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Impact of biostimulants on soil quality

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Abstract

The soil constitutes the basis for economic and cultural activities in our ecosystem. Nonetheless, factors such as population growth, climate change, intensive agriculture, and excessive grazing have led to deteriorating soil quality and health. Consequently, soil productivity and sustainability have decreased. Scientists have developed numerous soil quality models, and soil monitoring programmes have been initiated in response. The adoption of synthetic fertilisers has enhanced productivity. However, their prolonged use has resulted in leaching, leading to mixing with groundwater and consequent water pollution, poor water quality, and at times, eutrophication. Researchers have hence focussed on reducing synthetic fertiliser use and turning to biostimulants containing animal and plant material. This research investigated the effects of biostimulants, specifically ekofertile® and microfertile®, produced by the ECOLIVE corporation, on soil quality. The study was conducted in a controlled greenhouse environment, utilizing two distinct soil types—clayey and sandy-loam—each replicated three times. The experiment involved five treatments: control, inorganic fertilization, and two biostimulants at doses of 2.5%, 5%, and 10%, arranged in a complete randomized design. At the trial's conclusion, physical, chemical, and biological analyses were performed on the soil of each pot. Using the analytic findings, the soil qualities were determined using the SMAF model. Based on the results obtained, the most effective approach to enhancing soil quality in clayey soil was the application of 10% ekofertile®, which improved soil quality from 72.09 to 77.93. For sandy loam soil, the application of microfertile® at a 5% dose proved to be the most effective, resulting in a significant increase in soil quality from 76.53 to 78.19.

Keywords: Organic acids, Microbes, Ekofertile® and Microfertile®, SMAF model, Wheat

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Introduction

Soil is a vital natural resource on our planet, holding immense significance for humanity. The quality of soil significantly impacts plant growth, food production, water cycles, and the health of ecosystems. It is essential to take immediate measures to preserve the quality of soil for future generations to come. However, presently, soil quality is progressively declining. There are various factors that influence soil productivity, including agricultural practices, urban expansion, industrial activities, and climate change. These factors lead to a gradual decline in soil quality, which reduces its productivity and sustainability over time. Furthermore, the application of synthetic fertilizers to enhance agricultural productivity results in problems such as water pollution, lower water quality, and occasionally, the onset of eutrophication (Koli et al., 2019; Pahalvi et al., 2021). Moreso, the application of these fertilizers has been linked to the inclusion of harmful substances, such as cancer-causing agents, in the food supply (Zhang et al., 2018; Rahman and Zhang, 2018). In order to secure healthy food production, efforts have been made to decrease the application of synthetic fertilizers and identify sustainable substitutes. As a result, biostimulants have arisen as potential solutions to alleviate climate change stresses and lower reliance on synthetic fertilizers (Garcia-Fraile et al., 2017; Swift et al., 2018). While researchers continue to debate the definition of biostimulants, they generally comprise natural plant

and animal materials and have been grouped into various categories by the European Commission (European Parliament, 2019). Biostimulants are environmentally-friendly options intended to enhance agricultural productivity by boosting nutrient absorption, nutrient utilization efficiency, tolerance to non-biological stressors, and product quality. Moreover, they improve the accessibility of limited nutrients in the soil or plant rhizosphere (Garcia-Fraile et al., 2017; Chiaiese et al., 2018). The sustainability of biostimulants and their capacity to enhance soil properties has motivated researchers to include them in studies aimed at enhancing soil quality.

Soil quality is affected by various factors, which can be both challenging and expensive to determine. Therefore, it is crucial to choose appropriate indicators for evaluating soil quality (Negiş and Şeker, 2019). Currently, there are several methods available for assessing the quality of land and soil, such as the Land Quality Index method, Dynamic Multivariable Land Quality method, Land Test Kits, Soil Management Assessment Framework (SMAF), and Cornell Soil Health Assessment (Andrews et al., 2004; Gugino et al., 2009). Other approaches such as the Muencheberg Soil Quality Rating, LSRS (Land Suitability Rating Index), VSA (Visual Soil Assessment), and MicroLEIS DSS have been created to incorporate soil quality ratings on a global scale, resulting in more accurate assessments and close associations with crop yields [12, 13, 14] (Alaboz et al., 2022). The USDA's SMAF model is utilized to appraise quality indicators for soil quality analyses. This approach illustrates the dynamic quality of soil, which is more influenced by applied management than by genetic factors. It considers critical soil formation aspects, including climate, topography, parent material, and so on. SMAF includes various indices including electrical conductivity, pH, organic carbon, aggregate stability, sodium adsorption ratio, available potassium and phosphorus, microbial biomass carbon, bulk density, water-filled pore space, available water content, beta-Glucosidase enzyme activity, microbial biomass carbon, and potential mineralizable nitrogen (Andrews et al., 2004).

Thus, this study was setup to investigate the impact of two distinct biostimulants produced by ekolive, namely ekofertile® and microfertile® on soil quality as predicted by SMAF model under greenhouse cultivation of wheat.

Material and Methods

Study site description

The study was carried out at the greenhouse in the Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Ondokuz Mayıs University, Samsun, Turkey. The site coordinates are 264201 E and 4582754 N (WGS-84, Zone37 and UTM m). The average annual maximum and minimum temperatures range from 5°C to 27.7°C, while the relative humidity is 73%. The average annual precipitation is 937.26 mm.

Candidate Biostimulants

In this investigation, two products developed by the ekolive company in Slovakia were analyzed for their biostimulant activity in order to ascertain their potential as biostimulants, as indicated by the laboratory analysis of their composition, following the methodology of Yahkin et al. (2017). Table 1 presents the organic acid content of ekofertile® and microfertile® plant biostimulants. For ekofertile®, Tables 2 and 3 outline the chemical and biological constituents and their functions respectively. The same applies to microfertile®, where Tables 4 and 5 showcase the chemical and biological constituents and their functions respectively.

Table 1. Organic acid constituent of ekofertile® and microfertile® plant biostimulants

| Sample | Formic acid (mg/l) | Lactic acid (mg/l) | Acetic acid (mg/l) | Propionic acid (mg/l) | Butyric acid (mg/l) | Methanol (mg/l) | Ethanol (mg/l) |
|---------------------|--------------------|--------------------|--------------------|-----------------------|---------------------|-----------------|----------------|
| ekofertile® plant | <5 | 9320 | 1550 | 19* | 900* | 8.6** | 610 |
| microfertile® plant | <5 | <5 | <5 | <5 | <5 | <5 | <20 |

*= HS-GC-MS measurement with internal standard calibration (4-methyl valeric acid)

**=HS-GC-MS measurement with external standard calibration

Table 2. Chemical and microbial constituents of ekofertile® plant (sand based) biostimulant

| Chemical content | | | Microbial content | |
|------------------------------|------|----------|-------------------|---|
| Constituent | Unit | Quantity | Genus | Species |
| Dry matter | % | 0.91 | Lactobacillus | <i>Lactobacillus satsumensis</i> |
| Organic matter | % | 0.27 | | <i>Lactobacillus diolivorans</i> |
| Ash | % | 0.53 | | <i>Anaeromassilibacillus senegalensis</i> |
| Total Nitrogen | % | 0.040 | | <i>Lactobacillus bifermentans</i> |
| NH ₄ ⁺ | % | 0.01 | | <i>Lactobacillus perolens</i> |

| | | | | |
|--|---------|----------|---------------------------|--------------------------------------|
| NO ₃ ⁻ | % | < 0.01 | | <i>Lactobacillus nagelii</i> |
| Available Nitrogen | % | 0.01 | Clostridium_IV | <i>Clostridium tyrobutyricum</i> |
| Carbamide N | % | < 0.05 | | <i>Clostridium ljungdahlii</i> |
| P ₂ O ₅ mineral acid soluble | % | < 0.01 | Clostridium_sensu_stricto | |
| K ₂ O | % | 0.0840 | | |
| Total MgO | % | 0.0275 | Bifidobacterium | <i>Bifidobacterium mongoliense</i> |
| Total CaO | % | 0.0855 | | |
| Total Sulphur | % | 0.025 | Leuconostoc | <i>Leuconostoc fallax</i> |
| Sodium | % | 0.0895 | | |
| Silicon | % | < 0.0100 | Acetobacter | <i>Acetobacter indonesiensis</i> |
| Alkaline components | active% | 0.44 | Macellibacteroides | <i>Macellibacteroides fermentans</i> |
| Boron | mg/kg | < 2.00 | | |
| Cobalt | mg/kg | 0.117 | Bacteroides | <i>Bacteroides luti</i> |
| Iron | mg/kg | 142 | | |
| Copper | mg/kg | < 2.00 | | |
| Manganese | mg/kg | 6.58 | | |
| Molybdenum | mg/kg | < 0.100 | | |
| Zinc | mg/kg | < 2.00 | | |
| pH | | 4.5 | | |
| Salt content | % KCl | 0.782 | | |

Table 3. Role of beneficial microbes found in ekofertile® plant biostimulant

| | | Coal | |
|---|---------------------------|---|--|
| | Genus | Species | Function |
| 1 | Lactobacillus | <i>Lactobacillus satsumensis</i> | Catalyzes the hydrolytic depolymerization of polysaccharides in soil. Breakdown of complex polysaccharides, including starch, to a readily available form of glucose, extracellular polymeric substances secretion & fermentation |
| | | <i>Lactobacillus diolivorans</i> | Solubilize insoluble inorganic phosphate |
| | | <i>Anaeromassilibacillus Senegalensis</i> | |
| | | <i>Lactobacillus bifermentans</i> | |
| | | <i>Lactobacillus perolens</i> | |
| | | <i>Lactobacillus nagelii</i> | |
| 2 | Clostridium_IV | <i>Clostridium tyrobutyricum</i> | Free Nitrogen fixation release polysaccharides and carboxylic acids like tartaric acid and citric acid to solubilize K, breakdown organic matter releasing citric acid, formic acid, malic acid, and oxalic acid, making K available, fermentation |
| | | <i>Clostridium ljungdahlii</i> | Obligatory anaerobic heterotrophs only capable of fixing N ₂ in the complete absence of oxygen, isolated from rice fields |
| 3 | Clostridium_sensu_stricto | | Fermentation |
| 4 | Bifidobacterium | <i>Bifidobacterium mongoliense</i> | Degradation of non-digestible carbohydrates, protection against pathogens, production of vitamin B, antioxidants, and conjugated linoleic acids, and immune system stimulation. |
| 5 | Leuconostoc | <i>Leuconostoc fallax</i> | Catalyzes the hydrolytic depolymerization of polysaccharides in soil. Breakdown of complex polysaccharides, including starch, to a readily available form of glucose, fermentation |
| 7 | Macellibacteroides | <i>Macellibacteroides fermentans</i> | Fermentation |
| 8 | Bacteroides | <i>Bacteroides luti</i> | Pathogen-suppressing contributes prominently to rhizosphere phosphorus mobilization, express constitutive phosphatase activity, and organic matter degradation |

Table 4. Chemical and microbial constituents of microfertile® plant (milled silicified rock residues after coal mining based) biostimulant

| Chemical content | | | Microbial content | |
|--|---------|----------|---------------------------------|--------------------------------------|
| Constituent | Unit | Quantity | Genus | Species |
| Dry matter | % | < 0.32 | Thiobacillus | |
| Organic matter | % | < 0.01 | Shinella | |
| Ash | % | 0.4 | Comamonas | |
| Total Nitrogen | % | 0.020 | Bosea | |
| NH ₄ ⁺ | % | < 0.01 | Thermomonas | <i>Thermomonas koreensis</i> |
| NO ₃ ⁻ | % | < 0.01 | Clostridium_sensu_stricto | <i>Clostridium saccharobutylicum</i> |
| Available Nitrogen | % | < 0.01 | Pseudomonas | <i>Pseudomonas sp.</i> |
| Carbamide N | % | < 0.05 | Unclassified at the Genus level | |
| P ₂ O ₅ mineral acid soluble | % | < 0.01 | Castellaniella | <i>Castellaniella daejeonensis</i> |
| K ₂ O | % | < 0.0285 | Petrimonas | <i>Petrimonas sulfuriphila</i> |
| Total MgO | % | 0.0155 | Tepidibacillus | <i>Tepidibacillus fermentans</i> |
| Total CaO | % | 0.023 | | <i>Sedimentibacter saalensis</i> |
| Total Sulphur | % | 0.0465 | | |
| Sodium | % | 0.102 | | |
| Silicon | % | < 0.0100 | | |
| Alkaline components | active% | 0.555 | | |
| Boron | mg/kg | < 2.00 | | |
| Cobalt | mg/kg | 0.361 | | |
| Iron | mg/kg | 12.2 | | |
| Copper | mg/kg | < 2.00 | | |
| Manganese | mg/kg | < 2.00 | | |
| Molybdenum | mg/kg | < 0.100 | | |
| Zinc | mg/kg | 4.30 | | |
| pH | | 7.8 | | |
| Salt content | % KCl | 0.574 | | |

Table 5. Role of beneficial microbes found in microfertile® plant biostimulant

| | Coal | | Function |
|---|---------------------------|--------------------------------------|--|
| | Genus | Species | |
| 1 | Thiobacillus | | Release polysaccharides and carboxylic acids like tartaric acid and citric acid to solubilize K, breakdown organic matter releasing citric acid, formic acid, malic acid, and oxalic acid, making K available |
| 2 | Shinella | | Biosurfactant producers capable of degrading crude oil components within 14 days, bioremediations. |
| 3 | Comamonas | | Alleviate salinity stress, and degrade phenol and 4-chlorophenol mixtures completely through a meta-cleavage pathway, beneficial for enhanced cell growth and the biotreatment of both compounds, bioremediation, biofertilizer |
| 4 | Bosea | | Bioavailability of nutrients, N-fixation, denitrifier. |
| 5 | Thermomonas | <i>Thermomonas koreensis</i> | Nutrient cyclings, such as nitrogen respiration, nitrate reduction, nitrate respiration, fermentation, and cellulolysis |
| 7 | Clostridium_sensu_stricto | <i>Clostridium saccharobutylicum</i> | Fermentation |
| 8 | Pseudomonas | <i>Pseudomonas sp.</i> | Free Nitrogen fixation, solubilize insoluble inorganic phosphate and K Indole-3-acetic acid, wheat, A combined bio-inoculation of diacetylphloroglucinol producing PGPR and AMF and improved the nutritional quality of the wheat grain, organic compounds degradation, auxins |
| 9 | Castellaniella | <i>Castellaniella daejeonensis</i> | Acid phosphatase and invertase activities, |

| | | | |
|----|----------------|--|---|
| | | | available potassium and iron, and organic matter content |
| 10 | Petrimonas | <i>Petrimonas sulfuriphila</i> | Anaerobic and fermentative, Degradation of high insoluble organic molecules, plant residues decomposition |
| 11 | Tepidibacillus | <i>Tepidibacillus fermentans</i> <i>Sedimentibacter saalensis</i> | Ferment yeast extract and mono-, oligo-, and polysaccharides, including starch and xanthan gum |

Experimental Design

The experimental design of the greenhouse is a split-plot design, comprising two factors (Table 6). These factors pertain to dosage and biostimulant type and were evaluated on two soil types (loam soil from Samsun Turkey Bafra plain and clay soil from the Faculty of Agriculture practicing field). Technical term abbreviations are explained upon first use. Factor 1, dosage, was studied across 5 levels (control, inorganic fertilization, 2.5%, 5%, and 10% biostimulant), and biostimulant types included ekofertile® and microfertile® plant biostimulants. Ten treatments were applied to each soil type and replicated three times in the greenhouse. A total of 300kg of soil was collected from the field, with 150kg from the Faculty of Agriculture practicing field at Ondokuz Mayıs University and another 150kg from the Bafra plain in Samsun, Turkey. The soil was left in the shade to air dry for two weeks before being crushed and sieved through a 4mm sieve to obtain fine particle soil suitable for crop growth in the greenhouse. Three kilograms of soil were placed in a 5L bucket with no perforations to prevent leaching on a surface area of 0.031 m². The field capacity of the soil was estimated by measuring moisture content. Following the treatments detailed in Table 6 and the layout presented in Table 7, wheat seeds were sown accordingly. Each pot contained 15 seeds as 500 seeds are sown per square metre, and they were watered following seeding. The wheat crops were irrigated up to field capacity in the evenings, following a schedule of intervals of two days, to prevent drought stress. Manual weeding was also performed.

Table 6. Treatments combination

| Loam Bafra Soil | Biostimulant Dosage | ekofertile® | | | | | microfertile® | | | | |
|------------------|---------------------|-------------|--------------|------|----|-----|---------------|--------------|------|----|-----|
| | | Control | Inorganic F. | 2.5% | 5% | 10% | Control | Inorganic F. | 2.5% | 5% | 10% |
| Clay School Soil | Biostimulant Dosage | ekofertile® | | | | | microfertile® | | | | |
| | | Control | Inorganic F. | 2.5% | 5% | 10% | Control | Inorganic F. | 2.5% | 5% | 10% |

Inorganic F: Inorganic fertilization

Table 7. Greenhouse layout

| Replicate 1 | | Replicate 2 | | Replicate 3 | |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| ekofertile® | microfertile® | ekofertile® | microfertile® | ekofertile® | microfertile® |
| Control | Control | Control | Control | Control | Control |
| Inorganic F. 2.5% | Inorganic F. 2.5% | Inorganic F. 2.5% | Inorganic F. 2.5% | Inorganic F. 2.5% | Inorganic F. 2.5% |
| 5% | 5% | 5% | 5% | 5% | 5% |
| 10% | 10% | 10% | 10% | 10% | 10% |

Inorganic F: Inorganic fertilization

A 160-day trial, running from September 7th, 2022, to March 16th, came to a close with the harvest of plants. A set of soil samples were extracted from 48 pots to undergo biological analysis and then stored in a refrigerator at -4 degrees Celsius. The rest of the soil was properly dried, broken down with a wooden mallet, and sieved through a 2mm sieve for physicochemical analysis.

The soil samples' bulk density was determined using the approach reported by [Blake and Hartge \(1986\)](#). Meanwhile, [Klute's method \(1986\)](#) was employed to compute the field capacity and wilting point. The available water content in the soils was calculated by subtracting the moisture content at the wilting point from the moisture content at field capacity ([Klute, 1986](#)). Aggregate stability was evaluated by [Kemper and Rosenau \(1986\)](#). Analysis of available phosphorus was accomplished per the [Olsen et al. \(1954\)](#) method, while extractable potassium was evaluated employing a 1 N ammonium acetate solution[21] ([Bertsch, 1985](#)). Saturation extracts were used to measure soil pH and Electrical Conductivity (EC) values via a pH-EC meter, following [Rhoades et al. \(1999\)](#). Microbial biomass carbon was measured using [Anderson and Domsch's](#)

(1978) substrate-induced respiration method. The Sodium Adsorption Ratio (SAR) was calculated according to Soil Survey Staff (1996) (Equation 1), using the concentrations of Na, Ca, and Mg obtained from saturation extract filtrates.

$$SAR = \frac{Na^+}{\sqrt{\frac{[Ca^{+2}] + [Mg^{+2}]}{2}}} \quad \text{(Equation 1)}$$

Assessment of Soil Quality

The SMAF model assesses the ability of soils to satisfy both agricultural productivity and ecological functions. Within the SMAF model, the physical attributes of soil are evaluated, including bulk density (BD), water-filled pore space (WFPS), aggregate stability (AS), and available water content (AWC). In addition, chemical properties, which comprise organic carbon (OC), electrical conductivity (EC), pH, potential mineralizable nitrogen (PMN), sodium adsorption ratio (SAR), available phosphorous (P), and potassium (Ex-K), are also appraised. Furthermore, biological indicators, such as microbial biomass carbon (MBC), are taken into consideration (Andrews et al., 2004). Twelve indicators, excluding beta- Glucosidase enzyme activity, were used in this study. The model for scoring employs non-linear functions. The scoring curves employ three distinct approaches: "less is better," "the midpoint is optimum," and "more is better." To determine quality contributions for scoring, consideration is given to all three scoring functions. The model utilizes an algorithm or alternate algorithms for each property's non-linear scoring curve. Normalization and scoring for each indicator are computed by using the algorithms found in the model. The evaluations are executed on 150 crop varieties within the model. Scoring values pertaining to indicators may differ based on the crop variety, climate, and soil classification. Furthermore, regional climate data, mineralogical, and pedological properties, along with soil classification are taken into account. The SMAF model uses an incremental index called the Soil Quality Index (SQI) method (Equation 2) for this purpose.

$$SQI = \frac{\sum_i^i x_i}{n} \times 100 \quad \text{(Equation 2)}$$

Results And Discussion

Eight applications were performed on soils with two different textures, namely clayey and loam. The figures below display the distribution of scores for 12 parameters used to assess soil quality after application. Figure 1 presents AWC, BD, and WFPS, Figure 2 shows AGG, EC, and Ex-K, Figure 3 covers pH, PMN, and SoilP, while Figure 4 illustrates SAR, SOC, and MBC.

Upon analysis of the data in Figure 1, one can observe the distribution of soil quality parameters, including AWC, BD, and WFPS. It is evident that loamy soils demonstrate more favorable quality scores for wheat growing across all doses in comparison to clayey soils. These findings are based on the physical attributes of the soil, which vary according to soil texture. The SMAF model evaluates soil quality according to the specific plant's soil requirements, and hence suggests that loamy soils offer wheat cultivation with better quality conditions. Upon separate evaluation of clayey and loamy soils, the parameter with the highest quality score within clayey soils was found to be WFPS, whilst in loamy soils it was determined to be BD.

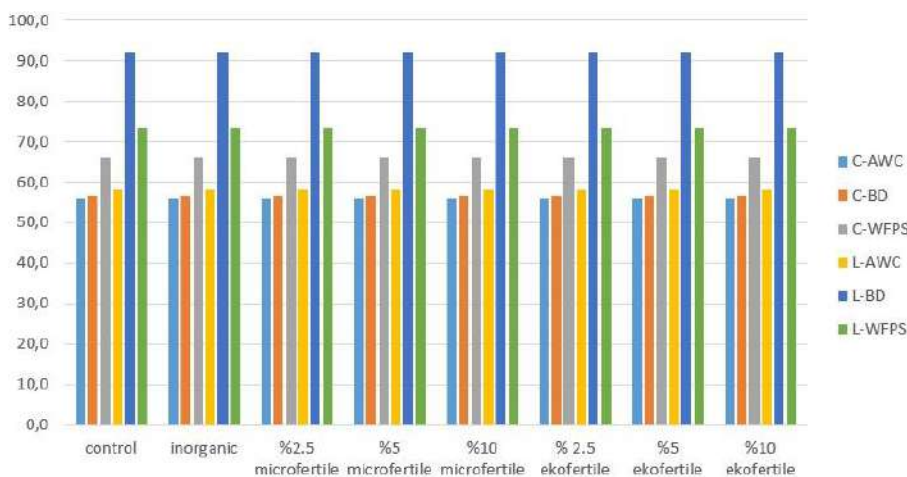


Figure 1. Distribution of quality scores for AWC, BD, WFPS parameters.

Upon examination of Figure 2, the distribution of soil quality parameters AGG, EC, and Ex-K are observed. It is evident that AGG is notably higher in clayey soils compared to loamy soils. Among clayey soils, 2.5% microfertile application has the highest AGG score among the doses, whereas among loamy soils, an inorganic application has the highest score. In clayey soils, EC scores are higher in the control group, and at 2.5% and 5% microfertile doses. However, in other doses, loamy soils yield higher quality scores. For both soil types, Ex-K values consistently demonstrate high quality scores across all doses.

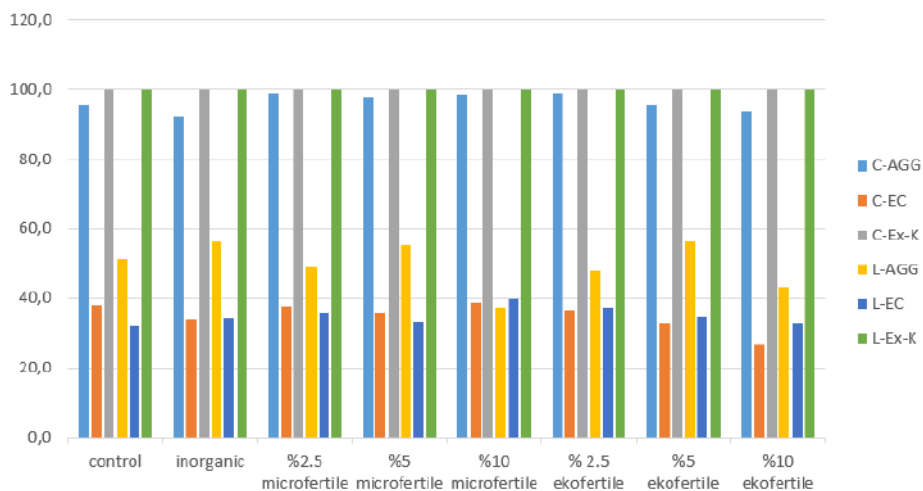


Figure 2. Distribution of quality scores for AGG, EC, Ex-K parameters.

When examining Figure 3, the distributions of soil quality parameters; pH, PMN, and SoilP can be observed. It is evident that the pH level is higher in clayey soils when compared to loamy soils. The application with the highest pH score among the doses for clayey soils is determined to be 5% ekofertile. On the other hand, for loamy soils, the highest score is associated with an inorganic application. The PMN quality scores of both soil types are high quality for all doses. SoilP quality scores are comparatively higher in loamy soils than in clayey soils. It is noteworthy that SoilP doses do not vary among loamy soils. On the other hand, the highest quality score among clayey soils is determined to be the 10% dose of ekofertile. Technical abbreviations such as SoilP and dose have been clearly explained upon their first use.

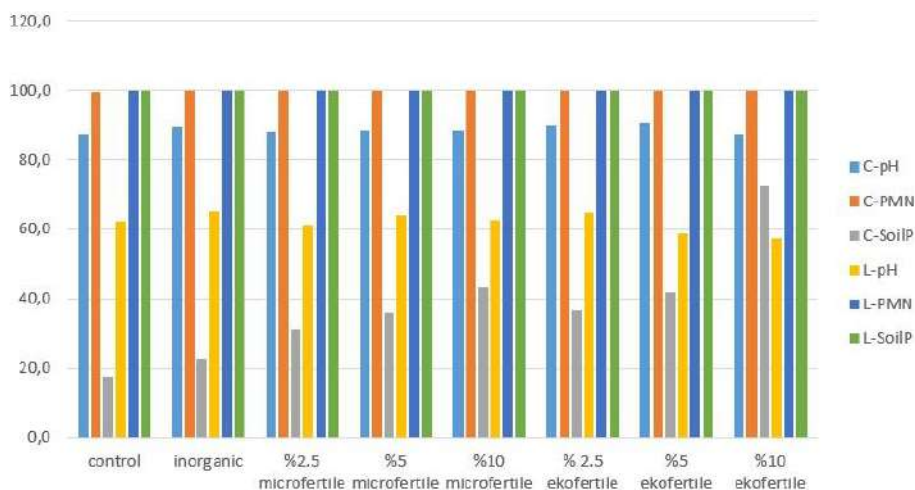


Figure 3. Distribution of quality scores for pH, PMN, SoilP parameters.

Upon examining Figure 4, it is apparent that SAR, SOC, and MBC are the soil quality parameters that can be seen. In terms of the SAR parameter, it is observable that the control condition without any applications yields a lower SAR in the clayey soil when compared to all other doses for both clayey and loamy soils. Noteworthy is that the highest quality score for SAR is achieved by an inorganic application in both clayey and loamy soils. In terms of SOC quality scores, it has been observed that the score is higher in clayey soils compared to loamy soils. However, it has been determined that in both clayey and loamy soils, the application with the highest SOC score is the 10% dose of ekofertile. With regards to MBC quality scores, although generally similar, it was

found that in clayey soil, all doses except for 2.5% microfertilizer, 2.5% ekofertilizer, and 10% ekofertilizer have lower scores than in loamy soils. In loamy soils, the application yielding the highest MBC quality score was found to be the 5% dosage of ekofertilizer.

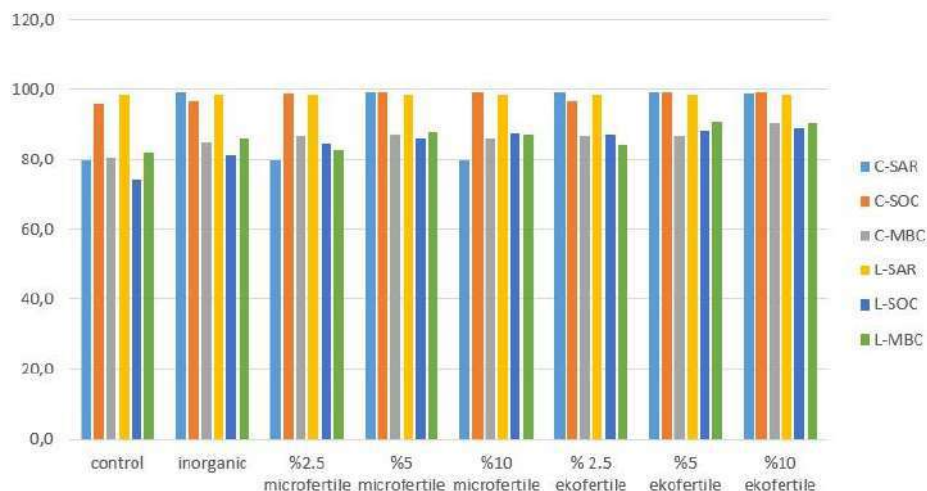


Figure 4. Distribution of quality scores for SAR, SOC, MBC parameters.

The comparison of soil quality values, obtained from twelve quality parameters for clayey and loamy soils, is depicted in Figure 5. The analysis suggests that loamy soils score higher in soil quality for wheat cultivation across all applications except for the 10% dose of ekofertilizer application. The most effective ekofertilizer dose turned out to be 5%.

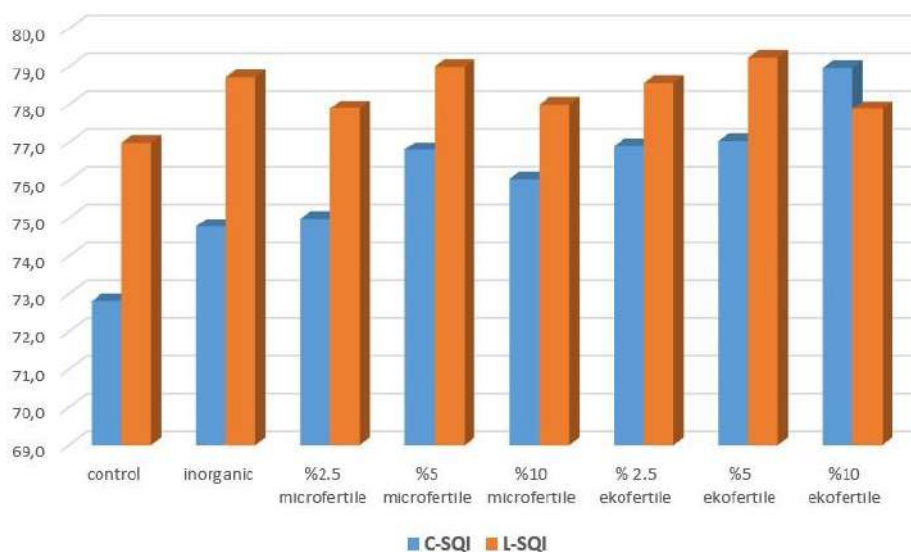


Figure 5. Soil quality scores for clay and loam soils at 8 different doses

Conclusion

The study showed that biostimulants are effective alternatives in enhancing soil quality at wheat rhizosphere. While 10% ekofertilizer® enhanced soil quality the most in clay soil, 5% microfertilizer® was most effective in general soil quality enhancement across the two soils and biostimulants dosages. We therefore recommend the usage of biostimulants to enhance soil quality.,

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