Differentiating biological roles of some organic amendments on some parameters for sandy and loamy soils cultivated with lettuce (*Lactuca sativa L.*) and final yield

ABSTRACT

Two-pot tests were carried out in the 2020–2021 and 2020–2022 years at the sandy Ismailia station of The Agricultural Research Center, Egypt, and the clayey Giza. In these tests, potassium humate (KH), vinasse, and olive pomace were used as partial potential replacements for mineral fertilizers when fertilizing lettuce.Effects of these alternatives on the development characteristics and green yield of lettuce, we are going to take at the physical and chemical characteristics of the soil as well as the enzymatic activity of the oxidoreductase enzymes (peroxidase and catalase) in the soil rhizosphere.A randomized complete block design (RCBD) with nine treatments was used to set up the experiments. Overall, the study's findings showed that the physical and chemical properties of the soil under study were improved by the application of vinasse 50 > k-humates 50 > k-humates 100 as a subsequence of these rates. The combination that used 50% olive pomace and 50% vinasse produced the highest average results across all metrics that were looked at. Increased growth resulted from this, and the yield parameter was positively impacted.

Keywords: organic fertilizer, olive pomace wastes, vinasse, k-humate, oxidoreductase enzyme, lettuce (Lactuca sativa L), yield

Introduction

The agricultural industry globally generates around 23.7 million metric tons of food daily, accounting for nearly 21% of greenhouse gas emissions **(Gerber *et al.*, 2013).** The growth in agricultural output impacts adversely soil, air and water resources. In the present era, the primary worldwide objective is to mitigate environmental degradation by using more ambitious and expedited methods to substantially enhance the productivity of economically relevant crops.

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Why use different soil textures, **biological roles, and lectures**?
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However, even extremely efficient systems had significant limitations in terms of sustainable fertility control. In the present ecological context, the decline in soil fertility is a significant concern for agricultural productivity. According to a report by the Global Environment

Facility **(GEF 2008)**, the reduction in soil nutrient reserves is primarily caused by a deficiency in soil organic matter. The Egyptian environment has seen significant yearly depletion of organic matter, mostly attributed to high rates of mineralization in recent decades and the extensive use of mineral fertilizers.

Comment [SA3]: The agricultural industry generates 23.7 million metric tons of food daily, contributing to 21% of greenhouse gas emissions. However, sustainable fertility control is limited, particularly in soil fertility. The Global Environment Facility reports soil nutrient reserves are reduced due to soil organic matter deficiency. Egyptian soil has experienced significant depletion due to mineralization and fertilizer use.

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Mineral fertilizers had satisfying results in term of crop yield worldwide. However, many concerns emerged from recent studies reviewing some health hazards beside production cost and yield quality that would prohibit inorganic fertilization excessive usage in coming future. Organic fertilization was studied in many researches as an indispensable alternative to inorganic forms and proved its reliability. **Kumar *et al.*, (2013)** clarified the role of using K-Humate as soil conditioner and growth promoter for plants, where it reduced leaching of macro and micronutrients while improving soil structure and increase its water retention. In lettuce production, **Moreira *et al.*, (2014)** studied the effect of different organic matter as fertilizers, of which poultry manure presented good results. **Hossain, M. and Ryu, K. (2017)** found that organic fertilizer did mitigate heavy metals in soil that did benefit lettuce yield over chemical fertilizers. Also, using organic fertilizers exceeded availability of nutrients it contained as by increasing soil porosity, aeration and maintaining soil moisture, as mentioned by **Ismail andObeid (2023)**.

Caused contamination in ecosystems, including the atmosphere, soil, and water. One potential solution to decrease the usage of chemical fertilizers is to use organic additions derived from recycling organic waste. Furthermore, sandy soils are characterized by a coarse texture and are mostly composed of individual grains. Due of the low clay content **(Khan, 2018**), they exhibit little shrinkage or expansion characteristics. The use of organic supplements in sandy soils is hindered by the issue of frequent turnover, since the decomposition rate is fast and the additional organic materials are often mineralized within short cropping seasons (Baiamonte *et al.* 2019).

The olive oil business was a significant economic sector in Mediterranean nations. Nevertheless, olives were mostly used for oil production. Furthermore, this process results in a substantial amount of garbage, including wood, branches, leaves, as well as by-products such as olive pomace, olive mill effluent, and olive stones. These waste materials have a detrimental environmental effect and need expensive management and disposal methods (Galanakis, 2017).The waste materials were often distinguished by elevated levels of chemical oxygen demand (COD) and biological oxygen demand (BOD), with values exceeding 200 and 100 g L-1, respectively.

The combined application of organic matter combined with a mineral fertilizer to olive grown on ethic camisoles positively affected the physical, chemical, and biochemical properties of the soil, due to the high organic matter content (Podgornik, *et al.* 2022).

Concentrated vinasse is an industrial by-product that contains important active ingredients that may be recycled for plant development**Debruck and Lewcki, 1990.** in the same pattern **Vadivel *et al.*, (2014**) It has been determined that the use of vinasse in agriculture has resulted in a substantial increase in nutrient content, improved the soil quality of degraded land, and boosted crop yields. Regarding this matter, **Osman *et al.*, (2014**) The study shown that the addition of diluted vinasse (20%) together with 25% of potassium mineral fertilizer to sandy soil resulted in a substantial increase in nutrient content, particularly potassium and organic matter. This led to an improvement in soil chemical characteristics, nutritional status, and crop output, **Seddik*et al.*, (2016)**Stated that the presence of vinasse (4%) greatly increased the amount of accessible NPK in the soil.Humic acid is the primary component of soil organic carbon and plays a crucial role in maintaining soil fertility. It is a key part of organic manures, which provide nutrients, enhance soil aggregation, and promote microbial diversity (**Seleem *et al.*,2022**).

Significant increases in microbial biomass carbon (MBC), organic matter (OM), NPK soil availability, and barley production were seen when poultry manures were added alone or in combination with vinasses at various rates. (**Chen *et al.*, 2004**). The use of humic acids resulted in favorable outcomes for the growth, production, and quality of durum wheat crops, as well as the photosynthetic metabolism (**Sebastiano *et al.*,2005**). Humic acid is a primary constituent of humic substances (HS).

Tejada *et al.*, (2005)It has been observed that humic acids have both direct and indirect impacts on plant development. The indirect effect of humic acid enhances the physical, chemical, and biological conditions of the soil. On the other hand, the direct benefits are related to its metabolic activity in promoting plant growth. **Abou Tahoun*et al.*, (2022**) Demonstrated that humic acid altered the composition of the soil by facilitating the quick development of large aggregates, reducing the density and acidity, and increasing the porosity and electrical conductivity, thereby enhancing the hydraulic capabilities of the soil.

More than 75% of Earth's land surface is affected by poor farming practices and changing climatic scenarios, and enzymes were crucial in maintaining soil degradation that resulted in (salinized, eroded, low organic matter), **Talukder *et al.*, (2021)**. In dry land areas, a variety of soil additives have been investigated to improve deteriorated soil. The effectiveness of applying soil amendments including olive pomace, vinasse, and potassium humate varies widely depending on the land use, soil type, and ecosystems globally. Extra or intracellular enzymes play an important role in maintaining loamy and sandy ecosystem quality functional diversity, and nutrient cycling **Sinsabaugh *et al.*, (2002).**

The current work aims at studying the potentiality of partially substituting mineral fertilizers by some organic additives on growth and yield of lettuce grown on a clayey soil and a sandy one as well as their chemical and physical properties

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Materials and Methods

The current research was conducted at the Agriculture Research Center over two consecutive seasons (2020/2021 and 2021/2022) to examine the impact of olive pomace, vinasse, and potassium humate as soil nutrientson vegetative development**,**fresh weight, head diameter, number of lettuce leaves, Photosynthesis pigments and chemical components of Lettuce*(Lactuca sativa L.)* as well as enzyme activity.

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Surface soil samples (0-30 cm) were collected from both Agricultural Research Center - Giza governorate and Ismailia agricultural stations - Agricultural Research Center to represent two different textured soils.

Organic fertilizer: Potassium humate was obtained from the Agricultural Research Center (ARC) at Giza governorate, Egypt.

Waste originated conditioner: Olive pomace was obtained from Olive Oil Production unit (OOP) at the Horticultural Research Institute, Agricultural Research Center (ARC), Giza governorate, Egypt. Vinasse was obtained from Hawamdia Sugar and Distillation Company. Egypt.

Experimental design

The current pot experiment was randomized complete block design. The pot experiments included Giza and Ismailia soils that had been distributed individually in pots with size capacity of 10 kg soil where only one lettuce transplant was cultivated individually in each pot. Uniform size seedlings of Lettuce seedlings *(Lactuca sativa L.)*of 40 days old were brought from Horticulture Research Institute at ARC- Giza governorate. They were transplanted during the second week of September in the two growing seasons into the aforementioned pots at a rate of one seedling/pot.

The NPK fertilization was followed after the recommendation of Agricultural Extension Office – Ministry of Agriculture – Egypt, at rates of 150: 45: 65 kg/ha as N: P2O5: K2O, respectively, as mentioned by **El-Mogy*et al.*,(2020).** NPK was applied as ammonium nitrate (33%N), calcium supper phosphate (15.5% P2O5) and potassium (48% K2O) equivalent to 63, 8 and 23 Kg/fed of NPK, respectively.Calcium supper phosphate was added once to soil combinations in each pot before transplantation, while either of ammonium nitrate and potassium sulfate was added as half dose at a time after 15 and 30 days of plantation. After harvest, both lettuce and soil samples were collected for determination of yield parameters, chemical and biochemical properties. The remaining agricultural procedures were executed in accordance with the prescribed techniques for lettuce cultivation(**El-Ghinbihi, and Mahmoud, 2007).**

The three types of organic fertilizers were applied individually in irrigation water (fertigation) either as 100% or 50% of recommended dose ($5m^3fed^{-1}$each of olive mill wastewater, vinasse and k-humate),

NPK recommended doses were 150: 45: 65 kg/ha as N: P_2O_5: K_2O, respectively, as mentioned by **El-Mogy*et al.*,(2020)**. NPK mineral fertilizers used in this study were ammonium nitrate (33%N), calcium supper phosphate (15.5% P_2O_5) and Potassium sulfate K_2SO_4 (48% K_2O) as recommended by Egypt ministry of agricultural. Calcium supper phosphate was added once to soil in each pot before transplantation of the lettuce plant seedlings. Each of the ammonium nitrate and potassium sulfate was added at two doses, the first one (half the dose) 15 days, while the second one 30 days after transplantation. The three types of organic fertilizers were applied individually in irrigation water (fertigation) either as 100% or 50% of recommended dose ($5m^3fed^{-1}$each of olive mill wastewater, vinasse and k-humate), while 50% of NPK recommended dose was combined with that 50% of organic fertilizers (**Hossain and Ryu, 2017).** However, the control was added at 100% of the recommended NPK dose According to the recommendations of Egyptian Ministry of Agriculture, plants were fertilized with no added organic fertilizer and were planned as shown in Table 1.

Table 1: The layout of the experimental design

Treatment	NPK%	Organic Fertilizer %	Treatment symbol
Control	100	0	T1
Olive mill waste	0	100	T2
	50	50	T3
Vinasse	0	100	T4
	50	50	T5
K-humate	0	100	T6
	50	50	T7

The other agricultural activities were implemented in accordance with the prescribed procedures for cultivating lettuce (**El-Ghinbihi, and Mahmoud, 2007)**. After harvest, both lettuce and soil samples were collected for determination of yield parameters, chemical and biochemical properties.

Chemical characteristics of soil

Random soil samples were obtained from the experimental field at a depth of 0 – 30 cm. then, the samples underwent air-drying, and were then crushed and sieved using a 2.0mm sieve. Following that, the soil samples underwent chemical and physical analysis, according to the defined methodologies specified by**Page *et al.*,(1982)** and **Klute**(**1986**). The objective of these investigations was to ascertain the many chemical and physical characteristics of the soil. An electrical conductivity meter was used to test the electrical conductivity (EC) of the soil paste extract.(**Rhoades *et al.*, 1989).**The soil pH was determined by measuring the pH of a 1:2.5 soil to water suspension using a pH meter, as per the specified method**Thomas and Devi (2018).**The presence of soluble carbonates and bicarbonates in a soil paste extract was measured by titrating it against 0.01M sulphuric acid, using phenolphthalein and methyl orange indicators to indicate the endpoint of the reaction. Calcium and magnesium were determined in a soil paste extract using the titration methods by versinate (0.01M) in presence of ammonium purpurate (murexide) and Eriochrome black T (EBT) indicators, respectively.Chloride concentration was determined in a soil paste extract using the silver nitrate (0.01M) in presence of potassium chromate as an indicator. Sulphate was calculated by subtracting total summation of total determined soluble anions from summation of total soluble cations. The concentration of sodium and potassium in a soil paste extract was analyzed using a flame photometer (**Page *et al.*, 1982)**. The organic matter was determinate by **Walkely and Black (1934).** Cations and anions were determined to have a complete knowledge about the experimental conditions (**Jackson, 1973**).

Three fertilizers (olive pomace, vinasse, and potassium humates) were analyzed as following: pH, electrical conductivity (EC), The contents of organic matter. Potassium (K), and phosphorus (P) were estimated using Atomic Absorption Spectrophotometer according to (**Cottenie*et al.*,1982**). The Kjeldahl method was used to calculate total nitrogen (T.N).

Vegetative growth characteristics

Various traits were assessed by randomly selecting sets of three plants from each experimental pot: vegetative growth, fresh weight, head diameter, number of lettuce leaves, chlorophyl II, carotenoids, TSS% and NPK of lettuce leaves. The plant leaf was then dried at 70°C until it reached a consistent weight, from which the dry weight per plant was calculated.

Enzyme activities

Assessment of soil and plant biological activities pertaining to enzyme activity in the soil rhizosphere and fresh plant. Biological activity was assessed in soil samples collected from the rhizospheres of lettuce plants. 105 days post-planting.

A 0.5g sample was well mixed with 10 ml of cold phosphate buffer (50 mM, pH 7). The homogenates were subjected to centrifugation at a speed of 4000 revolutions per minute (rpm) at a temperature of 20°C for a duration of 20 minutes. The liquid portion, known as the supernatant, was used as a raw extract for the enzymatic test. Reagents used in the following assays were prepared in the same previously indicated buffer.

Peroxidase activity (EC 1.11.1.7) was determined using methylene blue assay according to **Magalhaes *et al.*, (1996).**A single unit of peroxidase was determined as the quantity of enzyme necessary to oxidize 1.0 µmol of methylene blue during one minute.

The test for catalase activity (EC 1.11.1.6) included measuring the degradation of H2O2 using UV detection at 240nm, as stated by**Beers and Sizer (1952)**. The method stages were executed in accordance with**Pine *et al.*, (1984).**The units of catalase were determined by using a molar absorbance coefficient of 43.6 for H202. A single unit of catalase was determined as the quantity of enzyme needed to break down 1.0 µmol of H2O2 per minute.

The activity of dehydrogenase (DeH-ase) (EC 1.1.1.) was measured using the tri-phenyl tetra-zolium chloride (TTC) technique as described in**Casida *et al.*, (1964)**. A single unit of dehydrogenase was determined as the quantity of enzyme needed to catalyze the hydrolysis of TTC, resulting in the formation of 1.0 µmol of tri-phenyl formazan (TPF) per hour (µmol g-1h-1).

Para nitro phenyl phosphate was used to evaluate phosphatase activity (P-ase) (E.C 3.1.3) in accordance with **Tabatabai and Bremner (1969**). Phosphatase activity of one unit was defined as the amount of enzyme required to release 1µg of p-nitro phenol hydrolyze per hour.

Nitrogenase activity (E.C.1.18.6.1) was measured by acetylene reduction assay as described by (**Johnsen and Apsley, 1990**).

Statistical analysis procedure

The tests and analytical results were reproduced a minimum of three times, and the data reported represents the average values. The collected findings underwent a one-way analysis of variance (ANOVA) to establish the significance between treatments. The kind of analysis used depends on the variables that impacted the experiment. CoStat software was used for this study**(Stern, 1991**).

Results and discussion

Soil analytical data represented in Table (2) demonstrated the most popular analytes characterizing soil conditions that have been studied in researches worldwide up till now. Both loamy soil of ElGiza and the sandy soil of Ismailia had alkaline pH value of 8±0.05, while EC value and CaCO3 content in the former were nearly double than that in the latter soil type.

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Table 2:Chemical and physical properties of soil before plantation

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Analyte	El Giza	Ismailia	Analyte	El Giza	Ismailia
O.M (%)	7.50	6.2	Coarse sand %	7.45	12.80
pH	8.03	7.95	Fine sand %	20.85	73.20
EC (dS.m^{-1})	2.75	1.25	Silt %	30.44	8.30
CaCO$_3$ (mg.Kg^{-1})	29.00	18.5	Clay %	41.26	5.70
			Texture	Clay	Sand
Soluble Anions (mmolcl^{-1})			Available Micronutrients (mg.kg^{-1})		
$(HCO_3)^{-1}$	5.65	1.54	Cu	0.40	0.38
$(Cl)^{-1}$	12.33	3.22	Fe	3.40	1.49
$(SO_4)^{-2}$	9.55	7.74	Mn	2.33	0.89
Soluble Cations (mmolcl^{-1})			Zn	0.65	0.55
$(K)^{+1}$	0.87	0.88	Available Macronutrients (mg.kg^{-1})		
$(Ca)^{+2}$	10.90	3.85	N	38.55	33.50
$(Mg)^{+2}$	5.66	2.9	P	5.20	3.96
$(Na)^{+1}$	10.07	4.87	K	178.00	185.30

Organic wastes gathered from different agro-industrial activities were analyzed mainly for their NPK contents which were expected to partially substitute NPK mineral fertilization in lettuce cultivation. The three types of organic wastes used as organic fertilizers had considerable amount of organic matter %, while with insufficient NPK contents compared to required doses for plantation, as shown in Table (3). Vinasse and olive pomace were found to be acidic but K-Humate was alkaline, while vinasse had the highest EC value among organic wastes.

Table 3: Analysis of agroindustrial wastes used as organic fertilizes

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Organic fertilizer	pH	EC	O.M%	N%	P%	K%
Olive pomace	5.9	2.1	60.3	0.95	0.28	1.28
vinasse	4.3	12	35	0.23	0.35	5.46
K-humate	8.1	4	21.5	0.52	0.00	6.25

Substituting mineral NPK with organic fertilizers diminish the share of mineral NPK forms which were easily available for plant. Add to that, NPK contents in organic fertilizers were mostly in complex organic forms that would be released slowly depending on soil rhizosphere conditions including microbial activities. Low pH characterizing vinasse and olive pomace used as soil amendments resulted in a slight increment in the insoluble acid fraction **(Arafat and Abd-Elazim, 2002; Christofoletti*et al.*, 2013). Seddik*et al.* (2016)**

found that the application of vinasse often resulted in pH values decreasing due to the acidic influence of vinasse. Noticeably, all chemical, biological and physical analysis carried out for plant and soil samples were attributed only to their residual characterization at harvest.

Leaf Enzymes

Enzymatic activities measured in lettuce leaves at harvest, as illustrated in Figure (1), elucidated residual oxidation reduction activitiesthat might had been affected by their nutritional states.

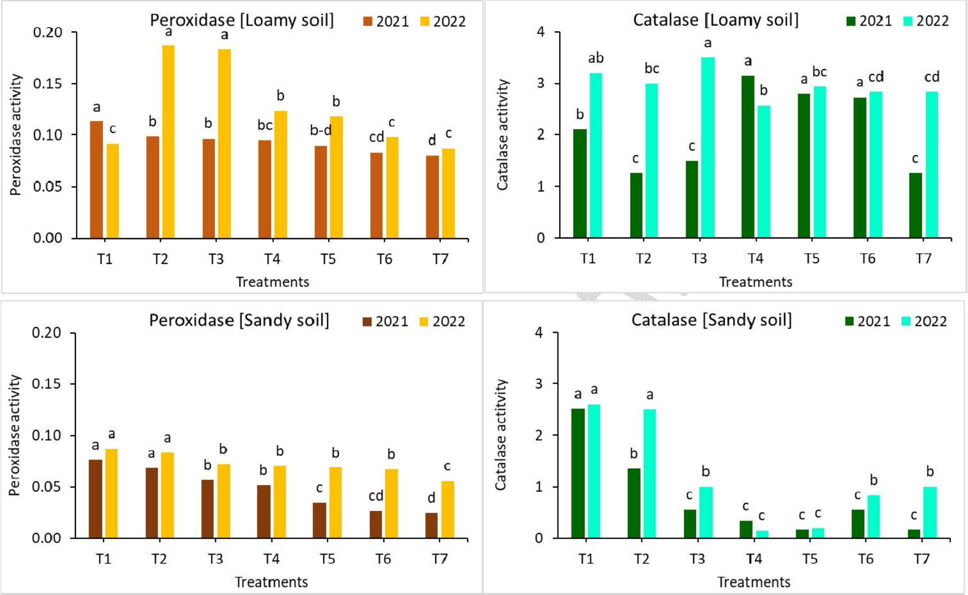

Figure (1): Catalase and Peroxidase activities in lettuce plant at harvest, where $LSD_{0.05}$ for Loamy soil peroxidase [0.011/0.015] and catalase [0.466/0.305], while for sandy soil peroxidase [0.009/0.009] and catalase [0.362/0.496] in 2021/2022 seasons, respectively. [T1: control, T2 & T3: OMW 100 & 50%, T4 & T5: Vinasse 100 & 50%, T6 & T7: K-Humate 100 & 50%]

Among all treatments, both catalase and peroxidase activities in lettuce harvested from loamy soil were mostly higher than those from sandy soil. Added to that, enzymatic activities were higher in second season than in first season from lettuce cultivated in both types of soils, that might be attributed to the accumulation of organic fertilizers used and consequently to any changes that had happened in the rhizosphere biological contents. In both types of soils and within most treatment cases, both peroxidase and catalase activities increased in the second season compared to the first.

In Tables (4) and (5) the photosynthesis pigments, NPK, fresh weight (Yield), head diameter and number of lettuce leaves results in lettuce leaves at harvest were shown, while in Table (5) their correlations were calculated.

Zhao *et al.* (2022)recognized in their work that catalase and peroxidase antioxidant enzymes increased in lettuce at early stage of growth while they decreased at the latter stage (harvest) in response to stress. Consequently, in the present work, the level of catalase and peroxidase activities at harvest time would be much less than that at the early stage of growth. Never the less, the variations in catalase and peroxidase activities correlated to applying different organic fertilizers could be considered as crucial evaluation for lettuce status in response.

Variations in catalase activities measured in lettuce sampled from loamy soil treated with organic fertilizers compared to control in the first and second seasons were difficult to describe as a fixed trait. For instance, catalase activities from OMW 100 and 50 % treatments were lower than control in the first season and increased in the second season, in the same time decreasing OMW share increased catalase activities proving that the OMW was beneficial for plant causing decrease in stress (catalase).

On the contrary, catalase activities from vinasse (100 & 50%)and K-Humate (100%) were higher than that in control in the first season and all vinasse and K-Humate (50 & 100%) were lower than that in control in the second season. In lettuce sampled from sandy soil, catalase activities in all organic fertilization treatments were lower than that in control in both seasons, being the most minimum in vinasse treatment. Catalase in case of OMW and K-Humate treatments increased in the second season and with less treatment sharing (50%).

Leitão *et al.* (2021) stated that peroxidase and catalase had an adequate role in quenching ROS resulting from oxidative stress. The formation of reactive oxygen species ROS needed to be removed boosted the plant to form those complex antioxidant enzymes **(Zhao *et al.*, 2022).**

As shown in Table (4), the effect of lowering the share of organic fertilizers on analytic level in leaves at harvest varied according to organic fertilizer and soil types. TSS contents in lettuce increased parallel to higher vinasse and K-Humate fertilizer shares in loamy soil, while increased OMW and vinasse shares in sandy soil led to decrease in TSS content.

Chlorophyll and Carotenoids

Worthy to notice that, the increase of chlorophyll and carotenoids in cultivated lettuce could be recognized in second season than in the first among all treatments in both soil types exceeding that of control in most cases.

On the other hand, catalase activities in leaves were negatively correlated to chlorophyll contents while positively correlated to carotenoidcontents in most cases of lettuce harveted from both soil types, as shown in Table (6).

Putting in consideration that the level of chlorophyll contents in any plant leave are negative sign for their health states when facing any type of stress while carotenoids are positive signs for those states. So, whenever the catalase increased as positive response for stress the carotenoids are positively correlated, while vice versa with chlorophyll. Lettuce chlorophyll content the use of several organic fertilizers, including molasses and vinasse, enhanced the crop **(Santos *et al.*,2010**; **Liu, *et al.*, 2014**). The increase in pigments responsible for photosynthesis could be attributed to successful tolerance mechanism in lettuce, as stated by **Deuner et al. (2020).**

N P K

Vinasse is beneficial because it contains chelated organic micro and macro nutrients, which are essential for plant growth, and because it may increase the bioavailability of NPK, which is essential for photosynthesis pigment synthesis (as mentioned by **Parnaudeau*et al.*, (2007).** Previous studies reported that leaf nutrient concentrations had increased when agro-industrial waste were applied to soil cultivated with several crops due to increased availability of nutrients in their rhizosphere **(Carvalho; *et al.* 2014; Mansour; 2018)**. Nitrogen decreased in leaf when treated by vinasse and olive than control, which could be clarified by N consumption in formation of head lettuce **(Mirdad, 2016).**

In loamy soil, the lower share of both K-Humate and OMW used increased chlorophyll and nitrogen while decreased the potassium contents, while they were all increased by decreasing shares in all organic fertilizer types used in sandy soil.

On the other hand, **Hossain and Ryu (2018)** stated that substituting chemical with organic fertilization did increase not only soil organic matter but also the total nitrogen and decreased EC that was positively correlated to increase in final lettuce yield. In the present study, addition of OMW might have shared in decreasing EC of loamy soil Increased OMW and vinasse did increase their effect of acidity and OM content in both soil types.

Correlations in leaf analysis and yield

In correlations described in Table (6), based on results in Tables (4) and (5), data were held to emphasize if head diameter or leave number were well correlated with final fresh weight. In sandy soil, both head diameter and leave number were positively correlated to final fresh weight (yield), while in loamy soil the leave number was the main contributor in fresh weight yield.

The positive correlation of leave no. with chlorophyll and carotenoids contents in lettuce from both types of soil and seasons was similarly noted by work of **Zhao *et al.* (2022)**. Worthy to mention that, the positive effect of both chlorophyll and carotenoids on fresh weight (yield) at harvest appearing as positive correlations, obviously in which the chlorophyll had the upper hand than carotenoids beside it had more impact on yield in sandy soil than in loamy soil (more positively correlated).

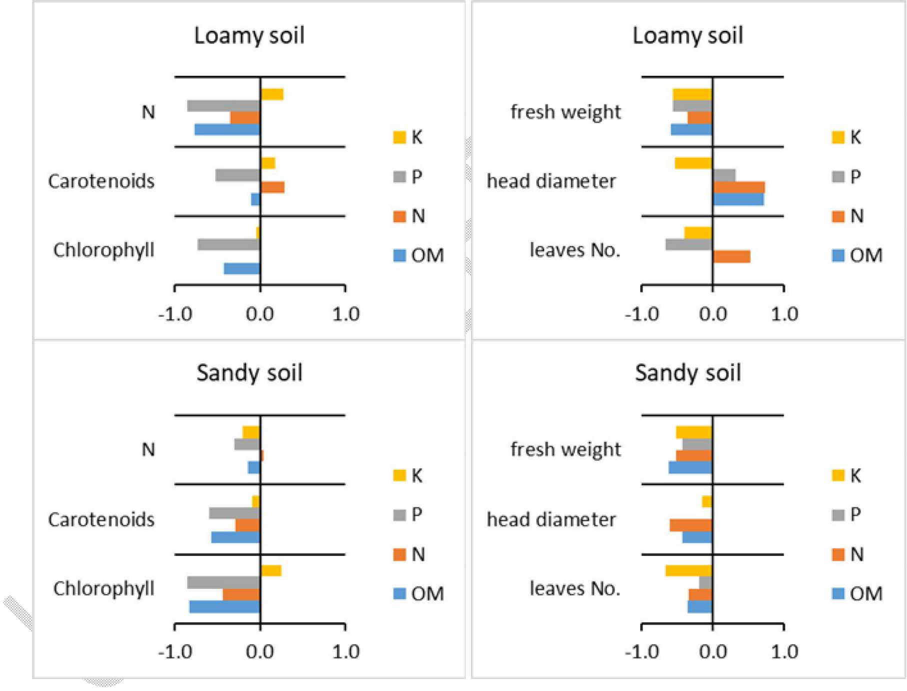

Figure (2): Correlating analytical contents in fertilizers used with those in lettuce and yield parameters measured. **Zhao*et al.* (2022)**.

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In case we were comparing organic fertilizers effect depending on their [NPK and OM] contents, we could get correlations shown in Figure (2), putting in regard that the more fertilizer we add the less mineral we put. In loamy soil the head diameters were positively correlated with increments in all organic fertilizer additions from 50 to 100%, based on their organic matter, N and P contents, while this relation was absent in sandy soil. As in loamy soil, important nutritional contents were said to be captured and slowly released for thebenefit of planted lettuce, while on the contrary the sandy soil loses so much nutrients **Garbowski,2023**

On the other hand, unusual increase in some of yield and analytical results in lettuce might be correlated to undetermined analytical characteristic of considerable role. For instance, characteristics that are beneficial for plant include low pH as in olive pomace; Cu, Zn, Fe, K and low pH as in vinasse, beside K, Zn Mg, Cu and Fe in K-Humates**Eletr and Hassan 2017**. On the contrary, characteristics with possible negative impact on plant growth as high EC, phenols in olive pomace; high EC, heavy metals, phenols and HMF in vinasse and high pH in humates **Diacono**. So, where ever the addition of more fertilizer positively affected the yield this might be due to previously mentioned beneficial characteristics of that fertilizer rather than its NPK or OM content.

Comment [SA12]: The unusual increase in lettuce yield and analytical results may be attributed to undetermined analytical characteristics. Beneficial characteristics include low pH, Cu, Zn, Fe, K, and low pH in olive pomace, while negative characteristics include high EC, phenols, heavy metals, phenols, HMF, and high pH in vinasse. The addition of more fertilizer may positively affect yield due to beneficial characteristics rather than its NPK or OM content. Studies have shown a positive relationship between nitrogen in leaves and chlorophyll concentration, with 50% vinasse having the strongest impact on growth and yield. The notable disparity in chlorophyll content in sandy soil may be due to accelerated nitrogen mineralization of fertilizer derived from olive pomace and the elevated nitrogen need during rapid growth.

Multiple investigations have shown a positive relationship between nitrogen in leaves and chlorophyll concentration **(Ding *et al.*, 2005 and DaMatta*et al.*, 2002)**.

Using 50% vinasse seemed to have the strongest impact on growth and yield**(Roig *et al.*, 2006)**.

Furthermore, the notable disparity in chlorophyll content in sandy soil may be attributed to the accelerated nitrogen mineralization of fertilizer derived from olive pomace, as compared to other treatments, and the elevated nitrogen need of plants during the rapid growth period**(Lawlor *et al.* 2004**).

Application of vinasse along with chemical fertilizers before lettuce plantation provides nitrogen with higher potassium and phosphorus **(Gómez-Muñozes *et al.* 2011; Ali, *et al* .2021).** Plant nutrient content was enhanced by applying vinasse.**(Schiavon *et al*, 2010)**.

Table (4): Photosynthesis pigments (µg/g FW) and NPK (%) in lettuce leaves at harvest

Soil type and year	Treat.	TSS%		Chlorophyll		Carotenoids		N		P		K	
		mean ± se	rank	mean ± se	rank	mean ± se	rank	mean ± se	rank	mean ± se	rank	mean ± se	rank
Loamy 2021	T1	0.10 ± 0.01	b	0.43 ± 0.02	de	0.47 ± 0.01	c	3.87 ± 0.13	a	0.12 ± 0.02	b	12.67 ± 0.17	a
	T2	0.13 ± 0.00	a	0.53 ± 0.04	cd	0.39 ± 0.03	cd	1.39 ± 0.06	d	0.15 ± 0.01	a	10.13 ± 0.13	b
	T3	0.12 ± 0.01	a	0.93 ± 0.04	b	0.72 ± 0.02	b	1.87 ± 0.13	c	0.15 ± 0.01	a	9.33 ± 0.09	c
	T4	0.12 ± 0.00	a	0.55 ± 0.03	c	0.31 ± 0.04	de	1.47 ± 0.03	d	0.15 ± 0.01	a	8.63 ± 0.19	d
	T5	0.11 ± 0.01	ab	0.33 ± 0.02	e	0.25 ± 0.03	e	1.12 ± 0.01	d	0.15 ± 0.00	a	8.40 ± 0.10	d
	T6	0.09 ± 0.00	b	0.87 ± 0.04	b	0.87 ± 0.04	a	2.17 ± 0.17	c	0.14 ± 0.00	ab	7.33 ± 0.08	e
	T7	0.07 ± 0.01	c	1.09 ± 0.06	a	0.34 ± 0.03	de	3.13 ± 0.12	b	0.13 ± 0.00	ab	6.00 ± 0.18	f
LSD			0.02		0.12		0.09		0.32		0.03		0.42
Loamy 2022	T1	0.08 ± 0.01	b	0.55 ± 0.03	c	0.61 ± 0.05	c	3.10 ± 0.05	ab	0.09 ± 0.00	d	12.83 ± 0.17	a
	T2	0.12 ± 0.01	ab	0.59 ± 0.06	c	0.42 ± 0.04	d	1.55 ± 0.05	c	0.12 ± 0.01	c	10.67 ± 0.17	b
	T3	0.13 ± 0.01	ab	1.10 ± 0.07	ab	0.77 ± 0.02	b	1.93 ± 0.07	bc	0.13 ± 0.01	bc	9.96 ± 0.04	c
	T4	0.10 ± 0.03	ab	0.59 ± 0.05	c	0.39 ± 0.03	d	1.86 ± 0.07	bc	0.14 ± 0.00	b	8.93 ± 0.18	d
	T5	0.14 ± 0.03	a	0.47 ± 0.04	c	0.38 ± 0.01	d	2.73 ± 0.15	a-c	0.14 ± 0.00	bc	8.77 ± 0.06	d
	T6	0.09 ± 0.02	ab	0.93 ± 0.04	b	0.90 ± 0.03	a	2.98 ± 0.03	ab	0.15 ± 0.00	a	7.77 ± 0.04	e
	T7	0.08 ± 0.01	b	1.23 ± 0.13	a	0.42 ± 0.02	d	3.65 ± 0.17	a	0.16 ± 0.00	a	6.23 ± 0.04	f
LSD			0.06		0.20		0.09		0.93		0.01		0.35
Sandy 2021	T1	2.65 ± 0.49	ab	0.41 ± 0.06	c	0.56 ± 0.02	a	2.68 ± 0.17	ab	0.10 ± 0.01	b	5.90 ± 0.10	a
	T2	2.11 ± 0.45	b	0.17 ± 0.00	d	0.23 ± 0.02	e	2.07 ± 0.07	a-c	0.13 ± 0.00	a	5.73 ± 0.15	ab
	T3	3.80 ± 0.52	a	0.66 ± 0.05	b	0.43 ± 0.04	b	2.25 ± 0.14	a-c	0.12 ± 0.01	a	5.50 ± 0.29	a-c
	T4	2.34 ± 0.37	b	0.43 ± 0.04	c	0.30 ± 0.02	de	1.61 ± 0.10	c	0.12 ± 0.00	a	5.10 ± 0.10	cd
	T5	3.39 ± 0.48	ab	0.47 ± 0.02	c	0.32 ± 0.01	cd	2.94 ± 0.07	a	0.11 ± 0.01	ab	4.83 ± 0.17	d
	T6	3.87 ± 0.50	a	0.76 ± 0.02	b	0.40 ± 0.03	bc	2.60 ± 0.05	ab	0.09 ± 0.00	b	4.13 ± 0.13	e
	T7	2.96 ± 0.40	ab	0.93 ± 0.02	a	0.33 ± 0.01	cd	2.00 ± 0.29	bc	0.07 ± 0.01	c	5.33 ± 0.17	bc
LSD			1.40		0.11		0.07		0.60		0.02		0.39
Sandy 2022	T1	3.12 ± 0.35	bc	0.49 ± 0.04	c	0.60 ± 0.02	a	2.93 ± 0.07	a	0.08 ± 0.01	b	5.97 ± 0.03	a
	T2	3.29 ± 0.68	bc	0.21 ± 0.01	d	0.31 ± 0.02	e	2.67 ± 0.17	a	0.12 ± 0.01	ab	5.93 ± 0.07	a
	T3	3.19 ± 0.40	bc	0.73 ± 0.04	b	0.51 ± 0.01	b	2.87 ± 0.13	a	0.13 ± 0.01	ab	5.67 ± 0.17	ab
	T4	1.97 ± 0.25	c	0.47 ± 0.02	c	0.34 ± 0.04	de	1.83 ± 0.09	b	0.10 ± 0.03	ab	5.57 ± 0.07	b
	T5	3.86 ± 0.26	b	0.49 ± 0.03	c	0.39 ± 0.01	cd	3.13 ± 0.13	a	0.14 ± 0.03	a	4.97 ± 0.03	c
	T6	3.92 ± 0.52	b	0.85 ± 0.04	b	0.41 ± 0.01	c	2.88 ± 0.12	a	0.09 ± 0.02	ab	4.97 ± 0.03	c
	T7	5.87 ± 0.49	a	1.13 ± 0.08	a	0.42 ± 0.02	c	1.88 ± 0.09	b	0.08 ± 0.01	b	4.93 ± 0.10	a
LSD			1.35		0.13		0.06		0.46		0.06		0.26

T1: control, T2 & T3: OMW 100 & 50%, T4 & T5: Vinasse 100 & 50%, T6 & T7: K-Humate 100 & 50%

Table (5): Fresh weight (Yield), head diameter and number of lettuce leaves at harvest

Soil type and year	Treat.	leaves No.				head diameter (cm)				fresh weight (g/plant)			
		mean original	±	se	rank	mean original	±	se	rank	mean original	±	se	rank
Loamy 2021	T1	25.0	±	0.29	d	1.50	±	0.29	b	483	±	4	c
	T2	27.7	±	0.15	b	2.20	±	0.12	a	327	±	8	e
	T3	27.0	±	0.29	c	1.40	±	0.06	b	851	±	5	a
	T4	19.7	±	0.15	f	1.40	±	0.12	b	228	±	10	f
	T5	23.7	±	0.15	e	1.73	±	0.15	ab	764	±	7	b
	T6	27.7	±	0.15	b	1.50	±	0.12	b	422	±	4	d
	T7	29.3	±	0.15	a	1.50	±	0.06	b	765	±	9	b
LSD					0.37				0.55				25.5
Loamy 2022	T1	28.0	±	0.29	c	1.80	±	0.06	bc	510	±	6	c
	T2	29.0	±	0.29	b	2.93	±	0.07	a	381	±	10	e
	T3	30.0	±	0.29	a	1.63	±	0.07	cd	857	±	12	a
	T4	24.5	±	0.29	d	1.33	±	0.09	e	228	±	10	f
	T5	24.3	±	0.20	d	1.90	±	0.10	b	775	±	8	b
	T6	28.4	±	0.23	bc	1.50	±	0.00	de	438	±	10	d
	T7	29.8	±	0.17	a	1.60	±	0.06	cd	784	±	8	b
LSD					0.77				0.21				28.4
Sandy 2021	T1	23.7	±	3.34	ab	1.24	±	0.12	bc	467	±	3	c
	T2	15.7	±	0.15	c	1.00	±	0.24	c	384	±	8	d
	T3	23.0	±	0.29	ab	2.00	±	0.19	a	784	±	12	b
	T4	15.0	±	0.29	c	1.50	±	0.24	a-c	354	±	7	e
	T5	20.0	±	0.58	b	1.73	±	0.12	ab	823	±	12	a
	T6	10.0	±	0.29	d	1.50	±	0.09	a-c	395	±	4	d
	T7	25.0	±	0.29	a	1.50	±	0.05	a-c	782	±	9	b
LSD					3.85				0.61				31.8
Sandy 2022	T1	17.5	±	0.29	d	1.83	±	0.09	bc	475	±	9	c
	T2	17.5	±	0.29	d	1.10	±	0.10	e	388	±	8	de
	T3	23.8	±	0.15	b	2.50	±	0.12	a	793	±	6	b
	T4	16.5	±	0.29	e	2.23	±	0.15	ab	365	±	6	e
	T5	21.6	±	0.30	c	1.72	±	0.12	cd	832	±	13	a
	T6	11.8	±	0.17	f	1.33	±	0.09	de	406	±	7	d
	T7	26.4	±	0.10	a	1.73	±	0.34	cd	794	±	14	e
LSD					0.72				0.50				37.1

T1: control, T2 & T3: OMW 100 & 50%, T4 & T5: Vinasse 100 & 50%, T6 & T7: K-Humate 100 & 50%

Table (6): Correlations between analyzed parameters in lettuce leaves at harvest

	Analyte	CAT	TSS	Chlor.	Carot.	N	P	K	Leave no.	HD	fresh wt.
2021 Loamy	POD	0.0	0.5	-0.6	-0.1	0.3	-0.3	1.0	-0.3	0.1	-0.3
	Catalase		0.1	-0.5	0.0	-0.3	0.1	0.0	-0.8	-0.4	-0.4
	TSS			-0.5	-0.1	-0.7	0.6	0.5	-0.5	0.4	-0.4
	Chlor.				0.5	0.3	0.0	-0.6	0.6	-0.4	0.4
	Carot.					0.1	-0.1	0.0	0.4	-0.3	0.1
	N						-0.9	0.3	0.3	-0.4	0.1
	P							-0.3	-0.2	0.3	-0.1
	K								-0.3	0.2	-0.3
	Leave no.									0.3	0.4
	HD										-0.2
2022 Loamy	POD	0.5	0.7	-0.1	0.0	-0.9	-0.1	0.3	0.2	0.6	0.0
	Catalase		0.3	0.2	0.4	-0.1	-0.4	0.5	0.6	0.2	0.6
	TSS			-0.3	-0.2	-0.6	0.0	0.1	-0.3	0.4	0.3
	Chlor.				0.4	0.4	0.6	-0.6	0.7	-0.4	0.5
	Carot.					0.1	0.1	0.1	0.5	-0.3	0.1
	N						0.2	-0.4	0.2	-0.4	0.4
	P							-1.0	0.1	-0.3	0.2
	K								0.0	0.4	-0.2
	Leave no.									0.2	0.3
	HD										-0.1
2021 sandy	POD	0.8	-0.5	-0.8	0.3	-0.1	0.6	0.8	0.2	-0.4	-0.4
	Catalase		-0.4	-0.5	0.6	0.2	0.1	0.6	0.2	-0.6	-0.4
	TSS			0.6	0.4	0.5	-0.3	-0.6	0.0	0.8	0.5
	Chlor.				0.2	0.0	-0.8	-0.4	0.2	0.5	0.5
	Carot.					0.5	-0.3	0.2	0.4	0.2	0.1
	N						-0.2	-0.2	0.1	0.2	0.3
	P							0.3	-0.3	-0.1	-0.3
	K								0.7	-0.3	0.0
	Leave no.									0.3	0.7
	HD										0.7
2022 sandy	POD	0.7	-0.6	-0.8	0.3	0.5	0.1	0.9	-0.4	-0.2	-0.5
	Catalase		0.0	-0.4	0.3	0.3	-0.3	0.7	-0.1	-0.4	-0.3
	TSS			0.7	0.0	-0.1	-0.3	-0.6	0.5	-0.3	0.6
	Chlor.				0.3	-0.3	-0.5	-0.7	0.4	0.2	0.5
	Carot.					0.4	-0.3	0.3	0.2	0.4	0.3
	N						0.5	0.1	-0.2	-0.2	0.2
	P							0.0	0.2	0.1	0.4
	K								-0.2	0.1	-0.5
	Leave no.									0.4	0.9
	HD										0.4

POD: Peroxidase, CAT: Catalase, TSS: total soluble salts, Chlor.: Chlorophyll, Carot.: Carotenoids, HD: Head diameter

Soil

The residual enzyme activities in soil at harvest in response to soil and organic fertilizer types used and their cultivation seasons shown in Table (7) and as correlated to fertilizer analytical contents were well represented in Figure (3). These enzyme activities were representing part of soil biological characters after harvest, which gave considerable expectations for future impacts of using organic fertilizers in soil ready conditions for the next plantation season.

Table (7): **Soil enzymes activities at harvest**

Soil and year	Treat.	DeHase			N-ase			P-ase		
		mean original	± se	rank	mean original	± se	rank	mean original	± se	rank
Loamy 2021	T1	2.81	± 0.12	e	40.17	± 0.31	e	14.53	± 2.12	b
	T2	33.56	± 0.30	b	67.83	± 0.38	b	23.83	± 2.67	a
	T3	22.83	± 0.51	c	60.73	± 0.45	c	20.73	± 3.22	ab
	T4	33.45	± 0.46	b	33.83	± 0.11	f	24.73	± 1.88	a
	T5	35.00	± 0.29	a	82.67	± 0.38	a	24.38	± 2.55	a
	T6	12.52	± 0.24	d	46.33	± 0.34	d	21.93	± 2.95	ab
	T7	21.86	± 0.07	c	68.17	± 0.56	b	24.90	± 1.21	a
LSD				0.97			1.16			7.44
Loamy 2022	T1	3.93	± 0.23	f	40.33	± 0.24	e	13.50	± 1.68	b
	T2	34.18	± 0.29	b	67.83	± 0.46	b	22.87	± 3.90	a
	T3	23.90	± 0.08	c	60.67	± 0.41	c	21.43	± 3.70	ab
	T4	36.47	± 0.29	a	33.73	± 0.41	f	25.23	± 2.26	a
	T5	35.83	± 0.27	a	82.33	± 0.24	a	23.90	± 1.82	a
	T6	13.83	± 0.17	e	46.17	± 0.44	d	22.57	± 2.42	a
	T7	22.80	± 0.20	d	68.10	± 0.17	b	25.13	± 2.68	a
LSD				0.70			1.08			8.35
Sandy 2021	T1	1.79	± 0.12	d	16.90	± 9.77	e	8.67	± 0.76	c
	T2	16.90	± 0.49	b	23.23	± 13.43	b	15.17	± 1.36	a
	T3	20.37	± 0.88	a	21.17	± 12.24	c	13.63	± 1.25	ab
	T4	12.57	± 0.90	c	24.95	± 14.42	a	11.63	± 1.59	bc
	T5	18.43	± 0.54	ab	20.17	± 11.66	d	13.90	± 0.96	ab
	T6	12.63	± 0.43	c	21.67	± 12.52	c	12.00	± 1.10	abc
	T7	18.53	± 0.40	ab	22.50	± 13.01	b	12.87	± 0.67	ab
LSD				1.80			0.74			3.43
Sandy 2022	T1	3.07	± 0.56	e	16.90	± 0.37	f	7.90	± 0.74	e
	T2	18.30	± 0.55	b	23.17	± 0.20	b	16.00	± 0.64	a
	T3	20.67	± 0.44	a	21.10	± 0.15	d	14.17	± 0.61	bc
	T4	13.83	± 0.46	c	24.90	± 0.19	a	12.57	± 0.67	cd
	T5	19.67	± 0.63	ab	20.20	± 0.21	e	14.73	± 0.47	ab
	T6	11.17	± 0.62	d	21.73	± 0.39	cd	12.11	± 0.52	d
	T7	14.33	± 0.63	c	22.53	± 0.29	bc	13.17	± 0.46	cd
LSD				1.70			0.83			1.79

T1: control, T2 & T3: OMW 100 & 50%, T4 & T5: Vinasse 100 & 50%, T6 & T7: K-Humate 100 & 50%

For instance, both phosphatase and nitrogenase were positively responding to nitrogen, phosphorous and organic matter contents in organic fertilizers while potassium was reversely correlated to phosphatase and dehydrogenase activities in sandy soil. On the other hand, the organic matter and phosphorous contents were beneficial for dehydrogenase activities, while nitrogenase activities were negatively correlated to potassium, phosphorous and organic matter contents in organic fertilizers used in loamy soil. Phosphatase activities in loamy soil was positively affected to potassium and phosphorous contents in organic fertilizers.

Phosphorous form in organic fertilizers were stated to be mostly in an organic form that needs increased microorganism phosphatase activities to be released for plant benefit in both soil types **Rawat*et al.*, 2021**

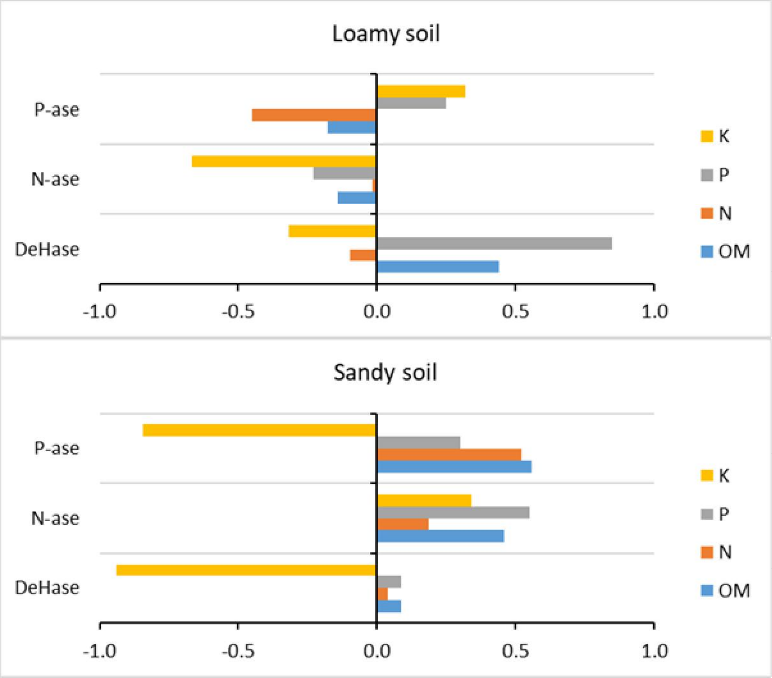

Figure (3): Correlation between soil residual enzymes activities and analytical contents of fertilizers used

One sensitive indication for changes in soil quality related to management and land uses is the activity of soil enzymes**,** (Bowles, et al., 2014).

Soil additive matter decomposes and plants release phenolic compounds, which reduce biological activity. The toxic effects of total phenol and hydroxyl methyl furfural (HMF) as a byproduct amendment inhibit dehydrogenase activity **(Natywa*et al.*, 2014; López-Piñeiro, *et al.*, 2011).**

Soil organic matter treatment, such as food waste compost, increased soil enzyme activity in lettuce growing, according to other research**(Lee,2004,Wojewódzki, *et al.* 2022).**

Potassium humate as an organic matter promoted microorganisms in soil **(Khaled and Fawy2011).**

Comment [SA13]: Please give the conclusion from this work.

Comment [SA14]: Soil enzyme activity is a sensitive indicator of changes in soil quality due to management and land use changes. Soil additives decompose, releasing phenolic compounds, reducing biological activity. Organic matter treatment, like food waste compost, increases enzyme activity in lettuce growing.

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