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Treatment of Türi sewage sludge from Heavy metals, organic pollutants and metal recovery by fungi

Bachelor's Thesis (12 ECTS)
Curriculum: Science and Technology

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English:

Abstract:

Sewage sludge is a biosolid formed as an end product of wastewater treatment and it is produced worldwide. Sewage sludge contain high amount of metals including the toxic ones. Therefore, an efficient treatment and recycling process is imperative due to the fact that it's loose or unguided application in agriculture and other fields can be hazardous to human, animals and plant lives. Sludges from Türi, a town in Järva County, Estonia were collected. The sludges samples included the raw, one year treated, two years treated, and four years treated. There are different methods currently in use in the treatment of sewage sludge including the use of bacteria. The use of fungi treatment can be employed in the treatment of sludge and recovery of metals and this method proves as a very effective approach. The main objective of this work was to examine the effectiveness of the removal of heavy metals using fungal treatment. The results showed that fungi were able to recover effectively the metals in the sludges as the concentration of metals decreased in the sludge with treatment.

Keywords: wastewater treatment, sewage sludge, fungi, heavy metals.

CERC: T270 Environmental Technology pollution control, T490 Biotechnology, B700 Environmental health

Raskmetallide, orgaaniliste saasteainete ja metallide regenereerimise Türi reoveesette töötlemine seente abil

Abstract:

Reoveesete on reoveepuhastuse lõppsaadusena moodustuv tahke aine ja seda tekib kogu maailmas suurtes kogustes. Reoveesetted sisaldavad suurtes kogustes metalle, sealhulgas keskkonnale mürgiseid metalle. Seetõttu on reoveesette tõhus töötlemisse ja ringlussevõtuprotsess hädavajalik, kuna vastasel korral on selle kasutamine põllumajanduses ja muudes valdkondades ohtlik inimeste tervisele, loomade ja taimede keskkonnale.

Antud lõputöös koguti Eestist Järvamaalt Türilt pärit setteid. Mudaproovid olid värskelt reoveepuhastuse protsessist tulnud reoveesete, üks aasta töödeldud, kaks aastat töödeldud ja neli aastat töödeldud kompost. On erinevaid meetodid, mida praegu kasutatakse reoveesette töötlemisel, sealhulgas bakterite ja seente kasutamine. Kasutades seente abil töötlemist muda käitlemiselja metallide taaskasutamisel võib antud meetod osutuda väga tõhusaks lähenemiseks. Selle töö peamine eesmärk oli uurida raskmetallide ekstraheerimise tõhusus valgehallitusseente abil. Tulemused näitasid, et seened suutsid metalle akumuleerida viljakehasse nin muda tõhusalt stabiliseerida vähendades bioloogilist aktiivsust mudas ning vähendades N ja P ühendeid.

Märksõnad: reoveepuhastus, reoveesette, seened, raskmetallid.

CERC: T270 keskkonnatehnoloogia reostustõrje, T490 biotehnoloogia, B700 keskkonna tervis

Contents

ABBREVIATIONS

EU-European union
WWTP-Waste water treatment plant
SRT- Sludge retention time

INTRODUCTION

Heavy metals as toxic substances are generally hazardous to the health of humans, animals, plants and other living creatures. They are poisonous even at minimal concentrations. These metals are described as heavy metals because they possess high densities or atomic weight. Examples of heavy metals include mercury, zinc,copper,lead, titanium, arsenic and chromium. On the one hand, heavy metals are trace elements needed by man and other living creatures. On the other hand, they can lead to poisoning when taken in high concentrations through water consumption, air intake near emission sources and via food chain. The essential heavy metals exert biochemical and physiological functions in plants and animals. They are important constituents of several key enzymes and play important roles in various oxidation-reduction reactions (WHO/FAO/IAEA.1996). In EU there are some specifications on the concentrations of some heavy metals in the soil.

Where the wastes come from?

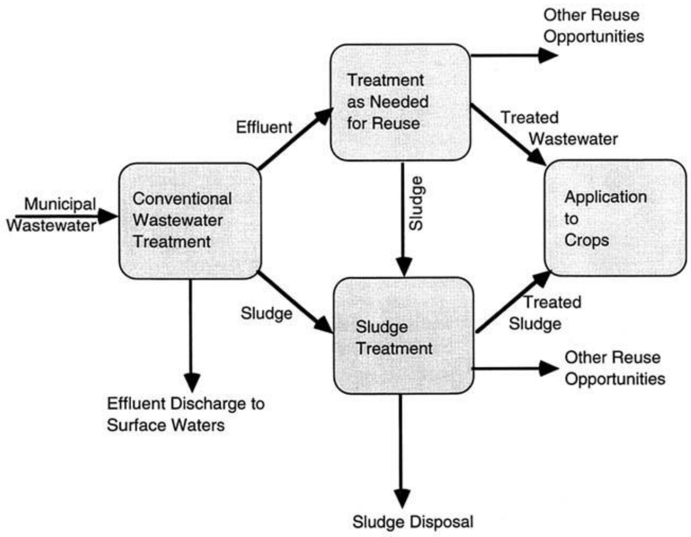

Figure 1 Showing the schemes of wastewater treatment (preliminary,primary and secondary) before further application(Use of reclaimed water and sludge in food crop production, Chapter 3, 1996)

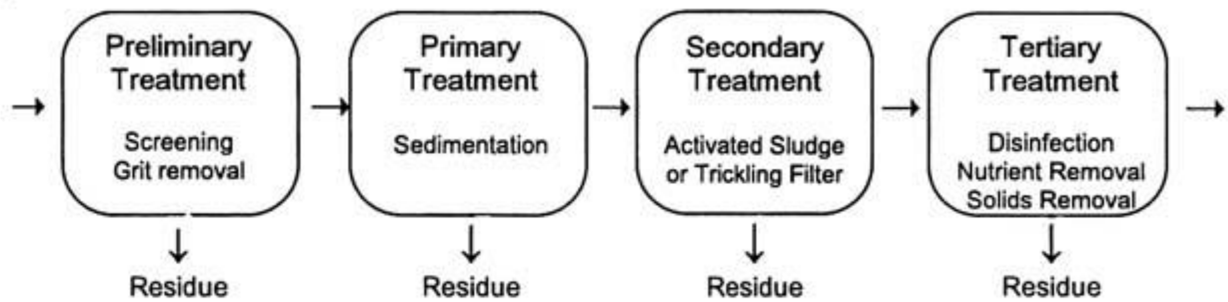

Figure 2: Showing the steps involved in wastewater treatment (Use of reclaimed water and sludge in food crop production, Chapter 3, 1996)

Sludge is a solid material obtained from industrial, domestic, agricultural wastewater and other sources, when the stones or grits have been removed and then stored for a couple of hours in tank. Raw municipal wastewater basically undergoes the preliminary,primary, secondary processes in addition to some other forms of treatment to become a sludge. The initial solids collected in the tank is a raw sludge and can be termed activated sludge. However, if it is left for a long time, anaerobic process can start with the release of a fetid odour. The reuse of wastewater for agricultural purposes such as irrigation is usually possible after secondary wastewater treatment and there could be the need for additional treatment. The sludges can be disposed of by being used in landfill sites or used in nonfood crop agriculture application. Preliminary wastewater treatment is done to prepare the wastewater for further treatment, which has literally no effective change on the quality of the wastewater. In the primary treatment, slightly more than 50% of suspended solids are removed by sedimentation, the residue from this treatment is called the 'primary sludge'. The secondary treatment process is usually a biological process, this is done by using microorganisms to remove biodegradable organic matter. The microorganisms cause aggregation to form a flocculent biomass with the biomass containing pathogens, trace elements and organic compounds. The tertiary process involves disinfection for reduce the pathogenic microorganism population and the concentration of suspended solids in treated effluents can be minimized by filtration and coagulation.The amount of sewage produced industrially is increasing at a fast pace. It was calculated that the total amount of sewage sludge produced in the EU would have reached 13 million tons in 2020. As a result, it is imperative to search for rational methods of its management (Wo1ejko et al. 2014). Several reports have said reports that, due to its properties, sewage sludge can be applied to reclaim biologically degraded soils. However, the toxic metals have to be removed from the sludge before being added to the soil. In the EU there are some specifications on the concentrations of some heavy metals in the soil.

THE AIM OF THE THESIS

- To develop an effective biological treatment method of sewage sludge using fungi
- To study how to extract heavy metals and possibly recover important metals from sewage sludge

1.LITERATURE REVIEW

Sewage sludge contain a high concentration of very important nutrients such as Nitrogen, phosphorus, as a result they can be effective fertilizer to the soil. Land application can also replenish organic carbon stock in the soil and prevent greenhouse gas emission. It is safest to apply it as a fertiliser for industrial or energy crops, as they are not used to produce food (Panasiewicz, K.2019)

Sewage sludge cannot be used for food crops because they can contain heavy metals which can build up in the soil and in the plants and spread in the food chain. Municipal wastewater is a major source of pollution in aquatic environments (Dai, J.2006). The composition of sewage sludge stems from the chemical composition of the influent wastewater and the method of treatment.

At the present, heavy metals are a major source of water and soil pollution. Due to various Industrial activities, majority of companies generate waste waters containing different metals, mainly Zn, Cr, Cu, Ni and Pb. Conducted studies have revealed that high levels of specific heavy metals in streams and soil can be identified due to the activities such as mining societies' effluents, agricultural activities, discharge from industrial parks and sewage works (U.A.S.L. Muthukalum,2020). Heavy metal contamination exists in aqueous wastes of many industries, such as metal plating, mining operations, tanneries, chloro-alkali, radiator manufacturing, smelting, alloy industries and storage batteries industries (Kadirvelu,k.2001).

The concentrations of the five heavy metals (Zn, Fe, Cd, Cu and Pb) in the sewage sludge samples are mostly higher than those in the wastewater and river water samples. This is not unusual because 80–90% of heavy metals in influent water are known to accumulate in sewage sludge (Agoro, M. 2020). It is therefore imperative to reduce the levels of heavy metals to rock bottom. The removal of toxic metals using biological treatments has become the most popular method due to it's cost effective and environmentally friendly methods.

1.The treatment processes

Metals have to be removed before the sludge can be applied to the soil in agriculture.

The interaction between metals and microbes can be described under the following different distinct processes. These bio-processes are as follows: bioremediation, biosorption, bioaccumulation, and bioleaching.

2.Fungi metal leaching and bacterial metal leaching.

Bioleaching involves the recovery of metals using micro-organisms to achieve this. Concentrated solutions of the metals can be obtained from their ores. The microbial extraction of metals is done through leaching by acidophilic sulfur oxidizing and iron-oxidizing bacteria.(Debaraj M,2005).Bioleaching is a process described as "the dissolution of metals from their mineral sources by certain naturally occur microorganisms" or the use of microorganisms to bring about change of form in the elements so that the elements can be extracted from a material when water is filtered through it (C. L. Brierly,1978;D. G. Lundgren,1983). Bioleaching can also be interpreted as the conversion of solid metals into a form in which they dissolve in water by the process of solubilization. The process of solubilization mechanisms provide
a means of metal biorecovery from solid matrices (Xinjin Liang,2017) Metal values are present in the aqueous phase and the remaining solids are discarded (Debaraj, M.2005).*Thiobacillus ferrooxidans* was the first bacteria used in the bioleaching process in 1961(L. U. Salkield, 1987) thereafter different bacteria have been used in the bioleaching process.Therefore, bioleaching is more of an effective bioprocess to recover metals from solid matrices, while a bioprecipitation or biomineralization approach seems to be effective for biorecovery from solution (Xinjin Liang,2017)

Most naturally occurring bacteria and fungi undergo some physiological processes that allows for their growth and proliferation. The effects of bacteria and fungi on minerals are mainly based on three principles, acidolysis, complexolysis, and redoxolysis (Debaraj,M.2005). Bacterial bioleaching using, e.g. Acidithiobacillus spp., is a well-established industrial process for several metals, e.g. Cu, from mineral ore resources (Johnson, 2014). Micro-organisms free metals to move using the following processes: Formation of organic and inorganic acids (proton formation), Excretion of complexing agents (ligand formation), Oxidation and reduction reactions (Debaraj,M.2005). The major inorganic acid produced during the leaching process is sulphuric acid, in addition to some other organic acids. Ferric ion is a very potent oxidizing agent that brings about dissolution of mineral sulfide. It is generated by the oxidation of reduced iron by chemolithotrophic bacteria under a slightly acidic condition.

Specifically, biosorption and bioaccumulation involves the action on heavy metals to remove or reduce the toxic elements in the environment where these metals accumulate or aggregate to the barest minimum. Sorption is a general term used for both the process of absorption and adsorption. Absorption describes the incorporation of a substance in one state into another of a different state e.g liquids being absorbed by a solid or gases being absorbed by water (Gadd GM,1999).

On the other hand, Adsorption is defined as the physical adherence or bonding of ions and molecules onto the surface of another molecule. (Gadd GM,1999).

Biosorption takes place due to the cell wall structure and composition of micro-organisms such as bacteria and fungi. Peptidoglycan carboxyl groups are the main binding site for metal cations in Gram-positive bacterial cell walls with phosphate groups contributing significantly in Gram-negative species (Beveridge TJ,1989; McLean JS,2002). Proteins and polysaccharides are other metal binding components. On the other hand, Fungal cell walls are complex macromolecular structures predominantly consisting of chitins, glucans, mannans and proteins, but also containing other polysaccharides, lipids and pigments, e.g.melanin (Gadd GM,1993;Gadd GM,1980;Gadd GM,1985). Chitin is a very important structural component of fungal cell walls and is an effective biosorbent for metals (Tobin J,1994). There is an avalanche of potential binding sites on fungal phenolic polymer and melanin with oxygen-containing groups including carboxyl, phenolic and alcoholic hydroxyl, carbonyl and methoxyl groups being distinct components for the biosorption process(Gadd GM,1993)

To describe the mechanism of bioleaching, there are 2 forms by which biosorption (bioadsorption) can occur. Microorganisms can oxidize metal sulfides via a direct mechanism where electrons are obtained directly from the reduced minerals (Debaraj, M.2005). This mechanism is described as a direct one because it requires direct contact with the metal surface. The other mechanism referred to as the 'indirect' mechanism, ferric (III) ion is employed in the oxidation of reduced metals. The ferric ion is formed as a result of the oxidation of ferrous ion in the minerals by the bacteria. In the indirect method, no physical contact is required between the bacteria and the mineral surface. It is generally opined that the direct mechanism over-rides the indirect mechanism because the direct mechanism involves direct physical contact of bacteria with the mineral surfaces (T. Gehrke,2005).

3.Biosorption

Specifically, biosorption and bioaccumulation involves the action on heavy metals to remove or reduce the toxic elements in the environment where these metals accumulate or aggregate to the barest minimum. Sorption is a general term used for both the process of absorption and adsorption. Absorption describes the incorporation of a substance in one state into another of a different state (e.g liquids being absorbed by a solid or gases being absorbed by water) (Gadd GM,1999).

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Fungi interact with metals and minerals in both natural and synthetic environments, altering their chemical and physical properties, as regard the metal speciation and mobility, and effecting mineral dissolution and formation through a variety of metal mobilization or immobilization mechanisms (Gadd, 2007;Gadd,2010). Fungi are organisms that produce energy from the oxidation of reduced organic compounds, i.e they are referred to as chemoorganotrophs. Electrons are being transferred from the sulphide or from cuprous copper in the case of Cu2S, involve Fe(II) bound in the cell envelope and exopolymer(C.Pogliani,1999).Fungi carry out the solubilization of insoluble metals by the excretion of organic acids such as oxalic acid and citric acid which reduces the P^H and form complexes around the metals so facilitate the solubility of the metal. In addition, fungi can also facilitate the formation of different kinds of minerals, including oxides, phosphates, carbonates and oxalates, also the formation of elemental forms of metals and metalloids such as Ag, Se and other precious metals such as Au and Pt. Such bio-precipitation largely depends on the organism modifying its local microenvironment to create appropriate physicochemical conditions for precipitation to take place (Xinjin Liang,2017). In comparison of fungi with bacteria, the ability of fungi to grow filaments provides a platform for them to support the mineralization while bacteria have a simple form of growth. Also, the production of reactive culture supernatants facilitates biorecovery/bioprecipitation of metals without the complication of biomass separation (Li et al. 2014). The ability of fungi to degrade organic substrates results in mineralization with the products of the biodegradation reacting with the available metals which is a major step towards biorecovery using precipitation or crystallization. For example, the activity of phosphatase enzymes on Phosphorus containing organic substrates results in the production of inorganic phosphate which can then precipitate

with available metals, as first demonstrated in bacteria (Macaskie et al. 1992, 2000). This process also is observed in fungi.

4.Fungal treatment

PLEOROTUS OSTREATUS

Fungi take more concentration of metals. They possess the ability to bioconcentrate metals and extract the metals.. The precipitation minerals of metals such as carbonates facilitated by microbes such as fungi and bacteria serve as an effective and desirable method for the biorecovery of toxic or valuable metals, e.g. Co and Ni. The metal carbonates are used industrially as precursors for some metal oxides with electrochemical properties e.g Fe_3O_4.

A number of fungi produce oxalates on interacting with a variety of different metals and metal-bearing minerals, e.g. Ca, Cd, Co, Cu, Mg, Mn,Sr, Zn, Ni and Pb (Fomina et al.2005;Gadd et al.2014)

It is a basidiomycete with simple and low production cost. It is also referred to as the Oyster mushroom.The Pleorotus specie is widely cultivated in Asia and Europe. They possess a very effective biological activity. Pleorotus species have the ability to excrete hydrolyzing and oxidizing enzymes which act on organic compounds in agricultural and industrial wastes. They grow quickly within a short period of time. The nutritional sources are mainly carbon, nitrogen with the substrates usually rice, wheat straw, cottonseed hulls, corncob, sugarcane and so on. The growths of diverse type of mushrooms require different type of substrates and availability of varied type of materials may dictate which type is used(S.T Chang,1988). sawdust is the most popular basal material used in substrates to cultivate shiitake mushroom in comparison to the wheat straw and rice or wheat bran.

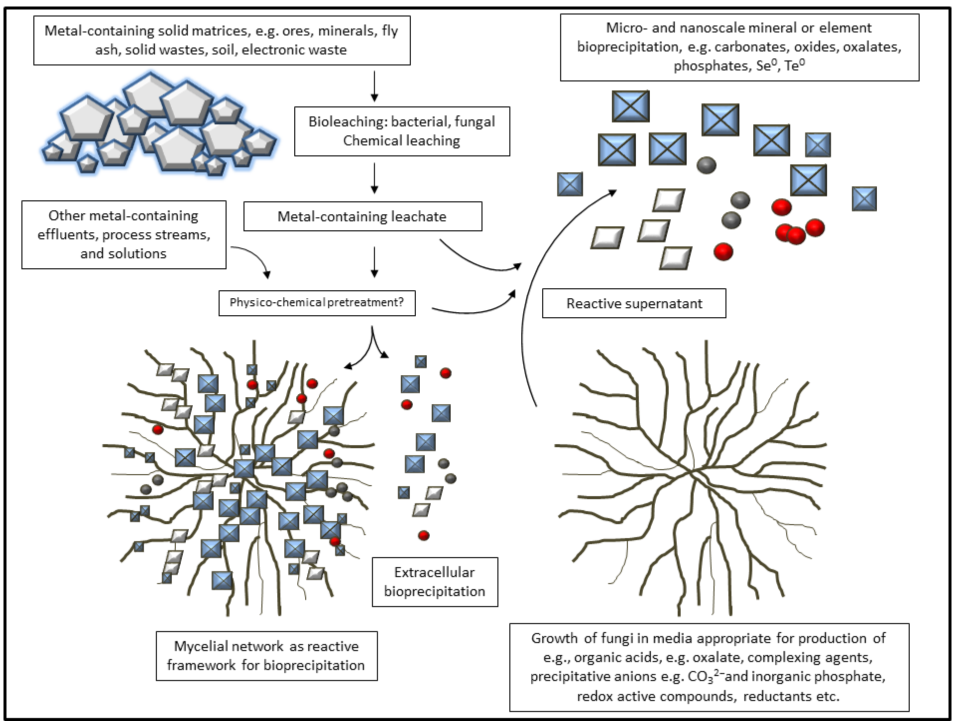


Fig 3: Showing the process of how fungi on contact with metals transform metals, metalloids and mineral in biorecovery with or without physicochemical treatment e.g pH adjustment (Xinjin Liang,2017).

2.Materials and methods

The experiment was carried out in the laboratory. Fruiting body *Pleurotus ostreatus* possess the ability to grow rapidly their mycelium and undergo saprophytic colonization on the starch granules substrate. The sludge was mixed in clean plastic containers with the fungi (*Pleurotus ostreatus*) and starch granules. The conditions is also made humid to allow for their growth.

Setup1: Sewage sludge: 101g

Fungi:101g

Starch granules:104.9g

Setup2: Sewage sludge: 101g

Fungi:101g

Starch granules:104g

1.Sterilization procedure

In the laboratory, all the apparatuses, materials and metallic instruments used were sterilized in an oven to maintain sterility. Sterilization was done for 3 hours under 2 atm pressure at a temperature of 120 ^{o}C.The metallic instruments were also cleaned with soap and also acetone regularly before use. Before inoculation of fungi, laminar airflow was turned on to avoid contamination.

2.Oxitop control system

The main mechanism of this set up is to determine the respiration rates in soil by the process of respirometry.

The continuous measurement of the CO_2 produced by the microbes in the soil is related to the state and activity of the soil. There is a proportionality between the CO_2 produced and the quantity of the degraded organic compounds, it is equally a measure of the rate of biological oxidation. This process also measures differences in the type, age, and concentration of organic contaminants according to the type or nature of soil. This method basically measures pressure. If oxygen is consumed in a closed vessel at a constant temperature, while CO_2 is absorbed by KOH, it leads to the generation of a negative pressure. The Oxitop measuring head measures and stores the pressure data during the whole duration of a measurement once started (Klebercz,2013). The Oxitop device measures the consumption of oxygen as a result of the respiratory activity of aerobic microbes in the sample. The microbes in the soil consume oxygen thereby generating generate CO_2 which is absorbed by the alkali KOH, resulting in negative pressure.

Figure 4: OxiTop OC110 controller (Envirocore,2014)

Figure 5: The measuring head (Envirocore,2014)

3.Determination total suspended solids and soil moisture:

Determination of moisture contents were performed in duplicate (two parallels) per soil sample.

A clean and dry crucible was weighed the mass was recorded

3g of the sludge was weighed into the crucible and the combined mass of the crucible and the sludge was recorded.

The crucible and the sludge were placed in an oven for 24 hours at a temperature of 105^{o} C.

After 24 hours, the crucible was removed from the oven and allowed to cool down.

At room temperature, the crucible and the dry sludge was reweighed.

The moisture content was calculated using this equation:

$$\textbf{\% Moisture} = \frac{(\textbf{Weight of crucible + fresh sludge}) - (\textbf{Weight of crucible + dry slugde})}{\textbf{\textit{Weight of slugde sample}}} \textbf{ x 100}$$

4.Method of determination of phosphate ($PO_4{}^{3-}$)

Prior to the commencement of this process, the dry sludge is being homogenized into fine powder using a mortar and pestle. The homogenized sludge is then transferred into plastic

The determination of phosphate is carried out from a centrifuged or filtered sample.

The graph of spectrophotometer is between 0.1 – 1.0 mg/L, therefore the sample is diluted when required for the spectrophotometer reading within this range. Preferably, the concentration of phosphate in the sample should be close to 0.5mg/L

5. ICP determination of metal content in the sludge

Induced coupled plasma (ICP) spectrometry is now the preferred method to determine metal content in the soil. This is because of it's fast measurement and it's efficiency to simultaneously determine the main part of the macro-and microelements, contained in soils and plants when compared to other methods such as colorimetric method. ICP-MS (inductively coupled plasma-mass-spectrometry) is a unique technique to measure low-concentrations (range: ppb = parts per billion = µg/l) and ultra-low-concentrations of elements. Atomic elements become ionized by passing through a plasma source. The ions are then sorted out based on their masses.

6.The principle:

The solution of the sample is introduced into the device by means of the peristaltic pump, where it becomes nebulized in a spray chamber. The aerosol that is generated is then passed down into an argon plasma with a temperature of about 7000K. A small portion of the ions generated in the torch migrate to the mass-spectrometer part.

The mass-spectrometer part consists of:

- The skimmer cone: This is the part where the small ions generated by the plasma migrate to.
- Electrostatic lenses: focus (positive) ions onto the entry to the true mass-spectrometer.
- The true mass-spectrometer: it is composed of 4 metal rods which separate the ions based on their mass by a kind of resonance principle.
- An electro-multiplier/detector: It facilitates the signal from one colliding ion so that a measurable pulse is produced.
- A counter: that counts and sorts the pulses and relates them to the corresponding mass.

Sample Preparation:

Samples were acidified (preferably with 1-5 % HNO_3 in order to keep metals in solution). Samples were collected in 12 – 15 ml tubes. The content of salts in samples were kept below 0.2% (=2 g/l).

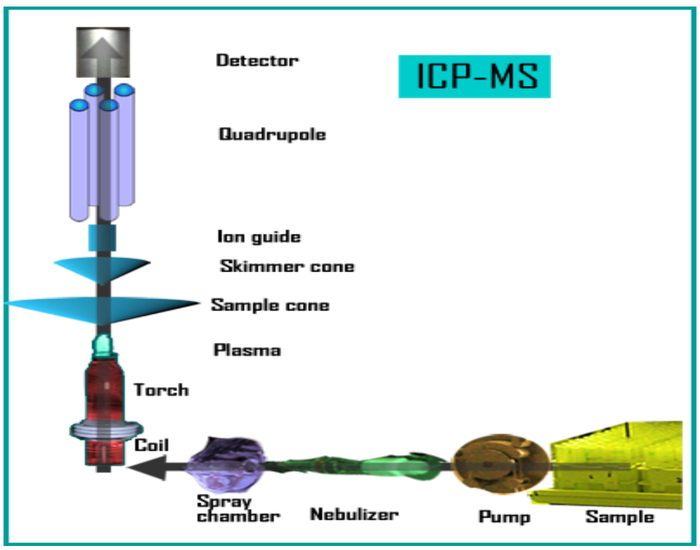


Figure 6: Induced coupled plasma set up (General Instrumentation, ICP-MS,Radbound University)

3.Result and Discussion

1.Metal analysis:

Sludges contain different amount of metals which can be essential for plant growth(Zhang, X.2020) while fungi has the ability to extract metals from sludges by the process of bio-absorption (Gadd GM,1993).An analysis was done to determine the extent at which fungi extract metals in different samples of sludges. The concentration of metals by the sludge at the initial state was found to have reduced after 2 months and 4 months while analysis of the fungi showed that the fungi was able to extract some metals at varying degrees. The result is represented in the table below.

Table 1: Showing the metallic content of the sludge at the initial state and after treatment within fungi in mg/kg Cd: Cadmium, Pb: lead, Cr: Chromium, Ni: Nickel, Cu: Copper, Zn: Zinc

Sludge Samples	Cr (mg/kg TSS)	Ni (mg/kg TSS)	Cu (mg/kg TSS)	Zn (mg/kg TSS)	Ag (mg/kg TSS)	Cd (mg/kg TSS)	Pb (mg/kg TSS)
Turi Raw	21.59	19.12	153.40	692.08	0.59	1.13	14.33
Turi 1 year	30.52	26.70	212.41	908.97	0.54	1.95	18.55
Turi 2 years	32.84	29.66	264.10	1235.77	0.51	1.84	21.92
Turi 4 years	30.35	29.81	242.91	1382.44	2.07	2.43	24.70
Metal in Fungi Turi Raw	8.56	6.12	35.78	154.22	0.38	0.32	4.22
Metal in Fungi Turi 1 year	9.82	6.80	62.36	275.87	0.72	0.63	6.88
Metal in Fungi Turi 2 years	4.20	3.66	41.02	169.93	0.32	0.22	2.11
Metal in Fungi Turi 4 years	9.05	7.71	76.29	428.20	0.83	0.63	5.74

The most abundant metals in the sludge samples were Cu, Pb and Zn while Ag and Cd are the least abundant. These observations are in concordance with the results from other research works (Álvarez, E.A et al. 2002;Turek et al. 2019). It is worthy of note that Cd and Hg (in excess

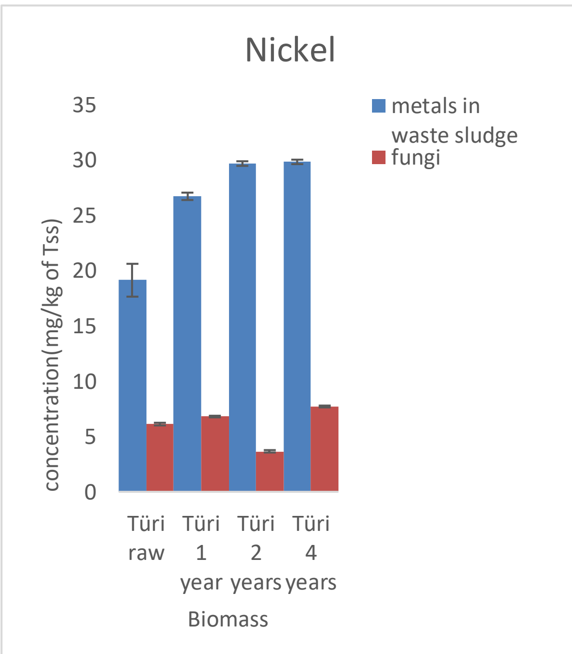
concentrations,above 10-35µg/L)) are highly toxic, mainly to humans and animals (and are less toxic to plants), while Zn and Cu inversely (Tiruneh,2014). It is imperative to note this because the main study of the concentration of sewage is due to its agricultural use. The toxic metals in the sludges did not exceed the permissible standards for sewage sludge in Estonia and EU (Final Implementation Report for Directive 86/278/EEC on Sewage Sludge: 2013 – 2015)
The metal removal process in the primary treatment stage is physical and the rate of removal is dependent on the insolubility of the metal (i.e. in particulate form) Metal size distribution therefore plays a significant role in influencing metal removal at this treatment stage.

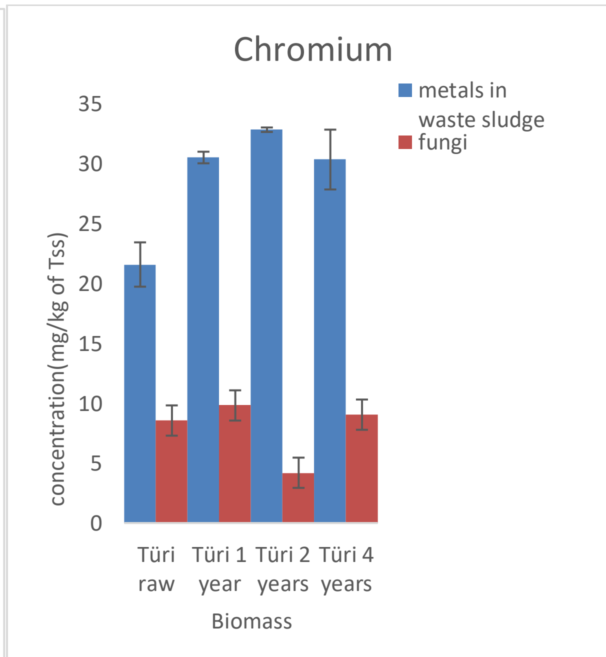
Metals are considered to exist in two size fractions namely the soluble fraction and the particulate fraction. Metals possess different characteristics in wastewater. Cu and Pb exist mainly in the particulate form and their behavior is supported by observations by removal correlations with total suspended solids (TSS) (Wagner-Döbler, 2000).

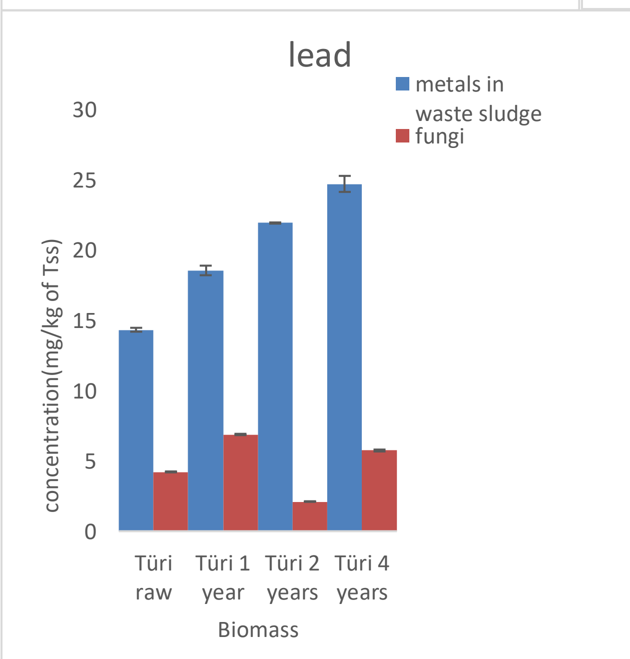
The result showed that four years sludge had the highest content of metals compared to others. It was likely because due to the partial decomposition of the organic matter in the sludge while the metals remained in the sludge. In addition, longer sludge retention time (SRT), diversity of microbial community and the binding availability of metals can also play a part. This is evident because there was higher removal of metals by fungi in the sludges that had higher SRT (i.e 1 year, 2 years and 4 years) than the raw sludges.

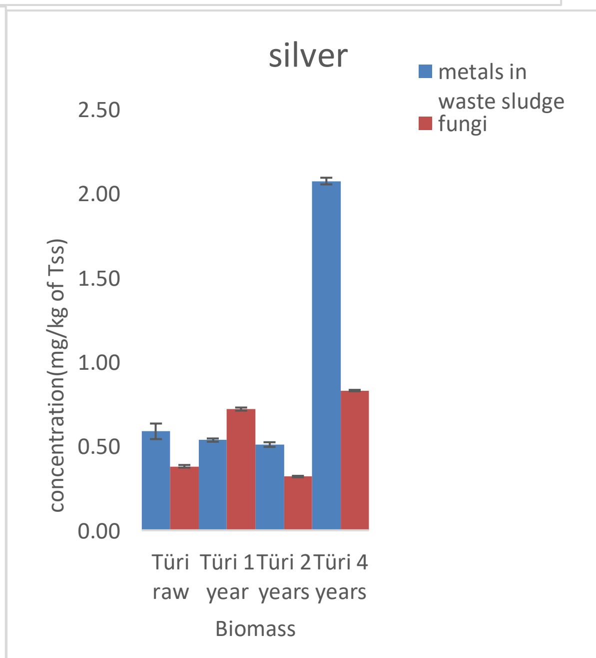
Microorganisms involved in metal removal include fungi, bacteria and algae. Fungi are considered as the most desirable because metals can move to the fruiting bodies of fungi. The fruiting bodies can be removed and the metals contained in them. Also, fungal biomass possess a high percentage of cell-wall material, which increases the variety of functional groups involved in metal binding (G.M. Gadd,1990; Paknikar, KM.1993) and thus increases the metal sequestration ability of fungi. The removal of different metals was substantially different in the sludge samples. The difference in the removal of metals can be due to metal species, surface complexation and ion exchange removal pathways (Andrew J,2018).
In most of the sludges, the fungi extracted the metals the highest in the Turi 4 years except for Cr and Pb. Also, the fungi were able to extract a large portion of the heavy metals such as Zinc and copper from the sludge. Mostly especially Zinc in Turi 4 years with a huge value of 1382.44

mg/kg TSS the fungi extracted about one-third of the metal with a value of 428.82 mg/kg. However, the percentage removal is lower when compared to silver and cadmium which can be due to the toxicity of the metal. From the sludge, the metal components values showed some decrease in values to 2.29mg/kg retained in the four years sludge (data not shown) while the fungi extracted 0.63mg/kg. In addition, mostly fungi in two years sample sludges had the lowest accumulation of metals, the reason for this is quite unknown.









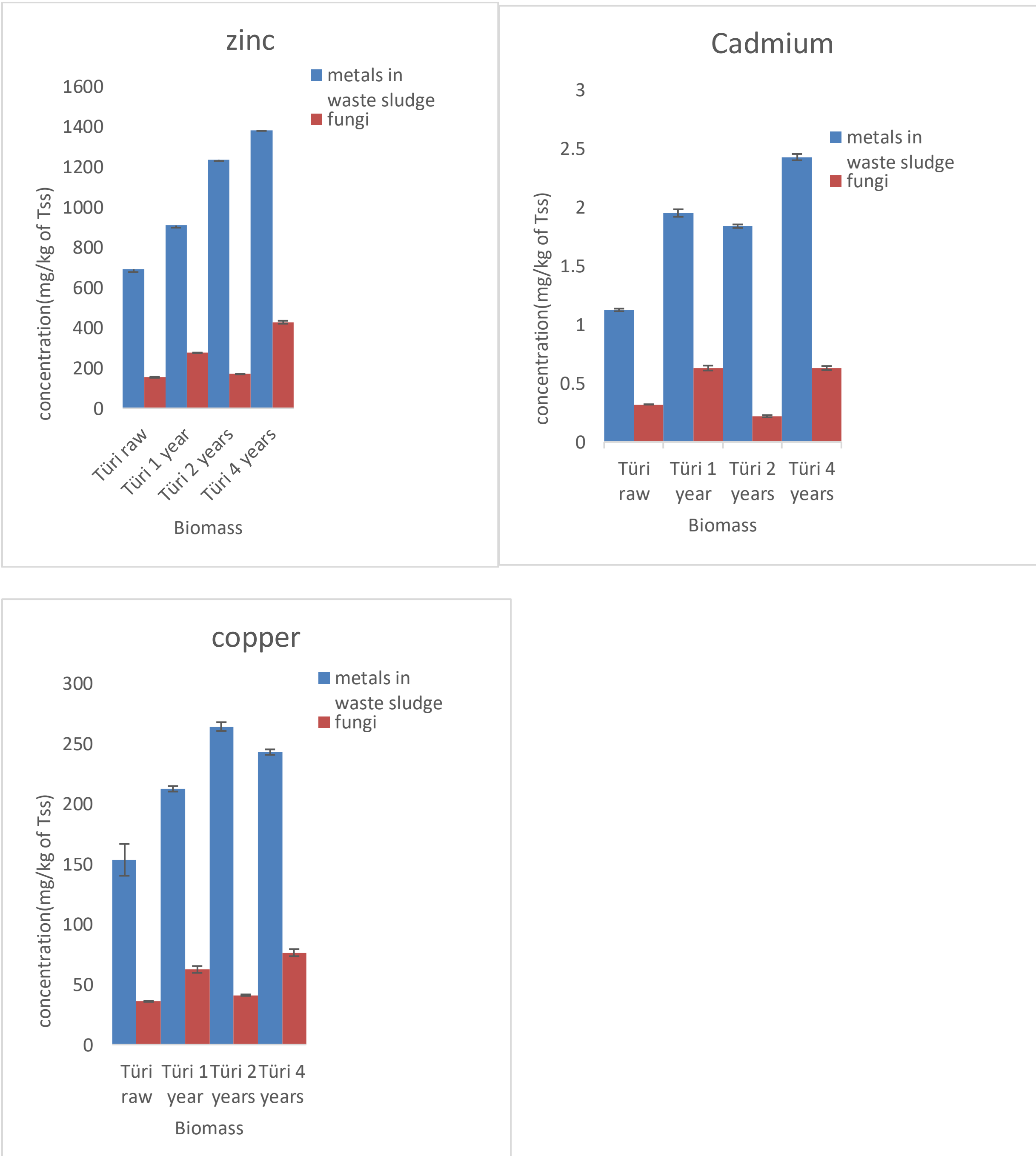


Figure 7: Metal concentrations of different metals in the sludges and those recovered in the fungi

2. Biological Activities in the sludge samples

This experiment was done in unsterile and sterile state to determine the correlation between respiratory activity.The respiratory activities were then plotted on a histogram for comparison.. Turi four years has been shown to contain the highest amount of metals.It is majorly due to the exhaustion of easily biodegradable organic matter and it could be slightly possible due to the fact that increased metal content might have exerted some inhibitory effect on the biological activity of older sludge while in fresh sludge the biodegradable organic matter is higher than that in old sludge. Therefore, the test on biological activities corroborates the fact that increase in toxicity results into reduction in biological activities(Figure 8).

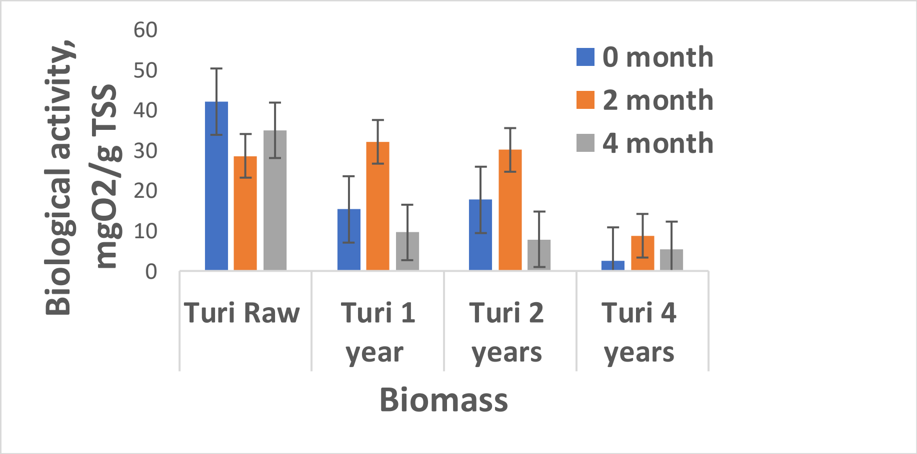


Figure 8: showing the biological activities of different samples of sludges for 0,2 and 4 months unsterilized.

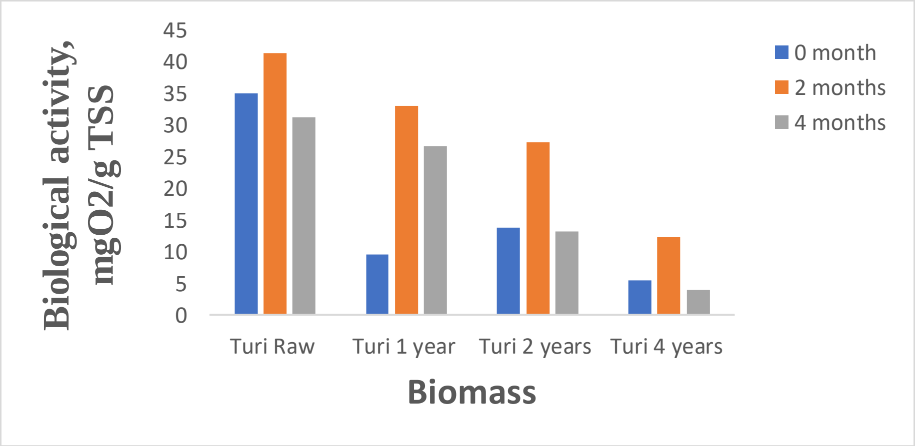
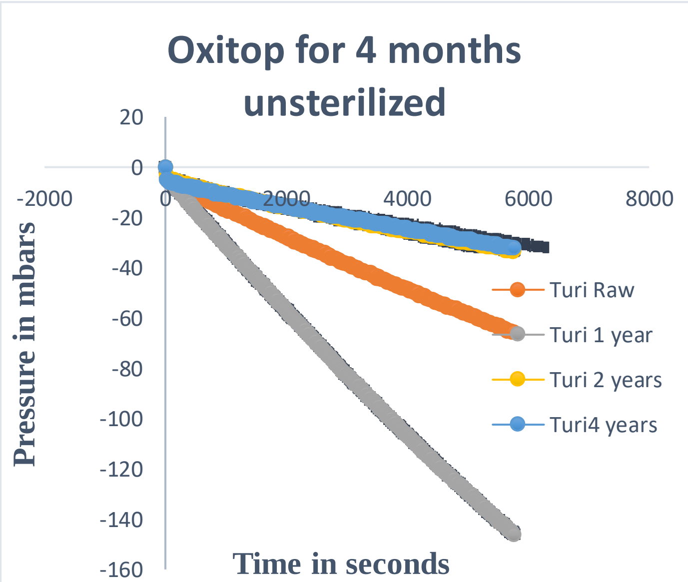
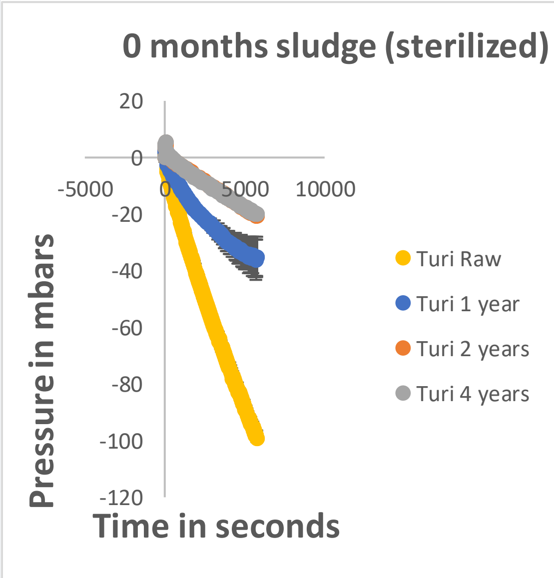
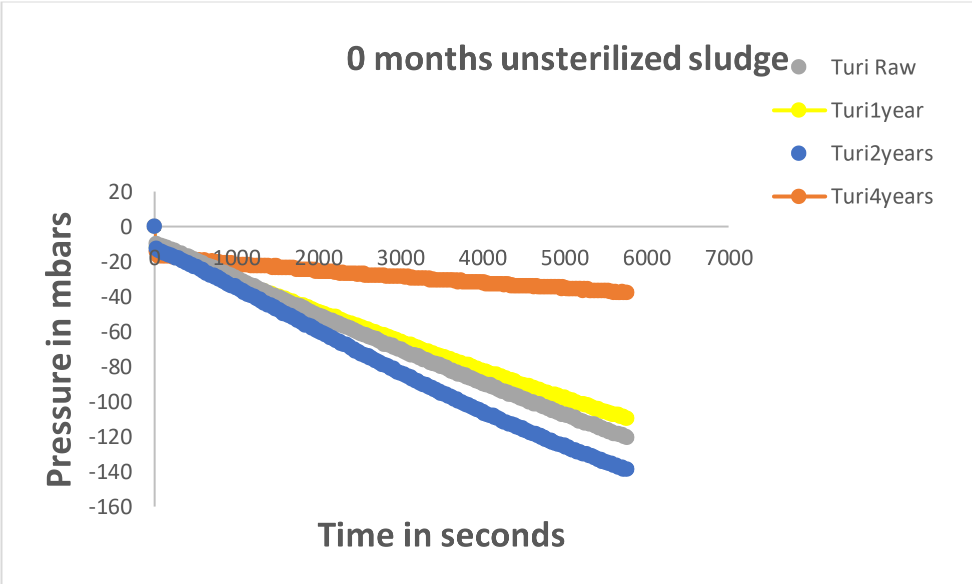


Figure 9: showing the biological activities of different samples of sludges for 0,2 and 4 months sterilized

This result is in tandem with other research works. the respiration of the activated sludge is inhibited in the presence of toxicants. Hence, activated sludge respirometry reduction is a direct indication of toxicity (Tzoris and Hall, 2006). These contaminants are toxic and are difficult to be degraded by activated sludge in a typical industrial wastewater treatment plant (WWTP) (Cai et al. 2010). When wastewater contains toxicants or inhibitors, the oxygen consumption rate of activated sludge decreases. The sludge samples were sterilized to avoid overgrowth of bacteria over fungi. This is because bacterial overgrowth can inhibit the fungal growth. The biomass of bacteria cannot be separated from sludge in the same process like fungal fruiting bodies. The removal of metals is complicated if the bacterial activity is very high. From the data in figure 8, the biological activities were higher in the samples of the sludges . Therefore, the higher biological activities in these sludges i.e Turi 1 year, Turi 2 years and Turi 4 years due to excess bacterial activity was removed by sterilization and this facilitated the ability of fungi to remove the toxic metals present in the sludges, most especially after 4 months treatment.







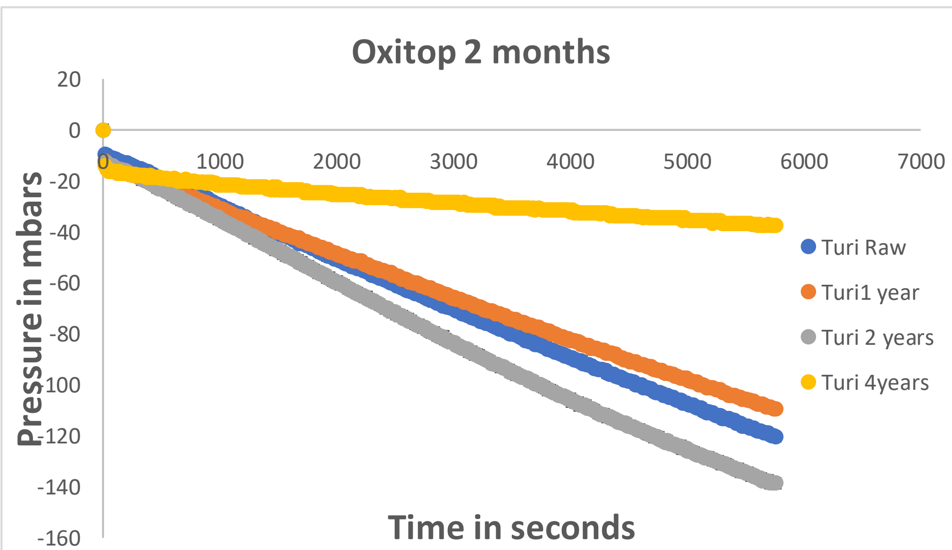
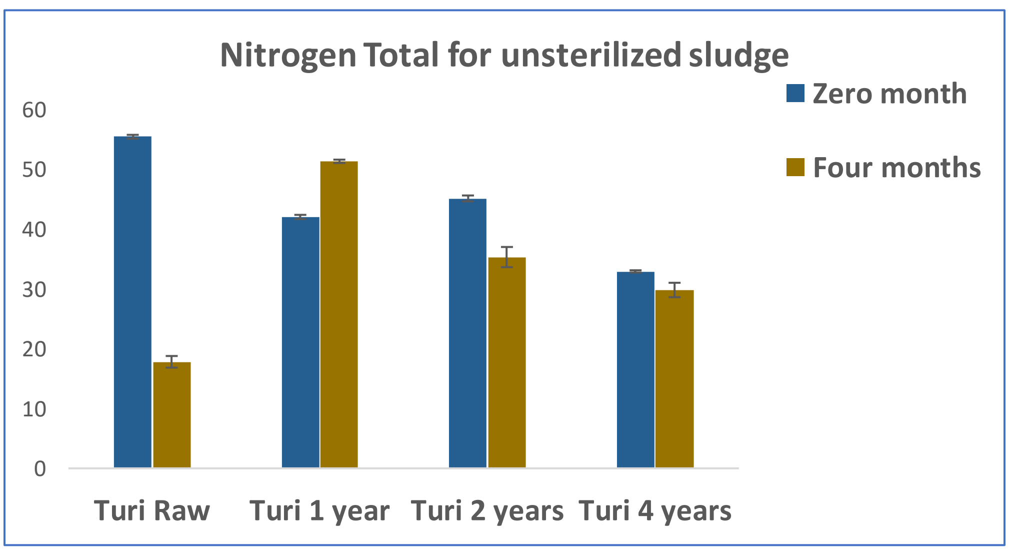


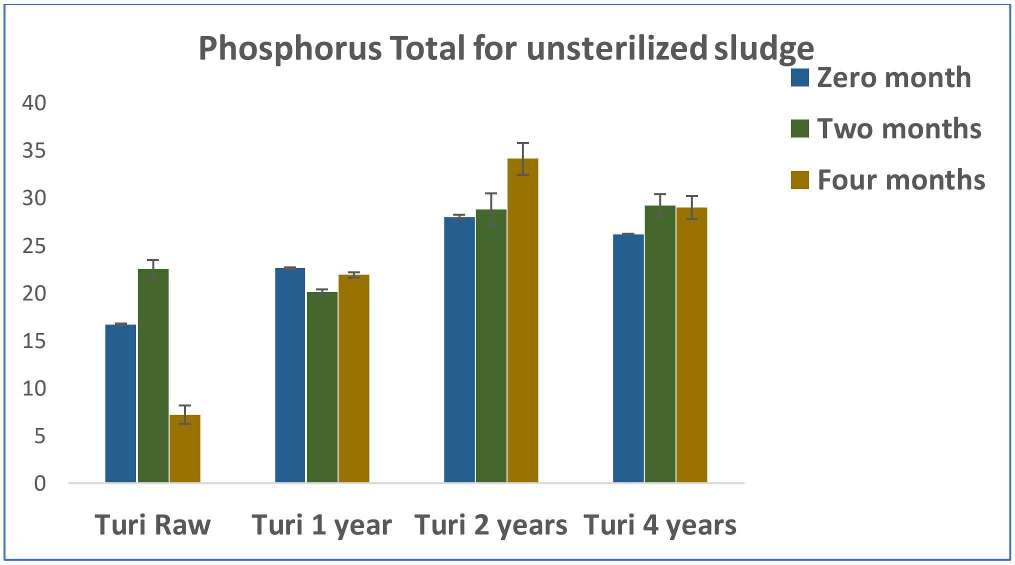
Figure 10: Showing oxitop graphs of different sludge samples in unsterilized and sterilized conditions.

Organic matter influence the physical, chemical and biological characteristics of the soil which include soil structure, water holding capacity and more importantly for provision food and energy for soil microbes. Respirometric test basically describes the state and characteristics of soil by determining the rate of oxygen consumption and carbon di oxide production by microbes in the soil which is related to the extent of degraded organic compounds in the soil. The Oxitop respirometric test result showed that Turi 2 years showed the highest level of respirometry intensity in the unsterilized state (zero month and Two months) except after 4 months.

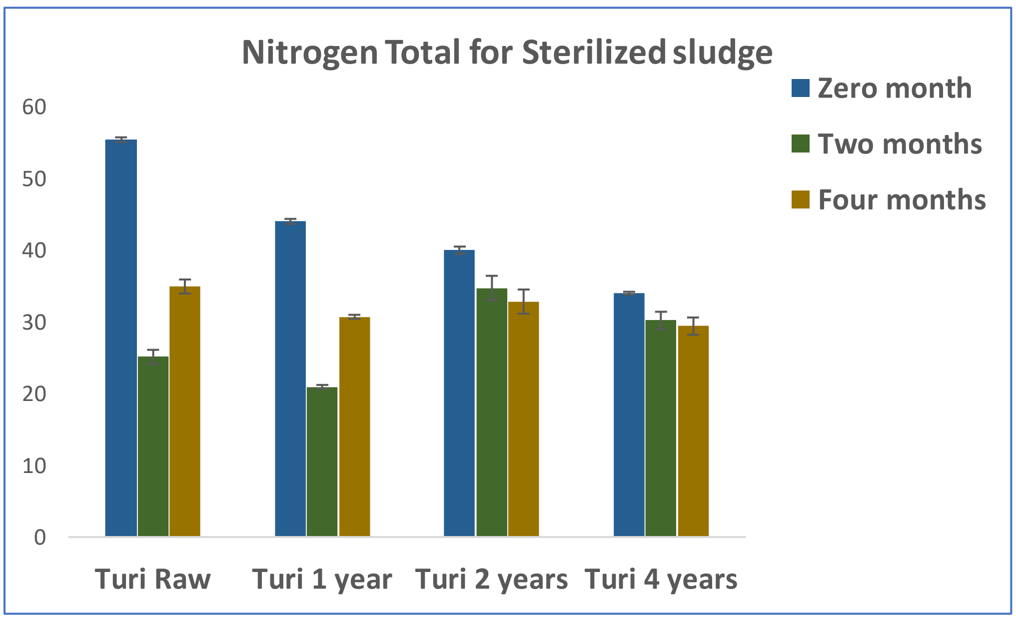
3. Nitrogen and phosphorus contents

Sewage sludge is known to contain a high amount of nutrients such as nitrogen and potassium and organic matter that are beneficial for plant growth and greater yield. Therefore, they are of great interest in agriculture. The fresh sludge contained the highest concentration of nitrogen in both the unsterilized and sterilized sets while four years sludge had the least concentrations. Nitrogen has been proved to be more easily available in non-composted sludge than that in composts which would be in more stable forms during the process of composting. The nitrogen in non-composted sewage sludge is somewhat degradable but after composting, the nitrogen is transformed into a more stabilized residual form, more difficult to decompose (Ciavatta C et al. 2001). Other authors have also observed that the concentration of nitrogen is higher in fresh sludge than in composted ones (Paul JW,1994;Ott P et al. 1983).





Phosphorus Total for unsterilized sludge
Zero month
Two months
Four months
40
35
30
25
20
15
10
5
0
Turi Raw
Turi 1 year
Turi 2 years
Turi 4 years



Nitrogen Total for Sterilized sludge
Zero month
Two months
Four months
60
50
40
30
20
10
0
Turi Raw
Turi 1 year
Turi 2 years
Turi 4 years

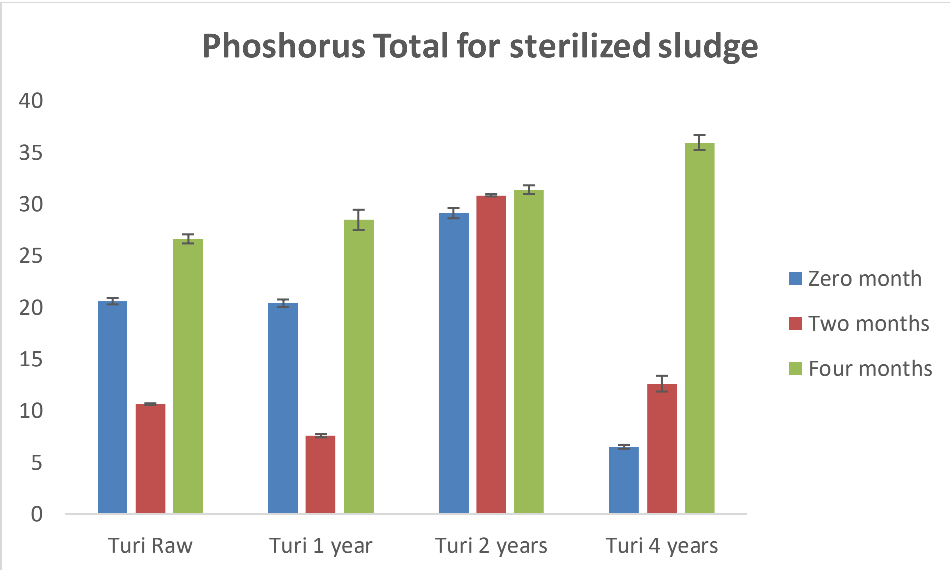


Figure 11: Showing the concentration of different sludge samples in mg/kgTSS

Phosphorus on the other hand,is at the lowest concentration in the one year sludge after 2 months treatment with fungi in both the unsterilized and sterilized states. The four years sludge after 4 months treatment with fungi contained the highest concentration of phosphorus. There was not significant reduction in nitrogen concentration in the Turi four years between 0 months and 4 months which shows that it still possessed it's ability to retain it's nitrogen content . However, there was a massive rise in the phosphorus concentration in the four years sludge from 0 month to 4 months. The reason for the increase is likely because of degradation of easily biodegradable organic matter. In terms of agricultural purposes, this is worthy of consideration, that caution needs to be taken in the application of the sludge to the soil. The Water Environment Research Foundation (1993) has recommended soil phosphorus levels be monitored where sludge applications are used continuously over time, and the rate of sludge application needs to be determined by crop phosphorus levels rather than the nitrogen needs of the crop.

In conclusion, Sludge retention time(SRT) plays a crucial role in sludges having a high concentration of metals and prolonged treatment of sludge with fungi facilitates the removal of metals, most especially the toxic ones from the sludge. However, considering application in agriculture there is a caveat. Sludges with a high SRT and prolonged treatment with fungi can possess a high concentration of phosphorus. Also, fungi removed some metals more than others.

This indicates that there is a possibility that fungi have affinity for some metals than others. Location and industrial activities play a significant role as there can be different concentrations of metals in different cities and towns due to varying industrial activities. Therefore, a lot of research still needs to be done to ascertain the possibility of preference of removal of metals by fungi using other species of fungi and longer treatment period.

SUMMARY

Biochemical treatment of sewage sludge using fungi proved to be suitable and efficient in metal recovery. The longer the longer the sludge treatment of the sludges with fungi, the more the fungi is able to recover metals from the sludges as it was observed that there was also decrease in biological activities, phosphorus and nitrogen concentrations in the sludges. However, more research still need to be done but the future of this method looks promising and provides a platform for a more resource efficient process than the current conventional methods employed in the treatment of sewage sludge.

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