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Treatment Of Sewage Sludge From Heavy Metals, Organics Pollutant And
Metal Recovery From Fungi

Bachelor's Thesis (12 ECTS)

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Abstract

Sewage sludge is the semi-solid substance that is released as an end product during the treatment of wastewater. More focus needs to be paid to the treatment and reuse or disposal of sewage sludge because of strictly controlled law. Sewage sludge can be used as a fertilizer in agriculture, but the use of sludge is prohibited since it contains heavy metals, poisonous chemicals, bacteria, etc. The use of fungi treatment can assist in the recovery of waste sludge and it's a substitute approach in the treatment of sludge. The aim of using this fungal treatment is to reduce a broad range of xenobiotics even at low concentrations due to the unspecific enzymatic system, especially in lignolytic fungi. This thesis focuses on sewage sludge-related topics such as substances, rules, treatment, and recycle. The main objective of the present work was to examine, in accordance with the law, the role of biological processes in the removal of heavy metals during fungal treatment.

Keywords: Wastewater treatment, Sewage sludge, Fungi, Heavy metal.

CERCS: T270 Environmental technology, pollution control; B700 Environmental health

Estonian

Reoveesete on pooltahke aine, mis eraldub lõppproduktina reovee puhastamisel. Rangelt kontrollitud seaduste tõttu tuleb rohkem tähelepanu pöörata reoveesette töötlemisele ja korduvkasutamisele või kõrvaldamisele. Reoveesetet võib kasutada põllumajanduses väetisena, kuid käitlemata liigmuda kasutamine on keelatud, kuna see sisaldab raskmetalle, mürgiseid kemikaale, baktereid jne. Seenetöötlus võib aidata kaasa jääkmuda taaskasutamisele ja see on asendusmeetodiks muda töötlemisel. Selle lõputöö eesmärk on vähendada ksenobiootikume kontsentratsioone ja siduda need seente abil. See lõputöö keskendub reoveesetega seotud teemadele, nagu saasteained, reeglid, töötlemine ja reoveesette ringlussevõtt. Käesoleva töö põhieesmärk oli uurida kooskõlas seadusega bioloogiliste protsesside rolli raskmetallide eemaldamisel seentega töötlemisel.

Keywords: Reovesi, reoveesete, seened, raskmetall

CERCS: T270 Keskkonnatehnoloogia, reostuskontroll; B700 Keskkonnatervis

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TERMS, ABBREVIATIONS, AND NOTATIONS

PHACs	Pharmaceuticals
WWTP	Waste Water Treatment Plant
PAHs	Polycyclic Aromatic Hydrocarbons
WRF	White Rot Fungi
SS	Sewage Sludge
HMI	Heavy metal ions

INTRODUCTION

Over the last couple of decades, the human population has increased rapidly causing our planet's overpopulation. This has a strong effect on the quantity of wastewater that needs to be treated to reduce its environmental impact. Heavy metals and organic pollutants are the major components that need to be removed from water bodies so that the water returns to the environment with little impact and prevents toxicity to the ecosystem.

Sewage sludge exists as a form of solid or liquid residue that has been produced from municipal or industrial wastewater treatment. It is considered to accommodate many pharmaceuticals and as a result, it has gotten a lot of publicity over the years. This has led to an increased need to determine and track their concentrations to create a safer biological environment. However, there is a need to determine their concentration with a high degree of certainty, one or more effective methods of extracting these substances from wastewater must be available.

Generally, the wastewater treatment approach needs high efficiency of energy. Several techniques have been used in the treatment of wastewater but one of the rising technologies is the fungal treatment of wastewater due to the broader enzymatic system that can degrade a wide range of xenobiotics at a lower concentration. Several amounts of research have been performed and hence, optimized fungus treatment for the elimination of organic pollutants and heavy metals is a very promising method that can generate a new substitute technology for the treatment of wastewater.

Importantly, there is a need to come up with an effective way to treat wastewater. A lot of studies have been recently carried out concentrating on the fungal treatment of pharmaceuticals (PHACs(Lucas et al., 2018; Rodriguez-Rodriguez et al., 2011b; Sankaran et al., 2010) with white-rot fungus (*Trametes*) showing especially good pharmaceutical removal efficiency. This further underscores the potentials of applying fungi in wastewater treatment.

1 THE AIMS OF THE THESIS

Due to the increased amount of sewage sludge produced every year in Estonia causing a threat to the ecosystem, there was a need to come up with an effective way to treat wastewater. Although the operation of this system is still unclear concerning the fungal treatment, which motivated this research.

- To develop the treatment of sewage sludge from heavy metals, emerging pollutants using biological methods that include fungi.
- To study how to apply the extraction of heavy metals from sludge, fungi mass, and the cultivation of fungi on sludge.

2 LITERATURE REVIEW

Wastewater is produced from domestic, industrial, and agricultural activities. The components represented in wastewater vary depending on the source of the wastewater. One of the major sources of wastewater is municipal wastewater. Municipal wastewater causes contamination to the aquatic world. Sewage sludge is a residue that is gotten from a byproduct of municipal or urban wastewater treatment (Rodriguez-Rodriguez et al., 2011a).

2.1 The Effect And Benefit of Sewage Sludge

The amount of sludges produced over the years is increasing (Wołejko et al., 2018) and it is stated that the European Commission's new strategy is to allow the beneficial usage of sewage sludge on land, as long as the sludge content meets public health and environmental criteria, as it appears to be a long-term sustainable solution to sludge disposal (Carbonell et al., 2009a). The most significant disposal of sewage sludge in Europe is agricultural lands, which account for about half of all sludge production. Land application has a lot of advantages because of its use as fertilizer, whereby improving fertility by adding nutrients such as Nitrogen (N), Phosphorus (P), sodium (Na), Potassium (K) and recovering the structure of the soil by introducing organic content (Singh et al., 2011). Land-applied sludges must be treated to reduce pathogens after being isolated from wastewater using one of many methods, including anaerobic digestion, lime stabilization, or composting. Each of these processes has an effect on the pathogens' and organic pollutants' fates in the sludge (Harrison et al., 2006). Research has shown that sewage sludge is the combination of organic, inorganic pollutants, and pathogens and they were able to indicate the presence of heavy metals which include pharmaceuticals, estrogens, UV filters, illicit drugs, brominated flame retardants, pesticides (Rodriguez-Rodriguez et al., 2011b) (Lucas et al., 2018). This component makes sewage sludge usage a main problem in the environment and it needs to be thoroughly checked to ensure that it meets the existing regulations and directives before being used.

According to (Smith, 2009), he reported that organic matter is abundant in sewage sludge, which is mainly composed of microorganisms. Organic matter is the main factor for soil productivity and the concentration of organic matter varies depending on the treatment of wastewater (Carbonell et al., 2009b). The availability of microorganisms in sewage sludge is important because it helps in soil fertility and improves soil properties.

2.2 Pollutants

The introduction of toxic products into the atmosphere is referred to as pollution, and harmful substances are referred to as pollutants. The Impurity that is released into the wastewater can either be biological or chemical. The chemical pollutant that is common in wastewater includes heavy metal cations, pesticides, nitrogenous compounds, pharmaceutical residues, and detergents while the microbiological contaminant could either be from animal or human feces which contain several kinds of protozoa, viruses, and bacteria which are toxic to human health(Germaine, 2014)

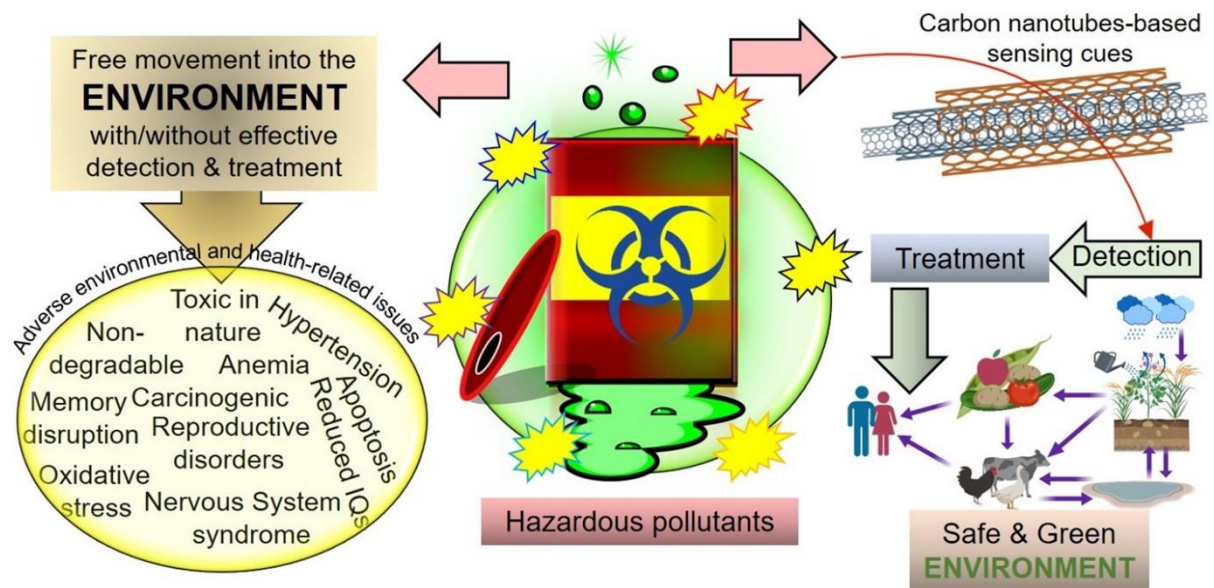


Figure 1. The problem of pollutants (Rasheed et al., 2019).

Studies have shown that pollutant known as a persistent organic pollutant (POP) are resistant to biodegradation, remaining in the bodies of the water for a long time and becomes a threat to the environment (Weil et al., 2000). A common health problem that is caused by the consumption of contaminated water is diarrhea, parasitic intestinal infections. The frequent use of pesticides in agriculture and domestic purposes; releases pesticides into the water in an unnoticed manner (de Figueirêdo Pitombeira et al., 2021). The popular pesticides used in both agriculture and household purposes are 1-1-bis(4-chlorophenyl)- 2-2-2-(trichloroethane) (DDT), which is organochlorine pesticides, which are extremely harmful because when they enter the food chain by water contamination it results in carcinogenic (potential to cause cancer). Research has shown (Gusain et al., 2020) that industries release millions of gallons of effluent into the river such as drugs, dyes, a heavy metal that is harmful to the ecosystem.

However, heavy metal ions (HMIs) are also widely used in cosmetics, vehicles, metal plating, electroplating, mining activities, and battery manufacturing. They consist of highly toxic metals which are produced in industrial wastewater (Rasheed et al., 2019). Pharmaceuticals effluents on the other hand contain medication precursors which include pathogenic organisms and radioactive elements, antibiotics and are discarded into water sources. They enter the water sources by urine and feces after the ingestion of drugs. They are non-biodegradable, containing radioactive elements, and have a negative impact on health when consumed in large quantities by polluted water (Lucas et al., 2018). The major organic pollutant that has been a concern in most countries is PAHs (Polycyclic aromatic hydrocarbons). It is very toxic and mutagenic which can be seen in Fig.1. They are the kind of pollutants that are difficult to reduce (degrade) due to the higher level of molecular weight. These pollutants are present in the air, water, and soil as a result of forest fires, volcanoes, and other natural disasters that penetrate the atmosphere, and they can also move through the surface of the water as a result of industrial plant discharge, pipelines, and other sources (Rasheed et al., 2019). As a result, eliminating organic and inorganic contaminants from wastewater and industrial effluent is important.

2.3 Heavy Metals

Heavy metals are regarded as any metals that contain more than 5g/cm³ of density. It is a serious environmental health issue. Sediment is major storage of heavy metals waste. The contamination of heavy metals in sediments has gotten a lot of attention because its toxicity is connected to various trophic levels and can damage several species once it gets into the food chain (Agoro et al., 2020). Generally, most of the heavy metals display toxicity at low concentrations due to the physical-chemical operation that is engaged in the treatment of wastewater for example metallic chemical elements of relatively high densities are found in varying quantities in nature (Huang et al., 2021). These metals can cause cancer while others can act as an agent such as chemical radiation which causes genetic mutation for both humans and animals depending on the duration of exposure (Mojeed A. Agoro, 2020). Research has shown that (Kremser et al., 2021) (Agoro et al., 2020) As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn are heavy metals that are of primary concern in the atmosphere due to their toxicity. A few trace elements (such as Zn, Cu, Mg, K, and others) are necessary to control biochemical processes and metabolism in the human body, but only to certain extents.

While heavy metal pollution is a global issue, contamination levels differ from place to place. Heavy metals are introduced into natural ecosystems by the discharge of commercial, urban, and agricultural wastewaters and sewage into waterways (Zhang et al., 2019). They readily adsorb to suspended particles in water, settle in the riverbed, and then are released into the water column, where they pose a secondary source of pollution, endangering ecosystems. Big increases in heavy metal concentrations have been observed in the natural world as a result of human activities, which is why their distribution and effects on the environment are being studied extensively (Sakshi & Haritash, 2020). According to a report conducted by the Estonian Centre for Environmental Research (EKUK) (2010.a), hitting the limit values for heavy metal concentrations in sewage sludge is a challenge in Estonia.

2.4 Treatment of Sewage Sludge

Sludge is the residue that builds up in water treatment plants. The by-product of wastewater treatment plants that are either solid or semisolid residue is referred to as sewage sludge (Harrison et al., 2006). Primary and secondary sludge are two common classifications for this residue. Chemical precipitation, sedimentation, and other primary processes produce primary sludge, while secondary sludge is activated waste biomass generated by biological treatments. In addition, household on-site wastewater treatment systems often send septage or septic tank solids to some sewage plants (Collivignarelli et al., 2019)

A mixture of thickening, digestion and dewatering processes may be used to treat sewage sludge. Thickening is the first step in sludge treatment since thin sludge, a slurry of solids suspended in water is difficult to manage. This is normally done in a tank known as a gravity thickener. A thickener will reduce sludge volume to less than half of its original size. Dissolved air flotation is an alternative to gravity thickening. The solids are carried by air bubbles in this process. Secondly, Organic solids are decomposed into stable substances during sludge digestion, which is a biological process. They help to destroy pathogens and reduces solid mass (Singh et al., 2011) while taking place in the presence of oxygen.

Thirdly is dewatering, they contain a small amount of water and can be treated as a solid substance. The easiest way of dewatering is by sludge-drying beds where evaporation and gravity drainage goes through the sand to dry (Haiba et al., 2016)

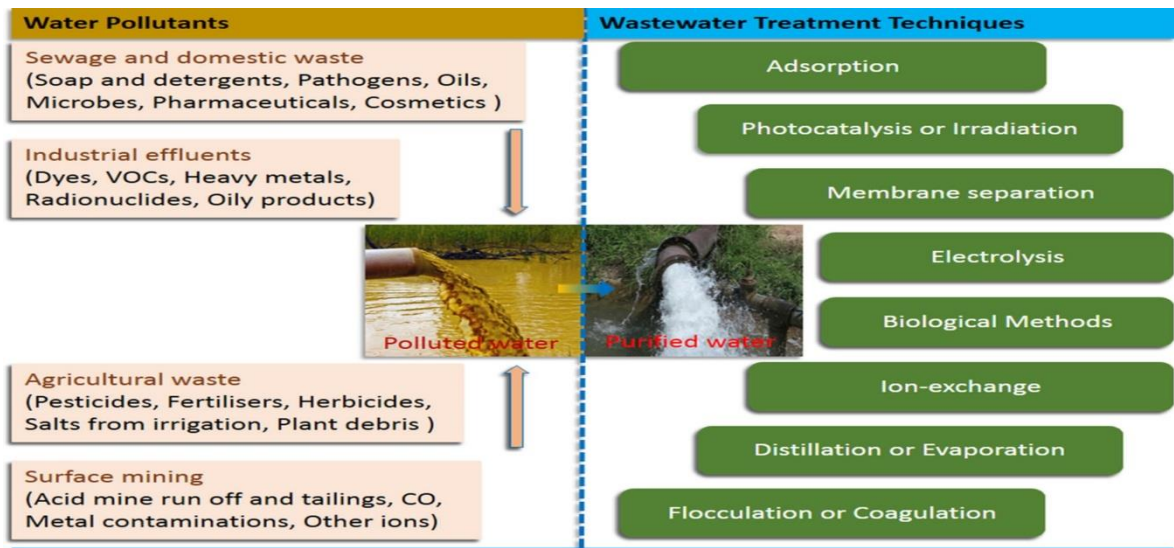


Figure 2. The amount of water contamination sources and the treatment techniques of wastewater (Gusain et al., 2020).

2.5 Treatment Techniques used in Wastewater

Adsorption is the most widely used method of eliminating harmful organic and inorganic contaminants from wastewater (Gusain et al., 2020). Adsorption is a cost-effective, fast, environmentally friendly, and simple process, and the adsorbent can be reused several times without losing effectiveness. Adsorbents for water pollution remediation have been studied on several products. Adsorption is the physical bonding of ions and molecules into another surface of molecules. Among other techniques used in the treatment of wastewater; adsorption has more advantages over several techniques.

Irradiation has been shown (Ahlstrom, 1985) to be an effective method for reducing pathogens in sewage sludge to levels that enable sludge reuse in public areas to meet public health standards. The disadvantage of irradiation is that it does not extract heavy metals from the sludge instead it acts as an agent in the removal of the pathogen when is being used in agriculture.

Biosorption is the dismissal of organic and inorganic substances from a solution by biological means(Huang et al., 2021). It has been used for the removal or recovery of pollutants due to its biomass availability. The mechanism used in biosorption is Adsorption, absorption, ion exchange, and precipitation. Most of the treated sewage sludge is mostly for landfilling or distributed on farmland to take advantage of its usefulness as a soil conditioner and fertilizer. Sludge is not spread on land where crops for human consumption are grown because it may contain harmful industrial chemicals.

2.6 Fungal Bioleaching and Bacterial Bioleaching

Bioleaching is a method that uses microorganisms like bacteria or fungi to remove precious metals from low-grade ores (Huang et al., 2021). Fungi are the most complex and it's the second group of rich species in nature (Pathak et al., 2021) due to their natural capacity to concurrently generate oxidants, such as reactive oxygen species, fungi are ideal organisms for metal bioleaching and absorption from the surrounding environment. The uses of fungi in the leaching of metals have attracted a lot of attention. In general, metal leaching by fungi requires an indirect bioproduction process for organic acids such as gluconic, citric, oxalic, etc. Bacterial metal extraction is not a modern technology; it has been used for ore leaching for decades, but the process has remained a mystery. *Acidithiobacillus ferrooxidans* is the most important bacteria in the bioleaching process (Brinza et al., 2021). It's a chemoautotrophic acidophile, which means it gets its energy from inorganic sources and fixes its carbon when living in an acidic setting.

Two key mechanisms cause bacterial leaching. The indirect mechanism is based on the ferric-ferrous cycle, while the direct mechanism is based on physical interactions between organisms and is unrelated to the indirect mechanism. Fungi are suitable organisms for metal bioleaching and uptake from the surrounding environment owing to their innate ability to simultaneously produce oxidants, e.g., reactive oxygen species (Hansel 2012). Biooxidation, acidolysis by organic acids, and complexolysis by chelation of materials produced during acidolysis are all ways that fungi can release metals from the solid matrix. This process includes biologically excreted amino acids, quinones, other carbonyl compounds such as cyanide and thiosulfate, or iodine redox cycling, and results in the appearance of gold and other precious metals as well as the formation of colloids compounds (Bohu et al 2019).

Fungi are more resistant species than bacteria in bioleaching and recovery, and they can be used in both cyanide/thiosulfate forming processes for metal release and biological recovery from PCB-s. As a consequence, fungi use a variety of fungi that grow at pH levels ranging from 1.5 to 9.8, produce a variety of organic acids, and recovers more metals. Both fungi, *Aspergillus niger*, and *Penicillium simplicissimum* have been used to successfully remove metals from several solid wastes, including power plant residual ash (Arshadi et al., 2020). Toxic metals can be removed from the environment by a diverse group of fungal strains, especially *Aspergillus* and *Penicillium* species. The use of fungal strains to remove heavy metals is a recent technology. The key benefit is that they are tailored not only to the presence of pollutants but also to the surrounding climate. Isolating fungal strains is necessary to

reduce the hazards and threats associated with their aggregation as a result of heavy industrialization and other human activities around the world(Turek et al., 2018).

2.7 Pleurotus Ostreatus And Lentinula Edodes

Pleurotus ostreatus, also known as oyster mushroom. The oyster mushrooms can grow in lignin and cellulose-rich environments, they are commonly found in weathered wood. It's also an edible mushroom. *Ostreatus* has medicinal properties due to bioactive compounds derived from the fruit body, which have been shown to inhibit the proliferation of cervix cancer cells(Mumpuni et al., 2017). *Lentinula edodes* (shiitake), on the other hand, is a type of fungus that uses a wide range of typical micronutrients and it was found to contain medicinal compounds such as polysaccharides, sterols, and lipids. Both of these mushrooms are known for their antitumor, antibacterial, and antiviral properties(Uluköy et al., 2016)

3 EXPERIMENTAL PART

The section below explains the material and methods that were used to perform the experiments. The use of sludge was combined with the fungi (*Pleurotus ostreatus*) and starch granules in clean plastic containers. To allow for their development, the conditions are kept in a moist state.

3.1 Materials And Methods

SAMPLING

Between June and September 2020, samples were collected, and three separate samples were taken from each wastewater treatment facility from the aged residue in Estonia:

- sewage sludge for less than one year (raw material);
- 1 year of sewage sludge ;
- 2 years of sewage sludge;
- 4 years of sewage sludge

Setup 1: The sewage sludge is not sterilized and it also includes granules and fungi and the amount that was used.

Sewage sludge : 10g

Starch granules:6.5g

Fungi:13g

Setup 2: This is where sewage sludge is being sterilized before the addition of granules and fungi.

Sewage sludge:101g

Starch granules:104g

Fungi:101g

3.1.1 Materials

- Oxitop respiratory
- Total Kjeldahl Nitrogen (TKN) and total phosphorus,

- ICP
- The use of Microsoft Excel spreadsheets was used in the analysis of data processing.

3.1.2 Oxitop Respiratory

The tests were carried out in the laboratory of the Chair of Colloidal and Environmental Chemistry of the University of Tartu during September 2020.

The OxiTop device is a respiration rate measurement tool. The method tests the amount of oxygen consumed by aerobic microbes in the sample due to their respiration. Soil microbes absorb oxygen and produce CO₂, which is sequestered by KOH, resulting in a pressure drop equal to the amount of CO₂ sequestered in the vessel.

The oxiTop respiratory also measuring pressure. A negative pressure arises as oxygen is ingested in a closed vessel at a constant temperature when CO₂ is absorbed by KOH. When a measurement is started, the OxiTop measuring head measures and stores the pressure data for the length of the measurement (Germaine, 2014)

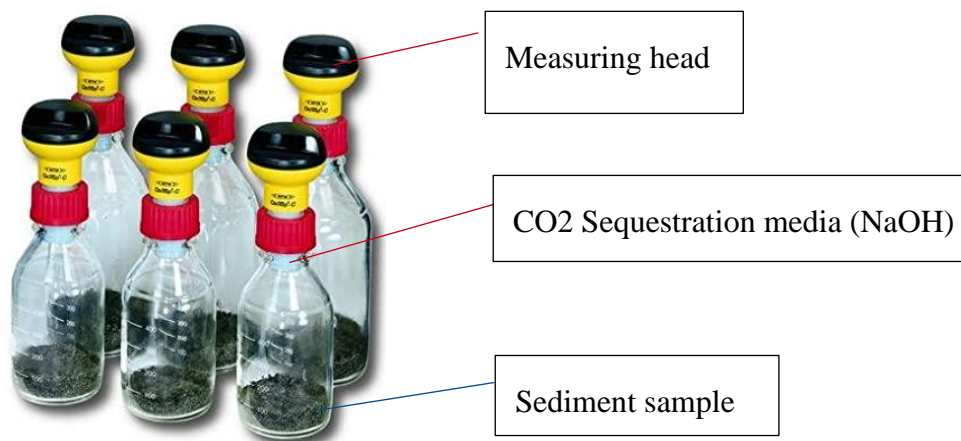


Figure 3. oxitop vessel (Germaine, 2014)



Figure 4. Handheld controller (Germaine, 2014)

3.1.3 Procedure

The materials used for the preparation of the oxiTop respirometry in determining respiration rates in soil are OxiTop measuring heads; OxiTop OC110 Controller; PC contact applications ACHAT OC; Data transfer wire; OxiTop vessels; Sodium hydroxide; Plastic 100ml beaker; Sewage sludge

3.1.4 Moisture Assessment In The Soil

The moisture content is done in two copies per sewage sludge

We used dry crucibles and record the mass

We measure 5g of the sewage sludge into the crucible and record the total mass present in the crucible and soil

The crucible and soil is been placed into an Oven at 105 degree Celsius for 24 hrs

After 24hrs we remove it from the oven and allows it to cool down for 45 mins.

Then we retake the measurement of the crucible and soil.

We determine the % moisture of the soil content by using this equation

$$\text{Total suspended solid} = \frac{(\text{Weight of crucible} + \text{fresh sludge}) - (\text{Weight of crucible} + \text{dry sludge})}{\text{Weight of sludge sample}} \times 100$$

3.1.5 How To Setup The Oxitop Vessel

A fixed volume of sewage sludge (10 g) was put in test bottles using a manometric measurement device OxiTop.

The bottle reported the system's relative pressure shift in hectopascans (hPa) over a predetermined period (96 hours);

Since microorganisms in the sediment decompose organic matter and use oxygen to do so, the pressure in the vessel changes (decreases). Potassium hydroxide (KOH) granules were added to measuring bottles to bind carbon dioxide (CO₂) emitted into the gas phase at biotic oxygen demand;

The zero pressure level was air pressure at the time the measurement bottles were closed.

Test sediment bottles are held at $20.0 \pm 1^\circ\text{C}$ during the test period

The formula 1 is used to turn from pressure shift to oxygen demand.

$$BA = \frac{M_R(O_2)}{R \times T} * \frac{V_{fr}}{m_{Bt}} * \Delta p$$

where: O₂ - Oxygen demand, mgO₂/KA

M_R - Molar mass of oxygen, 32 000 mg/mol

R - Universal gas constant, 83,14 L*mbar/mol*K

T - Measurement temperature, K

V_{fr} - Volume of air in the measuring vessel, litres (0,5 L)

m_{Bt} - Mass of dry matter in the measuring system, kg KA

Δp - Change in pressure in the measuring vessel, mbar (1 mbar = 1 hPa).

3.2 Inductively Coupled Plasma Mass Spectrometry

ICP-MS (inductively coupled plasma-mass spectrometry) is a technique for measuring trace quantities of elements in biological fluids. Some laboratories, however, continue to use ancient approaches including atomic absorption and atomic emission. The advantage of ICP-MS is that it provides a very high sample flow rate in the laboratory because of its multi-

element capability, which enables several elements to be calculated simultaneously in a single study.

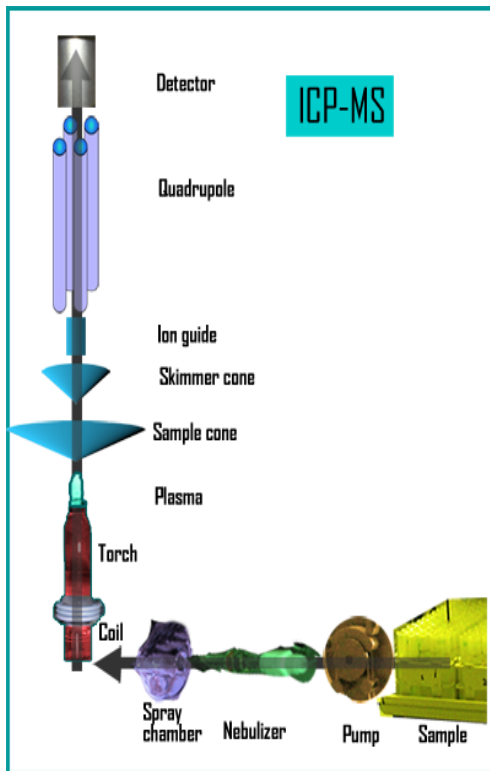


Figure 5. ICP- MS (*ICP-MS - General Instrumentation*, n.d.)

The sample, inductively coupled plasma (ICP), interface, ion optics, a mass analyzer, and detector are the six basic components of a single quadrupole ICP-MS. The instrument is depicted in Figure 5 as a simple diagram. Liquid samples are nebulized first in the sample introduction method, resulting in a fine aerosol that is then moved to the argon plasma. The high-temperature plasma atomizes and ionizes the sample, resulting in ions that are collected through the interface region and through the ion optics, a series of electrostatic lenses. The quadrupole mass analyzer's ion optics focuses and directs the ion beam. The mass analyzers divide ions into groups based on their mass-charge ratio (m/z), which are then calculated at the detector (Wilschefski & Baxter, 2019).

3.3 Total Kjeldahl Nitrogen

The mixture of ammonia and organic nitrogen is referred to as Total Kjeldahl Nitrogen (TKN). This test only looks for organic nitrogen compounds that appear as organically bound nitrogen in the negative state. The action of sulfuric acids and hydrogen peroxide transforms nitrogen in this form into ammonium salts. An updated Nessler system test is then used to measure ammonia.

- By filling both cylinders with deionized water to the 20 ml mark.
- To each cylinder, add three drops of mineral stabilizer.
- To each cylinder, apply three stops of polyvinyl alcohol dispersing agent.
- Fill both cylinders with deionized water to the 25ml level.
- Fill each cylinder with 1 mL of Nessler's reagent.
- When the timer goes off, pour the contents of each cylinder into separate squares and press zero; the display should show mg/L TKN.

3.4 Phosphate Determination

Phosphate is measured using a centrifuged sample and a spectrophotometry graph that ranges from 0.1 to 1.0 mg/L.

- By pipetting the appropriate amount of sample and filling the flask up to the volume mark with deionized water, the dilution is made into a 25mL volumetric flask.
- The flask is closed with a cap and shaken vigorously.
- The ascorbic acid solution is then pipetted into the flask in 1 mL increments.
- . The flask is then pipetted with 1ML of molybdate solution and the flask is closed with a cap and shaken vigorously so that the sample mixes well with reagents.
- After leaving the sample for 10 minutes, the result is determined with spectrophotometer program No. 952.

3.5 Determination of PH

ISO 10390:2005 describes an instrumental process for determining pH with a glass electrode in a 1:5 suspended in soil water.

3.6 Results and Discussion

The sections below explain the results that were generated from the experiments.

3.6.1 Results

Tests were done to assess the sludge stabilization throughout treatment. Pressure drops caused by bacterial activity utilizing oxygen mostly showed sludge stabilization enhancement along with treatment duration (0, 2, and 4 months). The unsterilized sludge from various sewage treatment plants had high-pressure drops at 0 months during process initiation.

Figure 6 depicts the change in pressure in measuring bottles holding 10 grams of sludge in the untreated sewage treatment throughout a different time. The pressure dropped on average for sediment in Tartu compost shitake as 110 hpa and Tartu raw Pleurotus for 70 hpa, Tartu raw shiitake, and Tartu compost Pleurotus for untreated sludge at 60hpa and 22 hpa respectively that is determined by the 4 days measurement period.

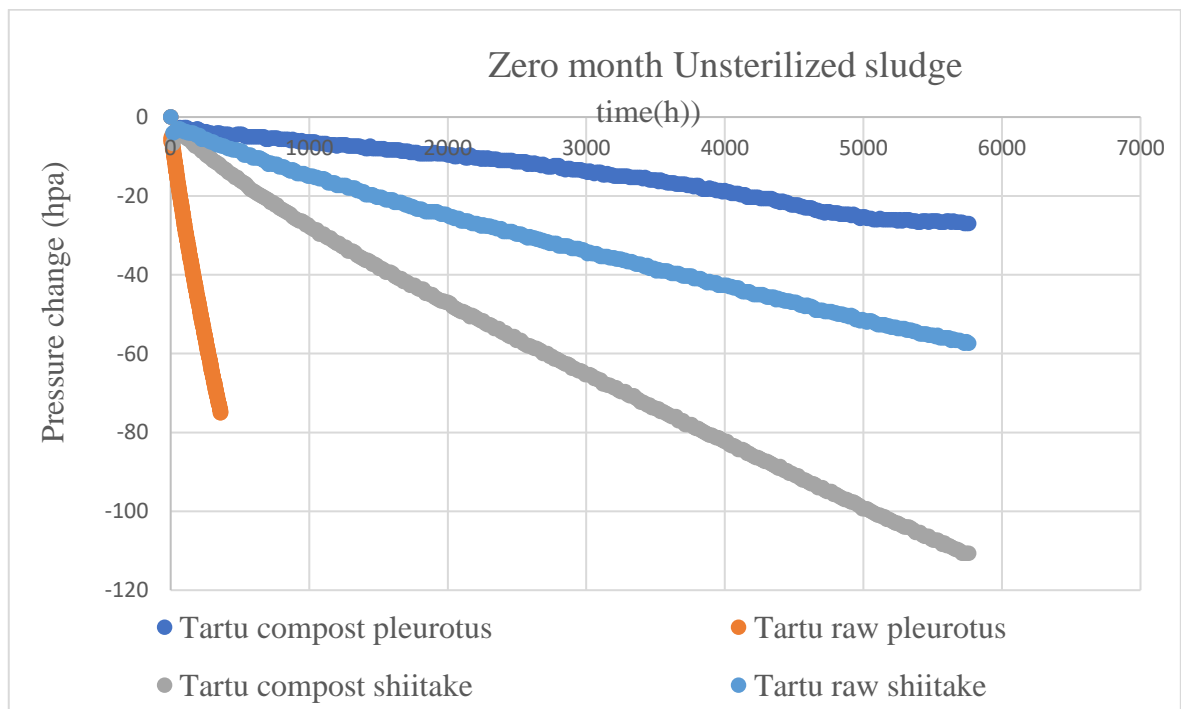


Figure 6. The unsterilized sewage sludge that is stated in oxygen demand in oxitop test during 4 days of measurement for zero month.

Figure 7 shows the change in pressure in measuring bottles holding 10 grams of sludge in the untreated sewage treatment throughout different times and it helps to compare the differences of the fungi used in the experiment. The pressure dropped on average for sediment in Tartu compost shitake as 65 hpa, whereas Tartu raw Pleurotus and Tartu compost Pleurotus both dropped as 50 hpa, Tartu raw shitake dropped at 40 hpa that is determined by the 4 days measurement period.

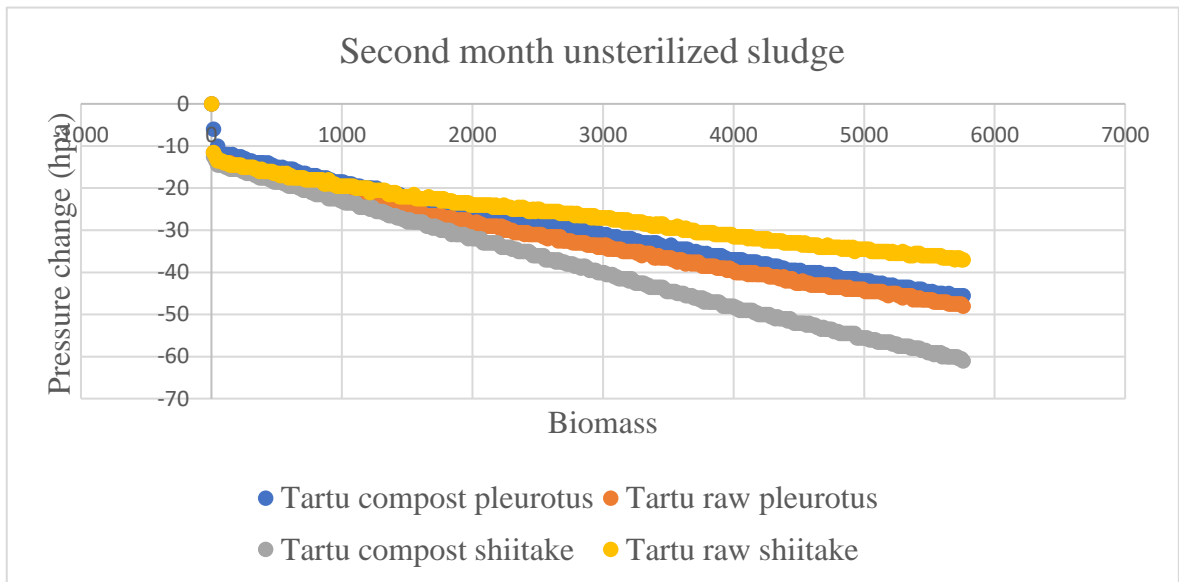


Figure 7: The unsterilized sewage sludge that is stated in oxygen demand in oxitop test during 4 days of measurement for two months.

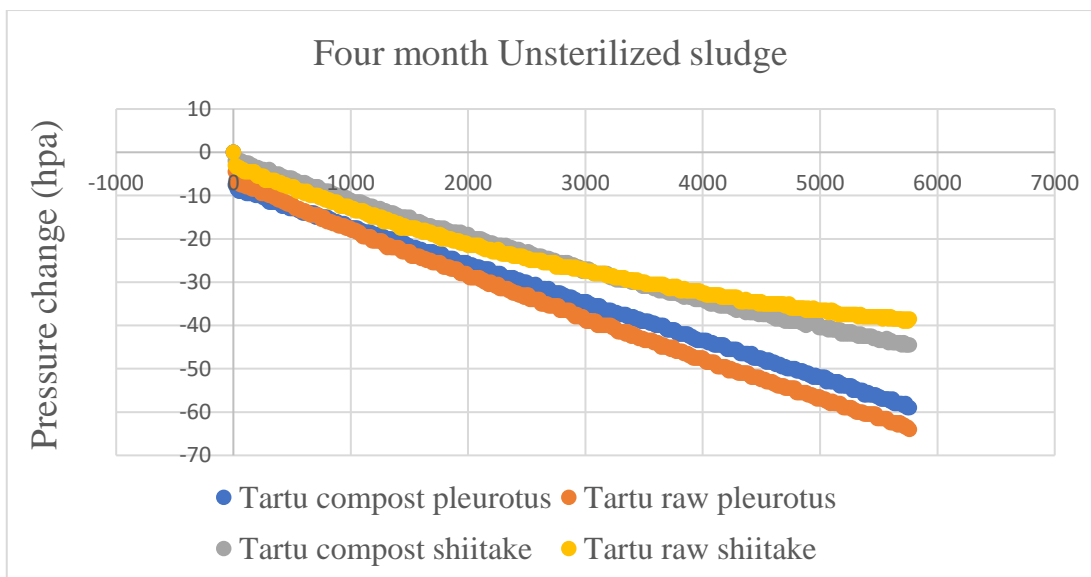


Figure 8. The unsterilized sewage sludge that is stated in oxygen demand in oxitop test during 4 days of measurement for four months.

The result from the oxitop analysis was used to determine the oxygen uptake rate during a 4-day respiration test in different unsterilized sludge. Biodegradability also plays a role in composting substances (Jędrzak 2007). From the graph shown above, it can be seen that 2 and 4 months sludges are mostly degradable, which are caused by the activity of microorganisms. The microorganism can exist in the form of bacteria, fungi, or algae, which helps in the removal of organic compounds. The pressure drop measurement from zero month can be seen to be varying from each other. Microorganisms use oxygen from the gaseous phase to degrade organic matter and produce carbon dioxide. The pressure drop reflects the intake of oxygen as carbon dioxide is extracted from the gaseous process by absorption on NaOH pellets (Myszograj et al., 2016).

In conclusion, it can be seen that 2 and 4 months shown in figure 7 and figure 8 all started at equilibrium at the beginning of the pressure measurement and later began to drop to reach a partly steady state because of the depletion of oxygen that limits the aerobic microorganism activity (Hubbe et al., 2010). While the zero month has the highest consumption of oxygen, based on the fact that the reduction of activated sludge in a respirometry test shows the presence of toxicity or contamination. Studies (Surerus et al., 2014a; Tzoris & Hall, 2006) has shown that when activated sludge contains contamination, the oxygen consumption of activated sludge decrease.

3.6.2 Biological Activity

Biological oxygen demand is used to measure the water quality and how the amount of oxygen will be used to biodegrade organic matter in sewage sludge (Roppola et al., 2007). The result from figure 9 shows the rate at which the unsterilized sludge was able to drop biological activity to some degree which is caused by the toxicity present in the sludges. A related studies shows (Surerus et al., 2014b) that activated sludge was used in the removal of organic pollutants and the theory behind this method is that in the presence of toxicants, activated sludge respiration is restrained, and therefore a decrease in activated sludge respirometry is a clear indicator of toxicity.

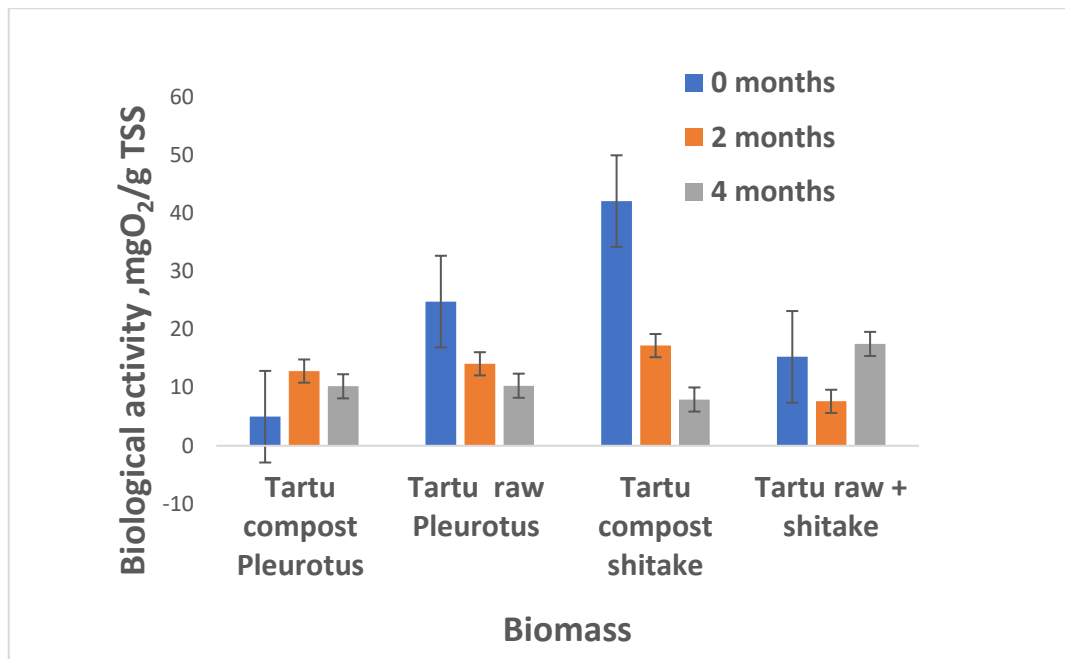


Figure 9. Tartu raw Pleurotus and Tartu compost of the unsterilized biological activity for 0,2,4 months (mgO₂/g of TSS).

Figure 10 shows the rate at which the biological activity for sterilized sludge for 0,2,4 months has been able to degrade toxic metals and reduces the effect of contaminants present in them. It can be seen that 4 months have the highest degradation of biological activity compared to the other month which is assisted by the sterilization process. Sterilization helps to kill microorganisms present in the soil including fungi that assist in the removal of toxicity in the soil. The EU authorities have regulated a law that biological activity should be below 10mg/kg of TSS, then sludge can be considered stabilized and safe for use in agriculture if other parameters are also under normative. In response to this, it can be seen that the sterilization of the sewage sludge in our research meets this regulation.

According to (Jose selis H., 2006) writes that increases in biodegradation are caused by the additional source of carbon that increases soil microorganisms in respiration rate that depletes over time. The main difference between both results of the sewage sludge was noticed to be caused by contamination in the unsterilized sludge which can be a result of the toxic substances which can be seen in figure 9 that it was not able to biodegrade most of the sludges and vice versa.

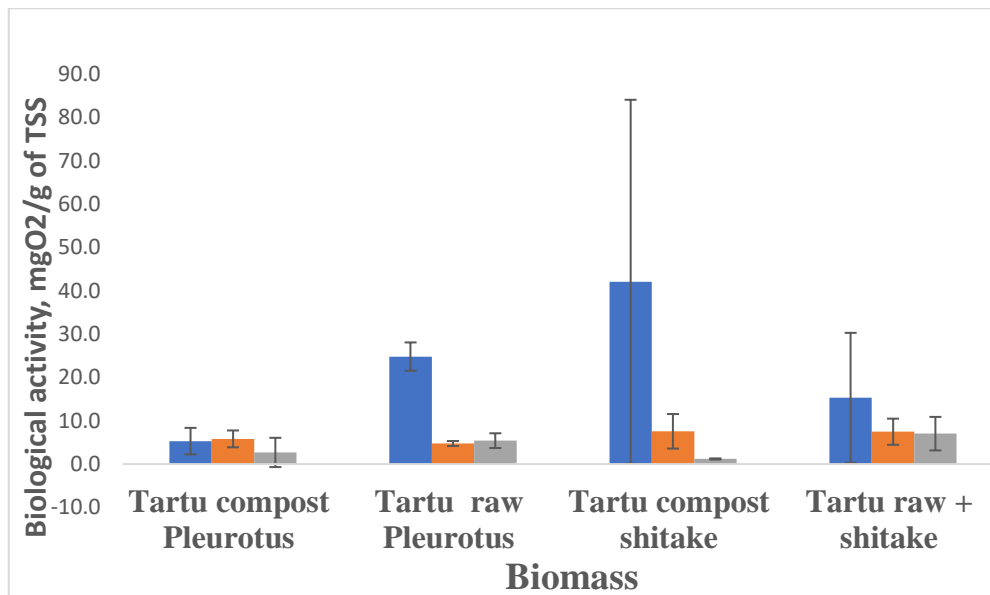


Figure 10. Tartu raw Pleurotus and Tartu compost of the sterilized biological activity for 0,2,4 months (mgO₂/g of TSS)

3.6.3 Nitrogen and Phosphorus

Studies (Bousselhaj et al., 2004; Salim Akhter, 1990) shown that Nitrogen and phosphorus are majorly common in sludge which is used as fertilizer in agriculture. It can be seen that the unsterilized sewage sludge and sterilized sludge contain a similar amount of nitrogen. The reason might be the soil type, climate, and water content. Nitrogen is common in the earth's atmosphere which is a highly stable and non-reactive form of gas. The zero month has the highest number of nitrogen present including the sterilized and unsterilized sludge. Research (Bousselhaj et al., 2004) shown that raw sewage sludge contains a large number of Nitrogen compared to the other month that the test was also conducted. (Lorenz and Maynard ,1988) explain that Nitrogen levels in industrial fertilizers, on the other hand, range from 11 to 82 percent compared to the experiment performed which ranges from 0 to 55 percent.

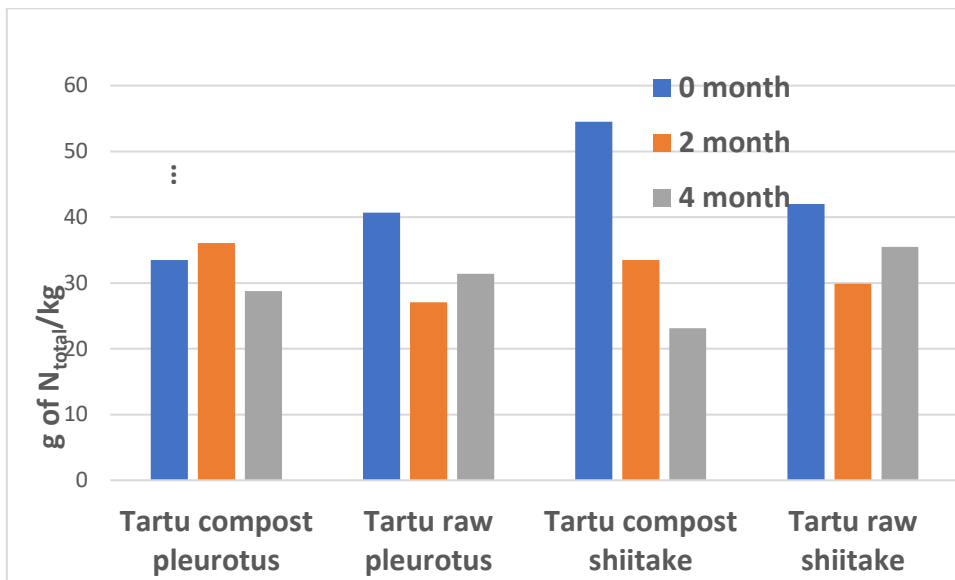


Figure 11. The unsterilized Nitrogen for 0,2,4 months.

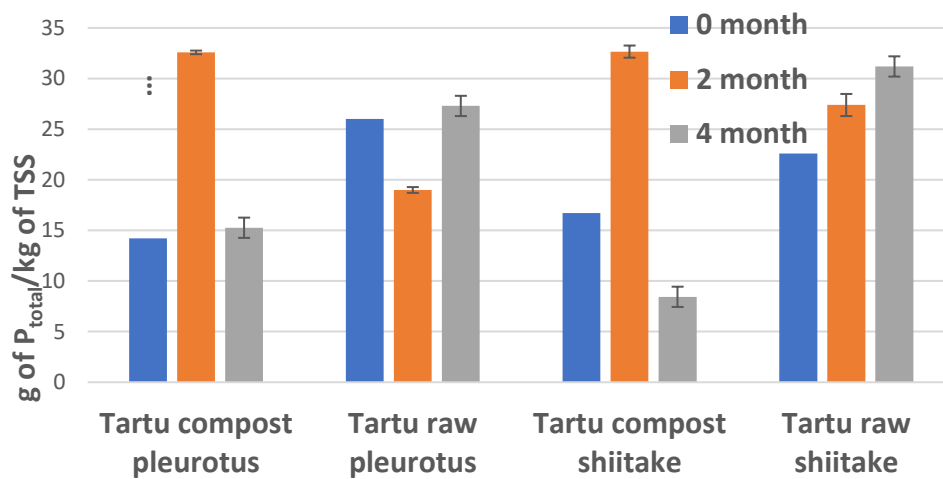


Figure 12. The unsterilized phosphorus for 0,2,4 months showing the error bars

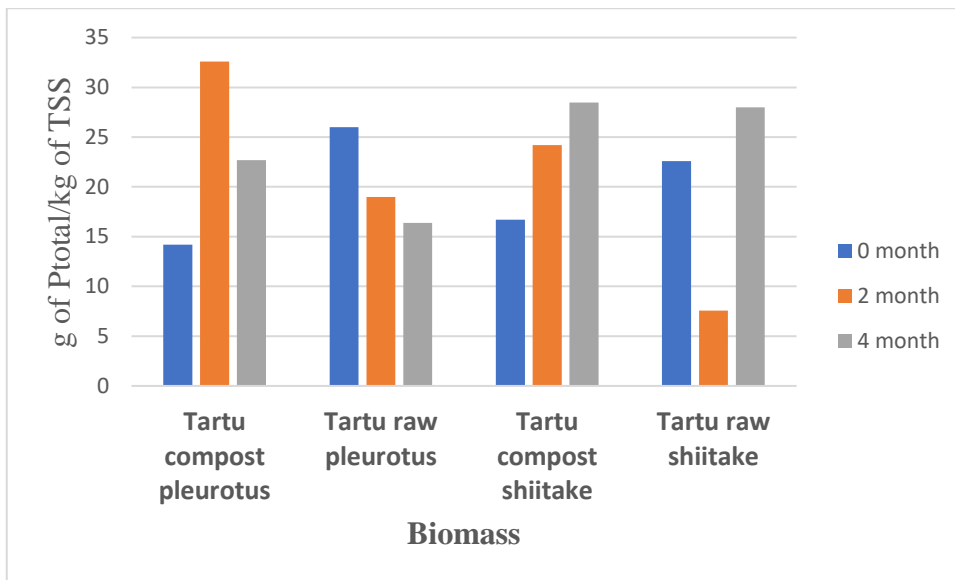


Figure 13. The sterilized phosphorus for 0,2,4 months

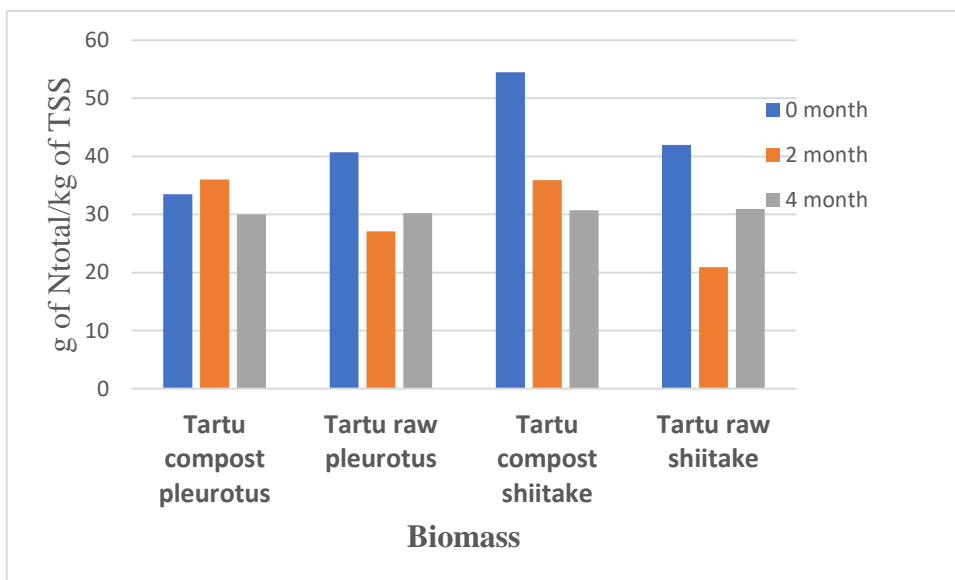


Figure 14. The sterilized Nitrogen for 0,2,4 months.

Although phosphorus is abundant in both organic and inorganic forms in soils, it is often a significant, if not the primary, limiting factor for plant growth (Khan et al., 2019). The presence of phosphorus in sewage sludge was investigated in sterile and unsterile conditions using 10g for each in both measuring bottles. The soil qualities and sterilization also contribute to phosphorus bioaccumulation after the treatment of soil. It can be seen that 2 month unsterilized and sterilized sewage sludge has the highest accumulation of phosphorus present in them due to their soil types and porosity used in the sample. The two-month unsterilized condition has a higher phosphorus concentration in the soil than the sterilized

condition, which may be due to microbial absorption in the nonsterilized soil, according to studies (Sinegani & Sedri, 2011). Mineral phosphate solubilizing processes are present in certain soil microorganisms and this capacity is linked to the release of organic acids and also the decreases in Ph. The fact that unsterile soils had more NaHCO₃-extractable P than sterile soils may be attributed to the activities of phosphate solubilizing microorganisms (Mahanty et al., 2017). The sterilized four-month of phosphorus was at equilibrium in the fact that they can possess some content according to their availability.

In conclusion, it is seen that fungi were able to treat sludge for 2 months more compared to the other month which to say it depends on location and sludges concentration in a particular area.

3.6.4 Recovery of Metals in Sewage Sludge

(Sandeep k. Malyan, 2021) said that the removal and recovery of heavy metals from wastewater is a long-term solution for wastewater treatment and environmental protection. In this research, we were able to determine different amounts of metals what was found in sewage sludge and how the fungi were able to extract metals from the sewage sludge by the process of water remediation. The initial concentration of Tartu raw in sewage sludge is twice the concentration of Tartu compost except for Ag (silver) and cadmium while the analysis of Fungi was the opposite from the Tartu compost there was able to extract more metals compared to Tartu raw from the sewage sludge, which is represented in the table below.

Sewage sludge sampling	Zn (mg/kg TSS)	Cd (mg/kg TSS)	Ni (mg/kg TSS)	Cr (mg/kg TSS)	Cu (mg/kg TSS)	Ag (mg/kg TSS)	Pb (mg/kg TSS)
Tartu raw	767.25	0.75	34.23	40.97	209.18	0.10	23.40
Tartu compost	475.37	0.57	19.62	27.55	134.73	0.12	14.75
Metals in Fungi treatment in Tartu raw sludge	64.51	0.10	2.52	3.92	20.63	0.33	1.87

Metals in Fungi for Tartu compost treatment	156.90	0.25	7.87	13.09	69.07	0.68	7.34
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Table 1. Metallic content found in sewage sludge (mg/kg) in the initial state and after treatment using fungi (mg/kg)

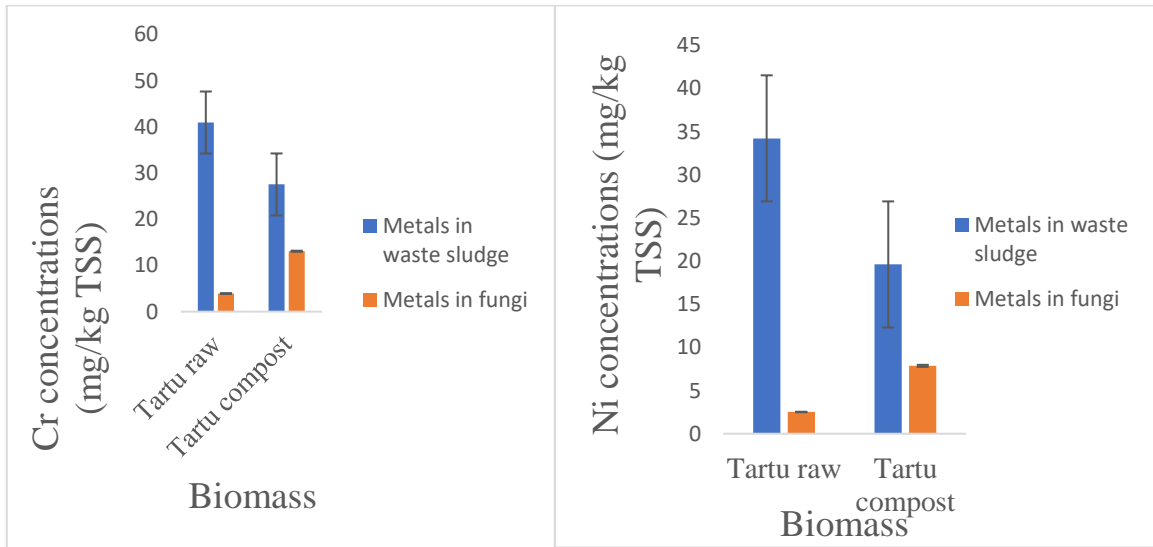


Figure 15a. Cr and Ni content found in sewage sludge (mg/kg) in the initial state and the treatment of sewage sludge using fungi.

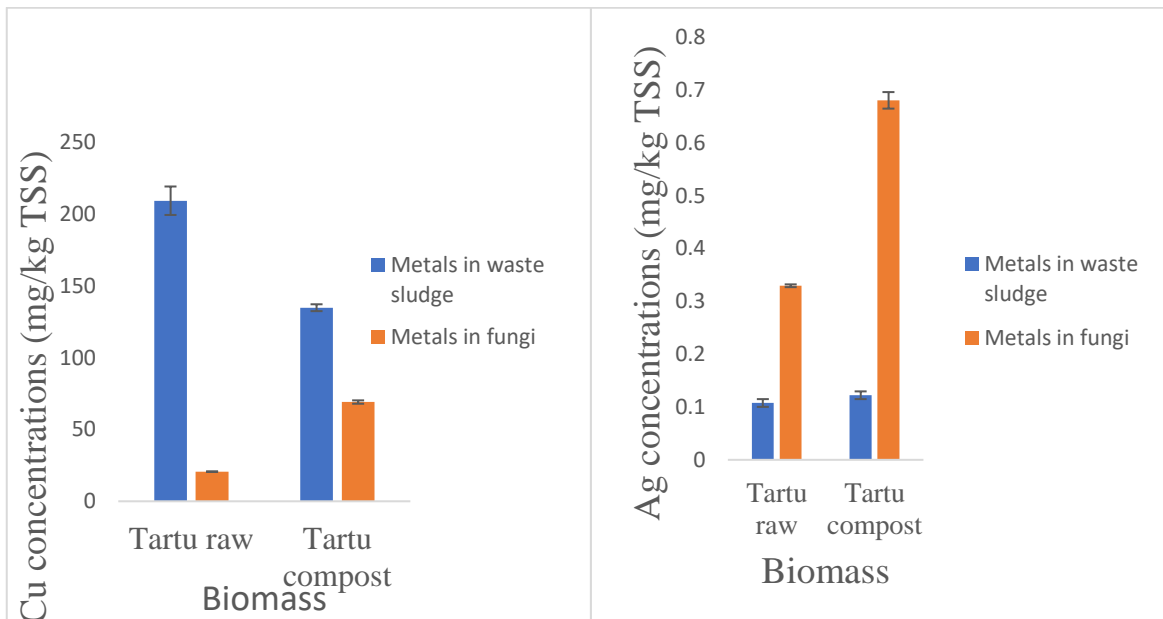


Figure 15b. Cu and Ag content found in sewage sludge (mg/kg) in the initial state and the treatment of sewage sludge using fungi.

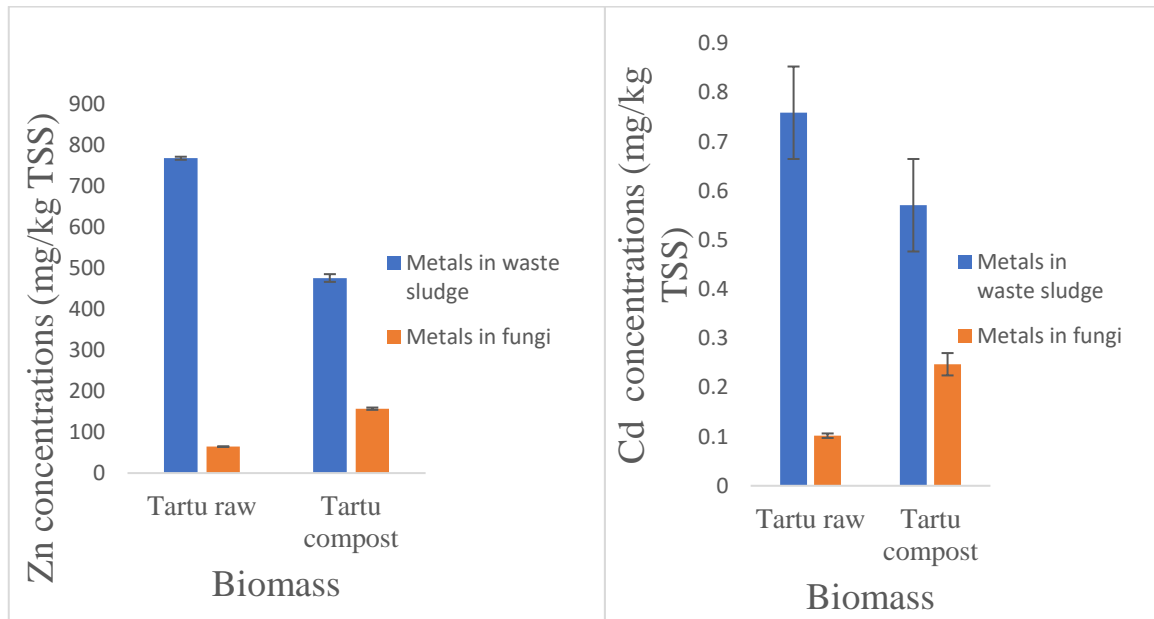


Figure 15c. Zn and Cd content found in sewage sludge (mg/kg) in the initial state and the treatment of sewage sludge using fungi.

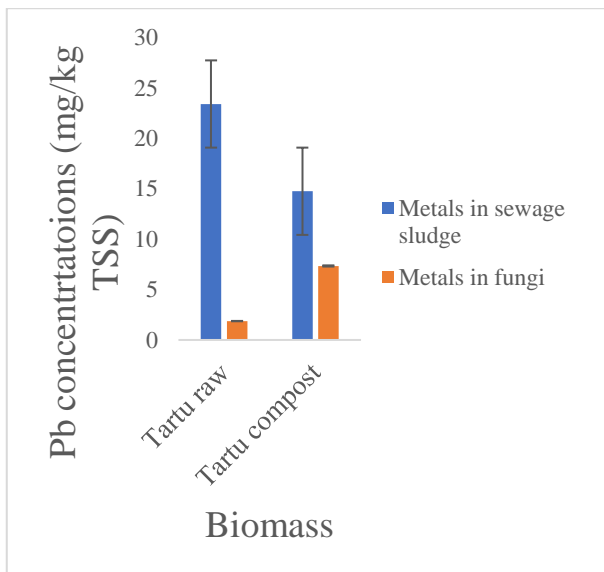


Figure 15d. Pb content found in sewage sludge (mg/kg) in the initial state and the treatment of sewage sludge using fungi.

Excessive concentration of heavy metals disrupts the ecosystem and has significant negative health consequences for animals including humans as can be seen in figure 15a, figure 15b, figure 15c, and figure 15d. However, zinc (Zn) and copper (Cu) has the highest concentration

ranging from 767mg/kg and 209mg/kg respectively, which is related to the research of (Kulbat et al., 2003). The fungi used in extracting the metals from the waste was having the higher amount in zinc and copper compared to the other heavy metals listed (chromium, nickel, lead, cadmium, silver) in the table. The reason was that zinc and copper are both essential in agricultural soil, but when in high concentration it can become toxic and a lower concentration of both metals could lead to deficiency in the environment (Shrivastava & Banerjee, 2015). Heavy metals in sewage sludge not only affect the efficiency of the sludge anaerobic digestion process but also limit the use of treated sewage sludge on the soil. To treat the sewage sludge containing heavy metals a fungi-mediated bioleaching process for simultaneous metal leaching was created (HAMAD et al., 2015).

It can be seen from Table 1 that Tartu raw has the highest concentration of metals compared to others due to its high organic matter and valuable nutrient content. The table is evidence that fungi were able to extract more metals in Tartu compost compared to Tartu raw because composting is mostly practiced in Estonia because it can be used in agriculture as a fertilizer and also helps to reduce soil erosion, which is assisted by the aerobic microorganisms that use organic matter to break down compounds into smaller units (Haiba et al., 2016)

The fungi were able to extract Ag ranging from 68% to 33% compared to the other metals which can be caused by several factors that influence the efficiency of fungal bioleaching such as fungus species, initial pH, temperature, and particle size (Chen & Wang, 2019). It is shown that fungi were able to extract cadmium ranging from 0.25% to 0.10%. The metals such as cadmium and silver show the lowest extraction because of the concentration of toxicity present in the metals.

The concentrations of all analyzed metals in treated sewage with fungi did not exceed the mandatory values set out in the Minister of the Environment's Regulation for sewage discharged to the soil. The accumulation of heavy metals in sewage sludge is one of the

essential measures of sludge quality in terms of its potential use inland farming because of their harmful impact on species and tend to accumulate in the atmosphere.



Figure 16. The result of the sterilized sludge with the combination of sludge, granules, and fungi

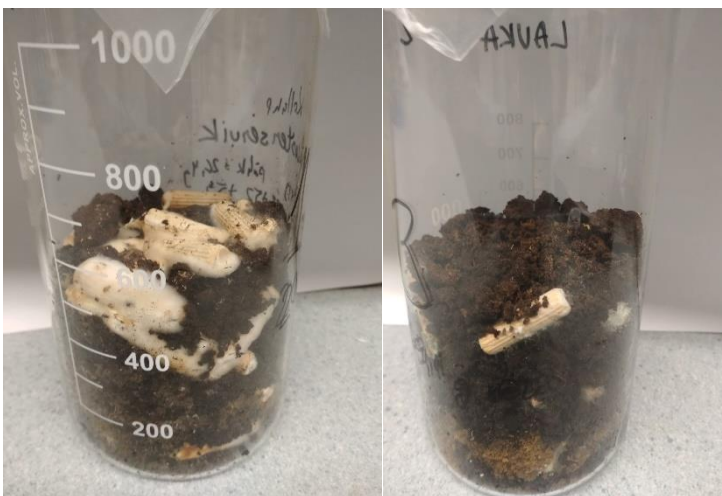


Figure 17. The result of the unsterilized sludge with the combination of sludge, granules, and fungi.

Summary

The sewage sludge is a by-product of the municipal wastewater treatment plant and the number of sludges producing over the years is increasing which needs to be regulated to provide a safer environment. The sludges also contain heavy metals, organic pollutants and are the challenges in which many countries are facing to subsidize its effect when used as a fertilizer in lands for agriculture. Then the aim of this research was able to provide a possible way to eliminate these substances from sewage sludge which are used as a means to develop treatment of sewage sludge from toxic metals, emerging pollutants using biological methods including fungi; secondly how to extract and monitor heavy metals from sludge using fungi.

A sludge study of two Estonian municipal wastewater treatment plants is used to help the practical part and discussion. The main goal of this project is to assess how well fungi treatment in sludge complies with the law. The fungi extraction method used was analytically good in recovering some metals especially in silver compared to the other metals and can be said that these biological methods (fungi) was effective for some substances and not for others and further research need to be taken to check if it depends on the age of the soil or from the climatic conditions. In raw sludge from the wastewater treatment sludge plant, it can be seen that they have higher metals present in them ranging from 200 to 700mg/kg compared to fungi ranging from 5-100 mg/kg.

Reoveesete on olmejäätmete reoveepuhasti kõrvalsaadus ja aastatega sette kogused kasvavad, mida tuleb turvalisema keskkonna tagamiseks reguleerida. Reoveesetted sisaldavad ka raskmetalle, orgaanilisi saasteaineid mis tekitavad probleeme, millega paljud riigid silmitsi seisavad. Reoveesette mõju on võimalik vähendada, kui neid kasutatakse väetisena põllumajanduses. Selle uurimistöö eesmärk oli pakkuda võimalikke viise nende ainete kõrvaldamiseks reoveesetest vahendina toksiliste metallide reoveesette töötlemiseks bioloogiliste meetodite, sealhulgas seente abil. Teiseks uuriti, kuidas setetest raskmetalle eraldada ja akumulierida seentesse. Samuti leiti N ja P ning bioloogilise aktiivsuse vähenemine settes seenekäitluse käigus.

Praktilise osa ja arutelu tarbeks kasutatakse kahe Eesti olmejäätmete reoveepuhasti setteproove. Selle projekti põhieesmärk on hinnata, kui hästi toimub seente abil töötlemine sette korral. Kasutatud seente ekstraheerimise meetod oli analüütiliselt hea mõnede metallide, eriti hõbedas eraldamiseks. Saagised võrreldes teiste metallidega olid head ja võib

öelda, et need bioloogilised meetodid (seenekäitlus) olid metallide jaoks tõhusad ja biogeenide jaoks mitte, ning selleks on vaja täiendavaid uuringuid, et kontrollida, kas see sõltub mulla vanusest või katsetingimustest. Reoveepuhastusjaama toores settes on näha, et neis on kõrgemaid metalle vahemikus 200–700 mg / kg, seentesse õnnestus akumuleerida metalle vahemikus 5–100 mg / kg.

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