ISSN : 1648–6897

# Journal of Environmental Engineering and Landscape Management

Volume No. 32
Issue No. 1
January - April 2024

ENRICHED PUBLICATIONS PVT. LTD

JE-18, Gupta Colony, Khirki, Extn, Malviya
Nagar, New Delhi-110017
PHONE : - + 91-8877340707
E-Mail : info@enrichedpublications.com

ISSN : 1648–6897

# Journal of Environmental Engineering and Landscape Management

## Aims & Scope

The Journal of Environmental Engineering and Landscape Management is a peer reviewed journal for the publication of original research on the environment with emphasis on changes caused by human activities and international research towards sustainability.

The scope is multidisciplinary and international and the subjects covered include:

atmosphere and air pollution control and pollution mitigation technologies,
water pollution control and pollution mitigation technologies,
soil pollution control and pollution mitigation technologies,
entrails of the earth and their protection,
waste management including zero-waste technologies,
environmental expertise,
control over physical and technological environmental impacts and reduction technologies,
nuclear hydro physics and radioactive pollution,
modelling environmental processes,
environmental monitoring,
landscape protection,
environmental ethics,
environmental law and management,
quality of indoor and outdoor environments

In addition, it publishes short reports and observations, reviews and bibliography, reports on conferences and workshops.

Publication of the Journal is funded by the Ministry of Education and Science of the Republic of Lithuania.
The journal is acknowledged by The Lithuanian Academy of Sciences and The International Academy of Ecological and Life Protection
Sciences (IAELPS), which is an associate member of the Public Information Department of the United Nations.

ISSN : 1648–6897

# Journal of Environmental Engineering and Landscape Management

ISSN : 1648–6897

# Journal of Environmental Engineering and Landscape Management

(Volume No. 32, Issue No. 1, January - April 2024)

## Contents

# ROLE OF THERMODYNAMIC PROCESSES IN PLANT LEAF GAS EXCHANGE SYSTEM FOR ASSIMILATION OF CO2 EMISSIONS FROM THE AMBIENT AIR

**Algimantas SIRVYDAS1, Tomas ŪKSAS2*, Paulius KERPAUSKAS1, Rasa ČINGIENĖ3**

1Experimental Station of Vytautas Magnus University Agriculture Academy, Kaunas, Lithuania

2Department of Mechanical, Energy and Biotechnology Engineering, Vytautas Magnus University Agriculture Academy, Kaunas, Lithuania

3Department of Agricultural Engineering and Safety, Vytautas Magnus University Agriculture Academy, Kaunas, Lithuania

## A B S T R A C T

*When temperature in the leaf gas exchange system changes, the thermodynamic parameters describing the condition of moist air also change. A temperature change of 1 oC in plant leaf tissues leads to a change in partial water vapour pressure of 144 Pa in the gas exchange cavities. Then a temperature decrease of 1 oC in a plant leaf produces 0.897 g of condensate, from 1 m3 of air in leaf ventilation cavities on the surface. When the temperature of plant leaves in the leaf ventilation system changes, the total water vapor state on the inner surface of the leaves changes, and the water vapor state in the stomatal cavities changes. The thickness of the formed condensate film on the plant leaf canal wall surfaces depends on the canal diameter and temperature change. The paper presents information about the mechanism of water formation and thermodynamic processes in the plant leaf gas exchange system participating in the process of assimilation. The formation and change of the internal surfaces of the stomatal cavities of the water film sheet allow the participation of chemical processes in the assimilation of CO2 emissions from the environment.*

***Keywords: CO2 assimilation process, thermodynamics of a plant leaf, leaf gas exchange system, leaf.***

## INTRODUCTION

The achievements of mankind in the field of new technologies have essentially changed living conditions on our planet and set new requirements for man himself. The current anthropogenic changes occurring in nature is the result of human activity and at the same time one of the greatest challenges to humankind. In nature they take place in all fields of human activities (Baltrėnas et al., 2008; Stravinskienė, 2009; Nobel, 1991; Zhang, 2015). The heaviest environmental anthropogenic pollution is caused by the burning of fossil fuels (coal, oil) in energy when heat, mechanical energy and electricity are produced. The anthropogenic pollution is a complex global problem encountered in all research fields while developing new technologies harmless to human health and preserving nature and its biological variety (Martin & Henrichs, 2010; Butkus et al., 2014; Shujie et al., 2015). In this respect it is particularly important to abate environmental pollution with CO2 gas and produce biomass as the result of the energy efficiency of the assimilation process.

No process in the physical world and in life can occur without movement. Therefore, the movement of $CO_2$ and metabolites between the plant leaf and the environment in the process of assimilation is only possible when a driving force is working. To create the driving force, energy is required. In a plant leaf, the share of the absorbed solar energy representing 98 to 96% is transformed into energy in the form of heat. Due to a small mass and biologically limited maximum temperature (58 °C) of their tissues thin plant leaves are not able to accumulate released heat. Therefore, the solar energy transformed into heat in plant leaves has to be released, in the form of heat and water vapour, into the environment as a metabolite. In the process of plant energy exchange with the environment low-value and low-potential heat released by plants into the environment continues to participate in the cycles of water and air circulation and forms climatic conditions on Earth. Scientists have long been interested in thermodynamic processes in plants (Stern, 1933), because the energy metabolism of the plant together with the assimilation process forms an important natural creation-restoration system, creating conditions for the existence of life on the Earth (Sirvydas et al., 2011a). In terms of thermodynamics, the plant is a bad user of the solar heat energy provided, in the form of rays, for the synthesis of organic compounds. Theoretically, the utilisation rate of the solar energy absorbed by plants for the production of organic matter could represent around 20 to 25%. In practice, only about 1 to 2% of the absorbed solar energy is used for photosynthesis (Šlapakauskas, 2006; Fitte & Hay, 2002). From another standpoint, the plant leaf is a unique, cheap and particularly complex laboratory. The plant is capable of utilising non-concentrated radiant energy from the Sun, creating organic matter, utilising the major part of technological pollutants emitted into the atmosphere, and supplying oxygen to the environment by creating conditions for life on Earth. Over a year the globe's vegetation assimilates around 640 billion tonnes of carbon dioxide and releases around 500 billion tonnes of free oxygen, thus reducing environmental $CO_2$ emissions, and transpires around 65 200 $km^3$ of water (Brazauskienė, 2004).

Plants exist in nature as open systems which uninterruptedly conduct metabolism and the exchange of energy with the environment. In order to ensure the exchange of $CO_2$ and metabolites during the productive stage of the assimilation process, it is particularly important to create and maintain intensive driving forces in the leaf ventilation system. The anatomical structure of a plant leaf suggests that complex thermodynamic and hydrodynamic processes take place in the plant leaf ventilation system. These processes can either stimulate or suppress biological processes in the leaf. The plant leaf ventilation system consists of leaf stomatal cavities a leaf surface area of 1 $mm^2$ from 50 to 400 units with nano, micro and mini channels in the mesophile (Šlapakauskas, 2006). Processes taking place in nano-, micro- and mini-channels are relevant in all fields of natural and technological sciences (Sirvydas et al., 2011a; Sajith et al., 2011). Research into these channels focuses on the properties of energy transporting fluids (Ide et al., 2007), their velocities in the channels (Sobhan & Garimella, 2003), the dependence of the internal boundary layer on channel parameters and (Sajith et al., 2011) heat exchange (Sobhan & Garimella, 2001; Boye et al., 2006). We have not found any data about thermodynamic processes taking place in biological and technical nano-, micro- and mini stomatal channels.

During a long way of its evolution the plant has, to the maximum extent, adapted to the natural conditions of its habitat. The anatomical structure of plant organs has maximally adapted to the biological processes inside them and environmental physical factors of the habitat. Plant leaf temperature and the difference of temperatures between the plant and the environment are those factors which can generate (or intensify) forces responsible for exchange between the plant and the environment (Sirvydas et al., 2011b). The leaf gas exchange system consisting of stomata, mini-, macro- and nano-channels forms among the spongy tissue cells in a plant leaf. The leaf gas exchange system takes a large surface area of mesophyll cells by which they have direct contact with air circulating in the gas exchange system. The ambient air is the main supplier of $CO_2$ for the process of assimilation. 90 to 95% of $CO_2$ together with the ambient air enters the spongy tissue of the leaf through open stomata; plant metabolites ($O_2$ and water vapour) are released into the environment also through open stomata (Šlapakauskas, 2006; Nobel, 1991). During the sunny day, a thermal stomata engine (the biological prototype of a heat engine) works in a plant leaf and generates mechanical energy at the expense of heat which intensifies the process of assimilation by activating leaf energy and gas exchange with the environment (Ūksas et al., 2016).

In the gas exchange system of a plant leaf the physical processes of heat and mass exchange with the environment take place through stomata. In leaf cavities the cell walls of ventilation system surfaces have contact with two different environments. From one side the cell wall has contact with the physical-gaseous environment (Co2 gas included), from the other side the cell membrane has contact with the cytoplasm environment of biochemical state. In the plant cell cytoplasm membranes have the selective permeability of dissolved materials and regulates their movement between the environment and the cell. It is evident that the physical processes occurring in a plant leaf as well as $CO_2$ gas transformations from the gaseousphysical state to the biochemical state have been insuff iciently examined (Šlapakauskas, 2006; Sirvydas et al., 2014). Quite different information is presented about the role of the anatomical structure of a plant leaf in the processes of gas ($CO_2$, $O_2$, $H_2O$ vapour) and energy exchange between the leaf and the environment (Wang et al., 2010). The purpose of the research is the analysis of the plant leaf gas exchange process in order to elucidate the mechanism of formation of aqueous medium in the leaf gas exchange system, $CO_2$ and $O_2$ gas transition from gaseous (physical) to biochemical (through chemical) and vice versa

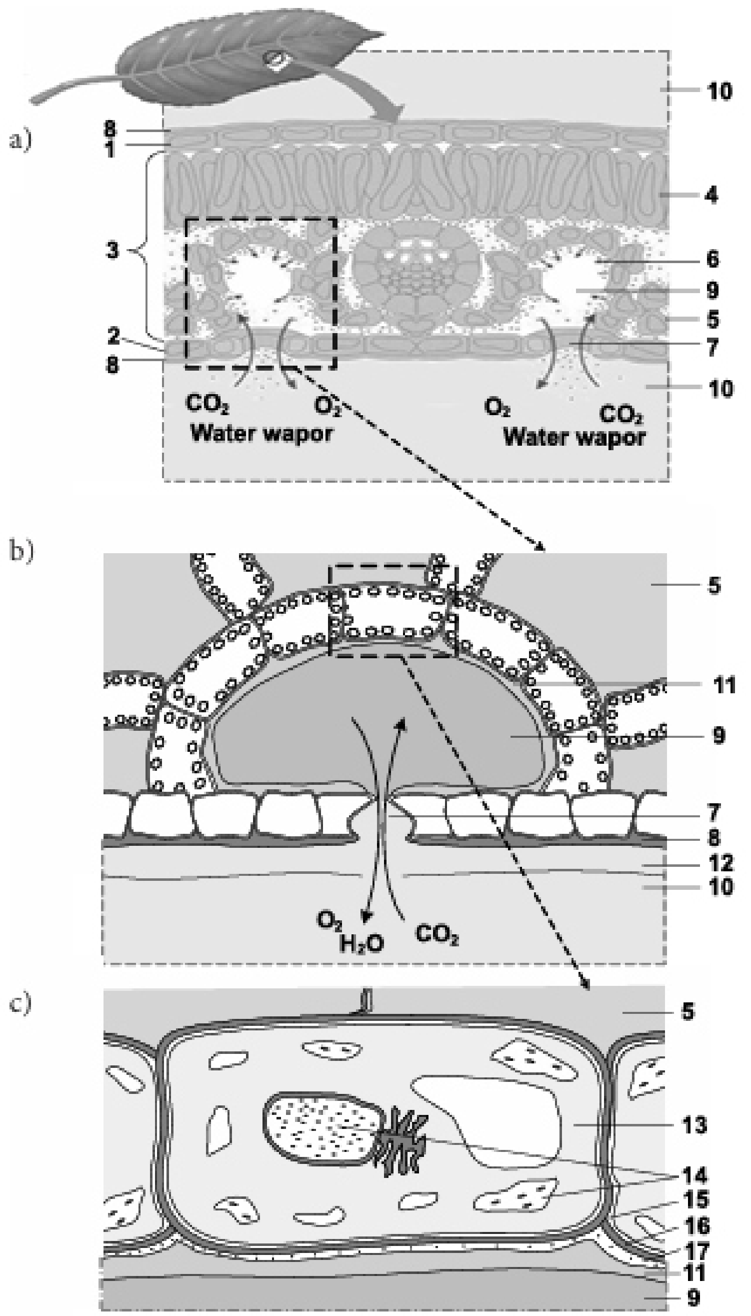
## 1. The structure and thermophysical model in the plant leaf gas exchange system

For bionic purposes plant leaf models are used to analyse the principles of vital activity in the leaf (Ye et al., 2013; Yuan et al., 2014; Sirvydas et al., 2013). Heat and mass exchange between the gaseous environment and the surfaces of a leaf occurs through the layer of transitional regimen which is also known as a internal boundary layer. Gas parameters therein gradually change from the parameters of the gaseous environment to those of the surface participating in the exchange (Sirvydas et al., 2011a; Incropera & DeWitt, 2001). This trend of physical processes applies to the surfaces of a plant leaf and other organs of the plant. The processes of heat and mass exchange with the environment in a plant leaf occur on the external and internal surface of a leaf. The process of assimilation involves both surfaces of a plant leaf with different environments and internal boundary layers of gaseous environments in which different heat and mass exchange processes occur.

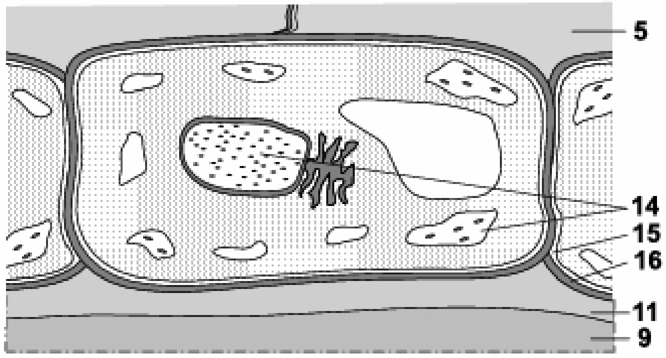
The upper epidermis (1) and the lower epidermis (2) with the cuticle (8) cover the external surfaces of a plant leaf from the top and the bottom (Figure 1). 5 to 10% of $CO_2$ used in the process of assimilation diffuses through the epidermis and cuticle. The epidermis with cuticle nearly does not allow water vapour and gas to pass through (Šlapakauskas, 2006; Nobel, 1991). Consequently, the internal boundary layers of $CO_2$, $O_2$, $H_2O$ vapour and other gas concentrations on the external surfaces of a plant leaf are unlikely to form. The external surfaces of a leaf participate in heat exchange with the environment; therefore they have the internal boundary layer of temperature on their surfaces (Šlapakauskas, 2006; Boye et al., 2006).

The internal surface of a plant leaf is in the spongy mesophyll layer of a leaf (5) (Figure 1a). The leaf gas exchange system of stomata (7), mini-, macro- and nano-channels is among the spongy tissue cells in a plant leaf. That forms a conditionally large contact cell area on the internal surface which has direct contact with the air circulating in the gas exchange system's mesophyll (9). The ratio between the area of the internal leaf surface (leaf ventilation system) and external leaf surface of plants exposed to shadow reaches 6.8–10.0, that of mesophytes – 11.0–20.0. In addition, the internal surfaces of a plant leaf evaporate the largest amount of water (80–98%) and release it into the environment through stomata (leaf ventilation system) (Šlapakauskas, 2006; Nobel, 1991).

The formation of the internal boundary layer of the leaf internal surface (Figure 1) is difficult to understand and experimentally immeasurable. It is natural because gas exchange processes take place in the system of leaf mini-, macro- and nano-channels, in the internal boundary layer (11) of leaf cell membrane (15) contact with gaseous environment (9). Metabolism and energy exchange on these cell surfaces take place when respective gradients participate. Engineering research in mini-channels shows the existence of the temperature internal boundary layer



d)




1 – upper epidermis; 2 – lower epidermis; 3 – mesophyll; 4 – palisade tissue; 5 – spongy tissue; 6 – intercellular duct with the system of nano-, micro- and mini-channels; 7 – stoma; 8 – cuticle; 9 – gaseous environment in the spongy tissue of a leaf; 10 – ambient air around the leaf environment; 11 – internal boundary layer of the internal surfaces of a leaf; 12 – internal boundary layer of the external surface of a leaf; 13 – cytoplasm; 14 – cell organoids; 15 – cell membrane; 16 – cell wall; 17 – water vapour condensate formed on the external surface of a leaf .

Figure 1. a – Chart of the plant leaf anatomical structure and gas exchange with the environment; b – Model of the internal boundary layer of the internal and external surfaces of a leaf (CO2, O2, H2O vapour); c – Model of the internal boundary layer of the internal surface of a leaf when water condensate forms on a cell surface in the period of temperature drop; d – Model of the internal boundary layer of the internal surface of a leaf when water condensate evaporates from a cell surface in the period of temperature rise (Sage & Monson, 1999; PirastehAnosheh et al., 2016)and its dependence on the physical parameters of channels and fluids (Sajith et al., 2011; Sobhan & Garimella, 2001; Boye et al., 2006). Therefore, the internal boundary layers of temperature, CO2, O2, H2O vapour and other gas concentrations may occur in the internal boundary layer of plant leaf internal surfaces (11). The internal internal boundary layer of a leaf participates in the process of Co2 assimilation and is distinguished by a large number and variety of processes occurring inside it. In the internal boundary layer (11) of the leaf ventilation system CO2 gas transits from the physical state to the biochemical level of a cell. A direct transition of CO2 gas from the physical state to the biochemical level of a cell is unlikely. It is realistic that this CO2 transition from the physical level to the biochemical level of a cell occurs with the help of chemical process. It is therefore necessary to analyse the thermodynamic processes in the internal boundary layer of the contact of plant leaf internal surfaces with the gaseous environment when plant leaf temperature changes.

## 2. The methods of research

The main object of research was research into the processes of exchange between the plant and the environment by applying the method of idealisation. This method involves the idealisation of the energy of biological processes occurring in a plant leaf as a means of discovering physical processes occurring in the plant leaf gas exchange system (in stomata with nano-, micro and mini-channels in the mesophyll) and in the layer of plant exchange with the environment during daylight hours when plant leaf temperature changes.

The temperature of a plant growing in natural environmental conditions was measured by thermocouple temperature sensors made of copper-constantan wires, 0.05 mm in diameter (Figure 2). The measurements were recorded with an instrument having a microprocessor data processing and accumulation system. Temperatures were recorded by necessity taking a maximum of 100

measurements per second.
Temperature sensors for all temperature measurements were used in observance of the requirements to be met by temperature measurements (in respect of the plant and its environment) (Sirvydas et al., 2006). The reliability of

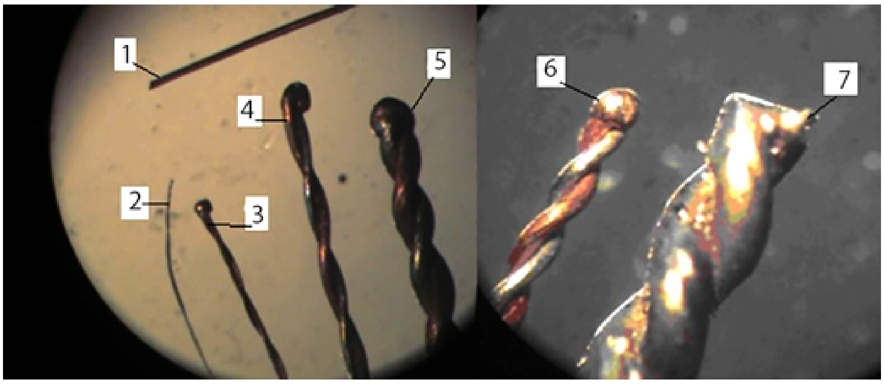



**Figure 2.** Individually manufactured temperature measuring sensors of various thicknesses: 1 – human hair 0.04 mm; 2 – temperature sensor 0.005 mm; 3 – sensor 0.03 mm; 4 – sensor 0.07 mm; 5 – sensor 0.18 mm; 6 – sensor 0.3 mm; 7 – factory sensor 0.45 mm

temperature measurements was evaluated by the describing the spread of values reasonably attributable to the temperature value concerned. The obtained total uncertainty of temperature measurements is 0.042 oC and relative uncertainty of temperature estimate – 0.07%, which to the confidence probability of 99% and demonstrates sufficient accuracy of temperature measurements.

Measuring the local temperature of a heat-impermeable 1mm thick plant leaf plate is an extraordinary problem. It is not possible to measure the temperature at the apex of a plant leaf with a leaf surface area of up to 100 units / mm2. The thermometer (sensor) does not show the temperature of the measured point, but the totality of its heat exchange with the environment.

**3. Results of investigation**

The local temperature of a plant leaf shows the total result of biological and energy exchange processes occurring in a leaf. Intensive metabolism and energy exchange in satisfying the needs of the assimilation process occur in a plant leaf during daylight hours. The satisfaction of the biological and physical environment needs of the assimilation process also depends on the processes taking place in the ventilation system of a plant leaf. We present the experimental measurement results of the temperature of a plant leaf and its surrounding which are useful for the analysis of thermodynamic processes in the plant leaf ventilation system. In natural environmental conditions during daylight hours air temperature around the plant is continually changing and chaotically pulsating (Figure 3). Air temperature pulsations are created by the vertical airflows of unequal temperature in a phytocenosis and the temperature fields of energy exchange in plant organs. Changing temperature around the plant (Figure 3), together with the change of the balance of plant energies, manifests itself in the local temperature through the process of plant leaf thermal accumulation. As theoretical studies show, a changing balance of energies in the plant can lead to variations in the local temperature of plant leaves (Sirvydas et al., 2011a). The measurements

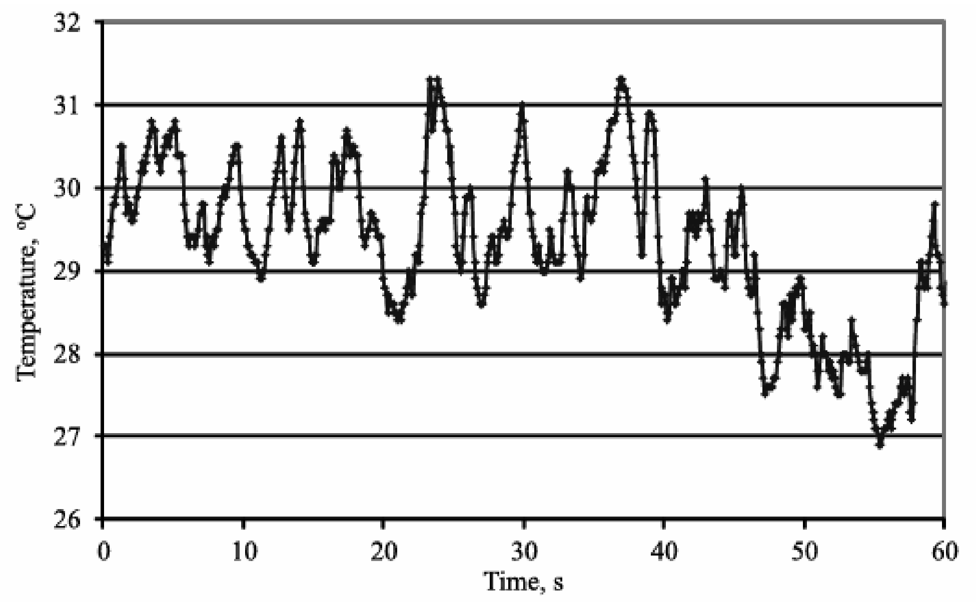



**Figure 3.** Temperature fluctuations in the air around the plant during the sunny period of the day under natural environmental conditions. Wind speed vm = 1.1 m/s

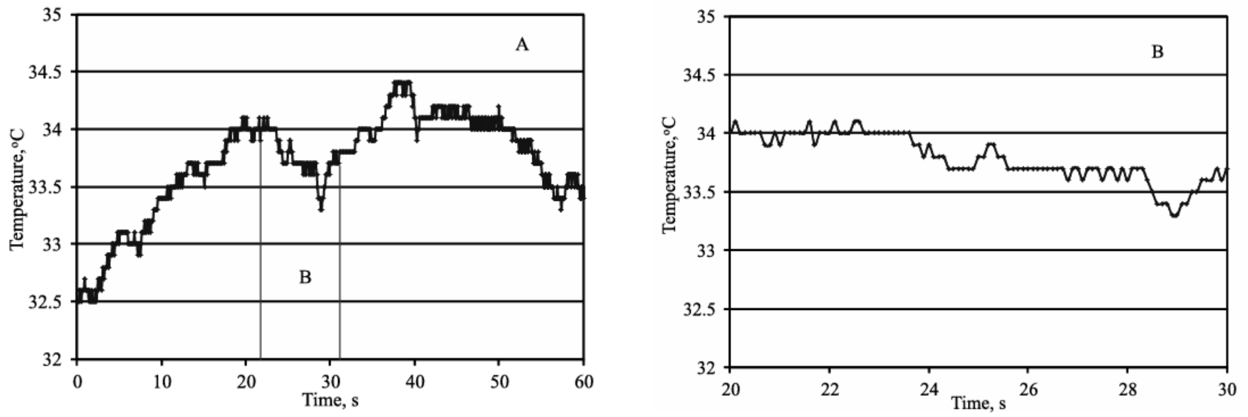



**Figure 4.** Temperature fluctuations in a plant leaf during the sunny period of the day under natural environmental conditions. Wind speed vm = 1.1 m/s

of temperature around a plant leaf during daylight hours in natural environmental conditions are presented (Figure 4). A large quantity of reports on plant leaf temperature measurement (Figure 4a) did not highlight the thermal accumulation process of a plant leaf. Upon changing the timescale, a change in plant leaf temperature within an interval of 10 s is shown in Figure 4b.

Figure 4 the experimental plant leaf temperature's pulsations under natural environmental conditions are confirmed by our previous theoretical studies of the plant leaf energy balance equation and theoretical modeling of the processes. We present a theoretical equation for the energy balance of the plant leaf that allows to examine the temperature pulsations Δt in the plant leaf by estimating environmental and plant biological factors (Sirvydas et al., 2011b).

$$\Delta t = \left(2\left(t_0 - t_{apl}\right) + \frac{wr}{\alpha}\right)\left(1 - \exp\left(\frac{-\alpha S}{\rho c \delta} \cdot \tau\right)\right).$$

Temperature pulsations in the plant leaf occur during the processes of thermal accumulation of leaf tissues and heat exchange with the environment.

## 4. Discussion

When leaf temperature changes other parameters describing the state of gas in the leaf gas exchange (ventilation) system also change. Gases (CO2, O2, N2 and others) in air content are in the state of superheated gas. In natural environmental conditions this gas in superheated state does not change the aggregate state in the leaf ventilation system; it remains superheated all the time. In natural environmental conditions water vapour in the plant leaf gas exchange system may change not only its aggregate state (gaseous↔liquid) but also the thermodynamic state of water vapour (moist↔saturated↔superheated vapour). Therefore while analysing the thermodynamic processes occurring in the system of plant leaf gas exchange (ventilation), it is necessary to observe the aggregate state of H2O and evaluate the state of water vapour. In natural environmental conditions the plant leaf exchange may have all possible forms of H2O (solid, liquid and gaseous) and states of water vapour (moist, saturated and superheated gas states in a mixture of gases). The evaluation of the water vapour state during leaf gas exchange is difficult as it requires complex observations and compatibility between the biological and thermodynamic processes taking place in the internal boundary layer of the leaf internal surface. Thermodynamic processes in the mixture of gases present in plant leaf ventilation cavities depends on the partial pressure of water vapour. When plant leaf tissue temperature changes, other parameters of the mixture of gases participating in the exchange also change. During daylight hours when leaf energy exchange with the environment takes place the change of the thermodynamic parameters of gases in the leaf gas exchange system first occurs in the internal boundary layer of gas contact with the surface. Since the leaf ventilation system consists of mini-, macro- and nano stomatal channels, changes in the state of water vapour occur in the internal boundary layer of the surface of plant leaf cell contact with the gaseous environment. Experimental research into thermodynamic parameters at the plant leaf cell level is not possible and we present, therefore, the theoretical analysis of thermodynamic processes occurring in the plant leaf gas exchange system by taking into account the thermodynamic trends of gas mixtures and water vapour.

During daylight hours when plant leaf temperature changes water vapour in the leaf gas exchange (ventilation) system changes its aggregate state and water vapour state j. That depends on the process of plant leaf temperature change. During the period of temperature rise the sequence of change of parameters for the H2O gas component of the mixture of gases in the gas exchange system is: liquid → moist → saturated (j = 100%) → superheated vapour (j < 100%). During the period of leaf temperature drop the sequence of H2O component parameter change is reverse. Essential changes in leaf energy exchange processes occur during these processes. The aggregate state of water changes, which leads to a change in volume of by around 1700 times. The values of heat transfer coefficient change by up to 1000 times.

For the numerical analysis of thermodynamic processes occurring in the plant leaf gas exchange system we assume that temperature in leaf cavities changes by up to 1 °C (19–20) °C. Based on the thermodynamics laws of moist air we state that when the temperature of plant leaf tissues decreases the process of moisture condensation on the surface of leaf gas exchange (ventilation) system walls will take place. Condensate will precipitate and moisten the surfaces of ventilation system cavities. A decrease of 1 oC in plant leaf tissue temperature will lead to a decrease in the partial pressure of water vapour in ventilation cavities by quantity Δpg. The partial water vapour pressure in the state of full saturation at 20 °C is equal to 2337 Pa. Partial pressure at 19 °C is 2196 Pa. When plant leaf temperature changes by 1 °C, partial water vapour pressure in leaf ventilation cavities changes by quantity Δpg = p20 °C – p19 °C = 2337 – 2196 = 141 Pa. Once change in partial water vapour pressure Δpg in the leaf ventilation system has been identified, the amount of condensate precipitation on the surfaces of leaf ventilation channels at a pressure of 98.1 kPa is determined. A change of 1 °C in plant leaf temperature produces 0.99 g of condensate from 1 m3 of air on the surface of the walls in leaf stomatal channels ventilation cavities.

Further, we will analyse the dependence of the surface area of the plant leaf ventilation system on its volume. The system of leaf ventilation nano-, micro- and minichannels is complicated. For the purpose of simplicity, we assume that the process of condensation takes place in ventilation cavities having a shape similar to a ball. The calculation data presented in Figure 5 show that the cavity volume (curve 1) increases more rapidly than the surface area (curve 2). This pattern shows that when the volume of plant leaf ventilation cavities increases (cavity diameter increases), thickness δ of the film of condensate having formed on cavity surfaces increases.

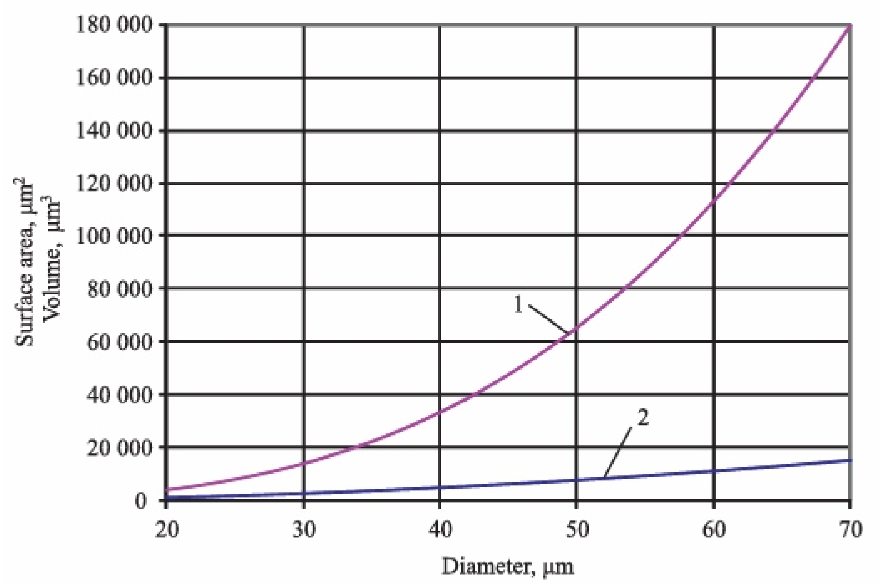



**Figure 5.** Dependence of the volume of cavities in the plant leaf ventilation system (curve 1) and the surface area of its walls (curve 2), on the channel diameter

Now, we determine the dependence of condensate film thickness δ on the volume of ventilation cavity. To that end, we assume that condensate uniformly distributes on the surface of ventilation cavities and forms a water condensate film of uniform thickness δ. Then, the easiest way to determine the dependence of condensate film thickness δ in ventilation cavities on cavity is to apply the ratio of cavity volume V to its surface area S. The thickness of condensate film for ball-shaped cavity δ = 0.149·10–6d. For cylinder-shaped channel δ = 0.224·10–6d. As we can see, the ratio of the volume of leaf ventilation channel cavity to its surface area V/S depends on cavity diameter d. The amount of condensate precipitated on cylindrically-shaped leaf channels is by 0.224/0.149 = 1.50 times thicker than that on ball-shaped channels. The thickness of the condensate film formed on the surface of plant leaf ventilation channel walls depends on cavity volume and temperature changes in plant leaf tissues and increases when they increase (Figure 6).

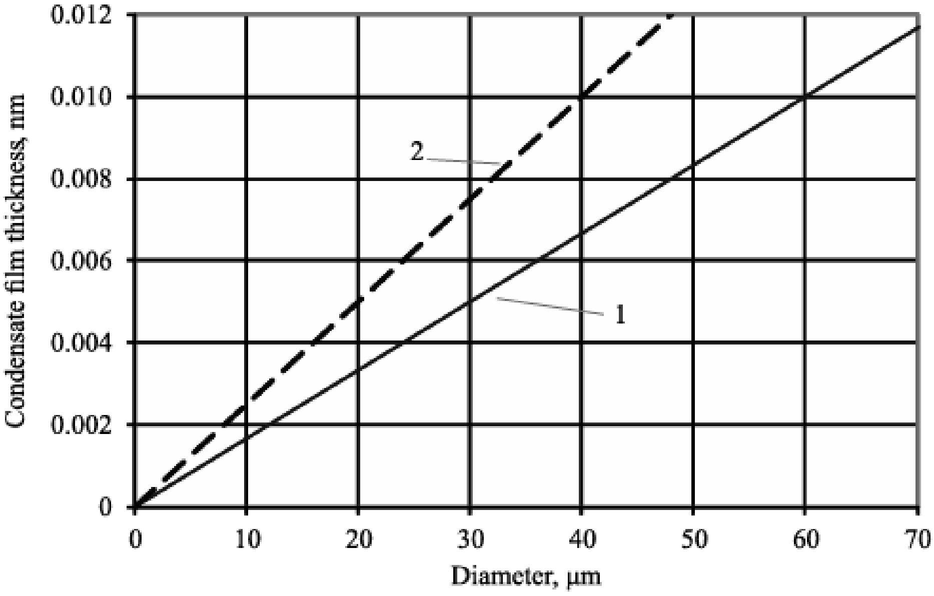



**Figure 6.** Dependence of condensate film thickness on the surfaces of cavity in the plant leaf ventilation system on the cavity diameter: curve 1 for a ball-shaped cavity; curve 2 – for a cylindrically-shaped channel when temperature changes by 1 °C

The thermodynamic analysis of the processes of formation of water medium on the internal surfaces of a leaf and gas exchange in the mini-, macro- and nano-cavities of a plant leaf has shown the following trends of leaf vital activities:

The CO2 assimilation process in a plant leaf involves leaf's internal and external surfaces of different biological purpose with different internal boundary layers and different heat and mass exchange processes. When plant leaf temperature changes in the mixture of gases in the cavities of the leaf ventilation system, water vapour changes its aggregate state (gaseous↔liquid) on the internal leaf surface and the state of water vapour (moist↔saturated↔superheated vapour) in the cavities.

During daylight hours when leaf energy and mass exchange with the environment takes place in a leaf the change of the thermodynamic parameters first occurs in the internal boundary layers of gas contact with leaf surfaces. The essential changes in leaf energy and gas exchange processes occur when the aggregate state of water vapour and the state of water vapour change. They are seen in the cavities of the leaf internal surface, the internal boundary layer of the surface of contact of plant leaf cell with the gaseous environment.

In the internal internal boundary layer of a leaf Co2 gas transits from the physical state to the biochemical level of a cell. A direct transition of CO2 and O2 gases from the physical state to the biochemical level of a cell is unlikely. It is realistic that the process of change of the water vapour aggregate state (gas↔liquid) participates in the exchange of CO2 and O2 gases between the physical state and the biochemical level of a cell in leaf gas cavities. The formation of water films on the internal surfaces of leaf cavities enables chemical processes to participate in CO2 and O2 exchange.

**Conclusions**

1. Under natural environmental conditions, on a sunny day, the air temperature washing the plant leaf and plant is chaotically pulsating.

2. As the leaf temperature of the plant changes, the water vapor in the leaf gas exchange (ventilation) system changes the physical state and condition parameters.

3. When the temperature of the plant leaf changes by 1 °C, 0.99 g of condensate falls on the surface of the walls of the leaf in the ventilation ducts of 1 m3 of air.

4. A film of water condensate forms on the surface of the ventilation duct walls of the plant leaf. It depends on the change in cavity volume and temperature in the plant leaf tissues.

5. The water vapor codensate formed on the membrane's cell surfaces in the leaf's ventilation channels enables chemical processes to participate in the exchange of $CO_2$ and $O_2$ gases from the physical state to the biochemical level of the cell.

**References**


• *Baltrėnas, P., Butkus, D., Oškinis, V., Vasarevičius, S., & Zigmontienė, A. (2008). Environmental protection. Technika.*

•*Boye, H., Staate, Y., & Schmidt, J. (2006). Experimental investigation and modeling of heat transfer during convective boiling in a mini-channel. International Journal of Heat and Mass Transfer, 35, 1237–1248.*

• *Brazauskienė, D. M. (2004). Agroekologija ir chemija. Naujasis lankas.*

• *Butkus, D., Lukšienė, B., & Pliopaitė-Bataitienė, I. (2014). Radio*

•*nuclides in plants. Vilniaus Gediminas Technical University. Fitte, A., & Hay, R. (2002). Environmental physiology of plants. Academic Press.*

•*Ide, H., Kariyasaki, A., & Fukano, T. (2007). Fundamental data on the gas–liquid two-phase flow in minichannels. International Journal of Thermal Sciences, 46(6), 519–530. https://doi.org/10.1016/j.ijthermalsci.2006.07.012*

•*Incropera, F. P., & DeWitt, D. P. (2001). Fundamentals of heat and mass transfer (5th ed.). Wiley.*

•*Martin, J., & Henrichs, T. (2010). The European environment. European Environment Agency.*

•*Nobel, P. S. (1991). Physiological and environmental plant physiology. Academic Press.*

•*Pirasteh-Anosheh, H., Saed-Moucheshi, A., Pakniyat, H., & Pessarakli, M. (2016). Chapter 3: Stomatal responses to drought stress.*

•*John Wiley & Sons. https://doi.org/10.1002/9781119054450.ch3 Sage, R. F., & Monson, R. K. (1999). C4 plant biology (1st ed.). Academic Press. https://doi.org/10.1016/B978-0-12-614440-6.X5000-9*

•*Sajith, V., Haridas, D., & Sobhan, C. B. (2011). Reddy Convective heat transfer studies in macro and mini channels. International Journal of Thermal Sciences, 50, 239–249. https://doi.org/10.1016/j.ijthermalsci.2010.04.005*

•*Shujie, M., Yunfa, Q., & Futao, Z. (2015). Conversion of cropland to grassland and forest mitigates global warming potential in Northeast China. Polish Journal of Environmental Studies, 24, 1195–1203. https://doi.org/10.15244/pjoes/33928*

•*Sirvydas, A., Kerpauskas, P., & Kučinskas, V. (2011a). Plant energy exchange. Akademija.*

• *Sirvydas, A., Kučinskas, V., Kerpauskas, P., & Nadzeikienė, J. (2011b). Theoretical modeling of temperature pulsations in plant leaf which are caused by leaf swing with respect to the sun. Journal of Environmental Engineering and Landscape Management, 19, 251–259. https://doi.org/10.3846/16486897.2011.606174*

•*Sirvydas, A., Kučinskas, V., Kerpauskas, P., & Ūksas, T. (2014). The principle of transforming the solar energy to mechanical energy in the plant leaf stomata engine. Energetics, 60, 19–26.*

•*Sirvydas, A., Nadzeikienė, J., Kerpauskas, P., & Ūksas, T. (2013). The principle of heat conversion into mechanical work. Mechanika, 19, 358–362. https://doi.org/10.5755/j01.mech.19.3.4661*

•*Sirvydas, A., Kerpauskas, P., Nadzeikienė, J., Stepanas, A., & Tereščiuk, V. S. (2006). Temperature measurements in research of thermal weed extermination. In Proceedings of the International Conference Development of Agricultural Technologies and Technical Means in Ecological and Energetic Aspects (pp. 321–331), Lithuania, Raudondvaris.*
•*Sobhan, C. B., & Garimella, S. V. (2001). Comparative analysis of studies on heat transfer and fluid flow in microchannels.*
•*Microscale Thermophysical Engineering, 5, 293–311. https://doi.org/10.1080/10893950152646759*
•*Sobhan, C. B., & Garimella, S. V. (2003). Transport in microchannels a critical review. Annual Review of Heat Transfer, 13, 1–50. https://doi.org/10.1615/AnnualRevHeatTransfer.v13.30*
•*Stern, K. (1933). Pflanzenthermodinamik. Berlin. Stravinskienė, V. (2009). Environmental bioindication. Kaunas. Šlapakauskas, V. (2006). Ecophysiology of plants. Lututė. Ūksas, T., Čingienė, R., Kučinskas, V., & Sirvydas, A. (2016, September 7–9). Solar energy conversion in plant leaf stomata as leaf temperature changes. In 6th International Conference on Trends in Agricultural Engineering (pp. 652–657), Prague, Czech Republic.*
•*Wang, S. J., Ren, L. Q., Liu, Y., Han, Z. W., & Yang, Y. (2010). Mechanical characteristics of typical plant leaves. Journal of Bionic Engineering, 7, 294–300. https://doi.org/10.1016/S1672-6529(10)60253-3*
•*Ye, H., Yuan, Z., & Zhang, S. (2013). The heat and mass transfer analysis of a leaf. Journal of Bionic Engineering, 10, 170–176. https://doi.org/10.1016/S1672-6529(13)60212-7*
•*Yuan, Z., Ye, H., & Li, S. (2014). Bionic leaf simulating the thermal effect of natural leaf transpiration. Journal of Bionic Engineering, 11, 90–97. https://doi.org/10.1016/S1672-6529(14)60023-8*
•*Zhang, Y. (2015). On the climatic uncertainty to the environment extremes: A Singapore case and statistical approach.*
•*Polish Journal of Environmental Studies, 24, 1413–1422. https://doi.org/10.15244/pjoes/31718*

# COMPARISON OF REMAINING COAL-BURNING ASH-BASED ON CD, PB, AND HG CONCENTRATION AT DIFFERENT TEMPERATURES: A CASE STUDY IN ACEH PROVINCE

**Asri GANI1, 2, Erdiwansyah ERDIWANSYAH2, 3*, R. E. SARDJONO4, Mariana MARIANA1, 2, Rizalman MAMAT5**

1Department of Chemical Engineering, Universitas Syiah Kuala, 23111 Banda Aceh, Indonesia

2Research Center for Environmental and Natural Resources, Universitas Syiah Kuala, 23111 Banda Aceh, Indonesia

3Faculty of Engineering, Universitas Serambi Mekkah, 23245 Banda Aceh, Indonesia

4Department of Chemistry, Faculty of Mathematics and Science, Universitas Pendidikan Indonesia,
40522 Bandung, Indonesia

5Faculty of Mechanical Engineering, Universiti Malaysia Pahang, 26600 Pahang, Malaysia

## ABSTRACT

*This study aims to investigate the efficiency level of absorption of heavy metals Cd, Pb, and Hg. Combustion is carried out using coal with the addition of absorbent ratios of 2%, 4%, 6%, 8%, and 10%. The adsorbent used is natural zeolite which is widely available and inexpensive. This study provides practical implications for the easy and inexpensive removal of heavy metal emissions during combustion. The results show that the maximum efficiency level for Cd metal reached 22.96% which was recorded at a temperature of 600 °C for an adsorbent ratio of 10%. The maximum efficiency level of Pb metal from the experimental results was obtained at a temperature of 600 °C with an adsorbent ratio of 10% to 10.83%. Meanwhile, the efficiency level for Hg metal produced was 0.05% which was recorded at the adsorbent ratio of 10% at 800 °C. The maximum total capacity of Pb metal for each tested combustion temperature was 600 °C 39.85 mg/kg, 700 °C 25.43 mg/kg, and 800 °C 7.21 mg/kg. On the other words, the higher the combustion temperature tested, the lower the absorption efficiency rate obtained.*

***Keywords: briquets, coal-burning, metal, combustion, efficiency.***

## Introduction

In recent years, haze resulting from burning has become increasingly common, especially in the largest cities around the world. The result of combustion using fossil fuels becomes a major source of emission production as described in several previous publications (Bi et al., 2007; Wei et al., 2018; Xie et al., 2008). The absorption of particulate pollution in most areas of Mainland China has also been investigated by (Wei et al., 2018). Combustion of biomass by municipal incineration can account for 9.8% of the annual mean mass concentration of PM10 as reported by (Xie et al., 2008). Coal is the largest source of electricity generation in the world amid global efforts to reduce the use of coal. The International Energy Agency or IEA stated in 2019 that coal holds 38% of the total share of power generation sources globally (Erdiwansyah et al., 2019b, 2021). The use of coal energy, especially in Indonesia, until 2025 is predictably high as reported in a study by (Erdiwansyah et al., 2019a). Since

2016, global coal production from year to year has continued to increase. Meanwhile, global coal reserves are recorded at more than one trillion tons. The IEA data report shows that total world coal production managed to reach 7.9 billion tons in 2019, higher than in 2014. Thermal and lignite coal account for about 86% of this production and the remainder is metallurgical coal. Indonesia is the fourth largest country in coal production after China, India, and the United States. In 2019, coal production in Indonesia reached 616 million tons, China had 3.7 billion tons, India had 783 million tons, and the United States had 640 million tons (Kementerian Energi dan Sumber Daya Mineral 2016, 2019). Meanwhile, the Chinese Department of Statistics in 2018 reported that China contributed the most to coal production which reached 69.3% and contributed 59% to national energy production (Cheng et al., 2019). The working substances of coal are potentially very dangerous (Shao et al., 2015).
Significantly, particulate pollutants have a major impact on atmospheric chemistry and climate change, especially for human health (Jones et al., 2009; Kan et al., 2012; Pian et al., 2016). Emissions resulting from burning coal in households used for heating and cooking are closely related to burning coal as in China (Li et al., 2012). Coal consumption processing and control systems must be a priority, especially for PM2.5 pollution (Xie et al., 2020).
Investigations regarding the composition of the elements of coal are very important to know geological information related to the environment. Toxic metals and metalloids found in coal can be released to the surface, groundwater, air, and soil during the combustion process (Tang et al., 2009). The toxic elements from coal can evaporate and condense on the ash particles for the energy production process (Yan et al., 2000). In general, cadmium (Cd) can be found in organs, pyrite, clay, and coal carbonate minerals (Yang, 2010; Shi et al., 2018). The Cd contained in coal is up to 80% lower than monosulfide, especially sphalerite. Meanwhile, 20% of Cd is bound to be the same as silicate and pyrite (Finkelman et al., 2018). Fly-ash particles containing Cd vapor obtained during off-gas cooling can be removed by electrostatic deposition (Goodarzi & Huggins, 2001). Mercury (Hg) found in coal can be associated with pyrite, calcite, marcasite, and cinnabar (Diehl et al., 2004; Hower et al., 2005). The toxic element in Hg cannot be broken down and accumulates in the human body so that it can cause the body's organs and nervous system (Tang et al., 2009). Power generation using coal can increase the Hg content of flying ash which can hurt the environment (Dittert et al., 2007). Bottom ash that accumulates from various toxic elements can create consequences for the health of people who live around the power plant area for a long time (Dai et al., 2012).

The purpose of this research was to study the residual ash from burning coal based on metal concentrations with different adsorbent ratios and temperatures. The mini-style zeolite adsorbent was chosen because it is widely available in Indonesia and is cheap and resistant to high temperatures. Selection of the three heavy metals Hg, Cd, and Pb because they are found in coal and are toxic. The adsorbent used is natural zeolite which is widely available and inexpensive. This study provides practical implications for the easy and inexpensive removal of heavy metal emissions during combustion. The experiments in this work were carried out three times with each combustion temperature of 600 °C, 700 °C, and 800 °C. Each experiment used five adsorbent ratios of 2%, 4%, 6%, 8% and 10%. The aim of this test is to determine the level of absorption, absorption, and absorption efficiency of the ratio of the adsorbent with different combustion temperatures. The results of this work can provide a scientific reference in the briquette production process by adjusting pollutants to the atmosphere. However, it also provides a huge potential for insights into the production that is obtained. The results of the comparison of each treatment with different adsorbent ratios and combustion temperatures are a special discussion in this work. The novelty of this research is that the natural adsorbent used can capture heavy metals during the combustion process. In addition, the weight of the natural adsorbent applied for combustion temperatures of 600 °C, 700 °C, and 800 °C can adjust to the given treatment.

## 1. Experimental setup and material

### 1.1. Experimental setup

The experiment used coal from Kaway XVI of West Aceh Sub-District in Aceh Province, Indonesia, while the natural adsorbent (zeolite) was obtained from West Java Province, Indonesia. Coal has low rank if it has high moisture content of 8.83%, sulfur content of 0.38%, and ash of 5.4% ash (db) with calorific value of 5904 cal/gr (Mahidin, 2009). The size of coal and zeolite was crushed and screened to 60 mesh using a crusher and ball mill, then was briquetted using a briquette holding machine without binder, while the rest of the sample was left in pulverized condition. The mercury content in the ash and sulfur were analyzed. The adsorbent was then added with various concentration ratios from 4 to 12 percent of the weight and formed into briquette and pulverized form. The briquette was molded in coal briquette molding equipment with pressure level of 10 ton/cm2 (SNI 047, 2006). Coal was crushed using a crusher until the particle size was homogenized up to 60 mesh. Then it was printed using a coal briquette printer with a pressure of 10 tons/cm2. The size of the coal briquettes was printed with variations in cylinder shape up to 6 in diameter and the same cylinder briquette height was 10 cm. The purpose of milling was to homogenize the size (60 mesh) for better mixing and a more perfect briquetting process. The furnace for the experiment in this study is shown in Figure 1. The specifications of the tools used in this study are shown in Table 1. The test experiments carried out in this work are shown in Table 2.

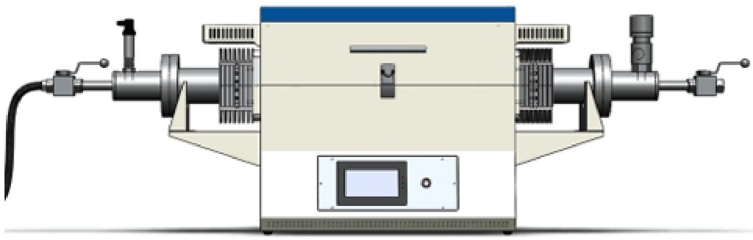


**Figure 1.** Combustion Chamber

**Table 1.** Specifications of tube furnace

| No | Properties | Range |
|---|---|---|
| 1 | Tube material | Ultrahigh temperature alloy |
| 2 | Tube diameter | Φ85 mm |
| 3 | Tube length | 1000 mm |
| 4 | Heating length | 440 mm |
| 5 | Constant heating zone | 200 mm |
| 6 | Sealing method | Pressure flange |
| 7 | Pressure measured | Semiconductor pressure gauge |
| 8 | Over-pressure protective | Safety relief valve |
| 9 | Pressure adjusts | Manual and auto adjustable |
| 10 | Over temperature alarm | Over-temperature power off |

| | | |
|---|---|---|
| 11 | Working temperature | ≦1100 °C |
| 12 | Working pressure | ≦20 MPa @800 °C<br>≦12 MPa @900 °C<br>≦6 MPa @1000 °C<br>≦4 MPa @1100 °C |
| 13 | Temper accuracy | ±1 °C |
| 14 | Temper control mode | AI-PID 30 Programmable control |
| 15 | Display | 7” LCD Touch Panel |
| 16 | Working gas | N2, Ar2, O2 and other non-corrosive gas |
| 17 | Power supply | AC110V/AC220V 50Hz/60Hz |

**Table 2.** Treatment and combustion temperature

| No | Briquet Batubara | Temperature (600, 700, and 800 °C) |
|---|---|---|
| 1 | 2% adsorbent | Treatment-I |
| 2 | 4% adsorbent | Treatment-II |
| 3 | 6% adsorbent | Treatment-III |
| 4 | 8% adsorbent | Treatment-IV |
| 5 | 10% adsorbent | Treatment-V |

From each of these combustions, there is residual ash that remains in the ceramics boat (bottom product) which is then temporarily stored in a desiccator to condition the temperature of the combustion in the furnace with the ambient temperature.

### 1.2. Material

The materials used in this research are coal, synthetic zeolite, and a strong acid reagent solution that functions for Hg, Pb, and Cd metals. In addition, the equipment used includes crusher, ball mill, vibration screen, coal briquette printer, compressor, tube furnace, electrically stainless-steel reaction tube, industrial gas combustion, and emission analyzer (E4400), desiccator, ceramics boat, multi ware, atomic absorption spectroscopy (AAS) 600, wet gas meter, chemical glass, Erlenmeyer, and flow meter. The uses and functions of each tool used in this study are described in Table 3. The structure of the process of materials used in this study is detailed in Figure 2. The process is carried out from the beginning of the preparation process to the final test.

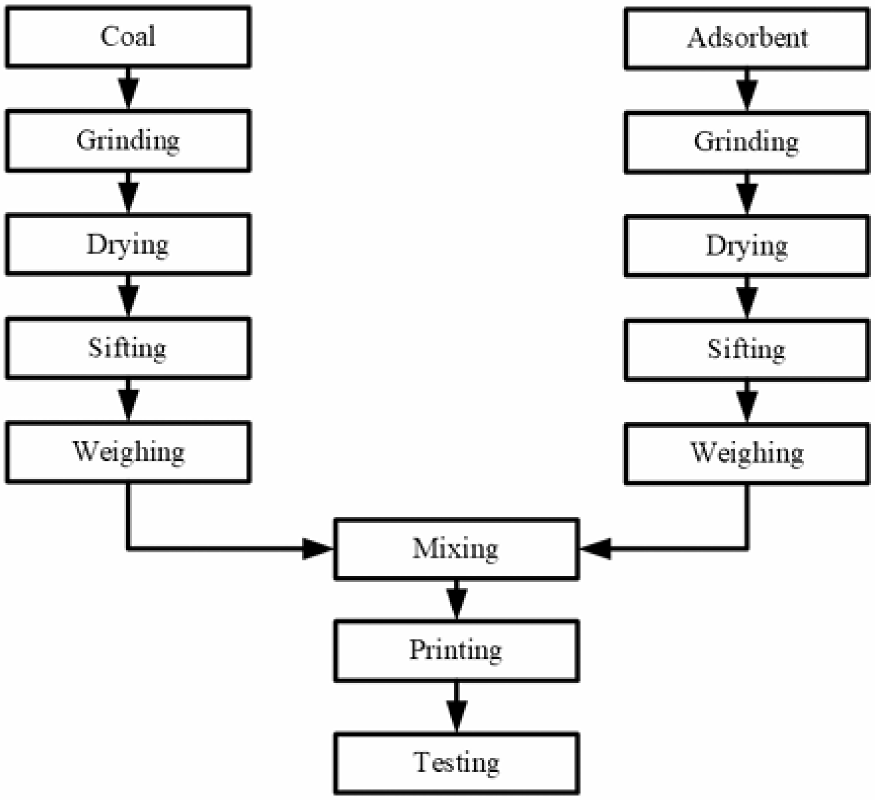



**Figure 2.** Diagram for material process

**Table 3.** Types of research tools and their respective functions

| No | Categories | Using |
|---|---|---|
| 1 | Crusher | Crushing of coal and zeolite |
| 2 | Ball Mill | Refine coal and zeolite |
| 3 | Vibration Screen | Coal and zeolite sieving |
| 4 | Coal Briquette Printer | To print briquettes |
| 5 | Compressor | Air combustion |
| 6 | Tube Furnace | Combustion Chamber |
| 7 | Electrically Stainless-Steel Reaction Tube | Pipe of combustion |
| 8 | Gas Combustion, and Emission Analyzer (E4400, E Instrument) | $SO_2$ and CO |
| 9 | Desiccator | Saver of sample |
| 10 | Ceramics Boat | Place the briquettes in the combustion chamber |

| | | |
|---|---|---|
| 11 | Multi ware | Sample and briquette storage containers |
| 12 | Atomic absorption spectroscopy (AAS) | Cd, Pb, and Hg |
| 13 | Wet Gas Meter | Flow rate calibration |
| 14 | Chemical Glass | Material analysis of heavy metals |
| 15 | Erlenmeyer | Container for analysis process |
| 16 | Flow meter | Flow meter |

There are two variables carried out in this study, namely fixed variables and random variables. Fixed variables include briquette weight, cylindrical briquette, coal particle size pressing pressure, zeolite, airflow rate, and combustion time. Meanwhile, the random variable include briquette combustion temperature and adsorbent ratio.

## 2. Result and discussion

The research in this paper was conducted to investigate the maximum point of absorption efficiency levels for cadmium (Cd), lead (Pb), and mercury (Hg) with the addition of adsorbents of 2%, 4%, 6%, 8%, and 10%. The test was carried out at a combustion temperature of 600 °C, 700 °C, and 800 °C for each treatment. The main fuel used is coal and adsorbents as pollutants. Residual samples from combustion products such as heavy metal content were analyzed in the laboratory of atomic analyzer spectrometer. Based on the results of the experiments carried out, it can be explained the comparison between each adsorbent ratio and the treatment is described below.

### 2.1. Comparison of bottom ash weight

The experimental results with combustion testing at temperatures of 600 °C, 700 °C, and 800 °C for different adsorbent ratios are shown in Figure 3a. It is shown that the higher the adsorbent ratio given, the higher the remaining combustion ash obtained. The highest remaining burning ash was recorded at the burning temperature of 600 °C. The results of the investigation show that when the combustion temperature increases, the remaining ash obtained will also be lower. This is because the fuel that is put at low temperature does not burn properly and vice versa; while if the combustion temperature is higher, then the fuel can burn optimally. The highest remaining burning ash at a temperature of 600 °C reached 2.01 grams. Meanwhile, the remaining ash produced when the temperature reached 800 °C was 1.62 grams, 0.39 grams lower than at 600 °C, and 0.3 grams lower than 700 °C. The higher the temperature, the more complete the combustion, and the more heavy metals emitted.The results of experimental measurements for mercury are shown in Figure 3b. It can be explained that the highest residual ash weight recorded at 600 °C reached 2.01 grams for an adsorbent ratio of 10%. The results of measurements carried out with temperatures of 700 °C and 800 °C of lower ash residue were 1.92 grams and 1.62 grams, respectively. Meanwhile, the results of measurements of the remaining metal ash of Pb and Cd with an experimental combustion temperature of 700 °C and 800 °C were the same as Hg shown in Figure 3a and 3c. However, the higher the temperature given, the more perfect the combustion rate will be. This is because the high combustion temperature can burn fuel optimally so that the resulting efficiency is better. While testing the combustion at a low temperature, the fuel that was entered did not burn out. However, the experiments carried out in this test show that the remaining combustion ash produced was almost the same for testing the entire temperature. However, the resulting levels of efficiency and absorption show a difference. Meanwhile, investigations for the recovery of coal fly ash

(CFA), coal bottom ash (CBA), and rice husk ash (RHA) for a partial replacement of ordinary Portland cement in translucent concrete have recently been discussed (Lo et al., 2021). The results of the investigation show that the carbon footprint from shifting ash can be reduced by 9.9–20.6%/m3 of translucent concrete. However, the substitution of the ash material carried out in their study aims to reduce the carbon footprint that can be found in translucent concrete. The use of adsorption for maximum metal absorption capacity for Pb (II) and Hg (II) ions can be generated up to 270.3 and 400.0 mg/g (Fu et al., 2019). These results were obtained from the application of XPS spectrum with high resolution. The results show that the investigation of the residual weight of ash against heavy metals Cd, Pb, and Hg with the addition of an adsorbent ratio of 2–10% as the results of research in this work have not been found.

### 2.2. Comparison of the total bottom metal

The results of the total metal comparison analysis based on the experiments conducted show that the phenomena that occur are almost the same as the results of the analysis for the remaining burning ash. The total heavy metal collected from the highest combustion product was recorded in Pb metal at 51.10 mg for an adsorbent ratio of 10% at 600 °C as shown in Figure 4c. While the total weight of Cd and Hg were 2.79 mg and 0.32 mg, respectively, with

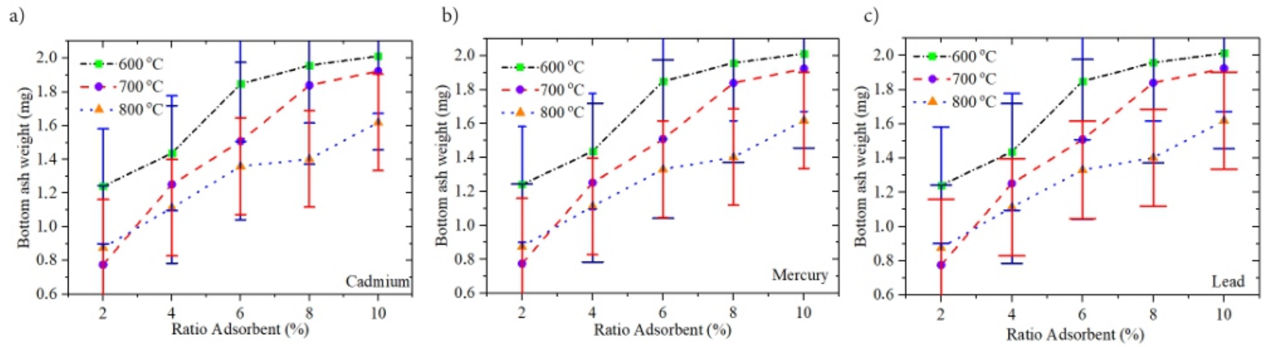



**Figure 3.** Remaining Cd, Hg, and Pb ash on the adsorbent ratio for different temperatures

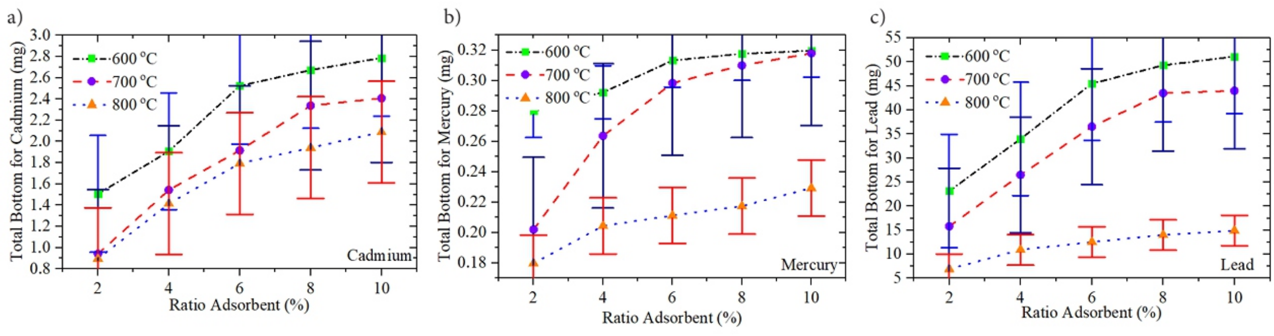



**Figure 4.** Total Cd, Hg, and Pb metal based on the adsorbent ratio for different temperatures

an adsorbent ratio of 10% as presented in Figure 4a and Figure 4b. A good and recommended standard adsorbent ratio for coal combustion binders is between 6–10%. If the adsorbent ratio is used too much, it is not optimal because the adsorbent system evaporates very quickly. Research using samples of fly ash, bottom ash, and surface soil from a waste incineration plant with the aim at investigating heavy metals in microplastic content has been studied (Shen et al., 2021). Whereas heavy metals such as Cr, Cu, Zn, Pb can be automatically adsorbed on the microplastic surface. The resulting Cr and Pb content reached

8.69, 2.15, 3.67 mg/kg and 9.67, 92.58, 7.27 mg/kg on bottom ash, fly ash, and soil. Their results were inferior to the results carried out in this work. However, the experimental treatment carried out showed a fundamental difference. The most widely used heavy metal investigation is to remove Cd2 +, Pb2 +, and Zn2 + ions as reported in a review study (Fouda-Mbanga et al., 2021). Meanwhile, investigations regarding the total metals Cd, Hg, and Pb based on the ratio of adsorbent and temperature are still lacking in various publications.

Particle size is very influential on combustion characteristics. Fine coal particles will make the coal burn faster and the process of releasing heavy metals is easier and faster so that more metals are emitted. Meanwhile, for adsorbents, the smaller the particle size, the larger the contact area, so that more heavy metal emissions can be captured. The importance of this research is to be able to absorb heavy metals during combustion in the presence of natural adsorbents used.

**2.3. Comparison of absorbed metal**

Five experimental captions based on the ratio of the adsorbent to each combustion temperature were carried out to investigate each metal that was absorbed for each combustion temperature tested. The results of the analysis show that the highest metal absorption was recorded for the Pb metal of 43.89 mg, which was obtained at the adsorbent ratio of 10% to 600 °C as shown in Figure 5c. Meanwhile, the absorption rate of Hg resulting from testing with different adsorbent ratios only reached 0.19 mg, much lower than heavy metal Pb as presented in Figure 5b. Meanwhile, the total Cd metal absorbed from the experiments with the highest different adsorbent ratios only reached 1.88 mg, which was obtained from the ratio of 10% for the combustion temperature of 600 °C as shown in Figure 5a. The results of the tests show that the higher the combustion temperature can reduce the absorption rate of heavy metals. This is because higher temperatures can burn coal completely. Meanwhile, the adsorbent ratio as a treatment which is a coal binder also looks better if the given ratio is higher. Meanwhile, the high efficiency produced, both absorption processes with higher adsorbent ratios and temperatures, showed a better level of efficiency. A better absorption efficiency level was obtained for heavy metal Cd than for Pb and Hg. The highest absorption efficiency level in heavy metal Cd reached 22.96% which was recorded at the adsorbent ratio of 10% to a combustion temperature of 600 °C as shown in Figure 6a.

**2.3. Comparison of absorbed metal**

Five experimental captions based on the ratio of the adsorbent to each combustion temperature were carried out to investigate each metal that was absorbed for each combustion temperature tested. The results of the analysis show that the highest metal absorption was recorded for the Pb metal of 43.89 mg, which was obtained at the adsorbent ratio of 10% to 600 °C as shown in Figure 5c. Meanwhile, the absorption rate of Hg resulting from testing with different adsorbent ratios only reached 0.19 mg, much lower than heavy metal Pb as presented in Figure 5b. Meanwhile, the total Cd metal absorbed from the experiments with the highest different adsorbent ratios only reached 1.88 mg, which was obtained from the ratio of 10% for the combustion temperature of 600 °C as shown in Figure 5a. The results of the tests show that the higher the combustion temperature can reduce the absorption rate of heavy metals. This is because higher temperatures can burn coal completely. Meanwhile, the adsorbent ratio as a treatment which is a coal binder also looks better if the given ratio is higher. Meanwhile, the high efficiency produced, both absorption processes with higher adsorbent ratios and temperatures, showed a better level of efficiency. A better absorption efficiency level was obtained for heavy metal Cd than for Pb and Hg. The highest absorption efficiency level in heavy metal Cd reached 22.96% which was recorded at the adsorbent ratio of 10% to a combustion temperature of 600 °C as shown in Figure 6a.

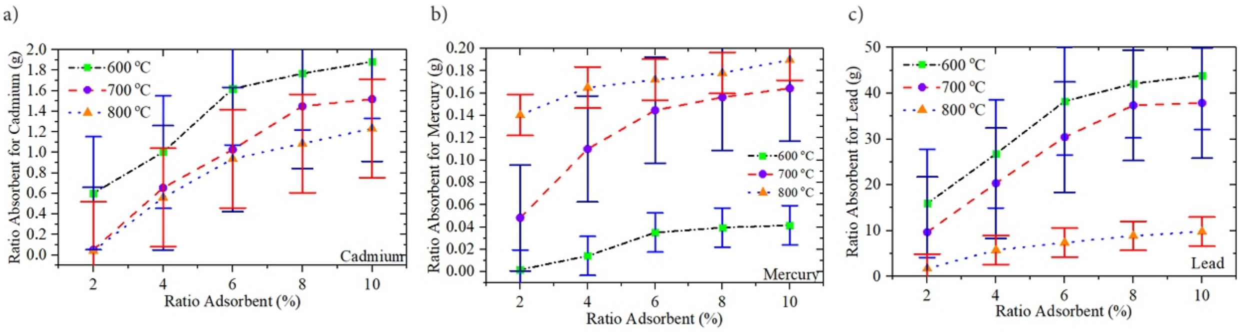



**Figure 5.** Total metal Cd, Pb, and Hg absorbed based on adsorbent ratio at different temperatures

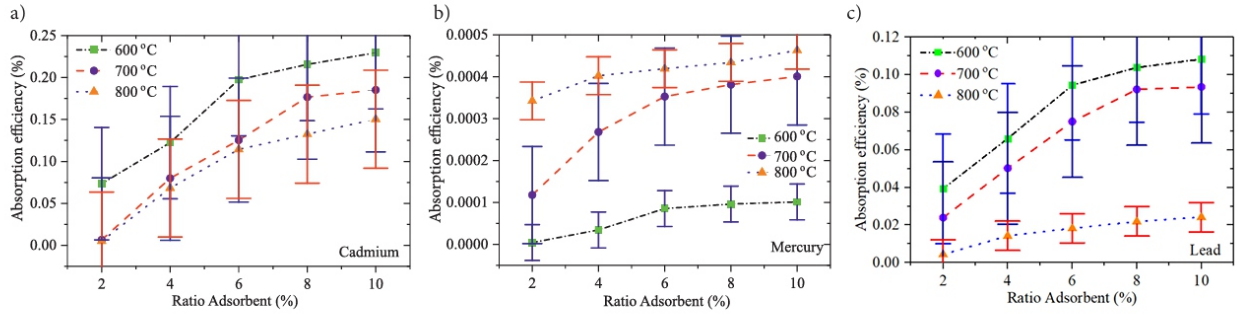



**Figure 6.** Efficiency absorption Cd, Hg, and Pb metal for ratio at different temperatures

## 2.4. Comparison of absorption efficiency

Furthermore, the analysis of the highest Pb absorption efficiency level of 10.83% was recorded at the adsorbent ratio of 10% for the combustion temperature of 600 °C. The absorption efficiency level of Pb metal was much lower than that of heavy metal Cd as shown in Figure 6c. Meanwhile, the absorption efficiency level resulting from the testing of heavy metal Hg was significantly lower than the heavy metal Cd and Pb. The absorption efficiency level of heavy metal Hg reaches 0.10% as shown in Figure 6b. T his shows that the efficiency level resulting from the testing process for heavy metal Hg is not good. Investigations regarding the mass absorption efficiency (MAE) of elemental carbon (EC) which were sampled as a measurement of emission sources through solid fuel combustion using thermal optical carbon analysis tools have also been carried out (Shen et al., 2013). The results of the measurements carried out show that the absorption efficiency of combustion reached 7.9 (4.8–11) mg/kg. Meanwhile, the use of four types of adsorbent material used has a significant removal efficiency for the treatment of sponges, especially for heavy metal Cr with each of (19.09%) and cotton (26.36%). Meanwhile, in this research, the level of absorption efficiency studied was the addition of the adsorbent ratio for the absorption efficiency of heavy metals Cd, Pb, and Hg.

## 2.5. Comparison of absorption capacity

The highest absorption efficiency level of mercury was obtained at an adsorbent ratio of 10% when the temperature reaches 800 °C of 0.46%. Meanwhile, the absorption efficiency level at 600 °C and 700 °C reach 0.10% and 0.40% as shown in Figure 7a. The absorption capacity obtained when the combustion temperature reaches 800 °C was 0.35 mg/kg, while when the combustion temperature of 600 °C and 700 °C were 0.03 mg/kg and 0.15 mg/kg, respectively. Meanwhile, previous studies show that the addition of the maximum adsorption capacity of Cu (II) and Pb (II) reached 61.96 and 138.11 mg/kg, respectively

(Jiang et al., 2020). However, the treatments and materials used in their study were significantly different from this study. There have been many studies on the use of coal fuel for various analyses, but investigations in particular for the addition of adsorbents to absorb heavy metals such as Cd, Pb, and Hg are still very rare in previous publications. Thus, this research can provide new references in terms of heavy metal absorption by adding different adsorbent ratios. This research is a continuation of our previous work which has been published recently (Gani et al., 2021).

The highest absorption capacity of Pb metal with different adsorbent ratios and combustion temperatures of 1.51 mg/kg was recorded at the adsorbent ratio of 2% to 600 °C. The absorption rate of Pb metal was higher than the heavy metal Hg as shown in Figure 6b. Overall, the Cd absorption rate for the three combustion temperatures tested was higher than the Hg variety. Meanwhile, the absorption rate for Pb metal was slightly better than Hg metal, especially at the combustion temperature of 600 °C and 700 °C. The maximum absorption capacity rate for Pb of 39.85 mg/kg is shown in Figure 8. This result is the

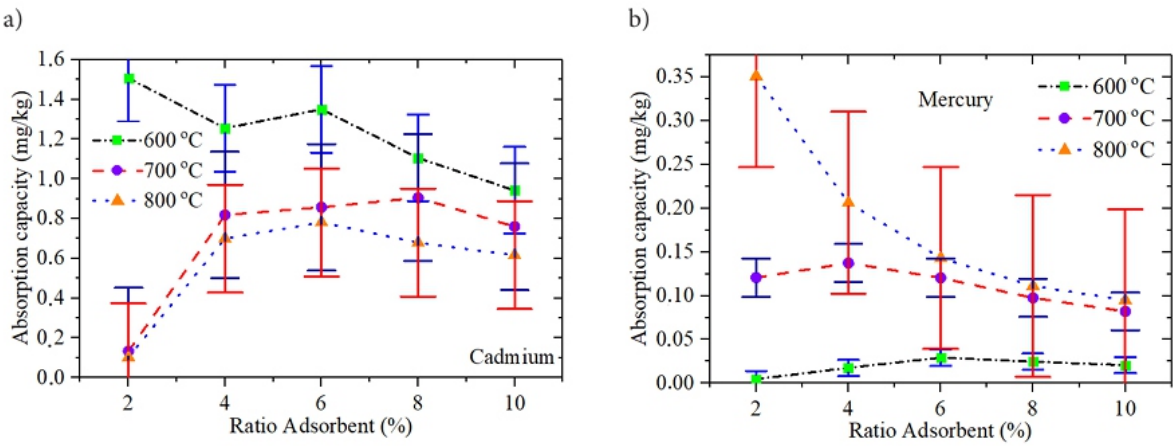



**Figure 7.** Total absorption capacity Hg and Cd with ratio and different temperatures

highest compared to Cd and Hg. The overall absorption capacity of Pb showed higher yields for all the different treatments and temperatures.

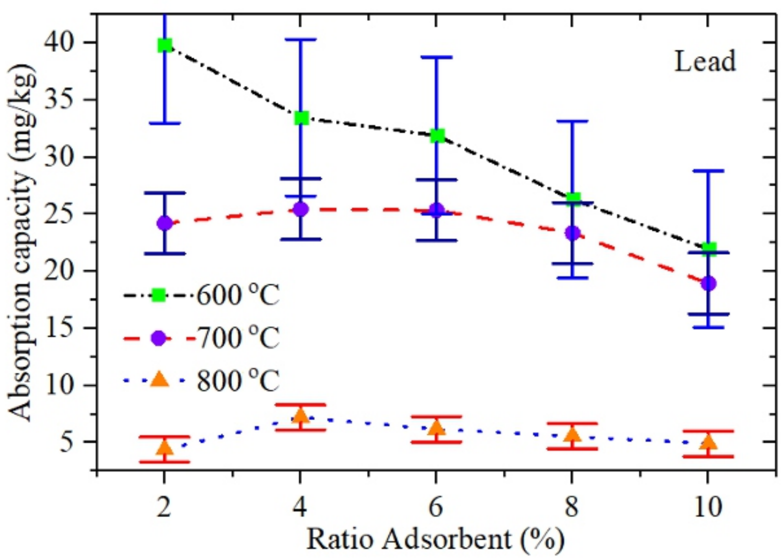


**Figure 8.** Total absorption capacity Pb with ratio and different temperatures

## Conclusions

Investigations on the experiments in this study were conducted to compare and find the most optimum results from different ratios and combustion temperatures. Heavy metals analyzed in this test include Pb, Hg and Cu. Heavy metal Pb produced the highest absorption capacity of 39.85 mg/kg at a combustion temperature of 600 °C with an adsorbent ratio of 2%. Heavy metal Pb also produced the highest absorption rate of 43.89 mg when the combustion temperature was 600 °C with an adsorbent ratio of 10%. Meanwhile, the best absorption efficiency was recorded for heavy metal Cd by 22.96% compared to heavy metal Pb and Hg. Based on several experiments, the results show that heavy metal Pb was more dominant in terms of absorption and absorption compared to heavy metals Cd and Hg. Meanwhile, the remaining amount of ash from the three tested combustion temperatures produced the same value.

## Acknowledgements

This research supported by Universitas Syiah Kuala, Research Institutions, and Community Service.

## Conflict of interest

There are no conflicts to declare.

## References

• *Bi, X., Feng, Y., Wu, J., Wang, Y., & Zhu, T. (2007). Source apportionment of PM10 in six cities of northern China. Atmospheric Environment, 41(5), 903–912. https://doi.org/10.1016/j.atmosenv.2006.09.033*

• *Cheng, X., Long, R., Chen, H., & Li, Q. (2019). Coupling coordination degree and spatial dynamic evolution of a regional green competitiveness system – A case study from China. Ecological Indicators, 104, 489–500. https://doi.org/10.1016/j.ecolind.2019.04.003*

• *Dai, S., Ren, D., Chou, C.-L., Finkelman, R. B., Seredin, V. V., & Zhou, Y. (2012). Geochemistry of trace elements in Chinese coals: A review of abundances, genetic types, impacts on human health, and industrial utilization. International Journal of Coal Geology, 94, 3–21. https://doi.org/10.1016/j.coal.2011.02.003*

• *Diehl, S. F., Goldhaber, M. B., & Hatch, J. R. (2004). Modes of occurrence of mercury and other trace elements in coals from the warrior field, Black Warrior Basin, Northwestern Alabama. International Journal of Coal Geology, 59(3–4), 193–208. https://doi.org/10.1016/j.coal.2004.02.003*

• *Dittert, I. M., Maranhão, T. A., Borges, D. L. G., Vieira, M. A., Welz, B., & Curtius, A. J. (2007). Determination of Mercury in biological samples by cold vapor atomic absorption spectrometry following cloud point extraction with salt-induced phase separation. Talanta, 72(5), 1786–1790. https://doi.org/10.1016/j.talanta.2007.02.012*

• *Erdiwansyah, E., Mahidin, M., Husni, H., Nasaruddin, N., Khairil, K., Zaki, M., & Jalaluddin, J. (2021). Investigation of availability, demand, targets, and development of renewable energy in 2017–2050: A case study in Indonesia. International Journal of Coal Science & Technology, 8, 483–499. https://doi.org/10.1007/s40789-020-00391-4*

• *Erdiwansyah, Mahidin, Mamat, R., Sani, M. S. M., Khoerunnisa, F., & Kadarohman, A. (2019a). Target and demand for renewable energy across 10 ASEAN countries by 2040. The Electricity Journal, 32(10), 106670. https://doi.org/10.1016/j.tej.2019.106670*

• *Erdiwansyah, Mamat, R., Sani, M. S. M., & Sudhakar, K. (2019b). Renewable energy in Southeast*

•Asia: Policies and recommendations. Science of the Total Environment, 670, 1095–1102. https://doi.org/10.1016/j.scitotenv.2019.03.273

• Finkelman, R. B., Palmer, C. A., & Wang, P. (2018). Quantification of the modes of occurrence of 42 elements in coal. International Journal of Coal Geology, 185, 138–160. https://doi.org/10.1016/j.coal.2017.09.005

• Fouda-Mbanga, B. G., Prabakaran, E., & Pillay, K. (2021). Carbohydrate biopolymers, lignin based adsorbents for removal of heavy metals (Cd2+, Pb2+, Zn2+) from wastewater, regeneration and reuse for spent adsorbents including latent fingerprint detection: A review. Biotechnology Reports, 30, e00609. https://doi.org/10.1016/j.btre.2021.e00609

• Fu, W., Wang, X., & Huang, Z. (2019). Remarkable reusability of magnetic Fe3O4-encapsulated C3N3S3 polymer/reduced graphene oxide composite: A highly effective adsorbent for Pb and Hg ions. Science of the Total Environment, 659, 895–904. https://doi.org/10.1016/j.scitotenv.2018.12.303

• Gani, A., Wattimena, Y., Erdiwansyah, Mahidin, Muhibbuddin, & Riza, M. (2021). Simultaneous sulfur dioxide and mercury removal during low-rank coal combustion by natural zeolite.

• Heliyon, 7(5), e07052. https://doi.org/10.1016/j.heliyon.2021.e07052 Goodarzi, F., & Huggins, F. E. (2001). Monitoring the species of arsenic, chromium and nickel in milled coal, bottom ash and fly ash from a pulverized coal-fired power plant in western Canada. Journal of Environmental Monitoring, 3(1), 1–6. https://doi.org/10.1039/b006733o

• Hower, J. C., Eble, C. F., & Quick, J. C. (2005). Mercury in Eastern Kentucky coals: Geologic aspects and possible reduction strategies. International Journal of Coal Geology, 62(4), 223236. https://doi.org/10.1016/j.coal.2005.02.008

• Jiang, S., Xi, J., Deng, W., Dai, H, Fang, G., & Wu, W. (2020). Low-cost and high-wet-strength paper-based lignocellulosic adsorbents for the removal of heavy metal ions. Industrial Crops and Products, 158, 112926. https://doi.org/10.1016/j.indcrop.2020.112926

•Jones, T., Wlodarczyk, A., Koshy, L., Brown, P., Longyi, S., & BeruBe, K. (2009). The geochemistry and bioreactivity of flyash from coal-burning power stations. Biomarkers, 14(Sup1), 45–48. https://doi.org/10.1080/13547500902965195

• Kan, H., Chen, R., & Tong, S. (2012). Ambient air pollution, climate change, and population health in China. Environment International, 42, 10–19. https://doi.org/10.1016/j.envint.2011.03.003

• Kementerian Energi dan Sumber Daya Mineral Republik Indonesia. (2016). Peraturan Menteri Energi dan Sumber Daya Mineral Nomor 13 Tahun 2016 tentang Organisasi dan Tata Kerja Kementerian Energi dan Sumber Daya Mineral. https:// peraturan.bpk.go.id/Home/Details/143302/permen-esdmno-13-tahun-2016

•Kementerian Energi dan Sumber Daya Mineral. (2019). Peraturan Menteri Energi dan Sumber Daya Mineral Nomor 11 Tahun 2019 Tentang Perubahan Kedua Atas Peraturan Menteri energi dan Sumber Daya Mineral Nomor 25 Tahun 2018

• Tentang Pengusahaan Pertambangan Mineral dan Batubara. https://peraturan.bpk.go.id/Home/Details/142232/permenesdm-no-11-tahun-2019 Li, W., Deng, G., Li, M., Liu, X., & Wang, Y. (2012). Roles of mucosal immunity against Mycobacterium tuberculosis infection. Tuberculosis Research and Treatment, 2012, 791728. https://doi.org/10.1155/2012/791728

• Lo, F.-C., Lee, M.-G., & Lo, S.-L. (2021). Effect of coal ash and rice husk ash partial replacement in ordinary Portland cement on pervious concrete. Construction and Building Materials, 286, 122947. https://doi.org/10.1016/j.conbuildmat.2021.122947

• Mahidin, M. (2009). Biomass utilisation in selected Asian countries: Policy, R&D and status. In National Conference on Biomass Utilization for Alternative Energy and Chemicals. Universitas Katolik Parahyangan, Bandung.

• *Menteri Energi Dan Sumber Daya Mineral. (2006). Pedoman Pembuatan Dan Pemanfaatan Briket Batubara Dan Bahan Bakar Padat Berbasis Batubara (SNI 047). https://jdih.maritim.go.id/en/peraturan-menteri-energi-dansumber-daya-mineral-no-47-tahun-2006 Pian, W., Cheng, W., Niu, H., & Fan, J. (2016). TEM study of f ine particles from coal-fired power plant ambient air. World Journal of Engineering, 13(4), 311–316. https://doi.org/10.1108/WJE-08-2016-042*

• *Shao, L. Y., Wang, J., Hou, H. H., Zhang, M. Q., Wang, H.,*

•*Spiro, B., Large, D., & Zhou, Y. P. (2015). Geochemistry of the C1 Coal of Latest permian during mass extinction in Xuanwei, Yunnan. Acta Geologica Sinica, 89(1), 163–79.*

• *Shen, G., Chen, Y., Wei, S., Fu, X., Zhu, Y., & Tao, S. (2013). Mass absorption efficiency of elemental carbon for source samples from residential biomass and coal combustions. Atmospheric Environment, 79, 79–84. https://doi.org/10.1016/j.atmosenv.2013.05.082*

• *Shen, M., Hu, T., Huang, W., Song, B., Qin, M., Yi, H., Zeng, G., & Zhang, Y. (2021). Can incineration completely eliminate plastic wastes? An investigation of microplastics and heavy metals in the bottom ash and fly ash from an incineration plant. Science of the Total Environment, 779, 146528. https://doi.org/10.1016/j.scitotenv.2021.146528*

• *Shi, J., Huang, W., Chen, P., Tang, S., & Chen, X. (2018). Concentration and distribution of cadmium in coals of China. Minerals, 8(2), 48. https://doi.org/10.3390/min8020048 Tang, Y., Chang, C., Zhang, Y., & Li, W. (2009). Migration and distribution of fifteen toxic trace elements during the coal washing of the Kailuan Coalfield, Hebei Province, China. Energy Exploration & Exploitation, 27(2), 143–152. https://doi.org/10.1260/0144-5987.27.2.143 Wei, L., Yue, S., Zhao, W., Yang, W., Zhang, Y., Ren, L., Han, X., Guo, Q., Sun, Y., Wang, Z., & Fu, P. (2018). Stable sulfur isotope ratios and chemical compositions of fine aerosols (PM2.5) in Beijing, China. Science of the Total Environment, 633, 1156–1164. https://doi.org/10.1016/j.scitotenv.2018.03.153*

• *Xie, S. D., Liu, Z., Chen, T., & Hua, L. (2008). Spatiotemporal variations of ambient PM10 source contributions in Beijing in 2004 using positive matrix factorization. Atmospheric Chemistry and Physics, 8(10), 2701–2716. https://doi.org/10.5194/acp-8-2701-2008*

• *Xie, X., Ai, H., & Deng, Z. (2020). Impacts of the scattered coal consumption on PM2.5 pollution in China. Journal of Cleaner Production, 245, 118922. https://doi.org/10.1016/j.jclepro.2019.118922*

• *Yan, R., Gauthier, D., & Flamant, G. (2000). Possible interactions between As, Se, and Hg during coal combustion. Combustion and Flame, 120(1–2), 49–60. https://doi.org/10.1016/S0010-2180(99)00079-6*

• *Yang, J.-y. (2010). Acid removal rate of trace elements and its organic-inorganic affinity in coal – in a case of the Late Paleozoic coal seam 5 from Weibei. Journal of Fuel Chemistry and Technology, 38(5), 522–527.*

# THE POTENTIAL FOR USING DIFFERENT SUBSTRATES IN GREEN ROOFS

**Handan CAKAR1*, Ozlem AKAT SARACOGLU1, Hulya AKAT2, Cenk Ceyhun KILIC1, Hakan ADANACIOGLU3**
1Bayindir Vocational School, Ege University, Bayindir-Izmir, Turkey
2Ortaca Vocational School, Mugla Sitki Kocman University, Ortaca-Mugla, Turkey
3Department of Agricultural Economics, Faculty of Agriculture, Ege University, Bornova-Izmir, Turkey

## A B S T R A C T

*This research was carried out in Izmir-Turkiye and investigated the potential of using three different substrates (cocopeat, loofah and perlite) in the design of green roofs with succulents (Crassula ovata) in aim to improve their performance. In this research, four different groups (G1: Soil-Cocopeat, G2: Soil-Loofah, G3: Soil-Perlite and G4: Soil) were created according to the plant growing media used in the planting layer. The researchers conducted measurements of the drained irrigation water's EC (Electrical Conductivity) value, pH value and drainage amount, the plant growing media's temperature and moisture, the plant's height and leaf number, and the amount of subsidence in the planting layer. In line with the results obtained from the evaluations of the analyses, it is possible to say that perlite (G3) offers more advantages than its alternatives in terms of many variables. However, according to the conclusive results, it has been understood that the use of a single type of substrate as plant growing media would not be sufficient to encourage the maximum performance of green roofs. To ensure that, considering the advantages of each substrate group, it is proposed that their combined use would be more beneficial.*

***Keywords: roof garden, vegetated roof, loofah, cocopeat, perlite, Crassula ovata.***

## INTRODUCTION

Several studies have been carried out to propose ways of reducing the effects of climate change, the symptoms of which we observe today, and preserving ecological balance. Among these studies, the ecosystem-based approach, green roof applications, have gained momentum in terms of ensuring the sustainability of ecological balance (Gülgün Aslan & Yazici, 2016). The aim of green roof designs, which are important elements of landscape architecture, has shifted from providing aesthetic pleasure to creating a component of urban ecology (Younis et al., 2020). As a result, different terms such as "roof garden", "vegetated roof" or "living roofs" have come to the fore today (Ekşi, 2014). Green roofs, which are classified as green space structures that offer important ecological benefits to cities, can be effective against the consequences of climate change, such as floods and overflows caused by heavy rains, high city temperatures, and atmospheric pollution (Dunnett & Kingsbury, 2008; Manso et al., 2021). At the same time, the vegetation in the green roofs can provide a living environment for birds and insects, clean the air, and positively improve the conditions of the habitat of all living things by cooling the air through evaporation (Wooster et al., 2022).

Besides providing micro-climate control, green roofs can also reduce the wind speed and act as a sound insulation layer by absorbing sound waves (Rowe, 2011; Manso et al., 2021). In addition, green roofs improve aesthetic value, increase work efficiency, and encourage the development of social relations by providing people with alternative and special resting areas, especially in buildings used for tourism, work, education, and health services. Green roofs also increase the economic value of the buildings, and make them preferable (Erkul & Sönmez, 2014).

In green roof applications, the climate conditions of the region such as wind, solar radiation duration and intensity, temperature, precipitation, and structural conditions, such as the roof's load carrying capacity, slope and direction, are important factors. Besides the static structure of the building, other factors affecting the success of a green roof can be named as; the qualities and properties of the substrate used in the growing of plants and the design of the roof cover. The roof cover consists of different layers; the planting layer, filter, drainage, protection, water and heat insulation, and structural layer (Reyes et al., 2016; Korol et al., 2018; Cascone, 2019). It is highly important to ensure the waterproofing and thermal insulation of the roof cover as it is essential, but increases the green roof's installation cost and is challenging to prepare.

The substrates examined in this study are materials that can be used in sufficient quantities for plants that grow in a limited area, can act as a buffer, do not carry diseasepests and weed seeds, and have high water holding capacity and nutrient contents. The pH value of the substrate may vary in relation to the requirements of the vegetation, but it should be between 6.0, and 8.5 and for turf substrates between 5.5 and 7.5 (Landscape Development and Landscaping Research Society e.V. [FLL], 2018). The use of soil as a sole substrate in green roofs is not highly recommended due to several disadvantages (the clogging of the filter layer, spreading of weed seeds and the high cost of controlling, loss of porous structure due to soil compaction, etc.) (Calheiros & Stefanakis, 2021). Nonetheless, lightweight textured soil can be preferred, as well as soil combining high organic matter content (farmyard manure, compost, peat, decomposed sawdust or bark, cocopeat, etc.) and inorganic materials (perlite, volcanic tuff, pumice, rockwool, schist, vermiculite, etc.) in certain proportions (Aslanboğa, 1988; Ürgenç, 1990; Johnston & Newton, 1993; Ampim et al., 2010). In recent years, mushroom compost and sewage sludge have been included in these materials. However, sewage sludge requires careful handling due to its heavy metal concentration (Woolley & Kimmins, 2000). Components such as recycled waste materials and by-products from foundries or incinerators could potentially be used, but contaminant concentrations must be taken into consideration (Rowe, 2011). In the case of a wrong substrate choice, the consequences could be compaction, imbalances between water and air, asphyxia of the root apparatus, increased weight, reduction in drainage, and the alteration of nutrients (Cascone, 2019).

The roof load bearing capacity is an important factor when deciding the plant species that will be used in the green roof and the thickness of the planting layer. Intensive green roofs, those with more than 15 cm of planting layer, can host a variety of plants such as herbaceous perennials, grass, and even trees, if the roof has adequate structural support. On extensive green roofs with a planting layer depth of 15 cm or less, the shallow planting layers cannot sustain most plants except those particularly adapted to drought and extreme temperatures (Vandegrift et al., 2019). Consistent with the FLL recommendations, the first accepted generalization is that succulent-dominated green roofs are well-suited to survive the extreme conditions of roofs and based on the many of the ecoregions investigated, they prefer shallow growing media, from 7 to 10 cm thickness. The second generalization is that herbaceousdominated green roofs appear to need growing media more than 10 cm deep across most ecoregions (Dvorak & Volder, 2010). Cascone (2019) has proposed that generally, the growing media weight of extensive roofs varies between 12–14 kg m–2 with a thickness of 8 cm, and of intensive roofs it is around 600 kg m–2 with a thickness of 50–60 cm.

Accordingly, this study has preferred to investigate the design of an extensive green roof by using succulents as they will not grow extensively tall, are drought resistant, and have low nutrient requirements. This study has aimed to demonstrate the potential use of three different substrates (mixed with soil; cocopeat, loofah, and perlite) in the green roofs.

## 1. Materials and methods

The research was carried out at Ege University Bayındır Vocational School in Izmir- Turkiye during the 3-month long summer period (from June 2021 to September 2021) as an open field pot experiment.

### 1.1. Plant materials

The plant species that was used to achieve the research target was Crassula ovata, which belongs to the succulent (fleshy-leaved) plant family and is suitable for extensive green roof designs. The plant seedlings that were chosen had reached a homogenous size, demonstrated healthy development, had 15 leaves, were 10 cm long, and did not show branching. The plants of the family Crassulaceae are often used in green roofs designs due to their low irrigation requirements and adaptability to sunny environments. The use of plants with low irrigation requirements is all the more advantageous in places where water availability is limited (Chagolla-Aranda et al., 2017). Thus, Crassula ovata species was chosen for the study due to its capability to develop quickly as a plant material and because the plant's oval leaves would provide easier measurement during the research.

### 1.2. Substrates

For the green roof designs in the research, 4 different groups (G1, G2, G3 and G0) were formed according to the plant growing media used in the planting layer. Kadıoğlu and Canbolat (2019), determined that the mixing ratio of perlite with soil of 25%:75% has higher water holding capacity than other mixing media. In this study, the substrate ratios of the plant growing media used in the groups were determined as 25%:75%. The substrate contents of the plant growing media used in these groups are as follows:– G1: The substrate used was S75:Co25 and consisted of soil (S) and cocopeat (Co) in a volumetric ratio of 75:25.

– G2: The substrate used was S75:Lo25 and consisted of soil (S) and loofah (Lo) in a volumetric ratio of 75:25.

– G3: The substrate used was S75:Pe25 and consisted of soil (S) and perlite (Pe) in a volumetric ratio of 75:25.

– G0: The substrate used was S100 and consisted of soil (S) in a volumetric ratio of 100:- (Control group).

The bulk density (g cm–3) and the visuals of the substrates featured in the study can be found in Figure 1.

| Substrates | Cocopeat | Loofah | Perlite | Soil |
| --- | --- | --- | --- | --- |
| |  | | | |
| g $cm^{-3}$ | 0.19 | 0.04 | 0.24 | 1.33 |

**Figure 1.** The bulk density and visuals of the substrates used in the experiment

The physical and chemical properties of the substrates in the research are as follows:– Cocopeat: EC < 0.5 ds m–1, pH 5.5–7;– Perlite: EC < 0.0 ds m–1, pH 6.5–7.5, particle size < 3.0 mm;– Soil: Texture loamy sand, pH 7.79, EC 0.12 ds m–1, organic matter content 0.45%, nitrogen content 0.022%. Loofah: Any literature and research on the use of loofah (Luffa cylindrica) as a substrate could not be found. Thus, the results obtained from this research could potentially contribute to the literature regarding the use of loofah as a substrate in green roof applications. The fibre part obtained from the matured dried fruit of the loofah plant was used in this study. The Loofah was divided into small pieces (3–5 mm) with the help of scissors before being mixed homogeneously into the soil.

**1.3. Experimental design**

The research area was designed in 3 replicates in accordance with the "random blocks experimental design". Transparent plastic pots with a diameter of 15 cm and a drainage outlet at the bottom were used as green roof models in the study area. Drainage containers with a volume of 1.5 litres were placed under the pots, which were connected to a drainage outlet preventing any possible leaks. Extensive green roof design principles were applied during the preparation process of the pots (Reyeset al., 2016; Korol et al., 2018; Cascone, 2019). To evaluate the potential of the substrates within the scope of this research, the pot arrangement in the experiment featured the planting layer, filter, and drainage layers, after each was considered sufficient. In this regard, the pebbles were placed in all the pots at a height of 5 cm first.
In green roof designs, a filtering layer should be used between the drainage and planting layer (Özdemir & Altun, 2010; Seçkin & Seçkin, 2016). Thus, the geotextile product named Polypropylene fabric (95 g m–2), also known as ground cover fabric in landscaping, was chosen as the filtration material. Accordingly, the polypropylene fabric was laid on the pebbles as a filtration material in all the pots.
Dvorak and Volder (2010) demonstrated that succulents performed best in the growing media at a depth of 7–10 cm. Cascone (2019) stated that generally for extensive green roofs, the growing media should have a thickness of 8 cm. Therefore, the depth of planting layer was an average of 8 cm. The four separately prepared plant growing medias were laid on the polypropylene fabric at a depth of 8 cm to form the planting layers. No nutrient solution was added to the plant growing media as the plants did not require fertilization throughout the research period.

After adding the plant growing media into the pots, Crassula ovata was planted as one plant per pot. The sample trial pot created for this study based on extensive green roof design principles is given in Figure 2.

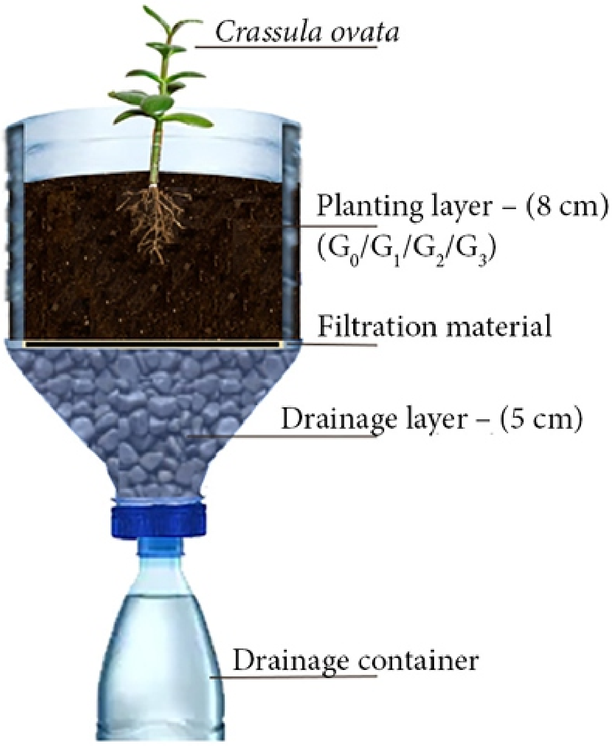



**Figure 2.** Cross-section of the sample pot used in study

**1.4. Measurments**

The study area, which is under the conditions of a climate with dry summers, received no precipitation in throughout this research process. The plants were watered under careful control. In the irrigation process, which was initiated after the planting, 800 mL of water per plant was used. The pots were watered twice a week at 9:00 a.m. with the use of a measuring cup to ensure they would receive the same amount (400 mL day–1). The measurements of the plant growing media's temperature and moisture were carried out 24 hours after the irrigation process was completed. These measurements were made at a depth of 5 cm from the surface, taking into consideration the plant root depth in the pot. A hand-held digital thermometer (TempLog Digital Thermometer) was used for temperature measurements, and a pot type moisture meter was used for moisture measurement.

Following the completed irrigation processes, the drained irrigation water collected in the lidded drainage containers was measured by volume every week before being removed from the system. After the determination of the drainage amounts, the EC (Electrical conductivity) value and pH value measurements were carried out in the drained water once a week. An EC meter (WTW, Cond 330i conductivity meter set) was used for the EC value measurements and a pH meter [WTW, pH 3210 (330i) pH meter set (portable)] was used for pH value measurements.

To determine the amount of subsidence in the planting layer, which was designed with a depth of 8 cm at the beginning of the study, the height of the planting layer was once again measured at the end of the experiment. In order to examine the effect of the 4 groups, (differing based on the substrates of their plant growing media) on the plant's growth, plant height (cm) and leaf number (number) measurements were carried out once a week to observe the plants' physical properties.

### 1.5. Statistical analyses

Various statistical analyses were applied to test whether the factors examined in this study differ in relation to substrates. The factors examined were EC value (µS cm–1), pH value, drainage amount (mL), in-pot temperature (°C), in-pot moisture (%), plant height (cm), number of leaves (number), and subsidence (mm) in the planting layer. Since the data can be variable, the Kolmogorov-Smirnov test was used to conduct a normal distribution test. While analysis of variance (one-way anova) was applied to the variants demonstrating normal distribution, KruskalWallis was applied to those that did not. The data in this study were analysed using the IBM SPSS Statistics (v21) software.

### 1.6. Hypotheses

The results obtained from this study aimed to determine the substrate that offers the most suitable conditions, among those examined in the study, to be used in the extensive green roofs. With this research, some hypotheses have been proposed regarding the substrates that could be used in extensive green roofs. These hypotheses are stated below:

H1: In extensive green roofs, EC value (µS cm–1), pH value, drainage amount (mL), in-pot temperature (°C), in-pot moisture (%), plant height (cm), number of leaves (number), and the amount of subsidence (mm) in the planting layer vary based on the performance of different types of substrates.

H2: To achieve the best performance in extensive green roofs, it is not sufficient to use a single type of substrate as plant growing media.

## 2. Results

### 2.1. EC value

The descriptive statistical results of the EC (Electrical conductivity) measurements, that were conducted on the groups with the differing substrates analysed in this study, can be found on Table 1. According to this, the highest salinity (EC) values within the irrigation water drained from the planting layer were spotted in the group (G1), in which cocopeat was used as the substrate. The lowest salinity values were found in the control group (G0), followed by the group using loofah as substrate (G2). Due to the normal distribution of the data, one-way ANOVA analysis was used to analyse the statistical differences between the substrates in terms of salinity (EC) values. According to the results obtained from the analysis, a significant statistical difference was found between the substrates in terms of salinity (EC) values at the 5% significance level ($F = 4.096$; $p = 0.007$).

**Table 1.** The salinity (EC) values (µS cm–1) of substrates

| Substrates | n | Mean | Minimum | Maximum | Std. Deviation |
|---|---|---|---|---|---|
| $G_1$ | 9 | 444.54 | 275.00 | 655.00 | 100.71310 |
| $G_2$ | 9 | 415.92 | 256.00 | 670.00 | 125.61636 |
| $G_3$ | 9 | 424.75 | 254.00 | 706.00 | 115.23709 |
| $G_0$ | 9 | 397.33 | 242.00 | 803.00 | 124.96457 |

## 2.2. pH value

The descriptive statistical results that were obtained by the pH measurement of the groups featuring the differing substrates are given in Table 2. Accordingly, the lowest pH value of 9.23 was obtained from the loofah group (G2). This was followed by the cocopeat group (G1) with 9.35. The highest values were found in the perlite group (G3) and control group (G0), which were close to each other, with 9.51 and 9.56 respectively. According to the results of one-way ANOVA analysis, no statistically significant difference was found between the substrates in terms of pH values (F = 1.485; p = 0.219).

**Table 2.** pH values according to substrates

| Substrates | n | Mean | Minimum | Maximum | Std. Deviation |
|---|---|---|---|---|---|
| $G_1$ | 9 | 9.35 | 8.57 | 10.46 | 0.49426 |
| $G_2$ | 9 | 9.23 | 8.50 | 10.29 | 0.50118 |
| $G_3$ | 9 | 9.51 | 8.81 | 10.69 | 0.60909 |
| $G_0$ | 9 | 9.56 | 8.75 | 10.74 | 0.65400 |

## 2.3. Drainage amount

The drainage amount measured from the pot drainage outlets of the groups featuring the differing substrates analysed in this study are shown in Table 3. According to these measurements the lowest drainage amount was found in the cocopeat group (G1) with 185.83 mL. This was followed by perlite (G3) with 217.63 mL, loofah (G2) with 257.42 mL, and control group (G0) with 342.54 mL. Because the data did not demonstrate normal distribution, the Kruskal-Wallis test was applied to determine the difference in the measured drainage amounts according to the substrates. According to the test results obtained, a statistically significant difference was found between the substrates in terms of drainage amounts (Chi-Square: 50.801; p = 0.000).

**Table 3.** Measured drainage amounts according to substrates (mL)

| Substrates | n | Mean | Minimum | Maximum | Std. Deviation |
|---|---|---|---|---|---|
| $G_1$ | 9 | 185.83 | 130.00 | 270.00 | 45.84915 |
| $G_2$ | 9 | 257.42 | 135.00 | 373.00 | 68.29407 |
| $G_3$ | 9 | 217.63 | 108.00 | 350.00 | 63.28735 |
| $G_0$ | 9 | 342.54 | 204.00 | 460.00 | 70.82126 |

## 2.4. In-pot temperature

The air temperature readings recorded during the study were between min. 34.14 °C – max. 46.08 °C. The average temperature values measured in the pot according to the groups with the differing substrates are given in Table 4. According to the findings, the lowest temperature value among the results was spotted in the perlite group he air temperature readings recorded during the study were between min. 34.14 °C – max. 46.08 °C. The average temperature values measured in the pot according to the groups with the differing substrates are given in Table 4. According to the findings, the lowest temperature value among the results was spotted in the perlite group (G3) with 34.10 °C. Besides that, the values measured in the cocopeat group (G1) and loofah group (G2) being 34.32 °C and 34.92 °C respectively, were close to the values observed in the perlite group (G3). It was determined that the average temperature value measured in the control group (G0) was 36.09 °C, which was the highest compared to the other groups. According to the Kruskal-Wallis test results, a statistically significant difference was found between the substrates in terms of temperature values (Chi-Square: 9.303; p = 0.026)

**Table 4.** Average temperature values measured by substrates (°C)

| Substrates | n | Mean | Minimum | Maximum | Std. Deviation |
|---|---|---|---|---|---|
| $G_1$ | 9 | 34.32 | 28.30 | 41.10 | 4.40773 |
| $G_2$ | 9 | 34.92 | 28.50 | 41.20 | 3.96799 |
| $G_3$ | 9 | 34.10 | 28.20 | 41.30 | 4.42879 |
| $G_0$ | 9 | 36.09 | 29.00 | 43.00 | 4.18630 |

### 2.5. In-pot moisture

The relative humidity readings recorded during the study were between min. 15.0% – max. 39.12%. The average moisture content values measured in the pots of the groups with the differing substrates are given in Table 5. According to these numbers, the highest moisture content value among the substrates was determined as 40.58% in the perlite group (G3). This was followed by the cocopeat group (G1) with 39.54%, the loofah group (G2) with 36.08% and the control group (G0) with 26.21%. As it can be understood from these values, a very low moisture value was observed in the control group, in which there was no intervention. As the data was not normally distributed, the Kruskal-Wallis test was applied to examine the difference in the moisture content values according to the substrates. According to the test results obtained, a statistically significant difference was found between the substrates in terms of moisture values (Chi-Square: 16.875; p = 0.001).

**Table 5.** Average moisture content values according to substrates (%)

| Substrates | n | Mean | Minimum | Maximum | Std. Deviation |
|---|---|---|---|---|---|
| $G_1$ | 9 | 39.54 | 22.00 | 66.00 | 12.23317 |
| $G_2$ | 9 | 36.08 | 15.00 | 54.00 | 11.16250 |
| $G_3$ | 9 | 40.58 | 18.00 | 62.00 | 12.52794 |
| $G_0$ | 9 | 26.21 | 12.00 | 42.00 | 10.57881 |

### 2.6. Plant height

The average plant height measurements of the groups with the differing substrates analysed in the study are given in Table 6. According to these, the highest value of plant height was determined as 14.04 cm in the perlite group (G3). This was followed by the control group (G0) with 12.00 cm, the cocopeat group (G1) with 11.80 cm, and the loofah group (G2) with 10.94 cm. Since the data did not demonstrate normal distribution, the Kruskal-Wallis test was applied to determine the difference in plant height values measured according to the substrates. According to the test results, a statistically significant difference was found between the substrates in terms of plant height values (Chi-Square: 25.262; p = 0.000).

**Table 6.** Average plant heights by substrates (cm)

| Substrates | n | Mean | Minimum | Maximum | Std. Deviation |
|---|---|---|---|---|---|
| $G_1$ | 9 | 11.80 | 10.00 | 14.00 | 1.21386 |
| $G_2$ | 9 | 10.94 | 10.00 | 12.30 | 0.63095 |
| $G_3$ | 9 | 14.04 | 10.00 | 18.00 | 2.67938 |
| $G_0$ | 9 | 12.00 | 10.00 | 15.80 | 1.69090 |

## 2.7. Number of leaves

The recorded leaf numbers of the groups with the different substrates can be seen in Table 7. According to the findings, perlite group (G3) and control group (G0) had the highest number of leaves per plant with 29.96. This was followed by the cocopeat group (G1) with 26.21. The lowest number of leaves per plant was determined as 18.67 in the loofah group (G2). According to the Kruskal-Wallis test results, a statistically significant difference was found between the substrates in terms of leaf number values (Chi-Square: 25.463; p = 0.000).

**Table 7.** Average number of leaves by substrates (number)

| Substrates | n | Mean | Minimum | Maximum | Std. Deviation |
|---|---|---|---|---|---|
| $G_1$ | 9 | 26.21 | 15.00 | 40.00 | 7.12606 |
| $G_2$ | 9 | 18.67 | 15.00 | 30.00 | 4.39037 |
| $G_3$ | 9 | 29.96 | 15.00 | 48.00 | 10.42355 |
| $G_0$ | 9 | 29.96 | 15.00 | 48.00 | 10.42355 |

## 2.8. Amount of subsidence in the planting layer

The amount of subsidence observed in the pots of the groups with the differing substrates analysed in the study are given in Table 8. According to these values, the group demonstrating the least subsidence was the loofah group (G2) with 3.83 mm. This was followed by the perlite group (G3) with 4.67 mm, the cocopeat group (G1) with 5.17 mm, and the control group (G0) with 11.83 mm. As it can be understood from these findings, the average amount of subsidence was the highest in the control group (G0) with only soil. According to the Kruskal-Wallis test results, a statistically significant difference was found between substrates in terms of subsidence values (ChiSquare: 26.094; p = 0.000).

**Table 8.** Average amount of subsidence in the planting layer according to the substrates (mm)

| Substrates | n | Mean | Minimum | Maximum | Std. Deviation |
|---|---|---|---|---|---|
| $G_1$ | 9 | 5.17 | 4.00 | 6.00 | 1.04083 |
| $G_2$ | 9 | 3.83 | 3.50 | 4.00 | 0.28868 |
| $G_3$ | 9 | 4.67 | 4.00 | 5.00 | 0.57735 |
| $G_0$ | 9 | 11.83 | 7.50 | 15.50 | 4.04145 |

## 3. Discussions

The data obtained during the study of the groups with differing substrates are summarized and given in Table 9 to demonstrate the substrates' superior properties. Among the groups with the differing substrates analysed in this study, the lowest EC value was observed in the loofah group (G2) following the control group (G0). It is known that the growth and development of plants are negatively affected by the high EC value since it indicates the presence of high levels of soil salinity (Ekmekçi et al., 2005). Thus, apart from the control group (G0), the loofah group (G2) was the best option in terms of salinity drainage among the substrates.

When the data was analysed in terms of the amount of drainage, the lowest amount of drainage among the groups with the differing substrates was observed in the cocopeat (G1) group, followed by the perlite group (G3). It is known that the amount of drainage water should be kept at a minimum to reduce the negative effects of excessive drainage on the environment, and minimize the cost of fertilizer (Başar, 2000). For this reason, it was concluded that the cocopeat group (G1) was better than the alternative substrates in terms of drainage amount, while the control group (G0) should not be preferred due to the very high drainage amount.

When the data was examined in terms of pot temperature, close values between the substrates were observed, except for the control group (G0), as its pot temperature was considered high. As is known, the planting layer's heat storage feature becomes beneficial in the summer due to the cooling effect of the plant layer on the surface, which also decreases the access of thermal load and reduces the cooling energy needs of buildings (Liu, 2004; Gaffin et al., 2006; Saiz et al., 2006; Ayçam & Kınalı, 2013). It has been observed that the addition of the substrates analysed in this research to the plant growing media has a positive impact since it protects the planting layer from high temperature, and hence, the building as well.

When the data was analysed in terms of the in-pot moisture, close values were observed among the substrates analysed in the study, except for the control group (G0) as the soil moisture of that group was low. It is known that plants initially get stressed due to the decrease in the amount of water in the soil and if the moisture decrease continues, they ultimately die (Tamsa, 2013). It has been concluded that adding the analysed substrates to the plant growing media would contribute positively in terms of moisture retention for plant roots.

The highest results for the number of leaves and plant height were observed in the perlite group (G3) among the groups with the differing substrates analysed in the study. While the control group (G0) showed the same success as the perlite group (G3) according to the number of leaves, it ranked second, behind the perlite group (G3), in terms of plant height. It is believed that the results obtained in terms of plant growth will vary according to the needs of the different plant species that can be used in extensive green roof designs. In this study where the Crassula ovata plant was used, the plant growth in all groups was considered sufficient and it was concluded that all the substrates included in the study can be used as plant growing media in extensive green roof designs.

When the data on the amount of subsidence in the planting layer was examined, the best result in terms of the amount of subsidence was observed in the loofah group (G2) with the least amount, while the highest amount of subsidence was seen in the control group (G0). Subsidence is a degradation process related to compression and occurs when the structural form of the planting layer gets disrupted because of rainfall or external mechanical forces. Compression in the planting layer may cause negativities in the physical and hydrological properties of the environment, but also lead to the physiological deterioration of plants, affect the plant's balance and its growth hormones, and limit its intake

**Table 9. Ordinal distribution of factors compared by substrates (1: best; 4: worst)**

| | EC | pH | Drainage | Temp. | Moist. | Plant height | Number of leaves | Subsidence |
|---|---|---|---|---|---|---|---|---|
| Substrates | | | | | | | | |
| $G_1$ | 4 | 2 | 1 | 2 | 2 | 3 | 3 | 2 |
| $G_2$ | 2 | 1 | 3 | 3 | 3 | 4 | 4 | 1 |
| $G_3$ | 3 | 3 | 2 | 1 | 1 | 1 | 1 | 3 |
| $G_0$ | 1 | 4 | 4 | 4 | 4 | 2 | 2 | 4 |
| Statistically significant difference among groups | yes | no | yes | yes | yes | yes | yes | yes |

of nutrients (Turgut, 2012). According to the results obtained, regarding the addition of the researched substrates to the plant growing media, it has been observed that they significantly contribute to the reduction of the amount of subsidence in the planting layer.

The data obtained by the groups with the differing substrates analysed in the study are evaluated on a scale from 1 to 4 and summarized in Table 9. Regarding all the data obtained in terms of the substrates included in the experiment, the best case was evaluated as 1 point and the worst case 4 points. In the evaluation carried out, those that demonstrated a statistically significant differences in terms of the studied factors were taken into account. According to this, it was understood that the best option among the substrates in terms of EC values was the control group (G0). Since there was no statistically significant difference between the substrates in terms of pH values, this factor was not taken into account. It was determined that the group with the best water holding capacity according to the measured drainage amount was cocopeat (G1). On the other hand, the perlite group (G3) turned out to be the best option in terms of in-pot temperature, in-pot moisture, plant height and number of leaves per plant. In regard to the amount of subsidence in the planting layer, the best result was observed in the loofah group (G2). According to all the results, it is possible to say that perlite (G3) offers more advantages than the alternative substrates in terms of many variables. In addition, in line with the hypotheses of the study, the performance of the groups according to the substrate types has varied regarding “EC value”, “pH value”, “drainage amount”, “in-pot temperature”, “in-pot moisture”, “plant height”, “number of leaves” and “the amount of subsidence in the planting layer”.

It is believed that the use of a single type of substrate as plant growing media would not be sufficient in terms of ensuring the best performance of extensive green roofs, and it would be more beneficial to consider the advantages that each substrate group demonstrated in the aforementioned subcategories.

## Conclusions

Green roofs and green areas are an important indicator of the quality of urban life. They establish a connection between nature and the city’s nostalgia for green spaces, and they incorporate nature within the city. This study has aimed to provide information regarding the ecosystembased approach of green roofs and discuss the potential use of the researched substrates in extensive green roofs. In this regard, recommendations were made according to the results obtained from the study.

One of the most important aspects in the design of extensive green roofs is the weight of the roof covering layer, which should be appropriate for low load-bearing roofs. In order to reduce the weight on the roof, the lightest possible substrates should be preferred within the planting layer. In this regard, it has been observed that the lightest material among the substrates analysed in the study is loofah, which could be a good choice. In addition, loofah was chosen as the substrate with the highest performance among the others in this study regarding the minimization of the subsidence in the planting layer. Since there is no existing literature on the use of loofah as a substrate, it is proposed that the results obtained from this study contribute to the literature on the use of loofah as a substrate in green roof applications. It is impossible to determine a single universal green roof substrate that could be preferred in all green roofs since its effects could change based on the green roof's design, climate conditions and geographical location, ecological suitability etc.

However, within the conditions that the research was carried out; although loofah has ranked behind the other substrates in terms of plant growth, it is a light material and creates a strong resistance against the substrate shrinkage that may occur in the planting layer. In addition to its success in in-pot temperature and moisture, perlite has shown the highest success in terms of plant growth among all the substrates included in the study. Cocopeat has the highest water holding capacity among the substrates studied.

To achieve the best performance in extensive green

roofs, it is recommended not to use the soil alone while preparing the plant growing media but to combine it with more than one substrate type, after considering the success rate of the substrates or according to the type of substrates.

It is believed that the results obtained from this study will benefit the relevant literature, initiate different studies, and shed light on future research.

**References**


• *Ampim, P. A. Y., Sloan, J. J., Cabrera, R. I., Harp, D. A., & Jaber, F. H. (2010). Green roof growing substrates: Types, ingredients, composition and properties. Journal of Environmental Horticulture, 28(4), 244–252. https://doi.org/10.24266/0738-2898-28.4.244*

•*Aslanboğa, İ. (1988). Ege Bölgesi iklim koşullarında çatı bahçesi yapımında kullanılabilecek yapısal ve bitkisel materyalin seçimi üzerine araştırmalar. Bilgehan Basımevi, Bornova-İzmir.*

•*Ayçam, İ., & Kınalı, M. (2013). Ofis binalarında yeşil çatıların ısıtma ve soğutma yüklerine olan etkilerinin analizi. Tesisat Mühendisliği, 135, 26–34.*

• *Başar, H. (2000). Bazı topraksız yetiştiricilik yöntemlerinin karşılaştırılması. Anadolu Ege Tarımsal Araştırma Enstitüsü Dergisi, 10(2), 169–182.*

• *Calheiros, C. S. C., & Stefanakis, A. I. (2021). Green roofs towards circular and resilient cities. Circular Economy and Sustainability, 1, 395–411. https://doi.org/10.1007/s43615-021-00033-0*

•*Cascone, S. (2019). Green roof design: State of the art on technology and materials. Sustainability, 11(11), 3020. https://doi.org/10.3390/su11113020*

•*Chagolla-Aranda, M. A., Simá, E., Xamán, J., Álvarez, G., Hernández-Pérez, I., & Téllez-Velázquez, E. (2017). Effect of irrigation on the experimental thermal performance of a green roof in a semi-warm climate in Mexico. Energy and Buildings, 154, 232–243. https://doi.org/10.1016/j.enbuild.2017.08.082*

•*Dunnett, N., & Kingsbury, N. (2008). Planting green roofs and living walls (2nd ed.). Timber Press Inc.*

• *Dvorak, B., & Volder, A. (2010). Green roof vegetation for North American ecoregions: A literature review. Landscape and Urban Planning, 96(4), 197–213. https://doi.org/10.1016/j.landurbplan.2010.04.009*

•*Ekmekçi, E., Apan, M., & Kara, T. (2005). Tuzluluğun bitki gelişimine etkisi. OMÜ Ziraat Fakültesi Dergisi, 20(3), 118125.*

•*Ekşi, M. (2014). Çatı bahçesi kavramı ve terim kullanımı üzerine bir değerlendirme. Avrasya Terim Dergisi, 2(2), 26–35.*

• *Erkul, E., & Sönmez, A. (2014). Çevre duyarlı mimarlık: Yeşil çatı sistemleri çevre etkileri. Mimarlık Dergisi, 375, 52–58.*

• *Gaffin, S., Rosenzweig, C., Parshall, L., Hillel, D., Eichenbaum Pikser, J., Greenbaum, A., Blake, R., Beattie, R., & Berghage, R. (2006, May 11–12). Quantifying evaporative cooling from green roofs and comparison to other land surfaces [Conference presentation]. The Fourth Annual Greening Rooftops*

*for Sustainable Communities Conference Awards and Trade Show, Boston.*
*• Gülgün Aslan, B., & Yazici, K. (2016). Yeşil altyapı sistemlerinde mevcut uygulamalar. Ziraat Mühendisliği, (363), 31–37.*
*• Johnston, J., & Newton, J. (1993). Building green: A guide to using plants on roofs, walls and pavements. London Ecology Unit.*
*• Kadıoğlu, B., & Canbolat, M. Y. (2019). Organik ve inorganik materyallerin ince bünyeli toprağa ilavesi ile hazırlanan yetişme ortamlarının hidrofiziksel özellikleri. Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 50(2), 107–114. https://doi.org/10.17097/ataunizfd.453748*
*•Korol, O., Shushunova, N., & Shushunova, T. (2018). Innovation technologies in Green Roof systems. MATEC Web of Conferences, 193(4), 04009. https://doi.org/10.1051/matecconf/201819304009*
*•Landscape Development and Landscaping Research Society e.V. (2018). Green roof guidelines: Guidelines for the planning, construction and maintenance of green roofs. Bonn, Germany.*
*• Liu, K. (2004). Engineering performance on rooftop gardens through field evaluation. Journal of Roof Consultants Institute, 22(2), 4–12.*
*• Manso, M., Teotónio, I., Silva, C. M., & Cruz, C. O. (2021). Green roof and green wall benefits and costs: A review of the quantitative evidence. Renewable and Sustainable Energy Reviews, 135, 110111. https://doi.org/10.1016/j.rser.2020.110111 Özdemir, E., & Altun, M. C. (2010, Nisan 15–16). Bitkilendirilmiş çatı sistemi tasarımı için bir kontrol listesi önerisi. In 5. Ulusal Çatı ve Cephe Sempozyumu, Dokuz Eylül Üniversitesi Mimarlık Fakültesi, Buca-İzmir. http://catider.org.tr/pdf/sempozyum5/Semp%205%20Bildiri%2022.pdf*
*•Reyes, R., Bustamante, W., Gironás, J., Pastén, P. A., Rojas, V., Suárez, F., Vera, S., Victorero, F., & Bonilla, C. A. (2016). Effect of substrate depth and roof layers on green roof temperature and water requirements in a semi-arid climate. Ecological Engineering, 97, 624–632. https://doi.org/10.1016/j.ecoleng.2016.10.025*
*•Rowe, D. B. (2011). Green roofs as a means of pollution abatement. Environmental Pollution, 159(8–9), 2100–2110. https://doi.org/10.1016/j.envpol.2010.10.029*
*•Seçkin, Y. P., & Seçkin, Y. Ç. (2016, Haziran 2–3). Mimari tasarımda yeşil çatıların gelişimi. In 8. Ulusal Çatı ve Cephe Sempozyumu, Mimar Sinan Güzel Sanatlar Üniversitesi, Fındıklı- İstanbul. http://catider.org.tr/pdf/sempozyum8/06MIMARI-TASARIMDA-YESIL-CATILARIN-GELISIMI.pdf Saiz, S., Kennedy, C., Bass, B., & Pressnail, K. (2006). Comparative life cycle assessment of standard and green roofs. Environmental Science and Technology, 40, 4312–4316. https://doi.org/10.1021/es0517522*
*•Tamsa, H. K. (2013). Sürekli solma noktasının tayininde yeni bir yaklaşım: difüzyon- toprak nem ilişkisi. Namık Kemal Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, Tekirdağ.*
*• Turgut, B. (2012). Ormanlık alanlarda toprak sıkışması sorunu.*
*•SDÜ Orman Fakültesi Dergisi, 13, 66–73. Ürgenç, S. (1990). Genel plantasyon ve ağaçlandırma tekniği. İstanbul Üniversitesi, Fakülte (Orman), Istanbul.*
*•Vandegrift, D. A., Rowe, D. B., Cregg, B. M., & Liang, D. (2019). Effect of substrate depth on plant community development on a Michigan green roof. Ecological Engineering, 138, 264273. https://doi.org/10.1016/j.ecoleng.2019.07.032 Woolley, T., & Kimmins, S. (2000). Green building handbook: Vol. 2. A guide to building products and their impact on the environment (1st ed.). Routledge.*
*• Wooster, E. I. F., Fleck, R., Torpy, F., Ramp, D., & Irga, P. J. (2022). Urban green roofs promote metropolitan biodiversity: A comparative case study. Building and Environment, 207(A), 108458. https://doi.org/10.1016/j.buildenv.2021.108458*
*•Younis, A., Zulfiqar, F., Ramzan, F., Akram, A., Wright, S. R., Farooq, A., Ahsan, M., & Sagu, A. H. (2020). Roof top gardening, a solution for landscape enhancement in urban areas: A case study of*

*Faisalabad, Pakistan. Pakistan Journal of Agricultural Sciences, 57(2), 333–337.*

# NITROGEN SEQUESTRATION DURING SEWAGE SLUDGE COMPOSTING AND VERMICOMPOSTING

**Aušra ZIGMONTIENĖ *, Vaida ŠEREVIČIENĖ**
Department of Environmental Protection and Water Engineering,
Vilnius Gediminas Technical University, Vilnius, Lithuania

## ABSTRACT

*Composting is the oldest and most natural form of organic material recycling. Technological parameters are very important because when the process is unbalanced, other gases are produced, some of which have objectionable odours (Nh3). Sewage sludge is a valuable material that has accumulated large amounts of nitrogen and phosphorus, which can contribute to improving soil quality. Optimal composting and vermicomposting conditions (C/N ratio, pH, and moisture) can reduce the emissions of gaseous pollutants in the environment. Experimental studies have shown that the volume of ammonia emitted into the environment during vermicomposting of sewage sludge is significantly lower (3 mg/m3 concentration was reached on the 28th day) than that resulting from traditional composting (3 mg/m3 concentration was reached on the 56th day). Vermicomposting of sewage sludge preserves higher amounts of total nitrogen (12.52 mg/kg) compared to traditional composting (10.35 mg/kg).*

***Keywords: sewage sludge treatment, composting, vermicomposting, earthworms, Kjeldahl nitrogen, C/N ratio, ammonia.***

## INTRODUCTION

Improved wastewater collection systems, which refer to modern purification and treatment technologies, lead to an increase in the amount of sludge produced during wastewater treatment (Zou et al., 2014). Most of this sludge is stored in the sludge sites of a sewage treatment facility, and only a small part of it is used in agriculture and the production of biogas. High-quality compost should not be applied to normal usage as e.g. landscaping/landfill cover (Van Fan et al., 2016). Sludge treatment by applying bioreactors or sludge drying systems is expensive. For this reason, the usage of sewage sludge in agriculture will reduce processing costs and contribute to the sustainability of the whole treatment cycle. The heavy metal content in sewage sludge is a limiting factor in sludge treatment and its continued use in agriculture (Ludibeth et al., 2012). Under these circumstances, composting (Frederickson et al., 2007) and vermicomposting (Alidadi et al., 2016) are environmentally acceptable processes for biological sequestration of nitrogen.

Vermicomposting – is a biotechnological composting process which uses earthworms to process organic waste and obtain a better final product compared to composting (Gupta & Garg, 2008). Earthworms process various mineral and organic matters into a complex organic-chemical mixture – a compost of high porosity and water retention. Vermicompost is rich in macro and microelements, growth-promoting substances, vitamins, antibiotics, amino acids and useful microflora. The rise of calcium, magnesium, phosphorus, trace elements, and enzymes is observed in the waste produced by earthworms (Alidadi et al., 2016). The chem earthworms (Alidadi et al., 2016). The chemical elements in vermicompost are changed into more accessible forms for the plants, nitrates, soluble phosphorus, potassium, and compounds of calcium and magnesium. T hese substances are easily absorbed by plants, and also help reduce soil pH and its salinity (Rodríguez-Quiroz et al., 2011).

Total nitrogen in sewage sludge consists of several nitrogen forms: total nitrogen; nitrites and nitrates; ammonium and ammonia; dissolved organic nitrogen gas; easily suspended biodegradable nitrogen and suspended (organic) nitrogen gas (Henze et al., 2001). The current studies (Gupta & Garg, 2008) analyse the parameters of the composting process to reach optimal conditions for high-quality compost production. Still, there is a data gap on emissions from different composting processes.

The purpose of this article is to evaluate the sequestration of nitrogen compounds from sewage sludge treated by different methods of composting by studying the transformation of nitrogen compounds into mineral and gaseous nitrogen compounds.

## 1. Materials and methods

Mechanically drained sewage sludge from the Vilnius wastewater treatment plant was used. Californian worms from a local worm farm were used in the vermicomposting process.

### 1.1. Preparation for the experiment

Mechanically dewatered sewage sludge accumulated in Vilnius Wastewater Treatment Plant was used for this research. To determine the moisture content, the sludge is dried at 105±2 °C for at least 1 h, until the weight is constant. Municipal sewage sludge with a moisture content of 70% was placed in two 56×39×28 cm plastic boxes.

Many biodegradable waste can be composted on their own if they have the right physical and chemical properties. However, other materials (additives) are usually used and play a role as supplements and fillers. In the case of sewage sludge, due to its low porosity, high moisture content, and low C/N ratio, it is necessary to add other wastes that provide structure and porosity (fillers) and improve moisture content and C/N ratio (Kulikowska & Gusiatin, 2015). Sewage sludge mixed with peat and tree leaves was composted and vermicomposted simultaneously. Californian earthworm vermiculture was added in the container with sludge. This species of earthworms was selected due to its ability to adapt easily to a new environment and process organic waste efficiently and rapidly and also due to its lower sensitivity to environmental fluctuations.

Before placing the earthworms in the studied sewage sludge, an acclimatisation process had been carried out according to the method described by (Shaymaa et al., 2010). Acclimatisation is necessary to adapt earthworms to their new environment, sewage sludge. The point of acclimatisation is the migration of earthworms from their original living environment to another. This process was carried out in the vermicomposting box that was divided into two sections. The first section contained the living environment of earthworms, compost, which consisted of cow manure, straws, and organic waste residues, while the second section contained the sewage sludge studied mixed with peat and tree leaves.

The experimental studies lasted for 91 days. Every 7th day samples of composted and vermicomposted sewage sludge were collected. The following parameters were measured: humidity of compost, temperature, pH, content of total carbon, Kjeldahl nitrogen, and ammonium concentration released.

The same conditions were used for vermicomposting and composting. The moisture content of the sludge of 60–80% and the compost temperature of 20±2 °C were ensured in a laboratory-executed experiment. Composting was carried out in an open aerobic system. Gaseous samples for ammonia concentration detection were collected from the surface of the piles.

### 1.2. Determination of total carbon content

The total carbon analyser of the Shimadzu TOC-V series SSM-5000A solid sample module (680 °C combustion catalytic oxidation/NDIR method) sample module total carbon analyser was used to determine the total carbon content. A sample was prepared for measurement as follows: the sample was prepared for measurement as follows: the sample was dried at a temperature of 105±2 °C for at least an hour until it was constant in weight and homogenised. 35 mg of dried and crushed sludge and 55 mg of vermicompost were poured into a constant volume of porcelain plates. The weights of the sludge and vermicompost samples differ due to their density. The carbon content within the sample measured by the carbon analyser is provided in %.

### 1.3. Photometric determination of ammonia

The method is based on a yellowish-brown compound (ammonium mercury iodate) produced during the reaction between ammonia and Nessler's reagent (Nesler spectrophotometrical method). The intensity of the colour produced during the reaction depends on the concentration of ammonia. An ammonia calibration curve was plotted during the investigation, showing the dependence of the optical density of the solution from the ammonia content in the volume of the analysed sample. T he air polluted with ammonia was pumped through an absorber filled with 6 ml of 0.01 N HCl solution. Traction time (20 min) was recorded after the aspirator was turned on. 5 ml samples were collected from the absorber into an empty test tube for analysis. 0.5 ml of Nessler's reagent was poured into the resulting solution and mixed.

After 5 minutes, the optical density of solutions in 10 mm cuvettes at a wavelength of 450 nm was measured. In comparison, a blank sample (5 ml of 0.01 N HCl) was used by pouring 0.5 ml of Nessler's reagent. The concentration of ammonia was determined using the calibration curve.

### 1.4. Determination of total Kjeldahl nitrogen (TKN)

The total concentration of Kjeldahl nitrogen (TKN) was measured by standard Kjeldahl digestion UDK-152 with a distillation unit (Velp Scientifica). During sample mineralisation, ammonium sulphate is produced; therefore, it should be isolated, distilled, and then titrimetrically determined. Nitrogen compounds were mineralised with sulfuric acid by mixing it with large amounts of potassium sulphate (necessary to raise the boiling temperature of the mixture) and using selenium as a catalyst. This is how nitrogen compounds were transformed into ammonium sulphate. After mineralisation, ammonia was separated from ammonium sulphate by adding alkali and distilling into a boric acid indicator solution. Ammonium ions in the distillate were determined through titration with a standard acid solution.

### 1.5. Quality control and statistical data analysis

The quality control procedure included collecting three samples in parallel during every sampling period. Statistical data analysis was performed using Microsoft Excel.

The graphically presented results include mean values with the values of standard deviations.

## 2. Results and discussion

### 2.1. Research and analysis of sludge stabilization indicators

To ensure the efficacy of the vermicomposting process and obtain good biohumus, it is very important to select optimal conditions: moisture content, temperature, and pH level of the compost (Table 1).

The vermicompost temperature during the entire experiment was around 20 °C and only occasionally dropped

**Table 1.** Optimal sewage sludge stabilization indicators

| Process | Composting | Vermicomposting |
|---|---|---|
| Temperature, °C | 19–45 | 20–21 |
| Moisture content, % | 50–60 | 60–80 |
| pH | 7–9 | 7–8 |

to 19.5 °C. The initial temperature of the compost was 19 °C and increased to 55 °C, after 7 days of decrease. It is vital to control temperature during vermicomposting and vermiculture processes to maintain its suitability for earthworms (not too high and not too low) (Munroe, 2007). Earthworms can survive even at temperatures as low as 0 °C; however, at such a low temperature, they stop breeding and process organic wastes in the compost. If the temperature is above 35 °C, earthworms leave the compost or die. A temperature of 20 °C is necessary to ensure an efficient vermicomposting process (Munroe, 2007).

Rynk et al. (1992) has determined that the ideal moisture content in traditional composting systems must be around 45–60%, while in vermicomposting processes it must be around 65–85%. In the current investigation, the moisture content of vermicompost was maintained between 60 and 80%. During the composting and vermicomposting processes, the moisture content was quite stable and met the optimal composting conditions throughout the research period (Table 1).During composting, the total carbon content generally decreases, as the microorganisms break down the organic material and use the carbon for energy. This process is known as mineralisation. During the sewage sludge composting experiment, the total carbon (TC) content decreased within the analysed samples from 35.49 to 26.21% (loss of TC 26.15%) (Figure 1). There were more fluctuations in total carbon content (from 35.49 to 20.33 %) in the vermicomposting experiment, the loss of TC content was 42.72%. The difference in TC content during composting and vermicomposting was significant ($p < 0.01$).

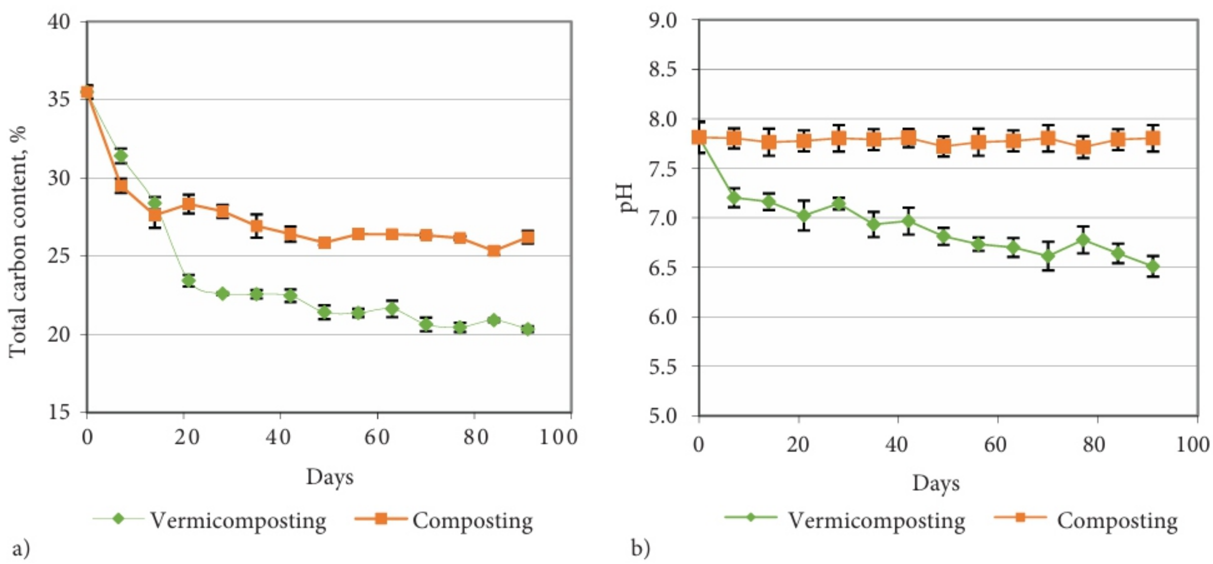



**Figure 1.** Changes of total carbon content (a) and pH values (b) during composting and vermicomposting

The general tendency during the vermicomposting process is that the total carbon content in sewage sludge decreases with time because earthworms use the carbon to obtain energy and grow (Alidadi et al., 2016). The percentage of carbon decrease at the end of composting versus vermicomposting can vary depending on a number of factors, such as initial carbon content, the type of organic matter that is composted, and the length of time that the composting or vermicomposting process is allowed to proceed. Hait and Tare (2011) have found that the loss of total carbon content in vermicompost was 6.1–13.8%, while the loss of TC in the control sample ranged from 0.8 to 6.1%. They suggested that significant differences in the TC contents of the control and vermicompost samples were due to rapid mineralisation of organic matter mediated by earthworms (Hait & Tare, 2011).

The pH level is also very important to ensure the efficient activity of earthworms. The pH values during the whole experiment (composting and vermicomposting) ranged from 6.44 to 7.89 (Figure 1b). The pH during vermicomposting decreased by almost 17%, while during the composting process it remained unchanged. The difference in pH between composting and vermicomposting was highly significant ($p < 0.001$). The decrease in pH during vermicomposting may occur due to CO2 and organic acids produced by microbial metabolism (Hait & Tare, 2011). The pH of compost could have a vital effect on the growth of bacteria and other organisms. The optimal pH range for bacteria growth is 6.0 to 7.5, for fungi 5.5 to 8.0 (Varma et al., 2015) and around neutral for earthworms (Manaf et al., 2009).

The most important materials that ensure the functions of microorganisms during composting are carbon and nitrogen, which excess or deficiency determine the value of the compost (Sharma & Garg, 2018). The decrease in organic carbon during the vermicomposting or composting process indicates complete degradation, maturity, mineralisation, and waste decomposition (Hait & Tare, 2011). C/N ratio determines maturity and stability of vermicompost. The reduction of the C/N ratio in the final vermicompost indicates rapid mineralisation and decomposition of the initial raw material and is used mostly as

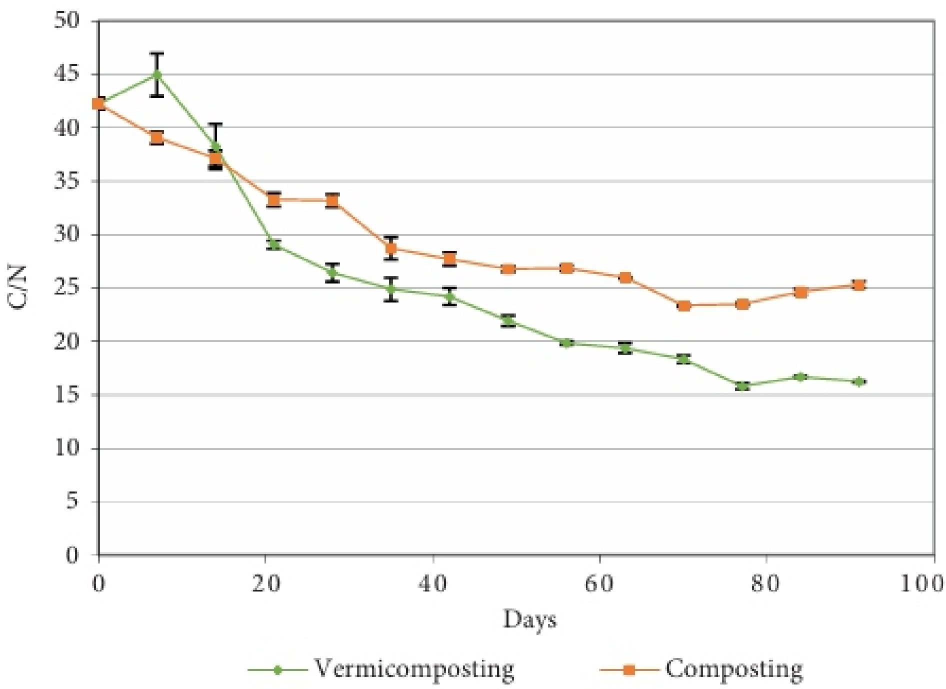



**Figure 2.** C/N ratio during composting and vermicomposting

the parameter for stability and maturity of organic wastes (Suthar & Singh, 2008). The results of the C/N ratio obtained during the research are provided in Figure 2.

The C/N ratio in the vermicomposed sewage sludge decreased throughout the research period and its average value at the end of the process 16.24. Compared to the vermicomposting process, the C/N ratio during sewage sludge composting fluctuated: it decreased from 42.2 of its initial value to 25.31. According to Haug (1993), the most suitable conditions for microorganisms to live are when this ratio is between 15 and 30. Huang et al. (2004) reported that composting at a low initial C/N of 15 would require a composting longer. Nigussie et al. (2016) have found that vermicomposting can reduce total nitrogen loss from the high C/N (30) substrate by 10 % and from the low C/N (24) substrate by 20%. Different composting substrate mixes would have different initial C/N ratio: dewatered fresh sewage sludge and wheat straw – 25 (Awasthi et al., 2016), vegetable waste and straw – 14–30 (Nigussie et al., 2016), food waste and dry leaves – 20–30 (Nguyen et al., 2020), green waste containing plant debris and cattle slurry – 24–53% (Cáceres et al., 2015), primary sewage sludge and cow dung – 32–69 (Gupta & Garg, 2008). Compost with a ratio of 10 to 15 can be considered stable, although the final ratio strongly depends on the starting materials used (Azim et al., 2018).

### 2.2. Nitrogen changes in vermicomposting and composting

The reactions associated with nitrogen in composting processes are complex, but the main processes that determine the formation of nitrogen species can all be divided into mineralisation, volatilisation, nitrification, immobilisation, and denitrification (Li et al., 2013). The nitrogen content changes during composting, typically increasing first as the microorganisms break down the organic material and release nitrogen. However, as the composting process continues and microorganisms consume more nitrogen for their own growth and reproduction, the nitrogen content may decrease again. This is because some of the nitrogen is lost through processes such as volatilisation or leaching. During other research, different profiles of total nitrogen were reported. Alidadi et al. (2016) reported that during their vermicomposting research, total nitrogen was increasing in all treatments (four mixing ratio of municipal solid waste and carbonaceous organic materials) from average 0.47 to 1.12 mg/kg. Awasthi et al. (2016) analysed the influence of zeolite and lime on emissions during sewage sludge composting and found a completely different profile of TKN. In treatments with zeolite + 1% lime, the TKN content initially decreased slightly in the first week and then increased sharply until 6 weeks, after which almost constant values were observed. Compared to the 1% lime treatments and the control, a decreasing trend in TKN content was observed until the 2nd and 4th week, which then slightly increased until the end of the composting process, resulting in significant differences between the treatments with zeolite and 1% lime. The initial decreasing trend of TKN in the treatments with zeolite + 1% lime addition can be attributed to the loss of ammonia, while the long decreasing trend in the control and the treatments with 1% lime addition due to the lack of zeolite addition indicates slow decomposition of organic matter and excessive loss of ammonia, as shown in the ammonia emission profile.

When composting sewage sludge in this research, the total Kjeldahl nitrogen (TKN) found in sewage sludge has increased over time (Figure 3a). TKN slightly decreased when sewage sludge vermicomposting: from 8.40 g/kg to 6.99 g/kg, after the first week and then increased to 12.52 g/kg 91 days. This determined the ability of earthworms to stabilise nitrogen in the form of nonvolatile compounds. Hence, vermicompost contains more nutrients, which are necessary to improve the properties of soil and activate its microbiological processes. Meanwhile, during the composting process, the TKN increment is less, up to 10.35 g/kg. Ludibeth and others (2012) also found out that nitrogen content (2.190 g/kg) in vermicompost is significantly higher than in sewage sludge (0.163 g/kg). Earthworms and microorganisms increase the decomposition of organic matter, accelerate the transformation of organic nitrogen into free nitrogen, and improve the properties of wastewater sludge. Greenhouse gas and an unpleasant smell are released during the decomposition and biodegradation of sewage sludge. Sludge storage can also be associated with emissions of high amounts of ammonia (NH3) and greenhouse gases (CH4, CO2) (Li et al., 2013). The proper management of biodegradable waste has become particularly important, as its decomposition products have a significant effect on climate change. As can be seen in Figure 3b, the concentration of ammonia released into the air decreases over time during both traditional composting and vermicomposting. When vermicomposting sewage sludge, the emissions of ammonia into the environment decreased much more rapidly. 25 mg/m3 of ammonia was released when vermicomposting sewage sludge on the first research day, i.e. 168.87 g/Mg/day. On day 56, the ammonia concentration in the air reached the minimum concentration – 0.061 mg/m³ (0.41 g/Mg/day), and on day 91, at the end of the composting process, the ammonia was no longer released into the environment.

A significant decrease in ammonia concentration was already observed within the first 28 days when composting with earthworms (the average ammonia concentration was 3 mg/m³ (20.26 g/Mg/d)). When composting without earthworms, the ammonia concentration reached the same level only on day 56 and did not fall below the average limit of 1 mg/m³ (6.75 g/Mg/d) during the research period. The results obtained suggest that significantly less ammonia is released into the environment when sewage sludge is vermicomposted rather than composted because while processing organic waste, earthworms stabilise nitrogen in the form of nonvolatile compounds. Similar results were obtained during the experiments carried out by Velasco-Velasco et al. (2011). It was found that, during the first days of experimental research, ammonia emissions into the environment reached 450 g/Mg/d when organic waste was processed by simple composting, while it reached only 253.2 g/Mg/d when vermicomposted.

After 45 days, ammonia concentration ranged from 0 to 21 g/Mg/d during the vermicomposting process. These results show that significantly less ammonia is released during the vermicomposting process compared to simple composting (Komakech et al., 2016). It can be assumed that the decrease in ammonia emissions is determined by the transformation of ammonia into organic nitrogen during the activity of earthworms.

During the vermicomposting process, earthworms use up a portion of carbon to obtain energy and absorb organic nitrogen when processing sewage sludge. This organic nitrogen is the main nutrient for living organisms; therefore, the nitrogen transformation process is reduced

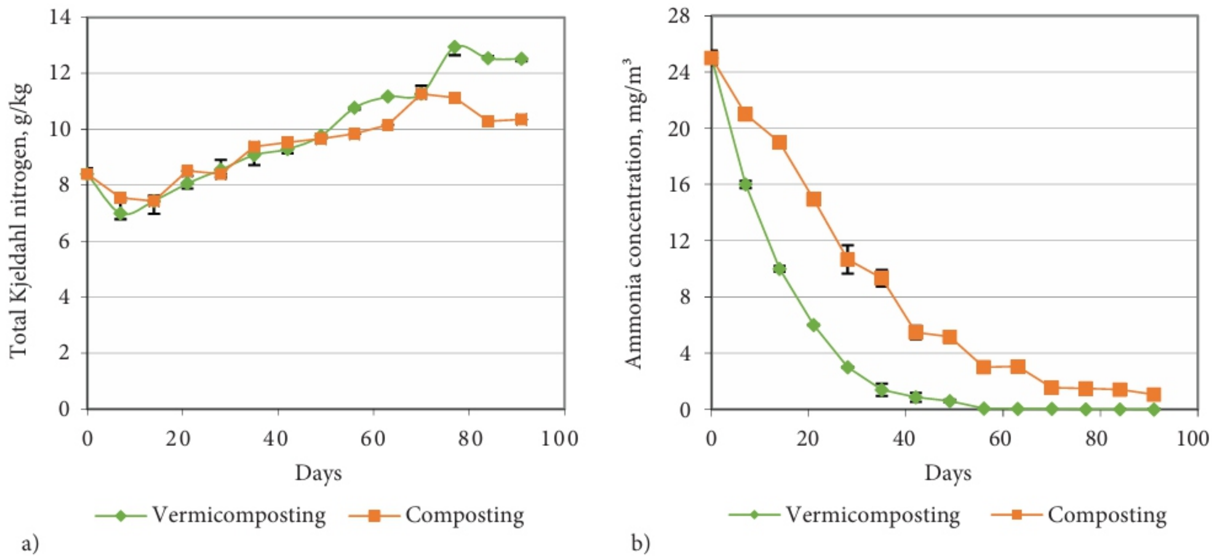


**Figure 3.** Concentration of a) total Kjeldahl nitrogen in compost and b) ammonia in the air during the research

to volatile ammonia. The positive correlation between ammonia concentration and total carbon content was determined (vermicompost $r = 0.99$, compost $r = 0.84$, $p < 0.05$) and the negative correlation between ammonia and TKN (vermicompost $r = –0.651$, compost $r = –0.889$, $p < 0.05$).

## Conclusions

Earthworms have a positive effect on mitigating Nh3 emissions. Throughout the experimental research period, the initial concentration of ammonia during the vermicomposting process decreased from 25 mg/m3 to 0 mg/m3, whereas during the traditional composting process it did not fall below the average limit of 1 $mg/m^3$.

This can be explained by the ability of earthworms to stabilise nitrogen in the form of nonvolatile compounds. Experimental research showed that a higher total nitrogen content (12.52 g/kg) is preserved when vermicomposting sewage sludge than during traditional sewage sludge composting (10.35 g/kg). Hence, vermicompost contains more nutrients, which are necessary to improve the properties of soil and activate its microbiological processes.

## References


• *Alidadi, H., Hosseinzadeh, A., Najafpoor, A. A., Esmaili, H., Zanganeh, J., Takabi, M. D., & Piranloo, F. G. (2016). Waste recycling by vermicomposting: Maturity and quality assessment via dehydrogenase enzyme activity, lignin, water soluble carbon, nitrogen, phosphorous and other indicators. Journal of Environmental Management, 182, 134–140. https://doi.org/10.1016/j.jenvman.2016.07.025*

• *Awasthi, M. K., Wang, Q., Huang, H., Ren, X., Lahori, A. H., Mahar, A., Ali, A., Shen, F., Li, R., & Zhang, Z. (2016). Influence of zeolite and lime as additives on greenhouse gas emissions and maturity evolution during sewage sludge composting.*

• *Bioresource Technology, 216, 172–181. https://doi.org/10.1016/j.biortech.2016.05.065*

• *Azim, K., Soudi, B., Boukhari, S., Perissol, C., Roussos, S., & Thami Alami, I. (2018). Composting parameters and compost quality: A literature review. Organic Agriculture, 8, 141–158. https://doi.org/10.1007/s13165-017-0180-z*

• *Cáceres, R., Coromina, N., Malińska, K., & Marfà, O. (2015). Evolution of process control parameters during extended cocomposting of green waste and solid fraction of cattle slurry to obtain growing media. Bioresource Technology, 179, 398406. https://doi.org/10.1016/j.biortech.2014.12.051*

• *Frederickson, J., Howell, G., & Hobson, A. M. (2007). Effect of pre-composting and vermicomposting on compost characteristics. European Journal of Soil Biology, 43, 320–326. https://doi.org/10.1016/j.ejsobi.2007.08.032*

• *Gupta, R., & Garg, V. K. (2008). Stabilization of primary sewage sludge during vermicomposting. Journal of Hazardous Materials, 153(3), 1023–1030. https://doi.org/10.1016/j.jhazmat.2007.09.055*

• *Hait, S., & Tare, V. (2011). Optimizing vermistabilization of waste activated sludge using vermicompost as bulking material. Waste Management, 31(3), 502–511. https://doi.org/10.1016/j.wasman.2010.11.004*

• *Hanc, A., & Chadimova, Z. (2014). Nutrient recovery from apple pomace waste by vermicomposting technology. Bioresource Technology, 168, 240–244. https://doi.org/10.1016/j.biortech.2014.02.031*

• *Haug, R. T. (1993). The practical handbook of compost engineering. Lewis Publishers.*

• *Henze, M., Harremoes, P., La Cour Jansen, J., & Arvin, E. (2001).*

• *Wastewater treatment biological and chemical processes.*

*Springer. https://doi.org/10.1007/978-3-662-04806-1*

• *Huang, G. F., Wong, J. W. C., Wu, Q. T., & Nagar, B. B. (2004). Effect of C/N on composting of pig manure with sawdust.*

•*Waste Management, 24(8), 805–813. https://doi.org/10.1016/j.wasman.2004.03.011*

• *Komakech, A. J., Zurbrügg, C., Miito, G. J., Wanyama, J., & Vinnerås, B. (2016). Environmental impact from vermicompost*

•*ing of organic waste in Kampala, Uganda. Journal of Environmental Management, 181, 395–402. https://doi.org/10.1016/j.jenvman.2016.06.028*

• *Kulikowska, D., & Gusiatin, Z. M. (2015). Sewage sludge composting in a two-stage system: Carbon and nitrogen transformations and potential ecological risk assessment. Waste Management, 38, 312–320. https://doi.org/10.1016/j.wasman.2014.12.019*

• *Li, Z., Lu, H., Ren, L., & He, L. (2013). Experimental and modeling approaches for food waste composting: A review. Chemosphere, 93(7), 1247–1257. https://doi.org/10.1016/j.chemosphere.2013.06.064*

• *Ludibeth, S.-M., Marina, I.-E., & Vicenta, E. M. (2012). Vermicomposting of sewage sludge: Earthworm population and agronomic advantages. Compost Science & Utilization, 20(1), 11–17. https://doi.org/10.1080/1065657X.2012.10737016*

• *Manaf, L. A., Lokman, M., Jusoh, C., Hanida, T., Ismail, T., Hafizan, H. R., & Jusoff, J. K. (2009). Influences of bedding material in vermicomposting process. International Journal of Biology, 1(1), 81–91. https://doi.org/10.5539/ijb.v1n1p81*

• *Mažeikienė, A., & Valentukevičienė, M. (2016). Removal of ammonium ions from digested sludge fugate by using natural zeolite. Journal of Environmental Engineering and Landscape Management, 24(3), 176–184. https://doi.org/10.3846/16486897.2016.1172075*

• *Munroe, G. (2007). Manual of on-farm vermicomposting and vermiculture. Organic Agriculture Centre of Canada.*

• *Nguyen, V.-T., Le, T.-H., Bui, X.-T., Nguyen, T.-N., Vo, T.-D.-H., Lin, C., Vu, T.-M.-H., Nguyen, H.-H., Nguyen, D.-D., Senoro, D. B., & Dang, B.-T. (2020). Effects of C/N ratios and turning frequencies on the composting process of food waste and dry leaves. Bioresource Technology Reports, 11, 100527. https://doi.org/10.1016/j.biteb.2020.100527*

• *Nigussie, A., Kuyper, T. W., Bruun, S., & de Neergaard, A. (2016). Vermicomposting as a technology for reducing nitrogen losses and greenhouse gas emissions from small-scale composting. Journal of Cleaner Production, 139, 429–439. https://doi.org/10.1016/j.jclepro.2016.08.058*

• *Rodríguez-Quiroz, G., Valenzuela-Quiñónez, W., & Nava*

•*Pérez, E. (2011). Vermicomposting as a nitrogen source in germinating kidney bean in trays. Journal of Plant Nutrition, 34(10), 1418–1423. https://doi.org/10.1080/01904167.2011.585200*

• *Rynk, R., van de Kamp, M., Willson, G. B., Singley, M. E., Richard, T. L., Kolega, J. J., Gouin, F. R., Laliberty, L., Kay, D., Murphy, D. W., Hoitink, H. A. J., & Brinton, W. F. (1992). Onfarm composting handbook. Northeast Regional Agricutural Engineering Service.*

• *Sharma, K., & Garg, V. K. (2018). Comparative analysis of vermicompost quality produced from rice straw and paper waste employing earthworm Eisenia fetida (Sav.). Bioresource Technology, 250, 708–715. https://doi.org/10.1016/j.biortech.2017.11.101*

• *Shaymaa, M., Ahmed, H., Norli, I., Morad, N., & Hakimi, I. M. (2010). Removal of aluminium, lead and nickel from industrial sludge via vermicomposting process. World Applied Sciences Journal, 10(11), 1296–1305.*

• *Suthar, S., & Singh, S. (2008). Feasibility of vermicomposting in biostabilization of sludge from a distillery industry. Science of the Total Environment, 394(2–3), 237–243.*

*https://doi.org/10.1016/j.scitotenv.2008.02.005*
- *Van Fan, Y., Lee, C. T., Klemeš, J. J., Bong, C. P. C., & Ho, W. S. (2016). Economic assessment system towards sustainable composting quality in the developing countries. Clean Technologies and Environmental Policy, 18, 2479–2491. https://doi.org/10.1007/s10098-016-1209-9*
- *Varma, V. S., Ramu, K., & Kalamdhad, A. S. (2015). Effects of waste lime sludge on nitrogen dynamics and stability of mixed organic waste using rotary drum composter. International Journal of Environmental Research, 9(1), 395–404. https://doi.org/10.22059/ijer.2015.911*
- *Velasco-Velasco, J., Parkinson, R., & Kuri, V. (2011). Ammonia emissions during vermicomposting of sheep manure. Bioresource Technology, 102(23), 10959–10964. https://doi.org/10.1016/j.biortech.2011.09.047*
- *Zou, H., Lu, X., & Li, T. (2014). A novel process for nutrients removal and phosphorus recovery from domestic wastewater by combining BNR with induced crystallization. Journal of Environmental Engineering and Landscape Management, 22(4), 274–283. https://doi.org/10.3846/16486897.2014.931284.*

# EFFECT OF IONISED (ELECTROLYSED) WATER ON THE RAT EMBRYO DEVELOPMENT

**Jolita STANKEVIČ1, Aistė AUDICKAITĖ1, Simona ŠILOVĖ1*, Valdas ŠIMČIKAS2, Henrikas CESIULIS3, Grita SKUJIENĖ4, Virginija BUKELSKIENĖ5, Violeta ŽALGEVIČIENĖ1, Janina TUTKUVIENĖ1**

1Department of Anatomy, Histology and Anthropology of the Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Čiurlionio g. 21/27, LT-03101, Vilnius, Lithuania

2 Joint Stock Company "Water research", Žirmūnų g. 106, LT-09121, Vilnius, Lithuania

3Department of Physical Chemistry of the Faculty of Chemistry and Geosciences, Vilnius University, Naugarduko g. 24, LT-03225, Vilnius, Lithuania

4Department of Zoology of the Institute of Biosciences, Life Sciences Center, Vilnius University, Saulėtekio al. 7, LT-10223, Vilnius, Lithuania

5Department of Biological Models of the Institute of Biochemistry, Life Sciences Center, Vilnius University, Saulėtekio al. 7, LT-10223, Vilnius, Lithuania

## A B S T R A C T

*The aim of this study was to investigate the effects ionised water has on embryonic development using Wistar rat animal model. For that purpose, alkaline and acidic water was prepared with a domestic water ioniser. It was found that the concentrations of Cl–, SO42– ions increased in acidic water, while in alkaline water, Ca2+ concentration decreased and halogenated hydrocarbon concentrations exceeded permitted levels. The animals were given test alkaline and acidic water, as well as tap water as control. After three months, female rats were mated. On the 21st day of gestation, they were euthanized and subjected to Caesarean sections; the number of live and dead fetuses was recorded. The fetuses were examined for external or visceral malformations and skeletal abnormalities. The data showed that embryo death was higher in acidic and alkaline experimental groups in comparison to the control group. The fetuses in both test groups were significantly shorter than in the control group. Long bones of fetal hind and front limbs were shorter in the acidic group in comparison to the control group. Retardation of limb osteogenesis was expressed in the acidic group fetuses. Therefore, in our model, ionised water had a negative effect on the embryonic development.*

***Keywords: alkaline and acidic water, electrolysis, rat, embryonic development, water cleaning technologies***

## Introduction

Water is the most important substance, which determines survival of all living beings. In the organism, it has a number of roles: it serves as a solvent, temperature buffer, metabolite, living environment, etc. However, natural water is not chemically pure $H_2O$. Atmospheric precipitation, rocks and sediments, through which water is filtered, play a key role in the formation of underground freshwater. Therefore, the variety of chemical compounds, found in drinking water, depends on the chemical composition of the rock (Lack, 1999). Consumption of water also determines the supply of any minerals it contains. Research suggests that 14 out of 21 mineral elements required for humans are essential for good health.

Different combinations of these elements are responsible for managing normal functioning of the body, i.e. bone and membrane structure (Ca, P, Mg, F), water and electrolyte balance (Na, K, Cl), metabolic catalysis (Zn, Cu, Se, Mg, Mn, Mo), oxygen binding (Fe), and hormone functions (I, Cr) (World Health Organization, 2005). However, though most of the chemical elements, present in water, are required for a healthy functioning of a human body, surplus in these elements may lead to various diseases and functional problems; thus, the amount of these elements allowed in drinking water is regulated and limited. Lithuania is considered among the leaders in EU in terms of water purity (LRP, 2016). Specialists claim that unpolluted deep water aquifers in Lithuania can be envied by most of the countries for groundwater quality, chemical composition and microbiological indicators. The water is not contaminated with such trace elements as iodine, while others, e.g. fluoride, are very unevenly distributed throughout the country (Klimas & Mališauskas, 2008). Nitrate, commonly associated with agricultural activities, is also found in very small quantities in Lithuanian groundwater. The same applies to other north European countries, such as Norway, Sweden, Finland, Estonia, and Latvia. It is believed that this is due to frozen ground in winter time, when the land is not arable, thus cannot be polluted with fertilisers and pesticides, the surplus of which might seep into the groundwater (Flem, Reimann, Birke, Banks, & Filzmoser, 2015).

Moreover, in Europe both surface (from rivers or lakes) and ground water is used for public water supply, while in some countries (Lithuania, Denmark, and Austria) mostly underground water is used for drinking (Volker & Borchardt, 2019; Flem et al., 2015). Most Lithuanian waters require no treatment, while those requiring some form of purification, are treated with natrium hypochlorite solution, which is considered safe for the environment and people (Vilniaus vandenys, 2016). Still, popular literature and the Internet abound with articles and advertising projects that recommend water treatment with water ionisers, which through the process of electrolysis produce two types of water: acidic (dead) and alkaline (living) (Laucevičius, 2009).

It is known that naturally, with the help of the solar energy, electrolysis occurs in plants and algae. In industry, electrolysis is performed to clean and preserve metallic objects from corrosion, while in the future even hydrogen might be produced by electrolysis as an alternative to fossil fuel (Santos et al., 2019; Rashid, Al Mesfer, Naseem, & Danish, 2015). Meanwhile, some people believe that electrolysis makes water better and that drinking it may cure many different illnesses. The somewhat popular alkaline diet adds to this believe. However, according to Fenton and Huang (2016), there is no proof that alkaline diet or alkaline water, as promoted by sales agents selling water alkalinisers, has any effect on cancer prevention or treatment. Still, authors who have been experimenting with living organisms in the hope that alkaline ionised water can work as a protection against diseases stemming from oxidative stress – diabetes, cancer, arteriosclerosis, neurodegenerative diseases, and adverse effects of hemodialysis – found that alkaline water is actually beneficial for human health (Xue et al., 2014; Ignacio, Joo, & Lee, 2012; Shirahata, Hamasaki, & Teruya, 2012). In addition, some authors brought acid-base hypothesis into view, which suggests that an overly acidic diet (high in protein and grains) leads to chronic diseases and bone health problems, thus, raising the body pH through alkaline diet and balancing the acid-base ratio could prevent osteoporosis and other related diseases (Dawson-Hughes, 2016; Xu et al., 2016; Fenton, Eliasziw, Lyon, Tough, & Hanley, 2008). However, as the authors state, the results on acid-base hypothesis are inconclusive and require further investigations (DawsonHughes, 2016; C. J. Fenton, T. R. Fenton, & Huang, 2017; Fenton et al., 2008)

In nature, high levels of either acidity or alkalinity can destroy life. Acid rains affect the soil, which in turn becomes less fertile and has a negative effect on microorganisms, flora and fauna living on it. Likewise, contaminants responsible for air pollution, such as nitrogen oxides and sulphur dioxides – waste product of manufacturing, transportation, mining and agriculture – when introduced into water, transform into acids and significantly affect water's pH levels (Osman, 2018; Weldeslassie, Naz, Singh, & Oves, 2018). In the presence of such information, the authors of this study found it important to investigate the effect electrolysis-altered water has on living organisms.

It is known that during electrolysis, ions distribute unevenly in the solution. Anions Cl–, SO42–, NO3– migrate towards the anode, where oxidation reactions take place, producing free chlorine and oxygen gas. Depending on the composition of the electrolysed solution, various salts, sulphuric or nitric acids form, and the pH value falls below 7. Meanwhile, cations migrate towards the cathode, where reduction occurs. Depending on the alkalinity of the solution, Ca2+ and Mg2+ ions might form low solubility hydroxides (resulting in turbidity) whereas sodium and potassium stay in the solution and form soluble sodium and potassium hydroxides. Potassium hydroxide begins to dominate the solution, and the pH value increases to 8−9.

Normally, halogenated hydrocarbons are not found in nature, as they are a product of human activity. In water, halogenated hydrocarbons can form after chlorination or by other means of water disinfection or electrolysis. Free chlorine, which forms during electrolysis, reacts with water, bromide, and organic substances, resulting in a variety of halogenated compounds – usually haloforms. Concentrations of free chlorine and haloforms are directly dependent on the composition of water being electrolysed (especially the initial concentration of Cl–), as well as on the duration of electrolysis.

According to the proponents of water ionisers, acidic water produced in these devices is a natural bactericide and pesticide (Bui et al., 2017; Sun, Zhang, Chen, & Han, 2012; Abbasi & Lazarovits, 2006). They also claim that alkaline water is better in neutralising stomach gastric acid, detoxifies the organism, and acts like a natural antioxidant and alkalizing substance due to a large content of oxygen and negative oxidation-reduction potential (Mousa, 2016; Laucevičius, 2009). There are many popular and scientific articles about the benefits of electrolysed water (Weidman et al., 2016; Koufman & Johnston, 2012; Shirahata et al., 2012; Laucevičius, 2009; Shirahata et al., 1997). Because of that, significant part of society now claims to have found a surprising "drug" for treating many different ailments.

However, there is little research on the adverse effects electrolysed water has on living organisms (El-Fiky, 2002; Merne, K. J. Syrjanen, & S. M. Syrjanen, 2001; Watanabe & Kishikawa, 1998a; Watanabe et al., 1998b; Watanabe, Kishikawa, & Shirai, 1997).

Thus, it can be argued that there is a lack of aggregated studies on electrolysed water effect on the whole organism. Likewise, not much is known how electrolysed water affects the development of offspring. Therefore, due to deficiency of scientific evidence supporting the safety of electrolysed water, and due to its popularity in Lithuania and other countries, the aim of this study was to assess the effects of ionised (electrolysed) water on the embryonic development using Wistar rats as a test model. Our goal was to evaluate the formation of the offspring of female rats, which were given acidic and alkaline water before and during pregnancy.

## 1. Materials and methods

### 1.1. Water electrolysis

Tap water provided by "Vilniaus vandenys" Ltd (Lithuania) was used in this study. To produce alkaline and acidic water, ordinary tap water was treated with a domestic water ioniser ("PTV-KL") for 10 and 20 min respectively, according to manufacturer's instructions.

### 1.2. Measurement of main ions and volatile

halogenated hydrocarbons in the water The ion analysis was carried out using Ion Chromatograph DIONEX IC-1000 according to Standards ISO 14911 (Cations) and ISO 10304-1 (Anions).

Haloform analysis was carried out by Gas Chromatograph DANI GC 1000 using Head Space injection technique according to Standard ISO 10301. The samples were taken from sealed vials in which the ratio of water volume to air volume was fixed. In this study, 20 mL-sized vials were filled with 10 mL of samples. The temperature of the vials was stabilized in a thermostatic system at 70 °C to achieve specified equilibrium conditions. After reaching equilibrium with water, an electron capture detector was used for gas chromatography in the sampling vials.

### 1.3. Animals

In this study, 3 months old Wistar rats were used. Approval of Ethics Committee and permission for the experimentation was received from the State Food and Veterinary Service of Lithuania, No 1; 14-01-2013. The experiment was designed in accordance to the requirements stated in 2010/63/EU Directive and Order of the Lithuanian State Food and Veterinary Service Director No B1-866; 31-12-2012. Wistar rats were obtained from and animal experiment was carried out in the Department of Biological Models, Vilnius University, Institute of Biochemistry (Lithuania).

### 1.4. Experimental methodology

Three months before mating, female rats were divided into three groups and given different types of electrolysed water: alkaline (n = 10) or acidic (n = 10), and tap water as control (n = 10). During this period and at the time of gestation, rats were provided with standard food for rodents and electrolysed (or tap) water ad libitum. The animals were weighed regularly. After three months, female Wistar rats were mated overnight with males of the same clone. The following morning, vaginal smears were subjected to a microscopic examination in order to determine the presence of sperm.

The day of sperm detection in vaginal smears was designated as day 0 of gestation. On the 21st day of gestation, the animals were euthanized and subjected to Caesarean sections. The uteri were removed and opened; dead and live fetuses were recorded in the uterine horn to determine post-implantation mortality indices.

### 1.5. Measurement of mother rat blood open circuit potential

Values of open circuit potential (OCP) were calculated from recorded time dependencies of potential differences measured directly between platinum electrode and reference electrode (saturated Ag, AgCl/Cl– electrode) immersed into the sample of blood. The volume of the sample was ~1 mL. The dependencies were recorded by means of programmable potentiostat/galvanostat AUTOLAB N302. Heparin was used as the anticoagulant of blood.

### 1.6. Analysis of fetuses

To assess the effects of ionised (electrolysed) water on the embryonic development, external and internal physical features of the fetuses were evaluated. For that purpose, the fetuses were weighed and measured; half fetuses of every female (alkaline (n = 35), acidic (n = 20), control (n = 47)) were fixed in Bouin's solution for subsequent examination for external and visceral malformations. In order to render the skeleton visible, another set of fetuses (alkaline (n = 34), acidic (n = 21), control (n = 48)) was fixed in ethanol. For visceral malformation analysis, fetuses were sliced using Hayes technique. Using an eyepiece micrometre attached to a stereomicroscope (MBS-1), internal organs of the fetuses (liver, kidney, heart, lungs, brain and other) were investigated, and length of ossification centers of the fetal limb bones was measured in the middle of diaphysis of long tubular bones (Hayes, 1994). The fetuses of the control group were prepared and examined in the same way as the experimental ones.

### 1.7. Statistical analysis

All the data are expressed as mean ± standard deviation (SD). Two-tailed Student's t-test was performed to ascertain statistical significance ($p < 0.05$) in differences between results of experimental and control groups. For the evaluation of ion concentrations in the water and water's effect on bone length, one-way analysis of variance (one way-ANOVA) was used, followed by Tukey's multiple comparisons post-hoc.

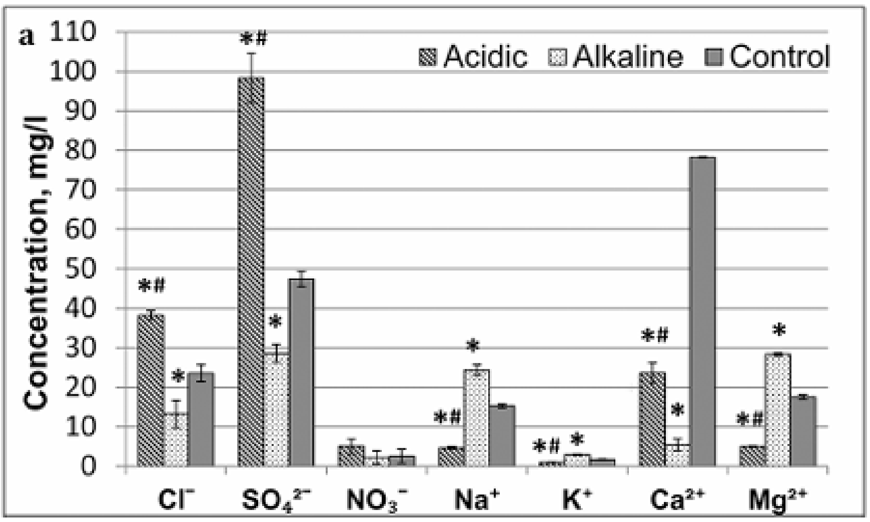
## 2. Results

### 2.1. Ion concentration in water and water pH after electrolysis

In the experiments, after tap water electrolysis for 10 and 20 min, concentration of main ions, volatile halogenated hydrocarbons and pH were analysed. The results revealed, that pH of electrolysed water assay was significantly different from the tap water (pH – 7.91) used: pH of acidic water was 3.38, and of alkaline – 10.14. Also, the concentration of Ca2+ was significantly reduced in alkaline water as compared to tap water (Figure 1a and 1b). The study of halogenated hydrocarbons in electrolysed water demonstrated increased concentrations of chloroform (CHCl3) and bromodichloromethane (CHBrCl2) in both acidic and alkaline water (Table 1). After ionisation, the concentration of chloroform in alkaline water increased from 0.38 (after 10 min) to 0.63 µg/l (after 20 min), while in acidic water, it decreased from 25.20 (after 10 min) to 23.10 µg/l (after 20 min). Similarly, the concentration of bromodichloromethane increased in acidic water from 10.50 to 16.30 µg/l. Also, an increase of bromoforms was detected in acidic water – from 0.89 to 1.49 µg/l.

### 2.2. Blood OCP, blood and urine pH in mother rats

In the medical, biological, and ecological literature, an OCP of platinum electrode placed into a test medium against a certain reference electrode is considered as the redox potential of the medium. In this case, "redox potential" means the OCP of the electrode immersed into studied biological medium, such as blood or urine. To evaluate the impact of ionised water on the physical status of mother rats, blood and urine samples were collected just before mating. It was found that for rats in acidic and alkaline groups, the values of OCP in blood decreased by ~0.03 V: from 0.165 V in control group to 0.135 V in groups, which consumed ionised water. Furthermore, urine pH of rats, which were given electrolysed water for 3 months, ranged as follows: 7.59±0.40 in acidic, and 7.23±0.53 in alkaline groups. Urine pH of control rats, which were given tap water, was 7.35±0.58. Therefore, generally urine pH did not differ significantly between the experimental and control groups ($p > 0.05$).



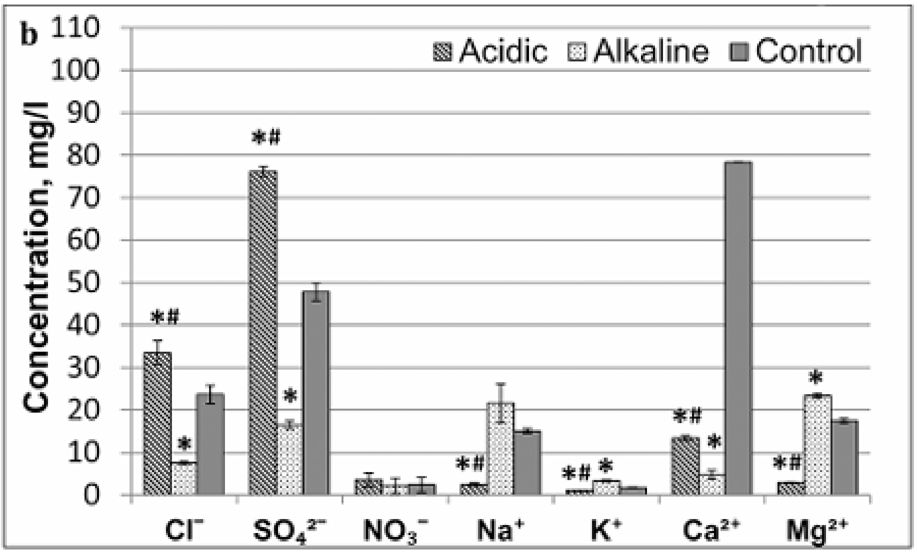



**Figure 1.** Ion (Cl–, SO42–, NO3–, Na+, K+, Ca2+, Mg2+) concentrations in acidic, alkaline and control tap water (mg/l); a − after 10 min.; b − after 20 min. of electrolysis; mean ± S.D; *p < 0.05 when compared to control; #p < 0.05 when compared to alkaline group

Similarly, mother rats' blood pH did not differ in experimental and control groups, when compared ($p > 0.05$): blood pH of alkaline group was 7.78±0.17, acidic group – 7.80±0.17, and control group – 7.80±0.10. Thus, the data suggests that ionised water did not affect the acidity or basicity of experimental rats' blood.

### 2.3. Body weight of differently watered rats

The analysis of rats' body weight gained during three months revealed such results: animals given alkaline water gained 40.5±18.05 g, acidic water – 32.4±16.90 g, while during the same time rats in the control group gained 52.4±17.52 g. Thus, rats in both experimental groups gained less weight than those in the control group, the lowest weight gain being observed in the acidic group. Nonetheless, there were no statistically significant

**Table 1.** Dssolved halogenated hydrocarbons (chloroform, bromodichloromethane, chlorodibromomethane, bromoform) in control (tap water), alkaline and acidic water after 10 and 20 min of electrolysis (μg/l)

| | | Chloroform (μg/l) | Bromodichloro-methane (μg/l) | Chlorodibro-momethane (μg/l) | Bromoform (μg/l) |
|---|---|---|---|---|---|
| Control | Tap water | <0.1 | <0.1 | <0.1 | <0.1 |
| Alkaline water | 10 min electrolysis | 0.38±0.02 | 0.13±0.04 | <0.1 | <0.1 |
| | 20 min electrolysis | 0.63±0.16 | 0.13±0.01 | <0.1 | <0.1 |
| Acidic water | 10 min electrolysis | 25.20±0.99 | 10.50±2.84 | 5.04±1.38 | 0.89±0.11 |
| | 20 min electrolysis | 23.10±6.08 | 16.30±4.66 | 12.40±1.41 | 1.49±0.01 |

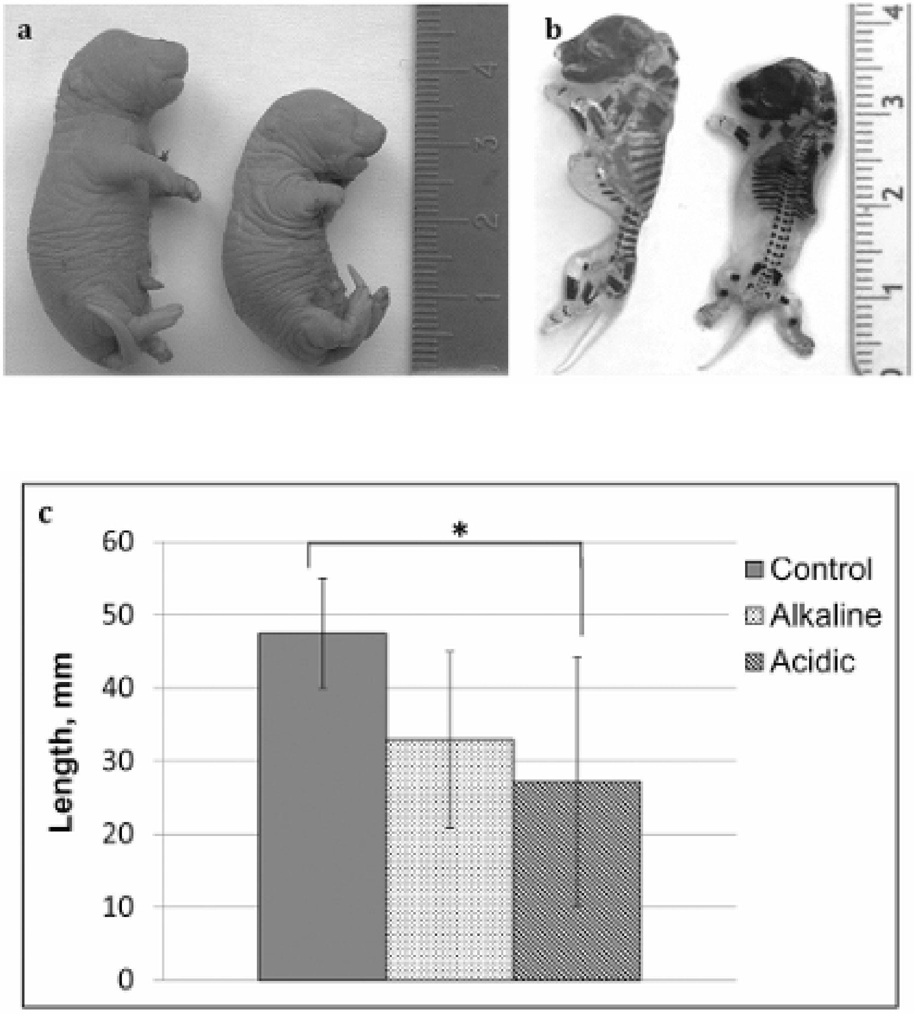



**Figure 2.** Ionised water effect on fetus length: a – general view of control (left) and acidic (right) groups fetuses; b – fetuses of control (left) and acidic (right) groups represent the retardation of bone development in acidic group fetuses; c – comparison of fetuses' length in control, alkaline and acidic groups; acidic group fetuses were significantly shorter ($p < 0.05$) compared to control differences between both experimental groups and control group ($p > 0.05$).

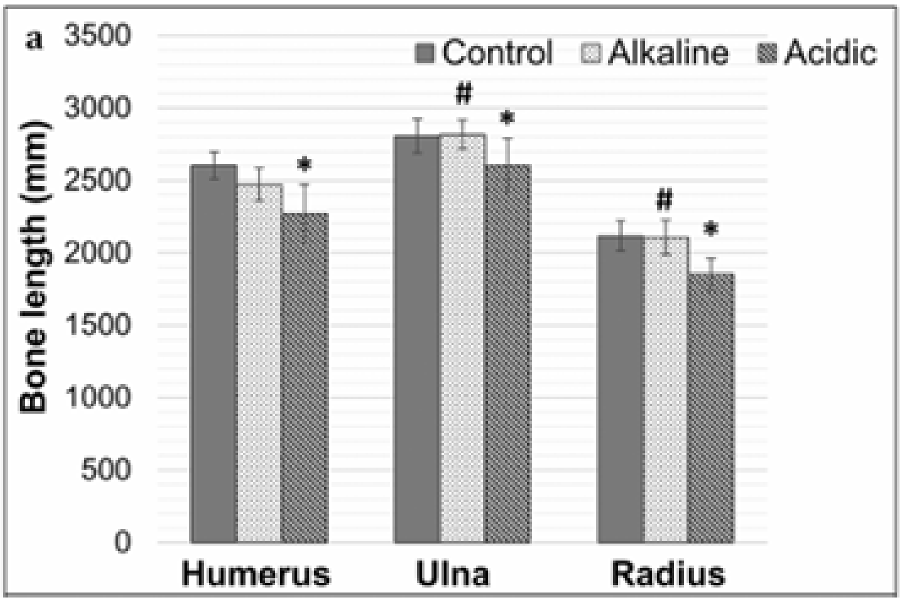
It also should be noted that rats in both experimental groups tended to consume less water (acidic or alkaline) than rats in control group, which consumed tap water.

**2.4. Embryonic development**

The main objective of this study was to assess the effects of ionised (electrolysed) water taken by the mother rats on the embryonic development. In assessing the viability of rat embryos, the data of this study revealed that embryo death rate increased in both acidic (27.0%) and alkaline (10.1%) groups compared to the control group (0%). In addition, it was detected that female rats, which had been given ionised water, had 10.14% of embryo resorptions. On the contrary, no embryo resorption was detected in rats, which consumed tap water. Moreover, electrolysed water did not cause any noticeable developmental defects of external or internal organs of the fetuses (Figure 2a and 2b). However, ionised water had a noticeable effect on the size of fetuses: rat fetuses of both experimental groups were shorter than those of the control group; even more, acidic group fetuses were significantly shorter ($p < 0.05$) compared to the controls (Figure 2c), even though their weight did not differ. While evaluating ossification centers of front and hind limb bones in coloured skeletons of the fetuses, it was found that acidic water did influence ossification of the long bones of limbs – they were shorter in comparison to the control group ($p < 0.05$) (Figure 3a and 3b). On theother hand, ossification of the long bones of the fetuses from alkaline group had no disruptions in skeletal development, and was not statistically different from the fetuses in the control group ($p > 0.05$).

## 3. Discussion

Water ionisation supporters claim that alkaline water neutralizes bodily acids and helps with the prevention of diseases, however, according to Vorman and Goedecke (2002), various buffer systems are responsible for a stable pH of body fluids, thus the acidity or alkalinity of food and drink is irrelevant, as it will not change the pH values of the body. In agreement with that, the results of this study indicate that urine and blood pH of the rats given alkaline water did not become more alkaline. Moreover, female rats which were given acidic and alkaline water gained less weight, though no statistically significant difference between experimental and control groups was found. These results could be compared to those of Watanabe et al. (Watanabe & Kishikawa, 1998a; Watanabe et al., 1998b; Watanabe et al., 1997), who investigated how alkaline water changed body weight, and analysed biochemical parameters of the blood, as well as hearts of pregnant rats and their offspring. Their study demonstrated that alkaline water leads to increased body weight of rats and some of their organs, erythrocyte haemolysis,



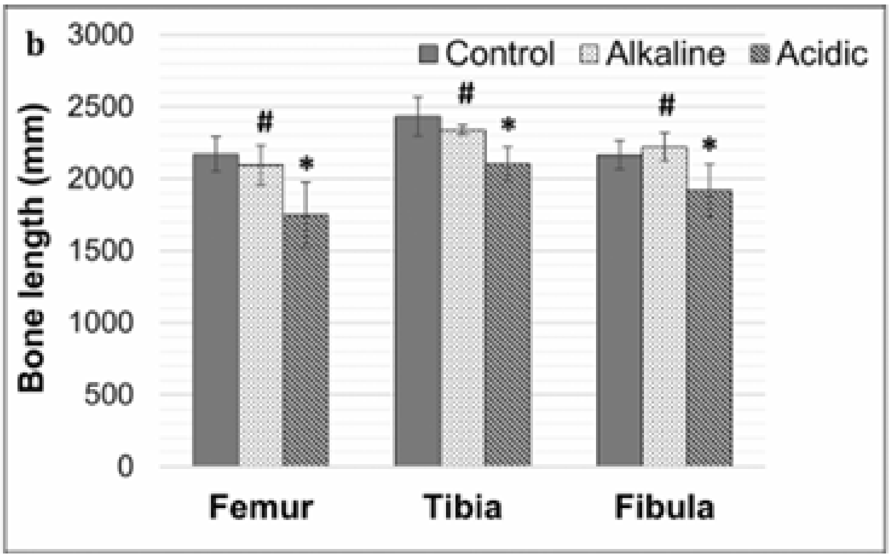



**Figure 3.** Effect of electrolysed water on the ossification of the fetuses long bones of limbs: a – length of front leg bud in control, alkaline and acidic group fetuses; b – length of hind leg bud in control, alkaline and acidic group fetuses; mean ±S.D; *$p < 0.05$ when compared to control; #$p < 0.05$ when compared to acidic group

elevated blood potassium, and induced myocardial necrosis and fibrosis.

On the other hand, Massimiliano Magro's et al. (2016) 3-year survival study of alkaline water consumption effects on a population of mice revealed that even though animals which were given alkaline water lived longer, there was no correlation between a risk of diseases and alkaline water consumption. After examining the animals' organs histologically, the authors found that there were no significant differences between mice treated with alkaline water and tap water (Magro et al., 2016). However, it must be taken into consideration that water ionisers produce both alkaline and acidic water, and that there is a permeable membrane between the cameras of the device, where this water is produced.

During electrolysis halogenated hydrocarbons (chloroform, bromodichloromethane, chlorodibromoethane, and bromoform) increase in concentration in the water. Out of these, the levels of chloroform are usually the most elevated. Through the aforementioned membrane, part of the chlorine ions migrate from acidic to alkaline water. It is well known that chloroform is used for water disinfection and that when its concentration increases in water, together with bromodichloromethane, it may become carcinogenic and increase the risk of malignancies – colon cancer, premature births or embryonic anomalies (Benmarhnia, Delpla, Schwarz, Rodriguez, & Levallois, 2018; Abdelhalim, Salaheldeen, Idris, Abdelsalam, & Sabahelkhier, 2016; Hrudey et al., 2015; Villanueva, Cordier, Font-Ribera, Salas, & Levallois, 2015; Chen, Lin, Duh, Chou, & Hsu, 2011; Florentin, Hautemaniere, & Hartemann, 2011; Legay et al., 2011; Mills et al., 1998). Our study revealed that even after 10 minutes of water electrolysation, halogenated compound levels of methane and ethane (haloforms) – toxic for living organisms – increased in the water. Thus, even though in the present study electrolysed water did not cause any noticeable developmental defects of the external or internal organs of the fetuses, nevertheless, this water had a significant impact on fetus size. Significant differences between ossification centers in long bones of fetuses were also detected.

Various literature sources point out that embryonic ossification depends on genetics or is affected by oxidative stress, diet, hormones, various environmental factors, and maternal physical activity (Hu et

2018; Jensen et al., 2017; Fadel, Sequeira, Abu-Hijleh, Obeidat, & Salem, 2012; Ornoy, Rand, & Bischitz, 2010; Rauch & Schoenau, 2001; Fadel & Persaud, 1993). Likewise, limb ossification disorders can be induced by teratogenic substances, such as azathioprine, which impairs the interaction between the apical ectodermal ridge and the progress zone, i.e., the morphogenetic zones located in the distal part of the limb (Zukiene, Zalgeviciene, & Rizgeliene, 2003). The results of this study demonstrated that, prior to mating, mother rats, which were given acidic water, tended to weigh less. Stress indicators, such as rough coat and bloody tears, were also evident in this animal group. Such physical changes of mother rats could have been the cause of embryo growth retardation and the slowing down of their bone development. In addition, reduced Ca2+ concentration in ionised water could also have led to abnormal ossification. Also, the possibility that halogenated hydrocarbons detected in water after electrolysis could be the cause of ossification retardation and other ossification disorders in the embryo must equally be taken into consideration.

Furthermore, in medical science, many attempts were made to estimate the redox properties of blood, other biological liquids, and tissues by measuring OCP. In a previous study done by the authors, rat blood OCP values decreased by ~0.03 V for rats in acidic and alkaline groups in comparison to the control group (Audickaitė et al., 2014). Usually, such values indicate no presence of pathology in human blood (Khubutiya et al., 2010). However, if pathology was detected, notable deviations from the average values of OCP would appear, and differences between OCP could reach up to 0.1 V (Vormann & Goedecke, 2002). Since many homeostatic processes are electrochemical, when their activity increases, and changes in the composition of potential forming system appear, OCP levels vary more significantly. However, evaluation according to the Nernst equation reveals that the ratio of oxidized molecules to reduced molecules in the rats' blood decreases up to 3 times, and such a decrease is probably enough to cause pathology in rats.

## Conclusions

1. The study revealed that after electrolysis the concentrations of Cl–, SO42– ions increased in acidic water, while in alkaline water Ca2+ concentration decreased and halogenated hydrocarbon concentrations exceeded permitted levels.
2. Rats, which were given alkaline and acidic water for 3 months, tended to gain less weight in comparison to those that drunk tap water.
3. Blood and urine pH differences were not statistically significant between groups; however, the urine of rats, which had been given alkaline water, had a tendency to acidify.
4. Neither alkaline nor acidic water had any effect on rat blood OCP.
5. Fetal analysis revealed that offspring of mother rats that had been watered with electrolysed water were smaller than those, whose mothers drank tap water.
6. Long bones of hind and front limbs of the fetuses from experimental groups (in particular – acidic) were noticeably shorter in comparison to the ones in control group.
7. In this model, ionised water had a negative effect on the development of embryos of mother rats which were given ionised (electrolysed) water.

## Conflict of interest

The authors declare no conflict of interest.

## References


- *Abbasi, P. A., & Lazarovits, G. (2006). Effect of acidic electrolysed water on the viability of bacterial and fungal plant pathogens and on bacterial spot disease of tomato. Canadian Journal of Microbiology, 52, 915-923. https://doi.org/10.1139/w06-048*
- *Abdelhalim, M. I., Salaheldeen, Y. M., Idris, O. F., Abdelsalam, E. B., & Sabahelkhier, M. K. (2016). Histopathological effect on different rat tissues induced by the trihalomethanebromoform administered in drinking water. Nova Journal of Medical and Biological Sciences, 5, 1-7. https://doi.org/10.20286/nova-jmbs-050280*
- *Audickaitė, A., Stankevič, J., Šimčikas, V., Cesiulis, H., Jarašienė, R., Bukelskienė, V., Žalgevičienė, V., & Tutkuvienė, J. (2014). Jonizuoto (elektrolizuoto) vandens įtaka žiurkių patelių fizinei būklei (eksperimentinis tyrimas) [The influence of ionized (electrolysed) water on physical status of female rats (experimental study)]. Sveikatos mokslai [Health Sciences], 24, 46-51. https://doi.org/10.5200/sm-hs.2014.024*
- *Benmarhnia, T., Delpla, I., Schwarz, L., Rodriguez, M., & Levallois, P. (2018). Heterogeneity in the relationship between disinfection by-products in drinking water and cancer: a systematic review. International Journal of Environmental Research and Public Health, 15(5), 979. https://doi.org/10.3390/ijerph15050979*
- *Bui, V. N., Nguyen, K. V., Pham, N. T., Bui, A. N., Dao, T. D., Nguyen, H. T., Trinh, D. Q., Inui, K., Uchiumi, H., Ogawa, H., & Imai, K. (2017). Potential of electrolysed water for disinfection of foot-and-mouth disease virus. Journal of Veterinary Medical Science, 79, 726-729. https://doi.org/10.1292/jvms.16-0614*
- *Chen, M. J., Lin, C. H., Duh, J. M., Chou, W. S., & Hsu, H. T. (2011). Development of a multi-pathway probabilistic health risk assessment model for swimmers exposed to chloroform in indoor swimming pools. Journal of Hazardous Materials, 185, 1037-1044. https://doi.org/10.1016/j.jhazmat.2010.10.011*
- *Dawson-Hughes, B. (2016). Acid-base balance of the diet: implications for bone. In C. M. Weaver, R. M. Daly, & H. A. Bischoff-Ferrari (Eds.), Nutritional influences on bone health (9th International Symposium). Cham: Springer International Publishing Switzerland. https://doi.org/10.1007/978-3-319-32417-3_9*
- *El-Fiky, N. K. (2002). The influence of water pH on the embryonic development of grass carp, Ctenopharyngodon idell.*
- *Egyptian Journal of Aquatic Biology and Fisheries, 6, 233-261. https://doi.org/10.21608/ejabf.2002.1760*
- *Fadel, R. A., & Persaud, T. V. (1993). Ossification of the vertebral column in the offspring of rats exposed to alcohol, acetaldehyde and caffeine. Experimental and Toxicologic Pathology, 45, 51-54. https://doi.org/10.1016/S0940-2993(11)80456-2*
- *Fadel, R. A., Sequeira, R. P., Abu-Hijleh, M. F., Obeidat, M., & Salem, A. H. (2012). Effect of prenatal administration of therapeutic doses of topiramate on ossification of ribs and vertebrae in rat fetuses. Romanian Journal of Morphology and Embryology, 53, 321-327.*
- *Fenton, T. R., Eliasziw, M., Lyon, A. W., Tough, S. C., & Hanley, D. A. (2008). Meta-analysis of the quantity of calcium excretion associated with the net acid excretion of the modern diet under the acid-ash diet hypothesis. The American Journal of Clinical Nutrition, 88, 1159-1166. https://doi.org/10.1093/ajcn/88.4.1159*
- *Fenton, T. R., & Huang, T. (2016). Systematic review of the association between dietary acid load, alkaline water and cancer. BMJ Open, 6, 1-5. https://doi.org/10.1136/bmjopen-2015-010438*
- *Fenton, C. J., Fenton, T. R., & Huang, T. (2017). Further evidence of no association between dietary*

*acid load and disease. The Journal of Nutrition, 147(2), 272. https://doi.org/10.3945/jn.116.242107*
•*Flem, B., Reimann, C., Birke, M., Banks, D., & Filzmoser, P. (2015). Inorganic chemical quality of european tap-water: 2.*
• *Geographical distribution. Applied Geochemistry, 59, 211-224. https://doi.org/10.1016/j.apgeochem.2015.01.016*
• *Florentin, A., Hautemaniere, A., & Hartemann, P. (2011). Health effects of disinfection by-products in chlorinated swimming pools. International Journal of Hygiene and Environmental Health, 214, 461-469. https://doi.org/10.1016/j.ijheh.2011.07.012*
• *Hayes, A. W. (1994). Principles and methods of toxicology. New York: Raven Press. Hrudey, S. E., Backer, L. C., Humpage, A. R., Krasner, S. W., Michaud, D. S., Moore, L. E., Singer, P. C., & Stanford, B. D. (2015). Evaluating evidence for association of human bladder cancer with drinking-water chlorination disinfection byproducts. Journal of Toxicology and Environmental Health, Part B, 18, 213-241. https://doi.org/10.1080/10937404.2015.1067661*
• *Hu, H., Zhao, X., Ma, J., Shangguan, Y., Pan, Z., Chen, L., Zhang, X., & Wang, H. (2018). Prenatal nicotine exposure retards osteoclastogenesis and endochondral ossification in fetal long bones in rats. Toxicology Letters, 295, 249-255. https://doi.org/10.1016/j.toxlet.2018.07.005 Ignacio, R. M. C., Joo, K.-B., & Lee, K.-J. (2012). Clinical effect and mechanism of alkaline reduced water. Journal of Food and Drug Analysis, 20, 394-397.*
• *Jensen, V. F. H., Molck, A.-M., Berthelsen, L. O., Andersen, L., Demozay, D., Renaut R., & Bogh, I. (2017). Gestational hypoglycaemia restricts foetal growth and skeletal ossification in the rat. Journal of Pregnancy and Child Health, 4, 1-11. Khubutiya, M. S., Evseev, A. K., Kolesnikov, V. A., Goldin, M. M.,*
•*Davydov, A. D., Volkov, A. G., & Stepanov, A. (2010). Measurements of platinum electrode potential in blood and blood plasma and serum. Russian Journal of Electrochemistry, 46, 537-541. https://doi.org/10.1134/S1023193510050071*
• *Klimas, A., & Directorauskas, A. (2008). Boron, fluoride, strontium and lithium anomalies in fresh groundwater of Lithuania. Geologija, 50(2), 114-124. https://doi.org/10.2478/v10056-008-0015-4*
• *Koufman, J. A., & Johnston, N. (2012). Potential benefits of pH 8.8 alkaline drinking water as an adjunct in the treatment of reflux disease. Annals of Otology, Rhinology & Laryngology, 121, 431-434. https://doi.org/10.1177/000348941212100702*
• *Lack, T. (1999). Water and health in Europe: an overview. BMJ, 318(7199), 1678-1682. https://doi.org/10.1136/bmj.318.7199.1678*
• *Laucevičius, T. (2009). Jonizuotas vanduo. Gyvenimas be ligų. [Ionised water. Life without diseases]. Kaunas: Obuolys (Lithuania).*
• *Legay, C., Rodriguez, M. J., Sadiq, R., Sérodes, J. B., Levallois, P., & Proulx, F. (2011). Spatial variations of human health risk associated with exposure to chlorination by-products occurring in drinking water. Journal of Environmental Management, 92, 892-901. https://doi.org/10.1016/j.jenvman.2010.10.056*
• *LRP. (2016). Lithuania's water resources and their protection receive attention in Davos. Vilnius. Retrieved from https://www. lrp.lt/en/press-centr/press-releases/lithuanias-water-resources-and-their-protection-receive-attention-in-davos/24575.*
• *Magro, M., Corain, L., Ferro, S., Baratell, D., Bonaiuto, E., Terzo, M., Corraducci, V., Salmaso, L., & Vianello, F. (2016).*
•*Alkaline water and longevity: a murine study. Evidence-Based Complementary and Alternative Medicine, 2016, 1-6. https://doi.org/10.1155/2016/3084126*
•*Merne, M. E., Syrjanen, K. J., & Syrjanen, S. M. (2001). Systemic and local effects of long-term exposure to alkaline drinking water in rats. International Journal of Experimental Pathology, 82, 213-*

*219. https://doi.org/10.1111/j.1365-2613.2001.iep188.x*
*• Mills, C. J., Bull, R. J., Cantor, K. P., Reif, J., Hrudey, S. E., & Huston, P. (1998). Workshop report. Health risks of drinking water chlorination by-products: report of an expert working group. Chronic Diseases in Canada, 19, 91-102.*
*• Mousa, H. A. (2016). Health effects of alkaline diet and water, reduction of digestive-tract bacterial load, and earthing. Alternative Therapies in Health and Medicine, 22, 24-33.*
*• Ornoy, A., Rand, S. B., & Bischitz, N. (2010). Hyperglycemia and hypoxia are interrelated in their teratogenic mechanism: studies on cultured rat embryos. Birth Defects Research. Part*
*•B, Developmental and Reproductive Toxicology, 89, 106-115. https://doi.org/10.1002/bdrb.20230*
*• Osman, K. T. (2018). Management of soil problems. Cham: Springer International Publishing AG. https://doi.org/10.1007/978-3-319-75527-4*
*• Rashid, M. M., Al Mesfer, M. K., Naseem, H., & Danish, M. (2015). Hydrogen production by water electrolysis: a review of alkaline water electrolysis, PEM water electrolysis and high temperature water electrolysis. International Journal of Engineering and Advanced Technology, 4, 80-93.*
*• Rauch, F., & Schoenau, E. (2001). The developing bone: slave or master of its cells and molecules? Pediatric Research, 50, 309-314. https://doi.org/10.1203/00006450-200109000-00003*
*• Santos, B. A. F., Serenario, M. E. D., Pinto, D. L. M. F., Simoes, T. A., Malafaia, A. M. S., & Bueno, A. H. S. (2019).*
*•Evaluation of micro-crack incidence and their influence on the corrosion resistance of steel coated with different chromium thicknesses. Revista Virtual de Quimica, 11, 264-274. https://doi.org/10.21577/1984-6835.20190019*
*• Shirahata, S., Kabayama, S., Nakano, M., Miura, T., Kusumoto, K., Gotoh, M., Hayashi, H., Otsubo, K., Morisawa, S., & Katakura, Y. (1997). Electrolysed–reduced water scavenges active oxygen species and protects DNA from oxidative damage. Biochemical and Biophysical Research Communications, 234, 269-274. https://doi.org/10.1006/bbrc.1997.6622*
*• Shirahata, S., Hamasaki, T., & Teruya, K. (2012). Advanced research on the health benefit of reduced water. Trends in Food Science & Technology, 23, 124-131. https://doi.org/10.1016/j.tifs.2011.10.009*
*• Sun, J. L., Zhang, S. K., Chen, J. Y., & Han, B. Z. (2012). Efficacy of acidic and basic electrolysed water in eradicating Staphylococcus aureus biofilm. Canadian Journal of Microbiology, 58, 448-454. https://doi.org/10.1139/w2012-005*
*• Villanueva, C. M., Cordier, S., Font-Ribera, L., Salas, L. A., & Levallois, P. (2015). Overview of disinfection by-products and associated health effects. Current Environmental Health Reports, 2, 107-115. https://doi.org/10.1007/s40572-014-0032-x*
*• Vilniaus vandenys. (2016). Water quality. Retrieved from https:// www.vv.lt/en/quality/#*
*• Volker, J., & Borchardt, D. (2019). Drinking water quality at risk: a European perspective. In M. Schroter, A. Bonn, S. Klotz, R. Seppelt, & Baessler, C. (Eds.), Atlas of ecosystem services. Drivers, risks, and societal responses. Cham: Springer International Publishing AG. Vormann, J., & Goedecke, T. (2002). Latent acidosis: overacidification as a cause of chronic diseases. Journal Suisse de Medecine Globale, 14, 90-96.*
*• Watanabe, T., Kishikawa, Y., & Shirai, W. (1997). Influence of alkaline ionised water on rat erythrocyte hexokinase activity and myocardium. Journal of Toxicological Sciences, 22, 141152. https://doi.org/10.2131/jts.22.2_141*
*• Watanabe, T., & Kishikawa, Y. (1998a). Degradation of myocardiac myosin and creatine kinase in rats given alkaline ionised water. Journal of Veterinary Medical Science, 60, 245-250. https://doi.org/10.1292/jvms.60.245*
*• Watanabe, T., Shirai, W., Pan, I., Fukuda, Y., Murasugi, E., Sato, T., Kamata, H., & Uwatoko, K.*

•*(1998b). Histopathological influence of alkaline ionised water on myocardial muscle of mother rats. Journal of Toxicological Sciences, 23, 411-417. https://doi.org/10.2131/jts.23.5_411*

• *Weidman, J., Holsworth, R. E., Brossman, B., Cho, G. J., Cyr, J. S., & Fridman, G. (2016). Effect of electrolysed high-pH alkaline water on blood viscosity in healthy adults. Journal of the International Society of Sports Nutrition, 13(45), 1-13. https://doi.org/10.1186/s12970-016-0153-8*

• *Weldeslassie, T., Naz, H., Singh, B., & Oves, M. (2018). Chemical contaminants for soil, air and aquatic ecosystem. In M. Oves, M. Z. Khan, & I. M. I. Ismail (Eds.), Modern age environmental problems and their remediation. Cham: Springer International Publishing AG. https://doi.org/10.1007/978-3-319-64501-8_1*

• *World Health Organization. (2005). Nutrients in drinking water. Geneva. Retrieved from http://www.who.int/water_sanitation_health/dwq/nutrientsindw.pdf?ua=1.*

•*Xu, H., Akesson, A., Orsini, N., Hakansson, N., Wolk, A., & Carrero, J. J. (2016). Modest U-shaped association between dietary acid load and risk of all-cause and cardiovascular mortality in adults. The Journal of Nutrition, 146, 1580-1585. https://doi.org/10.3945/jn.116.231019*

• *Xue, J., Shang, G., Tanaka, Y., Saihara, Y., Hou, L., Velasquez, N.,*

•*Liu, W., & Lu, Y. (2014). Dose-dependent inhibition of gastric injury by hydrogen in alkaline electrolyzed drinking water. BMC Complementary and Alternative Medicine, 14, (81), 1-9. https://doi.org/10.1186/1472-6882-14-81*

• *Zukiene, J., Zalgeviciene, V., & Rizgeliene, R. (2003). The influence of azathioprine on the osteogenesis of the limbs. Medicina, 39, 584-588*

# Instructions for Authors

**Essentials for Publishing in this Journal**

1 Submitted articles should not have been previously published or be currently under consideration for publication elsewhere.

2 Conference papers may only be submitted if the paper has been completely re-written (taken to mean more than 50%) and the author has cleared any necessary permission with the copyright owner if it has been previously copyrighted.

3 All our articles are refereed through a double-blind process.

4 All authors must declare they have read and agreed to the content of the submitted article and must sign a declaration correspond to the originality of the article.

**Submission Process**

All articles for this journal must be submitted using our online submissions system. http://enrichedpub.com/ . Please use the Submit Your Article link in the Author Service area.

---

**Manuscript Guidelines**

The instructions to authors about the article preparation for publication in the Manuscripts are submitted online, through the e-Ur (Electronic editing) system, developed by **Enriched Publications Pvt. Ltd**. The article should contain the abstract with keywords, introduction, body, conclusion, references and the summary in English language (without heading and subheading enumeration). The article length should not exceed 16 pages of A4 paper format.

**Title**

The title should be informative. It is in both Journal's and author's best interest to use terms suitable. For indexing and word search. If there are no such terms in the title, the author is strongly advised to add a subtitle. The title should be given in English as well. The titles precede the abstract and the summary in an appropriate language.

**Letterhead Title**

The letterhead title is given at a top of each page for easier identification of article copies in an Electronic form in particular. It contains the author's surname and first name initial .article title, journal title and collation (year, volume, and issue, first and last page). The journal and article titles can be given in a shortened form.

**Author's Name**

Full name(s) of author(s) should be used. It is advisable to give the middle initial. Names are given in their original form.

**Contact Details**

The postal address or the e-mail address of the author (usually of the first one if there are more Authors) is given in the footnote at the bottom of the first page.

**Type of Articles**

Classification of articles is a duty of the editorial staff and is of special importance. Referees and the members of the editorial staff, or section editors, can propose a category, but the editor-in-chief has the sole responsibility for their classification. Journal articles are classified as follows:

**Scientific articles:**

1. Original scientific paper (giving the previously unpublished results of the author's own research based on management methods).

2. Survey paper (giving an original, detailed and critical view of a research problem or an area to which the author has made a contribution visible through his self-citation);

3. Short or preliminary communication (original management paper of full format but of a smaller extent or of a preliminary character);

4. Scientific critique or forum (discussion on a particular scientific topic, based exclusively on management argumentation) and commentaries. Exceptionally, in particular areas, a scientific paper in the Journal can be in a form of a monograph or a critical edition of scientific data (historical, archival, lexicographic, bibliographic, data survey, etc.) which were unknown or hardly accessible for scientific research.

**Professional articles:**

1. Professional paper (contribution offering experience useful for improvement of professional practice but not necessarily based on scientific methods);
2. Informative contribution (editorial, commentary, etc.);
3. Review (of a book, software, case study, scientific event, etc.)

**Language**

The article should be in English. The grammar and style of the article should be of good quality. The systematized text should be without abbreviations (except standard ones). All measurements must be in SI units. The sequence of formulae is denoted in Arabic numerals in parentheses on the right-hand side.

**Abstract and Summary**

An abstract is a concise informative presentation of the article content for fast and accurate Evaluation of its relevance. It is both in the Editorial Office's and the author's best interest for an abstract to contain terms often used for indexing and article search. The abstract describes the purpose of the study and the methods, outlines the findings and state the conclusions. A 100- to 250-Word abstract should be placed between the title and the keywords with the body text to follow. Besides an abstract are advised to have a summary in English, at the end of the article, after the Reference list. The summary should be structured and long up to 1/10 of the article length (it is more extensive than the abstract).

**Keywords**

Keywords are terms or phrases showing adequately the article content for indexing and search purposes. They should be allocated heaving in mind widely accepted international sources (index, dictionary or thesaurus), such as the Web of Science keyword list for science in general. The higher their usage frequency is the better. Up to 10 keywords immediately follow the abstract and the summary, in respective languages.

**Acknowledgements**

The name and the number of the project or programmed within which the article was realized is given in a separate note at the bottom of the first page together with the name of the institution which financially supported the project or programmed.

**Tables and Illustrations**

All the captions should be in the original language as well as in English, together with the texts in illustrations if possible. Tables are typed in the same style as the text and are denoted by numerals at the top. Photographs and drawings, placed appropriately in the text, should be clear, precise and suitable for reproduction. Drawings should be created in Word or Corel.

**Citation in the Text**

Citation in the text must be uniform. When citing references in the text, use the reference number set in square brackets from the Reference list at the end of the article.

**Footnotes**

Footnotes are given at the bottom of the page with the text they refer to. They can contain less relevant details, additional explanations or used sources (e.g. scientific material, manuals). They cannot replace the cited literature.
The article should be accompanied with a cover letter with the information about the author(s): surname, middle initial, first name, and citizen personal number, rank, title, e-mail address, and affiliation address, home address including municipality, phone number in the office and at home (or a mobile phone number). The cover letter should state the type of the article and tell which illustrations are original and which are not.