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Fall-Winter 2023

Environmental management, land use, biodiversity

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APPLICATION OF HYSPLIT AND K-MEANS CLUSTERING IN TRAJECTORY ANALYSIS FOR IDENTIFYING SOURCE REGIONS OF SECONDARY INORGANIC AEROSOLS AT THE KECSKEMÉT BACKGROUND MONITORING STATION IN HUNGARY

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ABSTRACT

In this study, we conducted an investigation with the primary objective of identifying the source regions of secondary inorganic aerosols within the context of the Kecskemét Background Monitoring Station, situated in an agricultural area. Employing trajectory clustering through the HYSPLIT model, we subsequently applied K-means clustering utilizing CAMS reanalysis nitrate and ammonium data. Our findings revealed distinct clusters, with Cluster 3 encompassing 20% of air masses across various regions and Cluster 5 representing the second predominant source, constituting 12% of air masses within the region. This analysis provides valuable insights into the spatial origins of secondary inorganic aerosols in the specified agricultural setting.

Keywords: Hysplit, Air quality, CAMS, k-means clustering, Aerosols.

INTRODUCTION

Particulate matter (PM) in the atmosphere, stemming from both natural and human-induced sources, exhibits variations in composition and size. The interplay of local meteorological conditions and the potential impact of long-range transport significantly contribute to the recorded PM concentration levels at specific locations, as noted by (Abdalmogith & Harrison, 2005). However, this phenomenon remains inadequately documented in numerous European regions. Of particular significance in Europe's atmospheric composition are secondary inorganic aerosols (SIA), encompassing SO_4^{2-}, NO_3^-, and NH_4^+. Distinguished as secondary, these aerosols do not emanate directly into the atmosphere; rather, they arise from chemical reactions involving sulphur dioxides, nitrogen oxides, and ammonium, pollutants commonly emitted by transport, industry, and agriculture. Over recent decades, emissions of these gases have markedly declined in Europe, leading to a consequential reduction in ambient concentrations of secondary inorganic aerosols.

The occurrence of new particle formation (NPF) and subsequent particle growth has been documented across diverse settings, ranging from sub-arctic Lapland and remote boreal forests to urban and suburban environments. Findings indicate that H_2SO_4 plays a pivotal role in influencing NPF. However, preceding NPF, sulfuric acid clusters necessitate stabilization through interaction with other compounds. Prominent candidates for this role include amines, ammonia, and extremely low volatile organics, as outlined by (Tao et al., 2016). The integration of advanced theories on particle formation, along with analyses involving clusters and backward trajectory, enhances our comprehension of pertinent atmospheric processes. Furthermore, this approach serves as a valuable tool in the formulation of effective air quality management strategies.

METHODS AND STUDY AREA

Kecskemét, located in central Hungary and holding the status of a city with county rights, stands as the eighth-largest urban centre in the nation and serves as the county seat of Bács-Kiskun. Positioned equidistantly between the capital city Budapest and Szeged, it is situated 86 kilometres (53 miles) from both, and nearly equidistant from

Figure 1: Kecskemét location and agriculture lands.

the two major rivers of Hungary, namely the Danube and the Tisza (Fig. 1). Established at the convergence of a substantial sandy region and sandy yellow soil, Kecskemét is situated at an elevation of 120 meters (394 feet) above sea level. The western territory of the city is characterized by wind-blown sand, featuring nearly parallel northern-southern sand dunes and the plain nestled between them. Toward the conclusion of the 18th century and the commencement of the 19th century, the pastures faced depletion, with overgrazing by cattle causing damage to the natural vegetation cover. The movement of sand posed a serious threat to the town. To counteract these challenges, a concerted effort was undertaken, involving reforestation and the planting of fruit and vines, aimed at stabilizing the soil once again (Kováts et al., 2020).

The Hybrid-Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model, as established by the National Oceanic and Atmospheric Administration's Air Resources Laboratory (Qor-el-aine et al., 2022), was employed for analysis. HYSPLIT utilizes archived 3-dimensional meteorological fields derived from observations and short-term forecasts. Back trajectories spanning three days, calculated on an hourly basis, were generated for October 2022. These trajectories terminate at the sampling location Kecskemét, precisely at 0600 UTC and 100 meters above ground level.

The clustering of trajectories represents a challenge in the realm of time-series clustering. Time-series clustering approaches fall into three principal categories: feature-based, model-based, and raw-data-based, as outlined by (Warren Liao, 2005). Feature-based methods involve the transformation of time series data into static data, following which conventional clustering techniques like k-means, fuzzy c-means, and hierarchical clustering are applied to the static data. The efficacy of feature-based methods is notably contingent on the efficiency of two key processes: the conversion of time series into static values and the subsequent clustering of these static values. Within model-based methods, a probabilistic model is posited for the time series data, and the assessment of the time series data is gauged based on its degree of compatibility with the specified model. However, model-based approaches are deemed less appropriate for addressing the trajectory clustering problem, particularly when the clusters are not predefined. Raw-data-based methods, on the other hand, involve presenting time series data to the model in its original form, without undergoing reduction to distinct features. In this approach, each complete time series, from its initiation to conclusion, is treated as a sample for the model, and the subsequent clustering is executed based on the model's output.

Numerous raw-data-based techniques are documented in the literature, including Dynamic Time Warping (DTW), Self-Organizing Map (SOM), and various similarity/dissimilarity measures, as highlighted by (Karaca & Camci, 2010). In this study, the conventional clustering method of k-means has been opted for among the mentioned methods. It is imperative to acknowledge, however, that the sensitivity of k-means cluster analysis to the initial selection of cluster centres is a noteworthy consideration (Fig. 2.).

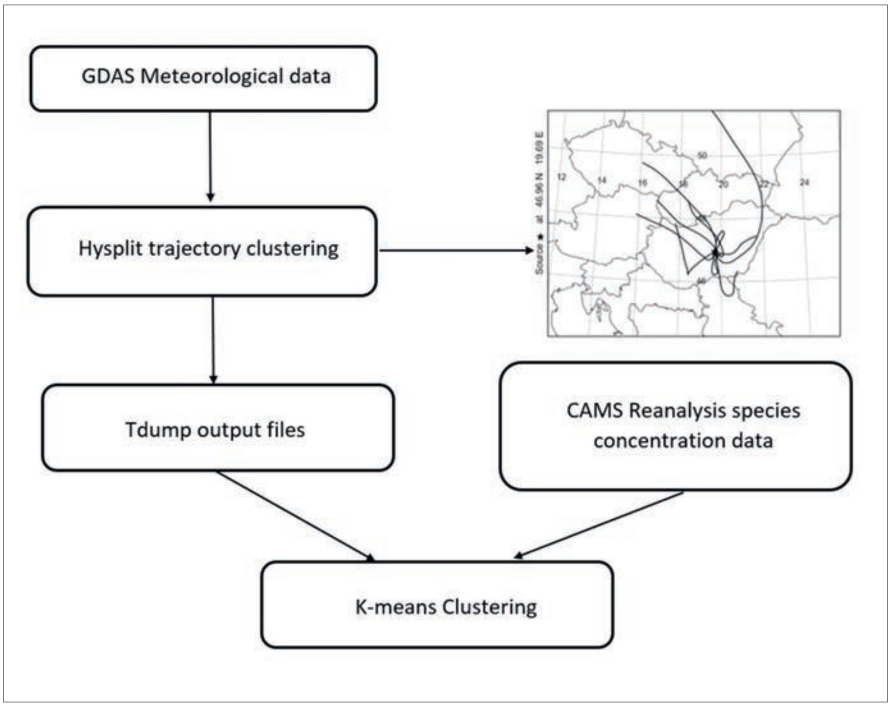

Figure 2: SIA source region identification process.

RESULTS AND DISCUSSION

The assessment of how synoptic-scale atmospheric transport patterns influence the observed levels of nitrate and ammonium entailed an initial examination of backward trajectories. Trajectories were assigned to clusters by selecting the trajectory with the smallest total distance from the corresponding cluster center. Each cluster center represents the arithmetic mean of all trajectories within the cluster. Figure 3 displays the ultimately determined cluster centers, which are utilized for investigating the origins of Secondary Inorganic Aerosols (SIA). Notably, the illustration indicates the existence of six major air mass paths reaching Hungary throughout October 2022.

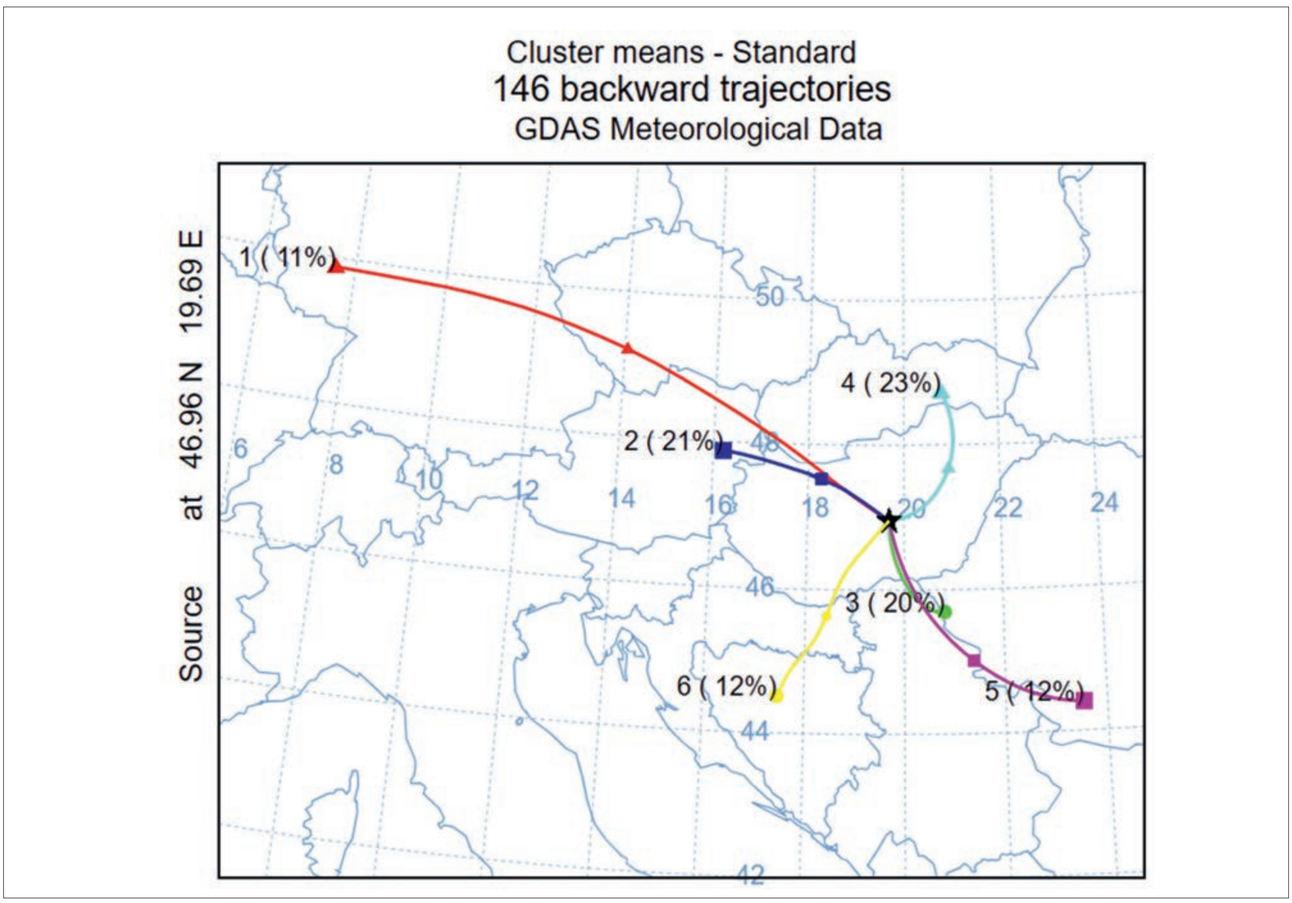

Figure 3: Trajectory clustering for October 2022.

On a yearly basis, the prevailing cluster patterns consistently aligned with the prominence of clusters 4 and 2, constituting 23% and 21% of the observed days, as illustrated in Fig. 3. These trajectories signify the primary continental source regions for the precursor gases of Secondary Inorganic Aerosols (SIA) in the east and west. The findings discerned three key source areas, each making substantial contributions to the presence of SIA in Hungary, as depicted in Fig. 4.

The trajectory class mean $NH4^+$ concentrations exhibited a range from 0.1 μg/m³ to 0.83 μg/m³. Notably, the highest concentrations were closely linked to air originating from southeastern Europe, particularly in cluster 3 and cluster 5. When considering nitrate concentrations at Kecskemét, cluster 5 (Southern) displayed the highest concentration at 2.46 μg/m³, while cluster 3 (from Germany) exhibited the lowest at 0.15 μg/m³. Elevated concentrations of Secondary Inorganic Aerosols (SIA) were notably associated with trajectories following eastern, southern, and western pathways, corresponding to clusters 2, 3, and 5. This suggests that these continental paths likely played a crucial role in collecting air pollution across the European continent and transporting it to the Kecskemét station. Drawing from the findings of (Ferenczi et al., n.d.), it can be inferred that concentrations peak when air parcels traverse the European source region. These trajectories, characterized by short transport patterns indicative of slow-motion air masses, reflect the influence of continental air masses passing over regions with concentrated industries.

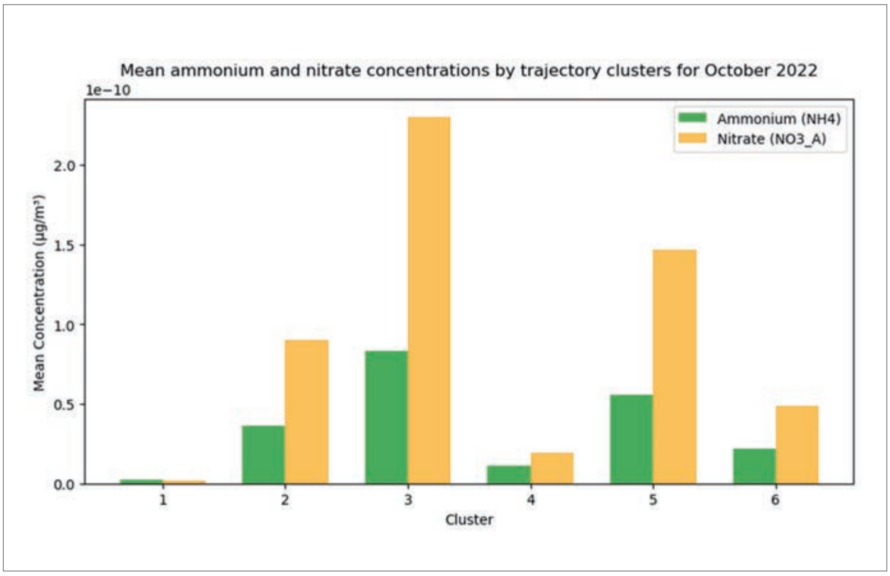

Figure 4: Mean ammonium and nitrate concentrations by trajectory clusters for October 2022.

CONCLUSION

This investigation employed air mass backward trajectory cluster analysis to examine the transport pathways and potential sources of Secondary Inorganic Aerosols (SIA) at the Kecskemét monitoring station. For October 2022, six clusters were identified, and among them, three featured air mass trajectories originating from the continental region. These trajectories collectively accounted for 53% of the observed SIA concentration at Kecskemét. Notably, the continental clusters (clusters 2, 3, and 5) demonstrated the most substantial daily contributions to the annual SIA concentration. Preliminary findings suggest that the primary origins of SIA are localized in the neighboring regions of Hungary.

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MONITORING AIR QUALITY IN FRUIT AND VEGETABLE STORAGE FACILITIES

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ABSTRACT

The aim of this research is to investigate the indoor air quality of the military camp kitchen complex and food storage facilities. This study presents an analysis of indoor air quality in fruit and vegetable storage facilities, with a focus on carbon dioxide (CO_2) concentrations, elevated levels of which can be harmful to humans but may have a favourable effect on the shelf life of fruits and vegetables. The first measurements were carried out to assess the CO_2 concentration changes in the indoor environment of a vegetable and fruit store in the kitchen of an operating military camp. The measurements were carried out under laboratory conditions, modelling storage over several days (3-4 days). Finally, our observations were made by modelling the respiration of a large quantity (2.5 tonnes) of apples. In longer-term storage (3-4 days), when 600 kg of apples are stored, CO_2 concentrations of up to 1100 ppm can be measured, which has a positive effect on fruit storage. Modelling the storage of 2500 kg Idared (*Malus domestica*) apples, CO_2 concentrations varying between 4000 ppm and 1500 ppm were measured. It is concluded that this value (between 3000 and 5000 ppm based on the literature) would be expected for fruit storage and that the natural filtration of an ISO storage container building at standstill is strongly influenced by external environmental effects.

Keywords: fruit, carbon dioxide, military camp kitchen storage, indoor air quality

INTRODUCTION

In the design of military camps, indoor air quality (hereafter: IAQ) scaling is clearly a key consideration for temporary installations. Pollutants affecting the quality of IAQ include gases and vapours, odours and aerosols. (Bánhidi–Kajtár 2000, Herczeg et al. 2000). Max von Pettenkofer (1858) studied the air quality of comfort spaces in the mid-19th century, classifying indoor air quality according to its CO_2 content.

One of the challenges of food storage is that food, whether of plant or animal origin, is living matter and its quality is constantly changing during storage. Human interventions (e.g. restocking, stock removal, cold chain interruption, etc.) also affect shelf life and product characteristics (Géczi et al. 2017, Kassebi et al. 2023).

Fruit respiration affects the atmosphere of enclosed spaces in a similar way to human respiration. During fruit storage, depending on the variety, a reduction in oxygen and an increase in carbon dioxide and ethylene levels caused by ripening and respiration processes can be observed. Since CO_2 is formed during respiration, if the partial pressure (concentration) of CO_2 in the atmosphere is high, the equilibrium process is shifted, i.e. the intensity of respiration is reduced, which results in extended storage. A low-cost technology for the storage of apple in an isolated "closed loop" has been developed, which allows the implementation of controlled atmosphere technology in typical environments (7-8% CO_2) and (13-14% O_2) and sub-normal environments (3% O_2 and 5% CO_2) (Varivoda et al. 2022). A decrease in oxygen levels affects the balance of respiratory processes in a similar way to an increase in CO_2 concentrations: it reduces the intensity of breathing (Rzhepakovsky et al. 2022). However, decreasing the oxygen concentration cannot be separated from increasing the CO_2 concentration. Reducing the oxygen concentration by half compared to normal air (~21% O_2) results in a reduction in respiratory intensity of about 10% (Ho et al. 2008). Too low an oxygen concentration <1% is already harmful, metabolic processes slow down, terminal oxidation stops and anaerobic processes start: alcoholic or lactic fermentation occurs (Barabanov et al. 2018). The continuous production of ethylene during storage is also detrimental because it increases respiration intensity, thereby increasing storage losses, and it is therefore advisable to remove it from the air or block its production (Dióspatonyi, n.a.). Furthermore, for example, Kustyawatin et al. (2020) reported in his study that sub-supercritical CO_2 processing for 10 min increased the shelf life of tempeh at storage temperatures of 30 °C because of a reduction in the number of bacteria, yeasts and moulds in the tempeh (tempeh is a traditional Indonesian food made from fermented soybeans.). The reason for this phenomenon is that the fungal growth produces enzymes that cause the softening of the beans by hydrolysis of various compounds during fermentation (Wati et al. 2020). At the same time, it is also true that the co-cultivation of mould fungi in soy fermentation has resulted in a pleasant "yeasty" aroma (Pleva et al. 2018). Similarly, there is a wealth of empirical evidence on the changes and impacts on people living in comfort spaces in fruit storage. Fagundes et al. (2013) found that the respiratory intensity of fresh-cut apples stored at 7°C is 25-30 mL·kg^{-1}•h^{-1} CO_2. Based on their experiments, at this temperature the O_2 level decreases from 21% to below 10% and the CO_2 concentration reaches 10% from 0.05%. Kádas and Frenyó (1985) have already found in 1984 that lemons stored at 20°C produce 14 mg·kg^{-1}•h^{-1} CO_2 by respiration. Bhande et al. (2008) showed a respiration intensity of ~15 mL·kg^{-1}•h^{-1} for bananas at 10°C, but this value exceeds 40 mL·kg^{-1}•h^{-1} at 30°C.

The technological solution for the long-term storage of fruit (for months) is the use of controlled atmosphere (CA) storage, there are two ways to achieve this. One method is to adjust the environmental conditions in the container so that the post-ripening process of the fruit is as decelerated as possible, but not completely stopped (Weber et al. 2017, Ramírez-Acosta et al. 2018, Wood et al. 2022). This requires a modern technological and IT system that constantly monitors and controls the parameters of the store's atmosphere and therefore the processes taking place in the fruit. This is called Dynamically Controlled Atmosphere (DCA) storage. The other method is to flood the container's atmosphere with a chemical substance (e.g. methylcyclopropene - MCP) once the fruit has been stored, which prevents the formation of ethylene in the fruit and thus prevents post-ripening (Butkeviciute et al. 2021, Thewes et al. 2017, Mditshwa et al. 2018). In either case, the use of perfectly sealed containers with minimal filtration is required. For temporary installations - containers - the effects of filtration, which generally reduce the concentration of substances released into the atmosphere during fruit ripening and respiration and do not lead to a drastic reduction in oxygen levels, must be taken into account.

Figure 1: Military camp facility on the southern border.

MATERIAL AND METHODOLOGY

On one hand, our research was carried out in real-life conditions at a border defence base in southern Hungary, where both the accommodation and storage containers are ISO 20' standard. (2m x 6m x 2.5 m) (Fig. 1).

The border base food warehouse was used to store fruit and vegetables for 4 to 10 days, and the temperature, humidity and CO_2 concentration of the air in the storage room were constantly monitored during continuous operation. On the other hand, in the storage area set up in the laboratory area (Figure 2), tests were carried out with Idared (*Malus domestica*) apples to obtain data on the variation of the CO_2 concentration in the air without opening the door of the storage area. The controlled atmosphere was set up in a 10' controlled enclosure container, located in the area of the Laboratory of Civil and Environmental Engineering of the Hungarian University of Agricultural Sciences and Life Sciences.

Figure 2: Idared apples in a container storage of the laboratory

The CO_2 load resulting from the respiration of larger quantities of fruit was achieved by introducing food-grade carbon dioxide controlled by a reductor. The adjustments were based on our own measurements and a extensive scientific literature (Fanguedes et al. 2013, Kádas-Frenyó 1985, Prichko et al. 2020, Melnyk et al. 2018, Crisosto et al. 1993, Bhande et al. 2008, Cervantes et al. 2020).

Instrumentation

For the laboratory measurements of temperature, humidity, carbon dioxide concentration, solar radiation intensity, wind speed, wind direction and atmospheric pressure, representing the outdoor conditions, and for the storage of the measured values, an ALMEMO 2590 measuring and storage unit and its associated sensors were applied (Ahlborn, Illmenau, Germany). For the measurements at the border defense base, an EBI300 temperature meter and data logger, supplemented with a THP 400 external capacitive humidity sensor (Ebro, Ingolstadt, Germany) and a PYLE PC02MT05 (Brooklyn N.Y., USA) and Wohler CDL 210 (Bad Wünnenberg, Germany) carbon dioxide sensors and data loggers were applied. Separate 24 h calibration measurements were performed to ensure a reliable and accurate comparison of the parameters recorded by the instruments.

Mathematical model

To validate the expected value of CO_2 concentration by calculation, we used the mathematical model presented by Herczeg et al. (2000), which simulates the concentration in the indoor air with the pollutant sources of the different measurements.

$$k_b = k_k + \frac{\dot{K}}{V_{sz}} \cdot (1 - e^{-n\tau}) \quad \textbf{(1)}$$

where: $\mathbf{V_{sz}}$ – ventilation air flow rate, - carbon dioxide point source, $\mathbf{k_b}$ – indoor air concentration rate, $\mathbf{k_k}$ – outdoor air concentration rate, **n** – air exchange rate, τ - time

RESULTS AND DISCUSSION

At the food storage facility at the border base, changes in air temperature {T_{indoor}}, humidity {H[%]} and carbon dioxide concentration were observed during the measurements. The primary objective was to observe the CO_2 concentration, the variation of which was influenced by the fresh air entering the container (removal-storage and filtration) in addition to the internal source (post-ripening and respiration of vegetables and fruits). Fig. 3 shows the air conditions in the 20′ storage ISO container.

Due to regular and continuous disposal and stacking, as well as filtering of the container, the expected increase in carbon dioxide concentrations did not occur. It can be concluded that during short-term storage, carbon dioxide concentrations do not reach the levels required to inhibit metabolic processes, but research on this topic has generally focused on changes during long-term storage, i.e. storage longer than 1 week. Esmer et al. (2021) has also done so and found that the shelf life of fresh sliced mushrooms packed in microperforated packaging was 8 days, while those packed in non-microperforated packaging was less than 7 days. For grapes, a period of 1 month was recorded (Jones et al. 1965), and experiments with apples have investigated storage periods of several months, up to half a year (Romero et al. 2019, Kassebi and Korzenszky 2021, Ghabour et al. 2021). In order to compare our research with similar studies (Selcuk and Erkan 2014, Nadeem et al. 2021), we continued our observations in the laboratory, modelling the behaviour of closed storage for more than 24 hours.

The laboratory experiments (Fig. 4) demonstrated that when 100 kg of fresh fruit were stored in a closed enclosure, the CO_2 concentration increased after 1 hour, but did not exceed a certain level without opening. The further increase in CO_2 concentration was determined by the filtration in the container and the external environmental parameters (wind speed, air pressure, temperature difference) affecting it.

The measured results correlate closely with the values obtained from the mathematical model (1), but it was also

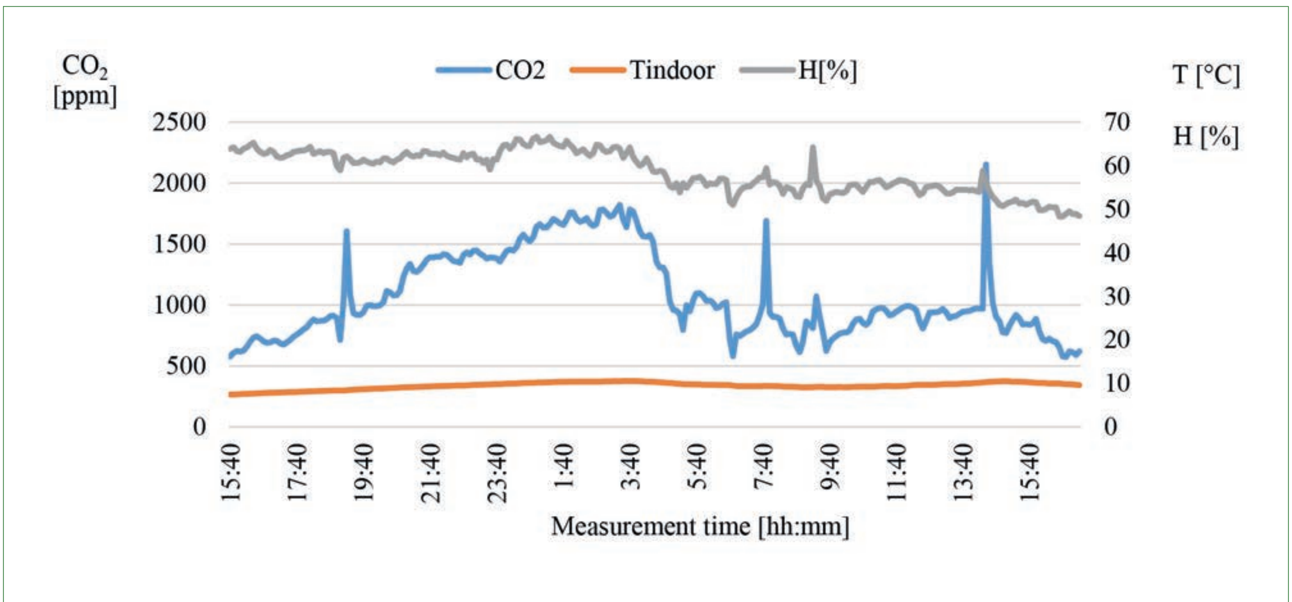

Figure 3: Measurement of the air quality of a military camp vegetable/fruit storage (14/02/2020)

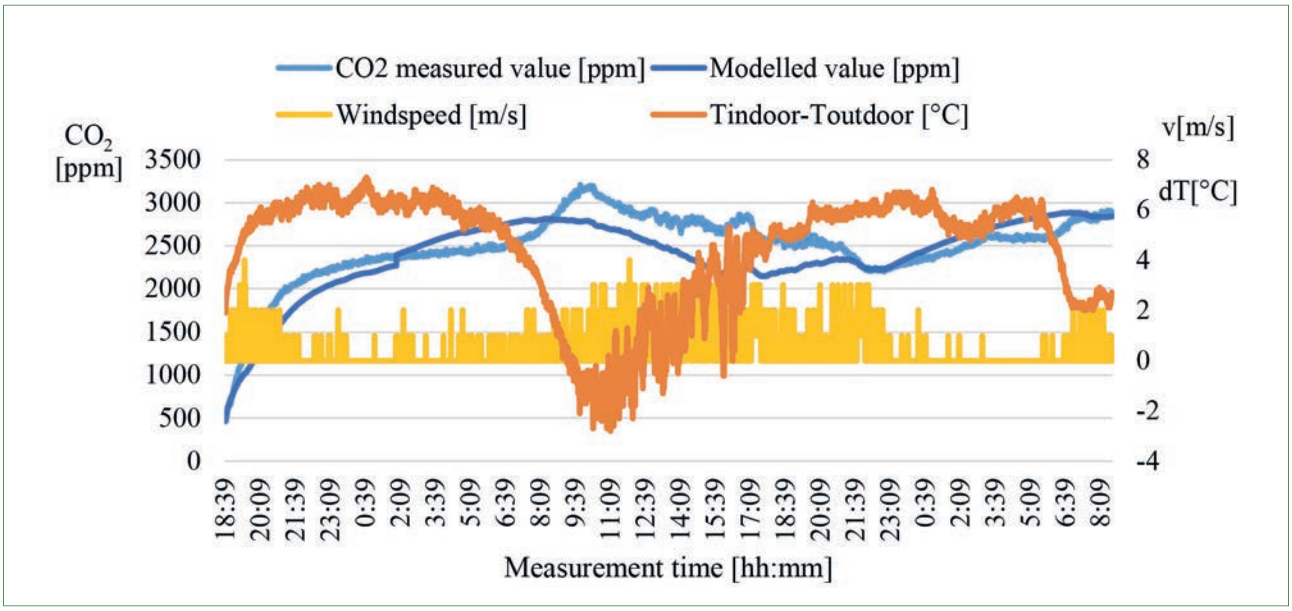

Figure 4: Measurement of air quality (CO2 concentration) and validation of the mathematical model in case of 100 kg apple storage

discovered that the CO_2 content of the container, which is already steady, varies with the surrounding environment. Former studies have already evaluated the effect of environmental stress on fruit, modelled the surface temperature dynamics of apples based on weather data, or the effect of other external forcing factors (Ho et al. 2008, Li et al.2014, Barbanov et al. 2018). Accordingly, we extended the study by storing increased quantities of fruit in closed long-term storage.

The food standard for border guards is 600 kg of fruit and vegetables per fortnight. Experiments have revealed that in a standard ISO 10′ container installed in a laboratory area, two and a half hours after the loading process begins, 600 kg of Idared apples develop a CO_2 concentration of 1100 ppm. (Fig. 5).

Although this value negatively impacts on human wellbeing (Pettenkofer 1858, Géczi et al. 2018) and interferes with several other processes of fruit metabolism (Rzhepakovsky et al. 2022, Phan et al. 2017, Corpas et al. 2018), it may have advantages in increasing shelf-life, such as texture preservation (Parentelli et al. 2007, Simón et al. 2005, Gonzalez-Fandos et al, 2001).

Long-term changes in CO_2 concentrations in response to external forcing were monitored using an artificial CO_2 source. With a constant CO_2 emission modelled with 2500 kg Idared apples, the long-term measurement se-

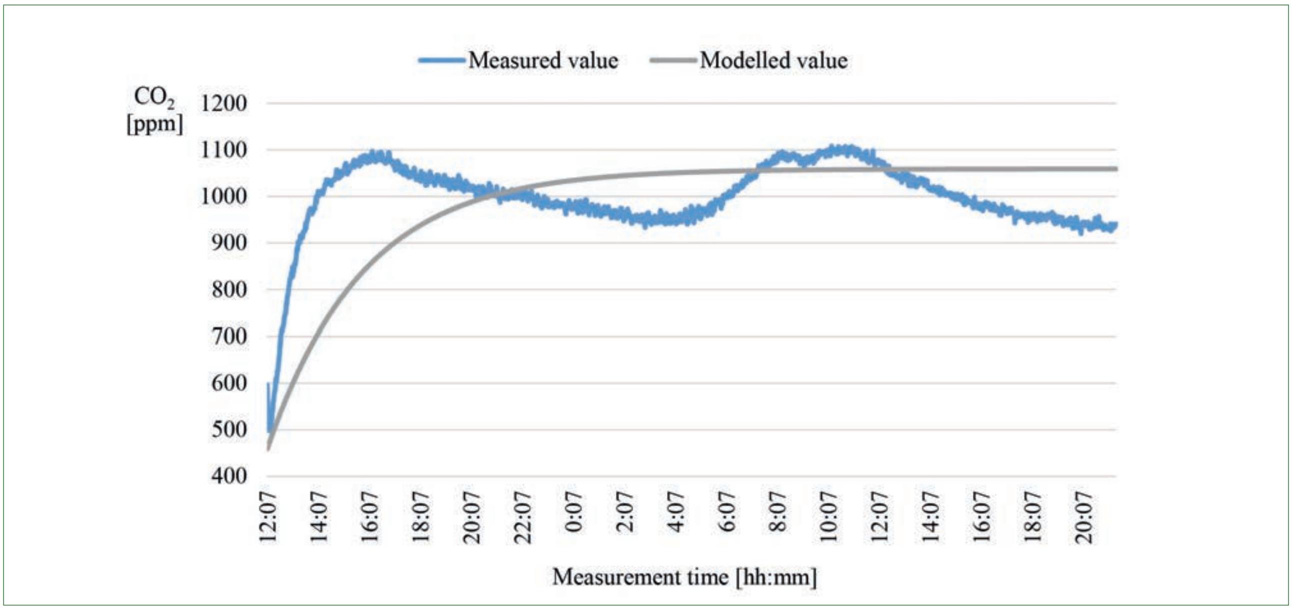

Figure 5: Increase in CO2 concentration during 600 kg apple storage

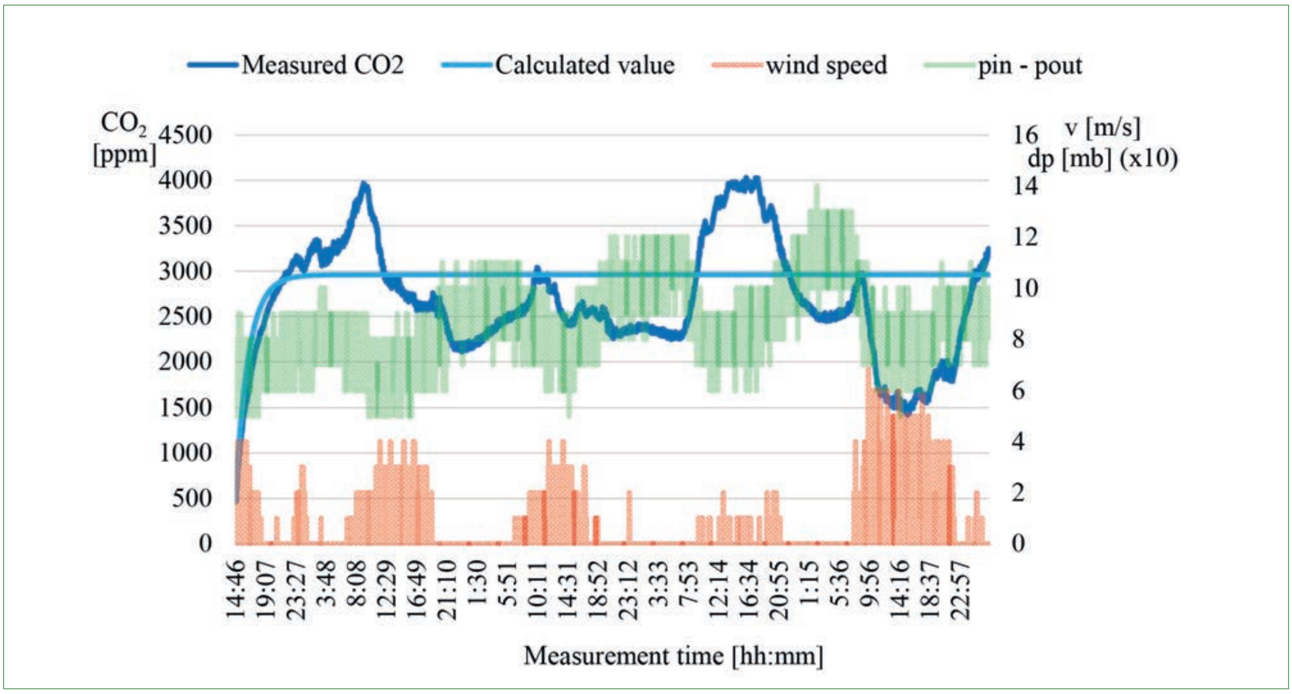

Figure 6: Change in CO2 content of air 2500 kg Idared apples in case of 5 days modelled storage

ries (Fig 6) shows strong deviations between 4000 and 1500 ppm.

CONCLUSION

The increase in CO_2 concentration due to fruit respiration is similar to the change due to human respiration. However, the hazardous concentrations in the comfort compartments for human use may benefit the storage compartment. The equations used for calculation can be applied in this context if the respiration rate of the fruit/vegetable is known or can be determined experimentally. The CO_2 concentration in the storage containers of temporary military installations is strongly influenced by environmental parameters (pressure differential, wind speed). Currently, there are no general mathematical models available that can provide a reliable estimate of the airspace conditions based on container size, container filtration, external influencing parameters and the amount of fruit and vegetables stored.

The change in filtration is influenced by the value of the difference between the internal pressure and the external pressure [p_{in}-p_{out}], which reaches a minimum value, then the CO_2 concentration starts to decrease, and when the difference between the internal pressure and the external pressure reaches a maximum value, the CO_2 concentration increases. The wind speed value should not be ignored, though, and has a significant direct impact on the scatter of the measurement data. The observed wind speed influences the filtration of a mobile food storage container of a military camp set up in a laboratory - measured to be relevant for measurements above 2 m/s wind speed - and therefore the selection of logistic deployment sites should take into account the military reconnaissance meteorological data of the geographical area. Models for predicting respiration during food storage exist (Ho et al. 2008, Finnegan et al. 2013, Fonseca et al. 2002, Géczi et al. 2019, Patonai et al. 2022) and improving their accuracy is essential to improve storage capacity efficiency and reduce food waste.

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MITIGATING ENVIRONMENTAL RISK FACTORS AND PROMOTING SUSTAINABLE AGRICULTURE THROUGH PÁLINKA SPENT WASH COMPOSTING

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ABSTRACT

This paper addresses environmental challenges in Hungary's Pálinka distillery industry and advocates for sustainable agriculture through Pálinka spent wash composting. Pálinka production generates substantial organic waste known as spent wash, posing environmental issues due to its high organic content, low pH and complex composition. Composting provides an eco-friendly solution by transforming this waste into humus-rich material and reducing phytotoxic substances. The study focuses on environmental concerns, especially copper in Pálinka spent wash. It explores the potential of Pálinka spent wash compost to mitigate these issues and enhance soil quality for sustainable agriculture. The investigation tracks copper reduction through co-composting with garden compost, diatomaceous earth and wood ash. Various analyses, including physicochemical assessments and germination tests, evaluate the compost's suitability for seed growth. This research highlights the importance of addressing environmental and health risks linked to Pálinka spent wash while emphasizing its potential as a valuable soil enhancer to improve soil health and fertility.

Keywords: composting, Pálinka spent wash, co-composting, copper, Bokashi Organico.

INTRODUCTION

Pálinka, a celebrated Hungarian hard liquor, yields around 300,000 tons of mash residue annually referred to as Pálinka spent wash (PSW). This organic byproduct is a blend of organic and inorganic components and constitutes a significant portion, about 85%, of the input material (Borges et al. 2022) With a low pH (3-4), substantial organic load, polyphenols and macro and micronutrients content, improper disposal of PSW can lead to water pollution, eutrophication and oxygen depletion (Borges et al. 2021).

The Pálinka production in Hungary employs two main distillation methods: traditional Pot-Still Double Distillation (PSDD) and Rectification Column Distillation Systems (RCDS) (Géczi et al. 2018; Korzenszky et al. 2020). Understanding these methods is essential for grasping the composition of PSW, including metals content, e.g., copper, moisture content, nutrients and polyphenols. Furthermore, the broader food industry is shifting towards sustainability, focusing on waste reduction and alternative energy sources (Géczi et al. 2016; Borges et al. 2015) Harnessing PSW for composting offers a solution to these environmental issues. Composting, whether aerobic or anaerobic, is a sustainable and effective approach for handling organic waste, alleviating odor and nutrient concern and enhancing various soil parameters, such as soil organic matter, nutrient availability and microbial activity, ultimately enhancing soil health and fertility. PSW compost further enhances soil structure, water-holding capacity and organic matter sequestration, stabilizing soil organic matter (SOM) (Marhuenda et al 2007; Bustamante et al. 2010). However, PSW has characteristics unsuitable for traditional composting due to its low pH and high moisture content. To optimize composting, mash residue can be combined with additives like diatomaceous earth, wood ash and garden compost, creating an ideal environment for microbial activity. These additives primarily serve as water-retaining materials and pH buffers. Moreover, alternative anaerobic composting, such as *Bokashi Organico*, offers a unique approach to food waste fermentation. It involves sealing food waste in a container known as a *Bokashi Organico* bin, preventing air contact and facilitating natural waste fermentation with the help of a bran-based starter material containing natural ingredients and effective microorganisms (Borges et al. 2022). Successful PSW composting follows best practices, including balanced material selection, appropriate system design and critical parameter monitoring (e.g., moisture, temperature and odor) (Borges et al. 2022). These practices ensure high-quality compost, enriching the soil, promoting nutrient cycling and enhancing soil structure and water-holding capacity. Composting PSW and exploring innovative methods like *Bokashi Organico* align with the discussion on food chain risk factors and offer an eco-friendly approach to environmental conservation and sustainable agriculture.

MATERIALS AND METHODS

In this research, we explore two distinct composting techniques for managing PSW:

1. Anaerobic Composting with Bokashi compost (BC): *Bokashi Organico* bin-produced compost was mixed with varying proportions of Pálinka spent wash (PSW) in 150 ml Mason Jars over a 4-week period. The study involved different BC and PSW ratios: 100% BC + 0% PSW, 75% BC + 25% PSW, 50% BC + 50% PSW, 25% BC + 75% PSW and 0% BC + 100% PSW, with each combination replicated three times, totaling 15 samples. Germination tests were conducted using lettuce, wheat, corn and mustard seeds, both before and after anaerobic composting. After the 4-week period, we collected samples, mixed them with distilled water, centrifuged them and filtered them to obtain an aqueous extract at a 1:10 ratio (w/v). For control treatments, drinking water was used, while Bokashi compost and the other treatments utilized the aqueous extract.

2. Aerobic Composting with Additives: We conducted a comprehensive 12-week composting experiment in controlled laboratory conditions. Perforated plastic boxes were used as incubation vessels for PSW composting. The initial conditions of the mash residue included a pH of approximately 3.5, a moisture content of 93.82% and an organic matter content of 99.60%. The treatments involved control (C), garden compost (GC), diatomaceous earth (DE), wood ash (WA) and combined treatments (CT). The diatomaceous earth, derived from Miocene-aged organic sedimentary rock, enhanced soil structure and moisture balance due to its water-retention properties, primarily consisting of silt-sized particles and containing amorphous silica (76–78%), calcite (15–17%) and montmorillonite (6–7%). Wood ash, known for its high potassium content, helped neutralize the acidic mash residue while improving water retention. The experiment also included wood ash-treated mash residue, revealing its potential as an organic fertilizer for acidic soils. Details of the composting treatments and additive quantities are presented in Table 1.

For both composting methods, we assessed a range of physicochemical parameters, including pH, electrical conductivity, total dissolved salts, nitrogen forms, phosphate, copper, sodium, potassium and organic matter.

RESULTS AND DISCUSSION

The chemical properties of PSW, Bokashi compost (BC) and their mixtures were analyzed (Table 2). Mixing Bokashi compost with PSW raised pH slightly, which re-

Table 1: Treatments and additive weights used in the PSW aerobic composting experiment (Unit: grams)

Treatments and additives	PSW	GC	DE	WA
Control	5000	-	-	-
GC+PSW	2500	2500	-	-
DE+PSW	5000	-	2500	-
WA+PSW	3750	-	-	2500
CT	4000	1000	1000	1000

Table 2: Parameters of PSW, Bokashi compost and the different treatments with Bokashi compost and spent wash.

Treatments and Parameters	pH (H_2O)/ pH (KCl)	EC (mS/cm)	Total Dissolved Salts (mg/L)
Pálinka Spent Wash (PSW)	3.5/3.44	3.59	1976.73
Bokashi Compost (BC)	4.27/4.27	3.27	1633
0% BC + 100% PSW	3.47/3.41	1.32	658.33
25% BC + 75% PSW	3.69/3.63	1.71	853.00
50% BC + 50% PSW	3.83/3.78	2.31	1155.33
75% BC + 25% PSW	4.01/3.93	2.78	1387.00
100% BC + 0% PSW	4.29/4.19	3.77	1886.33

Table 3: Seed germination percentages of anaerobic composting treatments and Bokashi compost after 4 weeks; control with only drinking water

Treatments	Lettuce	Wheat	Corn	Mustard
Control	41%	95%	67%	59%
Bokashi compost (BC)	0%	60%	61%	3%
100% BC + 0% PSW	0%	71%	45%	7%
75% BC + 25% PSW	3%	76%	52%	33%
50% BC + 50% PSW	3%	84%	52%	48%
25% BC + 75% PSW	1%	75%	30%	49%
0% BC + 100% PSW	0%	80%	45%	21%

Table 4: Chemical characteristics of the additives used for PSW aerobic composting

Chemical Parameters	Garden compost	Diatomaceous earth	Wood ash
$pH_{(H2O)}$/ $pH_{(KCl)}$	7.7/7.28	7.93/7.35	13.65/13.79
Total Dissolved Salts (mg/kg)	1480	356.5	104 500
NO_{2-}, NO_{3-}, NH_{4+} (mg/kg) of dry matter	42.3	6.15	5.5
P_2O_5 (mg/kg) of dry matter	2232.35	587.49	576.24
K_2O (mg/kg) of dry matter	3666	331	66 400
Cu (mg/kg) of dry matter	Under detectability	-	-
Organic material (%)	92.43	15.19	60.78

mained acidic. Total dissolved salts were noticeable, especially in treatments with higher Bokashi compost ratios. PSW featured significant potassium content.

Germination tests revealed that PSW, before anaerobic composting, did not support seed growth. After 4 weeks of anaerobic composting, improved germination occurred, particularly for wheat (Table 3).

For the second composting method, the mash residue underwent controlled fermentation before distillation. The process involved enzymatic apple pectin extraction and the introduction of special yeast with pH adjustment to 3.69 using phosphoric acid. The suspension had low total dissolved salts (1331 mg/l), with significant potassium (K_2O) (37,440 mg/kg) and phosphorus (P_2O_5) (1,231.2 mg/kg) in dry matter. Nitrite (NO^{2-}) and nitrate (NO^{3-}) were undetectable and ammonium (NH^{4+}) was minimal at 2 mg/kg. The suspension had 3.5% dry matter content, primarily composed of 93.2% organic matter. Treatment materials' physicochemical characteristics are summarized in Table 4.

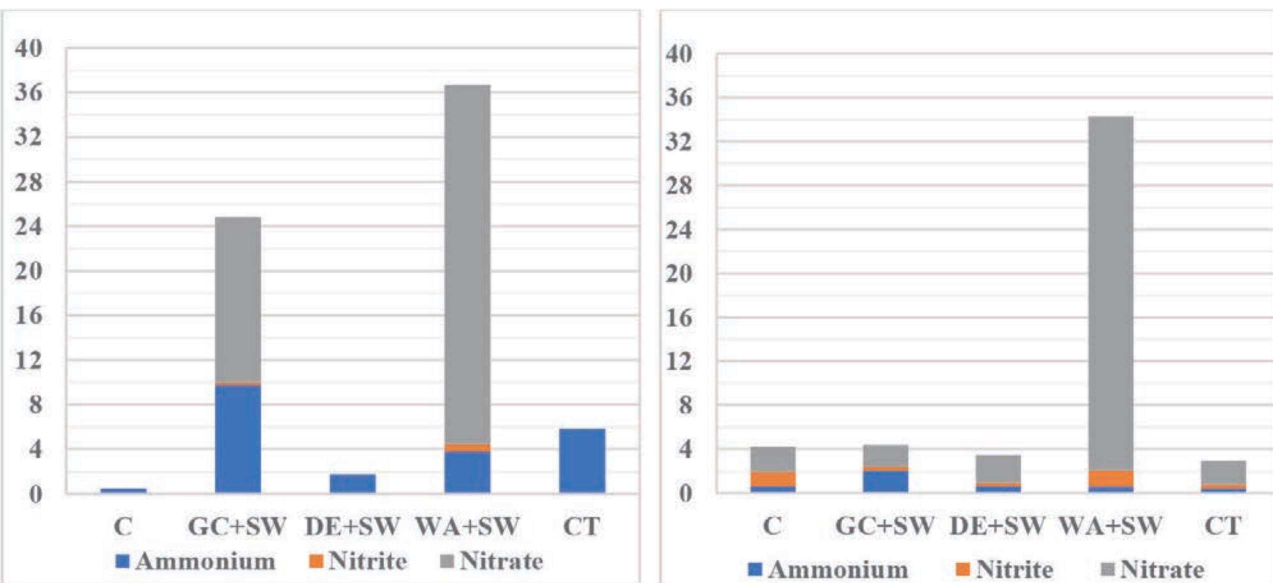

Figure 1: Nitrogen forms available to plants as a result of the treatments. Results from different times of the composting system, 6th week (left) and 12nd week (right). (Unit: mg/kg)

Tests on compost samples highlighted Bokashi compost's buffering capacity. All treatments maintained a near-neutral pH range, crucial for microbiota. Garden compost exhibited the highest mineralized nitrogen levels, while wood ash excelled in nitrogen supply and water retention (Figure 1).

The high copper content in PSW was a concern. The analysis revealed substantial variation and a decrease over time (Figure 2). Diatomaceous earth exhibited the lowest copper concentration but combining it with PSW increased it. Wood ash also showed an increase over time due to the blending of substances.

The analysis of organic matter content indicated the potential for humus formation. Humus formation was central to soil fertility and the addition of compost-mash residue significantly increased humus content. Garden compost excelled in humus content, as observed with diatomaceous earth and wood ash (Figure 3). Humus quality and stability were indicated by fulvic and humic acids. Furthermore, PSW compost plays a vital role in enhancing microbial activity and diversity in the soil, thereby promoting a healthy soil ecosystem. Composting PSW enriches the soil with organic matter, creating a nutrient-rich environment that supports the growth and function of beneficial soil microorganisms (Martinez-Sabater et al. 2009). Bacterial activity prevailed, especially with diatomaceous earth treatment, leading to improved soil mineralization. Wood ash-mash residue provided remarkable nitrogen provision with a suitable N:P:K ratio (Figure 4).

In summary, the diverse treatments offer promising options for sustainable PSW management, improving soil health and agricultural practices while mitigating heavy metal risks.

CONCLUSION

Composting PSW emerges as a potent strategy for environmental risk mitigation and the promotion of sustainable agriculture. This study confirms that composting effectively reduces copper levels in the mash and enhances humus and nutrient content in the resulting compost, ultimately improving soil fertility, structure and overall ecosystem functionality. Ongoing semi-industrial experiments, exploring various additives in mash residue composting, represent a forward-looking and ecologically sound approach, offering the prospect of scaling up

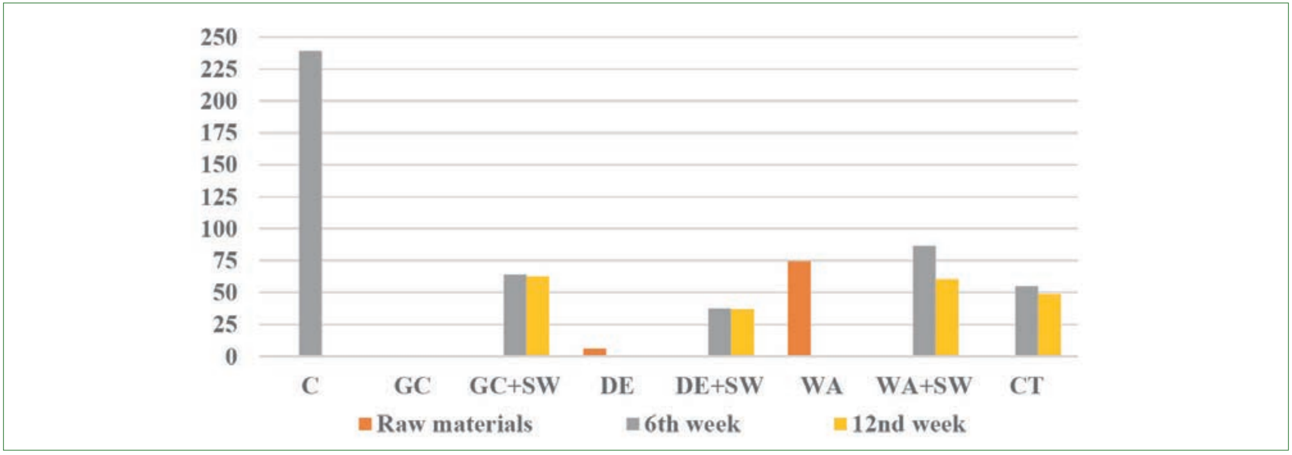

Figure 2: Effective reduction of higher copper content in mash residue by organic and mineral substances. (Unit: mg/kg)

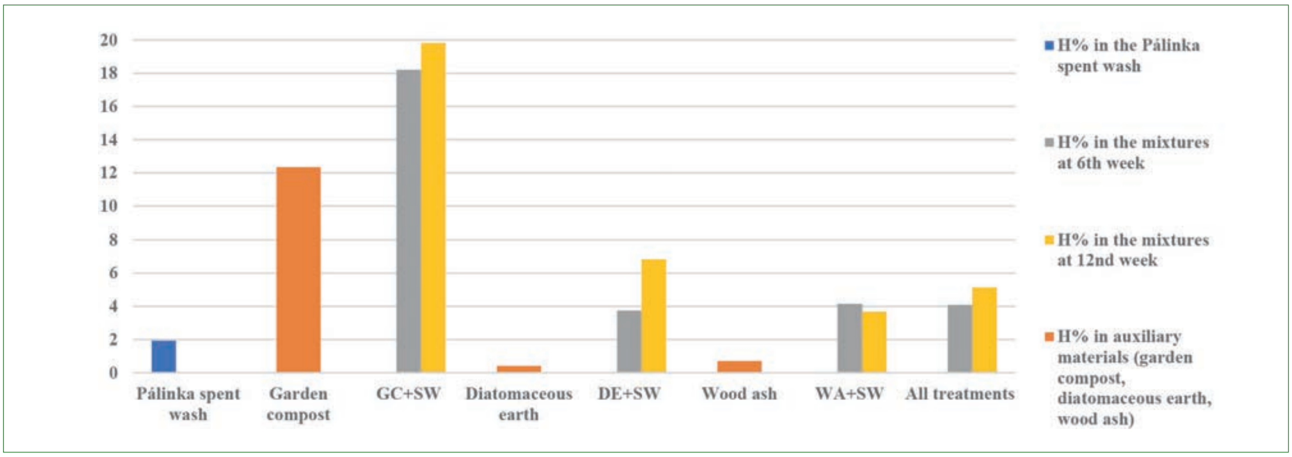

Figure 3: Humus quantity, in percentage, in Pálinka spent wash and additives, also in the treatments. (Unit: %)

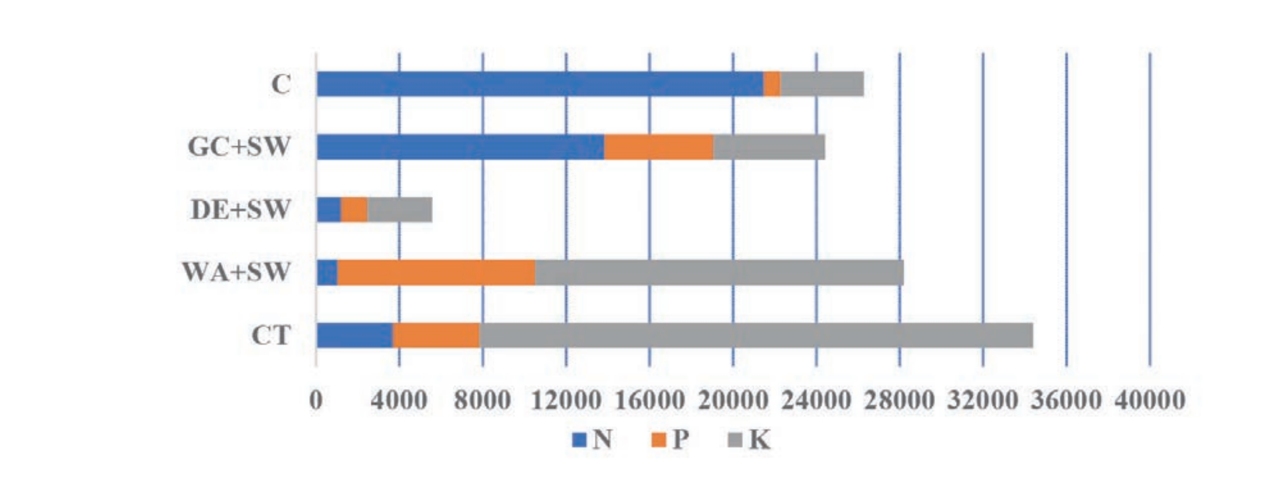

Figure 4: The ratio of macro elements (Nitrogen - N, Phosphorus – P and Potassium – K) in each treatment. (Unit: mg/kg)

composting technology for substantial quantities of mash residue. This marks a significant stride toward sustainable waste management and soil enrichment, highlighting the potential to enhance soil fertility and reduce environmental hazards through composting Pálinka mash residue. These findings offer support for environmental preservation and the advancement of enhanced agricultural practices. Composting PSW provides a practical path to greener, more productive and environmentally responsible agricultural systems.

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IN VITRO PROPERTIES OF ESSENTIAL OILS FROM AROMATIC PLANTS

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SUMMARY

Many natural products of plant origin have proved to be effective in prevention and therapy of several diseases such as bacterial and parasitic infections, and chronic diseases including cancer. In the search for alternative therapies, essential oils (EOs) can represent an excellent source of mixtures of biologically active natural compounds. In the present study, we investigated some biological activities of EOs extracted from seven aromatic plants including their antiparasitic property against *Toxoplasma gondii* tachyzoites: *Rosmarinus officinalis*, *Salvia somalensis*, *Thymus vulgaris*, *Achillea millefolium*, *Helichrysum italicum*, *Pistacia lentiscus*, and *Myrtus communis*. The cytotoxicity in A2780 cells and in Vero cells and the estrogenic/antiestrogenic activity in a yeast strain expressing the human estrogen receptor alpha (ERα) were evaluated. Based on the results of the *in vitro* cytotoxicity, three EOs with low, medium, and high toxicity (*S. somalensis*, *R. officinalis,* and *H. italicum*) were tested for their anti-parasitic property against tachyzoites of a *Toxoplasma gondii* RH strain. Our results showed that EOs may express a high to moderate cytotoxic effect on A2780 and Vero cells and a marked antiestrogenic activity. In addition, some EOs showed to influence at different degree the ability of *T. gondii* tachyzoites to infect Vero cells.

Keywords: essential oils, cytotoxicity, parasite, *Toxoplasma gondii*

INTRODUCTION

Essential oils (EOs) are complex, volatile, and fragrant molecules present in various plant species that play a fundamental role in the plant, often accumulated in specific organs or tissues with higher concentrations being found in the leaves or flowers, but also in the fruits, barks, roots, and rhizomes. For their numerous properties, they have used them since ancient times. The extraction of aromatic essences is, indeed, a very ancient art still carried out today with different techniques according to the part of the plant from which it is obtained.
The properties commonly attributed to EOs are numerous and include antioxidant, antiseptic, antibacterial, antiviral and in some cases, antiparasitic action. Depending on the species, they can exhibit a balsamic, expectorant, relaxing, stimulating, and nourishing action. Recently, they have been used in aromatherapy to support human health and well-being. Each EO has properties that makes it particularly suitable for specific disorders and systems: digestive, respiratory, nervous, and circulatory system (Hamidpour et al. 2017). Biological activities of several EOs and their components have been demonstrated also regard to human health (Valdivieso-Ugarte et al. 2019; Mancianti & Ebani, 2020). They have been described as effective antimicrobial agents against foodborne bacteria such as *Escherichia coli*, *Staphilococcus aureus* (Cosentino et al. 2003; Thielmann et al. 2019) as well as pathogenic fungi such as *Candida* spp. and *Aspergillus* spp. (Ebani et al. 2018; Mutlu-Ingok et al. 2020). Several EOs have also been found to exhibit antiparasitic potential against some parasites (Strothmann et al. 2022; Pereira Filho et al. 2023).
EOs are recognized as natural antioxidants (Mimica-Dukić et al. 2016), acting as free radical scavengers neutralising reactive oxygen species (ROS) (Diniz do Nascimiento et al. 2020; Nenadis et al. 2021). EOs also exert immunomodulatory (Sandner et al. 2020), and anti-cancer (Antrade et al. 2018; Abdoul-Latif et al. 2023). A pro-apoptotic activity of EOs in cancer cells has been reported (Navarra et al. 2015). Because of their antiproliferative effects, EOs may have a possible use as alternative or complementary cancer therapy (Russo et al. 2015). EOs also exhibit a weak estrogenic or anti-estrogenic action both *in vivo* and *in vitro* (Howes et al. 2002; Bartoňková and Dvořák, 2018; Contini et al. 2020). The typical (anti)estrogenic activity of plant compounds is determined by their ability to bind the estrogen receptor alpha (ERα) and/or beta (ERβ).
Because of the numerous above-mentioned properties, EOs can represent alternative therapies for beneficial purposes. New drugs are needed to potentiate the clinical efficacy of conventional chemotherapy and overcome

its relevant associated side effects. In the present study, we investigated some biological properties, includingy the antiparasitic activity, of EOs extracted from seven aromatic plants: *Rosmarinus officinalis, Salvia somalensis, Thymus vulgaris, Achillea millefolium, Helichrysum italicum, Pistacia lentiscus, Myrtus communis*.

MATERIALS AND METHODS

Essential oils

The EOs of *R. officinalis* (REO), *S. somalensis* (SEO), *A. millefolium* (AEO), *T. vulgaris* (TEO), *H. italicum* (HEO), and *M. communis* (MEO) were obtained from plants cultivated at the Centro Ricerca Orticoltura e Florovivaismo, Sanremo (Italy), and extracted by hydro distillation (Contini et al. 2020).

Host cells and culture conditions

A2780 cells (human cervical carcinoma cells) and Vero cells (African green monkey kidney fibroblast cells) were used to assess cytotoxicity. Vero cells were also used for *Toxoplasma* maintenance and the *in vitro T. gondii* growth inhibition assay. Cells were grown in RPMI-1640 supplemented with 10 % FBS (inactivated for experiments of *Toxoplasma* infection) and 1 % L-glutamine (Sigma Aldrich, Milan, Italy). The cells were maintained at 37 °C and 5 % CO2.

In vitro estrogenic/antiestrogenic activity

In a previous study, we evaluated the EOs agonistic/anti-agonistic activity (Contini et al. 2020). Briefly, yeast cell expressing the hERα were incubated with different concentrations of EOs (from 0.00001 µl/ml to 0.1 µl/ml) or with 10 nM E2 (agonistic effect); Yeast cells were co-incubated with EOs and 1 nM E2 to test the antagonistic effect. Results are expressed as percentage of the β-galactosidase activity induced by E2 (100%) (Contini et al. 2020).

Cytotoxicity assay in A2780 cancer cells and Vero cells

A2780 cells were exposed for 24 h to different concentrations of each EO (from 0.001 µl/ml to 1.0, to 5 µl/ml) obtained by serial dilutions of the extracts in DMSO (1% maximum final concentration) and cytotoxicity was evaluated by WST-1 assay (Roche Diagnostics, Milan, Italy). Vero cell viability in the presence of EOs was preliminary assessed to establish the non-toxic concentration of each compound in host cells. (0.001, 0.003, 0.006, 0.05, 0.5, 1, 5, 10 µl/ml). Optical densities were measured at 450 nm absorbance in a microplate reader (BioTek Synergy H1 Plate Reader, Agilent Technology). The cellular viability is expressed by the percentage of viable cells compared to control cells (cells maintained with only culture medium). Means ± SEM of three independent experiments with three replicates each.

Parasite strain

The virulent *T. gondii* RH strain (Type I) was maintained *in vitro* through serial passages in 25 cm^2 culture flasks of confluent Vero cells maintained at 37 °C and 5% CO2.

T. gondii growth inhibition assay

To evaluate the influence of EOs on *T. gondii* viability, Vero cells were cultured in 24 well plates (20.000 cells/ml, final V=2 ml) in appropriate medium and 5% CO2. After adhesion, cells were treated with different concentrations of EOs (0.001-10 µl/ml), infected with tachyzoites (parasite:cell ratio = 5:1; 2×10^5 parasites/ml were added to each well), and incubated overnight. Three EOs with low (REO), medium (SEO), and high (HEO) cytotoxicity at the two highest doses showed to be safe for host cells (0.5-0.25 µl/ml REO; 0.03-0.015 µl/ml SEO and HEO) were tested for their anti-*T. gondii* property. After 24 h, the supernatant from the treated cells was collected, centrifugated at 3700 rpm per 10 minutes, resuspended in 2 ml of fresh medium, and a volume of 100 µL was added to a subculture of cells (10×10^4 cells/mL, 100 µL; final V=200 µL). Vero cells infected with *T. gondii* alone, were used as positive control for the infection. The anti-*T. gondii* activity of EOs was determined by WST-1 assay.

RESULTS

Cytotoxicity in A2780 cell line

The results of the cytotoxicity assay in A2780 cells are shown in Fig. 1. Except for SEO, which was ineffective at all the tested doses, the others EOs significantly decreased survival of the HeLa cancer cells. In particular, the most effective extracts were HEO, MEO and PEO, which induced a 90% toxicity at 0.1 µl/ml.

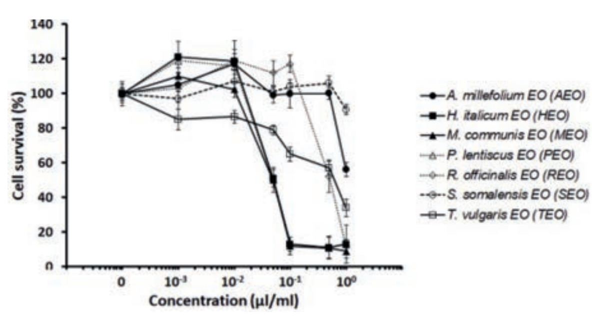

Figure 1: Evaluation of EOs cytotoxicity in the A2780 cell line

In vitro estrogenic/antiestrogenic activity

The majority of EOs tested showed no estrogenic activity in our system. PEO showed a weak estrogenicity (maximum β-gal activity, 29.3 ± 1.5% of E2). When EOs were tested for their ability to inhibit the β-galactosidase expression induced by 1 nM E_2, all the samples showed a dose-dependent antagonistic activity (Fig. 2). HEO, SEO and AEO exhibited the highest activity, with a maximum inhibition of 90.7% at 0.1 µl/ml for SEO. AEO and HEO reduced the E_2-mediated activity by 77.2% and 75.0%, at 0.1 µm/ml and 0.01 µl/ml, respectively.

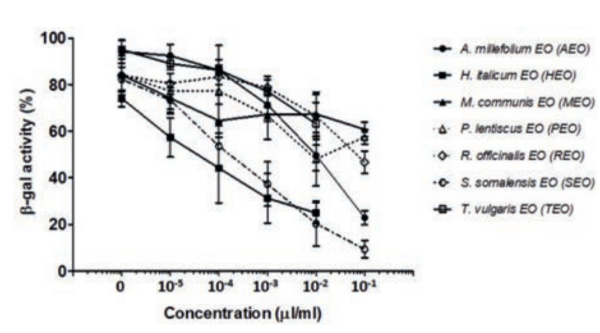

Figure 2: Evaluation of EOs inhibitory activity on the hERα

Anti parasitic activity

To verify whether EOs inhibit *T. gondii* proliferation without exerting a toxic effect on the host cells, Vero cells were treated with different concentrations of EOs (from 0.001 to 10 µl/ml). Based on the results of the *in vitro* toxicity on Vero Cells (data not shown), three EOs were chosen, with low (REO), medium (SEO), and high (HEO) cytotoxicity. When tachyzoites were treated with the two highest EOs concentrations (d1, d2) that showed to be safe for the host cells (0.5-0.25 µl/ml REO; 0.03-0.015 µl/ml SEO and HEO), an inhibitory effect on *T. gondii* proliferation was observed. Our results indicate that all three EOs were able to inhibit the growth of the parasite within safe concentrations. REO exhibited the highest anti-parasitic activity. The most inhibiting effect for REO was observed at a final dose 10-fold higher than SEO and HEO.

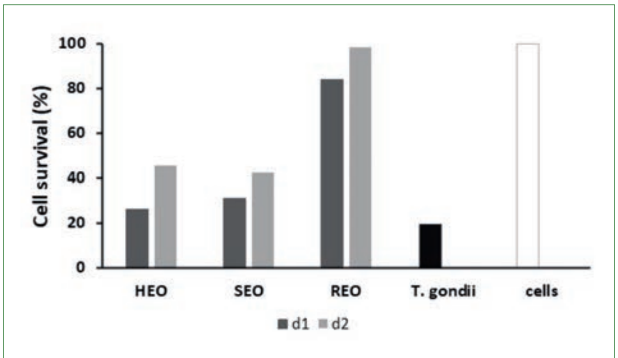

Figure 3: Evaluation of EOs inhibitory activity on *T. gondii* proliferation in Vero cells.

DISCUSSION

EOs are natural compounds widely used in cosmetic and food industry, but they are also tested for their potential therapeutic and pharmaceutical properties. For this reason, it is important to characterize their biological activity, which may also differ depending on the plant, and understand their therapeutical potential in humans. In a previous work, we determined the cytotoxic and genotoxic activity of seven EOs extracted by aromatic plants using the micronucleus assay (CBMN) in human peripheral lymphocytes, cytotoxicity in a human ovarian carcinoma cell line (A2780), and the estrogenic/antiestrogenic potential in a recombinant yeast strain expressing the human estrogen receptor alpha (hERα) (Contini et al. 2000). In the present study, we show some of the previous results and investigated the cytotoxicity on a monkey fibroblast cell line (Vero cells) and the ability of EOs to inhibit the proliferation of a virulent T. gondii RH strain (Type I). After some preliminary tests, we selected three EOs that showed different action on Vero cells.

CONCLUSIONS

Our results indicate that all three EOs were able to inhibit the growth of the parasite within safe concentrations. In particular, REO exhibited the highest anti-parasitic activity without being toxic for the host cells. This study can provide a useful contribution to better delineate the biological activities of EOs against cancer human cells, their potential use in hormone-therapy and their anti-parasitic effect.

ACKNOWLEDGMENTS

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COMPARISON OF WASTEWATER-BASED AQUACULTURE AND POND FERTILIZATION

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ABSTRACT

Municipal wastewater mostly considered unfeasible for farming, since its quality is inappropriate for farming fish, and presents a high risk due to the increased possibility of microbial infectivity and accumulation of heavy metals in fish products.
Fish farming, included pond fertilization using manure and human excrement exists since ancient times, as historical evidence suggests. The purpose of pond fertilisation is to increase fish production by enhancing the biological production of planktonic mass growth, thus providing more food for the fish stock during their development.
This fertilisation was carried out with plant mass or animal and human faecal waste, depending on availability. In some Southeast-Asian cultures municipal wastewater is still widely used for fish farming, while in Europe and North Africa this practice is limited to manure. Both practices have their benefits and risks, but overall, municipal wastewater contains more microbial and parasitic organisms of human origin, which are more liable to be human-specific and, therefore, present a more significant risk of infection. Although raw wastewater contains contaminants above tolerance levels for most fish species, disinfection and dilution can make it suitable for aquaculture purposes, while treating it as in purpose-built systems.

Keywords: wastewater, fish farming, manure, pond fertilization, dilution

BRIEF HISTORY OF POND FERTILIZATION

The animal manure application has a great history in fish farming and is still used as the cheapest form of fish mass production. The procedure partially aims to enrich microbial and thus planktonic life by providing necessary nutrients for bacteria and algae, feeding zooplankton which will serve as a considerable nutrient source for fish. Several species of aquaculture fish, such as the Nile tilapia (*Oreochromis niloticus*), feed directly on manure.
The practice has historical backgrounds in ancient China. In the year 473 B.C. Fan Li wrote a book, "Fish Breeding", which is the first known document on fish culture. Common carp (*Cyprinus carpio*) was widely used as subject. (Tapiador et al., 1977)
During the Tang dynasty, around 618, Emperor Li, whose name means 'carp', forbade farming the fish that bore his name. Farmers turned their attention to similar fish in the Cyprinidae family and developed the first form of polyculture. Liquid manure from livestock farming was also used to stimulate algae growth in ponds and make it more nutritious. The ponds were then drained and used as fertiliser. The first integrated agriculture-aquaculture systems emerged in China, where they are still implemented today (Wong, 1997; Nash, 2011).
The mixed fish stock was managed by feeding the grass carp (*Ctenopharyngodon idella*) with hay and freshly cut grass. The manure was used as a fertiliser in ponds where other species, mainly common carp and silver carp (*Hypophthalmichthys molitrix*), were fed. (FAO, 1983)
Also, pond fertilization is mentioned in the works of Shen Tung—fong of the Ch'ing dynasty: he cites from The Book on Agricultural Administration (by Hsu Kvang Chi, the Ming dynasty, 1368-1644), fish culturing methods in Kiangsi and discusses fish pond system, mixed culture of two cyprinid species, fish food, and the utilization of sheep manure for fertilization purpose (Chang et al., 1979).
The aquaculture of Nile tilapia (*Oreochromis niloticus*) dates back to ancient Egypt, where it was represented by the hieroglyph K1, of the Gardiner list: . Tilapia is traditionally fed by poultry and swine manure. The traditional way of farming tilapia is in ponds, where the fish can feed on naturally occurring food (Fig.1.). In several regions of the world, livestock such as poultry and swine are farmed alongside fish ponds, as their manure can be used to increase the pond's food supply for tilapia. (Belton et al., 2009; Ishikawa et al., 2013).

Figure 1: Central Garden Pool in the Garden of Nebamun's Tomb Painting, British Museum, late 18th Dynasty, circa 1350 BCE (Photo in public domain) http://www.electrummagazine.com/2012/09/ancient-egyptian-tilapia-fish-story/

Hungary also has a rich history in the use of manure in fish farming, which is documented in the work of Elek Woynarovich (2005), who received orders from the Communist Party to use manure from swine (*Sus scrofa*) farms that had been used to provide war compensation to the USSR. He developed a technology to utilize what was considered a wasted resource. His approach was to use manure not exclusively to enrich the macronutrient content for phytoplankton growth, but also to enrich the nutrients available for bacterial and zooplankton productivity by using the carbon content. This scientific approach increased fish production but also increased the risk of infection. Opposing opinions were expressed, as many lakes in Germany have a high humic content and carbon sources were less available for those cultures. The produced bacterial mass requires a sufficient dissolved oxygen (DO) supply to avoid putrefaction and an anaerobic environment that would lead to fish mortality. (Woynarovich et al., 2019; Woynarovich et al., 2020).

This practice is very close to the wastewater treatment process, where the degradation of complex compounds is mostly done by oxidative bacterial processes.

RECENT DEVELOPMENT IN WASTEWATER-BASED AQUACULTURE

A current emerging practice is Urban Aquaculture, where treated or untreated municipal wastewater is used for fish propagation. To avoid further damage to coastal ecosystems from increased nutrient loads, future urban ecosystems should plan for a greater percentage of wastewater to be diverted to inland areas and motivate water markets to develop new, innovative urban ecosystems that use wastewater for aquaculture and agriculture. A tool for this requirement is using wastewater for aquaculture.

A pilot project in California accomplished significant protein production while simultaneously removing 97% of the inorganic N in the original tertiary-treated wastewater. Pond effluents were treated effectively by the in-pond plants and wetland components of the ecosystem (Costa-Pierce et al., 2005).

This ecological and economic consideration aside, the practice of recycling wastewater into aquaculture systems is a common practice in several cities in Southeast Asia. People are taking advantage of places where they can farm fish or cultivate aquatic plants that are created during the urbanization process, such as borrow pits (holes left over from the removal of soil and used elsewhere for construction) and sewers. Catering waste from factories and by-products from slaughterhouses on the suburbs of Bangkok contribute to local intensive catfish farming. The relationship between aquaculture and wastewater use in urban areas is complex, partly as a high proportion of surface water is effectively contaminated around urban areas of developing countries because of inadequate sanitation. Additionally, wastewater may not be sewage alone, or at all, but rather constitute a mixture of run-off and local discharges. The best-developed and understood urban aquaculture in the SSEA region is based on semi-formal use of sewage that is introduced into ponds on the suburbs of cities. In urbanizing but unsewered areas outside urban infrastructure, de facto aquaculture due to eutrophication of surface water from overflowing septic tanks and septic tanks is common. Overhung latrines are also relevant features of urban life in some countries. The economics of zero nutrient costs are well known to contractors who place such ponds and associated latrines along major routes out of cities and around public gathering places (e.g. bus stops). These practices raise food safety concerns instantly. Albeit using wastewater for controlled eutrophication of fish culture ponds may reduce overall public health risks from pathogenic organisms, disease vectors, even risks of heavy metals and agrochemical residues still stand (Costa-Pierce et al., 2005).

The primary aim of informally developed wastewater use has been to utilize both water and nutrients for aquatic farming rather than just the treatment or disposal of

Table 1.: Water quality parameters in wastewater treatment plants

Location / Parameters	YSW-TP at intake	BC-WTP at intake	For pond fertilization with pig manure (calculated)
Biochemical Oxygen Demand (BOD) [mg/L]	45	325	n.a.
Chemical Oxygen Demand (COD) [mg/L]	115	583	3,267
Total Kjeldahl Nitrogen (TKN) [mg/L]	39	n.a.	n.a.
Ammonium nitrogen (NH4-N) [mg/L]	36	42	n.a.
Total Phosphorus (TP) [mg/L]	8,8	7	0,013
Total Nitrogen (TN) [mg/L]	75	58	0,065
Total Suspended Solids (TSS) [mg/L]	46	295	3,550
Sources/based	Nga et al., 2014	Budapest Waterworks, 2016	Woynarovich, 2005

wastewater. Wastewater is almost invariably used in aquaculture without any formal treatment, with inadequate attention given to public health. This may be contrasted with the formal design of wastewater treatment systems by engineers in which the primary aim is treatment and disposal, with concern for public health.

Most wastewater is used directly to propagate herbivorous and omnivorous fish, mainly carps, catfish and tilapia, or producing aquatic plants such as lotus, water mimosa and water spinach for human nutrition. There is also production of fish seed or fingerlings and aquatic plants, especially duckweed, to feed livestock and herbivorous fish (Costa-Pierce et al., 2005)

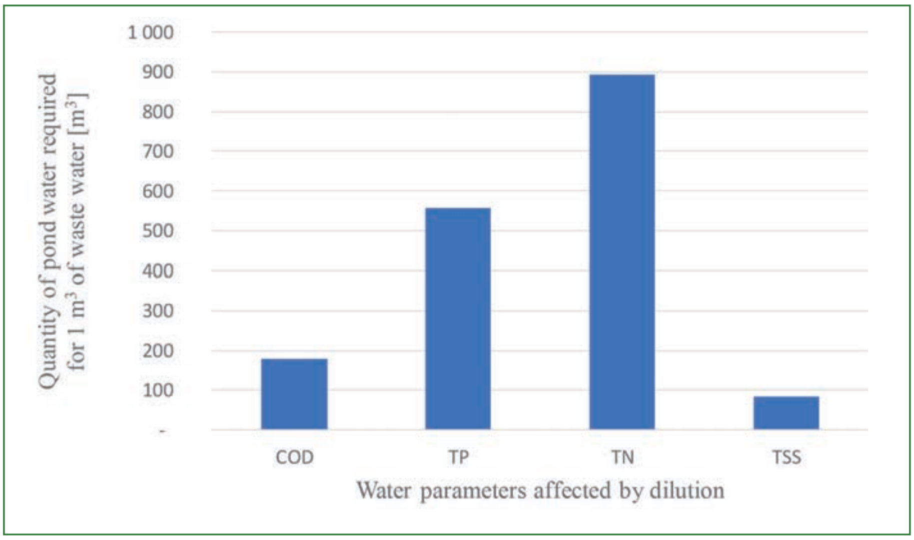

Figure 2: Dilution ratio [m3 pond water/ m3 wastewater] for wastewater used for pond fertilization

WATER QUALITY PARAMETERS DURING WASTEWATER TRAETMENT

Influent wastewater to Yen So Wastewater Treatment Plant (YS-WTP), Vietnam, Hanoi is arriving from a combined sewer system, collecting rainwater and wastewater. Budapest's largest wastewater treatment facility, the Budapest Central Wastewater Treatment Plant (BC-WTP) receives separated wastewater from the city's closed pipe network, with negligible infiltration.

These levels of contaminants in the wastewater of Hanoi are still way over most fish species used in aquaculture, for example the most tolerant species, tilapia can tolerate 7,41 mg/l for 48 hours (Benli et al., 2005). The wastewater-based fish production, might happen in any feasible water body, including ponds and channels, but based on those data direct effluent is not appropriate for fish production; therefore, fish sold as wastewater-raised are more probably originated from partially treated or mixed streams.

Wastewater from Budapest is even more concentrated than from Hanoi and cannot be recycled directly for aquaculture. Considering the well-known and established practice of pond fertilisation, in an pond with an average depth of 1.2 m, applying an average of 150 kg/ha of swine manure, with an average solids content of 22%, 5.2 g/kg nitrogen, 1 g/kg phosphorus and 245 g/kg organic matter, it is observed that the water produced by pond fertilisation contains much less contaminants than raw wastewater (Table 1.).

Figure 2 shows the dilution rate required to achieve similar contamination, especially for the plant nutrient composition used for springtime plant fertilization, based on the last two data columns in Table 1.

CONSUMER AND COMMERCIAL ATTITUDES

A survey in Ghana found that two-thirds of respondents were not concerned about the source of the fish in their diet - whether the catfish was reared in a traditional fish

Figure 3: Clarifier water surface disturbed by catfish, (Own photo, Vietnam, 2023)

farm or in a wastewater system did not influence their purchasing decisions. Factors such as price and taste played a more significant influence on purchase decisions. The results of the survey revealed that if the catfish was reasonably priced and if the respondents lived close to a water treatment plant, they preferred to purchase catfish raised in sewage. This suggests that consumers with more information about the water treatment process and the facility are more comfortable consuming fish (Suzette et al., 2021).

In Hungary, fish have been observed swimming up from the recipient into the effluent discharge of wastewater treatment plants, and fishermen often catch perch around the South-Pest (Budapest, Hungary) effluent, apparently undeterred by any taste or odour pollutants.

During extensive commissioning in the PRC and Vietnam, we have experienced that fish are farmed in clarifiers and even in aerobic reactors, that are inaccessible from external water bodies, making unintentional migration impossible (Fig.3.). The species identified as channel catfish (*Ictalurus punctatus*). The fish were cleaned and consumed, the operators reported they were not affected by any disease or parasite infection.

While fish and humans have numerous parasites in common, such as roundworms and tapeworms, Coliform and Salmonella bacteria infections represent a potential hazard and can have potential issues during farming or processing, thoroughly disinfected wastewater can be a significant way to provide nutrients to fish ponds - while also providing wastewater treatment (Fig.4.). Obviously,

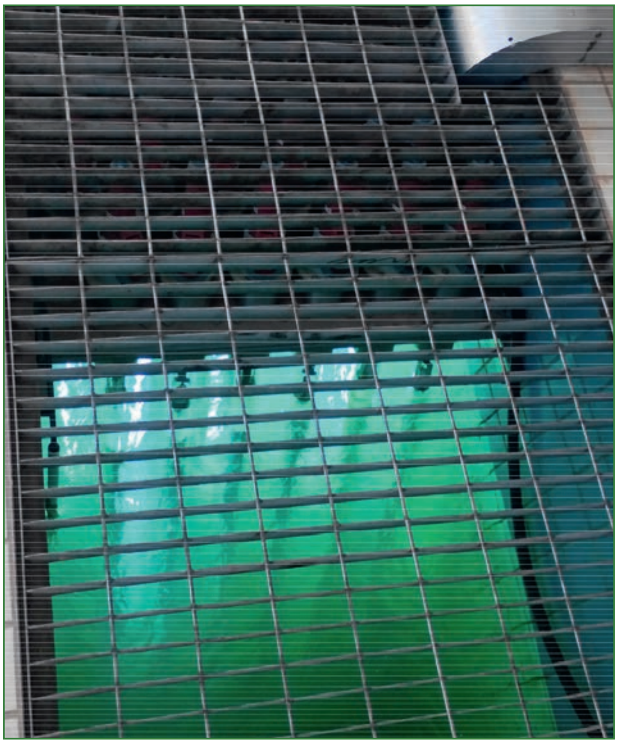

Figure 4: UV disinfection system in WTP effluent channel (Own photo, Budapest, 2023)

nutrient level monitoring is required and additional nutrient replenishment may be required, but these applications have their own advantages, especially in locations below the moderate climate belt where summer wastewater production is increased due to increased population - a practice that is widely used in subtropical and tropical climates.

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