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ORGANIC AND MINERAL SOIL IMPROVERS INTENDED FOR THE CULTIVATION OF BUTTERHEAD LETTUCE

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ABSTRACT: The aim of the research was to select soil improver which will enhance the soil fertility and to assess the effect on the growth and yielding of lettuce. Algal biomass from the species *Scenedesmus acutus, Chlorella vulgaris* and soil supplement – zeolite was used in the laboratory experiment. Four fertilizing combinations were used for each plant three times. Doses of fertilizers were established according to the content of nitrogen. Physicochemical analysis of the soil, tested substrates and plant growth parameters were examined. Algal biomass had the greatest impact on the improvement of soil fertility and plant productivity. Fertilization with *Scenedesmus acutus* caused the increase of almost all soil parameters, e.g. an increase in total organic carbon by 3694 mg kg-1, Kjeldahl's nitrogen by 1287 mg kg-1. It was found that algal can be used in organic farming, in which the use of soluble mineral fertilizers is impermissible.

KEY WORDS: algal biomass, fertilization, productivity, zeolite, sustainable agriculture

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Introduction

By the year 2050 the world population will reach 9.6 billion and generations are expected to be much wealthier and urbanized than nowadays (PRB, 2017). These changes result in rapid increase in food and feed demand and as a consequence may lead to environmental risks such as soil depletion. Approximately 52% of the land used for agricultural purposes worldwide is moderately or severely affected by soil degradation (FAO, 2015). Economic data indicates that since 2008 land degradation affects as much as 1.5 billion people on the world.

The value of the agricultural market is strongly dependent on the quality of soil which is assessed in Poland as one of the lowest in EU countries. Organic matter content in Polish soils oscillated between 0.5-10% (RDP, 2017). Soil acidity is another problem to face it because approximately 50% of soils are highly acidic (Stańczyk-Mazanek et al., 2012). Over 50% of arable land belongs to unfavorable farming conditions (OPR, 2017). Diminishment of soil fertility is caused by overusing of chemical fertilizers and its improper use. In Poland the consumption of mineral fertilizers is constantly increasing as in 2015/2016 amounted to 1895.4 thousand tons and it is projected to achieve in year 2025 approximately 2300 thousand tons (Sroka, Musial, 2015). Additionally, there is maintained unfavorable ratio of nitrogen, phosphorus and potassium in mineral fertilizers (NPK–1.00:0.30:0.48) with too high share of nitrogen content. Therefore, surplus nitrogen leakage from agriculture is the main reason of surface and groundwater pollution (CSO, 2017).

Considering the above facts it should be stated that sustainable agricultural approach is highly desirable to eliminate the adverse effect of chemical fertilizers for human health and the environment while ensuring increase the yield and quality of crops. Global programs such as those involved in Sustainable Development Goals and the Post-2015 Agenda confirm that sustainable management of natural resources provide opportunities to combat with land degradation (US, 2015). Also, the 7th Environment Action Programme adopted by the European Parliament, assumes that by 2020 soil will be managed in a sustainable way and soil remediation will be carried out at an advanced stage (GIOS, 2014).

Sustainable manner of agricultural practices consists in transition from conventional to organic farming which reduces negative impacts on the environment and as a key point prevents loss of organic matter content. There is increased financial support within Rural Development Program 2014-2020 which allocates near EUR 700 million for ecological farming. Sobczyk (2014) confirm that number of organic farms keeps constantly increasing, therefore sustainable development of rural areas in Poland may attain high level in future years. The sustainable fertility management may be achieved not only by popular organic fertilizers but by means of algal biomass which has great potential to provide nutritional requirements in ways similar or better than traditional green manures such as lupine, phacelia, wheat or barley (Waldenstedt, 2003). It is well documented by scientific research that algal biomass may be applied as biostimulant, biofertilizer, seed primer and stimulate growth and yield of different plants (Boghdady et al., 2016; Badry, Salim, 2016; Garcia-Gonzalez, Sommerfeld, 2016), improve nutrients availability and nutrients uptake from the soil (Turan, Köse, 2004), enhance tolerance to environmental stresses (Lichner et al., 2013; Abdel Aziz et al., 2011). The wide range of benefits of algal biomass used as organic fertilizer has been evidenced by high content of micronutrients (e.g. Fe, Cu, Zn) and macronutrients (N, P, K, Ca, Mg), plant growth promoting substances, amino acids and vitamins (Blunden et al., 2010; Challen, Hemingway, 1965; Khan et al., 2009; Stirk et al., 2004; Zabochnicka-Świątek, 2017). Unlike chemical fertilizers, algal biomass and their extracts are natural, fully biodegradable, non-polluting the environment, possible to use in sustainable and organic farming. Moreover, removal of heavy metals can also be achieved by algal biomass (Zabochnicka-Świątek, 2013).

The objectives of the present study were to take into investigation the following: (i) the influence of adding algal biomass in dry (dry biomass) and liquid (living biomass) phase on growth and yielding of butterhead lettuce, (ii) assessment of physicochemical characteristics of fertilized soil.

Research methods

Study site and soil characterization

The study area is located within the city of Czestochowa in the province of Silesia in Poland on Polish Jurassic Highland. The Czestochowa region is characterized by average vegetation period – 212 days, 60-80 days of snow cover in the wintertime and annual precipitation around 650-700 mm. Soils typical to this area are podzolic soils, brown soils, moor soils and carbonate soils. Most agricultural soils are characterized by periodic or permanent water scarcity (66.4%). The quality of soils within this region is relatively low. Most of arable land belongs to IV and V valuation class (EKO-LOG, 2017). Predominant soils are light or very light that occupy about 64% of arable land. Approximately 63% of soils are acidic or highly acidic. Statistical data of the Czestochowa region indicates on very low usefulness of agricultural production.

The soil used for the experiment is classified as podzolic soil and is assessed as low-productive and inhomogeneous. Alkaline character of the soil is probably caused by the human activity in this regions e.g. liming. According to grain size analysis dominant fraction occurring in the tested soil is sand. Sources as FAO/WRB (2006) and USDA (2006) allow classifying soil as sand; in turn other sources PTG (2008) and PSSS (2008) classify the soil as loose sand. Based on granulometric analysis the agronomic class was established as very light soil. Due to The Act from 12th September 2012 on soil classification the soil is classified as VI valuation class.

Materials and experimental procedure

The pot experiment was conducted in April 2017 under laboratory conditions at Institute of Environmental Engineering, Technical University of Czestochowa. The experimental soil samples were taken from the area of Steelworks and prepared for physicochemical analysis The experiment were performed under sandy soil conditions to study the effect of organic and inorganic improvers on growth of lettuce and mineral changes of soil. Butterhead lettuce, variety 'Attractie' was used as the object of the research due to short growing period of this plant ant its high popularity as green vegetable in Poland. The strains of unicellular green microalgae *Chlorella vulgaris* were obtained from the Culture Collection of Baltic Algae (CCBA) in Poland and had been cultivated in laboratory till the exponential phase and then harvested. Dry biomass of *Scenedesmus acutus* were obtained from University of Kentucky, USA.

Prior the main experiment, physical and chemical properties of soil and materials were subjected to analysis. Parameters of soil and materials were determined by following methods: pH was determined by means of norm PN-ISO 10390:1997 using ph-meter CyberScan 11, the analysis of dry matter content in compliance with PN-ISO 11465:1999. Total carbon (TC) was determined by using Multi N/C 2100 Analytik Jena, the results of Total Organic Carbon (TOC) was achieved on Spectrophotometer HACH DR/4000 V with wavelength of 600 nm. Kjeldahl's nitrogen was determined based on PN-ISO 11261:2002, using Büchi apparatus for mineralization K-435 and Büchi Labortechnik A6, Model K-355 for distillation with water vapour. Macro and microelements: P – phosphorus, K – Potassium, Ca – Calcium, Mg – Magnesium, Fe – iron, Zn – Zinc, Cu – copper were determined in accordance to PN-ISO 11047:2001 by using plasma spectrometer, Spectro Arcos ICP-OES. For the soil, determination of available forms of phosphorus and potassium was applied, in compliance with Egner-Riehm method. The available content of magnesium was also determined, using Schachtschabel method. Soil improvers used in the experiments are varied in terms of physicochemical properties and nutrients (table 1). Due to different state of materials of dry and liquid algal biomass, parameter of TOC was determined for *Scenedesmus acutus,* while DOC was tested for *Chlorella vulgaris*. The values of pH measured in H_2O of all soil improvers are placed within the range of slightly acidic and neutral values (pH from 6.0 to 7.0). Among organic based soil improvers the Scenedesmus acutus has relatively high organic matter content 254 390 mg kg^{-1}. Dry biomass – Scenedesmus acutus is characterized by relatively high Kjeldahl's nitrogen content – 70 387 mg kg^{-1}. *Chlorella vulgaris* contained significantly increased nitrogen content in the amount of 222 564 mg kg^{-1}. Zeolite as representative of mineral additive is especially rich in potassium and calcium content – 15 444 and 14 064 mg kg^{-1}.

Table 1. Physicochemical properties of soil improvers

Parameter	Unit	Results		
		Scenedesmus acutus	*Chlorella vulgaris*	*Zeolite*
pH H_2O	–	6.0	7.0	6.7
Kjeldahl's nitrogen	mg kg^{-1}	70387	222564	106
TOC	mg kg^{-1}	254390	nd*	nd*
DOC	mg kg^{-1}	nd*	179074	nd*
P	mg kg^{-1}	30529	7901	49
K	mg kg^{-1}	3116	16926	15444
Mg	mg kg^{-1}	4644	10103	3558
Ca	mg kg^{-1}	44484	19553	14064

* not detected

Source: author's own work.

The experiment was arranged in block consist of 4 combinations of fertilization with three replicates. There were planted 12 plants in 0.5 L plastic pots filled with previously mixed soil with appropriate fertilizer dose. The pots contained holes at the bottom to provide drainage and were lined with agrofabric avoiding the undesirable soil run-off. The doses of soil improvers were determined on nitrogen content which on mineral soils should be at the level of 3500 mg kg^{-1}. As the result of different physicochemical composition of each improver, the doses are varied.

Treatments were as follows:

- dry algal biomass *Scenedesmus acutus* in the dose of 35.5 g kg^{-1},
- living algal biomass *Chlorella vulgaris* in the dose of 11.6 g kg^{-1},
- zeolite in the dose of 35.5 g kg^{-1},
- control – tap water without fertilization.

The soil improvers were applied in time of plants transplantation. The whole fertilization procedure was conducted in accordance with good agricultural practice in order to avoid over-fertilization and its side effects.

Plants were cultivated thought the entire experiment in greenhouse conditions by means of phytotron chamber. They have grown in the period of one month with conditions such as 12 h daytime with temperature around 18°C and nighttime 12°C. The irrigation of plants was done regularly by hand. The plants were harvested at the end of experiment and growth parameters were measured. Also, physicochemical analysis of the soil subjected to fertilization was performed.

Results of the research

Determined pH analysis indicated that soil is alkaline. The Kjeldahl's nitrogen in the examined soil amounted to 806 mg kg^{-1} which ranks the soil below the average value of nitrogen for Polish mineral soils – 1200 mg kg^{-1}. Similarly, total organic carbon in the soil is as low as – 7493 mg kg^{-1} in comparison to the average value occurring in the soils – 11 200 mg kg^{-1}. Carbon to nitrogen ratio of tested soil (C/N = 9.64) is similar to the other soils of Poland. The nutrients levels of phosphorus, potassium, calcium and magnesium in the soil are lower than the average level of nutrients of Polish soils. The values of available phosphorus and potassium ranks the soil in the average fertility class for which range of limit numbers are 10.1-15.0 mg P_2O_5 100 g^{-1} and 7.6-12.5 mg K_2O 100 g^{-1}, respectively. The available magnesium content of the soil with the amount of 5.88 mg Mg 100 g^{-1} is reported as high due to limit numbers for high fertility class which are: 4.1-6.0 mg Mg 100 g^{-1}. Humic acids content extracted from the tested soil on the level of 4.75 g kg^{-1} is indicated as low. Most of above mentioned results point out that examined soil is characterized by low quality and low agriculture suitability. Therefore, fertilization is required to recover soil productivity and provide nutrients necessary for plants growth. The effects of fertilization on the soil are varied, depending on applied soil improvers (table 2).

Table 2. Effect of organic and mineral additives on the soil (Mean ± SD)

Parameter	Unit	Control	*Scenedesmus acutus*	*Chlorella vulgaris*	Zeolite
pHKCl	mg kg^{-1}	7.6±0.02	7.7±0.01	7.6±0.01	7.4±0.02
pHH_2O	mg kg^{-1}	7.8±0.02	7.9±0.02	7.7±0.01	7.6±0.01
Kjeldahl's nitrogen	mg kg^{-1}	939±61	2226±135	996±35	828±47
TC	mg kg^{-1}	17806±859	25984±1244	19010±788	nd*
TOC	mg kg^{-1}	8527±836	12221±317	12411 ±451	9393±1384
C/N ratio	–	9.08	5.49	13.12	11.34
P	mg kg^{-1}	140±1.1	561±70	176±5.6	143±13
K	mg kg^{-1}	574±17	611±79	716±10	1651±160
Mg	mg kg^{-1}	895±8	973±64	1079±32	1242± 99
Ca	mg kg^{-1}	4278 ±38	4675±231	4967 ±144	5389±25
Cu	mg kg^{-1}	20.5±0.4	17.4±0.2	24.4±1.0	20.8±1.4
Zn	mg kg^{-1}	403 ±5	305±48	494 ±15	409±36
Fe	mg kg^{-1}	13841±111	11359±624	15690±1034	13432±680
Available P	mg P_2O_5 100 g^{-1}	12.0 ±1	160.3±5	15.1 ±1	12.3±0.5
Available K	mg K_2O 100 g^{-1}	9.0±0.8	16.8 ±1	8.3 ±0.3	21.5±0.8
Available Mg	mg Mg 100 g^{-1}	6.4 ±0.3	12.6±0.9	6.1±0.1	6.7±0.5

* not detected

Source: author's own work.

The use of zeolite caused decrease of pH from 7.60 to 7.40, while algal based soil improvers maintained pH on similar range. However, the soil after treatment with different soil improvers maintained alkaline character. There was observed increase of Kjeldahl's nitrogen content among soil improvers excluding zeolite. The greatest increase was noticed after addition of dry algal biomass *Scenedesmus acutus* – 2.4 fold increase compared to control. The analysis of the soil pointed out that algal based soil improvers are rich in organic carbon content to similar degree. Among them, *Chlorella vulgaris* caused the greatest increase of organic carbon content, namely 1.4 increase compared to control soil. Carbon to nitrogen ratio decreased in case of using *Scenedesmus acutus* while addition of *Chlorella vulgaris*, and zeolite caused increase of C/N ratio at similar degree. As a result of fertilization, the content of macronutrients: phosphorus potassium, calcium and magnesium were changed in comparison to control soils. After application of *Scenedesmus acutus* the total phosphorus content increased up to 561 mg/kg which constitute 4 fold increase compared to control soil. The zeolite is considered to be the best additive in terms of enrichment the soil in potassium content – 1651 mg kg^{-1}, while control soil include only 574 mg kg^{-1}. The greatest increase of magnesium and calcium content was recorded after zeolite treatment. Relatively high content of magnesium was observed in the soil when *Chlorella vulgaris* was added – 1079 mg kg^{-1}. Given data of microelements showed that *Chlorella vulgaris* applied to the soil caused the considerable increase of copper, zinc and iron content compared to control and other soil improvers. For each type of applied treatment the content of available phosphorus increased. The range of limit numbers for available phosphorus content for average, high and very high fertility class are respectively: 0.1-15; 15.1-20.0 and from 20.1 mg P_2O_5 100 g^{-1}. The significant increase was observed in the soil treated with *Scenedesmus acutus* for which the value amounted to 160.3 mg P_2O_5 100 g^{-1} and soil fertility class changed from average to very high. In case of fertilization with *Chlorella vulgaris* the soil fertility class changed from average to high. The zeolite fertilization resulted in the highest increase of available potassium content (21.5 mg K_2O 100 g^{-1}) which caused change fertility class from average to very high. Limit numbers for average, high and very high fertility class for available potassium content are following: 7.6-12.5 mg K_2O 100 g^{-1}; 12.6-17.5 mg K_2O 100 g^{-1} and greater than 17.6 mg K_2O 100 g^{-1}, respectively. The application of *Scenedesmus acutus* caused change of the soil fertility class from average to high, while the use of *Chlorella vulgaris* caused slight decrease of available potassium, maintaining the fertility class at average level. The highest increase of available magnesium was found in case of application of *Scenedesmus acutus* for which the value was 12.6 mg Mg 100 g^{-1}, respectively. The soil is regarded as highly rich in available magnesium content when the values are greater than 6.1 mg Mg 100 g^{-1}.

The soil improvers differently influenced on growth parameters of lettuce (table 3). The pot experiment showed that generally all treatments influenced positively on growth of lettuce. Among all of soil improvers the greatest amount of leaves was recorded for *Chlorella vulgaris* treatment – 1.4 fold increase compared to control. Leaves length after treatment with zeolite and *Chlorella vulgaris* was the same (8.93 cm). It was observed that roots length in fertilized plants were better developed and branched compared to control. The longest roots and the best developed was characterized for plants fertilized with *Chlorella vulgaris*. The effects of applied *Scenedesmus acutus* are not presented in the table because plants did not survive the experiment. The probable factors could be physiological stress of plants in reaction for transplantation and overwatering which caused damage to plants, however over-fertilization is excluded.

Table 3. Effect of natural and organic soil improvers on growth parameters of lettuce (Mean ± SD)

Treatments	Number of leaves [-]	Leaves length [cm]	Leaves width [cm]	Roots length [cm]
Control	5.00±0	7.40±0.4	2.30±0.1	8.97±0.7
Chlorella vulgaris	7.00±0	8.93±0.1	2.80±0.2	9.27±0.6
Zeolite	6.33±0.9	8.93±0.2	2.23±0.1	7.67±1.3

Source: author's own work.

Discussion of the results

The experiment revealed that natural and organic amendments efficiently improved properties of low-productive and sandy soil. The literature sources confirm that optimum range of pH suitable for proper plants growth and development is: 5.5-7.2 (Dyśko et al., 2014) hence the studied soil is beyond this range. Soil improvers applied to the soil did not exhibit considerable changes of pH compared to control. Addition of zeolite caused decreased of pH while in other literature sources was found that zeolite usually is responsible for increase of pH levels (Ming, Boettinger, 2001). Algal based materials used for fertilization due to organic matter content and buffering properties allow for counteracting pH changes (Tiessen et al., 1994). The study results found out that algal biomass constitute big reservoir of nitrogen content–as much as 2.4 fold increase of Kjeldahl's nitrogen in the soil treated with *Scenedesmus acutus* compared to control soil. Addition of zeolite is favourable due to falling trend of nitrogen loss which was reported by Wang et al. (2017).

Algal based soil improvers incorporated similar and also significant amounts of total organic carbon to the soil. Addition of organic matter content is strongly associated with cation exchange capacity which was observed within study of Habashy and Abdel-Razek (2011) where cation exchange capacity increased the most in the soil treated with seaweed extract and zeolite. C/N ratio was subjected to changes in all of treatments. The narrowing of C/N was recorded when *Scenedemsus sp.* was applied. The increase of C/N occurred after treatment with *Chlorella vulgaris* and zeolite. The all soil improvers enhanced mineralization processes in the soil because it is reported that C/N ratio smaller than 15.0 favours mineralization while C/N>20.0 s cause immobilization processes (Fertilizers Europe, 2016). The applied natural and organic amendments resulted in increase of macro- and micronutrients to varying degrees. The highest increase of total P content was recorded for soil treated with microalgae *Scenedesmus acutus* which is 4 times more than result obtained for control. Similarly the available Mg and P content was the highest for *Scenedesmus acutus* which caused change of soil fertility class from average to very high. It was observed that high P content influenced on decrease of content of micronutrients – Cu and Zn compared to control soil. Such obtained data are in agreement with literature which reported that surplus of PO^{4-} ions cause immobilization of Zn and Cu and additionally may be the reason of formation sparingly soluble calcium-phosphate salt when pH>7 (Fang et al., 2012; Wandruszka, 2006).

Compared to other soil improvers, the application of zeolite resulted in the considerable increase of total K, Mg and Ca content. Also, the value of available potassium content for zeolite was the highest (21.52 mg K_2O 100 g^{-1}) which placed the soil in the very high fertility class. The calcium content was at similar level at fertilized soils. Taking into account the results of micronutrients: Zn, Cu and Fe, it can be concluded that the use of C*hlorella vulgaris* had the greatest impact on the increase of given nutrients in the soil followed by zeolite and *Scenedesmus acutus.* The stimulatory effects of dry and living algal biomass was tested in the experiment conducted *on Lectuca sativa* by Faheed and Abd-El Fattah (2008) and revealed that application of 2 and 3 g kg^{-1} of dry microalgae – *Chlorella vulgaris* significantly increased growth parameters of lettuce and content of chlorophyll. The other experiments used urea, poultry compost, dry and living blue green algae – *Anabaena sp.* as organic fertilizers and then recorded the highest increase of P, Zn and Fe content and all lettuce growth parameters for dry biomass of algae (Abuye, Achamo, 2016). The above results are in line with this experiment where dry algal biomass – *Scenedemsus acutus* achieved the highest nutritional values. The vast majority of literature sources focused of using algal extracts or algal compost as organic fertilizers and achieved favorable results concerning high plants growth and considerable improvement of physicochemical properties of soil (Michalak et al., 2016; Garcia-Gonzalez, Sommerfeld, 2016). The inorganic but of natural origin soil amendment – zeolite is proved to be good quality mineral fertilizer. For instance, the application of zeolite in the research of Eprikashvili et al. (2016) and Wang et al. (2017) resulted in increase of germination index and the nutrients content.

So far, the combination of mixed organic and chemical fertilizers were examined in different literature sources and resulted in the high yield of agricultural crops (Islam et al., 2017; Priyadarshani, 2013). Based on those results, the range of this experiment may be broaden by combining zeolite with algal based materials to sustain soil fertility and obtain high yields in a sustainable way without using chemical fertilizers. What is more, study of Habashy and Abdel-Razek (2011) revealed that application of algal extract is beneficial from the economic point of view because of the highest net income 4554 LE fed-1 compared to other fertilizers.

It is recommended to conduct further experiment under field and laboratory conditions to fully exploit high potential of algal based additives as organic fertilizers. Encouraging farmers to transition from conventional to organic farming is a key point in diminishment of environmental pollution and reduction of chemical fertilizers and hence practices sustainable agricultural approach (Mieszajkina, 2016). Data collected by Bryła (2015) indicates that Poland has a high place – 4th in terms of organic food producer and 5th place regarding the area of organic crops in Europe. Implementation of new methods of organic fertilization is crucial due to intense and dynamic growth in the market value of organic food in developed countries.

Conclusions

According to the obtained results the following conclusions can be drawn:

1. Algal based soil improvers are valuable source of organic matter content and may replace chemical fertilizers.
2. Organic and mineral soil improvers of natural origin significantly increased growth parameters of examined plants.
3. The application of *Scenedesmus acutus* considerably improved physicochemical parameters of soil, increasing its fertility.
4. In soil, increase of total organic carbon by 3694 mg kg^{-1}, Kjeldahl's nitrogen by 1287 mg kg^{-1}, total phosphorous by 421 mg kg^{-1}, available phosphorus by 148.3 mg P_2O_5 100 g^{-1} was found after application of *Scenedesmus acutus.*
5. Application of *Chlorella vulgaris* resulted in the greatest increase in the soil parameters: total organic carbon up to 12 411 mg kg^{-1}, Kjeldahl's nitrogen up to 996 mg kg^{-1} and C/N ratio up to 13.12.
6. Zeolite as mineral additive caused great contribution of macro nutrients in the soil: potassium – 1651 mg kg^{-1}, magnesium 1242 mg kg^{-1}, calcium – 5389 mg kg^{-1} and available potassium content – 21.5 mg kg^{-1}. Simultaneously, after the application of zeolite, no improvement in lettuce growth was observed.
7. The most beneficial effect on lettuce was observed after fertilization with *Chlorella vulgaris* due to the observed increase number of leaves up to 7, leaves length up to 8.93 cm, leaves width up to 2.80 cm and increase of roots length up to 9.27 cm.

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The contribution of the authors

Magdalena Zabochnicka-Świątek – 70% (concept and objectives, correction).
Roksana Kocela – 30% (literature review, research).

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