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Abstracts and full-text reports uploaded to the conference system undergo a review procedure. Authors will be notified of the application results in three weeks. Submitted abstracts will be evaluated on the basis of abstracts/proposals. The conference system allows you to submit the full text if your abstract is accepted. Please upload the abstract of your article to the conference system and wait for the results of the evaluation. If your abstract is accepted, you can upload your full text. Your full text will then be sent to at least two reviewers for review. The conference has a double-blind peer-review process. Any paper submitted for the conference is reviewed by at least two international reviewers with expertise in the relevant subject area. Based on the reviewers' comments, papers are accepted, rejected or accepted with revision. If the comments are not addressed well in the improved paper, then the paper is sent back to the authors to make further revisions. The accepted papers are formatted by the conference for publication in the proceedings.

Aims & Scope

Plant and animal health is closely related to human health. In this century, where the human population is rapidly increasing and technology is developing rapidly, the problem of food supply to the increasing population brings plant and animal health to the fore. Nowadays, when concepts such as artificial meat and capsule feeding are discussed, the process of growing plants and animals has begun to be discussed. For this reason, this conference focused on the changes and innovations in the field of Veterinary, Agriculture and Life Sciences.

The aim of the conference is to bring together researchers and administrators from different countries, and to discuss theoretical and practical issues of Veterinary, Agriculture and Life Sciences. At the same time, it is aimed to enable the conference participants to share the changes and developments in the field of Veterinary, Agriculture and Life Sciences with their colleagues.

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Biochemical Parameters Studying in Experimental Diabetes

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Abstract: Objective of study was biochemical parameters studying in experimental modeling of 2nd type diabetes in animals. To achieve the goal 2nd type induced diabetes was modeled in white male rats weighing on average 200.0±20.0 gr. Streptozotocin (MP Biomedicals, USA) administered intraperitoneally at 30 mg/kg dose was used as β-cytotoxin in modeling diabetes. After injection in experimental animals glucose concentration increased by 1.2 times ($p<0.05$), glycated hemoglobin level increased by 1.4 times ($p<0.05$). Lipid peroxidation outputs and antioxidant preservation enzymes in blood lymphocytes were studied: diene conjugates, malondialdehyde content, glutathione reductase, glutathione peroxidase, catalase activities. Studies have shown that in diabetes peripheral blood lymphocytes DC concentration increases by 20.68% ($p<0.05$), whereas MDA also increases by 23.07% ($p<0.05$). Antioxidant system enzymes underwent the following changes in diabetes: GlR activity in the blood decreased by 21.38% ($p<0.05$), catalase by 18.26% ($p<0.05$), whereas GlP activity was observed to decrease ($p>0.05$). Results show decrease in antioxidant system activation against the background of oxidative stress activation. AOP state studying seems crucial from the point of view of correctional therapy methods and determining pathological and adaptive alteration origin in the body. Conclusions. In diabetes mellitus oxidation process of chain lipids free radicals is activated entailing undesirable outputs accumulation in tissues having extremely harmful effects leading to cell membranes changes at different levels. Diabetes formation is accompanied by decrease in antioxidant system activation confirming by decrease in GlR, GlP, Kt expression against oxidative stress expression background.

Keywords: Diabetes, Lipid peroxidation, Antioxidant system

Introduction

It is clear that diabetes mellitus constitutes a complex metabolic disorder induced by a multifactorial etiology, mainly characterized by chronic hyperglycemia, a popular belief that is one of the most common diseases at present, occurring in approximately 8-9% of the world's population (Shimizu & Oniki, 2019; Roglic, 2016). R. Sherwin maintains (Sherwin & Jastreboff, 2012) that both the incidence and prevalence of diabetes continue to grow with the approaching tsunami force. Constantly the IDF (International Diabetes Federation) updates data, the number of people suffering diabetes rises.

Over the past 28 years, the number of patients bearing diabetes in the world has increased more than 12 times: from 30 million patients having diabetes in 1985 to 387 million in 2014, mainly as a result of patients suffering the 2nd type diabetes mellitus (DM II). It should be noted that the real rate of peak in incidence is tremendously ahead of even such disappointing forecasts of experts from the World Health Organization (WHO). According to IDF experts, in the number of patients bearing diabetes (in 2013 and 2014, respectively), China leads (98.4 million and 96.3 million), followed by India (65.1 million and 66.8 million), then USA (24.4 million and 25.779 million) (IDF, 2013; 2015). The number of patients suffering diabetes is constantly increasing due to the growth prevalence of obesity and sedentary lifestyle. Currently, it can be noted that diabetes is becoming sharply younger (an increase in the number of cases of DM II in children, adolescents and young adults) (Zimmet et al., 2014). The total quantity of patients having diabetes in Kazakhstan is growing steadily. Over the past 10 years, a progressive increase in the incidence of diabetes has been observed in Kazakhstan (an increase of 1.6 times). The number of sufferers tends to further increase, primarily in age groups over 40 years. Every 10-15 years, the number of human suffering diabetes doubles.

In accordance to IDF experts, the quantity of diabetes diseased in Kazakhstan in 2013 reached 526,000 people (IDF, 2013), in 2014 – 536,400 people (IDF, 2015). The significance of the medical and social problem of DM II is due not only to its high prevalence and the continuing trend towards an rise in the number of ailing, however also to high disability, mortality of sufferers owing to the development of micro- and macroangiopathies, additionally the need to organize a system of specialized care for patients. The development of chronic diabetic complications is the main problem of the 2nd type diabetes. Currently, there is convincing evidence of the direct involvement of hyperglycemia in the initiation of vascular complications of diabetes, while oxidative stress associated with increased generation of ROS plays a decisive role in their pathogenesis (Chandra et al., 2019).

Excessive production of ROS in β-cells can entail alterations in the shape, volume and function of mitochondria contributing to the breakdown of ATP-dependent K+ channels and impaired insulin secretion (Wang, 2017). These processes may be due to the fact that the content of antioxidant enzymes in β-cells is 10-20 times lower than in the cells of the liver, kidneys, heart, brain and other organs (Ceriello et al., 2016). Oxidative damage to β-cells caused by ROS as a result of hyperglycemia affects the quantity and quality of secreted insulin. There is evidence that β-cell dysfunction (impaired secretory capacity and increased insulin resistance) induced by oxidative stress plays a pivotal role in the pathogenesis of DM I and DM II. ROS can activate several other pathways, which, in turn, leads to one of the main complications of diabetes, namely endothelial dysfunction (Ighodaro, 2018).

Nowadays, the quality of the patients' life with diabetes is determined by the development and progression of chronic complications of this disease, caused by microangiopathies (injury to capillaries, arterioles and venules) and macroangiopathies (injury to medium and large vessels). Clinical manifestations of microangiopathies are diabetic retinopathy, nephropathy and neuropathy. Macroangiopathies result in myocardial infarction, stroke and gangrene of the lower extremities. The main culprit of disability and mortality in patients suffering diabetes are cardiovascular complications that develop as a result of progressive atherosclerotic vascular deterioration (Gracheva et al., 2012). The risk of developing coronary heart disease (CHD) is 2-4 times higher in the 2nd type diabetes sufferers compared to individuals out of diabetes (Wannamethee et al., 2011). In patients having DM II, diabetic nephropathy constitutes the second leading cause of death after CVD (Dedov, 2010). The culprit of the development and progression of vascular complications of diabetes is chronic hyperglycemia (American Diabetes Association, 2007). Numerous studies have confirmed the hypothesis of a causal association between hyperglycemia and oxidative stress (OS) (Esposito et al., 2002; Ikebuchi et al., 2010; Fiorentino et al., 2013).

In hyperglycemia, due to increased accumulation of advanced glycation end owtputs (advanced glycation end products - AGEs), pronounced changes are observed in the cytoplasm, nuclear structures and components of the extracellular matrix (Ramasamy et al., 2011). AGEs, by binding to their specific receptors (RAGE), are able to activate nicotinamide dinucleotide phosphate-NAD(P)H oxidase (NADPH) and elevate the oxygen free radicals production. Subsequently, by activating the transcription factor NF-kB (Nuclear Factor kappaB), they induce the production of vascular endothelial growth factor (VEGF) in monocytes/macrophages, epithelial cells, vascular smooth muscle cells and microvascular wall cells (Devi & Sudhakaran, 2011; Piarulli et al., 2013).

Study Objectives

Study of biochemical parameters in experimental modeling of the 2nd type diabetes in animals.

Methods

To achieve this goal, we modeled induced the 2nd type diabetes in experimental white male rats weighing on average 200.0±20.0 grams. All experimental procedures were carried out in accordance with the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, 2005; Position of the Council at first reading with a view to the adoption of a Directive of the European Parliament and of the Council on the protection of animals used for scientific purposes, 2010) and "Rules for Conducting Biomedical Experiments, Preclinical (Non-Clinical) and Clinical Research, as well as Requirements for preclinical and clinical bases" Order of the Minister of Health of the Republic of Kazakhstan dated April 2, 2018 No. 142. Before the start of the study, laboratory animals were kept for 14 days to adapt to group housing in cages. At the time of this period, the animals were monitored for clinical status by visual inspection every day. Laboratory animals with abnormalities detected during the examination were not included in the experimental groups. Before the start of the study, laboratory animals meet the criteria for inclusion in the experiment.

To induce diabetes in rats, the Mansor L.S. model was used (Mansor, 2013). When modeling diabetes, low doses of streptozotocin (CAT NO.100557, CAS NO. 18883-66-4. MP Biomedicals, USA) were used as a β-cytotoxin. The cytotoxin streptozotocin, characterized by increased tropism for β-endocrinocytes, is administered intraperitoneally to animals of group II on the 8th day of the experiment at a dose of 30 mg/kg in a solution (0.1 M) of citrate buffer (pH=4.4). A dose of 30 mg/kg of animal weight was chosen due to the fact that administration of a higher dose of streptozotocin to animals (>50 mg/kg) leads to injury to the vast majority of β-cells, simulating the 1st type diabetes (Srinivasan et al., 2005). A dose of 30 mg/kg of animal weight minimizes the detrimental effect of the cytotoxin. The administration of streptozotocin entails disruption of insulin signaling, contributing to disruption of carbohydrate metabolism. The intact group rats were intraperitoneally injected with a citrate buffer solution in the same volume as the diabetic groups. We combine the induction of diabetes using a diet associated a high fat content (food containing 61% saturated fatty acids, i.e. fats of animal origin) for 5 weeks.

The experimental model of DM II, induced by low doses of streptozotocin in combination with a high fat content, at the pathogenetic level most closely corresponds to the development of DM II in humans. This model of DM II, on the one hand, sufficiently imitates the stages of development of the disease, as well as the metabolic features of DM II in humans, on the other hand, it is less expensive, easily reproducible and suitable for research. In order to confirm the established experimental diabetes, 2 weeks after the injection of streptozotocin, fasting glucose levels were measured in order to identify animals that did not correspond to the model of diabetes. Blood for the study was taken from the tail vein. The main criteria for including animals in groups having experimental diabetes: the fasting glucose level was more than 7.0 mmol/l. Animals are removed from the experiment in accordance with ethical standards and recommendations for the humanization of work with laboratory animals.

Laboratory research. Determination of glucose concentration was carried out usage the express method using a glucometer. Blood for the study was taken from the tail vein.

Determination of HbA1c. The concentration of HbA1c, an indicator of chronic, stable hyperglycemia, was analyzed by enzyme immunoassay using the CEA190Ra ELISA Kit For Glycated Hemoglobin A1c (HbA1c), 96T, Cloud-Clone, Corp. (Weykamp et al., 2008).

Determination of C-peptide. C-peptide determinations were carried out using test kits, enzyme immunoassay using CEA447Ra ELISA Kit For C-Peptide (CP), 96T, Cloud-Clone, Corp.

Determination of insulin. To assess the degree of insulin resistance and functional activity of β-cells, the determination of immunoreactive insulin is used. Determination of insulin concentration was carried out by ELISA using test kits CEA448Ra ELISA Kit For Insulin (INS), Cloud-Clone Corp.

Determination of 1,5-anhydroglucitol. The concentration of 1,5-AG, an indicator of glycemic status, was analyzed by enzyme immunoassay using CEB046Ge ELISA Kit For 1,5-Anhydroglucitol (1,5-AG), 96T, Cloud-Clone, Corp.

Determination of lipid peroxidation products. The content of diene conjugates (DC) in lymphocytes was determined according to the method of Gavrilov and Mishkorudnaya (1983) and the content of malondialdehyde (MDA) was determined according to the method of Konyukhova et al. (1989).

Determination of the activity of enzymes of the antioxidant system. The activity of the enzyme glutathione reductase (GrR) and glutathione peroxidase (GlP) was determined according to the method of S.N. Vlasova (Vlasova, Shabunina & Pereslegina, 1990) and the activity of the enzyme catalase (CT) was determined according to the method Korolyuk et al. (1988).

The obtained research results were processed using the "STATISTICA 8.0" software package from StatSoft, Inc. USA. The arithmetic mean values of quantitative indicators presented in the text as M±SD were calculated, where M is the arithmetic mean, SD is the standard deviation. In all statistical analysis procedures, the significance level was assumed to be $p \leq 0.05$.

Results and Discussion

Metabolic Status

Changes in biochemical parameters under conditions of experimental diabetes mellitus were studied. At this stage, indicators of metabolic status were studied and the validity of the experimental model of the 2nd type diabetes was confirmed. The results of the metabolic status study in experimental rats of group II linked to the 2nd type diabetes demonstrated an increase in fasting glycemia, glycated hemoglobin, concentrations of 1,5-AG and C-peptide, insulin concentrations, relative to the intact group. It was found that the glucose concentration in experimental animals having the 2nd type diabetes mellitus increased 1.2 times ($p<0.05$), the glycated hemoglobin level rised from 2.85±0.22 to 3.91±0.23 ($p<0.05$). In comparing the concentration of C-peptide with the intact control group, a significant increase in this indicator was found in diabetic animals: in the control group - 2.01±0.2 ng/ml, whereas in the experimental group comes to 3.12±0.27 ng/ml ($p <0.05$).

1,5-anhydroglucitol (1,5-AG) is a medium-term indicator of glycemic status, reflecting fluctuations in the hyperglycemic range over a 2-week period. At normal glucose levels, the concentration of 1,5-AG in the blood plasma is maintained by a balance between dietary intake and renal excretion. Under conditions of hyperglycemia, when the renal threshold for glucose is exceeded, the plasma concentration of 1,5-AG falls owing to competitive inhibition of its reabsorption by glucose. The advantages of 1,5-AG as a marker of glycemic status are stability and lack of dependence on the physiological state at the time of blood sampling. When studying the hyperglycemia marker - 1.5-AG, a result was obtained showing constant hyperglycemia in experimental animals, which revealed a significant decrease: in the intact group - 24.21 ± 3.8 ng/ml, in the experimental group - 11.27 ± 3, 11 ng/ml ($p<0.05$).

When comparing the insulin concentration with the intact control group, a significant increase in this indicator was found in diabetic animals: in the intact group - 0.64±0.09 ng/ml, in the experimental group - 1.33±0.19 ng/ml ($p<0 .05$). Thus, summarizing the results of the metabolic status, we state that a model has been created that characterizes the typical pattern of established the 2nd type diabetes mellitus with a pronounced disturbance of the metabolic status.

Biochemical Status

Lipid peroxidation. Numerous studies in recent years indicate pivotal role of reactive oxygen species (ROS) in the development of pathological deviation caused by tissue factors (Johnson, 1981). Excessive production of ROS can lead to injury to one's own cells and damage to nuclear structures owing to oxidative modification of proteins, lipids and nucleic acids (Vladimirov, 1991; Dubinina & Shugaley, 1993). Activation of phagocytes tends to spontaneously increase, and a vicious circle of inflammation can form in inflammatory realm. Essential manifestations of the detrimental effects of free radicals in living systems are the mutagenic effect and disruption of the structural and functional state of cells through the initiation of lipid peroxidation processes (Soodaeva et al., 1982). Under physiological conditions, LPO is limited by antioxidant protection, which can be disrupted when exposed to harmful factors. Considering the importance of this above mentioned system in the formation of the pathological process, its lability, high sensitivity, as well as significant consequences when it is injured, we observed its manner in the formation of the pathological process in animals having the 2nd type diabetes interesting.

As studies have shown in experimental diabetes mellitus, the concentration of diene conjugates (Figure 1) significantly increases in peripheral blood lymphocytes from 0.29±0.02 to 0.35±0.02 ($p<0.05$) and the concentration of malondialdehydes also significantly increases in peripheral blood lymphocytes from 0.13±0.01 to 0.16±0.01 ($p<0.05$). The data obtained show that in diabetes mellitus, the process of chain free radical oxidation of lipids is activated leading to the accumulation of undesirable outputs in tissues having a very harmful effect, causing alterations in cell membranes at different levels.

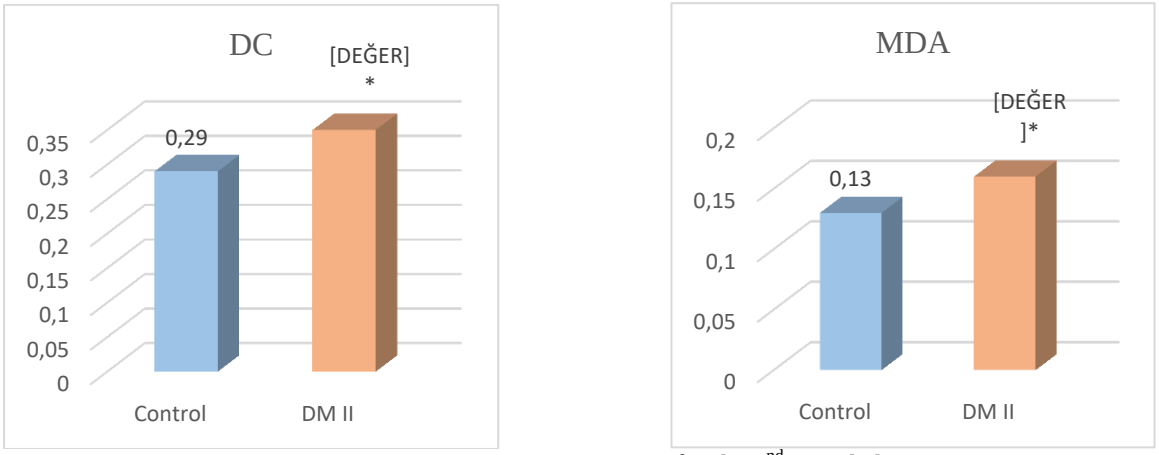
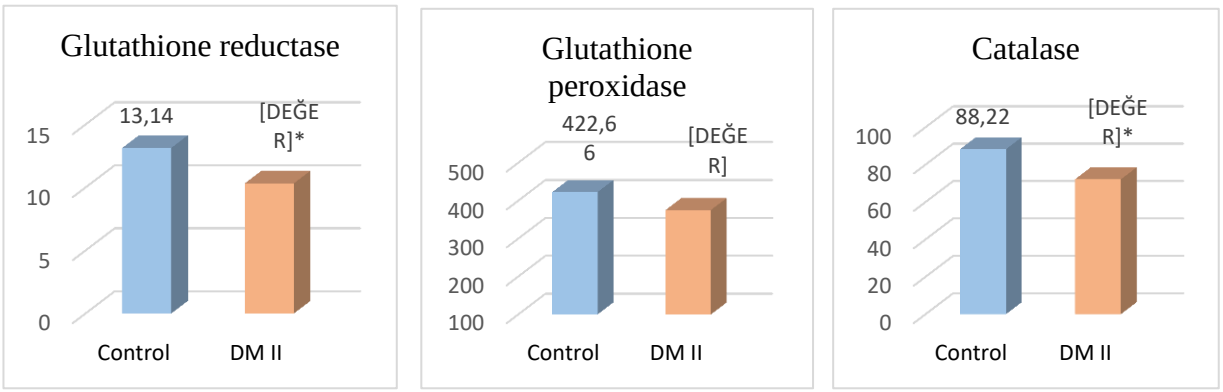


Figure 1. Deviations in LPO outputs for the 2nd type diabetes
Note: the difference is significant compared to the control group * - $p<0,05$.

The study conducted by Mrowicka M. (2005), Marjani A. (2010), Javed A, et al. (2023) found results consistent with the current study regarding MDA levels. Once again we are convinced that 2nd type diabetes is associated with increased lipid peroxidation. The latter is rised in the 2nd type diabetes mellitus and plays pivotal role in the pathogenesis and complications of diabetes. Antioxidants are known to prevent the progression and occurrence of diabetes. Free radical production can be reduced by preventing high blood glucose levels and controlling blood glucose instability. Patients suffering the 2nd type diabetes mellitus may have very high physiological requirements for antioxidants (Marjani, 2010; Mrowicka, 2005; Javed, 2023).

Findings from other researchers supported evidence that patients bearing diabetes were susceptible to oxidative stress, and higher blood glucose levels were associated with free radical lipid peroxidation. High levels of MDA in DM II indicate that oxidative stress plays an important role in the pathogenesis of cardiovascular complications. There is an imbalance between the oxidant and antioxidant systems in DM II (Davì et al., 2005; Likidlilid et al., 2010).

Antioxidant protection system. The research results obtained are reflected in Figure 2. Analysis of the research results indicates that the activity of the GlR enzyme in peripheral blood was significantly reduced from 13.14±0.97 to 10.33±0.81 ($p<0.05$). GlP activity in experimental animals changed slightly, this indicator was within the range of 375.39±28.34 in experimental animals, and in the control group 422.66±30.42 ($p>0.05$). Simultaneously, catalase activity was also significantly reduced from 88.22±6.27 to 72.11±4.37 ($p<0.05$).



Note: the difference is significant compared to the control group * - $p<0,05$.
Figure 2. Deviations in AOS enzyme activity in the 2nd type diabetes

Thus, the results of the studies indicate a decline in the antioxidant system activation against the background of oxidative stress activation in experimental DM II. Diabetes depletes the cellular antioxidant defense system and is associated with increased free radicals production. Oxidative stress can result from several pathways. Some of them are inextricably linked to substrate-mediated overproduction of reactive oxygen species in mitochondria, accelerated formation of advanced glycation end products, glucose autoxidation, and depletion of micronutrients and cellular elements with antioxidant properties. The gap between the robust experimental evidence for the pathogenetic role of increased oxidative load in diabetes mellitus and the overwhelming failure of antioxidants to demonstrate any health benefits can be described as the "antioxidant paradox" (Sheikh-Ali et al., 2011). High levels of free radicals and a simultaneous decrease in antioxidant defense mechanisms leading to injury to cellular organelles and enzymes increased lipid peroxidation and the development of insulin resistance. These oxidative stress effects may provoke the diabetes complications (Maritim et al., 2003).

Conclusion

1. In diabetes the oxidation of chain free radicals of lipids is activated leading to the accumulation of undesirable outputs in tissues having an extremely harmful effect entailing alterations in cell membranes at different levels

2. The development of diabetes is accompanied by a decrease in the activation of the antioxidant system confirming by a decrease in the expression of GlR, GlP, Ct against the background of the oxidative stress expression (impaired LPO-AOP processes).

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

* The authors declare no conflict of interest.

Acknowledgements or Notes

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Composition and Structure of Ectoparasites in Cattle in Setif Region - Algeria

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Abstract: This study was conducted to investigate the infestation of cattle by ectoparasites in the region of Setif (Algeria). Over a three-year period (2016-2018), a total of 2200 cattle were examined, revealing five species of insects (four species of lice and one myiasis) and seven species of ticks (Ixodidae). Our results indicate that 66.07% of cattle were infested with one or more ectoparasites. Among these, ixodid ticks were the most prevalent, accounting for an overall infection rate of35.30%. The dominant tick species were *Rhipicephalus turanicus* (12.89%)and *Hyalomma marginatum* (10.05%), followed by *Hyalomma excavatum* and *Rhipicephalus bursa* at (9.4% and 8.9%, respectively). The species *Hyalomma scupense*, *Rhipicephalus (Boophilus) annulatus*, and *Haemaphysalis sulcata* had the lowest prevalence rates (5.65%,2.02%, and1.47%, respectively). Additionally,783 cattle were affected by phthiriosis, with the dominant louse species being *Bovicola bovis* (19.82%), followed by *Haematopinus quadripertusus*, *Solenopotes capillatus*, and *Linognathus vituli* (at12.77%,8.15%, and7.7%, respectively). In contrast, only244 cattle were affected by a single species of myiasis (*Hypoderma lineatum*), with2034 larvae. The seasonal dynamics of ectoparasites were evident, with seven tick species showing activity during spring and summer. All four louse species caused winter disease, while Hypodermosis due to *Hypoderma lineatum* occurred predominantly in spring and summer, extending into autumn. Interestingly, the topographical region did not significantly influence lice and Hypodermosis infestations. However, it did create a favorable environment for tick activity. There was also a racial predisposition observed in lice and ticks, in contrast to Hypodermosis, where racial factors had no impact. Finally, the influence of age and sex on ectoparasite diseases closely correlated with the breeding system.

Keywords: Ectoparasites, Cattle, Veterinary

Introduction

Cattle breeding, especially dairy cattle, is a key strategic sector of Algerian agricultural policy, and according to Moufek (2007) , the number of cattle in Sétif is estimated at 10% of the national total, making it a pilot wilaya in this field, with 151 446 head of cattle in 2019, 74335 of them dairy cows. Of course, this livestock population is faced with a number of parasitic affections, more specifically external parasites, which are of great medical and veterinary importance, causing incalculable losses to livestock farming and representing a heavy burden for animals and human populations, and consequently losses for the national economy.

Knowledge of the etiological agents and their biological characteristics is essential for a rapid and accurate diagnosis, enabling the use of an appropriate curative and prophylactic therapeutic arsenal. With this in mind, we opted for this subject, the main aim of which is to inventory and identify the species of bovine ectoparasites in the southern region of the Wilaya de Sétif, and to study the influence of intrinsic (age, sex and breed) and extrinsic (season, rearing system and topography) factors on the infestation of these parasites. The work was carried out in three phases:

a) Field survey (collection of ectoparasites from animals).
b) Identification of ectoparasites in the laboratory and data processing.

c) Statistical analysis and discussion of the results obtained.

Finally, this study aims to monitor, over the course of a three years, the evolution of cattle infestation by the various species of ticks found in the southern region of eastern Algeria (Setif), as well as the influence of farming type and altitude on this infestation.

Method

In region of stat of Setif (Algeria) (fig 1). During three years (2016-2018) a total of 2200 cattle were examined, of different breeds (Montbeliarde, Fleckvieh, Pie Noire Holstein, and the Improved Dairy Cattle breed (BLA) resulting from the crossbreeding of various breeds with Montbeliarde). The animals were selected by random draw with an identical distribution (1100 in the mountainous region and 1100 in the lowland region). Throughout the study period, the sample remained the same with no loss or replacement, and no acaricide treatment was applied to the cattle. The farms in the study area are not strictly for dairy farming but are mixed farms with both dairy cows and fattening calves. There are therefore two types of farming: a semi-extensive system for dairy cows where the animals' diet is primarily based on hay and concentrated feed in the buildings during winter and on pasture in the good season (spring and early summer); and an intensive system for fattening cattle.Parasites were removed twice a month between January and December (2016- 2018), All fixed were preserved in 70° ethanol until identification. We based on the adult identification keys (Bouattour, 2002; Estrada-Peña et al., 2004, 2017).

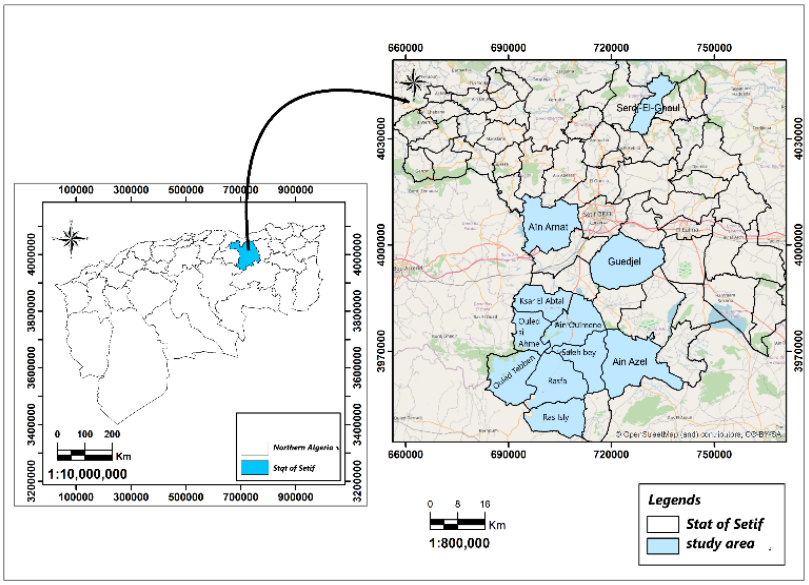

Figure 1. Geographic location of the study area

Statistical Analysis

Statistical analysis was carried out using the Chi-square test with SPSS 23 software (IBM Inc.). The data used in this study to determine the effect of altitude and type of rearing on tick infestation were based on a presence/absence criterion. Differences were considered significant at p values <0.05. Parasitological indicators were calculated according to Albert O & al (1997).

-Prevalence of tick infestation (%) =100× (the number of hosts infected with one or more individuals of a particular parasite species divided by the total number of hosts examined for that parasite species.
- Infestation intensity = the number of individuals of a particular parasite species on a single infected host, i.e. the number of ticks/number of heads infested.
-Infestation abundance = the number of individuals of a particular parasite on a single host, whether or not the host is infected, i.e. the number of ticks/number of heads examined.

Results and Discussion

After examining the cattle, five species of insect (four species of phtiriosis and one of myiasis) and seven species of tick (Ixodidae) were identified. Our results indicate that the infestation rate was 66.09% (1454 cattle were infested by at least one ectoparasite).

Using the determination keys of Price & al. (2003) for mallophagous lice and Taylor & al. (2016) for anoplurids, we identified the following species: *Bovicola bovis*, *Haematopinus quadripertusus*, *Solenopotes capillatus* and *Linognathus vituli*.We also used James' key (1947) to identify myiasis (*Hypoderma lineatum*). The cattle were infested with hard ticks, and for the determination of these parasites, we took into consideration the morphological characteristics, namely: the rostrum, the legs, and the dorsal and ventral sides of the body, as well as sexual dimorphism. The species identified are: *Rhipicephalus turanicus, Rhipicephalus bursa, Boophilus annulatus, Hyalomma marginatum Hyalomma excavatum and Hyalomma scupense and Haemaphysalis sulcata*

H. marginatum

H.excavatum

H.scupense

Rh.turanicus

Rh.bursa

Hae sulcata

Linognathus vituli

Solenopotes capillatus

Haematopinus quadripertusus

Bovicola bovis

Hypoderma lineatum

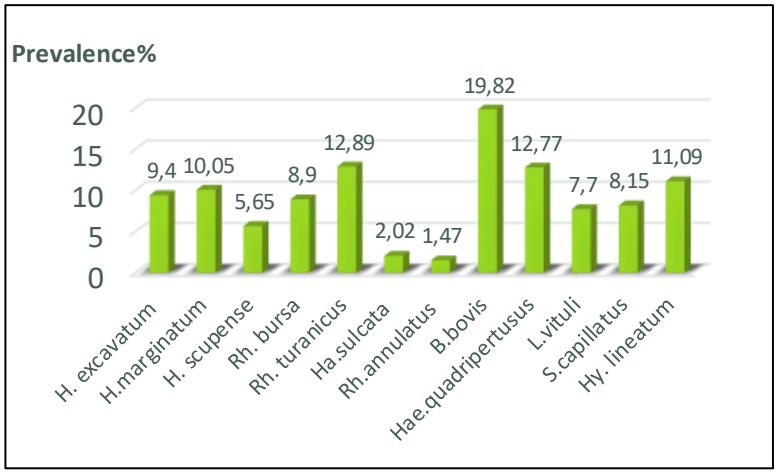

Figure 2. Prevalence of parasites

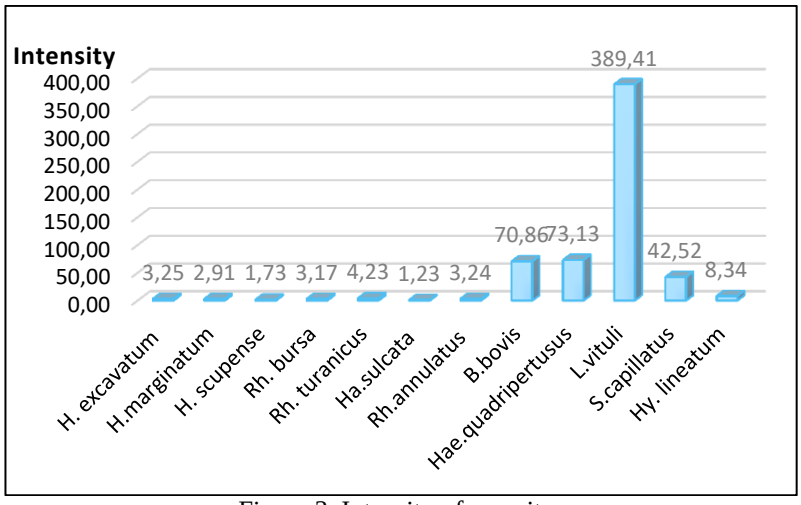

Figure 3. Intensity of parasites

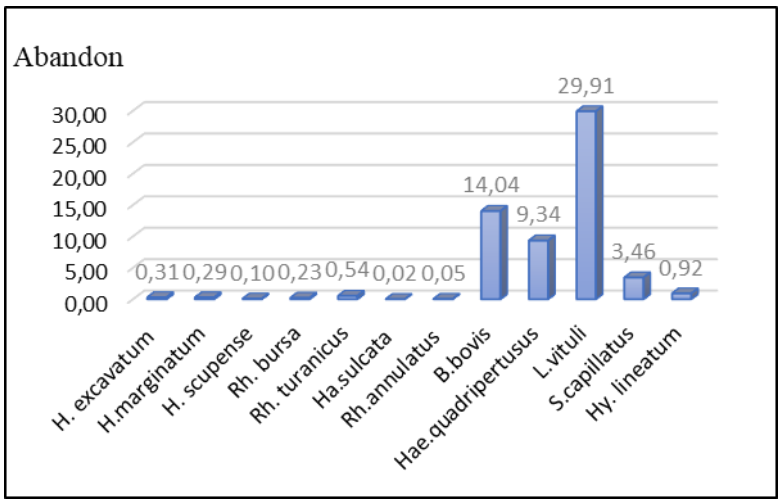

Figure 4. Prevalence of parasites

The total number of ticks collected shows a dominance of *Rhipicephalus turanicus*, followed by *Hyalomma marginatum*, then *Hyalomma excavatum* and *Rhipicephalus bursa* at the end, *Hyalomma scupense*, *Rhipicephalus (Boophilus) annulatus*, and *Haemaphysalis* sulcata have the lowest prevalence (Fig. 2). On the other hand, phthiriosis affected 783 cattle with a dominance of the mallophagan *Bovicola bovis* , followed by the anoplurans *Haematopinus quadripertusus*, *Solenopotes capillatus* , and *Linognathus vituli* . In contrast, only 244 cattle were affected by a single species myiasis (*Hypoderma lineatum*) with 2034 larvae.

Table 1. The statistical study of parasitic parameters

	Ticks		**Lice**		**myiasis**	
Factor	Prevalence %	Pv	Prevalence%	Pv	Prevalence %	Pv
Season		0.000		0		0.000
Autumn	26,63		29,4		7,5	
Summer	45,99		2,01		11,02	
Winter	0		56,5		3,7	
Spring	34,75		40,2		10,2	
Region		0.000		0,187		
Mountainous	30,6 %		31,8 %		8,1	0.297
Plaine	20 %		29,1 %		6,8	

Tab 1 shows that ectoparasites exhibit seasonal dynamics: seven species of ticks are active in spring and summer, while all four species of lice cause diseases primarily in winter. Hypodermosis, caused by *Hypoderma lineatum*, mostly occurs in spring and summer and can extend into autumn. The topographical region does not influence lice and hypodermosis occurrences but creates a favorable habitat for tick activity.

During our outings conducted over three years (2016-2018), *Rhipicephalus sp*. was the most abundant species. Moreover, its frequency was similar to that reported by Benchikh Elfegoun et al. (2013) and represented 47.6% of the collected ticks. Another study conducted by Boulkaboul (2003) showed that this genus is among the most abundant. We observed that the adults of the species *R. bursa* (vector of piroplasmosis), which is a monotropic species (Walker et al., 2014), are active in the summer while the immature stages are active in the cold season. Consequently, a peak was observed in April, where we found all three developmental stages present on multiple individuals at the same time. The species *R. turanicus*, a ditropic species, exhibits maximum activity between March and November; this species has been encountered in the semi-arid region of Mila (Benchikh Elfegoune et al., 2013), in the West (Yousfi-monod and Aeschlimann, 1996), and in (Boulkaboul, 2003), but with lower parasitic indices. This lower presence can be explained by the very high number of nymphs and larvae recorded on calves in the farms in September, which resulted in a second peak of infestation.

The species *R. annulatus (Boophilus)* (vector of bovine Babesioses) is a hydrophilic species commonly found in coastal regions with very high rainfall; its presence has been reported in the region of Tizi Ouzou (Abdul Hussain et al., 2004), in the Taher-Jijel region (Benchikh Elfegoune et al., 2007), and El Tarf (Benchikh Elfegoune et al., 2013) with very high prevalence rates of 79.96% and 77.08% respectively, compared to our result which is very low, at only 3.33%, aligning with similar findings in Mila (Benchikh Elfegoune et al., 2013) and Tiaret (Boulkaboul, 2003), both in semi-arid areas. However, this species is absent in Constantine (Benchikh Elfegoune et al., 2013). The low prevalence of this species is likely due to the dry climate effects in the region. The species *H. marginatum* and *H. excavatum* were present during the study period except during the cold months, from December to February. These two species are ditropic and have been found on cattle from March to November. Our results are similar to those of Boulkaboul (2003) and Benchikh Elfegoune et al. (2007 & 2013). Regarding the species *H. scupense*, it is an endophilic domestic tick of livestock, a two-host parasite, and other ungulates in the Maghreb region (Bouattour, 2002; Walker et al., 2014). This species transmits several pathogens, such as *Theileria annulata* (Ayadi, 2017), and various other pathogens are also transmitted by this species, including *Anaplasma phagocytophilum* and *Ehrlichia bovis* (Gharbi & Darghouth, 2014).

H. scupense is the least numerically representative species among the three belonging to this genus; however, this species was found in all three of its developmental stages. Larvae and nymphs were collected in October, and adults were present from March to September, peaking in October and absent in August. We note that the results obtained regarding the activity of this species are similar to those of Boulkaboul (2003) in Tiaret and Benchikh Elfegoune in Mila (2013). However, there is a notable difference between the prevalence obtained in our results (4.33%) and that of other authors, namely Benchikh Elfegoune et al. (2013), Boulkaboul (2003), and Laamari et al. (2012) with 13.6%, 28%, and 13.06% respectively.

Haemaphysalis sulcata is a species with a triphasic and polytropic life cycle (Estrada-Peña et al. 2004). It is characterized by a very low infestation rate (six individuals: three males and three females). Immature ticks mainly attach to reptiles such as lizards and adults to ungulates, but also to dogs (Estrada-Peña et al., 2004). Adults are active in autumn and winter from October to March. Nymphs and larvae are active in spring and summer. This low prevalence indicates occasional parasitism for this species. Our results are similar to those of Laamri et al. (2012) in Morocco.

The infestation depends on the study area: ticks are more numerous in mountainous regions. A similar observation was made during the study of the effect of altitude on tick dynamics in the Annaba region (Belabed et al., 2015) and in Constantine, where the authors Benchikh Elfegoune et al. (2019) demonstrated that altitude influences tick activity, more specifically the impact of vegetation presence and soil nature on the tick life cycle.

Conclusion

The results of our work aimed to provide an overview of the ectoparasite species of cattle during the period from January 2016 to December 2017 in the southern zone of the Wilaya of Setif, characterized by a semi-arid climate. This goal also highlighted the effect of risk factors, whether intrinsic (such as breed, sex, age) or extrinsic (such as season, type of breeding, and topography of the region), which are involved in the prevalence, abundance, and diversity of various parasites. The four species of lice exhibited seasonal activity. They caused winter infections with a peak in January. The topography of the study areas has no influence on lice infestation. It appears that the seven species of ticks have a seasonal dynamic, with activity in spring and summer. We observed that the topography of the study area created a favorable biotope for tick activity.

Recommendations

Note that the presence of these parasites on cattle is likely to create stress that could play a significant role in their well-being, production and their reproduction. In the end, we recommend further studies on the immunity status of cattle against parasitic diseases, in order to precisely determine the parasitic loads necessary for its maintenance.

Scientific Ethics Declaration

The author declares that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the author.

Acknowledgements or Notes

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Factors Influencing the Productive and Reproductive Performance of Local Rabbit Strain in Algerian

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Abstract: The objective of the present work was to study the factors influencing productive and reproductive performances of local rabbit strain in Algeria and to determine the influence of the factors (the mode of reproduction, the age of the mothers and the parity) on the number of total born rabbits, rabbits born alive and the number of weaned rabbits in a hutch of breeding of local rabbit strain. A follow-up of breeding was carried out on a total number of 60 rabbits of which 47 reproductive females inseminated naturally (31) or artificially (16) and 08 males for natural mating and 05 for artificial insemination. The herd is composed of rabbits of variable phenotype but of the same strain (different colors of the coat) of local strain. We found that: The breeding method in rabbits had a non-significant effect (P>0, 05) on the reproductive performance as well as the productive performance (slaughter weight) of the rabbits. A slight superiority was observed in the lot conducted under artificial insemination (AI) compared to the one conducted under natural reproduction. On the other hand, the reproduction mode had a significant effect on the number of weaned rabbits (P<0.01) with a remarkable superiority for the lot conducted under artificial insemination compared to the one conducted under natural reproduction (RN). The weights of the rabbits at slaughter resulting from natural or artificial insemination are very close for both reproduction modes.

Keywords: Local rabbit strain, Reproductive performances, Productive performances, Rtificial insemination.

Introduction

Rabbit farming developed in Europe from the middle Ages onwards, but has only really taken off worldwide in recent years. Its main purpose is to produce meat, and sometimes hair (Angora rabbits) or fur, or even to supply laboratories with animals for experimentation. Rabbits are considered to be the only animal species that produces the most meat in the shortest time (1.3 kg of carcass in just 4 months) and at the lowest cost, using only the fodder provided and food residues that have no immediate value for humans (Kpodekon et al., 2007).

However, rabbit meat production in Algeria remains modest compared with other countries, with an annual output of 5,000 to 19,000 tonnes (Lebas et al., 1996). A few years after this period, rabbit meat production was estimated at 27,000 tonnes per year, and could be greatly increased by using local populations in breeding (Gacem & Lebas, 2000).When we talk about rabbit farming in Algeria, we immediately imagine the image of traditional, family-run rabbit farms with small numbers of rabbits, compared with those in other sectors. Today, rabbit farming remains timid.

Rabbit farming in Algeria has developed considerably over the last few years in rural areas. Traditional rabbit farming has a long history in our country, whereas rational rabbit farming is a recent development. Our study was carried out in the Setif region, with the aim of determining the effect of various factors (such as the age of the female, parity and breeding method) on the number of total-born rabbits, the number of live-born rabbits, the number of weaned rabbits, and the slaughter weight; with the aim of proposing solutions to improve the production level of rabbit farms in our region.

- Selection and peer-review under responsibility of the Organizing Committee of the Conference

Materials and Method

Animal Materials

The experiment was carried out in a hutch belonging to a private breeder in the municipality of Hammam Guergour in the north of the wilaya of Sétif. In total, there are 60 rabbits of which 47 reproductive females (31 females for natural reproduction and 16 females for artificial insemination) and 08 males for natural mating reproduction and 05 for artificial insemination. All the rabbits used in this study were of Algerian local rabbit strain. The breeding rabbits (males and females) are kept in individual cages. They were in good health, weighed between 4 and 5.5 kg for females and between 5.5 and 6.5 kg for males, and were between 12 and 24 months old for males and between 8 and 24 months old for females. The female rabbits are reared in the same building and under the same rearing conditions as the male rabbits used.

Artificial Insemination and Natural Mating Methods

After the sperm collection operation, we visually check the volume of the ejaculate by direct reading using the graduations on the collection tube, as well as the color of the ejaculate (absence of blood and traces of gel). After dilution, the semen is placed in warm water, heated to maintain a temperature of 35°C, just prior to insemination. We suck up 0.5 ml of the diluted semen beforehand and hold the rabbit in an upright position. We lift the posterior part by the tail and then introduce the sheath to the end of the vagina where we reject the semen. After inseminating the rest of the rabbits in the same way, we inject 0.25 ml of GnRH hormone intramuscularly to induce ovulation. As for natural mating, we introduce the female at random into the male's cage, the moment mating ends with ejaculation and the rabbit throws itself back and emits the characteristic cry, we return the female to her cage.

Statistical Processing and Analysis

The variables of reproductive performance (number of total born rabbits, rabbits born alive and number of weaned rabbits) and productive performance (slaughter weight at 77 days of age) were subjected to a least squares analysis of variance using the univariate general linear model. This analysis was performed with SPSS software (version 21). The general linear model was used to test the effects of the factors (mode of reproduction and age of the dams) on the different variables. Significance and homogeneity between the different subsets (comparison test between the different means) were carried out by applying the S.N.K test (Student-Newman-Keules).

Results and Discussion

Effect of Reproduction Mode on the Productive and Reproductive Performance of Rabbits

The results in Table 1 show that the method of reproduction in rabbits had a non-significant effect (P>0.05) on reproductive performance (number of total born rabbits, number of rabbits born alive) as well as productive performance of the rabbits (slaughter weight, slaughter weight at 77 days of age).

Table 1. Effect of reproduction mod on the productive and reproductive performance of rabbits

Variables	Naturel reproduction	Artificiel insemination	Mean	Signification
Total born rabbits	8.44	8.50	8.47	0.93 (NS)
Rabbits born alive	6	7.17	6.44	0.19 (NS)
Weaned rabbits	5.74^{a}	7.13^{b}	6.26	0.04 (*)
Weight at slaughter	2035,85	2093,63	2057,35	0.21 (NS)

NS=Not significant, *Significant. The different exponents indicate significant differences (p<0.05).

A slight superiority is observed in the batch conducted by artificial insemination (AI) compared to that conducted by natural reproduction (NR) for the variables of total born rabbits (8.44 vs 8.50 in NR and AI respectively) and for the number of rabbits born alive (6 vs 7.17 in NR and AI respectively). Our results are similar to those obtained by Dekkiche et al (2024) who found that the effect of reproduction mode had no impact on the number of total rabbits born, the number of rabbits born alive and weaned rabbits. They found a

number of a total born rabbits of (7.4 vs 6.9 in NR and AI respectively) and a number of born rabbits alive of (4.55 vs 5.75 in NR and AI respectively) and a weaned rabbits of (4.55 vs 5.75 in NR and AI respectively). This difference can be explained by the type of ovulation induction method used during AI, as Boudour et al (2019) indicated that the stillbirth rate was the only parameter affected by the ovulation induction method used during AI, where rabbits treated with GnRH had a lower rate (16.53%) compared with rabbits treated with PMSG (29.4%). Therefore, the protocol and stages of ovulation induction and the practice of artificial insemination, as well as natural control, must be carefully followed to achieve good results.

Concerning the weights of the rabbits at slaughter at 77 days of age from natural reproduction or artificial insemination, the weight values are very similar for the two reproduction modes (2035.85 vs 2093.63 in AI and NR respectively). This weight performance is much better than the results reported by Gacem et al (2020), who reported a weight of 1506 g at 77 days of age in synthetic strain bred in Algeria.

On the other hand, the method of reproduction in rabbits had a significant effect on the number of rabbits weaned ($P<0.05$), with a remarkable superiority of the artificial insemination (AI) group over the natural reproduction (NR) group for the variable of rabbits weaned (5.74 vs 7.13 in NR and AI respectively). This difference can be explained as the result of mating frequency and its effect on the litter size of the female rabbits (in our study we used two collections in AI and more than two matings per male in natural reproduction).

Effect of Age and Parity of Female Rabbits on Productive and Reproductive Performance

The results given in Table 2 showed that the age of the breeding rabbits had a non-significant effect ($P>0.05$) on the reproductive performance (number of total born rabbits, number of rabbits born alive and number of weaned rabbits) as well as on the productive performance (slaughter weight at 77 days of age) of the rabbits.

Table 2. Effect of age and parity of female rabbits on the productive and reproductive performance of rabbits

Age	8 Mouths	12 Mouths	18 Mouths	24 Mouths	Mean	signification
Total born rabbits	8.06	8.59	9.25	8.80	8.47	0.72(NS)
Rabbits born alive	5.88	6.94	5	7.80	6.44	0.36(NS)
Weaned rabbits	5.71	6.76	5	7.40	6.26	0.38(NS)
Weight at slaughter	2025,2	2104,7	2005,6	2057,4	2057,3	0.37(NS)

NS=Not significant.

The age of the female rabbits is related to the parity of the female rabbits, so younger female rabbits, whose age is between puberty and 10 months are generally considered as primiparous and those older than 10 months are multiparous. In the present study, the best performances were recorded in multiparous rabbits aged 18 months for the number of total born rabbits, with an average of 9.25 , and in multiparous rabbits aged 24 months for the number of rabbits born alive and weaned (7.80 and 7.40 respectively). In terms of the weight of rabbits at the age of slaughter at 77 days, the best performances were rabbits from multiparous rabbits aged 12 months, with an average weight of at slaughter 2104.7 g. Our results presented in table 2, are similar to those published by Dekkiche et al (2024) , who found that the female had no effect on the zootechnical parameters. Fertility, number of total born rabbits and rabbits born alive. But our results differ from those reported by Belabbas et al (2023), who found a high rate of dead rabbits, in multiparous females more than nulliparous females and in lactating females more than non-lactating females.

Conclusion

In the light of the results obtained in our study, which was carried out to determine the impact of certain factors (insemination method, age of the female, parity) on the productive and reproductive performances, we can conclude that the reproduction method in rabbits had a non-significant effect on the reproductive performances (number of total born rabbits and number of live born rabbits) as well as the productive performances (weight at slaughter) of the rabbits. On the other hand, the method of reproduction had a significant effect on the number of rabbits weaned ($P<0.05$), with a remarkable superiority for the lot managed by artificial insemination compared with the lot managed by natural reproduction. In particular, we noted that the best performances were recorded in the 18-month-old multiparous female rabbits in terms of the number of total offspring born and in terms of the weight of the offspring at the age of slaughter at 77 days, while the best performances were recorded in the 12-month-old multiparous female rabbits. As a result, the parity of the mothers is considered to be one of the

most crucial factors in the success of artificial or natural insemination. The results obtained clearly show that the choice of breeding mothers and the reproduction mode used on these farms are the key to the success of breeding local strain rabbits in Algeria.

Scientific Ethics Declaration

The author declares that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the author.

Acknowledgements or Notes

* This article was presented as an oral presentation at International Conference on Veterinary, Agriculture and Life Sciences (www.icvals.net) held in Antalya/Turkey on November 14-17, 2024.

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Pervasive Microplastics and Zooplankton Abundance in Middle East Region of Java North Sea Indonesia: Spatio-Temporal of an Oceanic System

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Abstract: A significant threat to marine food webs is the ingestion of microplastics (MPs—plastic particles below 5 mm) by planktivorous organisms. Between February to August 2024, MPs samples from water coulomb and zooplankton were collected of Jepara and Rembang Region were related to Java North Sea Indonesia. This study was all related to the prey-predator linkage as a result of the size of microplastics affect plastic intake in oceanic islands' ecosystems collected from water samples. Microplastics collected from water sample then identified color, shape, size and abundance by microscope in this research. Microplastics type were analyzed used FTIR (*Fourier Transform Infrared Spectroscopy*) imaging. Zooplankton were identified and classified into their traits, size and abundance. The types of microplastics identified were fragments, fibers, films, and pellets. In water samples, black and fiber microplastics were increasingly common. Therefore, by analyzing the size and characteristics of zooplankton, comparing the abundance of microplastics and zooplankton in the marine environment, it is necessary to comprehend the probability of plastics invading the trophic web of the food chain.

Keywords: Marine, Prey-predator linkage

Introduction

The estimated annual emission of Indonesia microplastic pollution ranges from 0.48 to 1.29 million metric tons, which equates to a concentration of 30 to 960 particles per liter in Indonesian waters (Cordova et al., 2019). In 2021, Indonesia's solid waste volume reached 67.8 million metric tons, with a reported daily production of 31,200 tons of plastic waste (KLHK, 2022). Plastics have become an integral component of contemporary life, with a multitude of applications spanning various sectors, including clothing, electronics, furniture, and medical equipment. They are used extensively across the globe, are inexpensive to produce, and are available in a multitude of forms and qualities (Woods et al., 2019).

Microplastics represent a significant environmental concern, as they are among the most prevalent types of pollutants that can potentially harm living organisms and disrupt natural ecosystems. The provenance of microplastics can be attributed to anthropogenic activities that are deleterious to biota in both terrestrial and aquatic ecosystems. Microplastics have the potential to persist in the environment for extended periods, interacting with other hazardous pollutants, including heavy metals, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls (Rochman et al., 2015).

As indicated by the findings of studies conducted by Hakim et al. (2023) and Ismanto et al. (2023), the majority of microplastic research analyses in Indonesia have been conducted on the south coast of Java Island. In contrast, research on the abundance and toxicity effects of microplastics in the north coast of Java Island remains scarce. The potential for climate change to cause sea level rise on the north coast of Java represents a significant limitation to microplastic research in this location. Furthermore, the topography of northern Java is more undulating than that of southern Java, with high precipitation levels and the presence of significant tidal waves at all times. It is evident that human activities in the northern coastal areas of Java are more prevalent and have the potential to generate a greater quantity of waste than those in the southern regions. The northern coastal area is characterised by a prevalence of industrial activities, tourism, mining and fish markets (Ismanto et al., 2023). This indicates that the characteristics of microplastics are likely to differ between the northern and southern regions of Java Island.

The plains of the upstream and downstream areas of the North Coast of Java are characterized by the presence of plastic clays, which exhibit a low bearing capacity. Consequently, these regions are susceptible to subsidence, which may result in tidal flooding. The rapid industrial growth that has occurred in the inland and coastal areas of the North Coast has resulted in the deforestation of the surrounding mangrove swamp. The results of a study conducted in Jakarta and Central Java (Cordova et al., 2023) demonstrated that mangrove habitats adjacent to the coast have been contaminated with pollutants, and there is a significant presence of microplastics, along with clear indications of anthropogenic impact in the region.

In marine ecosystems, the predator-prey relationship between fish and plankton represents a significant aspect of biotic interactions, potentially influencing the structure of the ecosystem. Plankton have a function as a fundamental component of the food web. Barton *et al*. (2013) was observed that planktivorous organisms exhibited selective behaviour, demonstrating a preference for larger prey and a tendency to select prey with specific shapes and colours. As a prevalent zooplanktivorous predator in marine ecosystems, fish have been observed to ingest plastic particles inadvertently, erroneously identifying them as food sources (Boerger et al., 2010). Ory *et al*. (2017) observed a higher prevalence of blue microplastics within the gastrointestinal tract of fish (*Decapterus muroadsi*), suggesting that these plastic particles were misidentified as prey for blue copepods. It is therefore essential to analyse the size and characteristics of plankton, and to compare the abundance of microplastics and plankton in the marine environment, in order to describe the probability of plastic entering the trophic web of the food chain.

Method

Study Area

Central Java has a wide area from Rembang regency in the east to Brebes regency in the west, Central Java's northern coastline region stretches. From east to west, it was measured approximately 427 kilometers. Jepara regency was located at 110°9'48.02“ to 110°58'37.40” east longitude and 5°43'20.67“ to 6°47'25.83” south latitude. It is the northernmost region of Central Java Province. Jepara Regency is located on the East Coast of Central Java, which is bordered to the west and north by the sea. Furthermore, Rembang Regency is located at the northeastern tip of Central Java Province and is traversed by the Java North Sea, was located at the coordinates 111° 00'-111° 30' East Longitude and 6° 30'-7° 6' South Latitude. The selection of research site was determined by the anthropogenic activities of the surrounding community, the level of pollution generated, and the consumption fishing activities by the local community.

Sample Collection

The sampling sites were recorded using the global positioning system. The Indonesian National Standard for Water Quality Sampling Methods was used as a reference for point sampling. The measurement of microplastic abundance in seawater is based on the observation of microplastic particles under a microscope and the subsequent grouping of data according to the research location and sampling time. Furthermore, the calculation of microplastic abundance is conducted by accumulating all microplastic particle data observed at each location and its replication, thereby determining the total number of microplastic particles present in each research site. Seawater and plankton samples were obtained using a plankton net (Kitahara) with a mesh size of 80 microns, a mesh mouth diameter of 0.31 m,

and a length of 100 cm, connected to a 250 ml bottle. Samples were taken in 3 replicates at each station. The measurement of seawater samples was conducted using the NOAA (*National Oceanic and Atmospheric Administration*) WPO (*wet peroxide oxidation*) method. Seawater samples were filtered using a plankton net, with the filtered material transferred to a 50ml bottle. Furthermore, the sample was stored in a 140ml glass bottle and taken to the laboratory for microplastic analysis. The samples were transferred into Erlenmeyer and oven for 24 hours at a temperature range of 40-60°C. Subsequently, the sample was treated with 30% hydrogen peroxide and FeSO4 in the amount of 20ml respectively. Thus it was heated using a hot plate stirrer for 30 minutes at a temperature range of 40-60°C. The sample was filtered using a vacuum pump and filter paper. The filter paper was examined under a photomicroscope and evaluated for its physical and chemical properties.

Sample Analysis

The ISCC-NBS color system is a nomenclature for color based on a set of fundamental and derived colors. The basic colors of microplastics, as defined by the ISCC-NBS (*Inter-Society Color Council National Bureau of Standards*), are as follows: red, brown, yellow, green, blue, purple, white, black, and transparent. The color of microplastics can be either the original color of the plastic prior to degradation or the derived color resulting from degradation (Wu et al., 2018). The presence of colored microplastics is more frequently reported than that of transparent colors (Zhao et al., 2015). The information on the color of microplastics can be applied to determine the propensity of biota to ingest microplastics that closely resemble their prey (Wu et al., 2018).

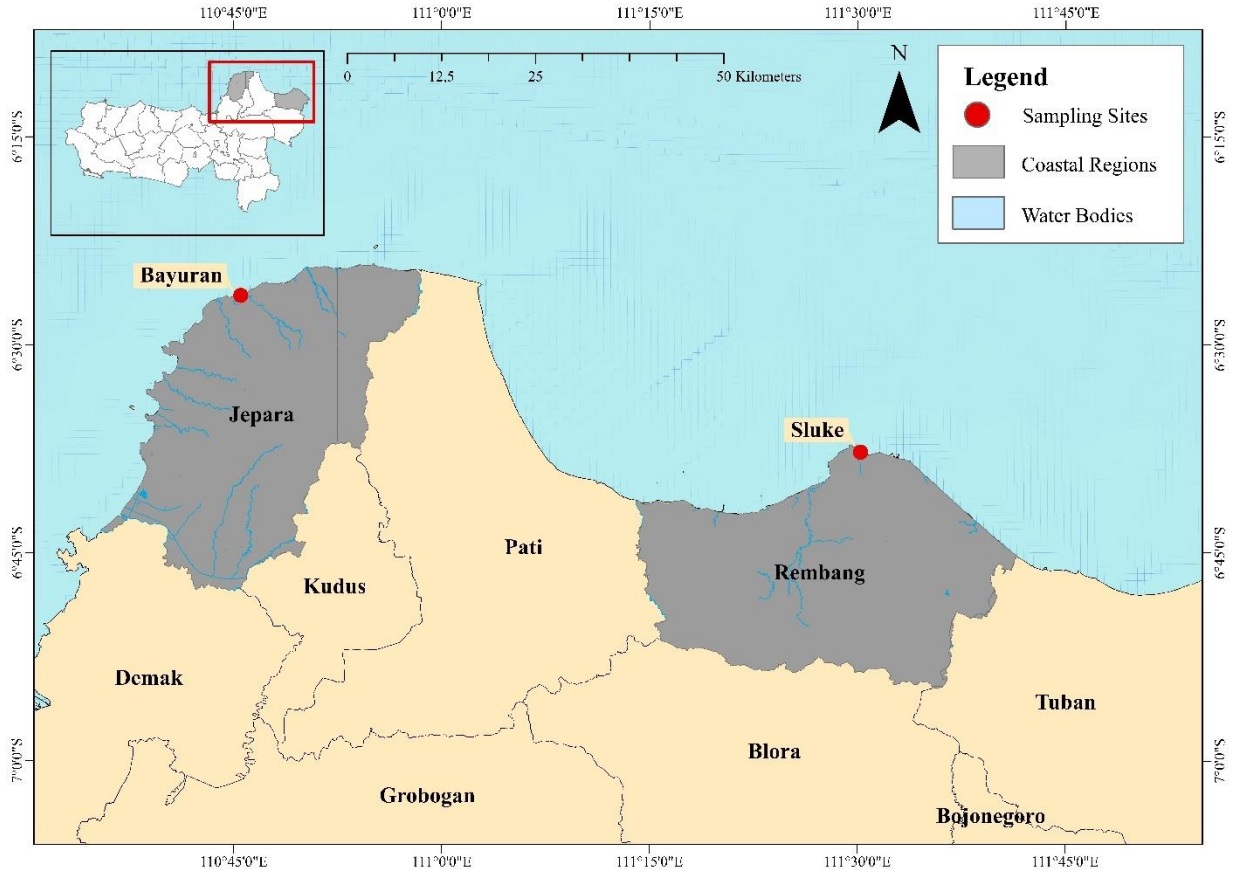

Figure 1. Location of sampling sites along the coast. Note the red dots represent points of water and plankton sampel collections.

Microplastics is defined as particles measuring less than 5 mm in size (Coppock et al., 2017). Pelleted microplastics have a reference diameter of 1 to 5 mm, with a typical diameter of 3.5 mm (Mato et al., 2001). In the case of

microplastic fragments, the size range is from 1 μm to 2 mm. The chemical characterization of microplastics can be accomplished through the implementation of a *Fourier Transform Infrared Spectroscopy*, which is designed to determine the specific type of microplastic polymer based on the wavelength that is reflected by the polymer.

The sample fraction method (2.5 ml per 150 ml sample) was applied to calculate the number of zooplankton individuals present in each sample bottle. Subsequently, the samples were transferred to a *Bogorov counting tray* and observed under a microscope with a magnification range of 4-40x. In order to identify the plankton taxonomic groups present in the samples, the reference provided by Nontji (2000) was used as a guide.

Contamination Control

To guarantee the integrity of the results, a series of measures were implemented to maintain a hygienic and uncontaminated working environment, both in the field and laboratory. Prior to the commencement of any experimental procedures, all apparatus, glassware, and work areas were meticulously sanitized using distilled water. It is preferred to use non-plastic research tools and materials. The solutions were filtered using GF/C Whatman 1,2 μm glass microfiber filters. Prior to use, the filter papers and petri dishes were examined under a photomicroscope for magnification. Additionally, procedural blanks (three in total) were conducted concurrently with the sample processing to ensure the absence of cross-contamination. The use of cotton lab coats and nitrile gloves was also implemented to further prevent contamination.

Data Analysis

The zooplankton composition was determined by classification into the following 7 taxonomic groups: Copepoda, Keratella, Chatognatha, Asplanchna, Crustaceans larvae, Siphonophora and Gelatinous zooplankton (others). For the purposes of the present analysis, only groups with an overall abundance proportion exceeding 1% were considered. Subsequently, the length of the longest body dimension for the zooplankton specimens was determined using a photomicroscope. The data has been organised in tables and graphs.

The MP samples were collected and classified according to their type and color. MPs are available in a variety of forms, including pellets, film, fiber or filament, and fragments. Meanwhile, MP types are identified according to Calcutt et al. (2018) by color (translucent, black, blue, red, green, etc.). The MPs were identified using FTIR transmission measurements based on the materials found through library search. The sample's FTIR reading is represented by peaks with different absorption ratings. A quality index of more than 0.7 (70%) is permitted, and the absorption score shows the comparability of particular plastic elements (Comnea-Stancu et al., 2017). The lowest possible score in this examination, as determined by comparison with the reference from the FTIR library for the first characterization study, was set at 60%. Before filtering MPs, a blank study utilizing filter paper was also carried out to ascertain the constituent materials and structure of the original filter paper. The procedure is used to remove bias from FTIR and SEM analysis.

Results and Discussion

Microplastics and Zooplankton Characteristics

A total of 162 particles in all samples were found and examined. All of the samples contained microplastics, with 36.8% of the particles made up of fibers (Fig. 2A). Nevertheless, various forms of plastic particles, such as fragments were present in 13.7%, films in 22.2% and pellets were present in 27.4% of the samples (Fig.2A). The most appearance color of microplastics were in black color (Fig.2B). The majority of the fibers were black, whereas the pellets and fragments were primarily (transculent and black) in color (Fig. 2B). According to Fig. 2B, the most favored color overall was black (56.8%), which was followed by translucent (33.1%), brown (3.4%), blue and orange (2.5%) respectively. There are three class sizes were categorized in each sample type. Their classes were classified based on their size found in microplastics. Furthermore, small size (1000–5000 μm) of pellets and medium size (100-1000 μm) of fibers, accounting for 24.57% of all particles, were frequently discovered. Thus, films were

commonly found in the medium size classes (100–1000 μm), followed by fragments were mostly represented in the medium size classes (100–1000 μm) (Fig. 2D).

In the zooplankton analysis, a total 105 zooplankton found in water sample. There are Asplanchna and Crustaceans Larvae were the most common appearance (representing 33.63% and 22.12% of the total sample respectively), followed by Gelatinous Zooplankton (18.58%) and Copepoda (17.69%), they represented over 85% of the total zooplankton community (Fig. 5). Asplanchna was the most favoured taxa in terms of size classes, in medium size classes (100–1000 μm). Furthermore both Chatognatha and Siphonophora were recorded only 3.39% of total samples.Other groups were recorded in low abundances (< 1 % of the total) was Keratella that only 0.84% in total samples.

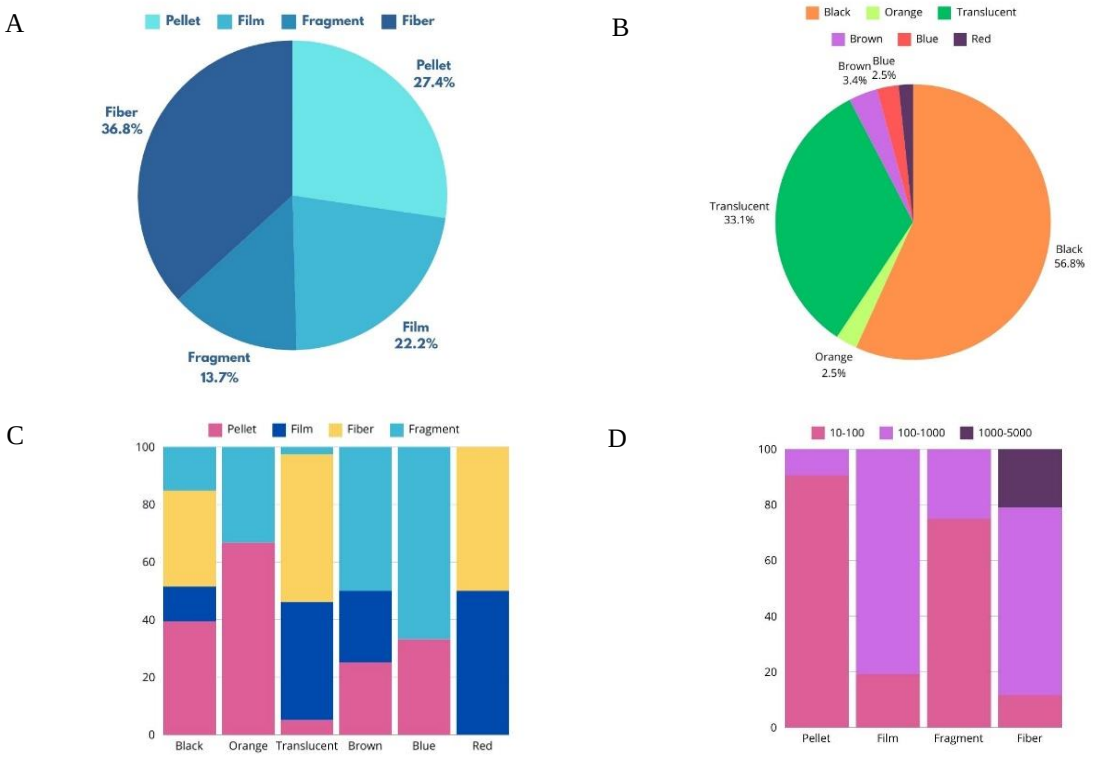

Figure 2. Characteristics of Microplastics found in the water samples: proportion of microplastic type categories (A), proportion of microplastic colors type (B), proportion of microplastics color composition(C) and proportion of size categories for types of microplastic (D).

The abundance of zooplankton and microplastics found was 0.33 individuals/m3 and 0.84 items/m, respectively. The seasonal factors may occur and depend for their abundant. In this research, sampling was conducted in the dry season. Jepara and Rembang were located in tropical region. The temperature showed 30^{o}C and low precipitation. Furthermore, the abundance of microplastics was not correlated with average precipitation, whereas a positive correlation was identified between precipitation and zooplankton abundance (Sambolino et al, 2022).

The prevalence of microplastics and zooplankton in seawater samples suggests a correlation between marine pollutants and the dietary habits of marine life. Such a conclusion may be drawn based on the size, colour, and groupings that are likely to be selected by natural processes. This phenomenon should be considered in relation to the potential impact of plastic pollution in open-ocean ecosystems. The coincidence in the variation of microplastics (MPs) and zooplankton traits increases the likelihood of MPs ingestion by planktivorous organisms. Moreover, it has been predicted that moderate to high levels of water turbulence will increase the ingestion rates of prey due to an increased frequency of particle contacts, thereby further increasing the possibility of ingestion (Botterell et al., 2019; Saiz et al., 2003).

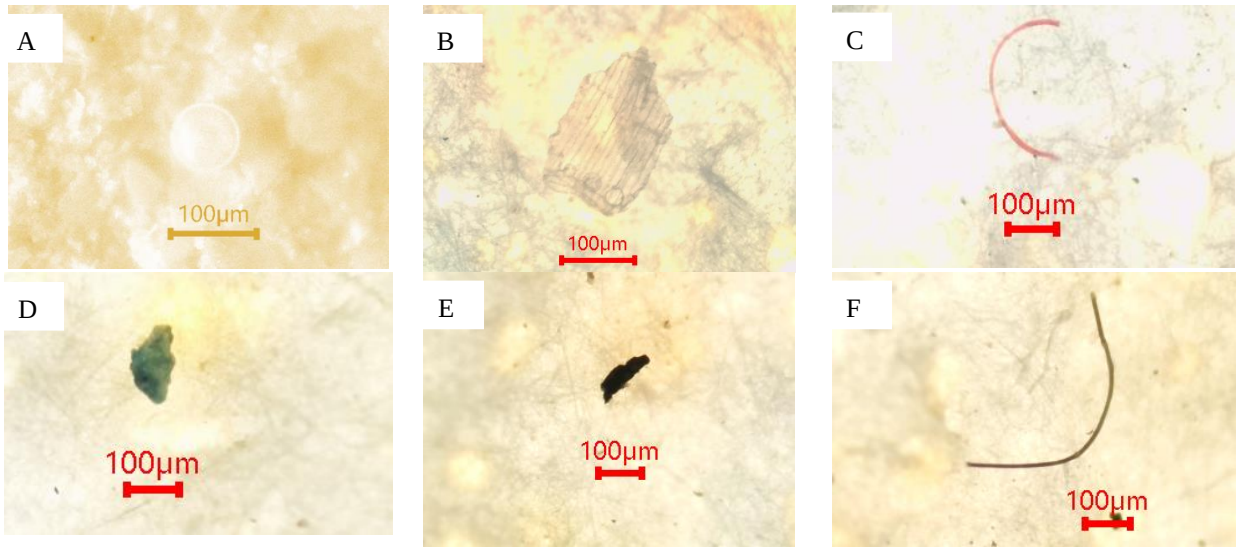

Figure 3. Microplastic identification using a photomicroscope: (a) transparant pellet, (b) transparant film, (c) red filament (d) blue film, (e) black fragment, and (f) black filament. *) (a), (b) with 10x magnification (c), (d), (e), (f) with 4x magnification.

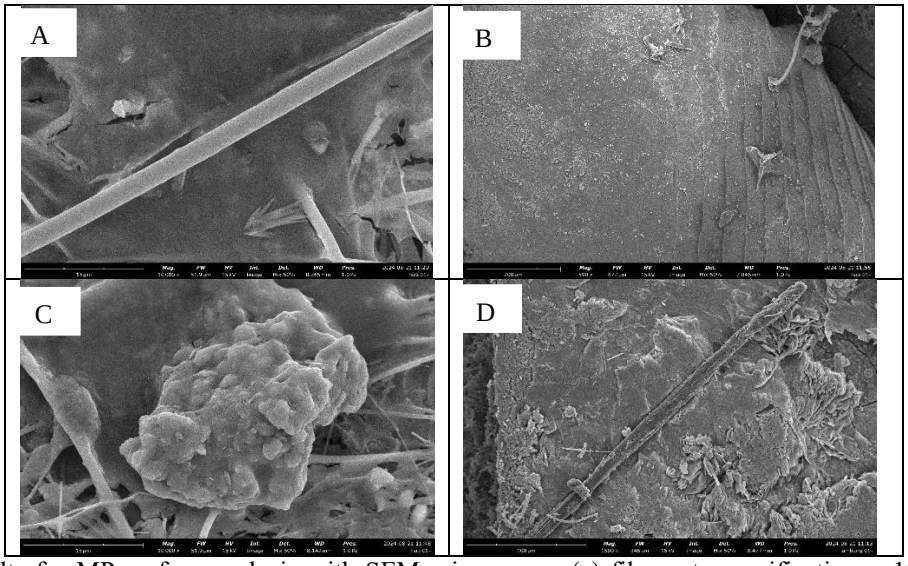

Figure 4. Results for MP surface analysis with SEM microscope: (a) filament magnification = 10.000×; (b) film magnification = 590×; (c) irregular fragment magnification = 10.000×; (d) filament magnification = 2000×.

A total of microplastics was identified type of polymers with Fourier Transform Infrared Spectroscopy (FTIR) shimadzu Irtracer 100. A total of seven types of polymer were identified: LDPE, Latex, Polystirena, Nylon Polyamides, Polypropilena and PET. The properties of Nylon Polyamides polymer are notable for their strength, durability, flexibility, elasticity, and lack of residual monomers. Nylon polymers are utilised in the production of clothing and rope products, as well as in the manufacture of toothbrush bristles. Furthermore Polypropilena is more robust than HDPE, with superior tensile durability and resistance to cracking. The properties of propylene permit the use of this type of polymer in the production of drinking bottles and food containers.

The application of Polystirena polymers is widely found in household appliances, insulators, and food wrapping materials. The properties of LDPE that make it particularly suitable for a number of applications are its weather resistance, strength, chemical resistance, crack resistance, and recyclability. LDPE can be identified as a constituent

of a number of commonly used consumer items, including shopping and newspaper bags, packaging for frozen food items, milk carton laminates, plastic wrap and laundry plastic. Latex is a polymer formed through the copolymerisation of several monomers, such as vinyl acetate monomer (VAM) and methacrylic monomer (MMA). The characteristics of latex polymers include resistance to oxidative degradation, ease of production, elasticity, and water resistance. Plastic bottles typically comprise PET polymers, which are used in a variety of packaging applications. These include cooking oil, mineral water, and chili sauce bottles, as well as cosmetic and drug packaging.

Microplastics profiling are necessary to describe the type of polymer. It's prior to conduct in each type of microplastics (fragment, fiber, film and pellet) that would be support the antropogenic activity by surrounding. To support the visualization of microplastics, projections were taken with visual SEM. This aims to determine the morphological structure of microplastics at the size of the nano structure (Fig.4).

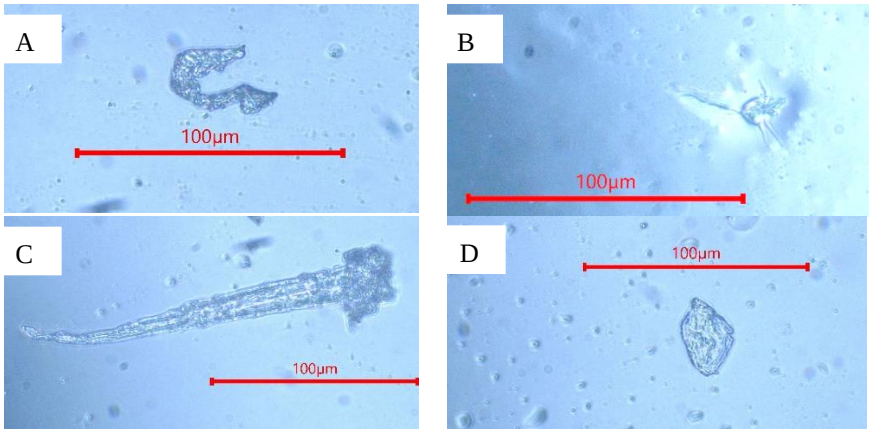

Figure 5. Zooplankton mapping based on their traits and morphology: (a) Copepoda; (b) Keratella; (c) Chaetognatha; (d) Asplancna *) with magnification = 40× respectively.

Conclusion

In the present study, we highlighted the importance of the influence of the occurrence of microplastics and zooplankton in a environment such as a deep ocean system. The most appearance color of microplastics were in fiber type and black color. Because of microplastics was found in black color, the degradation of plastics was occurred in oceanic system. Both microplastic and zooplankton size uptake occurred in the range of 100-1000µm. This provides of the prey-predatory relationship within the food chain, which is correlated with the uptake of microplastics in nature, both in their intact and degraded forms. Microplastics, acting as pollutants, are ingested by planktivorous organisms, which perceive them as food. Barton *et al*. (2013) was observed that planktivorous organisms exhibited selective behaviour, demonstrating a preference for larger prey and a tendency to select prey with specific shapes and colours.

Recommendations

In order to compare the quantity of zooplankton and microplastics in tropical oceans during the wet and dry seasons, this article suggests using seasonal fluctuations to measure these quantities.

Scientific Ethics Declaration

The author declares that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the author.

* The authors declare that none of the work reported in this study could have been influenced by any known competing financial interests or personal relationships.

Acknowledgements or Notes

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Exploring the Representation of Environmental Issues in an EFL Textbook for Secondary School: A Multimodal Analysis

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Abstract: This study intends to explore how environmental issues are represented in an EFL textbook for secondary school. The study obtained the data from the English for Nusantara book for 8th grade. By administering a qualitative textual analysis with a multimodal approach, the study analyzed verbal and visual complementarity of three worksheets. It utilized Ideational-intersemiotic Complementarity (Royce, 1998). The findings show that the verbal and visual texts represent flood-related environmental issue. There are three main points of flood-related environmental issues – the causes, the effects, and the solutions. Verbal and visual texts of the selected worksheets are completed each other. Hence, encouraging students' comprehension of the module, especially related to environmental issues, could be expanded by identifying both texts as a single whole.

Keywords: Environmental issues, Multimodal analysis, Environmental education

Introduction

Currently, people are discussing environmental issues extensively as they are concerned about environmental conditions that have reached an alarming level. Given the increasingly worrying environmental conditions, there are several fields that give their attention to environmental issues, one of them is ecocriticism, the study that academically popularized by an association arranged assemblies and published a journal about environmental education, the Association for the Study of Literature and the Environment (ASLE) (Romadhon, 2011). Ecocriticism has been defined as "the study of the relationship between literature and physical environment" (Glotfelty, 1996 p. xviii) or nature (Mishra, 2017). In addition, Glotfelty (1996) asserts that ecocriticism usually focuses on the connection between human culture and the physical world in literature. It can be assumed that ecocriticism is intended to see how people and nature are affected by each other in literature (Apriati, 2013). The purpose of discussing the interconnection between people and nature is to protect nature itself (Mirsha, 2017). It is in line with the statement of Saiful (2020) who asserts that ecocriticism study aims to instill a sense of love for nature to individuals' minds.

The way people perceive the environment are divided into two views, anthropocentric and ecocentric. Anthropocentric has been deemed as the view that locates people as the centre of the world and the environment as essential items that humans need (Casas & Burgess, 2012; Kopnina & Cocis, 2017). On the contrary, ecocentric has been considered as the view that positions people as not the centre of the world and the environment as items that have their own inherent values (Casas & Burgess, 2012; Kopnina & Cocis, 2017). In ecocriticism, the view that wants to be highlighted more is ecocentric as it promotes the notion that people and the environment are equally important in the world (Cocks & Simpson, 2015; Sardari, 2020). Nevertheless, this study does not focus on but acknowledges the current debate. The stance of this study is with Garrard (2004) who asserts that ecocriticism can help with defining, investigating, and addressing problems of the environment.

In addition, in the field of linguistics, the field that gives the attention to environmental issues is ecolinguistics. Ecolinguistics appeared in the 1990s as a new linguistics research that focuses on language and ecology (Zahoor & Janjua, 2019). Furthermore, ecolinguistics has been defined as the study that discusses the interaction

between language (linguistics) and nature (ecology in biological studies) (Crystal, 2008). According to Gul et al., (2021), ecolinguistics offers a framework to investigate discourses that discuss environmental issues along with its solutions. In such discourses, ecolinguistics tries to examine which belongs to ecological destruction pattern and which belongs to ecological assistance pattern (Faramarzi & Janfeshan, 2021). Otherwise stated, ecolinguistics can be considered as the study to see how language performs as a shaper, a nurturer, an influencer or a destroyer of relationship between human beings, living conditions, and the environment (Yuniawan et al., 2017).

Looking at the significance of ecocriticism, and ecolinguistics, it is considered really essential to incorporate environmental issues in English language teaching (ELT). It is in line with the statement of Alter (2015) who asserts that the teaching of English is concerned to not only improve students' language proficiency but also to familiarize them with issues around the world, including environmental issues. Furthermore, as the prospective English teachers have been introduced with "exploring literature" and "introduction to literature" courses in their English Education Departments, they are expected to distribute and infuse the sense of loving the environment in the English classroom (Saiful, 2020).

Likewise, in the context of teaching English as a foreign language (TEFL), apart from focusing on enhancing students' language skills, the teaching should also concentrate on expanding their critical thinking skills that can be beneficial to introduce environmental issues (Nkwetisama, 2011). Critical thinking skills in TEFL can help students perceive environmental issues and take actions as the solution. In addition, it is considered EFL teachers' function to help students discuss environmental global problems and lead them to find its solutions (Al-Jamal et al., 2014). As the leaders of the teaching and more knowledgeable others, EFL teachers have the opportunity to relate students' personal experiences and knowledge regarding environmental issues with the English classroom. Therefore, EFL teachers should be able to teach language skills, introduce environmental issues and generate them to solve the issues concurrently.

Furthermore, Brown (1991 as cited in Zahoor et al., 2019) asserts that the EFL curriculum should include ecological themes that discuss environmental issues as problems of the globe. The incorporation of ecological themes in EFL curricula has been deemed as a way to increase students' consciousness towards the global environmental crises. In Indonesia, education also has a focus on the issues of the environment, as seen in the 2013 curriculum that places environmental themes on integrated thematic lessons for the primary school level. Furthermore, the involvement of environmental issues in the Indonesian education system can also be seen in the new curriculum, Kurikulum Merdeka, projects students to be more concerned about the issues around them by reflection on Profil Pelajar Pancasila (Kurikulum Merdeka – Pusat Kurikulum Dan Pembelajaran, 2022).

Hence, the TEFL in Indonesia, especially at primary school level, will probably involve environmental themes. In addition, introducing environmental issues through TEFL for young learners is deemed very important. The younger the students are introduced to environmental issues, the better the results. It is in accordance with the statement of Kahyaoglu and Kiriktaş (2013) who assert that the concerns and attitudes shaped in pre-school and primary school levels frame the future behaviours, including the behaviours towards the environment.

Moreover, it also allows the students to gain new skills on environmental protection at a young age. For instance, through the use of language, students can learn how to plant as the actualization of their environmental protection (Dahniar et al., 2019). In addition, by allowing the students to express themselves in language about environmental issues, they can also have the opportunity to make language choices that can enhance their language development (Castillo & Rojas 2014). Therefore, it is also important to incorporate the topic of environmental issues in EFL materials.

EFL materials have an important role in the TEFL. Apart from leading students to achieve language proficiency, EFL materials are believed to be able to direct students to build positive behaviours in their life. It is because EFL materials supposedly can have unconscious affects towards students (Emilia et al., 2017). Likewise, Al-Jamal et al. (2014) asserts that the content of EFL materials can influence students' perceptions since language is considered as a medium of thinking and describing the world to themselves. Furthermore, they assert that to be powerful EFL materials, the materials should focus not only on generating students to communicate in English but also on expanding their environmental awareness. Therefore, it is considered essential to have a focus on incorporating environmental content of EFL materials in order to increase students' consciousness towards environmental issues unconsciously.

In selecting EFL materials for young learners that involve commander environmental content, the teachers can rely on the purpose of ecocritical teaching, which is embedding the perception of caring for the environment in

students' minds and building self-actualization of humankind towards nature in the classroom (Saiful, 2020). Such materials can be occupied from many sources. Veselinovska and Kirova (2013) proposed four materials that can be employed. First, there are articles, audios, or videos that have environmental issues content, from many resources like newspapers, TV shows, internet and so forth. The second is the content of English songs with environmental themes. The third is the discussion of environmental issues that occur around students (local level). The last is the results of surveys regarding environmental issues.

The selected material materials can be investigated further in terms of how the environmental issues are represented. In doing so, by relaying to the nature of ecocriticism, Gaard (2008) has formulated three basic questions as the guideline to expound how children's texts of the environment are written. The first one is "how does the text address the ontological question, 'who am I?' Is the human self-identity constructed in relation or in opposition to nature, animals, and diverse human cultures/identities?". This set of questions emphasizes the space of young learners and the environment that the texts try to build. The second is "how does the narrative define the ecojustice problem? Does the narrative conclusion offer an appropriate strategy for responding to the problem?". This set of questions highlights the roles of young learners in the context of the environmental issues presented in the texts. The third is "what kind of agency does the text recognize in nature? Is nature an object to be saved by the heroic child actor? Is nature a damsel in distress, an all-sacrificing mother, or does nature have its own subjectivity and agency?". This set of questions underlines the places disposed to the environment in the texts.

There are several researchers who have underlined the matter of integrating environmental issues in materials that can be used in TEFL. (See Apriati, 2013; Adugna, 2015; Zahoor & Janjua, 2019). Adugna (2015) explored ecological subjectivities in more than 50 Ethiopian children's narratives. He discovered that most of the narratives implied an anthropocentric view. In the same way, Zahoor and Janjua (2019) investigated how Transitivity patterns used in Pakistani English textbooks for primary school represents nature. They also found that the textbooks were more in the anthropocentric view. On the other hand, Apriati (2013) analysed the relationship between human and nature in a children's book through the perspective of ecocriticism. She found that the book presented both good and bad relationships between people and nature.

Despite myriad studies investigating the existence of environmental issues in EFL materials, most of the studies emphasized this matter in verbal texts of the materials and less attention paid to the visual texts. According to Damayanti (2014), visual texts are not considered as just illustrations to go with verbal texts since meaning construction is multimodal. Hence, to be able to fill in the gap, by using a qualitative textual analysis with multimodal approach, this study explores how environmental issues are depicted in verbal and visual texts of an EFL textbook for secondary school.

Ideational Intersemiotic Complementarity

This study uses the framework of Intersemiotic Complementarity formulated by Royce (1998) as the main framework of analysis of environmental issues in the verbal and visual texts of EFL textbook. This framework is the expansion of Halliday's SFL theory that has three constituents of metafunction (Damayanti & Febrianti, 2020). In the interest of space, this study will only focus on ideational metafunction.

In the analysis of ideational intersemiotic complementarity, the verbal items in Transitivity analysis are collated with visual message elements (VMEs), that can be gained when analysing Visual Grammar, to determine its similar or different meanings. In determining its relationships, Royce (1998) recommends the utilization of cohesion analysis in a text as proposed by Halliday and Hasan. There are Repetition (R), Synonymy (S), Antonymy (A), Hyponymy (H), Meronymy (M), and Collocation (C). This Royce's (1998) ideational intersemiotic complementarity can be seen in Table 1.

Method

Research Design

This study aimed to explore how environmental issues are represented in an EFL textbook for secondary school. A qualitative design was used in the study to generate the interpretations of the data (Creswell, 2014). In this study, the data interpretations included verbal and visual texts and their relations in an EFL textbook for secondary school. Particularly, this study employed textual analysis with a multimodal approach.

Table 1 Royce's (1998) ideational intersemiotic complementarity

Verbal Meanings	**Intersemiotic Complementarity**	**Visual Meanings**
Lexical elements which relate to the visual meanings These lexical items arise according to: *Identification (Participants):* who or what is involved in any activity or process? *Activity (Processes):* what action is taking place, events, states, type of behaviour? *Circumstances:* where, who with, and by what means are the activities being carried out? *Attributes:* what are the qualities and characteristics of the participants?	Various lexico-semantic ways of relating the experiential and logical content or subject matter represented or projected in both verbal and visual modes through the intersemiotic sense of: *Repetition:* identical experiential meaning *Synonymy:* the same or similar experiential meaning *Antonymy:* opposite experiential meaning *Meronymy:* the relation between the part and whole of something *Hyponymy:* the relation between a general class of something and its sub-classes *Collocation:* an expectancy or high probability to co-occur in a field or subject area	Variations occur according to the coding orientation. In the naturalistic coding we can look at: *Identification:* who or what the represented participants (actor, recipient, goal)? Who or what are they interacting with? Are the participants interacting? (vectors) *Activity (Process):* what action is taking place, events, states, type of behaviour (gestures, facial expressions, stance, physical moves)? *Circumstances:* where, who with, and by what means are the activities being carried out (setting, means, accompaniment)? *Attributes:* what are the qualities and characteristics of the participants?

(Royce, 1998:31)

This method allows the investigation of how verbal and visual modes as well as their relations in the content of language textbooks inscribe and convey concepts "about the world, treating meaning as representation" (Weninger, 2020:1). In addition, multimodal textual analysis has been considered as a tool that examines the singularity and the intricacy of a phenomenon while working out an issue and serving particular realistic examples (Lin, 2012). Therefore, a multimodal textual analysis in this study is used to explore how both visual and verbal texts as well as their relations construe the representation of environment issues in an EFL module for secondary school.

Research Context

This study involved an EFL textbook for 8th graders entitled English for Nusantara published by the Indonesian Ministry of Education, Culture, Research and Technology (Damayanti et al., 2022). The textbook becomes the primary resource that can be used by Indonesian students and the guidance for Indonesian teachers to conduct the English teaching and learning process based on the Emancipated Curriculum. Therefore, the textbook provides both verbal and visual texts to facilitate students in learning and mastering the six language skills that have to be learnt and achieved by students, based on the curriculum, including viewing and presenting skills. The book consists of five chapters. Each chapter also discusses different topics. In the interest of space, this study focuses on analyzing the fourth chapter of the book - No Littering. The fourth chapter focuses on recount texts and environmental issues. It provides several texts that tell the book characters' past experiences, both in house and school contexts.

Furthermore, this study only chooses three worksheets in the chapter. The chosen worksheets provide both verbal and visual texts. Worksheet 4.2 asks students to complete a story about the trash in a river. Worksheet 4.4 instructs students to guess what activities that would happen during a flood by circling pictures. All the verbs in the worksheet are made past as the focus of the chapter is recount text. Similarly, worksheet 4.5 asks students to complete a recount text that is in the form of a comic strip. In the text, the characters of the book discuss a flood that happened previously.

Data Analysis

In analyzing the data, this study followed three steps, which are verbal and visual meanings identification, ideational-intersemiotic complementarity identification, and description. The flow of the analysis can be seen in Figure 1.

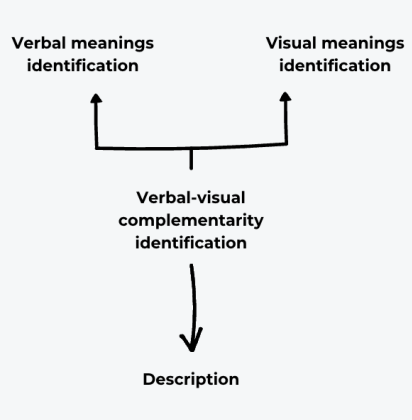

Figure 1. Data analysis

The first step of the analysis is verbal and visual meanings identification. Verbally, each clause was identified by using the Transitivity system of functional grammar developed by Halliday and Mathiessen (2014) to examine its Transitivity features, which comprise Processes, Participants, and Circumstances. Visually, the data were identified by using the framework of the 'Visual Grammar' developed by Kress and van Leeuwen (2006) to investigate the ways visual texts represent experiences through the represented Processes, Participants, and Circumstances. The second step of the analysis is ideational-intersemiotic complementarity identification. By utilizing the framework of Ideational Intersemiotic Complementarity developed by Royce (1998), the data were analyzed to determine the relationships between verbal and visual texts in making meaning. In the analysis, the verbal items in Transitivity analysis were collated with visual message elements (VMEs) gained when analyzing the visual texts, to determine its similar or different meanings. The relationships were, then, identified in Royce's (1998) work as cohesive mechanism embodied in Repetition (R), Synonymy (S), Antonymy (A), Hyponymy (H), Meronymy (M), and Collocation (C) between visual and verbal texts on panel. The third step of the analysis description. In this step, the findings found in the first and second steps were mapped and correlated with the previous studies and theories related to environmental issues. The study also provides several pedagogical implications from the findings.

Findings and Discussion

Based on the analysis, environmental issues are represented in the textbook. Specifically, in the selected worksheets, the issue that is raised is flood. As flood is one of the issues that commonly happen in Indonesia, the topic is deemed suitable for Indonesian students. How the topic of flood is represented in verbal and visual texts of the textbook can be seen in following sub-chapters.

Figure 2. Worksheet 4.2 Panel 3

The Causes of the Represented Environmental Issues

The findings show that there is a space between the environment and students that the texts try to build. It can be seen in the panels of Worksheet 4.2 where the characters of the story perceive the river as a dirty space (Figure 2).

Looking at the verbal text, the boy is questioning whether in the past the river was also dirty. The verbal text is completed each other by the relation of synonymy. It can be seen from a vector from the boy's sight to the trash as phenomenon. The trash in the river indicates that the river is dirty. The following question also indicate a space between the character and the river. Looking at the material process, he is questioning whether in the past the river can be swum. The answer of the question can be seen in the following panel.

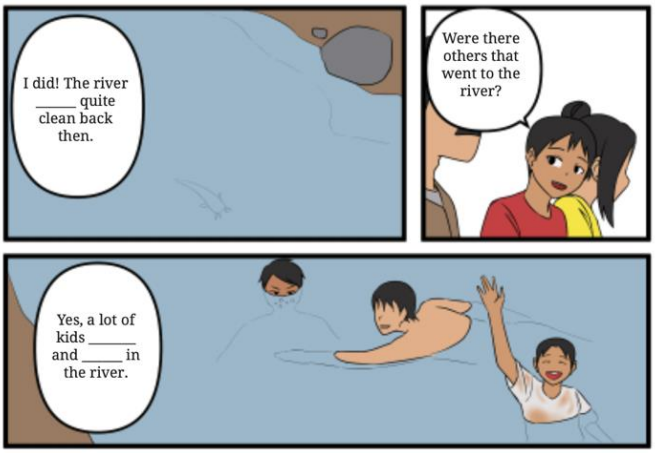

Figure 3. Worksheet 4.2 Panel 4, 5 and 6

The verbal texts show that there are several material processes indicating that in the past, the father used to play and swim in the river. It is completed in the visual text by the relation of repetition and synonymy. It can be seen in one of the panels, the represented participants are swimming and playing in the river. Both verbal and visual texts tell us that in the past, the characters have a good relationship with the river. However, it has changed as the river becomes dirty. The verbal and visual texts portray human as the main actor of this issue. It can be seen in the following panels.

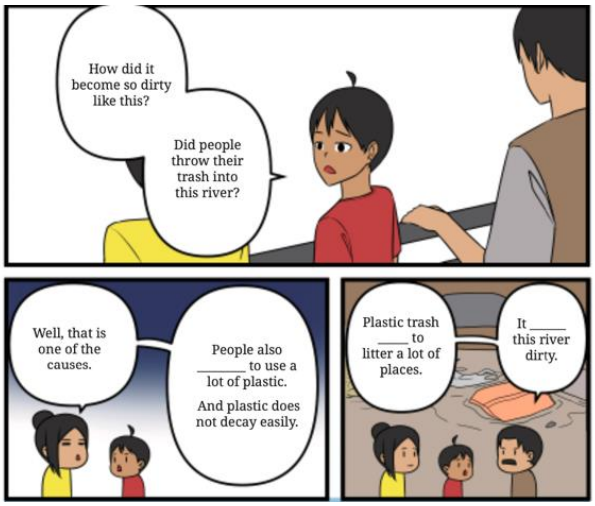

Figure 3. Worksheet 4.2 Panel 4, 5 and 6

Looking at the verbal texts, there is a material process where the boy is asking whether people are the ones that throw the trash into the river. The answer is yes. It can be seen from the following material process where the mother says that people are the actors that use a lot of plastic as the goals. However, how people use and throw the trash are not represented in the visual texts. Representing the ones who are responsible to the damage of the environment is considered important (Ibrahim & Damayanti, 2024). It can help students aware that using a lot of plastics and throwing them to the river can make the river dirty.

The Effects of the Represented Environmental Issue

Both verbal and visual texts of the selected worksheet also represent the effects of what is being done by humans to the environment. In the selected worksheets, the effect is flood. For example, it can be seen in the following panel.

Figure 4. Worksheet 4.4 Panel 3

Verbally, it is found that there is an Intensive Relational process where "almost underwater" is the Attribute. In the meantime, looking at the visual text, there is a locative circumstance as the neighborhood is slightly covered by the flood. According to the English Oxford Dictionary (2024), almost has been defined as "very nearly but not quite". Hence, the word almost is completely visually by the relation of Antonymy. It is because almost has the opposite meaning of slightly in visual text. Antonymy complementarity might cause confusion among students. Students might misinterpret the real meaning of the verbal text if they see the visual text that has the opposite meaning. Hence, there is a need for teachers to always check the suitability of verbal and visual meanings in students' learning material and tell students the true meaning of the texts.

Figure 5. Worksheet 4.4 Panel 1

The flood can also be recognized in the above figure. As can be seen in the figure, the verbal text is made in the form of Material process where "some children" as the Actors do a process of doing "played". Turning to the visual elements, it can be seen that there are several Actions. First, there is Unidirectional Transactional Action. It can be inferred from a vector emanating from two children that are sitting on a rubber tire on water. Second, there is Non-transactional Action where there is a boy who is soaking in water. Third, there is another Non-

transactional Action where there are three children standing in water with raised hands in the sky. Those Actions indicate that there are several children who are doing something like riding a rubber boat, soaking, and raising their hands in the sky in the middle of the flood.

In terms of complementarity, the verbal text is completed visually by the relation of Hyponymy. It is because the process of "played" is visualized by the subclasses of "playing" like riding a rubber boat, soaking, and raising their hands in the sky. This Hyponymy complementarity provides a space for teachers to discuss with their students about what activities that the characters of the book are playing. Hence, this type of complementarity can also increase students' vocabulary.

In the meantime, regarding its circumstances, verbal text contains a Location circumstance where it is mentioned "in the rain happily during the flood". Looking at the visual analysis, the Locative Circumstance is portrayed by the blue sky, indicating that there is a bright day. Moreover, there is also no raindrop seen in the visual text. Therefore, "the rain" in verbal text is completed by the relation of Antonymy where there is no rain in the visual text.

The Solutions to the Represented Environmental Issues

Besides portraying the causes and the effects of flood, the selected worksheets also represent the solutions to the flood. The portrayed solutions can be followed by students. In other words, they are given an agency of a role to save the environment. For example, it can be seen in the following panel.

Figure 6. Worksheet 4.5 Panel 8

Looking at the verbal text, the mother asks children to be the Actors of material process in which trash is the goal. The sentence is very important for the students as they can reduce one of the flood causes. Nevertheless, the verbal text is not completed by the visual texts. The visual text only portrays a plastic bag as the Goal of an action. Therefore, teachers have to show students, from the visual text, which thing that can usually be a trash. Better yet, teachers can ask students to tell how to reuse, reduce, and recycle the trash (Figure 7).

Another example of the solution to flood can be seen in Figure 7. Looking at the verbal analysis, the verbal text is in the form of Material processes where "the rescuers" are the Actors who took out "the trash" as the goal. The verbal text also includes a location circumstance mentioning "the gutters". Turning to the visual elements, the picture represents five men with orange uniforms. In the Indonesian context, the rescuers team also wears orange uniforms. Therefore, it can be inferred that the representation of five men means five rescuers. Focusing on what the two rescuers are doing in the front, there are two Unidirectional Transactional Actions. First, it can be seen from a vector emanating the below rescuer's hands that lift up a black blob that can be inferred as a pack of trash. Second, it can be seen from a vector emanating from the above rescuer's hands that take the same black blob. In the meantime, as the locative circumstance, the picture takes place in a gutter.

The rescuers took all the trash out of the gutters.

Figure 7. Worksheet 4.4 Panel 3

In terms of complementarity, all of the elements in verbal texts are completed visually. Most of them are picked up by the relation of Repetition in verbal texts. For example, in terms of participants, "the rescuers" is completed visually by five rescuers representation. In terms of process, "took out" is picked up visually by the representation of taking and lifting up the black blob. Similarly, both verbal and visual text also put gutter as the locative circumstance. Providing visual elements that are identical with verbal elements might help students identify each meaning of the written word directly. Teachers can utilize the visual elements to ask students to point out each element mentioned in verbal text. Furthermore, teachers can also emphasize the essence of work hand in hand to make the environment clean.

Pedagogical Implications

The findings indicate three pedagogical implications for its practical use in the classroom. First, paying attention to the visual and verbal texts can help teachers define the starting point in discussing the worksheets in the classroom. Based on the level of students, teachers can determine whether students should start discussing the worksheet from the verbal or visual texts (Damayanti & Febrianti, 2020). For example, the visual texts can be the right point of departure for low achievers as it can increase their curiosity of the texts. Mid achievers, on the other hand, might be able to start focusing on the verbal texts, in which the visual texts are supplementary, to see the flow of the texts. High achievers may be competent in discussing the worksheet panel by panel, considering both visual and verbal texts.

Second, both verbal and visual texts of the worksheets provide a space for teachers and students to discuss meanings. Both visual and verbal texts of the selected worksheets can be addressed to build students' glossary to better identify the concept of recounting a flood.

Firefighters and soldiers worked together to rescue the people.

Figure 8. Worksheet 4.4 Panel 2

For example, teachers can discuss each element of the panel in the above picture with students. In terms of process, teachers can ask about how the soldiers help the people. In terms of participants, teachers can ask students about why the soldiers are helping the people. Moreover, teachers can discuss the things brought by the soldiers. In terms of complementarity, besides discussing the flood, teachers can ask students the means needed to cross the flood if the flood is higher.

Third, the selected verbal and visual text can be utilized by teachers to increase students' awareness of environmental issues. EFL materials that include the content about environmental issues are considered very important. It is because EFL materials supposedly can have unconscious affects towards students (Emilia et al., 2017). Likewise, Al-Jamal et al. (2014) asserts that the content of EFL materials can influence students' perceptions since language is considered as a medium of thinking and describing the world to themselves. By utilizing the selected data, teachers can open a discussion about the reasons for the flood and how to prevent that phenomenon. By doing that, the teaching and learning process is not only focused on generating students to communicate in English but also expanding their environmental awareness. All in all, encouraging students' comprehension of the book could be expanded by identifying both visual and verbal texts.

Conclusion

This study has investigated how environmental issues are represented in three worksheets of the English for Nusantara textbook for 8th graders (Damayanti et al., 2022). The findings and discussion show that the selected worksheets portray flood as an environmental issue. Flood is portrayed in both verbal and visual texts of the worksheets. There are three main points of flood-related environmental issues that the verbal and visual texts portray. First, the verbal and visual texts represent the causes of flood-related environmental issues by representing humans as the Actor of the cause. Second, the verbal and visual texts represent the effect of what is being done by humans. Nevertheless, several verbal texts are completed visually by the relations of Antonymy. Hence, there is a need for teachers to always check the suitability of verbal and visual meanings in students' learning material and tell students the true meaning of the texts. Third, the verbal and visual texts represent the solutions to flood that students can do. The study also indicates three pedagogical implications for its practical use in the classroom. First, it can be used to determine the starting point of the students. Second, it can be used to discuss meanings in order to increase students' vocabulary. Third, it can be used to increase students' awareness about environmental issues.

Recommendations

Based on the findings of the study, theoretical, practical, and professional implications can be drawn. Theoretically, this study can be used as an example of the investigation of the representation of environmental issues in EFL materials. To be specific, this study provides the example of using several frameworks (Transitivity, Visual Grammar, and Ideational Intersemiotic Complementarity) to determine the representation of environmental issues across verbal and visual texts. Practically, this study provides input for the teachers in considering the representation of environmental issues when making, selecting, and arranging the teaching materials.

This textual analysis with a multimodal approach has achieved its purpose of exploring how environmental issues are represented across verbal and visual texts in an EFL textbook for primary school. However, the findings of the study cannot be generalized to other materials. The limitation of the study is the fact that only three worksheets chosen as the data source. Other materials of the book, thus, can be chosen as the data source for further study.

Scientific Ethics Declaration

The author declares that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the author.

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Optimization of Process Parameters for Experimental Production of Waste Frying Oil Based-Biodiesel by Taguchi Method

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Abstract: Recycling waste frying oils is an important issue concerning environmental and economic problems. Biodiesel production from waste oil is an effective method to obtain a sustainable energy source. Biodiesel attracts attention as a renewable and environmentally friendly alternative that can replace traditional fossil fuels in diesel engines. However, various process parameters such as process temperature, alcohol/oil ratio, and type of catalyst need to be carefully adjusted to increase the efficiency and optimize the quality of the biodiesel production process from waste oils. This study uses the Taguchi method to determine the most suitable process parameters for experimental biodiesel production from waste oils. The study's findings, which identified the optimum conditions for biodiesel production from waste frying oil, have the potential to impact the field of sustainable energy significantly. Three essential parameters were selected for investigation: the molar ratio of alcohol to oil, reaction temperature, and reaction time. Each parameter was examined at two levels, denoted as L-4 (23). Four experimental runs were conducted using the selected parameters and their respective levels to produce biodiesel from waste frying oil. Optimum conditions were found to be 1:6 for oil/methanol molar ratio, 60°C for reaction temperature, and 60 min for reaction time. Under optimum reaction conditions, biodiesel yield was an average of 97.7 %.

Keywords: Biodiesel, Waste frying oil, Taguchi, Design of experiment

Introduction

The rise in population and higher food consumption rates has led to a surge in waste frying oil (WFO) from various sources such as households, restaurants, hotels, schools, and industries. This issue is not just a local concern but a global one, increasingly prevalent in the TRNC (Turkish Republic of North Cyprus) and beyond. Due to its island status, Cyprus faces a particularly severe situation compared to mainland areas. Improper disposal of WFO through sinks and drains poses significant challenges for sewage treatment facilities, consequently driving up purification expenses. Indeed, WFO represents a valuable residue that holds potential as a raw material for various purposes such as soap manufacturing, energy generation through anaerobic digestion, thermal cracking, and biodiesel fuel production (Phan & Phan, 2008; Sabudak & Yıldız, 2010; Al-Shanableh et. al., 2023).

Biodiesel stands out as a popular alternative in liquid fuels, primarily due to its compatibility with conventional diesel engines with minimal or no adjustments required and its ability to be blended with petroleum diesel. Traditionally, Biodiesel has been produced from renewable sources such as edible or non-edible oils/fats (Al-Shanableh, 2017). The conversion of highly viscous oils/fats into less viscous biodiesel typically occurs through transesterification. In this process, which can be catalyzed by a base, acid, or enzyme, oil/fat reacts with an alcohol to produce fatty acid methyl esters (FAME), i.e., biodiesel, along with glycerol as a co-product with commercial value. Among the various transesterification methods, base-catalyzed transesterification (Encinar et

al., 2002) is regarded as the most promising approach for viscosity reduction. In this process, triglycerides undergo a transesterification reaction, converting them into a mixture of esters of the fatty acid in the oil/fat, utilizing a short-chain alcohol and a catalyst. Biodiesel is derived by purifying this mixture of fatty acid methyl esters. The following general equation can represent the transesterification reaction:

$$\text{Triglycerides} + 3\text{Monohydric Alcohol} \overset{Catalyst}{\Longleftrightarrow} 3FAME + \text{Glycerol}$$

Design of experiment (DOE) methods were used to determine the essential process parameters effective in the production of biodiesel from vegetable oils, such as catalyst type, alcohol type, reaction time, and reaction temperature (Priyadarshi & Paul, 2019; Yesilyurt & Cesur, 2022). The Taguchi method aims to evaluate the impact of individual parameters on processes (Taguchi & Konishi, 1987). Taguchi techniques of DOE are used to optimize various engineering applications, evaluating the most suitable and optimal condition of process parameters (Al-Shanableh et al., 2020). The method employs a set of orthogonal arrays that lead to a minimum number of experimental trials.

This study uses the Taguchi method to determine the most suitable process parameters for experimental biodiesel production from waste oils. Three parameters were selected for investigation: the molar ratio of alcohol to oil, reaction temperature, and reaction time. Taguchi orthogonal array analyzed each control parameter and their individual effects on the whole process. Each parameter was examined at two levels, denoted as L-4 (2^3). Four experimental runs were conducted using the selected parameters and their respective levels to produce biodiesel from waste frying oil. Produced biodiesel samples were evaluated for quality parameters using standard analytical methods.

Materials and Methods

Materials

The waste frying oil was sourced from the cafeterias of Near East University, where approximately 18 to 20 liters of WFO were gathered daily per cafeteria. Anhydrous methanol (MeOH) with a purity of 99.8% and high-purity sodium hydroxide (NaOH) were procured from Merck for the experiments. The feedstock's fatty acid (FA) compositions were analyzed according to the EN ISO 5508 method at the TRNC Ministry of Health, Directorate State Laboratory in Nicosia, utilizing Gas Chromatography (GC). The findings from the GC analysis are presented in Table 1.

Table 1. Fatty acid compositions of WFO

Fatty acid	**Molecular mass (g/mol)**	**% Composition of fatty acids**
Caprylic acid - C8:0	144.21	0.05
Capric acid - C10:0	172.27	0.33
Lauric acid - C12:0	200.32	1.18
Myristic acid - C14:0	228.38	0.10
Palmitic acid - C16:0	256.43	39.29
Palmitoleic acid –	254.41	0.14
Stearic acid - C18:0	284.48	4.04
Oleic acid - C18:1	282.47	40.42
Linoleic acid - C18:2	280.45	13.84
Linolenic acid - C18:3	278.44	0.18

Experimental Set-up for Base-Catalyzed Transesterification

Figure 1 outlines the sequence of experimental steps which were employed to produce biodiesel through a base-catalyzed one-step transesterification reaction. While transesterification constitutes the primary phase in biodiesel production, adhering to international standards requires additional procedures such as raw material pretreatment, separation of reaction products, and purification of the resultant products.

The characteristics of used frying oils are distinct from those of virgin oils. During the frying process, triglycerides undergo hydrolysis due to the combination of heat and water, increasing the free fatty acid (FFA) content. For successful base-catalyzed transesterification, the FFA levels in a sample should be below 3% (Sharma & Singh, 2009). Triglycerides with high FFA and water content required additional preprocessing steps, such as filtration and water removal. The amount of catalyst that should be used in the process is defined by titrating WFO samples against a standardized base solution such as NaOH and calculating the amount of FFA in the oil samples with Equation 1 (Wrolstad et al., 2005).

$$\% \, FFA \, as \, oleic \, acid = \frac{(V_{NaOH})(N_{NaOH})(MW_{oleic \, acid})}{Weight \, of \, sample} \quad (1)$$

where, V_{NaOH} is the volume of NaOH consumed in titration and N_{NaOH} is the normality of NaOH solution. % FFA found in WFO was 2.2 %, so base-catalyzed transesterification was suitable for WFO.

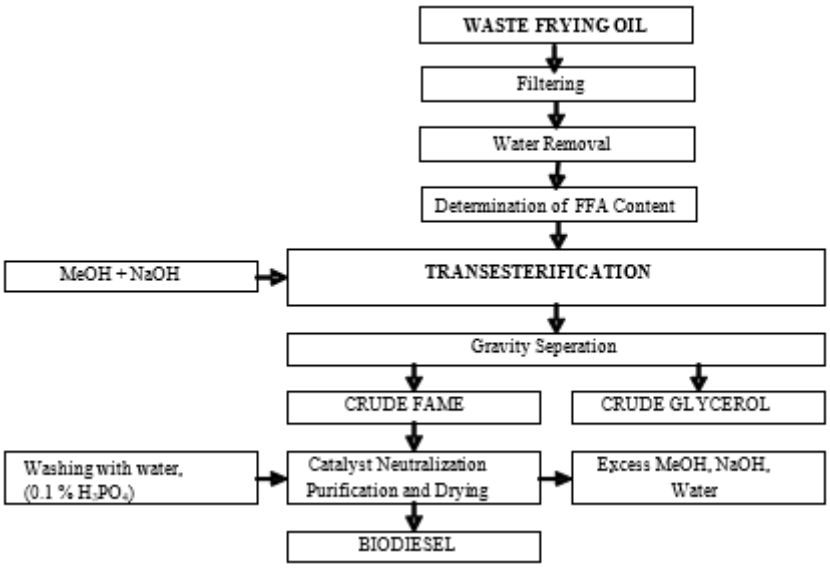

Figure 1. Flowchart of the experimental procedure for base-catalyzed transesterification

The reaction mixture was combined in a 2.0-liter three-necked flask equipped with a condenser, a magnetic stirrer, a T-type thermocouple, and a heater. The temperature of the transesterification reaction was either 50°C or 60°C. The molar ratio of methanol/oil was at two different levels of 1:4 or 1:6. The reaction time was fixed at 60 or 90 minutes. After the reaction was completed at the end of the specified time, separation and purification stages were utilized.

Design of Experiment by the Taguchi Method

The methodology devised by applying the Taguchi method to optimize process parameters for achieving the highest biodiesel yield via base-catalyzed transesterification is depicted in Figure 2 and can be readily followed.Three key control parameters were used to construct the Taguchi structure: the molar ratio of alcohol to oil, reaction temperature, and reaction time. Kim et al. (2010) and Buasri et al. (2009) worked on some other parameters, such as catalyst/alcohol type and catalyst concentration, which were utilized as control parameters, while those parameters were maintained at constant levels in this study. The three selected parameters with two levels denoted as L-4 (2^3), are listed in Table 2. Here, L-4 denotes a Latin square design with the replication number for the experiment.

Table 2. Parameters and their levels for DOE

Parameters	**Levels**	
	1	2
Molar ratio (oil/methanol)	1:4	1:6
Reaction temperature (°C)	50	60
Reaction time (min)	60	90

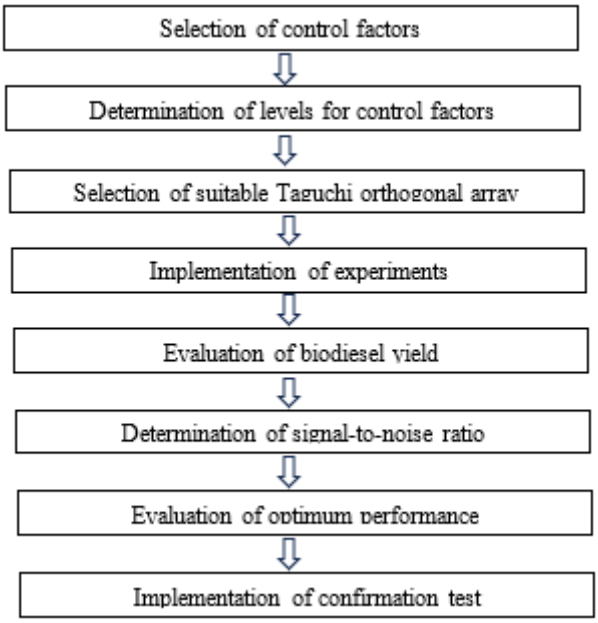

Figure 2. Taguchi's DOE procedure

To assess the impact of various control parameters on the efficiency of biodiesel production, an orthogonal array design was implemented with the experimental conditions outlined in Table 3, where numerical values represent the different levels of the parameters.

Table 3. The L-4 (23) orthogonal array for DOE of the current work

Experiment No	Control parameters and their levels		
	Molar ratio (oil/methanol)	Reaction temp. (°C)	Reaction time (min)
1	1	1	1
2	1	2	2
3	2	1	2
4	2	2	1

Using three independent variables, each with two levels would typically require 8 runs (i.e., 2^3). However, by employing Taguchi's DOE, the number of experiments can be reduced to 4.

Results and Discussion

The percent yield of WFO-based biodiesel (WFOME) produced was calculated using Equation 2 (Phan and Phan, 2008) and results are shown in Table 4 as % yield.

$$\%\,Yield = \frac{m_{ester}}{3 \times \frac{m_{oil}}{MW_{oil}} \times MW_{ester}} \qquad (2)$$

The mass percentage of fatty acids in the feedstock and fatty acid methyl esters in biodiesel produced were obtained by GC analysis.

The yields of waste frying oil methyl esters (WFOME) obtained under four different experimental conditions are presented in Table 4. All experiments were conducted three times according to the specified conditions of control parameters outlined in Table 3. Experiment Number 4 exhibited the highest mean yield of biodiesel production, reaching 97.7%, indicating it as the optimal experimental condition. Conversely, Experiment Number 1 yielded the lowest biodiesel output at 72.4%. As per Taguchi's recommendation, achieving the optimal conditions based solely on the mean yield of biodiesel produced is insufficient. It is also imperative to determine the signal-to-noise (S/N) ratio to evaluate the quality characteristics deviating from the desired value.

The S/N ratio was calculated using the 'Larger-the-best' approach based on following Equation 3. Findings are listed also in Table 4.

$$\frac{S}{N}\ ratio = -10\,log(MSD) \quad (3)$$

Where *MSD* is mean squared deviation and can be calculated as,

$$MSD = \frac{1}{n}\sum_{i=1}^{n}\left(\frac{1}{y_i}\right)^2 \quad (4)$$

where n is the number of repetitions of each experiment and y_i is the yield of biodiesel produced.

Table 4. WFOME yields and the S/N ratios in the four sets of experiments

Experiment no.	Yields of WFOME produced				
	1st trial	2nd trial	3rd trial	Mean	S/N Ratio
1	69.4	72.4	75.5	72.4	37.183
2	72.4	73.5	78.8	74.9	37.472
3	78.6	86.6	92.2	85.8	38.613
4	97.2	97.8	98.0	97.7	39.795

The control parameters from Experiment Number 4 could be considered optimal, as they yielded the highest mean biodiesel yield and the most significant S/N ratio.The mean S/N ratio can be utilized to display the effects of each level for every parameter. Each experimental parameter should be evaluated individually, and the interactions at the assigned levels should be determined by averaging all the S/N ratios. For example, considering the oil/methanol molar ratio and its first level (1:4), the mean S/N ratio (37.328) can be computed using the values (37.183 and 37.472) obtained from Experiment numbers 1 and 2. Conversely, when its second level (1.6) is being analyzed, the mean S/N ratio (39.204) can be calculated using the values (38.613 and 39.795) from Experiment number 3 and 4, and so forth. The mean S/N ratio for each level of the three influential parameters is summarized in Table 5.

Table 5. The mean S/N ratio of the three influential parameters

Parameters	S/N Ratio	
	Level 1	Level 2
Molar ratio (oil/methanol)	37.328	39.204
Reaction temperature (°C)	37.898	38.634
Reaction time (min)	38.489	38.043

The mean S/N ratio gave a perception about which parameter should be used at which level to achieve the highest biodiesel yield. The magnitude of the S/N ratio also defined the importance of control parameters. For this research, the molar ratio of alcohol to oil had the most crucial influence on process yield and was followed by reaction temperature and the last one was reaction time. The optimal reaction conditions based on the highest S/N ratio are as follows: for the first parameter (oil/methanol molar ratio), at level 2 (1:6); for the second parameter (reaction temperature), at level 2 (60°C); and for the third parameter (reaction time), at level 1 (60 min).

Biodiesel production from refined (unused) cooking oil (RCO) was conducted under optimized conditions to validate their effectiveness. The yield of refined cooking oil methyl esters (RCOME) was determined to be 98.1%. This yield closely resembles waste frying oil methyl esters (WFOME), produced using the optimal conditions of a 1:6 oil/methanol molar ratio, 60°C reaction temperature, and 60 minutes reaction time.

Conclusion

This study used the Taguchi method to determine the most suitable process parameters for experimental biodiesel production from waste oils. Optimum conditions found due to Taguchi's DOE were a 1:6 oil/methanol molar ratio, 60°C as reaction temperature, and 60 min as reaction time. The results constitute a critical step in optimizing the biodiesel production process from waste oils and producing environmentally friendly energy sources more effectively. Future studies can focus on validating the findings on a broader range of parameters and further improving the biodiesel production process.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Acknowledgements or Notes

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