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The Effect of Brown Seaweed and Cattle Manure Combinations on The Properties of *Eisenia fetida*'s Organic Fertilizer

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A B S T R A C T

In this study, the effect of brown seaweed (*Cystoseira barbata*) and cattle manure combinations were investigated as a worm food on the properties of *Eisenia fetida* worm manure. Seaweed *Cystoseira barbata* were collected from the coast of Giresun, desalted and dried and then ground. Cattle manure was obtained from cattle breeding dairy and had covered and fermented. Food groups; 0% (control group); 5.45%; 10.90%; 21.81% and 43.63% of the algae were prepared and containing three replicates were given to the worms. The experiment was established in Giresun University Faculty of Science and Letters Biology laboratories according to randomized plot design. The vermicompost groups were analyzed in terms of plant nutrients and mineral levels. The obtained data showed that as the amount of algae increased in the formula and vermicompost groups, the metal levels of Zn, Ni, Fe, Pb, Cr, Mn, Mg and Cu decreased and carbon, $CaCO_3$, N, P and K increased. This means that brown seaweed *Cystoseira barbata*, which grows naturally on our coasts, can be used in the structure of vermicompost.

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Kahverengi Deniz Yosunu ve Çiftlik Gübresi Kombinasyonlarının *Eisenia fetida* Organik Gübresi Özelliklerine Etkisi

M A K A L E B İ L G İ S İ

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Ö Z

Bu çalışmada, kahverengi deniz yosunu (*Cystoseira barbata*) ve sığır gübresi kombinasyonlarının solucan maması olarak kullanılmasıyla, *Eisenia fetida* solucanı gübresinin özelliklerine etkisi araştırılmıştır. *Cystoseira barbata* türü yosunlar Giresun sahillerinden toplanmış, tuzu arındırıldıktan sonra kurutulmuş ve öğütülmüştür. Sığır gübresi büyük baş hayvan yetiştiriciliği yapan mandıralardan temin edilmiş ve fermente edilmiştir. Yosun ve hayvan gübresinden elde edilen mama grupları; %0; %5,45; %10,90; %21,81 ve %43,63 oranlarında yosun içerecek şekilde hazırlanmış ve üçer tekerrür olarak solucanlara verilmiştir. Deneme tesadüf parselleri deneme desenine göre kurulmuştur. Elde edilen solucan gübresi (vermikompost) gruplarının bitki besin elementleri ve mineral düzeyleri açısından analizler yapılmıştır. Elde edilen veriler mama ve vermikompost gruplarındaki yosun miktarı arttıkça Zn, Ni, Fe, Pb, Cr, Mn, Mg ve Cu metal seviyelerinin düştüğünü, organik madde, karbon, kireç, N, P ve K gibi bitki besleyici maddelerin oranlarının arttığını göstermiştir. Bu durum sahillerimizde doğal olarak yetişen kahverengi deniz yosunu *Cystoseira barbata*'nın vermikompostun yapısında kullanılabileceğini ifade etmektedir.

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Introduction

Vermicomposting is a non-thermophilic biological process in which the organic material is transformed by worms into a peat fertilizer with high porosity, aeration, drainage, water holding capacity and rich microbial activities (Edwards, 1998; Atiyeh et al., 2000a; Arancon et al., 2004). Vermiculture is a cost-effective tool for environmentally friendly waste management (Banu et al., 2001; Asha et al., 2008). Studies show that worms contain substances with strong antimicrobial properties and that these substances are the most important elements of the worms' immune system. By using these substances, which worms use to protect themselves from pathogenic microorganisms, it may be possible to neutralize pathogens that cause great damage to cultivated plants and cause intense chemical use. In studies on this subject, it has been determined that when the liquid form of vermicompost is sprayed on the plants, insects do not approach the plants and the plants become resistant to diseases (Edwards, 1998; Atiyeh et al., 2000b; Arancon, 2004).

The positive effects of vermicomposting on pH, electrical conductivity (EC), C: N ratio and other nutrients have been documented. Worm activity reduced pH and C: N ratio in fertilizer (Gandhi et al., 1997; Atiyeh et al., 2000a). Studies have shown that vermicompost has lower pH, EC, organic carbon (OC) compared to the main material (Nardi et al., 1983; Albanell et al., 1988; Mitchell 1997), and has higher amounts of nitrogen and potassium total phosphorus and micronutrients (Hashemimajd et al., 2004). Vermicompost contains higher nutrient concentrations but produces more salinity than composts. EC indicates the salinity of the organic change. Compared to the main material used, vermicomposts have less soluble salt and greater cation exchange capacity (Holtzclaw and Sposito, 1979; Albanell et al., 1988).

Due to their different properties, marine algae have become the center of attention with many researches (Türkmen and Kütük, 2017; Dyo et al., 2018; Lauritano et al., 2020; Riccio and Lauritano 2020; Rosales-Mendoza et al., 2020; Silva et al., 2020; Türkmen and Akyurt, 2021; Türkmen and Aydın, 2021). Vermicomposts can significantly affect the growth and productivity of plants due to their micro and macro elements, vitamins, enzymes and hormones (Kale et al., 1992; Kalembasa, 1996; Edwards, 1998; Sinha et al., 2009). Vermicomposts have a special structure that provides strong microbial activity (Shi-Wei and Fu-Zhen, 1991). Mucus expelled from the worm digestive tract stimulates antagonism and competition between various microbial populations, accelerating the production of some antibiotics and hormone-like biochemicals and plant growth (Edwards and Bohlen, 1996). The aim of this study is to investigate the effects of *Eisenia fetida* worm fertilizer obtained by using brown seaweed (*Cystoseira barbata*) and cattle manure combinations as worm feed.

Material and Methods

The cattle manure used in this study was obtained from a cattle breeding barn in a village in Giresun Province. In this study, brown seaweed (*Cystoseira barbata*) used as research material was collected from the natural environment of Giresun province and district coasts (Piraziz; 40° 57' 12" N 38° 07' 56" E, Bulancak; 40° 56' 36" N 38° 15' 02" E, Giresun; 40° 54' 57" N 38° 25' 05" E, Tirebolu; 41° 00' 39" N 38° 51' 56" E, Eynesil; 41° 03' 58" N 39° 08' 26" E). *Cystoseira barbata* type of algae was detached from the rocks they held on and placed in nets and the sea water was awaited to escape. The algae brought to the laboratory environment were washed with tap water and other algae species, mussels and other undesirable substances were removed. These cleaned algae were spread and dried, and the powder was cut into 0.5 to 1.0 cm size with the help of a grinding tool (Türkmen and Su, 2019). Then, the mixtures in Table 1 were prepared from chopped seaweed and cattle manure, and food groups were formed by fermentation. After the initial samples were taken from the prepared food groups for analysis, each group was distributed in three replicates of 1500 g into the containers.

The average weight of *Eisenia fetida* worms in containers with food groups was calculated and left as 45 g per container (0.225 g / worm and an average of 200 pieces / container). Trial containers were placed in the laboratory environment according to the order of trial random plots and temperature and humidity controls were carried out every two days until the worms consumed the food. One month after the trial was established, it was observed that the worms consumed the formula and the trial was terminated. The vermicompost samples in the containers were dried separately, sieved and prepared for analysis after the worms were removed. The methods of humic fulvic acid, N, $CaCO_3$, organic C, P, Ca, Cu, K, Mg, Mn, Zn, Ni, Fe, As, Pb, Cr, Na, pH, EC and organic matter analyses were presented in Table 2 (Kacar., 2016).

Results and Discussion

1500 g of food and 200 worms (45 g) were left in each container, after 30 days it was observed that the worms consumed about their own weight daily. During the formation of vermicompost, the temperature and humidity of the environment were kept at optimum levels of 20-25°C and 60-70% humidity. Studies have stated that these values are the most suitable values for the activities of the worm (Sharma et al., 2005). EC and pH values of trial groups were given in Table 3 according to the groups.

The pH values increased as the amount of algae in the groups increased, except for the third group of food and the second group of vermicompost, but it was lower in vermicompost compared to the food groups. However, it is seen that the pH values of all food and vermicompost groups are neutral. It is considered that the lower EC values in the vermicompost groups compared to the food groups may be due to the lower amount of soluble salt in vermicompost. On the other hand, it is seen that all groups are slightly salty. Plant nutritional values of prepared food groups and obtained vermicompost groups are given in Table 4. The differences between the plant nutrition values of worm food and vermicompost groups were statistically significant ($P<0.05$). Organic matter and organic carbon were highest in V5 group, P and K WF3 group, $CaCO_3$ WF5 group, nitrogen V4 group and humic fulvic acid WF1 group. As the amount of algae in the food and vermicompost groups increased, the amount of organic matter and $CaCO_3$ increased, and no significant change was observed in carbon, nitrogen and potassium levels depending on the amount of algae. Nitrogen is one of the most essential nutrients that stimulate the formation of leaves and stems in plants. It affects important physiological developments in the plant body, product amount and product quality (Çepel, 1988). On the other hand, total humic fulvic acid amounts decreased except the fourth groups of food and vermicompost. This shows that there is a higher amount of humic-fulvic acid in composted animal manure compared to algae. While the total amount of phosphorus showed a change independent of the algae ratio in the food groups, it decreased in the vermicompost groups with the increase of algae, except for the fourth group.

The mineral substance levels of the food and vermicompost groups are presented in Table 5. The differences between the mineral substance levels of the food and vermicompost groups were statistically significant ($P<0.05$). As can be seen from the table, heavy metals such as Zn, Ni, Fe, Pb, Cr, Mn, Mg and Cu reached the highest levels in the food control group and their levels decreased as the amount of algae in the groups increased. This implies that these metals are at higher levels in animal manure than brown seaweed and that heavy metal levels decrease due to the increase in algae content. The group with the highest calcium content is the fifth group of vermicompost, that is, the group with the highest amount of algae. The group with the highest sodium content was the fourth group of vermicompost. This shows that Na and Ca elements are higher in algae material. In this case, it can be said that worms accumulate these metals in their bodies (bioremediation) during vermicomposting and cause a decrease in heavy metal levels in vermicompost.

Considering the analysis results, it was seen that the total amount of humic-fulvic acid decreased as the amount of algae in food mixtures increased and vermicomposting. The amount of organic matter increased due to the increase in the rate of algae, and after vermicomposting, it also increased compared to the food groups. It was observed that the amount of organic carbon was statistically insignificant in the food groups except for the first and third groups, but increased with the increase of algae in the vermicompost groups. The amount of $CaCO_3$ was statistically different in the food groups except for the second and fifth groups, but in the vermicompost groups, it increased with the increase in the amount of algae except the third group. It was observed that the total N amounts were statistically insignificant in the food and vermicompost groups. It was observed that the amount of phosphorus in the food groups showed an independent variation from the amount of algae, whereas in the vermicompost groups, it was statistically no different except for the fifth group. Again, the amount of potassium varied independently from the amount of algae in the food groups, but it was observed that the amount of algae increased in the vermicompost groups except for the third group.

Table 1. Mixing ratios of food groups

Groups	Mixes	Algae Ratio (%)
WF1	5.500 g Manure	0 %
WF2	5.200 g Manure+ 300 g Algae	5.45 %
WF3	4.900 g Manure + 600 g Algae	10.90 %
WF4	4.300 g Manure + 1.200 g Algae	21.81 %
WF5	3.100 g Manure + 2.400 g Algae	43.63 %

WF: Worm Food

Table 2. The Parameters, Methods and Units analyzed in Worm Food and Vermicompost

Parameters	Units	Methods
Humic-Fulvic Acid	%	TSE 5869 ISO 5073
$CaCO_3$	%	Scheibler Kalsimetrik
pH		TS ISO 10390
EC	dS/m	TS ISO 11265
N and C	%	Elemental Analyzer
Mn, Fe, Ni, Cu, Zn, As, Pb, Cr, K, P, Ca, Na, Mg	ppm	ICP-MS
Organic Matter	%	TS8336 (Walkley-Black)

Table 3. pH and Electrical Conductivity Values of Food and Vermicompost Groups

Groups	pH	EC (dS/m)
WF1	7.0±0.02	4.65±0.08
WF2	7.1±0.02	3.96±0.02
WF3	6.9±0.02	5.05±0.05
WF4	7.1±0.02	5.65±0.02
WF5	7.2±0.02	4.95±0.11
V1	6.8±0.02	4.80±0.05
V2	6.7±0.02	5.30±0.05
V3	6.8±0.02	5.56±0.01
V4	6.9±0.02	5.45±0.05
V5	7.1±0.02	5.72±0.07

WF: Worm Food, V: Vermicompost

Table 4. Plant Nutrition Values of Worm Food and Vermicompost Groups

Grp	Humic Fulvic Acid (%)	Organic Matter (%)	Organic C (%)	$CaCO_3$ (%)	N (%)	P (ppm)	K (ppm)
WF1	78.2±0.57^{h}	32.2±0.23^{b}	20.3±0.54^{ab}	1.70±0^{a}	0.02±0^{a}	1455±255^{abc}	5048±133^{abc}
WF2	66.2±0.05^{f}	26.8±0.77^{a}	19.9±0.06^{ab}	3.40±0^{b}	0.03±0^{a}	1057±136^{abc}	3370±526^{a}
WF3	62.2±0.57^{e}	30.9±0.83^{b}	21.3±0.60^{bc}	1.70±0^{a}	0.02±0^{a}	1671±35^{c}	5927±175^{c}
WF4	65.7±0.55^{ef}	33.3±0.57^{b}	23.7±0.11^{d}	1.70±0^{a}	0.02±0^{a}	1548±205^{bc}	5767±411^{c}
WF5	55.3±1.15^{d}	44.3±0.74^{e}	33.8±0.34^{f}	7.93±0^{d}	0.04±0.02^{a}	973±144^{ab}	5358±984^{bc}
V1	73.1±1.57^{g}	38.6±0.67^{c}	26.3±0.57^{e}	2.55±0^{ab}	0.04±0^{a}	1191±31^{abc}	3618±246^{ab}
V2	54.4±1.57^{d}	41.6±0.69^{de}	22.2±0.63^{cd}	3.40±0^{b}	0.02±0^{a}	1158±48^{abc}	4068±142^{abc}
V3	15.6±0.48^{a}	41.1±1.01^{cd}	19.4±0.34^{a}	2.55±0^{ab}	0.02±0^{a}	1008±25^{ab}	3539±153^{ab}
V4	45.3±1.03^{c}	42.8±0.91^{de}	23.7±0.11^{d}	5.10±0^{c}	0.04±0.01^{a}	1199±20^{abc}	4996±7.0^{abc}
V5	23.3±0.64^{b}	44.4±0.87^{e}	23.7±0.36^{d}	5.38±0.56^{c}	0.03±0.01^{a}	894±28^{a}	5693±33^{c}

*WF: Worm Food, V: Vermicompost, 1: Control, 2: 5.45 Algae %, 3: 10.90 % Algae, 4: 21.81 % Algae, 5: 43.63 % Algae, **Vertically, different letters show statistically significant differences between groups (P<0.05).

Table 5. Mineral Matter Levels of Food and Vermicompost Groups (ppm)

Grp	Zn	Ni	Fe	As	Pb	Cr
WF1	68.2±0.95^{d}	10.9±1.28^{c}	15371±429^{d}	5.38±0.84^{bcde}	5.76±0.28^{d}	14.2±0.41^{d}
WF2	47.8±6.82^{abc}	5.28±1.17^{ab}	8906±1414^{bc}	3.85±0.87^{abcd}	5.27±0.97^{bcd}	3.70±1.56^{abc}
WF3	63.1±1.71^{cd}	8.80±0.53^{bc}	12775±260^{cd}	4.78±0.18^{abcde}	4.96±0.17^{bcd}	10.4±0.73^{d}
WF4	61.5±5.68^{bcd}	7.74±0.69^{bc}	12864±1108^{cd}	6.51±0.83^{de}	5.43±0.48^{cd}	8.3±1.58^{bcd}
WF5	48.9±7.31^{abc}	5.48±1.30^{ab}	8716±1789^{bc}	6.16±0.91^{cde}	4.41±0.79^{abcd}	9.22±3.27^{cd}
V1	46.2±1.84^{abc}	3.81±0.20^{a}	6576±316^{ab}	2.01±0.17^{a}	3.65±0.12^{abcd}	2.61±0.22^{ab}
V2	46.6±1.79^{abc}	3.68±0.63^{a}	5795±231^{ab}	3.19±0.68^{abcd}	3.33±0.05^{abc}	2.11±0.02^{ab}
V3	39.5±0.30^{a}	3.28±0.36^{a}	5079±399^{ab}	2.42±0.02^{ab}	3.33±0.64^{abc}	2.30±0.21^{ab}
V4	45.9±1.55^{abc}	3.45±0.26^{a}	5047±158^{ab}	3.75±0.10^{abcd}	2.87±0.07^{ab}	2.58±0.22^{ab}
V5	42.8±0.36^{abc}	2.48±0.12^{a}	3293±177^{a}	6.97±0.54^{e}	2.08±0.02^{a}	1.88±0.22^{a}

Grp	Mn	Ca	Mg	Na	Cu
WF1	468±28.7^{e}	9461±226^{bc}	4264±69,0^{c}	736±71,9^{b}	28,4±2,30^{d}
WF2	293±47.9^{bcd}	6003±539^{a}	2719±371^{ab}	732±91,5^{b}	13,0±2,37^{abc}
WF3	364±11.4^{cde}	9366±205^{bc}	4199±112^{c}	825±46,7^{b}	18,1±1,02^{c}
WF4	401±21.8^{de}	10398±519^{bc}	4083±126^{c}	673±137^{b}	17,1±0,63^{bc}
WF5	289±50.3^{bcd}	13570±1541^{d}	3398±561^{bc}	14,3±2,37^{a}	13,4±2,43^{abc}
V1	284±14.7^{bcd}	6086±212^{a}	2397±91,9^{ab}	263±27,9^{a}	11,5±0,60^{abc}
V2	272±4.32^{abc}	8136±230^{ab}	2418±11,7^{ab}	121±22,5^{a}	10,1±0,73^{a}
V3	229±7.81^{ab}	8183±225^{ab}	2201±73,1^{a}	828±12,6^{b}	8,30±0,31^{a}
V4	241±3.11^{abc}	11601±147^{cd}	2664±35,4^{ab}	847±27,0^{b}	10,3±0,40^{ab}
V5	155±1.43^{a}	17051±300^{e}	2791±28,1^{ab}	637±51,6^{b}	7,37±0,49^{a}

*Vertically, different letters show statistically significant differences between groups (P<0.05).

In the study comparing traditional compost and vermicompost in the growth and yield of tomatoes, some differences in plant growth were noted. It has been stated that the difference between compost and vermicompost applications in plant development is due to the fact that most of the usable N form that the plant takes is released in the form of NO_3-N in vermicompost and in the form of NH_4-N in compost, which is due to the fundamental difference between the vermicomposting process using quite different microbial composts and composting (Atiyeh et al., 2000c). In the same study, it was stated that tomato plant showed more improvement in vermicompost application. In other studies, it has been reported that the increase in the total N content of soils as a result of applying organic wastes to the soil by vermicomposting is higher than the application of these organic wastes to soils without vermicomposting (Kaushik and Garg, 2003; Arancon et al., 2004). In a study conducted by Pattnaik and Reddy (2012), it was found that Pb, Zn, Cd, Cu, and Mn heavy metals were significantly removed by vermicomposting. In a study investigating the removal of Pb, Ni, Al heavy metals from industrial sludge by vermicomposting method, it was concluded that the removal of these metals was 97%, 86%, 72%, respectively (Shaymaa et al., 2010).

During vermicomposting, it forms complexes containing heavy metals, humic acids and other polymerized organic fractions, resulting in lower levels of heavy metals that are phytotoxic to plants (Dominguez and Edwards, 2004). Higher quality fruits and vegetables with less heavy metal or nitrate content were produced in the soil to which vermicompost was added (Kolodziej and Kostecka, 1994). According to the data obtained in the study, it was determined that heavy metal levels in both food mixtures and vermicompost groups were well below the organic fertilizer heavy metal limits reported by the Ministry of Agriculture. This shows that using vermicomposts in agricultural areas can prevent heavy metal pollution.

In terms of plant nutrients, it is concluded that all vermicompost groups in this study where brown seaweed *C. barbata* was used in the production of worm food will be beneficial for the plant, while the vermicompost groups that should be used according to the characteristics of the soil where the plant will be grown will be different. It would be more appropriate to use non-moss control group in soils with low total humic-fulvic acid and P amount. On the other hand, it would be more beneficial to use the fifth group of vermicompost group, which has the highest (43.63%) algae amount in soils with low organic matter, organic C, K and $CaCO_3$. It is recommended to use the fourth group of vermicompost group containing 21.81% moss in soils with low N ratio. In order to obtain more accurate results, it will be possible to perform bioassays using vermicompost groups obtained with a specific plant species to determine which group supports plant growth more.

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