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RESEARCH PAPER

Determining the Usage Properties of Some Natural Substrate Materials in Submerged Macrophyte *Vallisneria* sp. Culture

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Abstract: In this research, which targets removing nutrients resulting from the metabolic wastes of fish and unconsumed feed from the environment in aquarium systems where fish, plants, and ground material are used in the same environment and use them as plant nutrients, it was aimed to determine the usage properties of zeolite, leonardite and diatomite materials on the growth parameters of the *Vallisneria* sp. plant.
The study was conducted in three stages for 8 months. Trial groups were formed by adding the adsorbent mixture of the feed having a 40% protein value to the first group (G1), the adsorbent mixture of the feed having a 33% protein value to the second group (G2), and the adsorbent mixture without feed to the third group (G3).
When the SGR values at the end of the experiment were compared, there was no statistical difference between the G1 and G2 groups, which contained 40% and 33% protein (P>0.05). On the other hand, both groups (G1, G2) showed significantly higher (P<0.05) growth compared to the control group (G3). In this study, it was determined that the natural adsorbent mixture supported the growth of the *Vallisneria* plant for approximately 8 months and that adding adsorbent mixture enriched with nutrients is necessary at certain intervals.

Keywords: Adsorbent, ammonium, aquatic plants, leonardite, zeolite.

Bazı Doğal Substrat Malzemelerin Sualtı Makrofit Vallisneria sp. Kültüründe Kullanım Özelliklerinin Belirlenmesi

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Öz: Balık, bitki ve zemin materyalinin aynı ortamda kullanıldığı akvaryum sistemlerinde balıkların metabolik atıkları ve tüketilmeyen yemden kaynaklanan nütrientlerin ortamdan uzaklaştırılarak, bitki besini olarak kullanımı hedeflenen bu araştırmada, *Vallisneria* sp. bitkisinin büyüme parametreleri üzerine zeolit, leonardit ve diyatomit materyalinin kullanım özelliklerinin belirlenmesi amaçlanmıştır.
Araştırma üç aşamada 8 ay süreyle yürütülmüştür. Deneme grupları, birinci gruba %40 protein değerine sahip yemin adsorban karması (G1), ikinci gruba %33 protein değerine sahip yemin adsorban karması (G2) ve üçüncü gruba (G3) ise yemsiz olan adsorban karması eklenerek oluşturulmuştur.
Deneme başı ve sonundaki SGR değerleri karşılaştırıldığında %40 ve %33 protein içeren G1 ve G2 grupları arasında istatistiki bir fark görülmezken (P>0.05), her iki grubun (G1, G2) kontrol grubuna (G3) kıyasla istitistiki anlamda (P<0.05) daha iyi büyüme gösterdiği belirlenmiştir. Bu çalışmada, doğal adsorban karmasının yaklaşık olarak 8 ay süreyle Vallisneria bitkisinin büyümesine destek olduğu ve belirli aralıklar ile yeniden nütrientlerce zenginleştirilmiş adsorban karması ilavesinin gerekli olduğu belirlenmiştir.

Anahtar kelimeler: Adsorban, amonyum, leonardit, sucul bitki, zeolite.

INTRODUCTION

Aquatic plants play an active role in establishing the balance of aquatic life. In the aquatic ecosystem, aquatic plants are an important link in the food chain and on water parameters regulations, and they play a role in vital functions such as protection and reproduction for many living species. Aquarium plants are the most commonly used living species after fish in regulating freshwater aquariums. Their use for decorative purposes, in addition to maintaining the balance of aquatic life, is also a common practice (Les et al., 2008; Shelar et al., 2012). Macrophytes are used as turbidity-reducing agents, non-pollutant agents, fish feeding, and biofilters. Aquatic plants, in addition to removing nutrients for their own growth, contribute to nutrient transformation through a setting of physical, chemical and microbial processes (Sirakov et al., 2015; Zhang et al., 2019).

Valissneria L. (Hydrocharitaceae), a genus of submerged aquatic flowering plants, often form dense stands in natural habitats because of their stoloniferous growth habits. This genus is distributed in a wide area, especially temperate, tropical, and subtropical regions of Europe, North America, Australia, Asia, and Africa (Martin and Mort, 2023).

Among the most common submerged macrophytes in freshwater lake habitats is *Vallisneria natans*, which is a prominent factor in phytoremediation of contaminated sediments (Yan et al., 2022). Submerged macrophytes hold great significance in aquatic ecosystems due to their ability in the remediation of inorganic or organic contaminated sediments (Huang et al., 2021).

The use of various nutrients (Gue et al., 2016; Teğoğlu et al., 2017; Ünver, 2019) and ground materials for plants in aquatic plant culture (Xie et al., 2007; Qin et al., 2011, Gosselin et al.., 2018; Emirzeoğlu, 2020; Wang et al., 2023) has been the subject of many studies. Some of the natural substrate and adsorbent materials, such as zeolite, lava stone, leonardite, diatomite, perlite, etc., have been used for these purposes for many years and have become commercial products, while some of them have potential use and are the subject of current research (Yolcu et al., 2011; Ratanaprommanaee et al., 2016; Sava et al., 2019; Öz et al., 2022). These natural materials are used in regulating physical, chemical, and biological properties in the soil or aquatic environment through their physical and chemical properties (Wang et al., 2023). Moreover, it has been determined as a result of some studies that the efficiency of these natural materials can be increased by using them synergistically (Zang et al., 2019; Şahin, 2022; Zengin, 2013).

In the cultivation of aquatic plants, ground materials and some nutrients (such as nitrogen, phosphorus, and potassium) play a direct role. In aquatic ecosystems nutrients such as nitrogenous compounds, phosphorus, and potassium resulting from unconsumed feed and metabolic wastes of fish should be kept under control and prevented from reaching hazardous amounts (Kibria et al., 1997; Hua and Bureau, 2006; Munguti et al., 2021). The mentioned nutrients are used as food by plants. Hence, plants contribute to the preservation of aquatic balance (Loh et al., 2009; Sirakov et al., 2015). In order to protect the living community of aquatic ecosystems such as fish, invertebrates, plants, and bacteria, or the dynamic balance and interactions between these living groups and water or ground material, it is important to determine the characteristics of each contributor. A problem in any contributor has the ability to affect all other contributors. Therefore, it is important to use natural and harmless materials.

Zeolites, which are crystalline tectoaluminosilicates, are composed of $[SiO_4]$ $[AlO_4]_5$-tetrahedra linked at their corners, enabling them to form a well-defined 3D framework. Orientations of $[SiO_4]$ 4- and $[AlO_4]_5$- tetrahedra in their structure cause pores and channels to form on a molecular scale. Alkaline (Ca_2^+, Mg_2^+, etc.) or alkali (Na^+, K^+, etc.) earth metal ions encapsulated in surrounded water molecules within the void space compensate for the excess of negative charges produced by Al_3^+ (Sayehi et al., 2022).

Leonardite is highly enriched in HS, is characterised by well-known auxin-like effects and is an oxidised species of lignite (Barone et al., 2019). It contains a significant amount of organic matter (50 to 75%). Furthermore, its humic acid content ranges from 30 to 80%. Humic acid and other humic substances stimulate root and shoot development and soil aggregation, increasing nutrient availability and nutrient uptake from the soil (Ratanaprommanee et al., 2016).

The chemical formula of the porous frustules of diatomite is $SiO_2 \cdot nH_2O$ in the form of amorphous silica (opal-A). These do not contain crystalline silica; however, the impurities in diatomite deposits may carry quartz crystalline silica (Ersoy et al., 2022). Large specific surface area, high purity, chemical resistance, permeability of their structure, low bulk density, high porosity, and their use in the food industry as filtration materials (sugar syrup, water, fruit juices, etc.) are among the notable properties of diatomite (Reka et al., 2021).

Natural materials such as zeolite, besides its water and soil regulator properties, can retain nutrients such as nitrogen, phosphorus, and potassium in their structure and release them back in a controlled manner when they reach saturation (Yolcu et al., 2011; Moreno et al., 2017; Kalita et al., 2020; Szatanik-Kloc et al., 2021). With the help of these properties, natural adsorbent substances can be used in systems such as aquariums, where some nutrients are not desired to reach excessive amounts, by retaining (adsorbing) the excess of these nutrients and transporting them to environments where they can be consumed as nutrients by plants.

Plants such as *Centella asiatica*, *Ipomoea aquatica*, *Salvinia molesta*, *Eichhornia rassipes*, *Pistia stratiotes*, and *Vallisneria* sp., which are among the plant species of economic importance in aquaculture, can be used to improve the quality of the culture water (Nizam et al., 2020; Priya et al., 2022). In this research, which targets removing nutrients resulting from the metabolic wastes of fish and unconsumed feed from the environment in aquarium systems where fish, plants, and ground material are used in the same environment and use them as plant nutrients, it was aimed to determine the usage properties of zeolite, leonardite and diatomite materials on the growth parameters of the *Vallisneria* sp. plant.

MATERIAL AND METHOD

The study was carried out in three stages for 8 months at Sinop University, Faculty of Fisheries. The effects and using duration on the growth of the *Vallisneria* sp. plant of adsorbed nutrients from unconsumed feed by leonardite and zeolite mixture were investigated in aquarium condition.

A natural photoperiod was applied in the experiment. Water parameters (temperature, dissolved oxygen, pH, and NH_4^+) were measured with YSI Professional Plus Series instrument. The *Vallisneria* sp. plant and substrate materials (zeolite, leonardite, and diatomite) used in the study were supplied by local commercial companies. The *Vallisneria* is a freshwater macrophyte used for freshwater aquariums and has economic importance. Its suitable water parameter values are in the ranges of 15-30 °C, 6-9 pH, and 15-20 ppt salinity (Tootoonchi et al., 2020; Nagy, 2019).

In the study, powder-sized (100 microns) natural zeolite and leonardite and 1-3 mm sized diatomite were used without any processing (Figure 1).

Figure 1. Raw materials (diatomite, leonardite, and zeolite)

XRF (X-ray Fluorescence) analysis was conducted by the Central Research Laboratory of Kastamonu University. The results for the chemical compositions of these materials are given in Table 1.

Table 1. XRF results of natural diatomite, leonardite, and zeolite used in the study.

Chemical Compositions			
Chemicals	Diatomite	Leonardite	Zeolite
SiO_2 (%)	78.36	8.98	67.83
Al_2O_3 (%)	11.63	4.47	11.54
MgO (%)	3.81	0.19	1.24
K_2O (%)	1.15	0.34	2.11
CaO (%)	2.63	3.05	3.23
Na_2O (%)	1.46	0.01	0.57
Fe_2O_3 (%)	2.75	0.88	1.40
P_2O_5 (%)	0.37	0.31	0.22
SiO_2/Al_2O_3	6.73	2.00	5.88
pH	7.55±0.02	4.28±0.02	8.84±0.02

In the research, a preliminary study was carried out as the adsorption stage. In this adsorption stage, three groups were formed (G1 (40%), G2 (33%), Control, G3 (0%)) in triplicate. The leonardite and zeolite mixture used in the experiment was mixed in a 2 to 1 ratio, and a total of 3 g from this mixture was placed on the ground of the aquariums (Şahin, 2022). The L:Z (2:1) mixture was held for 12 days in an environment with 40% and 33% crude protein-containing feed and 500 ml tap water for the adsorption of nutrients in the feed, such as nitrogen, phosphorus, and potassium. In addition, for the control group, 3 g adsorbent mixture used in powder size was kept in freshwater without feed for 12 days. At the end of this period, these adsorbent mixtures were used to create the experimental groups (Figure 2).

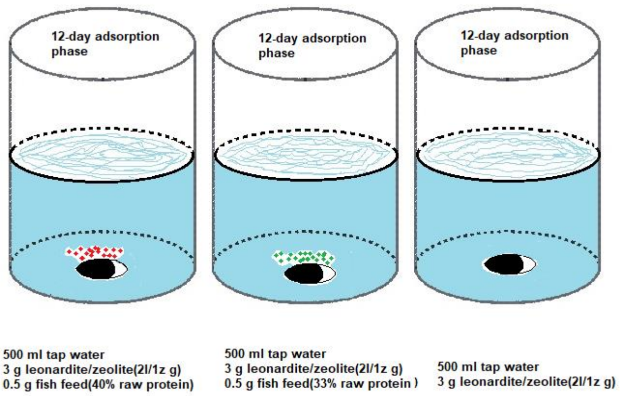

Figure 2. Adsorption phase of natural adsorbent mix during 12 days.

The first step of the three-stage study, created after the adsorption stage, was planned as the plant adaptation stage, and no fish were added to the environment. In the second step, fish was added to the environment. The third step was planned to determine the plant nutrient storage capacity (effective usage period) of the adsorbent mixture. At all stages, no plant nutrient or fertiliser other than fish food was used in the experimental aquariums.

In the first stage, three groups with three replications were formed. The first group (G1) received the adsorbent mixture obtained from the environment that contained the feed having 40% protein value, the second group (G2) received the adsorbent mixture obtained from the environment that included the feed having 33% protein value, and the third group (G3) received the adsorbent mixture obtained from the environment where there was no feed (the mixtures obtained after the adsorption stage). Furthermore, an equal amount of diatomite (4 g) was added to each 500 ml tap water cups as 1-3 mm diatomite as the same substrate material. The transparent containers in which the plants are planted have a 200 ml water capacity and were arranged with 3 cm of substrate material at the bottom (Li et al., 2012; Shelar et al., 2016; Ünver, 2019; Tang et al., 2021). Two *Vallisneria sp.* plants of equal weight and length were planted in experimental containers for each repetition (A total of 6 rooted plants for each group). The plants were cut with pruning shears to be arranged to have equal weight and length of roots and leaves (Li et al., 2012; Ünver, 2019; Tang et al., 2021). Trial containers were placed into a stock aquarium (50x30x25 cm) with 30 L of freshwater. The research setup was created using a heater and aerator in the stock aquarium (Figure 3). Additionally, 0.3 g of the feed containing 33% crude protein was added to the stock aquarium weekly as the plant food source. At the end of the 3-month-lasting phase, the effects of using natural adsorbent substances, which were previously submerged in water containing feed with two different protein contents, as ground material for plant growth were determined.

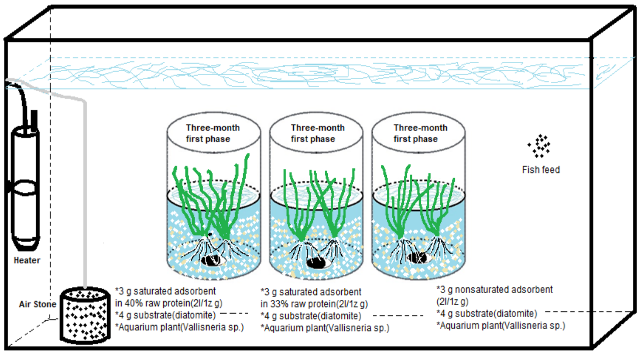

Figure 3. First stage of the experiment.

After the first stage, data were collected by measuring the plants. Afterwards, the roots and leaves were cut equally with pruning shears for the next step. They were again planted on the same ground material, and the second stage was initiated. At this stage, guppy fish were added to the stock aquarium (7 fish in total, 4 females and 3 males), and feeding was performed daily. The fish were fed with the feed containing 33% crude protein *ad libitum*. At this stage, the effects of natural adsorbent mixtures on plant growth parameters in aquarium water containing fish and feed were monitored for three months. At the end of this stage, plant weights and lengths were determined once again (Figure 4).

For the third stage, the plants, cut to equal lengths with pruning shears, were planted in equal weights in the same experimental containers, and the final step was started. Two months later, the research was completed with the observation of yellowing and detachment in the leaves of the plants. With the help of this stage, how long plant growth could be achieved by the nutrients retained by the adsorbent mixture was determined (Figure 5).

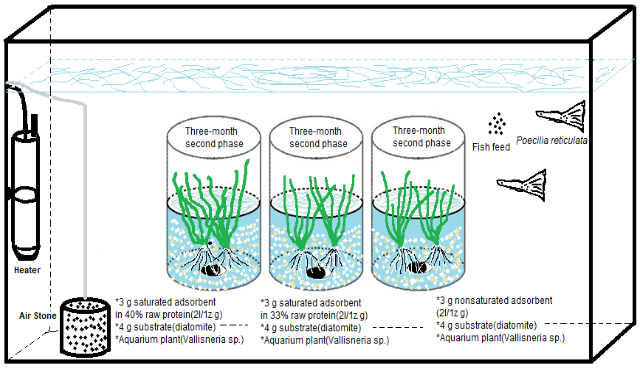

Figure 4. Second stage of the experiment.

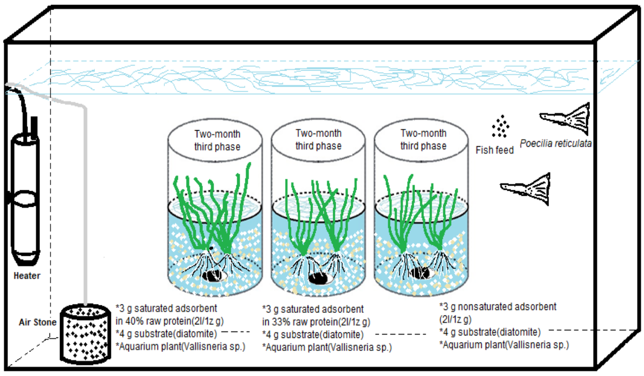

Figure 5. Third stage of the experiment

After the plants were lightly dehumidified with a paper towel, they were weighed and their weights were determined, and their weight gain and specific growth rates at the end of the study were determined. For weight measurement, individual roots and stems were weighed together, and for the length determination, individual stem and root lengths were measured (Tekoğul et al., 2017; Tootoonchi et al., 2020).

Weight gain and specific growth rate values were computed as per the following equations;

$$\text{Weight gain (g)} = \text{Final live weight (g)} - \text{Initial live weight (g)} \quad \text{(Eq.1)}$$

$$\text{Specific growth rate (\%)} = \frac{\ln Wf - \ln Wi}{t - ti} \times 100 \quad \text{(Eq.2)}$$

Where; Wf is the final weight (g), Wi is the initial weight (g) and (t-ti) is the duration of the experiment (days).

Statistical Analyses: Statistical analyses were performed using “Minitab for Windows” software (Release 17) with a 95% confidence interval. The data, presented as mean±standard error, were subjected to one-way analysis of variance (ANOVA). The means were compared using Fisher’s test when ANOVA indicated significant differences between groups.

RESULTS

The mean water temperature, pH, ammonium, and dissolved oxygen values throughout the study were determined as 23.55±0.50 °C, 8.51±0.01, 0.45± 0.03 mg/l, and 6.48±0.06 mg/l, respectively. At the beginning of all phases of the experiment, the stem and root lengths of the plants were cut equally and arranged to be 9 cm and 3 cm, respectively. Weight and length data obtained in the study are presented in Table 2. The appearances of the *Vallisneria sp.* plants used in the study at the beginning and end of the experiments are displayed in Figure 6.

Table 2. Weight and length data obtained from *Vallisneria sp.* plants during the trial stages.

	Groups	Mean Initial Weight (g)	Mean Final Weight (g)	Mean stem length at the end o the trial (cm)	Mean root length at the end of the trial (cm)	Weight gain (g)	Stem length gain (cm)	Root length gain (cm)
Period 1	G1	1.40±0.16^{a}	2.91±0.04^{a}	28.67±1.76^{a}	10.35±0.47^{a}	1.52±0.12^{a}	19.33±1.50^{a}	7.35±0.47^{a}
	G2	1.51±0.17^{a}	3.14±0.17^{a}	29.67±0.33^{a}	10.67±0.51^{a}	1.63±0.13^{a}	20.17±0.48^{a}	7.66±0.51^{a}
	G3	1.60±0.17^{a}	1.83±0.21^{b}	18.33±1.45^{b}	8.67±0.54^{b}	0.22±0.06^{b}	8.83±1.58^{b}	5.66±0.54^{b}
Period 2	G1	3.30±0.01^{a}	6.01±1.28^{a}	44.67±8.82^{a}	13.00±1.01^{a}	2.71±1.28^{a}	35.67±8.82^{a}	10.00±1.01^{a}
	G2	3.29±0.01^{a}	4.84±0.57^{a}	33.67±9.07^{a}	14.00±2.73^{a}	1.55±0.56^{a}	24.67±9.07^{a}	10.50±2.59^{a}
	G3	3.30±0.01^{a}	4.47±0.32^{a}	32.33±4.60^{a}	10.67±1.92^{a}	1.18±0.32^{a}	23.33±4.60^{a}	7.58±1.93^{a}
Period 3	G1	1.00±0.10^{a}	2.36±0.23^{a}	37.67±1.67^{a}	8.33±0.63^{a}	1.36±0.14^{a}	28.33±6.10^{a}	5.33±0.63^{a}
	G2	1.00±0.17^{a}	2.21±0.24^{ab}	31.33±5.61^{ab}	8.17±1.88^{a}	1.21±0.08^{a}	22.00±6.53^{a}	5.17±1.88^{a}
	G3	1.04±0.10^{a}	1.63±0.12^{b}	22.67±3.76^{b}	7.50±0.85^{a}	0.59±0.05^{b}	13.67±4.77^{a}	4.50±0.85^{a}

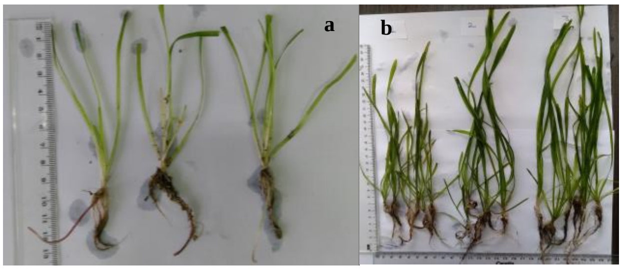

Figure 6. The appearance of the *Vallisneria sp.* plants used in the study at the beginning (a) and end of the trial (b)

The specific growth rates obtained at all stages of the experiment are shown in Figure 7. Accordingly, the SGR values obtained at the 1st, 2nd and 3rd stages from the G1, G2, and G3 groups were determines as 2.49±0.32, 2.47±0.30, 0.43±0.11; 1.86±0.66, 1.24±0.38, 0.99±0.22; and 2.86±0.12, 2.71±0.24, 1.49±0.09, respectively ($p<0.05$).

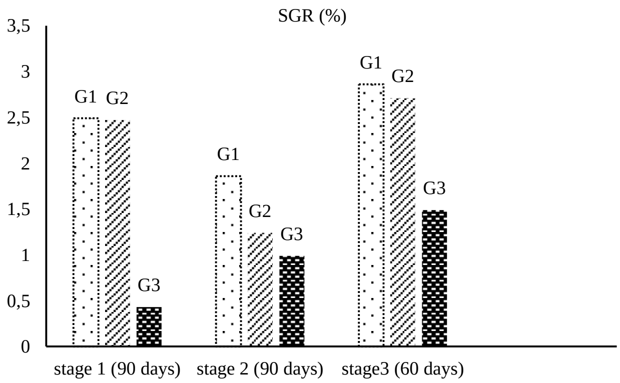

Figure 7. SGR (%) values of groups in different experiment stages.

DISCUSSION

In this research, when natural adsorbents that adsorb nutrients from fish feed were used as plant ground material, their effects on the controlled release of these nutrients and their availability to the *Vallisneria* sp. plant were examined.

At the end of the study, it was concluded that natural substrate materials can be used to maintain ecological balance in aquarium conditions, and nutrients resulting from unconsumed feed can be taken from the aquatic environment by natural adsorbents and can be used by aquatic plants in a controlled manner.

In some previous research, the effects of fertiliser addition and different substrate materials were investigated (Xie et al., 2007; Qin et al., 2011; Gue et al., 2016; Teğoğlu et al., 2017; Ünver, 2019; Gosselin et al., 2018; Emirzeoğlu, 2020). The use of fertiliser in aquariums where only plants are grown or in plant-dense aquariums may not be very suitable for aquariums prepared with a fish/plant mixture. Aquatic plants are one of the most important elements of aquariums, and it is mandatory to maintain balance in fish-plant interaction. There is a mutual benefit between fish and plants in aquatic ecology. However, disruption of balance causes vital problems for both organisms. Aquatic plants uptake some nutrients from the water column or substrate through their leaves or roots. It is typically accepted that submerged rooted aquatic macrophytes uptake nutrients from the substrate where nutrient concentrations are usually significantly greater than the surrounding water. On the contrary, plants can obtain nutrients from the water column through foliar uptake when the nutriet amount is greater in the water column than in the substrate. With that being said, there is no consensus on which nutrients are provided from water, substrate, or both. Furthermore, the nutrient uptake mechanism varies between species (Gosselin et al., 2018). In line with the mentioned literature, it was determined that the *Vallisneria* sp. plant developed by obtaining nutrients from water and substrate in all three stages of the study.

Li et al. (2012) reported in their study that the plant height of *V. natans* species varied between 20.44 cm and 44.52 cm at the end of the 7-month research. Zang et al. (2019) found that *V. spiralis* developed approximately 45-55 cm in length and 9.44-10.63 cm in root length after two months. Tekoğul et al. (2017) reported, using the same species that the total plant height varied between 46.9-92.7 cm in 2-month. The data recorded in stem height and root length in this study were determined to be similar to the mentioned literature.

The SGR (%) values obtained at the end of the experiment were found to be between 0.43 and 2.86 and were similar to the results of the study (0.62%-2.73%) conducted by Tekoğul et al. (2017). When the SGR values at the end of the experiment were compared, there was no statistical difference between the G1 and G2 groups, which contained 40% and 33% protein ($p>0.05$). In contrast, both groups (G1, G2) showed significantly higher growth ($p<0.05$) compared to the control group (G3).

Previous research has utilised zeolite for the filtration of fish wastewater and as bedding material in hydroponic systems designed for plant cultivation, and positive findings have been obtained (Rafiie and Saad, 2008; Yeşiltaş et al., 2021; Tanaya et al., 2021). Moreover, positive results have also been determined in studies on the use of leonardite in plant cultivation (Gardner and Al-Hamdani, 1997; Adiloğlu et al., 2018; Olego et al., 2022). Şahin (2022) reported that the use of zeolite and leonardite mixture in the aquatic environment was positively effective in regulating the ammonium values resulting from feed. In this study, the adsorbent mixture used to support the mutual symbiotic relationship between fish and plants adsorbs the nutrients in the aquatic environment and releases them when it reaches saturation. After this release, the plants use these nutrients to ensure aquatic balance. When the studies in the literature are reviewed, the fact that there is no similar study conducted in an aquarium environment increases the novelty of this investigation. Wang et al. (2023) obtained similar results to this study in their research examining the effect of vermiculite, another aluminosilicate like zeolite, on aquatic macrophytes.

According to the results of this research, it was determined that the *Vallisneria* sp. species can use nutrients from both the water column and the substrate, but enriching natural adsorbents with nutrients by keeping them in fish feed is much more effective on growth data. In this study, it was determined that the natural adsorbent mixture supported the growth of the *Vallisneria* sp. plant for approximately 8 months and that the addition of adsorbent mixture enriched with nutrients is necessary at certain intervals. In line with these data, nutrient enrichment at certain intervals can be investigated in future studies.

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