiou et al. (2021) furthermore confirm that the thermal treatment of seagrasses caters for an exceptional solution for the removal of phosphates from wastewater. It adds that an application of processed seagrass can be beneficial for soil



improvement as a fertilizer or conditioner. Asimakopoulos et al. (2021) suggest that KOH should be used for the activation of the carbon, leading to an interesting performance due to its high surface area. El Khames Saad et al. (2014) studied the cooperation between *Po* and activated carbon and concluded that it is a suitable material for the removal of anthracene and especially an economically available and suitable solution, which has been confirmed in other studies as well, that its low cost is one of the major benefits of this material (Ncibi et al. 2007, 2009; Pennesi et al. 2013; Photiou et al. 2021).

### **1.3.2.2. Compound extraction**

Benito-González et al. (2019) focused their research on bioactive extracts from the banquettes of *Po*. Basing their study on water and solvent extractions, mainly organically sourced ones. This research explored the valorisation process of this waste biomass throughout different fields. Creating a thorough evaluation of the material characteristics was conducted based on its antioxidant capacity, and antifungal & antiviral activity assays. Mainly, these tests were supported by a chemical analysis of the material, focusing on lipids, proteins, ash, phenols, carbohydrates etc. tested by multiple laboratory methods. Based on these tests and analyses it has been concluded that biomass has an interesting potential in the extraction of a variety of compounds with possible applications in the food industry. Interestingly, the research has focused mainly on water and organic-based solvents to avoid the use of toxic compounds.

The article by Coletti et al. (2013) suggests that the synthesis of cellulose acetate and glycidyl methacrylate grafted cellulose should be possible even on an industrial scale and shows, that the biomass has a high potential. Again, the use of the extracted materials can be found in wastewater treatment for water filtration. For further information on the use of SB for biobased materials, where the main focus is on cellulose the thesis of Malek Khadraoui (2022) shall be explored as it provides interesting insights into this topic, however, it will not be mentioned furthermore, as it is not the main topic of this thesis.

### **1.3.2.3. *Posidonia oceanica* as a material**

The use of fibres is ubiquitous and material science is searching for the new application of materials that are not exploited, therefore even *Po* found itself in the spotlight in various studies, exploring the viability of the use of this seagrass and its fibres for applications in numerous fields from packaging (Benito-González et al. 2018) to such

applications as composites fabrication (Khiari et al. 2011; Ntalos & Sideras 2014; Zannen et al. 2016; Rammou et al. 2021) and building construction industry (Herráiz et al. 2016; Olacia et al. 2020).

The study by Benito-González et al. (2018) conducted an exploration into the ability and potential of seagrass fibre biomass transformation into packaging materials and studied, whether its addition to the materials could improve the barrier and mechanical properties of the final products. The lignocellulosic fractions were obtained by elimination of the cell wall particles from the initial material and consequently studied and characterised. It suggests that the material properties are interesting and that it can be superior to such materials as commercially available corn starch, stating that the improvement in the final material can be achieved through the removal of lignin and hemicellulose.

Composites are a very important material in today's world, especially when it comes to replacing non-renewable components with more suitable renewable options. Some of the scientists turned to the sea in their search for answers. The results showed that *Po* displayed a variety of favourable characteristics as beneficial mechanical and thermal properties for its use in fibres-reinforced composites (Khiari & Belgacem 2017).

The promising finding has been confirmed in a previous study that focused on the flexural properties of composites and the relation between fibre weight ratio and its modification. Where it reached the best results at 10% of the fibre weight ratio and it suggests the use of such composites in various applications ranging from the automotive industry, packaging, and furniture (Zannen et al. 2016). Because of the aforementioned furniture, we can add the findings of the studies (Ntalos & Sideras 2014; Rammou et al. 2021) to conclude that particleboards could be another feasible use of SB with various ratios of wood and *Po*. It has been stated that the incorporation of 10% of *Po* in a particleboard will not significantly affect its mechanical properties, therefore could be suitable for such use with the use of a particular resin mixture a higher dose could be used (Rammou et al. 2021).

Additionally, interesting applications of SB can be found even in sectors such as building and construction. In the case of a recent study, it has been found that if compared to traditional materials used in the production of adobe bricks such as straw the bricks reinforced with seagrass fared well and in some aspects even improved the performance

of the final products as well as making the production cheaper and simpler, which has positive impacts on the suitability of the product, however, some deficiencies were found such as lower thermal efficiency and conductivity that need to be addressed further on (Olacia et al. 2020).

Another application of SB has been investigated by (Herráiz et al. 2016), exploring the suitability of the SB as an enhancer of performance in asphalt mixtures, where it has been compared to cellulose, hemp and polyester and the SB has been studied in detail doing 8 measurements to achieve a collection of representative data. From the initial phase of the research, it seemed that the material will not be suitable due to its high water absorption and lower thermostability, however, the second part of the experiment, which was practical, and it presented different results from the first phase. In the second phase, it has been proven that when the fibre content is kept between 1.5 and 2% it can be concluded that the lower contents of seagrass can positively influence the characteristics of the final material. Mainly affecting the higher stiffness, increasing the fatigue life and the rutting resistance.

All in all, *Po* is an interesting, highly available, renewable, sustainable, and economically accessible material that has a high potential in a variety of utilizations in different industries current scientific findings prove, that the material is suitable for many applications and that further studies should be conducted to examine the viability of industrial-scale operations to produce a variety of final products.

## **2. Aims of the thesis**

### **2.1. Hypothesis**

The main hypothesis of this Thesis was founded on knowledge and observations gained throughout the elaboration of the literature review part. The hypothesis has been formulated as follows: (i.) “The sea wrack is a suitable material for reuse as a biofuel and shall not be simply disposed of in landfills”.

### **2.2. Overall objective**

The main aim of the thesis was to identify the material, determine and analyse its properties, explore possibilities of how to improve its characteristics and find suitable solutions for its disposal and reuse.

### **2.3. Specific objectives**

The overall objective of the thesis is supported and supplemented by the specific objectives that are set to help to fulfil the main objective. The specific objectives of the thesis had been defined as follows:

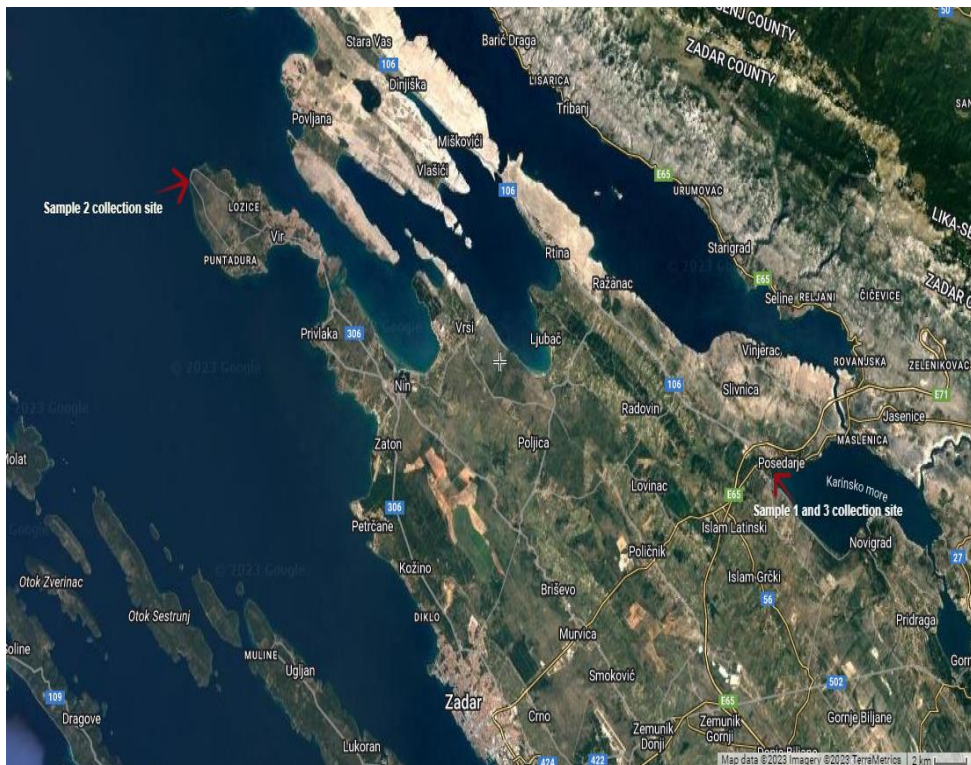
- (i.) to botanically identify the material to enable further research;
- (ii.) characterise the fuel-energy properties of the material (moisture content, ash content, calorific value of the selected biomass materials);
- (iii.) characterise the physiochemical properties of the material (volatile matter determination, CHN, S, Cl, major and minor elements, ash melting characteristics and its behaviour);
- (iv.) to determine the main deficiencies of the material that need to be addressed;
- (v.) to find appropriate solutions for enhancement of the material properties;
- (vi.) to produce biochar and characterise its properties;
- (vii.) to produce pellets and briquettes;
- (viii.) analyse the outcomes from all the phases of the experiment.

### **3. Methods**

Initially, the material for analysis was located, collected and sampled. The laboratory experiments with material were conducted on different specialized sites, mainly in the Laboratory of Biofuels at the Faculty of Tropical AgriSciences, secondly at the Laboratory of Organic Materials Analysis at the Faculty of Engineering (both at the Czech University of Life Sciences, Prague, Czech Republic), thirdly at the Laboratory of Biomass Characterisation located at CEDER-CIEMAT (Soria, Spain), and fourthly at external laboratories of the Research Institute of Agricultural Engineering (Prague, Czech Republic) and in the Optics Laboratory at the Institute of Botany of the Czech Academy of Sciences (Prague, Czech Republic).

#### **3.1. Sample collection**

The samples of various seagrass species of *Posidonia oceanica*, *Zostera noltii* (*Zo*) and *Cymodocea nodosa* (*Cy*) were collected in Croatia in the greater area of Zadar as it was a known area proposed by the staff of the University of Zadar. More specifically the first area is near the village of Posedarje found at the coordinates: 44.2054072N, 15.4710839E and the second mentioned location can be found on the island of Vir at these coordinates: 44.3146744N, 15.0210494E. Samples were collected in two batches, where the first collection happened on the 11<sup>th</sup> of September 2021 in Posedarje, which resulted in the collection of one sample (Sample no 1) and the consecutive second batch was collected on the 15<sup>th</sup> of November 2022 on the island of Vir and Posedarje creating two different samples (Sample no 2 and Sample no 3). A map has been created to show the locations, where the 3 samples were collected, and it can be seen in Figure 6. The location sites and sample collection can be seen in Figure 7, where collection on the island of Vir in Croatia is seen and in Figure 8, where collection in Posedarje, Croatia is shown.



**Figure 6 Sample collection locations**

Source: Google Earth adjusted by Author (2023)



**Figure 7 Sample collection Vir, Croatia**

Source: Author (2022)



**Figure 8 Sample collection Posedarje, Croatia**

Source: Author (2021)

To obtain a representative sample all three samples comprised a mixture of 1kg subsamples, which were extracted from the same beach, however, the places had to be at least 2 meters apart to ensure that the sample is not homogenous. Labelled plastic bags were used to collect the sample material and easily distinguish between the individual samples from the two sites. Afterwards, the samples were transported to the Laboratory of Biofuels at the Faculty of Tropical AgriSciences at CZU Prague to proceed with material treatment, drying and other laboratory tests. After the transport from the collection site to the laboratory, the material samples were stored and dried in the open air at room temperature and the moisture content was monitored. The samples underwent homogenisation, division, and additional drying.

### **3.2. Botanical identification of the samples**

The botanical identification of material samples was performed in the Optics Laboratory at the Institute of Botany of the Czech Academy of Sciences Prague. Identification of the material was needed for the characterisation of the samples and the purpose of further research for the literature review and discussion. The optical equipment used in the identification phase was the Stereo Microscope Olympus SZX12 (with

Olympus DF PLFL 0.5X PF Stereo Microscope Objective Lens) (Figure 9) paired with the Olympus KL1500 LCD Fibre Optic Cold Light Source. The Olympus DP70 Camera System was attached to the stereo microscope which enabled the capture of the pictures (in combination with the software Quick PHOTO MICRO 3.2).



**Figure 9 Work at the stereo microscope station**

Source: Author (2023)

The process of identification was based on three main factors: scientific expertise, scientific literature, and optical instruments. Therefore, the identification of the sample was consulted with a specialist in the field of marine botany (seagrasses). Furthermore, the microscopic images were made and compared to those found in (Green & Short 2003), enabling the precise identification of the material.



### 3.3. Biomass/biofuels characterization – Analytical tests

The properties analysed together with used analytical methods and standards are listed in Table 1.

**Table 1 The analysed properties with methods and standards**

Parameter	Unit	Standard	
Analytical sample preparation		ISO 14780:2017	
Proximate analysis	Moisture	% w.b.	ISO 18134–2:2017
	Ash	% d.b.	ISO 18122:2015
	Volatile matter	% d.b.	ISO18123:2015
Ultimate analysis	C, H, N	% d.b.	ISO 16948:2015
	S and Cl	% d.b.	ISO 16994:2016
Calorific value	GCV	MJ.kg <sup>-1</sup> d.b. and w.b. at constant volume	ISO 18125:2017
	NCV	MJ.kg <sup>-1</sup> d.b. and w.b. at constant pressure	
Major elements in ash	Al, Ca, Fe, Mg, P, K, Si, Na, Ti	% d.b.	ISO 16967:2015
Minor elements in ash	As, Cd, Cr, Cu, Ni, Pb, Zn, Hg	% d.b.	ISO 16968:2015
Ash melting behaviour	° C		ISO 21404:2020

#### 3.3.1. Analytical tests of fuel-energy properties of biomass/biofuels

##### 3.3.1.1. Analytical sample preparation

The grinding process for the preparation of an analytical sample was performed according to ISO 14780:2017 before the material could be analysed in laboratory equipment. The grinding was done using a knife mill Retsch GM 200 (see Figure 10).



**Figure 10** Device for grinding - knife mill Retsch GM 200

Source: Author (2022)

A metal screen with a diameter of holes of 0.5 mm was used to ensure the size of ground material particles according to the standard.

### **3.3.1.2. Moisture content determination**

The moisture content of the initial material samples was determined by the oven drying method (ISO 18134–2:2017). The samples were dried out to constant weight within 24 hours at  $105\pm 3$  °C in the oven Memmert UFE 500 (Mettler GmbH, Germany) (see Figure 11).



**Figure 11 Memmert UFE 500 oven**

Source: Author (2023)

The moisture content of analytical samples of material was determined according to ISO 18134-3:2015. To determine the weight of the analytical samples analytical balance KERN ABJ - 120 NM was used. The moisture content of analytical samples was determined by the equation:

**Equation 1 Moisture content of analytical samples**

$$MC_{ad} = \frac{(m_2 - m_3)}{(m_2 - m_1)} \times 100$$

where:

$MC_{ad}$  – moisture content as analysed, %;

$m_1$  – a mass of an empty dish and lid, g;

$m_2$  – a mass of a dish and lid with a sample before drying, g;

$m_3$  – a mass of a dish and lid with a sample after drying, g.

### 3.3.1.3. Ash content determination

The amount of ash in the analytical samples was determined according to ISO 18122:2015 by calcination at 550 °C in a laboratory muffle furnace LAC-LH 06/13 (Figure 12). Laboratory analytical balance KERN ABJ - 120 NM with a precision of 0.1 mg was used for the weighting of the analytical samples of biomass.



Figure 12 LAC-LH 06/13 muffle furnace

Source: Author (2023)

The following equation was applied:

Equation 2 Amount of ash in the analytical samples

$$A_d = \frac{(m_3 - m_1)}{(m_2 - m_1)} \times 100 \times \frac{100}{100 - M_{ad}}$$

where:

$A_d$  is ash content on a dry basis, %;

$m_1$  is a mass of an empty dish, g;

$m_2$  is the mass of the dish with a sample, g;

$m_3$  is the mass of the dish with ash, g;

$M_{ad}$  is the moisture content of the test portion used for the determination, %.

### 3.3.1.4. Calorific value

The gross calorific value was determined according to the standard ISO 18125:2017.

An automatic isoperibol calorimeter PARR 6400 (Figure 13) was used as well as isoperibol calorimeter LAGET MS-10A. Gross calorific value was determined in MJ.kg<sup>-1</sup> d.b. at constant volume and NCV at constant pressure. The results of GCV determination were displayed automatically or calculated by the equation 3 below.



Figure 13 Automatic isoperibol\_calorimeter PARR 6400

Source: Author (2023)

#### Equation 3 Gross calorific value

$$Q_{v.gr} = \frac{\varepsilon \times \theta - (m_{ign} \times Q_{ign} + m_{cb} \times Q_{cb})}{m_s}$$

where:

$Q_{v.gr}$  – a gross calorific value of a biofuel sample, J.g<sup>-1</sup>;

$\varepsilon$  – effective heat capacity of calorimeter, J.°C<sup>-1</sup>;

$\theta$  – corrected temperature rise, °C;

$m_{ign}$  – a mass of an ignition wire, g;

$Q_{\text{ign}}$  – a gross calorific value of an ignition wire (6,000 J.g<sup>-1</sup> for nickel-chromium), J.g<sup>-1</sup>;

$m_{\text{cb}}$  – a mass of a combustion bag, g;

$Q_{\text{cb}}$  – the gross calorific value of a combustion bag (16,279 J.g<sup>-1</sup> for paper), J.g<sup>-1</sup>;

$m_{\text{s}}$  – a mass of a biofuel sample, g.

### **3.3.2. Physicochemical characteristics of the biomass**

#### **3.3.2.1. Volatile matter determination**

The content of volatile matter (VM) was determined according to EN ISO 18123 (2015) standard as the loss in mass during calcination of the sample in a thermogravimetric analyser (LECO TGA-701 thermogravimetric analyser with multi-sample capacity) at 900 °C for 7 minutes.

#### **3.3.2.2. Carbon, Hydrogen and Nitrogen determination**

C, H, and N were determined by elemental analysis using the LECO CHN628 elemental analyser seen in Figure 14 (LECO Corporation, St. Joseph, MI, USA), following the ISO 16948:2015 standard. Where 1g of the sample was sealed into three replicates of a 502-186 Tin Foil Cup and set in the proper location on the sample carousel. The results were presented automatically following combustion at 1050 °C.



**Figure 14 LECO CHN628 elemental analyser and 628S analyser**

Source: Author (2023)

### 3.3.2.3. Sulphur and Chlorine determination

Sulphur and chlorine contents were measured by Ion chromatography (883 Basic IC Plus, Methrom), after sample combustion in a bomb calorimeter (PARR 6400 automatic isoperibol calorimeter) and the ulterior recovery of chloride and sulphate in an aqueous solution, in accordance with ISO 16994:2016. At the same time, sulphate determination was carried out by the LECO CHN628/628 S (Michigan, USA) in line with the provisions of BS EN ISO 16948:2015.

### 3.3.2.4. Major elements in ash

The determination of major elements (Al, Ca, Fe, Mg, P, K, Si, Na, Ti) can be used to evaluate the behaviour of the ash in a thermal conversion process or to evaluate the use of the ashes. These elements were determined according to the ISO 16967:2015 standard by microwave digestion (Ethos Pro, Milestone) and ICP-OES (Jarrell ash, model IRIS AP, Thermo Scientific). Biomass ashes, which were obtained at 550 °C, were acid digested in a microwave furnace using HNO<sub>3</sub>, H<sub>2</sub>O<sub>2</sub> and HF as a first step and H<sub>3</sub>BO<sub>3</sub> as a second step before being further analysed by ICP-OES.

### 3.3.2.5. Minor elements

#### 3.3.2.5.1 As, Cd, Cr, Cu, Ni, Pb, Zn



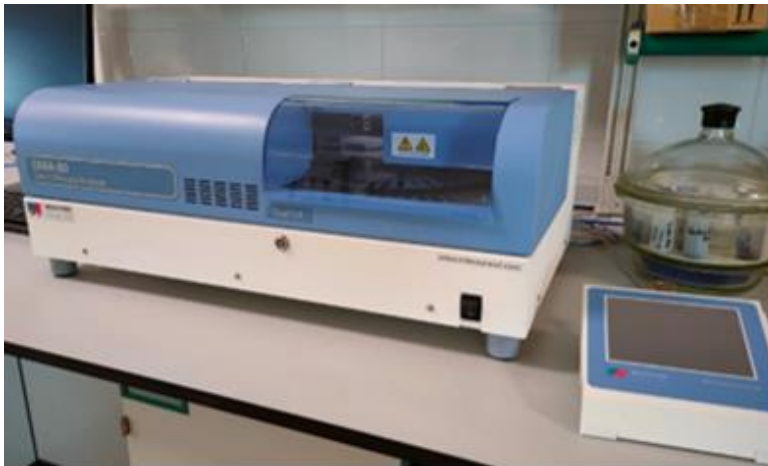
Figure 15 Microwave digester Ethos Pro

Source: Author (2023)

As, Cd, Cr, Cu, Ni, Pb, and Zn were determined by microwave digestion (Ethos Pro, Milestone) seen in Figure 15 and ICP-MS (inductively coupled plasma mass spectrometer Thermo Fisher Scientific iCAP Q).

#### **3.3.2.5.2 Mercury (Hg)**

Mercury was determined via thermal decomposition, gold amalgamation and absorption spectrophotometry by Milestone DMA-80 automatic mercury analyser, which can be seen in Figure 16.



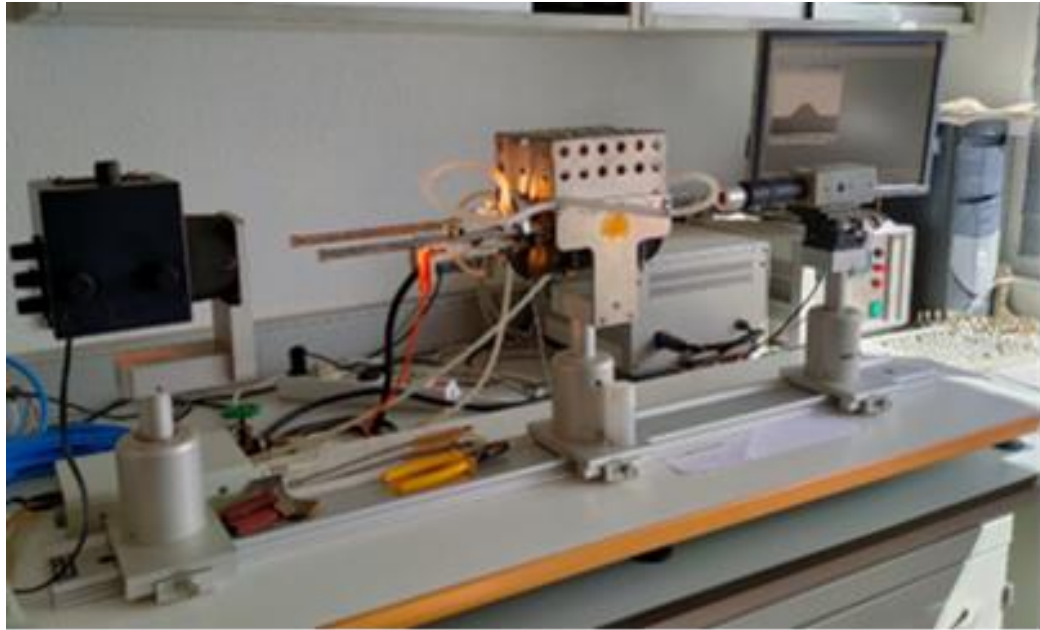
**Figure 16 Milestone DMA-80 automatic mercury analyser**

Source: Author (2023)

#### **3.3.3. Ash melting characteristics**

Hesse instrument HT15 optical heating microscope (Figure 17), the automated analyser was used to determine the ash melting behaviour of biomass samples applying the ISO 21404:2020 standard for solid biofuels.

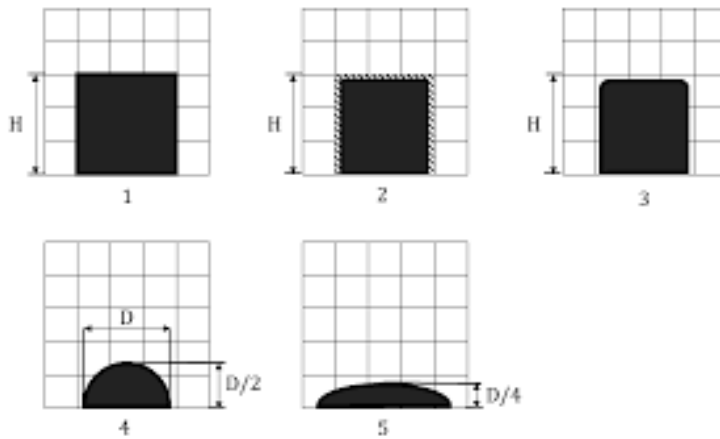




**Figure 17 Hesse instrument HT15 optical heating microscope**

Source: Author (2023)

Assessment of ash melting behaviour was based on the changes in shape (Figure 18) detected during the heating of a cylindrical biomass ash pellet (3 mm diameter and 3 mm height) from room temperature to 1400 °C in an air atmosphere. The following four characteristic temperatures were reported: shrinkage starting temperature (ST), deformation temperature (DT), hemisphere temperature (HT) and fluid temperature (FT). ST is defined as when the area of the test piece falls below 95 % of the original test piece area at 550 °C due to the shrinking of the test piece. DT is the temperature at which the first signs of melting occur. HT is the temperature at which the test piece forms approximately a hemisphere i.e., when the height is half of the base diameter (Figure 18:4). And FT is the temperature at which the ash is spread out over the supporting tile in a layer, the height of which is half of the height of the test piece at the hemisphere temperature (Figure 18:5) from which, all of the described behaviours can be found in Figure 18.



**Figure 18** Ash melting behaviour: first graph we can observe its original shape; in the second graph: shrinkage starting temperature (ST), in the third graph: deformation temperature (DT).

Source: ISO 21404:2020

### **3.4. Primary treatment by sieving and water washing**

In order to remove small solid impurities and to study the impact of biomass treatment via sieving, a horizontal sieve shaker (Retsch AS 200, Germany) was used. Each portion of biomass was sieved for 30 minutes at an amplitude of 3.0 mm/g applying a standard calibrated sieve with the diameter of 20 cm and opening sizes of 1.0 mm and the bottom pan. The procedure was performed according to the standard ISO 17827–2:2016. After sieving, the impurities collected on the bottom pan were removed, and the biomass remaining on the sieve was used for the further tests (characterization).

Preliminary waste treatment test was carried out by placing the samples in the plastic containers filled by water for 1.5 hours.

### **3.5. Biomass lixiviation**

The *Po* biomass material was afterwards water treated for different time periods in order to determine, whether and how much the ash content would decrease and what time of treatment is required. In this process the soluble substances were separated from the insoluble ones by dissolving them in two types of water: university tap water and river-sourced water. Each of the samples was treated in 11 l containers for different time periods. These ranged from 1 to 48 hours, which was the longest leaching period. The

periods were such: 1, 5, 12, 24, 36, and 48 hours. Each of the samples had the same weight of 25 grams, which was ensured using a KERN EMB 1200-1 laboratory scale. These 25 grams of material were put in the container and submerged in 2.5 litres of water such an amount of liquid ensured that all of the material was under the water and was exposed to the lixiviation for the given period. The liquid was always kept at room temperature. The containers were labelled multiple times to prevent mistakes and confusion and included the shortcut where the first letters included the type of material, two digits represented the duration of the process, and the last two letters represented the type of water e.g., PO48TW meant that the sample was *Posidonia oceanica* curing for 48 hours in tap water. A batch of samples during water treatment can be seen in the Figure 19.



**Figure 19** Water treatment of samples

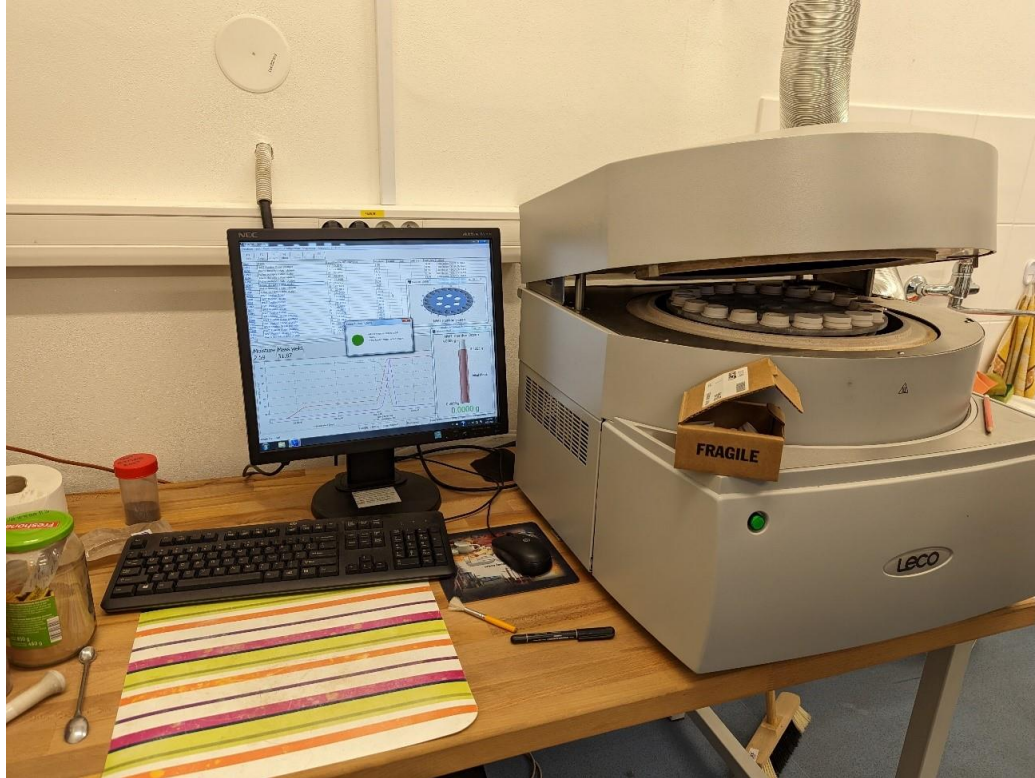
Source: Author (2023)

### **3.6. Torrefaction tests**

The LECO TGA701 Thermogravimetric Analyzer (LECO Corporation, St. Joseph, MI, USA) was used to perform torrefaction of *Po* samples seen in Figure 20.

The experiment conducted 5 distinct products consisting of 2 samples at 5 pre-set temperatures of 250°C, 300°C, 350°C, 450°C and 550°C to determine how these processes affect the calorific value and ash content. Therefore, in total 36 samples as it includes the untreated and treated sample at default temperatures.

Samples of studied material were loaded into the analyser in crucibles lined with aluminium foil and dried inside to constant weight. Before the torrefaction process, an inert atmosphere using N was introduced (the constant N flow rate was  $8.5 \text{ L}\cdot\text{min}^{-1}$ ).



**Figure 20 LECO TGA701 Thermogravimetric Analyzer**

Source: Author (2023)

### **3.7. Pelleting and briquetting**

Part of the material was transformed into pellets and briquettes. To produce pellets, the material was first milled with a hammer mill 9FQ – 40C with an energy input is 5.5 kW (Figure 21), by using of the sieve.

Subsequently, milled material was refined into pellets with the pelleting machine KovoNovák MGL200. Furthermore, the mechanical durability of produced pellets was determined by the pellet durability tester PT 500 according to standard ISO 17831-1:2015, and calculated via a following equation:

#### Equation 4 Mechanical durability of pellets

$$D_U = \frac{m_A}{m_E} \times 100$$

where:

$D_U$  – mechanical durability, %;

$m_E$  – pre-sieved pellets before the durability treatment, g;

$m_A$  – sieved pellets after the durability treatment, g.

From the initial material, the briquettes were produced as the material was suitable for use in its original form (without pre-treatment by milling) in the briquetting press BrikStar model CS 50.



Figure 21 Hammer mill 9FQ – 40C

Source: Author (2021)

### 3.8. Analytics

Analytics of the results was done with the help of descriptive analytics and basic statistical analyses in Microsoft Excel and results were compared to other studies in the discussion part.

## **4. Results and Discussion**

### **4.1. Botanical identification of the samples**

The botanical identification of material samples was conducted primarily, as it was essential for further research and analysis of the biomass to evaluate current findings and compare them appropriately.

With scientific expertise and guidance from RNDr. Martin Vohník, Ph.D.; optical instruments in the Optics Laboratory at the Institute of Botany of the Czech Academy of Sciences Prague and literature Green & Short (2003) & Boudouresque et al. (2012), we were able to identify 3 types of seagrasses that were included in the sample, which can be seen in Figure 22. Where, the microscopic images were made and compared to the description and characteristics of specific species included in the books, enabling the precise identification of the material.

The seagrasses were easily identifiable as the size differences between them and the limited number of seagrasses in the Adriatic Sea (Green & Short 2003) made it easier to classify each seagrass included in the sample. This gave us clear hints and thanks to the high-precision equipment we were able to work efficiently and precisely to make a strong foundation for the correct identification. Interestingly no algae species were found in the mixture as was previously expected and the material was fairly homogenous. Comprising only one species in the case of Sample 2 collected in Vir, Croatia, where only *Po* was present as could be seen in Figure 7 (Page 19).

In samples 1 and 3, collected in Posedarje, the situation varied and the mixture ratio changed in the two years, this can mainly be explained by the seasonality of the necromass, as during the first collection in 2021 the necromass was collected in September and in the case of the second sample collection we were able to collect the samples later on in the year 2022 in November, therefore the composition of the mixture varies, according to the findings this can be influenced by seasonality and mainly the weather in the area.

In the second case, the sample comprised two seagrass species namely *Cy* and *Zo* and as mentioned previously, the mixture ratio changed throughout the study, where in

the first collection *Cy* was prevalent in the mixture and comprised approximately 70% of the material in the later collection *Zo* prevailed with approximately 80%.

In Figure 22 we can observe the three mentioned seagrass species from the Adriatic Sea (Sample 2) and Karin Sea (Sample 1 and 3). Beginning from the left we can see *Po*, with *Cy* in the middle and *Zo* on the right.



**Figure 22 Three seagrass samples**

Source: Author (2023)

*Po* has significantly wider and longer leaves, which can be easily distinguished by its pattern of dark and light brown colour, which in the water has leaves that are between 30 and 140 cm long and up to 1 cm broad. The leaves have 13–17 parallel veins and are banded, that are obtuse or slightly rounded at the apex. They develop in 5-8 leaf bunches. Found in the middle is *Cy* a plant with leaves that measure from 30 to 100 cm long and from 3 to 9 mm broad. It can grow almost as large as *Po* under optimal circumstances. The leaves have 5–11 veins and are thin, elastic, banded, and veined. This seagrass is rather uncommon and can be found along Istria's northwestern shore, in the Bay of Rijeka and the Velebit Channel, close to the Novigrad and Karin Seas. And lastly, the smallest one of them is *Zo* or *Dwarf eelgrass*. A plant with light to dark green leaves that are 1

mm broad and 5–30 cm long. The leaves are mostly found growing in shallow muddy bays, calm stagnant lagoons, and estuaries. They are very slender, elastic, heart-shaped at the tips, and have one to three parallel veins (Boudoureque et al. 2012; Green & Short 2003).

## 4.2. Alien material characterisation

Throughout the material processing stages, many foreign objects of various origins have been found in the SB ranging from natural materials such as sand, feathers, branches and sticks and carcasses of *Emerita* to artificial materials that were mainly made up of plastics, fibres, bandages etc. In Figure 23 and Figure 24 we can observe these alien materials in a large variety.



**Figure 23 Sand, rock, plastics and sand fleas found in *Po***

Source: Author (2023)





**Figure 24** Fibres, feathers and wood found in *Po*

Source: Author (2023)

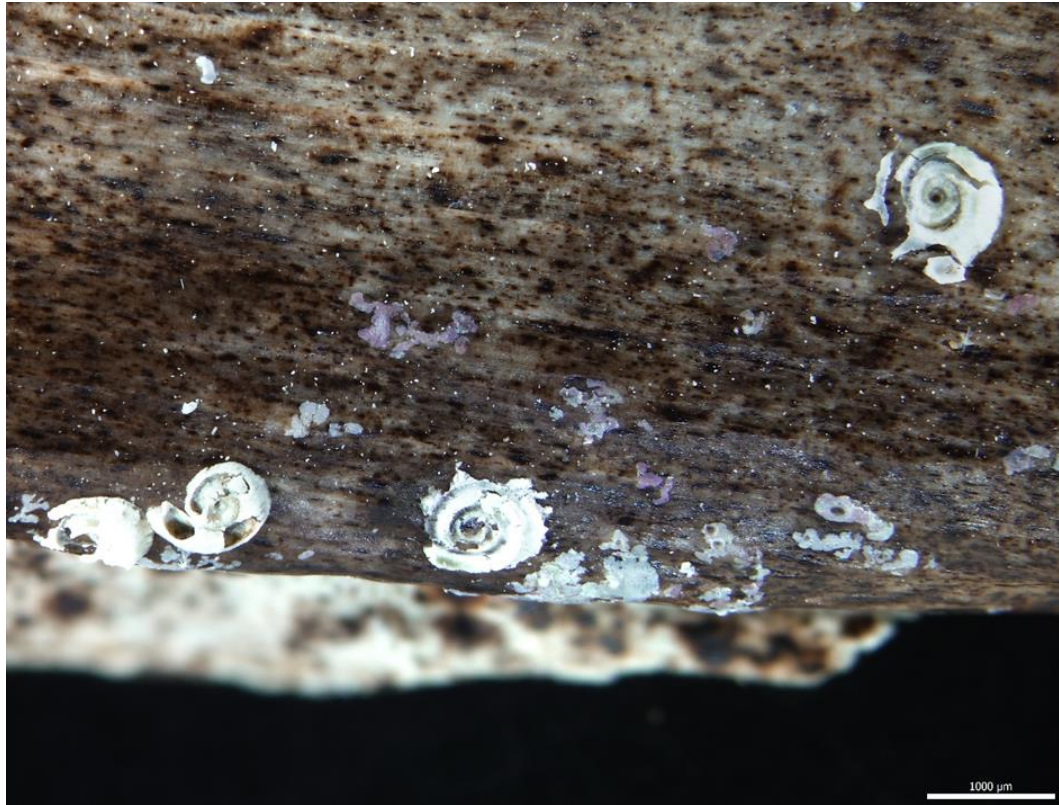
Furthermore, we can discuss the presence of large quantities of sand found under the microscope in Sample 3, which can have a high impact on the characteristics of the material. Potentially influencing the ash content and GCV of the materials. This can be observed in figure 25, where *Cy* and *Zo* sample has been studied closely and a large presence of sand and small rocks could be detected.



**Figure 25** Large quantities of sand found under the microscope

Source: Author (2023)

Another interesting find is a large presence of epiphytes on the leaves of *Po* and these shells rich in calcium could be another factor that impacts the ash content, these can be seen in Figure 26.



**Figure 26** Epiphytes on *Po* leaves

Source: Author (2023)

Lastly, a very impressive and interesting find was accidentally found during the study of the rhizomes of *Po*, when we encountered a rare species of Fungi in terms of occurrence in the Adriatic Sea. *Pontoporeia biturbinata* can be observed and admired in the Figure 27, where it forms bubble-like structures on the rhizomes of *Po*.



**Figure 27** *Pontoporeia biturbinata* found on the rhizomes

Source: Author (2023)

This occurrence has been reported to the experts at the University of Zagreb, Croatia to process this finding, at the same time, samples were collected for a planned donation of this material.

Identification of the samples was a crucial part for such work as it enables us to work more efficiently, find better articles and especially in my case, connect with experts from the field, that provide very important feedback and tips.

### 4.3. Primary treatment by sieving and water washing

#### 4.3.1. Evaluation of the main fuel-energy properties of seagrasses

We initially examined the effects of sieving and water treatment (for 1.5 h) on the chemical composition and other characteristics of seagrasses. As samples, were tested three of the Mediterranean's widely distributed seagrasses *Po* and a mixture of *Cy* and *Zo* further called *ZoCy*. These initial trials were conducted to determine, whether the application of sieving or water treatment cures would change the characteristics of SB. Obtained results are presented in Table 2.

**Table 2 Fuel-energy properties of biomass under different types of treatment**

Sample	MC (% w.b.)	Ash (% d.b.)	Volatile matter (% d.b.)	GCV d.b. (MJ/kg)	GCV w.b. (MJ/kg)	NCV d.b./w.b. (MJ/kg)
<i>Po</i> untreated	14.0	27.3	47.2	12.86	11.06	11.93/9.92
<i>ZoCy</i> untreated	14.5	33.6	32.8	11.87	10.15	11.00/9.05
<i>Po</i> sieved	13.4	27.3	45.5	12.76	11.05	11.82/9.91
<i>ZoCy</i> sieved	13.4	31.3	32.6	11.69	10.12	10.86/9.07
<i>Po</i> water-treated	12.9	12.6	69.7	15.77	13.74	14.64/12.43
<i>ZoCy</i> water-treated	11.8	20.7	52.1	14.05	12.39	13.07/11.24

From the Table 2 is clear, that seagrass biomass is characterized by very high ash content and low calorific values. As these properties are crucial for energy application, their enhancement and solution to these hurdles is needed.

From the results above it is also visible that *Po* has in general better properties if compared to *ZoCy* mix.

Starting with the AC we did not observe any major decrease or increase in the values in case of sieving treatment. Contrary, water treatment had a big impact on the Ash content. And the major effect that is more than half the ash content decrease was found in *Po*. In *ZoCy* the effect is still significant but not as prominent, the decrease is about one-third. Table 2 shows that the initial AC values of untreated *Po* were 27.3% d.b., which

decreased to 27.2% d.b. after sieving and to 12.6% d.b. after water treatment. The initial AC values of untreated *ZoCy* were 33.6% d.b., which decreased to 31.3% d.b. after sieving and to 20.7% d.b. after water treatment. According to the standard for commercialized graded non-woody pellets (ISO 17225-6:2014) and briquetted (ISO 17225-7:2014) produced from herbaceous biomass, fruit biomass, aquatic biomass and blends and mixtures, the AC should not exceed the limit of 6% d.b. for the best A class fuels, and  $\leq 10\%$  d.b. for B class. This confirms that AC of initial seagrass biomass is much higher comparing to the requirements. However, in case of *Po* after water treatment the AC is much closer to the maximum standard value.

The AC of *Po* in other articles varied from 26.1% in Plis et al. (2016), 17% in Chiodo et al. (2016), 22.07% in case of dry sample and 34.80 in wet samples in Voca et al. (2019). This means, that our results are comparable to those already published. Another biomass which is widely available and is having high AC is for example rice straw by Chaloupková et al. (2021) its AC is equal to 16.11 % d.b.). As it was already mentioned above, according to the results, water treatment has a very high impact on the AC especially in *Po* samples. Also, one of the reasons, why Chiodo et al. (2016) achieved lower AC in *Po*, as they employed water treatment method.

Looking into the Table 2, we can further see that in contrast to sieving, water treatment positively influenced the calorific values of treated biomass, too. For example, GCV d.b. of *Po* sample was improved by almost 3 MJ/kg, and nearly by 2.2 MJ/kg in case of *ZoCy*. In general, it should be highlighted, that according to standard requirements for non-woody biofuels (for both pellets and briquettes as well as all classes), the minimum required value of Net calorific value w.b. is stated to be 14.5 MJ/kg (ISO 17225-6:2014 and ISO 17225-7:2014). Calorific values of the studied seagrass species are found to be rather lower. Besides the fact that water treatment improves the calorific value, the resulting values are still not fully sufficient, that is why thermochemical treatment was also tested in our research.

As for moisture content, the initial moisture of biomass samples was about 14 % w.d. This moisture level was easily achieved by the passive drying. According to Akhmedov et al. (2019), the moisture content of biomass for the production of densified biofuels should not exceed 20%. And by ISO 17225-6:2014 and ISO 17225-7:2014), a maximum of 12% or up to 15% moisture content is recommended for different quality

classes of graded non-wood pellets and briquettes. Thus, it is expected that naturally dried biomass is suitable for further processing to biofuels.

It can be discussed that the MC of our *Po* material was higher if we would compare it with the result (MC of 10.7%) of Chiodo et al. (2016). In case of Plis et al. (2016) even lower MC of 5.5% was measured, which was comparable to the values of lignite (4.9%) in the mentioned research. Interestingly, Voca et al. (2019) achieved an average MC of 59.34% in an untreated sample, probably it was determined directly after the biomass collection.

#### 4.3.2. Further characterisation (chemical composition) of the material

The major chemical composition of the study materials, i.e., initial and after treatments, could be seen from the Table 3.

**Table 3 Chemical composition of the main elements**

	<b>C</b> (% d.b.)	<b>H</b> (% d.b.)	<b>N</b> (% d.b.)	<b>S</b> (% d.b.)	<b>Cl</b> (% d.b.)
<i>Po</i> untreated	34.7	4.3	0.59	0.71	5.70
<i>ZoCy</i> untreated	33.1	4.0	1.18	0.64	2.87
<i>Po</i> sieved	34.6	4.3	0.57	0.63	6.69
<i>ZoCy</i> sieved	33.0	3.8	1.18	0.63	2.57
<i>Po</i> water- treated	42.5	5.2	0.69	0.20	0.72
<i>ZoCy</i> water- treated	38.5	4.5	1.31	0.32	0.50

From the Table 3 is visible that the effect of sieving on percentage of C, H, N S and Cl in the samples is again negligible (although we can speculate on some impact in percentage of chlorine). Contrary, the water treatment has a huge impact on the composition. In water treatment there has been an increase in the content of C, H and N and very significant and important decrease in percentage of S and Cl. According to

Murcia Higuera (2019) the concentration of Cl and S is very undesirable in biofuels as it leads to the corrosion of the combustion chamber. The biggest decrease was observed in Cl where in both seagrass the presence of chlorine dropped below 1 percent (0.72 % d.b. for *Po* and 0.5 % d.b. for *ZoCy*) compared to initial nearly 6 or 3 percent, for *Po* and *ZoCy* respectively, corresponding to almost 8 times and 6 times reduction of Cl in wasted samples.

Again, achieved results on chemical composition of seagrasses showed better properties (more desirable for energy use) in case *Po* comparing to *ZoCy*, with the exception of high and almost two times higher Cl content.

In general, if comparing to the standard requirements of non-woody solid biofuels (ISO 17225-6:2014 and ISO 17225-7:2014), it can be concluded, that N content of studied biomass in initial form as well as treated totally correspond to the stated values of  $\leq 1.5$  % d.b. (for A class) and  $\leq 2.0$  % d.b. (for B class). It should be mentioned that N content has a direct impact on formation of harmful nitrogen oxides (NO<sub>x</sub>) during the fuel combustion (Ivanova et al., 2018). However, comparing our results to the standard limits for S and Cl, it was found that these compounds are problematic in the initial seagrasses, largely exceeding the limits, which are as: 0.20 % d.b. (for A class) and  $\leq 0.3$  % d.b. (for B class) in case of S, and 0.10 % d.b. (for A class) and  $\leq 0.3$  % d.b. (for B class) for Cl (ISO 17225-6:2014 and ISO 17225-7:2014). The water treatment experiment was found to reduce the values positively; thus, the resulting S in *Po* even fulfilled the border of A limit, *ZoCy* achieved the value close to the maximum of B class briquettes/pellets. Water treatment brought in significant Cl content reduction, nevertheless, the values are still about twice higher the limit, so it is necessary to count on this fact during biofuels combustion.

In the second part we came to the same conclusion as in the first part (data in the Table 2). There has not been any significant impact of sieving on the studied characteristics and composition of seagrass. In comparison there is strong evidence that water treatment has some impact on almost all of the studied properties.

Very detailed analysis of chemical composition (content of minor and trace elements) of seagrasses was carried out within the research; all our additional results can be found in the appendices (page XVI), and again similar trend related to both treatment methods is seen.

### 4.3.3. Ash melting characteristics

Taking into account the fact that seagrass biomass is typical by high ash content, therefore, the ash melting was also studied in order to get an idea of ash behaviour during the combustion process. In Table 4 we can observe a trend, where the shrinkage starting temperature is increased when different treatment methods are applied, this is mainly visible in the case of *Po*, where it increased from the initial value of 808°C to a final value of 946°C (after water-treatment). This trend, however, does not repeat in the case of *ZoCy*, where the values were not significantly affected and in one case the shrinkage temperature even decreased. If compared for example to the rice straw ash melting behaviour in Chaloupková et al. (2021), where the values of shrinkage phase were 750°C, we can say that the initial values of *Po* are very close, however, slightly higher than the measured value of their study.

In the case of DT *Po*, the deformation temperature changed from 1081°C negatively to 1021°C for the sieve-treated sample and rose to 1242°C in the water-treated sample. Where in the case of *ZoCy* the negative change happened throughout the whole process (both treatment) leading to a change from the initial value of 1171°C to the lowest value of 975°C for the water-treated sample. In this case rice straw (Chaloupková et al. 2021) achieved the value of 1063°C, which is the closest to the values of untreated *Po* and sieved *Zo*.

The hemispherical temperature did not represent any major differences in the case of *Po* where the initial (1332°C) and the water-treated values (1334°C) were almost the same, only the sieved material had a slight drop to 1278°C. In the case of *ZoCy*, a similar trend was shown, where all the values were between 1176°C and 1193°C. In the case of Chaloupková et al. (2021) the values for hemispherical temperature were 1225°C, where *Po* has positively higher results, and the lowest value of sieved *Po* is the closest. *ZoCy* showed lower hemispherical temperature in all cases, and the most different sample with the lowest temperature was water-treated *ZoCy*.

Lastly, the flow temperature was examined. The main takeaways from this analysis were that no significant changes were seen in the cases of both seagrass species after different treatments.



Overall, from the resulting values, it is visible that *Po* has higher temperatures of ash melting behaviour (especially positive is the high flow temperature) comparing to *ZoCy*.

**Table 4 Ash melting behaviour of seagrass samples under different treatments (in °C)**

	ST	DT	HT	FT
Posidonia original	808	1081	1332	>1450
Zostera original	980	1171	1193	1206
Posidonia sieved	861	1021	1278	>1450
Zostera sieved	954	1084	1184	1195
Posidonia water-treat.	946	1242	1334	1441
Zostera water-treat.	986	975	1176	1192

ST - Shrinkage starting temperature; DT - Deformation temperature; HT- Hemispherical temperature; FT- Flow temperature.

#### **4.4. Water treatment (lixiviation) of *Po* biomass**

After the evaluation of the results found in the previous subchapter (4.3.1), a water treatment trial was prepared to explore the relation between the period of water treatment and ash content reduction inspired by (Fernández et al. 2022).

In some articles, it has been already discussed that water treatment should be applied to the SB as it improves the characteristics of the material (Chiodo et al. 2016; Van Eldik et al. 2017, Moltó et al. 2022), however, not many studies stated the appropriate methodology of water leaching of the *Po* biomass as well as the suitable time of the water treatment was not previously well investigated. Thus, the present research will improve the current scientific findings, as in most of the cases, the only mentions were about flushing the material with water. Contrary, some articles did not mention water treatment of any form at all (Cocozza et al. 2011a; Cocozza et al. 2011b; Voca et al. 2019). Some articles used tap (Van Eldik et al. 2017) and some distilled water (Chiodo et al. 2016) and for example, Moltó et al. (2022) used a constant flow of tap water with a flow of 8.2 mL/s.

As the highest potential was identified in the water treatment methods and the better properties were achieved by *Po*, this became the foundation of our study and therefore a larger scale experiment with water treatment was conducted using *Po* biomass, which can be seen in the next Table 5.

**Table 5 Ash content (dry basis) of water-treated samples**

<b>Treatment duration</b> [h]	<b>AC river water</b> (%)	<b>AC tap water</b> (%)
1	11.72	11.50
5	11.72	11.95
12	12.02	11.67
24	11.58	10.88
36	11.05	11.73
48	11.35	10.57

The main findings of this experiment were that the biggest change in AC was between the untreated material and the material that was exposed to 1 h of water treatment, as longer periods did not achieve significantly enhanced results. To make the process of treatment as efficient as possible 1 h is suggested to be used as it had a significant improvement effect on the SB with a decrease from the initial 27.2% d.b. to the final value of 11.50% d.b. in the case of tap water and 11.72% d.b. in case of river water. It can be stated that the improvements in longer periods of water leaching are insignificant as well as there was not much deference found between the two types of water applied. Therefore, as an optimal samples for further torrefaction experiment, 1 h tap water treated *Po* material was used.

Following the finding discussed in the chapter 4.3.1, it was stated that additional treatment of seagrass biomass is needed to improve the calorific value, which is why the torrefaction test was conducted.

## 4.5. Torrefaction test with *Po* biomass

In the Table 6, we can see substantial growth in GCV values of treated samples, if we would compare the results with the initial values. Starting with GCV 15.98 MJkg<sup>-1</sup> d.b. as the sample was water treated., and the biggest difference between a raw state and the torrefied sample was found in the sample treatment of 350°C, where the difference in GCV is about 39.67 % with a value of 22.32 MJkg<sup>-1</sup> d.b., and the smallest is in a temperature of 250°C where the difference in the gained calorific value was smaller and equalled to 14.45% between the raw state and torrefied sample. However, even by applying the smallest temperature, the GCV of the tested biomass was improved by 2.31 MJ/kg.

**Table 6 Calorific value of torrefied biomass samples (water treated)**

Temp. [°C]	GCV (MJkg <sup>-1</sup> d.b.)	NCV (MJkg <sup>-1</sup> d.b.)
Non-torrefied	15.98	14.55
250°C	18.29	17.17
300°C	21.64	20.70
350°C	22.32	21.43
450°C	21.85	21.06
550°C	21.86	21.34

Our results of non-torrefied *Po* biomass have similar results as Plis et al. (2014), where it reached GCV of 13.5 MJkg<sup>-1</sup>. Interestingly bio-oil production from *Po* generated even higher GCV of 31.2 MJkg<sup>-1</sup> (Pattnaik et al. 2022). For comparison, in the case of Jeníček et al. (2022), 19.74 MJkg<sup>-1</sup> was measured as an initial value for spent coffee ground with the highest value achieved through torrefaction at 350°C, which was 31.26 MJkg<sup>-1</sup>, where similarly the highest increase in GCV could be seen.

Our results can be also compared to other studies and biomass materials, where the initial GCV of *Po* equal to 12.816 MJkg<sup>-1</sup> was measured and if we would compare it to standard materials as wood chips (18.660 MJkg<sup>-1</sup>) and lignite (17.716 MJkg<sup>-1</sup>) (Plis et

al. 2016), white pine (17.96 MJkg<sup>-1</sup>) and Lacustrine alga (14.78 MJkg<sup>-1</sup>) (Chiodo et al. 2016), we could say, that even the initial results are not bad if we take in account that we are comparing it to wood biomass, however in the whole image, *Po* is the worst studied sample. In Chiodo et al. (2016), the values of GCV after the torrefaction increased substantially as well.

In Table 7 we can see that the AC on a dry basis has been also increased greatly via torrefaction, moreover, by increasing the treatment temperature, the AC is always increasing. In smaller temperatures (up to 350 °C) by around 50 %. In higher temperatures, the difference is still quite high. Since the smaller ash content is desirable, the treatment seems to be beneficial in terms of GCV increase, however the higher AC is less desirable outcome.

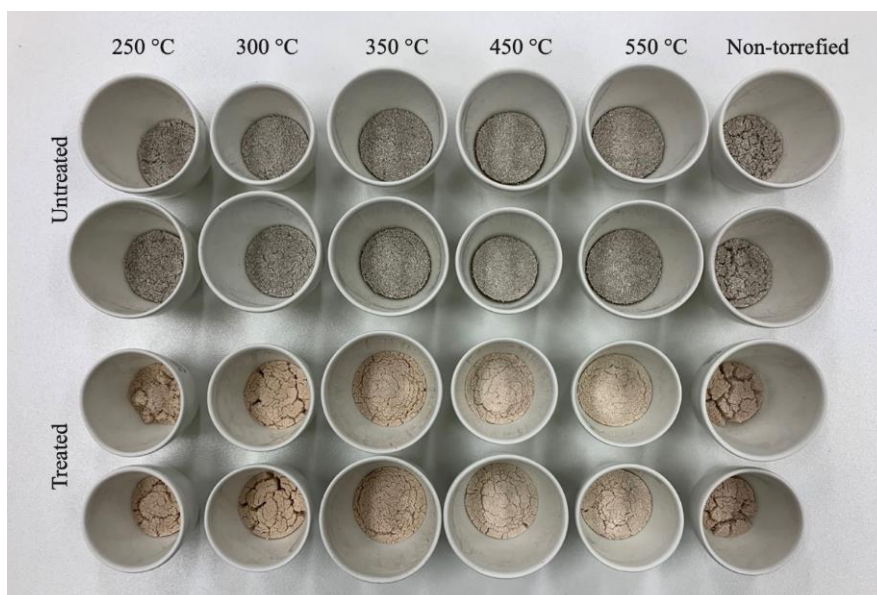
**Table 7 Ash content (dry basis) of torrefied samples**

<b>Temp.</b> [°C]	<b>AC not treated</b> (%)	<b>AC water treated</b> (%)
Non-torrefied	25.60	10.46
250	30.69	12.61
300	37.84	17.92
350	40.64	20.85
450	49.04	29.30
550	53.69	29.70

From the Table 7 is then visible that AC of torrefied non-water treated samples is extremely high, thus, water treatment prior to biochar production for energy is surely recommended.

Furthermore, we can compare the results of a recently published study from *Materials* where in the case of spent coffee ground the results in the ash content started with the initial value of 1.98 at 250°C and rose up to 6.59% at 550°C, where a similar steep rise can be seen (Jeníček et al. 2022).

From all these tests the resulting ashes of torrefied biomass samples and their colour differences can be seen in Figure 28.



**Figure 28 Dishes with the ash of torrefied biomass samples**

Source: Author (2023)

In Tables 8 & 9 we compare the chemical composition of samples in various high temperatures during torrefaction. We can again clearly see that treated samples have a higher percentual share of the desirable elements such as Carbon and Hydrogen, and more significant change in the value due to torrefaction. Nitrogen percentage is still higher in the treated sample, but the difference is not as significant as in the other two. The most prominent difference is in the highest temperature of 550 °C where the carbon share has risen up to 61.5% in treated samples; in case of non-treated *Po* the highest C value of 42.60 was observed at 300 °C.

**Table 8 Composition of torrefied samples at varying temperatures (not treated)**

<b>Temp.</b> [°C]	<b>C</b> [%]	<b>H</b> [%]	<b>N</b> [%]
Non-torrefied	32.06	5.14	0.58
250	39.30	3.82	0.78
300	42.60	3.15	0.89
350	41.85	3.17	0.90

450	40.43	2.21	0.79
550	41.59	1.51	0.74

**Table 9 Composition of torrefied samples at varying temperatures (water-treated)**

<b>Temp.</b> [°C]	<b>C</b> [%]	<b>H</b> [%]	<b>N</b> [%]
Non-torrefied	40.76	5.72	0.72
250	48.46	5.00	0.89
300	57.24	4.20	1.13
350	58.79	4.03	1.15
450	58.11	3.18	1.00
550	61.46	2.13	1.04

According to Moltó et al. (2022) results, the water treated *Po* material obtained the highest Carbon values at 300°C with 41.07%, followed by 28.31% at 400°C, and 26.60% at 500°C. In the case of Hydrogen the highest value of 2.50% was identified at 300°C, middle value of 1.17% at 400°C, and lastly 0.65% at 500°C. For Nitrogen the values at 300°C were 0.93% compared to our 0.89% in untreated sample and 1.13% in a treated sample. In the case of 400°C a N value of 0.61% was achieved and lastly at the temperature of 500°C N content of 0.56% was measured.

The above-mentioned researchers produced biochar at three different temperatures (300, 400, and 500 °C) and also analyzed the H/C ratio for all samples. They observed that the H/C ratio decreased with increasing temperature, indicating a carbonization process due to structural changes. Additionally, they measured the organic carbon content of all biochar, which was found to be greater than 10%, the minimum requirement for soil application. Comparing the characteristics of this biochar to other non-toxic biochar using germination tests, the researchers found no evidence of toxicity. This supports the potential use of this type of biochar for soil remediation, as it could help to reduce waste in landfills (Moltó et al. 2022). Torrefaction at lower temperatures is

preferred for the manufacture of and usage as solid biofuels since the lower ash concentration is desirable. Jeníček et al. (2022) claim that biochar made through torrefaction at higher temperatures has improved features (such cation exchange capacity) that can be used to boost soil quality and crop productivity.

The torrefaction test was carried out because, according to the findings described in chapter 4.3.1, extra treatment of seagrass biomass is required to increase the calorific value. To find the ideal temperature where both main parameters such as calorific value and ash content are in balance and best suited for energy utilisation, which in our case was achieved at 300°C or 350°C (where at the 350°C the calorific value is the highest and the ash content is slightly over 20%). It is necessary to take into account the fact that while the calorific value of the material is increasing during the torrefaction process, the ash content is also increasing.

#### **4.6. Experimental production of briquettes and pellets**

The mixed untreated biomass of *Zostera noltii* and *Cymodocea nodosa* was used as a feedstock for the experimental production of densified biofuels. As a result, briquettes and pellets were made. Production process was going smoothly without any complications.

As can be seen in Figure 29, it is visible that produced briquettes are well-made and look resistant to possible abrasion and damage (during handling processes and transport of biofuels).



**Figure 29 Pellets durability testing and *Po* briquette samples**

Source: Author (2021)

The mechanical durability of produced pellets was determined and resulted in 95.21%. In general, mechanical durability is the main indicator of solid biofuels' mechanical quality (strength). Biofuels of poor durability are characterized by high crumbling that contributes to losses during handling and transportation (Ivanova et al, 2018). By Novotný (2017) mechanical durability depends on moisture content, particle size, input material and compaction pressure.

According to ISO 17225-6:2014, the strict limitations of mechanical durability of graded won-woody pellets for the best A category are stated to be higher or equal to 97.5%, and for category B are stated to be higher or equal to 96%. The requirements for graded non-wooden pellets weren't slightly fulfilled. This parameter can be most probably also improved by water treatment and removal of impurities, optimizing moisture content or by using some additives – binders.



Depending on chosen technology for densified biofuels production (briquetting or pelleting), the initial feedstock can be ground in order to obtain the best quality of densified biofuels. Energy consumption of equipment for biomass grinding can be considered low due to seagrass's physical form. Soft and thin shoots can be ground and further compacted easily. These assumptions can be supported by the research conducted by Novotný (2017) where the energy consumption during processing (grinding and briquetting) of different types of biomass into solid fuels was investigated, and, it was found that soft, e.g., herbaceous biomass has the lowest energy consumption, and the highest consumption was recorded during grinding of hard wood. Nevertheless, the production of briquettes can be more economically viable when the non-ground feedstock is used for briquettes making (as it was made in our test). Production of pellets required pre-processing of feedstock (grinding, i.e., particle size reduction).

## 5. Conclusions

All in all, seagrass meadows are widely distributed throughout the Mediterranean and are considered to be a very productive ecosystem that provides numerous ecosystem services of an incalculable value. Therefore, seagrass necromass is a highly available material throughout the Mediterranean, which is commonly being removed from the beaches to create improved space for tourists, mainly to avoid the social, hygienic and economic impacts caused by the lack of beachgoers because of “polluted” beaches caused by the accumulation of banquettes. These are consequentially disposed of improperly, usually in a landfill. The removal of the biomass then only generates removal costs; however, they do not provide any benefits to the coastal communities.

Therefore, the main aims of this thesis were to explore the potentials of seagrass-based biomass and to evaluate, whether it would be a suitable resource for biofuel production. With a hypothesis of “the sea wrack is a suitable material for reuse as a biofuel and shall not be simply disposed of in landfills”. The specific objectives were to botanically identify the biomass to improve the efficiency of our research; characterise the fuel-energy properties of the material; determine the main deficiencies and try to improve them to enhance the suitability of the material.

The main deficiencies of the material were low Gross Calorific Value and high Ash Content. We tried to solve these problems with the application of two strategies. Firstly, we deployed water treatment to try and lower Ash Content in the material and we confirmed that this treatment has a positive impact on both characteristics its application lowers the Ash Content by more than -53%, while at the same time, it increases the Gross Calorific Value. Through the process of torrefaction, we managed to significantly increase the Gross Calorific Value, which was more than 36.7%. With the highest Gross Calorific Value of  $22.32 \text{ MJkg}^{-1}$  d.b. achieved during a torrefaction at  $350^{\circ}\text{C}$ , we considered this increase from the original value by an astonishing 73.56% a great success.

Thus, it can be said that the hypothesis can be accepted, as with some enhancements the seagrass wrack can be an unexpectedly great source of biomass.

Further research on optimization of seagrasses-based solid biofuels production as well as calculations of economic aspects are recommended.

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# Appendices

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## Appendix 1: Figures



Figure 1 Close-up of a briquette made from *ZoCy*

Source: Author (2021)

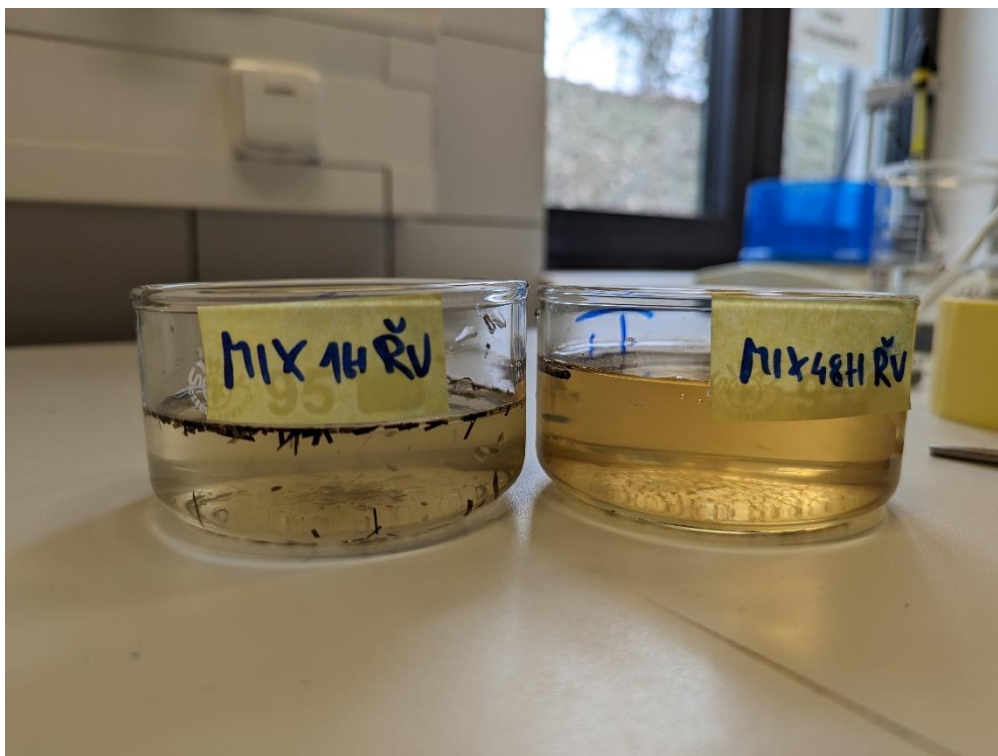


Figure 2 Difference in water colour between water treatments 1h and 48h

Source: Author (2023)



Figure 3. Water treatment method

Source: Author (2023)



Figure 4. Material pollutants

Source: Author (2023)



Figure 5. Material pollutants

Source: Author (2023)



Figure 6. Material pollutants

Source: Author (2023)



Figure 7. Fish abundance in the vicinity of *Po* meadows

Source: Author (2022)



Figure 8. Mechanical durability testing of ZoCy briquettes

Source: Author (2023)

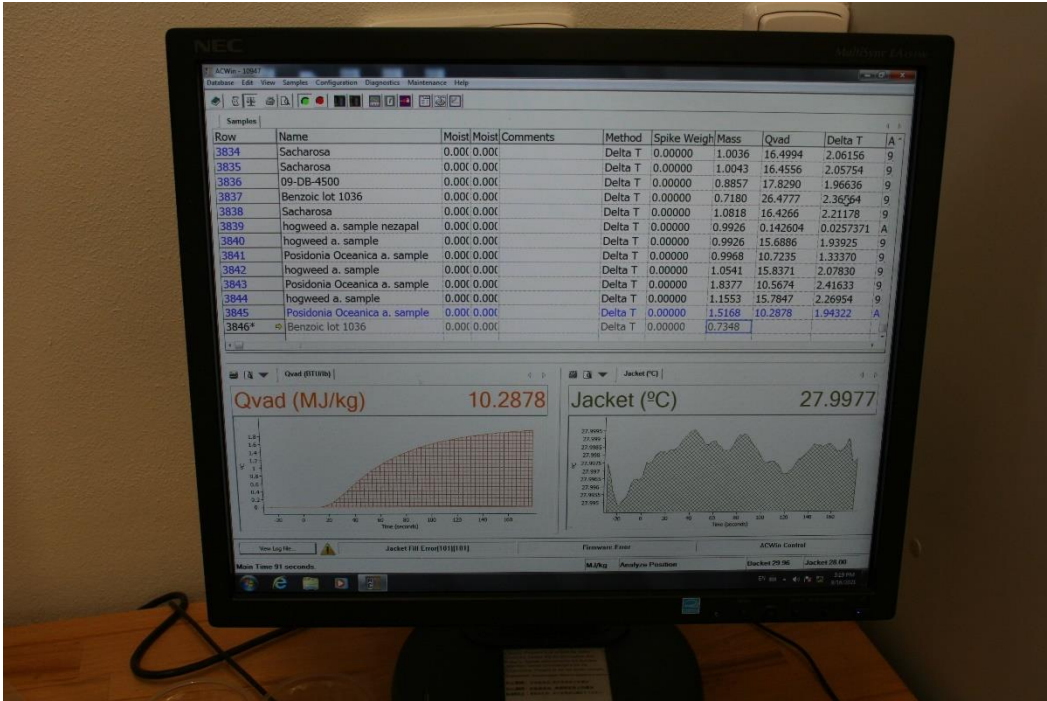


Figure 9. Calorific properties testing

Source: Author (2021)

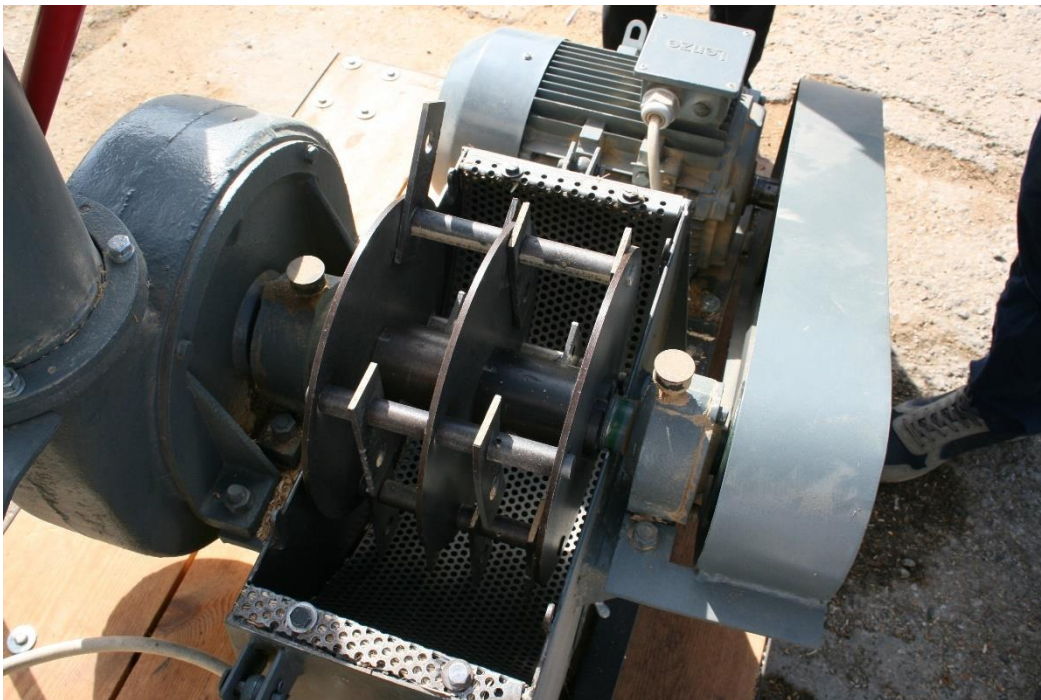


Figure 10. Hammer mill 9FQ – 40C

Source: Author (2021)



## Appendix 2: Tables

Table 1. The detailed determination of gross calorific value (dry basis) for torrefied water-treated biomass sample of *Posidonia oceanica*

Tested sample (temperature of torrefaction) (°C)	n. of repetition	The mass of ignition wire (g)	The mass of combustion bag (g)	The mass of sample (g)	Corrected temperature rise (°C)	Gross calorific value of the sample (J.°C <sup>-1</sup> )	Average value of GCV of the sample (J.°C <sup>-1</sup> )
250	1	0.0085	0.0579	0.2641	0.64165	18,344.64	18,292.65
	2	0.0086	0.0618	0.2688	0.65592	18,268.50	
	3	0.0082	0.0546	0.2040	0.51259	18,264.82	
300	1	0.0084	0.0614	0.2614	0.73830	21,682.71	21,643.53
	2	0.0084	0.0598	0.2655	0.74457	21,660.86	
	3	0.0086	0.0609	0.2617	0.73550	21,587.02	
350	1	0.0082	0.0581	0.2034	0.60675	22,250.78	22,323.90
	2	0.0083	0.0585	0.2066	0.61781	22,358.82	
	3	0.0084	0.0590	0.2046	0.61393	22,362.11	

450	1	0.0086	0.0442	0.2675	0.72881	21,907.70	21,848.55
	2	0.0082	0.0529	0.2645	0.73433	21,819.70	
	3	0.0084	0.0597	0.2627	0.74227	21,818.27	
550	1	0.0083	0.0571	0.2012	0.58946	21,790.09	21,856.19
	2	0.0084	0.0587	0.2070	0.60879	21,900.50	
	3	0.0084	0.0607	0.2012	0.59791	21,877.97	

Table 2. The detailed determination of gross calorific value (dry basis) for biomass sample of *Posidonia oceanica* treated with river-sourced water

Tested sample (duration of treatment) (h)	n. of repetition	The mass of ignition wire (g)	The mass of combustion bag (g)	The mass of sample (g)	Corrected temperature rise (°C)	Gross calorific value of the sample (J.°C <sup>-1</sup> )	Average value of GCV of the sample (J.°C <sup>-1</sup> )
1	1	0.0083	0.0573	0.2651	0.57359	15,980.79	15,952.93
	2	0.0080	0.0594	0.2652	0.57464	15,888.68	
	3	0.0087	0.0616	0.2699	0.59023	15,989.32	
5	1	0.0083	0.0656	0.2681	0.58969	15,844.41	15,855.68
	2	0.0085	0.0605	0.2657	0.57685	15,855.77	
	3	0.0085	0.0603	0.2626	0.57141	15,866.85	

12	1	0.0085	0.0656	0.2682	0.59129	15,888.31	15,940.95
	2	0.0085	0.0625	0.2677	0.58735	15,972.58	
	3	0.0083	0.0630	0.2666	0.58587	15,961.84	
24	1	0.0085	0.0605	0.2624	0.57640	16,039.57	16,071.53
	2	0.0084	0.0598	0.2620	0.57637	16,108.80	
	3	0.0085	0.0599	0.2643	0.57945	16,066.23	
36	1	0.0085	0.0610	0.2611	0.57466	16,027.62	16,048.83
	2	0.0085	0.0655	0.2691	0.59777	16,060.33	
	3	0.0086	0.0630	0.2711	0.59684	16,058.54	
48	1	0,0085	0.0583	0.2679	0.5777	15,888.12	15,911.23
	2	0.0087	0.0668	0.2648	0.58754	15,885.16	
	3	0.0086	0.0639	0.2658	0.58623	15,960.42	

Table 3. The detailed determination of gross calorific value (dry basis) for biomass sample of *Posidonia oceanica* treated with tap water

Tested sample (duration of treatment) (h)	n. of repetition	The mass of ignition wire (g)	The mass of combustion bag (g)	The mass of sample (g)	Corrected temperature rise (°C)	Gross calorific value of the sample (J.°C <sup>-1</sup> )	Average value of GCV of the sample (J.°C <sup>-1</sup> )
1	1	0.0083	0.0555	0.2210	0.49363	16,010.20	15,981.72
	2	0.0083	0.0567	0.2239	0.49998	15,973.64	
	3	0.0089	0.0572	0.2282	0.50851	15,961.32	
5	1	0.0084	0.0622	0.2640	0.58154	16,016.96	15,986.21
	2	0.0086	0.0630	0.2693	0.59099	15,968.22	
	3	0.0084	0.0649	0.2608	0.57949	15,973.44	

12	1	0.0086	0.0642	0.2529	0.56317	15,925.55	15,865.11
	2	0.0084	0.0617	0.2552	0.56061	15,854.92	
	3	0.0084	0.0622	0.2599	0.56855	15,814.86	
24	1	0.0087	0.0609	0.2536	0.56261	16,070.97	16,078.92
	2	0.0087	0.0569	0.2530	0.55456	16,076.94	
	3	0.0082	0.0540	0.2597	0.56122	16,088.85	
36	1	0.0087	0.0469	0.2500	0.52430	15,819.68	15,872.23
	2	0.0086	0.0656	0.2546	0.56767	15,890.52	
	3	0.0087	0.0679	0.2534	0.57020	15,906.49	
48	1	0.0092	0.0535	0.2568	0.56099	16,270.72	16,367.78
	2	0.0085	0.0591	0.2588	0.57799	16,406.65	
	3	0.0086	0.0570	0.2649	0.58586	16,425.96	

Table 4. The detailed determination of ash content (dry basis) for biomass sample of *Posidonia oceanica* treated with river-sourced water

Tested sample (duration of treatment) (h)	n. of repetition	The mass of empty dish (g)	The mass of the dish plus the test portion (g)	The mass of the dish plus ash (g)	Moisture content of the test portion (%)	The ash content (%)	Average value of the ash content of the test portion (%)
1	1	22.9586	24.1099	23.0932	0	11.69	11.7
	2	25.3823	26.4145	25.5032	0	11.71	
	3	22.3536	23.4838	22.4863	0	11.74	
5	1	25.3681	26.4706	25.499	0	11.87	11.7
	2	22.6764	23.9100	22.8218	0	11.79	

	3	22.9601	23.983	23.0778	0	11.51	
12	1	21.6785	22.949	21.8307	0	11.98	12.0
	2	24.6006	25.6964	24.7326	0	12.05	
	3	24.1792	25.2597	24.3093	0	12.04	
24	1	26.8716	28.0185	27.0049	0	11.62	11.6
	2	21.5174	22.5836	21.6388	0	11.39	
	3	25.7976	26.8815	25.9246	0	11.72	
36	1	26.4815	27.6520	26.6104	0	11.01	11.1
	2	20.7108	21.8630	20.8386	0	11.09	
	3	26.0849	27.2086	26.2091	0	11.05	
48	1	22.5744	23.7008	22.7013	0	11.27	11.4
	2	21.6238	22.7888	21.7566	0	11.40	
	3	21.3268	22.5695	21.4684	0	11.39	

Table 5. The detailed determination of ash content (dry basis) for biomass sample of *Posidonia oceanica* treated with tap water

Tested sample (duration of treatment) (h)	n. of repetition	The mass of empty dish (g)	The mass of the dish plus the test portion (g)	The mass of the dish plus ash (g)	Moisture content of the test portion (%)	The ash content (%)	Average value of the ash content of the test portion (%)
1	1	21.6222	22.6484	21.7389	0	11.37	11.5
	2	20.7090	21.7251	20.8276	0	11.67	
	3	24.1773	25.2286	24.2978	0	11.46	
5	1	25.3660	26.3176	25.4831	0	12.31	12.0
	2	26.0827	27.1509	26.2110	0	12.01	

	3	22.9583	23.9879	23.0769	0	11.52	
12	1	25.3798	26.4023	25.5000	0	11.76	11.7
	2	26.8695	27.8770	26.9866	0	11.62	
	3	22.5734	23.8468	22.7214	0	11.62	
24	1	24.5989	25.6125	24.7086	0	10.82	10.9
	2	22.3524	23.4113	22.4689	0	11.00	
	3	21.5171	22.5997	21.6341	0	10.81	
36	1	22.6757	23.6847	22.7944	0	11.76	11.1
	2	21.6769	22.6921	21.7965	0	11.78	
	3	26.4813	27.5133	26.6016	0	11.66	
48	1	22.9576	24.0437	23.0731	0	10.63	10.6
	2	25.7966	26.8411	25.9079	0	10.66	
	3	21.3258	22.3599	21.4337	0	10.43	

Table 6. The detailed determination of ash content (dry basis) for torrefied biomass sample of water-treated *Posidonia oceanica*

Tested sample (temperature of torrefaction) (°C)	n. of repetition	The mass of empty dish (g)	The mass of the dish plus the test portion (g)	The mass of the dish plus ash (g)	Moisture content of the test portion (%)	The ash content (%)	Average value of the ash content of the test portion (%)
Non-torrefied	1	24.1790	25.2184	24.2800	7.71	10.53	10.46
	2	20.3095	21.3400	20.4084	7.71	10.40	
250	1	21.8871	22.8896	22.0124	1.28	12.66	12.61
	2	16.1492	17.1561	16.2741	1.28	12.57	

300	1	18.0826	19.0990	18.2617	1.02	17.80	17.92
	2	20.8124	21.8270	20.9936	1.02	18.04	
350	1	21.5172	22.5202	21.7206	0.75	20.43	20.85
	2	24.6000	25.6017	24.8044	0.75	21.26	
450	1	18.6618	19.6664	18.9161	4.03	26.38	26.30
	2	26.4815	27.5093	26.7402	4.03	26.23	
550	1	16.7525	17.7613	17.0450	2.01	29.59	29.70
	2	15.6349	16.6688	15.9369	2.01	29.81	

Table 7. The detailed determination of ash content (dry basis) for torrefied biomass sample of untreated *Posidonia oceanica*

Tested sample (temperature of torrefaction) (°C)	n. of repetition	The mass of empty dish (g)	The mass of the dish plus the test portion (g)	The mass of the dish plus ash (g)	Moisture content of the test portion (%)	The ash content (%)	Average value of the ash content of the test portion (%)
Non-torrefied	1	26.8709	27.9109	27.1047	12.28	25.63	25.6
	2	25.3815	26.4031	25.6106	12.28	25.56	
250	1	22.5744	23.6383	22.8992	0.72	30.75	30.7
	2	21.3266	22.3882	21.6495	0.72	30.64	
300	1	20.2228	21.3034	20.6275	1.33	37.96	37.8
	2	22.3536	23.3898	22.7393	1.33	37.72	
350	1	22.9600	24.1012	23.4103	3.17	40.75	40.6
	2	23.0914	24.1046	23.4890	3.17	40.53	
450	1	16.7144	17.7445	17.2076	2.63	49.17	49.0

	2	25.3683	26.4379	25.8777	2.63	48.91	
550	1	24.8652	25.9340	25.4320	1.6	53.89	53.7
	2	22.6767	23.7729	23.2537	1.6	53.49	

Table 8. The detailed determination of C,H,N content (dry basis) for torrefied biomass sample of *Posidonia oceanica* (continues on another page)

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Name	Carbon %	Hydrogen %	Nitrogen %	Mass	Carbon mg	Hydrogen mg	Nitrogen mg	Analysis Date	Comments
Posidonia	32.094	5.1520	0.55126	0.1306	42.349	6.5609	0.71994	3/31/2023 4:54:04 PM	
Posidonia	31.960	5.1281	0.60733	0.1254	40.294	6.2413	0.76159	3/31/2023 5:57:54 PM	
Posidonia	32.138	5.1558	0.58329	0.1214	39.038	6.0662	0.70812	3/31/2023 7:01:51 PM	
Average	32.064	5.1453	0.58062	0.1258	40.561	6.2894	0.72988		
Std. Deviation	0.0925	0.01499	0.028129	0.005	1.6717	0.25085	0.028087		
RSD	0.289	0.291	4.845	3.667	4.122	3.988	3.848		

Name	Carbon %	Hydrogen %	Nitrogen %	Mass	Carbon mg	Hydrogen mg	Nitrogen mg	Analysis Date	Comments
Posidonia promyta	40.820	5.7396	0.67773	0.1193	49.204	6.6767	0.80853	3/31/2023 4:58:37 PM	
Posidonia promyta	40.614	5.7002	0.76013	0.1180	48.183	6.5281	0.89695	3/31/2023 6:02:27 PM	
Posidonia promyta	40.801	5.7153	0.72406	0.1076	43.928	5.9600	0.77909	3/31/2023 7:06:25 PM	
Average	40.745	5.7183	0.72064	0.1150	47.105	6.3883	0.82819		
Std. Deviation	0.1142	0.01987	0.041305	0.006	2.7983	0.37826	0.061343		
RSD	0.280	0.347	5.732	5.578	5.941	5.921	7.407		

Name	Carbon %	Hydrogen %	Nitrogen %	Mass	Carbon mg	Hydrogen mg	Nitrogen mg	Analysis Date	Comments
Posidonia 250C	39.118	3.8157	0.73587	0.1091	43.121	4.0592	0.80283	3/31/2023 5:03:10 PM	
Posidonia 250C	39.450	3.8593	0.81535	0.1005	39.861	3.7643	0.81943	3/31/2023 6:07:01 PM	
Posidonia 250C	39.330	3.7977	0.78405	0.1005	39.550	3.6990	0.78797	3/31/2023 7:10:59 PM	
Average	39.299	3.8242	0.77842	0.1034	40.844	3.8408	0.80341		
Std. Deviation	0.1680	0.03167	0.040041	0.005	1.9779	0.19191	0.015740		
RSD	0.428	0.828	5.144	4.803	4.843	4.996	1.959		

Name	Carbon %	Hydrogen %	Nitrogen %	Mass	Carbon mg	Hydrogen mg	Nitrogen mg	Analysis Date	Comments
Posidonia promyta 250C	48.447	5.0177	0.92975	0.0998	48.851	4.8829	0.92789	3/31/2023 5:07:43 PM	
Posidonia promyta 250C	48.405	5.0066	0.89572	0.0931	45.309	4.5238	0.83391	3/31/2023 6:11:34 PM	
Posidonia promyta 250C	48.533	4.9887	0.84783	0.0963	46.765	4.6559	0.81646	3/31/2023 7:15:34 PM	
Average	48.462	5.0043	0.89110	0.0964	46.975	4.6876	0.85942		
Std. Deviation	0.0655	0.01467	0.041155	0.003	1.7808	0.18163	0.059934		
RSD	0.135	0.293	4.618	3.476	3.791	3.875	6.974		

Name	Carbon %	Hydrogen %	Nitrogen %	Mass	Carbon mg	Hydrogen mg	Nitrogen mg	Analysis Date	Comments
Posidonia 300C	42.144	3.1161	0.88714	0.1209	51.481	3.6736	1.0726	3/31/2023 5:12:17 PM	
Posidonia 300C	42.925	3.1690	0.89380	0.1050	45.315	3.2294	0.93849	3/31/2023 6:16:08 PM	
Posidonia 300C	42.735	3.1611	0.90250	0.0987	42.203	3.0238	0.89077	3/31/2023 7:20:09 PM	
Average	42.601	3.1487	0.89448	0.1082	46.333	3.3089	0.96727		
Std. Deviation	0.4070	0.02850	0.007704	0.01	4.7221	0.33210	0.094247		
RSD	0.955	0.905	0.861	10.57	10.19	10.04	9.744		

Name	Carbon %	Hydrogen %	Nitrogen %	Mass	Carbon mg	Hydrogen mg	Nitrogen mg	Analysis Date	Comments
Posidonia promyta 300C	57.104	4.2033	1.1247	0.1008	58.158	4.1314	1.1337	3/31/2023 5:16:50 PM	
Posidonia promyta 300C	57.302	4.2068	1.1106	0.0971	55.941	3.9645	1.0784	3/31/2023 6:20:42 PM	
Posidonia promyta 300C	57.315	4.1865	1.1548	0.0938	53.793	3.8059	1.0832	3/31/2023 7:24:44 PM	
Average	57.241	4.1989	1.1300	0.0972	55.964	3.9673	1.0984		
Std. Deviation	0.1185	0.01082	0.02256	0.004	2.1827	0.16277	0.03062		
RSD	0.207	0.258	1.996	3.602	3.900	4.103	2.788		

Name	Carbon %	Hydrogen %	Nitrogen %	Mass	Carbon mg	Hydrogen mg	Nitrogen mg	Analysis Date	Comments
Posidonia 350C	41.415	3.1518	0.89096	0.1103	46.061	3.3789	0.98273	3/31/2023 5:25:58 PM	
Posidonia 350C	41.959	3.1701	0.91235	0.1060	44.617	3.2629	0.96709	3/31/2023 6:29:51 PM	
Posidonia 350C	42.162	3.1921	0.88965	0.1029	43.324	3.1816	0.91545	3/31/2023 7:33:54 PM	
Average	41.845	3.1713	0.89765	0.1064	44.668	3.2745	0.95509		
Std. Deviation	0.3865	0.02021	0.012744	0.004	1.3691	0.09914	0.035208		
RSD	0.924	0.637	1.420	3.493	3.065	3.028	3.686		

Name	Carbon %	Hydrogen %	Nitrogen %	Mass	Carbon mg	Hydrogen mg	Nitrogen mg	Analysis Date	Comments
Posidonia promyta 350C	59.455	3.9315	1.1959	0.0958	57.432	3.6607	1.1457	3/31/2023 5:30:32 PM	
Posidonia promyta 350C	59.577	3.9368	1.2024	0.0986	58.929	3.7692	1.1855	3/31/2023 6:34:25 PM	
Posidonia promyta 350C	57.346	4.2078	1.0536	0.0972	55.662	3.9616	1.0241	3/31/2023 7:38:28 PM	
Average	58.793	4.0254	1.1506	0.0972	57.341	3.7971	1.1184		
Std. Deviation	1.2541	0.15801	0.08411	0.001	1.6351	0.15238	0.08411		
RSD	2.133	3.925	7.310	1.440	2.852	4.013	7.520		

Name	Carbon %	Hydrogen %	Nitrogen %	Mass	Carbon mg	Hydrogen mg	Nitrogen mg	Analysis Date	Comments
Posidonia 450C	39.914	2.1755	0.79928	0.1047	42.138	2.2138	0.83685	3/31/2023 5:35:06 PM	
Posidonia 450C	40.948	2.2491	0.82497	0.0994	40.831	2.1708	0.82002	3/31/2023 6:38:59 PM	
Posidonia 450C	40.427	2.2119	0.74956	0.1053	42.510	2.2561	0.78929	3/31/2023 7:43:03 PM	
Average	40.430	2.2122	0.79127	0.1031	41.826	2.2136	0.81538		
Std. Deviation	0.5171	0.03682	0.038334	0.003	0.8816	0.04261	0.024114		
RSD	1.279	1.665	4.845	3.148	2.108	1.925	2.957		

Name	Carbon %	Hydrogen %	Nitrogen %	Mass	Carbon mg	Hydrogen mg	Nitrogen mg	Analysis Date	Comments
Posidonia promyta 450C	58.155	3.1923	1.0173	0.0899	52.717	2.7894	0.91451	3/31/2023 5:39:40 PM	
Posidonia promyta 450C	57.843	3.1711	0.99074	0.0867	50.309	2.6697	0.85898	3/31/2023 6:43:33 PM	
Posidonia promyta 450C	58.321	3.1873	0.99868	0.0974	56.725	3.0070	0.97272	3/31/2023 7:47:38 PM	



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Name	Carbon %	Hydrogen %	Nitrogen %	Mass	Carbon mg	Hydrogen mg	Nitrogen mg	Analysis Date	Comments
Average	58.107	3.1836	1.0022	0.0913	53.250	2.8220	0.91540		
Std. Deviation	0.2424	0.01109	0.01360	0.005	3.2409	0.17102	0.056877		
RSD	0.417	0.348	1.357	6.013	6.086	6.060	6.213		

Name	Carbon %	Hydrogen %	Nitrogen %	Mass	Carbon mg	Hydrogen mg	Nitrogen mg	Analysis Date	Comments
Posidonia 550C	41.587	1.5022	0.76316	0.1015	42.562	1.4820	0.77461	3/31/2023 5:44:13 PM	
Posidonia 550C	42.348	1.5321	0.73145	0.0972	41.293	1.4461	0.71097	3/31/2023 6:48:08 PM	
Posidonia 550C	40.844	1.4824	0.73182	0.0993	40.501	1.4258	0.72669	3/31/2023 7:52:13 PM	
Average	41.593	1.5056	0.74214	0.0993	41.452	1.4513	0.73742		
Std. Deviation	0.7524	0.02505	0.018204	0.002	1.0399	0.02847	0.033149		
RSD	1.809	1.664	2.453	2.165	2.509	1.962	4.495		

Name	Carbon %	Hydrogen %	Nitrogen %	Mass	Carbon mg	Hydrogen mg	Nitrogen mg	Analysis Date	Comments
Posidonia promyta 550C	61.494	2.1578	1.0302	0.0822	50.969	1.7240	0.84683	3/31/2023 5:48:46 PM	
Posidonia promyta 550C	61.459	2.1094	1.0335	0.0997	61.469	2.0421	1.0304	3/31/2023 6:52:43 PM	
Posidonia promyta 550C	61.425	2.1290	1.0607	0.0929	56.983	1.9157	0.98543	3/31/2023 7:56:48 PM	
Average	61.459	2.1321	1.0415	0.0916	56.474	1.8939	0.95422		
Std. Deviation	0.0347	0.02436	0.01676	0.009	5.2684	0.16020	0.095687		
RSD	0.056	1.143	1.610	9.631	9.329	8.458	10.03		

Element	Average	Std. Deviation	RSD	Count
Carbon %	46.887	9.3507	19.94	36
Hydrogen %	3.6058	1.25877	34.91	36
Nitrogen %	0.88506	0.170689	19.29	36
Mass	0.1029	0.01	10.43	36
Carbon mg	47.733	6.6253	13.88	36
Hydrogen mg	3.6612	1.52011	41.52	36
Nitrogen mg	0.89855	0.132082	14.70	36

Table 9. Minor elements composition of biomass samples

<b>Sample</b>	<b>As</b> (mg/kg)	<b>Cd</b> (mg/kg)	<b>Cr</b> (mg/kg)	<b>Cu</b> (mg/kg)	<b>Hg</b> (mg/kg)	<b>Ni</b> (mg/kg)	<b>Pb</b> (mg/kg)	<b>Zn</b> (mg/kg)
<i>Posidonia</i> untreated	1.2	0.06	1.6	4.5	0.036	23	1.8	15
<i>Zostera</i> untreated	4.0	0.45	19	6.6	0.036	15	7.1	27

Table 10. Physicochemical characteristics of the biomass of biomass samples with different types of treatment (is divided in three tables)

<b>Sample</b>	<b>C</b> (% d.b.)	<b>H</b> (% d.b.)	<b>N</b> (% d.b.)	<b>S</b> (% d.b.)	<b>Cl</b> (% d.b.)	<b>O</b> (% d.b.)	<b>Al</b> (% d.b.)	<b>Ba</b> (% d.b.)
<i>Posidonia</i> untreated	34.7	4.3	0.59	0.71	5.70	27.04	0.27	<0.002
<i>Zostera</i> untreated	33.1	4.0	1.18	0.64	2.87	24.80	2.8	0.009
<i>Posidonia</i> sieved	34.6	4.3	0.57	0.63	6.69	26.16	0.20	<0.002
<i>Zostera</i> sieved	33.0	3.8	1.18	0.63	2.57	27.71	2.9	0.009
<i>Posidonia</i> water- treated	42.5	5.2	0.69	0.20	0.72	38.09	0.43	0.002
<i>Zostera</i> water- treated	38.5	4.5	1.31	0.32	0.50	34.17	2.9	0.010

<b>Sample</b>	<b>Ca</b> (% d.b.)	<b>Fe</b> (% d.b.)	<b>K</b> (% d.b.)	<b>Mg</b> (% d.b.)	<b>Mn</b> (% d.b.)	<b>Na</b> (% d.b.)	<b>P</b> (% d.b.)	<b>S</b> (% d.b.)
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Posidonia untreated	12	0.30	1.1	6.1	0.025	20	0.035	2.6
Zostera untreated	15	1.8	1.7	4.8	0.13	7.2	0.21	1.9
Posidonia sieved	11	0.23	1.5	5.5	0.020	17	0.018	2.3
Zostera sieved	16	1.9	1.5	5.2	0.14	7.9	0.25	2.0
Posidonia water-treated	28	0.58	0.56	10	0.050	5.8	0.092	1.7
Zostera water-treated	19	1.1	0.95	6.8	0.16	3.3	0.29	1.6

<b>Sample</b>	<b>Si (% d.b.)</b>	<b>Sr (% d.b.)</b>	<b>Ti (% d.b.)</b>	<b>Zn (% d.b.)</b>
Posidonia untreated	5.4	0.11	0.006	0.011
Zostera untreated	17	0.12	0.18	0.013
Posidonia sieved	3.6	0.099	<0.002	0.007
Zostera sieved	15	0.13	0.19	0.014
Posidonia water-treated	4.2	0.26	0.013	0.019
Zostera water-treated	13	0.16	0.17	0.018