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https://doi.org/10.5109/22048

出版情報：九州大学大学院農学研究院紀要. 57 (1), pp.55-60, 2012-02. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **57** (1), 55–60 (2012)

Effect of Aqueous Extract of Biochar on Germination and Seedling Growth of Lettuce (*Lactuca sativa* L.)

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(*Received October 31, 2011 and accepted November 9, 2011*)

This study was carried out to confirm the effect of aqueous biochar extract on seed germination and seedling growth of lettuce (*Lactuca sativa* L.). Biochar produced from orange peel (OP), residual wood (RW), and water treatment sludge (WS) at pyrolytic temperatures of 300 and 700˚C were extracted with distilled water at final concentrations of 1 and 3%. The germination rate in the OP significantly delayed than in its biochar treatments and finally reached to 52 and 0% at 1 and 3% concentrations, respectively. Compared to the control containing only distilled water, the germination rate in all biochar treatment except for the WS biochar produced at 700˚C were similar or promoted. The OP inhibited the radicle as well as the hypocotyls and the inhibitory effect increased with increasing the concentration of aqueous extract. Similar results have been shown in the RW and RW biochar treatments, but the difference between radical growths was not statistically significant. In cases of the WS and WS biochar treatments, there was no significant difference on pyrolytic temperatures of the biochar and concentration of the aqueous extract between hypocotyl and radicle. These results suggest that the biochar had no negative effect on the germination rate and seedling growth. Therefore, biochar could be used not only as a valuable soil amendment to increase soil health and crop growth, but also as a carbon sink to mitigate global warming.

Key words: Biochar, germination, lettuce, seedling growth

INTRODUCTION

Today, an increasing number of wastes or by–products have been generated in agricultural and industrial fields with the qualitative improvement of human life. All over the world, practical management and safe disposal of these biomass have become one of the main environmental concerns. Pyrolysis has been used widely for centuries to solve these problems as an alternative way of managing a range of organic wastes (Shinogi and Kanri, 2003) because this process can reduce weight, volume, and foul odor of biomass and also make the product easier to handle (Shinogi *et al.*, 2003). In particular, biochar produced through the pyrolysis processing has drawn a lot of international attention as a useful material.

The International Biochar Initiative (IBI), according to its website, defined that the biochar is a solid material obtained from the carbonization of biomass. Recently, many researchers are studying about more effective use of the biochar in a variety of fields. Especially, a number of practical attempts and research efforts have been done to confirm potential benefits on the application of biochar to soil as a soil amendment. According to some previous studies, the biochar provides a unique opportunity to increase water retention in sandy soils (Gaskin *et al.*, 2007; Rasool *et al.*, 2008; Downie *et al.*, 2009), develop microbial activity (Thies and Rillig, 2009), ameliorate soil structure or aeration (Kolb, 2007; Chan *et al.*, 2008), enhance chemical fertility and nutrient–use efficiency (Lehmann, 2007; Lehmann and Joseph, 2009), and improve crop growth and yield (Lehmann *et al.*, 2003). In addition, the relatively stable nature of biochar shows a significant potential as a tool for carbon sequestration (Lehmann *et al.*, 2006). However, some biochar produced from feedstocks such as sewage sludge and tannery wastes have been reported to contain high contents of heavy metals and toxic substances (Muralidhara, 1982; Jones and Sewart, 1997; Bridle and Pritchard, 2004), which might reduce seed germination and seedling growth with consequent effects on crop establishment and yield (Free *et al.*, 2010). Therefore, in view of increasing public interest in the application of biochar to the soil, there is a need for more detailed studies with various biochar because biochar properties are governed by the type of feedstock and production conditions such as temperature and heating time (Novak *et al.*, 2009, Nguyen *et al.*, 2010).

In this study, for providing the basic information for effective crop production, we examined the effect of aqueous biochar extract on the seed germination and seedling growth of lettuce (*Lactuca sativa* L.) in laboratory bioassay condition. We also hypothesized that the effect of biochar may differ among the different pyrolytic temperatures and/or raw feedstock due to the variation of different biochar properties.

MATERIALS AND METHODS

Raw feedstock materials

Three different types of biomass, i.e. orange peel

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(OP), residual wood (RW), and water treatment sludge (WS), were prepared as raw feedstock for biochar manufacture. The orange was purchased in a local fruit market located in Fukuoka Prefecture, Japan, and the peels were stripped with a paring knife. The RW was collected from a dump site in Kyushu University, Japan. For easy storage and management, the OP and RW were washed with distilled water three times to remove dusts and then were cut into small pieces of an average of 1 cm^2. We also collected the WS, in which PAC (polyaluminium chloride) is used as coagulant, from Tatara drinking–water treatment plants located in Fukuoka Prefecture, Japan. These collected OP, RW, and WS were air–dried at room temperature for 3 days and subsequently oven–dried overnight at 75±5°C. For biochar production, the oven–dried feedstock were placed into a stainless steel dish in a muffle furnace (FM48, Yamaha, Japan), covered with fitting lid to restrict the access of air during carbonization and then pyrolyzed for 2 hours at different temperatures of 300 and 700°C. The resulting biochar were crushed, sieved into less than 2–mm size and stored for the following experiments in air–tight containers. For comparison, the oven–dried feedstock passed through 2–mm size sieve was also used in the study and is denoted by 0°C. In addition, the different biochar used in this study are denoted by OPB_{xxx}, RWB_{xxx}, and WSB_{xxx} represent the OP biochar, RW biochar, and WS biochar, respectively, and xxx to the pyrolytic temperature in °C.

Characterization of samples

To provide basic properties, the following analyses of selected chemical characteristics were performed on the raw feedstock and the biochar samples: pH and electrical conductivity (EC) by 1:10 (samples:distilled water) method using pH meter (F–21, HORIBA, Japan) and EC meter (B–173, HORIBA, Japan), respectively; heavy metal contents and exchangeable cations (Ex. cations) by inductively coupled plasma–optical emission spectrophotometer (ICP–OES, Varian 730–ES, USA and GBC Integra XL, Australia, respectively); carbon (C), hydrogen (H), and nitrogen (N) composition by an elemental analyzer (Yanaco CHN Corder MT–5, Japan), where the oxygen (O) content was determined by a mass balance; concentrations of total phosphorus (T–P) by a UV–visible spectrophotometer (UV 2450, Shimadzu Corp., Kyoto, Japan).

Bioassay for seed germination

Bioassay study was conducted to evaluate effect of aqueous biochar extracts on growth of crop such as germination and seedling growth. Lettuce (*Lactuca sativa* L.) as a testing plant was chosen for the study because it is one of the sensitive leafy vegetables and commonly consumed worldwide. Three–grams of dried powder from different biochar samples were placed in a 300 mL Erlenmeyer flask and then 100 mL of distilled water was added. After shaking for 2 hours at 120 rev min^{-1} in the dark, the mixtures were filtered through Whatman No. 2 filter paper and then through a membrane filter with 0.45 μM pore–size. The effect of the aqueous extracts in this study was examined at two concentrations of 1 and 3% (v/v). The pH and EC were measured with a pH meter (Orion 3–star, Thermo Scientific, USA) and an EC meter (Orion 3–star, Thermo Scientific, USA), respectively. Seeds of lettuce were surface–sterilized in sodium hypochlorite solution (2% active chlorine) with 0.01% Tween–20 for 15 min. For comparison, the oven–dried feedstock passed through 2–mm size sieve was also prepared by the same method. The different aqueous extracts were added into a Petri dish with Whatman No. 2 filter paper and twenty seeds were placed on the filter paper. They were incubated at 26°C in the dark conditions. We used distilled water as a control treatment. Germination of the lettuce seeds was measured every day and lengths of both hypocotyl and radical of lettuce seedlings were measured on all seedlings after 4 days of culture. The experiment was conducted with 3 dishes for each treatment.

Statistical analysis

Differences among treatment groups were evaluated by one–way ANOVA using the SAS program (SAS institute, ver. 9.2), with Tukey's LSD. A $p<0.05$ was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Characteristics of the prepared each sample

We investigated the main chemical properties of the different raw feedstock and biochar samples and the results were summarized in Table 1. The pH of the OP, RW, and WS were 4.1, 6.2, and 5.7, respectively. In this study, when the pyrolytic temperature increased from 300 to 700°C, the pH increased from 8.0 to 12.3 for the OPB, from 7.8 to 10.3 for the RWB, and from 5.8 to 6.8 for the WSB, respectively. It means that the biochar can be employed as a soil amendment to increase soil pH in acidic soils. Carbon content in the OP and RW treatments increased with increasing pyrolytic temperature, which was higher than that in the WS treatment. As expected in the pyrolytic process for all samples, the H and O contents decreased with increasing pyrolytic temperature. Ash content was higher in the RW (76.0%) than in the OP (2.9%) and WS (3.8%). Compared to the WS, the different biochar derived from the OP and RW had high organic matter (OM) and the high OM would contribute to an increase of water contents in a sandy soil (Troeh and Thompson, 2005) and productivity of crop. In addition, high concentrations of exchangeable cations such as K, Ca, and Mg may also become a valuable resource for crop growth when the biochar derived from the OP, RW, and WS was applied to soil.

In order to ensure safe and sustainable use of the biochar in agriculture, analysis of heavy metal content caused by the raw feedstock is very important. We also determined the heavy metal concentrations in different raw feedstock used in this study and various standards of the concentration are given in the Table 2. The heavy metals such as Cr, Cu, and Ni in the OP and RW and such as Ar, Cd, Cr, Cu, Ni, Pb, and Zn in the WS were

Table 1. Selected chemical properties of the different feedstock and biochar samples

Biochar sources	Pyrolytic temperatures (˚C)	pH (1:10)	EC (dS m^{-1})	OM (g kg^{-1})	Element content (%)				Ash (%)	Ex. Cations (cmol$_c$ kg^{-1})			Total–P (%)
					C	H	N	O		K	Ca	Mg	
OP	0[1]	4.1	1.7	7.1	41.9	6.4	1.0	47.9	2.9	12.7	8.4	5.5	0.1
	300	8.0	1.2	8.8	60.4	5.2	1.6	28.9	3.9	14.1	14.5	3.5	0.2
	700	12.3	7.2	5.1	74.8	1.6	1.7	13.4	8.5	8.9	14.2	3.6	0.2
RW	0	6.2	2.0	7.6	45.8	6.1	0.3	44.1	3.8	2.9	11.9	4.6	0.1
	300	7.8	0.5	8.5	62.1	4.4	0.9	24.8	7.8	19.5	16.4	4.9	0.2
	700	10.3	1.7	5.3	78.4	2.4	0.7	5.9	12.6	10.7	15.3	3.9	0.2
WS	0	5.7	1.3	1.2	6.6	1.8	0.3	15.3	76.0	0.1	7.1	1.9	0.3
	300	5.8	0.8	1.1	8.8	1.4	0.4	7.3	82.1	17.5	7.1	3.9	0.3
	700	6.8	0.3	1.0	8.1	0.5	0.2	0.6	90.6	12.5	4.0	3.7	0.3

[1] Raw material

Abbreviations: EC, electrical conductivity; OM, organic matter; Total–P, total phosphorus; Ex. Cations, exchangeable cations

Table 2. Heavy metal concentrations of each feedstock used for experiments and maximum allowable limits

(Unit : mg kg^{-1})

Properties	OP	RW	WS	Maximum allowable limits	
				Korea[1]	U. S.[2), 3)]
Chromium (Cr)	3.33	4.81	10.92	300	3000c
Copper (Cu)	5.60	11.50	26.54	300	4300b
Nickel (Ni)	2.39	1.69	7.53	50	420b
Lead (Pb)	n.d.	n.d.	8.54	150	840b
Zinc (Zn)	n.d.	n.d.	41.81	900	2500b
Arsenic (As)	n.d.	n.d.	9.46	50	75b
Cadmium (Cd)	n.d.	n.d.	0.27	5	85b

[1] source : Fertilizer management act., Republic of Korea (2009).

[2] source : Adapted from U.S. EPA (1995)

CCL (ceiling concentration limits) = maximum concentration permitted for land application

[3] source : Colorado Department of Health. Biosolids Regulation 4.9.0. (1996)

n.d. : not detected

detected. According to Dolgen *et al*. (2004), lettuce is very sensitive to Cu and Zn and also accumulates higher concentrations of heavy metals. However, the concentrations of heavy metals detected from OP, RW, and WS were significantly lesser than the maximum allowable limits of the Korea and US standards. It means that any biochar produced from the three types of feedstock used in this study can be safely used as agricultural purposes.

Bioassay for seed germination

Seed germination, one of the most important phases in the life cycle of a plant, is highly responsive to existing environment (Kuriakose and Prasad, 2008). As a first step to understand how biochar affect the ability of seed germination, we have examined the values of pH and EC in aqueous extract of different biochar and the results are shown in Table 3. The pH in aqueous extract of OP and RW treatments increased with increasing the pyrolytic temperature, while that in WS decreased. That is, the pH was higher in OPB_{300} and OPB_{700} than in OP, particularly in OPB_{700} significantly increased to 8.85 at 1% and 9.32 at 3%, respectively. The RW and RWB treatments show a similar trend to the OP and OPB treatments. Compared to the RW (pH 7.42 at 1% and pH 7.06 at 3%), the pH in RWB_{700} increased to 7.78 at 1% and 7.72 at 3%, respectively. Meanwhile, the pH in aqueous extract of WSB_{300} and WSB_{700} were significantly decreased to 7.61 and 7.65 at 1% and 7.32 and 7.40 at 3%, respectively. The result shown in Table 1 also indicates that the values of EC in the aqueous extracts of OP and WS treatments tended to increase with increasing the pyrolytic temperatures. However, there was no statistical significance in the variance between the pyrolytic temperatures at 1% in the OP and OPB treatments. Among the RW and RWB treatments, the EC was the highest in the WSB_{300}.

Effect of aqueous extract of the different raw feedstock and their biochar on germination rate of lettuce seed are shown in Table 4. The germination rates in control containing only distilled water were 100, 98 and 100% in OP, WS, and RW, respectively. There was no significant difference between the control and OPB treatments on concentration of aqueous extracts and pyrolysis temperatures. That is, lettuce in the aqueous extracts of OPB treatments was germinated in all seeds. However, the aqueous extracts of OP inhibited the germination of lettuce seeds. The inhibitory activity was proportional to the extract concentrations and higher concentration (3%) had the stronger inhibitory effect. Although the pH is not the only determining factor in seed germination, the lack of growth by the aqueous extract of OP used in this study might be due to a high level of acidity (Table 3). Indeed, high germinability of the lettuce seed was observed in the OPB_{300} and OPB_{700} treatments, which indicated pH increased by pyrolytic process (Table 1).

Table 3. Aqueous pH and EC values of biochars with different pyrolytic temperatures

Biochar sources	Pyrolytic temperatures (°C)	pH (Extract water)		EC (dS m^{-1})	
		1%[1)]	3%	1%	3%
Control (Distilled water)		6.67		0.57	
OP	0	5.72 c	4.83 c	0.01 n.s	0.03 b
	300	6.28 b	6.02 b	0.01 n.s	0.14 a
	700	8.85 a	9.32 a	0.01 n.s	0.19 a
RW	0	7.42 b	7.06 c	0.26 c	0.01 b
	300	7.40 b	7.21 b	1.19 a	0.09 a
	700	7.78 a	7.72 a	0.85 b	0.01 b
WS	0	7.93 a	7.71 a	0.01 c	0.01 b
	300	7.61 b	7.32 b	0.06 b	0.01 b
	700	7.65 b	7.40 b	0.32 a	0.46 a

[1)] Extract concentrations of each source.

Table 4. Germination rate of lettuce plants by aqueous extract of raw feedstock and biochar samples under different pyrolytic temperatures and concentrations

Pyrolytic temperatures	Concentration[1)]	Germination (%)		
		OP	RW	WS
Control (Distilled water)		100 a[2)]	98 a	100 a
0°C	1%	52 b	98 a	98 a
	3%	0 c	92 b	97 a
300°C	1%	100 a	100 a	100 a
	3%	100 a	100 a	98 a
700°C	1%	100 a	100 a	95 a
	3%	100 a	97 ab	89 b

[1)] Extract concentrations of each source.
[2)] Means in a column followed by the same letter are not significantly different at the 0.05 probability level.

Similar trend was observed in the aqueous extract of RW and RWB treatments. Unlike the OP and RW treatments, the germination rate in WSB_{700} was lower than that in the control and WS treatments. Although inhibition effect in the aqueous extracts of WSB_{700} was observed, compared to the control, the germination rate in WSB_{300} was similar or promoted.

Figure 1 shows the changes in germination rate of lettuce plant by aqueous extract of three types of raw feedstock and their biochar samples under different pyrolytic temperatures and extract concentration. The germination rate of lettuce seed in the aqueous OP extract significantly delayed than in the OPB_{300} and OPB_{700}. That is, the germination rate in the OPB_{300} and OPB_{700} increased rapidly and reached to 100% after 3 days, but only 52% and 0% of germination rate after 4 days were obtained at 1% and 3% concentrations of the aqueous OP extracts, respectively. According to Fujihara and Shimizu (2003), although extract from the peel of citrus fruits such as navel orange (*C. sinensis*) and lemon (*C. limon* Burm. f.) had very little or no effect, that from yuzu fruit peel (*Citrus junos* Sieb. ex Tanaka) strongly suppressed the germination of lettuce seeds and the inhibitory effect of yuzu peel depended on the extract concentration.

The seedling growth of lettuce by aqueous extracts of different samples is shown in Fig. 2. The results showed that the aqueous extracts with different concentrations and pyrolytic temperatures of three types of biochar samples were similar or promoted the hypocotyl growth of lettuce plants compared to the control. In cases of the OP and OPB treatments, the highest hypocotyls growth (23 mm at 1% and 21mm at 3%, respectivly) was observed in the OPB_{700} and the lowest (7 mm at 1% and 0 mm at 3%, respectivly) in the OP. Especially, the OP inhibited the radicle as well as the hypocotyls and the inhibitory effect increased with increasing the concentration of aqueous extract. Similar results have been shown in the RW and RWB treatments, but the difference between radical growths was not statistically significant. In cases of the WS and WSB treatments, there was no significant difference on the pyrolytic tempera-

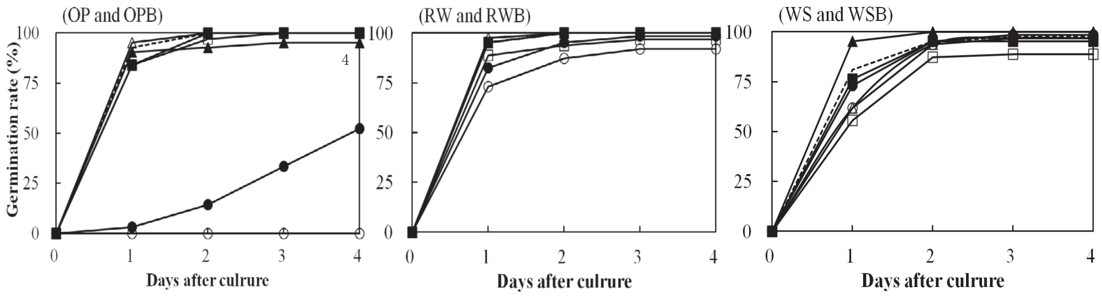

Fig. 1. Changes in germination rate of lettuce plant by aqueous extract of raw feedstock and biochar samples under different pyrolytic temperatures and concentration. Values are means of 3 Petri dishes with 20 seeds per a dish. (·····Control, ● 0°C–1%, ○ 0°C–3%, ▲ 300°C–1%, △ 300°C–3%, ■ 700°C–1%, □ 700°C–3%)

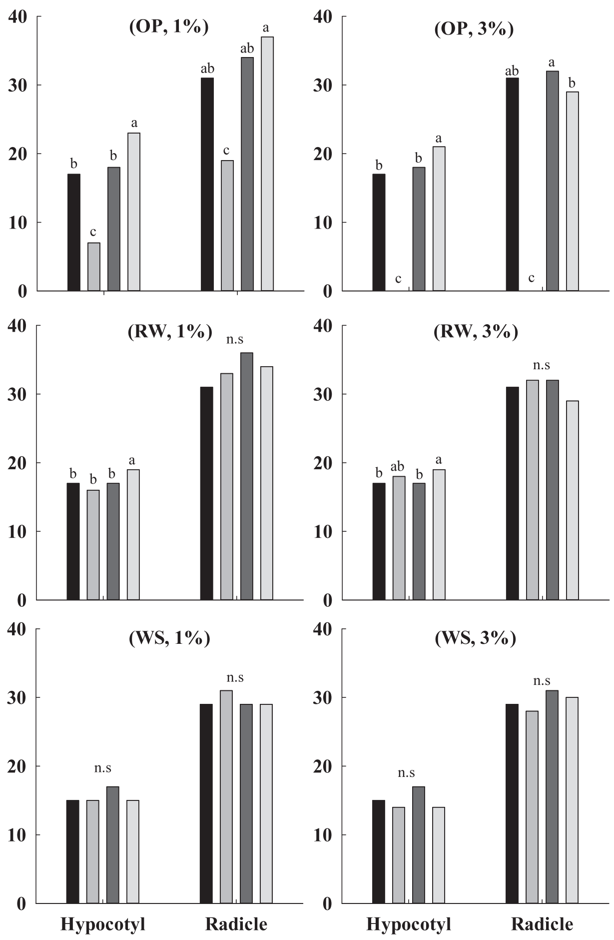

Fig. 2. Effects of aqueous extract of different raw feedstock and biochar samples on the seedling growth of lettuce plants. Means followed by the same letter are not significantly different at the 0.05 probability level.
(■ Control, ■ Raw material, ■ 300°C, □ 700°C)

tures and extract concentrations. The obtained results indicated that the use of WSB treatments did not affect the germination rate of lettuce. The present study with the three feedstocks revealed that biochar improved an inhibitory influence on tested lettuce plants. The aqueous extract of some feedstocks had a negative impact on lettuce seedling growth. The inhibition of lettuce seedling may be attributed to the reduced rate of cell division and cell elongation related to toxic substance in the extract. Some researcher has already reported that *Citrus* species should be considered as plants which cause allelopathic inhibition (Kato–Noguchi *et al.*, 2002; Kato–Noguchi and Tanaka, 2004). The results of this study provide evidence that biochar has the alleviation potential from feedstocks and it can be utilized as a soil amendment or fertilizer, at the same time, can be recycled the waste by incorporating it into the soil.

CONCLUSIONS

The biochar has a potential as a soil amendment for improving soil quality and increasing crop yields. However, careful evaluation of biochar on type and properties of raw feedstock before field scale biochar application is needed because negative impacts were also observed in some biochar. This study was carried out to provide basic information to the effective use of biochar derived from different biomass, i.e. orange peel, residual wood, and water treatment sludge, for crop production. The experimental results clearly show that aqueous extracts of different biochar samples seems to have a positive effect on germination rate and seedling growth of lettuce seeds, compared to control or raw feedstock (0°C) treatment. Especially, the increase of biochar pH by pyrolysis process is most likely to be beneficial as amendments in acidic soils where aluminium toxicity limits crop growth. In addition, unlike orange peel, the improvement of germination rate and seedling growth of lettuce plants by biochar derived from it may be because of the decrease or removal of some toxic substances by the pyrolysis process. Therefore, the biochar can be used as one of the possible sources for the improvement of soil fertility, nutrient–use efficiency, and crop productivity. However, practical field studies of the biochar on various soils and crops are necessary to understand the sustainability of biochar as a soil amendment.

REFERENCES

Bridle T. R. and D. Pritchard 2004 Energy and nutrient recovery from sewage sludge via pyrolysis. *Water Sci. Technol.*, **50**: 169–175

Chan K, L. Van Zweiten, I. Meszaros, A. Downie and S. Joseph 2008 Using poultry litter biochars as soil amendments. *Australian Journal of Soil Research*, /**46**: 437–444

Dolgen D., M. N. Alpaslan and N. Delen 2004 Use of an agro–

industry treatment plant sludge on iceberg lettuce growth. *Ecological Engineering*, **23**: 117–125

Downie A., A. Crosky and P. Munroe 2009 Physical Properties of Biochar, in: Lehmann S. and J. Joseph (eds.), *Biochar for Environmental Management.* Earthscan Ltd, London, UK

Free H. F., C. R. McGill, J. S. Rowarth and M. J. Hedley 2010 The effect of biochars on maize (*Zea mays*) germination. *New Zealand Journal of Agricultural Research*, **53**: 1–4

Fujihara S. and T. Shimizu 2003 Growth inhibitory effect of peel extract from *Citrus junos. Plant Growth Regulation*, **39**: 223–233

Gaskin J. W., A. Speir, L. M. Morris, K. Ogden, K. Harris, D. Lee and K. C. Das 2007 *Potential for pyrolysis char to affect soil moisture and nutrient retention status of a loamy sand soil*. in: Proceedings of the Georgia Water Resources Conference. 27–29 March 2007, Athens, Georgia, Institute of ecology, University of Georgia, Athens Georgia

Jones K. and A. Sewart 1997 Dioxins and furans in sewerage sludges: a review of their occurrence and sources in sludge and of their environmental fate, behaviour, and significance in sludge amended agricultural systems. *Critical Reviews in Environmental Sci. Technol.*, /**27**: 1–85

Kato–Noguchi H. and Y. Tanaka 2004 Allelopathic potential of *Citrus junos* fruit waste from food processing industry. *Bioresource Technology*, **94**: 211–214

Kato–Noguchi H., Y. Tanaka, T. Murakami, S. Yamamura and S. Fujihara 2002 Isolation and identification of an allelopathic substance from peel of *Citrus junos*. *Phytochemistry* **61**: 849–853

Kuriakose S. V. and M. N. V. Prasad 2008 Cadmium stress affects seed germination and seedling growth in *Sorghum bicolor* (L.) Moench by changing the activities of hydrolyzing enzymes. *Plant Growth Regulation*, **54**: 143–156

Kolb S. 2007 *Understanding the Mechanisms by which a Manure–Based Charcoal Product Affects Microbial Biomass and Activity*, PhD thesis, University of Wisconsin, Green Bay, US

Lehmann J. 2007 Bio–energy in the black, *Frontiers in Ecology and the Environment*, **5**(7): 381–387

Lehmann J., J. Gaunt and M. Rondon 2006 Bio–char sequestration in terrestrial ecosystems–a review. *Mitigation and Adaptation Strategies for Global Change*, **11**: 403–427

Lehmann J. and S. Joseph 2009 In: Johannes Lehmann and Stephen Joseph, Editors, *Biochar for environmental management: Science and Technology*. Earthscan Ltd, London, UK

Lehmann J., J. P. da Silba Jr., C. Steiner, T. Nehls, W. Zech and B. Glaser 2003 Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: Fertilizer, manure and charcoal amendments. *Plant and Soil*, **249**: 343–357

Muralidhara H. S. 1982 Conversion of tannery wastes to useful products. *Resources and Conservation*, **8**: 43–59

Nguyen B. T., J. Lehmann, W. C. Hockaday, S. Joseph and C. A. Masiello 2010 Temperature sensitivity of black carbon decomposition and oxidation. *Environ. Sci. & Tech.*, **44**: 3324–3331

Novak J. M., I. Lima, B. Xing, J. W. Gaskin, C. Steiner, K. C. Das, M. Ahmedna, D. Rehrah, D. W. Watts, W. J. Busscher and H. Schomberg 2009 Characterization and their effects on a loamy sand. *Annals of Environ. Sci.*, **3**: 195–206

Rasool R, S. Kukal and G. Hira 2008 Soil organic carbon and physical properties as affected by long term application of FYM and inorganic fertilizers in maize–wheat system. *Soil and Tillage Research*, /**101**: 31–36

Shinogi Y. and Y. Kanri 2003 Pyrolysis of plant, animal and human waste: physical and chemical characterization of the pyrolytic products. *Bioresour. Technol.*, **90**: 241–247

Shinogi Y., Y. Yoshida, T. Koizumi, M. Yamaoka and T. Saito 2003 Basic characteristics of low–temperature carbon products from waste sludge. *Advances in Environmental Research*, **7**: 661–665

Thies J. E. and M. C. Rillig 2009 Characteristics of biochar: biological properties. in: Lehmann S. and J. Joseph (eds.), *Biochar for Environmental Management*. Earthscan Ltd, London, UK

Troeh F. R. and L. M. Thompson 2005 *Soils and Soil Fertility*. Blackwell Publishing, Iowa, US