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# SELECTION OF SUBSTRATE AMENDMENTS IMPROVING HYDROLOGICAL PROPERTIES OF EXTENSIVE GREEN ROOFS IN JAPAN

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**TRAVAIL DE FIN D'ÉTUDES PRÉSENTÉ EN VUE DE L'OBTENTION DU DIPLÔME DE MASTER BIOINGÉNIEUR EN SCIENCES ET TECHNOLOGIES DE L'ENVIRONNEMENT**

**ANNÉE ACADÉMIQUE 2018-2019**

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# Abstract

Stormwater management constitutes a major problem in cities. The increase in impermeable surfaces due to urbanization reduces the absorption of this water and exacerbates the problem. Green roofs are one of the solutions that would lower these volumes of runoff water. Nevertheless, they can act as a source of pollutant. Therefore, this study is conducted to analyze the impact of several substrates on runoff water in order to reduce its volume and optimize its quality. Different mixtures composed of conventional substrate, coco peat and rice husk were tested. While the results showed that different substrates did not have significant impacts on the sum of drainage water collected during the entire period, some collections enable drawing conclusions. In these latter, the RH50 emerged as the most suitable to retain water. Quality analyses highlighted that the conventional substrate was a source of $NO_3$ and absorbed Ca, Mg and Na. The increase in the percentage of coco peat leads to an elevation of the C and Fe concentration while decreasing the Cl concentration. At the same time, the rise in the percentage of rice husk induces a source of $SO_4$, Ba, K and V while it absorbs Ca, Mg and Na.

**Résumé**

La gestion des eaux de ruissellement est un problème majeur dans les villes. L'augmentation des surfaces imperméables dues à l'urbanisation diminue les possibilités d'absorption de cette eau et ne fait qu'accroitre la problématique. Les toits verts constituent l'une des solutions permettant de réduire les volumes d'eaux de ruissellement. Néanmoins, ils peuvent être source de pollution. . L'objectif de cette étude est donc d'étudier l'impact de différents substrats sur les eaux de ruissellement afin d'en réduire le volume et d'en optimiser la qualité. Différents mélanges composés de substrat conventionnel, de tourbe de coco et de balle de riz ont été testés. Bien que les résultats aient démontrés que les différents substrats n'avaient pas d'impact significatif sur l'eau de drainage récoltée durant l'ensemble de la période étudiée, certaines collectes permettent de tirer des conclusions intéressantes. Dans ces collectes, le RH50 a été identifié comme le plus apte à retenir l'eau. Les analyses de qualité ont démontrées que le substrat conventionnel était source de $NO_3$ et absorbait le Ca, Mg et Na. L'augmentation du pourcentage de tourbe de coco entraine une augmentation de la concentration en C et Fe tout en diminuant la concentration Cl. Parallèlement, la croissance du pourcentage de balle de riz induit une source de $SO_4$, Ba, K et V tandis qu'il absorbe le Ca, Mg et Na.

# List of abbreviations

| | |
|---|---|
| CEC | Cation exchange capacity |
| S | Substrate with 100% of conventional soil |
| CC10 | Substrate with 90% of conventional soil and 10% of coco peat |
| CC25 | Substrate with 75% of conventional soil and 25% of coco peat |
| CC50 | Substrate with 50% of conventional soil and 50% of coco peat |
| CC100 | Substrate with 100% of coco peat |
| RH10 | Substrate with 90% of conventional soil and 10% of rice husk |
| RH25 | Substrate with 75% of conventional soil and 25% of rice husk |
| RH50 | Substrate with 50% of conventional soil and 50 % of rice husk |
| AV2 | Two-ways ANOVA |

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# 1. Introduction

The current population on earth exceeds 7.6 billion people and continues to grow day after day. Among them, 55% of the world's population lives in urban areas. Although some cities in low-fertility countries experienced population decline between 2000 and 2018, it is now expected that this demographic trend will reverse by 2030 and increase again. The percentage of inhabitants in cities is expected to increase to 68% by 2050 (United Nations, 2018). This phenomenon is called urbanization.

At the moment, Tokyo is the largest city in the world with a population of 37 million. By 2030, the world is expected to have 43 megacities of more than 10 million people. (European Commission, sd) As the world becomes increasingly urbanized, the sustainability of its development will depend on the proper management of this growth.

In Japan, a period of strong economic growth has followed urbanization. Due to a continuous rural exodus between 1950 and 2000, the urban population reached 78.6% (ECOSOC, 2014). The exodus and growth of cities have induced an increase in impermeable surfaces. This waterproofing reduced infiltration and evaporation of rainwater. It therefore increased runoff to sewers which can also be overwhelmed by high-intensity hydrological events (Carpenter et al., 2016). This can lead to complications with water management in cities.

It seems essential to implement actions to improve the living conditions of urban dwellers. Three dimensions must be taken into account: economic, social and environmental. The advantages of the agglomeration must be maximized while minimizing environmental degradation and all negative aspects. Policies to manage urban growth must ensure access for all to social infrastructure and services, with a focus on housing, education, health care, work and a safe environment (United Nations, 2018).

Implementing green roofs is part of the actions to improve the city dwellers quality of life. Indeed, they provide many benefits both privately and for the community. These benefits include improved air quality, reduced heat island effect or better water management (Townshend, 2007). However, the implementation of green roofs can also lead to deterioration in the quality of runoff water with the leaching of nutrients or metals during rainfall (Buffam et al., 2016). Indeed, when water passes through the soil, it can carry some soil elements or ions that will end up in the drained water. For example, green roofs can contain phosphorous in significant amount. Unfortunately excessive phosphorus concentrations in the environment can induce eutrophication of water (Karczmarczyk et al., 2018).

Unfortunately, little attention is paid to the water quality, especially the pollutants that can emanate from the construction elements of green roofs (Karczmarczyk et al. , 2018). Excessive concentrations of certain elements can be harmful to humans or the environment. According to Roesner (1999), it is essential to combine water quality as well as design of green roofs in order to improve runoff water quality. Further research is required to improve this quality. Therefore, it is necessary to analyse the quality of runoff water from different substrate in green roofs. These different substrates can have distinct proprieties with impact on the water quality, but also on the amount of drained water. Indeed, the bigger the retention capacity of a substrate, the less water it will reject in the environment. This criterion is even very much studied throughout the scientific literature.

This study will test the retention and purifying capacities of different substrates and substrate amendments simultaneously. It is known that coco peat has good water retention and a high Cation Exchange Capacity (CEC) ratio. This prevents the leaching of nutrient ions and makes them available to the plant (Nature's Bounty PLC, sd). Rice husk, on the other hand, is known to increase the waters nutrient charge and eliminating metals (Huang et al., 2013). To explore these properties, we will mix different percentages of rice husk or coco peat with a conventional substrate and analyses will be carried out on water quality and quantity.

# 2. State of the art

## 2.1 Green roof

A green roof is defined as a flat or low-pitched roof covered with vegetation and the layers necessary for its development (Noirfalisse, 2015). The first green roofs were created centuries ago. Indeed, in ancient times, primary green roofs were already in place. An obvious example is the hanging gardens of Babylon, built around 500 BC (Getter and Rowe, 2006). They were generally composed of sod roofs covered with soil and plants often used for agricultural, residential and ceremonial purposes. They provided shelter from the elements, but unfortunately were not waterproof and problems with wildlife could be encountered (Jörg Breuning, sd).

Thereafter, modern green roof technologies only began to develop in the 20th century. Germany was the pioneer of the first green roofs (Oberndorfer et al., 2007). Indeed, some roofs were made of highly flammable materials and were covered with sand and gravel for their non-combustible property. Then, plants colonized its roofs and many years later, it was observed that 50 of them were still intact and waterproofs (L'Etudiant, sd). Subsequently, 1970s were a pivotal period in international environmental awareness. Many ecological disasters, such as oil spills, have helped to raise awareness (Bodson, 2010). Thereafter, interest in green roofs is growing in many countries and they have been developed over the years to reach the current level of performance.

### 2.1.1 Technical aspects of green roof

Green roofs can be classified into two categories, "intensive" and "extensive" green roofs. On the one hand, intensive green roofs are characterized with a heavier weight and a greater depth (from 200 mm to up to 2000 mm). It allows a greater variety of plants, including shrubs and trees, which require a greater depth. Their implementation requires greater maintenance and therefore higher capital costs. They can also be used as gardens. On the other hand, extensive green roofs are characterized by their low weight and a thin soil (between 50 mm and 150 mm). They generally consist of herbs and Sebums. They require little or no irrigation and do not require a lot of maintenance. Indeed, maintenance two to three times a year is sufficient to avoid the growth of undesired plants. It reduces capital costs compared to the intensive roof. Nevertheless, in green beds, intensive green roofs are more efficient than extensive green roofs in terms of outflow quality (Beecham and Razzaghmanesh, 2015).

The choice of roof type therefore depends on the expectations and budget of the applicant. If required, a combination of the two is also possible. The components are basically the same for both of them and are shown in Figure 1.


Figure 1 - Representation of the different layers of a green roof

They are located directly on the roof and commonly consist of the following layers: a roof deck, a waterproofing membrane and an insulation layer, a root barrier and a protection layer, a drainage element and a filter fabric, to finish with substrate and plants (Vijayaraghavan, 2016).

### 2.1.2 Advantages

Green roofs make an environmental, social and visual contribution. They are very useful in improving the quality of life in cities and have many benefits both privately and collectively. The designs vary according to the regions and the desired objectives. Studies have demonstrated the many services that green roofs can provide to improve the quality of life in cities. Santamouris (2014) studied the impacts of green roofs on the urban heat islands effect and concluded that applied on a city scale, it can permit to reduce the temperature between 0.3 and 3K. They can also reduce air pollution. For example, a city like Chicago could remove up to 2049.89 metric tons of pollutants if all the rooftops were covered with intensive green roofs (Jun Yang et al,. 2008).

At the building level, various advantages are also observed. Indeed, these roofs provide better insulation of the building for heat and sound and therefore better energy efficiency. It may be useful to specify that the insulation may be more or less important depending on the plants used on the roof (Cox, 2010). Green roofs are also an opportunity for developing useful open spaces. They can be used for food production purposes and bring ecological, wildlife and aesthetic value to roofs (Townshend, 2007). In addition, it has been proven that the green space view increases human health. It reduces stress, lowers blood pressure and increases positive feelings (Ulrich et al., 1991).

Finally, one of the most valuable benefits is water management. Indeed, for a given rainfall, a green roof can reduce the water peak and delay runoff compared to a traditional roof. This is illustrated in Figure 2. Part of this water will be drained while the other part will be retained by the soil. This is due to the retention capacity of substrate in green roofs. The retained water can be used and/or transpired by the plants or evaporated. This explains the difference in volume runoff between the different roofs (Berndtsson, 2010).

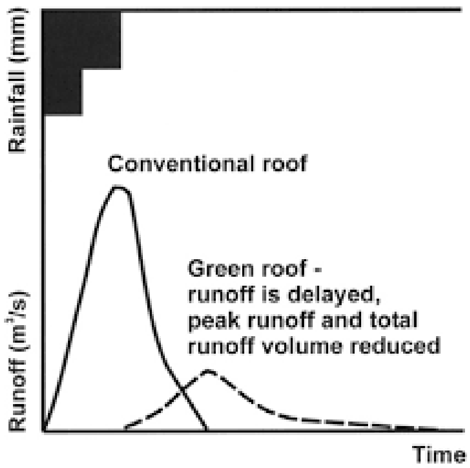


**Figure 2 - Example of runoff peak with green and traditional roof by a given rain event (S.Muhammad et al., 2018)**

Water absorption helps to counter the increase in impermeable surfaces due to urbanization and reduce flooding in cities. For optimal retention, various factors must be taken into account: slope, plants, design and soil depth. DeNardo et al. (2005) and VanWoert et al. (2005) even show that some intensive green roofs would achieve 100% water retention. Carter and Jackson (2007) have shown that the impact of green roofs in cities depends on the size of rainfall events. Green roofs can be useful for managing small storms in developed areas. However, they are not sufficient on their own, for a complete stormwater management. In addition, the passage of water through the soil can filter out certain elements or pollutants. It also reduces the negative effects that rainwater can have after it has run off onto the traditional roofs such as acidification. Unfortunately, the substrate can also be a source of pollution with some elements.

### 2.1.3 Water runoff studies on green roofs

Over the years, many studies have been carried out to optimize the performance of green roof components. Variations may be observed depending on the depth of the substrate, the kind of substrate, the type of plant or the local weather.

Schultz et al. (2018) analyzed the water runoff performance differences between a 75 mm deep substrate and a 125 mm deep substrate. The most obvious difference was when the soil was below saturation level before precipitation and for small rains events. In both cases, the deepest substrate absorbed significantly more water. This suggests that longer the dry period before an event is, better the performance will be. This can therefore cause problems in wet climate regions because it makes it more difficult to retain large quantities of rainwater. At the same time, Moran et al. (2005) show that some green roofs with a substrate depth greater than 10 cm could have water retention of 66% to 69%.

It has also been published that the different seasons induce variations in the performance of green roofs. Some elements, like major base cations (K, Na, Mg, Ca) or bioactive element (N, C, P) are found in higher concentrations during summer and usually less in winter. At the same time, other elements do not seem to be affected by these variations, such as the pH and some dissolved metals (Fe, Al and Zn). It may be important to note that these variations are more present in water from green roofs than from traditional roofs (Buffam et al, 2016). These variations should be correlated with environmental variables, especially the temperature that can influence biological activity, chemical processes, evapotranspiration or plant absorption (Wang et al., 2012).

Following these studies, it is very important to remember that green roofs, because of the leaching of elements, can act as a source of water pollution (Beecham et al., 2014). The materials and substrates used to install a green roof can have a significant impact on water quality. For example, a substrate composed of 15% compost will leach phosphorus and nitrogen into its runoff water (Moran et al, 2005). Unfortunately, these two elements released into the environment can induce eutrophication in surface water (Carpenter 1998) which leads to a decrease in biodiversity. It therefore seems very important to optimize water quality.

Finally, Hatt et al. (2007) and Henderson et al. (2007) identified that without plants, soil filtration can act more as a source than a sink of pollutants. In the same direction, Beecham and Razzaghmanesh (2015) observed that the pollutant concentration in non-vegetated bed runoff water was higher. The benefit of plants could therefore be linked to the leaching from the growing media and the uptake of plants into green roofs. The selection of plants can be made according to several criteria. Succulent plants can be favoured for extensive green roofs because of their ability to retain water. Their crassulacean acid metabolism (CAM) allows them to minimize water loss (Technical Preservation Services, sd). It avoids the need for an irrigation system. Unlike sebum, which is favoured for its transpiration capacity and therefore for reducing runoff water, but may require irrigation. (Nagase and Dunnett, 2012). The presence of plants during the experiment therefore seems essential, although attention is focused on the substrate.

The choice of substrates and plants are crucial and must be adapted to the desired objectives as well as to the local climate. Indeed, the climate will undeniably influence water retention and plant growth, which will have a direct impact on the efficiency of the green roof.

### 2.1.4 Green roof substrate

Some studies, such as Dunnett et al. (2008) and VanWoert et al. (2005) show that the most significant influence on the water retention capacity of green roofs comes from the type of substrate and its depth. It is very important to combine properties that are useful for plants (plant available nutrient and high CEC) as well as beneficial soil properties (low bulk density and rapid drainage) (Ampim et al., 2010). This study aims at optimizing both water retention and drainage water quality on green roofs. For this purpose, two substrates have been selected: Rice husk and coco peat. They will be added to a conventional substrate. The use of recycled materials and by-products promotes sustainable, eco-friendly development and reduces costs. It is important to specify that the values given below are theoretical values and not those of the soils used.

### *Rice Husk*

Rice is a very important crop worldwide. It feeds more than half of the population, mainly in Asia and more importantly in some countries such as China, India and Japan (Hu, 2012). Considering the example of Japan, although consumption has tended to decline over the past 30 years, rice production remains high (Chern et al., 2003). On average, 12,000 thousand tonnes were produced during year 2000 (Perspective monde, sd). This large production can induce a large amount of by-products or waste. For example, rice husk is the coating on a grain of rice. It is a by-product of rice production and is produced during the rice milling stage. It was considered for a long time as a waste and was often thrown away or burned down. It is composed of silica and lignin and protects the seed during the growing season. One kg of milled white rice produces about 0.28 kg of rice husk (Rice Knowledge Bank , sd). If we take into account the production of the year 2000, Japan had about 3,360 thousand tonnes of rice husks. Fortunately, we know now that rice husk can be used in many forms. There are rice husk in its loose form, which is mainly used for energy production. Rice husk briquettes and pellets, to increase materials density and combustion performance. Charcoal rice husk ash, used in smaller quantities, as a soil amendment and as additive in some construction materials. Finally, Carbonized rice husk which can be used as soil amendment, for processing fertilizer, etc (Rice Knowledge Bank , sd). We will focus our attention on this latter.

Production is carried out by thermal decomposition of the rice husk at low temperatures (less than 700°C) and under a restricted supply of oxygen ($O_2$) (Rice Knowledge Bank , sd). The rice husks carbonization permits improvement of the water-holding capacity (Oshio et al., 1981). Following this carbonization, rice husks has a micro-porous structure and a bulk density of 150kg/$m^3$ (Haefele et al., 2011). Williams et al. (1972) have shown that the use of rice husk in soil can improve soil properties. It can enhance soil pH and decreasing general soil bulk density. Its presence increases the availability of the elements and allows removing heavy metals from the system (Williams et al., 1972). Moreover, its presence on the island is an advantage: there is no need to import. Nor can it be overlooked that being in the past considered as a waste from rice production, it is therefore a cheaper substrate.

These many characteristics make it an interesting substrate for our study. Indeed, its water retention capacity and water pollution control are significant assets.

### *Coco peat*

Coco-peat is a ligno-cellulosic light fluffy biomass produced throughout the fibre separation from the ripened coconut husk. While long fibres are used in different sectors, such as automotive or brushes, shorter fibres (≤ 2mm) will be used as a planting medium. Short fibres will be cut, crushed and washed to produce coco peat (Alzorg et al., 2013). It is useful to know that coco peat is not “peat” at all. Its name comes from its similarity to the "peat moss" in appearance and function. Unlike the peat moss, which emits billions of tonnes of greenhouse gases per year, the coco peat is renewable and sustainable (HortGrow, 2018).

In recent years, coco peat has been used in green roof substrates or biofilters (Vijayaraghavan, 2016). Its air-filled porosity (≈ 11%) and its high water holding capacity (≈ 46%) makes an ideal growth medium for the plants. A study by Vijayaraghavan et al. (2016) explained different criteria of the coco peat such as its high CEC (≈ 51 meq/100 g) and low bulk density (≈ 115 kg/m3). Its high CEC potentially allows the material to act as sorbent for metal cations. Bound ions in the soil structure, are less leachable and more available for the plant. (Nature's Bounty PLC, sd) Then, this CEC can potentially contribute to a clean-up of water. Moreover, its high value of hydraulic conductivities (≈ 3280 mm/h) allows avoiding water ponding on the surface of the substrate (Vijayaraghavan et R.S. Praveen, 2015). Beyond its physical characteristics, coco peat is eco friendly (Nature's Bounty PLC, sd).

The many uses of this substrate make it, as well as rice husk, an inevitable interest in our study.

### 2.1.5 Green roof drainage

#### *Water amount*

First of all, all precipitation does not reach the ground: drops can be intercepted by the foliage. Then, the water that reaches the ground runs off, infiltrates and moistens the soil. A quantity of water, corresponding to the field capacity, will be absorbed in the substrate. In other words, water in the soil is found in three different forms: gravitational, capillary and hygroscopic water (Susha Lekshmi et al., 2014). Only capillary and hygroscopic water will be kept in the soil, while gravitational water will be drained.

The water retained in the soil will be absorbed through the roots, transpired by the plants or evaporated. This transpired and evaporated water explain the runoff amount reduction compared with conventional roofs. Finally, a reduced fraction will be drained after crossing the substrate. The speed at which drainage water exits depends on the permeability of the soil. This explains the decrease in stormwater runoff and the peak delay mentioned above.

Globally, the maximum water retention capacity of a soil corresponds to its field capacity. It is the soil moisture after drainage of macropores water by gravity (De Oliveira et al., 2015). Soil moisture before rain provides information on the remaining soil retention capacity. This water content can be measured with a gravimetric (% of weight) or a volumetric (% of volume) method (Berndtsson, 2010). Water retention capacity manages the storage of water in the soil but also its availability and distribution within the soil (Yang et al., 2014). It is influenced by many factors: previous soil water history, soil texture and structure, type of soil (clay or organic matter), temperature, depth of wetting, presence of impeding layers and evapotranspiration (Kirkham, 2005).

To optimize water retention in green roofs, the following factors are considered: slope, plants, climate, kind of substrate (texture, structure) and soil depth. Different studies contradict each other with the importance of the various factors, some show that substrate type, depth, vegetation and climate are the most important factors, while others assert that roof slope and rain properties are the most influential (Beecham and Razzaghmanesh, 2015). The impact of soil depth and the choice of plant species have already been discussed in the previous section. It will therefore not be repeated and other factors will be favoured.

First, a steep slope increases total runoff amount (Shishegar, 2015). Getter et al., (2007) shown that runoff retention capacities decrease as slope increase by analyzing runoff retentions quantities of different extensive green roofs with slopes of 2, 7, 15, 25 %. In addition, a roof is considered inaccessible if its slope exceeds 30° (Townshend, 2007).

Then, within the different kinds of substrates, soil particles, quantities of organic matter and bulk density are really important criteria. Indeed, they are widely used in pedotransfer functions (PTFs) to predict soil water retention (Kern, 1995). The closer the soil texture is to clay, the greater its water retention capacity will be. It is due to its adsorptive effects. Conversely, the closer it gets to the sand, the weaker its retention capacity will be (Agralis, sd). Its particles are more spaced apart and water circulates more quickly. In the same way, the presence of organic matter increases the water retention owing to its affinity to water. It also has an influence on soil structure and bulk density. At the same time, bulk density is related to porosity (Yang et al., 2014).

Finally, climate is impacted by different factors, such as rainfall intensity. Indeed, the intensity of the rains as well as the intervals between them has a major impact on water retention. As rainfall intensity increased, the water-retaining capacity decreased (Lee, 2013). Then, the climate directly impacts evapotranspiration. Evapotranspiration includes transpiration by plants and evaporation from open water surfaces, soil, snow, ice and vegetation. It varies with temperature, wind, atmospheric pressure, soil moisture, water quality and depth, soil type, vapour pressure gradient, solar radiation. The more water is evaporated during periods of drought, the more water the soil will be able to absorb at the next rainfall. As mentioned above, periods of drought before rain have a significant impact on the soil's retention capacity (World Meteorological Organization, 2008). Nevertheless, the amount evaporated decreases with the amount retained in the soil. The capillary forces prevent water from leaving and the energy required to extract water is growing as the soil becomes poorer in water (Beauchamp, 2003).

### *Water quality*

Once the retention capacity of a soil is exceeded, the water drained from green roofs will be discharged into the environment. Unfortunately, little attention is paid to water quality although it can be a source of environmental damage. Indeed, some of the elements it contains can be detrimental to the environment or to humans. Of course, water quality is taken in comparison to water applied before its exposure to contaminants through the soil or surfaces encountered.

Two methods of analysis stand out in the various studies analyzing the effect of roofs on water quality. First, it is possible to compare the concentration of pollutants in the input water and the output water. A decrease in concentration would therefore mean that the green roof acts as a well. Then, it is possible to analyze the mass of pollutant released in the total volume of water passing through a $1m^2$ green roof during a given period. A decrease in pollution would result in a decrease in contaminant loads compared to the load present in same rainwater during the same period of time (Berndtsson, 2010).

To check the usefulness of a green roof, it may seem wise to compare the impact of its chemistry water runoff and that of a traditional roof. Berndtsson, Bengtsson, and Jinno (2009) and Zhang et al. (2015) agree that green roofs have a better ability to neutralize water acidity than impermeable roofs. Nevertheless, their opinions differ with regard to the concentration evolution of $NO_3^-$ and Cl−. Other elements would be found in higher concentrations in green roofs, such as F-, $SO_4^{2-}$, $K^+$, $Ca^{2+}$ and $Si^{4+}$. Zhang et al. (2015) compared stormwater quality results between green roofs and asphalt roofs and found that green roofs could neutralize the pH of rainfall and reduced the concentration of total suspended solids (TSS). However, as mentioned above, since water retention is more important for a green roof, the amount of water discharged into the environment will be less. The general quantity of released elements is therefore reduced (Berndtsson, Bengtsson, and Jinno, 2009).

The impact of a green roof on water quality is largely due to its components. The materials used, such as substrate composition, drainage layer and maintenance compounds (ex: fertilizer), can greatly influence the output water. Subsequently, precipitation dynamics, wind direction and local pollution sources also influence quality. The factors that can be used to improve quality and quantity are therefore the depth and kind of substrate, the vegetation and the physicochemical properties of pollutants (Berndtsson, 2010).

On the one hand green roofs can be used as a pollutant remover thanks to their filtration and pollutant absorption capacity. As mentioned above, green roofs have a good ability to neutralize acidity, which helps to reduce the acidity of rainfall. This should protect the downstream receiving waters from acidification and thus preserve underwater life from possible changes in their environment (Beecham and Razzaghmanesh, 2015). In addition, a reduction in TSS is visible. This may be due to the presence of soil and filter layers that prevent particles from flowing into the runoff water (Zhang et al., 2015). The impact of green roofs on the different forms of nitrates is more controversial. While some studies show that green roofs act as nitrogen wells (Berndtsson et al., 2006), (Berndtsson, Bengtsson, and Jinno, 2009), others consider them as a source (Zhang et al., 2015). This nitrogen concentration would be related to the type of soil, age and roof maintenance (Berndtsson, 2012).

On the other hand, green roofs can be source of pollutants due to releases from building components, plants and fertilizers (Beecham and Razzaghmanesh, 2015). Phosphorus, generally found in the $PO_4$-P form, is found mainly in runoff water from extensive green roofs. In contrast, intensive green roofs do not show any significant difference (Berndtsson, Bengtsson, and Jinno, 2009). Phosphorus retention will increase proportionally with the planting of the green roof (United States Environmental Protection Agency, 2009) and inversely proportional with the addition of fertilizer (Moran et al., 2005).

Green roofs are also a source of carbon. Indeed, many studies have shown increases in concentration between green roofs drained water and rainwater. Berndtsson, Bengtsson, and Jinno (2009) indicated that an extensive green roof studied, composed of 5% organic components, received a 20 times higher carbon concentration its water than in precipitation. Carbon would come mainly from soil organic matter and plant decomposition.

Green roofs also increase the concentration of metals. Vijayaraghavan et al. (2012) indicated that drained water from green roofs may contain concentrations of heavy metals such as Fe, Cu and Al. Nevertheless, Berndtsson (2010) and Berndtsson, Bengtsson, and Jinno (2009) agree that green roofs are generally not significant sources of metals.

Green roofs can also be a source of some anions such as $F^-$ $Cl^-$ $SO_4^{2-}$ or some cations like $K^+$ $Ca^{2+}$ $Si^{4+}$, probably from substrate material. Vijayaraghavan et al. (2012) also found high concentrations of Na and Mg in runoff water. For example, Berndtsson, Bengtsson, and Jinno (2009) demonstrated that in his study, the concentration of K in green roofs drained water was seven times higher than in rainwater. Simultaneously, the concentrations of Ca were 10 times higher. This significant increase would be due to the dissolution of the soil material.

# 3. Material and Methods

## 3.1 Study site

The experiment is implemented in Japan, in Nishi-Chiba on the site of Chiba University. The site is located at latitude of 35.6270 and longitude of 140.1043 as shown in Figure 3.


**Figure 3 - Location of the study site in Nishi-Chiba, Japan (35.6270; 140.1043)**

Japan is an island country in East Asia. It consists of 4 districts seasons with a climate varying from subarctic in the north to subtropical in the south. More specifically, the eastern part of Japan, where the study area is located, experiences cold winters and hot and humid summers (Japan Meteorological Agency, sd). To illustrate the weather, the average temperature and rainfall over the last 10 years during each month in Chiba is shown in Figure 4.

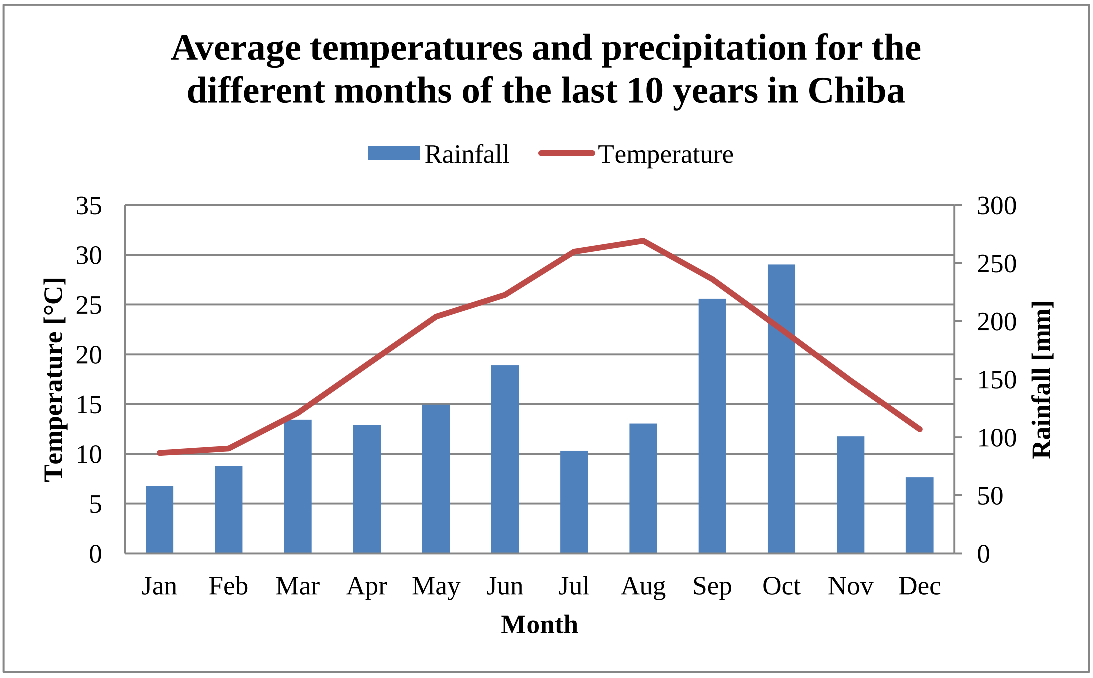


**Figure 4 - Average monthly temperature and precipitation of the last 10 years in Chiba (Japan Meteorological Agency, sd)**

It can be observed that September and October were the rainiest month. This is related to the typhoon season that takes place from July to October in south of Japan.

The experiment takes place on the roof of a 10-storey building. On the roof, architectural elements on two sides of the site induce a variation in shading during the day. The area is shown in Figure 5.

**Figure 5 - Zone of implementation of the experiment**

## 3.2 Experimental setup

The experiment aims at quantifying the impact of different extensive green roof substrates on quantity and quality of water drainage. To do this, we reproduced miniature extensive green roof modules in 70x40x21cm (length, width and height) containers. They were subject to artificial rainfall events, which allow accurate quantification of the incoming and outgoing water quantity and composition. The modules are placed outside and subjected to the normal meteorological conditions. We therefore monitor substrate moisture contents throughout the experiment, so that we can relate rainfall, initial moisture status and outflow characteristics to each other.

Rainwater, sometimes considered non-polluting, can be acidic and contain nitrates. It may also contain pollutants depending on winds and local pollution sources. Tap water, which is often used for irrigation of green roofs, also has specific characteristics that vary locally. Next to the properties of the green roof substrate, the composition of the water source is therefore decisive for the drainage water quality.

### 3.2.1 The green roof modules

We placed the modules 25 cm above the ground level to provide space for the water collection system. Water is collected at the bottom of this container with a hole. This hole is connected with a pipe to send water in a closed container, the water collector. It is placed below the green roof container to protect it from wind and rain. A diagram illustrates this module in Figure 6. Moreover, an adapted closure has been made to close the collector and let the pipe in. This implementation avoids the presence of insects or extra water into the container. They have been realized in polystyrene with the Hotwire Foam Cutter HCM-S Plus. The evolution of the cap and the machine used are shown in Figure 7.

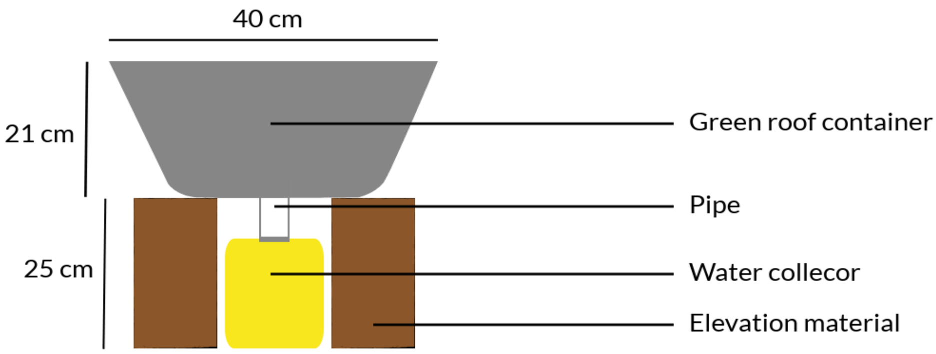


Figure 6 - Illustration of an experimental unit

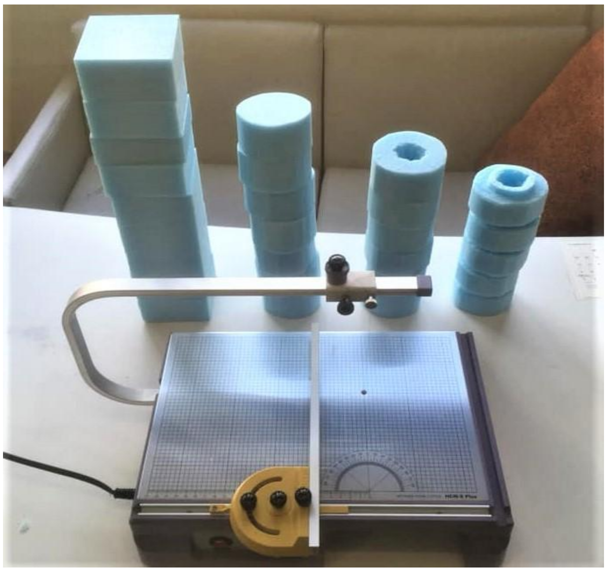

Figure 7 - Evolution of the production of polystyrene closures and the tool used

Green roofs are composed from the bottom to the top of a drainage layer, a 15cm substrate layer and turf. The drainage layer is a 33cmx50cm plastic grid. It is used to reproduce the passage of water inside a real green roof and decrease the soil transfer inside the pipe and the water collector. For the substrate layer, different soil mixtures are analyzed to see their amount of water runoff and their quality. Then, a layer of grass in the form of a mat is placed on the substrate. The grass was selected because of its high transpiration capacity and its sufficiently high growth, which reduces runoff water. (Nagase and Dunnett, 2012). The purpose is to optimise the retention capacity and water quality of extensive green roofs.

### 3.2.2 The substrate layer composition

Three different substrates are mixed at different percentages: conventional green roof substrate, coco peat and rice husk. The conventional green roof substrate that has been chosen is the most commonly used substrate for green roofs in Japan. Viva soil is a mixture of organic nutrients, moist porous minerals and other ingredients necessary for plant growth. It is a moist, light and porous artificial soil composed of 10% of organic matter (Toho Leo, 2018). Coco peat and rice husk have been selected on the basis of their various physical criteria that could potentially improve soil properties.

Firstly, a substrate composed exclusively of conventional soil has been installed. Secondly, some substrates consist of conventional soil mixed with 10, 25 ad 50% of rice husk. The test cannot be performed with 100% of rice husk because its weight is composed of 67% volatile matter (Aquaculture, accessed 2016) and its installation in green roof would be complicated. Thirdly, some substrates consist in a mixture of conventional soil with 10, 25 and 50% of coco peat. Finally, the last substrate is composed strictly of coco peat. Each substrate is reproduced five times. The percentages have been achieved by added volumes from the different soils until a soil depth of 15 cm is reached. These different substrates are represented in Table 1. A colour code for each type of substrate is used to simplify the understanding of the implementation of the experiment shown in Table 1.

**Table 1 - Composition of the different substrates**

| Substrate | Conventional soil | Coco peat | Rice husk | Abbreviation |
|---|---|---|---|---|
| **1** | 100% | - | - | S |
| **2** | 90% | 10% | - | CC10 |
| **3** | 75% | 25% | - | CC25 |
| **4** | 50% | 50% | - | CC50 |
| **5** | - | 100% | - | CC100 |
| **6** | 90% | - | 10% | RH10 |
| **7** | 75% | - | 25% | RH25 |
| **8** | 50% | - | 50% | RH50 |

### 3.2.3 The spatial lay-out of the modules

The available space on the roof is divided into different homogeneous areas. Indeed the presence of shadow on some places can influence the evapotranspiration and thus the water amount. These different areas are representing on the Figure 8 with the thick black lines and each of them is represented by a number placed next to the block. Once the blocks were made, the different substrates were randomly placed inside. It is also represented at the Figure 8.

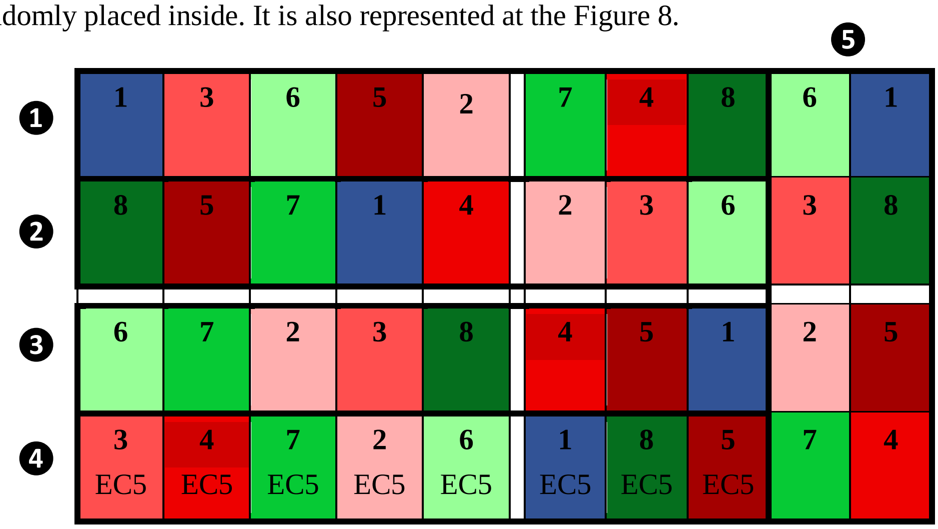


**Figure 8 - Repartition plan of the different substrates and blocks**

A photo of the final implementation of the experiment is shown in Figure 9.

Figure 9 - The spatial layout of the extensive green roof modules

## 3.3 Data Collection

We collected drainage water each week during six weeks from April 22 to May 28 2019. Once a week, the various containers are irrigated with four litres of tap water to simulate rain using a watering can. Unfortunately, the uncertain rainfall during the planned study period made us prefer tap watering. However, a comparison between theoretical rainwater in Japan and the tap water was made to see the quality differences. In addition, the watering was carried out in order to get as close as possible to the usual rainfall in terms of quantities. Then, each bed has been watered two litres by two litres. The two watering are spaced about an hour apart (time required to water the entire area a first time). This reduces the intensity of rainfall in order to get closer to a natural rainfall. Each two litres application is completed in 25 seconds. This corresponds to a total rainfall of 15 mm. We therefore obtain two rains at approximately 1 hour intervals with an intensity of 1080 mm/h each. Irrigation less intense and therefore longer over time would be preferable to approach normal rainfall but manually this was not achievable. In parallel to the watering, 500ml of the tap water have been kept to allow a comparison between the water that penetrate the soil and the water that come out. Water quality depends on the quality of the water source and its interaction with the different substrates materials and their respective geochemical properties.

The amount of added water chosen was based on a compromise between regional rainfall and soil retention capacity. Indeed, according to data from the Japan Meteorological Agency over the past 10 years, the average rainfalls in May and June in Chiba are 128 mm and 162 mm respectively. In addition, the probability of a precipitation of at least 1 mm occurring during May and June is 35% and 42% respectively (Cedar Lake Ventures, Inc., sd). These data gives us an average rainfall of 12.2 mm per day of rain over the considered months. It would mean adding 3.3 litres of water.

At the same time, conventional soil information revealed that the soil had a water retention capacity of 100 (±20) l/m³ at PF between 1.8 and 3. Considering that the soil volume of the container is approximately 0.038 m³, we can deduce that it would be able to retain 3.8l of water. It is important to notice that the supply of drained water should also be sufficient for analysis. The added water was therefore decided at four litres to ensure sufficient water collection. On a large scale, water runoff may be present due to the high intensity of watering. However, the side walls of the container prevent water from flowing.

Thereafter, the water is collected 24 hours later for reasons of convenience and to homogenize all the collections despite their different watering hours. This also allows the water to drain off and allows several soil moisture measurements during the collection period. The watering date is preferably each Monday, but depending on the rains, the day may change. Indeed, if Monday is rainy, the water brought in is not homogeneous with the other harvests. It is therefore essential to select a period of the week with two consecutive dry days. The dates of the waterings are shown in Table 2.

**Table 2 - Dates of the various collections**

| | Collection 1 | Collection 2 | Collection 3 | Collection 4 | Collection 5 | Collection 6 |
|---|---|---|---|---|---|---|
| Date | April 22 | April 28 | May 8 | May 12 | May 22 | May 26 |

The quantity of water was measured directly at harvest using 500ml graduated containers and a 50ml pipette. 500ml of each container is kept for analysis. Drained water samples were preserved in the freezer at a temperature of 4°C. It is important to notice that since the experiment takes place outdoor, it is also influenced by rainwater that falls outside harvest periods. Among other things, the amount of water absorbed by the soil also depends on the length of the previous dry period and the rain intensity.

To measure the soil moisture, EC-5 or 5TE sensors have been vertically placed in the centre of each substrate in order to provide a general overview of its evolution. The containers concerned and there place are shown in Figure 8. EC-5 sensors only measure moisture while 5TE sensors measure moisture, temperature and conductivity. However, although two different sensors are used, only moisture dataset have been used. Moreover, as the sensors were insufficient to analyze all the tanks, a manual measurement was carried out in addition to this in order to cover all the experimental units. The soil moisture content is measured four times with a Delta-T Devices Ltd brand HH2 moisture meter which measures the volumetric water content. The first time is before the water application. The second time is after this one. Then, it is measured twice the next day, once in the morning and one in the afternoon. During each measurement, the moisture is measured in five parts of the container and only their average is used in order to use homogenized data. The difference in moisture between two periods of a roof can also be used to estimate evapotranspiration.

To complete the soil capacity analysis, photos of each green roof were taken and their percentages of green grass have been evaluated. The grass planted was in the form of a grass mat, its survival may differ between different substrates. This evaluation was carried out using an 8x13 grid of boxes positioned on the photos. Each box composed exclusively of plants have been evaluated as being composed of green grass or dead grass. A percentage was then calculated.

## 3.4 Samples analyses

Quality analyses were carried out on only 2 collections for financial and timing reasons. These collections were selected according to the greatest variations in water quantity during the various collections. Water quality is defined using its pH and concentrations of different ions and heavy metals. The list of elements and their analysis method are summarized in Table 3.

**Table 3 - Summary of analyses**

| Analyses | Elements measured |
|---|---|
| Total Organic carbon analyser | DOC |
| Portable pH meter | pH |
| Inductivity coupled plasma optical emission spectrometer | Al |
| | As |
| | Ba |
| | Be |
| | Bi |
| | Ca |
| | Cd |
| | Co |
| | Cr |
| | Cu |
| | Fe |
| | Ga |
| | K |
| | Li |
| | Mg |
| | Mn |
| | Na |
| | Ni |
| | Pb |
| | Se |
| | Sr |
| | V |
| | Zn |
| Ion chromatography | Cl |
| | $NH_4$ |
| | $PO_4$ |
| | $NO_2$ |
| | $NO_3$ |
| | $SO_4$ |

Firstly, Organic Carbon analyses were realized with a TOC-L Total Organic carbon analyser from Shimadzu brend. Before analyses, samples were filtered with Cellulose filter papers Advantec NO.131. They were sparged with N2 gas after putting acid to remove the inorganic Carbon. Secondly, pH was measured with a Portable pH meter HM-30P DKK-TOA. Finally, for the other analysis, the samples had to be centrifuged at 15,000 rpm for 6 min and the supernatant liquid was filtered. Then, metals were filtered through a 0.45µm filter (Millipore MFT Membrane filter; Millipore, Biller-ica, MA) and 0.25 ml of nitric acid (60 % - 61%) was added to 10 ml of each samples. They were analysed using Inductivity coupled plasma optical emission spectrometer; ICP-OES (Avio 500, PerkinElmer Japan). The analysis of several wavelengths ensures consistency of the results. An average of the consistent results of the different wavelengths of each element is used. At the same time, ions were filtered through a 0.2µm Chromato discfilter (GL science, Japan) and they were analysed using ion chromatography ( ICS-1100, Thermo Scientific Dionex Japan).

### 3.5 Statistical analyses

Statistical analyses of the different water quantities and their qualities are carried out in parallel. For water quantities, a two-ways Analysis of Variance (AV2) is performed using RStudio software. ANOVA is used to check the influence of substrate and block factors on the amount of final runoff water. Then, a Dunnett test is performed to verify if a significant difference exists between the most commonly used soil type in Japan (S) and other substrates with Microsoft Office Excel. A Tukey test is also performed to see the differences between the different substrates. For the quality study, an AV2 is also performed on each component analyzed. Again, a Dunnett test is performed, to determine the differences (significant or not) between the average of a control group (tap water) and the average of the water drained by the various substrates. The vegetated surfaces as well as the evaporation have also been subjected to AV2 to see their link with the different substrates.

# 4. Results and discussion

## 4.1 Complementary data

It is really important to give a general vision of climate during the collect to have a better comprehension of the different results. The Figure 10 shows the temperature and the rain during this period (Tenki, sd). The dates of watering and their water level are also included. It is important to remain cautious about this weather as it corresponds to the city of Chiba, so the impact may vary slightly in the results.

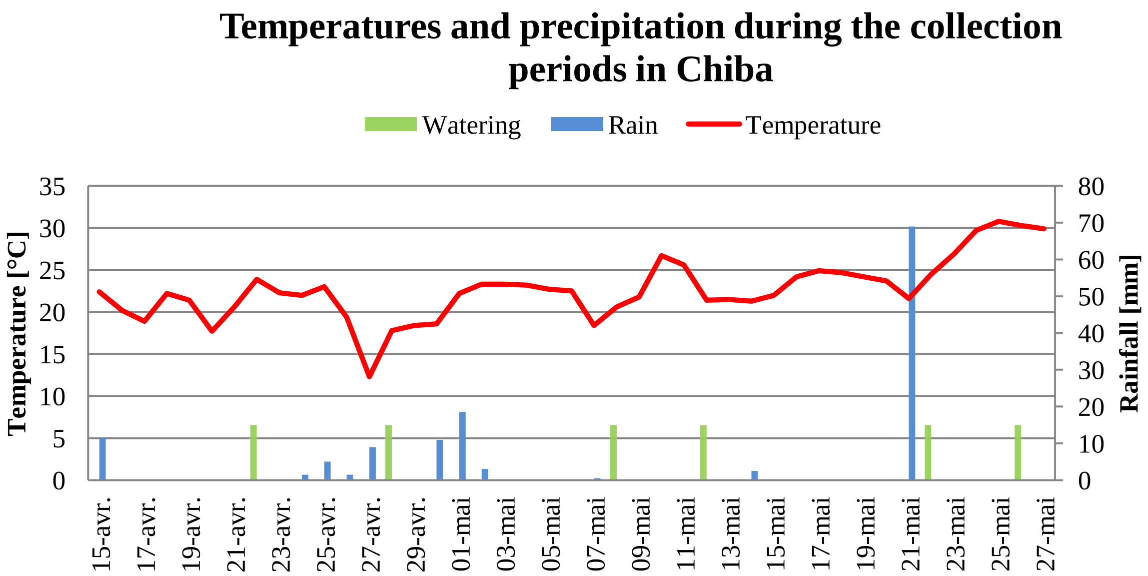


Figure 10 - Waterings, temperatures and precipitation in Chiba during the study period

It can be observed that the rains are heterogeneous. Indeed, some collections took place after rather dry periods, such as collections 1, 3, 4 and 6. While collection 2 is carried out after a period of moderate rainfall over several days and collection 5 takes place after a very intense rainy day. The temperature also varies during the study period. The minimum temperature is around collection 2, while collection 6 has the highest temperatures. It is useful to remember that retention capacity depends on periods of drought and rainfall before watering. These differences in climate around the collection periods should therefore be reflected in the results of water volume.

## 4.2 Soil moisture

As mentioned in the methodology, soil moisture was measured using two methods: one continuous using sensors inserted into the soil and the second manually, four times by collection using a moisture meter.

Firstly, here is an illustration of the results measured by the sensors (Figure 11) in order to have a general overview of soil moisture throughout the study period. Only EC-5 sensor data are used because they are the only ones in sufficient number to compare all the different types of substrates with each other with the same device. These sensors are placed in the containers of block 4, it is the closest to the building and parallel to it. It is not one of the blocks most impacted by the shadow.

It is worth noticing that the colour rule imposed for each type of substrate will be respected in all results.

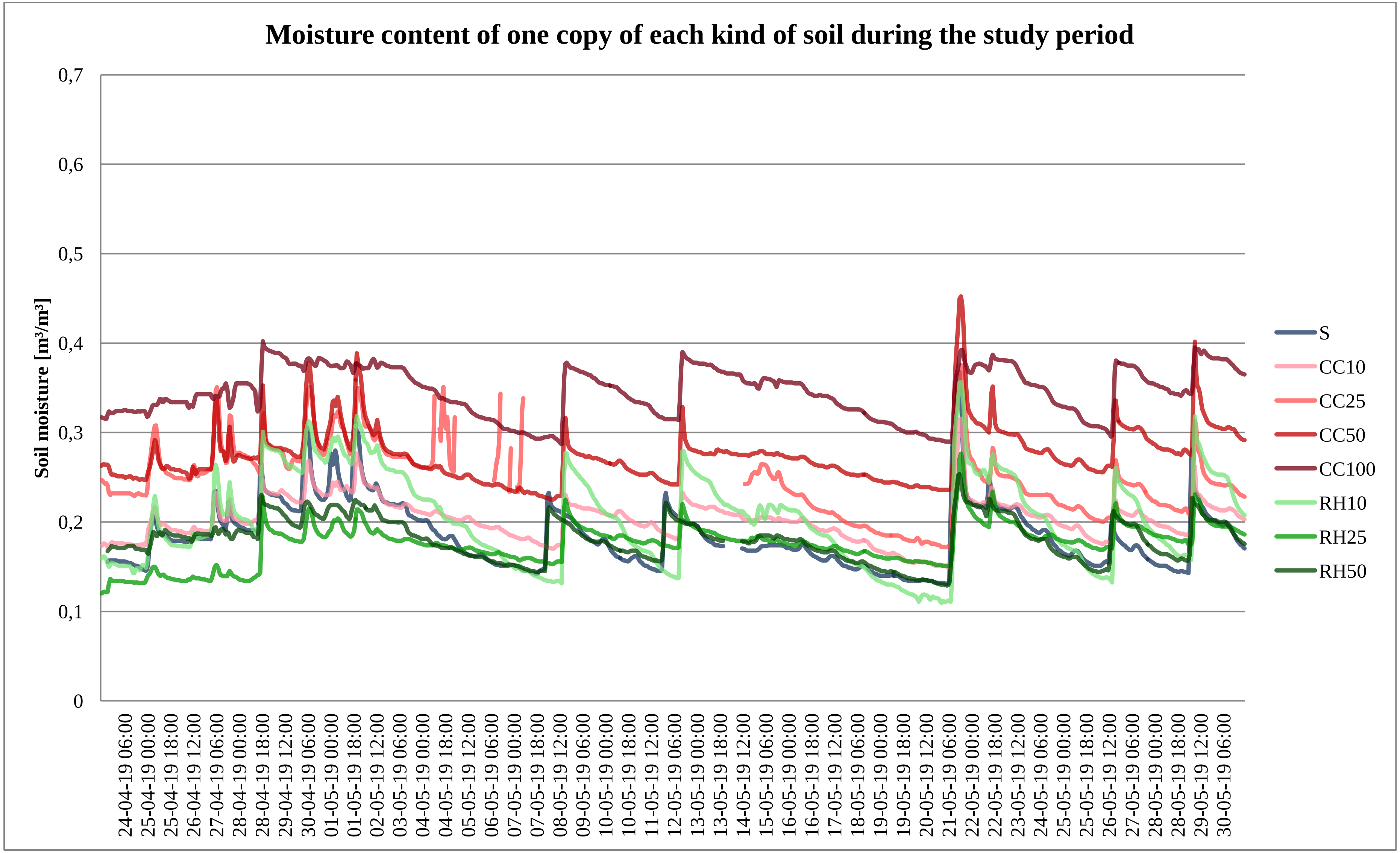


Figure 11 - Average moisture content of the different soils during the study period

The data collection started on 23/04/19 and therefore the day after the first collection.

Considering the general trends of the different moistures, different peaks in water content can be observed between April 25 and 29 due to the first rains and the watering of the 2nd week. Rainfall between April 30 and May 2 has a mixed impact on soil moisture. It can be observed that the soil moisture with 100% coco peat remains almost constant with these rains. It can be assumed that the maximum water content was almost reached. However, the other types of substrates were more affected by the new rains. The peaks due to humidification are more or less visible depending on the different soils.

On May 21, following the intense rainfall, significant peaks are visible for all substrates. These moistures are decreasing very rapidly. It can be assumed that free water stagnated in the sensor depression during the measurement and was subsequently drained. Thereafter, humidification and drying cycles are visible depending on rainfall and dry periods. Water is absorbed during rainy periods and evapotranspirated during dry periods.

If we focus on the differences between the different substrates, we can see that the soil with 100% coco peat stands out with the highest humidification capacity. This water content decreases when the volumes of coco peat in the mixture are reduced. K. Ankenbauer and S.P. Loheide (2016) showed that the higher the percentage of organic matter, the greater the moisture content. This study was carried out up to 20% of organic matter in the soil. Moreover, the affinity of organic matter with water reinforces this idea.

Most coco peat substrates have a better water content than traditional substrates or substrates with rice husk. It may be useful to remember that the moisture content of the different soils depends on their retention capacity, which is influenced by soil porosity and bulk density. The porosity of the conventional substrate is reduced with the addition of coco peat or rice husk. At the same time, the two soil substitutes allow a low bulk density, which induces a greater shrinkage (Dec et al., 2008). According to theory, coco peat has a lower bulk density than rice husk. This suggests that substrates with a percentage of coco peat will have better water retention than a substrate with the same percentage of rice husk. Traditional soil should therefore have the lowest moisture. This is respected for the majority of substrates. However, this is more controversial once the percentage of rice husk increases to 25%. It can be assumed that optimum water retention is present between the concentrations of 10 and 25% rice husk. Once exceeded, it would stagnate at a value close to the moisture capacity of the S substrate.

Then, less precisely, graphs with the manual measurements of each collection are illustrated in Figure 12 to Figure 17.

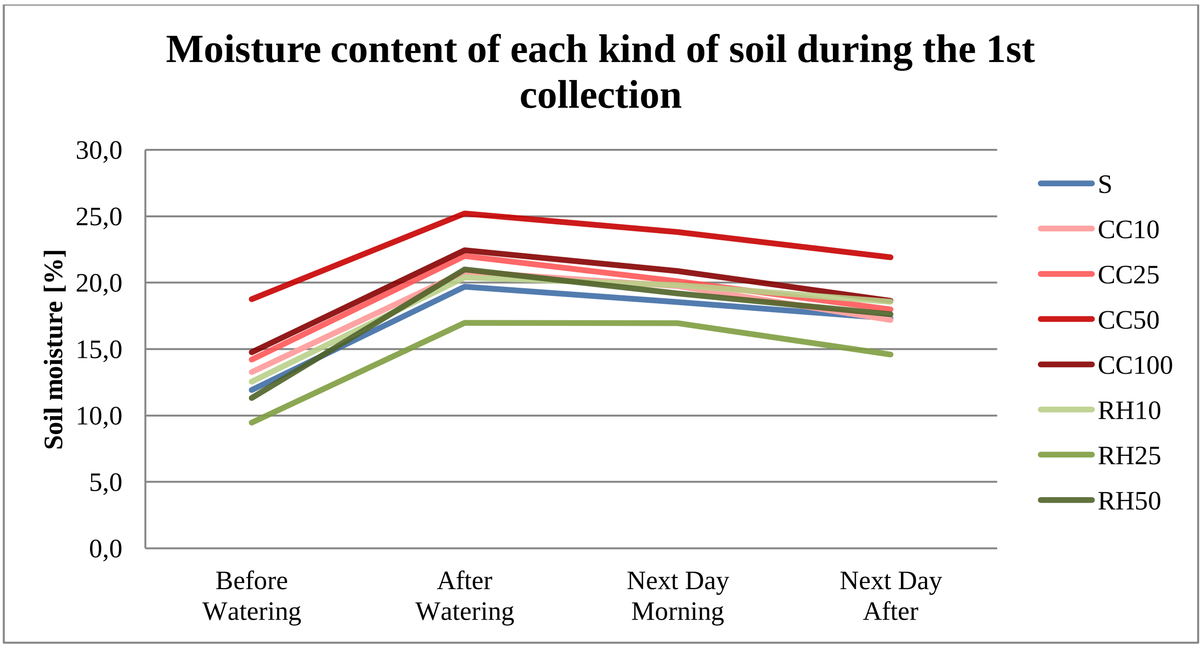


Figure 12 - Moisture content of each kind of soil during the 1st collection

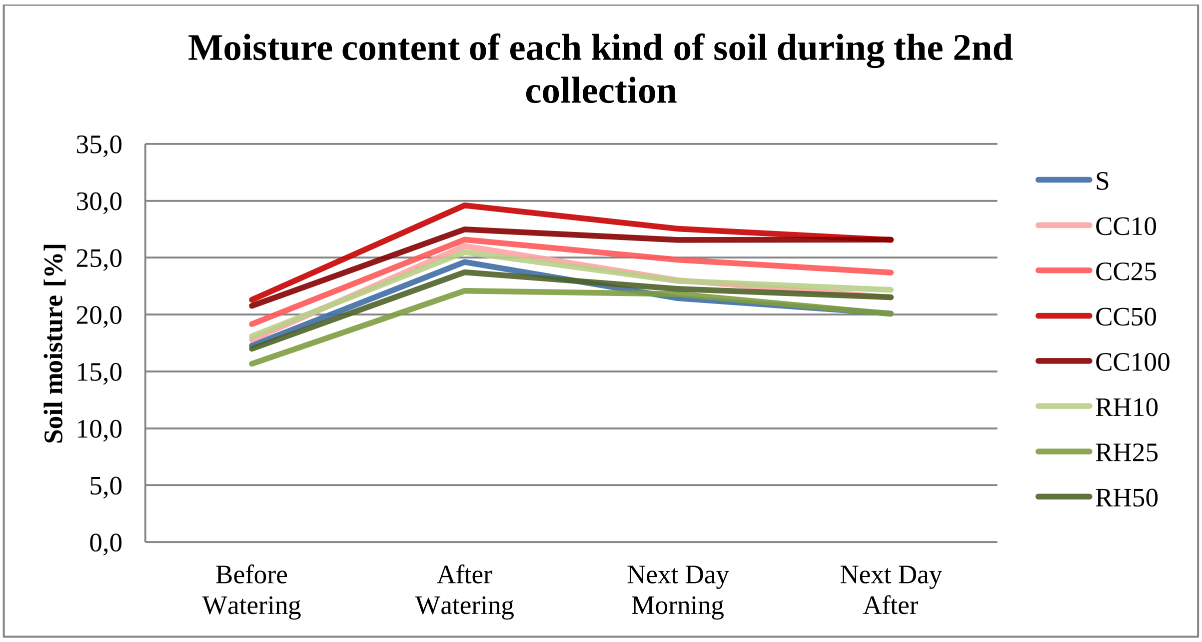


Figure 13 - Moisture content of each kind of soil during the 2nd collection

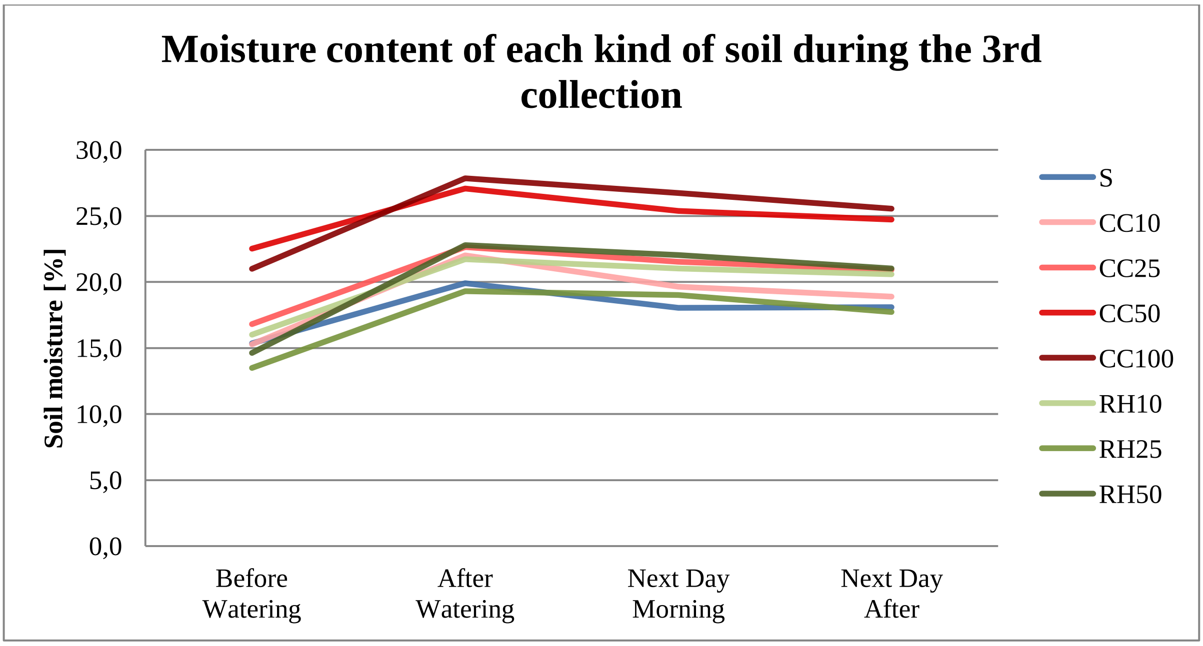


Figure 14 - Moisture content of each kind of soil during the 3rd collection

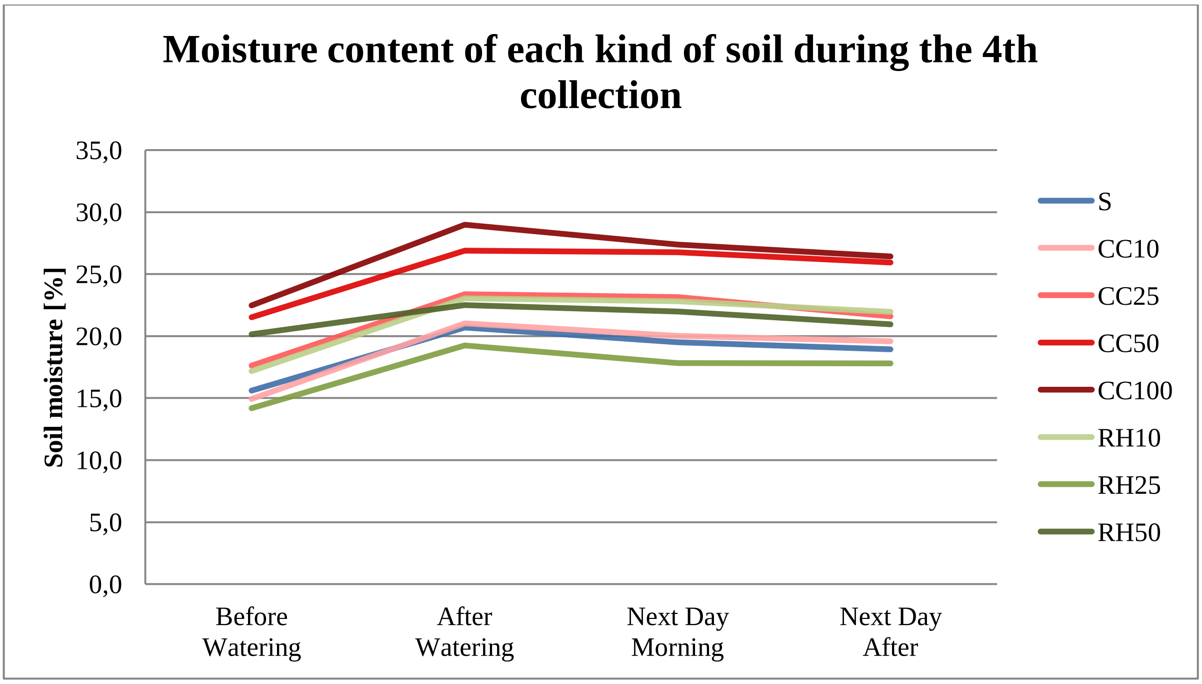


Figure 15 - Moisture content of each kind of soil during the 4th collection

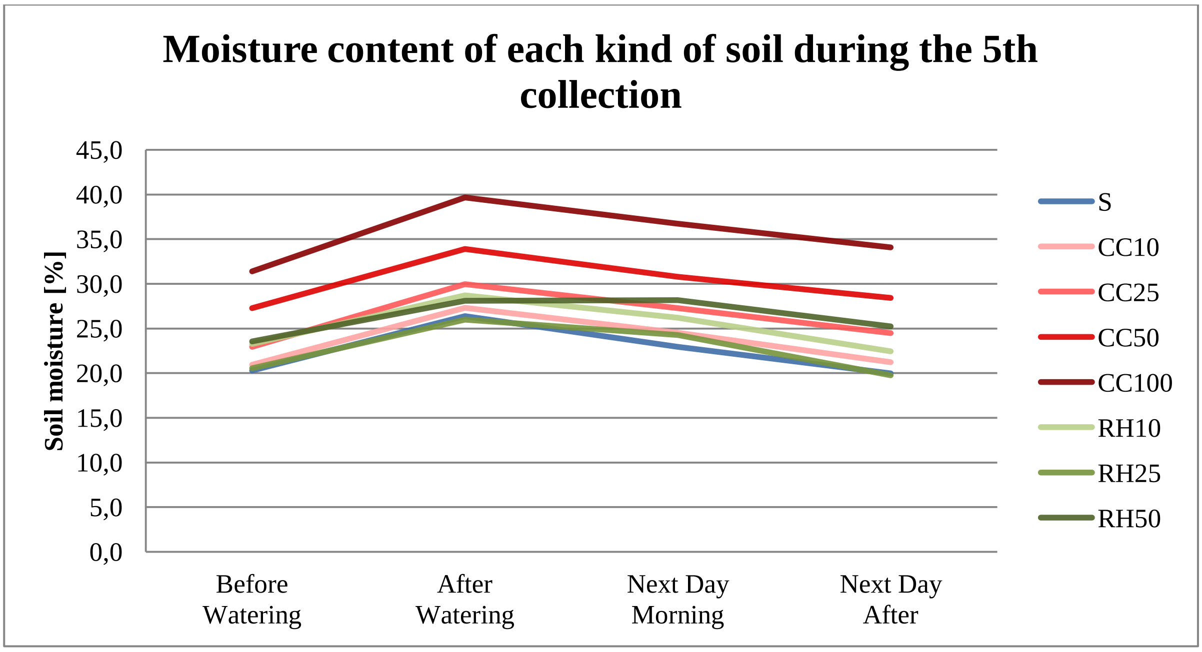


Figure 16 - Moisture content of each kind of soil during the 5th collection

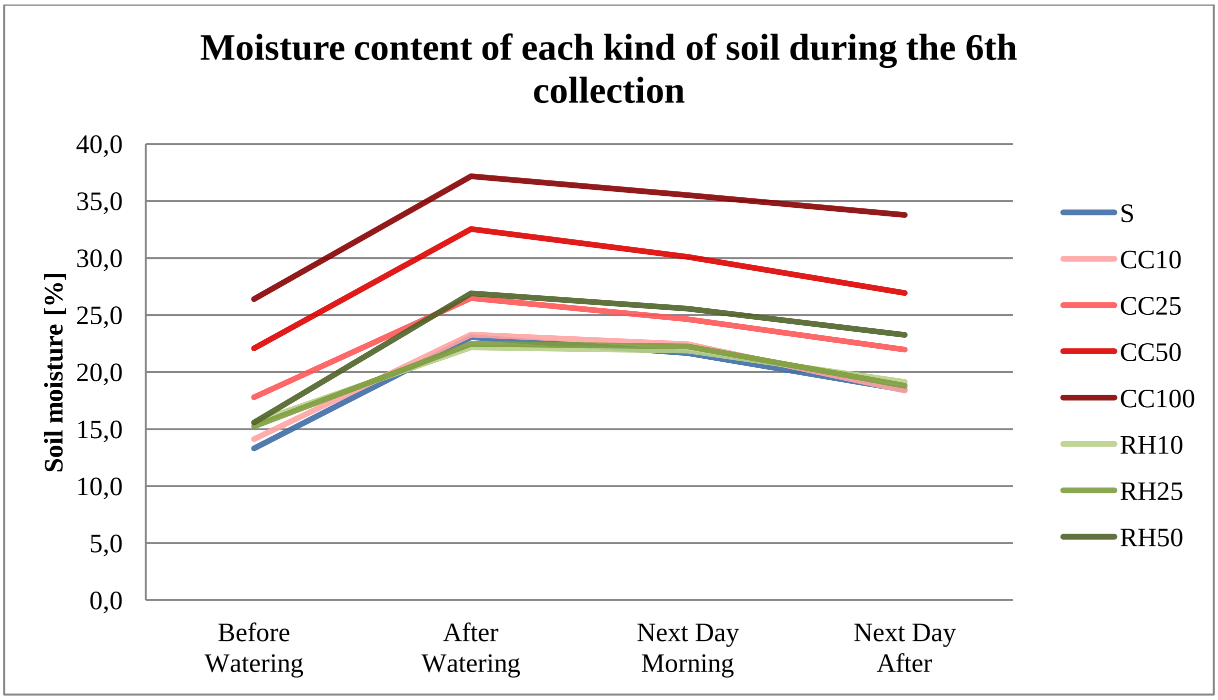


Figure 17 - Moisture content of each kind of soil during the 6th collection

As represented in Figure 11, CC100 substrate has the highest water content in most collections. Nevertheless, two collections (Figure 12 and Figure 13) show better soil moisture for the CC50 Substrate. It is possible that the water content curve of the soil as a function of its organic matter content may stabilize above a certain percentage or in certain climates. It is also possible that too much fibre residue in the coco peat may reduce its wettability (Awang et al., 2009).

On the basis of these differences in moisture, it is possible to measure the evaporated water during the 24 hours following watering. This period did not receive any additional rainfall as the watering period was chosen deliberately. Since the measurements were taken on the top of the roof after watering each of them, the gravity water had time to drain. The evaporated water was calculated using Equation 1.

**Equation 1 - Volume of water evaporated during the 24 hours following watering**

$$Vevap = \left(\frac{\Delta M}{100}\right) * Vcont$$

M: Soil moisture (%)
Vcont: Green roof volume ($m^3$)
Vevap: Evaporated water ($m^3$)

A graph illustrating the average water evaporated for each soil type during the different collections is shown in Figure 18.

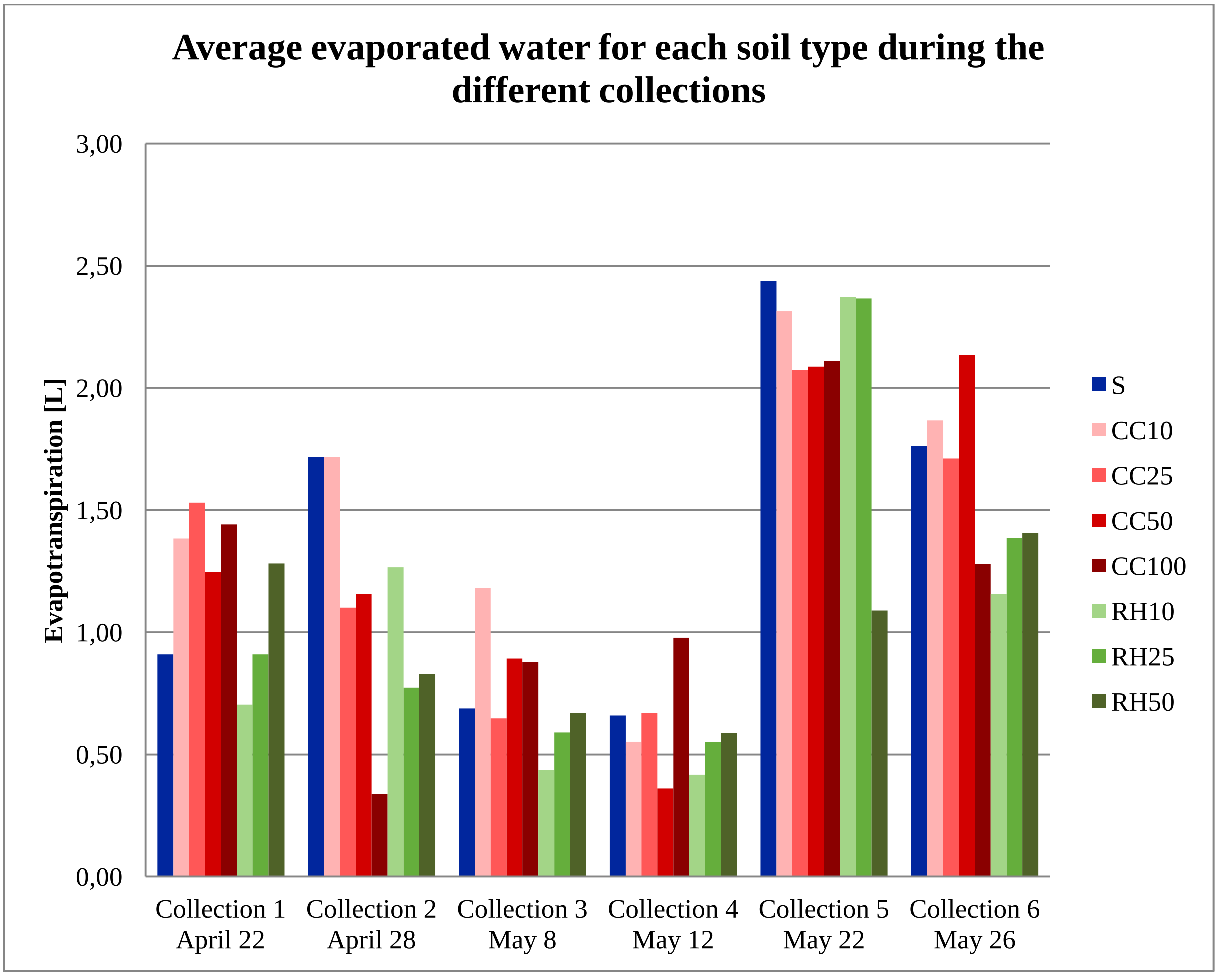


**Figure 18 - Average evaporated water for each soil type during the different collections**

It is important to note that the evaporated water can vary depending on the temperature but also on the amount of soil moisture before the drying period. Indeed, the closer a soil is to saturation, the less it will retain its water, which can therefore be more easily evaporated. On the other hand, a drier soil will have higher capillary forces, which will retain water more strongly (Musy and Soutter, 1991). It is therefore too complicated to compare the collections between them, only the substrates can be compared between them, within the same collection. In order to verify an impact of the substrates and blocks on this evaporated water, an AV2 is performed. The results are shown in Table 4.

**Table 4 - ANOVA results for evapotranspiration**

**Collection 1**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 7 | 3.113 | 0.4447 | 1.749 | 0.138 | |
| bloc | 4 | 1.210 | 0.3025 | 1.190 | 0.337 | |
| Residuals | 28 | 7.121 | 0.2543 | | | |

**Collection 2**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 7 | 7.780 | 1.1114 | 2.413 | 0.0457 | * |
| bloc | 4 | 3.667 | 0.9168 | 1.991 | 0.1233 | |
| Residuals | 28 | 12.896 | 0.4606 | | | |

**Collection 3**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 7 | 1.824 | 0.2606 | 0.497 | 0.8287 | |
| bloc | 4 | 5.907 | 1.4766 | 2.815 | 0.0442 | * |
| Residuals | 28 | 14.685 | 0.5245 | | | |

**Collection 4**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 7 | 1.236 | 0.1766 | 0.787 | 0.604 | |
| bloc | 4 | 1.686 | 0.4215 | 1.878 | 0.142 | |
| Residuals | 28 | 6.285 | 0.2245 | | | |

**Collection 5**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 7 | 6.616 | 0.9451 | 2.280 | 0.057 | . |
| bloc | 4 | 1.091 | 0.2728 | 0.658 | 0.626 | |
| Residuals | 28 | 11.604 | 0.4144 | | | |

**Collection 6**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 7 | 83.4 | 11.91 | 0.981 | 0.465 | |
| bloc | 4 | 66.5 | 16.62 | 1.369 | 0.270 | |
| Residuals | 28 | 340.0 | 12.14 | | | |

Only the 2nd collection considers a significant impact of the substrate on the evaporated water. This may seem strange with the marked differences in moisture curves between the different substrates. Nevertheless, it is possible that the general moistures are varied but not the differences between the moisture after watering and 24 hours later. Collecting 2 occurred after a period of light rainfall. The volumes of collection 2 are represented in Table 5. In parallel, the blocks significantly impacted the evaporated water from the collection 3.

**Table 5 - Volume of evapotranspirated water of the 2nd collection**

| Substrate | Volume of evaporated water |
|---|---|
| S | 1.72 |
| CC10 | 1.72 |
| RH10 | 1.27 |
| CC50 | 1.16 |
| CC25 | 1.10 |
| RH50 | 0.83 |
| RH25 | 0.77 |
| CC100 | 0.34 |

It can be seen that the S and CC10 substrates were the most efficient in evaporated water while the CC100 substrate retained the most water. It is possible that the affinity of the CC100 for water may induce a higher capillary force. Unfortunately, it is not possible to define a real conclusion with the result of a single collection.

## 4.3 Vegetated surface

Green roof plants have an impact on the volume and quality of water (World Meteorological Organization, 2008). They allow a greater or lesser water and nutrient absorption and transpiration of water according to its needs. The plants used during the experiment were planted as grass mats. After their placement, it was therefore necessary for the roots to develop in order to penetrate the substrate mixtures to meet its needs. Unfortunately, some areas have not survived. It is therefore useful to know the surface of the grass capable of absorbing in order to verify a potential impact on water absorption (Appendix 1). Indeed, a dead zone does not allow water to be absorbed and transpired. In addition, its coverage on the ground can impact its evaporation. These percentages are shown in Figure 19.

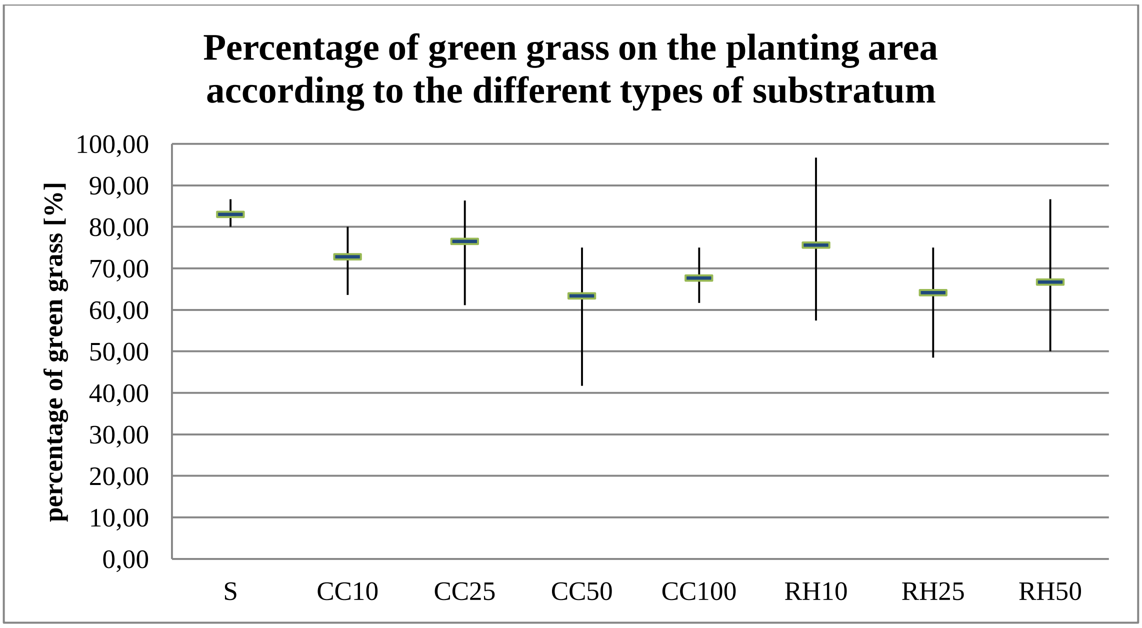


**Figure 19 - Green grass percentage on the planting area according to the different types of substrate**

This graph represents the different percentages of green grass on the different green roofs. The marker represented by a transverse line represents the average of its percentages for each substrate. Nevertheless, some substrates with varied results, the minimum and maximum have been represented in order to visualize the differences between the different green roofs of each substrate.

The availability of water and nutrients from different soils may have impacted plant survival. A two-ways ANOVA (AV2) is performed to check the impact of substrates and blocks. The results are shown in Figure 20.

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Sub | 7 | 1646 | 235.14 | 1.909 | 0.107 |
| bloc | 4 | 364 | 91.09 | 0.740 | 0.573 |
| Residuals | 27 | 3326 | 123.17 | | |

Figure 20 - ANOVA results testing the impact of substrates and blocks

The different substrates and blocks therefore do not have a significant impact on plant survival.

Nevertheless, it can be assumed that this impact is not visible due to the youth of the green roofs and the plants. Once the plant will be correctly inserted into the substrate, we can imagine that the vegetation will develop in a different way.

## 4.4 Water Amount

The impact of the different substrates is the main objective of this study. Therefore, a graph of the mean of water quantities for each substrate during the different collections was made. It is illustrated in Figure 21. All collections are taken into account for the statistics of water quantity analysis. The raw data for the collection can be found in Appendix 2.

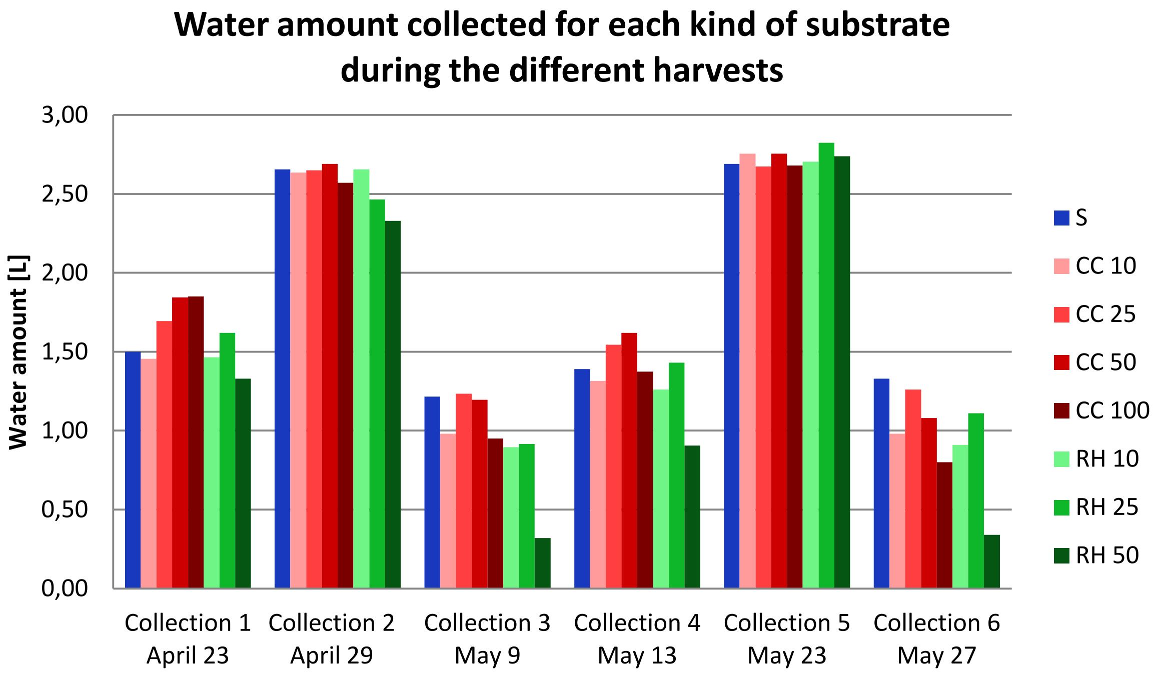


Figure 21 - Water amount collected for each kind of substrate

It is useful to remember that the harvest dates differ from the watering dates because they are carried out with a 24-hour interval.

It can be seen that during some collections, the quantities of water were higher (Collection 2 and 5). If we observe the temperature and rain graph of the previous point, we can confirm that the rains preceding the collection have a direct impact on the water collected. Indeed, it is known that the longer the dry period before rainfall, the greater the soil's retention capacity will be. As a result, the retention capacity of each substrate decreases if rainfall events occur before watering. It is also noticed that the difference in retention of the different soils is mainly noticeable during events with a previous rainfall further away in time or lower.

Then to check the impact of the different substrates and blocks on the results, an AV2 is performed. Table 6 synthesizes these results.

**Table 6 - ANOVA results for water quantities**

**Collection 1**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 7 | 1.270 | 0.1814 | 3.029 | 0.016805 | * |
| bloc | 4 | 1.954 | 0.4884 | 8.153 | 0.000172 | *** |
| Residuals | 28 | 1.677 | 0.0599 | | | |

**Collection 2**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 7 | 0.5411 | 0.07730 | 2.487 | 0.0405 | * |
| bloc | 4 | 0.1980 | 0.04951 | 1.593 | 0.2037 | |
| Residuals | 28 | 0.8702 | 0.03108 | | | |

**Collection 3**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 7 | 3.0607 | 0.4372 | 34.430 | 4.01e-12 | *** |
| bloc | 4 | 0.1999 | 0.0500 | 3.935 | 0.0117 | * |
| Residuals | 28 | 0.3556 | 0.0127 | | | |

**Collection 4**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 7 | 1.6335 | 0.23336 | 21.616 | 1.02e-09 | *** |
| bloc | 4 | 0.1607 | 0.04018 | 3.722 | 0.015 | * |
| Residuals | 28 | 0.3023 | 0.01080 | | | |

**Collection 5**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 7 | 0.0905 | 0.01293 | 0.626 | 0.730 |
| bloc | 4 | 0.1062 | 0.02656 | 1.286 | 0.299 |
| Residuals | 28 | 0.5785 | 0.02066 | | |

**Collection 6**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 7 | 3.244 | 0.4635 | 23.053 | 7.78e-10 | *** |
| bloc | 4 | 0.194 | 0.0485 | 2.411 | 0.0737 | . |
| Residuals | 27 | 0.543 | 0.0201 | | | |

Different impacts are visible in the results. The impact of the substrate on water amount is highly significant for collections 3, 4 and 6. It is significant for collections 1 and 2 and has no impact on collection 5. Regarding to water quantities, we can check that the two collections with the highest rainfall are in the least significant. It is also confirmed that collections with lower water quantities, during drier periods, are the 3 collections with the highest impact. Therefore, it can be concluded that the more frequent and intense the rainfall, the less the retention capacities will differ between the different substrates. Indeed, if the soil moisture is almost at its maximum it will no longer be able to increase and therefore the soil will only retain a little water. In a too rainy area or with a climate that limits evapotranspiration, the different kinds of soil will not be able to absorb the water needed to manage runoff. The use of green roofs should therefore be encouraged in regions with less intense rainfall or climates that favour evapotranspiration.

At the same time, collections 1, 3 and 4 show that the blocks also impact the water amount collected. External elements such as surrounding buildings, shade, wind, etc. must be taken into account.

### *Impact of blocks*

A ranking of the retention averages of the different blocks is carried out in order to have an idea of the most advantageous areas. Table 7 summarizes the different blocks and the average of their volumes of drained water from harvests where the impact is significant.

**Table 7 - Water quantities according to collections and blocks**

| Collection 1 | | Collection 3 | | Collection 4 | |
|---|---|---|---|---|---|
| 3 | 1.28 | 3 | 0.87 | 3 | 1.28 |
| 5 | 1.48 | 2 | 0.91 | 5 | 1.30 |
| 4 | 1.57 | 5 | 0.97 | 4 | 1.34 |
| 2 | 1.70 | 4 | 1.00 | 2 | 1.39 |
| 1 | 1.94 | 1 | 1.07 | 1 | 1.46 |

Reminder: Figure 8 lists the different blocks.

Unanimously, block number 3 has the best water retention while block number 1 has the highest volume of water. Block 1 being the block most subject to shade, it can be assumed that its larger volume comes from a lower evapotranspiration. As the day progresses, the shade also spreads to the 2nd block. Its evapotranspiration can therefore also be impacted. It may explain the large volume during these two collections. For the other three blocks, it is more difficult to draw conclusions. Blocks 3 and 4 are the least shaded, however, it is possible that structures and wind direction and intensity may have an impact on the amount of water that reaches the green roofs.

### *Impact of substrate*

Since conventional soil is the most common type of soil in Japan, it seems wise to compare the difference between the different mixtures and conventional soil in order to check the usefulness of improve it.

For this purpose, a Dunnett test will be carried out using the conventional soil (S) as a control. Only collections where the substrates have a significant impact on water will be used. This is represented in Table 8. The volume difference between the substrate S and the others is in brackets next to its respective substrate.

Table 8 - Useful Dunnett test results

| Collection 1 | Collection 4 |
|---|---|
| No significant difference | RH50 (0.485)<br>CC50 (-0.230) |
| Collection 2 | Collection 6 |
| RH50 (0.326) | RH50 (0.990)<br>CC100 (0.525)<br>RH10 (0.420)<br>CC10 (0.345) |
| Collection 3 | |
| RH50 (0.895)<br>RH10 (0.320)<br>RH25 (0.300)<br>CC100 (0.265)<br>CC10 (0.235) | |

Firstly, we can see that all collections have different results. In major collections, the RH50 has the best significantly different water retention. Only the first collection has no significant difference with respect to the reference substrate. This result may be questionable given the maximum moisture level as well as the evaporation capacity after 24 hours of this substrate which is lower than other substrates. It can be assumed that its porosity is lower. CC100, RH10 and CC10 substrates have significant differences in collections 3 and 6. These two collections are those with the lowest drained volumes and therefore, with the highest retention averages. RH25 has a significant difference only during collection 3. All the above-mentioned substrates absorbed more water than the conventional substrate. Unlike the CC50 substrate which had a negative difference during collection 4. This means that it absorbs less water than conventional substrate.

RH50 can therefore be considered as the substrate with the best retention capacity, it would significantly reduce the drainage of water from green roofs compared to the conventional substrate (S) used mainly in Japan.

### *Average of the different collections*

In order to visualize the general impact of the different substrates, a sum of the water quantities of the different collections for each substrate is carried out. These averages are illustrated in Figure 22.

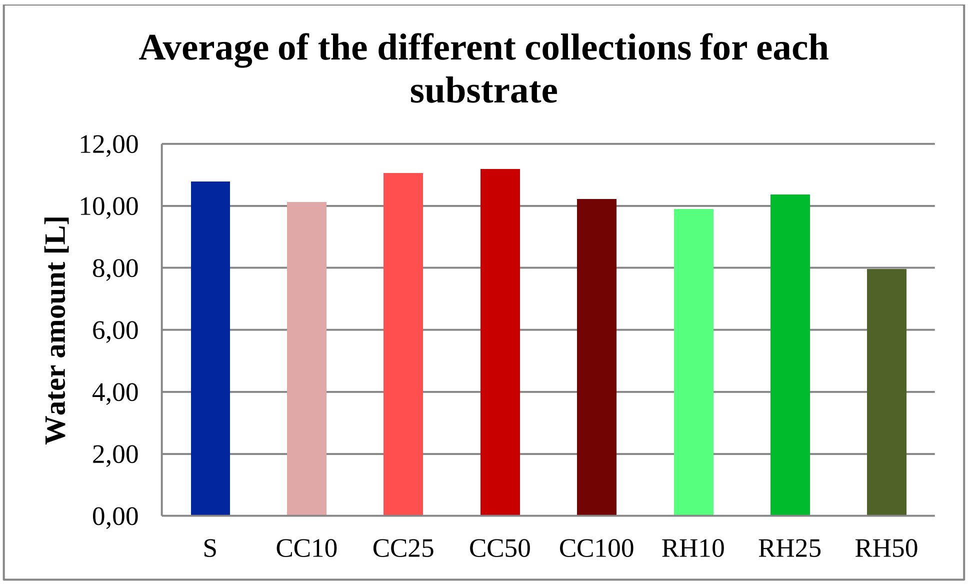


**Figure 22 - Sum of the water quantities of the different collections for each substrate**

The general trend confirms the previous conclusion that RH50 is the soil with the best water retention. The other substrates have at least 2l additional drainage water. A Tukey test is performed to see if the differences between the sum of the drained water from different substrates is significant.

| Contraste | Différence | Différence standardisée | Valeur critique | Pr > Diff | Significatif |
|---|---|---|---|---|---|
| CC50 vs RH50 | 0,641 | 1,500 | 3,239 | 0,80 | Non |
| CC50 vs RH10 | 0,249 | 0,583 | 3,239 | 0,99 | Non |
| CC50 vs CC10 | 0,213 | 0,498 | 3,239 | 1,00 | Non |
| CC50 vs RH25 | 0,178 | 0,417 | 3,239 | 1,00 | Non |
| CC50 vs CC100 | 0,177 | 0,414 | 3,239 | 1,00 | Non |
| CC50 vs S | 0,068 | 0,160 | 3,239 | 1,00 | Non |
| CC50 vs CC25 | 0,009 | 0,021 | 3,239 | 1,00 | Non |
| CC25 vs RH50 | 0,632 | 1,480 | 3,239 | 0,81 | Non |
| CC25 vs RH10 | 0,240 | 0,562 | 3,239 | 1,00 | Non |
| CC25 vs CC10 | 0,204 | 0,478 | 3,239 | 1,00 | Non |
| CC25 vs RH25 | 0,169 | 0,396 | 3,239 | 1,00 | Non |
| CC25 vs CC100 | 0,168 | 0,393 | 3,239 | 1,00 | Non |
| CC25 vs S | 0,059 | 0,139 | 3,239 | 1,00 | Non |
| S vs RH50 | 0,573 | 1,341 | 3,239 | 0,88 | Non |
| S vs RH10 | 0,181 | 0,423 | 3,239 | 1,00 | Non |
| S vs CC10 | 0,145 | 0,339 | 3,239 | 1,00 | Non |
| S vs RH25 | 0,110 | 0,257 | 3,239 | 1,00 | Non |
| S vs CC100 | 0,109 | 0,255 | 3,239 | 1,00 | Non |

| | | | | | |
|---|---|---|---|---|---|
| CC100 vs RH50 | 0,464 | 1,086 | 3,239 | 0,95 | Non |
| CC100 vs RH10 | 0,072 | 0,168 | 3,239 | 1,00 | Non |
| CC100 vs CC10 | 0,036 | 0,084 | 3,239 | 1,00 | Non |
| CC100 vs RH25 | 0,001 | 0,002 | 3,239 | 1,00 | Non |
| RH25 vs RH50 | 0,463 | 1,084 | 3,239 | 0,96 | Non |
| RH25 vs RH10 | 0,071 | 0,166 | 3,239 | 1,00 | Non |
| RH25 vs CC10 | 0,035 | 0,082 | 3,239 | 1,00 | Non |
| CC10 vs RH50 | 0,428 | 1,002 | 3,239 | 0,97 | Non |
| CC10 vs RH10 | 0,036 | 0,084 | 3,239 | 1,00 | Non |
| RH10 vs RH50 | 0,392 | 0,918 | 3,239 | 0,98 | Non |
| CC50 vs RH50 | 0,641 | 1,500 | 3,239 | 0,80 | Non |

The sum of the water content shows us insignificant differences between substrates. The results therefore show that, although during some rains the differences in water quantities are significant, the differences in the sums of water collected during this study are not. The impacts of different substrates can therefore change over the year depending on the climate. A full year analysis will allow a better vision of the utility of the different soil.

## 4.5 Water Quality

As explained above, only two collections were analyzed in this section. The quality analyses were carried out on two collections with opposite climates, the fifth and the sixth collection. The fifth collection took place after a heavy rainfall while the sixth followed a drier and warmer period. This allows us to identify the different elements leached during periods of soil saturation and drier periods.

A two-ways ANOVA is carried out to check the impact of the substrates and blocks. The elements significantly impacted by the different substrates are listed in Tables 9 and 11. Once the elements impacted by the substrates identified, a Dunnett test will be applied to them to assess if their difference with the control (tap water) is significant or not.

### 4.5.1 Collection 5

The fifth collection is the one with heavy rainfall preceding it. It is therefore with an almost saturated soil that the watering took place. Its volume of drained water was the most important and was not significantly impacted by the different substrates. The raw quality data can be found in Appendix 3. The elements with a concentration significantly impacted by substrates are listed in Table 9. The results of the AV2 performed can be found in Appendix 4. Then, Table 10 will summarize the results of the Dunnett test. Each substrate will be connected to elements that have a significant difference from the control. The difference between the concentration of the control and the substrate average will also be written.

**Table 9 - Elements significantly affected by substrates**

| Elements significantly affected by substrates | | | | |
|---|---|---|---|---|
| pH | DOC | Al | As | Ba |
| Bi | Ca | Cl | Fe | Ga |
| K | Mg | Mn | Na | $NH_4$ |
| $NO_3$ | $PO_4$ | $SO_4$ | Sr | V |

**Table 10 - Elements with significant differences with tap water and their differences according to different substrates**

| Substrate | Element impacted | Difference (mg/l) | Element impacted | Difference (mg/l) |
|---|---|---|---|---|
| S | $NO_3$ | -1.074 | Ga | 0.020 |
| | Bi | 0.204 | Mg | 3.072 |
| | Ca | 10.43 | Na | 16.622 |
| CC100 | pH | 1.364 | Ga | 0.020 |
| | DOC | -252.000 | K | -32.034 |

| | | | | |
|---|---|---|---|---|
| | Cl | 0.954 | Mg | 3.421 |
| | Bi | 0.168 | Mn | -0.038 |
| | Ca | 15.259 | Sr | 0.029 |
| | Fe | -2.162 | | |
| CC50 | DOC | -74.800 | Fe | -1.293 |
| | Cl | 0.814 | Ga | 0.018 |
| | Al | -2.381 | Mg | 4.879 |
| | Ba | -0.117 | Na | 13.218 |
| | Bi | 0.194 | Sr | 0.038 |
| | Ca | 16.87 | | |
| CC25 | Al | 1.487 | Mg | 4.939 |
| | Bi | 0.202 | Na | 12.520 |
| | Ca | 17.054 | Sr | 0.04 |
| | Ga | 0.020 | | |
| CC10 | $NO_3$ | -0.948 | Ga | 0.018 |
| | Bi | 0.200 | Mg | 4.096 |
| | Ca | 15.717 | Na | 12.020 |
| RH50 | $SO_4$ | -2.158 | K | -67.434 |
| | Ba | -0.102 | Mg | 4.939 |
| | Bi | 0.196 | Na | 17.852 |
| | Ca | 16.618 | Sr | 0.03 |
| | Ga | 0.018 | V | -0.018 |
| RH25 | Cl | 0.858 | Ga | 0.02 |
| | Al | -2.291 | K | -18.900 |
| | Ba | -0.098 | Mg | 4.875 |
| | Bi | 0.166 | Na | 18.184 |
| | Ca | 16.546 | V | -0.012 |
| RH10 | $NO_3$ | -1.014 | Ca | 14.351 |
| | $SO_4$ | -2.142 | Ga | 0.02 |
| | Ba | -0.085 | Mg | 4.012 |
| | Bi | 0.210 | Na | 16.430 |

Firstly, it is remarkable that only the CC100 substrate has a significant difference with the control in pH. The drained water has a low pH of 5.75 while the tap water pH was 7,11. This difference means that the CC100 substrate acidifies tap water. This is against the interest of green roofs which neutralize the acidity of the water (Berghage et al., 2007).

Secondly, in order to compare the impact of different substrates, we can focus on the elements whose concentration has increased during water passage through the soil. The evolution of the percentage rate of coco peat and rice husk additions is illustrated in Figure 23. Significantly different elements are stated below the box of their respective substrates. The arrow in brackets indicates the trend of the element in relation to the previous box. Each box is compared to the previous one to see their evolution. If an element has no arrows, it is because its presence was not significant in the previous percentage of substrate. This practice is used for all absorption and reserve diagrams.

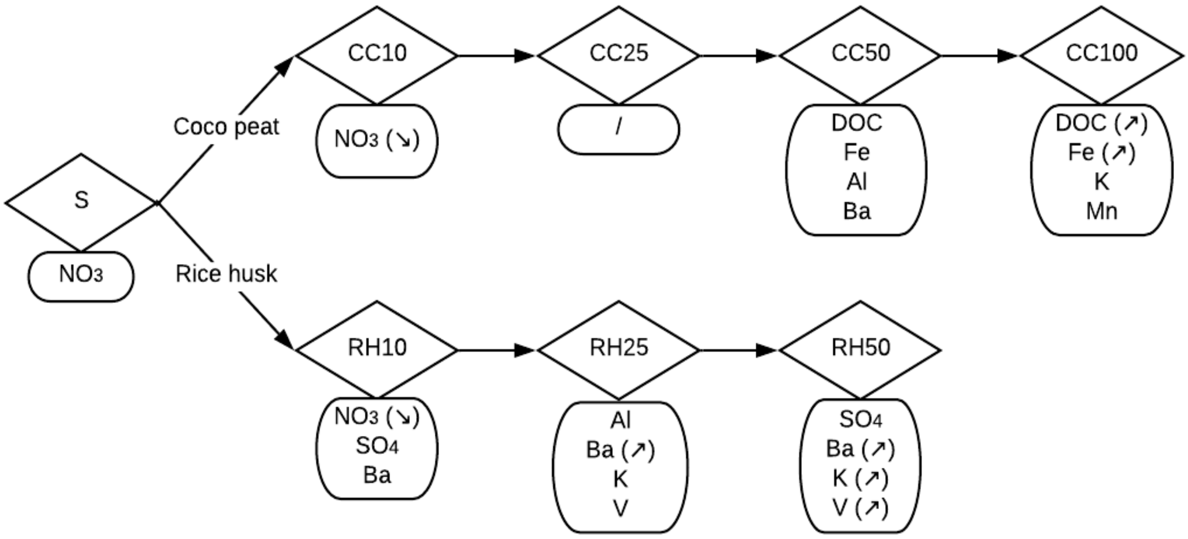


**Figure 23 - Supply of elements due to the substrate**

It can be observed that the traditional soil (S) is source of $NO_3$, which disappears as coco peat and rice husk are added. Ghorbani, Asadi and Abrishamkesh (2019) have shown that a supply of biochar can reduce $NO_3$ leaching. Its high superficial levels and anionic adsorption potential can improve the adsorption of nitrate and maintain it in the soil. When adding coco peat, there is an increasing concentration of different elements such as C and Fe. They are not yet significant with the CC10 and CC25 substrates but are important with the CC50 and CC100. More punctual elements are also present such as Al and Ba in the CC50 substrate and K and Mn in the CC100 substrate. The addition of rice husk goes in a completely different direction. We can see the increase in concentration of Ba, K and V. $SO_4$ appears to grow between the substrate RH10 and RH50. However, it is absent from the RH25 substrate, which does not allow us to be clear on this growth. There is also a presence of Al in the RH25 substrate.

Then, it may be interesting to create the same diagram for the elements absorbed by green roofs. This would indicate if they retain elements that are harmful to the environment. This diagram is shown in Figure 24.

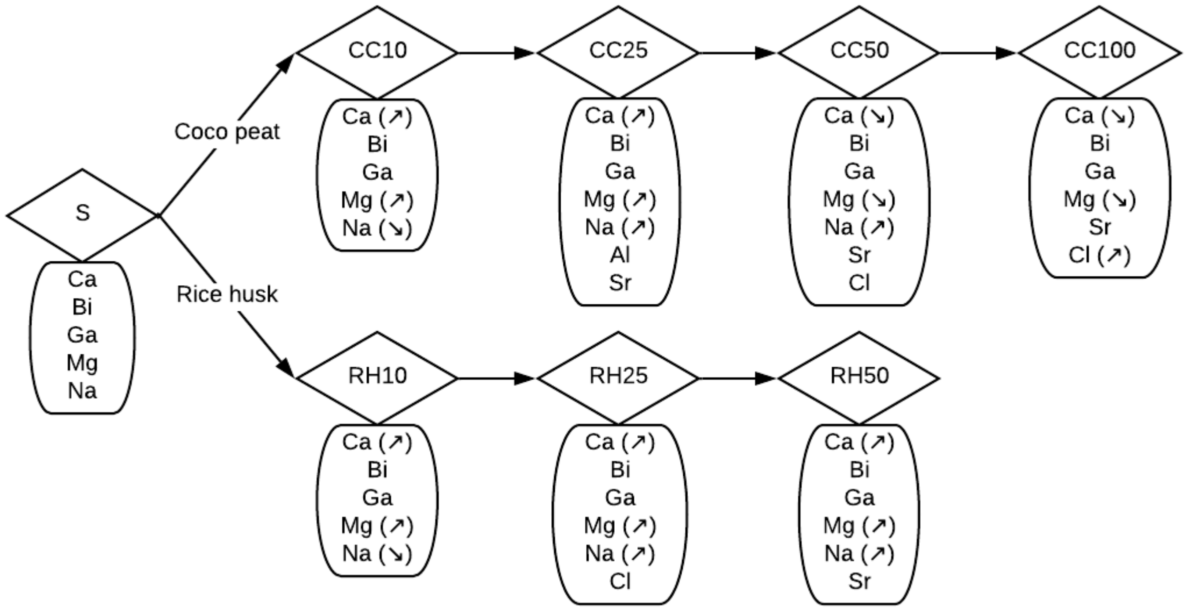


**Figure 24 - Absorption of the elements by the substrate**

A common trend can be observed towards Ca and Mg. Both elements increase with the addition of coco peat to the CC25 substrate and will decrease thereafter. When the percentage of rice husk increases, the two elements follow the same pattern again, but increase proportionally. These similar trends come from their very similar cycles. Although Mg is less retained by the CEC than Ca (Cornelis, 2016). Na has a more random flow. Indeed, it is decreased in drained water from the CC10 substrate but will subsequently increase to the CC50 substrate. Its presence will no longer be significantly different in the water of the CC100 substrate. At the same time, its presence also decreases in the RH10 substrate and then increases to RH50. The other elements are punctual or remain stable in their concentration.

The substrates containing rice husk or coco peat increase the ability of conventional soil to retain exchangeable cations. This growth explains why cations such as Ca, Mg and Na are found in greater quantities in soils with these substitute substrates (Soil Quality Pty Ltd, 2019).

### 4.5.2 Collection 6

The sixth collection took place after a dry period and high temperatures. Its amount of drained water was significantly impacted by the different substrates and was one of the lowest. The raw quality data can be found in Appendix 5. The elements of this collection significantly impacted by the different substrates are in Table 11. The results of the AV2 conducted can be found in Appendix 6. As observed in the previous point, each element with a substrate impact was subjected to the Dunnett test to check if the difference between the substrates water and tap water was significant. Elements and substrates where the drained water is significantly different from tap water are included in Table 12.

Table 11 - Elements significantly affected by substrates

| Elements significantly affected by substrates | | | | |
|---|---|---|---|---|
| pH | DOC | Al | As | Ba |
| Ca | Cl | Cu | Fe | K |
| Mg | Mn | Na | $NO_3$ | $PO_4$ |
| $SO_4$ | Sr | V | Zn | |

Table 12 - Elements with significant differences with tap water and their differences according to different substrates

| Substrate | Element impacted | Difference (mg/l) | Element impacted | Difference (mg/l) |
|---|---|---|---|---|
| S | Na | 1.308 | Ca | 7.234 |
| | $NO_3$ | -0.620 | Mg | 2.014 |
| CC100 | pH | 1.140 | Fe | -2.897 |
| | DOC | -322.2 | K | -40.726 |
| | Cl | 0.692 | Mg | 1.852 |
| | Ca | 10.982 | Mn | -0.053 |
| | Cu | -0.012 | Zn | -0.014 |
| CC50 | Al | -2.646 | Fe | -1.523 |
| | Ba | -0.167 | Mg | 3.648 |
| | Ca | 13.024 | | |
| CC25 | Ca | 13.393 | Sr | 0.032 |
| | Mg | 7.494 | | |
| CC10 | Ca | 12.657 | $SO_4$ | -1.012 |
| | Mg | 3.293 | | |
| RH50 | Ba | -0.110 | $SO_4$ | -1.964 |
| | Ca | 13.118 | Mg | 7.656 |
| | K | -61.324 | V | -0.022 |
| | Na | 1.736 | | |
| RH25 | Ca | 12.976 | $NO_3$ | -0.542 |
| | Mg | 3.775 | $SO_4$ | -1.500 |
| | Na | 1.572 | | |
| RH10 | Ca | 11.412 | Na | 1.320 |
| | Mg | 3.111 | $SO_4$ | -1.476 |

Firstly, as observed previously, CC100 is the only one to significantly impact the pH variation. Its pH is 5.56 while tap water has a pH of 6.70. The drained water is therefore more acidic than during the previous collection, but tap water is also. Finally, the difference between the two is smaller.

Then, we focus on the elements with an increasing concentration after the passage through the soil. The evolution over time of the coco peat and rice husk additions is illustrated in Figure 25.

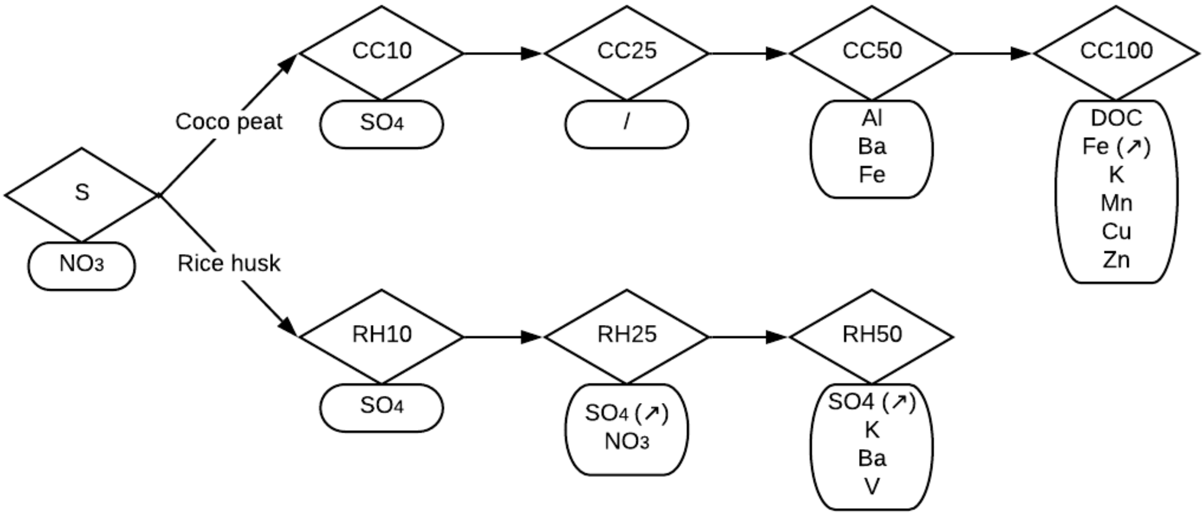


Figure 25 - Supply of elements due to the substrate

As in the previous collection, the traditional soil is a source of $NO_3$. However, its presence is no longer significant in drained water from other substrates. When increasing the volume of coco peat, the elements are very varied. The CC10 substrate leaches $SO_4$ while the CC25 substrate does not transmit anything significantly. The number of elements increases once the substrate contains 50% coco peat and Al, Ba and Fe are leached. The substrate composed exclusively of coco peat has an increasing concentration of Fe and induces an appearance of C, K, Mn, Cu and Zn. At the same time, the $SO_4$ increases as the volume of rice husk in the substrate expands. As for coco peat, specific elements are present in the various drained waters. The substrate RH25 has $NO_3$ while the RH50 increases its concentrations of K, Ba and V.

Then, the diagram for the elements absorbed by green roofs is shown in Figure 26.

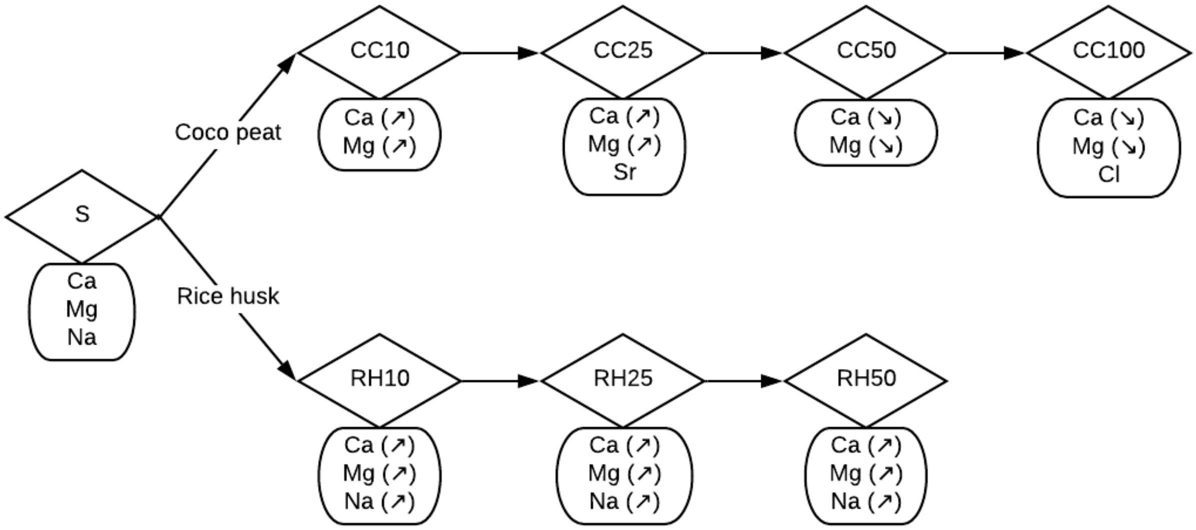


Figure 26 - Absorption of the elements by the substrate

It is noticeable that the diversity of elements is less than in the previous collection. Only five elements were significantly absorbed in tap water. Ca, Na and Mg are retained by the traditional soil. On the one hand, this trend increases with the addition of rice husk. On the other hand, the growth of the volume of coco peat increases the concentration of Ca and Mg to RH25 substrate and after, a decrease is observed. It is possible that the growth of coco peat increases the competition between metal ions and protons for the binding sites (Vijayaraghavan et al., 2016).

### 4.5.3 Comparison to drainage water standards

In order to verify the conformity of concentrations within the drained water from the different roofs, the Japanese drained water quality standards will be compared to the different measured concentrations (Ministry of the environment Government of Japan, sd). Table 13 represents the standards for the elements present in this study. Absent elements have either not been significantly present or are not considered harmful. It is also important to remember that only elements impacted by the variety of substrates are treated. It is therefore not excluded that some non-impacted elements may be present in quantity. Nevertheless, the aim being to improve the substrates, their impact or not is not negligible.

Table 13 - Comparative table of elements concentrations and Japanese standards.

| | S | | CC10 | | CC50 | | CC100 | | RH10 | | RH25 | | Standards mg/l |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Collectes | 5 | 6 | 5 | 6 | 5 | 6 | 5 | 6 | 5 | 6 | 5 | 6 | |
| Hazardous substance | | | | | | | | | | | | | |
| Nitrate compounds | 1.22 | 0.75 | 1.10 | | | | | | 1.16 | | | 0.67 | 100 |
| Other substances | | | | | | | | | | | | | |
| Copper content | | | | | | | | 0.01 | | | | | 3 |
| Zinc content | | | | | | | | 0.01 | | | | | 2 |
| Soluble iron content | | | | | 1.29 | 1.52 | 2.16 | 2.90 | | | | | 10 |
| Soluble manganese content | | | | | | | 0.04 | 0.05 | | | | | 10 |
| Chromium content | | | | | 1.17 | | 1.03 | 1.19 | | | 1.12 | | 2 |

It is clear that none of the source elements exceed the limit set by the Japanese government for the concentration of hazardous elements in drainage waters. The different substrates, although having different impacts on the presence of elements, are therefore not considered harmful to the environment or humans. However, it may seem wise to favour a substrate with a minimal presence of these monitored elements.

### 4.5.4 Difference between tap water and theoretical rainwater

As this study used artificial rain with tap water, it is obvious that the reality of rainfall will be different. Takeda et al. (2000) conducted a study on rainwater in Japan. This study measured element concentrations in rainwater at Higashi-Hiroshima. Of course, this rain can differ from the rainwater in Chiba, depending on local pollution. Nevertheless, this gives an idea of the composition of rainwater. A comparison with tap water can be found in Table 14.

Table 14 - Tap water and theoretical rainwater element concentrations

| Element (mg/l) | Al | Ba | Ca | Cd | Cu | K | Mg | Mn | Na | Pb | Sr | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rainwater | 0.14 | 0.01 | 2.30 | 0.00 | 0.01 | 0.66 | 0.84 | 0.03 | 6.61 | 0.03 | 0.01 | 0.07 |
| Collection 5 | 0.00 | 0.01 | 17.87 | 0.00 | 0.00 | 7.52 | 5.36 | 0.00 | 35.21 | 0.00 | 0.05 | 0.00 |
| Collection 6 | 0.00 | 0.00 | 14.22 | 0.01 | 0.00 | 6.74 | 4.19 | 0.00 | 28.50 | 0.00 | 0.04 | 0.00 |

It is clear that the concentrations of Na, Mg, K and Ca are very strongly decreased in rainwater. Their contribution to the soil will be lower. This could potentially free up space for other cations on the

CEC. Other elements such as Ba, Cd, Cu, Pb and Sr are found in similar concentrations. While elements absent in tap water appear in rainwater in small quantities (Al, Mn and Zn). Concentrations of the elements in rainwater remain low and are not expected to pose any real problems.

# 5. Conclusion

The world urbanization has brought many disadvantages for city dwellers: countless buildings, air pollution and difficulties in managing runoff water. This expansion of cities has led to an increase in impermeable surfaces that inhibit the absorption of water in cities. This runoff water ends up in the sewers and can lead to flooding.

Green roofs can be a solution to these management problems. Their presence reduces the volume of runoff water thanks to the retention capacity of their substrate. However, they can act as a source of pollutant. Therefore, this study is conducted to analyze the impact of several substrates on runoff water in order to reduce its volume and optimize its quality.

The retention capacity and water drained quality of a green roof depends on the composition of its substrate. Different mixtures of conventional soil, coco peat and rice husk were tested. One kind of substrate composed exclusively of conventional soil (S). Three kinds of substrates composed of conventional soil mixed with 10 (RH10), 25 (RH25) and 50% (RH50) of rice husk. Three kinds of substrates composed of conventional soil with 10 (CC10), 25 (CC25) and 50% (CC50) of coco peat and one kind of substrate composed strictly of coco peat (CC100). The various substrates were sprayed with 4l of tap water. The volumes of drained water were measured and their concentration in elements was analyzed. Many components were analyzed such as: pH, DOC, Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga K, Li, Mg, Mn, Na, Ni, Pb, Se, Sr, V, Zn, Cl, $NH_4$, $PO_4$, $NO_2$, $NO_3$, $SO_4$.

The results showed that the different substrates did not have significant impacts on the sum of drainage water collected during the entire period. Nevertheless, by analysing the collections separately, we can draw conclusions. Only the volumes of water collected during collection preceded by heavy rainfall are not affected by the substrates. Obviously, despite the absence of impact of the different substrates, the volume of runoff water is reduced after absorption. Among the collections showing a significant impact of the substrates, the mixtures were compared to the most commonly used soil in green roofs in Japan (S). Within the different mixtures, only the RH50 showed a significant difference in most collections. Its retention capacity is higher in each collection than any other substrate. Then, CC100, RH10 and CC10 substrates allows a significant improvement in water absorption in 2 collections. It was noted that a climate with dry periods and sufficient evaporation favours the retention capacities of the different substrate. Therefore, RH50 distinguishes itself, although its volume difference in the total amount of water collected is not significant.

Various factors such as evaporation, moisture and vegetation surface were also measured to assess the impact of substrates on them. Nevertheless, none of these factors are significantly impacted by the substrates. This is certainly due to the youthfulness of the roofs which is a limiting factor in this study.

Although the decrease in water volume limits the possibility of element leaching, it is still important to ensure that drained water is not harmful to the environment or humans. In general, the conventional substrate is a source of $NO_3$ and absorbs Ca, Mg and Na. Then, the increase in the percentage of coco peat has led to an increasing source of C and Fe while its absorption of Cl is rising. Ca and Mg are also absorbed, but this sorption increases up to CC25 and then decreases in CC50 and CC100 substrates. At the same time, the increase in rice husk will expand the concentration of $SO_4$, Ba, K and V while Ca, Mg and Na concentrations decrease. A comparison of the results with standard concentrations of drainage water in Japan showed that none of the drained water from the different substrates would be damaging to humans or the environment. Substrates releasing smaller concentrations of elements still need to be favoured. However, it has been shown that the CC100 substrate tends to acidify the pH of the water. This substrate will therefore not be selected because it will not neutralize acid rain.

# Perspectives

First, a longer study may be useful for a better understanding of climate impacts. Indeed, since Japan's climate is varied throughout the year (rainy season, typhoons, high temperatures, etc) it is difficult to select a substrate on the basis of a single month of observation. The results of the drained water volumes showed that some collections had significant differences with the conventional substrate used of Japan. Nevertheless, the sum of the collections throughout the study shows no significant differences between the different substrates. A longer study will allow to target periods when some substrates would be more useful than others or simply to prove that the different substrates do not have significant differences under the annual climate in Japan. In addition, seasonal variations between concentrations of different elements have been identified. It may therefore be interesting to carry out the experiment more than one year.

In the same context, it seems essential to me to re-examine these criteria later, once the grass has been properly developed. This will make it possible to better link the different components of the different green roofs and probably to see more significant impacts of substrates on different criteria (vegetation, evapotranspiration, etc).

Then, I think that texture and structure analyses of the different substrates could be useful in order to better interpret and understand the results of the data like the water retention. A better knowledge of their respective CEC could also be beneficial. Indeed, few data on substrates were present in this study other than theoretical data found in the literature.  This leaves the field open to misinterpretation.

Finally, an analysis on rainwater will allow a better understanding of the real conditions and should be encouraged.

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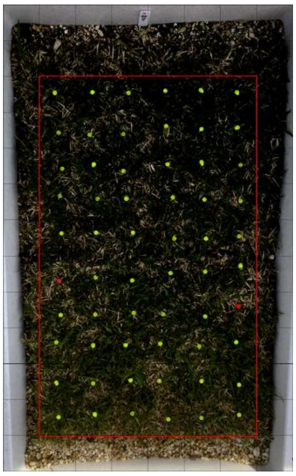
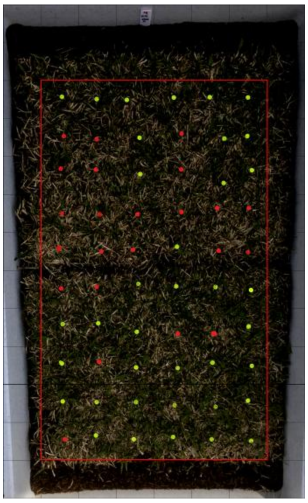
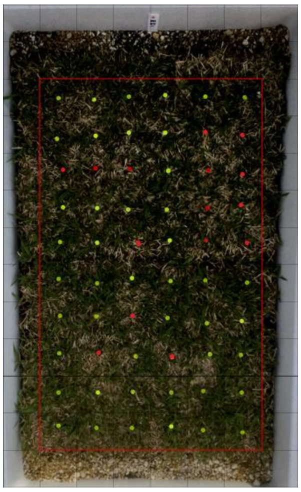
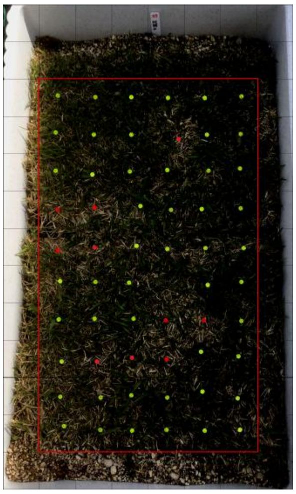
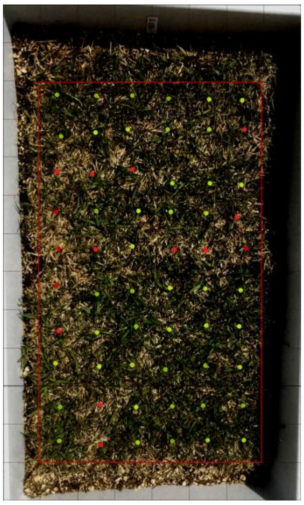
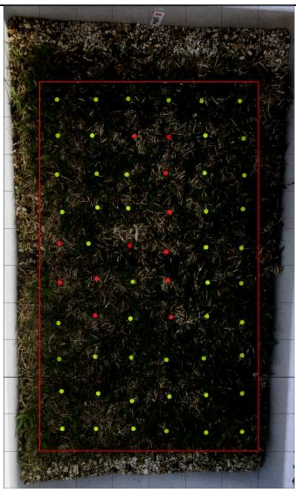
# Appendix

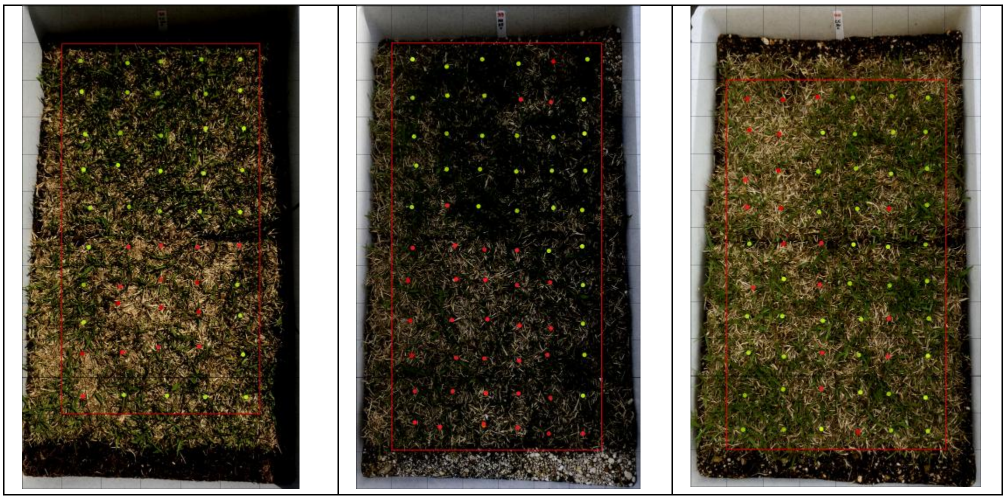
Appendix 1 - Photos of the different containers

| | | |
|---|---|---|
| 1 | 2 | 3 |
|  |  |  |
| 4 | 5 | 6 |
|  |  |  |
| 7 | 8 | 9 |
|  |  |  |
| 10 | 11 | 12 |

| | | |
|---|---|---|
|  |  |  |
| 13 | 14 | 15 |
|  |  |  |
| 16 | 17 | 19 |
|  |  |  |
| 20 | 21 | 22 |

| | | |
|---|---|---|
|  |  |  |
| 23 | 24 | 25 |
|  |  |  |
| 26 | 27 | 28 |
|  |  |  |
| 29 | 30 | 31 |

| | | |
|---|---|---|
|  |  |  |
| 32 | 33 | 34 |
|  |  |  |
| 35 | 36 | 37 |
|  |  |  |
| 38 | 39 | 40 |



Appendix 2 – Water Quantity

| Cont. | Bloc | Substrate | Water amount Week 1 | Water amount Week 2 | Water amount Week 3 | Water amount Week 4 | Water amount Week 5 | Water amount Week 6 |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | S | 2.03 | 2.68 | 1.425 | 1.625 | 2.7 | 1.35 |
| 2 | 1 | CC25 | 1.95 | 2.75 | 1.2 | 1.55 | 2.75 | 1.35 |
| 3 | 1 | RH10 | 1.70 | 2.88 | 1.075 | 1.45 | 2.95 | 1.075 |
| 4 | 1 | CC100 | 2.20 | 2.70 | 1.025 | 1.35 | 2.7 | 0.9 |
| 5 | 1 | CC10 | 1.95 | 2.78 | 1.025 | 1.4 | 2.85 | 1.1 |
| 6 | 1 | RH25 | 1.90 | 2.75 | 1 | 1.575 | 3 | 1.15 |
| 7 | 1 | CC50 | 2.30 | 2.63 | 1.3 | 1.675 | 2.8 | 1.2 |
| 8 | 1 | RH50 | 1.50 | 2.45 | 0.525 | 1.05 | 2.85 | 0.65 |
| 9 | 5 | RH10 | 1.80 | 2.83 | 1.025 | 1.35 | 2.9 | 1.1 |
| 10 | 5 | S | 1.52 | 2.73 | 1.2 | 1.375 | 2.575 | 1.15 |
| 11 | 2 | RH50 | 1.13 | 2.18 | 0.125 | 0.85 | 2.7 | 0.15 |
| 12 | 2 | CC100 | 2.05 | 2.60 | 0.975 | 1.5 | 2.725 | 0.85 |
| 13 | 2 | RH25 | 1.60 | 2.65 | 0.975 | 1.525 | 2.925 | 1.1 |
| 14 | 2 | S | 1.50 | 2.65 | 1.125 | 1.375 | 2.65 | 1.75 |
| 15 | 2 | CC50 | 2.30 | 2.78 | 1.25 | 1.725 | 2.725 | 1.1 |
| 16 | 2 | CC10 | 1.73 | 2.70 | 0.875 | 1.275 | 2.825 | 0.925 |
| 17 | 2 | CC25 | 1.83 | 2.68 | 1.25 | 1.575 | 2.65 | 1.3 |
| 18 | 2 | RH10 | 1.50 | 2.55 | 0.7 | 1.275 | 2.45 | 0.725 |
| 19 | 5 | CC25 | 1.35 | 2.68 | 1.3 | 1.575 | 2.625 | 1.2 |
| 20 | 5 | RH50 | 1.52 | 2.25 | 0.325 | 0.8 | 2.65 | 0.225 |
| 21 | 3 | RH10 | 0.95 | 2.40 | 0.825 | 1.1 | 2.55 | 0.825 |
| 22 | 3 | RH25 | 1.40 | 2.53 | 0.7 | 1.35 | 2.9 | 1 |
| 23 | 3 | CC10 | 1.15 | 2.48 | 1.075 | 1.325 | 2.575 | 1.1 |
| 24 | 3 | CC25 | 1.55 | 2.65 | 1.225 | 1.575 | 2.65 | 1.225 |
| 25 | 3 | RH50 | 0.73 | 2.30 | 0.075 | 0.85 | 2.725 | 0.3 |
| 26 | 3 | CC50 | 1.83 | 2.68 | 1.15 | 1.6 | 2.7 | 0.975 |
| 27 | 3 | CC100 | 1.48 | 2.55 | 0.825 | 1.25 | 2.625 | 0.725 |
| 28 | 3 | S | 1.15 | 2.63 | 1.075 | 1.225 | 2.825 | 1.05 |
| 29 | 5 | CC10 | 1.10 | 2.60 | 0.8 | 1.125 | 2.8 | 0.825 |
| 30 | 5 | CC100 | 1.63 | 2.40 | 0.925 | 1.3 | 2.6 | 0.775 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 31 | 4 | CC25 | 1.80 | 2.50 | 1.2 | 1.45 | 2.7 | 1.2 |
| 32 | 4 | CC50 | 1.45 | 2.80 | 1.125 | 1.675 | 2.825 | 1.05 |
| 33 | 4 | RH25 | 1.60 | 1.75 | 0.9 | 1.25 | 2.3 | 1.3 |
| 34 | 4 | CC10 | 1.35 | 2.63 | 1.125 | 1.45 | 2.725 | 0.95 |
| 35 | 4 | RH10 | 1.38 | 2.63 | 0.85 | 1.125 | 2.675 | 0.8 |
| 36 | 4 | S | 1.30 | 2.60 | 1.25 | 1.35 | 2.7 | 1.325 |
| 37 | 4 | RH50 | 1.78 | 2.48 | 0.55 | 0.975 | 2.775 | 0.35 |
| 38 | 4 | CC100 | 1.90 | 2.60 | 1 | 1.475 | 2.75 | 0.75 |
| 39 | 5 | RH25 | 1.60 | 2.65 | 1 | 1.45 | 3 | 1 |
| 40 | 5 | CC50 | 1.35 | 2.58 | 1.15 | 1.425 | 2.725 | 1.05 |

Appendix 3 – Water quality data of collection 5

| Cont. | Bloc | Substrate | pH | Carbon | Al | As | Ba | Be | Bi | Ca | Cd | Co | Cr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | S | 6.71 | 8 | 0.04 | 0 | 0.03 | 0 | 0 | 6.31 | 0 | 0 | 0.01 |
| 2 | A | CC25 | 7.04 | 27 | 1.54 | 0 | 0.06 | 0 | 0 | 0.7 | 0 | 0 | 0.01 |
| 3 | A | RH10 | 6.76 | 9 | 0.67 | 0 | 0.08 | 0 | 0 | 3.29 | 0 | 0 | 0.01 |
| 4 | A | CC100 | 5.81 | | 0.6 | 0 | 0.04 | 0 | 0 | 2.67 | 0 | 0 | 0.01 |
| 5 | A | CC10 | 5.88 | 8 | 0.5 | 0 | 0.04 | 0 | 0 | 1.89 | 0 | 0 | 0.01 |
| 6 | A | RH25 | 6.58 | 13 | 2.56 | 0 | 0.09 | 0 | 0 | 1.15 | 0.01 | 0 | 0 |
| 7 | A | CC50 | 6.73 | 63 | 1.71 | 0 | 0.04 | 0 | 0 | 0.41 | 0.01 | 0 | 0 |
| 8 | A | RH50 | 7.48 | 30 | 0.75 | 0.02 | 0.07 | 0 | 0 | 0.99 | 0 | 0 | 0.01 |
| 9 | E | RH10 | 6.92 | 11 | 0.57 | 0 | 0.08 | 0 | 0 | 3.08 | 0 | 0 | 0.01 |
| 10 | E | S | 6.8 | 6 | 0.16 | 0 | 0.04 | 0 | 0 | 7.34 | 0 | 0 | 0.01 |
| 11 | B | RH50 | 7.87 | 19 | 0.93 | 0 | 0.11 | 0 | 0 | 1.11 | 0 | 0 | 0.01 |
| 12 | B | CC100 | 5.53 | 329 | 0.73 | 0 | 0.04 | 0 | 0 | 2.64 | 0 | 0 | 0.01 |
| 13 | B | RH25 | 6.47 | 17 | 2.42 | 0 | 0.12 | 0 | 0 | 1.23 | 0 | 0 | 0.01 |
| 14 | B | S | 6.31 | 4 | 0.17 | 0 | 0.04 | 0 | 0 | 7.17 | 0 | 0 | 0.01 |
| 15 | B | CC50 | 6.54 | 97 | 2.52 | 0 | 0.21 | 0 | 0 | 1.29 | 0 | 0 | 0.01 |
| 16 | B | CC10 | 6.33 | 6 | 0.51 | 0 | 0.04 | 0 | 0 | 1.9 | 0 | 0 | 0.01 |
| 17 | B | CC25 | 6.57 | 25 | 1.33 | 0.01 | 0.05 | 0 | 0 | 0.88 | 0 | 0 | 0.01 |
| 18 | B | RH10 | 6.27 | 5 | 0.34 | 0 | 0.09 | 0 | 0 | 4.69 | 0 | 0 | 0.01 |
| 19 | E | CC25 | 6.64 | 22 | 1.61 | 0 | 0.07 | 0 | 0 | 0.9 | 0 | 0 | 0.01 |
| 20 | E | RH50 | 7.53 | 12 | 1.15 | 0.01 | 0.13 | 0 | 0 | 1.27 | 0 | 0 | 0.01 |
| 21 | C | RH10 | 7.22 | 5 | 0.73 | 0 | 0.11 | 0 | 0 | 2.64 | 0 | 0 | 0.01 |
| 22 | C | RH25 | 7.26 | 9 | 1.1 | 0 | 0.08 | 0 | 0 | 1.17 | 0 | 0 | 0.01 |
| 23 | C | CC10 | 7.15 | 6 | 0.38 | 0 | 0.05 | 0 | 0 | 2.73 | 0 | 0 | 0.01 |
| 24 | C | CC25 | 7.25 | 21 | 1.4 | 0.01 | 0.06 | 0 | 0.01 | 0.79 | 0 | 0 | 0.01 |
| 25 | C | RH50 | 7.4 | 13 | 1.45 | 0.02 | 0.12 | 0 | 0.01 | 1.7 | 0 | 0 | 0.01 |
| 26 | C | CC50 | 6.9 | 85 | 2.1 | 0 | 0.17 | 0 | 0 | 1.06 | 0 | 0 | 0.01 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 27 | C | CC100 | 5.39 | 258 | 0.7 | 0 | 0.03 | 0 | 0.01 | 2.02 | 0 | 0 | 0.01 |
| 28 | C | S | 6.09 | 5 | 0.35 | 0 | 0.03 | 0 | 0.01 | 7.37 | 0 | 0 | 0.01 |
| 29 | E | CC10 | 6.32 | 11 | 0.63 | 0.01 | 0.04 | 0 | 0.01 | 1.88 | 0 | 0 | 0.01 |
| 30 | E | CC100 | 5.71 | X | 0.87 | 0 | 0.05 | 0 | 0.01 | 4.12 | 0 | 0 | 0.01 |
| 31 | D | CC25 | 6.31 | 23 | 1.57 | 0.01 | 0.07 | 0 | 0.02 | 0.83 | 0 | 0 | 0.01 |
| 32 | D | CC50 | 6.26 | 59 | 2.18 | 0.01 | 0.08 | 0 | 0.02 | 0.72 | 0 | 0 | 0.01 |
| 33 | D | RH25 | 6.17 | 10 | 1.66 | 0.01 | 0.1 | 0 | 0.02 | 1.54 | 0 | 0 | 0.01 |
| 34 | D | CC10 | 6.1 | 12 | 0.76 | 0 | 0.04 | 0 | 0.02 | 2.37 | 0 | 0 | 0.01 |
| 35 | D | RH10 | 6.19 | 9 | 0.5 | 0 | 0.1 | 0 | 0.02 | 3.9 | 0 | 0 | 0.01 |
| 36 | D | S | 6.24 | 5 | 0.16 | 0 | 0.04 | 0 | 0.01 | 9.01 | 0 | 0 | 0.01 |
| 37 | D | RH50 | 7.41 | 10 | 2.23 | 0.01 | 0.13 | 0 | 0.02 | 1.21 | 0 | 0 | 0.01 |
| 38 | D | CC100 | 6.29 | 172 | 0.42 | 0.01 | 0.02 | 0 | 0.07 | 1.61 | 0 | 0 | 0.01 |
| 39 | E | RH25 | 6.82 | 8 | 3.72 | 0.01 | 0.14 | 0 | 0.06 | 1.55 | 0 | 0 | 0.01 |
| 40 | E | CC50 | 6.34 | 75 | 3.4 | 0.01 | 0.12 | 0 | 0.02 | 1.54 | 0 | 0 | 0.01 |
| 41 | | TW | 7.11 | 1 | 0 | 0.01 | 0.01 | 0 | 0.07 | 17.87 | 0 | 0 | 0.01 |

| Cont. | Bloc | Substrate | Cu | Fe | Ga | K | Li | Mg | Mn | Na | Ni | Pb | Se |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | S | 0 | 0 | 0 | 4.13 | 0 | 1.89 | 0 | 14.86 | 0 | 0.01 | 0 |
| 2 | A | CC25 | 0 | 0.53 | 0 | 13.32 | 0 | 0.35 | 0 | 18.94 | 0 | 0 | 0 |
| 3 | A | RH10 | 0 | 0.13 | 0 | 18.86 | 0 | 1.19 | 0 | 17.25 | 0 | 0 | 0 |
| 4 | A | CC100 | 0 | 2.12 | 0 | 43.66 | 0 | 2.02 | 0.04 | 32.22 | 0 | 0.02 | 0 |
| 5 | A | CC10 | 0 | 0.13 | 0 | 8.95 | 0 | 1.11 | 0 | 20.27 | 0 | 0 | 0 |
| 6 | A | RH25 | 0 | 0.59 | 0 | 24.11 | 0 | 0.37 | 0 | 14.51 | 0 | 0.01 | 0 |
| 7 | A | CC50 | 0 | 0.82 | 0 | 17.33 | 0 | 0.26 | 0 | 17.65 | 0 | 0.01 | 0 |
| 8 | A | RH50 | 0 | 0.26 | 0 | 86.28 | 0 | 0.43 | 0 | 15.28 | 0 | 0 | 0 |
| 9 | E | RH10 | 0 | 0.13 | 0 | 20.45 | 0 | 1.08 | 0 | 17.29 | 0 | 0 | 0 |
| 10 | E | S | 0 | 0 | 0 | 4.99 | 0 | 2.12 | 0 | 16.63 | 0 | 0.02 | 0 |
| 11 | B | RH50 | 0 | 0.23 | 0 | 69.16 | 0 | 0.36 | 0 | 15.96 | 0 | 0 | 0 |
| 12 | B | CC100 | 0 | 2.31 | 0 | 39 | 0 | 1.91 | 0.05 | 28.03 | 0 | 0 | 0 |
| 13 | B | RH25 | 0 | 0.57 | 0 | 26.16 | 0 | 0.38 | 0 | 14.68 | 0 | 0 | 0 |
| 14 | B | S | 0 | 0.02 | 0 | 5.04 | 0 | 2.16 | 0 | 17.64 | 0 | 0.01 | 0 |
| 15 | B | CC50 | 0 | 1.42 | 0 | 20.81 | 0 | 0.58 | 0 | 22.73 | 0 | 0 | 0 |
| 16 | B | CC10 | 0 | 0.14 | 0 | 8.22 | 0 | 1.18 | 0 | 22.97 | 0 | 0 | 0 |
| 17 | B | CC25 | 0 | 0.6 | 0 | 12.81 | 0 | 0.46 | 0 | 22.17 | 0 | 0 | 0 |
| 18 | B | RH10 | 0 | 0.09 | 0 | 22 | 0 | 2.03 | 0 | 22.19 | 0 | 0 | 0 |
| 19 | E | CC25 | 0 | 0.64 | 0 | 14.75 | 0 | 0.49 | 0 | 25 | 0 | 0 | 0 |
| 20 | E | RH50 | 0 | 0.3 | 0 | 62.54 | 0 | 0.4 | 0 | 19.22 | 0 | 0 | 0 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 21 | C | RH10 | 0 | 0.17 | 0 | 22.94 | 0 | 1.1 | 0 | 16.79 | 0 | 0 | 0 |
| 22 | C | RH25 | 0 | 0.28 | 0 | 25.92 | 0 | 0.42 | 0 | 15.31 | 0 | 0 | 0 |
| 23 | C | CC10 | 0 | 0.1 | 0 | 10.63 | 0 | 1.56 | 0 | 21.94 | 0 | 0 | 0 |
| 24 | C | CC25 | 0 | 0.56 | 0 | 11.55 | 0 | 0.41 | 0 | 22.18 | 0 | 0.01 | 0 |
| 25 | C | RH50 | 0 | 0.46 | 0 | 92 | 0 | 0.59 | 0.02 | 17.73 | 0 | 0.01 | 0 |
| 26 | C | CC50 | 0 | 1.31 | 0 | 22.1 | 0 | 0.51 | 0 | 23.34 | 0 | 0 | 0 |
| 27 | C | CC100 | 0 | 1.78 | 0 | 36.2 | 0 | 1.58 | 0.04 | 28.22 | 0 | 0.01 | 0 |
| 28 | C | S | 0 | 0.08 | 0 | 5.22 | 0 | 2.62 | 0 | 21.17 | 0 | 0 | 0 |
| 29 | E | CC10 | 0 | 0.17 | 0.01 | 8.19 | 0 | 1.12 | 0 | 24.37 | 0 | 0 | 0 |
| 30 | E | CC100 | 0.01 | 3.18 | 0 | 48.97 | 0 | 3.07 | 0.07 | 38.05 | 0 | 0.01 | 0 |
| 31 | D | CC25 | 0 | 0.69 | 0 | 15.55 | 0 | 0.41 | 0 | 25.16 | 0 | 0 | 0 |
| 32 | D | CC50 | 0 | 1.17 | 0 | 21.42 | 0 | 0.35 | 0 | 23.46 | 0 | 0 | 0 |
| 33 | D | RH25 | 0 | 0.44 | 0 | 31.89 | 0 | 0.54 | 0 | 19.47 | 0 | 0.01 | 0 |
| 34 | D | CC10 | 0 | 0.25 | 0 | 8.5 | 0 | 1.37 | 0 | 26.4 | 0 | 0.01 | 0 |
| 35 | D | RH10 | 0 | 0.14 | 0 | 23.49 | 0 | 1.36 | 0 | 20.38 | 0 | 0.01 | 0 |
| 36 | D | S | 0 | 0.03 | 0 | 6.07 | 0 | 2.67 | 0 | 22.64 | 0 | 0 | 0 |
| 37 | D | RH50 | 0 | 0.6 | 0.01 | 64.79 | 0 | 0.34 | 0 | 18.6 | 0 | 0 | 0 |
| 38 | D | CC100 | 0 | 1.44 | 0 | 29.94 | 0 | 1.12 | 0.01 | 24.17 | 0 | 0.01 | 0 |
| 39 | E | RH25 | 0 | 1.86 | 0 | 24.02 | 0 | 0.72 | 0 | 21.16 | 0 | 0.01 | 0 |
| 40 | E | CC50 | 0 | 1.75 | 0.01 | 24.22 | 0 | 0.71 | 0 | 22.78 | 0 | 0 | 0 |
| 41 | | TW | 0 | 0 | 0.01 | 7.52 | 0 | 5.36 | 0 | 35.21 | 0 | 0 | 0 |

| Cont. | Bloc | Substrate | Sr | V | Zn | Cl | $NO_2$ | $NO_3$ | $PO_4$ | $SO_4$ | $NH_4$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | S | 0.06 | 0 | 0 | 1.4 | 0.02 | 1.09 | | 2.08 | |
| 2 | A | CC25 | 0.01 | 0 | 0 | 1.33 | 0.02 | 0.24 | 0.18 | 1.69 | |
| 3 | A | RH10 | 0.07 | 0 | 0 | 1.3 | 0.02 | 1.34 | 0.26 | 3.14 | |
| 4 | A | CC100 | 0.02 | 0 | 0 | 1.28 | 0.01 | 0.03 | | 1.06 | |
| 5 | A | CC10 | 0.03 | 0 | 0 | 1.21 | | 1.13 | | 2.45 | |
| 6 | A | RH25 | 0.02 | 0.01 | 0 | 0.98 | 0.01 | 0.72 | 0.32 | 2.58 | 0.03 |
| 7 | A | CC50 | 0 | 0 | 0 | 1.24 | | 0.26 | 0.21 | 1.55 | 0.35 |
| 8 | A | RH50 | 0.01 | 0.01 | 0 | 2.49 | 0.04 | 0.96 | 2.57 | 3.98 | |
| 9 | E | RH10 | 0.06 | 0 | 0 | 1.29 | 0.02 | 1.18 | 0.25 | 3.13 | |
| 10 | E | S | 0.08 | 0 | 0 | 1.3 | | 1.33 | | 2.07 | |
| 11 | B | RH50 | 0.02 | 0.01 | 0 | 1.53 | 0.02 | 0.34 | 1.48 | 2.29 | 0.03 |
| 12 | B | CC100 | 0.02 | 0 | 0 | 0.94 | 0.01 | 0.07 | | 0.75 | 0.01 |
| 13 | B | RH25 | 0.02 | 0 | 0 | 1.12 | | 0.5 | 0.26 | 2.41 | |
| 14 | B | S | 0.07 | 0 | 0 | 1.29 | | 1.05 | | 1.73 | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | B | CC50 | 0.02 | 0 | 0 | 1.25 | 0.02 | 0.1 | 0.34 | 1.46 | |
| 16 | B | CC10 | 0.03 | 0 | 0 | 1.3 | | 1.23 | | 2.24 | |
| 17 | B | CC25 | 0.01 | 0 | 0 | 1.38 | 0.02 | 0.29 | 0.22 | 1.49 | |
| 18 | B | RH10 | 0.1 | 0 | 0 | 1.6 | | 1.74 | 0.25 | 3.16 | 0.34 |
| 19 | E | CC25 | 0.01 | 0 | 0 | 1.5 | | 0.34 | | 1.81 | |
| 20 | E | RH50 | 0.03 | 0.01 | 0 | 1.94 | | 0.57 | 1.08 | 2.02 | 0.04 |
| 21 | C | RH10 | 0.07 | 0 | 0 | 1.25 | | 0.84 | 0.25 | 2.83 | |
| 22 | C | RH25 | 0.03 | 0 | 0 | 1.04 | | 0.6 | 0.39 | 2.07 | |
| 23 | C | CC10 | 0.05 | 0 | 0 | 1.26 | | 1.28 | 0.24 | 2.06 | |
| 24 | C | CC25 | 0.01 | 0 | 0 | 1.25 | | 0.06 | | 1.33 | |
| 25 | C | RH50 | 0.02 | 0 | 0 | 3.23 | | 0.41 | 2.65 | 4.9 | 0.06 |
| 26 | C | CC50 | 0.01 | 0 | 0 | 1.1 | | | 0.27 | 1.41 | |
| 27 | C | CC100 | 0.02 | 0 | 0 | 0.98 | | 0.07 | | 0.78 | 0.03 |
| 28 | C | S | 0.08 | 0 | 0 | 1.21 | | 1.67 | | 1.98 | |
| 29 | E | CC10 | 0.03 | 0 | 0 | 1.15 | | 1.12 | | 2 | |
| 30 | E | CC100 | 0.04 | 0 | 0.01 | 1.01 | | 0.04 | | 0.8 | 0.06 |
| 31 | D | CC25 | 0.01 | 0 | 0 | 1.18 | | 0.15 | | 1.79 | |
| 32 | D | CC50 | 0.01 | 0 | 0 | 1.08 | | 0.22 | 0.32 | 1.33 | |
| 33 | D | RH25 | 0.03 | 0 | 0 | 1.31 | | 0.63 | 0.29 | 1.89 | |
| 34 | D | CC10 | 0.04 | 0 | 0 | 1.17 | | 0.73 | | 2.15 | |
| 35 | D | RH10 | 0.06 | 0 | 0 | 1.37 | | 0.72 | | 2.8 | |
| 36 | D | S | 0.08 | 0 | 0 | 1.39 | | 0.98 | | 1.8 | |
| 37 | D | RH50 | 0.02 | 0.01 | 0 | 1.59 | | 0.8 | 0.91 | 1.95 | |
| 38 | D | CC100 | 0.01 | 0 | 0 | 0.92 | | 0.04 | | 0.69 | |
| 39 | E | RH25 | 0.02 | 0 | 0 | 1.16 | 0.01 | 0.17 | 0.33 | 1.49 | |
| 40 | E | CC50 | 0.02 | 0 | 0 | 1.16 | | 0.15 | 0.32 | 1.47 | |
| 41 | | TW | 0.05 | 0 | 0 | 1.98 | | 0.15 | | 0.87 | |

Appendix 4 – ANOVA results of the water quality data of collection 5

pH

```
          Df Sum Sq Mean Sq F value   Pr(>F)    
Sub        8  8.934  1.1167   9.617 2.68e-06 ***
bloc       4  0.965  0.2412   2.077    0.111    
Residuals 28  3.251  0.1161                     
```

C

```
          Df Sum Sq Mean Sq F value   Pr(>F)    
Sub        8 168123   21015   48.84 1.02e-13 ***
bloc       4   2564     641    1.49    0.234    
Residuals 26  11187     430                     
```

Al

As

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 25.543 | 3.193 | 14.740 | 3.45e-08 | *** |
| bloc | 4 | 1.253 | 0.313 | 1.447 | 0.245 | |
| Residuals | 28 | 6.065 | 0.217 | | | |

Ba

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.0005756 | 7.195e-05 | 2.985 | 0.015 | * |
| bloc | 4 | 0.0001250 | 3.125e-05 | 1.296 | 0.295 | |
| Residuals | 28 | 0.0006750 | 2.411e-05 | | | |

Be

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.05082 | 0.006352 | 8.882 | 5.65e-06 | *** |
| bloc | 4 | 0.00498 | 0.001244 | 1.739 | 0.169 | |
| Residuals | 28 | 0.02002 | 0.000715 | | | |

Bi

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 8 | 0 | 0 | | |
| bloc | 4 | 0 | 0 | | |
| Residuals | 28 | 0 | 0 | | |

Ca

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.00472 | 0.0005900 | 3.655 | 0.00495 | ** |
| bloc | 4 | 0.00356 | 0.0008900 | 5.513 | 0.00211 | ** |
| Residuals | 28 | 0.00452 | 0.0001614 | | | |

Cd

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 397.8 | 49.72 | 134.208 | <2e-16 | *** |
| bloc | 4 | 1.5 | 0.37 | 1.008 | 0.42 | |
| Residuals | 28 | 10.4 | 0.37 | | | |

Co

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 3.024e-05 | 3.780e-06 | 0.882 | 0.5434 | |
| bloc | 4 | 4.000e-05 | 1.000e-05 | 2.333 | 0.0802 | . |
| Residuals | 28 | 1.200e-04 | 4.286e-06 | | | |

Cr

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 8 | 0 | 0 | | |
| bloc | 4 | 0 | 0 | | |
| Residuals | 28 | 0 | 0 | | |

Cu

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 3.024e-05 | 3.780e-06 | 0.882 | 0.5434 | |
| bloc | 4 | 4.000e-05 | 1.000e-05 | 2.333 | 0.0802 | . |
| Residuals | 28 | 1.200e-04 | 4.286e-06 | | | |

Fe

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 8 | 1.756e-05 | 2.195e-06 | 0.878 | 0.547 |
| bloc | 4 | 1.000e-05 | 2.500e-06 | 1.000 | 0.424 |
| Residuals | 28 | 7.000e-05 | 2.500e-06 | | |

Ga

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 18.920 | 2.3650 | 22.958 | 2.3e-10 | *** |
| bloc | 4 | 1.048 | 0.2621 | 2.544 | 0.0617 | . |
| Residuals | 28 | 2.884 | 0.1030 | | | |

K

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.000121 | 1.512e-05 | 2.117 | 0.0681 | . |
| bloc | 4 | 0.000040 | 1.000e-05 | 1.400 | 0.2596 | |
| Residuals | 28 | 0.000200 | 7.143e-06 | | | |

Li

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 17877 | 2234.6 | 65.012 | 4.84e-16 | *** |
| bloc | 4 | 54 | 13.4 | 0.391 | 0.813 | |
| Residuals | 28 | 962 | 34.4 | | | |

Mg

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 8 | 0 | 0 | | |
| bloc | 4 | 0 | 0 | | |
| Residuals | 28 | 0 | 0 | | |

Mn

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 37.29 | 4.661 | 40.040 | 2.53e-13 | *** |
| bloc | 4 | 0.33 | 0.082 | 0.701 | 0.598 | |
| Residuals | 28 | 3.26 | 0.116 | | | |

Na

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.007610 | 0.0009512 | 13.764 | 7.18e-08 | *** |
| bloc | 4 | 0.000265 | 0.0000663 | 0.959 | 0.445 | |
| Residuals | 28 | 0.001935 | 0.0000691 | | | |

Ni

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 848.7 | 106.09 | 14.246 | 4.98e-08 | *** |
| bloc | 4 | 87.7 | 21.93 | 2.944 | 0.0377 | * |
| Residuals | 28 | 208.5 | 7.45 | | | |

Pb

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 8 | 0 | 0 | | |
| bloc | 4 | 0 | 0 | | |
| Residuals | 28 | 0 | 0 | | |

Se

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 8 | 0.0003951 | 4.939e-05 | 1.563 | 0.181 |
| bloc | 4 | 0.0001150 | 2.875e-05 | 0.910 | 0.472 |
| Residuals | 28 | 0.0008850 | 3.161e-05 | | |

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 8 | 0 | 0 | | |
| bloc | 4 | 0 | 0 | | |
| Residuals | 28 | 0 | 0 | | |

**Sr**

```
          Df   Sum Sq   Mean Sq F value   Pr(>F)
sub        8 0.022995 0.0028744   34.62 1.57e-12 ***
bloc       4 0.000475 0.0001187    1.43     0.25
Residuals 28 0.002325 0.0000830
```

**V**

```
          Df   Sum Sq   Mean Sq F value   Pr(>F)
sub        8 0.000279 3.488e-05   7.234 3.52e-05 ***
bloc       4 0.000025 6.250e-06   1.296    0.295
Residuals 28 0.000135 4.820e-06
```

**Zn**

```
          Df    Sum Sq   Mean Sq F value Pr(>F)
sub        8 1.756e-05 2.195e-06   0.878  0.547
bloc       4 1.000e-05 2.500e-06   1.000  0.424
Residuals 28 7.000e-05 2.500e-06
```

**$NH_4$**

```
          Df  Sum Sq Mean Sq F value   Pr(>F)
sub        4 0.14769 0.03692 105.492 0.00941 **
bloc       2 0.00103 0.00052   1.476 0.40385
Residuals  2 0.00070 0.00035
```

**$SO_4$**

```
          Df Sum Sq Mean Sq F value   Pr(>F)
sub        8 21.168  2.6460  10.648 9.96e-07 ***
bloc       4  1.553  0.3882   1.562    0.212
Residuals 28  6.958  0.2485
```

**$PO_4$**

```
          Df Sum Sq Mean Sq F value   Pr(>F)
sub        5  8.278  1.6556   9.771 0.000653 ***
bloc       4  0.697  0.1743   1.029 0.431812
Residuals 12  2.033  0.1694
```

**$NO_3$**

```
          Df Sum Sq Mean Sq F value  Pr(>F)
sub        8  7.912  0.9890  17.355 8.6e-09 ***
bloc       4  0.153  0.0382   0.671   0.618
Residuals 27  1.539  0.0570
```

**$NO_2$**

```
          Df    Sum Sq   Mean Sq F value Pr(>F)
sub        6 0.0005667 9.444e-05   2.125  0.286
bloc       2 0.0000667 3.333e-05   0.750  0.544
Residuals  3 0.0001333 4.444e-05
```

**Cl**

```
          Df Sum Sq Mean Sq F value   Pr(>F)
sub        8  4.694  0.5868   7.406 2.88e-05 ***
bloc       4  0.158  0.0394   0.498    0.738
Residuals 28  2.218  0.0792
```

Appendix 5 - Water quality data of collection 6

| Cont. | Bloc | Substrate | pH | Carbon | Al | As | Ba | Be | Bi | Ca | Cd | Co | Cr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | S | 5.96 | 5 | 0 | 0.01 | 0.04 | 0 | 0.02 | 7.59 | 0 | 0 | 0.01 |
| 2 | A | CC25 | 6.45 | 20 | 0.45 | 0.01 | 0.06 | 0 | 0.04 | 1.05 | 0 | 0 | 0.01 |
| 3 | A | RH10 | 6.48 | 6 | 0.12 | 0.01 | 0.08 | 0 | 0.03 | 2.82 | 0 | 0 | 0.01 |
| 4 | A | CC100 | 5.72 | 333 | 0.53 | 0.01 | 0.05 | 0 | 0.02 | 3.47 | 0 | 0 | 0.01 |
| 5 | A | CC10 | 6.05 | 10 | 0.26 | 0.01 | 0.04 | 0 | 0.03 | 1.75 | 0 | 0 | 0.01 |
| 6 | A | RH25 | 6.44 | 11 | 0.75 | 0.01 | 0.1 | 0 | 0.03 | 1.46 | 0 | 0 | 0.01 |
| 7 | A | CC50 | 6.64 | 47 | 0.95 | 0.01 | 0.08 | 0 | 0.03 | 0.58 | 0 | 0 | 0.01 |
| 8 | A | RH50 | 7.47 | 16 | 0.68 | 0.02 | 0.11 | 0 | 0.02 | 1.11 | 0 | 0 | 0.01 |
| 9 | E | RH10 | 6.94 | 6 | 0.13 | 0.01 | 0.07 | 0 | 0.03 | 3.3 | 0 | 0 | 0.01 |
| 10 | E | S | 6.77 | 5 | 0 | 0 | 0.05 | 0 | 0.01 | 7.94 | 0 | 0 | 0.01 |
| 11 | B | RH50 | 7.48 | 25 | 0.56 | 0.02 | 0.14 | 0 | 0 | 1.5 | 0 | 0 | 0.01 |
| 12 | B | CC100 | 5.3 | 457 | 1.01 | 0 | 0.07 | 0 | 0 | 5.02 | 0 | 0 | 0.01 |
| 13 | B | RH25 | 6.54 | 10 | 0.52 | 0.01 | 0.1 | 0 | 0.01 | 1.41 | 0 | 0 | 0.01 |
| 14 | B | S | 6.3 | 5 | 0.12 | 0 | 0.05 | 0 | 0.01 | 7.38 | 0 | 0 | 0.01 |
| 15 | B | CC50 | 6.56 | 112 | 4.06 | 0.01 | 0.33 | 0 | 0.01 | 1.94 | 0 | 0 | 0.01 |
| 16 | B | CC10 | 6.47 | 8 | 0.89 | 0 | 0.05 | 0 | 0.02 | 1.6 | 0 | 0 | 0 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | B | CC25 | 6.7 | 24 | 0.87 | 0 | 0.04 | 0 | 0 | 0.91 | 0.01 | 0 | 0 |
| 18 | B | RH10 | 6.43 | 6 | 0.23 | 0 | 0.06 | 0 | 0 | 3.56 | 0.01 | 0 | 0 |
| 19 | E | CC25 | 7.14 | 26 | 1.02 | 0 | 0.05 | 0 | 0 | 0.82 | 0.01 | 0 | 0 |
| 20 | E | RH50 | 7.53 | 20 | 2.16 | 0 | 0.13 | 0 | 0 | 1.02 | 0.01 | 0 | 0 |
| 21 | C | RH10 | 7.09 | 6 | 0.58 | 0 | 0.07 | 0 | 0 | 1.81 | 0.01 | 0 | 0 |
| 22 | C | RH25 | 7.16 | 9 | 1.41 | 0 | 0.08 | 0 | 0 | 1.14 | 0.01 | 0 | 0 |
| 23 | C | CC10 | 6.95 | 9 | 0.37 | 0 | 0.02 | 0 | 0 | 1.62 | 0.01 | 0 | 0 |
| 24 | C | CC25 | 7.06 | 23 | 1.3 | 0 | 0.04 | 0 | 0 | 0.67 | 0.01 | 0 | 0 |
| 25 | C | RH50 | 7.47 | 17 | 1.27 | 0.01 | 0.06 | 0 | 0 | 0.95 | 0.01 | 0 | 0 |
| 26 | C | CC50 | 6.46 | 114 | 2.46 | 0 | 0.21 | 0 | 0 | 1.25 | 0.01 | 0 | 0 |
| 27 | C | CC100 | 5.31 | 308 | 0.48 | 0 | 0.02 | 0 | 0 | 2.26 | 0.01 | 0 | 0.01 |
| 28 | C | S | 6.38 | 4 | 0.19 | 0 | 0.01 | 0 | 0 | 5.02 | 0.01 | 0 | 0 |
| 29 | E | CC10 | 5.92 | 6 | 0.9 | 0 | 0.02 | 0 | 0 | 1.14 | 0.01 | 0 | 0 |
| 30 | E | CC100 | 5.57 | 416 | 0.66 | 0 | 0.03 | 0 | 0 | 3.86 | 0.01 | 0 | 0.01 |
| 31 | D | CC25 | 6.15 | 24 | 2.19 | 0 | 0.06 | 0 | 0 | 0.67 | 0.01 | 0 | 0 |
| 32 | D | CC50 | 6.07 | 79 | 1.82 | 0 | 0.07 | 0 | 0 | 0.8 | 0.01 | 0 | 0 |
| 33 | D | RH25 | 6.26 | 9 | 1 | 0 | 0.08 | 0 | 0 | 1.22 | 0.01 | 0 | 0 |
| 34 | D | CC10 | 6.24 | 9 | 1.15 | 0 | 0.02 | 0 | 0 | 1.69 | 0.01 | 0 | 0 |
| 35 | D | RH10 | 6.43 | 6 | 0.69 | 0 | 0.07 | 0 | 0 | 2.53 | 0.01 | 0 | 0 |
| 36 | D | S | | | | | | | | | | | |
| 37 | D | RH50 | 7.25 | 14 | 1.01 | 0 | 0.09 | 0 | 0 | 0.91 | 0.01 | 0 | 0 |
| 38 | D | CC100 | 5.9 | 107 | 0.33 | 0 | 0.01 | 0 | 0 | 1.56 | 0.01 | 0 | 0 |
| 39 | E | RH25 | 6.58 | 10 | 5.3 | 0 | 0.11 | 0 | 0 | 0.98 | 0.01 | 0 | 0 |
| 40 | E | CC50 | 6.27 | 78 | 3.88 | 0 | 0.16 | 0 | 0 | 1.4 | 0.01 | 0 | 0 |
| 41 | | TW | 6.7 | 2 | 0 | 0 | 0 | 0 | 0 | 14.22 | 0.01 | 0 | 0 |

| Cont. | Bloc | Substrate | Cu | Fe | Ga | K | Li | Mg | Mn | Na | Ni | Pb | Se |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | S | 0 | 0 | 0.01 | 6.79 | 0 | 2.31 | 0 | 28.05 | 0 | 0 | 0 |
| 2 | A | CC25 | 0 | 0.38 | 0 | 18.27 | 0 | 0.57 | 0 | 28.62 | 0 | 0 | 0 |
| 3 | A | RH10 | 0 | 0.03 | 0 | 21.75 | 0 | 1.05 | 0 | 26.06 | 0 | 0 | 0 |
| 4 | A | CC100 | 0.01 | 2.99 | 0 | 56.32 | 0 | 2.5 | 0.05 | 43 | 0 | 0 | 0 |
| 5 | A | CC10 | 0 | 0.14 | 0.01 | 11.28 | 0 | 0.99 | 0 | 27.27 | 0 | 0 | 0 |
| 6 | A | RH25 | 0 | 0.31 | 0 | 30.65 | 0 | 0.43 | 0 | 24.69 | 0 | 0.01 | 0 |
| 7 | A | CC50 | 0 | 0.82 | 0.01 | 23.12 | 0 | 0.29 | 0 | 26.64 | 0 | 0 | 0 |
| 8 | A | RH50 | 0 | 0.39 | 0 | 89.25 | 0 | 0.44 | 0 | 22.44 | 0 | 0 | 0 |
| 9 | E | RH10 | 0 | 0.04 | 0 | 19.84 | 0 | 1.2 | 0 | 24.74 | 0 | 0 | 0 |
| 10 | E | S | 0 | 0 | 0 | 7.4 | 0 | 2.28 | 0 | 27.05 | 0 | 0 | 0 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | B | RH50 | 0 | 0.29 | 0 | 77.78 | 0 | 0.44 | 0 | 23.16 | 0 | 0 | 0 |
| 12 | B | CC100 | 0.03 | 4.85 | 0 | 64.08 | 0 | 3.73 | 0.12 | 45.08 | 0 | 0 | 0 |
| 13 | B | RH25 | 0 | 0.22 | 0 | 34.81 | 0 | 0.45 | 0 | 23.68 | 0 | 0 | 0 |
| 14 | B | S | 0 | 0.03 | 0 | 7.17 | 0 | 2.3 | 0 | 28.22 | 0 | 0.01 | 0 |
| 15 | B | CC50 | 0 | 2.3 | 0.01 | 27.17 | 0 | 0.81 | 0 | 30.64 | 0 | 0 | 0 |
| 16 | B | CC10 | 0 | 0.25 | 0.01 | 9.86 | 0 | 1 | 0 | 28.12 | 0 | 0.01 | 0 |
| 17 | B | CC25 | 0 | 0.36 | 0 | 12.32 | 0 | 0.51 | 0 | 20.33 | 0 | 0.01 | 0 |
| 18 | B | RH10 | 0 | 0.06 | 0 | 17.12 | 0 | 1.5 | 0 | 19.43 | 0 | 0.01 | 0 |
| 19 | E | CC25 | 0 | 0.47 | 0 | 13.61 | 0 | 0.44 | 0 | 21.89 | 0 | 0.01 | 0 |
| 20 | E | RH50 | 0 | 0.59 | 0 | 51.41 | 0 | 0.3 | 0 | 14.92 | 0 | 0 | 0 |
| 21 | C | RH10 | 0 | 0.13 | 0 | 17.79 | 0 | 0.75 | 0 | 15.8 | 0 | 0.01 | 0 |
| 22 | C | RH25 | 0 | 0.35 | 0 | 26.01 | 0 | 0.41 | 0 | 14.45 | 0 | 0.01 | 0 |
| 23 | C | CC10 | 0 | 0.11 | 0 | 9.13 | 0 | 0.91 | 0 | 18.5 | 0 | 0 | 0 |
| 24 | C | CC25 | 0 | 0.51 | 0 | 11.88 | 0 | 0.35 | 0 | 19.9 | 0 | 0 | 0 |
| 25 | C | RH50 | 0 | 0.4 | 0 | 74.57 | 0 | 0.36 | 0.01 | 14.67 | 0 | 0 | 0 |
| 26 | C | CC50 | 0 | 1.52 | 0 | 20.97 | 0 | 0.59 | 0 | 21.08 | 0 | 0.01 | 0 |
| 27 | C | CC100 | 0.01 | 2.13 | 0 | 37.47 | 0 | 1.63 | 0.04 | 25.35 | 0 | 0 | 0 |
| 28 | C | S | 0 | 0.04 | 0 | 4.95 | 0 | 1.8 | 0 | 20.49 | 0 | 0.01 | 0 |
| 29 | E | CC10 | 0 | 0.2 | 0 | 6.95 | 0 | 0.64 | 0 | 21.09 | 0 | 0.01 | 0 |
| 30 | E | CC100 | 0.01 | 3.03 | 0 | 47.75 | 0 | 2.74 | 0.06 | 34.68 | 0 | 0 | 0 |
| 31 | D | CC25 | 0 | 0.79 | 0 | 14.23 | 0 | 0.34 | 0 | 21.5 | 0 | 0 | 0 |
| 32 | D | CC50 | 0 | 1.18 | 0 | 18.87 | 0 | 0.4 | 0 | 19.7 | 0 | 0.01 | 0 |
| 33 | D | RH25 | 0 | 0.29 | 0 | 28.11 | 0 | 0.42 | 0 | 17.91 | 0 | 0 | 0 |
| 34 | D | CC10 | 0 | 0.31 | 0 | 7.33 | 0 | 0.93 | 0 | 21.67 | 0 | 0 | 0 |
| 35 | D | RH10 | 0 | 0.16 | 0 | 17.2 | 0 | 0.89 | 0 | 18.85 | 0 | 0 | 0 |
| 36 | D | S | | | | | | | | | | | |
| 37 | D | RH50 | 0 | 0.31 | 0.01 | 47.31 | 0 | 0.27 | 0 | 16.93 | 0 | 0.01 | 0 |
| 38 | D | CC100 | 0 | 1.5 | 0 | 31.71 | 0 | 1.09 | 0.01 | 23.34 | 0 | 0 | 0 |
| 39 | E | RH25 | 0 | 1.17 | 0 | 26.26 | 0 | 0.35 | 0 | 16.53 | 0 | 0.01 | 0 |
| 40 | E | CC50 | 0 | 1.81 | 0 | 21.62 | 0 | 0.61 | 0 | 18.29 | 0 | 0 | 0 |
| 41 | | TW | 0 | 0 | 0 | 6.74 | 0 | 4.19 | 0 | 28.5 | 0 | 0 | 0 |

| Cont. | Bloc | Substrate | Sr | V | Zn | Cl | $NO_2$ | $NO_3$ | $PO_4$ | $SO_4$ | $NH_4$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | S | 0.07 | 0 | 0 | 1.45 | | 0.55 | | 1.46 | 0.24 |
| 2 | A | CC25 | 0.01 | 0 | 0 | 1.43 | | 0.15 | 0.23 | 1.26 | 0.07 |
| 3 | A | RH10 | 0.07 | 0 | 0 | 1.32 | | 0.7 | 0.28 | 2.12 | 0.01 |
| 4 | A | CC100 | 0.03 | 0 | 0.01 | 1.37 | | 0.05 | | 1.2 | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | A | CC10 | 0.03 | 0 | 0 | 1.65 | | 0.43 | | 1.93 | |
| 6 | A | RH25 | 0.03 | 0.01 | 0 | 1.35 | | 0.71 | 0.32 | 2.32 | |
| 7 | A | CC50 | 0 | 0 | 0 | 1.66 | | 0.13 | 0.22 | 1.44 | |
| 8 | A | RH50 | 0.01 | 0.01 | 0 | 2.28 | | 0.82 | 1.98 | 3.31 | |
| 9 | E | RH10 | 0.06 | 0 | 0 | 1.66 | | 0.68 | | 2.23 | 0.16 |
| 10 | E | S | 0.08 | 0 | 0 | 1.68 | | 0.9 | | 1.94 | 0.02 |
| 11 | B | RH50 | 0.03 | 0.02 | 0 | 1.76 | | 0.06 | 1.43 | 2.6 | 0.01 |
| 12 | B | CC100 | 0.05 | 0 | 0.03 | 1.05 | | 0.07 | | 0.88 | |
| 13 | B | RH25 | 0.03 | 0.01 | 0 | 1.62 | | 0.5 | 0.26 | 2.26 | 0.01 |
| 14 | B | S | 0.08 | 0 | 0 | 1.7 | 0.01 | 0.61 | | 1.58 | 0.01 |
| 15 | B | CC50 | 0.04 | 0 | 0 | 1.46 | | 0.07 | 0.28 | 1.35 | |
| 16 | B | CC10 | 0.04 | 0 | 0 | 1.61 | | 0.54 | | 1.84 | |
| 17 | B | CC25 | 0.01 | 0 | 0 | 1.68 | | 0.23 | 0.15 | 1.25 | |
| 18 | B | RH10 | 0.07 | 0 | 0 | 1.84 | 0.01 | 1 | 0.26 | 2.47 | |
| 19 | E | CC25 | 0.01 | 0 | 0 | 1.75 | | 0.17 | 0.17 | 1.7 | |
| 20 | E | RH50 | 0.02 | 0.01 | 0 | 1.92 | | 0.52 | 1 | 2.26 | |
| 21 | C | RH10 | 0.04 | 0 | 0 | 1.61 | | 0.4 | | 2.23 | |
| 22 | C | RH25 | 0.03 | 0.01 | 0 | 1.37 | | 0.85 | 0.33 | 2.49 | 0.02 |
| 23 | C | CC10 | 0.03 | 0 | 0 | 1.56 | | 0.34 | 0.15 | 1.88 | 0.03 |
| 24 | C | CC25 | 0.01 | 0 | 0 | 1.56 | | 0.15 | 0.18 | 1.3 | |
| 25 | C | RH50 | 0.01 | 0.01 | 0 | 2.77 | | 0.42 | 2.59 | 3.71 | |
| 26 | C | CC50 | 0.01 | 0 | 0 | 1.36 | | | 0.19 | 1.45 | |
| 27 | C | CC100 | 0.01 | 0 | 0.01 | 1.39 | | 0.08 | | 0.97 | |
| 28 | C | S | 0.05 | 0 | 0 | 1.63 | | 0.94 | 0.22 | 1.57 | |
| 29 | E | CC10 | 0.02 | 0 | 0 | 1.5 | | 0.57 | | 1.84 | |
| 30 | E | CC100 | 0.03 | 0 | 0.01 | 1.06 | | | | 0.89 | |
| 31 | D | CC25 | 0 | 0 | 0 | 1.51 | | | | 1.65 | |
| 32 | D | CC50 | 0.01 | 0 | 0 | 1.3 | | | 0.24 | 1.46 | |
| 33 | D | RH25 | 0.02 | 0.01 | 0 | 1.73 | | 0.62 | 0.29 | 1.94 | |
| 34 | D | CC10 | 0.02 | 0 | 0 | 1.59 | | | | 1.62 | |
| 35 | D | RH10 | 0.04 | 0 | 0 | 1.67 | | 0.36 | | 2.38 | 0.06 |
| 36 | D | S | | | | | | | | | |
| 37 | D | RH50 | 0.02 | 0.01 | 0 | 1.98 | | 0.55 | 0.62 | 1.99 | 0.15 |
| 38 | D | CC100 | 0.01 | 0 | 0 | 1.07 | | | | 0.81 | 0.13 |
| 39 | E | RH25 | 0.02 | 0.01 | 0 | 1.1 | | 0.68 | 0.34 | 2.54 | 0.01 |
| 40 | E | CC50 | 0.02 | 0 | 0 | 1.23 | | 0.3 | 0.24 | 1.31 | |
| 41 | | TW | 0.04 | 0 | 0 | 1.88 | | 0.13 | | 0.81 | |

Appendix 6 - ANOVA results of the water quality data of collection 6

**pH**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 9.553 | 1.1941 | 14.591 | 5.46e-08 | *** |
| bloc | 4 | 0.860 | 0.2149 | 2.626 | 0.0566 | . |
| Residuals | 27 | 2.210 | 0.0818 | | | |

**C**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 423806 | 52976 | 22.146 | 5.74e-10 | *** |
| bloc | 4 | 12190 | 3047 | 1.274 | 0.305 | |
| Residuals | 27 | 64588 | 2392 | | | |

**Al**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 24.333 | 3.0417 | 3.979 | 0.00318 | ** |
| bloc | 4 | 6.721 | 1.6803 | 2.198 | 0.09603 | . |
| Residuals | 27 | 20.641 | 0.7645 | | | |

**As**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.0002625 | 3.281e-05 | 2.211 | 0.0589 | . |
| bloc | 4 | 0.0006744 | 1.686e-04 | 11.362 | 1.55e-05 | *** |
| Residuals | 27 | 0.0004006 | 1.484e-05 | | | |

**Ba**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.08384 | 0.010480 | 6.618 | 8.63e-05 | *** |
| bloc | 4 | 0.01228 | 0.003070 | 1.939 | 0.133 | |
| Residuals | 27 | 0.04275 | 0.001584 | | | |

**Be**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 8 | 0 | 0 | | |
| bloc | 4 | 0 | 0 | | |
| Residuals | 27 | 0 | 0 | | |

**Bi**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.000338 | 4.22e-05 | 0.89 | 0.538 | |
| bloc | 4 | 0.004080 | 1.02e-03 | 21.52 | 4.52e-08 | *** |
| Residuals | 27 | 0.001280 | 4.74e-05 | | | |

**Ca**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 265.52 | 33.19 | 85.926 | <2e-16 | *** |
| bloc | 4 | 5.99 | 1.50 | 3.877 | 0.0129 | * |
| Residuals | 27 | 10.43 | 0.39 | | | |

**Cd**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.0000850 | 1.063e-05 | 1.128 | 0.377 | |
| bloc | 4 | 0.0006207 | 1.552e-04 | 16.477 | 6.01e-07 | *** |
| Residuals | 27 | 0.0002543 | 9.420e-06 | | | |

**Co**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 8 | 0 | 0 | | |
| bloc | 4 | 0 | 0 | | |
| Residuals | 27 | 0 | 0 | | |

**Cr**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.0001825 | 2.281e-05 | 1.921 | 0.0979 | . |
| bloc | 4 | 0.0004744 | 1.186e-04 | 9.987 | 4.29e-05 | *** |
| Residuals | 27 | 0.0003206 | 1.188e-05 | | | |

**Cu**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.0006300 | 7.875e-05 | 5.102 | 0.000617 | *** |
| bloc | 4 | 0.0000632 | 1.580e-05 | 1.024 | 0.412841 | |
| Residuals | 27 | 0.0004168 | 1.544e-05 | | | |

**Fe**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 33.88 | 4.235 | 16.083 | 1.95e-08 | *** |
| bloc | 4 | 1.41 | 0.353 | 1.339 | 0.281 | |
| Residuals | 27 | 7.11 | 0.263 | | | |

**Ga**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 8 | 1.150e-04 | 1.438e-05 | 1.249 | 0.310 |
| bloc | 4 | 8.437e-05 | 2.109e-05 | 1.834 | 0.151 |
| Residuals | 27 | 3.106e-04 | 1.151e-05 | | |

**K**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 15550 | 1943.8 | 37.090 | 1.32e-12 | *** |
| bloc | 4 | 732 | 183.0 | 3.491 | 0.0201 | * |
| Residuals | 27 | 1415 | 52.4 | | | |

**Li**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 8 | 0 | 0 | | |
| bloc | 4 | 0 | 0 | | |
| Residuals | 27 | 0 | 0 | | |

**Mg**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 30.834 | 3.854 | 29.579 | 2e-11 | *** |
| bloc | 4 | 1.507 | 0.377 | 2.892 | 0.041 | * |
| Residuals | 27 | 3.518 | 0.130 | | | |

**Mn**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.013598 | 0.0016997 | 7.962 | 1.84e-05 | *** |
| bloc | 4 | 0.000836 | 0.0002091 | 0.980 | 0.435 | |
| Residuals | 27 | 0.005764 | 0.0002135 | | | |

**Na**

**Ni**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 879.5 | 109.93 | 9.36 | 4.33e-06 | *** |
| bloc | 4 | 565.7 | 141.43 | 12.04 | 9.59e-06 | *** |
| Residuals | 27 | 317.1 | 11.75 | | | |

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 8 | 0 | 0 | | |
| bloc | 4 | 0 | 0 | | |
| Residuals | 27 | 0 | 0 | | |

Pb

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 8 | 0.0001300 | 1.625e-05 | 0.625 | 0.750 |
| bloc | 4 | 0.0000779 | 1.946e-05 | 0.748 | 0.568 |
| Residuals | 27 | 0.0007021 | 2.600e-05 | | |

Se

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 8 | 0 | 0 | | |
| bloc | 4 | 0 | 0 | | |
| Residuals | 27 | 0 | 0 | | |

Sr

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.014177 | 0.0017722 | 22.950 | 3.82e-10 | *** |
| bloc | 4 | 0.002235 | 0.0005588 | 7.236 | 0.000427 | *** |
| Residuals | 27 | 0.002085 | 0.0000772 | | | |

V

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.0009175 | 1.147e-04 | 44.293 | 1.51e-13 | *** |
| bloc | 4 | 0.0000101 | 2.520e-06 | 0.974 | 0.438 | |
| Residuals | 27 | 0.0000699 | 2.590e-06 | | | |

Zn

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 0.0006300 | 7.875e-05 | 5.102 | 0.000617 | *** |
| bloc | 4 | 0.0000632 | 1.580e-05 | 1.024 | 0.412841 | |
| Residuals | 27 | 0.0004168 | 1.544e-05 | | | |

$NH_4$

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| sub | 6 | 0.01619 | 0.002698 | 0.209 | 0.951 |
| bloc | 4 | 0.01663 | 0.004156 | 0.322 | 0.849 |
| Residuals | 3 | 0.03871 | 0.012903 | | |

$SO_4$

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 13.393 | 1.6742 | 17.94 | 5.99e-09 | *** |
| bloc | 4 | 0.351 | 0.0877 | 0.94 | 0.456 | |
| Residuals | 27 | 2.519 | 0.0933 | | | |

$PO_4$

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 6 | 6.481 | 1.0802 | 7.336 | 0.00181 | ** |
| bloc | 4 | 0.690 | 0.1725 | 1.172 | 0.37142 | |
| Residuals | 12 | 1.767 | 0.1473 | | | |

$NO_3$

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 1.8559 | 0.23198 | 6.499 | 0.000271 | *** |
| bloc | 4 | 0.0727 | 0.01818 | 0.509 | 0.729441 | |
| Residuals | 21 | 0.7496 | 0.03569 | | | |

$NO_2$

Lack of data

Cl

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| sub | 8 | 2.7154 | 0.3394 | 7.293 | 3.89e-05 | *** |
| bloc | 4 | 0.1188 | 0.0297 | 0.638 | 0.64 | |
| Residuals | 27 | 1.2567 | 0.0465 | | | |