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Shooting Range Contamination: Effect of Trace Elements on Photosystem II on Peat Moss (Sphagnum)

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Contents

Abstract

Shooting ranges in Norway deposit large amounts of trace metals to the environment mainly by leaching out metal ions that accumulate over time. Trace metals are potentially toxic to many plants and animals and even metals that are nutrients can be toxic at high enough concentrations. Many shooting ranges are placed in peatlands and therefore peat moss growing there are exposed to higher levels of trace metals than normal. Peat mosses have an affinity for binding metals, which may be harmful to the moss at high concentrations and can reduce their capacity for growth and reproduction and threaten peatlands as a habitat. Peat moss of the genus Sphagnum. This paper aims to examine the effects of metal contamination on the genus *Sphagnum* from Steinsjøen shooting range in eastern Norway by using chlorophyll fluorescence, a technique which measures light emitted by plants at a certain wavelength as an indirect measure of photosynthetic capacity. When plants experience stress or damage it usually occurs in the protein complex of photosystem II and thus chlorophyll fluorescence may register harmful effects on peat moss. In combination with analysing *Sphagnum* moss from a shooting range, this paper also examines the effect of four trace elements Sb, Pb, Cu, and Zn on *Sphagnum medium* in separate solutions ranging from 0 µM up to 1000µM for each element, and how that effects photosynthesis by using chlorophyll fluorescence. Results suggest that peat moss from the shooting range is not experiencing any stress or damage to photosystem II when using the chlorophyll fluorescence parameter Fv/Fm. Most samples lie within moderately high Fv/Fm values for peat moss at 0.6-0.7 and most contaminated samples were not significantly different than the uncontaminated control when using chlorophyll fluorescence. Exposing *Sphagnum medium* to Sb, Pb, Cu, Zn and their effects on photosynthesis could not be determined due to other factors significantly contributing to the observed Fv/Fm values. However, the results from Steinsjøen shooting range seem to indicate the peat is able to handle the local metal contamination which could support attempts for non-invasive methods of metal removal and filtration and help give renewed focus on regrowth and restoration.

1. Introduction

Despite its low population, Norway contains many shooting ranges which see both civil and military use. There are about 1700 active shooting fields out of an estimated 3000 shooting fields that exist in total in Norway (Bolstad, 2015). A country like the USA, which dwarfs Norway in population, has approximately 3000 military and 9000 civil shooting areas (Hockmann et al., 2014). Ammunition from firearms introduce metals to the environment by leaching metals from bullets as well as particulates from gun barrels and gunpowder after a shot is fired.

These metals can accumulate over time and leach into the soil, water or in tissues of plants and animals and can remain in an ecosystem for several centuries (Ackerman, 2011, Shotyk et al., 1998). This holds true even for shooting fields that no longer see active use. As such, metals from shooting ranges can pose a serious health risk to flora and fauna. Many shooting ranges in Norway are placed in peatlands due to their open and mostly flat geography and have historically been viewed as areas of little value. Bryophytes like peat moss, has a large capacity to adsorb ions and metallic compounds, and they create anaerobic conditions with low pH which generally leads to higher adsorption of metals and a very slow rate of decomposition of organic matter (Flatberg, 2013, Kalmykova et al., 2007). Studies have shown that *Sphagnum* sp. is quite an efficient adsorbent and has several proton-binding sites on its surface (0.65 mmol g^{-1}) (Gonzales & Pokrovsky, 2014). Because of this, peatlands could be more susceptible to metal pollution as they take in more metals coming from spent ammunition. In shooting areas that are no longer in use, restoring the area and removing local metal pollution is desirable in order to maintain an areas biodiversity and ecological function.

However, there are many challenges involved in peatland restoration. Remediation of peatlands is associated with high economic cost and can cost up to 5-10 million Norwegian kroner depending on the size and complexity of the field (Bolstad, 2015). Contaminated soil is usually removed and deposited at an approved waste site, but there are restrictions on organic matter in Norwegian law (Directive FOR-2004-06-01-930), which makes peat not suitable for this method as there a large volumes of contaminated matter that need to be deposited (Mariussen et al., 2017, Strømseng 2014,). Additionally, removal of peat is linked with high emissions of greenhouse gasses as peatlands is a large storage of carbon (Flatberg, 2013) and they also serve a vital role in maintaining biodiversity, which restoration efforts would permanently alter.

One possible option is to minimize intervention in the area and focus on revegetation and reestablishing close to natural conditions. Run-off water has been effectively treated with sorbents that filter out metals (Okkenhaug et al., 2017) and similar methods may be deployed in shooting areas to limit contamination spreading to other areas. This is ideal if the goal is to keep the peatland as is and not for any other development like housing, agriculture or industry where much stricter regulations regarding contamination would be required. It is very much possible for peatmoss to live with higher-than-normal metal concentrations and as such some peatlands with decommissioned shooting ranges may require much less removal of contaminated peat than has been done in the past. Methods to ascertain the health and vitality of plant life growing in contaminated peatlands could prove important in assessing the damage caused by metal pollution and developing a strategy for remediation.

1.1 Background

According to the Norwegian Defense Research Establishment (FFI), in 2023 the Norwegian army reportedly used 17 384 762 individual units of ammunition across 62 shooting- and training fields and 520 shooting ranges (Lausand et al. 2023). Ammunition in this context includes bullets, grenades, artillery shells, tank shells, mines and even signal flares (Lausand et al. 2023). However, the great majority came from small arms fire using 5.56 mm bullets, but 9 mm and 7.62 mm bullets were also common (Lausand et al. 2023). Between 1970 and 2005 Norway used 700 t of ammunition containing mainly Pb and Cu, per year (Okkenhaug et al., 2017) and in 2010 Pb from ammunition contributed to 55% of lead pollution in Norway (Bolstad, 2015). While some metals function as essential micronutrients, they can become toxic as high concentrations (Singh et al., 2022). Metal pollution can lead to reduced immune system, disease, organ and tissue damage, oxidative damage, hormone disruption, reduced growth and many more (Zamora-Ledezma et al., 2021). Some metals are toxic to plants even at low concentrations (Vardhan et al., 2019). As metals accumulate in the environment, they can be a source of stress to plants and can affect the overall health of an ecosystem. As stated, several shooting fields are in peatlands, but not much is known about what affect that has on the peat moss growing there.

One way of assessing the vitality of plants is by measuring the state of photosynthesis, as one of the earliest signs of stress in plants tend to show up in ways that hinder photosynthesis (Maxwell and Johnson, 2000). One way this can be achieved is by using chlorophyll fluorescence. Chlorophyll fluorescence provides an indirect measure of how well plants utilize light energy for photosynthesis. By shining light at a given wavelength at a leaf, a small amount light that cannot be seen by humans will be emitted as chlorophyll fluorescence which can be captured and measured by a camera. This emitted light can provide useful information about photosynthetic activity and performance (Chen et al., 2019) and is a fast, non-invasive and easy to use method to acquire information and there are numerous chlorophyll fluorescence parameters that are used. This paper focuses on the F_v/F_m parameter, which is sensitive to plant stress and gives a good indicator of the maximum quantum yield that is produced by photosystem II (Murchie & Lawson, 2013). F_v/F_m gives a value between 0 and 1 and in most plants under optimal conditions reach an F_v/F_m value of approximately 0.83 (Murchie & Lawson, 2013). For unstressed bryophytes, the values are somewhat lower of around 0.75 (Grau-Andrés et al., 2017).

1.2 Objectives

The aim of this paper is to compare an observational study done on peat moss from shooting areas where a cocktail of different metals may contribute to observed Fv/Fm values with and experimental study, where four different metals are introduced to nonpolluted peat moss in varying concentrations to examine if one metal affects Fv/Fm values more than another. This paper used light-adapted moss when measuring the fluorescence of samples from shooting areas and dark-adapted moss from samples in the experiment. Both light-adapted and dark-adapted moss give insight into the efficiency of PSII by measuring Fv/Fm values. Relevant to this paper is exploring comparative effects of heavy metals on photosynthesis.

- How does lead zinc, copper, antimony affect peat moss and photosynthetic activity?

- How does the viability of different species of peat mosses respond to Pb, Sb, Zn, Cu. Combining an observational study with an experimental study of natural conditions

with multiple heavy metals versus natural conditions with only one heavy metal at a time with different concentration levels.

2. Theory

2.1 Shooting ranges and contamination

In shooting ranges, firearms can introduce metals to the environment via gunshot residue or GSR which is the particles that are expelled from the barrel of a gun after a gunshot (Shukla et al, 2023). Other than the gunpowder, the residue can be traces of the barrel, bullet or the casing or any other particle that is expelled from the muzzle when a gun is fired because of a highly pressurized explosion (Warlow, 2012). The size of a GSR particulate can range from 0.3 − 8.10 μm in size (Shukla et al. 2023). Depending on what type of shot is used, the extent of metal pollution can be different between shooting ranges. Bullets from rifles have been shown to accumulate in natural barriers (Sorvari, 2007), and when hitting the ground, penetrating the soil causes abrasion between sandy particles within the soil and the bullet which leads to an immediate release of metals (Sorvari, 2007). There exists a degree of traceability of rifle shots through sandy soils and rifles tend be concentrated on a smaller area (Sorvari, 2007). Others, such as shotguns spread their ammunition over a wide area and can contaminate an area up to ten hectares (Sorvari, 2007).

Bullet casings and other metal objects present in water or soil may also corrode over time and leak metal ions into their surrounding environment (Sorvari, 2007) (Strømseng et al. 2009). The discharge of heavy metals to the environment can pose a serious health risk to animals and plants as well as for humans. In plants specifically, toxic metals can serve as a source of stress and thus can harm a plants ability to grow and reproduce. Toxic metals can disrupt normal metabolic functions by causing oxidative stress (Kupper & Andresen, 2016) and are able to harm a plants' ability to perform photosynthesis (Kupper & Andresen, 2016). For example, zinc ions can replace other ions that are less electronegative, like Mg^{2+} that normally bind to chlorophyll (Chl) (Kupper & Andresen, 2016). This [Zn]-Chl combination is less stable while in an excited state located in the antenna of the Chl, making the entire photosystem apparatus

less efficient by making it less likely that electrons will be transferred to a reaction centre for photosynthesis (Kupper & Andresen, 2016). In addition to shooting ranges used by the military, public shooting ranges with small arms fire also lead to significant discharge of heavy metals (Ackerman et al. 2009, Okkenhaug et al. 2017, Shukla et al. 2023). The challenge in studying the effects of metal pollution lies in the fact that metals have a broad range of different factors affecting their mobility through the environment and how they interact with other elements and molecules. Topography, precipitation, climate, soil and groundwater properties affect the mobility of metals (Barker et al. 2021, Strømseng et al. 2009). Vegetation plays a part by promoting conditions that favour either sorption or desorption of different metal ions that can be positively charged like Pb or negatively charged metalloids like antimony (Sb) (Barker et al. 2021). The most important factors are likely water saturation and pH (Strømseng et al. 2009), since whether the conditions are acidic or alkaline greatly affect metal corrosion and leakage as well as how metals and metalloids bind to organic matter (Rognerud, 2003, Sorvari, 2007, Strømseng et al. 2009). In peatlands and marshes, the soil and/or water is acidic which can increase leaching, and they contain organic matter with high capacity for binding with metal compounds (Strømseng et al. 2009). Metals often found in conjunction with military activity include Pb, Cu, Cd, Sb, tungsten (W), zinc (Zn), nickel (Ni), chromium (Cr) and arsenic (As) and many others (Shukra et al. 2023, Barker et al. 2010). This paper will be focusing mainly on Pb, Sb, Zn and Cu and are among the most common metals found in shooting ranges from small arms fire in Norway (Strømseng et al. 2009).

2.1.2 Metals in ammunition

Ammunition in small arms fire contain about 60% Pb, 29% Cu and 8% Sb and 3% Zn (Strømseng et al. 2009). Lead has long been used in bullets due to its high density, however it is also soft for a metal (Lausand et al. 2023) and is therefore made with antimony as a hardening agent (Ackerman et al. 2009, Lausand et al. 2023), and a bullet can contain anywhere from 0.7% to 10% of antimony (Barker et.al, 2021). Many bullets are also coated with a copper jacket and cartridges or casings tend to be made of brass (Lausand et al. 2023), which is an alloy of zinc and copper. This gives us the presence of all four of our metallic suspects just from bullets. FFI estimates that in 2023 the Norwegian military released 55 840 kg of Cu, 6 030 kg of Zn, 4 548 kg of Pb and 66 kg of Sb to the environment from ammunition (Lausand et al, 2023). Over time Pb has been phased out and the Norwegian army now uses bullets made of a steel core which is covered with an alloy of Zn and Cu (Mariussen et al., 2017). However, since

complete oxidation of Pb bullets may take 100 to 300 years (Ackerman, 2011), Pb from bullets is still a serious concern.

2.1.3 Behaviour of Sb, Pb, Cu and Zn in the environment

Lead, copper and zinc occur as positively charged cations while antimony is a negatively charged anion (Rognerud & Rustadbakken, 2006). Since peatmoss creates peat, which contains larges amount humic and fulvic acids, adsorption of cations is greatly favoured (Mariussen et al., 2017). Lead has long been known to be a toxic element, and high levels of lead are commonly found in leaching from shooting ranges. In mires the Pb^{2+}-ions bind easily to peat under acidic conditions (Kalmykova et al., 2008) and is strongly attached to organic matter. While Pb tend to be the main pollutant in shooting ranges, Sb is a more toxic metal and is potentially dangerous even at relatively low concentrations (Evangelou et al, 2012). Sb is more soluble than the other metals at higher pH levels, near neutral pH (Evangelou et al, 2012), and antimony can form oxyanions which makes Sb quite mobile and can be transported quickly through soil and water (Sorvari et al., 2006). Cu differs from the other cations Pb and Zn by often forming inner-sphere complexes (Mariussen et al., 2017), binding directly to a surface. Zinc is considered less dangerous than the other trace elements discussed and Zn concentrations is not generally found in nature (Vardhan et al., 2019). Damage done by zinc resembles chlorosis done by iron deficiency but sorption of Zn ions in peat tends to be lower than other metals which usually has a lower affinity to binding sites as compared to Pb, Cu and Sb (Bucher & Shenk, 1999).

2.2 Life cycle and morphology of peat moss

Peat moss are non-aquatic land-based organisms, yet they live and thrive in very wet environments and are dependent on water as a medium to reproduce. The peat moss life cycle consists of two stages; one gametophyte stage which is haploid and one sporophyte stage which is diploid. When an individual moss starts its life, it grows first from single haploid spore. Green and threadlike protonema emerges from the spore accompanied by rhizoids which anchors the protonema to a nearby surface. Out from the protonema grows a green and leafy shoot called a gametophore, which grows to become the most visible and recognizable part of

the plant. The protonema and the gametophore together form the gametophyte. The gametophyte develops egg bearing gametes called archegonium and sperm producing gametes called antheridium. The sperm from the antheridium have two flagella which enable them to swim to reach the eggs in an archegonium. Fertilization leads to the formation of a zygote that develops into a diploid sporophyte. The sporophyte houses spores in a capsule where mitosis occurs, and a great number of haploid spores are made. The spores are eventually released, the sporophyte is discarded, and the sporophyte stage ends (Flatberg, 2013).

Most peat mosses found in Norway are dioecious, meaning individuals usually produce either female or male gametes. The distance traveled by spermatozoids to reach an archegonium tends to be quite short, only a few centimeters (McQueen, 1985, Flatberg, 2013), however they can travel much further depending on the flow of water and wind. From a gametophore grows a central stem with apical growth. Out from the stem in sections called fascicles, grow 2-3 spreading branches out of the steam and 1-3 pending branches (Flatberg, 2013, Michaelis, 2019). The spreading and pending branches have 30-50 small leaves tightly packed together (Flatberg, 2013). Around the stem are also small and ovate stem leaves arranged in a helix (see **Error! Reference source not found.**). At the growth point, stubby and newly developed branches are densely packed together in an area called capitulum, which forms the characteristic head of the moss (Michaelis, 2019). The leaves are a single cell layer thick, lacking a central midrib and consist of two types of cells, namely cells with chlorophyll with engage in photosynthesis, and large empty hyaline cells used for storing water (Flatberg 2013). The hyaline cells are reinforced by bands of fibrils, a cell wall structure which keeps the cells form bursting when water is abundant and retains shape and form when dry. Fibrils have pores in them the regulate flow of water but are reduced or absent in moss that have been submerged (Michaelis, 2019). The stem itself has an outer cortical layer with large empty cells which help with absorbing water and serves as an effective barrier (UCMP, n.d), while the inner layer consists of thin walled and unspecialized cells that are adaptable to a variety of functions such as photosynthesis (FNA n.d., UCMP n.d.).

Figure 1: Kjell Ivar Flatberg, Norges Torvmoser (2013). Typical morphology of Sphagnum centrale (left) with a leaf growing by the stem and a leaf form a branch (right)

2.2.1 Variations in morphology

Morphological variation in peat moss is quite common and can be seen in a vast array of different traits, both to the naked eye and under the microscope (Flatberg, 2013, p.75). Such variations include differences in size and shape, speed of growth, branch orientation, branch density, length of leaves, visibility of the head, colour, and many others (Flatberg, 2013, p.75). Such variations are apparent even between individuals of the same species and make members of the sphagnum genus very challenging to correctly identify (Flatberg, 2013, p.75).

Most of the morphological variation in peat mosses is thought to be due to phenotypic plasticity (Flatberg, 2013, p.76), that is changes and adaptions to an organism's phenotype as a response to its local environment, utilizing the same genotype (Flatberg, 2013, p.76). Therefore, a lot of variation can be found along different gradients. Such as the dry-wet gradient, light-dark gradient, hot-cold gradient and poor-rich gradients (Flatberg, 2013, p.76). However, it is quite difficult to determine whether a single morphological trait is brought on by phenotypic plasticity due to conditions of the local environment or simply genetic variation (Flatberg, 2013, p.78).

2.2.2 *Sphagnum* bioavailability

Peat mosses are well suited as bioindicators due to their ability to easily take in water and absorb the molecules within. Moss can contain as much as 16-26 times their own dry weight as water (Gonzales & Pokrovsky, 2014) and they have a very high capacity for ion exchange

(Flatberg, 2013). This is due to the cell walls containing uronic acids, which harbour various functional groups, like carboxyl and carbonyl groups (Flatberg, 2013, Astolfi et.al, 2024). Uronic acids in cell walls lie within a pectin-like carbohydrate called sphagnan and can be up to 21% of the dry biomass in *Sphagnum fuscum* for example (Flatberg, 2013, p.83). This high capacity for exchanging ions enables peat moss to grow in relatively nutrient poor areas and can survive of of minerals obtained just from rainwater and the atmosphere (Flatberg, 2013). When the moss form layers of peat, other organic acids like fulvic and humic acid become present as the moss breaks down and form active functional groups (Astolfi et.al 2024). This is relevant in the distinction between peat moss such as *Sphagnum* with living cells and the organic matter called peat which are decaying remnants of moss. However, both peat and living peat moss interact with metal ions through chemical processes. The interactions can be sorted into ion exchange, complexation and adsorption (Leiviska et al. 2018). In ion exchange, dissolved cations in surrounding waters are exchanged with ions from sphagnan in the cell walls by releasing H_3O^+ into the environment (Flatberg, 2013, p83) (Leiviska et al. 2018). In complexation reactions, metal ions may form a complex compound, where a central metal or atom is being surrounded by ligands (Leiviska et al. 2018). Adsorption is a mechanism where metal ions can be present on moss directly and happens when metal ions attach to a surface by adhesion and without any exchange of ions or electrons (Brown et al. 2000) (Leiviska et al. 2018). Peat mosses have an affinity for nutrient poor conditions (Flatberg, 2013, p.82), and are known to lower the pH of the surrounding waters, primarily as a consequence of releasing H^+-ions to the environment, as mentioned above. Peatlands which receive most of their water from precipitation, the pH is about 3.5-4.2 (Artsdatabanken, 2016). In peatlands which receive most of their water from groundwater, the pH can be anywhere from 4.0 pH to 8.0 pH (Artsdatabanken, 2016) depending on how nutrient rich the peatland is in calcium. Lowering the pH in water can increase the motility of metal ions through a system and the optimum range for metals uptake by peat is generally around 3.5-6.5 pH (Brown et al. 2000). While the presence of several metals can create competition for the same binding sites limiting the rate of ion exchange, the total capacity for absorption has been found to increase (Brown et al. 2000).

2.3 Chlorophyll fluorescence

Chlorophyll fluorescence is a common method for analysing the state of photosystem II (PSII) and can be observed as a far-red light with wavelengths of 650-800 nm emitted nanoseconds after light is absorbed in photosynthetic tissue (Chen et al., 2019). In short, when light enters a plant and reaches chlorophyll molecules within chloroplasts, the chlorophyll uses light energy to excite electrons into higher unstable states of energy (Kalaji et al., 2016), which are donated to electron carriers in photosystem II (PSII) or in photosystem I (PSI). Ultimately, light energy absorbed by chlorophyll have pathways, one of which is photochemistry primarily under the well know process of photosynthesis, however plants are not 100% efficient in their use of light and not all absorbed light goes to photosynthesis. Light can also be emitted as fluorescence, as well as heat (Maxwell and Johnson, 2000) (Kalaji et al., 2016). The sum of all three pathways, heat, photochemistry and fluorescence, is always equal to 1 and are in competition of each other, such that the increase in one decreases the other two (Maxwell and Johnson, 2000). The yield of fluorescence is measured by exposing photosynthetic tissue to light and analysing fluorescent light being emitted (Maxwell and Johnson 2000). Fluorescence produces light of longer wave lengths than absorbed light, and so the yield of fluorescence can be measured by controlling the wavelength of light a plant is exposed to and measuring the light emitted at longer wavelengths (Maxwell & Johnson, 2000). Even though only a small percentage of the total absorbed energy is emitted as fluorescence (Kalaji et al., 2016), studies have shown that the amount of fluorescence emitted is inversely proportional to the energy that goes to photochemistry as well as inversely proportional to the heat being emitted (Kalaji et al., 2000), meaning that heat emission may also be used during fluorescence experiments as a measure of photosynthetic activity (Kalaji et.al, 2000). Chlorophyll fluorescence also gives insight into how well different plant species utilize light energy efficiently and can be sensitive to changes affecting photosynthetic performance, such as pollution (Chen et.al, 2019).

Fluorescence can be observed when reaction centres in PSII cannot receive further electrons as they are waiting for electrons do be passed along electron carriers along the photosynthetic pathway (Maxwell & Johnson, 2000). PSII enters then a closed state and leads to a reduction in converting light into energy and a corresponding increase in fluorescence (Maxwell and Johnson, 2000). Therefore, chlorophyll fluorescence can be used as an indirect measure of

photosynthesis by measuring how much of the light a plant is able to utilize for electron transport in PSII (Chen et. al, 2019).

When going from darkness to light, reactions centres get progressively closed. (Mazwell & Johnson, 2000). Closed reaction centres leads to a reduction in the efficiency of photochemistry and an increase in fluorescence (Maxwell & Johnson, 2000). For samples used in a dark-adapted state, exposing such samples to light yields a sudden increase in fluorescence in a very short time (Maxwell and Johnson, 2000) (Kautsky et al., 1960). This is the so called Kautsky-effect and explains how when electron carriers, such as plastoquinone (Q_A), accepts an electron, it can no longer accept any more until it has reduced other electron carriers (Q_B) further along PSII (Kautsky et.al 2016). Now the reaction centre is closed and the following decrease in photosynthetic activity turns into an increased yield in fluorescence (Maxwell and Johnson, 2000). This increase is observed during the course of one or two seconds, after which fluorescence decreases and seem to stabilize over time (Maxwell and Johnson, 2000). This is usually due to plants response to light, where they will increase the rate at which electrons are transported in PSII, called photochemical quenching, and an increase in the amount of energy which is converted into heat, which is non-photochemical quenching or NPQ (Maxwell and Johnson, 2000). This response is a mechanism to prevent damage by too much light (Aro et al., 1993) and will in most plant species stabilize after 15-20 minutes (Maxwell & Johnson 1993) where a steady state is attained. This results in measurements that can be used in dark-adapted samples as well as light-adapted samples that have reached a steady state. The difference between dark-adapted and light-adapted samples is such that the emitted light of fluorescence will be different due photochemical and non-photochemical quenching in reaction centres in light-adapted samples, but the efficiency of PSII is still comparable between the different samples, such as with F_v/F_m. F_v/F_m is the maximum efficiency or quantum yield of PSII and has been shown to be a strong indicator of the maximum yield produced by photochemistry (Murchie & Lawson, 2013). It is given by the formula:

$$Fv/\,Fm\; = (Fm - \mathrm{Fo}\,)/Fm$$

Where F_m is the maximum value of fluorescence observed, F_o is the starting point of fluorescence, in other words the minimum value required to observe fluorescence. F_v is the difference between the maximum and starting value (F_m-F_o). Dividing F_v with F_m gives a value between one on zero (Figure 1).

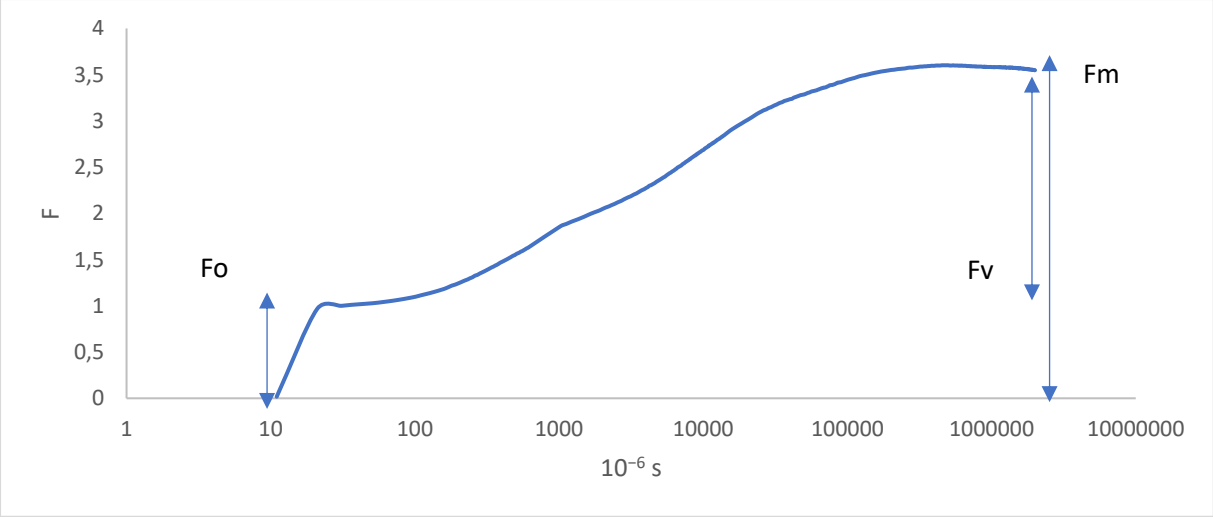


Figure 1: OJIP-curve showing a visual representation of Fo, Fm and Fv. The starting point Fo is the amount of light required to achieve chlorophyll fluorescence, which peaks at a maximum value called Fm. The difference between starting values and max values is called Fv. The Y-axis displays fluorescence yield, but the units are arbitrary.

For most leaves under optimal conditions, F_v/F_m tend to be around 0.83 (Maxwell & Johnson, 2000, Murchie & Lawson, 2013). Other studies indicate that for mosses specifically, optimal values are slightly lower (Jägerbrand & Kudo, 2017, Grau-Andrés et al., 2017). Any stress or damage apparent in PSII may lead photoinhibition or sustained quenching of reaction centres in PSII, both of which may lower the value of F_v/F_m (Murchie & Lawson, 2013).

3. Methods and materials

3.1 Field locations

Peat moss (*Sphagnum sp.)* was collected on 23. September 2024. All samples were taken from *Steinsjøen skyte- og øvingsfelt* (SØF), a shooting range in the municipality of Østre Toten in Innlandet county in Norway and is owned by Mathiesen Eidsvoll Verk ANS (MEV) where it is mainly used by the Norwegian army. The shooting range sits in a boreal forest with spruce (*Picea* sp) and birch (*Betula* sp) trees in conjunction with many species of moss, some of which form layers of peat. It has a continental subarctic climate with long and cold winters, and relatively warm and short summers. The entire field encompasses an area of 11 km^2 (see figure 3) and has several dedicated shooting ranges, usually in open areas with peat, with metal boards put up at different distances, either mounted on mounds of gravel or sand, or put up directly in the peat. On older ranges, a large stone protruding from the ground or a nearby hill which surrounded the shooting range may have also served as a target.

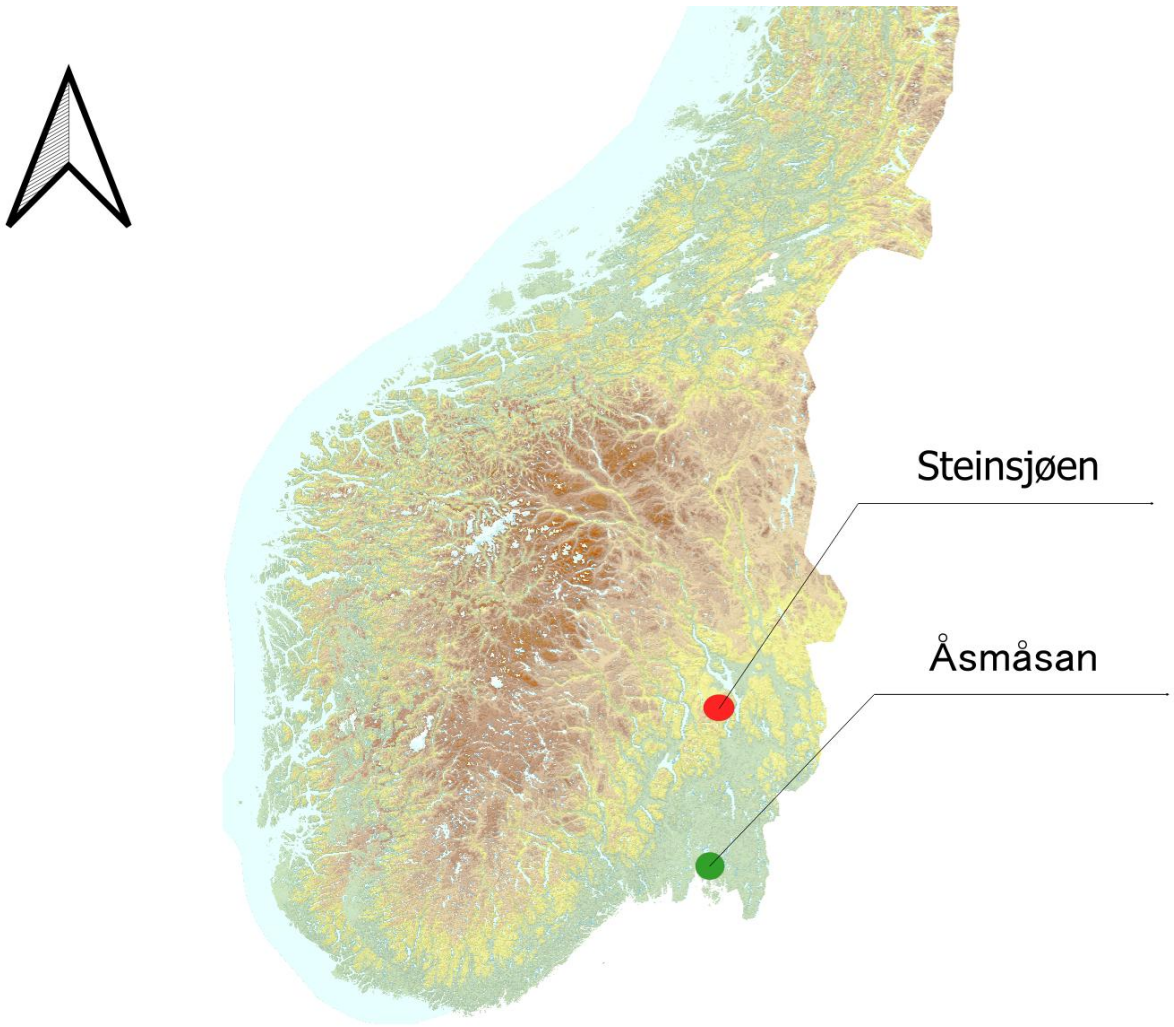


Figure 3: Map showing central Norway and the location Steinsjøen for the observational study and Åsmåsan for the experimental study. Map projection from GEONORGE.no.

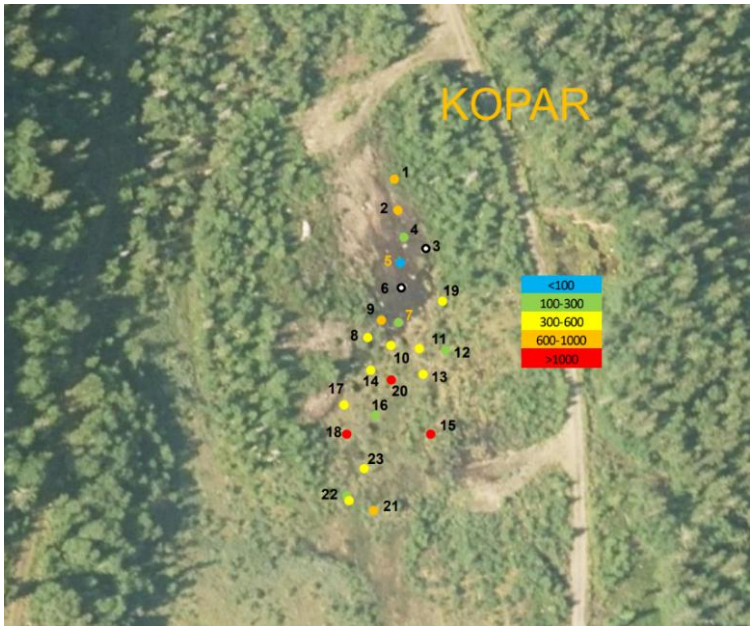


Figure 4: A birds-eye-view of shooting ground 6 (SF6) with water samples taken at various intervals. Concentrations of copper (Cu) are displayed from low to high respectively with the colours blue at <100 µg/L, green from 100-300 µg/L, yellow from 300-600 µg/L, orange from 600-1000 µg/L and red at >1000 µg/L.

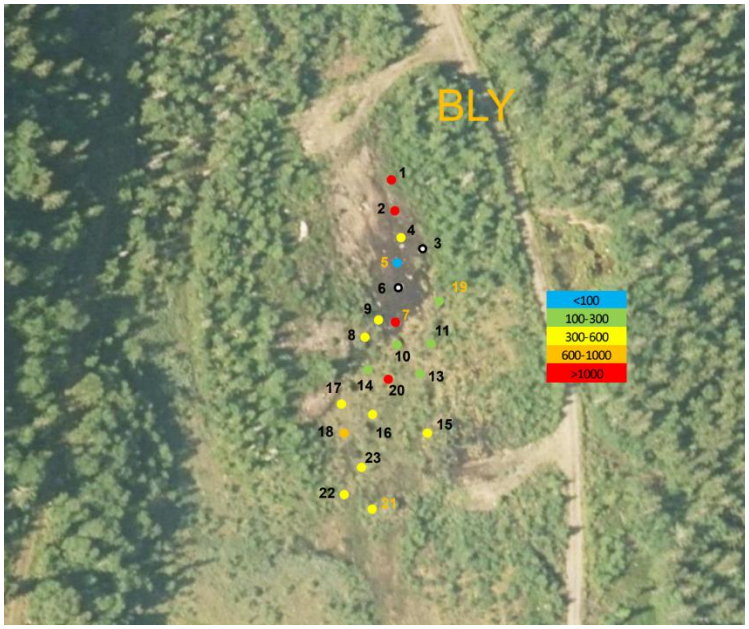


Figure 5: A birds-eye-view of shooting ground 6 (SF6) with water samples taken at various intervals. Concentrations of lead (Pb) are displayed from low to high respectively with the colours blue at <100 µg/L, green from 100-300 µg/L, yellow from 300-600 µg/L, orange from 600-1000 µg/L and red at >1000 µg/L.

Figure 4 and 5 served as a guideline for collecting samples with known metal contamination. Attempts were made the collect samples in areas that had moderate to high levels of metal

contamination; however, no such map was available for shooting fields 25 and 26. When gathering samples at the 25 and 26, the focus shifted towards getting a good representation of the area as a whole and samples were collected at across each field.

Åsmåsan is a bog or a geogenous mire, also known as a fen (IPS, n.d) in Ås municipality located in Akershus county in south-eastern Norway (Figure 6). The bog contains several common species of moss such as *Sphagnum medium, Sphagnum fuscum* and *Shagnum tenellum* to name a few. Åsmåsan has been producing peat for thousands of years (Tveraa, 2022) and receives many of its nutrients from marine sediments below and much of its water as groundwater (Tveraa, 2022). It has been shown to have low PH values of 3.74 in the top 2 m and reaches depths of 6 m before reaching marine sediments below which can be traced to the last ice age (Tveraa, 2022). The bog is close to urban areas (image) and has long been studied by students and employees from the local university, The Norwegian University of Life Sciences (NMBU).

Figure 6: The mire in Åsmåsan.

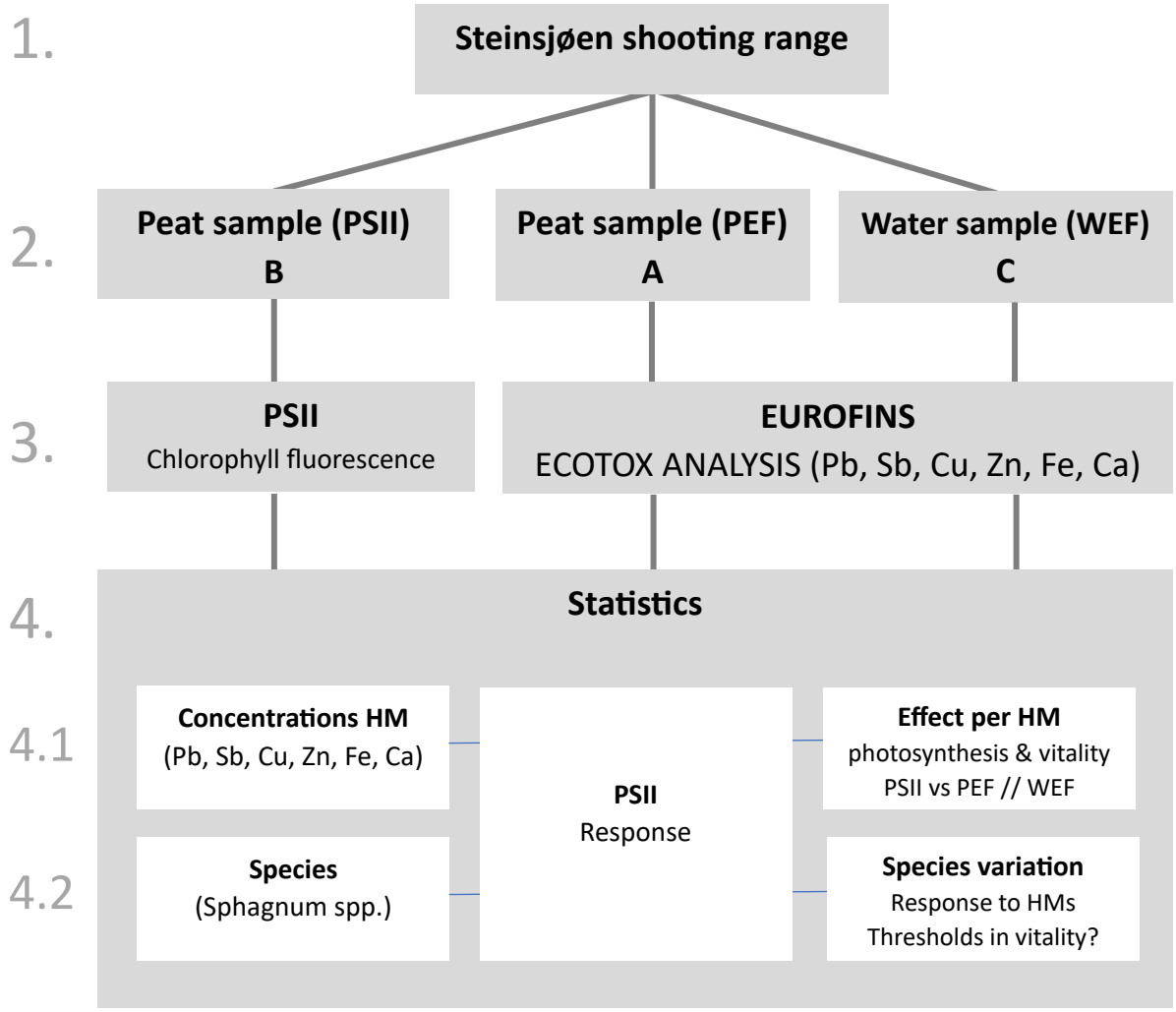
3.2 Data sampling

3.2.1 Observational study

Water quality in shooting areas used by the army have been monitored since the early 1990's, with water samples being taken every year in fields that have known high levels of heavy metal pollution or in fields that are in active use by the Norwegian army and help to locate certain areas that had known high levels of heavy metals. For the experiment, peat moss was taken from three different shooting grounds which were no longer in use and had not seen military activity for several years, still both low and high concentrations of heavy metals were present. Most relevant for the experiment were zinc (Zn), copper (Cu), lead (Pb) and antimony (Sb). A separate batch of uncontaminated peat moss that was not from a shooting area was used as a control group.

Samples were collected either as organic samples or as water samples. In total 2x30 plastic bags of peat moss and 30 bottles of water were collected from Steinsjøen. The peat moss collected was in duplicates, one set of 30 was returned to the university for analysis using chlorophyll fluorescence, while the other set was sent to Eurofins for analysing heavy metal composition in the dry biomass, by using ecotox analysis. Samples were given an identification, e.g. SF6_13, where SF6 is shooting range number six, and the latter number corresponds to the numbers used by Forsvarsbygg on previous water samples taken on range 6 (see figure 4, 5). Each sample was given either the letter A or B at the end to denote whether it was sent to Eurofins for analysing how much heavy metals was absorbed (A) or the university for chlorophyll fluorescence (B), e.g. SF6_13A and SF6_13B respectively. Water samples were given the same system of identification and were identified by the letter C at the end, e.g. SF6_13C. Water was acquired by squeezing water by hand from the samples of moss with plastic gloves and drained into plastic containers containing approximately 250 ml of water per sample. Control samples used the same system but were given the letters KP with 01 for the one control area and then numbered in sequence as they were taken first to last.

Table 1: A process diagram describing in simple steps the design of the experiment. 1: From Steinsjøen shooting range, 2: how the samples were divided into three groups, 3: the methods that were used for analysis, chlorophyll fluorescence and ecotox analysis, 4: how the samples were used in statistics



The samples of moss were each put into plastic bags with identification, date and project name written on each bag (see figure 7). The water bottles were also marked accordingly. A few handfuls of moss were taken in each sample to ensure there was a sufficient amount of moss gathered which could be used for analysis. The A and C samples were sent off to Eurofins while the B samples at the university was being stored in a cooler at around 5°C in low light environments. Experiments started the day following collection, at 24. September 2024, using light-adapted samples with chlorophyll fluorescence using IMAGING PAM. A small selection of moss was taken out of each plastic bag and laid out on medium-sized petri dishes, lightly covering it.

Analysis of trace elements in the water and pH was measured in compliance with the International Organization for Standardization (ISO). The pH was analysed using the standard

NS-EN ISO 10390:2022 treatment of soil, sludge and biowaste (Standard Norge, n.d). Water quality was analysed with the NS-EN ISO 17294-2:2023 standard for water quality. Dry biomass was analysed by the Swedish standard SS-EN 12880 for determining dry tissue samples and trace elements in the dry tissue was determined by the standard SS 28311:2017 (SiS, n.d.).

Figure 7: Collected samples from Steinsjøen shooting range. Marked and sorted into plastic bags.

3.2.2 Experimental Study

Samples of peat moss was collected from Åsmåsan by hand and subsequently put into plastic bags. Shortly after, members of *Sphagnum medium* Limpr (see figure 10) could later be identified and separated from the rest, which was discarded. Great care was taken to preserve individuals with an intact head, branches and stem and individuals which lacked functioning photosynthesis tissue, were discarded. The moss was kept cool and dark for a couple of days in preparation for the experiment. See figure 8 for an overview of the experiment.

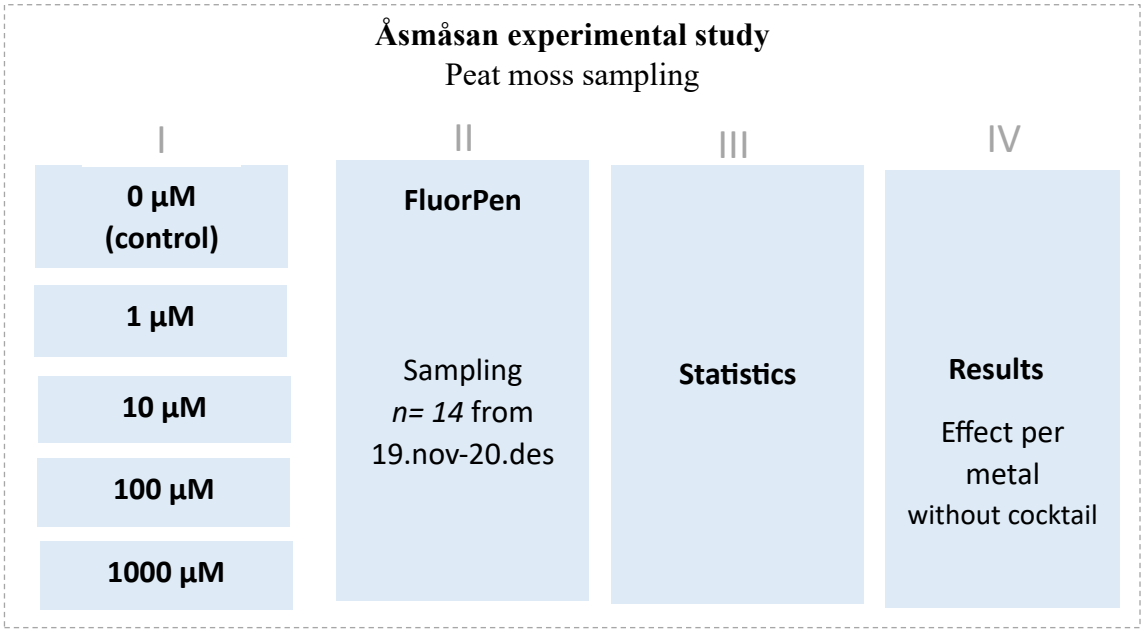


Figure 8: An overview over the experiment done in the lab at the university, showing how every single concentration goes through chlorophyll fluorescence, then analysed and compared.

Large glass bottles with a plastic cap were used to contain solutions of lead, antimony, copper and zinc, and were prepared in the lab using salts. Lead (II) nitrate was used for lead, potassium hexahydroxy antimonate was used for antimony, Copper (II) sulphate for copper and zinc chloride for zinc. Glass bottles with 400 ml of water were used to create lead, copper and zinc solutions and 800 ml for antimony (different volume lies in practical reasons), while the salts were weighed on a scale in order the achieve the right concentrations. When the amount of

water, concentration and molecular weight of every salt are known, the necessary weight needed to make every concentration could be calculated using the following equation:

$$C = \frac{m}{V}\ x\ (\frac{1}{MW}) \quad \text{(i)}$$

Where C is the desired concentration using molarity, in this experiment micromolar (µM) was used, m is the weight in grams, V is the volume of water in the solution either 400 ml or 800ml, and MW is the molecular weight of each salt (which in the case of copper sulphate for example, would be 159,609 g/mol). Solve this equation for m and the following equation gives the weight needed:

$$m = CV\ x\ (\frac{1}{MW}) \quad \text{(ii)}$$

For every metal, four solutions were made starting at 1[µM], then 10[µM], 100[µM] and 1000[µM] at the highest concentration. A single stem of *Sphagnum medium* with branches and a head was laid out on five petri dishes for every concentration, accompanied by five control dishes (0 [µM]) with regular tap water per solution. This tallied up to 25 petri dishes per metal combining into a total of 25x4=100 dishes in total (figure 9).

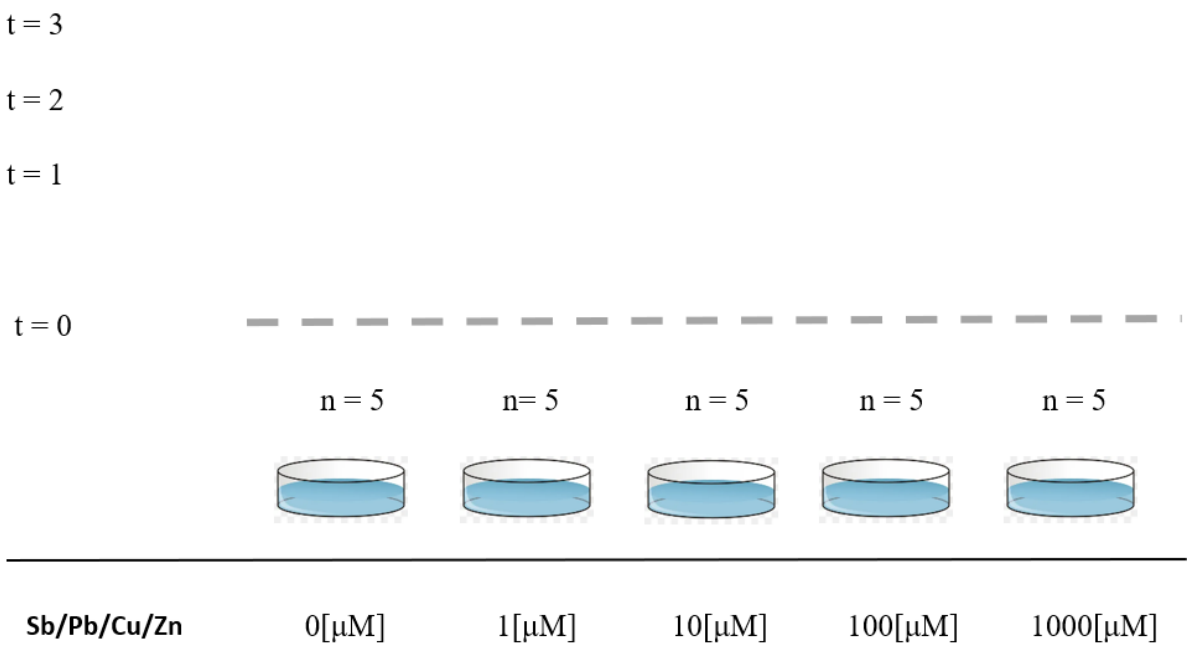


Figure 9: Simple overview of the experiment. Every metal is divided into five concentrations which contain five samples of moss on a petri dish to be analysed by chlorophyll fluorescence up to 14 times (n=14).

Every moss preparation contained approximately 20 ml of prepared liquid and was refilled in case of spilled water and evaporation. They were measured by chlorophyll fluorescence every other weekday from November 19th to December 20th. They were kept in the dark for 15 minutes to unsure dark adaption before being measured. When not used to measure chlorophyll fluorescence, the sample were kept in a fridge with temperatures at 10°C during the day and 6°C during the night in 12-hour cycles.

Figure 10: Spagnum medium Limpr. Collected from Åsmåsan.

3.3 Instruments

Mainly the head of the moss was measured when the FluorPen was used, and polygons were drawn around the head using IMAGING PAM. The FluorPen gave one Fv/Fm value per sample when it was used. For the IMAGING PAM machine, multiple polygons were drawn around the head and was used to acquire an average for Fv/Fm values representing Fv/Fm values for each sample (see figure 11).

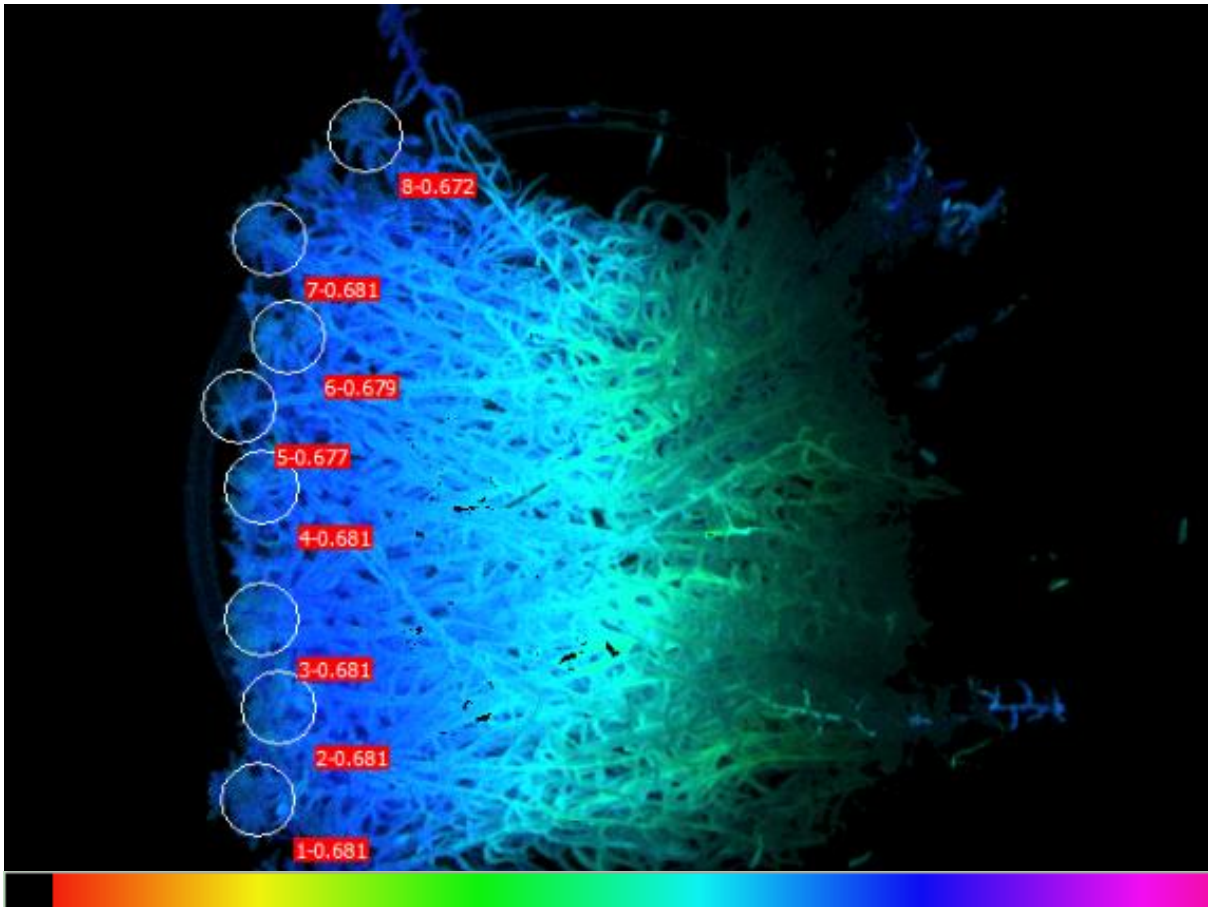


Figure 11: Sample SF26-05B under the IMAGING PAM machine with areas of interest drawn around the heads of the moss. The image is displaying Fv/Fm values shown in red textboxes and the colour spectrum is showing Fv/Fm going from 0 to 1.

Moss from Steinsjøen shooting range were laid out to fill a petri dish and analysed in a low-lit room using an IMAGING PAM M-series machine from Walz Photosynthesis Instruments (IMAGING PAM. Maxi red version, Walz Effelfrich, Germany) Essentially, a medium sized box with a camera and a light source on the inside. The machine's software ImagingWinGigE V2.56zn (updated April 2023) was downloaded directly from Walz Photosynthesis Instruments own website. The machine combines the camera with an easy-to-use software interface which allows the samples to be viewed visually on a screen while being measured for chlorophyll fluorescence (see figure 12). Different points of interest could then be drawn manually on every sample, covering the capitulum or head of the moss and served as the basis for chlorophyll fluorescence in every sample.

Moss from Åsmåsan were analysed using a FluorPen FP 100 by Photon System Instruments using the OJIP system, which is well suited for analysing dark-adapted samples. The FluorPen is a battery charged handheld device which makes for a very quick and precise measurement chlorophyll fluorescence and can easily be utilized in a laboratory (see figure 12). Both IMAGING PAM and the FluorPen can give data visually in images but also as numbers, such as values for Fv/Fm, which was then further analysed in Microsoft Excel, Jamovi and R which formed the basis for most of the statistics used in this thesis.

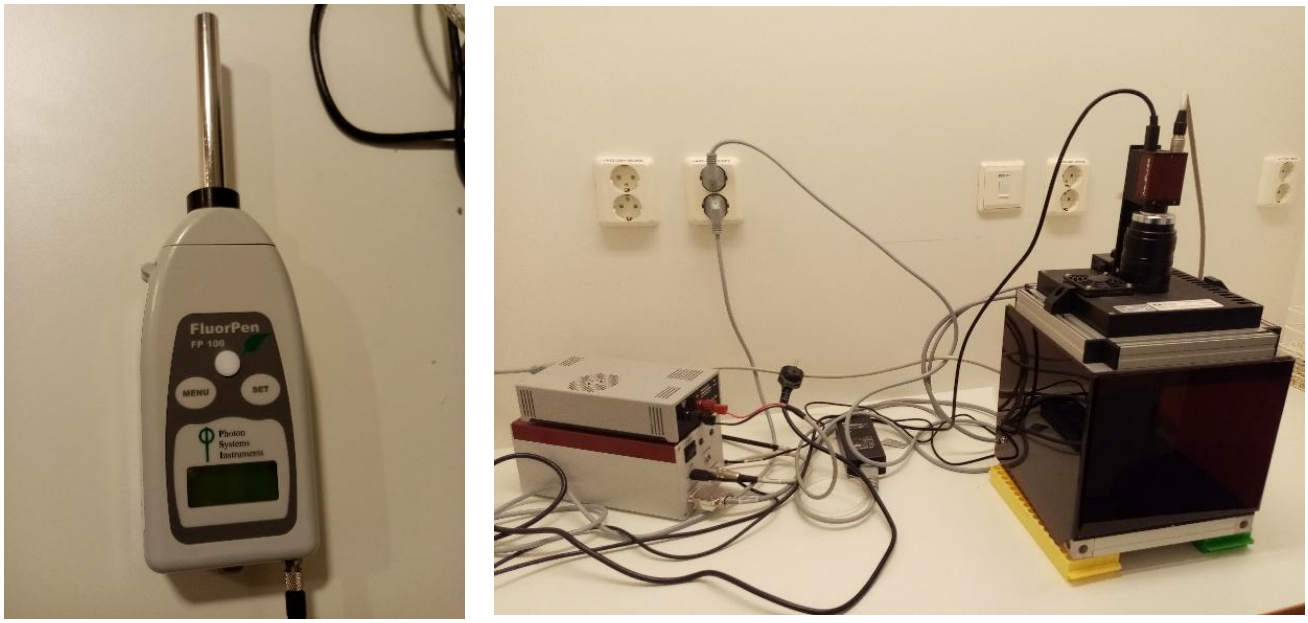


Figure 12: Left: FluorPen FP 100 by Photon System Instruments. Right: IMAGING PAM M-series machine from Walz Photosynthesis Instruments.

3.4 Statistical analysis

Microsoft Excel was used to organize the data into spreadsheets. The data from the IMAGING Pam M-series was saved as CSV-files and the data from the FluorPen was saved as TXT-files, both of which was converted into spreadsheets in Microsoft Excel. Here the data was organized, and averages, standard deviations and standard errors were calculated. Bar and line graphs were made in Excel. The program Jamovi (version 2.6. 2024) was used to create scatter plots and run ANOVA-test. Jamovi is a software that uses R but allow statistical analyses to be run with putting in minimal commands or coding from the user. From a datasheet, modules come installed in the program which enable the user to select the desired method for analysis from the user interface. Jamovi was used to create scatterplots and to run one-way & repeated measure ANOVA-tests.

4. Results

4.1 Shooting range results

Table 2: *Filtered water analysis of samples taken from three shooting fields and control samples, showing metal-ions of antimony (Sb), lead (Pb), Copper (Cu), Zinc (Zn) and iron (Fe) in µg/L, pH and dissolved organic carbon (DOC).*

ID	Sb (filtered) µg/L	Zn (filtered) µg/L	Pb (filtered) µg/L	Cu (filtered) µg/L	pH	Dissolved organic carbon (DOC) mg/L	Fe (filtered) µg/L
SF6_07 C	2,5	32	30	21	5	72	4400
SF6_08 C	0,87	97	7,4	28	4,5	60	230
SF6_11 C	6,40	100	160	81	4,7	120	8100
SF6_13 C	2,10	60	23	45	4,7	48	1200
SF6_15 C	3,5	280	100	59	4,6	150	1300
SF6_19 C	4,7	80	210	34	5,3	52	22000
SF6_20 C	2	160	41	22	4,7	67	3800
SF6_21 C	2,2	49	22	4,8	4,5	130	760
SF26_1 C	0,94	660	57	35	4,1	140	110
SF26_2 C	0,6	46	9,8	28	4,8	44	220
SF26_3 C	0,43	44	3	6,5	5,6	22	44
SF26_4 C	0,58	140	18	37	4,4	67	93
SF26_5 C	1,7	67	13	31	4,3	43	160
SF26_6 C	0,81	130	18	21	4,7	130	69
SF26_7 C	0,69	39	8,6	12	5,5	31	83
SF25_1 C	14,00	230	340	890	4,7	67	3800
SF25_2 C	2,6	38	34	57	4,9	39	1400
SF25_3 C	0,96	120	6,4	1,3	4,5	28	66
SF25_4 C	0,33	96	9,5	6,5	4,4	82	53
SF25_5 C	0,91	98	24	30	4,3	110	75
SF25_6 C	2,3	76	19	73	4,5	40	180
SF25_7 C	0,38	82	7,4	2,3	4,3	170	63
SF25_8 C	2,1	28	27	31	5,2	41	6000
SF25_9 C	23,00	29	49	47	5,6	27	110
KPO1_1 C	0,49	100	3,2	1,3	4,6	53	960
KPO1_2 C	0,19	200	1,6	0,36	5,3	66	76
KPO1_3 C	0,068	93	1,1	0,68	4,7	60	33
KPO1_4 C	0,087	19	0,56	0,4	4,6	28	87
KPO1_5 C	0,06	22	0,39	0,4	4,3	22	86
KPO1_6 C	0,069	13	0,15	0,78	4	35	30

This table contains the analyses done by EUROFINS on water samples and shows the ID of all 30 samples taken from Steinsjøen shooting range and how much of Sb, Pb, Cu and Zn the water squeezed out of the peat moss contained, shown in µg/L. The table includes pH of the water as well as dissolved organic carbon (DOC) and Fe contents.

Table 3: *Dry biomass analysis of samples taken from three shooting fields and control samples, showing metal-ions of antimony (Sb), lead (Pb), Copper (Cu), Zinc (Zn) in µg/kg and pH.*

ID	Antimony Sb µg/kg	Lead Pb µg/kg	Copper Cu µg/kg	Zinc Zn µg/kg	pH
SF6_07 A	7,3	93	34	36	4,6
SF6_08 A	7,2	53	79	79	4,7
SF6_11 A	7,2	210	140	110	4,3
SF6_13 A	8,5	31	77	260	4,5
SF6_15 A	6,4	640	290	300	4,6
SF6_19 A	10	930	110	230	5
SF6_20 A	7,4	370	160	140	4,7
SF6_21 A	8,3	65	41	71	4,5
SF25_01 A	18	1000	850	650	4,6
SF25_02 A	7,5	15	21	41	5,2
SF25_03 A	8,3	4,2	23	62	4,5
SF25_04 A	6,9	43	63	83	4,9
SF25_05 A	14	260	320	200	4,8
SF25_06 A	11	13	18	45	4,5
SF25_07 A	6,3	8,4	16	110	4,6
SF25_08 A	7,2	65	60	110	5,3
SF25_09 A	14	1400	470	180	5,6
SF26_01 A	6,9	49	35	95	4,1
SF26_02 A	7	32	150	120	4,6
SF26_03 A	7,9	52	42	70	5,6
SF26_04 A	7,9	26	110	180	4,3
SF26_05 A	9,7	39	89	82	4,6
SF26_06 A	7,1	37	48	190	5,4
SF26_07 A	8,6	220	90	77	5,6
KP01_1 A	7,9	4	4	22	4,4
KP01_2 A	8	4	4	32	5,3
KP01_3 A	8,7	4,4	4,4	20	4,7
KP01_4 A	8,8	4,4	4,4	26	4,7
KP01_5 A	13	6,5	6,5	40	4,9
KP01_6 A	8,3	4,2	4,2	27	4,3

Table 3 is also showing analysis done by EUROFINS and is now showing metal contents of the analysed dry biomass of the collected peat moss, in µg/kg. The pH shown is also from the biomass, not to be confused with the water that was collected separately in table 2.

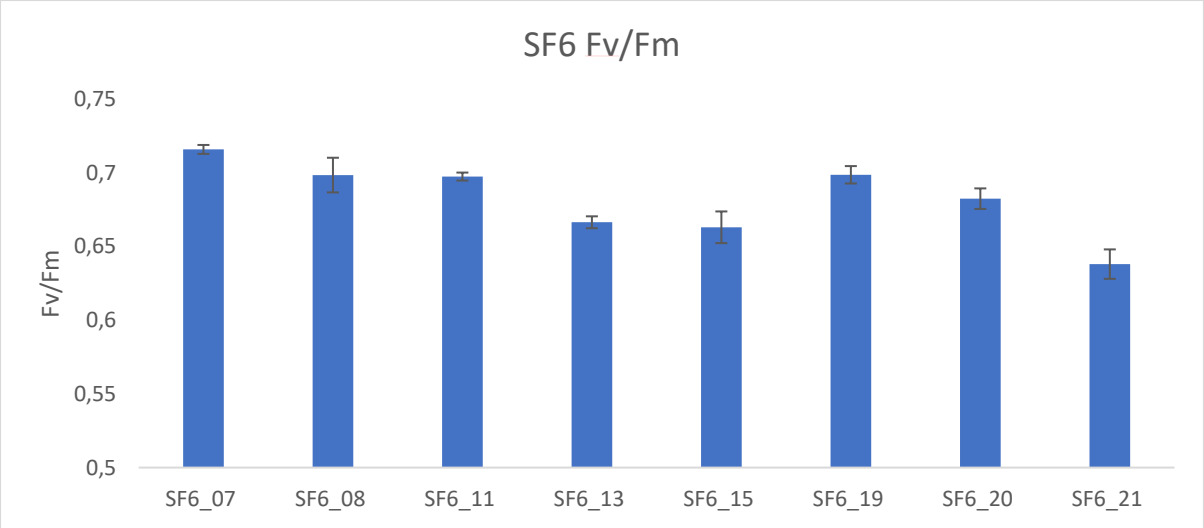


Figure 13: Graph showing Fv/Fm values of the samples from shooting range 6 (SF6).

Samples taken from shooting field 6 are shown in figure 13 and after chlorophyll fluorescence gives Fv/Fm values ranging from approximately 0.72, at the highest for sample SF6_07, to 0.64 for sample SF6_21, at the lowest.

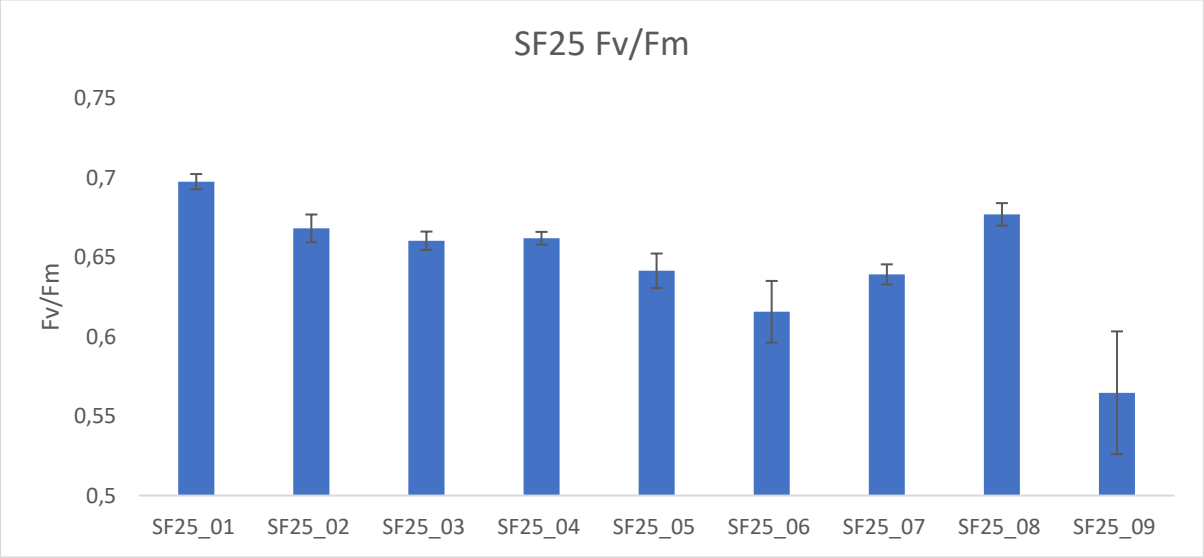


Figure 14: Graph showing samples taken from shooting field 25 (SF25) and their Fv/Fm values.

The figure 14 displays Fv/Fm values after chlorophyll fluorescence on samples from shooting field 25. Most Fv/Fm values was within 0.65-0.69, except sample SF25_09 which reached an Fv/Fm value of 0.56.

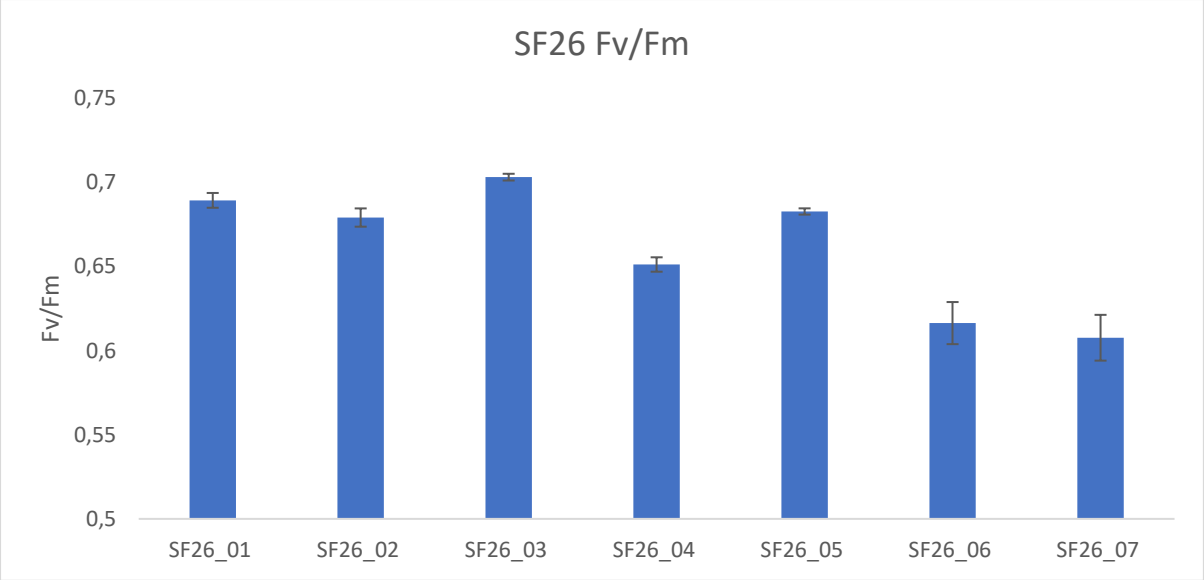


Figure 15: Graph showing Fv/Fm values from samples taken from shooting field 26

Fv/Fm values taken after analysing samples from shooting range 26 with chlorophyll fluorescence shown in figure 15 shows that Fv/Fm reaches 0.7 for sample SF26_03, the highest from this field. The lowest value was achieved by sample SF26_07 at 0.61.

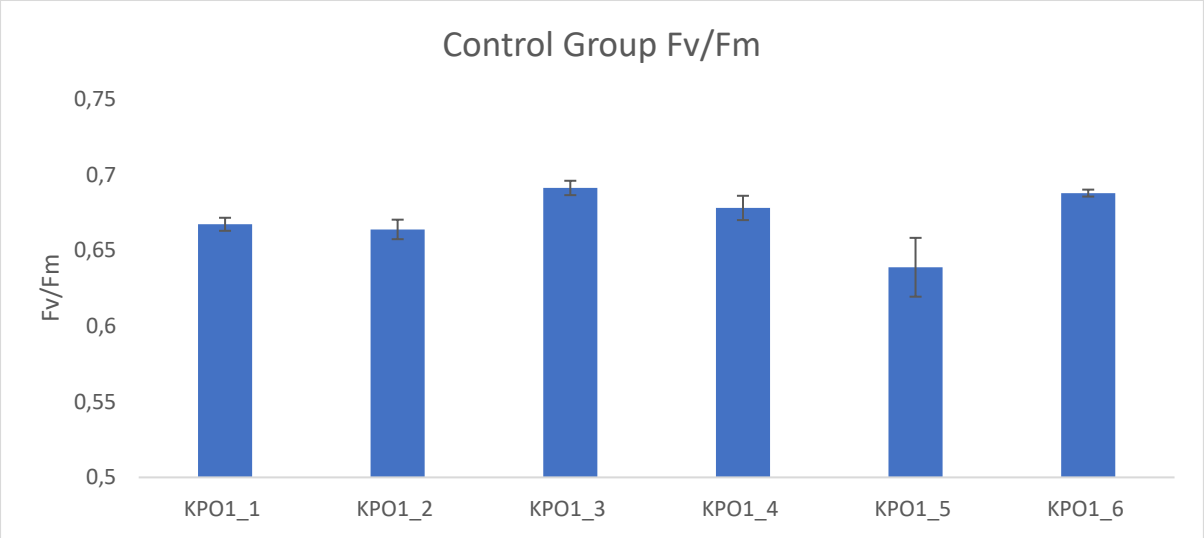


Figure 16: Graph showing Fv/Fm values for samples from the control group.

The control group shown in figure 16 gives a difference in Fv/Fm values from 0.69 for KP01_3 to 0.64 form sample KP01_6.

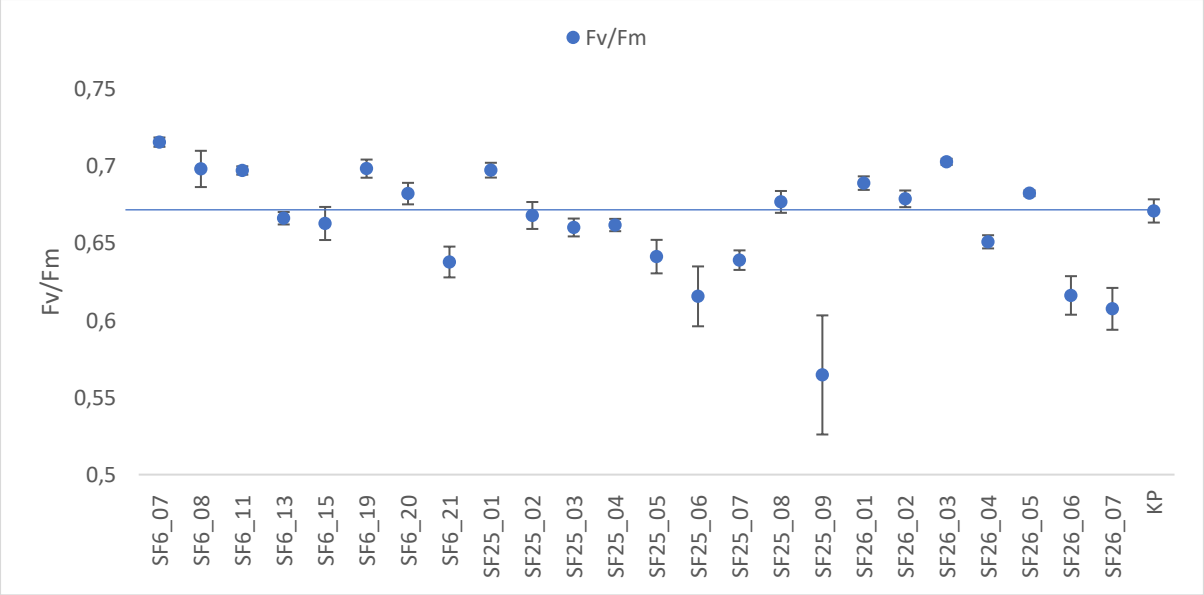


Figure 17: Graph showing samples analysed from three shooting fields, SF6, SF25, and SF26 and their Fv/Fm values. The average Fv/Fm value from the control group is shown as the point KP.

Figure 17 above combines the Fv/Fm values for all three fields from Steinsjøen shooting range together with the average Fv/Fm values from the control group which is represented by the data point KP. The average Fv/Fm value at KP is approximately 0.67 and is where the line on the graph is drawn.

4.2 Experimental results

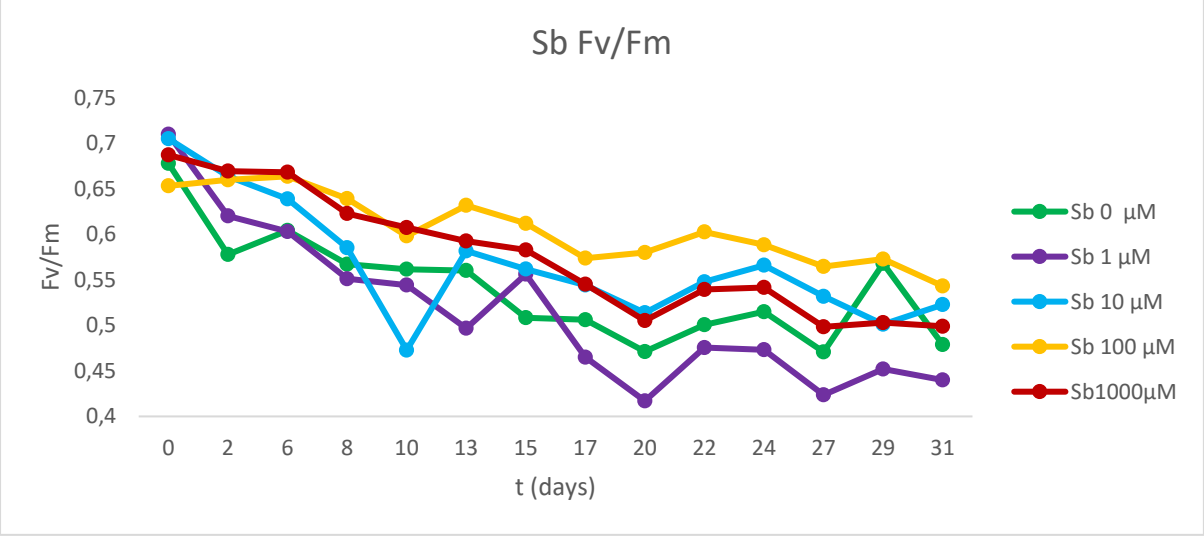


Figure 18: Fv/Fm values for Antimony (Sb) using five different concentrations, taken over the span of 30 days.

After 30 days Sb show a clear decrease in Fv/Fm values for every concentration (Figure 18). Sb concentration of 100 µM seems to have the highest on average Fv/Fm values overall while the 1 µM concentration has the lowest Fv/Fm values.

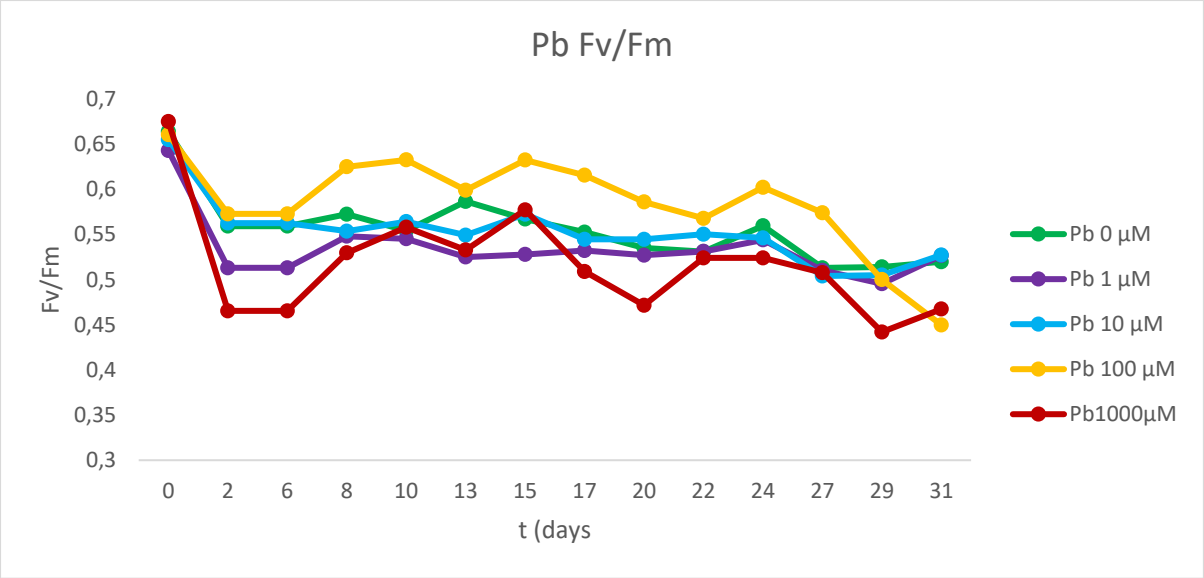


Figure 19: Fv/Fm values for Lead (Pb) using five different concentrations, taken over the span of 30 days.

Figure 19 shows that all Pb samples regardless of concentration drop sharply in Fv/Fm values after two days. Afterward, Fv/Fm increases somewhat and appears to stabilize slightly over the course of the experiment until Fv/Fm decreases after day 27. The concentration of 100 µM Pb seem to perform the best with the highest Fv/Fm level, before it decreases again and becomes the lowest result at around 0.45. Pb at 1000 µM also performs poorly, while concentrations 0,1 and 10 µM remain close to 0.55 Fv/Fm.

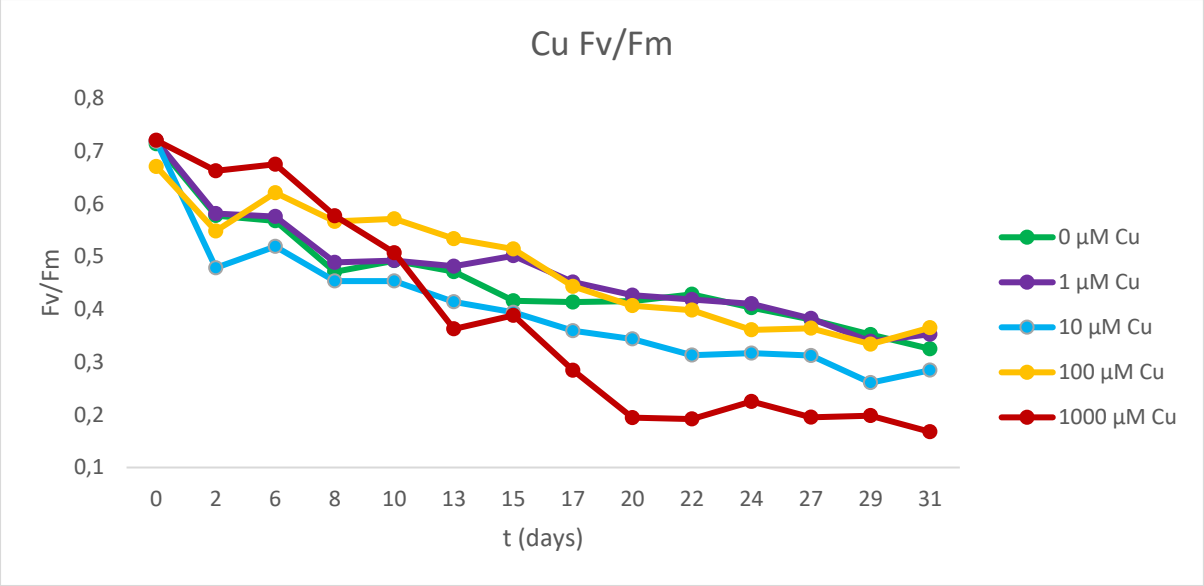


Figure 20: Fv/Fm values for copper (Cu) using five different concentrations, taken over the span of 30 days.

Copper shows a clear difference in Fv/Fm values at 1000 µM which decreases rapidly after six days (figure 20). While the concentration 0,1,10 and 100 µM has decreasing Fv/Fm levels over time, they remain near each other and end up with Fv/Fm in between 0.3-0.4. The highest concentration 1000 µM reaches the lowest Fv/Fm value at the end of the experiment around 0.17.

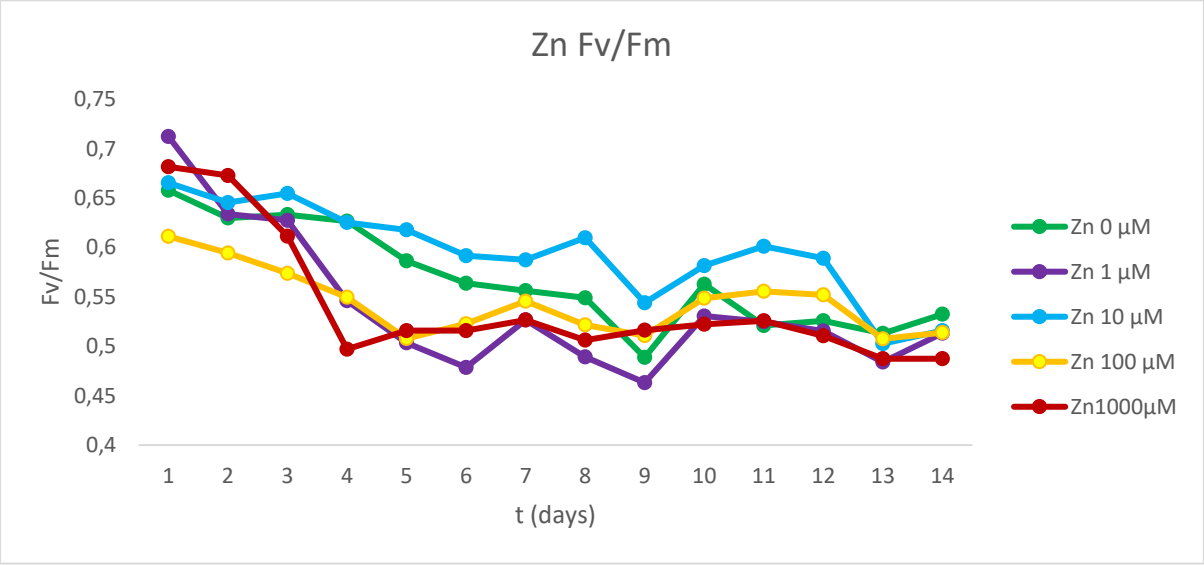


Figure 21: *Fv/Fm values for Zinc (Zn) using five different concentrations, taken over the span of 30 days.*

Figure 21 shows Fv/Fm values for all samples of Zn analysed by chlorophyll fluorescence over the course of the experiment. All concentrations display Fv/Fm levels closer to each other at the end of the experiment when compared to the beginning. The five different concentrations of Zn show somewhat high variance at the start in their Fv/Fm values. While every concentration has decreasing Fv/Fm values over time, 0 µM has the highest Fv/Fm value of 0.53 while 1000 µM of Zn ends up having the lowest of around 0.49.

5. Discussion

5.1 Trace element contamination at Steinsjøen shooting range

5.1.1 Concentrations of trace elements in peat moss and pore water

The majority of metals from metal pollution tend be located in the top soil column, regardless of mire type (Mariussen et al., 2017). Thus, there is reasonable that samples gathered form the top layers contain the majority of metal contamination in each shooting range. When comparing the metal concentration of the water squeezed form the peat moss with metal taken up in the peat moss tissue, there doesn't appear to be a strong difference (see figure 22), and the data is skewed by a few individual samples that happen to have much higher values than average.

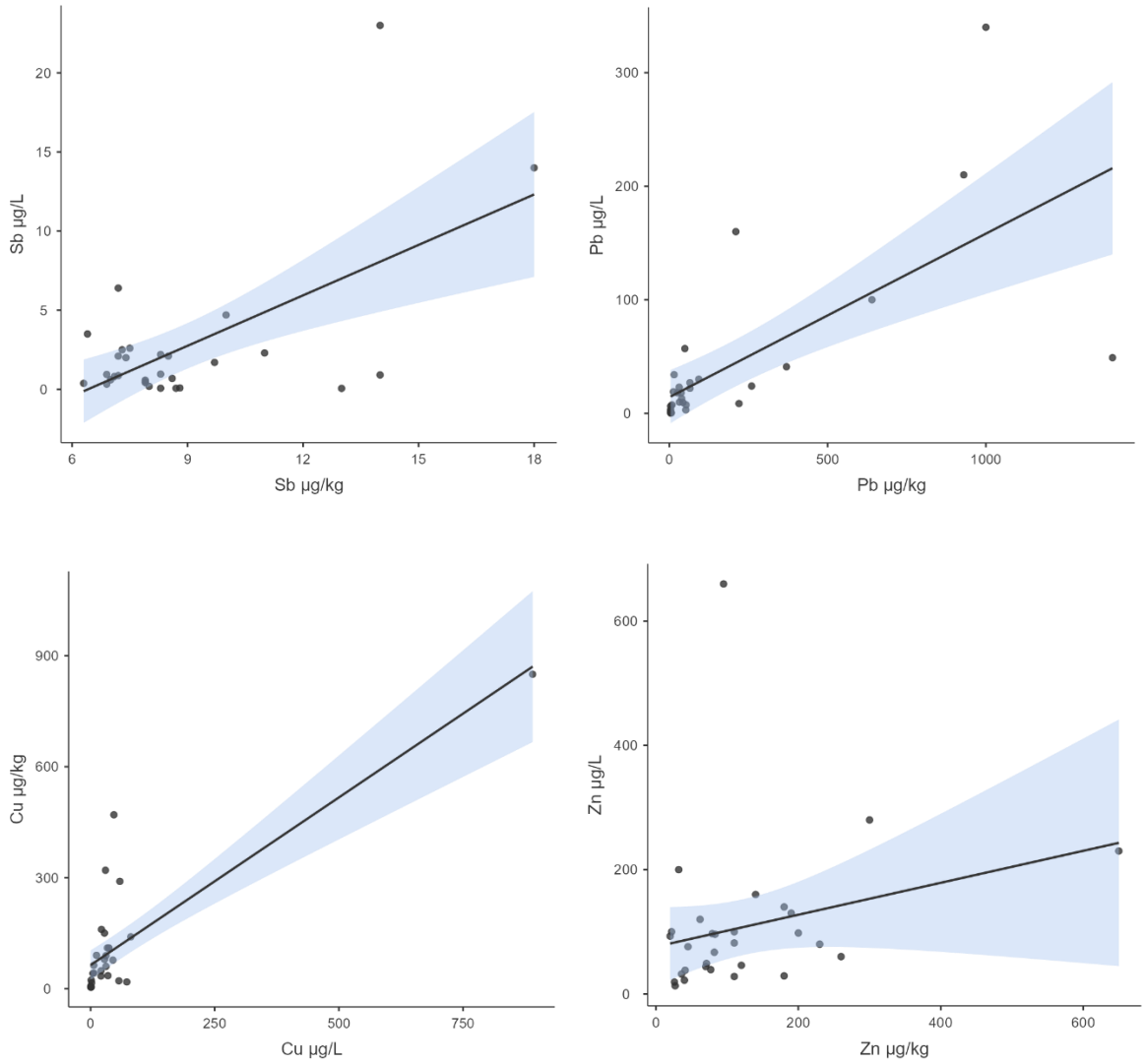


Figure 22: Scatterplots comparing metals analysed in water with metals in dry biomass. From top left to right; Sb, Pb, Cu & Zn.

When the pH is low at approximately 3.5-6.5, it reaches an optimal range for metal capture by active binding sites on peat moss (Brown et al., 2000). A pH above 3 is needed in order to prevent metal ions from leaching out of the peat moss, and a pH below 8 is required for sorption to be significant enough (Brown et al., 2000). Adsorption of metals usually peak at around a pH of 6 (Gonzales & Pokrovsky, 2014) but depending on the metal, maximum sorption may be different. For *Sphagnum sp.,* Cu^{2+} reaches the highest percentage of adsorption at pH 6.1, Pb^{2+} is highest at around pH 5.5 while Zn is highest at pH 7.8 (Gonzales & Pokrovsky, 2014).

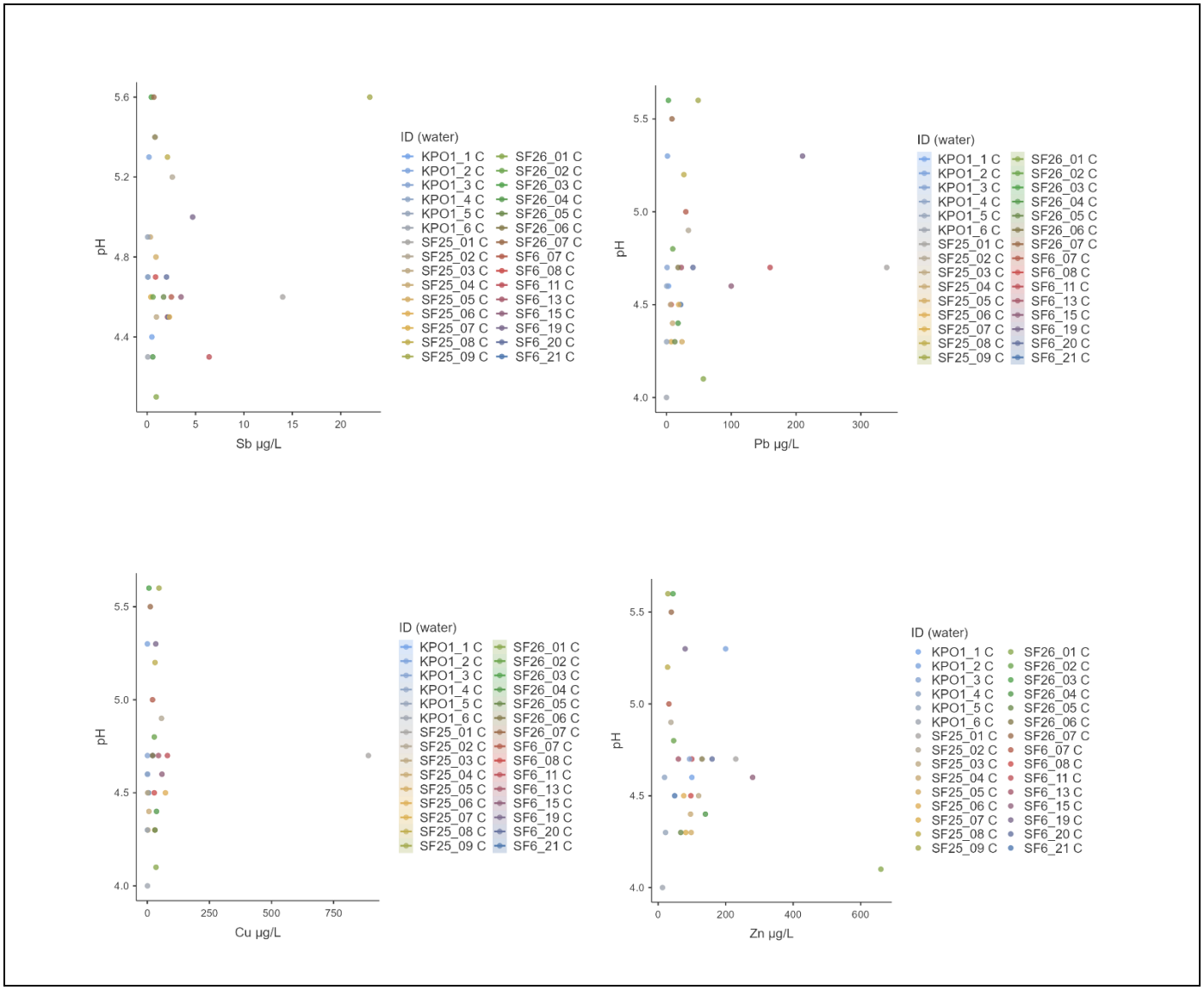


Figure 23: Scatterplots for every metal with comparing pH.

At low concentrations metal ions occupy the sites they have high affinity for and can be said to engage in preferential adsorption (Kalmykova et al., 2007). At higher concentrations, this preference decreases as metals occupy sites of lower affinity (Kalmykova et al., 2007). The uptake of metals decreases slightly with higher concentrations and the binding sites get saturated over time (Kalmykova et al., 2007). While the presence of more than one metal may create competition for available binding sites, the presence of several metals in the same solution may increase total adsorption over all (Brown et al., 2000). As such a cocktail of

various metals in a solution may lead to higher uptake of metals. Figure y shows that the pH in the water from the *Sphagnum* moss, remains quite acidic and lies in the range of 4.0-5.6. However, this appears to be a deciding factor on how high or low the metal concentration is in the water.

5.1.2 Comparison between shooting ranges

Data from Steinsjøen shooting range show a marked difference in metal content in the peat moss between moss taken from contaminated fields with moss taken from a nearby uncontaminated area. This holds true regarding both in the water contents drained from the peat moss, as well as how much was taken in the tissue directly. This can be illustrated further by comparing the different fields with each other and the uncontaminated control group.

Table 4: Dwass-Steel-Critchlow-Fligner pairwise comparisons for drained Sb, Cu, Pb, and Zn contents from three shooting ranges SF6, SF25 and SF26 including an uncontaminated area KP.

Pairwise comparisons - Sb µg/L

		W	p
KP	**SF25**	4.167	0.017
KP	**SF26**	4.041	0.022
KP	**SF6**	4.382	0.011
SF25	**SF26**	-2.171	0.417
SF25	**SF6**	0.885	0.924
SF26	**SF6**	4.255	0.014

Pairwise comparisons - Cu µg/L

		W	p
KP	**SF25**	4.425	0.010
KP	**SF26**	4.248	0.014
KP	**SF6**	4.387	0.010
SF25	**SF26**	-0.825	0.937
SF25	**SF6**	0.000	1.000
SF26	**SF6**	1.148	0.849

Pairwise comparisons - Pb µg/L

		W	p
KP	**SF25**	4.50	0.008
KP	**SF26**	4.05	0.022
KP	**SF6**	4.38	0.011
SF25	**SF26**	-1.42	0.746
SF25	**SF6**	1.43	0.743
SF26	**SF6**	2.95	0.158

Pairwise comparisons - Zn µg/L

		W	p
KP	**SF25**	1.167	0.843
KP	**SF26**	1.414	0.750
KP	**SF6**	1.371	0.767
SF25	**SF26**	0.823	0.938
SF25	**SF6**	0.816	0.939
SF26	**SF6**	0.327	0.996

Table 5: Dwass-Steel-Critchlow-Fligner pairwise comparisons for dry matter analysis for Sb, Pb, Cu and Zn from three shooting ranges SF6, SF25 and SF26 as well from an uncontaminated area KP.

Pairwise comparisons - Antimony Sb µg/kg

		W	p
KP	**SF25**	-0.250	0.998
KP	**SF26**	-2.438	0.311
KP	**SF6**	-2.470	0.300
SF25	**SF26**	-1.200	0.831
SF25	**SF6**	-1.298	0.796
SF26	**SF6**	0.164	0.999

Pairwise comparisons - Copper Cu µg/kg

		W	p
KP	**SF25**	4.508	0.008
KP	**SF26**	4.254	0.014
KP	**SF6**	4.391	0.010
SF25	**SF26**	0.674	0.964
SF25	**SF6**	1.089	0.868
SF26	**SF6**	0.737	0.954

Pairwise comparisons - Lead Pb µg/kg

		W	p
KP	**SF25**	3.9272	0.028
KP	**SF26**	4.2543	0.014
KP	**SF6**	4.3914	0.010
SF25	**SF26**	0.0748	1.000
SF25	**SF6**	1.5659	0.685
SF26	**SF6**	2.9459	0.159

Pairwise comparisons - Zinc Zn µg/kg

		W	p
KP	**SF25**	4.504	0.008
KP	**SF26**	4.243	0.014
KP	**SF6**	4.199	0.016
SF25	**SF26**	0.150	1.000
SF25	**SF6**	0.682	0.963
SF26	**SF6**	0.818	0.939

Table 4 and 5 above shows a clear difference in P-values when comparing the control KP with the other three shooting areas; SF6, SF25 and SF26 regarding every metal, while comparisons between the shooting ranges with each other show little significance. This was to be expected; however, this is not apparent when comparing Fv/Fm values. When comparing Fv/Fm values, there appears to be no significant difference on Fv/Fm values whether the sample was from a contaminated shooting area or from an uncontaminated area.

5.2 Effects of trace elements on PSII in peat moss

As mentioned in section 2.3, leaves of most plants yield F_v/F_m values of around 0.83 under optimal conditions. While chlorophyll fluorescence has been widely used with vascular plants, bryophytes such as *Sphagnum* are much less studied and is reflected in comparatively few studies using chlorophyll fluorescence in conjunction with mosses. Nevertheless, some studies have used moss where control groups have F_v/F_m values of 0.65-0.73 (Jägerbrand & Kudo, 2016) while others claim healthy samples of *Sphagnum* moss when F_v/F_m is at or exceeds 0.75 (Grau-Andrés et al., 2017). The control group on this study measured F_v/F_m values of 0.66-0.69 (fig 5). The results show that for *sphagnum* moss from Steinsjøen shooting range (fig 13-15.), Fv/Fm values do not appear to be significantly lower overall compared to the control group (fig 16.). Indeed, some F_v/F_m values are higher than the control group which does not support the assumption that F_v/F_m would decrease in samples with higher metal concentration. Interestingly, the sample SF25_09 has an F_v/F_m value of 0.56, the lowest measured (see fig 17.), and has the highest lead concentration of 1400 µg/kg, in addition to 14 µg/kg Sb, 470 µg/kg Cu and 180 µg/kg Zn (see table 3). Compare this to another sample from the same field, SF25_01. Even though SF25_01 has a somewhat lower lead concentration at 1000 µg/kg, it also has higher amounts of Sb, Cu and Zn at 18, 850 and 650 µg/kg respectively and still has a considerably higher F_v/F_m value of approximately 0.7. There appears to be large differences from sample to sample which might be explained by site-specific conditions, different species, plasticity and hybridization (Flatberg, 2013) (Jägerbrand & Kudo, 2016). The results seem to indicate that the peat moss from the three shooting ranges, have functioning PSII reaction centres that are not worse off or damaged when compared to peat moss from non-polluted areas (see table 6).

Results from the experiment (fig 18-21) showed that for all metals and concentrations, F_v/F_m went down over time but there didn't appear to be any significant differences in concentration of the metal on F_v/F_m, except for copper (fig 20). Copper displayed a significant ($P< 0.001$) difference between the five concentrations during the end of the experiment, with samples containing 1000 µM of Cu having the lowest F_v/F_m values. The results from the experiment may indicate that *Sphagnum medium* is more sensitive to damage done by copper, more so than the other metals. This could be explained by the fact that copper differs from other metals by forming ring-shaped compounds and binding to phenolic functional groups, while lead and

zinc bind to carboxylic groups (Kalmykova et al., 2008). Thus, a higher proportion of phenolic groups may lead to greater uptake of copper as compared to the other metals in the experiment. However, it should be noted that in both the experiment and for the shooting ranges, there exist a high degree of uncertainty regarding the accuracy of chlorophyll fluorescence combined with other factors which may affect metal adsorption in each sample. It is therefore very relevant to discuss how to interpret the data and what conclusions can be draw from F_v/F_m values and chlorophyll fluorescence as a method.

Table 6: Pairwise comparison of Fv/Fm values on shooting ranges SF6, SF25, SF26 and uncontaminated control group KP using Dwass-Steel-Critchlow-Fligner pairwise comparison.

Pairwise comparisons - Fv/Fm

		W	p
KP	**SF25**	-1.833	0.566
KP	**SF26**	-0.202	0.999
KP	**SF6**	1.278	0.803
SF25	**SF26**	1.272	0.805
SF25	**SF6**	2.858	0.180
SF26	**SF6**	1.473	0.725

The Fv/Fm parameter has been widely used in numerous studies on photosynthesis however, it is possible there exists plant damage or stress that the Fv/Fm method does not register. Singh et al., suggest that because fluorescence is due to Fo and Fm levels, the Fv/Fm parameter is not sensitive enough to detect differences between samples (Singh et al., 2018). There are also several types of stress that can occur and the Fv/Fm method may not be sensitive to all of them. For instance, water stress does not consistently alter Fv/Fm values unless combined with high intensity light (Chen et al., 2019). Mosses are less affected by cold, salinity and osmotic stress than other plants and more vulnerable to high light intensity and high temperatures (Chen et al., 2019). It is possible the peat moss from the shooting ranges does experience some stress from metal contamination but that it is not detected by chlorophyll fluorescence using the Fv/Fm parameter. However, it seems just as likely that the peat moss is largely unaffected by the metals and does not experience any form of notable stress at all. Further analysis would be required to determine the presence of stress or damage to PSII.

5.3 Experimental study - Åmåsan

Table 7: Anova One-Way tests done with all 31 days of the experiment showing concentrations of 1,10,100 and 1000 µM together with p-values of Fv/Fm on Sb, Pb, Cu and Zn for every concentration. The coefficient of determination is given by R^2 (see appendix).

Concentration (µM)	Sb P-value	Pb P-value	Cu P-value	Zn P-value
1	0.107	0.340	0.441	0.015
10	0.080	0.930	<.001	0.018
100	<.001	0.115	0.276	0.042
1000	0.019	0.075	<.001	0.025
R^2 (adjusted)	0.309	0.0358	0.596	0.300

The results from the experimental study done with *Sphagnum medium* collected from Åsmåsan show little difference between increasing concentrations from either 1 µM up to 1000 µM, regardless of metal. Fv/Fm values are consistently dropping over time for all samples, and while some p-values are different between concentrations (see table U), it didn't follow the expected outcome of Fv/Fm values being lowest with the highest concentration of 1000 µM. Furthermore, the control group containing 0 µM of any metal did not have any noticeably higher Fv/Fm values than the other concentrations, regardless of metal, suggesting that the amount of each metal in this experiment was not relevant to the observed Fv/Fm value and neither was the type of metal compound. Cu appears to show the clearest trend toward 1000 µM being the most harmful and does reach the lowest Fv/Fm value on average, however further study would be required to support the claim that Cu if more harmful to *Sphagnum medium* than Sb, Pb and Zn. The explanation for these results is likely the insufficient conditions the *Sphagnum* moss was kept in during the experiment. The water used to create the metal solutions for Sb, Pb, Cu and Zn was simply water from the tap in the lab. The problem with this method is now the moss is kept in neutral water with different minerals and lacking in organic matter which in other words do not accurately reflect the conditions the moss normally lives in. The pH of the water affects how effectively metal ions bind to peat moss (Rognerud, 2003) and neutral pH could indicate a lower affinity for Pb, Cu and Zn ions in the different samples and thus less effective binding of each metal. This could help explain the lack of any noticeable difference between each metal and the different concentrations. When the conditions are near

7 pH, Sb is more soluble than the other three metals (Evangelou et al, 2012) which could suggest a more effective binding of Sb in theory, but this was not observed during the experiment.

Another potential issue is that the moss was kept in a fridge in a day-night system with 12-hour of darkness and 12 hours of UV-light. The temperature should be no issue for the moss, staying at 6-10 degrees depending on day or night, however the light intensity could be harmful. Although mosses are autotrophs and as such utilize light as a source of energy, moss tend to prefer shaded areas where they are not exposed to direct sunlight for long periods of time. Therefore, mosses are vulnerable to overexposure of sunlight which causes damage mainly in Photosystem II (PSII) by inactivating electron carriers and causing oxidative stress in reaction centres (Aro et al., 1993). The light in the fridge was likely too intense for the moss as they require little direct sunlight, and this could mask the results observed when using chlorophyll fluorescence. Any potential damage metal ions may have cause could therefore be covered up by other factors such as light damage and might explain the overall decrease in Fv/Fm values across all samples with negligible difference between the metal solution or the concentration. This makes it difficult to compare the results from the experiment to the observational study at Steinsjøen shooting range and the conclusions that can be drawn from the experiment are limited.

5.3 Contamination and restoration

It is important to put the levels of metal contamination from Steinsjøen shooting range into context. The amount of metal pollution that was analysed is consistent with previous studies done in Steinsjøen (Rognerud, 2003) and the Norwegian authorities defines contaminated soil and water (TA-2553) with values far exceeding what was observed at Steinsjøen. For example, Soil containing >100 mg/kg Cu is considered very good (Tremoen, 2009), which corresponds to >100 000 µg/kg Cu, far above the highest levels of Cu that were analysed from any sample collected in the observational study. Keep in mind the analysis was of the metal in the water drained out of peat moss or of the moss biomass, not the local water directly. However, this suggest that the three fields at Steinsjøen shooting range were not contaminated to a large extent and this is perhaps reflected in the results, which show no significant difference in Fv/Fm values when comparing contaminated peat moss with uncontaminated moss, suggesting the moss is not experiencing stress or damage to the photosynthetic apparatus. Given that the three

shooting fields are no longer in use but are surrounded by fields that are and there is still metal leaching and corrosion from objects in the fields that will leak metals for centuries, it might seem desirable to remove as much contaminated soil as possible and restore the area. However, this approach poses many challenges. Removing contaminated soil from peatlands is quite time consuming and invasive and there are large distances to transport vast amounts of organic matter and challenges in storing large amounts of contaminated soils (Mariussen et al., 2017). Removing contaminated peat is also very costly both in terms of economic costs and the heavy burden to the environment (Bolstad, 2015). Permanent damage occurs in the process of removing contamination, such as changing the natural hydrology of the area, impact by heavy machinery, leaking metals into other unintended area, draining large amounts water when drying peat and the release of greenhouse gasses (Bolstad, 2015).

Peat moss has in many cases been used as a natural adsorbent to treat pollution precisely because they easily bind metal ions and thus function as an effective filter (Astolfi et al., 2016). Thus, metal contamination may be less of a threat to the peat moss itself than other plants. Chlorophyll fluorescence used in this paper suggest that the vitality of the peat moss is not affected by the metal contamination. Other less invasive methods of removing metals may be desirable and a greater focus perhaps should be placed on limiting metal contamination spreading to other areas.

Conclusion

The results show no apparent negative effects on photosystem II by metal contamination by using Fv/Fm values. The Fv/Fm values for most samples were not sufficient to argue that the samples from the shooting range differed significantly from the uncontaminated control group, in fact some Fv/Fm values from the shooting range were higher than what was observed in the control group, which was the opposite of what was expected. Likewise, the results failed to demonstrate any clear difference in what type of trace element the peat moss is exposed to and what that does to PSII. Fv/Fm values decreased overtime in all samples, regardless of concentration or meal, except for possibly Cu which at 1000 µM displayed the lowest observed Fv/Fm values. Factors such as high light intensity and neutral pH water likely interfered with the results. Therefore, this paper argues for non-invasive methods of metal removal, cleaning and restoration. The results suggest that the peat moss is not harmed by the current metal contamination levels at the fields 6, 25 and 26 at Steinsjøen shooting range.

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Appendix

Table A-1: One-way Anova Kruskal-Wallis test with Sb samples. Comparing the different concentrations with the control at 0 µM (intercept) with Fv/Fm being the dependent variable.

Experiment - Sv Fv/Fm

Predictor	Estimate	SE	t	p
Intercept[a]	0.62671	0.0132	47.35	<.001
t (days)	-0.00538	4.94e-4	-10.90	<.001
Concentration (µM):				
1 – 0	-0.02429	0.0150	-1.62	0.107
10 – 0	0.02636	0.0150	1.76	0.080
100 – 0	0.06589	0.0150	4.39	<.001
1000 – 0	0.03531	0.0150	2.35	0.019

[a] Represents reference level

Model Fit Measures (Sb)

Model	R	R^2	Adjusted R^2
1	0.564	0.319	0.309

Table A-2: One-way Anova Kruskal-Wallis test with Pb samples. Comparing the different concentrations with the control at 0 µM (intercept) with Fv/Fm being the dependent variable.

Experiment - Pb Fv/Fm

Predictor	**Estimate**	**SE**	**t**	**P**
Intercept[a]	0.56624	0.0176	32.1171	<.001
t (days)	-0.00152	6.58e-4	-2.3176	0.021
Concentration (µM):				
1 – 0	-0.01911	0.0200	-0.9556	0.340
10 – 0	-0.00176	0.0200	-0.0879	0.930
100 – 0	0.03163	0.0200	1.5813	0.115
1000 – 0	-0.03570	0.0200	-1.7849	0.075

[a] Represents reference level

Model Fit Measures (Pb)

Model	**R**	**R^2**	**Adjusted R^2**
1	0.223	0.0496	0.0358

Table A-3: One-way Anova Kruskal-Wallis test with Cu samples. Comparing the different concentrations with the control at 0 µM (intercept) with Fv/Fm being the dependent variable.

Experiment - Cu Fv/Fm

Predictor	**Estimate**	**SE**	**t**	**P**
Intercept[a]	0.6513	0.0150	43.476	<.001
t (days)	-0.0120	5.56e-4	-21.540	<.001
Concentration (µM):				
1 – 0	0.0131	0.0170	0.772	0.441
10 – 0	-0.0592	0.0170	-3.488	<.001
100 – 0	0.0185	0.0170	1.090	0.276
1000 – 0	-0.0777	0.0170	-4.577	<.001

[a] Represents reference level

Model Fit Measures (Cu)

Model	**R**	**R^2**	**Adjusted R^2**
1	0.776	0.602	0.596

Table A-4: One-way Anova Kruskal-Wallis test with Zn samples. Comparing the different concentrations with the control at 0 µM (intercept) with Fv/Fm being the dependent variable.

Experiment - Zn Fv/Fm

Predictor	Estimate	SE	t	p
Intercept[a]	0.63461	0.0102	62.15	<.001
t (days)	-0.00419	3.81e-4	-10.99	<.001
Concentration (µM):				
1 – 0	-0.02844	0.0116	-2.46	0.015
10 – 0	0.02749	0.0116	2.37	0.018
100 – 0	-0.02367	0.0116	-2.04	0.042
1000 – 0	-0.02600	0.0116	-2.24	0.025

[a] Represents reference level

Model Fit Measures (Zn)

Model	R	R^2	Adjusted R^2
1	0.557	0.310	0.300

Note. Models estimated using sample size of N=350

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