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TABLE OF CONTENTS

TABLE OF CONTENTS	3
COMMITTEES	5
DEAN'S GREETING	7
FOREIGN LANGUAGES SECTION	9

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DEAN'S GREETING

Fifty years ago, in 1972 was founded the Technical College of Light Industry, the legal predecessor of the Rejtő Sándor Faculty of Light Industry and Environmental Engineering of Óbuda University.

Rejtő Sándor Faculty of Light Industry and Environmental Engineering, and its legal predecessors, has undergone a number of serious transformations during their half-century of operations.

We have constantly adapted to new situations and challenges presented to us, and we continue to do so. However, our mission has not changed: to train professionals who can ensure our environment is more comfortable and beautiful.

Our Faculty combines an accumulated high level of professional knowledge, real ongoing relations to industry and a high proportion of qualified instructors. These factors guarantee the preservation of knowledge. Together, they also allow the new scientific discoveries and innovations to progress.

As a university faculty, one of our main tasks is education. As such, we are proud of our highly regarded vocational training, which has been chosen by more than 1,200 students. We offer three basic courses in Hungarian and two in English, as well as two at Master's level. After their Master's studies, our students can continue their studies at the Doctoral School of Materials Science which was founded by the faculty.

Due to our close ties to industry, the faculty started dual training in 2017. This is an excellent and ongoing example of the close and effective collaboration between education and industry.

We are known for the tangible educational resources we offer students, as well as the intangible, most notably the enthusiasm that we impart in creating professionals in our field. Supplementing this with accumulated knowledge of our faculty, a knowledge that we ourselves are committed to continuously expanding. We believe that these core ideals are the key to the preservation, and extension, of our technical and cultural values.

I wish you successful browsing of our book!

November 4, 2022
Dr. Habil. László Koltai Ph.D.
Dean

FOREIGN LANGUAGE SECTION

PHYSIOCHEMICAL PARAMETERS AND MICROBIOLOGICAL INDEX OF BLUE DANUBE WATER RIVER: BUDAPEST CITY AS STUDY CASE

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Abstract: *Faecal coliform bacteria are present in water when bacterial pathogens from faecal contamination are present. The bacteriological parameters used for monitoring the quality and eco-status of the River Danube surface water in Budapest were examined according to the Hungarian Standard Methods. The basic bacteriological indicators are investigated once a month throughout June, August and October in 1998, 2005, 2010, 2011, 2012, 2013, 2014 and 2015 at five different sites on the Danube River run throughout Budapest in three replicates per site. The analysis of the results showed noticeably lower quality on 2015 comparing with the earlier years throughout the entry and exist points of the Danube River into Budapest. The results recorded changes in bacterial contamination in Danube surface water samples. The bacteriological analyses indicated that Escherichia coli, total coliform, Pseudomonas aeurginosa and enterococci reached to above 300, 200, 200 and 300 CFU/ml, respectively at summer season. The bacterial indicators composition was related to changes in environmental conditions and eco-physicochemical parameters. Results were realized a small, non-constant, and unstable correlation between enteric bacteria and some eco-physicochemical parameters. These correlations were not sufficiently consistent to establish a reliable association; therefore, this study corroborates that only the bacterial assay itself is reliable for the diagnosis of faecal contamination by enteric bacteria in the collected surface water samples. Thus the challenges to continuous physical, chemical, biological and bacteriological monitoring will be immense.*

Keywords: *Monitoring; Budapest; Danube River; water quality; Coliforms; Physicochemistry; Heavy Metals, bacteriological parameters*

INTRODUCTION

According to the global environmental changes, water quality problems of the developed and undeveloped nations such as pollution, eutrophication, toxicity development, ecosystem dysfunction, acidification from air pollution now aggravated through long standing forest fires. Monitoring the environment with different systems is absolutely essential to identify human health and ecosystem hazards, to assess environmental cleanup efforts, and to prevent further degradation of the ecosystem. The worldwide degradation of surface water has resulted in decreasing water availability for different specific uses (Zhang et al., 2010). Biological, chemical and sediment deposits in the rivers have resulted in high levels of pollutant. Water availability and quality are of critical concerns due to its importance to humans. Industrialization, urbanization and municipal activities have contributed to the quantities of wastes generated which include: solid, liquid and gaseous emissions deposited in the environment that may result in the pollution of our environment. Microorganisms are resident flora of all ecosystems, but microbiological contamination with feacal bacteria due to human activities is considered to be a critical issue for surface waters (Bayoumi Hamuda and Patko, 2012). Assessment of surface and groundwater quality continues to be of great public interest in the developed world. There is a great need for monitoring water quality (Pekárová et al. , 2009); therefore, the assessment for the presence of pathogenic bacteria in water represents a major concern for human and animal health protection (WHO, 2011). Cabral (2010) mentioned that microbiological water analysis is mainly based on the concept of fecal indicator bacteria. It was concluded that safe drinking water for all is one of the major challenges of the 21st century and that microbiological control of drinking water should be the norm everywhere. Routine basic microbiological analysis of drinking water should be carried out by assaying the presence of *Escherichia coli* by culture methods. Whenever financial resources are available, fecal coliform determinations should be complemented with the quantification of enterococci. More studies are needed in order to check if ammonia is reliable for a preliminary screening for emergency fecal pollution outbreaks. Financial resources should be devoted to a better understanding of the ecology and behavior of human and animal fecal bacteria in environmental waters. Benedek et al. (1996) mentioned that the Danube flowing across Hungary is a moderately polluted river, at least in its middle stretch, where its greatest polluter is Budapest with 2 million inhabitants and a large industrial complex. Serving the river for drinking water supply, as well, it is obvious that the pollution control for the capital is of paramount importance. There is a 20 year planning concept for the improvement of the sewerage, wastewater treatment and storm water outfall. Frîncu (2021) mentioned that the Danube River is the second longest in Europe and its water quality is important for the communities relying on it, but also for supporting biodiversity in the Danube Delta Biosphere Reserve, a site with high ecological value. He presented a methodology for assessing water quality and long-term trends based on water quality indices (WQI), calculated using the weighted arithmetic method, for 15 monitoring stations in the Lower Danube and Danube tributaries in Romania, based on annual means of 10 parameters for the period 1996–2017. A trend analysis is carried out to see how WQIs evolved during the studied period at each station. Principal component analysis (PCA) is applied on sub-indices to highlight which parameters have the highest contributions to WQI values, and to identify correlations between parameters. Factor analysis is used to highlight differences between locations. The results show that water quality has improved significantly at most stations during the studied period, but pollution is higher in some Romanian tributaries than in the Danube. The parameters with the highest contribution to WQI are ammonium and total phosphorus, suggesting the need to continue improving wastewater treatment in the studied area. The methodology and the results of the study may be very useful instruments for specialists and decision makers in updating river basin management plans and prioritising intervention measures.

In recent years, a much stronger focus is on ecological assessment and on emerging micropollutants, like pharmaceuticals and endocrine disruptors, which may have a strong environmental impact even in small amounts (Kortenkamp et al., 2019). There are several studies that analyzed individual parameters in the Danube and how they evolved over longer periods of time and a number of studies that looked at the complex interaction between parameters and between parameters and their drivers (Azara etal., 2018; Frincu et al., 2020). Surface water is more vulnerable to pollution than ground water resource especially in developing countries where the heavy industrialization, increasing urbanization, and adaptation of modern agricultural practices play an important role in improving the living standard but at the same time cause severe environmental damage (Mulk et al., 2015). Tu (2013) reported that human activities associated with land uses can cause surface water degradation. Nutrient loading into rivers is generally increased by human-induced land-use changes and can lead to increased surface water pollution. With the optimistic estimate; one in four people could not be reached to adequate drinking water in 2050. One of the most important factors that will determine humanity's future quality of life within the scope of the environmental pollution is water pollution, and it is updated day by day with more important. Water quality is a concept that includes taste, odour, colour, appearance, softness, temperature and bacteriological and chemical characteristics, and it is being affected by climatic and meteorological changing as well. Water quality in the Danube has improved during the last decade, but further improvement is still needed. Most stretches of the Danube can be described as moderately polluted, but some tributaries and stretches of the lower Danube fail to achieve this status. In some areas harmful substances from farmland and heavy industries pollute the rivers and severely undermine the quality of the water. Monitoring the Danube's environment with biological and chemical systems is absolutely essential to identify human health and ecosystem hazards, to assess environmental clean-up efforts, and to prevent further degradation of this ecosystem. Adequate supplies of fresh water of acceptable quality are a prerequisite for human health, food security, industrialization and economic development. Danube River represents an important touristic and economical resource. Danube River is the second largest river in Europe after the Volga River. Danube River flows 2,860 km with a total area of 801,463 km². The river rises in the Black Forest Mountains of Germany and flows eastward into the Black Sea. It is an important international waterway, flowing through or forming a part of the borders of ten countries and through major cities such as Vienna, Bratislava, Budapest, and Belgrade. Geographically (Figure 1) the river basin includes the territories of 19 countries: Germany (Baden-Württemberg and Bavaria), Austria, Czech Republic, Slovakia, Hungary, Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, Serbia, Bulgaria, Romania, Moldova and Ukraine, with catchment areas larger than 2000 km²; and Switzerland, Italy, Poland, the Former Yugoslav Republic of Macedonia and Albania with smaller areas. Danube region is heterogeneous area in the economic, ecological and cultural aspects and inhibited by 83 million. Hungary is located at the "meeting point" of weather fronts but overall has a moderate climate with strong continental influence. Seasons are usually well defined, with July and August averaging 28-32°C and December and January down in average to about –5°C. Annual precipitation is 600 mm with ranges of 300-1200 mm; while evapotranspiration rates are similar at 500-600 mm/year. Hungary is located in the lowest part of the Carpathian Basin, 84% of the country lies below 200 m, with only 2% above 400 m and river gradients are generally low.

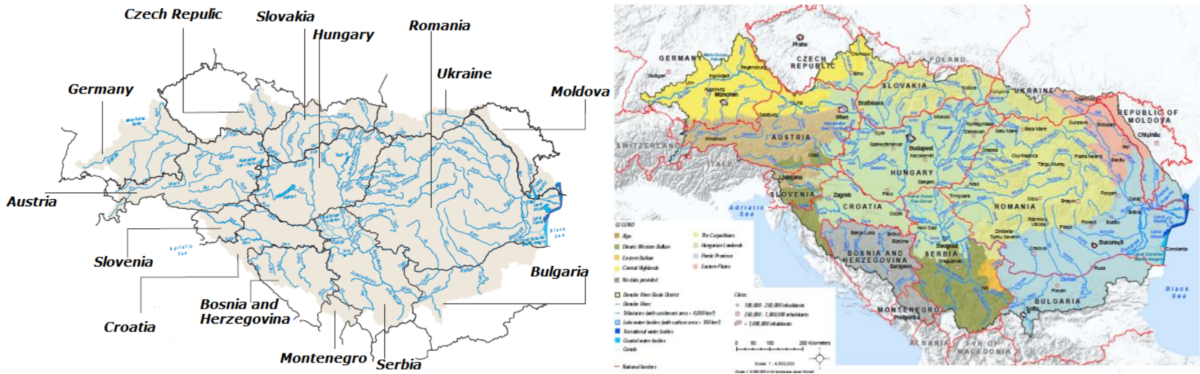

Figure 1. Map of the Danube River Basin Ecoregions in 2015 (Maps of the Danube River Basin District Management Plan).

According to the International Commission for the Protection of the Danube River (ICPDR, 2014), the largest environmental investment to be implemented in Central Europe will fundamentally modernise the wastewater treatment system of Budapest, ensuring cleaner waters for all those living along the banks of the Danube. The Hungarian Danube traverses 417 km, forming the border with Slovakia in the North-West and thereafter flowing south. In the east, also flowing southwards is the Tisza, covering 595 km before reaching Serbia where it later flows into the Danube (ICPDR, 2014). The investigation of changes of the surface water quality is one of the major questions of environment protection. Extreme floods occurred in June 2013 on the upper and lower Danube River and the strength and intensity of this flood event reminded to floods in 2002. Since Danube River is among the most endangered ecosystem in Europe, there is urgent demand for comprehensive methodological approaches to evaluate the actual state of this ecosystem and to monitor its rate of changes. Danube Day was held annually on 29 June is an international day honouring the Danube and the rivers that flow into it, paying tribute to the vital role they play in providing water, food, power, recreation and livelihood. Environmental health and water quality are important determinants of human health, especially the water borne diseases in local communities like the bacterial contamination of surface water of Danube River in Budapest territory remains a central local and global problem. In cities where inefficient sewage system, the domestic wastewater discharge (Figure 2) is one of the main sources of pollution, stimulating the growth of bacteria and adding other microorganisms to the environment, including those found in faecal matter (Silva et al., 2010). Danube River ecosystem contains lot of various types of animals and plants; from fish and ducks to tiny water beetles and worms, and from algae to water plants as well as the planktons (zoo-, phyto- and bacterioplanktons). Analysis of eco-physicochemical (Figures 3a, b, c) and bacteriological parameters of water is essential to assess water quality for the best usage. Public and environmental health protection requires safe water, free of pathogenic bacteria. Among the pathogens disseminated in water sources, enteric pathogens which are the most frequently encountered. The sources of faecal pollution in water due to anthropological activity must be strictly controlled and the most significant factors that affecting water quality of Danube River Basin are organic, nutrient, hazardous substances, and microbial pollution, alterations due to the hydro-morphological pressure (Gasparotti et al., 2013).

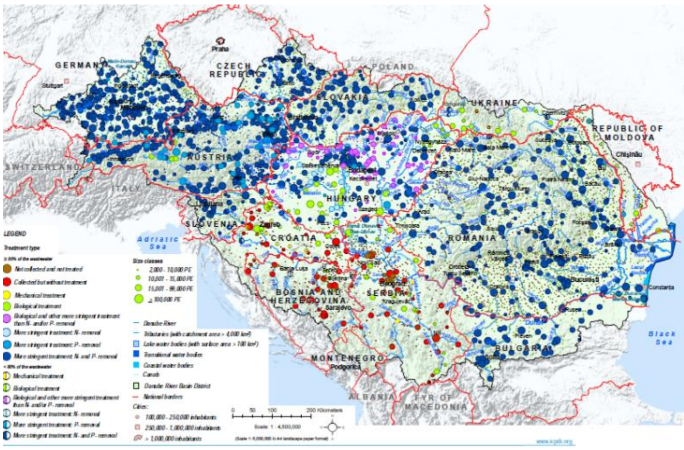

Figure 2. Map of the Danube River Basin District. Urban Wastewater Discharges: Baseline Scenario – Urban Waste Water Treatment Directive (UWWT) 2015).

Drainage basins of most affluent have the same predominant lithology of the Danube course, probably with a greater contribution from sedimentary lithologies (Comero et al. 2014). Water quality in Danube River basin is under a great pressure due to the diverse range of the human activities including large urban centre, industrial, agriculture, transport and mining activities. Faecal microbial pollution is a major problem throughout the Danube River Basin, posing a threat to various types of water use, including drinking water production from river bank filtrates, water supply for agricultural and industrial use, and the role of the river as a recreational space. It is introduced into the river by point sources, such as discharges of treated or untreated sewage from anthropological sources or livestock, and by nonpoint sources, such as urban and agricultural runoff. Higher levels of faecal pollution were found in the middle part of the Danube, particularly downstream of major cities (Budapest, Beograd) as far as 1.100 river km and in the Lower Danube to the Danube Delta (Kavka et al., 2006). In addition, faecal input from wildlife may be of importance in specific regions. Despite huge efforts to improve wastewater management in the past decade, in many sections, the river and its tributaries exhibit very high levels of faecal microbial pollution. The main groups of bacteria used to indicate pollution levels in water are total and faecal coliform, and more specifically Escherichia coli, has been indicated to evaluate the anthropogenic contamination of a wellspring (Silva et al., 2010). Total coliform and faecal coliform indicator tests are common public health tests of the safety of water and wastewater which might be contaminated with sewage or faecal material (APHA, 1998). Most coliforms are present in large numbers among the intestinal flora of anthropological and other warm-blooded animals, and are thus found in faecal wastes. As a consequence, coliforms, detected in higher levels than pathogenic bacteria, are used as an index of the potential presence of entero-pathogens in water environments. Coliform includes members of the family Enterobacteriaceae. Pathogenic organisms are normal species of all ecosystems, but microbiological contamination with faecal bacteria is considered to be a crucial issue throughout the rivers (Bayoumi Hamuda & Patkó, 2012).

Figure 3a. Nutrient Pollution: Nitrogen

Figure 3b. Nutrient Pollution: Phosphorus

Figure 3c. Vulnerable zones

Figure 3. Map of the Danube River Basin District: Nitrogen, phosphorus and nitrate status in vulnerable zones during 2015.

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Attempts to comprehensively monitor bacteriological quality in large, international rivers have been tackled by a number of organisations in Europe. For the River Danube, the only large-scale bacteriological investigation published to date deals with the changes of bacterial community composition along the whole river (Winter et al., 2007). The combination of the faecal indicator data sets of the two Danube surveys (JDS 2001, 2007) with major environmental parameters and the integration of long term observations at 16 representative sampling stations enabled us to draw for the first time a clear picture of the faecal pollution patterns along the longitudinal profile of this important international river. With a novel microbial source tracking approach, it was recently shown that about

70% and 80% of E. coli/FC variation observed during JDS 2001 and JDS 2007 in the tributaries could be explained by a faecal human specific Bacteroides marker (Reischer et al., 2007). In comparison to Budapest and Bucharest, E. coli concentrations downstream Vienna was 25 times and 425 times lower, respectively. Surprisingly, no such increase could be observed for example after Bratislava. Farnleitner et al. (2010) concluded that E. coli and enterococci are reliable faecal indicators for alpine mountainous water resources monitoring, although E. coli is the more sensitive one and suggested a conservative microbial source tracking marker for anthropogenic faecal influence. One main reason for this observation is likely that during both Joint Danube Surveys samples were taken only from the middle of the river. In large rivers like the Danube, wastewater or highly polluted tributaries produce a sewage plume on the respective side where they merge, which is not mixed with the main river water for tens of kilometres. Water of Danube River Basin could be chemically, physically, or bacteriologically contaminated. Each of which is linked to various sources and health related problems and consequences. Two main factors determine the chemical and bacteriological conditions of water quality: artificial and natural contamination. Any bacteriological or chemical analysis of water reveals the joint effects of both sources of contamination, and it is usually impossible to fully identify and separate these sources. Kittinger et al. (2015) reported that the stretch of the River Danube between Vienna, Bratislava and Budapest passes a region that is highly industrialized, intensively used for agricultural purposes and also highly populated. The elevated values may indicate these influences. This study set out to evaluate the occurrence of some enteric bacteria in surface water samples of Danube River at Budapest territory, and its relation to some eco-physicochemical parameters. So, the purpose of this monitoring effort is to monitor and evaluate the changes in surface water quality resulting from changes in environmental changing practices. The work also, is to summarize the historical biomonitoring in Danube River surface water quality during the period between 1998 and 2015, and to determine the faecal bacterial pollution status of the Danube River at Budapest territory.

MATERIALS AND METHODS

To make sure that the investigated water samples of Danube River in Budapest region were representative, the water samples were taken from the Pest and the Buda sides of the river's bank at every sampling location. Water samples were collected in June, August and October of 1998, 2005, 2010, 2011, 2012 and 2013 from five locations: Rákóczi, Petőfi, Szabadság, Erzsébet and Árpad bridges with three replicates. All samples for physical, chemicals and bacteriological analysis were collected in (~ one litre capacity) sterile dark bottles without air bubbles and immediately placed on dark cooling boxes and processed within 6 h of collection. Temperature, pH and electroconductivity were measured according to the Hungarian standards (MSZ EN 27888:1998, MSZ 448-22-1985 and MSZ EN 27888: 1998, respectively) immediately after the water samples were collected. The chemical (CODMn) and biological (BOD5) oxygen demands were detected by MSZ ISO 6060-1991 and MSZ ISO 5815-1998, respectively. The bacteriological quality of surface water was carried out according to the Hungarian method: MSZ 12749:1993. Total and faecal coliform bacteria: MSZ ISO 9308-2-1994 and MSZ ISO 5541-1 (1994). Detection of E. coli: MSZ 448-44-1990 in comparison with ISO 11866-(1997). Enterococcus sp.: MSZ 12749-1993. Counting the total aerobic mesophile heterotrophs was carried out by MSZ ISO 6610 (1993). Different cultural media were used for the isolation of different faecal indicator organisms were carried out using the membrane filtration method: this is based on the filtering, under negative pressure, of the water sample through a cellulose acetate membrane with a porosity of 0.45 µm and connecting with glass filtration unit. Bacteria are retained on the filter, which is then placed on a suitable nutrient medium. Bacterial colonies growing on the medium can then be counted. When a selective or differential medium is used, desired colonies will have a distinctive appearance. Total aerobic mesophile heterotrophic bacteria were enumerated by standard plate count (tryptone glucose yeast agar) by serial dilution of the sample, followed by the conventional spread plate method (Chen & Kueh, 1976) and the colony count were measured after incubation at 37°C for 24 hr. Detection and enumeration of E. coli and coliform bacteria were done on Endo Agar. Typical coliform colonies count pink to dark red colonies with metallic sheen. Atypical coliform colonies Count dark red or nucleated without metallic sheen. Detection and enumeration of intestinal enterococci on Brain heart infusion agar. Detection and enumeration of Pseudomonas aeruginosa was done on modified M-PA Agar and Cetrimide-agar. Colonies appear as brownish to green black centres on filters. Eosin Methylene Blue agar, MacConkey agar and m-Endo agar were used to count the total and faecal coliforms, E. coli and Enterobacter. Enumeration of cellulose decomposers was done by using carboxymethylcellulose (CMC) agar medium. One ml of serial dilution of Danube water sample (10-5) was spread over the CMC agar plate and cultivated at 28 ± 2°C for 3 days. The plate was flooded with 0.1% Congo red for 15 to 20 min, washed with 1 M NaCl for 15-20 min, and observed for clear zone around the colony (Hendricks et al., 1995). The clear zone formed around the bacterial colonies is used as indicator for cellulase activity. The bacterial population was expressed in term of log10 of the CFU/100 ml.

STATISTICAL ANALYSIS

The mean and standard deviations of the microbiological counts and physicochemical parameters were calculated. The Duncan test was used to segregate means in regions where there were significant differences at 95% confidence intervals, and the ANOVA was performed to assess for significant differences.

RESULTS AND DISCUSSION

The Danube territory is a major international water catchment area and ecological corridor. Therefore, a nature conservation, territorial development and water management approach is needed. The environmental impact of transport links, tourist developments, or new energy-producing facilities must also be considered. Major flooding, droughts, and industrial pollution events are all too frequent. Prevention, preparedness and effective reaction require a high degree of cooperation and information sharing. With current increasing trends in population growth and socio-economic development, the quality and quantity of water is gaining widespread attention worldwide. This increasing concern about water quality and quantity necessitates the interventions in water systems to meet the objective of sustainable water supply and prevent potential environmental deterioration. Water could be chemically, physically or bacteriologically polluted. Each of which is linked to various sources and health related problems and consequences. The major factors determine the chemical and bacteriological consist of water quality: artificial and natural pollution. The results of monitoring of some physical, chemical and bacteriological parameters of the surface water samples along Danube River in Budapest region are presented in the average of 3 replicates of 5 investigated sites on both sides of the river basin during June, August and October in 1998, 2005, 2010, 2011, 2012, 2013, 2014 and 2015 are presented in Figures 4, 5, 6, 7 and 8. Physical and chemical parameters: The values are favourable to growth of microorganisms which could have contributed to high total coliform count as observed in this study. Water temperature in this study ranges between 19.55°C (1998) to 24.2°C in 2015 (Figure 4a). This was found to be within the permissible limit of the world health organization (WHO, 2003). Water bodies will naturally show changes in temperature seasonally and daily; however, man activities can also contribute to changes in surface water temperature. The high temperature could also be as a result of urban, industrial and agricultural activities around the River. The temperatures observed were higher than 18°C in all the locations and all through the various months which favour the growth of bacteria. Aquatic organisms are sensitive to changes in water temperature and it is an important water quality parameter which is relatively easy to measure. Temperature also influences the rate of photosynthesis by algae and aquatic plants. As water temperature rises, the rate of photosynthesis increases thereby providing adequate amounts of nutrients (Boulton, 2012). The temperature values obtained throughout the investigation period fall within the optimal growth range for mesophilic bacteria including human pathogens. The results illustrated that pH value (Figure 4b) of all the water samples range from 7.32 in the monitoring year 2011 to 7.95 in 2005. Generally, the pH values obtained fell within the WHO standard of 6.5-8.5 (WHO, 2003). There was no significant difference between the pH values for the three months in all the collection points at $P<0.05$ significant level. According to the pH values obtained, majority were in the trend of slightly alkaline. Therefore, the water samples were unlikely to cause health problems such as acidosis (Asamoah & Amorin, 2011).

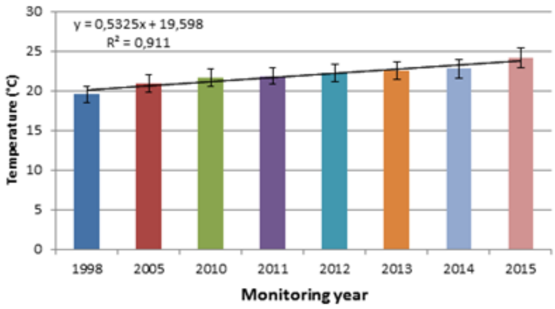

Figure 4a Temperature

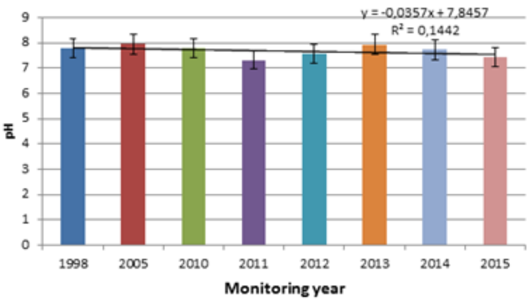

Figure 4b pH

Figure 4. Monitoring the average temperature and pH in Danube River at Budapest city

However, pH played a significant role in determining the bacterial population growth. Increases in the observed pH, could be attributed to the production of basic metabolic waste products by increasing bacterial population. Prescott et al. (1999) stated that microorganisms frequently change the pH of their own habitat by producing acidic or basic metabolic waste products. Electroconductivity is the degree to which a water sample can carry an electric current. The magnitude of the electroconductivity of a sample is a function of the amount of ions present in the sample. High electroconductivity can be an indicator of excessive mineralization from either natural or industrial sources. The results indicated that the electroconductivity of water samples were ranged between 328.1 in 2010 and it was at maximum (35.2 m/Sm) in 2014 (Figure 4a). Turbidity typically composed of fine clay or silt particles, plankton, organic comp compounds and microorganisms. Sources include erosion, storm water runoff, industrial discharges, microorganisms, and eutrophication. Monitoring of turbidity is an important criterion of water. The turbidity profile varied throughout the study period and ranged from 36.9 in 2010 to reach the maximum 56.1 mg/l in 2005. The Month of June had the highest turbidity level while the lowest was recorded in the month of October. The high level of turbidity could be due to industrial effluents, improper disposal of sewage, animal waste and wastewater from domestic activities among others. The CODMn values ranged from 2.17 mg/l in 2011 and 4.42 mg/l in 2013 (Figure 5a). High CODMn values could be due to high organic loading resulting in high total solid materials within the water body. CODMn differs from BOD in that it measures the O2 demand to digest all organic content, not just that portion which could be consumed by biological processes. Figure 5b highlights the result so presented for BOD5 which indicates the amount of organic waste in the water and measure of the O2 used by microorganisms to decompose this waste. Growth of aerobic and facultative anaerobic bacteria will be enhanced by the presence of dissolved oxygen in any water body. A decrease in dissolved oxygen was generally observed in 2012 with 1.91 and 3.63 mg/l in 2013. WHO (1996) reported that there is tendency for the level of BOD5 values range from 10.00 to 35.89 mg/l. All the values of BOD5 in the river samples are lower than the permissible standard the WHO standard of 50 mg/l for waste water. The more organic material presents in the river the higher the BOD5.

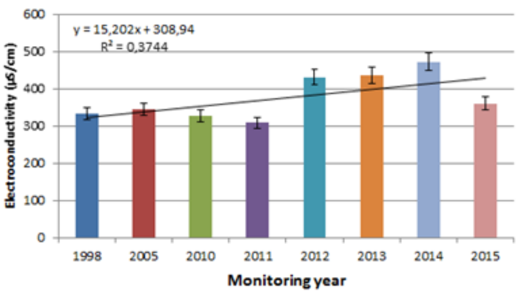

Figure 5a Electroconductivity

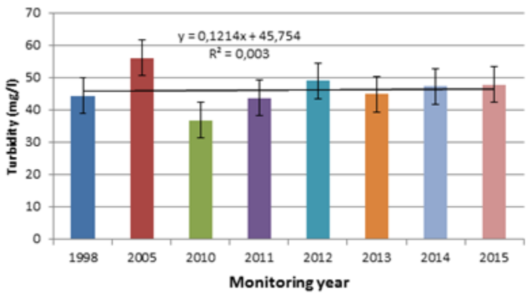

Figure 5b Turbidity

Figure 5. Monitoring the average electroconductivity and turbidity in Danube River at Budapest city

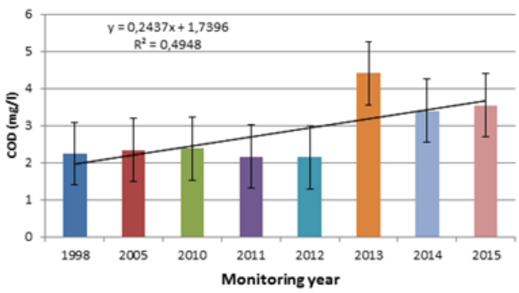

Figure 6a COD

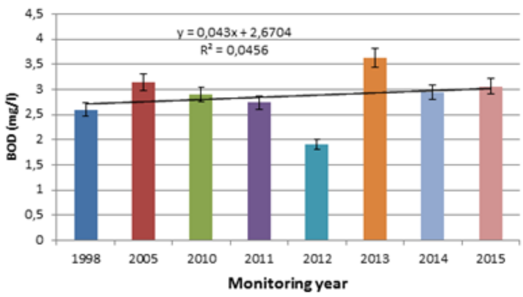

Figure 6b BOD

Figure 6. Monitoring the average COD and BOD in Danube River at Budapest city

Bacteriological parameters: The number of bacterial colonies can be influenced by weather and seasonal effects. This variability makes the bacterial concentrations in natural water difficult to predict at any one time. The WHO standards for total and faecal coliforms are 1 to 10/100 ml and 0/100 ml, respectively (WHO, 2003). The results in Figures 7 and 8 revealed that all the water samples had very high counts of total and faecal coliforms which could be attributed to human and animal activities on the river because coliforms are of intestinal origin. Therefore, a potential health risk exists due to their presence in water and the result is in agreement with Poonkothai & Parvatham (2005) in India that revealed the presence of bacteria at high concentration in automobile wastewater. Industrial waste and the municipal solid waste have emerged as one of the leading cause of pollution of surface and ground water in many parts of this country, thus available water is rendered non-potable because of the presence of industrial effluents and high microbial contamination. The bacteriological assessments expressed in the term of log10 of the colony forming unit showed that the total counts of aerobic mesophile heterotroph bacteria and the total coliforms were gradually increased from 1998 and reach the maximum in 2015. It was found that this group of bacterial population is changed from 4.66 (log10 of CFU/100 ml) in 1998 to 7.97 in 2015. Coliform bacteria have long been used to indicate faecal contamination of water and thus a health hazard. The population of total coliforms was increased gradually from 4.34 (1998) to reach 5.52 in 2015. Meanwhile, the population of faecal coliforms was changed to be the lowest in 2013. Similar indication was obtained by Enterococcus that it was lowest bin 2013 with 1.913 and highest in 2005 with 2.95. The highest log10 value of E. coli/ 100 ml was found during 2005 season (2.04), followed by the 2011 (1.76), 2010 (1.47), 2012 (1.45), 2013 (1.38) and 1998 season (1.37). The measured bacteriological indicator parameters provided a consistent picture of faecal pollution in the Danube River in Budapest region throughout the investigation periods. Highest enterococci population was observed in 2005, followed by 2010 and 1998. Faecal indicators were all highly significantly intercorrelated in the investigated periods. The lowest population was recorded with Enterobacter sp. Followed by the faecal *Streptococcus* sp. and *P. aeruginosa*. The population of cellulose-decomposers was higher than E. coli and lower than Enterococcus. Figure 8 shows that the ratio between total coliforms and total aerobic mesophile heterotrophs was at lowest value in 1998 with 0.749 and the highest was in 2012 to 1.385. Also, the ratio between the faecal coliforms and total coliforms was at highest in 2005 and the lowest value was detected in 2013. The ratio between *E. coli* and total coliforms had the similar pattern as in case of the ration between faecal coliform and total coliforms.

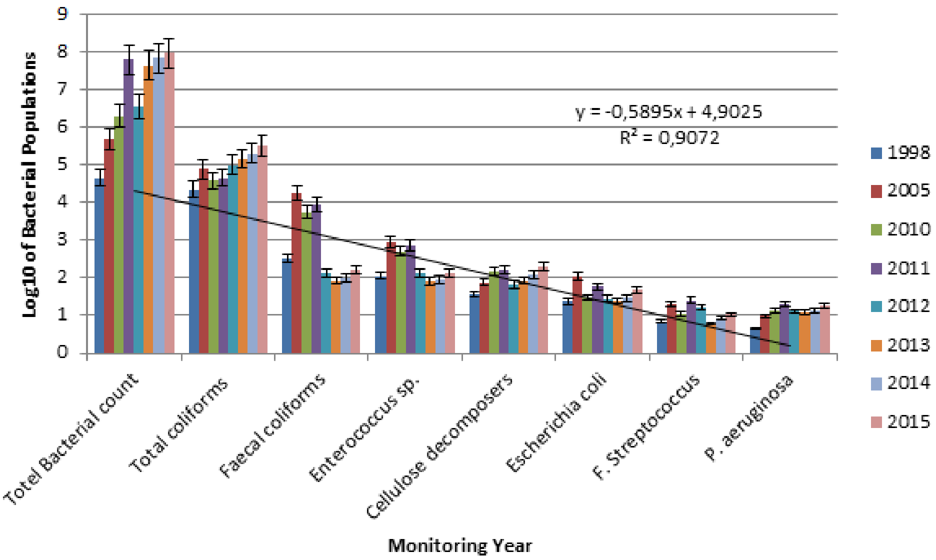

Figure 7. Monitoring the average log10 of some bacterial communities in Danube River at Budapest city

It was found that the lowest ratio was detected in 2013 (0.267) and the highest ratio was found in the monitoring year 2005. The ratio between *E. coli* and faecal coliform was varied between 0.392 (2010) and 0.758 (2015). The results indicated that the bacteriological assessments and bacterial populations were low in 1998 in comparison with the results of the other monitoring years. In 2012, investigations illustrated the highest faecal Streptococcus pollution, wherein 2010 was the lowest. Bacterial pollution of water continues to be a widespread problem across the World and is a major cause of illness and deaths by water-borne diseases. The slight alkaline pH values and turbidity levels in the Danube water varied in accordance with the increased primary productivity and degradation of the organic pollution. Over the past few years, the application of different methods to monitor faecal pollution in diverse water sources has become very important; however, there is no universal approach which fits all requirements to allow completely reliable faecal source identification (Stricker et al., 2008). Bacteria are ideal sensors for the indication of microbial pollution of surface water because of their fast response to changing environmental status. Faecal coliforms E. coli and intestinal enterococci (faecal streptococci) are good indicators for the assessment of faecal pollution mainly caused by raw and treated sewage and diffuse impacts from farmland and pastures. The concentrations of heterotrophic bacteria correlate commonly to organic pollution.

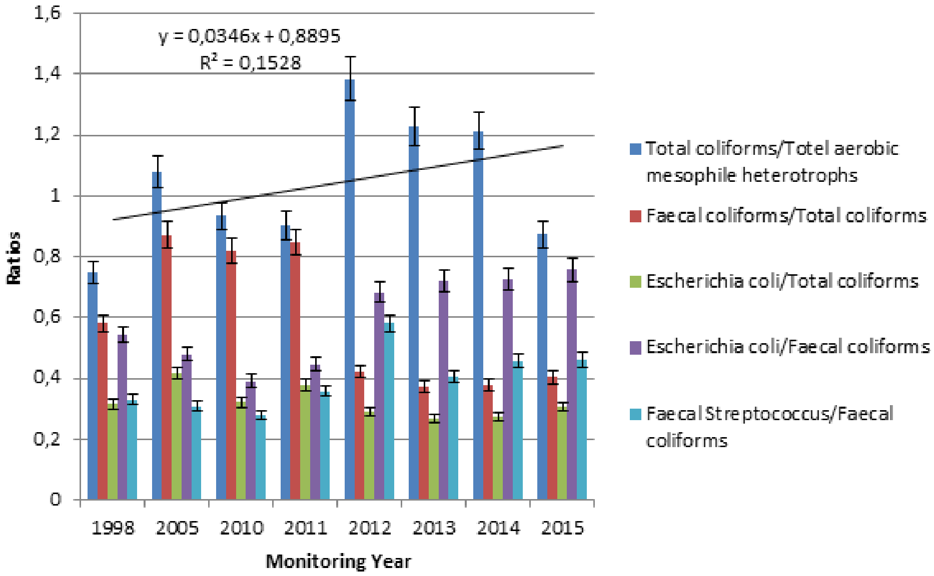

Figure 8. Monitoring the average ratios between some bacterial communities in Danube River at Budapest city

In the Danube river basin total coliforms, faecal coliforms and E. coli indicate persistent contamination, with lower values of total coliforms in July and the highest value in August. Variations in these parameters could be spatiotemporarily linked to the number of visitors in this ecosystem (Ajeagah et al., 2012). Our investigation can support the conclusion of Ajeagah et al. (2012) as it presented in Figure 3. Bacterial numbers positively correlated with enterococci and total coliforms. A high ratio of E. coli to faecal streptococci suggests a human source and a ratio less than one suggests an animal source. A differential count of the actual streptococcal species present in water can help to find out the exact source of contamination. These variations in bacterial counts among the different service reservoirs and consumer ends may be attributed to the general management practices for maintenance of service reservoirs and the possibility of reroute water pollution. Numerous factors affect bacterial concentrations in the investigated locations of Danube River. The values for all investigated groups of bacteria in the river water showed a great variability, which can be attributed to unequal loading with wastewater during the sampling seasons. Faecal coliforms to Enterococcus ratio indicated a human origin of the pollution. E. coli and faecal coliforms are the best indicators for the assessment of recent faecal pollution, mainly caused by raw and treated sewage and diffuse impacts e. g. from farm land and pasture. *E. coli* and faecal coliforms indicate also the potential presence of pathogenic bacteria, viruses and parasites (Kavka & Poetsch 2002). Kavka et al. (2006) mentioned that the higher levels of faecal pollution were found in the middle part of the Danube, particularly downstream of major cities (Budapest and Beograd) as far as 1.100 river km and again in the Lower Danube from stream-km 500 to the Danube Delta. Bacteriological contamination from faecal pollution by anthropogenic sources is considered to be a crucial problem throughout the Danube River basin imposing a threat to all kinds of water uses (Kirschner et al., 2009). Here, our investigations suggested that the Danube surface water quality was highly contaminated in 2010 more than in 2005 due to the sewage effluents. E. coli and intestinal enterococci are used worldwide as indicators for the assessment of faecal pollution in the aquatic environment. Faecal coliforms to Enterococci ratio was used to indicate the origin of pollution. A ratio lower than 1.5 indicates pollution by water flow, while a ratio higher than 4 is typical for anthropogenic pollution (Geldreich & Kenner, 1969). Our observations are confirmed by Liang et al. (2014) and Gupta et al. (2012) who indicated the cellulase activity of the organisms, by measuring the diameter of clear zone around the colony and hydrolytic value on cellulose Congo Red agar media. Kirschner et al. (2015) summarized the historical developments in microbiological water quality research and to reflect the most recent publicly available data on the faecal microbial pollution status of the Danube River. Moreover, the first results on faecal microbial source tracking by molecular biology methods are presented along with their applicability in river water quality monitoring, including the monitoring of riparian wells and alluvial groundwater resources. Construction of urban waste water treatment systems and development of existing systems in Danube basin should be continued receiving priority, including planning measures related to enhancing the capacities of the territorial and local levels. Douglas and Longjohn (2022) mentioned that the physicochemical parameters and heavy metals of the surface water samples were determined using standard methods for the examination of water. Results of the total heterotrophic bacterial counts for A, B, C, D and E are: $1.13 \pm 0.42 \times 10^6$, $5.6 \pm 0.55 \times 10^5$, $1.6 \pm 0.50 \times 10^6$, $6.6 \pm 0.70 \times 10^5$ and $1.02 \pm 0.25 \times 10^6$ cfu/ml, respectively. Results of the coliform counts for A, B, C, D and E are $1.48 \pm 3.08 \times 10^5$, $8.6 \pm 0.73 \times 10^4$, $8.3 \pm 1.04 \times 10^4$, $3.4 \pm 2.63 \times 10^4$ and $8.4 \pm 0.20 \times 10^4$ cfu/ml. Total heterotrophic bacterial counts were higher in samples collected from location A followed by locations E while the least counts were recorded in location B. Thirty-six bacterial isolates belonging to: *Pseudomonas* sp, *E. coli, Enterobacter* sp, *Alcaligenes* sp, *Serratia* sp, *Staphylococcus* sp, *Bacillus* sp, *Shigella* sp, *Salmonella* sp and *Klebsiella* sp were isolated from the different locations. The results showed that the mean range of Physicochemical properties were; pH 8.2 to 8.4, temperature: 26.5-28°C, electrical conductivity: 45600-122000µs/cm, turbidity: 0.71 to 1.45NTU, salinity: 3.06 to 8.18 mg/l, nitrate: 0.02 ± 0.03 to 0.35 ± 0.27 mg/l, phosphate: 0.41 ± 0.52 to 0.72 ± 0.05 mg/l, DO: 1.05 to 2.10 mg/l, BOD: 1.40 to 3.70 mg/l and THC: 8.0 ± 14.14 to 47.0 ± 14.14 mg/l. The results for the heavy metal analyses showed that only the cadmium concentration obtained in locations A, C and E (0.00386 ± 0.00, 0.00249 ± 0.00 and 0.00196 ± 0.00 mg/l) were within the WHO limits (0.005 mg/l). High concentrations of lead, chromium and nickel which exceeded WHO permissible limits were recorded in all the samples. The results of this investigation revealed that the Bonny River is highly contaminated with feacal bacteria, Total Hydrocarbon Content, and heavy metals, which may potentially cause bioaccumulation in organisms. These contaminants have made the water unfit for domestic and other recreational uses. There is need for regular monitoring of these parameters for early detection of any major pollution issues. Water pollution may originate in point sources (municipal and industrial discharges of treated or untreated wastewater) or diffuse sources, like run-off from agricultural land (Manassaram et al., 2006). After treatment in the drinking water supply facilities, tap water may be contaminated in the distribution network with algal toxins and heavy metals from pipes corrosion, which are directly linked with a wide range of diseases. Moreover, some of the products used for water disinfection may have negative health impacts if not removed (Mueller et al., 2004). This has lead in many regions of Europe to distrust in drinking tap water and a preference for bottled water (Dettori et al., 2019). In 2020, the EU Council has approved a proposal to revise the directive, updating quality standards and including endocrine disruptors and pharmaceuticals in the monitoring programme (König et al., 2016). Turbidity is a measure of suspended minerals, bacteria, plankton, and dissolved organic and inorganic substances and it is often associated with surface water sources. This parameter is important for photosynthetic aquatic organisms, as high turbidity affects light penetration into the water column. The values obtained for turbidity in the locations were within the WHO limits indicating that despite the turbulence of the water influenced by human activities, the water was still within acceptable limits. The presence of salts in the Danube River may be due to dissolution of wastes, erosion and surface run off, ions and majorly from the proximity of the river. This agreed with Sikoki and Akpiri **(2017)** who in a similar study attributed the saline nature of the Bonny River to be due to closeness to the sea. The levels of BOD in the samples are within the WHO limits (15 mg/l). The BOD is the quantity of oxygen needed for microbial decomposition of organic materials (Obire et al., 2021). These organic contaminants may enter the water body from municipal and industrial effluents due to urban life and many industrial establishments. These organic materials eventually get broken down by bacteria, which require oxygen for the decomposition process, leading to depletion of DO, hence the low DO content of the water. Nitrate and phosphate concentrations in all times were within the WHO limits during investigations. These inorganic nutrients are referred to as nutrient elements and their presence linked to anthropogenic sources such as organic and inorganic wastes associated with the presence of make-shift public conveniences scattered all over the area, soil run-off (as phosphorus bound in the soil will be released, fertilizers from farm lands), synthetic materials which contain organophosphates such as insecticides and livestock waste (Obire et al., 2021). Cadmium plays important roles in surface water monitoring studies, due to its toxicity to fish and other aquatic organisms. This metal is widely found in the aquatic environment which bioaccumulates along the trophic levels, building up in the internal organs such as kidneys and livers in fish and has a very high carcinogenic effect on humans (Graves et al., 2000) Cadmium can get into human body through the use of contaminated water that could lead to painful degenerative bone diseases, respiratory and digestive diseases and kidney failure (Chikere and Ekwuabu, 2014; Nishijo et al., 2017). Chromium is naturally occurring heavy metal found in the sea water, earth crust and introduced from industrial activities releasing it to soil, air, surface and ground water; which could cause renal failure, dermal, gastrointestinal, respiratory, and neurological and several other cancers when ingested through water and food by humans (Vasile et al., 2020). The WHO (2004) also reported that chromium in its hexavalent form ($Cr6+$) is a known carcinogen in humans, implicated in several health issues. Hence, the high values obtained from this study, may be detrimental to living things in that environment. Nickel is widely distributed in the environment found in soil, air and water. Elevated quantities found as a result of anthropogenic activities mostly from industrial waste, especially battery waste. Exposure to this heavy metal in man can result in respiratory, cardiovascular, renal, and nasal cancer (Nishijo et al., 2017). Finally, a discussion of the general state of water quality and public health is presented concerning (i) the current situation and potential limitations of the Water Framework Directive regarding the microbiological quality elements, (ii) further improvements regarding sampling and monitoring strategies, and (iii) the recently introduced concept of “integrated framework of faecal pollution monitoring and management” and expected further methodological developments in the context of the Danube watershed. Rapid progress in research and development is currently being made in the area of faecal microbial source tracking, pathogen detection, and health risk assessment and these innovations are also likely to complement basic faecal pollution monitoring programs for river systems such as the Danube in the near future.

CONCLUSION

The physicochemical and microbiological parameters of the Danube River were studied. The total aerobic heterotrophic bacterial counts and the coliform bacterial counts in all the water samples were very high and exceed WHO recommended limits. The effects of climate change, pollution, population increase and with large numbers of new chemicals entering the river system, continuous monitoring regime for their detection will become increasingly important with respect to ecological impacts they produce. Much effort has to be directed towards the detection of such pollutants in river. The frequency of bioindicators in the river is significantly influenced by the flow condition, temperature and turbidity of the water, habitat conditions and variations as well as the chemical status of the water environment. Results indicated that the improvement of water quality of the investigated locations of Danube River in Budapest is mainly depending on the many ecological factors and the quality and quantity of the available impurities carried out by the river from the source. The presence of E. coli in water is nearly always associated with recent faecal pollution. So, bacteriological monitoring should be carefully chosen with respect to designated functional uses and/or the intrinsic ecological value of the river ecosystem. Monitoring program and methods should be upgraded especially with reference to the updated EN ISO Standards for isolating indicator bacteria; sampling sites should include river banks to increase the chance of detecting focal points of pollution.

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CARBON DIOXIDE EMISSION FROM HEAVY MEAL POLLUTED SOIL

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Abstract: *Carbon dioxide emissions losses from cultivated soil amended with cadmium (Cd) and lead (Pb) were studied in closed microcosm at various incubation temperatures (15 and 37 ℃) conditions were analysed by gas chromatographic methods. Results indicated that the production of carbon dioxide (CO_2) released did not significantly increase by the increasing time of incubation up to the 15th day and then it decreased gradually. On the other hand, the amount of gas emission in Cd contaminated soil was more than soil amended with Pb. The results indicated that the CO2 was more emitted at 37 ℃ than at 15 ℃, and the high concentration of Pb more inhibited the emission process than Cd concentration.*

Keywords: *CO_2 emission; heavy metal; microcosm; temperature; soil contamination.*

INFORMATION

Economic growth has accelerated energy demand, and the resulting rapid increase in carbon emissions has become a prominent problem restricting the green and sustainable development of the economy. As global warming has a profound impact on economic development, ecological environment, and human health, taking urgent action to deal with climate change and its impact has been listed as a sustainable development goal of the United Nations. According to the Emissions Gap Report 2020 (UNEP 2020), despite the decline in global carbon dioxide emissions in 2020 due to the impact of COVID-19, the world is still heading for a 3.2°C temperature rise this century. Failure to achieve the Paris Agreement goal of limiting global warming to well below 2°C will have irreversible impacts on a wide range of areas, such as an increase in extreme weather, especially for developing countries with weak economic foundations

Nowadays, the carbon emitted by fossil fuel–based vehicles is seriously threatening the environment and directly impacts the climate. Global warming and climate alterations are caused by pollution due to the extensive burn of diesel by diesel-based vehicles.

In IEA (2020), it has been reported that transportation is at the origin of 24% of CO2 emissions worldwide. Still worst, in 2020, the European Environment Agency suggested that about 27% of CO_2 is emitted by the transport sector, while more than 70% of emissions are mainly caused by vehicles (EEA 2020). From the 1970s, fossil fuel combustion increased the emission of CO_2 , CO, SO_2 , and NO by 90% to reach 36.1 Gt in 2014 (Tran et al. 2021).

Since the 1970s, global carbon emissions have basically shown a positive correlation with global economic development. With the development of the global economy, both overall and per capita carbon emissions have increased significantly. Nowadays, the issue of carbon emissions and the resulting climate change has become the focus of the international community. In 2019, the carbon emissions of major countries accounted for as high as 56.55% (Xi and Niu, 2021); the details are as follows: China (9.826 billion tons of CO_2, accounting for 28.76%), the USA (4.965 billion tons of CO_2 , accounting for 14.53%), India (2.480 billion tons of CO_2 , accounting for 7.26%), Russia (1.533 billion tons of CO_2 , accounting for 4.49%), and Japan (1.123 billion tons of CO_2: accounting for 3.29%), and it is clear that global carbon emission reduction still faces severe challenges. There are significant differences between countries in the factors affecting carbon emissions. In Japan and Germany, changes in the structure of international input have led to increased carbon emissions (Jiang et al. 2021). A study based on carbon decoupling and economic growth in 25 African countries found that primary energy intensity and population are the main drivers of carbon emissions (Tenaw and Hawitibo 2021), at the same time, referring to OECD countries data; Zaidi et al.(2021) pointed out that there is a positive correlation between financial inclusion and carbon emissions.

Circular economy collectively refers to the reduction, reuse, and management of material resources in the processes of production, circulation, and consumption. Developing the circular economy presents an important way to achieve sustainable development and serves as a fundamental means to effectively utilize resources and protect the ecological environment. The European Union has made recycling a prerequisite for climate neutrality and has strengthened the synergy between recycling and reducing greenhouse gas emissions.

The aims of this research were: (1) to evaluate the effect of different concentrations of Cd or Pb on trace gases emissions; (2) to evaluate differences in the trace gases emissions from heavy metals contaminated soil samples incubated at low and high temperatures.

MATERIAL AND METHODS

The microcosm experiment conducted in glass vessels covered tightly by silicone septa. A 200 g homogenized (< 2 mm) soil samples (the soil is slightly alkaline solonchak obtained from Keszthely, Hungary) of low humus content were placed into the vessels of 1200 cm3. The soil samples collected in the upper 0-300 mm layer after removing the top 20-30 mm from a sample site. The soil is a sandy-loam texture in the upper (0-300 mm) horizon. The main physico-chemical properties of the (0-300 mm) soil were: $pH_{(KCl)}$ 7.55, total salt content 0.054%, humus 1.48%, total organic C 1.08%, total N 0.08%, NH_4^+-N 0.53 mg 100 g^{-1} soil, NO_3^--N 0.18 mg 100 g^{-1} soil, K_2O 136 mg 100 g^{-1} soil, P_2O_5 130 mg 100 g^{-1} soil, soil density 2.45 g cm^{-3} and C/N ratio 13.5. The water filled pore space (WFPS) of soil samples was 60%. The soil samples were contaminated by the addition of different doses of $Pb(CH_3COO)_2.3H_2O$ at 40, 80 and 160 mg Pb kg^{-1} soil or $CdCl_2.2.5H_2O$ at 6, 12 and 24 mg Cd kg^{-1} soil. The vessels were incubated in a laboratory thermostat at 15 or 37°C for 35 days. During the experiments, CO_2 gas samples were taken from the headspace of each vessel and determined regularly by gas chromatographic method. A 250 µl gas sample was taken by gas tight Hamilton syringes and injected to the HP 5890 gas chromatography. Thermal Conductivity Detector (TCD) detected CO_2 concentrations, respectively. The separated gas content was analysed three times per day whenever measurements carried out using external standard and one point linear calibration. The experiment conducted with three replicates. Results were statistically evaluated using analysis of variance and using SPSS 10.0 statistical programme. The LSD test (Sváb 1981) was used for multiple comparisons of means at $P < 0.05$.

RESULTS AND DISCUSSION

In the first period of the experiment the CO2 emissions from soil raised due to the increase of microbiological activity and the acceleration of soil organic matter mineralization after drying and rewetting of soil.

High significant differences in the production rates of CO2 recorded between microcosms contaminated with Cd or Pb soil and incubated at 37°C (Figure 1) or 15°C (Figure 2). The incubation of microcosms at 37°C stimulated three times the production rates of soil contaminated with Cd or Pb over the production rates at 15°C.

Comparatively, no significant difference in the production rates of CO2 observed between microcosms contaminated with Cd or Pb soil. Also, no significant difference found in the production rates of CO_2 between the various concentrations of Cd or Pb. The results demonstrated that 40 mg Pb kg^{-1} soil stimulate the production rate of the gas compared with control (Figure 1).

Moreover, the all applied Cd or Pb concentrations improved the emission of CO2 from the contaminated soils than control. During the first 28 days of the incubation, no significant differences were observed between the heavy metal treatments and the rates of gas emission especially at 15°C (Figure 2).

The results indicated that at 15°C, Pb had more toxic effect on the rate of gas production in comparison to Cd at the same incubation temperature. According to our findings here, temperature may be playing the main role in the gas emission as shown in Figures 1 and 2. As the amount of CO_2 uptake of plants in photosynthesis and the rate of the emitted CO_2 during plant respiration correspond to each other annually (Guo & Zhou 2007), soil microbial processes are presumed to contribute significantly to the agricultural CO_2 emission. Although the basic factors of determining the rate of these microbial events are soil moisture, temperature and C and N availability (Chu et al. 2007).

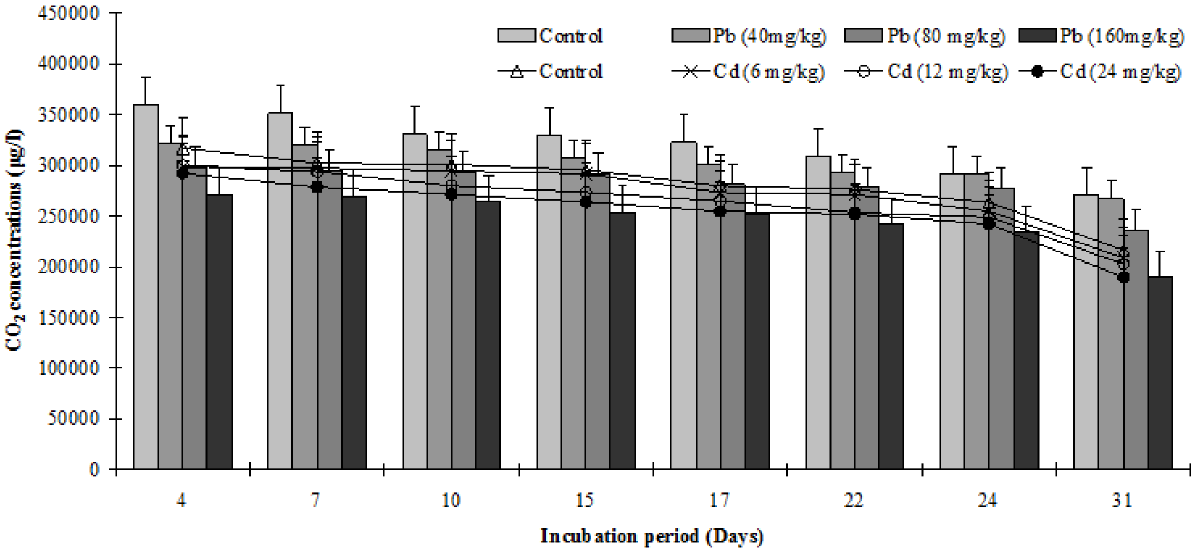

Figure 1. Figure 2. Emissions of CO2 from soil polluted by different concentration of Pb and Cd and incubated at 37℃

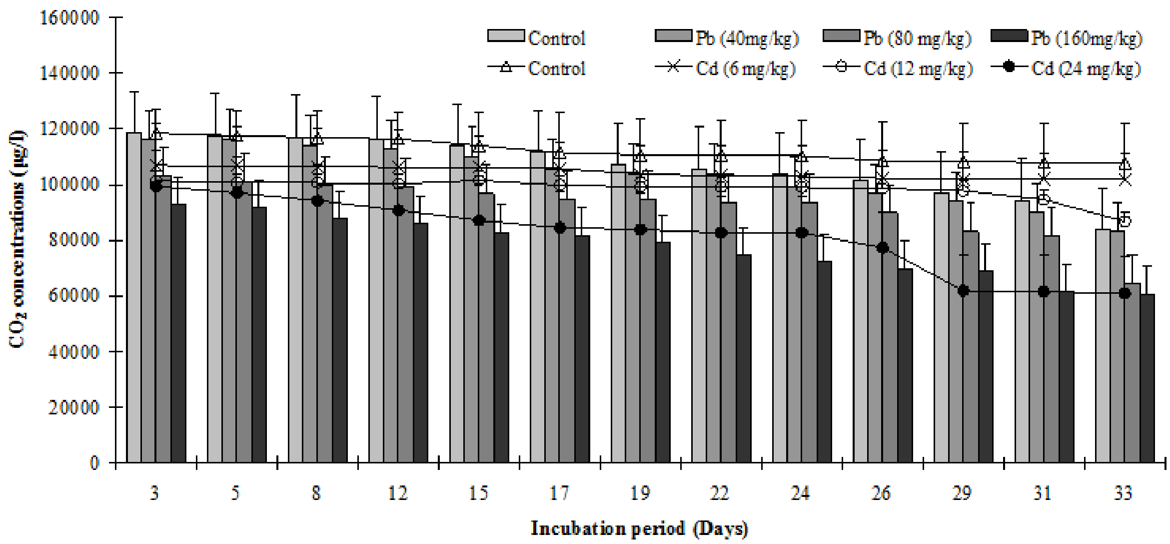

Figure 2. Emissions of CO2 from soil polluted by different concentration of Pb and Cd and incubated at 15℃

Conclusion

In conclusion, it can be stated that the applied microcosm experimental model proved to be a suitable tool for detecting the effect of factors influencing the CO_2 release from agricultural soil. This study underlines the key role of contamination of soil sample with different concentrations of two heavy metals (Cd and Pb) in emissions of CO_2 from 60% WFPS soils with low organic matter contents under different incubation temperatures conditions. The management of soil contamination and the temperature are key considerations for mitigating trace gases emissions from these micro-agroecosystems.

It is necessary to take into account the incubation temperature at 37°C increased the production rates of the CO_2 more than at 15°C., Pb concentration over 40 mg kg^{-1} soil caused a reduction in trace gas production.

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SCREENING OF MICROBIAL INOCULANTS USED IN AGRICULTURAL SOIL AS PLANT BIOSTIMULANTS

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Abstract: Antimicrobial resistance within populations of different infectious agents is a worldwide public health threat not only for human and animal but also for plant. *Today, with the gradually increases in the global population and climatic changes as well as the increasing the global environmental problems, one of the most important topics is to protect our plant crops biologically as a part of Innovations in biotechnology and toxicology and pharmaceutics in agriculture. Regarding to the healthcare and human nutrition, the antagonism is the most effective objective in the plant protection using the microbiomes which inhibit the growth of the plant pathogens instead of using agrochemical protective agents like fungicides, insecticides, etc which laterally can enter the food web and chain and indirectly affect the human health. Some microorganisms are able to produce antimicrobial agents as secondary metabolites that are able to kill or inhibit the plant pathogens. Many methods were used to investigate the interactions between the soil useful microbiomes and plant pathogens as well as the crop production and soil health.*

Keywords: *Soil microbiomes, PGPR, mechanism of action, plant-microbe interation*

INTRODUCTION

Sustainability in agricultural systems without compromising the environmental quality and conservation is one of the major concerns of today's world. The excessive use of agrochemicals (fertilizers and pesticides) is posing serious threats to the environment. Furthermore, the long-term sustainability of agricultural systems depends most likely on effective handling of the internal/ indigenous resources of agro-ecosystems. During the last couple of decades, the new biotechnologies have opened new vistas for enhancement of agriculture productivity in a sustainable manner. Advances in understanding of soil microbiology and biotechnology have made possible exploitation of soil microorganisms for improving crop productivity and, in turn, have offered an economically attractive and ecologically viable supplement to reduce external inputs to some extent. The plant rhizosphere is a remarkable ecological environment as myriad microorganisms colonize in, on, and around the roots of growing plants. It is well known that a considerable number of bacterial species, mostly those associated with the plant rhizosphere, are able to exert a beneficial effect upon plant growth. Therefore, their use as biofertilizers or control agents for agriculture improvement has been a focus of numerous researchers for a number of years. This group of bacteria has been termed 'plant growth promoting rhizobiomes' (PGPR), and among them are strains from bacterial genera such as *Pseudomonas*, *Azospirillum, Burkholderia, Bacillus, Enterobacter, Rhizobium, Serratia, Alcaligenes, Arthrobacter*, *Acinetobacter*, *Flavobacterium* and also from fungi such as *Trichoderma*, Mycorrhizae, etc.

The protection of plants from diseases caused by various pathogens is an economically and socially important problem; the losses in crop production constitute 20% of the harvest in different parts of the world. The use of chemical pesticides is the main method of plant protection. However, chemical preparations have a number of serious disadvantages. Biologicals for plant protection (BPP) are currently being developed more intensely. The world biggest chemical companies, BASF, Bayer and Syngenta, show great interest in the market for preparations of biological control (Azizbekyan, 2019).

Enzymes of microbial origin are of immense importance for organic material decomposition leading to bioremediation of organic waste, bioenergy generation, large-scale industrial bioprocesses, etc. The market demand for microbial cellulase enzyme is growing more rapidly which ultimately becomes the driving force towards research on this biocatalyst, widely used in various industrial activities (Sahoo et al., 2020).

Stimulation of different crops by PGPR has been demonstrated in both laboratory and field trials. Strains of Pseudomonas putida and Pseudomonas fluorescens have increased root and shoot elongation in canola, lettuce, and tomato as well as crop yields in potato, radishes, rice, sugar beet, tomato, lettuce, apple, citrus, beans, ornamental plants, and wheat (Kloepper, 1994).

The diverse groups of bacteria and fungi in close association with roots and capable of stimulating plant growth by any mechanism(s) of action are referred to as plant growth-promoting rhizobiomes (PGPR). They affect plant growth and development directly or indirectly either by releasing plant growth regulators (PGRs) or other biologically active substances, altering endogenous levels of PGRs, enhancing availability and uptake of nutrients through fixation and mobilization, reducing harmful effects of pathogenic microorganisms on plants and/or by employing multiple mechanisms of action. Recently, PGPR have received more attention for use as a biofertilizer for the sustainability of agro-ecosystems. Selection of efficient PGPR strains based on well-defined mechanism(s) for the formulation of biofertilizers is vital for achieving consistent and reproducible results under field conditions. Numerous studies have suggested that PGPR-based biofertilizers could be used as effective supplements to chemical fertilizers to promote crop yields on sustainable (Khan et al., 2009).

Mechanisms of Action

PGPR affect growth and development of plants by direct or indirect mechanisms. The direct mechanisms include N_2-fixation, mobilization of nutrients via production of phosphatases, siderophores, or organic acids, and production of phytohormones and enzymes. Indirectly, the microorganisms may exert a positive influence on plant growth by lessening certain deleterious effects of a pathogenic organism by inducing host resistance to the pathogen or by knocking out the pathogen from root surfaces or by producing chitinases or other pathogen-suppressing substances. Certain soil microbiomes possess multiple traits to affect plant growth where one trait may dominate the other one. A bacterium influencing plant growth by releasing PGRs can play a role in controlling plant pathogens and diseases, and vice versa. So, plant response to PGPR is a complex phenomenon, and recent advances in research at the molecular level have provided a sufficient basis to understand these mechanisms more precisely. The major mechanisms of PGPR action involved in the improvement of plant growth and development.

Fixation, Mobilization and Uptake of Nutrients

Nutrients are one of the extremely important factors which influence growth, yield, and quality of different crops. Soil microbiomes can provide nutrients to plants either through the fixation of atmospheric N_2 or by enhancing nutrient mobilization/uptake through their biological activities, such as mineralization and through siderophore, organic acid and phosphatase production, etc. Biological N_2-fixation by rhizobia and associative diazotrophic bacteria is a spontaneous process and one of the widely studied mechanisms by which plants benefit from the interacting partners. The bacteria benefit the plants by fixing N_2 in exchange for fixed carbon either provided directly to the bacteria or indirectly by releasing carbon as root exudates. However, obtaining maximum benefits on farms from diazotroph PGPR biofertilizer requires a systematic strategy designed to fully utilize all these beneficial factors, allowing crop yields to be maintained or even increased while fertilizer applications are reduced (In addition to biological N_2-fixation, PGPR are also known to affect the nutrient availability to the plant through acidification and redox changes or by producing iron chelators and siderophores, and/or mobilizing the metal phosphates Several reports have suggested that PGPR can stimulate plant growth through their P-solubilizing activity Furthermore, Wu et al. (2005) reported increased assimilation of nutrients, such as N, P, and K, in plants, in response to inoculation with P-solubilizer *(Bacillus megaterium*) and K-solubilizer (*Bacillus mucilaginous*). Likewise, Orhan et al. (2006) reported that inoculation with a phosphate-solubilizing Bacillus strain M3 significantly improved P, Fe, and Mn contents of the leaves of raspberry, suggesting that *Bacillus* M3 alone or in combination with some other strains had the potential to increase the nutrition of raspberry plants, in addition to growth and yield.

Production of Plant Growth-Regulating Substances

Plant growth-regulating substances are naturally occurring organic compounds that influence various physiological processes in plants, such as cell elongation and cell division. They perform these functions at concentrations far below the levels at which nutrients and vitamins normally affect plant processes. It is now well established that the majority of soil microorganisms can produce plant growth regulating substances, including phytohormones (auxins, gibberellins, cytokinins, ethylene, and abscisic acid) and enzymes. Production of PGRs by microorganisms is affected by the presence of suitable substrate(s)/precursor(s) as well as type and concentration of exudates. The inocula in the presence of a specific physiological precursor of a PGR and/or inocula that produce physiologically-active concentrations of a phytohormone can be highly effective in promoting plant growth and enhancing consistency and reproducibility (Arshad and Frankenberger, 2002). Some PGPR can influence plant growth by altering the synthesis of endogenous phytohormones through the production of specific enzymes. Among these enzymes, bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase plays a significant role in the regulation of a plant hormone, ethylene, and thus the modification of the growth and development of plants (Arshad and Frankenberger 2002)

Biological Control

In modern agronomic practices, a huge amount of chemicals (insecticide/fungicide) are used to offset various pathogens inflicting severe losses to crop yields. Although these chemicals are vital for controlling the pathogens on the one hand, on the other hand they can drastically affect the microbial diversity and functional properties of natural microbial communities of soils, leading thereby to imbalanced agro-ecosystems. It is therefore important to discover the most viable and economical means for effective disease management in an environment-friendly manner. Currently, biopesticides are receiving worldwide attention and considered important for the sustainability of the agricultural system. Furthermore, the WTO guideline that suggests that only residue-free agricultural produce can be exported has further created a great interest and demand for the use of biopesticides in crop protection systems. In recent times, PGPR have emerged as potential candidates with wide scope for inducing systemic resistance in crop plants against many pathogens (Masoud and Abbas 2009). Various species of bacteria including P*seudomonas fluorescens*, *P. putida*, *P. cepacia*, *P. aeruginosa*, *Bacillus* spp., *Rhizobium* and many other PGPR like *Trichoderma* sp exhibit biological control activity and inhibit pathogens by synthesizing chitinase, and by production of hydrogen cyanide, protease, siderophores, and cellulase, and/or indirectly by promoting plant growth and health through any mode of action.

Multiple Mechanisms of Action

Some PGPR can stimulate plant growth through multifarious activities. Although PGPR have been reported to influence plant growth by an array of mechanisms, the specific traits by which PGPR facilitate plant growth, yield, and nutrient uptake were limited to the expression of one or more of the traits expressed simultaneously in a given environment of plant–microbe interaction. A PGPR can promote plant growth by improving plant nutrition, modifying root growth architecture, and by plant responses to external stress factors, simultaneously (Shaharoona et al., 2008). However, one trait may dominate the other one of the same PGPR when exposed to certain environmental conditions. For instance, Dey et al. (2004) reported more than one mechanism of PGPR responsible for growth promotion. They suggested that, besides ACC deaminase activity, expression of one or more of the traits such as suppression of phytopathogens, solubilization of tricalcium phosphate, production of siderophore and/or nodulation promotion by the PGPR might have simultaneously contributed to the enhancement of growth, yield, and nutrient uptake.

PGPR for Bioremediation

The addition of PGPR increases the removal of pollutant most likely by enhancing germination, and by stimulating plant growth including root biomass and survival of plants, in soils that are heavily contaminated. Some rhizobacteria can enhance phytoremdiation by promoting plant growth through the synthesis of siderophores, phytohormones, enzymes, and antibiotics and/or through stimulation of certain metabolic pathways, such as nitrogen fixation and the uptake of N, P, S, Mg, Ca, and other nutrients Similarly, PGPR can increase the tolerance of plants to contaminants; the PGPR–plant system cannot survive in comparatively extreme environments such as with high concentrations of heavy metals (Wani et al. 2008a). Usually, rhizosphere soil is more conducive to remediation due to high concentrations of nutrients exuded from the roots and dense bacterial populations. Many workers have reported bioremediation of both organic (Muratova et al. 2005) and inorganic contaminants in the environment by using PGPR (Wani et al. 2008b).

Formulations of Effective Biofertilizers

A number of steps are involved in developing effective PGPR-based biofertilizers for achieving consistent results in terms of crop productivity under field conditions (Figure 1). The most critical steps involved in the development of biofertilizers include isolation of bacterial strains from the same habitat and/or crop followed by screening of PGPR under axenic conditions by conducting repeated trials. The strains showing better results under controlled conditions should be tested further for their performance under natural conditions by conducting pot and field trials.

Finally, the PGPR strain selected for biofertilizer formulation should be investigated thoroughly to maintain its quality. Quality of biofertilizers is one of the most critical factors which determine their success or failure and acceptance or rejection by end users, the farmers. The functionality of selected PGPR must be defined well before using it as a candidate for biofertilizer formulation, which could be achieved by employing biochemical and molecular tests in the laboratory and then determining correlations between PGPR traits with the growth promotion of inoculated plants under axenic and natural conditions.

The production of biofertilizer and its acceptance by farming communities are closely linked. For their use to expand globally at the farmers' end, quality management is essential and must be performed consistently in order to supply contaminant-free bioproducts to the users. For this, skilled personnel are required who know how to work with these materials and be able to respond to the modern conditions of agricultural production. In addition, they should be well aware of the sustainability and environmental protection measures. Furthermore, proper guidelines for the production and commercialization of biofertilizers should be framed in order to popularize the use of such bioagents for maintaining the sustainability of agro-ecosystems across the globe.

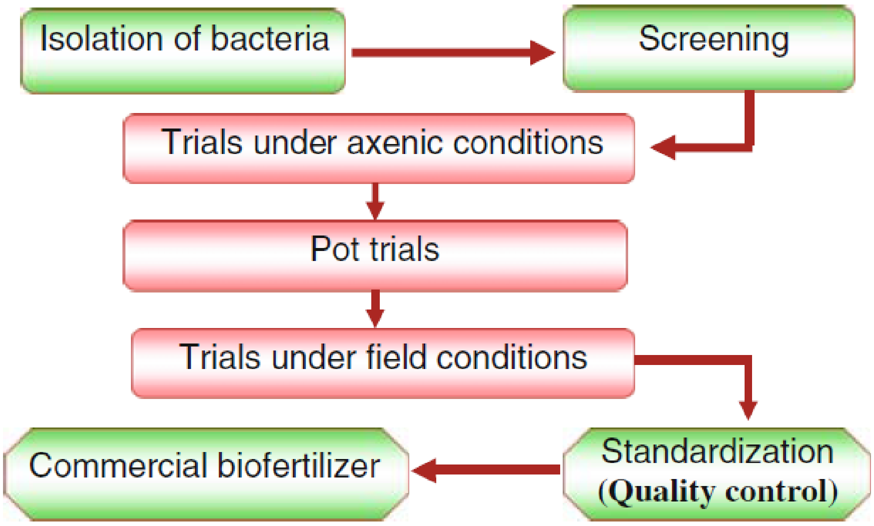

Figure 1. Steps involved in the development of an effective biofertilizer product

CONCLUSION

Enhancement in the use of PGPR is one of the newly emerging options for meeting agricultural challenges imposed by the still-growing aggregate demand for food. Moreover, this biotechnology is also likely to ensure conservation of our environments. However, before PGPR can contribute to such benefits, scientists must learn more about them and explore ways and means for their better utilization in the farmers' fields. Future research should focus on managing plant–microbe interactions, particularly with respect to their mode of actions and adaptability to conditions under extreme environments for the benefit of plants. Furthermore, scientists need to address certain issues, like how to improve the efficacy of biofertilizers, what should be an ideal and universal delivery system, how to stabilize these microbes in soil systems, and how nutritional and root exudation aspects could be controlled in order to get maximum benefits from PGPR application. Biotechnological and molecular approaches could possibly develop more understanding about PGPR mode of actions that could lead to more successful plant–microbe interaction. Efforts should also be directed towards the use of PGPR to reduce pesticide applications. In brief, PGPR biotechnology provides an excellent opportunity to develop environment-friendly biofertilizer to be used as supplements and/or alternatives to chemical fertilizers.

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SUSTAINABLE POLYESTER FIBRE POLY(LACTIC ACID) (PLA) – RECENT DEVELOPMENT

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Abstract: *Poly(lactic acid) appeared, as a promising sustainable and advantageous material with potential to replace conventional petrochemical-based polymers, more than twenty years ago. Its possible application in fibre form to replace conventional polyethylene terephthalate (PET) polyester fibre was also widely discussed. That time and later many questions came up in the field of polymer manufacturing and application for various purposes, including fibres. The present paper gives a short insight into the contemporary development of poly(lactic acid) technologies, especially in the field of fibres.*

Keywords: *poly(lactic acid) fibre, biobased, biodegradation, sustainability, textile technology*

INTRODUCTION

Protection of the environment and sustainability are very important considerations in various areas. Poly(lactic acid) (PLA) is a linear aliphatic thermoplastic polyester derived from 100% renewable sources such as corn, and the polymer is biodegradable.

The monomer used to manufacture poly(lactic acid) is obtained from annually renewable crops. (Low quality, contaminated crops are suitable for this purpose.) Energy from the sun promotes photosynthesis within the plant cells; carbon dioxide and water from the atmosphere are converted into starch. This starch is readily extracted from plant matter and converted to a fermentable sugar (e.g. glucose) by enzymatic hydrolysis. The carbon and other elements in these natural sugars are then converted to lactic acid through fermentation.

The primary mechanism of degradation of PLA is hydrolysis, catalysed by temperature, followed by bacterial attack on the fragmented residues. In composting, the moisture and the heat in the compost pile attacks the PLA polymer chains and splits them apart, creating smaller polymer fragments, and finally, lactic acid. Microorganisms, found in active compost piles, consume the smaller polymer fragments and lactic acid as energy source. Since lactic acid is widely found in nature, a large number of naturally occurring organisms metabolize lactic acid [1].

Figure 1: Lyfe cycle of polylactic acid [2]

Chemistry of PLA

Lactic acid (2-hydroxypropanoic acid), also known as milk acid plays role in several biochemical processes. Lactic acid is chiral (exists as 'mirror images') and has two optical isomers. One is known as L-(+)-lactic acid or (S)-lactic acid and the other, its mirror image, is D-(−)-lactic acid or (R)-lactic acid. L-(+)-Lactic acid is the biologically important isomer (Fig 2). The properties of the polymer depend on the ratio of these isomers.

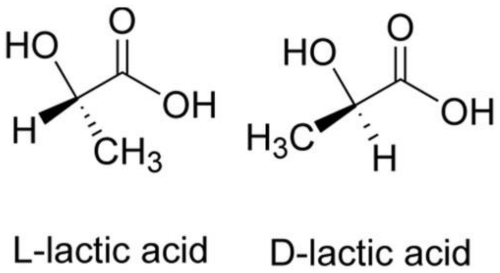

Figure 2: The stereoisomers of lactic acid

Polycondensation of lactic acid by removal of water produces polymer (Fig. 3)

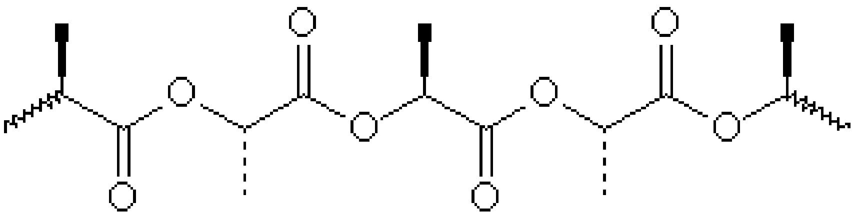

Figure 3: Polylactic acid

The various methods of reactions giving poly(lactic acid), the reaction mechanisms and the most important properties of PLA resulted in various technologies were reviewed in detail by Garlotta in 2001 [3]. PLA physical properties including hydrolysis were summarized by Henton et al. in 2005 [4] and Lim et al. in 2008 [5].

The chemical technology of the PLA manufacture was followed by the study of the possible applications like extrusion, injection moulding, foaming, fibre spinning, composites' matrix etc. [5, 6, 7]. Moreover, the modifications of the polymer were studied very soon [6].

PLA TEXTILES

Fibres

Fibres were in the focus of interest at the very first time of PLA history [8, 9]. PLA fibres can be manufactured by melt spinning and by dry spinning methods, wet spinning and electrospinning are also suitable processes.

Different melting points of PLA, varying from 130°C to 220°C, can be obtained: the melting point depends on the lactic acid's L and D isomer content. P(L)LA having only L-lactic units has a melting temperature of 180ºC. A blend of poly(L-lactic acid) and poly(D-lactic acid) can lead to a polymeric stereocomplex with higher melting point than either L- or D-polymers alone and with improved mechanical properties [10]. The melting points of pure poly(L-lactic acid) (PLLA), pure poly(D-lactic acid) (PDLA) and poly(D,L-lactic acid) (combination with highest melting point) are 180°C, 180°C and 220°C, respectively [11, 13]. As in the case of other fibre forming polymers, crystallinity of the fibres can be influenced by drawing ratio [9, 12]. Very detailed results can be found in the field of wet and dry spinning and melt spinning as well [13]. PLA filaments were textured by false-twist method [13].

Yarns

Many studies have demonstrated that staple spun PLA yarn can be manufactured with both pure PLA fibre and blends of PLA with other fibres such as cotton, lyocell and rayon, etc. Both the ring spinning system and rotor spinning system can be used to manufacture staple spun PLA yarns. In the staple fibre spinning system, the spinning process and parameters are strongly affected by the fibre properties. The spinnability of commercially available PLA fibre is similar to that of PET fibre [13, 14].

The PLA fibre has low moisture regain and high mass specific resistance, leading to the easy accumulation of static electricity on its surface during the spinning process. Therefore, the environmental conditions (temperature and humidity), machine speed, roller material and roller pressure, and so on, need to be well controlled and designed. The PLA fibre has high tensile strength, low inter-fibre friction force and low cohesive force, which also influence the spinning process to a large extent [13].

Fabrics

The most commonly used fabric-producing techniques are knitting and weaving. Like other synthetic fibre yarns, PLA staple fibre yarns and filaments could be knitted or woven into fabrics using currently existing knitting or weaving machines. Blended fabrics have been developed using blended yarns or combining PLA staple yarns or filaments with other yarns such as natural cotton, wool, silk yarns, as well as man-made Tencel and lyocell yarns. The machine parameters for PLA fabric knitting and weaving are often set referring to PET fabrics, but the properties of PLA yarns and filaments should be considered, such as higher elongation, relatively lower strength and instability (becoming less elastic) after long-term storage in air [9].

Wet processing

As PLA fibre is a kind of synthetic fibre and has broadly similar properties as PET fibre, PLA fabrics have been conventionally treated with the same dyes and chemicals as for PET fabrics in wet processing. On the other hand, PLA has much lower glass transition temperature (55–60°C) and crystalline melting point (165–180°C) than PET, and hydrolytic degradation easily occurs under high temperature and strong alkaline conditions, and thus the wet processing condition should be adjusted accordingly. For instance, PLA fabrics are normally dyed with disperse dyes as for PET fabrics, but in lower temperature at 120°C. NatureWorks suggests to treat PLA fabric following this process flow: pre-heat-setting (depending on yarn type), pretreatment (desizing and scouring), dyeing, reductive clearing, post-heat-setting, finishing [9, 15]. The most important practical advices for various wet processes (scouring, bleaching, and dyeing) of PLA fabric can be found on the website www.fibre2fashion.com and the websites of the fibre manufacturers, especially Toray and NatureWorks (Ingeo™).

Polymer properties and dyeing

The dye exhaustion is affected by the polymer properties of PLA. PLA fibre generally has a moderate affinity with disperse dyes. PLA fibre with high crystallinity (50%) was found to have lower exhaustion than PLA fibre with low crystallinity (21%). The fibre with high crystallinity had a lower degree of amorphous and fewer interstices that dye molecules could penetrate, which limited the dye saturation capacities. The dyes with multiple carbonyl groups did not exhaust well on the high-crystallinity PLA, whereas their exhaustion on low-crystallinity PLA was high [13, 16]

The concentration of D-isomer in PLA affects its crystallinity, and further influences its dyeability. PLA fibres with higher D-isomer concentration have greater fibre entropy, more amorphous and less crystalline regions in the polymer, with respect to low D-isomer fibres. Therefore, high D-isomer fibres presented greater dye exhaustion and colour strength for all kinds of dyes and concentrations. Beside, high D-isomer fibres could be dyed to an excellent black shade, whereas low D-isomer fibres appeared brown. The wash fastness of the two fibres was very similar [13, 17].

PLA blends

As cotton fibre needs much stronger alkaline conditions and higher temperature for scouring and dyeing, hydrolysis and strength loss of PLA fibre are the critical problems for PLA/cotton blended fabrics. Another challenge is the reductive clearing after dyeing under alkaline conditions, which may also affect PLA fibre.

Application of PLA fibres

At first, medical applications were investigated due to the biocompatibility of PLA: the degradation product, lactic acid, is metabolically innocuous. The fibres may be fabricated into various forms and may be used for implants and other surgical applications such as sutures and tissue engineering as one of the most favourable matrix material [9].

PLA fibres can be used and processed as textiles similarly to PET fibres (see before).

PLA has been widely used as matrix in a composite [7]. Two types of unidirectional biodegradable composites, PLA self-reinforced and PLA reinforced polybutylene succinate (PBS) matrix composites, were produced [18].

PLA is used in 3D printing alone and as matrix in natural fibre reinforced composites as well [19].

SUMMARY

PLA issue regarding the textile industry is presented on Figure 4 [13].

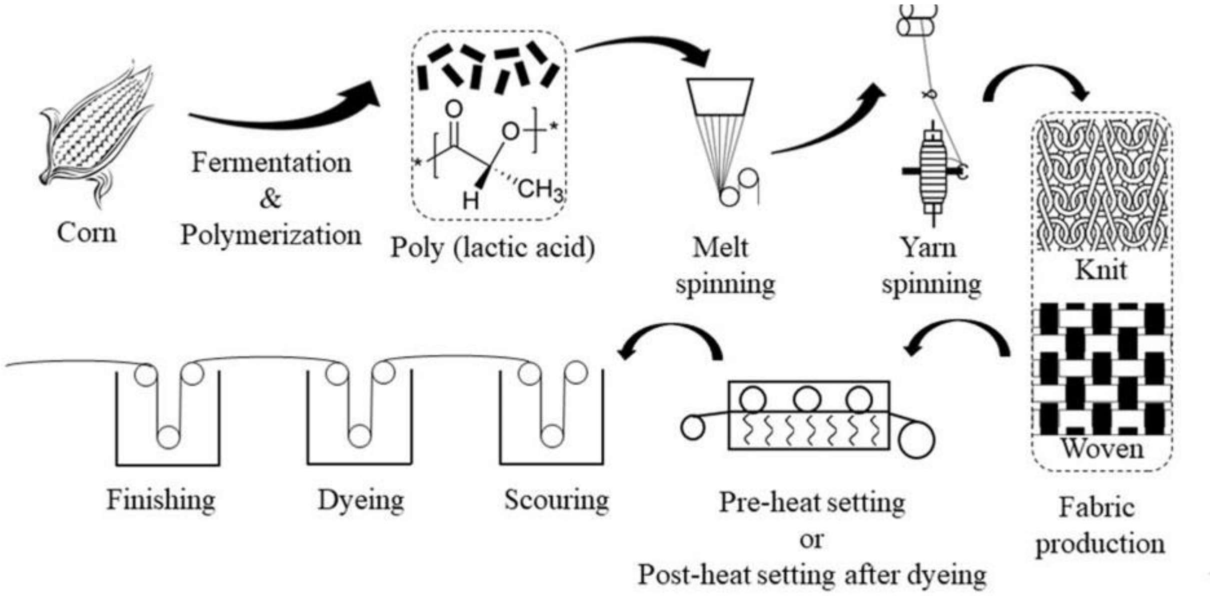

Figure 4. The production and processing stages of PLA textiles [13]

PLA products were developed by NatureWorks first, starting their work in 1989. Their PLA Ingeo is the most known type of PLA. NatureWorks produce PLA for various purposes [20].

Toray Company (Japan) produces PLA (Ecodear™), moreover, other bio based fibres are developed, too: Ecodear™ N510, will be the first 100% plant-based nylon fibre in Toray's Ecodear™ lineup [21, 22].

CONCLUSION

Significant part of the technological problems of PLA have been solved in the last decade(s). The environmental demand must speed up the research on sustainable materials, including PLA.

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INHALER DESIGN

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Abstract: *The result of the design process is a medical product design ('Releaf'), an inhaler for asthmatic and other respiratory diseases suffering patients. The purpose of the personally motivated innovation was to use a new form, function and material to move the inhaler design away from being a strange medical device to a product in which the patients trust and can rely on even in the most unexpected situations.*

Keywords: *inhaler, product design, asthma attack*

INTRODUCTION

Comprehensive researches pointed out the fact that the currently used inhaler designs have less comfortable portable functions. Furthermore, they can be easily contaminated by their surroundings, the most used inhalers are complicated to use, and also they are aesthetically not too pleasing for the users.

The Releaf inhaler [Figure 1.] can easily be operated in one hand, it also has an electronic measuring device, so it allows the exact amount of the medication available to be read from the display. The new design is more ergonomic than the most available ones on the market and also environmentally conscious: the carefully selected materials can be separated and recycled in an easy way.

Releaf is a metered dose portable inhaler that can be used quickly, so it provides safety and a feeling of security for patients who have asthma or suffer from other respiratory diseases.

Respiratory diseases

The product was designed to help patients with asthmatic respiratory diseases. The cause of the disease is complex and difficult to diagnose, on the other hand, the treatment is complicated, as different medications are recommended for each individual. Also, it is worth mentioning that this type of respiratory disease can develop at any stage of life, and unfortunately, due to environmental influences and changes in living conditions, the number of patients having this disease is increasing both in Hungary and around the world.

The most common solution for preventing and helping asthma attacks is the inhaler, which is mainly a bronchodilator. Based on previous observations and surveys, we have found that the functionality of the products on the market is not the most effective, their use is not customized at all, and last but not least, their appearance does not create a positive experience among users, they are present in the household as strange, impersonal objects. In our opinion, a more aesthetically pleasing appearance with a variety of color options would make the inhaler for medical use more stylish and user-friendly. In addition, the treatment of emergencies is also an important consideration. It may happen that a patient has an unexpected attack and needs help and medication as soon as possible, even within seconds.

We have seen similar cases among our acquaintances, so we think it is important that the product, in addition to its aesthetic appearance, has an innovation that can significantly improve its quick and efficient use in case of emergency, helping the patient to recover quickly and treat symptoms immediately. Currently, the majority of portable inhalers on the market cannot fulfil this task. Apart from the complexity and time-consuming nature of using such products, there is another problem: let's face it, they are not pretty to look at. Talking to several users and professionals, it was revealed that patients who use the product are reluctant to take it with them to social gatherings and events. This further reduces the chances of getting proper help in the event of an attack. The problem mentioned could be prevented by designing an aesthetically pleasing product for them that they could use in public without discomfort.

Important to note that many inhalers are not environmentally friendly, as they contain composite materials, such as metal and plastic parts, which cannot be separated or are very difficult to separate, and therefore cannot be recycled. In addition, they are easily contaminated, so that the patient cannot avoid inhaling dust or other harmful substances.

Also an important problem is that the cartridges used in metered dose inhalers cannot be purchased separately, so every time the medicament runs out, the inhaler can be bought only together with the primary packaging, generating unnecessary accumulation of plastic waste in households.

Design process

During the design process numerous criteria were taken in account. Economic, environmental, aesthetic, ergonomic, marketing, legal, design and manufacturing aspects were combined to create a list of requirements for selecting the optimal product from the sketches.

It was most important that the product should be visually appealing, environmentally friendly and inexpensive, and that it should be reliable, obvious and easy to use, so there should be no hindrances when people need it the most. Based on these considerations the final product was an inhaler with a safety lock that met most of the requirements previously established.

Its user-friendly design is very different from the inhalers on the market. That helps to make people with respiratory illnesses feel welcome to use it in public. Releaf can be carried in a bag or pocket, as the safety lock prevents the cartridge from being activated accidentally, and the attachable plastic cover ensures that the product remains hygienic. The capsular shape is advantageous for several reasons, firstly because it has a rounded surface, making it difficult to get stuck in a bag or pocket. In addition, the rounded end form made it easy to add to it the mouthpiece design, which makes the inhaler comfortable to use.

As far as the material is concerned, after taking every possibility in consideration, plastic was selected, particularly polycarbonate, which, although not environmentally friendly as such, is nowadays easily recyclable. It has the advantage of being moisture-tolerant, aroma- and vapor-proof, and has a high tensile strength. It is malleable, making it suitable for precise shaping of more complex forms, has good heat and impact resistance and can be cold bent. Its expected lifespan of 15-20 years was also an important design consideration, as one of my aims was to make the product suitable for multiple use, i.e. to act as a multi-purpose packaging.

After evaluating the surveys, it was revealed that the vast majority of users were not satisfied with the hygienic closure of the mouthpiece. Many consider the size of the cap and the mouthpiece to be too large, and the cap is easily lost as it is not fixed to the inhaler. To remedy this, a customized mouthpiece cover was designed [Figure 2.]. The closure cap is made of thermoplastic polyurethane (TPU) and is shaped like a plug on a beach mat [Figure 3.]. TPU is thermoplastic, it is mainly used to make dashboards, roller wheels, sports equipment, medical devices, footwear, inflatable rafts and phone cases. They are highly wear resistant, flexible, transparent, also resistant to oil and grease. The customized mouthpiece cover ends in a ring that is fixed to the product and cannot be lost during use or transport.

The disadvantage of most inhalers currently on the market is that they can be triggered when not in use, so precious medicine is wasted in bags and pockets. Releaf has a safety lock button that does not allow the inhaler top to be pushed down accidentally. When in use, the lower opening of the inhaler must first be placed against the mouth opening, then the green lock button must be pushed and held in while the top of the inhaler is pushed down, so the medicament can be released. After releasing the safety lock button, it returns to the original position and stops the top of the inhaler to be pushed down.

There is also an LCD display [Figure 4.] on the device and a micro-switch connected to it. When releasing the inhaler the activated micro-switch sends an electrical signal to the display, which eventually steps the counter that informs the user about the number of doses remaining in the cartridge. A second button is used to reset the counter when the empty cartridge is replaced.

Conclusion

The result of the design process is a medical product design ('Releaf'), an inhaler for asthmatic and other respiratory diseases suffering patients. The finished product solves many problems found in inhalers already on the market. *Releaf* is easy to use, even by one hand, safely and hygienically portable, visually pleasing, so it can be used in public without feeling unease, environmentally conscious because it is easily recyclable, and also it has a meter so the amount of the remaining drug is always known.

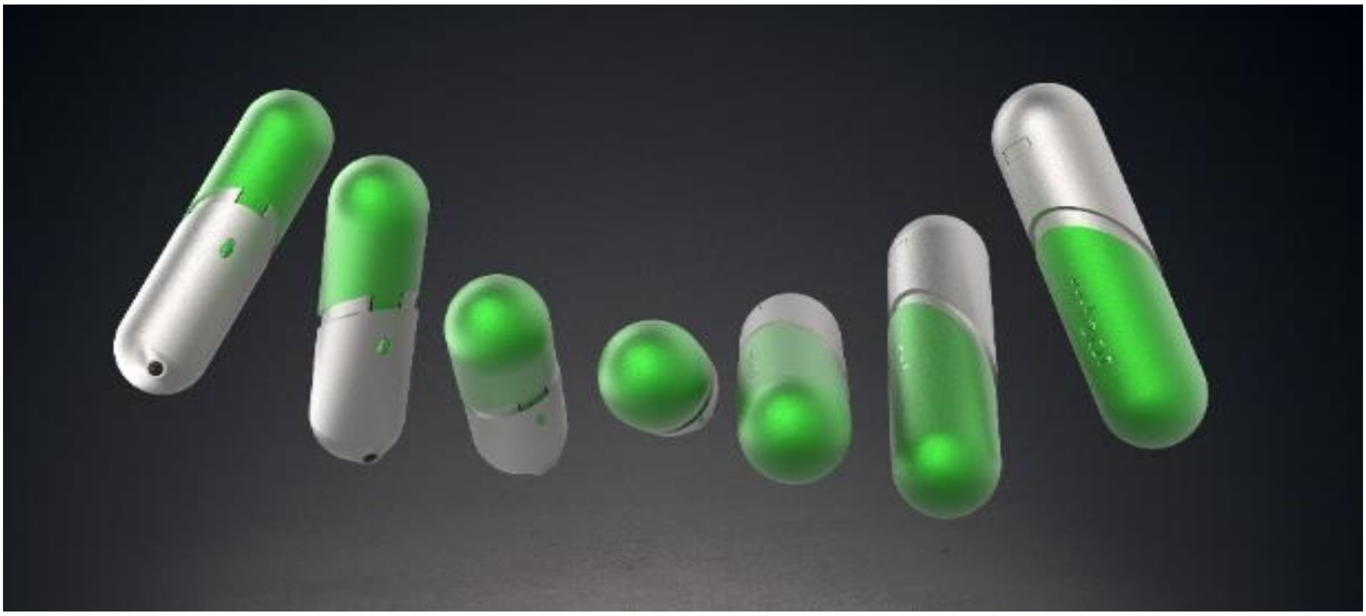

Figure 1: Releaf inhaler (© Liza FEIL)

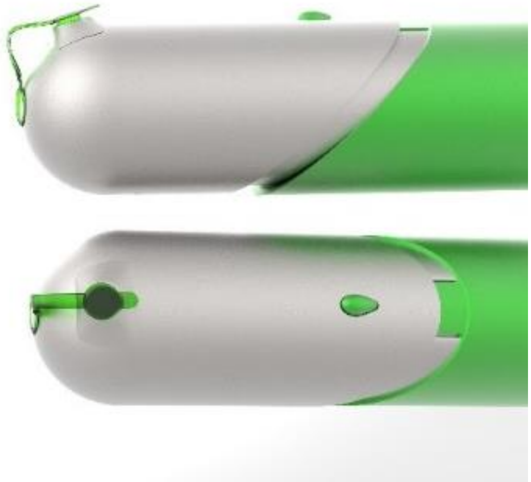

Figure 2: Customized mouthpiece cover (© Liza FEIL)

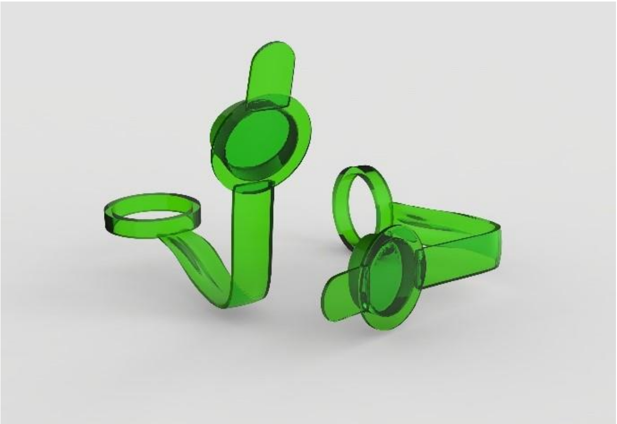

Figure 3: Customized mouthpiece cover (© Liza FEIL)

Figure 4: LCD screen (© Liza FEIL)

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SOLVENT RETENTION OF CELLULOSE-BASED PACKAGING RELATION TO FOOD SAFETY

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Abstract: *Packaging has a barrier function between the product and the environment. Food packaging has nowadays become a critical part of the food product. The presence of residual solvents into gravure printed cellulose-based packaging could potentially migrate into the packaged food product or packaging headspace* *causing health issues or food quality problems, most notably might change the taste and flavour of packed products that should be avoided. Recently the migration of solvent residues in gravure printing inks and varnishes into food products has been a source of increased concern. In order to avoid off-flavour and off-taste effects, the solvent retention values should be kept at low level and consequently, on the one hand there is a need for measuring solvent retention in gravure printed packaging materials by gas chromatography, on the other hand to identify the factors influencing the cartonboard solvent resistance by investigations. In this study we review the solvent retention of cellulose-based gravure printed packaging relation to food safety.*

Keywords: *folding boxboard, solvent retention, gravure printing, gas chromatography, food safety*

INTRODUCTION

Food packaging has nowadays become a critical part of the food product. In addition to functionally storing and protecting food, it provides a brand image that allows the product to attract attention and stand out from its competitors. The primary purpose of packaging is undoubtedly to ensure that food reaches the consumer in the best quality. Although packaging is used to protect food from contamination and spoilage, it can also be a source of odour and taint. The printed substrate that comes in contact with food is required to be manufactured according to strict food industrial standards mainly due to health reasons. Another issue is the sensory property, a slightly unpleasant smell or taste can be enough to cause the consumer to reject the food, which can result in significant quality decrease and financial losses. For some people, a certain taint is pleasant, while for others it is unacceptable. These factors make it difficult to test and control odour and taint from packaging, therefore analytical measuring methods have to be used to determine the exact amount of contaminants in the food packaging. In a series of investigation we study the factors related to the solvent retention of cellulose-based FBB (folding boxboard) type paperboard packaging materials for the food industry, produced by gravure printing, in order to improve the solvent retention and release of printed packaging materials to avoid food safety problems. In this study we review the solvent retention of cellulose-based gravure printed packaging relation to food safety.

PAPERBOARD – A CELLULOSE-BASED PACKAGING MATERIAL

Cellulose-based packaging materials – paper and paperboard made of virgin or recycled fibres - are currently the most commonly used food packaging materials due to their light weight, environmentally friendly characteristics, stability and affordable price.

Grade classification

The paperboards are classified into three categories: cartonboards, containerboards and specialty boards as shown in Figure 1. Cartonboard is a common name for paper products used for packaging cartons, consisting of three or more pulp layers - simultaneously manufactured on a multilayer paperboard machine. Cartonboards are divided into five subgrades: folding boxboard (FBB), solid bleached board (SBB), solid unbleached board (SUB), liquid packaging board (LPB) and white lined chipboard (WLC). [1,2]

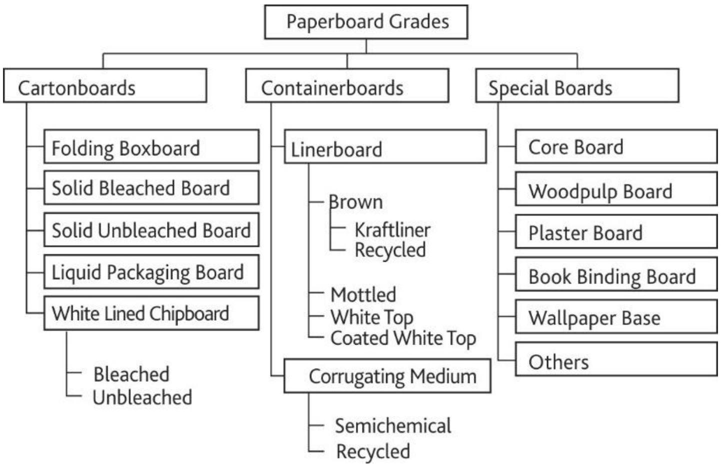

Figure 1: Paperboard grades classification

The basis weight of cartonboard is usually higher than 150 g/m^2. Cartonboard packaging materials are mainly used for food, cigarettes, milk and pharmaceutical products.[1,2]

Properties of folding boxboard

FBB type cartonboard is a cellulose-based and versatile packaging material used for many packaging and non-packaging solutions (e.g. book covers, postcards) worldwide, the total consumption in 2021 was nearly 3000000 tonnes in Europe.[3] The grammage range of FBB is 160-450 g/m^2. The folding boxboard is a multiply cartonboard, typically made of three pulp plies: top ply – chemical pulp (bleached softwood or bleached hardwood pulps); middle ply – mechanical pulp (groundwood, pressure groundwood, thermo-mechanical pulp) or CTMP (chemi-thermo-mechanical pulp); back ply – chemical pulp (bleached softwood or bleached hardwood pulps). Depending on the end-use requirements, the top side of FBB can be single, double or triple coated, while the back side can be single coated or uncoated.[1,2,4] A typical multilayer structure of FBB type cartonboard can be seen on Figure 2.

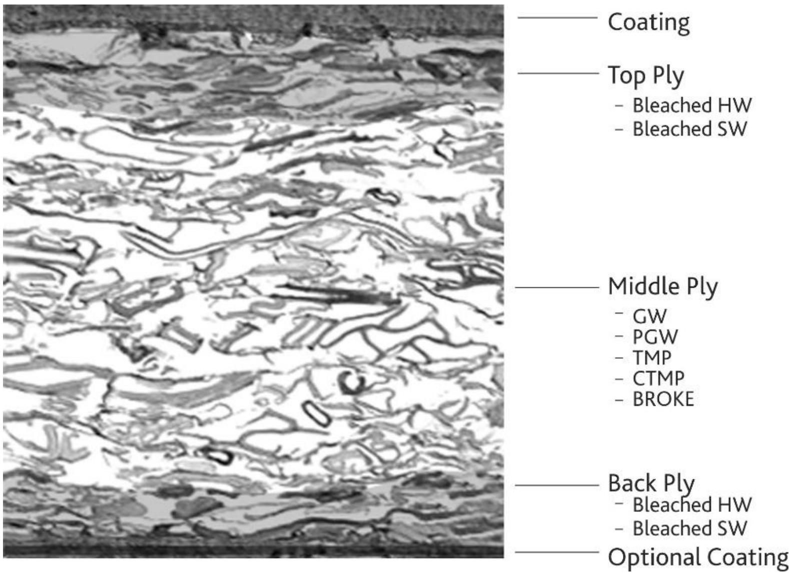

Figure 2: A typical multilayer structure of FBB type cartonboard

Fully coated FBB grades have a smooth surface and as a result, FBB has excellent printing characteristics. This type of paperboard is a primary (virgin fibre) product with consistent purity for food product safety and mostly suitable for the packing of aroma and flavour sensitive products.[5]

GRAVURE PRINTING

Almost all packaging materials contain printings. The printing quality requirements are defined by the package design. Gravure printing is considered the best printing method to achieve high quality printed results if designs with especially metallic colours are requested to be printed. In gravure printing method, the liquid ink is transferred from a metal based cylinder to the surface of the substrate. Metal based image cylinder is rotating in the ink tank, consequently the full surface of the cylinder is covered by ink that is removed by a doctor blade from the non-image areas. Ink from the image cells are transferred to the substrate surface by high pressure generated by the impression roller (Figure 3). Each colour is printed by one printing unit, after each printing unit the ink layer is dried and solvents are evaporated in the drying unit by heating the substrate with hot air.[6]

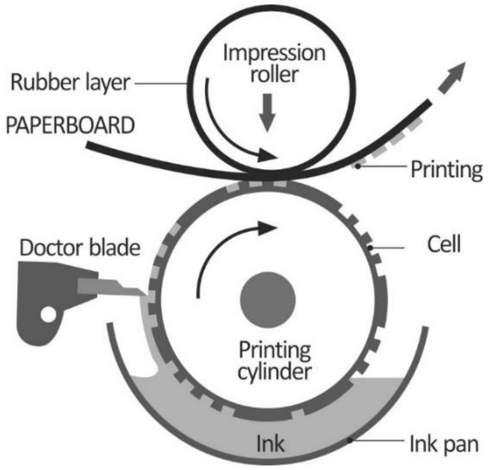

Figure 3: The principle of gravure printing

PACKAGING MATERIALS AS A SOURCE OF ODOUR AND TAINTS

Packaging not only has to protect the food from external contamination, but it also has to ensure that the flavours and aromas of the food stay inside the packaging. Care must also be taken to ensure that the packaging itself is not a source of substances that could affect the organoleptic (sensory) properties of food or to be toxic and dangerous to humans. Migration of packaging components from paperboard to food is generally low.[7] Migration is the transfer of chemical compounds from or to the packaging film that occurs upon contact with the food. We have considered mostly the transfer of chemical substances from packaging to food. The chemical substances can potentially come from packaging substrates (such as paper, cardboard, or plastics), but other packaging components (such as printing inks, adhesives, or coatings) could also be sources of chemical migrants.[8] Where the contamination does occur, it usually results from inks and solvents with the paperboard was printed or chemicals in coatings or adhesives.[7] Prints are almost always applied to the outer surface of a food packaging and are not intended to come into direct contact with food. However, low molecular weight substances in ink easily migrate (permeate) through the packaging material into the food. Only a few packaging materials, such as glass and aluminium foil, prevent all the substances in the ink from migrating. Fibrous materials and most plastics are not barriers to migrants. Solvents in particular can easily pass through packages made of paper, cardboard or plastic. Many substances in the ink can have a noticeable odour or they can penetrate through the substrate and cause taint problems to the packed food. Food producers should therefore regularly check the deliveries of packages before accepting the material, especially when a new grade is introduced. Solvents often contain smelly substances.[9] Solvent-based gravure and flexo inks used in packaging always retain some amounts of solvents in the final package.

Excess solvent retention might cause the package to have an odour and can spoil the taste of food products.[10]

Food contact regulations

Packaging materials in contact with food must not transfer components from the packaging to the food product in amounts that could harm the consumer.[5]

In Europe, food contact materials are generally regulated under the EU Framework Regulation EC 1935/2004 on materials and articles intended to come into contact with food, which allows for further regulation being made on paper and board materials. In 2012, a voluntary Industry Guideline for the Compliance of Paper and Board Materials and Articles for Food Contact was published by the Confederation of European Paper Industries (CEPI) and the International Confederation of Paper and Board Converters in Europe (CITPA). In 2015, the German Federal Institute for Risk Assessment (BfR) released a recommendation on paper and board in contact with food. In the U.S., paper and paperboard components are regulated as indirect food additives under the Code of Federal Regulation (21 CFR 176).

Types and features of packaging materials for food industry

To protect the food from all the possible contaminants and to protect the food's quality, texture and flavour, functional barriers are used.[11] Functional barriers are materials that are placed between the food and its packaging to prevent, on the one hand, the unwanted migration from the packaging into the food and, on the other hand, to prevent, for example the losses of aroma. Materials that are used currently are mostly synthetic ones, which are often used in combinations of multi-layered materials, such as polyethylene (PE), polypropylene (PP) or aluminium. Disadvantages of these commonly used coating materials are that they make the recycling of the resulting packaging material more complex and expensive.[12,13,14]

In order to avoid the use of an extra barrier material, and to ensure that there is no setback in terms of environmental protection and economy, it is a sensible step to investigate and improve the solvent retention and release of the existing cellulose-based raw materials.[15]

As well known, the primary purpose of food packaging is to preserve the safety and quality of food during transportation and storage and to preserve the shelf life of food by avoiding undesirable conditions such as moisture, spoilage microorganisms, external force, mechanical shock and vibration, odour, gases, oxygen and chemical contamination etc., as Figure 4 shows.[16]

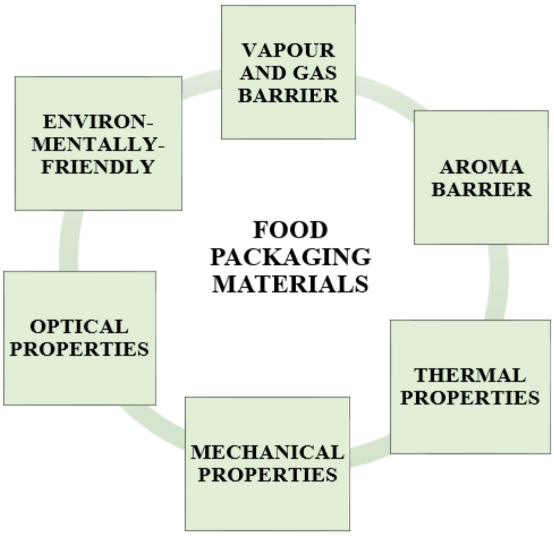

Figure 4: Common features required for food packaging materials

Glass, plastic, metal and paper have been used for many packaging purposes and these are the common categories of food packaging materials. It is important to consider several factors when choosing the suitable package for a particular food product. In general, the packaging material can be either rigid or flexible. Rigid containers include glass and plastic bottles, jars, cans, ceramics, wooden boxes, barrels, tins, plastic jars and tubes. These types of food packaging provide physical protection to the food inside that is not provided by flexible packaging. Flexible packaging is a group of materials, including plastic films, papers, foils, certain types of plant fibres and cloths, which can be used to make packaging materials, wrappings and sealed or unsealed bags. Both flexible and rigid packaging materials, alone or in combination with other preservation methods, have been developed to provide the barrier, inactivation and containment properties required for successful food packaging. The combination of rigid packaging materials made of metal, glass or plastic with heat has been proven to be the most effective and widely used method for inactivating micro-organisms.[17] Key properties of paper and paperboard are: good stiffness, tear easily, low-density, poor barriers to light without coatings or laminations, can be creased, folded and glued, excellent substrates for printing, can be grease resistant, absorbent to liquids and moisture vapour, poor barriers to liquids, gases and vapours unless they are barrier coated, laminated or wrapped.[5]

Solvent retention

During the printing process of polymer materials, the inks and adhesive formulations used are diluted or dissolved in organic solvents that are later removed through evaporation as the printed films are passed through dryers. Printing solvents that are retained on the packaging films after the drying process at significant residual levels – called solvent retention - could form off-odours that may migrate through the packaging and interact with the food. The solvents used in printing consist of alcohols, hydrocarbons, glycol ethers, ketones, and esters.[18] Regularly used solvents, such as ethanol and ethyl acetate are considered as volatile organic compounds (VOCs) and can be detected by gas chromatography method that has been widely used for detection of residual solvents in packaging materials at laboratories.[19]

The compounds involved in flavour degeneration of a product from the ingress of adhesives and organic solvents used printing inks usually contain four to twelve carbons and may often contain several functional groups. The residual compounds migrate through into the product headspace or the product itself. The odour threshold of the human nose for many of these volatile compounds is well below the levels at which they are toxicologically significant. Therefore, there is a health risk if the solvent compounds used during printing are retained at toxicological levels. However, the larger issue of off-odour and off-flavour development and their effect on food product quality is the concern. This is because off-odours develop at retained solvent levels that are much lower than levels that pose a health threat. Current trends in the food industry indicate a widespread emphasis on product quality, and the monitoring of any potential source of off-odours is a serious concern.[20]

SUMMARY

Packaging has a barrier function between the product and the environment. Flavour and taste changes in food products due to the transfer of undesirable volatile compounds such as organic solvents from gravure printed cardboard packaging should be avoided. Over the past 20 years, the migration of solvent residues in printing inks and varnishes into food products has been a source of increased concern, resulting in a number of studies assessing consumer safety and exposure. The presence of residual solvent into gravure printed cellulose-based packaging could potentially migrate into the

packaged food product or packaging headspace causing health issues or food quality problems, most notably might change the taste and flavour of packed products. In order to avoid off-flavour and off-taste effects, the solvent retention values should be kept at low level and under control, consequently, on the one hand there is a need for measuring solvent retention in gravure printed packaging materials by gas chromatography for the detection of residual solvents in packaging materials, on the other hand to identify the factors influencing the board solvent resistance by investigations.

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THE OXIMORON OF TEACHING FREEHAND DRAWING ONLINECHANGES IN MY TEACHING METHODS DURING COVID

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Product Design Institute

Abstract: *During the Covid-19 pandemics the author had to change her teaching methods because of online lessons. Given the fact that one of the author's subjects, freehand drawing, a definitely sensual subject, absolutely necessitates presence, certain materials and a special studio environment, the new situation enforced unusual, unexpected and atypical solutions. Therefore, each task had to be broken down into individual steps. Especially difficult was the evaluation of the students' work: to prevent and eliminate the situation where students try to cheat by handing in the same drawing under different names while streaming.*

Keywords: *freehand drawing, online teaching methods, atypical solutions, individual steps*

INTRODUCTION

During the temporary lockdown due to the pandemics the teaching staff had to introduce online training instead of the traditional face-to-face lessons, which required new attitudes and solutions. It was necessary to reconsider how to perform the practical subject of freehand drawing online and substantial changes had to be introduced in the teaching methods. The unexpected situation necessitated new solutions: for each task the main criteria had to be defined in a way so that they could be passed down online. The tasks had to be broken down into parts and step by step had to be presented on different drawings in a way that they could be followed up even by those students who had never learnt drawing before. The execution of the tasks was difficult to evaluate on the screen. Very close attention had to be paid so that a well-done drawing should not be "handed in" by several students. The experienced artistic visual capacity was a great help for the author to locate immediately if students tried to cheat, because unfortunately they had tried it several times. Whenever the author pinpointed such a trick, she warned the student and told them that she had already seen this very drawing earlier. At the same time the student was given an opportunity to perform their own task/artwork. During Covid both students and teachers were under great pressure: fear from the infection and stress on account of the new ways of performing our tasks. On the other hand, the unexpected situations enhanced our creativity and brought about ad hoc, atypical task solutions.

METHODICAL AND TECHNICAL SPECIFICS: THE WORK ON A LONG DRAWING AND SHORT-TERM FREEHAND DRAWING

Short-term freehand drawing or sketch is the most individual way of artistic thinking and includes an analysis of creative and psychological selection. Such qualities of the short-term freehand drawing as the freshness of the form, the immediate expression of a complex thought complex with the help of a laconic graphic language, require a certain professional approach to perception. It ensures conscious, solid, error-free decisions at work. "The training of art students should therefore mainly consist of sharpening their sense of these expressive qualities or teaching them to consider expression as the main criterion every time they touch the pencil, brush or cutter," as Arnheim puts it. [1] The work on a long drawing and sketch has its own specifics, but they are one and cannot be separated. In both cases, they are carried out from the general to the specific, but the main thing is emphasized in the sketch, and the details should be summarized - movement, dynamics and expressiveness become the main ones. The sketch is the most individual way of artistic thinking and includes an analysis method of creative and selection. Such qualities of a short-term freehand drawing as freshness of the form, instant expression of a complex set of thoughts with the help of a concise graphical language, requires for its perception a certain professional approach, conscious, solid, error-free decision-making process. Sketches help develop creative abilities, with the help of minimal concise tools: lines, tonal spots, strokes. In the short-term freehand drawing, there is a constant search for their own technical methods, style, system, content, themes, it is both creative and educational at the same time. A coherent and distinct vision, the distribution of masses and spots, compositions of light and shadow, the solution of spatial composition remain important tasks of a sketch. That is, due to a sketch, a student forms their views on the choice of composition, foreshortening, identifies material and actual conception of an image. Similarly, the perception of a bulk form is experimentally proved that it can only be perceived by changing different sides. Getting a complete picture of an image from one point of view is impossible, that is, without sketches. In the process of working on drawings, one not only adjusts a figurative plan, but often changes all the conception of the artistic image. [2] As Atiana Nilovna said about the creative process: “You can take any colour, but the drawing must be accurate. And if the drawing is accurate, the colour will be found. ..., what form is, how this form can be represented on a plane, what volume there is in space, how it can be conveyed with various materials. And above all, attention was focused on the drawing as such. “ [3]

PRACTICAL TEACHING METHODS REDRESSED ONLINE

To demonstrate my online teaching methods, I chose the practical tasks of drawing geometric bodies with drapery – freehand drawing in coloured pencils. The time frame was two weeks, the drawing had to be made in four steps. (*Figure 1) First step*: I set up a still life, took a picture of it (*Figure 2)*, and sent the photo to my students online.

Figure 1: Steps of methods to online teaching

Figure 2: Still life photo

The second step (*Figure 3*) was to sketch the still life loosely with a black pencil (e.g., Rotring, 2B, 0.5 or grey coloured pencil). Here the students had to sketch the whole composition by measuring the proportions of height and width. They had to measure the objects and compare them to each-other (e.g., the proportions of the ball and the big cube; the cylinder and the cube; the cube and the ball.)

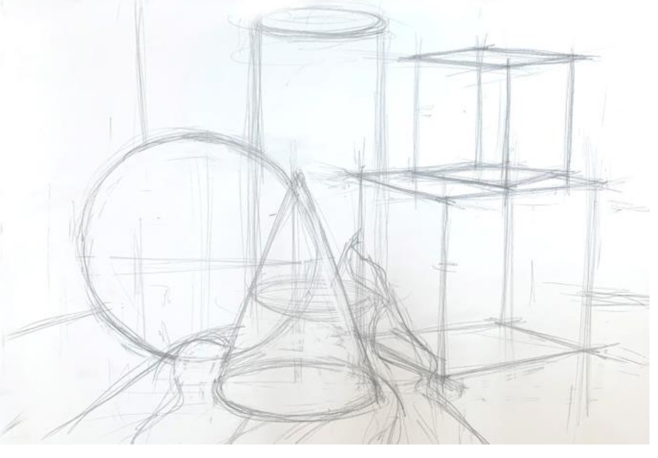

Figure 3 The second step: *sketch the still life (instructor's drawing)*

The *third* step was to continue drawing with coloured pencil. (*Figure 4)* The students had to build up the colours of each object and integrate them in the composition. They had to develop each object, while keeping the whole picture in mind! First, they had to look which side of the cube was the darkest and then they had to draw them with coloured pencils.

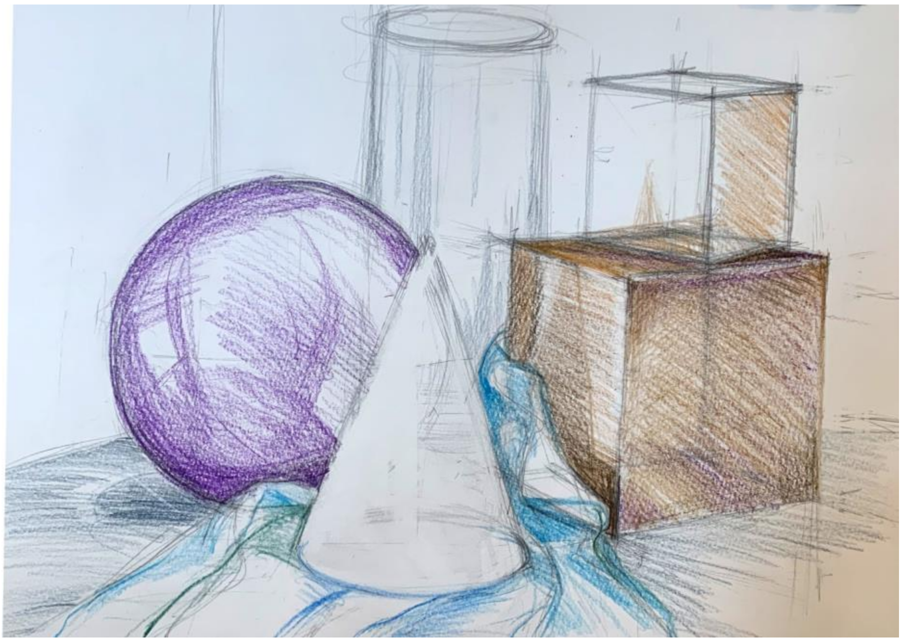

Figure 4: The third step: *continue drawing with coloured pencil (instructor's drawing)*

Finally, the students had to further develop and finish the coloured drawing (*Figure 5)* by putting on the shades in the same way as with the black pencil. Here they had to draw a little lighter, loosely, as if the objects were translucent – this makes the drawing more attractive and artistic. They also had to show the invisible back edges and planes. They had to keep in mind to continuously develop the objects related to each-other and pay attention to the cast shadows!

Figure 5: The fourth step: *putting the shades (left: instructor's drawing, right: student's drawing, Zorigt Ariunzaya)*

CONCLUSION

During Covid both students and teachers were under great pressure: fear from the infection and stress on account of the new ways of performing our tasks. On the other hand, the unexpected situations enhanced our creativity and brought about ad hoc, atypical task solutions. During the pandemics I had to introduce changes in my teaching methodology I used to apply during my Freehand drawing lessons. This is also an asset contributing to the 50th anniversary of the university. It is gratifying that the celebration of this half a century history of the university renders the opportunity for me to affirm that I could cope with the difficulties in teaching caused by Covid. Moreover, the students did not receive less tuition and education and the changes did not leave them short in any respect as they got the curriculum material in full details and they could gain proper knowledge.

The article sheds light on the problem of the requirements and implementation of short-term freehand drawing tasks in order to develop students' ability to use graphic means of expression and the plastic characteristics of drawing. Due to the current teaching practice due to the pandemic, it was urgently necessary to create and develop the short-term freehand drawing tasks of the educational program in an online system, in accordance with the modern problems of art education. The author's development includes the improvement and search for methodological and technical features of short-term freehand drawing in the educational program, where the author takes into account the skills and techniques of sketching, offers examples of educational tasks, exercises and questions for design students. Visually analyses the sequence of conducting a short-term freehand drawing and is based on the topics of the exercises and the analysis of the works of her students, illustrated with the works of the author.

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EXPERIMENTAL INVESTIGATION AND KINETIC MODELLING OF THE SOIL DERIVED NO, N_2O AND CO_2 EMISSIONS IN A MICROCOSM SYSTEM

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Abstract: *The movement and transformation of the different forms of nitrogen and carbon in the soil/plant/atmosphere system are governed by interconnected cycles. The aim of our study was to describe a part of this complex system and how the human activities are the sensitive balance of these cycles and how interventions are damaging the environment and the human health.*
To achieve our goal, a laboratory microcosm experiment was set up to investigate the greenhouse gas (GHG) emissions of N_2O, NO and CO_2 using gas chromatography. The tendency in the mathematical approximation of the modelling of nitrogen transformation processes and therefore the carbon concentration changes in soils is to use the simplest possible mathematical formalism together with chemical kinetic equations. When modelling a result of the microcosm experiment, mainly first-order chemical kinetic equations were the best approximation to describe the chemical processes under investigation. The system of equations prescribed is solvable, but the applied equations are not well handled due to their complexity, therefore so the equations are mostly simplified. In most published literature, soil processes are modelled separately using simple chemical kinetic equations and, the first-order chemical kinetic equation has also been used, as was done in our studies, too.

Keywords: *Greenhouse gas, emission, microcosmos, kinetic modelling*

INTRODUCTION

Nitrogen occurs in nature both in its elemental state and in compounds. It is a permanent, essential component of the proteins of living organisms. The biogeochemical cycling of nitrogen compounds in the soil/plant/atmosphere system is carried out through multiple interconnected cycles [1]. Human interference in the nitrogen cycle [2] (intensive agriculture, fossil fuel use, forest burning, etc.) is increasing. This is putting increasing strain on the delicate balance of these cycles and creating a whole series of ecological problems. The managing and controlling these problems is a major challenge for science and technology. In this paper, our aim is to illustrate the complexity of the biogeochemical cycles of nitrogen and the environmental problems caused by human interference in these cycles (air and water pollution, greenhouse effect, etc.). The model is a simplified, idealised representation of a complex system, which can be described in a mathematically rigorous way and which reflects more or less faithfully some (but not all) of the properties of the system under study. The model facilitates a more complete understanding of the system under study [3]. This method has been chosen to study and describe the processes of the nitrogen cycle in soil. The results of a microcosm experiment were used for the modelling work. The importance of laboratory experiments was perhaps best expressed by FAUST [4] as follows: "Laboratory studies are important to understand the basic mechanisms of soil/plant interactions in the system". A better understanding of the processes involved in the N-cycle in soils and the importance and sensitivity of the different processes is necessary to allow, for example, rational, environmentally sound fertilization practices in the field of N nutrient addition [5]. The nitrogen transformation processes [6] (immobilisation, mineralisation, nitrification, denitrification) in soil have been approximately modelled by chemical kinetic equations. In addition to modelling of the individual sub-processes, the aim of our work was to describe mathematically the temporal variation of the amount of each form of nitrogen in the soil by taking several sub-processes into account.

Materials and methods

Laboratory microcosm experiment

Microcosm system is a "laboratory version" [7] of the chamber method: gas chamber over soil samples (brown forest soil, in Gödöllő) in soil sample cylinders placed on a sand column of a given matrix potential (water content). The measurements were performed in 3 replicates.

a b

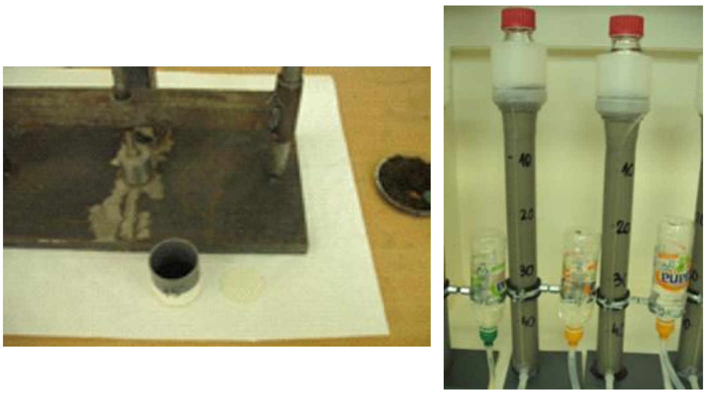

Figure 1: a) Sample b) Incubation of microcosms

The next treatments were used:

- Control measurement (loose soil structure was used and without fertiliser)
- Other group: Using compactor to create more intensive compaction in layers. Compaction (Figure 2.) in 3 layers, 5 strokes per layer, falling from a height of 46 cm; compaction work: approximately: 0.029 kJ/dm^3. One series was used without fertiliser and other one with fertiliser).

In all cases it was changed of matrix potential (water content): -1kPa

Figure 2: Compactor

Finally: the mass of filled sample containers was measured.

Microcosm system: Incubation of microcosms

The setup of the microcosm experiment can be seen in Figure 1b. The next system was used during the experiment: in the first days of the experiment, the water content was set to a matrix potential of about -1kPa corresponding to -10 cm, then increased to 0 cm corresponding to total saturation.

Gas emission testing over soil samples covered by gas field:

- First series of measurements: 0 minutes (sampling immediately after closure)
- Second series of measures: sampling about 14-15 minutes after the coverage
- Third series of measurements: sampling about 23-28 minutes after coverage

The gas sample amount was 1 ml with Hamilton syringe, manually. The gas sample was immediately injected into the gas chromatograph during sampling. (The total amount of sample is injected into the column = direct injection)

Table 1: Analysis of CO_2 and N_2O in gas samples with HP 5890 Series II and HP 5890 gas chromatograph

Single units\ Measured chem., occurrence conc.	**CO_2 ca 2-0,04 %**	**N_2O ca 5-0,32 ppm** (ppm: 10^{-6})
Injector	105 °C	
Precolumn	Nafion (drying gas N_2)	
Analytical Column	2 x 1,82 m, 80/100 mesh 2 x 1.82 m, 80/100 mesh grain size Porapak Q; column space: 30 °C(4)	
Detectors (Lined up in a row)	TCD (150 °C) (Thermal conductivity detector)	ECD (300 °C) (Electron Capture Detector)
Carrier gas	50 cm^3/min flow rate; 4.6 purity He	50 cm^3/min flow rate; 4.6 purity He; 4.6 purity N_2 auxiliary gas
Retention times: **CO_2** **N_2O**	2.6 min 3.5 min	2.6 min 3.5 min

The NO emission was measured using a chemiluminescent detector (ANTEK 7050). In CO_2 measurements, the presence of water vapour was a disturbing factor. The interference was eliminated by using a Nafion (ascarids, Nafion) pre-column.

Theoretical method

Chemical kinetic equations approximated the description of the different nitrogen and carbon transformation processes in the modelling work. As it is known the reaction rate is the amount of material converted in a unit of time. It depends on the chemical quality and concentration of the reactants and temperature. Its magnitude is well defined, when related to a change in concentration over a relatively short time [8].

First order reactions

The rate of first-order reactions is proportional to the concentration of a single component. The rate equation for the concentration of starting material [9]:

$$c = c_0 \cdot e^{-k \cdot t}$$

Modelling of sub-processes:

The processes are approximated by chemical kinetic equations. The results of the experiments described above were used to determine the kinetics of the transformations. Thus, chemical kinetic equations of different orders were fitted to the measured data by nonlinear regression. This was done using “Origin” and "MyCurveFit" statistical software [10, 11].

RESULTS

CO_2 amount

Loose soil *Compacted soil*

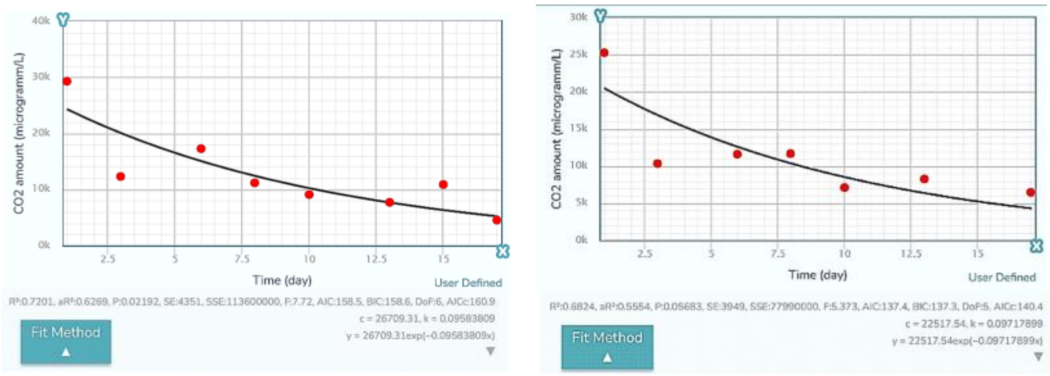

Figure 3: Fit of CO_2 concentration by "MyCurveFit" [11] and First-order kinetics

NO amount

Loose soil Compacted soil

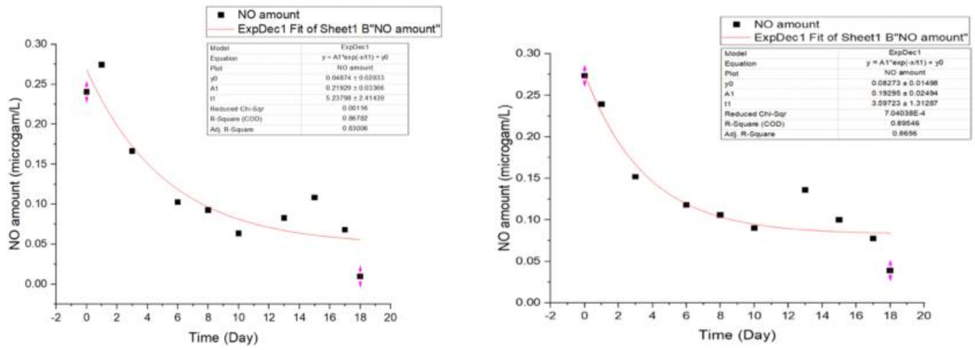

Figure 4: Fit of NO concentration by Origin [10] and First-order decay

N_2O amount

Without KNO_3, fertiliser

Loose soil (without fertiliser) Compacted (without fertiliser)

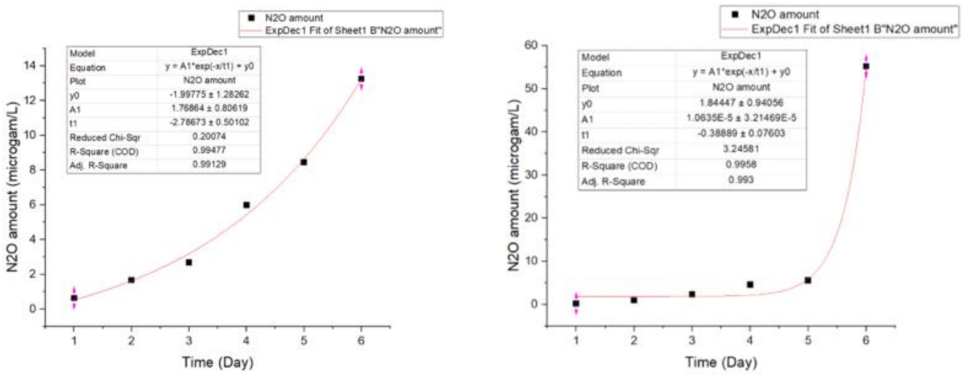

Figure 5: Fit of N_2O concentration by Origin [10] and First-order decay (without KNO_3)

With KNO_3, fertilizer

Loose soil (with fertiliser) Compacted soil (with fertiliser)

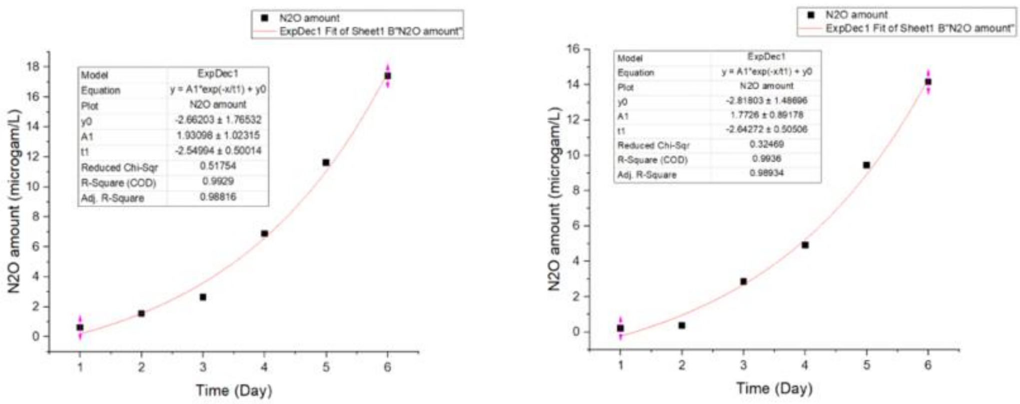

Figure 6: Fit of N_2O concentration by Origin [10] and First-order decay (with KNO_3)

EVALUATION

Laboratory model experiments are a relatively simple and good way of modelling the structural change in soil when heavy machinery is used, equivalent to a compacted microcosm experiment. Attempts have been made to restore soil structure in practice, e.g., by planting black algae, but unfortunately so far this regeneration has not been successful in terms of GHG emissions [12].

CO_2 emission in the soil incubation experiment

The emission of CO_2 varied among the treatments corresponding with each date of sampling. The change in CO_2 (Figure 3.) could be approximated by the first-order chemical kinetic equation. From the trend of CO_2 emission, it was observed that, emission drastically reduced in 17 days. The maximum emission was recorded on the first day of the experiment. On the 17th day, CO_2 emission came down sharply and approached its minimum in all kinds of soil (compacted or loose soil).

The trend of CO_2 emission from loose and compacted soil, which decreased after 17 days of the experiment, pushed us to reconsider their direct usage in agriculture. Anaerobic digestion and composting could be an environmentally beneficial solution that minimizes greenhouse gas emissions while also producing biogas to replace fossil fuels and nutrient-rich compost for use as fertilizer. Scientists and governments have recently become interested in anaerobic digestion to address climate change mitigation [13]. In general, it takes time for microbial activity to begin after residues have been incorporated into soils [14]. Because of the richness and complexity of the environment, the results collected in the lab may not be comparable to those gathered in the field.

NO emission in the soil incubation experiment

The change in NO (Figure 4.) amount could be approximated by a first-order chemical kinetic equation. The emission of NO varied among the treatments corresponding with each date of sampling. From the trend of NO emission, it was observed that, emission drastically reduced in 18 days. The maximum emission was recorded on the first day of the experiment. On the 18th day, CO_2 emission came down sharply and approached its minimum in all kinds of soil (compacted or loose soil). No significant difference was found between loose and compacted structures in terms of NO reduction, in contrast to the literature, where it was found that NO emissions were drastically reduced in the case of compaction [15].

N_2O emission in the soil incubation experiment

In group without KNO_3, fertiliser and with KNO_3, fertiliser in each treatment, the change in the amount of N_2O could be approximated by a first-order chemical kinetic equation. The emission of N_2O varied among the treatments corresponding with each sampling date. From the trend of N_2O emission, it was observed that in all groups, emission drastically reduced in 6 days. The maximum emission was recorded on the first day of the experiment. On the 6th day, N_2O emission increased significantly and approached its maximum in all kinds of soil (compacted or loose soil, with fertiliser or not). Our results are consistent with experimental data in the literature [16].

Let's compare the amount of N_2O from fertilizer-treated and untreated soils in the case of compacted soil. We see that although both processes can be described by first-order kinetics, the solution functions are very different. In the fertilizer-treated case, there is a fairly steep increase in N_2O, while in the untreated case, there is almost no change at the beginning and a significant increase in N_2O production in the last sampling.

Table 2. Main findings of the statistical analysis

Tested gases	**Time, significance level**	**Soil structure, significance level**	**Parallel measurements, significance level**	**Replication effect (concurrent treatments), significance level**
NO	P < 0,001	P < 0,001	–	P < 0,01
		Loose > compacted		
N_2O	P < 0,001	P < 0,001	–	–
		Loose < compacted		
CO_2	P < 0,001	P < 0,001	P < 0,001	–
		Loose > compacted		

Soil structure (compacted/loose) and time progression had a significant effect for all three gases. CO_2 and NO: higher emissions from loose soil samples, suggesting an aerobic origin (more air-filled pores). N_2O: higher emissions from soil samples with a compacted structure, suggesting an anaerobic (denitrification) origin (fewer air-filled pores).

Conclusions

On the basis of the presented data, it can be concluded that the conditions of formation and emission of CO_2, N_2O and NO gases are different in the loose and compacted soils and these processes are significantly influenced by the microbiological activity of the soil. Therefore, real estimation of GHG emissions from soils can be obtained in such joint experimental systems as it was presented in this paper.

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