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PREFACE

Effective pretreatment plays a pivotal role not only in reducing sludge volume from wastewater treatment plants but also in stabilizing sewage sludge properties during anaerobic digestion, thus facilitating biogas production. This book details anaerobic sludge digestion process, the process chemistry, and chemical-based methods.

Bacillus aryabhattai is a bacterial species with potential bioremediation and industrial applications due to its metabolic versatility and ability to produce enzymes. This book discusses the bioremediation efficiency of B. aryabhattai on glyphosate herbicide.

This book discusses the changes in Artvin Çoruh University's resource utilization as a significant model for sustainability and efficiency.

Climate change has become a problem that closely affects livestock performance in many continents, countries, and regions around the world. This book explains the effects of climatic change on animal production.

The topics covered in this book are presented to the readers as a fundamental resource containing current information in the field of Environmental Sciences and Engineering.

Editor

Prof. Dr. Ali BİLGİLİ

Content

CHAPTER I

Maximizing biogas production: Chemical disintegration for improved sludge digestion

Fatma Olcay TOPAÇ[1]

1-Introduction

Over the past 20 years, efforts have intensified to improve wastewater treatment plants and upgrade current systems. This push is fueled by heightened environmental consciousness, which stresses the importance of reducing or eliminating toxic chemical contamination in sewage. This means that wastewater treatment plants now concentrate toxic substances and pathogens in the waste sludge created during the treatment process. As a result, the qualities and quantities of such sludge have shifted, resulting in increased expenses for handling it. Although the sludge produced is a small part of the treated wastewater, it accounts for half of the wastewater

[1] Prof. Dr., Bursa Uludag University, Engineering Faculty, Department of Environmental Engineering, Bursa/Turkey, Orcid: 0000-0002-6364-4087, olcaytopac@uludag.edu.tr

treatment plant's operating costs (USEPA, 2008). Different methods have been used to handle waste sludge, and government rules and regulations for general sludge management have also changed. Legal pressure requires all nations to adhere to the waste management hierarchy. Within this hierarchy, prevention, recycling, recovery and reuse are considered the most preferred routes, while storage and disposal are strictly bounded (Rorat and Kacprzak, 2017). Conducting innovative research to develop more sustainable scenarios for sludge management emerges as a priority. A significant portion of this research focuses on the anaerobic digestion process of waste sludge.

Anaerobic digestion is regarded as a low-cost, environmentally benign technique used for the treatment of waste sludges and several organic type wastes (Obileke et al., 2021). This process stabilizes waste sludge, eliminates odor problems, provides pathogen removal, and reduces the amount of solid matter. Furthermore, it offers a solution to today's energy problems by producing renewable energy in the form of biogas. In this way, a portion of the cost of processing sludge can be covered, contributing to efforts to reduce dependence on fossil fuels (Khan et al., 2017).

Biogas generation by anaerobic digestion of wastewater sludge is a highly profitable process due to the methane content ranging from 50% to 70% in the biogas. The digested sewage sludge can be used as a mineral fertilizer alternative, and the biogas produced can be used straight away as a source of heat or energy (Cristina et al., 2020). It has been estimated that treating sewage sludge with anaerobic digestion could decrease the emissions of greenhouse gases by 75 to 100 million tons of CO_2 equivalent per year, primarily by replacing energy derived from fossil fuels. Estimates indicate that applying anaerobic digestion to capture and process the entirety of current organic waste has the potential to result in a 10% decrement in annual global greenhouse gas emissions by the year 2030 (WBA, 2019; WBA, 2021).

Due to all these advantages offered by the anaerobic digestion process, a lot of research is being done to increase the efficiency of the process and to raise the potential for methane production by enhancing biogas output. Many effective sludge pretreatment procedures have been developed to boost methane generation. With the help of these pre-treatment technologies, extracellular polymeric substances (EPS) and resistant cell walls are broken down and thus liberation of intracellular compounds is encouraged. Pretreatment techniques under investigation include thermal, chemical, biological, and physical/mechanical treatments, which can be used singly or in appropriate combinations (Mitraka et al:, 2022).

In this book chapter, firstly, details about the anaerobic sludge digestion process are given and the process chemistry is explained. Current studies on chemical-based methods, among the pre-treatment methods applied to increase process performance and biogas production, are compiled and presented.

2-Anaerobic Digestion of Sewage Sludge

Obtaining bioenergy through the anaerobic digestion of waste sludge is considered a suitable waste management approach that contributes to mitigating climate change. Additionally, anaerobic digestion systems offer numerous advantages, including relatively low maintenance costs and simplicity, as well as the ability to easily adapt to various climatic conditions. The advantages of anaerobic digestion systems are summarized in Figure 1.

For anaerobic digestion to be effective, a group of microbes must interact reciprocally and syntrophically in order to break down complex organic compounds into soluble monomers like glycerols, simple sugars, fatty acids, and amino acids. Anaerobic digestion has been utilized as a suitable method for processing a wide range of raw materials obtained from various sources. Any organic material that degrades biodegradably can, in theory, be broken down anaerobically to create biogas. Table 1 is a collection of examples of anaerobic digestion basic materials that came from various sources (Uddin and Wright, 2023). The raw material used to make anaerobic digestion should be readily biodegradable and free of any harmful substances that can harm microorganisms.

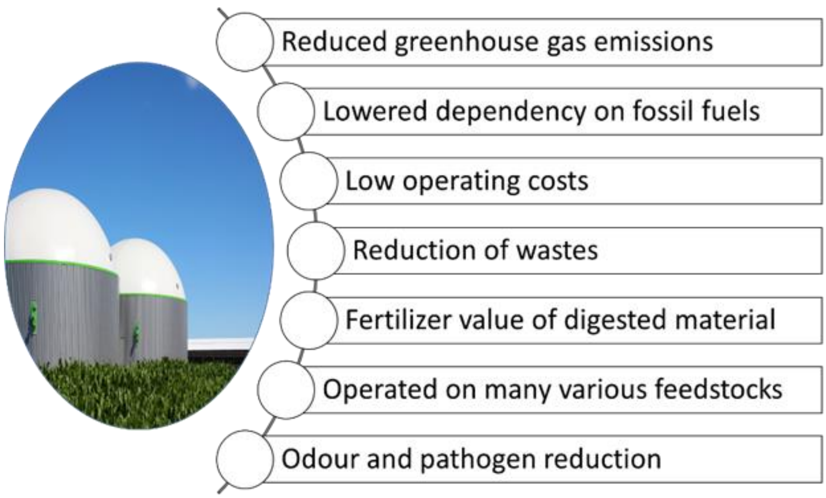

Figure 1: The advantages of anaerobic digestion systems

Tablo 1: Common feedstocks suitable for anaerobic digestion

Common Waste Group	Origin
Industrial Residues	-Agroprocessing -Food and beverage industry -Slaughterhouse -Pharmaceutical industry -Dairy production
Agricultural Residues	-Harvest wastes -Energy crops -Farm mortality -Livestock residues
Municipal Residues	-Sewage sludge -Garden rubbish -Municipal solid waste -Restaurant/cafeteria wastes -Supermarket wastes

The ability to digest sewage sludge with high water content anaerobically without the need for any pre-treatment is considered an important advantage of this technology. The characteristics of waste sludge change significantly during the anaerobic digestion process. With anaerobic digestion, not only biogas is obtained, but also many positive results are obtained in terms of the sludge management steps that follow the process. Anaerobic digestion increases the stabilization of sewage sludge, reduces odor emissions, pathogenic bacteria, and dry matter content, leading to a remarkable decreament in the final volume of the sludge (Zhang et al., 2023; Xu et al., 2020). These advantages have led several nations to adopt anaerobic digestion of sewage sludge as a common technological practice.

3-Chemistry of Anaerobic Digestion

The anaerobic digestion process entails a number of phases and intricate interactions between several microbe species. Different microbial communities work together to break down complex biomass polymers into a gaseous mixture at different stages of degradation. As illustrated in Figure 2, biochemical anaerobic digestion processes can be categorized into four distinct stages: I) hydrolysis, II) acidogenesis, III) acetogenesis, and IV) methanogenesis.

I)Hydrolysis*:* In essence, the process of hydrolysis is the initial step in the digestion process. Water and extracellular enzymes degrade the complex polymeric structures of proteins, cellulose, and starch to liberate the equivalent simple units, which include glucose, fatty acids, and amino acids. Generally speaking, hydrolytic enzymes include pectinase, protease, cellulase, and amylase. Hydrolytic bacteria typically develop at a very rapid pace. On substrates rich in lignin, however, the polymer breakdown becomes the rate-limiting step. While most chemicals at this stage require additional breakdown through other stages, some are ready to be turned into biogas. Hydrolysis is frequently recognized as the rate-limiting stage in anaerobic digestion, signifying its role as the slowest step and its significant influence on the duration of feedstock residence in a digester (Appels et al., 2008). Consequently, pretreatment techniques in anaerobic digestion, like chemical disintegration, concentrate on enhancing this phase (Zhen et al., 2017).

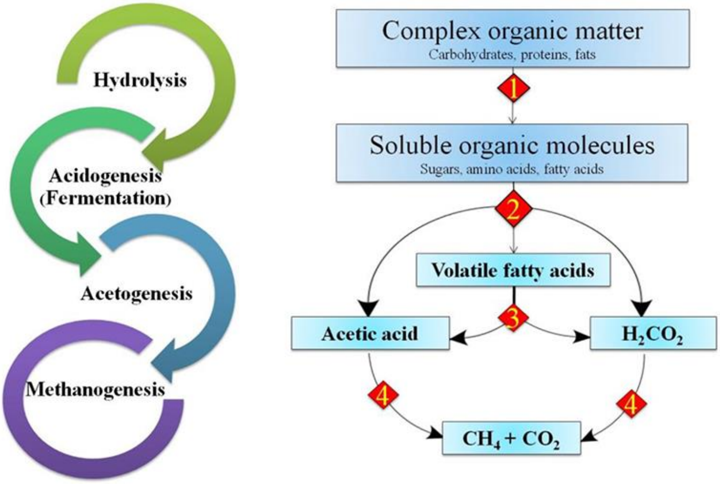

Figure 2: Flow diagram of the anaerobic digestion process (Dussadee et al., 2016).

II)Acidogenesis: In the acidogenesis phase, acidogenic bacteria further break down the products of hydrolysis. These hydrolytic products are primarily converted into short-chain volatile fatty acids (VFAs) such as acetic acid, propionic acid, formic acid, and lactic acid, as well as alcohols like ethanol and methanol, and ketones such as glycerol and acetone. By-products like CO_2, H_2, NH_3, alcohols, and small amounts of other substances are also produced. Certain products like CO_2, H_2, acetate, and formates are readily utilized by methanogens in the final stage. However, other substances require additional decomposition for methane production. If acidogenesis is not well controlled, there is a chance that VFAs will build up in the digester and cause toxicity (Akuzawa et al., 2011)

III)Acetogenesis: A fraction of the original substrate becomes eligible for acetoclastic methanogenesis when the process of turning substrate into acetate occurs during acidogenesis. However, acetogenesis is responsible for producing hydrogen and converting other more VFAs produced during this phase into acetate. Acetate, carbon dioxide, and hydrogen are produced by acetogenic bacteria from the byproducts of acidogenesis and the hydrolysis of specific long-chain fatty acids. If the partial pressure of hydrogen is more than 10-4 atm, the acetogenesis reactions are not naturally spontaneous. However, methanogenic bacteria consume the hydrogen created, which lowers this partial pressure. Because of the syntrophic relationships that exist between hydrogenotrophic methanogens and acetogenic bacteria, acetogenesis and methanogenesis are closely related processes that contribute to the overall process' thermodynamic favorability (Detman et al., 2021).

IV)Methanogenesis: In this final stage, all intermediate products from the preceding phases combine to produce methane. Since oxygen prevents methanogenic bacteria from thriving, this stage is strictly anaerobic. A group of obligate anaerobic archaea known as methanogenic microorganisms was found to be extremely sensitive to oxygen; 99% of Methanococcus voltae and Methanococcus vannielli cells were found to have died within 10 hours of being exposed to oxygen (Kiener and Leisinger, 1983) Acetate, or CH_3COOH, and H_2 are transformed into CO_2 and CH_4 by two distinct bacterial species: hydrogenophilic and acetophilic. Acetate is converted into CH_4 and CO_2 by acetophilic bacteria, whereas hydrogenophilic bacteria convert H_2 and CO_2 into CH4.

4-Available Sludge Pretreatment Methods for Enhanced Biogas Production

The quantity of biogas generated per treated unit of raw material determines a biogas plant's profitability. Since only half of the organic material is actually converted, there is a lot of potential to grow the sewage sludge biogas yield. Since the organic matter in sewage sludge is primarily present in particulate form and hydrolysis is the process's rate-limiting step, optimizing the anaerobic digestion process heavily depends on increasing hydrolysis efficiency Innovative pre-treatment techniques have been created to break up the sludge's flock structure, increase the solubility of solid organic materials, and ultimately boost the production of methane (Mitraka et al., 2022).

Pre-treatment methods applied to sludge prior to anaerobic digestion with the aim of increasing biogas production encompass independent procedures or suitable combinations of physical/mechanical, thermal, chemical, and biological processes (Figure 3).

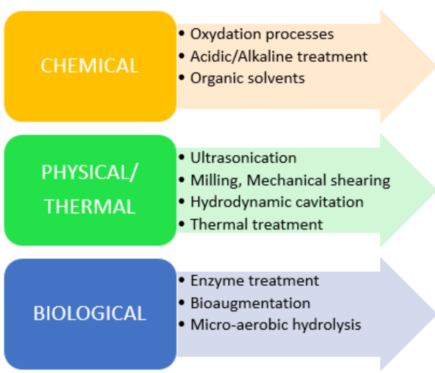

Figure 3: Sludge pre-treatment methods with different approaches

These pre-treatment methods are intended to elicit modifications in the characteristics of sludge, aiming for:

- Release of organic compounds trapped in waste sludge into the liquid phase and providing access to intracellular enzymes

-Providing faster digestion and increasing biogas production and efficiency

-Enhanced COD solubility and organic substrate release, suggesting an easily assimilated supply of organic C for denitrification reactions

-Removing activated sludge foam and eliminating the foaming problem

5-Chemical Pretreatment Strategies for Improved Anaerobic Digestion

Chemical pretreatment can improve the rate-limiting hydrolysis step of anaerobic digestion of sewage sludge, which in turn facilitates the biodegradation of complex polymers by solubilizing the particulate fraction of the substrate. The primary goal of chemical pretreatment techniques is to break up the sludge's floc structure. As a result of the interaction between microbial cells and strong chemicals, cell walls and membranes dissolve. Thus, enzymatic attacks on intracellular content are supported Acids, organic solvents, alkaline solutions, and ionic liquids are among the substances that have demonstrated beneficial effects in dissolving the structure of substrates that are difficult to work with, like lignocellulosic materials. It is known that cellulose, an intricate polymer carbohydrate, poses challenges in anaerobic digestion due to its resistance to breakdown. Additionally, lignin acts as a protective barrier, forming a crust-like material and providing mechanical resilience (Syaichurrozi, 2018). Chemical pre-treatments are favored over physical and biological methods for enhancing the biological degradation of lignocellulosic substrates due to their superior effectiveness in substrate degradation and often greater economic viability (Song et al., 2014, Pellera ve Giderakos, 2018).

5.1-Acidic Treatment

The study conducted by Devlin et al. (2011) investigated the impact of pre-treatment using HCl on sludge digestion performance. The process, conducted under conditions ranging from pH 6 to pH 1, yielded the most effective results when acid was added until pH 2 was reached. This method increased biogas yield by 14.3% compared to untreated waste activated sludge (WAS). Furthermore, aside from enhancing biogas potential, it was observed that the method also led to the destruction of Salmonella present in the sludge.

Ren et al. (2024) investigated the impact of applying Peracetic acid (PAA), recognized as a novel and efficient chemical pre-treatment approach, on the anaerobic digestion process of sludge, focusing on physicochemical and microbiological parameters. The study findings revealed that the highest biogas efficiency was achieved with PAA application at a dosage of 2 mM/g VS. Furthermore, mechanistic investigations indicated that reactive oxygen species are the primary products of PAA decomposition, effectively enhancing sludge solubility. Another notable discovery from the study was the enrichment of certain functional microorganisms associated with different stages of anaerobic digestion in PAA-treated sludge. This enrichment was found to be significantly correlated with biogas production. In a similar study previously conducted, it was determined that the application of a peracetic acid dosage equivalent to 25 g/kg of the total solids in the sludge increased biogas production by approximately 21% (Appels et al., 2011).

While numerous studies indicate that acidic pre-treatment enhances the performance of anaerobic sludge digestion, it is generally emphasized in research that in the context of breaking down and dissolving organic matter, an alkaline pre-treatment proves more effective compared to an acidic approach. (Chen et al., 2007). However, since this method supports the accumulation of hydrolytic microorganisms under acidic conditions, it is more effective in the pretreatment of sludges rich in lignocellulosic substances (Neumann et al., 2016).

5.2-Alkaline Treatment

Alkaline pretreatment is a commonly employed method to disrupt sludge cells and EPSs, facilitating the dissolution of organic matter without leaving harmful residues in subsequent processes. This reaction can be conducted at ambient temperature and pressure, requiring minimal energy input (Bakry et al., 2022).

Grübel et al. (2013) reported in their study that the application of alkaline substances to waste activated sludge resulted in the breakdown of flocs and microorganisms, leading to a significant increase in the concentration of organic matter in the supernatant. Experimental results demonstrated that adjusting the pH value of waste sludge to 9 increased the SCOD value from 101 to

530 mg/dm3. In a similar study, it was emphasized that even with an increase in the sludge pH to 8, the SCOD concentration in the sludge increased significantly, thereby promoting the production of volatile fatty acids. It was stated that enhancing the hydrolysis step would be greater by increasing the pH up to 10. However, considering the corrosion effects, it was suggested that a pre-treatment bringing the sludge pH to 9 would be technically appropriate and sufficient (Suschka et al., 2015).

Wei et al. (2017) assessed the efficacy of a pre-treatment technique involving the utilization of free ammonia to enhance methane recovery from waste sludge through anaerobic digestion. The study revealed that the solubility of sludge treated with free ammonia (up to 680 mg NH_3-N/L) was tenfold higher compared to untreated sludge. The optimal dose of ammonia for enhancing biogas production was identified as 250 mg NH_3-N/L, with the maximum increase in biogas observed at doses ranging from 420 to 680 mg NH_3-N/L. Based on these findings, free ammonia-based methods were underscored as economically viable and environmentally friendly approaches.

In another study, experiments were conducted using HCl and NaOH to assess the impact of acidic and alkaline conditions on sludge solubility. These experiments investigated the increase in solubility and evaluated the effect of reaction time and total solids level. The results revealed that acidic and alkaline pretreatment led to the release of various compounds from the floc structure into the sludge water. The highest degree of solubility was achieved under alkaline conditions with a pH adjusted to 12. Organic carbon, carbohydrate, and protein concentrations in the sludge supernatant increased by 15, 41, and 40 times, respectively, due to the applied treatment. Evaluation of biomethanization tests showed a 3.6-fold increase compared to untreated sludge (de Souza et al., 2021)

5.3-Ozonation

Ozone (O3) has garnered significant interest as a potent oxidant in the pretreatment of waste activated sludge (WAS). It has the ability to harm the cell membrane, break down the zoogloea structure, and has been effectively implemented in the solubilization and reduction of excess sludge. The efficiency of sludge solubilization depends on the dosage of ozone applied, and it shows a linear correlation within a moderate range (Campos et al., 2009).

Tuncay et al. (2022) examined the impact of various ozone doses (0.03, 0.06, and 0.09 grams of ozone per gram of total suspended solids (TSS)) on the performance of a laboratory-scale anaerobic digester operating under mesophilic semibatch conditions. The digester, with a hydraulic retention time of 15 days and organic loading rates ranging from 1.45 to 1.80 g VS/l/day, was utilized in the investigation. The study concluded that the optimal ozone dose for enhancing sludge properties was identified as 0.06 g O_3/g TSS. Sludge pretreated with ozone exhibited a 48% increase in methane production. Moreover, the rise in methane content was linked to the abundance of acetotrophic Methanosaeta species in the study.

In a similar study conducted by Hodaei et al. (2021), the effects of applying ozone, a strong oxidizing agent, to sludge before digestion, on anaerobic sludge digestion performance, biogas production, and dewaterability were examined with two ozone dosages of 0.05 and 0.1 g O_3 g^{-1} TS. The results obtained showed that the effects of the application largely depend on the ozone dosage and sludge retention time. When high doses of ozone were applied to sludge, energy consumption increased, and anaerobic sludge digestion performance and biogas production rate decreased. The acceleration of the hydrolysis phase due to the effect of high ozone concentration, the increase in the concentration of volatile fatty acids in the acidogenesis phase, and the decrease in the performance of methanogenic bacteria have been shown to be the reasons for this decrease. As a result, ozone application at a dose of 0.05 g O_3 g^{-1} TS and a sludge retention period of 10 days were recommended as suitable conditions for sludge pretreatment.

In another study (Pazdzior et al., 2022), the objective was to ascertain how ozonation affected the efficiency and kinetics of producing biogas from waste activated sludge. The results showed that, due to the pre-treatment process, some organic compounds within the sludge flocs underwent hydrolysis, leading to an increase in biogas production efficiency. It was determined that the amount of biogas obtained through ozonation increased by 21%, with a corresponding increase of 2.5% in methane content within the biogas.

5.4-Fenton's Reagent

The Fenton reaction, categorized as an advanced oxidation process, arises from the interaction between H_2O_2 and Fe^+; in this context, Fe^+ functions as a catalyst for generating potent free radicals like OH^- .Concerning oxidation-reduction potential, the free radicals generated through the Fenton reagent (+ 2.33 V) exhibit greater strength compared to those produced solely by H_2O_2 and ozone (Hallaji et al., 2018). According to Zhen et al., (2014) pretreatment with Fenton's reagent leads to the fragmentation of sludge aggregates, disruption of microbial cell membranes, and the release of organic matter contained within the cells. Additionally, using Fenton's reagent significantly lowers the number of pathogenic bacteria, improving the sludge's hygienic quality. In their study, Şahinkaya et al. (2015) assessed the efficacy of traditional Fenton (CFP-Fe^{2+} + H_2O_2) and Fenton-type (FTP-Fe^0 + H_2O_2) processes as methods for pretreating sludge, focusing on sludge disintegration efficiency and anaerobic digestibility. Optimal conditions for both processes were determined as follows: iron dosage = 4 g/kg TS, H_2O_2 dosage = 40 g/kg TS, pH = 3, and oxidation time = 1 hour. The impact of pretreatment processes under optimal conditions on anaerobic digestion was evaluated using biochemical methane potential analysis. Results revealed that total methane production in reactors pretreated with CFP and FTP increased by 26.9% and 38.0%, respectively, compared to the control group. Furthermore, it was stated that reductions in total chemical oxygen demand in the pretreated reactors were more pronounced.

To determine the influence of Fenton pretreatment application on the anaerobic digestion of secondary sludge, laboratory-scale experiments were conducted by Pilli et al. (2016). The results obtained indicated that production of methane increased by approximately 15% with the applied pretreatment approach. While the amount of methane produced was 430 m^3 CH_4/Mg VS in the control sludge, this value was found to be 496 m^3 CH_4/Mg VS in the Fenton-treated trial. While the net energy increased by 3.1 times with the applied pretreatment, greenhouse gas emissions were found to be lower than the control. In line with the results, the Fenton pretreatment method was recommended as a cost-effective approach.

In the context of another study, the application of Fenton reagent was assessed to enhance the anaerobic digestion process and increase biogas production. Iron ion dosages ranging from 0.02 to 0.14 g Fe^{2+}/g TS were tested, while hydrogen peroxide was utilized at ratios ranging from 1:1 to 1:10 per iron ion. Consequently, the most suitable iron ion dose was determined to be 0.08 g Fe^{2+} / g dry matter, with an optimal Fe^{2+} : H_2O_2 ratio of 1:5. Under these conditions, the degree of digestion of sludge pretreated with Fenton reagent doubled compared to untreated sludge. Additionally, there was a 35% increase in the efficiency of biogas production (Zawieja and Brzeska, 2019).

5.5-Sulfate Radicals

Although sulfate radicals are commonly employed in wastewater treatment for oxidizing organic pollutants (Uran-Duque et al., 2021; Hassani et al., 2023), their use in degrading waste activated sludge is still regarded as a novel approach. These radicals are generated from oxone ($2KHSO_5$•$KHSO_4$•K_2SO_4), with its active species being peroxymonosulfate (PMS). Sulfate radicals exhibit a higher oxidation potential (2.5-3.1 eV) in comparison to hydroxyl radicals (2.8 eV) (Shi et al., 2012). To generate sulfate radicals, persulfates typically require activation. The typical methods for activating persulfate can be categorized into two groups: physical methods, which involve ultrasonic (US), heat and ultraviolet (UV) radiation; and chemical methods, such as transition metals, alkali and carbonaceous materials. (Song et al., 2019).

Sun et al. (2012) examined the impact of sulfate radical pretreatment on mesophilic anaerobic sludge digestion. Their findings showed that this application effectively enhanced the performance of anaerobic digestion process. It was observed that following sulfate radical pretreatment, the removal rate of total chemical oxygen demand (TCOD) increased by 11.5%, accompanied by a 44.9% increase in cumulative gas production. The highest methane yield attained through this method was 0.27 m^3 CH_4/kgVS, representing a 180.0% increase compared to the control. These results suggest that persulfate/$K_2S_2O_8$ pretreatment could serve as an efficient approach for enhancing methane yield.

Ren et al. (2015) studied the impact of sludge pre-treatment with sulfate radicals on the degradation of waste activated sludge at various peroxymonosulfate (PMS) dosages. Through statistical analyses aimed at identifying parameters indicative of sludge disintegration, it was determined that COD and total phosphorus (TP) were not suitable predictors of sludge degradation by oxidants. Conversely, total nitrogen, total organic carbon, polysaccharides and UV254 demonstrated a good correlation with each other and could serve as reliable indicators of sludge disintegration. Analysis of 3D-EEM fluorescence spectra obtained during the study revealed that high doses of sulfate radical oxidation resulted in the degradation of aromatic and tryptophan protein-like substances. Application of PMS to the sludge at a dosage of 12 mg (g SD)$^{-1}$ led to sludge degradation degrees of 24.8% and 29.9% for TOC and TN, respectively.

In another study on sludge disintegration, the effects of applying potassium monopersulphate (PMS) to low organic content sludge were examined. It was observed that the pre-treatment application stimulated enzyme activities and led to an increase in short-chain fatty acids. Short-chain fatty acids, initially at 16.40 mg COD/L in the untreated sludge, increased to 716.72 mg COD/L after applying PMS at a dose of 0.08 mg PMS/mg SS. The application of PMS increased protease, α-glucosidase, alkaline phosphatase, acidic phosphatase, and dehydrogenase activities in the sludge by 1.42, 4.38, 2.1, 1.7, and 1.37 times, respectively. The study concluded that using PMS as a sludge pre-treatment method positively affected the microorganisms responsible for hydrolyzing sludges with low organic matter content and for producing short-chain fatty acids (Jin et al., 2018).

Hu et al. (2019) investigated the effects of the zero-valent iron (ZVI)-persulfate oxidation method on the performance of anaerobic sludge digestion and biogas efficiency. It was determined that applying the method with the optimal ZVI-persulfate dosage increased biogas production by 53.6% compared to the control. Additionally, the applied pre-treatment enhanced sludge dewaterability, reducing the capillary suction time in the treated and digested sludge by 42% compared to the control.

6-Conclusion

Anaerobic digestion technology is an effective and proven technique for producing renewable energy and the utilization of organic wastes. The utilization of sewage sludges as a source for methane production aligns with the principles of a circular economy, promoting resource optimization and environmental sustainability. However, to enhance methane yields, it is imperative to implement technical modifications and optimize operating parameters to foster synergistic interactions among various microorganisms involved in the digestion process.

Effective pretreatment plays a pivotal role not only in reducing sludge volume from wastewater treatment plants but also in stabilizing sewage sludge properties during anaerobic digestion, thus facilitating biogas production. Various sludge pretreatment technologies have been globally developed and studied to reduce sludge generation and expedite anaerobic digestion rates. Chemical pretreatment methods offer numerous benefits, including operational flexibility, reduced digestion time and reactor size, increased biogas production, improved sludge dewaterability, decreased excess sludge generation, performance stability, and simplified management. Additionally, chemical disintegration might be prioritized over alternative disintegration approaches in specific instances owing to its impact on particular hard-to-degrade species present in sludge. However, challenges such as equipment corrosion and the need for re-neutralization of acid or alkali-pretreated sludge for anaerobic digestion should be addressed, as they may result in higher operational and maintenance costs. Nonetheless, these costs can be mitigated by substantial reductions in post-treatment expenses such as transportation, handling, dewatering, drying, storage, or incineration, as well as by improved biogas yields.

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CHAPTER II

The bioremediation of herbicide glyphosate in soil media by *Bacillus aryabhattai*: Use of Gammarus pulex as Bioassay

Nuran CIKCIKOĞLU YILDIRIM[1]
Gökhan Önder ERGÜVEN[2]
Yusuf SÖYLEMEZ[3]

1.Introduction

Glyphosate(C3H8NO5P) is a widely used herbicide that belongs to the class of chemicals known as organophosphates. Glyphosate is known for its effectiveness in killing a wide range of weeds and unwanted plants, making it a valuable tool for agricultural and non-agricultural purposes (Bareille and Gohin, 2020).

[1] Prof. Dr, Munzur Unıversıty, Pertek Sakıne Genc Vocatıonal School, Tunceli, Türkiye, Orcid: 0000-0003-3975-6705, nurancyildirim@munzur.edu.tr
[2] Assoc. Prof. Dr., Munzur Unıversıty, Faculty of Economıcs And Admınıstratıve Scıences, Department of Urbanization and Environmental Issues, Tunceli, Turkey, Orcid: 0000-0003-1573-080X, goerguven@munzur.edu.tr (*Corresponding author)
[3] Msc., Munzur Unıversıty, Graduate School of Institude, Tunceli, Turkey, Orcid: 0000-0001-7357-0061, y.sylmz62@gmail.com

Glyphosate's effectiveness, coupled with its relatively low environmental persistence compared to other herbicides, made it a preferred choice for both large-scale agriculture and home gardening. However, over the years, concerns about the potential environmental and health impacts of glyphosate have emerged, leading to debates and regulatory evaluations (Bocker et al., 2020).

Environmental concerns primarily revolve aroundthe potential for glyphosate to persist in soil and water, impacting non-target plants and organisms. The herbicide's broad-spectrum nature raises concerns about its impact on crops, native vegetation, and wildlife. Additionally, there is apprehension about the potential development of glyphosate-resistant weeds, leading to increased herbicide use (Ye et al., 2021).

Herbicide bioremediation is a process that involves the use of microorganisms, such as bacteria and fungi, to degrade or detoxify herbicides present in the environment. This approach aims to reduce the harmful impact of herbicides on ecosystems, water sources, and non-target organisms by utilizing the natural abilities of certain microorganisms to break down and transform these chemicals into less harmful substances (Masotti et al., 2023). Several microorganisms have been identified for their ability to degrade herbicides through enzymatic reactions. These microorganisms can utilize herbicides as a carbon and energy source for their growth and metabolism. Some common herbicides that have been targeted for bioremediation include atrazine, glyphosate, and 2,4-D) (Sviridov et al., 2015).

Bacillus aryabhattai is a bacterial species with potential bioremediation and industrial applications due to its metabolic versatility and ability to produce enzymes. Its discovery and characterization contribute to our understanding of microbial diversity and their roles in various ecological processes (Farooq et al., 2022).

Gammarus pulex, commonly known as the freshwater shrimp or signal crayfish, is a small crustacean species found in freshwater environments. It has gained significant attention in biomonitoring studies due to its sensitivity to environmental changes and pollutants, making it a valuable indicator species for assessing water quality and ecosystem health (Tanyol et al., 2021).

In this study, the bioremediation efficiency of *B. aryabhattai* on glyphosate herbicide by using the *G. pulex* bioassay was aimed to investigate.

2. Materials and methods

2.1. Chemicals

Glyphosate was obtained from the local pesticide market in 500g packages. Test kits used for cytochrome P4501A1 (CYP1A1) biochemical analyzes were provided by Cusabio company (catalog number: CSBEL006395FI). CAT and GST test kits were purchased from Cayman Chemical company. The catalog numbers of these kits are 707002 and 703302 respectively. The preferred kits for COD analyzes were obtained from the Turkish distributor of Hach Lange company with the product code LCK114.

2.2. Soils used in artificial feld setup

Total salt (%), pH, saturation (%), organic matter (%), lime (%), available phosphorus (P2O5 kg/da) and available potassium (K2O kg/da) on the studied soil samples were analyzed in the Soil Analysis Laboratory of the Tunceli Provincial Directorate of Food, Agriculture and Livestock. The methods used and the results are presented in Table 1.

Table 1. Analysis results of agricultural soil used in bioremediation studies and bacterial isolation

Analysis	**Result**		**Reference of method**
Saturation %	77	Clay	Tuzuner (1990)
pH	7.78	Sightly alkaline	Yurdakul (2017)
Total salt %	0.03	Salt - free	Richards (1954)
Lime %	26.35	Too much	Tuzuner (1990)
Organic material %	0.87	Very low	Walkley and Black (1934)
Useful Phosphorus P_2O_5 kg/da	4.18	Very low According to Olsen et al (1954) Very high according to Bray and Kurtz (1945)	Olsen et al (1954) Bray and Kurtz (1945)
Useful Potasium K_2O kg/da	575.87	High	Kacar (1995)

2.3. Isolation and identification of bacteria

In the study, soil samples were collected from an agricultural area alongside the Tunceli-Elazig highway, where bacterial samples were discovered. The soil was obtained from depths of 0-30 cm and carefully transfered in sterile glass jars following the methodology described by Zelles et al (1991) (Zelles et al., 1991). Specifically, around 10 g of soil sample was initially diluted to a concentration of 106 in isotonic water containing 0.8% sodium chloride. From this dilution, 0.1 ml portions were aseptically spread onto plate count agar medium, following the procedures detailed by Travers et al

(1987). Subsequently, the Petri dishes were placed in a 25°C incubator to facilitate bacterial growth. Colonies that developed on the Petri dishes were then transferred to subaraud dextrose broth for further enrichment, utilizing sterile loops within a controlled cultivation environment, according to the metdods given by Cruikshank (1972).

For molecular species identification, Sentebiolab Biotech conducted the genomic DNA extraction from Gram-positive and Gram-negative bacteria, employing the Wizard Genomic DNA Purification kit method by Beutler et al (1990). The molecular characterization process encompassed various steps, including nucleic acid extraction, Polymerase Chain Reaction (PCR), Denaturing Gradient Gel Electrophoresis (DGGE), and subsequent nucleic acid sequence determination.

2.4. Preparation of glyphosate herbicide solutions

In order to assess the biochemical response of untreated media including inital glyphosate concentration, the LC50 value of glyphosate on *G. pulex* was determined. The lethal concentration (LC50) value for glyphosate was precisely determined as 422.49±35.65. The initial glyphosate concentration added to the soil medium was 106.6 ppm, which is 1/4 of the LC50 value.

2.5. Bioremediation of glyphosate in soil media

In order to determine the bioremediation activities of bacteria in glyphosate media, firstly, *B. aryabhattai* isolated from plate count agar media were added to the enriched medium (sabouraud dextrose broth) and enriched in about 3 days. When the COD and TOC values calculated nearly as COD and TOC values of used Glyphosate pesticide concentration, the bioremediation studies started. These enciched media seperated to 10, 20 and 40 ml in each Erlenmayer flasks and transfered to each bottles including Glyphosate and agricultural soil. 7 sterile plastic bottles were used in the bioremediation study. The contents of these bottles are presented in Table 2. COD and TOC analyzes were performed on the filtrates of these media taken every 24 hours. Closed reflux 5220C method for COD and 5310B high temperature burning method for TOC used (SMC, 2009). Studies carried out for 1, 3, 5, 7, 9, 11 and 13 days.

Table 2. The content of the bottles

Bottle no	Mediums
x	Soil + 10 ml *B. aryabhattai*
y	Soil + 20 ml *B. aryabhattai*
z	Soil + 40 ml *B. aryabhattai*
t	Soil + LC50/4 Glyphosate + 10 ml *B. aryabhattai*
q	Soil + LC50/4 Glyphosate + 20 ml *B. aryabhattai*
r	Soil + LC50/4 Glyphosate + 40 ml *B. aryabhattai*

The actual COD and TOC values are calculated according following formula;

CODactual t = CODt - CODx

CODactual q = CODq – CODy

CODactual r = CODr - CODx

TOCactual t = TOCt - TOCx

TOCactual q = TOCq – TOCy

TOCactual r = TOCr - TOCx

2.6. Model organism *G. pulex*

G. pulex samples were collected from Munzur River (390, 10ı, 28ıı N; 390, 27ı, 37ıı E) (Figure 1). These individuals were quickly taken to Laboratory of Department of Environmental Engineering in Munzur University in plastic bottles. The *G. pulex* samples kept in a 20 L aquarium, maintained at 18°C, under a 12:12 light:dark cycle, and were nourished with willow leaves for a duration of 15 days before commencing the experimental (DE lange et al., 2005) (Figure 2).

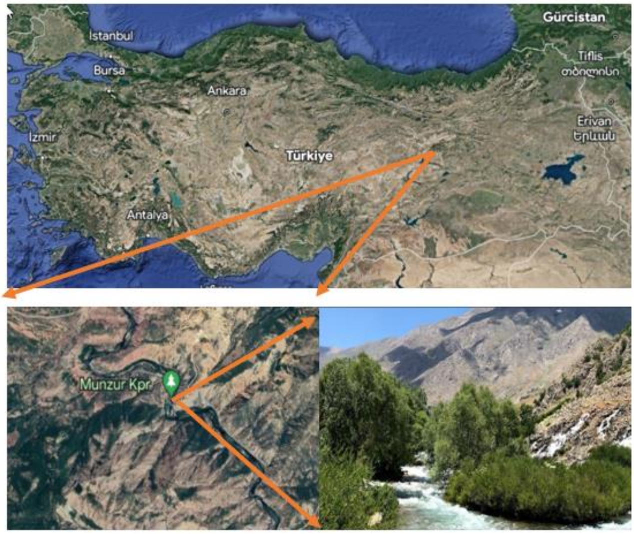

Figure 1. G. pulex location

Figure 2. G. pulex samples in aquarium

2.7. Exposure of *G. pulex* to treated and untreated glyphosate solution

The lethal concentration (LC50) value for glyphosate was precisely determined as 422.49±35.65. this untreated glyphosate solutions were prepared at three different sublethal doses, specifically 1/16, 1/8, and 1/4 of the LC value previously established for glyphosate. These treated and untreated solutions were subsequently used to expose the organisms for both 24 and 96 hours. Each group consisted of 10 organisms, and all applications were executed with three replications (Figure 3). No feeding material was given during exposure. Any deceased individuals were promptly removed from the experiment. The treated and untreated groups to which *G. pulex* was exposed are shown in Table 3.

Figure 3. G. pulex experimental setup

Table 3. The treated and untreated groups to which G. pulex was exposed

Groups	Mediums
Control	Natural living Water
A (Untreated)	1/4 Glyphosate + 10 ml *B. aryabhattai* + Soil
B (Untreated)	1/8 Glyphosate + 10 ml *B. aryabhattai* + Soil
C (Untreated)	1/16 Glyphosate + 10 ml *B. aryabhattai* + Soil
D (Treated)	1/4 Glyphosate + 10 ml *B. aryabhattai* + Soil
E (Treated)	1/8 Glyphosate + 10 ml *B. aryabhattai* + Soil
F (Treated)	1/16 Glyphosate + 10 ml *B. aryabhattai* + Soil

2.8. Preparation of tissues and procedures of dissection

A 1/5 w/v phosphate-buffered saline solution (PBS) was added to *G. pulex* samples and homogenized with a homogenizer. Then, these samples were centrifuged in a refrigerated centrifuge at 17,000 rpm for 15 minutes, and the supernatants were stored in the deep freezer at -86 °C until the experimental stage

2.9. Biochemical analysis

For biochemical analyses; SOD, CAT, AChE activities and GSH, MDA levels were analyzed. The kits used in the study were purchased from CAYMAN. Catalog number of kits were as CAT:

707002, SOD: 706002, AChE: CSB-E17001Fh, GSH:703002 TBARS:10009055.

2.9.1.SOD Enzyme activity

In this method, in which the kit content is determined, xanthine oxidase and hypoxanthine tetrazolium salt is used to determine the superoxide radicals produced. A unit of Superoxide showing the dismutation of 50% of the free radical is defined as SOD. This method is used to determine the activity of three types of SOD (Cu/Zn, Mn, Fe - SOD).

2.9.2.Catalase enzyme activity

The basic principle of the method is that the enzyme is accompanied by an optimal H2O2 concentration by reacting with methanol. The generated formaldehyde transforms into 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald), and its spectrophotometric measurement is conducted as a chromogen. Purpald, particularly in the presence of aldehydes, forms a bicyclic heterocycle. The alteration from a colorless to purple hue is observed based on the oxidation transformation.

2.9.3. Acetylcholinesterase enzyme activity

The prepared homogenates were centrifuged at 3500 rpm for 15 minutes. AChE enzyme activity studied by taking supernatants CUSABIO brand CSB-E17001Fh catalog. It was determined with a microplate reader according to the ELISA method with kits No.

2.9.4. Glutathione levels

The sulfhydryl group of GSH reacts with DTNB (5,5'-dithio-bis-2-nitrobenzoic acid, Ellman's reagent), resulting in the formation of a yellow TNB (thionitrobenzoate). The recycling process involves the disulfide mixture of GSH and GSTNB (between GSH and TNB), catalyzed by glutathione reductase, leading to the generation of more TNB. The rate of TNB production is directly proportional to the GSH concentration in the sample, indicating a proportionate relationship to the recycling reaction.

Measuring the absorption of TNB at either 405 or 412 nm offers a precise estimation of GSH in the sample. The GSH disulfide dimer to GSSG is readily oxidized. The inclusion of glutathione reductase in the GSH test kit allows for the measurement of both GSH and GSSG, reflecting the total glutathione content due to the enzymatic reduction of GSSG back to GSH. GSH levels were measured with Elisa Kits purchased from the company. Included in the kit GSH using the calculation formulas of the company whose absorbance curve was created with the standards levels were calculated.

2.9.5.TBARS (Thiobarbituric Acid Reagent) levels

Lipid peroxidation levels are a well-known marker of cellular damage in living organisms. It is employed as an indicator of oxidative stress taking place in cells and tissues. Polyunsaturated lipid peroxides, originating from fatty acids, are unstable and give rise to reactive carbonyl compounds. MDA (malondialdehyde) can be quantified through a controlled reaction with thiobarbituric acid, resulting in the formation of TBARS (thiobarbituric acid reactive substances). The TBARS assay, using a kit from Cayman Company, is conducted under high temperatures (90-100°C) and acidic conditions. The MDA-TBA adduct formed as a result of the MDA and TBA reaction is colorimetric based on 530-540 nm measurement.

2.10. Statistical analysis

Statistical analyzes of all data were performed using SPSS 24.0 statistical program. Probit analysis used for calculatingthe LC50 value of the glyphosate hebicide in *G. pulex.* For biochemical parameters, the statistical distinction among all control, untreated, and treated groups within the same application period was assessed using Duncan's multiple range test. Additionally, an independent t-test was employed to identify differences between the application times (24 and 96 hours).

3. Results

3.1. Soil analysis

According to the soil quality results obtained, the soil type used in the bioremediation study was clayey with 77% in terms of saturation; alkaline with a pH value of 7.8; Salt-free with a total salt content of 0.03%; very chalky with a value of 26.35% in terms of lime percentage; When the ratios of nutrients in the soil are examined, it is found that it contains very little organic matter with a value of 0.87% in terms of organic matter; Considering the amount of available phosphorus, low values of 4.18kg/da indicate that the value of 575.9 kg/da, which is also calculated as available potassium, is high. Considering these values, according to Shanahan (2004), this soil type is suitable for bioremediation with nutrient supplementation. Nutrient supplement was also given to the system with *B. aryabhattai* and sabouraud dextrose broth (Shanahan, 2004).

3.2. Removal efficiencies

Removal efficiencies were associated with the 1400 mg/l COD and 680 mg/l TOC value of Glyphosate, which is recommended for use in agricultural land and added to the bioremediation mechanism created at the same time. Accordingly, the removal efficiencies were calculated over these two values. According to these results, 10 ml *B. aryabhattai* reduced the COD value from 1400 mg/l to 400 mg/l with a 71.1% removal rate in 13 days, while the decrease in TOC value was from 680 mg/l to 97.4 mg/l. It was found to be 85.6%. According to these results, the best removal real efficiency for both parameters is in the media of 10 ml *B. aryabhattai*, as in the system removal efficiency (Figure 4).

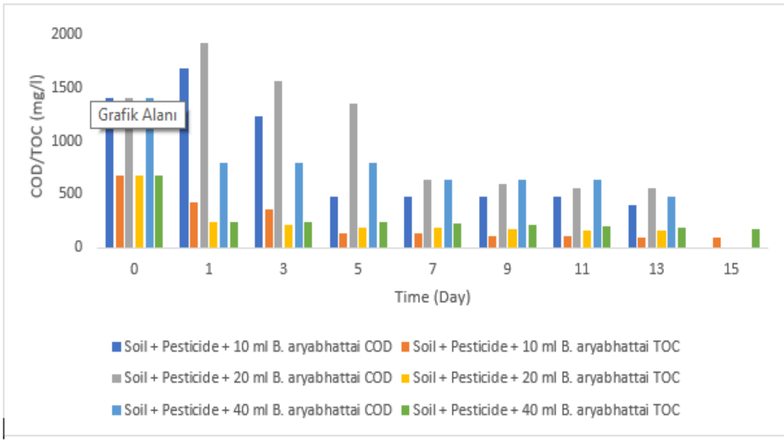

Figure 4. Actual COD-TOC removal amounts of filtrate obtained from the assembly consisting of soil + glyphosate + various concentrations of B. aryabhattai.

3.3. Biochemical biomarkers

3.3.1. AChE results

The AChE enzyme levels were assessed in *G. pulex* exposed to various concentrations of glyphosate solution before and after bioremediation. At 24 hours, it was observed that AChE levels increased compared to the control group, depending on the glyphosate application concentration ($p<0.05$). A significant increase was detected at the 96th hour due to glyphosate administration compared to the control group ($p<0.05$). When comparing enzyme levels before and after treatment, a decrease was observed at the 24th hour after treatment ($p<0.05$). There was a statistically significant decrease in the D group, but an increase in the E and F groups at the 96th hour after treatment ($p<0.05$). When comparing exposure times, a statistically significant difference was found in the D and E groups ($p<0.05$) (Figure 5).

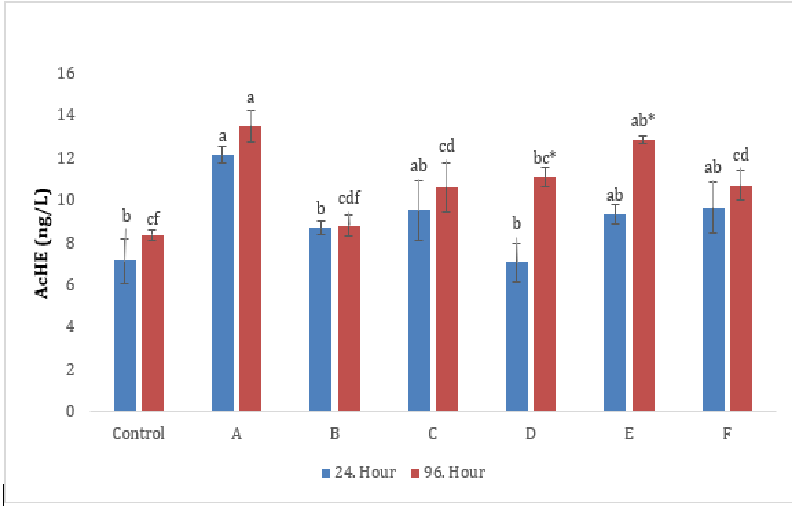

Figure 5. AcHE(ng/L) enzyme levels in G. pulex exposed to different concentrations of glyphosate solution before and after treatment

3.3.2. MDA results

MDA levels increased both at the 24th hour and at the end of the 96th hour in the three groups exposed to different sublethal doses of glyphosate compared to the control ($p<0.05$). However, it was observed that MDA levels decreased at 24 and 96 hours after treatment ($p<0.05$). A significant difference was found in the groups C and D when comparing exposure times ($p<0.05$) (Figure 6).

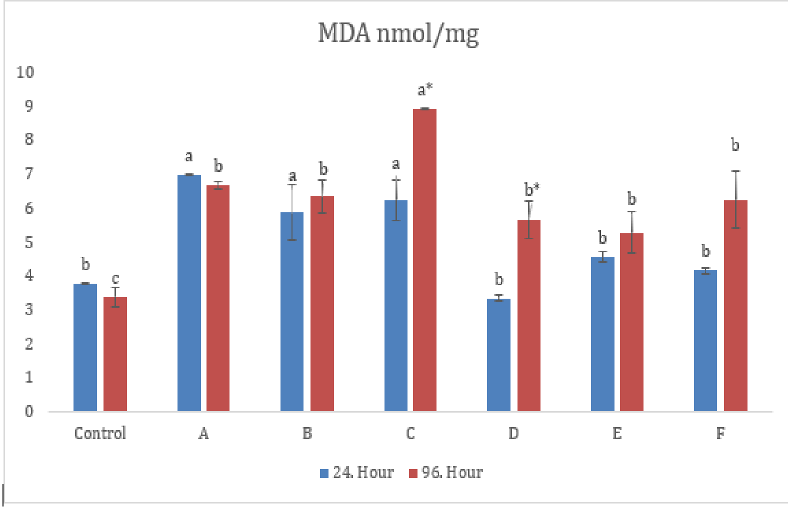

Figure 6. MDA (nmol/mg) levels in G. pulex exposed to different concentrations of glyphosate solution before and after treatment

3.3.3. GSH results

It was noted that GSH levels decreased at 24 hours, depending on the application concentration of glyphosate, compared to the control ($p<0.05$). A significant decrease was observed at the 96th hour due to glyphosate administration compared to the control ($p<0.05$). Moreover, GSH levels were observed to increase at the 24th hour after treatment ($p<0.05$), and a statistically significant increase was noted at the 96th hour after treatment ($p<0.05$). When comparing exposure times, statistically significant differences were found in the groups B, C, D, and F ($p<0.05$) (Figure 7).

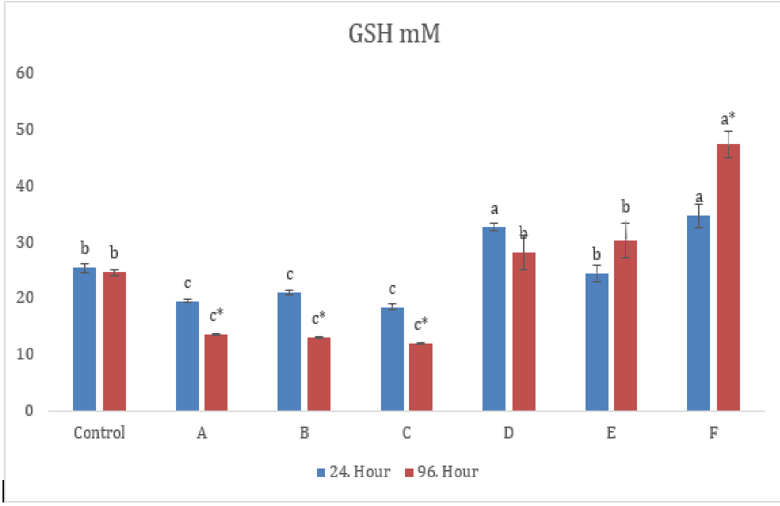

Figure 7. GSH (mM) levels in G. pulex exposed to different concentrations of glyphosate solution before and after treatment

3.3.4. SOD results

While SOD enzyme activities decreased in groups A and B at the end of the 24th hour compared to the control, at the end of the 96th hour, a decrease was detected in group A ($p<0.05$). When comparing SOD enzyme activities before and after treatment, it was observed that SOD enzyme activity increased at the 24th hour after treatment ($p<0.05$). Following treatment, SOD enzyme activity increased in the D group but decreased in the E and F groups at the end of the 96th hour ($p<0.05$). A significant difference was found in the groups B, C, D, and E when comparing exposure times ($p<0.05$) (Figure 8).

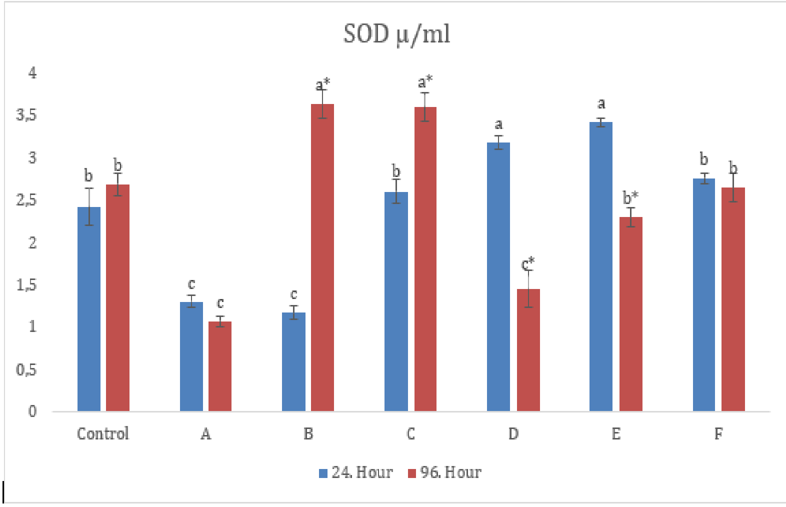

Figure 8. SOD (µ/mL) enzyme activities in G. pulex exposed to different concentrations of glyphosate solution before and after treatment

3.3.5. CAT results

CAT enzyme activities decreased both at the end of the 24th hour and at the end of the 96th hour in the groups treated with three different sublethal doses of glyphosate compared to the control ($p<0.05$). When comparing CAT enzyme activities before and after treatment, it was observed that CAT enzyme activity increased at the 24th hour after treatment ($p<0.05$). It was also found to increase at the end of the 96th hour after treatment ($p<0.05$). When comparing exposure times, a statistically significant difference was found in the control, A, B, D, E, and F groups ($p<0.05$) (Figure 9).

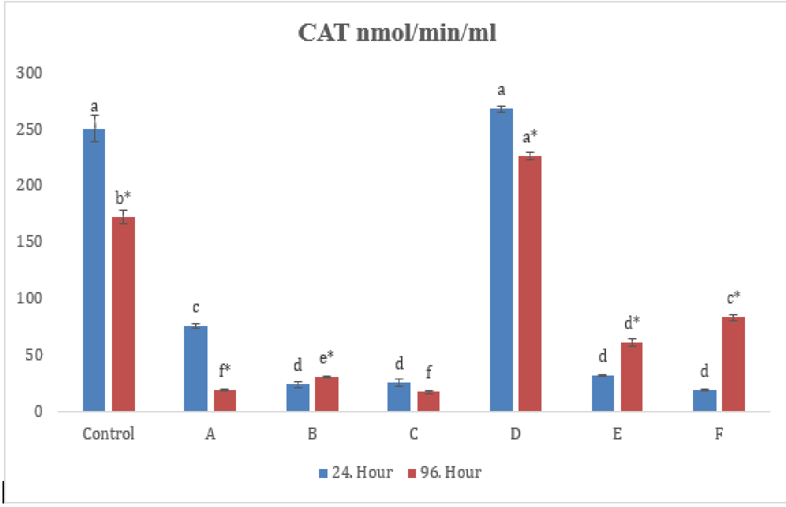

Figure 9. CAT (nmol/min/mL) enzyme activities in G. pulex exposed to different concentrations of glyphosate solution before and after treatment

4. Discussion

There is high demand to increase crop production and hence use of herbicides to meet the increasing nutritional requirement worldwide. However, excessive use of herbicides causes negative effects such as selection of resistant weeds, partial degradation of needed plants, production of toxic metabolites, degradation of soil microorganism communities, changes in biogeochemical cycles, changes in plant nutrition and soil fertility, and permanent environmental pollution (Pileggi et al., 2020). One of the most commonly used pesticides in both agricultural and non-agricultural areas is glyphosate with the active ingredient N-phosphonomethyl glycine, which is a non-selective but systemic type of herbicide (Koppenberg et al., 2023). Góngora-Echeverría et al. (2020) conducted a study on the degradation of glyphosate using pure strains of *Pseudomonas nitroreducens*, Ochrobactrum sp. B18, and *Pseudomonas citronellolis*, as well as their microbial consortium.

ADA-23B strain were used. According to the mortality results, they showed that it was quite capable of detoxifying glyphosate. Zhao et al. (2015) conducted a study on Pseudomonas spp. and identified that strains GA09, GA07, and GC04 exhibited the highest biodegradation efficiency against glyphosate. Given the widespread use of glyphosate as a herbicide globally, evaluating its potential toxicity to non-target species is crucial for the control of terrestrial and aquatic plants (Bareille and Gohin, 2020).

In a bioremediation study involving another insecticide, chlorpyrifos, it was observed that *B. cereus*, *B. subtilis*, *B. melitensis*, *P. aeruginosa*, *P. fluorescence*, and *S. marcescens* removed active material of this insecticide by 46-72% (Lakshmi et al., 2008). The chemical degradation of chlorsulfuron by Aspergillus niger was studied by Boschin et al. (2003), revealing approximately 30% degradation by the end of the 30^{th} day. Mohamed (2009) conducted a bioremediation study that Stenotrophomonas maltophilia M1 strain has the ability to degrade glyphosate insecticide. Erguven and Yildirim (2016) investigated the biodegradation rate of chlorsulfuron pesticide using only the COD parameter. Their findings, after 4.5 days, indicated that *B. simplex*, *B. muralis*, *M. luteus*, and *M. yunnanensis* exhibited COD removal efficiencies ranging from 70 to 93%. In another bioremediation study focused on the herbicide acetochlor by Erguven (2018), the researcher achieved removal efficiencies between 91 and 50% in COD, TOC, and BOD_5 using different soil microorganisms in agitated culture media.

Furthermore, Erguven and Yildirim (2019) investigated imidacloprid remediation employing *M. radiotolerans* and *M. arthrosphaerae* strains. After 18 days, for the COD parameter, the improvement of imidacloprid was 52, 96, and 99% with 20, 40, and

80 ml bacterial consortia, respectively. Alternatively for COD; BOD5 removal efficiencies were found to be 88, 79 and 50% in the same bacterial volumes. On the other hand, in our study, glyphosate removal was studied with *B. aryabhattai* and the removal rate determined as 71.1 and 85.6% for COD and TOC respectively.

According to our bioremediation results of this study, when the COD removal efficiencies of the system are examined, the best removal efficiency seen in 10 ml *B. aryabhattai* medium. The removal amount of the *B. aryabhatai* consortium in the soil was initially 1400 mg/l, but with the effect of the bacterial load, the COD load decreased to 404 mg/L. The efficiency of this removal was found to be 71.1%. The bacteria *B. aryabhattai*, in a 10 ml solution, decreased the TOC value from 680 mg/l to 97.4 mg/l, indicating a removal efficiency of 85.6%. This suggests that bioremediation can serve as an effective and alternative method for mitigating environmental pollution caused by glyphosate insecticide. The favorable enhancement of bioremediation using different bacterial strains in recipient environments implies that *B. aryabhattai* is a suitable microorganism for reducing the negative effects of pesticides in agricultural areas.

Antioxidant defense mechanisms involve enzymes such as SOD and CAT, along with non-enzymatic systems like GSH. Due to their heightened sensitivity to environmental pollutants, GSH is utilized as a marker for assessing the toxic effects of exposure to xenobiotics, as noted by Manno et al. in 1985 Manno et al., 1985). MDA (malondialdehyde) is indicative of a radical chain reaction in lipid peroxidation, potentially disrupting fundamental cell functions and leading to cell death. SOD plays a protective role against oxidative stress caused by free radicals, catalyzing the conversion of the superoxide anion to O_2 and subsequently facilitating the conversion of H_2O_2 to H_2O, as described by the reference given by Oruc et al (2000).

CAT mitigates oxidative stress induced by hydrogen peroxide by converting it into O_2 and H_2O (Meng et al., 2014). Antioxidant enzymes are responsive to contaminants and can be rapidly induced, making them relatively sensitive indicators of environmental damage compared to other toxicity parameters. Oxidative stress was observed in Oreochromis niloticus exposed to 2, 20, and 200 μg/L of metomyl (Meng et al., 2021).

In a study by Mansour et al. (2009), the effects of Methomyl on lipid peroxidation and antioxidant enzymes in rat erythrocytes were investigated. The results indicated that methomyl led to a decrease in AChE, SOD, and GST activities while increasing the level of lipid peroxidation (LPO) and the percentage of hemolysis. The antioxidant response occurred in a concentration-dependent manner. The antioxidant capacity of hepatic cells decreased in terms of SOD, CAT, glutathione reductase (GR), glutathione-S-transferase (GST) and GSH content activities (El-Khawaga, 2002). The bioremediation capacity of *Sphingomonas melonis* for methomyl-contaminated soil media was studied. The effectiveness of bioremediation was assessed by examining oxidative stress and neurotoxic responses in Dreissena polymorpha. GSH, MDA levels and CAT, SOD, AChE activities were determined before and after bioremediation. According to the results obtained, it has been suggested that methomyl can effectively ameliorate with *S. Melonis*, and changes in CAT, AChE activities, and GSH, TBARS levels can be useful biomarkers to evaluate the bioremediation capacity (Erguven et al., 2020). In another study, the efficacy of chlorpyrifos-ethyl healing was evaluated by *Methylobacterium radiotolerans* and *Microbacterium arthrosphaerae* using the response of some biochemical biomarkers. In this study, detoxifying and antioxidant enzyme response before and after bioremediation in *G. pulex* was investigated. Depending on chlorpyrifos-ethyl, the activity of catalase enzyme decreased before bioremediation and increased after it. SOD activity increased at the end of the 96th hour of chlorpyrifos-ethyl exposure. After bioremediation, SOD enzyme activity initially decreased at 24 hours but increased by the end of the 96^{th} hour, as reported by Tatar et al. in 2020. According to the study results, when MDA levels were compared before and after treatment, a significant decrease was observed at both the 24^{th} and 96^{th} hours after treatment ($p<0.05$). Comparing GSH levels before and after treatment revealed an increase at the 24^{th} hour ($p<0.05$), with a statistically significant increase observed at the 96th hour after treatment ($p<0.05$). When comparing SOD enzyme activities before and after treatment, an increase was noted at 24 hours after treatment ($p<0.05$). At the end of the 96^{th} hour, SOD enzyme activity increased in the D group after treatment but decreased in the E and F groups ($p<0.05$). Similarly, when comparing CAT enzyme activities before and after treatment, an increase was observed at the 24^{th} hour ($p<0.05$) and also at the end of the 96^{th} hour after treatment ($p<0.05$). The comparison of SOD and CAT enzyme activities before and after treatment, where enzyme activities increased again after treatment, serves as an indicator of the efficiency of the bioremediation method. In present study, it was found that oxidative stress was induced and MDA levels, an indicator of lipid peroxidation, increased due to exposure to the herbicide gyphosate. The antioxidant defense system was induced against oxidative stress that occurred after herbicide exposure, and SOD and CAT enzyme activities and GSH levels were therefore decreased. After an effective bioremediation; It was observed that SOD and CAT levels and GSH levels increased again.

Farrukh et al. (2017) conducted a study on the inhibitory effects of various phosphorothioates, including Ethyl parathion and Chlorpyriphos, and phosphates such as Dichlorvos, Monocrotophos, and Phosphamidon on acetylcholinesterase (AChE) in Sea Mackerel (*Rastrelliger kanagurta*) captured off the coast of Goa. Their findings indicated that among the five organophosphorus pesticides tested, dichlorphos exhibited the highest inhibitory potential, followed by Chlorpyriphos, Ethyl Parathion, Monocrotophos, and Phosphamidone. Moreover, in vitro studies revealed that muscle AChE inhibition in mackerel serves as an early warning signaling system for neurotoxic pollutant parameters in the environment, and AChE inhibition can be utilized as a biomarker (Farrukh, 2017).

Xuerep et al., (2009) investigated the response of organophosphorus chlorpyrifos and carbamate methomil to both feeding and locomotor behavior at the living organism level in *G. fossarum*, and according to their results, approximately 65% AChE inhibition levels not caused deaths in living tissues in the short term. The inhibitory effects of glyphosate, imazalil, imidacloprid and lambda-cyhalothrin insecticide on AChE, butyrylcholinesterase (BChE) and tyrosinase activity were evaluated using direct in vitro enzymatic inhibition methods. All pesticide applications inhibited the enzyme in a dose-dependent manner. The lowest neurotoxicity was observed as a result of Glyphosate administration with low acetylcholine inhibition rate (Sarkar et al., 2022). According to the results, it was observed that AChE levels increased due to glyphosate administration at 24 and 96th hours compared to the control ($p<0.05$). When comparing enzyme levels before and after treatment, it was observed that enzyme levels decreased at the 24th hour after treatment ($p<0.05$). At the 96th hour after treatment, there was a statistically significant decrease in group D, but an increase in groups E and F ($p<0.05$). Changes in AChE (acetylcholinesterase) activity are thought to result from the direct binding of glyphosate to the enzyme, alterations in the electrical charge of the membrane layer, an increase in reactive oxygen species (ROS), and modification of the membrane, as suggested (Bukowska and Hutnik 2006). The results showed that glyphosate administration causes toxic effects and AChE can be used as a suitable biomarker.

5. Conclusions

In this study, synthetic solutions containing glyphosate herbicide were treated by bioremediation method by *Bacillus aryabhattai*, then bioremediation efficiency was evaluated by determining the biochemical changes in model organism *G. pulex* before and after treatment. In the bioremediation experiments carried out in the first stage, the bioremediation system was used in order of Soil + Pesticide, Soil + Pesticide + 10 ml *B. aryabhattai*, Soil + Pesticide + 20 ml *B. aryabhattai* and Soil + Pesticide + 40 ml *B. aryabhattai* bacteria consortium. Bioremediation results consist of two stages: system efficiency and actual system efficiency.

When the COD and TOC removal efficiencies of the system were examined, the best removal efficiency was found in the soil of 10 ml *B. aryabhatai* consortium. The efficiency of this removal for COD; It was found to be 75.9%. Considering the TOC parameter, a removal efficiency of 76.8% was found. According to the dates obtained from this loboratory scale study, it was shown that glyphosate herbicide can be effectively removed by *B. aryabhattai*.

In the second phase of the study, *G. pulex* were exposed to treated and untreated synthetic glyphosate solutions for 24 and 96th hours to investigate the biochemical response. According to the results, it has been shown that bioremediation of glyphosate herbicide using *Bacillus aryabhattai* can be done and changes in MDA, GSH levels and SOD, CAT, AChE activities can be used in the evaluation of bioremediation efficiency.

CRediT authorship contribution statement

Nuran Cikcikoglu Yildirim: Investigation, Writing – original draft, Project administration, Funding acquisition editing. Gokhan Onder Erguven: Investigation, Validation, Writing – review & editing. Yusuf Soylemez: Conceptualization, Data curation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in the present study.

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CHAPTER III

Energy and Natural Resources at Artvin Çoruh University: Sustainability and Efficiency Perspectives

Adalet KESKİN[1]
Elanur ADAR YAZAR[2]
Kadir DOĞANŞAHİN[3]

1.Introduction

Natural resources refer to all kinds of substances and energy resources found in nature and used by humans (Zaharia & Suteu, 2011). Natural resources are water, air, soil, minerals, forest, oil, natural gas and coal. Soil, water, and forest are renewable resources, while fossil fuels and minerals are non-renewable resources (Doğanşahin & Demirarslan, 2021). Natural resources are used to

[1] Lecturer, Artvin Çoruh University, Borçka Acarlar Vocational School, Department of Electric Power Generation, Transmission and Distribution, Artvin/Turkiye, Orcid: 0000-0002-7778-7337, adalet.keskin@artvin.edu.tr
[2]Assoc. Prof., Artvin Çoruh University, Faculty of Health Sciences, Department of Occupational Health and Safety, Artvin/Turkiye, ORCID ID: 0000-0002-9609-0439, aelanur@artvin.edu.tr
[3] 3Assist. Prof., Malatya Turgut Özal University, Faculty of Engineering and Natural Sciences, Department of Electrical and Electronics Engineering, Malatya/Turkiye, ORCID ID: 0000-0002-6763-058X kadir.dogansahin@ozal.edu.tr

sustain people's lives, meet their basic needs and use them in economic activities. However, the fact that natural resources are limited and not managed sustainably can cause environmental problems and economic imbalance. In addition, population growth, development of technology and industry, rising living standards and rapid production and consumption increase the use of natural resources. Therefore, it is important to use natural resources effectively and efficiently, conserve them and transition to renewable resources (Ekins et al., 2016). In order to achieve success in the efficiency of natural resources, it is of great importance to understand the behaviors in the consumption of these resources. In the 2015 Global Status Reports, it was stated that the second highest share in global primary energy consumption was in buildings with 30%, while the share of industrial consumption was 31% (Global Status Reports 2017; Doğanşahin & Demirarslan, 2021).

Coal is a traditional fossil fuel and is commonly used to generate heat and electricity. However, the combustion of coal releases harmful emissions into the atmosphere, contributing to climate change and adversely affecting air quality. Fuel oil is another fossil fuel based on petroleum and is generally preferred for heating and power generation. However, the combustion of fuel oil can also release harmful pollutants into the atmosphere and factors such as its limited reserves and price fluctuations mean that it is not a sustainable energy source. Problems such as deteriorating air quality and air pollution pose serious threats to the environment and human health. Natural gas is considered a clean fossil fuel. It is widely used for heating, electricity generation and industrial uses. It has lower emissions compared to other fossil fuels, but there are environmental concerns such as methane leaks and hydraulic fracturing (Arı, 2023;

Kazanasmaz et al., 2023). Water is an indispensable part of life and is used in many areas such as drinking, agriculture, industrial production and energy generation. However, factors such as depletion and pollution of water resources can lead to water scarcity and environmental problems. Water is also one of the cleanest sources of energy and is used to generate electricity through hydroelectric power plants. However, the impacts of hydroelectric facilities on ecosystems and water resources should be considered (Pehlivan et al., 2017). Electrical energy is an indispensable part of modern life and is used in many areas. Electricity from renewable energy sources offers a more environmentally friendly and sustainable option compared to fossil fuels. Energy has become an indispensable requirement in every aspect of our lives today (Arı, 2023; Kazanasmaz et al., 2023). Factors such as increasing population, technological advances and industry's dependence on machine power rather than manpower continuously increase the demand for energy and the amount of its use. Existing energy resources are insufficient to meet this increasing demand. Therefore, the exploration of alternative energy sources and the development of energy management policies are gaining importance. Over the last 20 years, global primary energy demand has increased by more than 50% to 162194.44 TWh in 2019. In the same period, electricity consumption reached 27004.7 TWh in 2019 (Bernard, 2020).

Among natural resources, water and fossil fuels are critical for meeting our basic needs such as heat and electricity. However, these resources need to be used sustainably and efficiency needs to be increased. Universities, which host many buildings and facilities, also consume more natural resources. The number of employees and the number of students served at the university directly affect the consumption of natural resources used to meet basic needs such as lighting, water supply and air conditioning (Oyedepo et al., 2015). In universities, the effective and efficient use of natural resources is critical for minimizing environmental impact and sustainability. Understanding behaviors on natural resource consumption in universities is fundamental to efforts to improve natural resource efficiency. However, interest in natural resource sustainability in universities has not yet increased sufficiently, which requires raising awareness among university administrators and experts.

The consumption of natural resources (coal, fuel oil, natural gas, water and electricity) on Artvin Çoruh University (AÇU) campuses between 2016 and 2023 has been analyzed in detail. Rates of electricity, fossil fuel, natural gas and water use are evaluated considering factors such as campus size and number of students, and some recommendations are presented. This study plays an important role in identifying energy consumption trends on campuses and their potential for improvement. It also contributes to the knowledge of the institution on how changes in the number of students can affect consumption. The findings of the study can be a reference for similar institutions to understand their natural resource and energy consumption behavior.

2.Artvin Çoruh University

The university is located in the province of Artvin in the Black Sea Region of Turkiye. It consists of 8 campuses and 1 botanical garden. Data were obtained from internal authorized units and annual reports (AÇU, 2024). As for the use of electrical energy, water and natural gas, which was transformed in the central campus as of 2020, monthly usage amounts with more realistic values could be obtained on a campus basis since monthly invoices are made by the suppliers of these resources. In campuses using coal and fuel oil, monthly usage and consumption data are not available in the campus shares since these resources are purchased wholesale through tenders in certain periods. The use of these resources for heating purposes starts especially in the late autumn months and continues until the spring months of the following year.

Seyitler Campus is located in Seyitler Village of Artvin City Center. In this campus, there are Faculties of Forestry, Engineering, Business Administration, Sports Sciences and Tourism, Artvin Vocational School and Graduate School of Education as academic unit buildings. The Rectorate and administrative departments were also located in the Faculty of Forestry building and moved to the central campus in June 2022. Other buildings on the campus are the Building Works, Furniture Decoration Workshop, Sports Hall, Social Culture Center, Technology Transfer Office and Science Technology, Application and Research Center.

The central campus was established on a total area of 78572.03 m^2 at the entrance of Artvin City Center, on the site of the old chipboard factory. This campus includes the Rectorate, 4 faculties, 1 central library, 1 congress culture center and other units. The Rectorate building has been in the central campus since June 2022.

Hopa campus has 1 education building and 1 administrative building. Hopa Vocational School, Faculty of Economics and Administrative Sciences and Faculty of Theology operate on the campus. There is also a fitness center with a closed area of 406.64 m^2 on this campus.

Arhavi campus moved to its new building in 2018. There is Arhavi Vocational School and the Faculty of Art and Design, which moved to Arhavi in 2018.

The other campuses are the Vocational School buildings in Borçka, Şavşat and Yusufeli districts, the education building with two associate degree programs at Artvin Vocational School in Ardanuç district, and the Ali Nihat Gökyiğit Botanical Garden in Artvin Center Salkımlı Village. Technical information about the campuses is given in Table 1.

Currently, three different sources are used to provide heat energy in AÇU campuses. These are coal, fuel oil and natural gas. The status of the sources used to obtain heating energy according to the campuses is presented in Table 1.

Table 1: The technical informations of Artvin Çoruh University campuses (AÇU, 2024)

Open Area (m^2)	Campuses/ Units	Year of foundation	Closed Area (m^2)	Electric Installed Power/Contracted Power (kW)	Number of Students								Fuel Type
					2023	2022	2021	2020	2019	2018	2017	2016	
78572.03	Central Campus	2008	**47906.17**	2000/1200 MV Transformer Subscription	5439	5239	5188	4916	4617	4131	3599	3201	Fuel-Oil, Natural Gas (Converted in January 2020)
183952.23	Seyitler Campus	2009	**40796.47**	2x1000/600 MV Transformer Subscription	4161	3869	3391	2812	2361	2367	2992	2962	Coal, Fuel Oil
21846.23	Hopa Campus	2010	**9050.74**	1600/960 Transformer Subscription	1407	1386	1354	1496	1680	1782	1948	1979	Fuel Oil
12815.73	Arhavi Campus	2018	**8989.80**	630/378	631	631	584	588	642	634	712	719	Fuel-Oil
28391.00	Borçka Campus	2014	**792284**	845.26/507.156	952	856	687	557	539	476	491	472	Fuel-Oil
7821.64	Şavşat Campus	2016	**4566.24**	5.0/5.0	330	325	304	271	243	163	80	40	Coal
7698.36	Ardanuç Campus		**734.01**	14.739/8.843	-	-	178	121	76	41	-	-	Coal
-	Yusufeli Campus*			28.954/17.372	121	9	23	57	131	163	162	131	Coal
120000.00	ANG Botanical Garden		**2856.61**	250/150									Natural Gas

*: The campus in Yusufeli district was closed. Current students continue their education in the central city campus

3.Use of energy and natural resources at Artvin Çoruh University

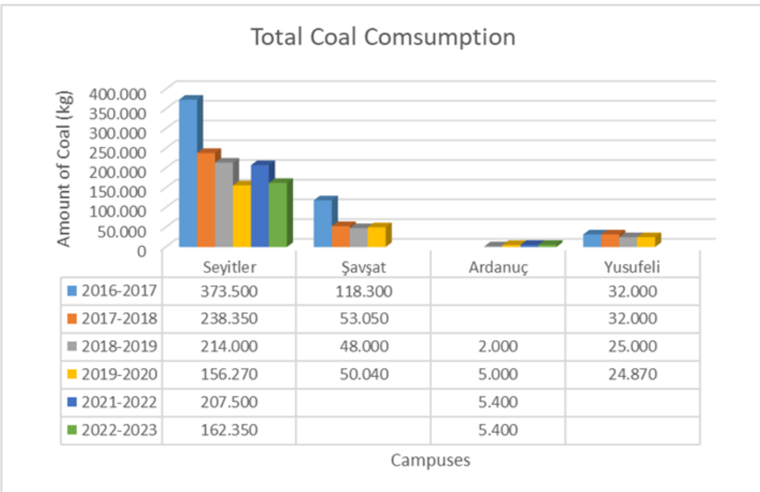
3.1.Coal consumption values by years

In Seyitler campus, the heating method of the buildings except the gymnasium is coal. The total closed area heated with coal in Seyitler campus is around 35000 m^2. The amounts of coal consumed per m^2 by years are 10.67 kg, 6.81 kg, 6.11 kg, 4.46 kg, 5.92 kg and 4.64 kg, respectively (Figure 1 and Table 2). In the last two periods, the absence of formal education due to the pandemic decreased the consumption values. After 2016, the average coal consumption is 5.59 kg, which is approximately half of 2016. This may be due to climate change, the pandemic and the increasing number of dams in Artvin. Compared to the past, winters are shorter and the climate can be quite variable.

The second largest campus using coal is the Vocational School building in Şavşat district. As of the establishment year of this building, the heating method was determined as coal according to the district conditions. However, with the arrival of natural gas in the district in 2022, the building was converted to natural gas. Şavşat Vocational School was opened in the 2016-2017 academic year. The high value of the 2016-2017 period in the graph is due to the amount of coal purchase (Figure 1 and Table 2). There is no record of consumed coal data. Coal from this period was also used in the following periods. The annual average amount of coal per m^2 is 12.5 kg. Since the number of students and classrooms increased between 2017-2020, there was an average level of use. Coal consumption decreased with each passing year.

The third campus that uses coal is the Ardanuç district education building. Education in the district started in the 2018-2019 academic year. The amount of coal purchased for the building as of this period is given in Figure 1 and Table 2. The annual average amount of coal per m^2 is 6.32 kg.

Yusufeli Vocational School moved its students to Artvin Central Campus as of 2020 due to the relocation of the district due to the Yusufeli Dam works. When the status of the district is clarified, the Vocational School will continue education in its new building in the district. Due to this situation in the district, a new department was not opened in the new vocational school and no students were admitted. There was an average level of coal use during the academic year. Coal utilization data for the four-year period are given in Figure 1 and Table 2.



	Seyitler	Şavşat	Ardanuç	Yusufeli
2016-2017	373.500	118.300		32.000
2017-2018	238.350	53.050		32.000
2018-2019	214.000	48.000	2.000	25.000
2019-2020	156.270	50.040	5.000	24.870
2021-2022	207.500		5.400	
2022-2023	162.350		5.400	

Figure 1: Total coal consumption for some campuses of AÇU

According to the graph, which shows the situation of all campuses heated with coal, it is seen that Seyitler Campus has the highest coal use according to the size of the ratio of indoor area and number of students. Since there is no regular record of the amount of coal consumed in the coal data, an evaluation was made based on the amount of coal purchases.

Table 2: Coal consumptions for some campuses of AÇU

Seyitler	**kg**	**kcal**	**toe**
2016-2017	373.500	2390400000	239.04
2017-2018	238.350	1525440000	152.55
2018-2019	214.000	1369600000	136.96
2019-2020	156.270	1000128000	100.01
2020-2021	181.740	1163136000	116.31
2021-2022	207.500	1328000000	132.80
2022-2023	162.350	1039040000	103.90
Şavşat	**kg**	**kcal**	**toe**
2016-2017	118.300	851760000	85.18
2017-2018	53.050	381960000	38.20
2018-2019	48.000	345600000	34.56
2019-2020	50.040	320256000	32.03
2020-2021	15.240	97536000	9.75
2021-2022	2.175m^3 Natural Gas		
2022-2023	35.228m^3 Natural Gas		
Ardanuç	**kg**	**kcal**	**toe**
2016-2017			
2017-2018			
2018-2019	2.000	14400000	1.44
2019-2020	5.000	36000000	3.60
2020-2021	5.400	38880000	3.88
2021-2022	5.400	38880000	3.88
2022-2023	5.400	38880000	3.88
Yusufeli	**kg**	**kcal**	**toe**
2016-2017	32.000	230400000	23.04

2017-2018	32.000	230400000	23.04
2018-2019	25.000	180000000	18.00
2019-2020	24.870	179064000	17.9
2020-2021			

3.2.Fuel-oil consumption values by years

The highest rate of fuel oil use is in the central campus. The central campus has a closed area 4-5 times larger than the closed areas of Hopa, Arhavi and Borçka campuses that use fuel oil. Since the number of students in the central campus is higher than in other areas, fuel oil usage values are at the highest level. While the number of students in the central campus and Borçka campus has increased over the years, the number of students in Hopa and Arhavi campuses has decreased over the years. However, we cannot say that there is a decrease in fuel oil usage due to the decrease in the number of students. In 2019-2020 and 2020-2021 periods, the lack of students due to the pandemic affected the results. Since natural gas conversion was made in the central campus as of 2020, fuel oil was used only in the last months of 2019 in the 2019-2020 period, and natural gas started to be used as of 2020. In the fuel data, an evaluation was made based on the amount of fuel oil purchased for the campuses. Consumption values were calculated approximately based on the purchase amounts (Figure 2 and Table 3).

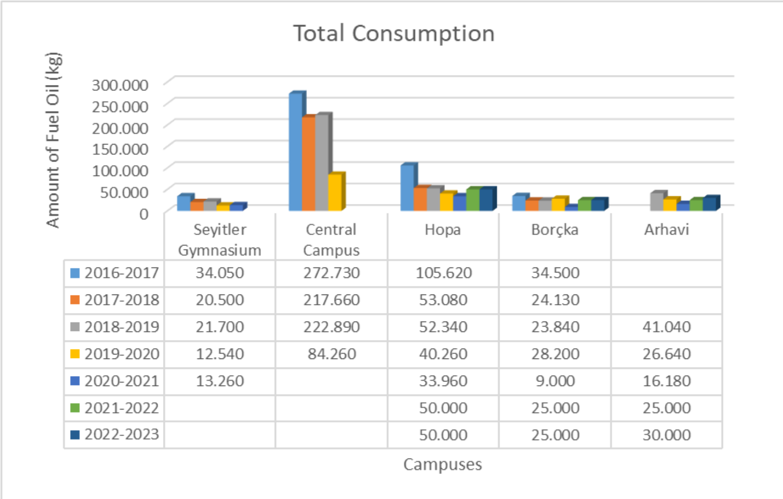


Figure 2: Total fuel-oil consumption for some campuses of AÇU

Table 3: Fuel-oil consumptions for some campuses of AÇU

Central	**kg**	**kcal**	**toe**
2016-2017	272.730	2618208000	261.82
2017-2018	217.660	2089536000	208.95
2018-2019	222.890	2139744000	213.97
2019-2020	84.260	808896000	80.89
2020-2021			
2021-2022	**Natural gas conversion done in February 2020**		
Borçka	**kg**	**kcal**	**toe**
2016-2017	34.500	331200000	33.12
2017-2018	24.130	231648000	23.16
2018-2019	23.840	228864000	22.89
2019-2020	28.200	270720000	27.07
2020-2021	9.000	86400000	8.64
2021-2022	25.000	240000000	24.00
2022-2023	25.000	240000000	24.00
Hopa	**kg**	**kcal**	**toe**
2016-2017	105.620	1013952000	101.40

2017-2018	53.080	509568000	50.96
2018-2019	52.340	502464000	50.25
2019-2020	40.260	386496000	38.65
2020-2021	33.960	326016000	32.60
2021-2022	50.000	480000000	48.00
2022-2023	50.000	480000000	48.00
Arhavi	**kg**	**kcal**	**toe**
2016-2017		0	
2017-2018		0	
2018-2019	41.040	393984000	39.40
2019-2020	26.640	255744000	25.57
2020-2021	16.180	155328000	15.53
2021-2022	25000	240000000	
2022-2023	30000	288000000	
Seyitler Gymnasium	**kg**	**kcal**	**toe**
2016-2017	34.050	326880000	32.69
2017-2018	20.500	196800000	19.68
2018-2019	21.700	208320000	20.83
2019-2020	12.540	120384000	12.04
2020-2021	13.260	127296000	12.73
2021-2022			
2022-2023	A different LPG system converison was made		

3.3.Natural gas consumption values by years

Natural gas is the primary energy source both in electricity generation and heating of houses in the world and in Turkiye. Natural gas has reached all provinces in Turkiye and is now used as heat energy in residences and workplaces. Due to its geographical structure and strategic location, Artvin province has been a province that has reached natural gas late. Natural gas was brought to the city with a pipeline at the beginning of 2020, and AÇU was the first institution to transform the city center due to the location of its central campus. Ali Nihat Gökyiğit Botanical Garden is the other campus of AÇU that benefited from the natural gas pipeline and was transformed as a priority. In early 2022, Şavşat District also received natural gas. Thus, natural gas transformation was realized at the Şavşat campus.

Natural gas usage values at AÇU are given in Figure 3 and Table 4 according to the annual and monthly values of the central campus for the years 2020-2023.

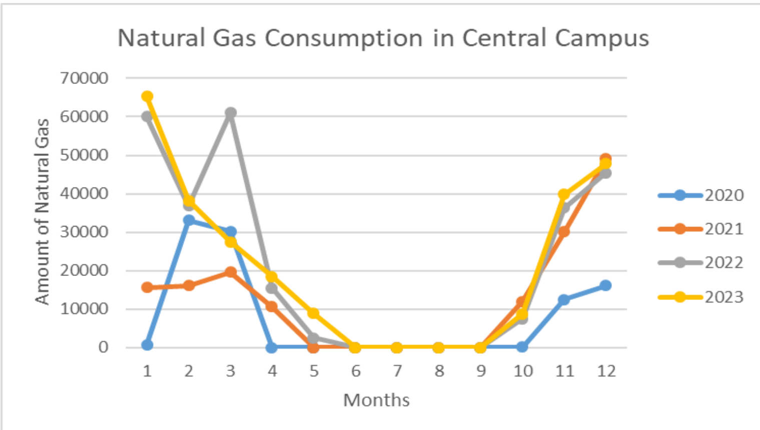


Figure 3: Natural gas consumption for Central campus

Table 4: Natural gas consumption values by years (Central campus)

Months	**2020(m^3)**	**2021(m^3)**	**2022(m^3)**	**2023(m^3)**
January	792	15610	60070	65364
February	33110	16141	36881	38164
March	30215	19617	61073	27504
April	0	10632	15423	18413
May	0	0	2533	8875

June	0	0	0	0
July	0	0	0	0
August	0	0	0	0
September	0	0	0	0
October	143	11988	7536	8714
November	12432	30081	36288	39833
December	16054	49026	45508	47864
Total	**92746**	**153095**	**265312**	**254731**
Number of Students	4916	5188	5239	5439
m^3 per student	**18.87**	**29.50**	**50.64**	**46.83**

At the beginning of 2020, natural gas conversion took place in the Central Campus. Since there were no students due to the pandemic during this period, the amount of natural gas per student remained at the level of 18.87 m^3. In 2021, with the ongoing distance education process and the start of face-to-face education in October 2021, the usage value per student increased to 29.50 m^3. Since the winter period of 2021-2022 was cold and long across the country, the natural gas utilization value in this period was realized at a high slope compared to the previous periods starting from the end of 2021.

3.4.Water consumption values by years

Water is a primary and indispensable natural resource for living things to survive. It is not possible for all living things to survive without water. Today, apart from being a source of life, the most common use of water is for cleaning needs. Water services are provided by local governments in homes and wherever there is life. Municipalities and special administrations, which provide water services in order to provide uninterrupted water and operate water installations, invoice water usage through meters. Annual and monthly water consumption assessments for the years 2019-2023 for the Seyitler and Central campuses in the city center of Artvin, where the highest water use is provided at AÇU, are given in Figure 4 and Figure 5.

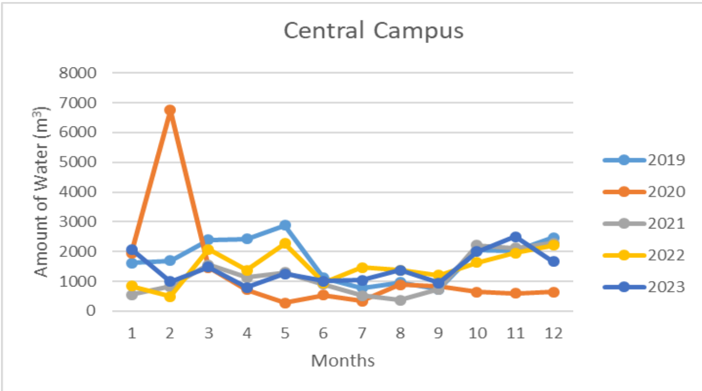


Figure 4: *Water consumption for Central campus*

When the water consumption values in the central campus are examined, again due to the pandemic, the water consumption curve did not change much from March 2020 until the end of the year. In February 2020, there was a 6744 m^3 water consumption in the central campus due to a problem with meter reading failure. Until October 2021, there were no students at the campus, which kept the water consumption curve at constant levels. With the resumption of face-to-face education in the 2021-2022 academic year, the water consumption curve increased. This period is at the same level as the 2019 period. In the first 6 months of 2019, the curve was at high levels, unlike 2020 and 2021, due to normal education and student presence before the pandemic. In June 2022, the Rectorate units moved from Seyitler campus to the Central campus. Therefore, as of this period, there is a decrease in Seyitler campus water use and a slight increase in Central campus water use. In February 2023, after the major earthquake in February, the transition to distance education decreased the water usage rate again.

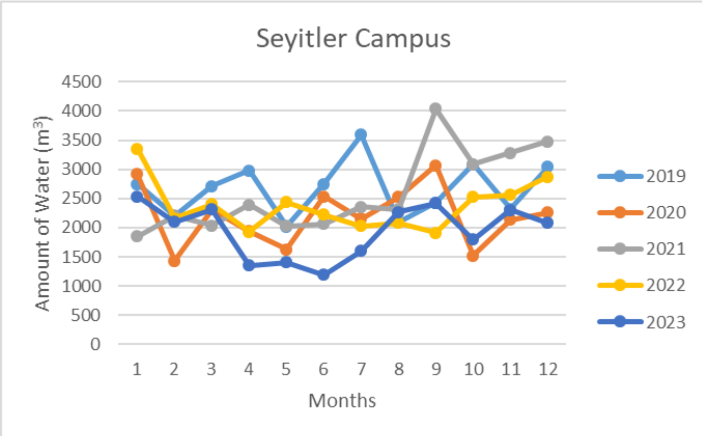


Figure 5: Water consumption for Seyitler campus

When we look at the water consumption values of Seyitler campus, it cannot be said that there is no pandemic-related impact (Figure 5). Until 2023, water consumption values were at average levels due to the fact that the Rectorate and administrative units were located in this campus. However, as of October 2021, when face-to-face education started after the pandemic, water use increased.

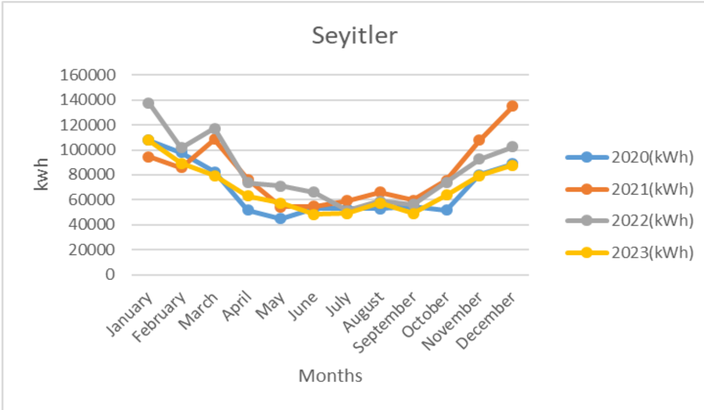
Since the monthly water use indices of the campuses in Artvin Center are regular, they can be graphed on a monthly basis. Monthly water consumption of the settlements in Hopa, Arhavi, Borçka and Şavşat districts could not be compared on a monthly basis since the municipalities in these districts do not regularly invoice water consumption every month. Instead, the total water consumption values for 2019-2023 are given in Table 5. In 2019, the presence of students in the campuses again affected the total water consumption values in direct proportion. However, according to the table, considering the physical size of Arhavi Campus, it is seen that there is more water consumption compared to other campuses.

Table 5: Water consumption for all campuses

Years	Central			Seyitler			Şavşat		
	Number of Students	**Water (m^3)**	**m^3 per student**	**Number of Students**	**Water (m^3)**	**m^3 per student**	**Number of Students**	**Water (m^3)**	**m^3 per student**
2019	4131	21125	**5.11**	2361	31978	**9.31**	163	561	**3.44**
2020	4617	15636	**3.39**	2812	26432	**9.39**	243	199	**0.82**
2021	4916	14476	**2.94**	3391	31148	**11.57**	271	844	**3.11**
2022	5239	17822	**3.40**	3869	28504	**8.05**	325	926	**2.85**
2023	5439	17104	**3.14**	4161	23398	**5.62**	330	1103	**3.34**
Years	**Hopa**			**Arhavi**			**Borçka**		
	Number of Students	**Water (m^3)**	**m^3 per student**	**Number of Students**	**Water (m^3)**	**m^3 per student**	**Number of Students**	**Water (m^3)**	**m^3 per student**
2019	1782	5469	**3.07**	634	2816	**4.44**	476	1081	**2.27**
2020	1680	1959	**1.17**	642	2483	**3.87**	539	565	**1.05**
2021	1469	924	**0.63**	588	2542	**4.32**	557	641	**1.15**
2022	1386	2363	**1.70**	631	3036	**4.81**	856	1164	**1.36**
2023	1407	1743	**1.24**	631	1768	**2.80**	952	652	**0.68**

3.5.Electric energy consumption values by years

In addition to coal, fuel oil, natural gas and water, which are used as natural resources at AÇU, electricity energy use values, which are secondary energy sources that we constantly need in our home and business activities, which have become an indispensable energy source today, are given on a campus basis (Figure 6-9). Central Campus and Seyitler Campus were prioritized according to campus size (Figure 6).



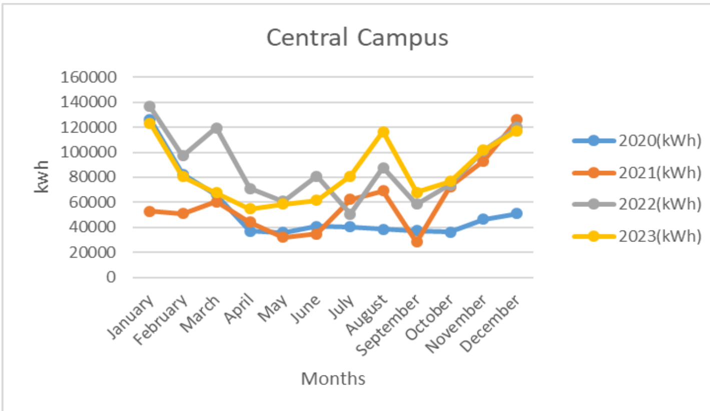
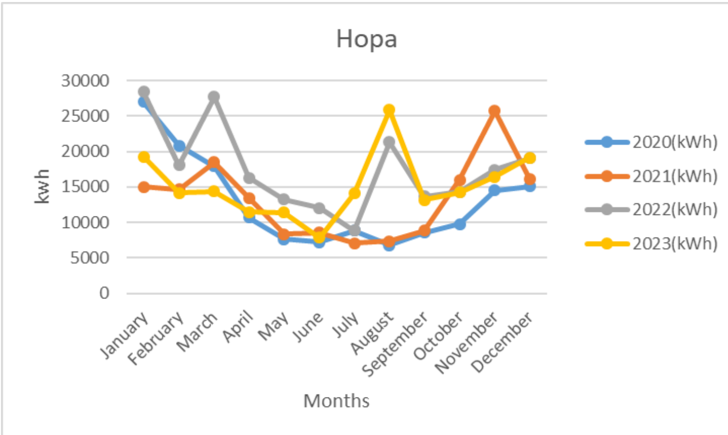
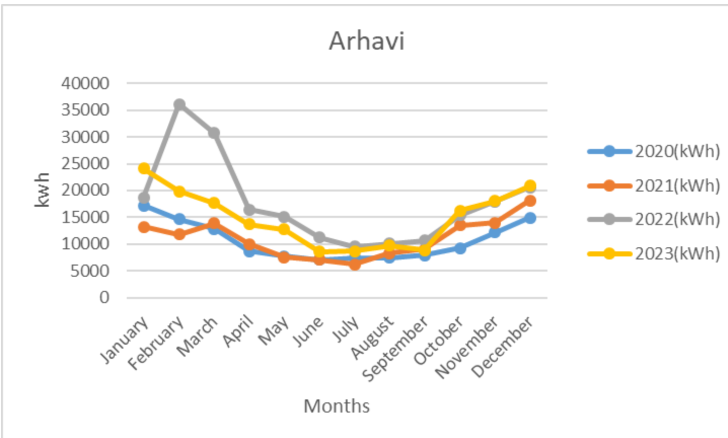


Figure 6: Electrical energy consumption for Central and Seyitler campuses

When looking at the electric energy consumption rates of Seyitler and Central campuses for the years 2020-2023, it is seen that there is approximately the same consumption in the months corresponding to the education period. In the summer period, consumption values are higher than the central campus due to the presence of administrative units in Seyitler and the continuation of their activities. It is also observed from the data that usage values decrease during the pandemic period.

In Figure 7, it is given the results of electricity use in Hopa, Arhavi, Borçka, Şavşat, Ardanuç and Yusufeli district campuses, respectively. The ranking is based on campus size. After Artvin central campus, evaluations were made based on monthly electricity consumption indices for the years 2020-2023 according to the size of the electricity installed capacity.





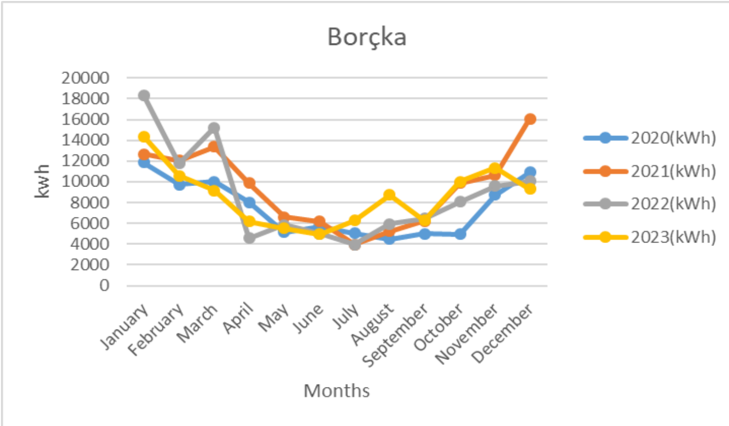


Figure 7: Electrical energy consumption for Hopa, Arhavi and Borçka campuses

Looking at the graphs of the other three major campuses following the main campuses in the center of Artvin, it is seen that the usage rates are low during the pandemic effect and periods without students. As of October 2021, with the start of face-to-face education, the utilization values for this period increased compared to previous periods. In Arhavi campus, the number of students is gradually decreasing compared to previous periods, so electricity usage rates are expected to decrease. However, in 2022 values, although the number of students is lower than the others, it is seen that there is more usage. It is thought that this usage is based on personnel and building needs.

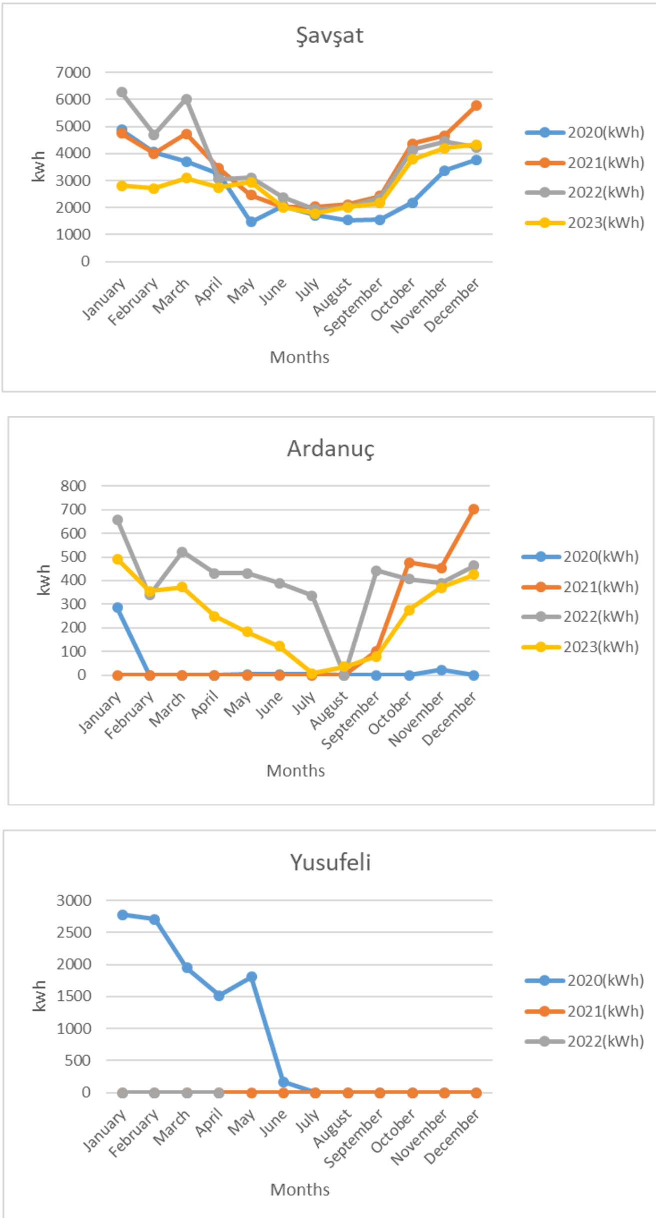


Figure 8: Electrical energy consumption for Şavşat, Ardanuç and Yusufeli campuses

Since Ardanuç, Şavşat and Yusufeli campuses do not have a complete campus and building organization, their utilization rates are low. Yusufeli campus was not used because it was closed. In Ardanuç, there was no use during the pandemic period. In the 2021-2022 academic year, due to the increase in the number of students in Şavşat and the transition to face-to-face education, the rate of electrical energy use increased significantly compared to previous periods (Figure 8).

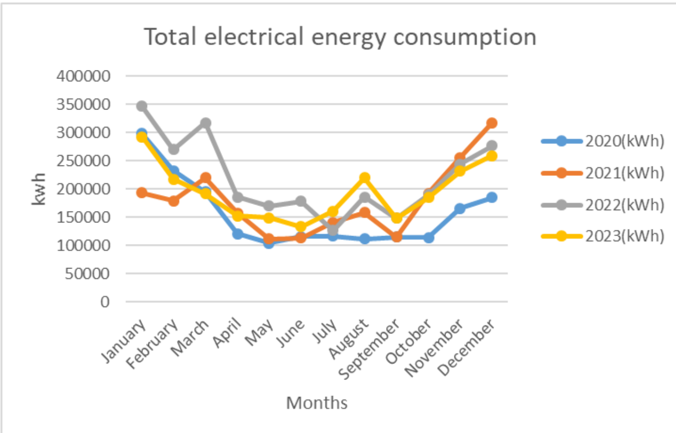


Figure 9: Total electrical energy consumption for AÇU

Table 6: Electrical energy consumption per student by year for AÇU

Year	**2020**	**2021**	**2022**	**2023**
Number of Students	10670	11531	12315	13041
Kwh per student	**175.57**	**186.66**	**214.30**	**179.43**

The total electric energy utilization data of AÇU for 2020-2023 are as above (Figure 9 and Table 6). According to these data, it is observed that the utilization values have increased as of October 2021, while the utilization value was low during the pandemic period. In the first four months of 2022, the utilization rate is very high compared to the first four months of previous years. In February 2023, post-earthquake distance education decreased the kWh usage per student.

In a similar study, Doğanşahin and Demirarslan (2021) compared the consumption of natural resources (electricity, water and fuel consumption for heating purposes) at AÇU in the 1-year period before the coronavirus pandemic that emerged on March 11, 2020 (2019-2020) and during the pandemic period (2020-2021) (only administrative and academic staff consumed natural resources during this period). Electricity and water consumption decreased significantly during the pandemic period. This leads to the conclusion that students also have a significant impact on natural resource consumption. In addition, this result shows that conducting theoretical courses remotely, excluding applied courses, would be beneficial in terms of natural resource consumption.

In their study, Alagöz et al. (2022) aimed to calculate and evaluate the carbon footprint of the Electricity Generation Corporation Central Campus (EGC), to determine measures to reduce the carbon footprint, to carry out training/awareness studies and to contribute to the more sustainable use of natural resources. For 2021, they calculated the total amount of carbon generated in EGC Central Campus as 2570 tons of CO_2 equivalent and the carbon footprint value per person as 2.28 tons of CO_2 equivalent. In order to reduce the amount of carbon and consumption of natural resources, they suggested increasing energy efficiency studies, including building insulation, hybridizing vehicles in the service network, conducting training/awareness studies, increasing CO_2 sequestration capacity by keeping the number of ground cover plants and green areas around the campus alive, and using solar energy panels on the campus.

Conscious water consumption of 139 students studying in the final year of biology, physics and chemistry teaching at the Department of Secondary Education, Kazım Karabekir Faculty of Education, Atatürk University was determined with a questionnaire study. In the study, it was emphasized that pre-service teachers did not pay much attention to water saving and that their water consumption behaviors varied according to gender, age, the department they studied and where they lived (Alaş et al., 2009). It can be concluded from the results of this study that education and awareness studies should be carried out more and periodically.

The results of the present study, although not conclusive, provide useful information for making approximate consumption estimates based on future student numbers. While providing information on the consumption of natural resources per student in formal education, it can suggest an approach to monitoring consumption in different institutions. The data obtained can help authorities in internal planning, consumption monitoring, efficiency and savings efforts.

When we look at the academic studies on natural resource consumption at the university, it can be said that they are limited. Saving studies on electricity use are especially intensive at the university. AÇU pays attention to energy conservation and protection of water resources. It takes measures to ensure energy efficiency on campuses. With this approach, it attaches importance to environmental sensitivity and resource conservation.

To ensure conscious consumption of natural resources at the university;

- Training seminars should be organized and projects should be developed to raise awareness on sustainability, water harvesting, renewable energy production, zero waste management, global climate change, etc.

- Environmentally friendly, energy-efficient products with low carbon emissions should be purchased and encouraged.

- More savings should be made in water, transportation, electricity and heating.

- Artvin is a rainy province and this feature should be turned into an opportunity by harvesting rain on campuses.

- Zero waste management approach should be adopted by staff and students

- Renewable energy sources (wind, solar and biomass energy) should be used for power, water heating, etc.

- Waste management should be carried out efficiently. Campus waste should be composted and used as fertilizer in gardens

- New buildings should be environmentally friendly green buildings.

4.Conclusion

With the impact of the pandemic process, Artvin Çoruh University has experienced significant changes in resource use.

Especially in the period from March 2020 to October 2021, there was a significant decrease in consumption values. However, with the start of face-to-face education in 2021-2022, an increase in resource use was observed, especially in the cold and long fall, winter and spring months. As the Rectorate and administrative units affiliated to the Rectorate, which were located in Seyitler campus, moved to the new building in the city campus in June 2022, the utilization values in Seyitler and Central campus have changed as of this period.

The impact of changes in the number of students on resource utilization rates is also noteworthy. While the number of students is expected to decrease in some units, higher resource utilization was observed in growing campuses and units with an increasing number of students. In particular, the fact that the administrative units at the Seyitler campus were active in the summer months until they moved to the central campus in 2023 caused resource utilization to be higher than other units during this period. However, it is important to evaluate these changes in terms of sustainability and efficiency. The decreases experienced during the pandemic period have shown that resources can be used effectively and efficiently. The saving measures and environmentally friendly practices implemented during this period can be adopted in the future and contribute to the conservation of resources and the reduction of environmental impact. On the other hand, increases in the number of students and increased resource utilization in growing campuses require more effective management and planning processes. Campus planning and infrastructure development strategies can ensure a balanced and efficient use of resources.

In conclusion, changes in Artvin Çoruh University's resource utilization can provide a more significant model for sustainability and efficiency. These observations emphasize the need to adopt more effective and environmentally friendly practices in the future. With the cooperation of the university management and stakeholders, it should be aimed to use resources sustainably and reduce environmental impact.

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CHAPTER IV

Effects of Climatic Change on Animal Production

Emre Aydemir
Nilgün Yapıcı
Medine Kaya
Songül Şahin

Introduction

Climate change has become a problem that closely affects livestock performance in many continents, countries and regions around the world. Especially in recent years, with the increasing population around the world, the need for nutrients has also increased. The most important sources of this increasing nutritional need are products of plant and animal origin. Especially animal-derived products meet a large part of the required protein needs. However, in recent years, the production potential does not meet the supply of population increase. One of the most important reasons for this is climate change. This change negatively affects animal production, which has a large share of approximately 70% in agricultural production (Gaughan and Cawdell-Smith 2015). This effect is the climatic differences observed in the atmospheric events of the earth. There are natural and unnatural factors in the formation of these differences.

As a result of these factors, warming and cooling are observed in the air mass in the atmosphere. While the global cooling is occurring as a result of lower than normal temperatures during the winter months; in the summer months, the temperature increase above normal causes global warming. Increasing temperature with global warming closely affects the livestock sector. In order to prioritize this effect, precautions are taken, such as choosing businesses that provide the environmental standards required by the model animal and choosing breeds with high genetic adaptation.

But; while there is a possibility to improve environmental conditions; is more difficult to make the genetic structure comply with the desired standards (Mitlöhner et al. 2001; Bernabucci et al. 2010). Many model animals are exposed to thermal stress against high temperatures As a result, it results in loss of productivity and performance, and even death, in the model animal (Mitlöhner et al. 2001; Bernabucci et al. 2010).

Temperature and habitat of the model animal

In animal production, the ideal temperature and humidity values required for the life of the model animal must be provided. In the comfort zone where these values are met; the model animal provides optimum performance and consumes minimum energy. But; as the temperature increases, the model animal is exposed to heat stress (Nardone et al. 2006). The heat stress that occurs in this situation occurs when the model animal is unable to dissipate sufficient heat to maintain homeothermia. As a result, it causes respiration, pulse and heart rate to increase (Schneider et al. 1988; Daramola et al. 2012).

Temperature and stress effects

Heat stress may occur as a result of the increase or decrease in the temperature of the comfort zone required for the model animal to survive. Changes in this temperature value may cause loss of efficiency and performance, such as a decrease in sperm quality, sexual behavior and fertility rate, embryonic deaths, deterioration in physiological, psychological and chemical structures. It is also stated that due to heat stress, the function of the reproductive system of the preovulatory follicle may be impaired, gonadotropin secretion may decline, the development of body luteum may decline, and the balance between biochemical and endometrial factors may be disrupted (Parsons 1994; Ullah et al. 1996; n Wolfenson et al. 1997). In particular, can result in death as well as production losses (Nardone et al. 2010; Tanizawa et al. 2014).

Relationship between temperature factor and animal production

The most common problem in the livestock industry due to global warming is heat stress. Cattle and poultry are especially sensitive to sudden changes in environmental conditions. For example; cattle with higher live weight, thicker hair, and animals with darker fur are very sensitive to heat. Dobson et al. According to the results of a study conducted by (2000), black cows; they found that they absorb much more heat than white colored ones. When the efficiency characteristics are examined; is known that milk yield in dairy cows decreases due to heat stress. The reason for this is; is stated that high milk yielding breeds are more sensitive to heat stress.

Moreover; it has been determined that the decrease in milk yield is caused by the heat they emit metabolically (Sprott et al. 2001; Hahn et al. 2003; Bernabucci et al. 2003; Rhoads et al. 2009; Elam et al. 2009; Salama et al. 2020). Mitloehner et al. (2001) in a study conducted by, depending on high temperature and solar radiation; they observed that there was a decrease in daily dry matter intake and average daily gain, carcass weight loss, lower fat thickness and an increase in disease cases in calves. In another study, Kadim et al. (2004) reported that the quality characteristics of beef were negatively affected during periods when air temperature increased (average temperature 34.3 ± 1.67 °C and 48.8 ± 7.57% relative humidity).

Examining the effects of heat stress on another yield characteristic, Roth et al. (2000), Rutledge (2001) observed that only 10% to 20% of inseminations performed during periods of exposure to heat stress resulted in normal pregnancies.

In another study, Dobson et al. (2001) reported that while pregnancy rates exceeded 50% in the winter months, this rate decreased to 20% in the summer months. Additionally, Dobson et al. (2001) stated that the Jersey breed is more tolerant to heat stress than the Holstein breed.

A decrease in feed consumption and conversion rate and daily live weight gain is observed due to heat stress. Consequently; has been found to have negative effects on productivity traits and reproductive performance such as milk, meat and eggs (Syafwan et al. 2011; Yadav et al. 2013; Herbut et al. 2019).

Unlike cattle and poultry, species such as goats and sheep are more sensitive to heat stress (Berman, 2005Berihulay et al. 2019; Gonzalez-Rivas et al. 2020). Goats, on the other hand, are much less sensitive to heat stress than others.

In poultry such as chicken, due to heat stress; decreases are observed in daily feed intake, feed conversion rates, protein concentration and breast muscle weight, egg mass, shell quality and production (Lin et al. 2006; Mignon-Grasteau et al. 2015; He et al. 2018). In the results of the study conducted by Yahav and Hurwitz (1996), they reported that exposure to high temperatures at an early age could trigger thermotolerance in chickens.

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In another study, Lin et al. (2006) stated that the productivity and performance characteristics of broilers, such as growth rate, were negatively affected when exposed to high temperatures. Another formation effect is on reproductive performance. Depending on the temperature increase, sperm and egg quality, number and ability decrease.

Besides; By negatively affecting the estrus period and fertility; causes anoestrus and embryonic deaths (Ross et al. 2017).

Relationship between climate change and disease

Many animal species in animal production are sensitive to climate change. For example; is stated that significant changes are observed in the intestinal microphones of a broiler or layer chicken breed under high temperatures (heat stress) (Wang et al. 2018; Dahl et al. 2020). As the temperature increases, dairy cows may become vulnerable to diseases such as mastitis (Wang et al. 2018; Dahl et al. 2020). Common to many species; is observed that it also negatively affects the immune system. Moreover; is known that the incidence of pathogens and parasites increases due to rapid temperature changes. Another important reason is economic losses resulting from health problems and deaths due to sudden temperature changes (Paull et al. 2015; Wang et al. 2018; Dahl et al. 2020). In addition, as the temperature increases further, the death of the model animal is possible. For example; For chickens, it does not show a stress response to 27 ◦C ambient temperature or 41 ◦C body temperature. On the other hand, causes death as a result of a 4 ◦C increase in body temperature (Saeed et al. 2019). This is a serious economic loss for industrial production.

The relationship between nutrients and climate change in animal production

Due to greenhouse gas emissions that cause the atmosphere to warm with global climate change; soil, air, water pollution and decreases in biodiversity occur. Animal production is also directly and indirectly affected by this situation. It negatively affects performance characteristics such as milk, meat, eggs and wool, as well as productivity and growth, directly depending on the nutrient consumption of animals. Indirectly, the decrease in plant and feed production and quality causes the decrease in pasture quality as a result of the decrease in biological diversity. Therefore, it causes the model animal's nutritional needs to be inadequately met. As a result, it negatively affects the efficiency and performance characteristics (Hai et al., 2000; Naqvi et al., 2015). For example; in the study conducted by De Waal (1994), they reported that mohair yield decreased as a result of feeding goats with plants growing in arid pastures.

Relationship between temperature and nutrition

Daily feed consumption of animals may decrease due to heat stress. As a result, it can cause many problems such as metabolic disorders, oxidative stress, infections and impairment of the immune system (Gaughan et al., 2009). For example; Nardone et al. (2010); in the results of the study conducted by Yadav et al. (2013), they reported that they observed metabolic disorders such as deterioration in rumen physiology and activity and various digestive problems due to heat stress. In the results of another study, Yadav et al. (2013) reported that high temperature caused an increase in lactic acid concentration in ruminants. In the results of another study, Rhoads et al. (2009) stated that the decrease in feed consumption due to heat stress caused a decrease in metabolic body weight. They also reported that there was a decrease in body condition score. In another study, Rana et al. (2014) due to insufficient water supply as a result of heat stress; result of dehydration; they reported that the deterioration of the structure of myofibrils causes a decrease in meat quality and loss of weight and taste. Kadzere et al. (2002), Van Laer et al. (2015) reported that due to heat stress, they negatively affected the yield characteristics by causing a decrease in milk fat, protein and short-chain fatty acids, and a decrease in lactose, protein and protein levels. In the results of another study, Hai et al. (2000) reported that they observed a decrease in live weight, egg quality and number of laying hens due to decreased feed consumption due to heat stress.

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CHAPTER IV

Thermal Pollution

Emre Aydemir
Berk Arkın
Bület Efe Mollaahmetoğlu

Introduction

One of the most important problems observed with the increasing population around the world is environmental pollution. Environmental pollution is increasing, especially with industrial production and industrialization (Imhoff et al. 2010). In addition, migration and unplanned urbanization, solid waste and garbage, erosion and fires, mine lime, stone and sand quarries, motor and marine vehicles, destruction of natural vegetation, natural events, drying of wetlands such as rivers and lakes, radioactivity, heavy metals (Factors such as Hg, Cd, Pd, Cr, Cu, Ni, Zn, Co), oil and petroleum products, detergents, organic and inorganic wastes, microorganisms, poaching, solid materials and waste heat cause environmental pollution.

Moreover; chemicals such as insecticides, fungicides and herbicides are among the reasons for the increasing environmental pollution. As a result, the negative effects on air, water and soil disrupt the ecological balance (Imhoff et al. 2010; Tewari et al. 2019).

Climate change

Climate change; is the change of seasonal conditions specific to a region over a long period. The change in these sags is due to natural and unnatural reasons.

Naturally, there are many factors such as volcanic events, solar radiation, and deviations in sea currents. In addition, global warming and climate change are observed as a result of unnatural causes of greenhouse gases released into the atmosphere due to human-based problems such as the use of fossil fuels, more widespread and unconscious use of agricultural lands, and the destruction of forests and green areas (Nordell 2003; Nandy et al. 2006). In addition, it has many negative effects on living species. For example; Zhao et al. (2013) seasonally; due to differences in thermal pollution areas; they observed that it causes differences in living species and diversity, number, shape and size. In addition, it causes premature death, respiratory disorders, cardiovascular diseases and various psychological disorders in living beings (Herb et al. 2008; Jinqi et al. 2020).

Global warming

It has been observed that human activities such as the increase in the use of fossil fuels, the destruction of forests and the acceleration of urbanization with the industrial revolution have significantly increased the amount of greenhouse gases in the atmosphere. Accordingly, it causes heat increase as a result of reasons such as the natural greenhouse effect. Moreover; as a result of global warming, the melting of glaciers and the rise in sea levels cause changes in climate zones, increased floods and floods, erosion, drought, desertification and an increase in agricultural pests (Imhoff et al. 2010; Tewari et al. 2019).

Waste heat

One of the biggest reasons for the generation of waste heat is the pollution caused by the cooling water of thermal and nuclear power plants (Imhoff et al. 2010; Tewari et al. 2019). As a result, due to the increase in the temperature of the water body in which it is located, it causes a decrease in the diversity of the living population in the water.

In addition, deterioration of the biochemical structure of water, increased oxygen consumption in water, acceleration of reactions, and increase in organic pollution load. Heat pollution is similar to the climatic warming of water. Moreover; surface water temperatures that cause air temperature to increase cause climate changes such as global warming (Nordell 2003).

Urban heat island

It is a temperature increase that has come to the fore especially in recent years; occurs in urbanized areas that are exposed to higher temperatures than outer regions (Santamouris et al. 2015; Li et al. 2018; Manoli et al. 2020).

The reasons for this are due to structures such as buildings, roads and other infrastructures being constructed; causes it to absorb and re-radiate more solar heat into natural environments such as forests and water bodies.

As a result, is observed that it has effects such as increased air temperature in urban neighborhoods, thermal disturbances, higher energy consumption and deterioration of public health, reducing the quality of environment and life (Keerekoper et al. 2012; Santamouris et al. 2015; Manoli et al. 2020). For example; Qaid et al. (2016), Salata et al. (2017) reported that the results of the studies conducted by urbanization lead to a decrease in thermal comfort and deterioration of public health (Sen and Roesler 2020; Shi et al. 2019; Paolini et al. 2020; Balany et al. 2020). In another study, Ulpiani et al. (2020) stated that the disproportionate growth of megacities, together with the increasing urban population until 2040, is a clear indication that thermal pollution will gradually increase.

The relationship between urbanization and heat pollution

During the rainwater flow that occurs as a result of the increase in the number of surface layers and construction with urbanization; is stated that the temperature of the water body causes a temperature increase between 5 ◦C and 12 ◦C (Arsmon et al. 1999; Herb et al. 2008; Jinqi et al. 2020).

One of the biggest reasons for this is that the asphalt in the surface layer prevents the temperature exchange and causes the water mass accumulated under the surface to warm up (Arsmon et al. 1999; Herb et al. 2008; Jinqi et al. 2020). For example; Jinqi et al. (2020) stated that the water temperature of rivers and lakes in urban areas increased significantly after summer rains. Moreover; researchers have reported that the amount of dissolved oxygen in water and water quality decrease. In another study, Herb et al. (2008) stated that the heat generated as a result of friction during precipitation and runoff; they reported that it caused the river temperature to rise instantly. In another study, Nelson et al. (2008) reported that the temperature of river water after rain increased significantly between 3.5 ◦C and 7 ◦C. Moreover; researchers have stated that the increase in temperature negatively affects the plant and animal population diversity in the river. Due to this temperature difference; 'Thermal shock' is observed in living things in river waters (Goel, 2006; Laws 2017). As a result, is stated that the biodiversity of species may decrease and new thermophilic species may occur (Goel, 2006; Laws 2017). In contrast, Nakayama et al. (2011), Wong et al. (2010), Zhao et al. (2010b), Memon et al. (2008) reported that urban planning and reduction of thermal pollution can help reduce waste heat and increase vegetation and water bodies.

The relationship between industrial waste and thermal pollution

Another important cause of heat pollution is industrial waste. The results of a study conducted in the United States indicate that approximately 75 to 80 percent of heat pollution is produced by power plants (Goel, 2006; Laws 2017).

In another study, Zhao et al. (2013) found that seasonal changes are caused by urbanization; in addition, they reported that it causes thermal pollution as a result of industrial and energy conversion. In the results of a study conducted by Nordell (2003), they reported that thermal pollution is caused by heat emission resulting from the widespread use of fossil fuels and nuclear energy, especially industrial hot gas and water discharges after intense energy consumption and use on a local scale. Verone et al. (2010), Teiveira et al. (2009) reported that the population structure and richness of living organisms in the aquatic ecosystem decreased.

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