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Dursun S, Ineson P, Frankland JC, Boddy L, (1993) Sulphite and pH effects on CO_2 evolution from decomposing angiospermous and coniferous tree leaf litters. *Soil Biology & Biochemistry* **25**, 1513-1525.

Ergas SJ, Schroeder E, Chang D, Scow K, (1994) Spatial distributions of microbial populations in biofilters. In: Proceedings of the *78th Annual Meeting and Exhibition of the Air and Waste Management Association*, pp. 19-24, Cincinnati, OH.

Hickey M, King C, (1988) *100 Families of Flowering Plants*. Cambridge University Press, Cambridge.

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Research Paper

Biodegradation of 2,4-D and Trifluralin Herbicides by the Bacteria *Pseudomonas spp.* Using Factorial Design of Experiments

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Abstract: Herbicides are commonly used to control unwanted weeds in fields, gardens, airports, parks, and railways. In addition to the benefits of herbicides that are applied to the ground with the help of agricultural tools, they also may be observed to have some damaging effects on the ecosystem. Herbicides may cause death and birth defects by getting mixed into drinking water. Studies show that numerous Pseudomonas spp. species isolated from various environments degrade hydrocarbon compounds. Degradation processes increase when environmental conditions become extreme. My purpose is to treat Pseudomonas ssp. isolated from environmental and clinical specimens. To clean herbicides by bacteria and contribute to cleaning nature economically. This study aims to establish the biodegradation of bacteria in the most effective medium in a statistical 23 multi-factorial testing apparatus created from four environmental and four clinical isolates selected from Pseudomonas aeruginosa, *Burkholderia cepacia*, Pseudomonas fluorescens, and Pseudomonas putida species. *Burkholderia cepacia* species was observed to degrade 2,4-D at a rate of 99.7% in the presence of activated carbon in the medium, and Pseudomonas aeruginosa species was found to degrade trifluralin at a rate of 99.3% in the presence of activated carbon in the medium. The presence of activated carbon and succinic acid in the medium increased the efficiency of bacteria in herbicide biodegradation. Consequently, it is believed that the use of Pseudomonades for eliminating toxic residues left by 2,4-D and Trifluralin herbicides may provide some benefits environmentally, clinically, and economically.

Keywords: *2,4-dichlorophenoxyacetic acid(2,4-D), Trifluralin, Pseudomonas aeruginosa, Burkholderia cepacia, Pseudomonas fluorescens, Pseudomonas putida.*

Introduction

2,4-dichlorophenoxyacetic acid (2,4-D) and trifluralin herbicides are commonly used in weed control in our country and around the world. These herbicides, which are found in soil, water, and factory wastes, cause unwanted mutations in various organisms in the environment. Some microorganisms in soil degrade 2,4-D, especially trifluralin under aerobic conditions. Some herbicides remain in the soil in varying amounts based on the variety of the soil. The residue is mixed in streams through rain and irrigation water and accumulates in lakes and seas. Anaerobic microorganisms degrade 2,4-D and trifluralin herbicides that permeate into the deep soil. In addition, microorganisms that degrade such herbicides are found in sediments in sea and lake bottoms (Kerner, 1971; Berry *et al.,* 1987).

The demand of human beings for food increases due to the upsurge in the world population. Agricultural production is not at the desired level. The main reason for low yield in agricultural production is due to weeds. In addition, damage by weed diseases and pests also causes low yield (Loser *et al*., 1999). In addition to their benefits, various chemical substances (e.g., pesticides and insecticides) used in the control of weeds and pests that lead to the loss of yield in agricultural produce cause environmental pollution and threaten the health of living organisms due to their toxic effects (Loser *et al*., 1999; Leahy *et al*., 1990).

For a chemical substance to create an effect, it must first be taken into the body in a certain way and then absorbed by the body. When given to a living organism a certain way and at a sufficient dose, every chemical substance can create detrimental effects. The severity of the effect is due to the amount of substance reaching the effect zone and the physical structure of an organism. When establishing the toxicity risk of a chemical substance, knowing just the type of effect is not sufficient. The main factors

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affecting toxicity are the dose of the toxic substance, the manner of administration, contact time, and frequency (Gomes *et al*., 2009).

The zone where Pseudomonas species bacteria are colonized, and the condition of the host are the most significant factors affecting their pathogenicity. The primary criterion for the disease to occur is the settlement of the pathogen in a suitable area. Colonization occurs when the skin and mucosa structure deteriorates or the immune system is suppressed, causing systemic disease through local invasion. Cellular damage plays a deterministic role in the colonization of Pseudomonas species bacteria in epithelial cells. Damage occurring in epithelial cells because of infection through a virus or endotracheal intubation causes similar results. This phenomenon is called "opportunistic adherence". Pseudomonas bacteria are a significant trigger in the pathogenesis of infections. Pseudomonas bacteria rarely cause any diseases in healthy persons. However, they can establish infection in every system and organ in persons with a deficient immune response and defense system, especially in a hospital environment; in other words, whenever they find an opportunity (Koneman *et al*., 1997).

Studies conducted on the genes of microorganisms that degrade pesticides demonstrated that these genes are carried on plasmids, transposons, and chromosomes. Catabolic genes are modified. Means of metabolic degradation were achieved through the purification of enzymes found in microorganisms (Colpella *et al*., 1990). Today, some Pseudomonas bacteria species commonly found in clinical environments (hospitals, burn units, and any environment with infection and wound risks) and environmental areas (soil, water, food, *etc*.) are popular for their ability to degrade pesticides biotechnologically. Another dimension of this research is the production of biotechnological metabolites by Pseudomonades through degradation to maintain life (Balows, 2003).

Photosynthetic microorganisms are commonly used for the purification of nitrogen, phosphorus, heavy metals, and other toxic products from industrial wastewater. The use of photosynthetic microorganisms that can develop without the need for any carbon source is economical for industrial research (Kerner, 1971).

Pseudomonas spp. bacteria that will constitute the basis of this study were isolated from various clinical and environmental areas. The fact that these bacteria are economical and easy to work with is a significant motivation in investigating their biodegradation abilities. Advanced statistical methods and HPLC measurements were used to establish the resistance of bacteria against toxic environments and to reveal the herbicide biodegradation capabilities of the most resistant strains. The biological capability of a combination of a commonly found herbicide and *Pseudomonas* spp. under different environmental conditions shall be discussed in light of experimental findings. In the next section, some important biodegradation studies shall also be reviewed in short. In the third section, information on materials and methods shall be summarized. Experimental results and findings shall be discussed in the fourth section.

A Brief Look at Biodegradation Studies

It can be seen in the literature that many varieties of pesticides and chemicals are broken down by different microorganisms. In a study conducted with Chlorobenzene, the Pseudomonas spp. RHO1 bacteria used the biodegradation end products of 2-chlorophenol and 3-chlorocatechol as carbon and energy sources (Fritz *et al*., 1992).In many studies conducted with pure and mixed cultures, it was reported that 2,4-D could be used as a carbon and energy source by species belonging to the Artrobacter, Pseudomonas, Xanthobacter, and Alcaligenes genera (Fisher *et al*., 1978). In a ten-day study conducted under light, the cyanobacteria *Microcystis aeruginosa* was able to survive at a 2,4-D dose of 1000-1500 ppm (Hoffmann *et al.,* 1996). The LC50 of two phytoplankton against the 2,4-D herbicide was determined. According to this evaluation, the LC50 of *Phaeodactylum tricornutum* against 2,4-D was determined as 362±9 ppm, and the LC50 of *Dunaliella tertiolecta* against 2,4-D was determined as 185±11ppm (Okay et al. 1996).In a 44 days study conducted with *B. cepacia* on sterile and non-sterile soil, it was reported that while 5 ppm of 2,4-D was toxic, bacterial growth continued at a 2,4-D dose of 500 ppm (Jacobsen *et al*., 1992).

By isolating the dioxygenase gene that breaks down naphthalene from the *Klebsiellaoxytoca*, *Herbaspirillum seopedicae*, and Bacillus megaterium bacteria, it was investigated whether the same gene would be effective for breaking down trifluralin. This article is the first to investigate the metabolic breakdown pathways for the pesticide trifluralin. However, because of sequence analysis with PCR and the use of other techniques (Clear zone), it was determined that the genes that breakdown naphthalene and trifluralin are not the same, and that these genes do not entirely breakdown trifluralin (Bellinaso *et* *al*., 2003). It is seen in the literature that Pseudomonas bacteria breakdowns trifluralin at doses of approximately 500 ppm. In a study on the biodegradation of trifluralin, oxadiazon, and norflurazon in soil sediments, it was determined that pesticides fully absorbed into the sediments were biodegraded at a faster rate than pesticides that did not mix with the sediments. biodegradation of 2,4-D and trifluralin herbicide was studied with P. fluorescens and P. putida isolated from the Mississippi river. Although these bacteria did not break down 2,4-D, trifluralin was broken down to a considerable extent (Zablotowicz *et al.*, 2001). The trifluralin herbicide, once used against the plant disease *Rhizoctonia solani* in cotton, was broken down by the *B. cepacia* bacteria (Heydari et al. 1998). A study was conducted in which succinic acid was added to the media while evaluating the effect of *B. cepacia* on the biodegradation of the 2,4-D herbicide. According to the HPLC results, the addition of 0.2% succinic acid accelerated the bacterial breakdown of 2,4-D by 95% (Daugherty et al. 1994). In a study conducted with five pesticides and the *R. spharoides* and *R. pallustris* bacteria, 0.2% maltose was added as an inducer to the media, and it was observed that the rate of the breakdown reaction accelerated as a result (Chalam *et al*., 1997). In wastewater with high carbon content (COD=584.11), it was reported P. putida broke down more than 90% of 2,4-D and paraquat herbicides within 24 hours (Kopytko et al. 2002).

In a study that evaluated the effects of active carbon (NP5) on the biodegradation of phenoxy acid, it was observed that the 2,4-D, MCPA, and MCPP herbicides were effectively broken down (Wattanaphon *et al.,* 2008). It was reported that the rate of breakdown for soil samples contaminated with benzene increased with the aid of the biosurfactant obtained from the BSP3 strain of *Burkholderia cepacia* and by adding glucose to the media (Ignatowicz, 2009). Also, a study was published in 2008 that demonstrated the enzymatic breakdown of the 2,4-D herbicide by the *B. cepacia* bacteria (Smith et al. 2008). In a study that investigated the changes in the morphology, structure, and capability of removing the target contamination of the aerobic sludge granules cultured with mixed substrates of glucose and 2,4-dichloro phenoxy acetic acid (2,4-D) in a long-time running sequence batch reactor (SBR), when the carbon source transformed into the sole carbon source of 2,4-D (Ma et al. 2010).

In a study by Elizangela *et al*. (2021), Pseudomonas strains were isolated from environments with 2,4-D and its derivatives and the original Pseudomonas CMA-7.3. strain and all antioxidant capacity activities were studied. In the studies, it was observed that 36% of the bacteria cleaned the herbicide in the tanks with a biofilm layer. It is recommended that it can be used in ports or warehouses.

Studies investigated the potential genotoxic and retinal developmental effects of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) on zebrafish (*Danio rerio*) during their early life stages. To assess genotoxicity, we measured DNA damage using the comet assay and analysed the mRNA expression of genes involved in apoptosis and DNA repair. Additionally, we evaluated retinal developmental toxicity through a histological approach. The results of our study revealed that exposure to 2,4-D caused alterations in the DNA integrity of zebrafish larvae. Furthermore, the transcriptomic data showed a significant increase in the expression of p-53 and casp-3 genes, which are associated with apoptosis, and a noteworthy reduction in the expression of the lig-4 gene, involved in DNA repair, in the larvae exposed to the highest tested concentration of 2,4-D (0.8 mg/L). These findings suggest that 2,4-D exposure may have detrimental effects on the genotoxicity and retinal development of zebrafish during their early life stages (Gaaied *et al*., 2022).

Microbial elimination of these herbicides is economically and environmentally feasible. Mycoremediation (bioremediation by fungi) is recognized as an effective approach to cleaning up harmful chemicals and converting them to non-toxic metabolites through various enzymatic pathways. Various fungi including *Phanerochaete chrysosporium, Phlebia aurea, A. niger, Phoma glomerata, Chrysosporium pannorum,* and *Trichoderma sp.* have shown that they have the potential to convert or break down harmful pesticides into harmless or less harmful compounds (Magnoli et al. 2020).

According to EPA regulations, the use of 2,4-D herbicides at currently recommended concentrations (< 2 ppm whole lake treatment) may pose a risk to freshwater fish (Dehnert *et al*., 2021).

Soil samples were taken from different fields in studies with Trifluralin herbicide, which is frequently used in cotton fields in China. Trifluralin residues in these soils were not sufficient to kill soil worms but were risky for barley, wheat, and alfalfa (Yang *et al*., 2021).

Recent studies have found that trifluralin may potentially affect mitochondrial function (Oliveira *et al.*, 2020), exhibit genotoxicity (Hakala et al., 2010), and act as a persistent biotoxin in mammalian cells (Bisceglia et al., 2018). Additionally, it has been associated with higher cancer incidence rates in agricultural workers exposed to the chemical (Weichenthal et al., 2012).

In the study of Kumar *et al*., (2016) significant progress has been made in understanding the biodegradation mechanisms of 2,4-dichlorophenoxyacetic acid (2,4-D). The 2,4-D biodegradation pathway has been elucidated in several microorganisms, including strains of *Cupriavidus necator* JMP134 (previously known as *Wautersia eutropha*, *Ralstonia eutropha*, and *Alcaligenes eutrophus*) and Pseudomonas. An alternative approach involves introducing suitable plasmid-derived catabolic genes into established and competitive natural bacterial populations. Therefore, further characterization of new local bacterial populations is needed for possible application in the bioremediation of 2,4-D. That's why it's an important article (Kumar *et al*., 2016).

Trifluralin is a widely used herbicide with significant environmental persistence and ecotoxicity, especially for aquatic organisms. It is insoluble in water and highly volatile, leading to rapid loss from soils when applied on the surface. The herbicide strongly binds to soil organic matter and shows minimal leaching into water. Trifluralin's structure contains a tertiary amino group, two nitro-groups, and a trifluoromethyl-group. Despite its xenobiotic nature, it can undergo biodegradation through dealkylation or nitro-group reduction by specific bacteria and fungi (Coleman *et al*., 2020).

Materials and Methods

Pseudomonas samples used in the research were divided into two groups clinical and environmental. Water and soil samples were taken from 10cm below the surface in sterile containers and polyethylene bags. The locations of the samples were recorded, and the samples were taken immediately to the laboratory environment. After the samples were taken, they were maintained at 4ºC for 1-2 days until analyses were carried out (Brock, 1979). Clinical samples were supplied by the Microbiology Laboratory of the Medical Faculty of Gazi University in sterile Eppendorf tubes containing 0.5ml glycerol maintained at -80ºC and were activated (Rajmohan *et al*. 2002).

Pseudomonas spp. bacteria were isolated from the environmental water and soil-based samples obtained from the surroundings of Ankara Province (Govan, 1989; Pier *et al*., 2005). In addition, 11 *Pseudomonas* spp. isolates (clinical samples) were supplied by the Microbiology Laboratory of the Medical Faculty of Gazi University (GUMF). The total number of isolated samples was 121 which is shown in Table 1.

Bacteria belonging to the genus Pseudomonas; Pseudomonas P (King A) giving pyocyanin pigment and Pseudomonas F (King B) giving Pyoverdin and Fluoressin pigment were incubated for 24 hours at 37° C in a selective medium (Kristiansen, 1983). Studies were conducted on environmental (4 isolates) and clinical (4 isolates) samples of *Pseudomonas aureginosa*, *Burkholderia cepacia*, *Pseudomonas fluorescens*, and *Pseudomonas putida* bacteria belonging to Pseudomonas species. For biochemical tests, the definition of isolate identifications was conducted by Analytical Profile Index (API20NE; Biomérieux, Marcy I' Etoilé, France) and "Microscan Auto-analyser".

For three days after isolation, 2,4-D herbicides in 25, 50, and 100ppm concentrations and trifluralin herbicides in 100, 250, and 500ppm concentrations were pipetted onto Elisa plates along with bacteria (0.5 MacFarland) in live colony count experiments. Live colony count was carried out by cultivating samples taken every 24 hours for three days on plate count agar (PCA) media (Bauer 1982; Claus 1989). Results were compared with control samples that did not contain herbicides.

Statistical Probit Analysis and Lethal Concentration (LC) values were determined, and a new experiment set was set up by selecting two herbicide-resistant strains (Levesque, 2007; Finney, 2009). In the statistical 23 testing apparatus designed to find out in which media bacteria best degrade herbicides (Hicks *et al*., 1999; Yates, 1937), the results were established by HPLC measurements (Bresolle *et al*., 1996) and were analysed and interpreted by Statistical Yates Algorithm (Yates, 1937).

The 23-design used in the study aims to establish statistically the differences between the biodegradation capabilities of herbicide-resistant bacteria under various media conditions. Independent variables of the design were established as 2,4-D or trifluralin herbicide concentration (low and high doses), succinic acid usage rate (0.2% and 0.4%), and the presence of activated carbon in media (present or absent) (Daugherty *et al*., 1994; Kopytko *et al*., 2002). In the multi-factor experiment, there were three factors (independent variables) and two levels of each factor. The dependent variables of the experiment design (responses) are 2,4-D and trifluralin biodegradation percentages. The 23 experiment was designed to answer the following questions: How does succinic acid affect biodegradation? Does activated carbon have the feature of absorbing (physicochemical) substances in the environment it exists? What kind of effects does an increase or decrease in herbicides in the environment pose?

Table 1. Areas from where Pseudomonades were isolated and isolation count.

Areas from where pseudomonades were isolated Environmental samples	Isolation count
1) WATER SAMPLES	
Lake Mogan	12
Ankara River	24
Lake Eymir	16
Kecioren Waterfalls 1 and 2	8
Gazi Medical Faculty Bayrakkale Pool	4
Total	64
2) SOIL SAMPLES	
Kecioren and Cubuk Municipalities Vegetable Gardens	18
AUAF Pulse Plantation (Barley, Wheat, Chickpeas) Areas	13
House Plants Pot Soil and Agricultural Mold	8
Agricultural Soil from the Surroundings of Lake Eymir	7
Total	46
CLINICAL (HOSPITAL SAMPLES)	
Cerebrospinal Fluid (CSF)	1
Wound culture	4
Blood Culture	2
Urine Culture	1
Sputum Culture	2
Abscess Culture	1
Total	11
Grand Total	121

Table 2. 2^3 experimental design set for 2,4-D

Experiment Numbers	Herbicide Consantration (X)	Succinic Acid (Y)	Activated Charcoal (Z)	Trial Combination	Bacteria
1	14 ppm	%0,2	NO	(1)	YES
2	27 ppm	%0,2	NO	X	YES
3	14 ppm	%0,4	NO	Y	YES
4	27 ppm	%0,4	NO	XY	YES
5	14 ppm	%0,2	YES	Z	YES
6	27 ppm	%0,2	YES	XZ	YES
7	14 ppm	%0,4	YES	YZ	YES
8	27 ppm	%0,4	YES	XYZ	YES
9	27 ppm	%0	NO	9	YES
10	27 ppm	%0	YES	10	YES
11	14 ppm	%0	NO	11	YES
12	14 ppm	%0	YES	12	YES

LC90 results were taken into consideration for the highest concentration of herbicides in the testing set. The division of high concentration by two obtained low herbicide concentration used in the experiment. Low and high values of succinic acid were established to be 0.2% and 0.4%, respectively. In the test design, 0.225gr activated carbon (1-disc 15gr/l) was added to the 15ml medium by calculation. A contrary result would indicate the absence of activated carbon (Kopytko *et al*., 2002). Tests 9,10, 11, and 12 were for control purposes (Tables 2 and 3).

HPLC measurements were conducted by Thermo Finnigan Surveyor brand HPLC device at the Instrumental Analysis Unit of Ankara University. For 2,4-D, analyses were conducted with RP18column, UV50 detector, 230nm wavelength, Methanol-0.1% Phosphoric Acid (60/40) at mobile phase, and flow rate of 1ml/minute (Yadav *et al*., 1993). For trifluralin, analyses were conducted with a C18 column, UV50 detector, 275nm wavelength, 80% acetonitrile, and 20% distilled water at mobile phase, and a flow rate of 1ml/minute (Bellinaso *et al*., 2003). During the biodegradation phase of bacteria, a minimal salt medium was used to conveniently observe the products of degradations by not seeing a molecule that might arise from the media (Nam *et al*., 2003).

In the minimal salt medium of the experiment, the shaker continued to operate with 15ml tubes (3 parallels) at 100rpm and 37ºC incubator temperature for three days. A sample was taken every 24 hours and herbicide was extracted by the necessary method (Yadav *et al*., 1993; Bellinaso *et al.,* 2003; Nam *et al*., 2003). The biodegradation level of the bacteria was measured with the HPLC device. The results were calculated with a [y=ax+b] linear regression model based on the calibration values taken before the test (Bresolle *et al*., 1996). These were then transformed into percentile results by comparing them with the controls according to Abbott's formula (Abbott, 1925). HPLC tests were conducted for three days, however, the results for the third day in which the herbicides were best degraded by bacteria were taken into consideration. Yates' method was applied to the results of the two repeated results of the third day and an F statistic was calculated as a statistical significance control (Yates, 1937).

Table 3. 2^3 experimental design set for Trifluralin

Experiment Numbers	Herbicide Concentration (X)	Succinic Acid (Y)	Activated Charcoal (Z)	Trial Combination	Bacteria
1	180 ppm	%0,2	NO	(1)	YES
2	360 ppm	%0,2	NO	X	YES
3	180 ppm	%0,4	NO	Y	YES
4	360 ppm	%0,4	NO	XY	YES
5	180 ppm	%0,2	YES	Z	YES
6	360 ppm	%0,2	YES	XZ	YES
7	180 ppm	%0,4	YES	YZ	YES
8	360 ppm	%0,4	YES	XYZ	YES
9	360 ppm	%0	NO	9	YES
10	360 ppm	%0	YES	10	YES
11	180 ppm	%0	NO	11	YES
12	180 ppm	%0	YES	12	YES

Results

The live colony count results were compared with the controls, and the resistance of bacteria to herbicides was estimated (Figures 1, 2and 3). When conducting probit analysis, percent inhibition results from the second day, in which bacterial growth levels were higher, were used (Table 4). The lower the percent inhibition results were the higher the herbicide's resistance to bacteria. These results were inversely proportional to live colony count results. For instance, for trifluralin herbicide, P41 (*P. fluorescens*) bacteria in 100ppm concentration (66,73) were observed to be more resistant compared to P11 (*B. cepacia*) bacteria (71,35). During the evaluation stage, percent inhibition results were used, and LC levels on which the bacteria were the most resistant to the herbicide were established by SPSS statistical software.

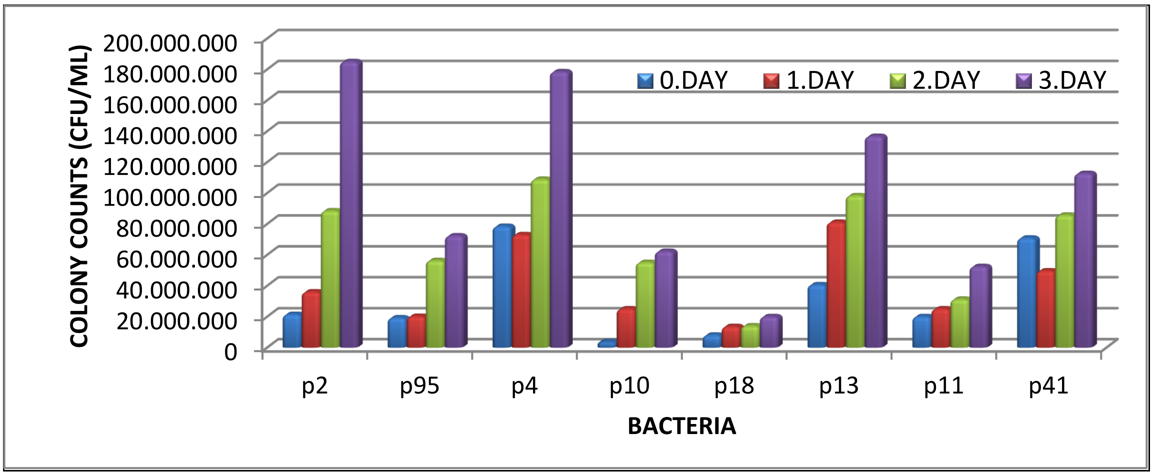

Figure 1. Control table for Pseudomonas spp. strains

LC 10, 50, 90, and 95 results were taken as the basis for probit analysis data. Figures 4 and 5 demonstrate the second-day probit analysis results of 2,4-D and trifluralin. Based on the results of probit analysis conducted with 2,4-D herbicide, the P11 (LC 95; 46,19ppm) bacteria strain was established to be the most resistant strain. In the probit analysis conducted with trifluralin herbicide, the P4 (LC 95; 609,41ppm) strain was found to be the most resistant.

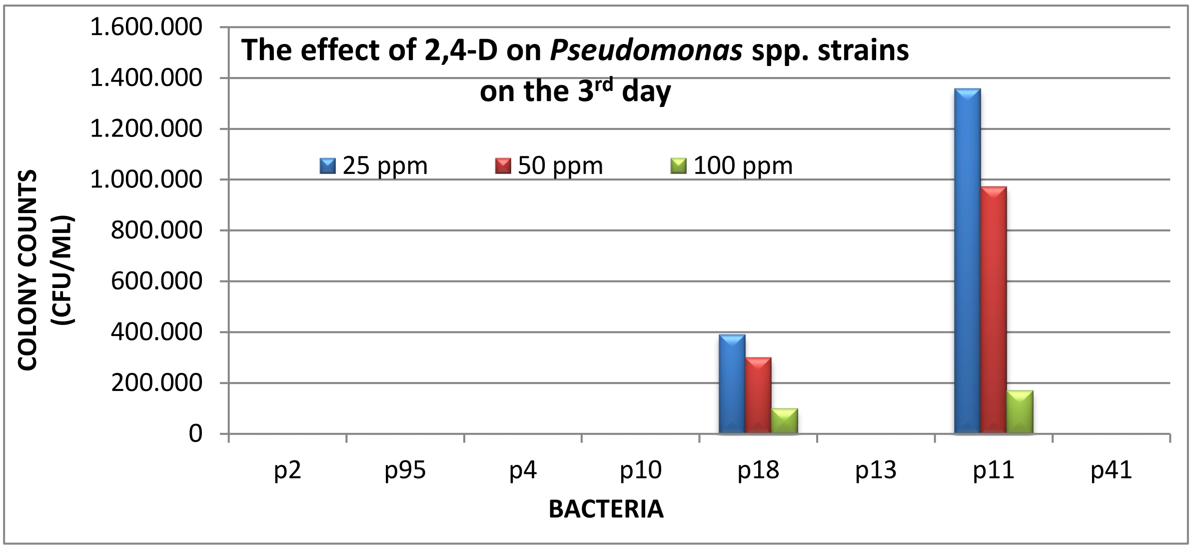

Figure 2. The effect of 2,4-D on Pseudomonas spp. strains on the 3rd day

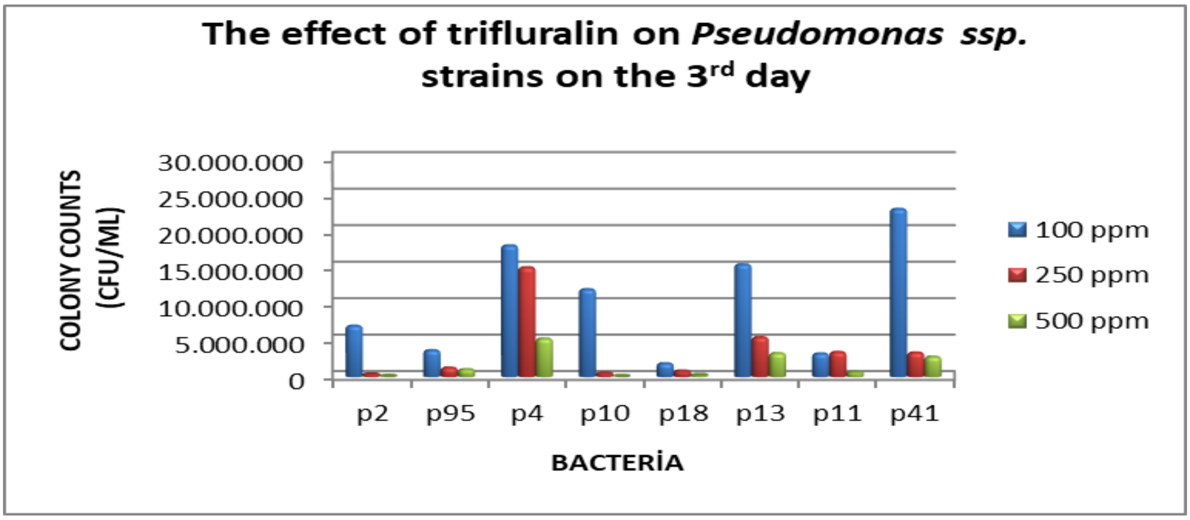

Figure 3. The effect of trifluralin on Pseudomonas spp. strains on the 3rd day

Table 4. 2nd day % inhibition results of 2,4-D and trifluralin herbicides

Second day Bacteria	2,4-D 25 PPM	50 PPM	100 PPM	Trifluralin 100 PPM	250 PPM	500 PPM
p2	100	100	100	90,82	99,15	99,88
p95	100	100	100	90,72	96,12	94,64
p4	100	100	100	68,71	77,98	96,86
p10	100	100	100	77,84	98,52	99,99
p18	94,24	95,20	99,07	77,21	94,21	97,12
p13	100	100	100	75,56	94,08	94,62
p11	89,66	94,16	99,13	71,35	91,23	94,39
p41	100	100	100	66,73	95,49	96,74

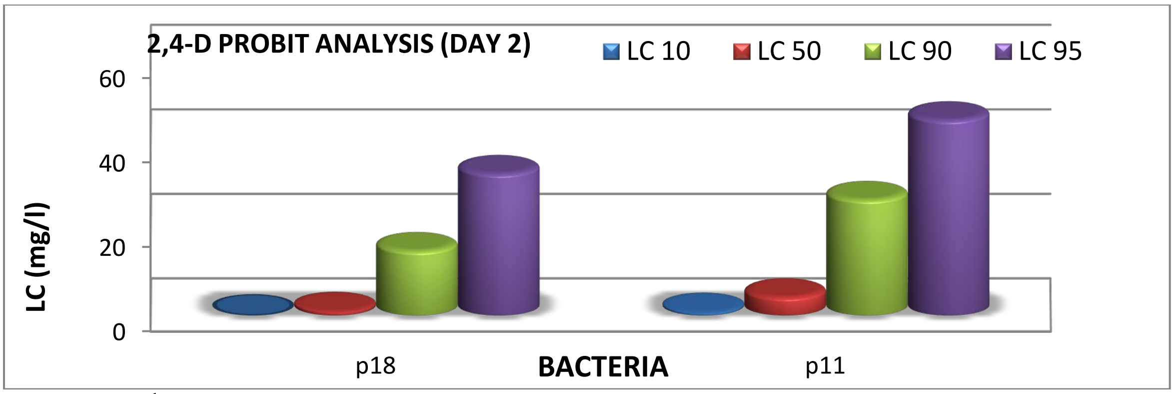

Figure 4. 2nd day probit analysis of 2,4-D

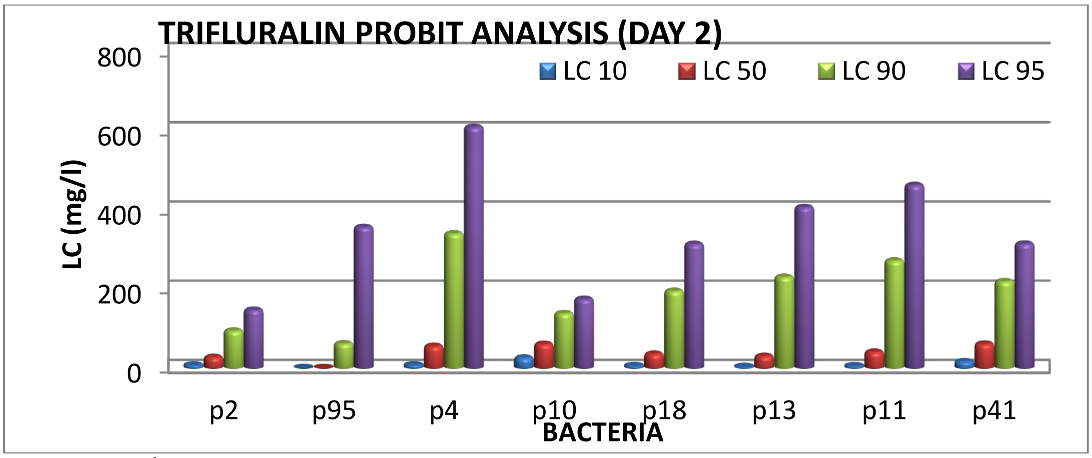

Figure 5. 2nd-day probit analysis of trifluralin

Clinical sample P11, which was resistant to the 2,4-D herbicide, was *Burkholderia cepacia*; the environmental sample P4 was *Pseudomonas aureginosa*. The HPLC method was adopted to measure the resistance of bacteria to herbicides. Table 5 shows the 2,4-D biodegradation percentages of Burkholderia *cepacia*. As 2,4-D herbicide is toxic, *B. cepacia* bacteria demonstrated significant degradation at a 14ppm low dose. As activated carbon showed a physicochemical absorption effect in the study, biodegradation occurred at over 95% in testing sets with activated carbon (5th - 8th experiments). Succinic acid being at 0.2% in the medium increased the biodegradation compared to its absence. It was observed in some sets that biodegradation was more effective at higher levels of succinic acid (0.4%). In testing sets in which activated carbon was absent (1st, 2nd, 3rd, and 4th experiments) the highest rate of 2,4-D degradation of P4 bacteria on the third day was only 33.3%.

Table 5. Biodegradation of 2,4-D herbicide by *B. cepacia*

	2,4-D Biodegradation (%) Experiment numbers											
Day	1	2	3	4	5	6	7	8	9	10	11	12
1	14,5	10,3	21,7	25,7	98,1	94,3	94,2	98,3	16,0	80,9	17,4	95,3
2	15,7	10,5	30,8	26,9	98,8	94,7	95,1	98,5	16,4	81,5	24,4	96,2
3	**16,4a**	**14,2**	**33,3**	**28,4**	**99,7**	**99,3**	**97,5**	**98,3**	**28,8**	**82,3**	**22,9**	**97,0**

a= Inhibition percent rate

Yates algorithm was applied to the estimated third-day biodegradation percentages (two-repetition) and results are shown in Table 6. According to the results in Table 6, herbicide concentration (X), succinic acid (Y), and activated carbon (Z) were established to be single significant factors in the 2,4-D biodegradation of *B. cepacia* (0.95F1.8= 5.32). The combination of herbicides and succinic acid (XY) was not found to be significant in terms of biodegradation, but bacteria demonstrated biodegradation at higher levels when herbicides and activated carbon (XZ) and succinic acid and activated carbon (YZ) were in combination. The combination of herbicides, succinic acid, and activated carbon (XYZ) did not have a significant effect on the biodegradation of B.cepacia.

Table 6. Yates algorithm of 2,4-D herbicide

Exp.	response	[1]	[2]	[3]	SSTb	Trial Combination	Fc
1	32,86	61,3	184,86	974,92	59404,31	Identify Average	
2	28,44	123,56	790,06	-13,68	11,6964	X	6,817127
3	66,74	398,22	-14,34	55,88	195,1609	Y	113,7475
4	56,82	391,84	0,66	-2,96	0,5476	XY	0,319163
5	199,58	-4,42	62,26	605,2	22891,69	Z	13342,19
6	198,64	-9,92	-6,38	15	14,0625	XZ	8,196184
7	195,12	-0,94	-5,5	-68,64	294,4656	YZ	171,6263
8	196,72	1,6	2,54	8,04	4,0401	XYZ	2,354731

***a**= Total biodegradation percentage (3rd-day results of a study in 2 parallels)*
***b**= Squares total; **c**= F statistics; The significance level is 5% and the standard deviation is 1.31.*

In the trifluralin herbicide biodegradation of Pseudomonas aeruginosa, the medium in which the bacteria showed the most degradation was determined by creating different media through experimental design (Table 7). Since, in the study, activated carbon demonstrated a physicochemical absorption effect, biodegradation occurred at over 95% in testing sets that especially contained activated carbon (experiments 5, 6, 7, and 8). The succinic acid present in the medium at 0.2% increased biodegradation compared to its absence. In some sets, it was observed that biodegradation was more effective at higher levels of succinic acid (0.4%). The highest P11 degradation rate of trifluralin on the third day was at only 58.4% in test sets in which activated carbon was not present (experiments 1, 2, 3, and 4).

Table 7. Biodegradation of trifluralin by *P. aeruginosa*

	Trifluralin Biodegradation (%) Experiment Numbers											
DAY	1	2	3	4	5	6	7	8	9	10	11	12
1	32,4	26,0	27,0	34,9	98,7	96,3	97,9	97,4	27,2	98,2	6,4	93,7
2	34,1	36,0	40,9	41,3	99,0	96,8	98,4	97,9	30,5	98,7	14,9	93,9
3	**44,9a**	**47,9**	**58,4**	**54,2**	**99,3**	**98,9**	**99,0**	**98,7**	**40,1**	**99,3**	**24,4**	**93,8**

a= Inhibition percent rate

The Yates algorithm evaluated the significance of the established experiment design sets, single, double, and triple. According to the results given in Table 8, herbicide concentration (X) was not a significant factor by itself in the trifluralin biodegradation of P. aeruginosa (0.99F1.8= 11.3). If only succinic acid (Y) or only activated carbon (Z) is present in the medium, bacteria degrade trifluralin at higher levels. The combination of herbicide and succinic acid (XY) or the combination of herbicide and activated carbon (XZ) is not significant in terms of biodegradation. However, bacteria demonstrate higher levels of biodegradation when succinic acid and activated carbon (YZ) are in combination. The triple interaction of herbicide, succinic acid, and activated carbon (XYZ) is not significant in the biodegradation of *P. aureginosa*.

Table 8. Yates algorithm of Trifluralin herbicide

Exp.	Response	[1]	[2]	[3]	SSTb	Trial Combination	Fc
1	89,7	185,52	410,76	1202,54	90381,4	Identify Average	
2	95,82	225,24	791,78	-3,78	0,893025	X	0,120792
3	116,92	396,36	-2,48	38,78	93,99303	Y	12,71366
4	108,32	395,42	-1,3	-14,42	12,99603	XY	1,757865
5	198,58	6,12	39,72	381,02	9073,515	Z	1227,299
6	197,78	-8,6	-0,94	1,18	0,087025	XZ	0,011771
7	197,96	-0,8	-14,72	-40,66	103,3272	YZ	13,97622
8	197,46	-0,5	0,3	15,02	14,10003	XYZ	1,907194

a= Total biodegradation percentage (3rd-day results of a study in 2 parallels); **b**= Squares total; **c**= F statistics; The significance level is 1 % and the standard deviation is 2.72.

Discussion

Biological interventions for the degradation of pesticides would greatly contribute to the food chain, and thus, the natural cycle of nature. Biological intervention methods are more cost-effective and less harmful to the environment compared to other intervention methods. This and other similar studies conducted in a laboratory setting are a model for other large-scale studies. The application of bacteria in solution via pump to herbicide-intensive areas as the bacteria reproduce in a way that does not threaten other living organisms, is considered effective.

This study investigated the effectiveness of various Pseudomonas species in eliminating toxic residues left in the environment by 2,4-D and trifluralin herbicides used for agricultural pest control in our country. Only two of four environmental and four clinical isolates, succinic acid and activated carbon used in the study were established to have high degradability activity. It was found that B.cepacia (P11; clinical isolate, Blood Culture) degraded 2,4-D in the presence of succinic acid and activated carbon best in 72 hours and that the degradation reached 99.7%. Again, P. *aeruginosa* (P4; environmental isolate, Kecioren Vegetable Garden) was established to degrade trifluralin in the presence of succinic acid and activated carbon best in 72 hours, and the degradation reached 99.3%. Based on these results, both tested bacteria species were observed to be quite effective in the destruction of herbicides with toxic effects. It is recommended to conduct more comprehensive studies for their commercial uses. Contributions to the use of advanced experimental designs and analyses in biodegradation issues have been emphasized in this paper.

Compliance with Ethical Standards Ethical responsibilities of Authors: *The author has read, understood, and complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors".*

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Conflict of Interest: *The author declares that he has no conflict of interest. Availability of data: All data used for this study are included within the manuscript.*

Change of Authorship: *The author has read, understood, and complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors and is aware that with minor exceptions, no changes can be made to authorship once the paper is submitted.*

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Research Paper

Compressive Strength Prediction Using Linear Regression Method

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Abstract: This study investigates the compressive strength of cement kiln replacement mixtures using a mechanistic modeling approach. The objective is to establish a mathematical model that predicts the compressive strength based on the percentage of cement kiln replacement. The study analyzed data from various replacement percentages ranging from 10% to 35% and their corresponding compressive strength values. A linear regression model was developed to capture the relationship between the replacement percentage and compressive strength. The model exhibited a good fit to the data, with a mean squared error of approximately 0.0254. Confidence intervals were calculated to provide a range of predicted compressive strength values at different replacement percentages. The findings of this study contribute to understanding the mechanical behavior of cement kiln replacement mixtures and offer insights for optimizing mixture designs. The developed mathematical model can serve as a valuable tool for engineers and researchers in the construction industry, aiding in the estimation of compressive strength for various cement kiln replacement scenarios.

Keywords: *Cement kiln Replacement, Compressive Strength, Mechanistic Modeling, Mathematical model, linear regression.*

Introduction

Cement kiln replacement has gained significant attention in the construction industry as a sustainable alternative to traditional cement production. By replacing a portion of cement with alternative materials, such as fly ash, slag, or pozzolans, the environmental impact of cement production can be reduced while maintaining the desired engineering properties of concrete (Abukhashaba *et al.,* 2014). One crucial property that needs to be assessed in cement kiln replacement mixtures is compressive strength (Ahmad *et al*., 2014).

Compressive strength is a fundamental mechanical property of concrete and is often used as a measure of its structural integrity (Yang *et al.,* 2014). Understanding the relationship between the percentage of cement kiln replacement and compressive strength is essential for optimizing mixture designs and ensuring the performance of concrete structures (Rodríguez Viacava et al., 2012).

In recent years, there has been a growing interest in developing mathematical models to predict the compressive strength of cement kiln replacement mixtures (Rodríguez Viacava *et al*., 2012; Zeyad *et al*., 2022). These models provide valuable insights into the mechanical behavior of such mixtures and aid in decision-making processes related to material selection and mixture design. Mechanistic modeling, which involves establishing a mathematical relationship between input variables and the output response based on underlying scientific principles, offers a robust approach for developing predictive models for compressive strength (Siddique, 2006).

This study aims to develop a mechanistic model to predict the compressive strength of cement kiln replacement mixtures based on the percentage of replacement. The model will be derived using a linear regression approach, assuming a linear relationship between the replacement percentage and compressive strength. By analyzing experimental data from various replacement percentages, a mathematical equation will be established to estimate the compressive strength of cement kiln replacement mixtures. The findings of this study will contribute to the existing knowledge on cement kiln replacement and provide a useful tool for engineers and researchers in the construction industry.

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The developed model and its associated confidence intervals will assist in optimizing mixture designs, evaluating the suitability of different replacement percentages, and ensuring the desired compressive strength of concrete structures while considering sustainable practices.

Methodology

The experiments were conducted in the Civil Engineering Laboratory at Career Point University, Kota. The laboratory is equipped with the necessary facilities and equipment to perform concrete testing and analysis (Kunal *et al.,* 2012). The study focused on investigating the compressive strength of cement kiln replacement mixtures. The materials used in the experiments included Ordinary Portland Cement (OPC) as the control mix, along with alternative materials for the cement kiln replacement. The specific type and properties of these replacement materials were selected based on their availability and suitability for the study (Maslehuddin *et al.,* 2009). A series of concrete mixtures were prepared by varying the percentage of cement kiln replacement. The replacement percentages studied included 10%, 15%, 20%, 25%, 30%, and 35%. The mix proportions were determined based on a mixture design approach to ensure consistent water-cement ratio and workability across all mixtures. Cubical concrete specimens were cast using the prepared mixtures (Shoaib *et al.,* 2000). The specimens were cast in accordance with relevant standards and guidelines to ensure uniformity and consistency. After casting, the specimens were subjected to a standard curing regime, which involved moist curing at a controlled temperature for a specified duration (Shoaib *et al.,* 2000).

The compressive strength of the concrete specimens was determined using a compression testing machine. The specimens were carefully positioned in the testing machine, and a gradual load was applied until failure occurred (Udoeyo & Hyee, 2002). The maximum load at failure was recorded, and the compressive strength was calculated based on the cross-sectional area of the specimens. It should be noted that three major experiments were conducted in the laboratory, in this paper, emphasis are laid on compressive strength and the modeling of it so the authors are silent on flexural and tensile strength tests. The authors proposed to prepare the results for flexural and tensile strength tests for other articles as combining all in this paper will lead to too many pages.

The compressive strength values obtained from the testing were recorded for each replacement percentage. The data collected included the average compressive strength values for each replacement percentage, along with their corresponding standard deviations (Utsev *et al*., 2022).

A mechanistic approach was employed to develop a mathematical model for predicting the compressive strength of cement kiln replacement mixtures based on the percentage of replacement. A linear regression analysis was conducted to establish the relationship between the replacement percentage and the compressive strength. The model parameters, including the slope and y-intercept, were calculated using the least squares method (Gauch *et al*., 2003).

The methodology described above provides a detailed overview of the experimental setup, sample preparation, testing procedures, data collection, mathematical modeling, and statistical analysis involved in the study. These steps were followed to ensure the accurate assessment of the compressive strength of cement kiln replacement mixtures and the development of a reliable mathematical model. Experimental results are presented in the tables below:

Table 1. Compressive Strength Results of Cement Kiln Replacement in Concrete Mix

Cement Kiln Replacement Percentage	Compressive Strength (MPa) - Experiment 1	Compressive Strength (MPa) - Experiment 2	Compressive Strength (MPa) - Experiment 3	Average Compressive Strength (MPa)
Conventional OPC	24.3	23.4	24.0	23.9
10%	25.0	24.6	24.9	24.8
15%	24.9	24.5	25.3	24.9
20%	26.8	25.1	24.9	25.6
25%	24.1	24.6	23.9	24.2
30%	24.2	24.1	24.2	24.2
35%	24.0	24.1	22.9	23.7

Table 1 presents the experimental results of compressive strength for different cement kiln replacement percentages in a concrete mix. The table includes the cement kiln replacement percentage, as well as the compressive strength values obtained from three separate experiments. The average compressive strength column displays the average value calculated from the three experiment results.
Each row corresponds to a specific cement kiln replacement percentage, ranging from Conventional OPC (0% replacement) to 35% replacement in increments of 5%. For each replacement percentage, three experiments were conducted, and the compressive strength values (measured in megapascals, MPa) from each experiment are recorded in the respective columns.
To determine the optimal values, we can look for the cement kiln replacement percentage that yields the highest average compressive strength. From the provided table, the cement kiln replacement percentage of 20% exhibits the highest average compressive strength of 25.6 MPa.

Table 2. Tensile Strength Results of Cement Kiln Replacement in Concrete Mix

Cement Kiln Replacement Percentage	Tensile Strength (MPa) - Experiment 1	Tensile Strength (MPa) - Experiment 2	Tensile Strength (MPa) - Experiment 3	Average Tensile Strength (MPa)
Conventional OPC	3.5	3.6	3.4	3.5
10%	3.3	3.1	3.2	3.2
15%	3.0	2.9	3.1	3.0
20%	2.8	2.7	2.9	2.8
25%	3.6	3.7	3.9	3.7
30%	2.4	2.3	2.5	2.4
35%	2.2	2.1	2.3	2.2

Table 2 presents the experimental results of tensile strength for different cement kiln replacement percentages in a concrete mix. The table includes the cement kiln replacement percentage, as well as the tensile strength values obtained from three separate experiments. The average tensile strength column displays the average value calculated from the three experiment results.
Each row corresponds to a specific cement kiln replacement percentage, ranging from Conventional OPC (0% replacement) to 35% replacement in increments of 5%. For each replacement percentage, three experiments were conducted, and the tensile strength values (measured in megapascals, MPa) from each experiment are recorded in the respective columns.
To determine the optimal values, we can look for the cement kiln replacement percentage that yields the highest average tensile strength. From the provided table, the cement kiln replacement percentage of 25% exhibits the highest average tensile strength of 3.7 MPa.

Table 3. Flexural Strength Results of Cement Kiln Replacement in Concrete Mix

Cement Kiln Replacement Percentage	Flexural Strength (MPa) - Experiment 1	Flexural Strength (MPa) - Experiment 2	Flexural Strength (MPa) - Experiment 3	Average Flexural Strength (MPa)
Conventional OPC	4.2	4.3	4.1	4.2
10%	4	3.9	4.2	4
15%	3.7	3.6	3.8	3.7
20%	3.5	3.4	3.6	3.5
25%	3.8	3.7	3.9	3.8
30%	3.2	3.1	3.3	3.2
35%	3	2.9	3.1	3

The provided data presents the flexural strength values for different cement kiln replacement percentages. The flexural strength indicates the ability of a material to resist bending or deformation under applied loads (Al-Harthy *et al.,* 2003). The measurements were conducted in three separate experiments (Experiment 1, Experiment 2, and Experiment 3), and the average flexural strength was calculated for each replacement percentage.

In the case of the conventional Ordinary Portland Cement (OPC) without any replacement, the flexural strength was consistently recorded at 4.2 MPa in all three experiments. This serves as a reference point for comparison with the replacement mixtures. For the 10% replacement, the flexural strength values ranged from 4.0 MPa to 4.2 MPa across the three experiments, with an average of 4.0 MPa. As the replacement percentage increased to 15%, 20%, and 25%, the flexural strength decreased gradually, with average values of 3.7 MPa, 3.5 MPa, and 3.8 MPa, respectively. Further increasing the replacement percentage to 30% and 35% resulted in a continued decrease in flexural strength. The average flexural strength values for these replacement percentages were recorded at 3.2 MPa and 3.0 MPa, respectively. The data highlights the trend of decreasing flexural strength with an increase in the percentage of cement kiln replacement. This indicates that higher replacement percentages may result in reduced bending resistance and potentially lower structural performance.

Table 4. Average Compressive, Tensile, and Flexural Strengths for Concrete Mixes with Varying Cement Kiln Replacement Percentages

Cement Kiln Replacement Percentage	Average Compressive Strength (MPa)	Average Tensile Strength (MPa)	Average Flexural Strength (MPa)
Conventional OPC	23.9	3.5	4.2
10%	24.8	3.2	4.0
15%	24.9	3.0	3.7
20%	25.6	2.8	3.5
25%	24.2	3.7	3.8
30%	24.2	2.4	3.2
35%	23.7	2.2	3.0

In this evaluation, we analyze the average compressive, tensile, and flexural strengths of concrete mixes with different cement kiln replacement percentages. Each row in the table represents a specific cement kiln replacement percentage, while the columns display the average values of compressive, tensile, and flexural strengths for each concrete mix.
Key observations from the data are as follows:

- The conventional OPC (0% replacement) has an average compressive strength of 23.9 MPa, average tensile strength of 3.5 MPa, and average flexural strength of 4.2 MPa.
- As the cement kiln replacement percentage increases, there is a slight variation in average strength values.
- The highest average compressive strength of 25.6 MPa is observed at a cement kiln replacement percentage of 20%.
- The highest average tensile strength of 3.7 MPa is observed at a cement kiln replacement percentage of 25%.
- The highest average flexural strength of 4.2 MPa is observed with the conventional OPC (0% replacement).

This evaluation provides insights into the effect of cement kiln replacement percentages on the average compressive, tensile, and flexural strengths of concrete mixes. It aids in understanding the strength characteristics associated with different replacement percentages and assists in making informed decisions in concrete mix design and selection.

Developing Mathematical Model

To mathematically model the compressive strength, we can use a linear regression approach (Tiza et al., 2023). It was established that the relationship between the replacement percentage and the compressive strength is linear.
Let us denote the replacement percentage as "x" and the compressive strength as "y." We can write the equation for the linear regression model as follows:

$y = mx + b$

where "m" is the slope (representing the change in y for each unit change in x) and "b" is the y-intercept (representing the value of y when x is zero).

To find the values of m and b, we need to calculate them using the given data points. We'll use the method of least squares to minimize the sum of the squared differences between the predicted values and the actual values.
First, let us calculate the mean values for the replacement percentage ($\bar{x}$) and the compressive strength ($\bar{y}$):
$\bar{x} = (10 + 15 + 20 + 25 + 30 + 35) / 6 = 22.5$ $\bar{y} = (23.9 + 24.8 + 24.9 + 25.6 + 24.2 + 24.2 + 23.7) / 7 = 24.22857143$
Now, let us calculate the deviations from the mean for both x and y:
$\Delta x = x - \bar{x}$ $\Delta y = y - \bar{y}$
Next, we need to calculate the sum of the products of the deviations:
$\Sigma(\Delta x * \Delta y)$
Let us calculate this value:
$\Sigma(\Delta x * \Delta y) = (10 - 22.5) * (23.9 - 24.22857143) + (15 - 22.5) * (24.8 - 24.22857143) + (20 - 22.5) * (24.9 - 24.22857143) + (25 - 22.5) * (25.6 - 24.22857143) + (30 - 22.5) * (24.2 - 24.22857143) + (35 - 22.5) * (24.2 - 24.22857143) + (0 - 22.5) * (23.7 - 24.22857143)$
$\Sigma(\Delta x * \Delta y) = -11.25 * -0.32857143 + -7.5 * 0.57142857 + -2.5 * 0.67142857 + 2.5 * 1.37142857 + 7.5 * -0.02857143 + 12.5 * -0.02857143 + -22.5 * -0.52857143$
$\Sigma(\Delta x * \Delta y) = 3.69857143$
Next, we need to calculate the sum of the squared deviations for x:
$\Sigma(\Delta x^2)$
Let us calculate this value:
$\Sigma(\Delta x^2) = (10 - 22.5)^2 + (15 - 22.5)^2 + (20 - 22.5)^2 + (25 - 22.5)^2 + (30 - 22.5)^2 + (35 - 22.5)^2 + (0 - 22.5)^2$
$\Sigma(\Delta x^2) = 12.5^2 + 7.5^2 + 2.5^2 + 2.5^2 + 7.5^2 + 12.5^2 + 22.5^2$
$\Sigma(\Delta x^2) = 1562.5$
Now, we can calculate the slope (m) using the formula:
$m = \Sigma(\Delta x * \Delta y) / \Sigma(\Delta x^2)$
$m = 3.69857143 / 1562.5$
$m \approx 0.0023659$
Next, we can calculate the y-intercept (b) using the formula:
$b = \bar{y} - m * \bar{x}$
$b = 24.22857143 - 0.0023659 * 22.5$
$b \approx 24.1746191$
Therefore, the equation for the linear regression model is:
$y \approx 0.0023659x + 24.1746191$
This equation represents the mathematical model using the mechanistic method for the compressive strength based on the replacement percentage.
Now that we have the mathematical model for the compressive strength based on the replacement percentage, one can use this equation to predict the compressive strength for different replacement percentages.

Example
For example, if one wants to predict the compressive strength for a 40% replacement percentage, one can substitute the value of x into the equation:
$y \approx 0.0023659 * 40 + 24.1746191$
$y \approx 0.094636 + 24.1746191$
$y \approx 24.2692557$
Therefore, the predicted compressive strength for a 40% replacement percentage would be approximately 24.27 MPa.
One can continue to use the equation to predict the compressive strength for any other replacement percentages within the range of the data one have (10% to 35%). Just substitute the desired value of x into the equation and solve for y.
Please note that this mathematical model assumes a linear relationship between the replacement percentage and the compressive strength. If one has data points beyond the range of the given data, it is important to exercise caution when extrapolating the model.

Limitation of the study

The developed mathematical model for predicting compressive strength based on the replacement percentage has several limitations (Abukhashaba et al., 2014).These include a limited data range (10% to 35%), an assumption of linearity in the relationship, potential lack of generalizability to different conditions and materials, reliance on specific statistical assumptions, the possibility of confounding variables, the quality of data used, the need for external validation, and subjective model selection. It is important to consider these limitations when interpreting the results and applying the model. Further research, validation, and sensitivity analysis are recommended to improve the model's accuracy and reliability.

Result and Discussion

The result of the mathematical model using linear regression indicates that there is a linear relationship between the replacement percentage and the compressive strength. The equation obtained, $y \approx 0.0023659x + 24.1746191$, represents the relationship between the two variables. In the example provided, when the replacement percentage is 40%, the predicted compressive strength is approximately 24.27 MPa. This value is obtained by substituting $x = 40$ into the equation. It is important to note that the accuracy of the predictions relies on the assumption that the relationship between the replacement percentage and the compressive strength is linear, as established in the model. However, it is crucial to exercise caution when extrapolating the model beyond the range of the given data. Extrapolation may introduce uncertainties and potential inaccuracies. Additionally, it is worth considering that linear regression assumes certain assumptions, such as linearity, independence of errors, and homoscedasticity. It would be prudent to assess whether these assumptions hold true for the given data and adjust the model accordingly if needed. Overall, the developed mathematical model provides a starting point for predicting compressive strength based on the replacement percentage, but further evaluation and validation are recommended before relying on the model for critical applications.

Future of the Research

While this study establishes a mechanistic model for predicting compressive strength of cement kiln replacement mixtures through linear regression, future research could explore non-linear modeling to capture complex relationships, analyze multi-factor influences such as curing conditions and aggregate properties, validate the model across diverse scenarios, study long-term durability and sustainability metrics, develop optimization strategies for desired strength and sustainability goals, conduct field studies for real-world validation, investigate innovative replacement materials, extend the model to predict other concrete properties, and perform life cycle assessments for a comprehensive understanding of environmental impact. These avenues would collectively enhance the model's accuracy, practicality, and contribution to sustainable concrete technology and construction practices.

Conclusion

In conclusion, a mathematical model based on linear regression was developed to predict compressive strength using the replacement percentage. The model provides an initial approximation of the relationship between these variables. However, it is important to be aware of the limitations of the study, such as the limited data range, the assumption of linearity, and potential lack of generalizability. The model's accuracy and reliability should be further evaluated through external validation and consideration of other influencing factors. Additionally, alternative modeling approaches may be explored to improve the predictions (Agwa & Ibrahim, 2019). Ultimately, the developed mathematical model serves as a starting point for understanding the relationship between replacement percentage and compressive strength, but further research and refinement are necessary for practical applications.

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Research Paper

Hazard Profile in Landscaping: Determination of Operators Noise Exposure for Work Process Safety

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Abstract: The first step in dealing with noise in the workplace is to identify the nature, processes, and areas where excessive noise exposure occurs, regardless of the use of hearing protection. In this study, the intensity of noise emitted by lawnmowers operated by groundskeepers in Abeokuta, Nigeria, was measured and evaluated under the National Institute for Occupational Safety and Health (NIOSH) recommended workplace exposure limit using a digital noise meter (Model Benetech GM 1351). The study observed a range of 85.78-90.55 dBA for an 8-hour TWA. The lawnmowers evaluated were 100% unsafe for noise exposure at work. This, therefore, required the effective use of personal protective equipment by workers to protect their hearing.

Keywords: *Exposure, noise, hearing, occupation, safety*

Introduction

In every work environment, there is a predominant and potentially dangerous problem (Azodo *et al*, 2018). Safety and health risk assessment typically begins with identifying the nature, operations, and areas that may be at risk and providing appropriate control measures to create an ideal safe workplace. The classification of noise as hazardous at work is a function of a combination of its frequency, intensity, and duration, with due regard to worker safety and health. Although noise is associated with work processes involving mechanised equipment and tools, it is often one of the most common preventable occupational health hazards prevalent in various occupational dispensations.

Groundskeepers's work routines include the use of mechanized equipment and tools in their mowing and trimming duties (Balanay *et al*. 2016; Bureau of Labour Statistics, 2014; OSHA, 2015). Studies have found that power tools produce continuous noise that may be less intense when compared to intermittent, regular, and irregular noise sources. Chung *et al*. (2012) expressed that exposure to continuous noise carries a greater risk of hearing loss than intermittent exposure, even if the mean range in A-weighted decibels is similar. Exposure to noise levels well over the established exposure standard could be harmful to exposed workers (Plontke and Zenner, 2004). For any workplace exposed to noise, there are established guidelines on the limit of A-weighted equivalent sound pressure level (LAeq) and exposure time for a 100 percent dose of noise that an unprotected worker should be exposed to in the work environment. Given the focus of this study, Table 1 shows the National Institute for Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration (OSHA), and Federal Environmental Protection Agency (FEPA) specifications for relative noise exposure limits for exposure. From Table 1, it can be seen that the noise intensity varies inversely with the duration of exposure concerning the hearing safety of workers. The reassessment and confirmation of occupational noise exposure at or above which noise levels are classified as hazardous by NIOSH is 85 dBA as an 8-hour time-weighted average (NOISH, 1998).

Noise, whether irregular, intermittent, or statistically random, changes the air pressure in the natural environment that is transmitted to the ear by sound waves. The received sound waves are then converted into electrical signals by sensitive hair cells called cilia in the inner ear or cochlea. These signals or nerve

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impulses are transmitted from the auditory nerve to the brain and interpreted as sound. The properties of noise that are important in the workplace are frequency, sound pressure, sound power and temporal distribution. The classification of noise as dangerous is a combination of frequency, noise intensity and duration that can lead to permanent hearing loss. Concerns about the impact of noise on workers as a result of intensity and time distribution are impaired communication, problems concentrating, stress from overwork, safety risks, productivity and profitability, acoustic shock and ototoxic chemicals. These effects contribute to accidents and injuries in the workplace by making it difficult for workers to hear warning signals.

Table 1. NIOSH, OSHA and FEPA specification for noise exposure limits for a 100% noise dose

A-weighted equivalent sound pressure level (dBA)		**Noise exposure duration (hours)**
NIOSH	**OSHA and FEPA**	
85	90	8
88	95	4
91	100	2
94	105	1
97	110	0.5
100	115	0.25

Source: NOISH, 1998; FEPA, 1991; OSHA, 1983

Noise exposure is one of the most common health problems in the workplace. Every year, thousands of workers are exposed to workplace noise hazards that result in preventable hearing loss. Statistical reports on noise exposure at work are not available for most developed and non-developed countries; Tips on the status of exposure to noise at work in countries such as South America, Africa and Asia were summarized from various studies that Nelson *et al*. carried out in these countries Nelson *et al*., 2005. with high noise exposure at the workplace. Studies on occupational noise exposure conducted in various professional organizations in Nigeria included: automobile assembly (Oleru, 1980), textile mill (Oleru *et al*., 1990 ; Osibogun *et al*., 2000), cable and wire production industry (Anjorin *et al*., 2015), wood processing factory (Anjorin *et al*., 2015), sawmill (Eziyi *et al*., 2015), soft drink- Bottling industry (Oyedepo and Saadu, 2010), tobacco industry (Oyedepo and Saadu, 2010), mineral crushers (Oyedepo and Saadu, 2010), beer brewing and bottling industry (Oyedepo and Saadu, 2010), sack manufacturing industry (Ismaila and Odusote, 2014). Other areas of noise investigation were traffic noise (Onuu, 1992), environmental noise (Adeke *et al*., 2018; Akinkuade and Fasae 2015; Anomohanran, 2013; Oyedepo, 2012; Ibhadode *et al*., 2018) and generator noise (Azodo and Adejuyigbe, 2013; Azodo *et al*., 2018; Otutu, 2011). The hazard profile in landscaping must be established to determine the acoustic exposure of the operator to the safety of the work process. Therefore, in this study, the maximum output intensity of noise emanating from a lawnmower used by the groundskeeper at Abeokuta and the associated safe exposure level were evaluated using the combination of noise exposure levels and duration criteria for a recommended standard occupational exposure limit.

Materials and Methods

This study was conducted to measure and evaluate the intensity of noise emitted by lawnmowers to represent the noise exposure levels of operators whose 8-hour TWA noise exposure may be 85 dBA or more. Physical measurements were carried out for the quantitative assessment of noise pollution from lawnmowers at groundskeepers in Abeokuta, Nigeria. The design instrument used for data collection was a digital noise level meter (DNLM) (model Benetech GM 1351). The DNLM operates with an A-weighted frequency in the frequency range of 31.5 to 8 kHz and measures a sound level in a range of 30 to 130 dBA. The consistency of the sound level measurements was made possible by the precise internal calibration of Benetech's DNLM and set to a slow response corresponding to a time constant of 1 s. The resolution setting of the digital noise level meter was 0.1 dB with an accuracy of ±1.5 dB. A total of 14 lawnmowers were used by the groundskeepers, five of which were weed killers, seven push lawnmowers and two tractor lawnmowers. To assess and record noise levels, each of the 14 lawnmowers evaluated in this study was assigned an alphanumeric code from G1 to G14. A digital sound level meter carrier has been designed to be carried by the worker during his work process. The improved design attached the DNLM to the worker's clothing with the microphone close to the ear. Measurements were taken and recorded after an exposure time interval of 5 minutes every hour for each of the designated lawnmowers at the maximum option setting. Working hours were 8:00 a.m. to 12:00 p.m. (4 hours) and 1:00 p.m. to 3:00 p.m. (2 hours). The collection of data on the noise level of the individual groundskeepers extended over a total period of five weeks. This resulted in a total of 6 measurements per day for 6 hours of work and a total of 30 measurements per participant for the 5-week work exposure. The A-weighted equivalent sound pressure level (LAeq) has been calculated to give a single constant noise level value representing an equivalent total sound energy to which groundskeepers are exposed while on duty during the assessment period. This calculation was in the form of an A-weighted equivalent sound pressure level (LAeq) using Eq. (1) below (Oyedepo *et al.*, 2019).

$$L_{Aeq} = 10\log_{10}\left[\frac{1}{N}\sum_{i=1}^{N}\left(anti\log\frac{L_{Ai}}{10}\right)\right] \quad (1)$$

Where
L_{Aeq} = A-weighted equivalent sound pressure level
L_{Ai} = A-weighted sound pressure level in dB
i = 1, 2, 3… N
N = total number of measurements

In addition, the daily duration of each groundsman's working time was recorded for an 8-hour conversion representing the daily noise exposure level using the equivalent International Standards Organization (ISO) (3) formula. The time-weighted average (TWA) noise level and noise dose were calculated to indicate workers' exposure to occupational noise, normalized to 8 hours (hrs) per day, taking into account the calculated A-weighted equivalent sound pressure level (LAeq) (equation 4) and the exposure time during the work process (Eq. 2). Occupational noise exposure, which is a combination of exposure level (L) and duration (T), was assessed using the expression (NOISH, 1998).

$$T_n(\text{min}) = \frac{480\ (min)}{2^{(L-85)/3}} \quad (2)$$

Where
L = The combination of exposure level
T_n = Exposure duration for which noise at this level becomes hazardous
3 = the exchange rate

Whereas the daily dose (D) of the noise exposure for each of the sessions at different noise levels obtained was calculated according to (NOISH, 1998) the following formula:

$$D = \left[\frac{C_1}{T_1} + \frac{C_2}{T_2} + \cdots + \frac{C_n}{T_n}\right] \times 100 \quad (3)$$

Where
C_n = Total time of exposure at a specified noise level, and
T_n = Exposure duration for which noise at this level becomes hazardous
The daily dose was converted into an 8-hour TWA using (NOISH, 1998) the formula

$$TWA = 10.0 \times log\left(\frac{D}{100}\right) + 85 \quad (4)$$

The assessment of noise intensity levels from lawnmowers on groundskeepers for safety and health risk analysis was performed with reference to the revised recommended standard criteria for noise exposure at work from the National Institute for Occupational Safety and Health (NOISH, 1998) (see Table 1). The NIOSH specification for noise exposure limits for a noise dose of 100% was adopted for the safety analysis in this study because OSHA amended its noise standard to include specific hearing protection program provisions for occupational exposures at 85 dBA or greater (Department of Labour, 1981; US Department of Labour, 1983). The amended OSHA noise standard does not cover all industries (NOISH, 1998). In addition to comparing excessive risk estimates developed by the National Institute for Occupational Safety and Health (NIOSH), the Environmental Protection Agency (EPA), and the International Standards Organization (ISO) for material hearing damage caused by an average daily exposure to noise in the workplace over a period of 40 to 30 years. According to NIOSH, working years resulted in a higher excess risk percentage (NOISH, 1998). The time-weighted average (TWA) noise levels obtained for each of the workers were classified as safe and unsafe noise exposure levels according to the interpretation guide in Table 1.

$$Spl = \frac{\sum(wf_i.t_i)}{\sum t_i} \in [0,1] \qquad (1)$$

$$\begin{bmatrix} Spl_{unsafe} \text{ with } wf_i = \begin{bmatrix} 1 \; if \; I_{sound\ level} > I_{85\ dBA} \\ 0 \; if \; I_{sound\ level} \le I_{85\ dBA} \end{bmatrix} \\ Spl_{safe} \text{ with } wf_i = \begin{bmatrix} 1 \; if \; I_{sound\ level} < I_{85\ dBA} \\ 0 \; if \; I_{sound\ level} \ge I_{85\ dBA} \end{bmatrix} \end{bmatrix}$$

Where

Spl = Sound pressure level
t_i = Time in hours
wf_i = A-weighting factor variable which depends on $I_{sound\ level}$ values

Table 4. Acoustic risks and safety analysis interpretation of the noise intensity level from lawnmowers on groundskeepers for 85 dBA as an 8-hour TWA

TWA noise levels	85 dBA as an 8-hour TWA exposure level interpretation
≤85dBA	safe noise exposure levels
≥85dBA	unsafe noise exposure levels

Results and Discussions

Table 5 shows the noise level measurement recorded with the noise level meter and the evaluated noise descriptors of the 14 lawnmowers used by the groundskeepers. The average range of the measured noise level (Lav) was 94.11 - 99.8 dBA. The lowest noise level measured (Lmin) was 86.1 dBA, while the highest (Lmax) was 109.9 dBA. The noise descriptor ranges were 89.7–95.46 dBA, 93.2–100.25 dBA, 97.19–105.31 dBA and 95.16–102.89 dBA for the 10th percentile (L10), the 50th percentile (L50), the 90th percentile (L90) and the A-weighted equivalent sound pressure level (LAeq) respectively (Table 5). The assessment of the potential for lawnmower occupational hearing loss in lawnmowers was performed by reference to the National Institute for Occupational Safety and Health's revised recommended standard occupational noise exposure criteria for a combination of noise exposure levels and duration criteria. This was assessed using the NIOSH recommended occupational noise exposure limit of 85 A-weighted decibels (85 dBA) for an 8-hour time-weighted average exposure period (8-hour TWA). The Time-Weighted Average (TWA) criterion is the measured noise levels and safety levels at the workplace for each worker. If a worker's exposure exceeds 85 dBA on the 8-hour time-weighted average (TWA), this simply means that the exposure level is unsafe, and therefore a hearing loss prevention program is required. Analysis of the data obtained for five weeks assessment presented in Table 5 showed that the occupational exposure levels for the assessed groundskeepers were all above the NIOSH recommended exposure limit for occupational noise exposure of 85 decibels, A-weighted, as an 8-hour time-weighted average (85 dBA as an 8-hour TWA).

The range of an 8-hour time-weighted average over five (5) weeks was 85.78 - 90.55 dBA. The high baseline noise intensity observed in this study at 85 dBA as an 8-hour TWA is similar to other studies that have assessed workplace noise exposure (Kelly *et al*, 2012; Lao *et al*., 2013). Due to the health effects of the intensity and duration of worker exposure to noise, an analysis of individual users' safety regimens for safe to unsafe operational characteristics (equal to or above 85 dBA) revealed a 100% unsafe level of occupational noise exposure among participants as a total time-weighted average (TWA) The Noise levels measured for the mower evaluated exceeded 85 dBA. This is of concern as continuous and prolonged exposure to excessive or repetitive sound above 85 dB in everyday work life is potentially dangerous, often resulting in hearing loss (Azodo & Adejuyigbe, 2013; Azodo *et al*., 2018; Green and Anthony, 2015). The damaging effect of noise is insidious and only becomes apparent when the victim has been impaired over the years while maintaining normal hearing, as dangerously loud noises are hazardous even if they are not painful, and pain only occurs at 120-140 decibels (Roland-Mieszkowski, 1994).

Table 5. Average noise descriptors from the lawnmowers to the groundskeepers over a period of five weeks

Groundskeepers (G)	L_{max}	L_{min}	L_{10}	L_{50}	L_{90}	L_{av}	L_{Aeq}	T_n	DOSE (%)	*TWA*
1	102.4	90.7	91.87	95.95	101.5	95.92	97.59	6.86	1373	86.37
2	109.9	90.6	94.02	100.25	105.31	99.8	102.89	27.75	5551	90.55
3	101.8	90.4	91.66	95.35	101.71	96.18	97.67	7.01	1403	86.40
4	104.6	91.8	91.89	93.20	104.42	96.73	99.97	11.42	2284	87.28
5	98.7	89.9	91.16	95.35	98.07	94.68	95.47	4.17	834	85.83
6	102.1	91.8	92.43	95.65	100.39	95.89	97.14	5.98	1197	86.20
7	100.2	91.4	91.94	96.00	97.59	95.68	96.33	5.31	1061	86.06
8	101.0	94.4	94.76	95.95	100.55	96.78	97.44	6.04	1208	86.21
9	99.7	86.1	89.7	94.75	98.08	94.11	95.52	4.02	804	85.80
10	100.5	93.9	94.35	98.00	99.96	97.37	97.91	7.17	1435	86.43
11	98.0	90.4	92.11	95.05	97.19	94.67	95.16	3.89	778	85.78
12	104.4	94.2	95.46	97.95	102.96	98.56	99.76	11.24	2248	87.25
13	101.4	90.1	91.18	97.85	100.68	97.09	98.25	7.74	1548	86.55
14	103.9	92.9	92.9	98.70	102.19	98.18	99.77	11.16	2233	87.23

Conclusion

In this study, the intensity of the noise emitted by a lawnmower used by the groundskeeper was measured and the associated safe exposure level was assessed using the combination of noise exposure levels and duration criteria for a recommended occupational standard. Analysis of the data obtained using the criteria for a recommended occupational exposure standard revealed that the occupational exposure levels for the groundskeepers assessed were all above the NIOSH recommended occupational noise exposure limit of 85 decibels, A-weighted, as an 8-hour time-weighted average (85 dBA as an 8-hour TWA). Due to the health effects of the intensity and duration of worker exposure to noise, an analysis of individual users' safety regimens for safe to unsafe operational characteristics (equal to or above 85 dBA) revealed a 100% unsafe level of occupational noise exposure among participants as a total time-weighted average (TWA) The Noise level of the evaluated lawn mower exceeded 85 dBA. This requires proactive safety measures through the use of personal protective equipment such as earmuffs or earplugs for workers' hearing protection.

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Research Paper

The role of the Media in Raising Ecological-Environmental Awareness in Tetova and the Surrounding area

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Abstract: Citizen protests were started in 2013 by NGOs against air pollution which lasted for several years and initially had a small number of participants but over the years this number began to increase continuously. In this context, the purpose of this paper was to see how traditional and social media have influenced the information and awareness of the population about ecological-environmental problems. To conduct this paper, a questionnaire was prepared with different questions from the field of environmental problems and the way of getting informed about them. Applying the theoretical methods of analysis, synthesis, induction, and deduction as well as the main survey method, we surveyed casual citizens, high school students, undergraduate students, and experts. A total of 136 respondents participated in the survey, of which 15 citizens, 32 students, 84 high school students, and 5 environmental experts. Respondents to the questions posed were able to answer according to the answers required: do not agree at all, do not agree, do not know, agree, and completely agree. The survey showed that the majority of respondents to the questions posed answered with agree. This shows that the media have played a positive role in properly and objectively informing the population about environmental-ecological problems which enabled the increase in the number of protesters and forced the authorities to accept the situation and shut down one of the largest air pollutants in the city.

Keywords: *pollution, social media, ecological awareness, air, survey.*

Introduction

The changes that began in many parts of the world during the nineties also affected the Balkan countries, in which case the transition from a totalitarian to a democratic system began. This was of course accompanied by legal changes in various areas and in this regard in the field of environment as well. The Republic of North Macedonia brought and adapted almost all standardized laws of the EU that were related to the environment.

Tetova and the surrounding area, as well as many other cities in North Macedonia, were facing numerous problems in the field of environment. But, one of the many problems faced by the citizens of Tetova and the surrounding area, which most affected the life and health of the population was the enormous air pollution. However, due to the old legal standards for air pollution, they did not have the opportunity to protest, as according to the law everything was considered normal. But, with the adoption of the new standards which came into force in January 2012, the population was given the legal right to react to the extremely high air pollution in Tetova. The first reactions started as a civic initiative with the organization of protests in December 2013. This initiative enabled the formation of the first NGOs such as "Eco guerila". It should be noted that the first protests had a very small participation of the population, and that is not because they were against protesting but there was no high awareness of participating in protests and reacting.

The demands of the protesters were not even heeded by the government, they even opposed them. It was this arrogant and irresponsible behavior of the government that mobilized even more now the leaders of the NGO "Eco guerila" who in a way became the leaders and forerunners of the protests.

The protest organizers launched an extensive campaign collaborating primarily with experts in the field, students, pupils, citizens and the media. This enabled the forthcoming protests which lasted until 2016, to force the state bodies not only to sit down, talk and take measures to prevent air pollution, but

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also to accept and close one of the main sources of air pollution pollution which did not respect the working standards. In this regard, we must emphasize that in addition to the persistence of the organizers, an extremely large role has been given by experts who with their knowledge and scientific results not only showed the main source of pollution but with these scientific results forced the leading bodies of the plant and state to accept them. But, we must emphasize that all this activity has been constantly followed and stimulated by the media, which have correctly informed citizens about environmental pollution, their rights as citizens, obligations and duties, through informative programs, interviews with environmental experts, as well as various articles and interviews with citizens.

Methodology of Research

Subject of research

Given the fact that North Macedonia had already brought all the laws in the field of environment starting from the ***law on the environment*** and all other laws deriving from this law adapted and harmonized with those of the EU and the specifics of the country, where the limit values of pollutants in a certain environment are clearly defined, and the law on free access to public information, as can be seen below from Articles 17 and 18 of the Law on Environment.The principle of public participation and access to information(URL.01) *The government bodies and the municipal bodies of the City of Skopje and of the municipalities in the City of Skopje, are obliged to provide all necessary measures and describe the procedures that ensure the realization of the right of access to information and public participation in decision-making, regarding the state of the environment, as well as to provide a public statement in the decision-making process* (Government of the Republic of Macedonia, 2006).

The principle of raising public awareness of the importance and protection of the environment (URL.02). *Scientific, educational, health, information, cultural and other legal institutions, including citizens' associations, within their activities, promote and ensure public awareness of the environment, its importance, as well as the need for active participation in its promotion and protection* (Government of the Republic of Macedonia, 2006).

Based on these data arising from the legal regulations, the purpose of this paper was to see how much the population has knowledge about: environmental problems, rights, duties and obligations provided by law, how much, and by whom they are informed about these environmental problems. How much the media have influenced the promotion, information and raising awareness of environmental problems. Starting from this purpose and to answer the questions posed we have used various non-experimental empirical methods; theoretical method of analysis, synthesis, methods of induction and deduction. The main method which was applied in this research was the survey method. So, to see what was the role of the media in raising public awareness of ecological-environmental problems, we have submitted a questionnaire with questions from the field of environment and how they are informed about these issues. The questions asked in the questionnaire are mainly related to obtaining knowledge of respondents on environmental issues and problems, their rights, duties and obligations arising from environmental laws and the ways they were informed about environmental problems. The survey included citizens from different categories of society, starting from: high school students, undergraduate students, casual citizens across the city and experts. Respondents to the questions posed were able to give their answers through these required answers as: ***strongly disagree, disagree, don't know, agree and strongly agree.*** The questionnaire as in Table1 was submitted and completed directly, but also through emails. Data were collected and processed according to the questions asked and the category of respondents.

Table 1. Questionnaire for: high school students, university students, citizens, experts

No.	Institution	Strongly disagree	Disagree	Don't know	Agree	Strongly agree
	High school, Faculty, institution:	**1**	**2**	**3**	**4**	**5**
1	Do you think the main parameters of the living environment: soil, air, water today are more endangered than ever before?					
2	***The current pollution situation in Tetova and the surrounding area today is better than a few years ago but not yet at the right level.***					
3	Do you think that with the cessation of the work of the Jugokrom plant the level of air pollution has decreased?					
4	***The main source of pollution of the environment, air, water and soil in Tetovo and the surrounding area comes from the use of fossil fuels.***					
5	The main activities of air, water and soil pollution in the city of Tetovo are, industry, traffic, solid waste and household heating.					
6	Air pollution in Tetovo poses the greatest risk to human life and health and requires priority solutions.					
7	Municipal solid waste pollution is the most dangerous pollution and requires priority solutions.					
8	Do you think the lack and supply of drinking water is the most important problem in the city?					
9	Are we informed about our legal rights guaranteed by the law on environmental problems?					
10	***Informing platforms, social media influenced your information on environmental problems.***					
11	I am mostly informed about environmental problems and activities from social media.					
12	The informing platforms and social media are captured, do not inform correctly?					
13	How much are you interested in and contributing to environmental problems?					
14	Have you participated in actions, organized protests for environmental problems, cleaning, voluntary actions?					
15	***Are the bodies working towards solving environmental problems?***					
Total						

Results

Analysis and discussion of the answers given by the respondents about the environment

The questions asked to the respondents were formulated in such way that we wanted to get information about:

- Knowledge that the population has about environmental problems and issues,
- Rights, duties, obligations and their participation in environmental activities and actions guaranteed by law,
- Their way of being informed about environmental problems.

We have arranged and analyzed all the collected answers according to the group of respondents. The data are presented in tables and diagrams.

Analysis of the answers given by high school students

In Table 2 below we have presented the answers given by the students to all the questions posed, expressed as numbers and as percentages (%).

Table 2. Students' answers according to the the approximate questionnaire for all questions indicated in numbers and in %

No	Respondents		Answers to survey questions expressed in numbers															Total	
	Category	No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Ank.	%
I	*Students*	*84*																	
1	*Strongly disagree*		0	6	2	1	0	1	1	0	4	6	5	6	3	8	23	66	5
2	*Disagree*		1	20	7	1	0	0	1	12	7	3	9	17	3	15	30	126	10
3	*Don't know*		4	11	16	24	8	4	13	5	41	14	16	33	30	6	20	245	20
4	*Agree*		25	35	38	43	30	22	37	34	23	35	36	15	28	24	3	428	**34**
5	*Strongly agree*		54	12	21	15	46	57	32	33	9	26	18	13	20	31	8	395	31
Total			84	84	84	84	84	84	84	84	84	84	84	84	84	84	84	126 0	
No	**Respondents**		**Answers to survey questions expressed in %**															**Total**	
I	*Students*	*84*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Ank.	%
1	*Strongly disagree*		0	7	2	1	0	1	1	0	5	7	6	8	4	10	27	79	5
2	*Disagree*		1	24	9	1	0	0	1	15	8	4	11	20	4	18	36	152	10
3	*Don't know*		5	13	19	29	10	5	16	6	49	16	19	39	36	6	24	292	20
4	*Agree*		30	42	45	51	36	26	44	40	27	42	43	18	32	29	3	508	**34**
5	*Strongly agree*		64	14	25	18	54	68	38	39	11	31	21	15	24	37	10	469	31
Total %			10 0	10 0	10 0	10 0	10 0	10 0	10 0	10 0	10 0	10 0	10 0	10 0	10 0	10 0	10 0	**150 0**	10 0

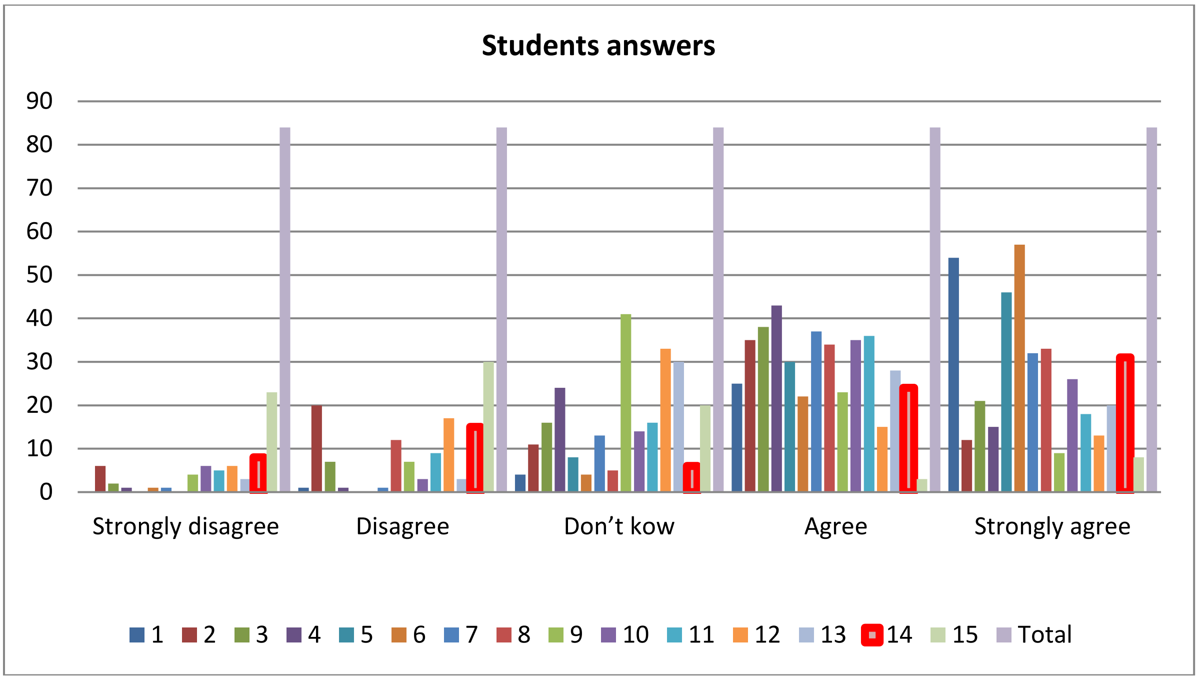

Figure 1. Answers given by students according to the approximate questionnaire for all questions indicated by numbers

As can be seen from Table 2 expressed in percentage, respondents with a higher percentage-which is marked in green in all questions posed answer Agree with 34% and Strongly Agree with 31%.

Analysis of the answers given by undergraduate students

Table 3. Students' answers given according to the approximate questionnaire for all questions indicated in numbers and in%

No	Respondents		Answers to survey questions expressed in numbers															Total	
	Category	No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Ank	%
II	***Students***	***32***																	
1	*Strongly disagree*		1	2	0	0	0	0	0	0	2	3	6	0	0	3	14	31	7
2	*Disagree*		1	9	7	4	0	2	2	2	0	2	1	2	1	4	6	43	9
3	*Don't know*		0	5	2	12	2	1	0	3	20	8	6	11	8	0	8	86	18
4	*Agree*		12	11	16	12	20	9	24	9	9	16	16	12	14	12	2	194	**40**
5	*Strongly agree*		18	5	7	4	10	20	6	18	1	3	3	7	9	13	2	126	26
Total			32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	**480**	
No	**Respondents**		**Answers to survey questions expressed in %**															**Total**	
II	***Students***	***32***	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**Ank**	%
1	*Strongly disagree*		3	6	0	0	0	0	0	0	6	9	19	0	0	8	44	95	7
2	*Disagree*		3	28	22	13	0	6	6	6	0	6	3	6	3	13	19	134	9
3	*Don't know*		0	16	6	37	6	3	0	10	63	26	19	34	25	0	25	270	18
4	*Agree*		38	34	50	37	63	28	75	28	28	50	50	38	44	38	6	607	**40**
5	*Strongly agree*		56	16	22	13	31	63	19	56	3	9	9	22	28	41	6	394	26
Total %			**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**1500**	**100**

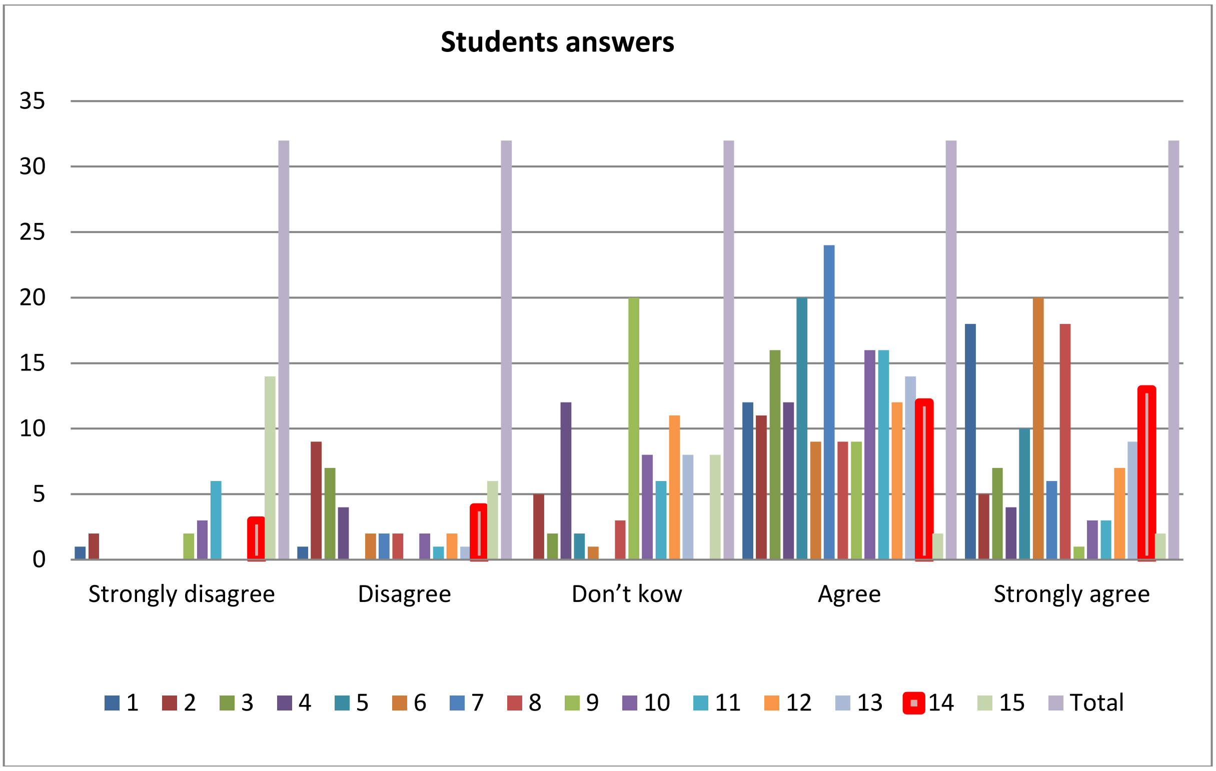

Figure 2. Answers given by students according to the approximate questionnaire for all questions indicated by numbers

Table 3 provides the answers of the respondents - students to all the questions posed. In the table, the answers with the highest percentage to each question are marked in green. Students answered **Agree** with 40% .

Analysis of the answers given by citizens

Table 4. Answers given by citizens according to the approximate questionnaire for all questions indicated in numbers and in %

No	Respondents category	No	Answers to survey questions expressed in numbers															Total	
II	***Citizens***	***15***	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**Ank**	**%**
1	*Strongly disagree*		0	3	2	3	0	2	0	0	6	0	0	0	2	0	6	24	11
2	*Disagree*		0	3	2	3	0	4	3	0	5	0	0	4	4	0	3	31	14
3	*Don't know*		3	4	3	4	3	4	2	0	4	0	0	6	5	3	5	46	20
4	*Agree*		7	3	5	3	7	2	6	10	0	10	7	3	4	7	1	75	33
5	*Strongly agree*		5	2	3	2	5	3	4	5	0	5	8	2	0	5	0	49	22
	Total		15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	**225**	
No	**Respondents**		**Answers to survey questions expressed in %**															**Total**	
III	***Citizens***	***15***	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**Ank**	**%**
1	*Strongly disagree*		0	20	13	20	0	13	0	0	40	0	0	0	13	0	40	159	11
2	*Disagree*		0	20	13	20	0	27	20	0	33	0	0	27	27	0	20	207	14
3	*Don't know*		20	27	20	27	20	27	13	0	27	0	0	40	33	20	33	307	20
4	*Agree*		47	20	34	20	47	13	40	67	0	67	47	20	27	37	7	493	33
5	*Strongly agree*		33	13	20	13	33	20	27	33	0	33	53	13	0	43	0	334	22
	Total %		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	**1500**	100

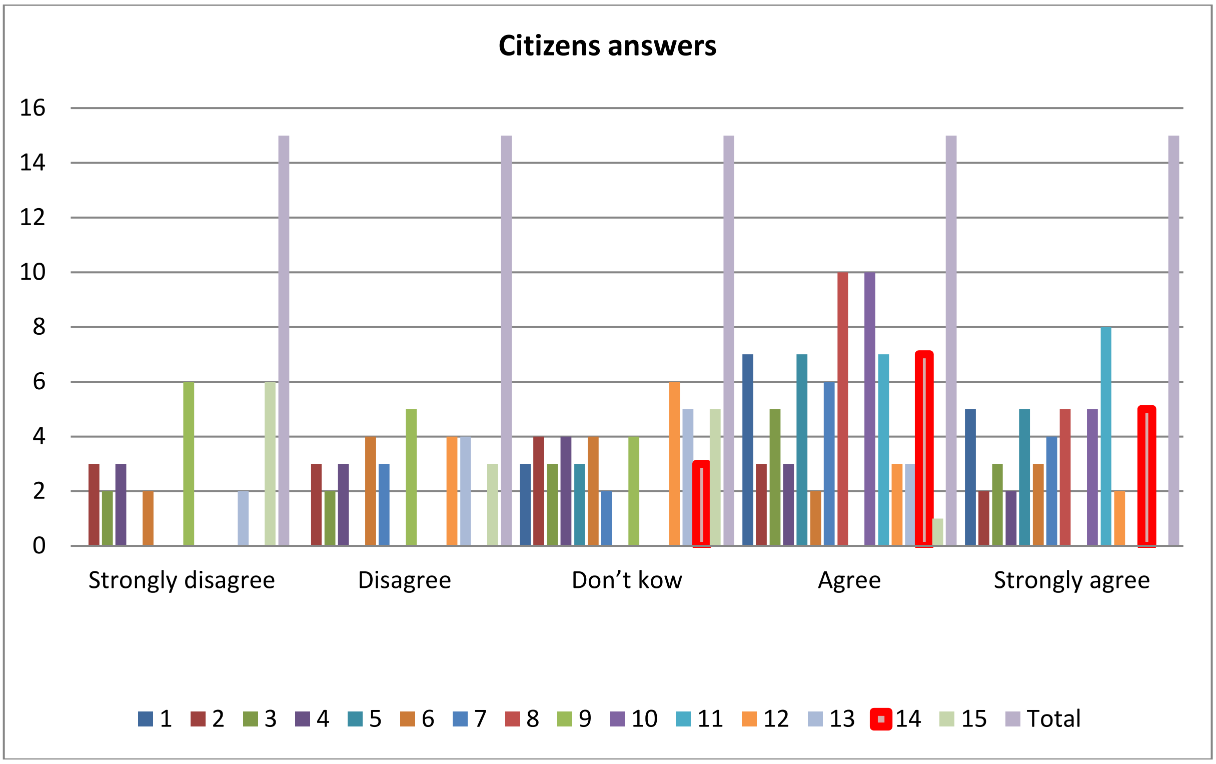

Figure 3. Answers given by citizens according to the approximate questionnaire for all questions indicated by numbers. Citizens answered **Agree** with 33% .

Analysis of the answers given by experts

Table 5. Answers given by experts according to the approximate questionnaire for all questions indicated in numbers and in%

No	Respondents		Answers to survey questions expressed in numbers															Total	
	Category	No																	
IV	***Experts***	***5***	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**Ank**	%
1	*Strongly disagree*		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	*Disagree*		0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	3
3	*Don't know*		0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	3	4
4	*Agree*		3	0	0	2	0	3	2	1	3	4	2	2	3	4	4	33	44
5	*Strongly agree*		2	5	5	3	5	2	1	4	2	0	3	1	2	1	1	37	**49**
Total			5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	**75**	
No	**Respondents**		**Answers to survey questions expressed in %**															**Total**	
IV	***Experts***	***5***	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**Ank**	%
1	*Strongly disagree*		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	*Disagree*		0	0	0	0	0	0	40	0	0	0	0	0	0	0	0	40	3
3	*Don't know*		0	0	0	0	0	0	0	0	0	20	0	40	0	0	60	120	8
4	*Agree*		60	0	0	40	0	60	40	20	60	80	40	40	60	80	20	600	40
5	*Strongly agree*		40	100	100	60	100	40	20	80	40	0	60	20	40	20	20	740	**49**
Total %			100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	**1500**	100

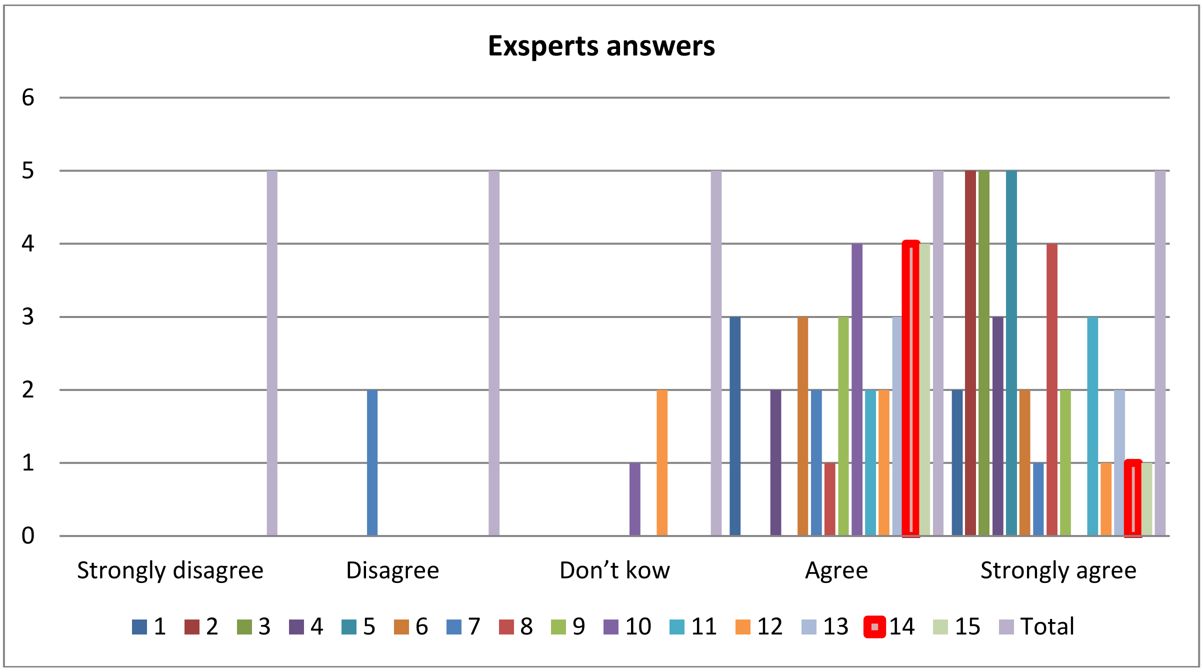

Figure 4. Answers given by experts according to the approximate questionnaire for all questions indicated by numbers

Experts, in contrast to other respondents who respond with a higher percentage of **Agree,** answered with a higher percentage of 49% with **Strongly Agree.**

Analysis and comparison of answers given by respondents

To compare and analyze how the respondents answered the questions posed, the results from their answers are presented in a common table. But, since the number of respondents was not the same to do this analysis, the answers to the given questions will be presented in percentage (%).

Table 6. Answers given to the respondents according to the approximate questionnaire for all questions expressed in percentage %

No	Respondents		Answers to survey questions expressed in%															Total
I	*Students**	84	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Ank.	%
1	*Strongly disagree*		0	7	2	1	0	1	1	0	5	7	6	8*	4	10	27	79	5
2	*Disagree*		1	24	9	1	0	0	1	15	8	4	11	20	4	18	36	152	10
3	*Don't know*		5	13	19	29	10	5	16	6	49	16	19	39	36	6	24	292	20
4	*Agree*		30	42	45	51	36	26	44	40	27	42	43	18	32	29	3	508	34
5	*Strongly agree*		64	14	25	18	54	68	38	39	11	31	21	15	24	37	10	469	31
	Total %		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	1500	100
II	*Students*	22	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Ank.	%
1	*Strongly disagree*		3	6	0	0	0	0	0	0	6	9	19	0	0	8	44	95	7
2	*Disagree*		3	28	22	13	0	6	6	6	0	6	3	6	3	13	19	134	9
3	*Don't know*		0	16	6	37	6	3	0	10	63	26	19	34	25	0	25	270	18
4	*Agree*		38	34	50	37	63	28	75	28	28	50	50	38	44	38	6	607	40
5	*Strongly agree*		56	16	22	13	31	63	19	56	3	9	9	22	28	41	6	394	26
	Total %		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	1500	100
III	*Citizens*	15	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Ank.	%
1	*Strongly disagree*		0	20	13	20	0	13	0	0	40	0	0	0	13	0	40	159	11
2	*Disagree*		0	20	13	20	0	27	20	0	33	0	0	27	27	0	20	207	14
3	*Don't know*		20	27	20	27	20	27	13	0	27	0	0	40	33	20	33	307	20
4	*Agree*		47	20	34	20	47	13	40	67	0	67	47	20	27	37	7	493	33
5	*Strongly agree*		33	13	20	13	33	20	27	33	0	33	53	13	0	43	0	334	22
	Total %		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	1500	100
IV	*Experts*	5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Ank.	%
1	*Strongly disagree*		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	*Disagree*		0	0	0	0	0	0	40	0	0	0	0	0	0	0	0	40	3
3	*Don't know*		0	0	0	0	0	0	0	0	0	20	0	40	0	0	60	120	8
4	*Agree*		60	0	0	40	0	60	40	20	60	80	40	40	60	80	20	600	40
5	*Strongly agree*		40	100	100	60	100	40	20	80	40	0	60	20	40	20	20	740	49
	Total%		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	1500	100
No	Respondents		Answers to survey questions expressed in %															Total
Total Respondents		136	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Ank.	%
1	*Strongly disagree*		1	8	3	3	0	3	1	0	9	6	8	4	3	8	32	89	5
2	*Disagree*		1	23	12	6	0	4	5	10	9	4	7	17	6	13	29	146	10
3	*Don't know*		5	15	15	29	10	7	11	6	48	17	16	38	32	7	26	282	19
4	*Agree*		35	36	44	44	42	26	51	40	25	48	45	24	36	35	5	536	36
5	*Strongly agree*		58	18	26	18	48	60	32	44	9	25	24	17	23	37	8	447	30
	Total %		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	1500	100

* *High school students*

From Table 6 we see that all respondents to all questions asked with a large percentage (green squares) answered **Agree with 36%**. In particular: high school students with 34%, undergraduate students with 40% and citizens with 33% with a higher percentage answered with **Agree**, while experts with a higher percentage of 49% answer with **Strongly Agree**.

Conclusion

From this research we can draw the following conclusions:

- North Macedonia turns out to be among the countries with high environmental pollution, according to reports from world institutions, such as WHO and World Bank.
- We are mainly dealing with air pollution, and the highest pollution at the state level was in Tetovo.

- With the entry into force of new laws and standards harmonized with those of the EU, the state is obliged to respect them. But, in fact, although the observance of the new standards for air had to start from January 2012, air pollution continued to be alarming in the city of Tetovo.

Precisely, based on these laws according to which citizens have the right:

- *To live in a clean and healthy environment,*
- *For information and free access to information,*

The informing platforms, social media and television continued to inform the general public on a daily basis about the situation with the environment, through the presentation of the situation on the ground, the reaction of the population, as well as interviews conducted by environmental and health experts.

This enabled:

- Raising awareness of the population,
- Establishment of NGOs (Eco Guerila), and
- The start of civil protests in December 2013 which continued to intensify and lasted for several years until they forced the government to take appropriate measures for air protection.

Today, we can freely say, as we saw from the results of the respondents that:

- *The citizens of Tetovo and the surrounding area are well informed about environmental problems, their rights and obligations, and*
- *Due to this and their reaction in Tetovo we have much cleaner air, but not yet according to the allowed limit values.*
- *That the media-social networks with which citizens are constantly connected through electronic devices, have made a very important contribution to informing and sensitizing the population about environmental problems.*

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Research Paper

The Effects of Different Humic Acid and Seaweed on Some Yield and Yield Components of Ryegrass

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Abstract: Livestock farm need forage crops that have high yields and good nutritional value per unit area. One of the plants that can contribute to covering this need is ryegrass. In the present study, the purpose was to determine the effects of biostimulants as organic fertilizer and their doses on some yield and yield components of ryegrass. The study was conducted under Konya conditions in 2017 with 3 replications according to the randomized blocks design. According to the results of the study, stem length was found to be 45.9-51.4 cm, stem diameter was 2.07-3.07 mm, the number of leaves on the main stem was 3.8-4.3, leaf length was 31.3-36.1 cm, leaf width was 5.8-6.8 mm, green fodder yield was 5303-6166 kg/da, and hay yield ranged between 989 and 1157 kg/da. Although a difference was detected between the variable that were observed, it was not found to be statistically significant. Since the yield and nutritional values of ryegrass may vary according to varieties, regions and seasons, the data obtained are preliminary information for researchers working on this subject. It would be useful to conduct more studies on location, year, etc. to confirm the findings of the study.

Keywords: *Humic acid, Lolium multiflorum, organic fertilizer, ryegrass, seaweed, yield*

Introduction

Lolium multiflorum (annual ryegrass or Italian ryegrass) is native to central and southern Europe, north-west Africa and south-west Asia (Hubbard, 1968; Soya et al., 1997). It is also found naturally in Türkiye. Today, it is an important fodder plant which is cultivated almost all over the world. Ryegrass is an annual, sometimes biennial at high altitudes, dense-tillering grass-forming forage crop used for green fodder, hay, silage, and grazing purposes. It has a long growing period and is highly productive when adequate fertilization and maintenance are made under suitable climatic conditions (Aganga et al., 2004). The fact that it can be mowed more than once a year, has a high nutritional value, is eaten by animals with appetite, is suitable for grazing and frequent mowing, and it also can be silaged as a mixture or pure makes ryegrass valuable.

The yield and nutritional values of ryegrass vary among cultivars, regions, and growing techniques. Fertilization, in particular, has the effect of increasing yield and quality. It is necessary to use more fertilizers, especially nitrogen fertilizers for high yields in ryegrass, which has a negative effect on the production costs and the environment. It is necessary to reduce the use of excessive inorganic fertilizers for sustainable production in ryegrass and to increase the use of organic fertilizers.

The deficiency of organic matter is common in the soils of the region with a continental climate. Agricultural techniques and climate are effective in this. Soil organic matter loss is caused by many factors such as wrong tillage techniques, erosion, stubble burning, and overgrazing (Demiray et al., 2023). The low organic matter of the soil is among the most important obstacles to sustainability in agriculture. Organic matter affects the physical, chemical, and biological characteristics of soils positively (Adhikariet al., 2023; Demiray et al., 2023). Inadequate supply of organic fertilizers (i.e., peat, sheep manure, and compost from municipal solid wastes) and high transportation application costs limit their use. Also, the problem with the use of organic fertilizers is the large amount to be used and the difficulties in applying them together with other fertilizers (Asenjo, 2000). However, environmental awareness, rising costs of synthetic fertilizers, and high transportation costs require the use of renewable fertilizers such as seaweed extracts (Hunter, 2004). In our present day, as well as mineral fertilizers, organic matter of different origins, humic substances, seaweed extracts, amino acids, and biostimulants are employed as organic fertilizers. Humic acid and seaweed extracts are also

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classified as biostimulants, which positively affect plant growth, plant nutrition, product quality, and yield as materials that can be applied to plants from leaves, soil, or seeds to increase the resistance of plants to stress and may contain organic or inorganic compounds, microorganisms, and some of them also have regulating effects of the soil (Külahtaş and Çokuysal, 2016). As well as providing good root development, more nutrients, and water intake in plants, seaweed increases the resistance of the plant to diseases and pests, stress factors such as frost, drought, inadequate sun, excessive water, heat, and extreme cold (Yağmur et al., 2021). The effects of humic acid in agriculture facilitate the uptake of nutrients in the soil by plants, increasing the activities of microorganisms and the water holding capacity and air permeability of the soil as well as the resistance of plants to stress conditions with positive effects on resistance to pests and diseases under stress conditions (Külahtaş and Çokuysal, 2016; Yılmaz and Boz, 2022; Eryiğit and Husamalddin, 2023).

Organic fertilisers are better than chemical fertilisers instead of the use of the environmental pollution and prevent environmental pollution and utilisation of farm waste is very important (Demiray and Özaslan Parlak, 2023). In recent years, efforts to reduce the use of mineral fertilisers by increasing the use of organic fertilisers have accelerated. Therefore, in the present study, the effects of humic acid and seaweed fertilizers applied in different mowings and doses on the yield and yield components of ryegrass were determined.

Materials and Methods

The study was conducted to determine the effects of different organic fertilizers on yield and yield components of ryegrass under Konya conditions at the Research and Application Station of Field Crops Department, Faculty of Agriculture, Selcuk University, Konya, Türkiye, in 2017. Barsmultra II variety of ryegrass (*Lolium italicum* L. Syn. *L. multiflorum* Lam) obtained from a private brand was employed as a plant material.

The trial area where the study was conducted is located at the coordinates 38°02'N dan 31°30'E and at an altitude of about 1016 m above sea level. Konya is located in the southern part of the Central Anatolian Region and has a continental climate with harsh, cold, and snowy winters and hot and dry summers.

The climate data of 2017 and the long-term average of the months in which the study was conducted are given in Table 1. The average monthly temperature was the lowest in April at 10.8 °C, the highest in July and July at 25.2 °C, and the average temperature was 19.8 °C during the trial period. Compared to the long-term average (18.8 °C), it was warmer (19.8 °C) during the 2017 trial period. The average relative humidity was 46.4% in the months of the trial period in 2017, and lower than the long-term average (48.6%). Monthly rainfall was the lowest in July with 0.0 mm, the highest in May with 43.7 mm, and the total amount of precipitation was 131.1 mm in 2017. The total precipitation during the trial in 2017 was slightly higher than the long-term average (129.0 mm).

Table 1. Climate Data of Konya Province for 2017 and Long-term (LT) Average*

Month	Precipitation (mm)		Air Temperature °C		Relative Humidity (%)	
	2017	LT	2017	LT	2017	LT
Apr	39.3	32.7	10.8	11.1	53.0	58.1
May	43.7	44.4	15.4	15.7	57.9	56.2
Jun	25.4	24.8	20.4	20.1	54.6	49.0
Jul	0.0	6.9	25.2	23.5	35.6	41.3
Aug	19.4	6.7	24.3	23.3	45.3	41.0
Sep	3.3	13.5	22.4	18.8	31.7	46.9
Total	131.1	129.0	-	-	-	-
Mean	-	-	19.8	18.8	46.4	48.6

Konya Climate Data for 2017. T.R. Ministry of Agriculture and Forestry 8th Regional Directorate of Meteorology, Konya.

According to the soil analysis, the area was identified to be clayey-loamy texture and alkaline characteristics (pH: 7.7). Organic matter amount 1.19%, EC (µS / cm): 193, P_2O_5:10.86 ppm, K_2O: 221.16 ppm, Zn: 2.12 ppm, Fe: 1.30 ppm, Cu: 0.82 ppm, Mn: 4.95 ppm, Ca: 5800.00 ppm and Na: 65.49 ppm were determined (Table 2).

Table 2. Some Physical and Chemical Characteristics of Trialal Field Soil*

Soil depth (cm)	pH	EC (µS/cm)	Texture	Organic substance (%)	P_2O_5 (mg/kg)	K_2O (mg/kg)
0-30	7.7	193	clayey-loamy	1.19	10.86	221.16
Soil depth (cm)	Ca (mg/kg)	Na (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)
0-30	5800.00	65.49	4.95	2.12	1.30	0.82

**Soil analyses were made in Konya Commodity Exchange Laboratories (KLD).*

Three different organic fertilizers were employed in the study. Liquid humic acid, granule humic acid, and foliar fertilizer obtained from seaweed were employed as organic fertilizers. Fertilizer contents are given below (the contents declared by the brands).

Liquid humic acid (LH)

Total organic matter	: 15%
Total humic + fulvic acid	: 15%
Water-soluble K_2O	: 2.5%
pH	: 9-11
Raw Material	: Leonardite + KOH

Granule Humic Acid (GH)

Organic matter	: 90%
Humic acid	: 50%
Amino acid	: 10%
Nitrogen	: 16%
Potassium (K_2O)	: 1%
Phosphorus (P_2O_5)	: 2%
Humidity	: 1%
pH	: 3-5

Seaweed (SW)

Organic matter	: 40%
Alginic acid	: 8%
Water soluble K_2O	: 15%
EC (dS/m)	: 36.2%
pH (with 10 distilled water)	: 7-9
Raw material	: Seaweed

The trial was set up according to the Random Block Design with 3 replications. The observations and measurements were made according to TTSM (Variety Registration and Seed Certification Center, Republic of Türkiye Ministry of Agriculture And Forestry) Technical Instructions (Lolium L. species) (Anonymous, 2001). The soil was plowed and then harrowed. During the soil preparation, 20 kg/da of DAP fertilizer was given as base fertilizer and mixed with the soil on October 7, 2017, by hand to a depth of 2-3 cm on the rows opened with a marker. The rows were then closed and pressed with a soil roller.

Plot dimensions were as follows

Row spacing	: 20 cm
Number of rows per plot	: 10 rows
Plot row length	: 4 m
Plot area in sowing	: 0.20 x 10 x 4 = 8 m^2
Plot area at harvest	: 0.20 x 8 x 3 = 4.8 m^2

The cultivation area of each plot was 8 m^2 and the data, yield and samples were taken from 4.8 m^2 areas, taking into account the edge effects at harvest. It is recommended to mow at the beginning of spike emergence in Italian ryegrass (Özköse et al., 2015). The Italian ryegrass variety used in the study did not show a tendency to spike. Therefore, plant height was taken as a basis in determining the harvest. Three doses of 3 different organic fertilizers were tried. In determining the doses, the manufacturer's recommendations for Italian ryegrass were taken into consideration. The characteristics of the fertilizers and their doses were as follows:

Liquid humic acid : 5 L/da; 10 L/da 15 L/da
Granule humic acid : 5 kg/da; 10 kg/da 15 kg/da
Seaweed : 50 g/da; 100 g/da; 150 g/da

Liquid and granule humic acids were applied to the soil before planting, and seaweed fertilizer was applied to the leaves one week after each mowing. Also, pure nitrogen (N) is given to all plots, including the control, at 5 kg/da after each mowing. Irrigation and weed control were performed as cultural processes. The weeds in the plots were controlled by hand picking or hoeing. Irrigation was done according to the water needs of the plant and rainfall. There was no tendency to spike in the cultivar employed. The plants were mowed three times on 19 June, 27 July, and 21 September 2017. Plant height (cm), stem thickness (mm), number of leaves (pieces), leaf length (cm), leaf width (mm), green fodder yield (kg/da), and hay yield (kg/da) observations and measurements were performed in the study.

The data obtained in the study were subjected to analysis of variance in the MSTAT-C program with 3 replication according to the experimental design in randomized block design. The LSD test was employed to compare the means, and the means were grouped at $p<0.01$ or $p<0.05$ according to the significance level determined as a result of the variance analysis.

Results and Discussion

Three doses of three different organic fertilizers were tried, and three mowings were conducted during the study. Plant height, stem thickness, number of leaves, leaf length and leaf width were determined by taking the average of three mowings, while green fodder yield and hay yield were determined by taking the sum of three mowings. The effects of fertilizers and doses employed in the study on all the varisbles examined were not found to be significant (Table 3).

Table 3. Yield elements of the ryegrass to which different organic fertilizers and doses

Fertilizer	Plant Height (cm)	Stem Thickness (mm)	Number of leaves (pcs)	Leaf Length (cm)	Leaf Width (mm)	Green Fodder Yield (kg/da)	Hay Yield (kg/da)
Control	47.2	3.03	4.3	34.0	6.8	5629	1088
LH1	46.1	2.73	4.1	32.0	6.3	5469	1065
LH2	49.5	2.90	4.2	35.8	6.3	5808	1075
LH3	46.6	2.97	4.1	34.9	6.0	5714	1066
GH1	51.4	2.93	4.3	36.1	6.8	5916	1132
GH2	49.9	3.07	4.0	35.7	6.5	6166	1114
GH3	49.2	2.87	4.0	33.1	6.3	6166	1157
SW1	45.9	2.70	4.0	31.3	5.8	5703	1109
SW2	46.0	3.03	4.1	32.9	6.3	5303	989
SW3	48.2	2.77	3.8	35.1	6.1	5814	1102
LSD	ns	ns	ns	ns	ns	ns	ns

LH= Liquid Humic Acid; LH1: 5 L/da; LH2: 10 L/da; LH3: 15 L/da; GH= Granule Humic Acid; GH1: 5 kg/da; GH2: 10 kgt/da; GH3: 15 kg/da; SW= Seaweed: SW1: 50 g/da; SW2: 100 g/da; SW3: 150 g/da; ns= Not Significant

Plant Height (cm)

The lowest plant height was measured in SW1 treatment at 45.9 cm and the highest in GH1 treatment at 51.4 cm. However, the effect of the fertilizer types and doses employed on the average plant height was not statistically significant. Plant height in ryegrass was determined by some other researchers to be between 48.1 cm (Özdemir, 2017) and 123.8 cm (Sever, 2021). The differences in plant height of ryegrass depended to nitrogen fertiliser doses (Kesiktaş, 2010; Çolak, 2015; Çetin, 2017; Pak Örün, 2019), the number of mowings (Kuşvuran and Taysı 2005), inter-row distance (İnce, 2000), mixture rates with legumes (Özkan, 2017; Sever, 2021), varieties (Aktar, 2019; Acar, 2020) and mowing times (Özköse et al. 2015). The Italian ryegrass cultivar used in the study had a short plant height. Because the Italian ryegrass cultivar used in the study did not show a tendency to spike.

Main Stem Thickness (mm)
The stem diameter varied between 2.70 and 3.07 mm. However, the effect of the fertilizer types and doses employed on the average stem thickness was not statistically significant. This result was similar with several previous study that has been reported. Özköse et al. (2015) reported that the stem diameter of ryegrass ranged from1.69 to 3.75 cm, Çolak (2015) reported results ranged form 2.92 to 3.69 mm, Çetin (2017) reprted results ranged from 3.20 to 3.80 mm, Pak Örün (2019) reported the results ranged from 2.74 to 3.32 mm and Sever (2021) also reported the results ranged from 2.42 to 3.19 mm. Although there were similarities between the results of the present study and the results of the other researchers, there were also some differences. The reason for this may be the different cultivars employed in the studies, the effect of the trials, and the climate, soil, and growing conditions in which the trials were conducted.

Number of Leaves (pcs/stem)
The number of leaves was determined instead of the number of nodes on the main stem since the ryegrass variety used in the study did not have stem emergence. Italian ryegrass cultivar used in the study has vernalisation request. When it is sown as summer in spring, it does not show a tendency to spike. In the above-ground part of the plant, the number of leaves is the same as the number of internodes since one leaf emerges from each node. In the study, the number of leaves on the main stem varied between 3.8 and 4.3. The number of leaves obtained in the study was similar to the number of nodes obtained by Darvishi (2009) (3 – 4), Özköse et al. (2015) (4.2 – 5.9), Anonymous (2015) (3 – 7), and Anonymous (2016) (4 – 7) were close to or slightly lower than the number of nodes.

Leaf Length (cm)
The leaf length obtained in this study was 31.3 - 36.1 cm, higher than Darvishi (2009) (14.0 - 20.0 cm) and similar to Özköse et al. (2015) (10.2 - 37.2 cm), Anonymous (2015) (16.8 - 36.3 cm) and Anonymous (2016) (16.7 - 42.5 cm). In the present study, the variation range of the average leaf length is narrow because of the use of a single variety and the small effect of fertilizer applications. However, the variation ranges were high because of the use and applications of many cultivars in the studies of other researchers.

Leaf Width (mm)
The leaf width varied between 5.8 and 6.8 mm. The data obtained were lower than the flag leaf width of 7.2 – 9.5 mm obtained by Darvishi (2009), 8.0 – 17.0 mm determined by Anonymous (2015) and 8.4 – 15.0 mm determined by Anonymous (2016). The difference between the studies may be due to the genotype, climate and soil conditions of the growing region and differences in agricultural practices.

Green Fodder Yield (kg/da)
Green fodder yield of ryegrass varied between 5303 – 6166 kg/da and the effect of the fertilizers and their doses on yield was statistically insignificant. There may be many reasons for this result. These are; insufficient doses of the fertilisers used, deficiency of other elements in the soil limiting the yield, poor response of the variety used to the fertilisers applied, climatic conditions in the study year, especially high temperature, and many factors may have separate or combined effects. Compared to the results in the literature, the green fodder yield obtained from the study result was similar to the green grass fodder reported by Gültekin (2008) with 3313.7 – 6591.8 kg/da; higher than the results obtained by Darvishi (2009) with 2626.4 – 3439.0 kg/da, Çolak (2015) with 845.4 – 1931.7 kg/da, Kesiktaş (2010) with 1334.6 – 1814.5 kg/da, Kuşvuran and Taysı (2005) with 2984.1 – 3102.0 kg/da, Demiray and Özaslan Parlak (2023) with 2481.7 – 4948.3 kg/da and Rahetlah et al. (2013) with 1468.0 kg/ha; but lower than the results obtained by Anonymous (2015) with 7033.1 – 12758.5 kg/da and Anonymous (2016) with 9620.1 – 11293.5 kg/da.

Hay yield (kg/da)
The hay yield varied between 989 and 1157 kg/da. However, the effect of the fertilizer types and doses employed on the average hay yield was not statistically significant. The fact that the difference

between the hay yields according to the applied fertiliser and doses was not statistically significant may be due to the fact that the applied doses were not sufficient to affect the yield of Italian ryegrass, which has a high yield capacity. Italian ryegrass variety used in the research has vernalisation request. When it was sown as summer in spring, it did not show a tendency to spike. Although the plant formed abundant leaves, the dry matter content of the leaves was low and this affected the herbage yield. The region where the experiment was conducted is hot and low relative humidity during summer months. Even if enough irrigation is done, water loss is high with evapotranspiration. The growth of Italian ryegrass, which is a cool climate plant, slows down in summer. High temperature and humidity is one of the most important factors limiting the yield. This situation limits the effect of applied fertiliser doses on yield. When compared with the results in the literature, the hay yield obtained in the present study was similar to the yield reported in the study by Özköse et al. (2015) (812.2 – 1855.6 kg/da), Gültekin (2008) (781.4 – 1294.2 kg/da), Darvishi (2009) (922.7 – 1643.2 kg/da), and higher than that reported by Çolak (2015) (224.4 – 455.9 kg/da), Kesiktaş (2010) (398.7 – 550.2 kg/da), Kuşvuran and Tansı (2005) (642.2 – 731.0 kg/da), Pavinato et al. (2014) (485 – 525 kg/da), Rahetlah et al. (2013) (303 kg/da), and lower than that reported by Anonymous (2015) (1678.3 – 2902.4 kg/da) and Anonymous (2016) (2219.0 – 2580.8 kg/da). Genetic potential of the variety used; soil characteristics; climatic conditions such as temperature, relative humidity, precipitation; agricultural practices such as irrigation, types and amounts of fertiliser applied, amount of seed sown, weed and pest control are effective on the yield of Italian ryegrass. These factors individually or together may have an effect on the similarity or difference with the results of other researchers.

Conclusion

The study was conducted in Konya conditions for one year in 2017 and three different doses of three different organic fertilizers were used. However, the effects of fertilizers and doses on all yield and yield components were not found to be significant. The applied doses of organic fertilizers did not increase the yield even compared to the control group. It would be useful to conduct more studies on location, year, etc. to confirm the findings of the study. In order to make more reliable conclusions according to these results, further studies should be carried out for a few more years under different climatic and soil conditions, detailed soil analysis of the experimental area according to different depths and supplementing the missing macro and micro element content, and using more ryegrass varieties instead of a single variety.

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