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Preliminary Study of Phytoremediation and Biomass Production by *Salix* Species on Abandoned Farmland Polluted with Heavy Metals

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Abstract: The objective of this study was to clarify the growth properties of *Salix* species on abandoned farmland polluted with heavy metals in order to use such an area for cultivation as well as land remediation. Four clones of willow, *Salix pseudolinearis* (FXM), *S. pet-susu* (HB471, KKD), and *S. sachalinensis* (SEN) were cultivated within two sites ("HM" and "LHM") with different heavy metal content. AT site HM, the total Cd was 2.57 ± 0.18 mg/kg dry weight (DW) and total Zn was 227 ± 22 mg/kg DW, and at site LHM, the total Cd was 0.114 ± 0.036 mg/kg DW and total Zn was 3.48 ± 0.96 mg/kg DW. The fresh weight of *Salix* after one season of growth at site HM was approximately one-thousandth that of plants at LHM. The poor growth in HM was due to dry soil lacking plastic mulch. KKD was the fastest growing of the four clones at both sites. Although soil Cd and Zn content at site HM was approximately 22-92 times higher than their corresponding content at site LHM, the range of heavy metal content of plants at both sites was almost the same. The accumulation capability differed. Of the four clones, FXM showed the highest Cd and Zn content. Compared with hyperaccumulating plants (*Arabidopsis halleri* ssp. *gemmifera*), FXM salvaged 38.9% of the Cd accumulated by the hyperaccumulator, even when the site was contaminated with only low levels of heavy metal. We concluded that the FXM clone performed the best as a heavy metal collector, and the KKD clone was the best for biomass production.

Key Words: Biomass production, Phytoremediation, *Salix*, Willow.

1. Introduction

As the combustion of fossil fuels is becoming widely understood as resulting in both global warming and exhaustion of these fuels, there is increasing focus on biomass as a source of renewable energy. C4 plants such as maize or sugarcane are well known as energy crops; however, it is a concern that their use conflicts with food security because of their high value as staple foods. Therefore, lignocellulosic feedstocks offer the potential to provide “second generation” biofuels (Simpson-Holley *et al*., 2007, Naik *et al*., 2010) because they are free from conflicting demands for food. Willow, seaweed, galingale, Japanese pampas grass, sunflower, and rape seed are listed as second generation biomass crops in Japan (EX Research Institute, 2011a).

In Sweden and Canada, willow species have already been used to produce commercial fuel (Juga *et al*., 1999). Use of this biomass is currently limited to direct combustion and formation of biogas, though fermentation for bioethanol production is being studied (Sassner *et al*., 2006). Willow and *Salix* species are widespread in the Northern Hemisphere and can grow in diverse environments. Various trials have been conducted for use of willows as biomass resources in Japan. In Hokkaido, some studies on a business model for cultivation and ethanol production from willow have been carried out (Hokkaido Development, 2011, Orihashi *et al*., 2012). A trial in Saitama was conducted to convert a boiler from heavy oil/coal oil to wooden chips in greenhouses for horticultural use and to grow willow on abandoned farmlands as sources of wooden chips (EX Research Institute, 2011b). In the cold climate of the southern Tohoku region, Mitsui *et al*. (2010) selected four superior clones for biomass production.

Along with an increase in the average age of farmers, the area of abandoned paddy fields in Akita Prefecture increased from 4,002 ha in 2000 to 6,789 ha in 2005. Most farmers renounced cropping rice not only because of their age, but also because of falling rice prices. Abandoned farmland can cause problems such as the spread of pests and weeds to neighboring farms, wild animal invasion, and difficulties managing irrigation and drainage facilities; therefore, it is critically urgent to develop other uses for the abandoned land to maintain community environments.

Heavy metal contamination of cropland and pollution of agricultural products are other environmental problems found downstream of mining areas worldwide. In the case of Japan, 7,575 ha of agricultural land has been assigned as polluted areas due to contamination with heavy metals, especially Cd,

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of which 794 ha remain untreated (Environment Management Bureau, Department of Environment, Japan, 2012).

A main countermeasure to heavy metal contamination of soil is covering the land with unpolluted soil; however, this is increasingly difficult because of a lack of fresh soil. Another technology for land rehabilitation from pollution is phytoremediation. Willow species have been selected for phytoremediation of heavy metal pollution in some studies (Berndes *et al*., 2004; Maxted *et al*., 2007). On the other hand, very few trials have been conducted with willow plantations for phytoremediation in Japan.

From the above background, use of heavy metal polluted land for biomass resource production is meaningful for both agricultural land rehabilitation and use of the land for production of inedible species. The objective of this study was to clarify the growth properties of *Salix* species on heavy metal polluted abandoned farmland in order to use the area for cultivation as well as land remediation.

2. Materials and Methods

2.1. Site outline

Two sites were selected for study; one site is a heavy metal contaminated area previously used for rice cropping in Daisen city, Akita Prefecture, Japan (site HM) and the other site is a low-pollution fallow land, used as a control, at the Center of Field Education & Research, Akita Prefectural University, Ogata village, Akita Prefecture, Japan (site LHM). Typical topsoil properties of the two sites are shown in **Table 1**. The heavy metal content at site HM was total Cd 2.57 mg/kg DW and total Zn 227 mg/kg DW, and at site LHM was total Cd 0.114 mg/kg DW and total Zn 3.48 mg/kg DW.

Table 1. Typical topsoil properties at the two experimental sites.

Site		HM	LHM
Soil type		Gray Lowland Soil	Gray Lowland Soil
pH (H_2O)		6.26 (0.059)	6.53 (0.028)
Total C*	mg/g	0.329 (0.032)	0.111 (0.003)
Total N*	mg/g	0.0273 (0.0028)	0.0111 (0.0040)
Total Cd**	mg/kg	2.57 (0.184)	0.114 (0.036)
Total Cu**	mg/kg	1070 (232)	N.T.***
Total Mn**	mg/kg	1200 (304)	N.T.***
Total Zn**	mg/kg	227 (22.2)	3.48 (0.958)

The values in parentheses mean standard deviation.
*: Total carbon and nitrogen were determined by combustion method.
**: Total Cd, Cu, Mn, Zn were determined by an ICP atomic emission spectrometer (ICAP 6000, Thermo Fischer Scientific, K. K.).
***: Not tested.

2.2. *Salix* cultivation

Four clones of *Salix* were cultivated in the study: *Salix pseudolinearis* (FXM), *S. pet-susu* (HB471), *S. pet-susu* (KKD), and *S. sachalinensis* (SEN), selected by Mitsui *et al*. (2010). Branches were cut into 30 cm long segments 2-7 days before transplanting. Cuttings of each clone were stuck into soil up to 20 cm deep in the ground. At site HM, cultivation started May 8, 2012 with no fertilizer. Three cuttings of each clone were planted randomly at one-meter intervals. Insecticide was applied around each stem on July 31. During the growing season, no irrigation was used. The whole shoot was collected on October 15. At site LHM, cultivation started May 8, 2010, without fertilizer but with plastic mulch. For each clone, 66 cuttings were placed at 0.5 m intervals with 1.4 m between clones (i.e. a planting density of 12,000 trees per hectare). Insecticide was applied around the stem in July of 2010 and 2011. During the growing season, no irrigation was used. The whole shoot was collected to survey growth before new sprouts emerged in April 10, 2012.

2.3. Sample preparation and analysis of heavy metal content

At site HM, collected shoots were divided into leaves and stems, and the fresh weight was measured before oven-drying. The samples were dried in a 70°C oven for 3 days before determining dry weight, and then cut with scissors into 2-3 cm sections. The samples were powdered with a ball mill. At site LHM, collected shoots were divided into timber and bark and were air-dried in a greenhouse for a week, then weighed to determine fresh weight. Dry samples were weighed again to determine dry weight and were then milled with a cutting mill and sieved through a 40-100 µm mesh. Litter was collected between lines of clone of KKD and FXM on the same day, April 10, 2012. Fresh weight and dry weight were determined in the same manner as timber and bark. Dry leaves were powdered with a coffee mill.

Samples were digested with nitric acid and perchloric acid on a hot sandbath. Cd, Mn, and Zn contents were determined with an ICP mass spectrometer (ICAP Q, Thermo Fisher Scientific, K. K.). Uptake of Cd, Mn, and Zn was calculated as dry weight multiplies by each heavy metal contents.

3. Results and Discussion

3.1. Growth of trees

The fresh weight of the four *Salix* clones at the two sites is shown in **Table 2**. The fresh weight in site HM ranged from 0.44 to 3.6 g per a plant was approximately a thousandth lower than those in LHM ranged from 0.7 to 1.5 kg per a plant. *Salix* species are known as a fast growing species and thus

Table 2. Fresh weight of four clones of *Salix* at two sites.

Line	Site HM		Site LHM	
	stem	leaf	1st year stem	2nd year stem
	g/plant	g/plant	kg/plant	kg/plant
FXM*	0.44	0.19	0.8±0.3	9.3±5.5
HB471	2.6±2.4	1.7±2.1	1.0±0.3	7.9±1.1
KKD	3.6±2.8	4.5±3.4	1.5±0.6	14.6±3.7
SEN	2.5±0.5	2.5±0.2	0.7±0.3	8.1±1.8

The values show means±standard deviation.

* Only one FXM cutting at site HM survived during the study.

trees in LHM showed same ranged growing as other trials (Mitsui *et al*., 2010). Heavy metal content may affect a negative effects on their growth, however in another trial with plastic mulch adjacent to the site HM, all the four clones showed the almost same growth (2.02, 1.83, 2.31 and 2.14 m of height for FXM, HB471, KKD and SEN, respectively for eleven month growing) as in LHM (2.1, 1.8, 2.7 and 2.2 m of height for FXM, HB471, KKD and SEN, respectively for twelve month growing). Therefore the poor growth in HM caused from soil water condition being dry because of lack of plastic mulch in the moment.

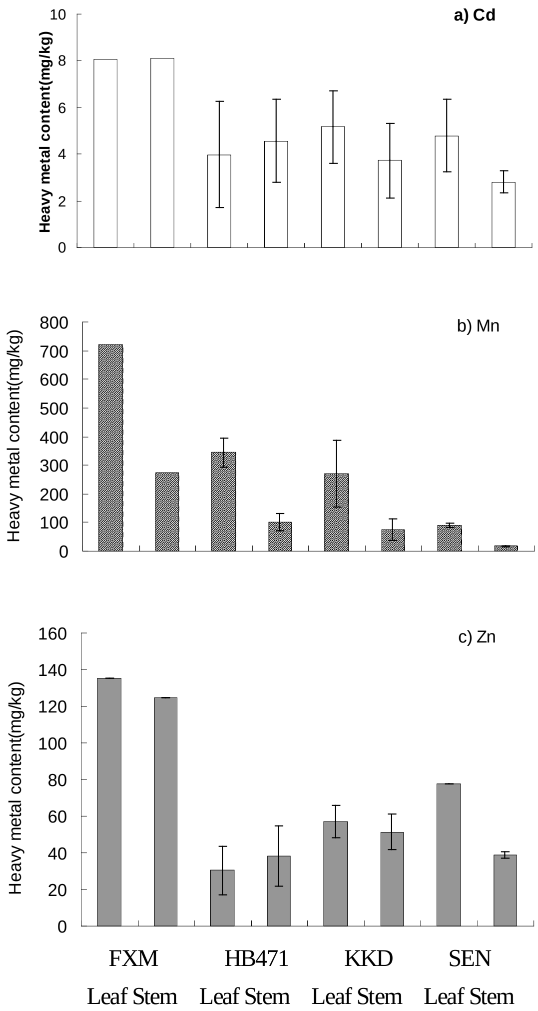

Fig. 1. a) Cadmium, b) Manganese, and c) zinc content in leaf and stem of four Salix in site HM. Error bar means standard deviation.

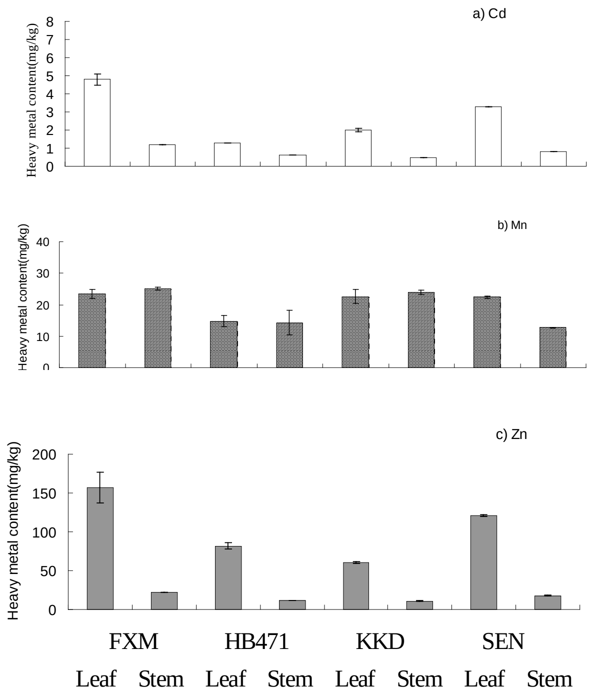

Fig. 2. a) Cadmium, b) manganese, and c) zinc content in leaf and stem of four Salix in site LHM. Error bar means standard deviation.

KKD grew the fastest of the four clones at both sites. As Mitsui *et al*. (2010) concluded, KKD produced the most biomass; the tendency of growth for the four clones was the same at the two sites.

3.2. Heavy metal content in *Salix*

Figures 1 and 2 show the Cd, Mn, and Zn content in the four *Salix* clones at site HM and LHM, respectively. The content of Cd and Zn was very similar in every clone. At site HM, the content ranged from 2.8 mg/kg in stems of SEN to 8.1 mg/kg in stems of FXM for Cd and from 30.3 mg/kg in leaves of HB471 to 135 mg/kg in leaves of FXM for Zn. At site LHM, the content ranged from 0.5 mg/kg in timber of KKD to 4.8 mg/kg in bark of FXM for Cd and from 10.7 mg/kg in timber of KKD to 157 mg/kg in bark of FXM for Zn. Cd and Zn belong to the same group of elements, therefore, their behavior is quite similar. Plants take up Cd by the same pathway as Zn; consequently, plants tend to accumulate a certain level of Cd if Cd is present in the soil. The Mn content showed a different trend from those of Cd and Zn. The soil Cd and Zn content at site HM were respectively approximately 10 and 30 times higher than at site LHM, whereas the range of content of these heavy metals in plants at

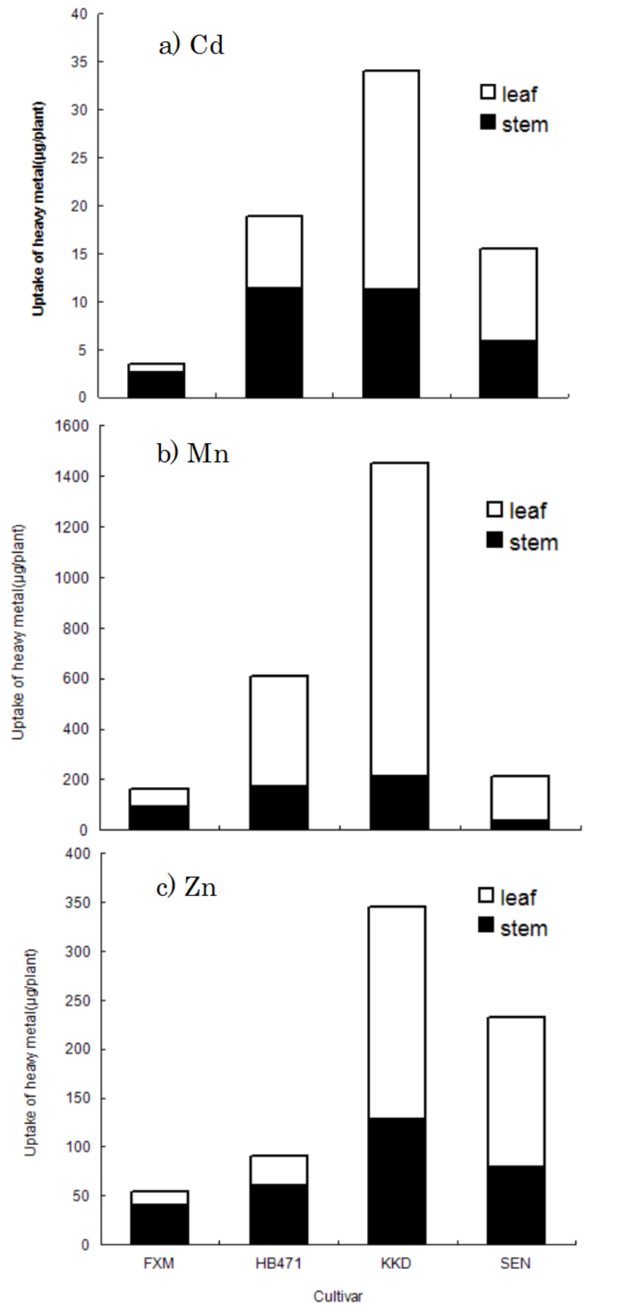

Fig. 3. Uptake of a) cadmium, b) manganese, and c) zinc by four Salix in site HM.

both sites was almost the same. However, the accumulation capability differed among cultivars and clones. Maxted *et al.* (2007) reported the results of field trials of phytoremediation by *S. caprea* × *cineria* × *viminalis* on soil that contained 41.6±0.58 mg Cd/kg and 2418 ± 81.8 mg Zn/kg; the plants contained 8.20 mg/kg Cd and 12 mg/kg Zn. Compared to the study by Maxted *et al.* (2007), the four clones we tested showed from one-tenth to the same range of heavy metal content, although the soil content was 20 to 40 times lower. In the four clones, FXM showed the highest Cd and Zn content. Harada *et al.* (2010) reported that *Salix* accumulates Cd and Zn at the tips of the serrations in leaves. The four *Salix* lines

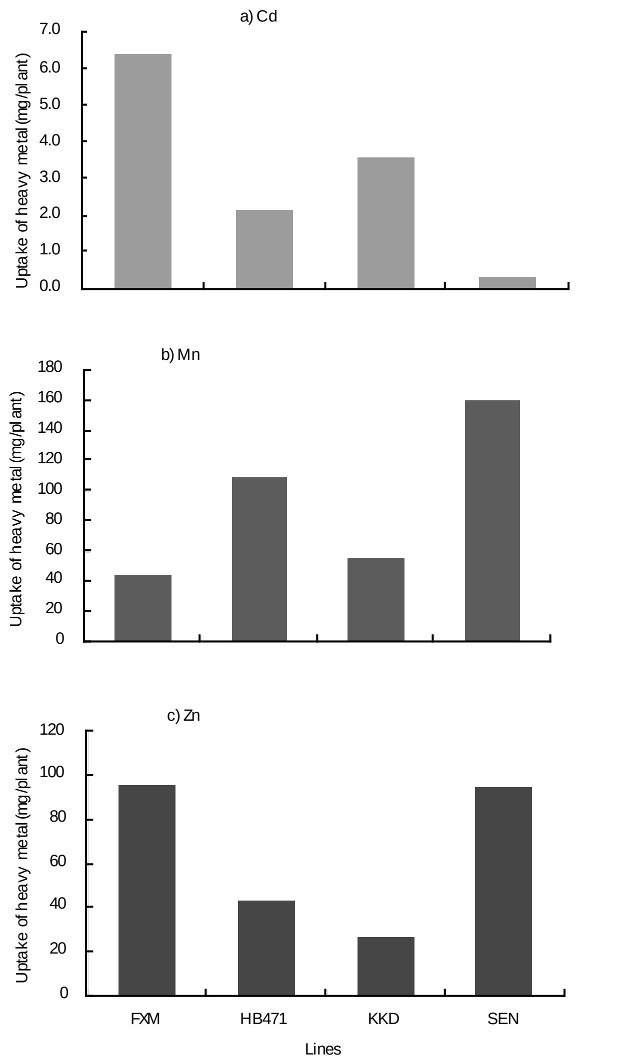

Fig. 4. Uptake of a) cadmium, b) manganese, and c) zinc by four Salix in site LHM.

have differently shaped leaves; therefore, the different range of accumulation may be a result of the shapes of their leaves.

3.3. Cd uptake by *Salix*

Figures 3 and 4 show Cd, Mn, and Zn uptake in the four *Salix* lines at sites HM and LHM, respectively. At HM, uptake of Cd and Zn was very similar in every clone. At site HM, uptake of Cd was in the order KKD > HB471 > SEN >> FXM and uptake of Zn was KKD > SEN > HB471 > FXM, whereas at site LHM, uptake of Cd was FXM > KKD > HB471 > SEN and uptake of Zn was FXM = SEN > HB471 > KKD. The heavy metal uptake at site HM was exponentially smaller than at site LHM because of the difference in plant growth between the sites. The difference in the order between the four clones arose from the growth performance at HM; in particular, FXM grew only to one-fifth the weight of the others.

The salvage of heavy metals from the soil can be calculated by multiplying the uptake of heavy metals by planting density.

The salvage of Cd from site LHM amounted to 77, 26, 43, and 4.0 g Cd/ha by clones FXM, HB471, KKD, and SEN, respectively. Ishikawa *et al*. (2009) found that 199 g/ha of Cd was collected by hyperaccumulating plants (*Arabidopsis halleri* ssp. *gemmifera*) from site HM. FXM salvaged 38.9% of the Cd at site LHM, as high as the hyperaccumulator, even when the site was only contaminated with low levels of heavy metals.

One use of *Salix* biomass is as material for formation of particle board. Yamada *et al*. (2013) produced willow particle board from wood chips obtained in the first year of cropping at site LHM, and reported that willow particle board showed a lower modulus of elasticity and modulus of rupture, but the same internal bonding strength and thickness swelling on water absorption compared to cedar boards. Direct combustion is another option for its use, but these boards contain more heavy metal than common boards; i.e. Ono (2005) showed that the median heavy metal content in wood chips from construction waste is 0.05 mg Cd/kg, 45 mg Mn/kg, and 29 mg Zn/kg. Use as particle board is preferable to avoid the chemical hazards of combustion.

4. Conclusion

All four clones selected in this study showed growth comparable to that found in previous studies and a yield as high as >10 t/ha・y, even in a cold snowy area in the northern Tohoku region when appropriate treatments such as mulching were given. From the viewpoint of phytoremediation, the FXM clone was concluded to be the best accumulator of heavy metals because its heavy metal content was almost five times that of the other three clones. To produce the maximum biomass, the KKD clone was the best because it had the highest plant weight. The study did not prove phytoremediation performance directly in heavy metal contaminated land; therefore, further study is needed to clarify whether the performance can be maintained on such land.

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