Study on the Physical, Chemical and Biological Properties of Water Treatment Residuals and Their Applicability to a Plant Growth Medium

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Study on the Physical, Chemical and Biological Properties of Water Treatment Residuals and Their Applicability to a Plant Growth Medium

（浄水汚泥の物理的、化学的、微生物的性質とその植栽基盤への適用性に関する研究）

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Abstract

Water treatment residuals (WTRs) are the sludge generated from water purification plants (WPPs) in tap water making process, and are composed of the suspended solids contained in river water and the coagulants to remove the solids. WTRs are disposed from WPPs in huge amounts. This study aimed to utilize the disposed WTRs as a plant growth medium. This paper is comprised of the following six chapters.

In the first chapter, the situation of disposal of WTRs as industrial waste, problems with the disposal management, and various recycled uses of the WTRs were introduced, and then, the previous studies on the physical and chemical (i.e., physicochemical) properties of the WTRs and the utilization of these as a plant growth medium were reviewed by referring to the references collected from around the world. At the end, the key issues that should be addressed in the study of the utilization of the WTRs as a plant growth medium were introduced.

In the second chapter, location environment, intake source of raw water for water purification, the kind and amount of chemicals for water purification treatment, dewatering method of WTRs (i.e., mechanical dewatering and solar drying methods), produced amount of purified water, disposed amount of the WTRs, etc. were summarized for the seven targeted WPPs that are located in Fukuoka and Saga prefectures. For improving the function of the WTRs as a plant growth medium, soil conditioner (bark compost) and phosphate (P) fertilizer were added to the WTRs. Their addition rates and the reason of the additions were mentioned.

In the third chapter, the physicochemical analysis was performed on WTRs and the

mixture of WTRs with bark compost and P fertilizer. Significant differences were observed between the respective WRTs (including their mixtures) on their physicochemical properties. The dewatering method of the sludge affected largely on the physicochemical properties of the WTRs in a WPP. However, there were almost no correlations between the physicochemical properties across the WTRs, indicating that the properties are independent from each other. By the additions of bark compost and P fertilizer, the pH, electric conductivity and cation exchange capacity changed to some extent and maintained suitable values for plant growth. While the amount of plant available manganese (Mn) decreased and the possibility to suffer from Mn excess (Mn toxicity) in plants was reduced. The P absorption coefficient maintained a high value unsuitable for plant growth, despite the additions of bark compost and P fertilizer.

In the fourth chapter, the community structure of microorganisms (bacteria) that live in the WTRs were analyzed by the denaturing gradient gel electrophoresis method. As a result, differences in the number of microbial colony were observed between the kind of WTRs and between the addition and no addition of P fertilizer. When bark compost was added to WTRs, microbial colony grew, but composition of microbial species was the same for the same WTR. This growth of microbial colony was considered to be related to the enhancement of the function of WTRs as a plant growth medium.

In the fifth chapter, a plant growth experiment was performed by using Japanese mustard spinach (*Brassica rapa* var. *perviridis*) that is a plant species widely used as a test plant. The plant was planted in the WTRs (including their mixtures) of a plant growth medium. Regarding the plant growth (foliage weight) for a period in relation with the additions of bark compost and P fertilizer, the growth was better when the bark compose/

P fertilizer was added than when these were not added. However, the growth did not increase always with the increased addition of these materials, and the best growth was observed when the additions were moderate. This plant growth characteristic was mainly affected by the properties of the original WTRs that did not contain the additional materials. When the Mn concentration of the original WTRs was large, Mn excess was clearly observed in plants, despite the additions of bark compost and P fertilizer.

In the sixth chapter, a general discussion was made. Namely, the relationships between the physical, chemical and biological properties on the WTRs (including their mixtures) and their reasons were discussed. There were almost no mutual relationships between the physicochemical properties. The chemical properties (Mn concentration, etc.) changed by not only the addition of bark compost but also the method of sludge dewatering. Though the addition of P fertilizer did not change the P absorption coefficient, the fertilizer addition affected positively on the plant growth. When the bark compost was added to the WTRs, number and species of microorganisms in WTRs increased and plants showed a better growth. In order to use WTRs as a plant growth medium, addition of both bark compost and P fertilizer is required in some amount, and the respective addition amounts are variable depending on the WTRs.

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Chapter 1 General Introduction

1.1 Generation Process of Water Treatment Residuals (WTRs)

Water treatment residuals (WTRs) or water purification sludge are generated in the process of treating tap water. The process is mentioned below with an illustration in Fig.1.1.

At a water purification plant (WPP), raw water to make purified water is taken from river, lake, dam reservoir, or underground water. After taking the raw water, large contaminants such as plants, wood, and/or fish are filtered out by a screen (Wolters, 2015). Coarse sands in the raw water are deposited by gravity in the sand basin.

After that, coagulants such as poly aluminum chloride (PAC) and ferric sulfate are added to the raw water in the flocculation & sedimentation basin. Then, the water is stirred up for hours in a day. With this stirring up, flocks are formed by combining small particles in the water. The flocks grow larger with time, and the large flocks are sunk down to the bottom of the basin.

At the next stage, the above mentioned treated water flows into the rapid filtration basin. In this basin, the water that still contains small flocks is filtered passing through the filtering layers composed of gravel and sand. In some WPPs, the filtered water further proceeds to the advanced treatment basin, where the filtered water is treated by ozone and activated carbon to remove organic and odor substances.

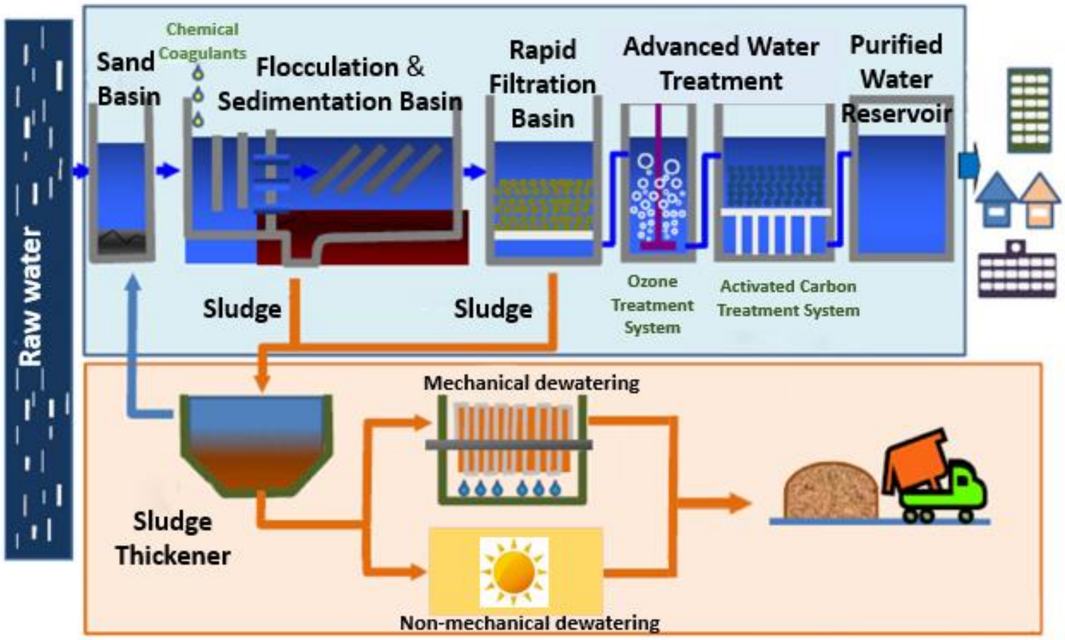

Fig.1.1 Drinking water treatment process (modification from Water Suppy Division MHLW, 2013)

Microorganisms exist in the water are removed by membrane filtration and the microorganism-removed water is sterilized by chlorine in the rapid filtration and/or advanced water treatment basin. Thus, the purified water is produced. The purified water is sent to the purified water reservoir to distribute it to the customers.

During the water purification process, sludge composed of small sand, silt and clay etc. are generated from the flocculation & sedimentation and rapid filtration basins.

These sludge are sent to a sludge thickener that is shown in Fig.1.1. In the thickener, solids are separated from water, and the separated water is sent back to the sand basin. Solids in the sludge are taken out from the thickener, and dewatered either by mechanical dewatering or non-mechanical dewatering (solar drying) methods. By this dewatering of the sludge, water treatment residuals (WTRs) are generated.

The main methods of mechanical dewatering are belt filter press, chamber filter press, and centrifuges. The mechanical dewatering method is a sophisticated one, requiring a high degree of operator supervision and operator training. Costs of the facility construction, maintenance and repair works for the method are higher than those for non-mechanical dewatering method. (Stauffer, 2016).

In the mechanical dewatering method, dewatering is done quickly within a day. The WTR generated by the mechanical dewatering method is comprised of flat solid blocks. WTR has a uniform bulk density with a uniform moisture content. The color of WTR is affected by the color of the source materials contained in raw water and/or the chemicals added to the water during water purification process.

Non-mechanical dewatering method is advantageous where a large drying space is available. In the non-mechanical dewatering, moisture in the WTR is removed either by natural evaporation, gravity induced drainage, or a combination of these. The process of the no-mechanical method is less complex, easier to operate and require less energy than that of the mechanical method. However, the dewatering by non-mechanical method requires a large space for drying, in addition, the drying needs a long period of time with several months or more. The success of the dewatering operation depends very much on the local climatic conditions (Alturkmani, 2012). A typical non-mechanical dewatering method in Japan is the solar drying. Concerning the WTRs generated from solar drying, the shape, thickness and size of the fractions of WTRs are different between the WPPs. The moisture content of the WTR is higher in the bottom than in the upper part, and the color of the bottom part is darker than the upper part.

In either case that the WTR is generated by the mechanical or non-mechanical method, the WTR is composed of organic and inorganic substances originally contained in the raw water and in the added chemicals during water purification process. In Japan, WPPs are usually located in the upper basin of the river, where there are almost no factories that discharge hazardous wastes into rivers. Therefore, the WTRs contain almost no hazardous materials except manganese (Mn). Mn is a naturally occurring substance as mentioned later, and WTRs are disposed as a non-hazardous material.

1.2 Generation, Disposal and Recycling of WTRs in Japan and Other Countries

1.2.1 Generation, Disposal and Recycling of WTRs in Japan

In Japan, there are 5,221 WPPs that provide drinking water with an amount of 14.7 billion m^3 to 120 million people annually (JWWA, 2015). In the water purification process, a large amount of WTRs (360,000 tons) are generated annually from these WPPs.

Fig.1.2 shows the amount of generation, disposal and recycling of WTRs in major WPPs in Japan during 2003 and 2013. According to Fig.1.2, the amount of generation ranged from 250,000 to 300,000 tons annually except in 2007, and it did not increase largely with years during 2003 and 2009, but increased a little during 2010 and 2013. In 2013, the amount of generation exceeded 350,000 tons, among which the landfill disposal purposes occupied 24.5% and the recycling purposes 66.2%.

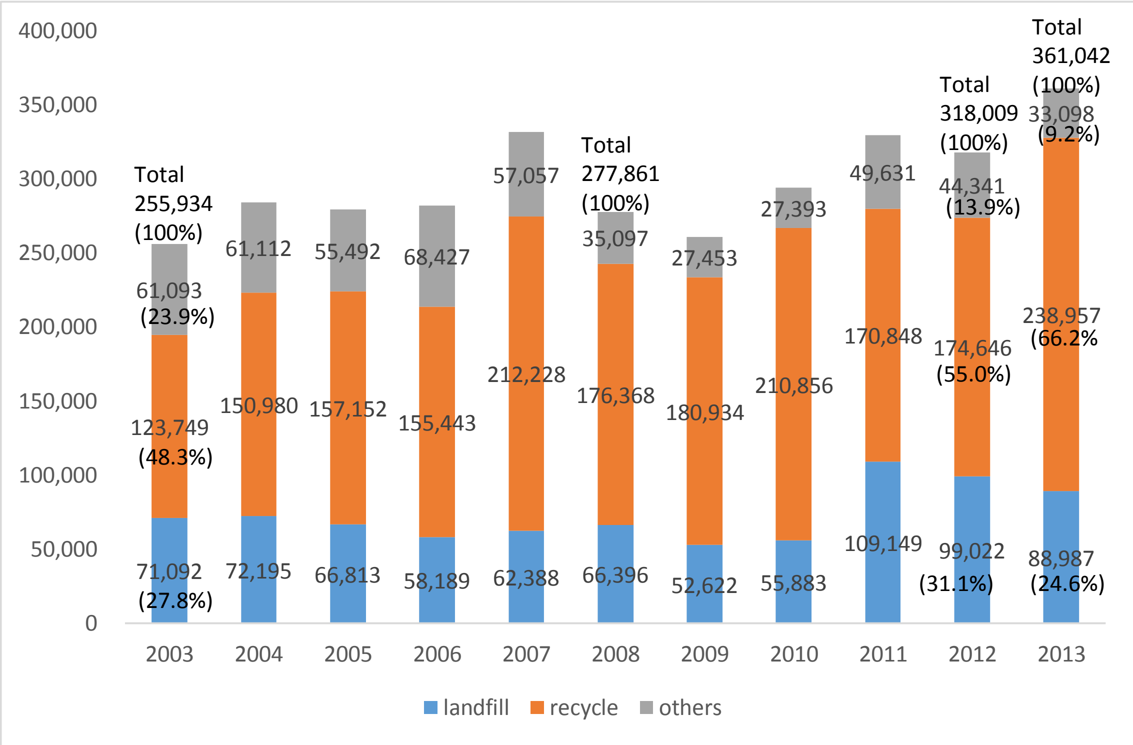

Fig.1.2 Annual amount of generation, disposal and recycling of WTR in Japan during 2003 and 2013 (JWWASCCWWS, 2005-2015)

For recycling purposes, WTR is used for cement manufacturing, ground covering, agricultural soils, etc. in Japan.

Since WTR has a high potential to be used for various recycling purposes, further development of recycling uses is a challenging issue.

Major uses for disposal and recycling purposes of WTRs are mentioned below.

Landfill

Landfill is a traditional disposal method of WTRs. According to Fig.1.2, 50,000-100,000 tons of WTR are disposed annually. Landfill of WTRs requires low skills and no special procedures. A large amount of WTRs is likely to be disposed in future as well. According to the Japanese law, WTRs of a moisture content less than 85 % must be disposed at a designated landfill site after transported from the WPPs. However, since the landfill site is limited in location, there is a shortage of landfill site by the increased disposal amount (Tamagami, 2005).

According to JWWA (2015), the rate (or amount) of the disposal of WTRs in the region in Japan was 49.8% (7,584 DS-t) in Hokkaido, 18.5% (6,311 DS-T) in the Kanto, 74.8% (3,173 DS-t) in Shikoku, 11.6% (2,525 DS-t) in northern Kyushu, which shows that the disposal rate differs widely with the region.

Raw Materials for Cement Manufacturing

The aluminum-based WTRs that are composed of small solid particles are suitable as the raw materials for cement manufacturing. Among the recycling uses, 20 % (or 13.8 kilo tons) of the WTRs is used for cement manufacturing in Tokyo (Water supply division of MHLW, 2010), and 48% (or 10.5 kilo tons) in Osaka (Osaka City Waterworks Bureau, 2013), respectively in Japan.

Ground Soil Material

Since WTRs have a good water permeability, water retention capacity, and compaction degree, WTRs are often used as a ground soil material (Towa Sports Facility Corporation, 2010). A ground soil material made by WTR was recognized as an excellent material by the evaluation and recognition committee of the recycling materials in Okinawa Prefecture in 2013 (Okinawa Prefectural Enterprise Bureau, 2016).

During 1996 and 2010, Kitakyushu City in Japan used WTRs with a large amount of 26,597 tons in total for ground construction/maintenance work (Kitakyushu City Water and Sewer Bureau, 2016). While in Tokyo, 2,301 tons of WTRs (or 3% of the total generated amount of WTRs) was used as a ground soil material in 2013 (Bureau of Waterworks Tokyo Metropolitan Government, 2016). In Japan, WTRs are used widely as ground soil material, though there may be a difference in the amount depending on locations.

Agricultural Soils

Since the WTRs are composed of organic and inorganic substances and have characteristics similar to soils, a large amount of WTRs is used as agricultural soils recently (Kakuta et. al, 2003).

For instance, WTRs were used as a greening base material for levees in Hokkaido Region (Sakamoto, 2004). Mixing of WTRs into gravel rich levee soils can support plant growth, because WTRs contain a lot of clay and silt that can absorb and maintain the nutrients required for plants.

Usually for greening levees, adhesive materials are sprayed onto the surface of levees in order to fix the soils, but when WTRs are mixed into the soils, less or no spraying is needed, because WTRs contain a lot of adhesive material of polymer coagulant that was used in the water purification process.

There are some uses of WTRs for growing of plants, including the plants of bottle gourds (Kakuta et al., 2003), strawberries (Ohta et al., 2011), and rice (Mochizuki et al., 2011).

In Osaka City, 2,027 tons of WTRs (corresponding to 9% of the total generated WTRs) were used as agricultural soils in 2007 (Osaka City Waterworks Bureau, 2013). In Kitakyushu City, the annual amounts of WTRs used for agricultural purposes were: 2,425 tons in 2008; 1,929 tons in 2009; 1,278 tons in 2010, respectively (Kitakyushu City Water and Sewer Bureau, 2016).

The Mino WPP located in Okayama City produces a gardening soil by using WTR along with peat moss and perlite. The soil has been sold under the name of “Okayama sando” since 2013 (Okayama City Waterworks Bureau, 2016).

In Kurume City, a gardening soil called “Yoka baido,” made from WTRs, is sold commercially (Oishi Bussan Co., Ltd., 2009). In Matsue City, WTR alone is sold commercially after crushing and drying (Matsue City Water and Sewer Bureau, 2016).

In Japan, a total WTR amount of 361,000 tons are generated annually from WPPs, among which 66.2% are recycled, 24.6% landfilled, and 9.2% used for the other purposes. Annual cost for sludge treatment and disposal is 17.7 billion yen (corresponding to 160 million US dollars), where 34% of the cost is for disposal (JWWA, 2015).

1.2.2 Generation, Disposal and Recycling of WTRs in Other Countries

The situations of the generation, disposal and recycling of WTRs in other countries are not largely different from those in Japan. The generated amount and recycling method of WTRs, and the problems to be solved for reusing WTRs in some countries are summarized by Babatunde et al. (2007), Wendling and Douglas (2009), and Zhao et al. (2011).

In Italy, approximately 750,000 tons of WTRs are generated annually. These WTRs are disposed mostly in landfilling, where the total cost of transportation and disposal is 50 million euro/year. Some amount of WTRs is used for cement manufacturing (Verlicchi et al., 2002).

In The Netherlands, disposal cost of WTRs is a high of £30-£40 million. While in Ireland, the estimated disposal cost is 15,000 to 18,000 tons/year and the disposal cost is predicted to be double in 2021(Evuti et al., 2011). According to Zhao (2011), the total annual amount of WTRs generated in Ireland is 15,679 tons of dry solids, among which, only 8% of WTRs is recycled or reused by composting, land spreading, cement manufacturing, wetland construction and quarry remediation.

In Taiwan, the annual generation amount of fresh water sludge (same with WTR) is approximately 120 kilo tons, and most of them are provided for landfilling (Pan et al., 2004).

In USA, the main disposal ways of WTR are land application, land disposal and deep well injection (EPA, 2011). The final solid waste residuals (WTR) were mostly disposed in landfill in 40% WPPs in USA. Thirty-five percent of WPPs in USA have established the pathways to reuse the wasted solids through topsoil manufacturing and agricultural land application practices (Roth et al., 2009).

In worldwide, there are thousands of WPPs that use coagulants for efficient removal of particulate solids and colloids thereby several tons of sludge per year were produced, thus their disposals were necessary with associated costs (Evuti et al., 2011).

1.3 Use of WTRs for Agriculture and its Problems

In present day, WTRs are used for agriculture in Japan as well as in some other countries.

Concerning the properties of WTRs, the water retention and drainage properties are more important than the nutrient properties (Skene et al., 1995). Park et al. (2010) showed that the intra- and inter-aggregate pores formed uniquely in the WTRs contributed to improve the physical properties. In addition, WTRs have good chemical properties, i.e., high values of cation exchange capacity, organic carbon and organic matter contents (Razali et al. 2007; Ippolito et al., 2011). Therefore, WTR can be used as a soil conditioner.

Addition of WTRs into degraded soils was found to improve soil physical properties and soil pH (Wendling and Douglas, 2009).

Oh (2010) compared the growth of lettuce cultivated in the decomposed granite soil (DGS) that had less organic matter with that cultivated in WTR added with DGS. The growth was better in WTR added with DGS than in DGS alone, indicating that WTR was useful as a soil conditioner.

Some favorable results in plant growth were achieved by the addition of WTRs to soils as indicated below. According to Ulen et al. (2012), Italian ryegrasses showed a better growth with the addition of WTRs to the loam sand and clay loam soils. Co-addition of WTRs and vermicompost to the salt-affected soils improved the soil properties and contributed to a better growth of barley (Mahmoud and Ibrahim, 2012). Mahdy et al. (2007) showed that the increased crop growth was achieved after the addition of 30-40 g/kg WTRs to soils.

Further, WTRs contain a lot of available manganese (Mn) (Roppongi et al., 1993;

Titshall and Hughes, 2005; Trollip et al., 2013), and the Mn is easily absorbed by plants, therefore, Mn excess (Mn toxicity) is likely to occur in plants when using WTRs as a plant growth medium.

1.4 The Purpose of the Present Study

At first, WTRs are collected from seven WPPs located in Fukuoka and Saga prefectures. These WTRs are considered to have different characteristics due to their different locations.

Next, the physical, chemical and biological properties are measured on these WTRs. In addition, bark compost and P fertilizer are added to the WTRs in order to improve the properties as a plant growth medium. The physical, chemical and biological properties are also measured on these mixtures.

By using the above-measured values, the differences between the WTRs are compared on the properties of WTRs including their mixtures, and correlations between the respective properties are analyzed. The relationship of the properties to the geological conditions of the WPP is also examined.

Further, a plant growth experiment is performed by using WTRs including their mixtures, and the relationship of plant growth to the physical, chemical and biological properties of WTRs is analyzed.

Finally, the adaptability of the WTRs to a plant growth medium is clarified, based on the above-derived results.

Chapter 2 Water Purification Plants and Water Treatment Residuals Targeted

2.1 Water Purification Plants (WPPs) Targeted in this Study and Collection of WTRs

Not only the generated amount but also the physicochemical properties of WTRs may differ from the location and the operation method of WPPs. In more details, the location of the river/dam reservoir from which the raw water is taken, and the method of sludge dewatering may affect the generated amount and properties of the WTRs. Therefore, the locational condition and the operation method of the target WPPs are introduced at first.

In this study, a total of 7 WPPs were targeted for collecting WTRs. These WPPs were Tatara, Takamiya, Meotoishi WPPs located in Fukuoka City in Fukuoka Prefecture, Zuibaiji WPP located in Itoshima City in Fukuoka Prefecture, Kouno WPP in Saga City in Saga Prefecture, and Anou and Ideura WPPs in Kitakyushu City in Fukuoka Prefecture, respectively, Japan.

Among which, WTRs collected from Anou and Ideura WPPs were provided as cultivation soils in plant growth experiment. Table 2.1 shows the general information of the WPPs. Table 2.2 shows the amount of water treatment chemicals used in water purification process in the respective WPPs (in 2013).

Table 2.1 General information of the WPPs targeted in this study

WPP	Location (city)	Source of raw water	Dewatering method	Water purification capacity m^3/day	Amount of tap water production m^3/day*	Generated amount of WTR	Disposal method of WTR
Tatara	Fukuoka	Tatara River, Kubara Dam, Nagatani Dam, Ino Dam, Narufuchi Dam	mechanical dewatering	100,000	65,819	3-5 ton/day, 650 ton/year	plant growth medium
Takamiya	Fukuoka	Minamihata Dam, Naka River	solar drying	199,000	78,644	5151 ton/year	landfill
Meotoishi	Fukuoka	Sefuri Dam, Magarifuchi Dam, Muromi River, Naka River	mechanical dewatering	174,000	78,427	800-1000 ton/year	ground soil, plant growth medium, landfill
Zuibaiji	Fukuoka	Zuibaiji Dam	solar drying	15,000	10,128	200 ton/year	landfill
Kouno	Saga	Tahuse River	mechanical dewatering & solar drying	50,000	15,860	44 m^3/day	plant growth medium, civil engineering material
Anou	Kitakyushu	Onga River, Tonda Dam, Rikimaru Dam.	mechanical dewatering	300,000	100,710	9.3 ton/day	ground soil, seedling soil, cement raw material
Ideura	Kitakyushu	Aburagi Dam, Masufuchi Dam, Yabakei Dam, Murasaki River	solar drying	255,200	149,085	2000 ton/year	road construction material

*on average in 2013, from Database of Water Quality of Aqueduct (2014)

Table 2.2 The amount of water treatment chemicals used in water purification process at the respective WPPs targeted (2013)

WPP	PAC g/m^3	Activated carbon g/m^3	Sodium hypochlorite g/m^3	Sodium hydroxide g/m^3	Sulfuric acid g/m^3	Carbon dioxide g/m^3	Slaked lime g/m^3
Tatara	31.47	1.9	8.43	18.8	7.4	-	-
Takamiya	43.11	2.9	9.5	18.1	-	-	-
Meotoishi	31.32	1.0	10.96	7.0	-	-	-
Zuibaiji	27.08	5.0	8.54	8.4	-	-	-
Kouno	29.9	13.6	1.77	-	-	3.2	2.9
Anou	39.13	-	18.01	0.33	-	0.38	-
Ideura	23.22	-	12.34	-	-	-	-

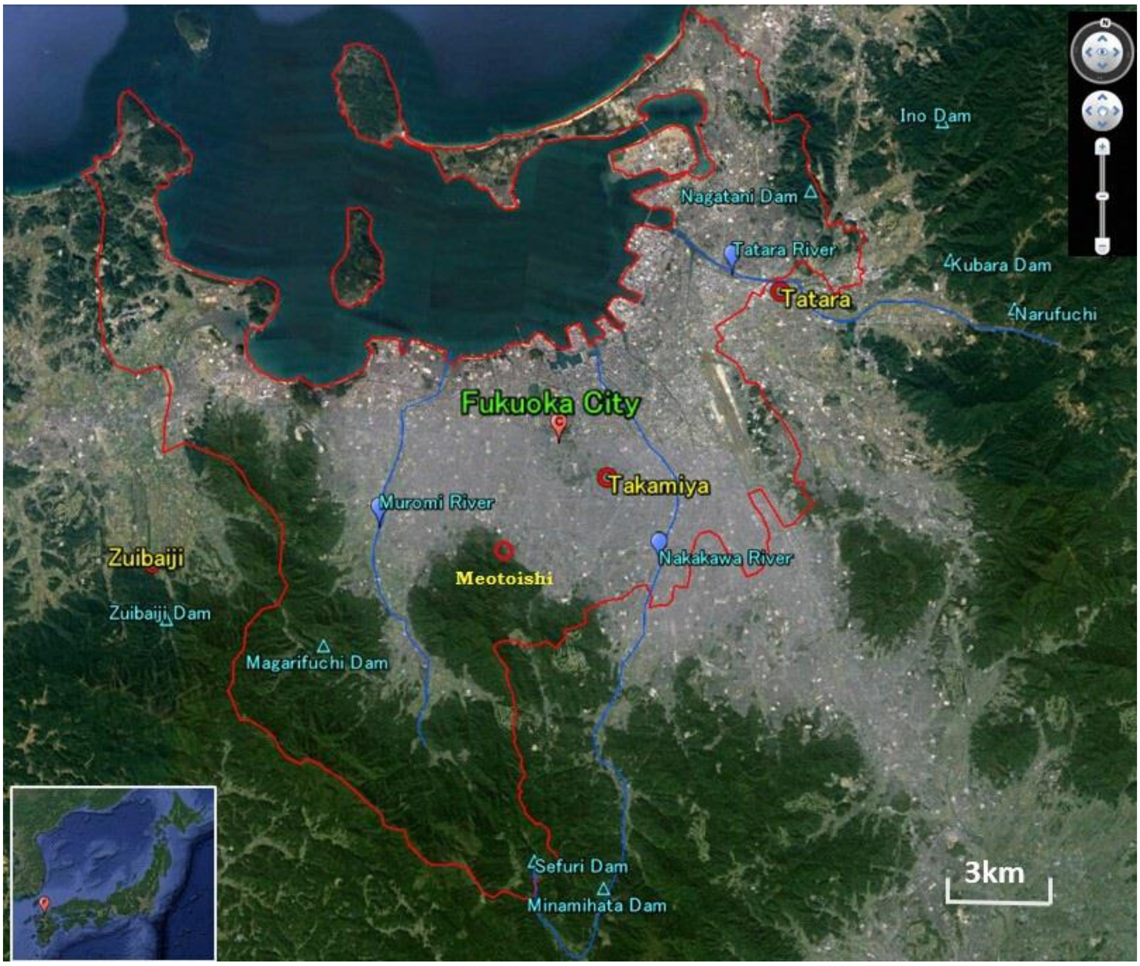

Fig. 2.1 Location map of the Tatara, Takamiya, Meotoishi WPPs located in Fukuoka City and Zuibaiji WPP in Itoshima City. (modified from Google Earth (2016))

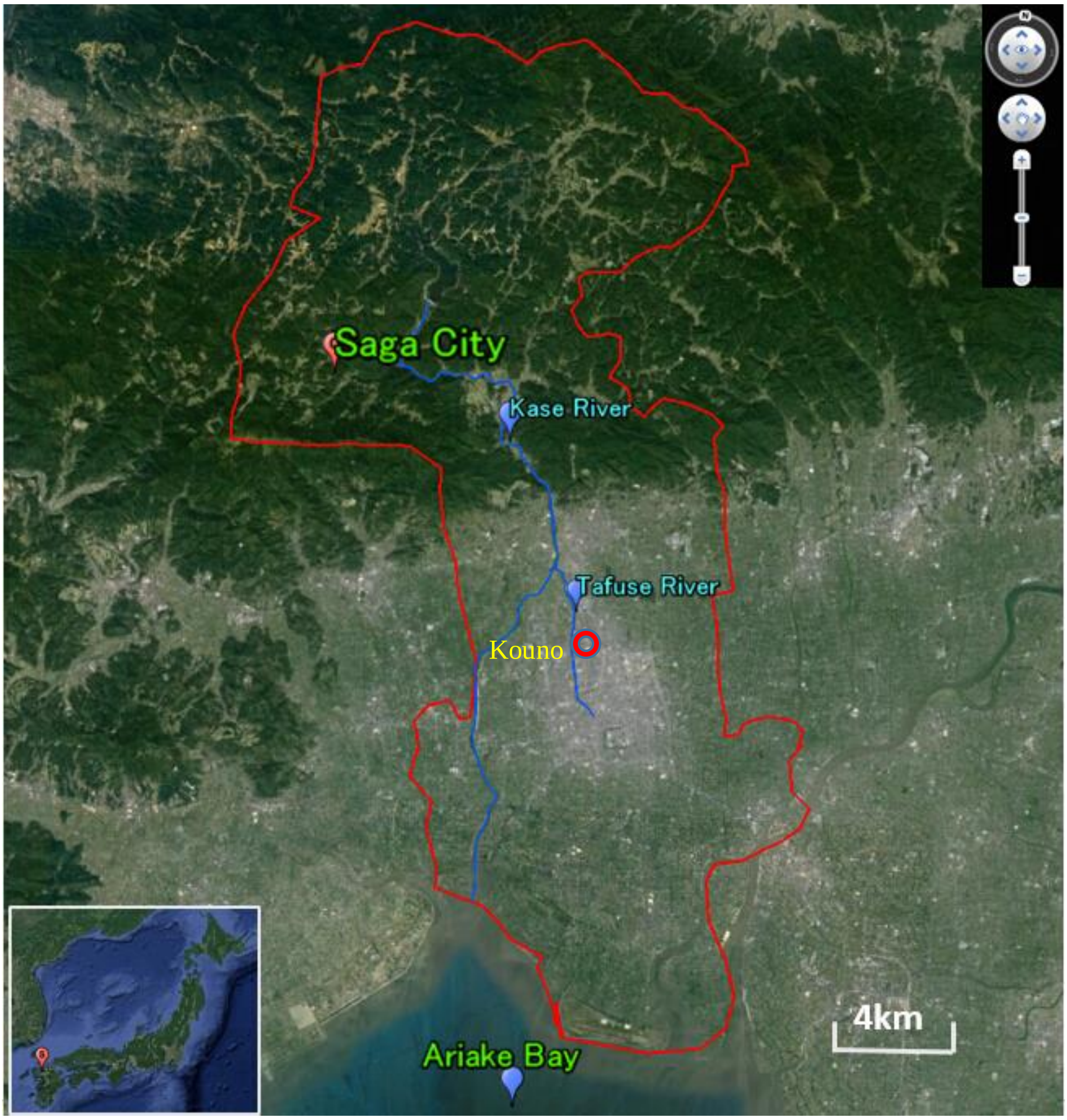

Fig. 2.2 Location map of Kouno WPP in Saga City. (modified from Google Earth (2016))

Fig. 2.3 Location map of Anou and Ideura WPPs in Kitakyushu City. (modified from Google Earth (2016))

The general information on the 7 WPPs is described below based on Tables 2.1 - 2.2 and Figs 2.1-2.3.

The Tatara WPP

The Tatara WPP, operated by Fukuoka City Waterworks Bureau, is located beside the Tatara River at the eastern part of Fukuoka City (Fig. 2.1). As shown in Table 2.1, the raw water is taken mainly from the Tatara River and partly from the reservoirs of the Kubara, Nagatani, Ino, and Narufuchi dams.

In the Tatara WPP, poly-aluminum chloride (PAC) is used as coagulant and sodium hypochlorite is as disinfectant in water purification process. In addition, activated carbon is used for adsorbing organic compounds, and odor and anionic substances. Sodium hydroxide and sulfuric acid are also used to control pH of the treating water. Further, ozone is used to decompose organic materials and odor substances. According to Fukuoka City Waterworks Bureau, the Tatara River water contains a lot of organic matters compared to the water in other rivers in Fukuoka City. In order to remove trihalomethane and musty odor efficiently, an advanced water treatment system is applied in the WPP. By which, the amount of chemicals including disinfectant can be reduced. According to Table 2.2, 31.47 g PAC, 1.9 g activated carbon, 8.43 g sodium hypochlorite, 1.88 g sodium hydroxide and 7.4 g sulfuric acid were used per 1 m^3 treating water in the WPP.

The water supplying capacity of the WPP is 122,000 m^3/day. In 2013, the water purifying amount on average is 65,819 m^3/day (CWB Fukuoka, 2014). Filter press is used for dewatering the sludge. After the dewatering, the WTR is produced. The WTRs have an angular sheet shape, uniform brownish color and uniform thickness with around 4 mm, and the length and width with around 12-35mm (Fig2.4). The moisture content of the WTRs in the Tatara WPP is about 60%. Three to five tons of the WTRs are generated daily in the WPP. Under the influence of the weather, the raw water quality is different with years, and the total generated amount of WTRs is different with years, too. Usually, the generated amount of the WTRs in the Tatara WPP is less than 1,000 tons/year. In 2015, 650 tons of the WTR were generated. The WTRs are sold commercially for various purposes.

Fig. 2.4. The WTR in the Tatara WPP (collected in March 2014).

The Takamiya WPP

The Takamiya WPP, operated by Fukuoka City Waterworks Bureau, is located in the southern part of Fukuoka City (Fig. 2.1). The raw water is taken from the Minamihata Dam and the Naka River.

As shown in Table 2.2, 43.11 g PAC and 2.9 g activated carbon per 1 m^3 treating water are used for coagulating impurities contained in the raw water. Further, 9.5 g sodium hypochlorite for disinfection, and 18.1 g sodium hydroxide for pH adjustment were used per 1 m^3 treating water as of 2013.

Fig. 2.5. The situation of solar drying of sludge at the Takamiya WPP.

Fig. 2.6 WTR generated from the Takamiya WPP (collected in June 2014).

The water supplying capacity in the Takamiya WPP is 199,000 m^3/day. In 2013, the water treated amount in the WPP was 78,644 m^3/day, and 5,151 tons of WTRs were generated in a year. Solar drying method is used for drying sludge as shown Fig. 2.5. The WTRs generated in the WPP with solar drying have brownish to dark color and were relatively soft (Fig.2.6). The moisture content of the WTR after drying should be less than 85% in Japan (Japanese Ministry of Environment, 2016).

Due to aging of the facilities together with some other reasons, the Takamiya WPP is scheduled to be closed in 2024. Therefore, Fukuoka municipal government do not invest to build a new facility of the mechanical dewatering system that requires a lot of cost, and solar drying operation has been continued up to now and will be continued in future. There are 9 solar drying beds in the WPP with the total sludge receiving capacity of 5,703 m^3. Since the solar drying operation is influenced by the weather, it usually takes 3-5 months for complete drying. Moreover, due to restriction by the law, the WTR generated from the Takamiya WPP can not to be used for recycling purposes, thus the WTR is transported to the designated landfill sites.

The Meotoishi WPP

The Meotoishi WPP, operated by Fukuoka City Waterworks Bureau, is located in the southern part of Fukuoka City (Fig. 2.1). The raw water is taken from the Sefuri and Magarifuchi dams, and the Muromi and Naka rivers. As shown in Table 2.2, 31.62 g PAC and 1.0 g activated carbon per 1 m^3 treating water are used for coagulating impurities contained in the raw water, and 10.96 g sodium hypochlorite for disinfection, and 7.0 g sodium hydroxide for pH adjustment were used per 1 m^3 treating water as of 2013.

Fig. 2.7 Sludge dewatered by the mechanical dewatering method in the Meotoishi WPP

Fig. 2.8 WTR generated from the Meotoishi WPP (collected in July 2013)

The tap water supply capacity of the Meotoishi WPP is 174,000 m^3/day. The water purification amount was 78,427 m^3/day in 2013. The generated amount of the WTR is 800-1000 ton/year (personal communication). The sludge dewatering is done by mechanical dewatering with a filter press. The WTRs look similar with the Tatara WTRs, and have an angular sheet shape, uniform brownish color and uniform thickness (Fig2.8). The moisture content of the WTR after the dewatering is 60-70%. Most of the generated WTR is sold to construction companies for ground maintenance purposes and farmers for agricultural purposes. The remained WTR is transported to the disposal site for landfilling.

The Zuibaiji WPP

The Zuibaiji WPP, operated by Fukuoka City Waterworks Bureau, is located in Itoshima City that is adjacent to Fukuoka City (Fig. 2.1). The raw water is taken from the Zuibaiji Dam.

From Table 2.2, 27.08 g PAC and 5.0 g activated carbon were used for coagulating impurities contained in the raw water. Further, 8.54 g sodium hypochlorite for disinfection, 8.4 g sodium hydroxide for pH adjustment were used for 1 m^3 treating water on average in 2013.

Fig. 2.9 Solar drying of sludge by solar drying beds at the Zuibaiji WPP

Fig. 2.10 WTR generated from the Zuibaiji WPP (collected in July 2013)

The water purification capacity of the Zuibaiji WPP (1,500 m^3/day) is several times smaller than those of the other WPPs located in Fukuoka City. In 2013, the average water purified amount is 10,128 m^3/day. Currently, the generated amount of WTR at the Zuibaiji WPP is 200 ton/year. The solar drying method is used for dewatering sludge. There are 9 solar drying beds, and the total capacity volume of these beds is 1,296 m^3. It takes 3-5 months for complete solar drying. The moisture content of the sludge (WTR) reaches 70-80%, when the drying is completed. This WTR has an irregular shape, uneven density and dark color with a partly high moisture content (Fig.2.10). The WTR is transported from the WPP to the final landfill site.

Because of small water purification capacity and comparatively clean raw water, generated amount of the WTR at the Zuibaiji WPP is small. The mechanical dewatering is an effective method, but construction of the facility needs a high cost, therefore, there is no plan to construct the facility now, and current solar drying system will be continued in the WPP.

The Kouno WPP

The Kouno WPP, operated by Saga City Waterworks and Sewerage Bureau, is located beside the Tafuse River in Saga City (Fig. 2.2). The raw water is taken from the Tafuse River that is a tributary of the Kase River.

According to the results of the operation in 2013 shown in Table 2.2, 29.9 g PAC and 13.6 g activated carbon were used for coagulating impurities contained in the raw water, and 1.77 g sodium hypochlorite for disinfection, 3.2 g carbon dioxide for assisting the work of PAC, and 2.9 g slaked lime (calcium hydroxide) for disinfection and pH adjustment were used for 1 m^3 treating water. The tap water supplying capacity of the Kouno WPP is 50,000 m^3/day. The average amount of water purification in 2013 was

15,860 m^3/day.

Fig. 2.11 Sludge dewateing by the mechanical dewatering method in the Kouno WPP

Fig. 2.12 Sludge dewatering by solar drying method in the Kouno WPP

Fig. 2.13 WTR generated by the mechanical dewatering in the Kouno WPP

Fig. 2.14 WTR generated by solar drying in the Kouno WPP

The two dewatering methods are used in the Kouno WPP. One of them is mechanical dewatering with filter presses, the other is solar drying.

Concerning the mechanical dewatering of sludge in the WPP, sludge is filter pressed after the condensation of sludge in the condensation tank, and 44 m^3/day of WTR is generated on average. The moisture content of the dewatered WTR is around 65%. The WTRs look similar with the Tatara and Meotoishi WTRs, and have an angular sheet shape, uniform thickness and dark color. The dark color is caused by the activated carbon used with a huge amount in the Kouno WPP (Fig. 2.13). All the mechanically dewatered WTRs are transported out from the WPP and provided for a plant growth medium through marketing.

In the WPP, solar drying facility is used when the mechanical dewatering facility is overhauled, i.e., the filter press machine is overhauled, and the sand basin, sedimentation basin, and distributing reservoir are cleaned up. This WTR has an irregular shape with dark color (Fig. 2.14). The solar dried WTRs are transported to an industrial waste treatment facility, and then recycled as a civil engineering construction material.

The Anou WPP

The Anou WPP, operated by Kitakyushu City Water and Sewer Bureau, is located in Yahata-Nishi Ward of Kitakyushu City, Fukuoka Prefecture (Fig. 2.1). The raw water is taken from the Onga River, the Tonda Reservoir and the Rikimaru Dam.

According to the operation results of the WPP in 2013, 39.13 g PAC was used for coagulating impurities in the raw water, 18.01 g sodium hypochlorite for disinfection, 0.33 g sodium hydroxide and 0.38 g carbon dioxide for pH adjustment were used for 1 m^3 treating water.

Fig. 2.14 Mechanical dewatering facilities (with a filter press method) in the Anou WPP

Fig. 2.15 The Anou WTR (collected in July 2014)

The water purification capacity of the Anou WPP is 300,000 m^3/day. The average amount of water purification was 101,000 m^3/day in 2013. The generated amount of the WTR is 9.3 ton /day. Dewatering of the sludge is done by the filter press mechanical dewatering method. The moisture content of the WTR after drying is around 65%. The WTRs look similar with the Tatara WTRs, and have an angular sheet shape, uniform thickness with around 4 mm, and two different colors of brown and dark brown. The length and width of the WTRs were about12-35 mm (Fig2.15). All WTRs generated from the Anou WPP are sold at low prices. Seventy percent of the WTRs are used as a ground improvement material, and the others are as agricultural soils or cement raw materials.

The Ideura WPP

The Ideura WPP, operated by Kitakyushu City Water and Sewer Bureau, is located at Kokura-Minami Ward of Kitakyushu City, Fukuoka Prefecture (Fig. 2.1). The raw water is taken from the reservoirs of the Aburagi Dam, the Masufuchi Dam, the Yabakei Dam, and the Murasaki River.

According to Kitakyushu City Water and Sewer Bureau (2014), only 23.22 g PAC and 12.34 g sodium hypochlorite are used for purifying 1 m^3 raw water, because of the rather clean raw water.

The water purification capacity of the Ideura WPP is 255,200 m^3/day. The average amount of water purification in 2013 was 149,085 m^3/day.

Fig. 2.16 Solar drying of sludge at the Ideura WPP

Fig. 2.17 WTRs collected from the Ideura WPP (July, 2014)

The sludge dewatering is done by the solar drying method using 12 drying beds. The total capacity volume of the beds is 14,420 m^3. Currently, the WTR amount generated in the WPP is 2,000 ton/year. This WTR has an irregular shape with uneven density. The color was dark with a partly high moisture content (Fig.2.17). The moisture content of the WTR after drying is different with seasons, ranging from 20-75% with an average of 60%. It usually takes 3-5 months for sludge drying. The WTRs generated from the WPP is not used for landfill purposes but used as a road construction material. According the Kitakyushu City Water and Sewer Bureau, there are a lot of land for solar drying operation with a low cost, therefore, there is no plan to construct a mechanical dewatering facility in the Ideura WPP.

Comparison of the Locational Conditions and the Use of Chemicals between the WPPs

In the seven WPPs targeted, the sources of the raw water were river or reservoir water, and the quality of the raw water is different with the location. Due to this difference, the kind of water treatment chemicals and its amount are different with the location. According to Table 2.2, input amount of activated carbon in the Kouno WPP is much higher than those in the other WPPs. For this reason, the color of WTR in the Kouno WPP is darker and softer than those in the other WPPs.

On the other hand, the same purification procedures were taken in all WPPs except in the Tatara WPP in terms of the use of PAC. The Tatara WPP used an advanced water treatment system. The used amount of PAC in each WPP is shown in Table 2.2. The raw water taken in the Takamiya WPP was more impure than those in the other WPPs, and the raw water in the Ideura WPP was comparatively pure. The more impure in the raw water, the more the PAC needed. Therefore, the amount of the PAC used in the Takamiya WPP was the highest, and that in the Ideura WPP was the lowest as shown in Table 2.2.

2.2 Collection of WTRs and Additions of Bark Compost and P Fertilizer to the WTRs for Plant Growing Purposes

2.2.1 The Preliminary Analysis on the Properties by the Additions of Bark Compost and P Fertilizer

During 2011-2013, WTR was collected from the Tatara WPP, and bark compost was added to the WTR with three levels of 0, 20, and 40 percent volumes of the WTR. In addition, P fertilizer was added with three levels of 0, 5 and 10 g per liter to the WTR after adding bark compost. After these additions, physicochemical properties (pH, electric conductivity (EC), oxidation-reduction potential (ORP), P absorption coefficient, carbon to nitrogen radio (C/N radio) and concentrations of water-soluble and exchangeable manganese etc.) were measured. These WTRs including their mixtures were referred as cultivation soils, and provided to the growth experiment of tomato, bottle gourd and komatsuna.

According to the physicochemical properties analysis on the cultivation soils, no significant difference was observed in the properties between the 20% and 40% addition of bark compost to WTR, and between the 5g and 10g addition of P fertilizer in our previous study. (Xie et. al, 2013).

2.2.2 The Addition Levels of Bark Compost and P Fertilizer in the Present Study

Based on our preliminary analysis, the growth experiment was performed by using WTRs collected from Anou and Ideura WPPs located in Kitakyushu City, Fukuoka Prefecture, which were the mechanically dewatered and solar dried WTRs, respectively.

The cultivation soils by using WTR generated from the Anou WPP are referred to as "Anou soils", and those from the Ideura WPP are as "Ideura soils" hereafter.

Addition of bark compost to WTR was done with three levels of 0, 15, and 40 percent volumes of the WTR. Namely, the concentration of WTR in the cultivation soils was 100, 85 and 60% for the respective levels. These are hereafter denoted as WTR-100, WTR-85 and WTR-60, respectively.

In our preliminary analysis, difference in plant growth with the different levels of P fertilizer addition was not confirmed probably because of their large amounts, therefore, in the present study, P fertilizer was added with three levels of 0, 0.5 and 1.5 g per one liter of cultivation soils in order to clarify the effect of P fertilizer addition to WTR on the plant growth.

The addition was done before the experiment of the plant growth. The above P-fertilizer additions are hereafter denoted as P0, P0.5 and P1.5, respectively. The P fertilizer used was manufactured by Seiwa Fertilizer Ind. Co., Ltd. in Japan, which contained 17.5% weight of P.

In the following, the additions of 0 percent bark compost and 0 g P fertilizer to WTR, which means that the mixtures are composed of WTR alone, are also included into the cultivation soils as long as it is used for plant growing purposes.

Chapter 3 Physical and Chemical Properties of Water Treatment Residuals as Plant Growth Medium

3.1 Introduction

Since WTRs have soil-like properties, WTRs can be used as a soil substitute (or plant growth medium) (Dayton EA and NT Basta, 2001). For using WTRs as a plant growth medium, an assessment of the properties of WTRs is necessary. The properties that should be assessed are physical, chemical, and biological ones (Arshad et al., 1992).

In this chapter, the applicability of WTRs to use it as a plant growth medium is assessed through the analysis of WTRs chemically and physically. The biological analysis is mentioned in Chapter 4. The items for assessment are pH, electrical conductivity (EC) (these two are physical properties), effective cation exchange capacity (ECEC), P absorption coefficient, and the concentrations of water-soluble and exchangeable manganese (Mn) (these four are chemical properties). In the following, physical and chemical properties are called as physicochemical properties simply.

The respective properties are discussed from the viewpoint of using it as a plant growth medium.

(1) pH

The soil pH is a numerical expression of the intensity of acidity or alkalinity of soil, and is probably the single most informative measure of soil properties. Soil pH affects not only the physical and chemical, but also biological properties of soils and soil processes, as well as plant growth.

Every plant has its optimum pH for the growth, but many plants grow best if the soil pH is nearly neutral (pH 6 to 7.5). Bacterial populations and activity decline at low pH levels, whereas fungi adapt to a wide range of pH (acidic and alkaline). Most microorganisms have an optimum pH range for survival and function (Smith JL et al., 1996).

(2)EC

There are many soluble salts in soil solution (e.g., Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, H^{+}, $NO_3{}^{+}$, $SO_4{}^{2-}$, Cl^{-}, $HCO^{3}{}^{-}$, $CO_3{}^{2-}$, OH^{-}). Soil electrical conductivity (EC) is a measure of the amount of the salts in soils. The more the nutrients in soils, the more the soluble salts becomes, and the higher the soil EC value becomes. Soil EC correlates with the other soil properties that affect plant nutrient availability, and activity of soil microorganisms. (Grisso et al.; USDA-NRCS).

Usually, before fertilizer application, the EC value in vegetable field is 0.1 ~ 0.3 mS/cm, and that in pasture is less than 0.1 mS/cm. The EC of 0.3 - 1.0 mS/cm is suitable for the growth of most crops（MPAFFFD, 1997）.

(3) ECEC

Physicochemical properties of the most soils are influenced by their ion-exchange characteristics, including the amount and balance of individual ions present. Cation-exchange capacity (CEC) is the total capacity of soils to hold exchangeable cations. ECEC is an important soil property influencing soil structure stability, nutrient availability, soil pH and the soil's reaction to fertilizers and other ameliorants (Hazelton and Murphy 2007).

Cations in soils are positively charged ions such as sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), hydrogen (H^+), aluminum (Al^{3+}), iron (Fe^{2+}), zinc (Zn^{2+}) and copper (Cu^{2+}). The main ions associated with CEC in soils are the exchangeable cations Ca^{2+}, Mg^{2+}, Na^+ and K^+ (Rayment and Higginson, 1992), and are generally referred to as the base cations. The effective cation exchange capacity (ECEC) of soils (theoretically equal to the CEC) is calculated as the sum of these exchangeable cations.

(4) P Absorption Coefficient

The P (phosphate) in soils, which is supplied mainly from P fertilizers, binds with calcium (Ca^{2+}), iron (Fe^{2+}), aluminum (Al^{3+}) etc., composing lime phosphate (Ca_3PO_4), iron phosphate ($FePO_4$), aluminum phosphate ($AlPO_4$), etc. These P are sparingly soluble, and not easy to be absorbed by plants. The P-absorption coefficient is determined as the P fixing ability of soils (Yamazaki, 1966). The higher the coefficient, the stronger the fixation of P becomes, and fertilization of P is less effective for plant growing purposes (NFACA, 2014).

As mentioned in chapters 1 and 2, water treatment chemicals that contain aluminum and iron are added to the treating water during the flocculation process, therefore, WTR contains aluminum and irons. Aluminum and iron are positively charged and attract negatively charged P. As a result, only a small amount of plant available P remains in WTRs. Therefore, a large amount of P fertilization is necessary when using WTRs as a plant growth medium.

(5) The Water-Soluble and Exchangeable Manganese (Mn) Concentrations

Mn naturally occurs in rocks, soils, and water, and an is an essential trace element for plant growth. Mn is found in a number of general chemical forms in soils. Water-soluble Mn includes cations complexed with organic and inorganic ligands. Water-soluble Mn may be the critical parameter where Mn toxicity is suspected, especially in acid, poor aerated or flooded soils. Exchangeable Mn refers to the Mn that is weakly held on the cation exchange sites of clay minerals. Exchangeable Mn is probably a good estimate of readily available Mn in most soils (Gambrell, 1996).

According to Tamaue (2005), plant growth is severely limited when WTR alone is used as a growth medium. As such, soil (Oh et al., 2010; Mahdy et al., 2007), and composted bark (Kakuta et al., 2003) were added to WTR. According to Roppongi (1993), the exchangeable Mn in a plant growth medium decreased with time. The decrease was perhaps due to the change in the chemical form of Mn present in the medium. Kenneth (2006) indicated that the change in chemical form of Mn was caused by the microbial activity in the soil medium. When bark compost is added to the soils, Mn is absorbed by the compost and the Mn toxicity for plants is reduced (Maher, 1991).

However, for the combined growth medium, the physicochemical properties and its changes with time have not been clarified yet.

3.2 Materials and Methods

3.2.1 Materials

1. Eight kinds of WTRs were used for measuring the physicochemical properties. These WTRs were collected from 7 WPPs (water purification plants) of Tatara, Takamiya, and Meotoisi, Zuibaiji, Kouno, Anou and Ideura located in Fukuoka and Saga prefectures.

2. For the following plant growth mediums that is a mixture of bark compost and P fertilizer with WTRs (i.e., cultivation soils), the physicochemical properties were also measured. In the following, the cultivation soils are indicated by both the WTR containing rate (after the addition of bark compost) and P fertilizer addition rate. For example, cultivation soils with WTR containing rate 85% and P fertilizer addition rate 0.5 liter per 1kg are indicated as WTR85-P0.5. The cultivation soils used here are as follows.

Anou soils: WTR100-P0, WTR100-P0.5, WTR100-P1.5

WTR85-P0, WTR85-P0.5, WTR85-P1.5

WTR60-P0, WTR60-P0.5, WTR60-P1.5

Ideura soils: WTR100-P0, WTR100-P-0.5, WTR100-P1.5

WTR85-P0, WTR85-P0.5, WTR85-P1.5

WTR60-P0, WTR60-P0.5, WTR60-P1.5

3.2.2 Methods

All samples of WTRs and cultivation soils were air-dried, milled and passed through a 2 mm sieve, and then the physicochemical properties were measured for the samples.

(1) pH and EC

The pH and EC were determined by the procedure of CAMSE (2003). To be exact, 10 g weight of air-dried soil was put into a 100-mL Erlenmeyer flask, and 50 mL deionized water was poured into the flask and mixed well. The flask was mechanically shaken for 30 minutes, and then left it for 1 hour. In order to measure the pH and EC, the pH and EC electrodes (Horiba D51 and ES-71, Horiba Co., Ltd.) were immersed in the solution at least 3 cm below the surface. And then, the pH and EC values were read and recorded. An example of the pH measurement is shown in Fig. 3.1.

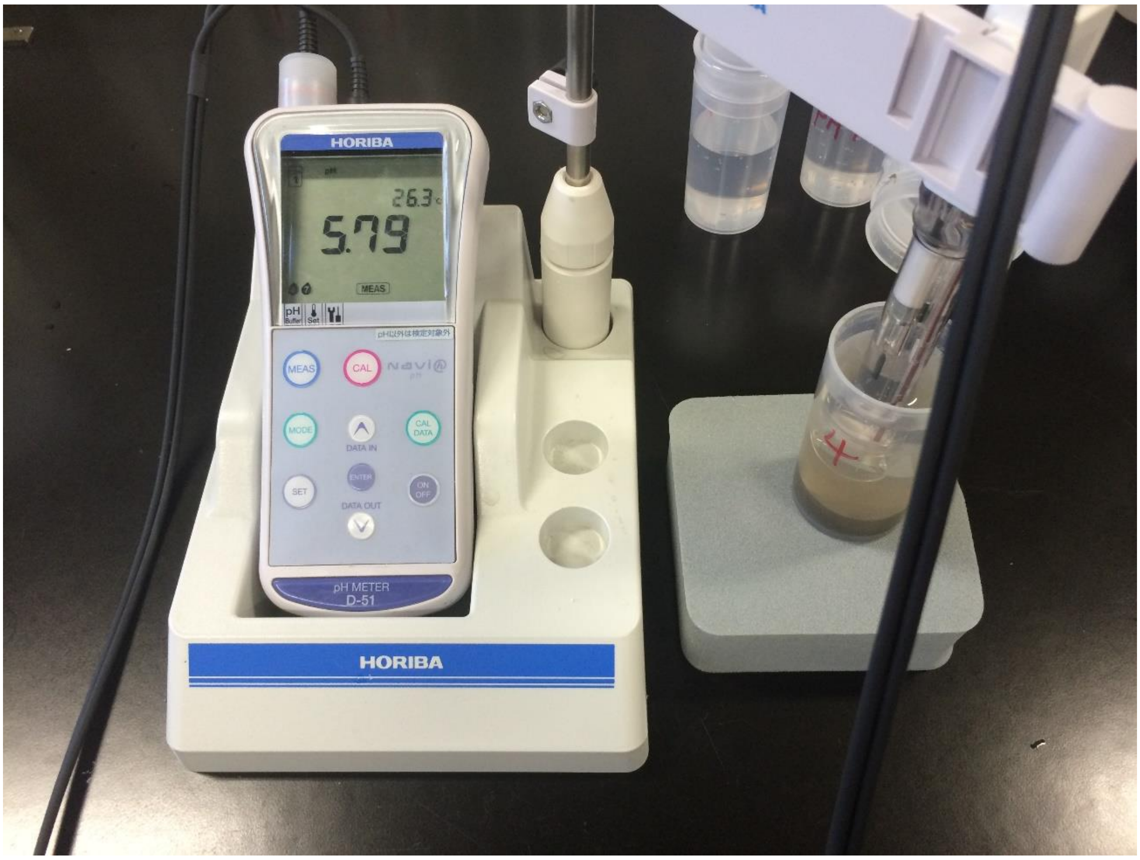

Fig. 3.1 Measurement of pH using a pH meter (Horiba D51, Horiba Co., Ltd.)

(3) ECEC

ECEC was determined by the procedure of Muraki (1992). A 2 g soil was put into a 85-mL centrifuge tube and 1 M ammonium acetate was added there. The tube was shaken for 15 minutes, and then centrifuged for 3 minutes at 2,500 rpm. The supernatant liquid was decanted to a 100-mL volumetric flask. This process was repeated 3 times, and the supernatant liquids were mixed in the same volumetric flask, making a volume of 1 M ammonium acetate solution. By using the solution, cations of Na, K, Mg and Ca in concentration are determined by atomic absorption spectrophotometry (AAS) (Hitachi Z-2300, Hitachi Co., Japan) (Fig. 3.2).

Fig. 3.2 Measurement of cation concentrations by using atomic absorption spectrophotometry (Hitachi Z-2300, Hitachi Co., Japan)

(4) The P Absorption Coefficient

P absorption coefficient was determined by the procedure of CAMSE (2003). A 25 g soil was put into a 100-mL Erlenmeyer flask, and 50 mL ammonium phosphate dibasic ($(NH_4)_2HPO_4$) (containing 13.44 g/L P_2O_5) was poured into the flask, and then the flask was mixed well and was left for 24 hours. The suspension in the flask was filtered through a paper filter (Advantec No. 5A, Advantec Co. Ltd. Japan). The concentration of P_2O_5 of the derived filtrate was analyzed by spectrophotometer (Hitachi U-2910, Hitachi Co., Japan).

Fig.3.3 Spectrophotometer used in the analysis (Hitachi U-2910, Hitachi Co., Japan)

(5) Water-Soluble and Exchangeable Mn Concentrations

The water-soluble and exchangeable Mn concentrations were determined by the procedure of Gambrell (1996) as shown below.

Water-soluble Mn: A 10 g soil was put into a 250-mL Erlenmeyer flask, and 100 mL high-purity water was added to it. The flask was shaken for 30 minutes on a mechanical shaker, and then the solution was filtered through a paper filter (Advantec No. 5A, Advantec Co. Ltd. Japan). Derived filtrate was poured into a 100-mL volumetric flask, and the volume was made up to 100 mL by adding high-purity water. The Mn concentration of the solution was determined by AAS (Hitachi Z-2300, Hitachi Co., Japan).

Exchangeable Mn: A 10 g soil was put into a 250-mL Erlenmeyer flask, and 100 mL neutral 1N ammonium acetate was added to it. The mixture was shaken continuously for 30 minutes on a mechanical shaker and then shaken intermittently for at least 6 hours. The suspension was centrifuged and a known volume of the solution was filtered through a paper filter (Advantec No.5A, Advantec Co., Ltd., Japan). The concentration of Mn was determined by AAS (Hitachi Z-2300, Hitachi Co., Japan).

For the relationship analysis between the physicochemical properties, Pearson's correlation coefficient analysis was used.

For clarifying the effects of the additions of bark compost and P fertilizer (i.e., two factors) on the physicochemical properties of the WTRs, a two-way ANOVA was used. When an interaction was observed between the two factors, a simple main effect test was performed to clarify the effect of one factor depends on the level of the other factor.

3.3 Results and Discussion

3.3.1 The Physical and Chemical Properties of the WTRs

Table 3.1 shows the physicochemical properties measured for the eight WTRs collected from the respective water purification plants (WPPs). In the following, WTRs collected from each WPP is shown by putting each WPP's name in front of WTR, for example, the WTR collected from Tatara WPP is shown as the Tatara WTR. From Table 3.1, the following characteristics were found.

Table 3.1 Physical and chemical (physicochemical) properties of the WTRs collected from the respective water purification plants.

Water purification plant	Tatara	Takamiya	Meotoishi	Zuibaiji	Kouno I*	Kouno II*	Anou	Ideura
pH	6.6	6.7	7.4	6.8	6.4	6.5	6.7	7.1
EC (mS/cm)	0.24	0.29	0.22	0.34	0.25	0.28	0.36	0.29
ECEC (cmolc/kg)	6.4	12.1	3.4	4.4	9.5	10.6	14.8	11.9
P absorption coefficient	2234	2206	2196	2244	1932	2212	2231	2183
Water-soluble Mn conc. (mg/kg)	6.3	21	10.5	14.3	4.8	12.6	30.1	141.7
Exchangeable Mn conc. (mg/kg)	55.1	64.5	139.2	80.9	48.3	131.1	80.7	1479.7

*Kouno I and II WTRs were the mechanical dewatered and solar dried WTRs.

(1) pH

The pH values were nearly neutral ranging from 6.4-7.4 that have no major differences depending on WPP. Since the pH range of 5.5 - 7.5 is suitable for most crops (Liu and Hanlon, 2012), pH of these values are thought to be favorable for crop growth.

(2) EC

The EC (mS/cm) values were low with a range of 0.22-0.36. According to Rayment and Lyons (2011), these values are acceptable for the growth of most plants.

(3) ECEC

The ECEC (cmolc/kg) values ranged widely from 3.4 – 14.8. As mentioned previously, ECEC theoretically equals to CEC for non-acidic soils and relates to the sum of the bases plus aluminum in acidic soils. Since the pH of the WTRs is near neutral, ECEC is thought to be equal to CEC. According to Price (2006), soils have a low nutrient retention capacity, when the soil CEC is lower than 10 cmolc/kg. The ECEC of the Tatara, Meotoisi, Zuibaiiji, and Kouno I WTRs were lower than 10 cmolc/kg. These WTRs are unsuitable for plant growth in terms of nutrient retaining capacity of soils, and the WTRs must be utilized carefully. The other four WTRs of Takamiya, Kouno II, Ideura and Anou WTRs having a CEC higher than 10 cmolc/kg are suitable for plant growth.

(4) The P Absorption Coefficient

The P absorption coefficient ranged from 1,932 – 2,244 with a minor difference with WPP. According to Yamasaki (1966), the P absorption coefficient is 600-750 in ordinary crop fields. If the coefficient exceeds 1,200, the P fixing ability is very strong. If the coefficient exceeds 1,500, most of the P in soils can be adsorbed onto soil particles, becoming unavailable for plant growth. In Table 3.1, all P absorption coefficient values exceeded 1,500, therefore, plants grown in these WTRs could suffer from P deficiency, and the application of P fertilizer is necessary.

(5) The Water-Soluble and Exchangeable Mn Concentrations

The critical concentration of water-soluble Mn to cause the Mn toxicity in plants is 5 mg/kg (Watanabe, 2002). The water-soluble Mn concentrations (mg/kg) of the eight WTRs ranged from 4.8 - 141.7 with difference with WPP. The concentration of the Kouno I WTR was the lowest (4.8) that was slightly lower than the critical value for the Mn toxicity. The water-soluble Mn concentration of the other seven WTRs exceeded severalfold the critical value. The highest one observed in the Ideura WTR that exceeded 28 times the critical value. As a whole, the water-soluble Mn concentrations were nearly equal to or larger than the critical value, which could cause the Mn toxicity in plants.

The exchangeable Mn concentration of the eight WTRs were different from each other, ranging from 48.3 - 1479.9. Similarly to the water-soluble Mn concentrations, the lowest one was observed in the Kouno I WTR, and the highest one in the Ideura WTR, and the highest one was 31 times larger than the lowest one. According to Takahashi (1980), Mn toxicity occurs when the exchangeable Mn concentration exceeds 10 mg/kg. Therefore, all the exchangeable Mn concentrations were high enough to produce the Mn toxicity, and the WTRs cannot be used without treatment for plant growth.

According the water purification process shown in Table 2.2 (Chapter 2), no chemicals that contain Mn was used. There is no industrial factory to use Mn in the watershed of the WPPs. Therefore, the Mn is not originated in the WPP. Mn is thought to be natural origin and is contained in soils. The soils were transported by river water and thought to be reached the WPPs. Soil chemical properties are affected by parent material, landscape position, climate, vegetation, land use practices (Acosta et al , 2005). Lundy (2012) reported that hydrogeologic setting may influence Mn concentration in groundwater.

Fig.3.4 shows the watershed area of the targeted water purification plants. Fig.3.5 shows the distribution of MnO (%) in northern Kyushu region. From these figures, the MnO concentrations in the WPPs located in Kitakyushu City are higher than those in other cities (Fukuoka, Saga and Itoshima cities). Since the available Mn concentration of the WTRs generated from Anou and Ideura WPPs in Kitakyushu City are higher than the WTRs generated from WPPs in Fukuoka, Saga and Itoshima cities.

Therefore, the available Mn concentration of the WTRs was likely to be related to a geological condition of the distribution of the MnO concentration in watershed.

On the other hand, from Table 3.1, the plant available Mn concentration (i.e., water-soluble plus exchangeable Mn concentrations) in the Kouno II WTR was 2.6 -2.7 times higher than that of Kouno I. In addition, the highest plant available Mn concentration in all WTRs was recorded in the Ideura WTR. In both Kouno II and Ideura WPPs, the solar drying method was used for dewatering. Therefore, the WTRs generated by solar drying method were thought to have higher plant available Mn concentration than those generated by the mechanical dewatering method.

More specifically, Mn in the WTRs was perhaps converted to plant available (water-soluble and exchangeable) forms in the process of solar drying, due to the inundated condition (i.e., reducing condition) of the sludge, resulting to have a higher available Mn concentration in the solar dried WTRs.

Therefore, the plant available Mn concentration appeared to be influenced not only by the distribution of MnO concentration in watershed but also by dewatering method of the WTRs.

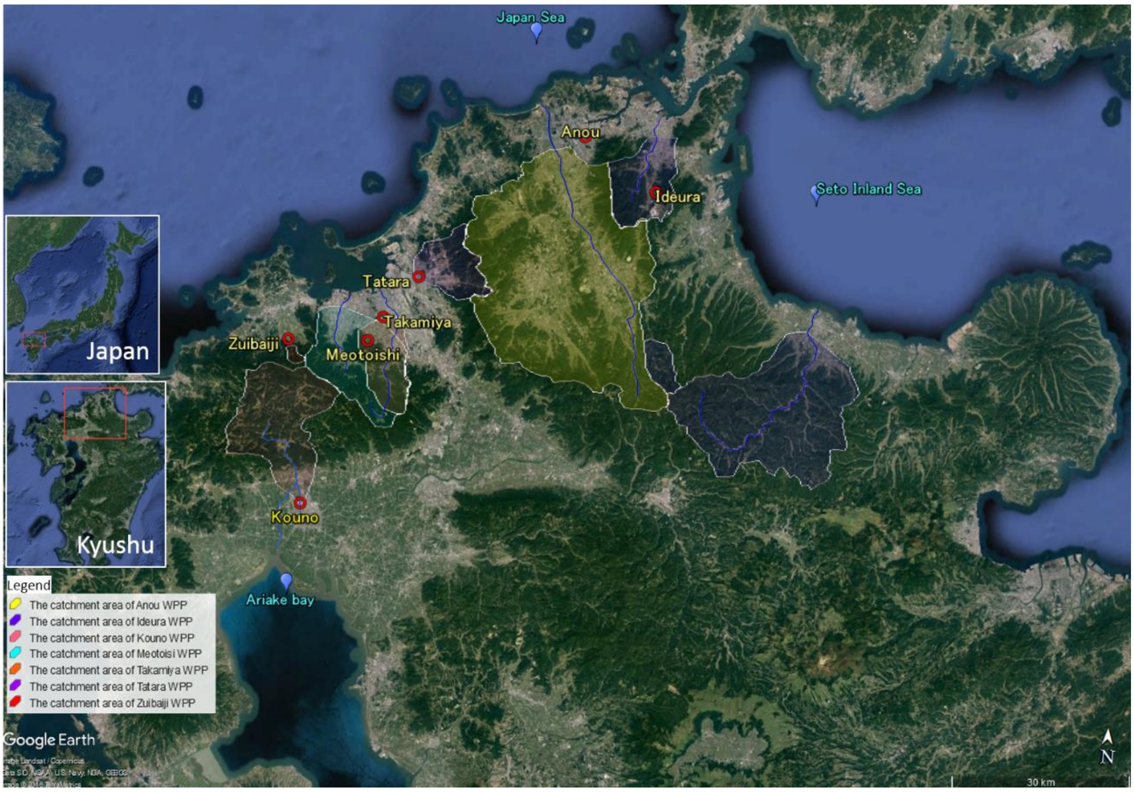

Fig.3.4 The catchment area of targeted water purification plant

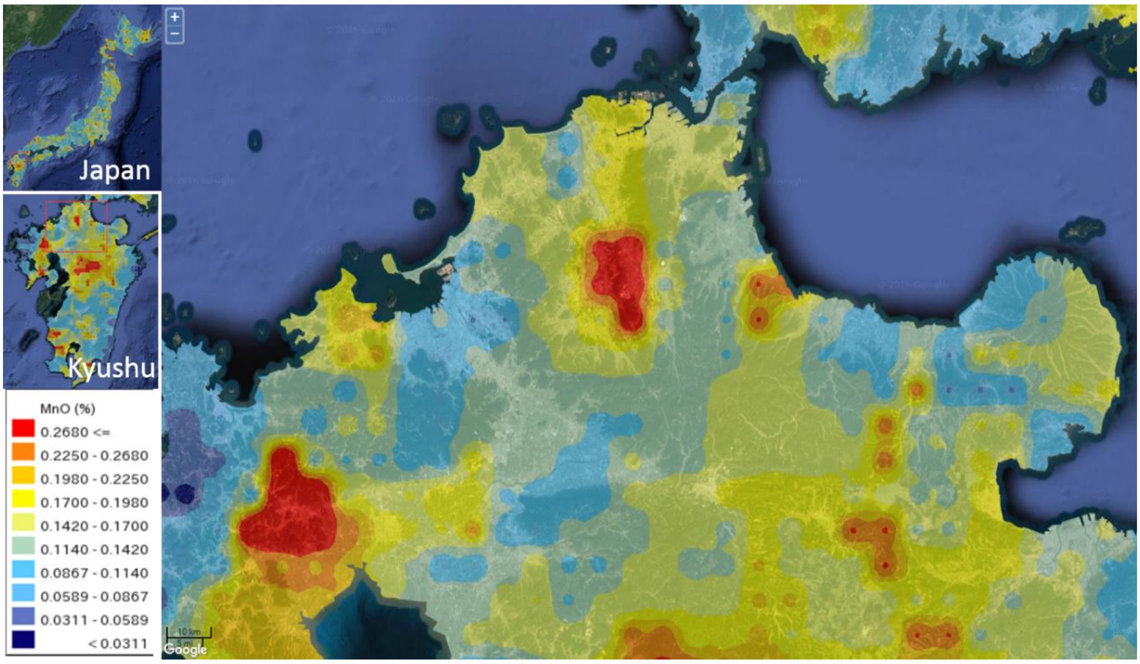

Fig.3.5 Distribution of MnO concentration in northern Kyushu region

(6) The Physical and Chemical Properties of the WTRs and their Mutual Relationships

Table 3.2 The correlation coefficient values between the physicochemical properties of the WTRs

	EC	ECEC	P absorption coefficient	Water-soluble Mn conc.	Exchangeable Mn conc.
pH	-0.19	-0.38	0.35	0.41	0.44
EC		0.49	0.36	0.19	0.04
ECEC			-0.07	0.39	0.26
P absorption coefficient				0.10	0.04
Water-soluble Mn conc.					0.98**

** significant at 1% level.

Table 3.2 shows the correlation coefficient values between the respective physicochemical properties of the WTRs. According to Table 3.2, there is no significant correlation between the physicochemical properties except between the exchangeable and water-soluble Mn concentrations. The exchangeable Mn concentration was positively correlated with the water-soluble Mn concentration with a 1 % level of significance.

From the above results, the respective physicochemical properties have independent characteristics except between water-soluble and exchangeable Mn concentrations and should be assessed independently.

If the available (water-soluble plus exchangeable) Mn concentration is high, Mn excess occurs in plants, but, there were no properties showing possible occurrence of Mn excess in the target physicochemical properties other than the available Mn concentration.

3.3.2 The Physical and Chemical Properties of WTR as a Plant Growth Medium

The physicochemical properties measured on the respective cultivation soils are shown in Table3.3. Here, average values are only shown. Further, the results of ANOVA on the effect of the additions of bark compost and P fertilizer on these properties are shown in Table 3.4.

In Table 3.4, the cases having significant differences on the effects of the additions were only shown. However, the cases with no significant difference were discussed in the text when needed.

Table3.3. Physicochemical properties of the cultivation soils of the Anou and Ideura WTRs

Physicochemical properties	Cultiv. soils	WTR100 (Bark compost 0%)			WTR85 （Bark compost 15%）			WTR60 （Bark compost 40%）		
		P0	P0.5	P1.5	P0	P0.5	P1.5	P0	P0.5	P1.5
pH	Anou	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.8	6.8
	Ideura	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
EC (ms/cm)	Anou	0.36	0.40	0.43	0.38	0.37	0.43	0.39	0.40	0.45
	Ideura	0.29	0.30	0.31	0.28	0.24	0.30	0.26	0.27	0.35
ECEC (cmol/kg)	Anou	14.8	14.5	16.7	17.5	17.8	19.8	21.2	22.2	24.6
	Ideura	11.9	12.5	14.3	14.9	15.1	17.2	19.7	21.4	23.4
P absorption coefficient	Anou	2231	2219	2257	2157	2204	2234	2244	2281	2348
	Ideura	2183	2181	2238	2292	2293	2347	2331	2349	2326
Water-soluble Mn conc. (mg/kg)	Anou	30	27	25	20	19	18	12	13	14
	Ideura	142	141	136	116	88	90	64	53	54
Exchangeable Mn conc. (mg/kg)	Anou	81	82	82	81	81	81	81	80	80
	Ideura	1480	1465	1429	1437	1420	1406	1311	1306	1253

P0, P0.5, P1.5: Level of P-fertilizer mixture; WTR: Water treatment residual.

Table3.4. Results of ANOVA and the simple main effect test for the effect of the additions of bark compost and P fertilizer on the physicochemical properties in the respective experiments.

Physicochem. properties	Cultiv. soils	Effects					Interaction and the simple main effect test
			Mixture of bark compost		Mixture of P fertilizer		
EC	Anou	*	WTR100, WTR85 < WTR60	**	P0, P0.5 < P1.5		-
	Ideura	*	WTR100>WTR85	**	P0, P0.5 < P1.5		-
ECEC	Anou	**	WTR100 < WTR85 < WTR60	**	P0, P0.5 < P1.5		-
	Ideura	**	WTR100 < WTR85 < WTR60	**	P0 < P0.5 < P1.5		-
P absorption coefficient	Anou	**	WTR100, WTR85 < WTR60	*	P-0, P0.5 < P1.5		-
	Ideura	**	WTR100 < WTR85, WTR60	*	P0, P0.5 < P1.5		-
Water-soluble Mn conc.	Anou					**	WTR100: P0 > P0.5, P1.5; P0, P0.5, P1.5: WTR100 > WTR85 > WTR60
	Ideura					**	WTR85,WTR60: P0 > P0.5, P1.5; P0, P0.5, P1.5: WTR100 > WTR85 > WTR60
Exchangeable Mn conc. (mg/kg)	Anou					**	WTR100: P0 < P0.5, P1.5; WTR60: P0 > P0.5, P1.5; P0.5,P1.5: WTR100 > WTR85> WTR60
	Ideura	**	WTR100 > WTR85 > WTR60	**	P0 > P0.5 > P1.5		-

**and *: Significant at 1% and 5% levels; -: No significant difference was observed.

(1) pH

According to Table3.3, pH ranged from 6.7- 6.8 in Anou, and ranged at 7.1 in Ideura soils, showing the neutrality of the soils. There was no significant effect of the addition of bark compost and P fertilizer on the pH.

(2) EC

EC（ms/cm）were 0.36-0.45 in Anou, and 0.26-0.35 in Ideura soils. These values are also favorable for plant growth based on Rayment and Lyons (2011). According to Table 3.4, EC was larger in WTR60 than in WTR85 and WTR100 for Anou soils. While for Ideura soils, EC was larger in WTR100 than in WTR85, and no difference was observed in the other comparisons for WTRs. EC was increased in P1.5 compared to P0 and P0.5, and was not increased in P0.5 compared to P0 in both Anou and Ideura soils (Table 3.4).

(3) ECEC

ECEC (cmol/kg) ranged from 14.5-24.6 for Anou, and from 11.9-23.4 for Ideura soils, showing that there appears to be no major difference in ECEC between the two cultivation soils. According to the ANOVA results (Table 3.4), ECEC increased from WTR100 to WTR85 and to WTR60 for Anou and Ideura soils that show an effect of the addition of bark compost. While ECEC increased from P0 (or P0.5) to P1.5 for Anou, and increased from P0 to P0.5 and then to P1.5 for Ideura soils that show the effect of the addition of P fertilizer.

(4) The P Absorption Coefficient

In Table 3.3, the P absorption coefficient ranged from 2,181 - 2,349 in both Anou and Ideura soils. According to Table 3.4, the coefficient was higher in WTR60 than in WTR85 and WTR100 for Anou, and higher in WTR60 and WTR85 than in WTR100 for Ideura soils; and the coefficient was higher in P1.5 than in P0 and P0.5 for both soils. Here, the coefficient was likely to be increased with the respective additions. The reason was considered to be that P in bark compost and P fertilizer made to increase the amount of P adsorbed in soil particles, resulting to increase the coefficient.

(5) The Water-Soluble and Exchangeable Mn Concentrations

The water-soluble Mn concentration (mg/kg) ranged from 12-30 for Anou, and from 53-142 for Ideura soils. The exchangeable Mn concentration (mg/kg) ranged from 80-82 for Anou, and from 1,253-1,480 for Ideura soils (Table 3.3). Here, both Mn concentrations, in particular the exchangeable Mn concentration, were higher in Ideura than in Anou soils.

In Table 3.4, the interaction between the effects of the additions of bark compost and P fertilizer was observed for the water-soluble and for the exchangeable Mn concentrations in Anou soils. In Ideura soils, the interaction was observed in the water-soluble Mn concentration only.

In the case when the interaction was observed, the simple main effect test was performed to investigate the effect of the respective factors. The results were shown in Table 3.4.

From Table 3.4, the water-soluble Mn concentration decreased in P0.5 and P1.5 compared to P0 for WTR100 of Anou soils. No significant change in the concentration was observed by the P fertilizer addition for both WTR85 and WTR60 for Anou soils.

In Ideura soils, the water-soluble Mn concentration decreased in P0.5 and P1.5 compared to P0 for WTR85 and WTR60. However, no significant change in the concentration was observed by the addition of P fertilizer for WTR100.

The water-soluble Mn concentration decreased with the increased addition of bark compost, i.e. decreased from WTR100 to WTR86 and then to WTR60 for both Anou and

Ideura soils at P0, P0.5 and P1.5, respectively.

For Anou soils, the exchangeable Mn concentration was higher in P0.5 and P1.5 than in P0 for WTR100, while the concentration was higher in P0 than in P0.5 and P1.5 for WTR60. No significant change was observed between P0, P0.5 and P1.5 for WTR85. Here, the magnitude of the effect of P fertilizer addition on the concentration varied with the added amount of bark compost.

The exchangeable Mn concentration decreased with the increased addition of bark compost at P0.5 and P1.5 in Anou soils, though the decrease was small (Table 3.3).

In Ideura soils, the exchangeable Mn concentration decreased with the increased additions of bark compost and P fertilizer (Table 3.4).

There are two possible reasons for the decrease in Mn concentrations by the addition of bark compost to WTRs as follows. (1) By the addition of bark compost, permeability of cultivation soils could be increased, due to the increase in pore spaces in the soils. According to Gilkes et al. (1988), Mn exists as a trivalent or tetravalent oxide in soils under aerobic conditions. By the increase in permeability, the oxygen in soils also increases, and water-soluble and exchangeable Mn that is divalent Mn, can be decreased by their conversion to trivalent or tetravalent Mn by the oxidation. (2) The addition of bark compost to the WTRs increases the number and distribution of microorganisms (which will be mentioned in Chapter 4). Among the multiplied microorganisms, there could be some Mn oxidizing microorganisms to convert divalent Mn to trivalent or tetravalent Mn .

Moreover, the addition of P fertilizer might decrease the concentrations of water-soluble and exchangeable Mn according to the results in Table 3.4. Further examination is necessary on this point.

Chapter 4 Biological Properties of Water Treatment Residuals as a Plant Growth Medium

4.1 Introduction

Torsvik et al. (1990) reported that the natural soil microbial communities contain a lot of microbial species, i.e., there are about 4,000 genetically distinct species in 100 g of beech forest soil. Moreover, soil microorganisms play an important role in soil ecosystem. Nowadays, the study on soil microbial diversity and the role of microorganisms has increasingly been paid attention in ecosystem functioning and environmental protection.

Numerous studies show that microbial colonies and microbial functional diversity can be a good indication to assess the quality of soils (Kennedy et al., 1999; Zhang et al., 1998; Xu et al., 2001). According to Elton's hypothesis (1958), most researchers agreed that the ecosystem functioning and sustainability of the ecosystem may depend on the soil microbiological diversity. (David et al., 1996; Marcel et al., 1998; Ananyeva et al., 1999).

In recent years, with the development of molecular biological technology, PCR amplification of the 16S ribosomal DNA and diversity detection technology have developed for the analysis of microbial variability. Denaturing gradient gel electrophoresis (DGGE) is one of the molecular fingerprinting method that separates polymerase chain reaction (PCR)-generated DNA products. DGGE was first proposed by Fisher and Legman (1979) for detecting DNA mutations. Muzyers (1993) applied the DGGE technique in the field of molecular microbiology, and confirmed the great advantages of DGGE in the research on genetic diversity of microorganisms and the difference in microorganisms in natural microbial flora.

The principle of DGGE is the use of a denaturing gradient gel to separate DNA fragments. At the beginning of electrophoresis, the rate of migration of DNA in the gel is only related to the size of the molecule. During DGGE, DNA encounter increasingly higher concentrations of chemical denaturant as they migrate through a polyacrylamide gel. Once the DNA reaches a certain point of DNA denaturation, double-stranded DNA begins to denature at which time migration slows dramatically. Differing sequences of DNA will denature at different denaturant concentrations, resulting in a pattern of bands (Debabrata et al., 2014).Theoretically, each DNA band represents a different bacterial population present in the community. Once band was generated, fingerprints can be uploaded into databases in which fingerprint similarity can be assessed to determine microbial structural differences between environments and/or among treatments (Marco, 2011).

However, the diversity of microorganisms in WTRs has not been investigated and reported. In this study, bacterial DNA was amplified and subjected to DGGE to analyze the soil microbial diversity of the 6 WTRs collected from different WPPs in Fukuoka and Saga prefectures in Japan. The microbial diversity was also analyzed for the plant growth mediums made by WTRs to which bark compost was added.

The experiment in this chapter aimed to investigate the relationship between the colony development and the species composition of microorganisms in WTRs, and to assess the biological diversity of soil microorganisms with difference in WTRs.

4.2 Materials and Methods

4.2.1 Samples of Cultivation Soils

1. Six WTRs collected from the Takamiya, Meotoishi, Zuibaiji, Kouno, Anou, and Ideura WPPs were used.

2. Details of the cultivation soils (mentioned in 2.2.2) used in this experiment are as follows:

Anou soil: WTR100-P0, WTR100-P0.5, WTR100-P1.5

WTR85-P0, WTR85-P0.5, WTR85-P1.5

WTR60-P0, WTR60-P0.5, WTR60-P1.5

Ideura soil: WTR100-P0, WTR100-P0.5, WTR100-P1.5

WTR85-P0, WTR85-P0.5, WTR85-P1.5

WTR60-P0, WTR60-P0.5, WTR60-P1.5

All the soil samples were air-dried, milled and passed through in a 2 mm sieve before their use.

4.2.2 Microbial Culturing

The culture medium for growing the soil microorganisms were consisted of: 20 g hipolypeptone (Wako Pure Chemical Industries Ltd., Japan), 1 g dried yeast extract, 0.5 g glucose in 1L distilled water. The culture medium was adjusted to pH 7.0, and 15 mL of the medium was dispensed to 50 ml volume centrifuge tube and then autoclaved for 20 minutes at 121°C. After cooling in a clean bench, a 10 g soil sample was added to the culture medium, and the tube was capped. The media was incubated in a shaker (shaking machine) at 30°C for 2 weeks for growing soil microorganisms.

Fig 4.1 The soil sample and the sterile culture medium in a clean bench

4.2.3 DNA Extraction

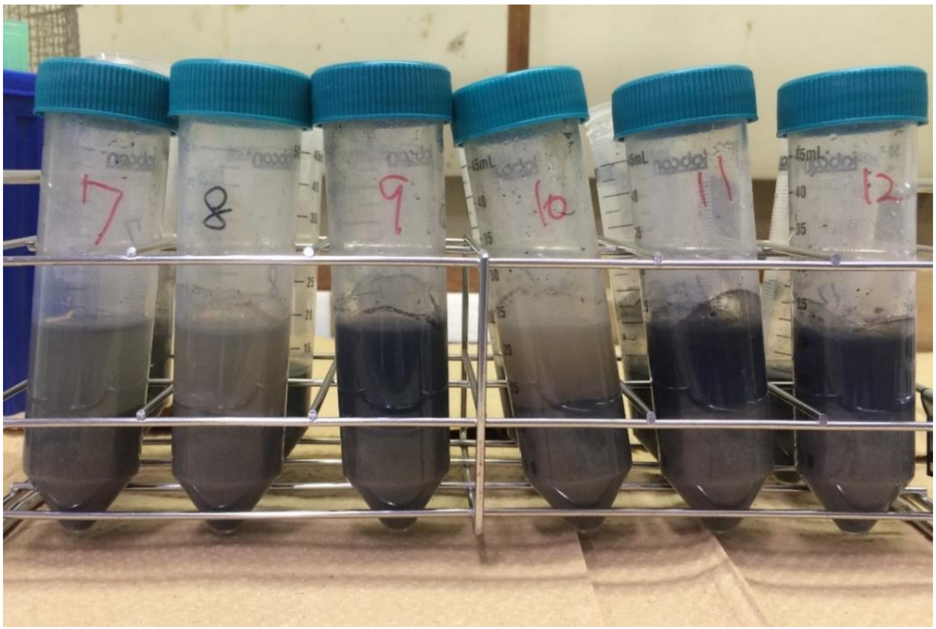

Fig. 4.2 The centrifuge tube for 2 weeks after incubation

After incubation, the medium was stratified. The upper suspension was transferred to a new 15 ml volume centrifuge tube, and was centrifuged at 40,000 rpm for 15 minutes, then discard supernatant, and the precipitate was dried at 30 ° C for 24 hours. For PCR amplification, DNA was extracted by using the Qiagen DNeasy Plant Mini Kit with manufacture protocols.

4.2.4 PCR and DNA Purification

PCR amplification was performed with KOD-plus DNA polymerase (TOYOBO) using F984GC (5'-cgc ccg ggg cgc gcc ccg ggc ggg gcg ggg gca cgg ggg g aa cgc gaa gaa cct tac-3', the underline is GC clamp) and primer R1378 (5'-cgg tgt gta caa ggc ccg gga acg-3') for the target bacteria possessing V6-8 region of 16S rRNA fragment.

For each PCR reaction, the mixture solution contained 33 µl sterile deionized water, 5 µl 10x buffer, 5 µl dNTPs (2mM), 2 µl $MgSO_4$ (25mM), 1 µl F984GC (10µM), 1 µL R1378 (10µM), 1 µl bovine serum albumine (BSA, 20mg/ml), 1 µl KOD-Plus-, and 1µl template DNA in a 100µl tube. The PCR program selected for this reaction started initially at 94°C for 5 minutes, followed by 34 cycles at 92°C for 30s, at 55°C for 30s and at 68°C for 72s, with final extension cycle at 72°C for 15 minutes. The PCR product was purified using a QIAquick PCR purification kit (Qiagen) and purified solution was store at 5 °C until use.

4.2.5 Denaturing Gradient Gel Electrophoresis (DGGE)

PCR products were run on a 6 % Acrylamide/Bis 37.5:1 gel in a 50–70% denaturing gradient of urea and formamide (deionized) using the Bio-Rad DCode^{TM} Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA). DGGE were carried out at 50 V and 58°C for 18 h in 1x TAE buffer. After electrophoresis, the gels were stained with SYBR Green I (Lonza, Rockland, ME, USA) and photographed under UV light using the ChemiDoc™ XRS+ system (Bio-Rad Laboratories, Hercules, CA, USA). DGGE image was processed and analyzed with BioNumerics ® software package (Version 7.0; Applied Maths, Sint-Martens-Latem, Belgium). Calculation of the pair-wise similarities of densitometric profiles was conducted based on Pearson's correlation coefficients for comparison of band patterns among WTRs. Cluster analysis based on the similarity matrix was carried out with UPGMA (unweighted pair-group method with arithmetic averages, also known as average linkage) method.

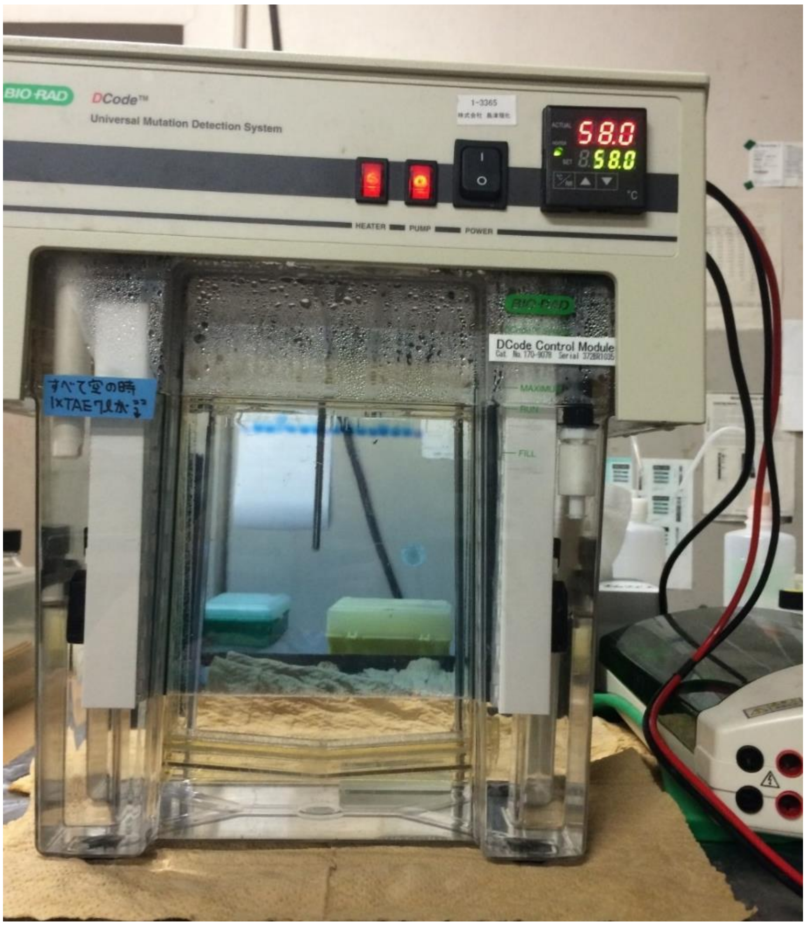

Fig. 4.3 DGGE electrophoresis

4.3 Results

4.3.1 Result of the DGGE Analysis for the 6 WTRs

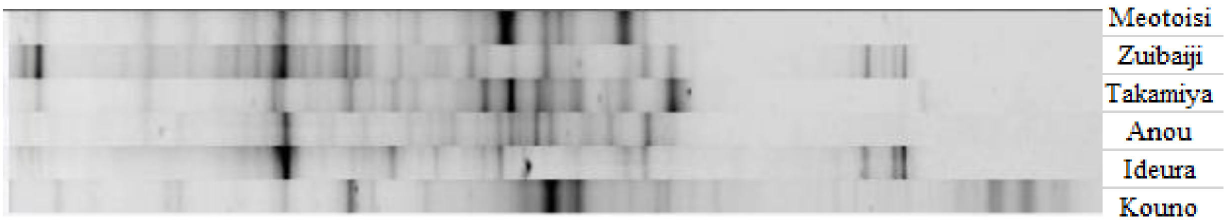

Fig.4.4 DGGE profiling of patterns of bacterial communities in the 6 WTRs

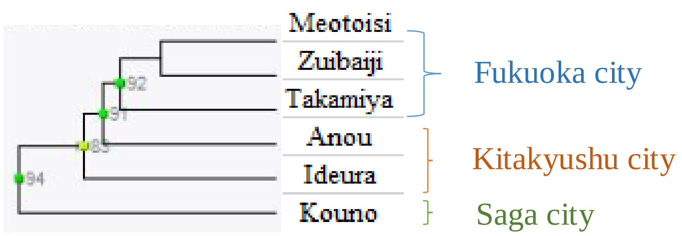

Fig.4.5 Dendrogram analysis (UPGMA) based on the DGGE banding patterns obtained from the 6 WTRs

Table 4.1 The similarity (%) of the communities between the 6 WTRs

	Zuibaiji	Takamiya	Anou	Ideura	Kouno
Meotoisi	68.4	54.5	51.3	48.5	31.3
Zuibaiji		60	56.5	56.4	25.8
Takamiya			50	44.3	25.6
Anou				43.9	38.1
Ideura					28.6

For amplification of the soil bacteria in the V6-8 region of 16S rRNA fragments, F984GC and R1378 primer were used. DGGE separation patterns of bacterial communities based on the PCR products is shown in Fig.4.4. Diversity of microbial communities in WTRs, based on DGGE profiles, was estimated by the number of amplified 16S rDNA bands (Fig. 4.4), because each band was assumed to represent as a single operational taxonomic unit (OTU). Therefore, each band may represent one microorganism.

All 6 WTRs displayed a different or similar length and number of bands. Partitioning profiles of microbial community structure can be seen in the dendrogram that was created by illustrating the similarity of microbial communities in the 6 WTRs (Fig.4.5). And the dendrogram was constructed by the unweighted-pair group method using arithmetic averages (UPGMA). The similarity of the bacterial communities among the 6 WTRs is shown in Table 4.1.

From these results, there were several groups of the WTRs according to their similarities, and the community structure of bacteria was similar in a group of WTRs composing homological microbial species.

WTRs collected from Meotoishi and Zuibaiji WPPs, which are closely located with each other in Fukuoka and Itoshima cities, respectively, revealed the highest similarity (68.4%) in the community structure. In addition, the Takamiya WPP is also located in Fukuoka city, and the Takamiya WTR has a high level of similarity compared with the Meotoishi and Zuibaiji WTRs respectively (54.5% and 60%). Anou and Ideura WPPs, which are located in Kitakyushu City, and their WTRs displayed the divergent communities with only 43.9% of the similarity. This similarity is lower than that between Zuibaiji and Takamiya WTRs. The Kouno WPP located in Saga City, and the Kouno WTR showed a low similarity of less than 40 % in community structure compared with the other 5 WTRs.

These results indicate that the geographical location of the WPPs did not affect the similarity of the bacterial community structure in this experiment.

Dewatering method of the Meotoishi and Anou WPPs are solar drying method. On the other hand, Zuibaiji, Takamiya and Ideura WPPs used a mechanical dewatering method. In this experiment, the Kouno WTR was generated by both solar drying and mechanical dewatering methods. Based on the Fig.4.5 and Table 4.1, excluding the Kouno WTR, the dewatering method did not affect the similarity of the bacterial community structure.

Meotoishi and Takamiya WPPs use the same river water (Naka River) as the raw water. However, the Meotoishi and Takamiya WTRs did not show the similarity in the community structure (Table 2.1). From the result of Fig.4.5 and Table 4.1, the similarity in the community structure between the WTRs is not thought to be influenced by the source of raw water. Moreover, the 6 WTRs were grouped differently in clusters not only the source of raw water, but also the location and dewatering method.

4.3.2 DGGE Analysis Results of Anou and Ideura Soils

In Case of Anou Soils

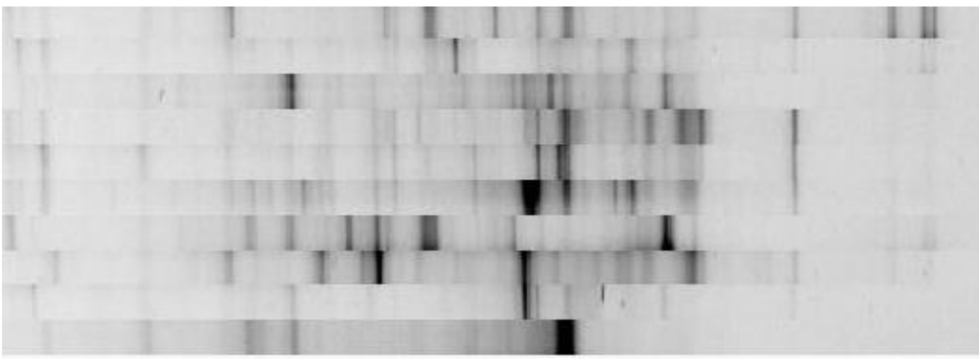

Fig.4.6 DGGE profies of the bacterial communities in Anou soils and bark compost

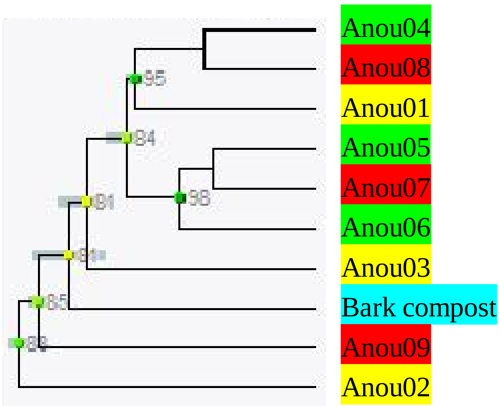

Fig.4.7 Dendrogram profiles (UPGMA) of DGGE banding patterns from the obtained Anou soils and bark compost

Table 4.2 The similarity (%) of the bacterial communities between the Anou soils incuding the addition of bark compost to it

	Anou 08	**Anou 01**	**Anou 05**	**Anou 07**	**Anou 06**	**Anou 03**	**Bark compost**	**Anou 09**	**Anou 02**
Anou04	76.0	58.3	68.2	57.8	52.2	53.7	43.2	41.2	34.3
Anou08		64.0	60.9	68.1	58.3	55.8	46.8	44.4	43.2
Anou01			54.5	57.8	56.5	58.5	48.9	47.1	40.0
Anou05				78.1	71.4	37.8	62.5	40.0	22.3
Anou07					69.8	57.9	51.4	45.2	43.8
Anou06						41.0	51.4	43.6	36.4
Anou03							46.2	37.0	35.7
Bark compost								26.1	24.0
Anou09									38.1

The DGGE profiles of the Anou soils are shown in Fig. 4.6. Each soil can be separated by a DGGE with different numbers of electrophoretic bands, and the intensity and mobility of each band are divergent. UPGMA clustering from the DGGE profile generated based on the individual PCR products showed that the duplicate samples of specific ages were clustered together (Fig.4.7). The similarity of the communities among the 6 WTRs is shown in Table 4.2.

In Figs. 4.6 and 4.7 and Table 4.2, Anou01, Anou02 and Anou03 soils were the cultivation soils of WTR100-P0, WTR100-P0.5 and WTR100-P1.5, which did not contain bark compost; Anou04, Anou05 and Anou06 soils were the cultivation soils of WTR85-P0, WTR85-P0.5 and WTR85-P1.5, which contained 15% bark compost; Anou07, Anou08 and Anou09 soils were the cultivation soils of WTR60-P0, WTR60-P0.5 and WTR60-P1.5, which contain 40% bark compost.

According to Fig. 4.6, in Anou01, Anou02 and Anou03 soils, which did not contain bark compost, the number of ladder bands was small and the intensity of bands was weak; in contrast, the soils containing bark compost showed less quantity and number of microbial species compared to those without containing bark compost in Anou soils.

Comparison of the soils between the addition (Anou04 - Anou09) and no-addition (Anou01- Anou03) of bark compost showed that the band patterns were different profiles between them. This fact indicated that the addition of bark compost diversified the community structure of microorganisms in Anou soils.

According to Fig. 4.7, there was a similarity between the bark compost (alone) and Anou soils, indicating that the community structure of microorganisms in bark compost and Anou soils was similar with each other, and the comunities were consisted of comparatively homological microbial species. In addition, excluding the WTR60-P1.5 (Anou09), the Anou soils, containing 15% (Anou04, Anou05 and Anou06) and 40% (Anou07 and Anou09) bark compost, showed a close relationship with each other, and belonged to the same group based on the dendrogram. From Table 4.2, the similarity reached at 52.2% -76%. The amount of bark compost itself did not affect the species and number of microorganisms.

Ideura Soils

Fig.4.7 DGGE profiles of bacterial communities in Anou soils and bark compost

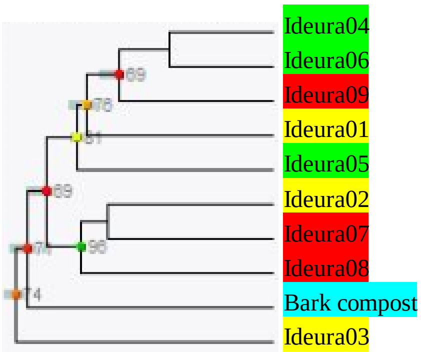

Fig.4.8 Dendrogram analysis (UPGMA) based on the DGGE banding patterns obtained from Ideura soils including the addition of bark compost to it

Table 4.3 The similarity (%) of the microbial communities between the Ideura soils

	Ideura 08	**Ideura 01**	**Ideura 05**	**Ideura 07**	**Ideura 06**	**Ideura 03**	**Bark compost**	**Ideura 09**	**Ideura 02**
Ideura04	71.8	51.2	57.9	51.4	40.0	46.2	28.6	30.3	36.1
Ideura06		66.7	51.4	50.0	36.7	51.9	46.0	38.7	34.2
Ideura09			41.0	44.4	44.4	46.7	36.7	41.2	36.1
Ideura01				45.2	51.6	42.0	45.6	20.7	38.2
Ideura05					40.2	30.8	8.7	41.4	38.4
Ideura02						56.0	47.6	22.2	27.7
Ideura07							50.0	34.8	31.0
Ideura08								47.8	14.5
Bark compost									30.8

The DGGE profiles obtained from the analysis of Ideura soils are shown in Fig. 4.7. The dendrogram is also shown in Fig.4.8. The similarity of the communities between the Ideura soils is shown in Table 4.3.

From Figs. 4.7 and 4.8 and Table 4.3, the DGGE profile and the similarity rate of Ideura soils showed the results were the same with those of Anou soils. 1) In the Ideura WTR (without adding the bark compost), the number and density of microbial species were less than those in bark compost. 2) The community structure of bacteria in bark compost and Ideura soils showed some similarities. The reason is probably that they were consisted of homologous microbial species. 3) The addition of bark compost affected the community structure of bacteria in Ideura WTR. 4) The magnitude of the amount of bark compost addition to the WTR did not relate to the number and density of microbial species.

Anou and Ideura Soils

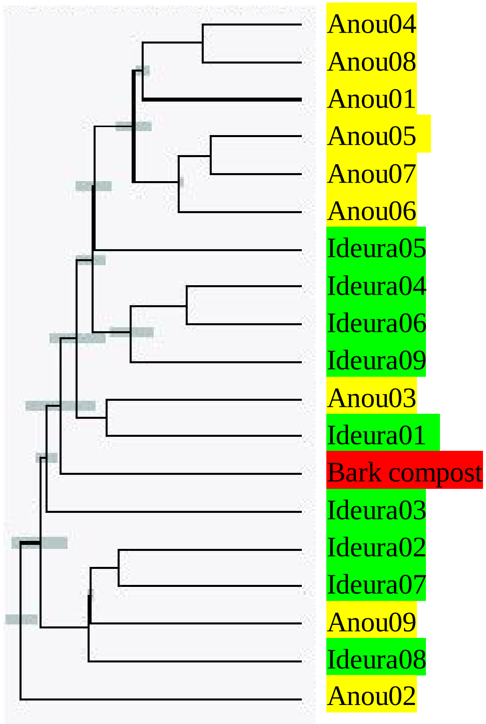

Fig.4.9 Dendrogram analysis (UPGMA) based on the DGGE banding patterns obtained from Anou and Ideura soils and bark compost

Fig.4.9 showed that the dendrogram (UPGMA) based on DGGE banding patterns obtained from Anou and Ideura soils and bark compost. Excluding Anou03, Anou09 and Anou02, Anou and Ideura soil were assigned to a divergent cluster in this dendrogram. By the addition of bark compost, Anou and Ideura soils showed a significant difference in the community structure of bacteria. In other word, the addition of bark compost to WTRs had a limited systemic impact in the community structure of bacteria.

4.4 Discussions and Conclusions

In this experiment, the community structure of microorganisms in the WTRs showd the similarities, however the same and different patterns of microbial species in the WTRs were also observed. The similarity of the WTRs were not likely to be affected by the location, dewatering method, and the source of the raw water from the result of DGGE analysis.

b ん m v ん m.

The community structure of bacteria observed in bark compost and Anou and Ideura soils showed some similarities, where the bacteria were consisted of almost homologous microbial species.

The addition of bark compost to the WTR diversified the community structure of bacteria in both Anou and Ideura WTRs, but the bacterial multiplication was limited.

This experiment revealed that the magnitude of the added amount of bark compost did not relate to the number and species of microorganisms.

The diversity of microorganisms in soil is one of the criteria to maintain soil health and quality (Garbeva et al., 2004). After the addition of bark compost to WTRs, the diversity of microorganisms becomes larger, which could improve the soil quality, and contribute to the plant growth. In addition, as mentioned in Chapter 3, the diversification of the community structure of microorganisms might contribute to the reduction of available Mn concentration, thereby to the reduction of the risk of Mn excess and to the improvement of soil quality.

Chapter 5 Plant Growth Experiment using Water Treatment Residuals

5.1 Introductio

As mentioned in Chapter 1, approximately 300,000 tons of WTRs are disposed in Japan, and the disposed amount is tended to increase. In recent years, proper disposal/reuse of a huge quantity of WTR is a growing concern in the world. Kim (2002) mentioned that utilization of WTR as a soil substitute is one of the most cost-effective method alternative to the disposal, because WTRs have favorable properties for plant growing purposes (Dayton EA and NT Basta, 2001). When the WTRs were used for agricultural purposes, soil physical properties must be improved before its use (Moodley and Hughes, 2005), the amount of P leaching from farmland becomes smaller (Ulen et al., 2012), and the discharge of P from an animal husbandry area to a river is prevented （Novak and Watts, 2005). These properties give some advantages in terms of the reduction of agricultural production cost and environmental protection.

As mentioned previously, there are a lot of researches on the utilization of WTR, most of which focus on the physicochemical properties. When WTRs are used for crop growing purposes, bark compost and P fertilizer are usually added, however, the change in the properties with the addition have less studied.

Here, the changes in the physicochemical properties with the additions of bark compost and P fertilizer are studied, where the difference in the properties according to the dewatering method of WTRs were taken into consideration.

5.2 Materials and Methods

5.2.1 Materials

WTRs provided for plant growth experiment were as follows, where bark compost and P fertilizer were added to the WTRs:

Anou soils: WTR100-P0, WTR100-P0.5, WTR100-P1.5

WTR85-P0, WTR85-P0.5, WTR85-P1.5

WTR60-P0, WTR60-P0.5, WTR60-P1.5

Ideura soils: WTR100-P0, WTR100-P0.5, WTR100-P1.5

WTR85-P0, WTR85-P0.5, WTR85-P1.5

WTR60-P0, WTR60-P0.5, WTR60-P1.5

The abbreviations of the above were mentioned in 2.2.2.

Plant used for the experiment

Komatsuna (*Brassica rapa* var. *perviridis*) (Japanese mustard spinach) (Misaki Series Species) (Sakata Seed Co., Ltd.) was used for the experiment. Komatsuna is widely used for plant growth experiment. Komatsuna is a fast growing leafy vegetable that is native to Japan. In a mature plant, Komatsuna is dark green with slender light green stalks, around 30 cm long and 18 cm wide. The plant can be grown all year round in temperate and subtropical areas.

Komatsuna is easy to grow in a home garden due to less replant failure. Suitable temperature for the growth is 10-25℃, and cultivation period is around 30 days when the sawing is done in summer, while the period is around 90 days in winter.

Komatsuna is a plant that is particularly sensitive to Mn excess (AFFRC, 1977). No other plants are so sensitive to Mn excess. Though only plant species of Komatsuna was tested in this experiment, the test results were producible to identify the effect of the physicochemical properties and the additions of P fertilizer and bark compost on the plant growth.

5.2.2 Methods

The growth experiment of Komatsuna was done with four pots for the Anou soils, and five pots for the Ideura soils for replication. The difference in the number of pots between the Anou and Ideura soils was owing to the difference in the collected amount of WTRs from the respective WPPs. In each pot, five Komatsuna seeds were sown. Seedlings were grown from the seeds. Among the seedlings, three well-grown seedlings were left and two others were thinned out at seven days after the sowing. The remaining three seedlings were grown for 55 days in Anou and 28 days in Ideura soils after the sowing. The seeds of Komatsuna used for the experiment were the commercially available ones purchased from Sakata Seed Corporation in Japan.

The plant growth experiment was performed in a phytotron facility in Kyushu University, where the air temperature was maintained at 20 ºC and the relative air humidity was maintained at 70 %. During the experiment, water in an amount corresponding to the field capacity was supplied to the plants once in two days. Just after the 55 and 28 days of the growth, foliage weight of the plants (above ground biomass weight; raw weight), was measured as a representative growth.

In order to clarify the effect of the additions of bark compost and P fertilizer on the foliage weight, the two-way ANOVA was applied by using the Excel Software.

5.3 Results and Discussion

5.3.1 Results of the Plant Growth Experiment using Anou Soils

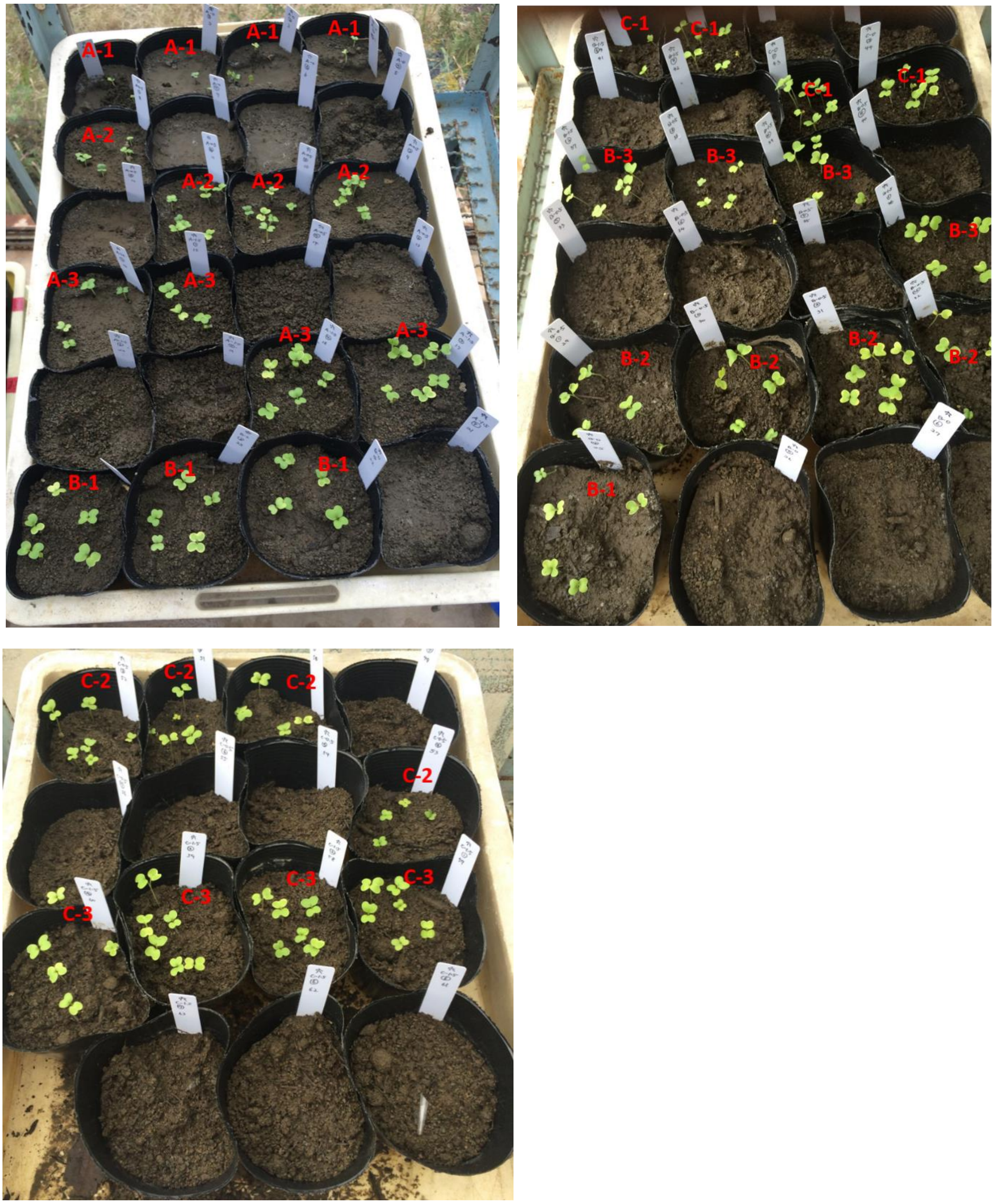

Fig. 5.1 The situation of the plant growth on day 6 of the experiment.

The marks shown in Fig. 5.1 show the following cases:

A-1: WTR100-P0; A-2: WTR100-P0.5; A-3: WTR100-P1.5

B-1: WTR85-P0; B-2: WTR85-P0.5; B-3: WTR85-P1.5

C-1: WTR60-P0; C-2: WTR60-P0.5; WTR60-P1.5

Fig. 5.2 The situation of the experiment on day 27.

Fig. 5.3 The situation of the experiment on day 55.

The situation of the plant growth experiment using Anou soils on day 6, 27 and 55 after the sowing are shown in Fig. 5.1, Fig. 5.2 and Fig.5.3, respectively.

According to Fig. 5.1, all the Komatsuna grew well on day 6 and their growths on that day were similar between them.

According to Fig.5.2, the growth of Komatsuna in the soil of WTR100-P0 (WTRs occupied 100%) was smaller than in other soils, due to that Komatsuna plants in the soil were withered and died. In other soils of WTR100-P0.5 and WTR100-P1.5, Komatsuna grew well and the growth did not look different from that in WTR85 with any addition amount of P.

In the soils of WTR85-P0, WTR85-P0.5, and WTR85-P1.5, Komatsuna grew well and these showed no difference with each other. While in the soils of WTR60-P0.5, WTR60-P0.5 and WTR60-P1.5, the Komatsuna growth was smaller than in WTR85 group with the addition of P, and showed a yellowish color on plant leaves.

According to Fig.5.3, most of Komatsuna plants grown in WTR100-P0 were dead, and the plants in other soils showed a tendency to wither. The Komatsuna grown in the soil of WTR100-P0.5 showed a large growth without any dead plant. The growth was larger than in WTR100-P0, but some leaves showed an unhealthy color of yellow. The plants grown in WTR100-P1.5 showed the best growth among the plants grown in all the soils. In the soils of WTR85-P0, WTR85-P0.5 and WTR85-P1.5, the plants grew well and healthy, and did not look different from each other. In the soils of WTR60-P0.5, WTR60-P0.5 and WTR60-P1.5, the plants did not grow well with yellowish leaves and tended to be dead.

As a whole, on day 6, all plants grew well and they did not look different from each other. On day 27, the plant growth was different with the soils. On day 55, the difference became very clear, i.e., the plants grown in WTR100-P0 showed the worst growth and most of them were dead. This indicated that the plants grown in Anou soils without adding both of bark compost and P-fertilizer grew unhealthy, resulting to die. When the P-fertilizer was added to Anou soils, the plants grew larger than in using WTR alone, and the growth increased with the increased P-fertilizer addition. While, when the bark compost was added to Anou soils (WTR85-P0 and WTR60-P0), the plants grew better than in using WTR alone, but the plants in using WTR85-P0 grew better than in using WTR60-P0, showing that the higher addition of bark compost, the better the plant growth became. When both of bark compost and P-fertilizer were added, the plant growth become better.

Table 5.1 The foliage weight of Komatsuna plants grown in Anou soils (on average)

P fertilizer addition	WTR100	WTR85	WTR60
P0	0.005	1.548	0.548
P0.5	0.865	2.312	0.357
P1.5	2.624	2.167	0.557

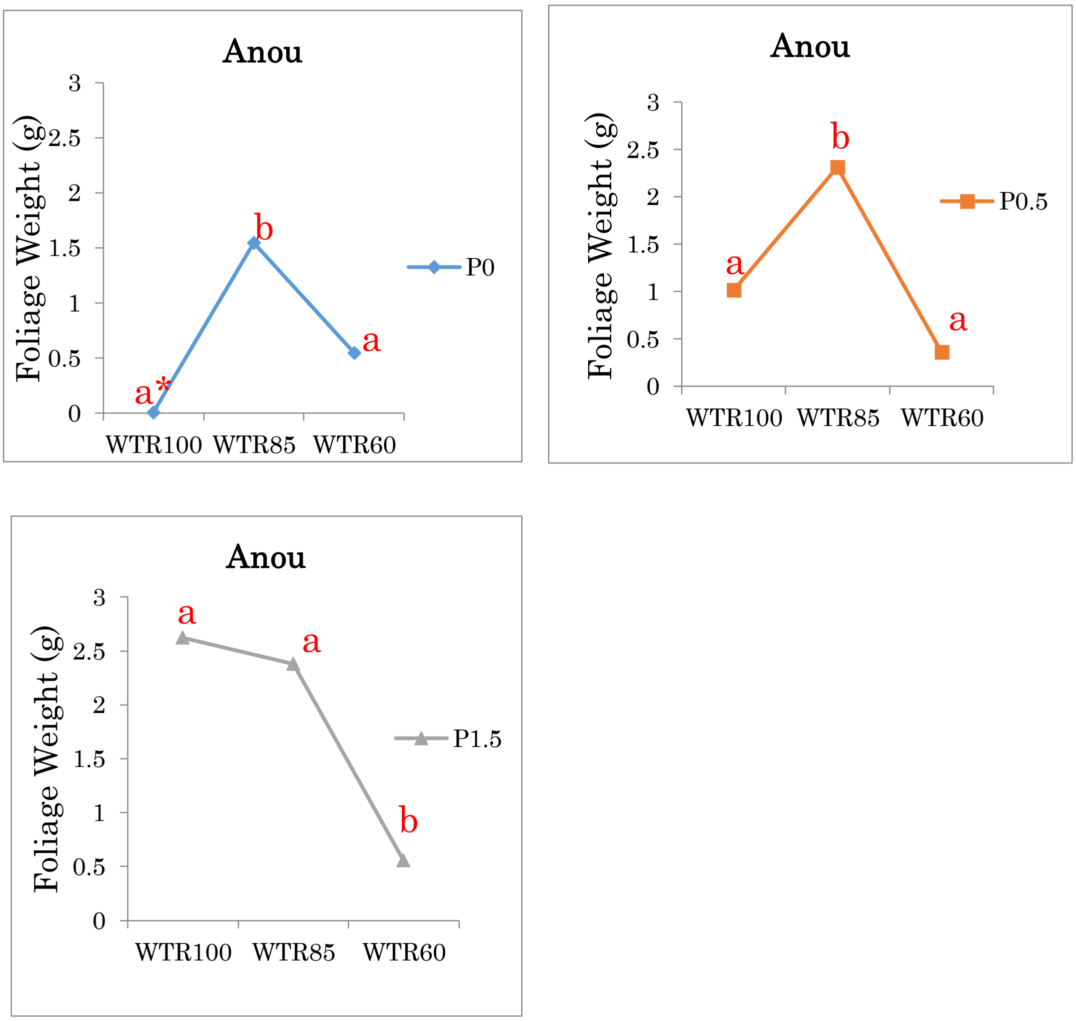

Fig. 5.4 The simple main effect of the addition of bark compost at each addition level of P-fertilizer on the foliage weight

*: Different letters indicate a significant difference among different levels, and same letters indicate no significant difference among different levels

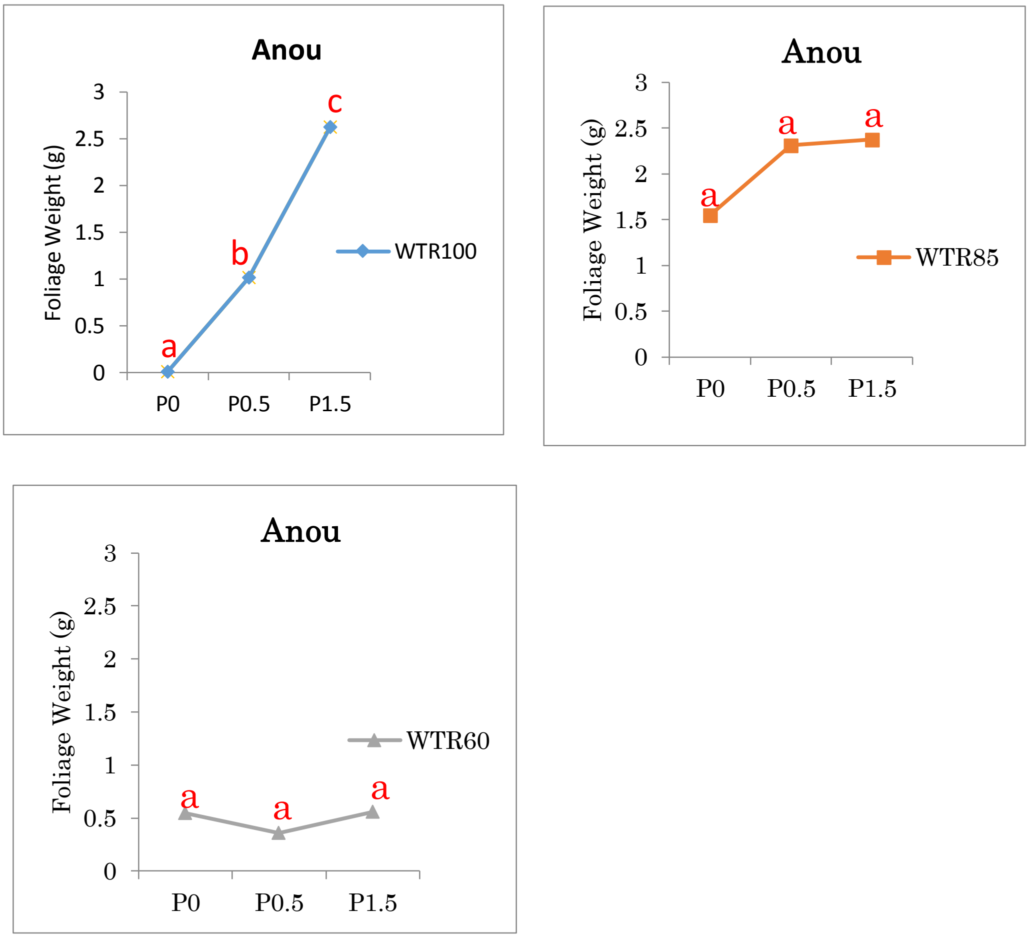

Fig. 5.5 The simple main effect of the addition of P-fertilizer at each addition level of bark compost on the foliage weight

The foliage weight of Komatsuna plants grown in Anou soils (on average) was shown in Table 5.1. According to Table 5.1, the foliage weight (g) was 0.01-2.62 g. At the soil of WTR100-P0 (i.e. the use of WTR alone), the foliage weight was 0.00-0.01 g, showing almost no growth of the plants.

According to the ANOVA results, there was an interaction between the effect of the additions of bark compost and P-fertilizer on the Komatsuna growth in Anou soils.

Therefore, the simple main effect test on the addition of bark compost at each level of P-fertilizer addition, and that on the addition of P-fertilizer at each level of bark compost addition were performed. The results were shown in Fig.5.4 and Fig.5.5.

Fig.5.4 shows the simple main effect of the bark compost addition at each level of P-fertilizer addition (addition levels were P0, P0.5 and P1.5). According to Fig.5.4, the foliage weight was higher in the soils of WTR85 group (WTRs containing 15% bark compost) than in WTR100 and WTR60 groups at P fertilizer addition levels of P0 and P0.5, however, no significant difference was observed between WTR100 and WTR60 groups. The foliage weight was higher in WTR100 and WTR85 than in WTR60 groups, when the P fertilizer addition was P1.5, no significant difference in the foliage weight was observed between WTR100 and WTR85 in the groups. It indicated that when 0 g or 0.5g P-fertilizer was added to 1L Anou soils, the growth of Komatsuna was increased significantly by the addition of the bark compost with 15% in amount (WTR85), but was not increased by the addition of bark compost with 40 % (WTR60) from the addition of 15% in amount (WTR85). While, when 1.5g P fertilizer was added to 1L Anou soils (P1.5), the growth in 15 % addition of bark compost (WTR85) was increased from WTR100, but on the contrary, that for 40 % addition of bark compost (WTR60) was decreased from the addition of 15% in amount (WTR85).

Fig.5.5 shows the simple main effect of the addition of P-fertilizer at each level of bark compost addition (the levels are: WTR100, WTR85 and WTR60). According to Fig.5.5, the foliage weight was higher in P1.5 than in P0.5, and higher in P0.5 than in P0, when the addition of bark compost was 0 of WTR100. For both WTR85 and WTR60, no significant difference in the foliage weight was observed by the addition of P fertilizer. It indicated that when no bark compost was added to Anou soils (in the case of WTR100), addition of P-fertilizer (P0.5 or P1.5) affected significantly on the growth of Komatsuna. Here, the foliage weight was higher in P1.5 than in P0.5. When the bark compost was added to Anou soils with 15% or 40% (WTR100 or WTR60), the addition of P fertilizer (P0.5 and P1.5) showed no significant effect on the growth of Komatsuna.

From the above, the effect of the additions of bark compost and P fertilizer to the WTRs on the foliage weight was recognized but to some extent. For the recognition of the effect of bark compost addition, not only the nutrient supply for plant growth but also improvement of water holding capacity by the addition of bark compost are conceivable.

5.3.2 Plant Growth Experiment Results using Ideura Soils

Fig.5.6 The situation of the plant growth using Ideura soils on day 5.

Fig.5.7 The situation of the plant growth using Ideura soils on day 12.

Fig.5.8 The situation of the plant growth using Ideura soils on day 34.

The situations of the plant growth using Ideura soils on day 5, 12 and 34 after sowing are shown in Fig. 5.6, Fig. 5.7 and Fig.5.8, respectively. According to Fig. 5.6, all the Komatsuna plants grew well except the case in WTR100-P0 on day 5 after sowing that is an early growth stage, and which are similar to the experiment with using Anou soils. In case of WTR100-P0, the growth appeared to be weak, and a weak water holding capacity and a deficiency of P are conceivable for the weak growth.

According to Fig.5.7, which shows the situation of day 12, all Komatsuna plants grew a little larger than that on day 5, however some leaves turned yellow at the margins and necrotic area. This phenomenon could be an apex symptom, showing Mn toxicity in plants. Komatsuna plants grown in WTR100 (including WTR100-P0.5, WTR100-P0.5, and WTR100-P1.5) showed the worst growth among all plants, and the plants grown in WTR85 group showed the second-worst growth, however, the plants grown in WTR60 group showed a better growth compared to those grown in WTR100 and WTR85 groups. For all the cases of WTR100, WTR85 and WTR60, no clear differences were observed between P0, P0.5 and P1.5 of P fertilizer addition.

According to Fig.5.8, which shows the situation of growth on day 34, most of Komatsuna plants were dead in WTR100-P0.5, WTR100-P0.5, and WTR100-P1.5. Plants in the other cases appeared to be weak, resulting to decay. In the cases of WTR85-P0, WTR85-P0.5, and WTR85-P1.5, Komatsuna plants were stunted with chlorotic leaves, resulting also to decay. A symptom of Mn toxicity was observed in plant leaves. Among the cases, the growth of Komatsuna plants was the best in WTR85-P1.5, followed by WTR85-P0.5, and the worst in WTR85-P0. In the cases of WTR60-P0.5, WTR60-P0.5, and WTR60-P1.5, Komatsuna plants were stunted with chlorotic leaves, but grew better than the other cases.

When WTRs alone were used in Ideura soils, the addition of P-fertilizer (WTR100-P0.5 and WTR100-P1.5) showed no better growth than those with no addition of P fertilizer (WTR100-P0). While, the addition of bark compost only (WTR85-P0 and WTR60-P0) showed a better growth than those with no addition of bark compost. Here, the growth became better with the increased amount of the addition.

Table 5.2 The foliage weight of Komatsuna plants using Ideura soils (on average)

	WTR100	WTR85	WTR60
P0	0.000	0.036	0.395
P0.5	0.000	0.044	0.539
P1.5	0.004	0.081	0.578

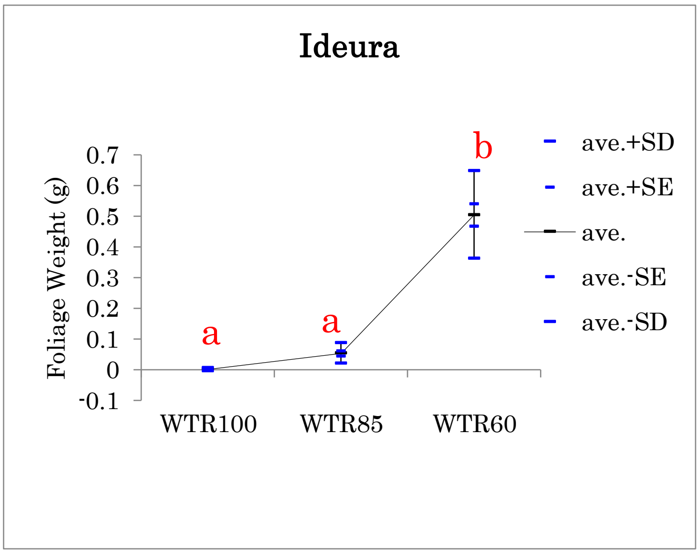

Fig. 5.9 The effect of the addition of bark compost on the foliage weight in Komatsuna plants grown in Ideura soils

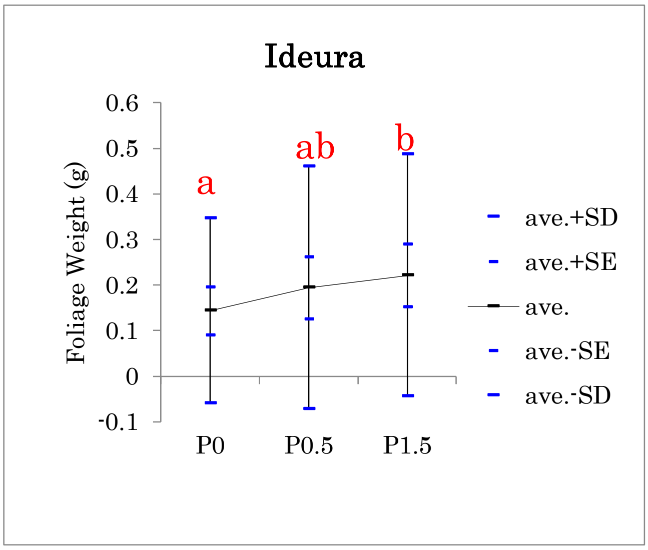

Fig. 5.10 The effect of the addition of P-fertilizer on the foliage weight in Komatsuna plants grown in Ideura soils

The foliage weight of Komatsuna plants grown in Ideura soils (on average) was shown in Table 5.2. According to Table 5.2, the foliage weight of the plants was 0.00-0.58 g. At the case of WTR100-P0, the weight was 0.00 g that shows no growth of the plant, as well as the case in Anou soils.

According to ANOVA results (Figs. 5.9 and 5.10), the effects of the additions of bark compost and P-fertilizer were significant without interaction between the respective effects for the growth of Komatsuna plants in Ideura soils.

Fig.5.9 shows a positive effect of the addition of bark compost on the plant growth, i.e., the foliage weight became higher in cases from WTR100 to WTR85 and to WTR60, though there was no significant difference between WTR100 and WTR85. Therefore, when using Ideura soils for plant growth, much amount of the addition (40 % addition) of bark compost is preferable.

According to Fig.5.10, the foliage weight was higher in P1.5 than in P0 regarding the effect of P fertilizer addition, however, no significant difference was observed between P1.5 and P0.5 and between P0.5 and P0. It indicated that when using Ideura soils, some amount of the addition of P-fertilizer (P0.5 or P1.5) is required for plant growing purposes.

5.4 Conclusions

The following conclusions were drawn from the plant growth experiments.

At first, WTR alone was used in the growth experiment for both Anou and Ideura soils, In the experiment, the planted plants of Komatsuna were stunted and died. Thus, the use of WTR alone was not considered to be suitable for plant growth.

In the next experiment, bark compost and P fertilizer were added to WTRs in soils. ANOVA was applied to identify the effect of the respective additions on the plant growth (foliage weight). In case of the use of Anou soils, there was an interaction between the effect of the additions of bark compost and P-fertilizer on the plant growth. According to the simple main effect test, a positive effect of bark compost addition on the growth was found. Namely, 15 % (volume) addition of bark compost increased the growth with compared to 40 % addition of the compost. Further, a positive effect of the addition of P-fertilizer was found. Namely, when the P-fertilizer addition increased from 0 g/L to 0.5 g/L and to 1.5 g/L, the foliage weight of plants also increased, respectively.

In case of the use of Ideura soils, there was no interaction between the effect of the additions of bark compost and P-fertilizer on the plant growth. For the effect of the addition of bark compost, the marked increase in plant growth was observed when the bark compost was added with 40%. While for the effect of P-fertilizer addition, the plant growth increased from 0 to 0.5 and to 1.5 g/L in P-fertilizer addition level.

From the above results, Anou soils are possible to be used as a plant growth medium with the additions of bark compost and P-fertilizer. For Ideura soils, the plants showed a certain growth when bark compost was added with 40% and P-fertilizer with 1.5g /L P-fertilizer, however, the plants showed a symptom of Mn toxicity and some other growth inhibition symptoms. Therefore, further improvement is necessary to use Ideura soils for plant growing purposes.

Bark compost is often used for soil improvement. Bark compost contributes to improve soil structures, to increase the soil nutrients and the nutrient holding capacity, and to improve the water drainage (Barney et al., 1991). In this study, bark compost was found to increase the soil microbial diversity, and reduce the available Mn content. Thus, the addition of bark compost has a positive effect on the growth of crops. In contrast, the addition of P fertilizer also contributed to crop growth, but limitedly in solving the P deficiency problem.

From the above results, it not accurate that as the addition of bark compost becomes higher, the higher the plant growth becomes. Addition of 15% bark compost to the WTRs was found to be enough to improve the productivity of soils.

Chapter 6 General Discussion

6.1 The physical, Chemical and Biological Properties of the Water Treatment Residuals in Terms of Plant Growth and Their Differences with Location of Water Purification Plant

According to the physicochemical measurements of WTRs, pH was near neutral with little difference by the water purification plant (i.e., location). EC (mS/cm) ranged from 0.24-0.36, where only two values exceeded 0.3 and there were little differences by the location. Both pH and EC were in an acceptable range for plant growth.

While, ECEC varied by the location and the difference between the maximum and the minimum was 4.3 times. However, most ECECs were low, showing a low nutrient retention capacity of the WTRs.

The P absorption coefficient was high with minor differences by the location. The high coefficient values were caused by aluminum compounds input in the water purification process and showed possible inhibition of plant growth.

Water-soluble Mn concentration differed with the location. The variation between the maximum and the minimum in the concentration was about 30 times. The water-soluble Mn exceeded the threshold of 5 mg/kg for causing the Mn excess in most locations, and the Mn excess could occur in plants. While, the exchangeable-Mn concentration was high, and 3-13 times higher than the concentration of the water-soluble Mn. The exchangeable Mn concentration exceeded the threshold of 10 mg/kg for causing Mn excess and the Mn excess could occur in plants. The Mn concentration was influenced by hydrogeologic settings (Lundy et al, 2012).

The distribution of microorganisms in WTRs collected from 6 locations was classified into several groups. The derived classification did not relate to the source of water or dewatering method of the WTRs.

6.2 The Mutual Relationships of the Physical, Chemical and Biological Properties of the Water Treatment Residuals and Their Reasons

According to the correlation analysis on the physicochemical properties, there were almost no properties to correlate with the other properties. Therefore, the properties targeted are the independent properties.

The water-soluble Mn concentration was positively correlated to the exchangeable Mn concentration as a whole, however, these two concentrations were not correlated with each other, when the concentrations were in a small range where the most concentrations were located.

The Mn concentrations appeared to be influenced mostly by geology and partly by the dewatering method of the WTRs. The water-soluble plus exchangeable Mn (i.e. plant available) concentration was higher in WTRs derived from solar drying than that from mechanical dewatering.

There is no clear conclusion that whether/how the distribution of microorganisms relates to the physicochemical properties in the WTRs.

6.3 Effect of the P Fertilizer and Bark Compost Additions to WTRs on the Properties and Plant Growth

For the effect of P fertilizer addition, pH of the WTRs did not change with the addition, but EC increased with the increased addition of P fertilizer.

For the effect of bark compost addition, EC of the WTRs did not show clear increasing or decreasing trends. The pH of the WTRs did not change with the bark compost addition. The P absorption coefficient increased with the increased P fertilizer and bark compost additions, respectively.

The water-soluble Mn concentration decreased with the increased addition of bark compost. P fertilizer addition affected the water-soluble Mn concentration but intricately.

Plant growth (foliage weight) was larger as the P fertilizer addition became larger, under the condition of no bark compost addition. When no P fertilizer was added, the effect of the addition of bark compost was rather complex.

Plant growth was better when bark compost was added moderately, while the growth was better as the P fertilizer addition was larger. The interaction between the effects of P fertilizer and bark compost additions was found in some cases but not found in other cases. The Mn excess occurred in plants, when the Mn concentrations were large in the WTRs.

By the addition of bark compost to the WTRs, the number and kind of microorganisms were increased to some extent. The magnitude of the addition did not affect the number and kind of microorganisms. The addition of bark compost to the WTR varied the distribution pattern of microorganisms in both Anou and Ideura soils.

There were some similarities in characteristics between the addition of bark compost, distribution pattern of microorganisms, and plant growth, i.e. (1) the number of microorganisms in the WTRs increased when the bark compost was added to the WTRs, but the number was not proportional to the amount of the added bark compost, and (2) plants grew better when the bark compost was added to the WTRs, but the growth was not necessarily proportional to the amount of added bark compost. Therefore, bark compost addition might contribute to enhance microbial activity and to promote plant growth.

Among the microorganisms that multiplied by the addition of bark compost, some microorganisms could be present that might act to decrease the available Mn in the WTRs.

6.4 Assessment of WTRs Including Their Mixtures as a Plant Growth Medium

WTRs were found to have large values of P absorption coefficient and available Mn concentration by their measurements that might affect negatively the plant growth. In order to use WTRs as a plant growth medium, these defects must be corrected. Available Mn concentration was decreased with the increased bark compost addition, but P absorption coefficient increased with the increased additions of P fertilizer and bark compost. Though the addition of P fertilizer is highly necessary, to diminish the P absorption coefficient is difficult and a challenging task to use it as a plant growth medium.

Some properties varied largely with the location such as available Mn concentration, ECEC, and the distribution pattern of microorganisms was also varied, though some other properties did not vary with the location (pH, EC). The properties that have characteristics to change with the location (including dewatering method) must be assessed respectively according to the location of WPPs.

The biological property (community structure of microorganism) was affected by the addition of bark compost. However, the property may also change with the location though to some extent.

By the plant growth experiment, the effect of the properties on the plant growth and the method to improve the properties were clarified, thus WTRs are concluded to have a good potential to be used as a plant growth medium with some improvements.

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