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Reduction of cadmium uptake in spinach (*Spinacia oleracea* L.) by soil amendment with animal waste compost

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ABSTRACT

A field experiment was conducted to evaluate the efficacy of animal waste compost (AWC) in reducing Cd uptake by spinach (*Spinacia oleracea* L.). Spinach was grown in a field that had been treated by having cattle, swine, or poultry waste compost incorporated into the soil before each crop throughout 4 years of rotational vegetable production. Cadmium concentration was 34–38% lower in spinach harvested from the AWC-treated soils than in the chemical fertilizer-treated soil. Although the repeated application of swine and poultry compost caused significant P accumulation in the cropped soils, that of cattle compost did not. These results indicate that cattle compost with high affinity for Cd and low P content should be the preferred soil amendment when used to reduce Cd uptake by spinach.

1. Introduction

Cadmium (Cd) has long been recognized as toxic to humans. In Japan, Cd was found to cause the *itai–itai* disease in the 1960s, a condition characterized by osteomalacia in combination with renal tube dysfunction [1]. Public concern about the potential for Cd toxicity has been increasing over the past decade, and the Codex Alimentarius Commission [2] adopted a new international standard for Cd concentration of less than 0.4 mg kg^{-1} wet weight (WW) in brown rice, 0.2 mg kg^{-1} WW in wheat and leafy vegetables, 0.1 mg kg^{-1} WW in stem and tuber vegetables and 0.05 mg kg^{-1} WW in other vegetables.

Extensive studies have been conducted in Japan on methods of reducing Cd concentrations in rice. Methods include flooding management [3,4], identification of genotypic variations in Cd accumulation [5,6], soil replacement [7], raising soil pH by the addition of lime, silicate, or phosphates [8], chemical washing of paddy soils [9,10] and phytoextraction [11–13]. However, few practical techniques to reduce Cd uptake have been reported for vegetables. Surveys revealed that Cd concentrations in 3% of spinach (*Spinacia oleracea* L.), 10% of taro (*Colocasia esculenta* Schott), 30% of garlic (*Allium sativum* L.) and 22% of okra (*Abelmoschus esculentus* Moench) samples produced in Japan exceeded the Codex regulations for spinach (0.2 mg kg^{-1} WW), taro (0.1 mg kg^{-1} WW), and garlic and okra (0.05 mg kg^{-1} WW) [14]. Tlustos et al. [15] reported that spinach contained higher Cd concentrations than radish (*Raphanus sativus* L.), carrot (*Daucus carota* L.), or green bean (*Phaseolus vulgaris* L.). Alexander et al. [16] also reported that spinach appeared to be a relatively high Cd accumulator. Spinach is one of the most common vegetables cultivated worldwide, and Japan ranks as the third largest producer of spinach in the world (300,000 Mg WW [17]). Therefore, effective techniques to reduce Cd uptake by spinach are urgently needed.

The application of alkaline materials such as limestone ($CaCO_3$), a method often used in rice to reduce Cd uptake, is a potential low-cost method of increasing soil pH and reducing Cd uptake by vegetables. Although a large decrease in crop Cd is commonly observed when the soil pH is raised from 5.5 to 6.5 or above, decrease is seldom observed when the initial pH is raised to 7 or higher [18,19]. Recently, the application of organic material has been reported to reduce Cd extractability from soil; such materials include grape marc (skins) and mushroom compost by pot experiment [20], peat moss extract by pot experiment [21], and commercial compost by lysimeter experiment [22]. However, little information exists about the effect of applying animal waste composts (AWCs) on Cd phytoavailability in soils. Besides, most of the studies by other researchers were carried out by hydroponics, pot or lysimeter experiment and thus field experiment is needed to confirm the effectiveness of AWC to decrease Cd phytoavailability of a crop.

AWC is regarded as one of the main Cd sources in agricultural fields due to widely used feed additives. The main Cd source

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Table 1
The rotation of vegetable crops in conjunction with three animal waste compost (AWC) treatments, a chemical fertilizer control, and a no addition control prior to the growth of a spinach crop to examine the effect of repeated AWC addition on Cd accumulation in spinach.

Year	Season	Crop
2002	Autumn	Broccoli (*Brassica oleracea* var. *italica* Plenck)
2003	Spring	Lettuce (*Lactuca sativa* L.)
	Autumn	Chinese cabbage (*Brassica rapa* var. *glabra* Regel.)
2004	Spring	Carrot (*Daucus carota* L.)
	Autumn	Cabbage (*Brassica oleracea* var. *capitata* L.)
2005	Spring	Lettuce
	Autumn	Cabbage
2006	Spring	Sweet corn (*Zea mays* L.)
	Autumn	Lettuce

in AWC is from the phosphate rock that is added in the diet to supply inorganic phosphorus to animals [23,24]. However, annual application of fresh manure since 1850 at Rothamsted increased soil Cd compared to the untreated plot, but manure fertilization caused decreased grain Cd over time, while the chemical fertilizers generally caused increased grain Cd over time [25]. Li et al. [26] demonstrated that Cd phytoavailability in soils to which AWC high in Cd was added was less than in soils to which soluble Cd salt was applied at the same Cd dosage by pot experiment. Although this report might encourage the use of AWC as a low-cost method of suppressing Cd phytoavailability in soil, the authors used AWC containing extremely high Cd content (10.5 mg kg^{-1} Cd in poultry and 20.4 mg kg^{-1} Cd in swine waste compost) that was inappropriate for agricultural use. The major types of AWC used in Japan are derived from cattle, swine, and poultry waste, and the mean Cd contents in these are 0.38 mg kg^{-1}, 0.50 mg kg^{-1} and 0.57 mg kg^{-1}, respectively [27]. Hence, field experiments are needed to evaluate the efficacy of AWC with low Cd concentration as a soil amendment to decrease Cd uptake by vegetables.

Repeated waste application at rates based on crop nitrogen (N) requirements has been reported to cause phosphate (P [28,29]) and potassium (K [30,31]) accumulation in cropped soils. We therefore decided to assess the effect on Cd phytoavailability of the maximum sustainable application rate of AWC based on crop nutrient requirements and predicted P and K accumulation.

The objectives of this study were (1) to evaluate the effects of three kinds of AWC as soil amendments on Cd uptake by spinach grown in soils with low Cd content, and on the Cd phytoavailability from soils, and (2) to evaluate the amounts of P and K applied in chemical fertilizer that can be replaced by AWC.

2. Materials and methods

2.1. Site and soil

The experiments were conducted in a field of the Horticultural Research Center (Seiro, Niigata, Japan). The soil classification was a Fluvisol [32].

The field experiment was arranged in a randomized block design with three replications per treatment. Each block was 30 m^2 (1.5 m × 20 m) and comprised five plots of 1.5 m × 4.0 m. The five treatments were the addition of cattle compost, swine compost, poultry compost, chemical fertilizer (CF), or no addition (NA) for 4 years. Compost or fertilizer was applied just before cultivation and incorporated thoroughly by rotary cultivator and the furrows were made. Vegetables were cultivated twice a year rotationally as shown in Table 1 under ambient conditions. Each AWC was obtained from farmers located near the experimental field, and the same batches were used.

Base fertilizer containing 150 kg ha^{-1} each of N, P_2O_5 and K_2O was applied to the soil of the CF control; this matched the conventional fertilization practice for spinach in this area [33]. The amount of each AWC applied was determined based on the P and K content to assess their potential for accumulation after repeated AWC application (Table 2). As there is no established standard for allowable P and K input to agricultural soils derived from AWC in Japan, we adopted the standards for single cultivation of each vegetable in the rotational production as follows: P input could not exceed double the amount necessary for vegetable cultivation because accumulation of P does not cause physiological disorders of crops in the short term and 50–66% of P from manure is phytoavailable form [28] and K could not be more than 8.3 kg ha^{-1} in excess of requirements (approximately 5% excess of vegetable requirements) because 90% of K from manure is phytoavailable form [34]. Where the N and K contents of the AWC were lower than those in the control, we added ammonium sulfate and potassium chloride respectively to ensure that the total nutrient contents were comparable in all treatments; we did not adjust the P content. The pH values and the elements contained in the AWC are shown in Tables 3 and 4.

2.2. Plant growth and yield

Compost or fertilizer was applied just before cultivation and incorporated thoroughly by rotary cultivator and the furrows were made on 9 April 2007. Seeds of spinach 'Pandora' (Sakata Seed Co., Yokohama, Japan) were sown directly into the soil of each plot on 13 April. The plants were grown to an average density of 80 plants m^{-2} in one furrow on which 4 inter-rows were allocated with 25 cm spacing, and supplementary spray irrigation was sup-

Table 2
The amount of compost applied in this study and the cumulative amount for 4 years, and the available N, total P, and total K contents in the three AWCs tested (fresh weight basis).

Compost	The amount of compost applied in this study				The amount of composts applied for 4 years prior to spinach cultivation			
	Amount applied (Mg ha^{-1})	Available N (kg)	Total P (kg)	Total K (kg)	Amount applied (Mg ha^{-1})	Available N (kg)	Total P (kg)	Total K (kg)
Cattle	7.75	2.3	41.9	133.1	101.2	30.4	547.4	1738.1
Swine	6.50	34.5	130.5	94.5	74.5	394.9	1495.5	1082.2
Poultry	6.10	29.9	104.1	132.2	74.9	367.0	1278.0	1622.8

Table 3
The pH values and macro-elements contained in the AWCs used in the experiment (air-dried weight basis).

Compost	pH	Total C (g kg^{-1})	Total N (g kg^{-1})	Available N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})	Total Ca (g kg^{-1})	Total Mg (g kg^{-1})
Cattle	7.52	260.6	21.6	0.6	9.9	31.5	30.1	8.0
Swine	6.02	381.6	44.8	7.6	28.8	20.8	45.7	11.9
Poultry	7.10	342.0	33.4	5.9	20.5	26.1	70.7	6.6

Table 4
Trace elements contained in the AWCs and chemical fertilizer (CF) used in the experiment (air-dried weight basis).

Compost	Total Cd ($mg\,kg^{-1}$)	Total Cu ($mg\,kg^{-1}$)	Total Zn ($mg\,kg^{-1}$)	Total Mn ($mg\,kg^{-1}$)	Total Fe ($mg\,kg^{-1}$)
Cattle	0.42	27.8	145.3	480.2	2776.7
Swine	0.53	297.6	739.1	693.6	8940.0
Poultry	0.74	34.5	304.0	340.9	1561.1
CF	1.96	4.7	58.2	32.0	980.0

Table 5
The growth indices and yields of spinach grown with the addition of three types of animal compost, a chemical fertilizer control (CF), and a no addition control (NA).

Treatments	Fresh weight (g)	Leaf length (cm)	Number of leaves	Yield ($Mg\,ha^{-1}$)
Cattle compost	35.9 ± 1.8 b[a]	30.1 ± 0.4 b	10.1 ± 0.1 b	21.7 ± 1.6 b
Swine compost	32.9 ± 2.6 b	29.8 ± 0.9 b	10.4 ± 0.1 b	20.9 ± 0.8 b
Poultry compost	33.0 ± 3.8 b	28.3 ± 1.0 b	10.0 ± 0.2 b	21.0 ± 2.0 b
CF	28.4 ± 2.4 b	26.2 ± 1.4 b	10.0 ± 0.4 b	18.5 ± 1.4 b
NA	4.1 ± 0.6 a	9.0 ± 0.5 a	7.9 ± 0.3 a	2.4 ± 0.4 a

[a] Means ± SE. Means in the same column followed by the same letter are not significantly different at $P < 0.01$ based on Tukey's multiple-comparison test.

plied as required. The pesticides and fungicides were applied to match the conventional pest management practice for spinach in this area [33]. All spinach leaves were harvested on 21 May 2007.

Yield was calculated from the total weight of fresh leaves harvested from the central 1.0 m × 1.0 m width of each plot. The mean fresh weight, leaf length and number of leaves per plant were also obtained from the mean values of 20 plants at harvest time.

2.3. Plant analysis

The harvested spinach leaves were thoroughly washed with distilled deionized water and oven-dried at 70 °C for 72 h and then ground in a stainless steel mill (TI-100, Heiko Seisakusho, Ltd., Tokyo, Japan). Samples were digested with a mixture of concentrated HNO_3 and 60% $HClO_4$ (4:3, v/v) on a heating block and analyzed by graphite furnace atomic absorption spectrophotometry (AAS) (Z-5010, Hitachi, Tokyo, Japan). The Cd concentration in fresh spinach leaves was calculated from the dry weight values.

2.4. Soil, AWC and fertilizer analysis

Soil samples were collected from the plow layer of each subplot. All samples were air-dried and were then passed through a 2 mm mesh sieve before use. The soils before sowing were collected on 2 April and those after harvesting on 28 May.

The pH values of soil and AWC were measured in distilled deionized water (1:2.5, w/v) using a pH meter (HM-40V, TOA, Tokyo, Japan). Total soil carbon (C) and N were determined using an NC analyzer (MT-700, Yanaco, Kyoto, Japan). Exchangeable K was extracted with 1 mol L^{-1} CH_3COONH_4 (1:12.5, w/v) and analyzed by flame AAS (Z-6100, Hitachi, Tokyo, Japan). Available P was analyzed according to the method of Truog [35]. Available N was determined according to the method of the Japan Soil Association [36] based on a 4-week incubation. Total Cu and Zn were determined by digestion with concentrated HNO_3, 60% $HClO_4$ and 48% HF [37]. To identify whether the Cd phytoavailability were influenced by the repeated application of AWC, we employed the extraction method using 0.05 mol L^{-1} $Ca(NO_3)_2$ for the exchangeable Cd form [11]. This mild neutral salt extraction has been reported to be the preferred predictor of plant Cd concentrations [38]. The procedure of this extraction method is as follows: 3.0 g of soil was placed in a 40 mL-polypropylene (PP) tube and added 30 mL of 0.05 mol L^{-1} $Ca(NO_3)_2$. The PP tube was shaken side by side for 24 h and centrifuged (1710 × g, 20 min). The supernatant was filtrated using disposable 0.2 μm PTFE syringe filters (Millex-LG, Millipore, USA). The exchangeable Cd in soil extracts were determined by means of inductively coupled plasma-optical-emission spectroscopy (ICP-OES; Vista-Pro, Varian, Mulgrave, Australia). Certified reference material for soil (JSAC 0401) was included to ensure the accuracy of soil analysis.

Samples of AWC were digested with concentrated HNO_3 and $HClO_4$ according to the method of the Japan Soil Association [36]. Elements in the digests except P were analyzed by flame AAS. Phosphate was analyzed by the vanadomolybdate method [39]. A sample of chemical fertilizer was digested with concentrated HNO_3 and HCl (1:3, v/v) and analyzed by flame AAS.

3. Results

3.1. Plant growth and yield

The spinach growth indices and yields of each treatment are shown in Table 5. The yields of spinach from all treatments except NA were higher than the standard yield of spinach grown in the Niigata region of Japan (15 $Mg\,ha^{-1}$ [33]). The yields of spinach from the AWC treatments were greater than that from the chemical fertilizer control but the difference was not significant. The yield of spinach from the NA control was only 16% of that of the regional standard.

3.2. Cd concentration in spinach leaves

The cadmium concentrations in the leaves of spinach grown in soils amended with all three AWCs were significantly lower than those from the CF and NA controls (Fig. 1). Thus, the Cd concen-

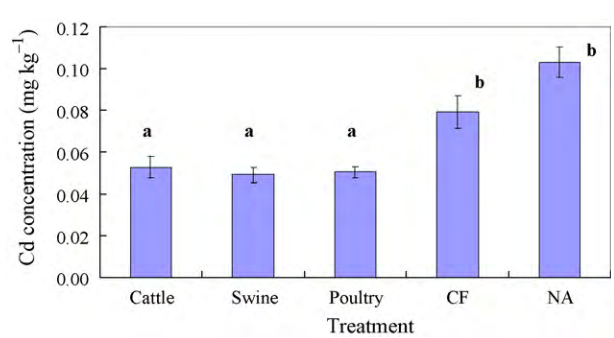

Fig. 1. Cadmium concentration in spinach leaves. Cattle: cattle compost, Swine: swine compost, Poultry: poultry compost, CF: chemical fertilizer (control), and NA: no addition. Bars represent standard errors ($n = 3$). Different letters indicate significant differences at $P < 0.05$ based on Tukey's multiple-comparison test.

Table 6
Chemical properties of soils before sowing, and pH values before sowing and after harvesting in experimental treatments comprising addition of three types of animal compost, a chemical fertilizer control (CF), and a no addition control (NA).

Treatments	pH (H_2O) (Before sowing)	pH (H_2O) (After harvesting)	Total C ($g\,kg^{-1}$)	Total N ($g\,kg^{-1}$)	Available N ($mg\,kg^{-1}$)	Available P[b] ($mg\,kg^{-1}$)	Exchangeable K^+ ($cmol\,kg^{-1}$)	CEC ($cmol\,kg^{-1}$)	Total Cd ($mg\,kg^{-1}$)	Total Cu ($mg\,kg^{-1}$)	Total Zn ($mg\,kg^{-1}$)
Cattle compost	6.60 ± 0.03 a[a]	6.31 ± 0.11 a	11.4 ± 0.3 b	1.0 ± 0.0 b	20.7 ± 0.4 a	288 ± 1 a	0.44 ± 0.03 b	11.1 ± 0.3 c	0.35 ± 0.01 a	20.5 ± ± 1.0 ab	146.8 ± 4.8 a
Swine compost	6.53 ± 0.09 a	6.38 ± 0.15 a	10.9 ± 0.3 b	1.1 ± 0.1 b	27.2 ± 1.1 b	417 ± 12 c	0.46 ± 0.06 b	10.5 ± 0.0 bc	0.34 ± 0.01 a	22.9 ± 0.5 b	150.6 ± 3.0 a
Poultry compost	6.89 ± 0.04 b	6.73 ± 0.02 ab	11.0 ± 0.3 b	1.0 ± 0.0 b	29.2 ± 2.0 b	459 ± 7 d	0.37 ± 0.03 ab	9.7 ± 0.2 ab	0.32 ± 0.01 a	19.0 ± 0.5 a	140.7 ± 4.1 a
CF	6.61 ± 0.02 a	6.47 ± 0.09 a	8.6 ± 0.2 a	0.8 ± 0.0 a	17.6 ± 0.7 a	324 ± 5 b	0.48 ± 0.01 b	9.6 ± 0.3 ab	0.35 ± 0.03 a	19.2 ± 0.5 a	139.1 ± 5.2 a
NA	7.32 ± 0.06 c	7.10 ± 0.04 b	8.6 ± 0.3 a	0.8 ± 0.0 a	18.3 ± 1.2 a	304 ± 6 ab	0.22 ± 0.03 a	9.5 ± 0.1 a	0.31 ± 0.01 a	19.7 ± 0.2 a	145.7 ± 6.0 a

[a] Means ± SE. Means in the same column followed by the same letter are not significantly different at $P < 0.05$ based on Tukey's multiple-comparison test.
[b] The mean value of available P in the upland field and the proposed maximum allowable P level for vegetable production in Japan are 292 $mg\,kg^{-1}$ and 325 $mg\,kg^{-1}$, respectively [57].

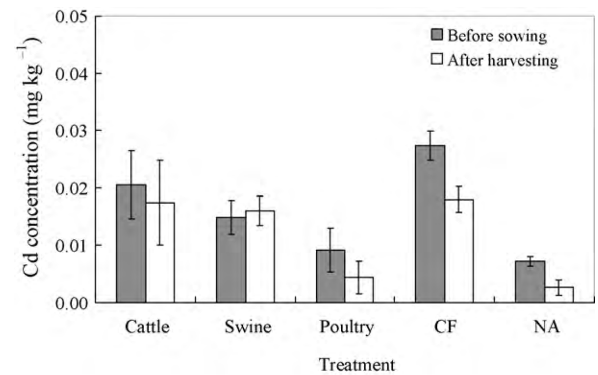

Fig. 2. Cadmium concentration in exchangeable form extracted with 0.05 $mol\,L^{-1}$ $Ca(NO_3)_2$. Cattle: cattle compost, Swine: swine compost, Poultry: poultry compost, CF: chemical fertilizer (control), and NA: no addition. Bars represent standard errors ($n = 3$).

tration in spinach was reduced compared to that in the CF control by 34%, 38%, and 37% by the application of cattle, swine, and poultry compost, respectively. The highest Cd concentration in spinach leaves was observed in the NA control, and the value was 30% higher than in the CF control.

3.3. Chemical properties of the soils

The chemical properties of the soils in all treatments before sowing with spinach are shown in Table 6. The soil of the poultry compost treatment had a significantly higher pH than the soil of the other two AWC treatments and the CF control. Total C and N were not significantly different between AWC treatments but were significantly higher than both controls. Available N was significantly higher in the soils treated with swine and poultry compost than in both control soils. Significant levels of available P accumulation occurred in the soils treated with swine and poultry compost. The soil treated with cattle compost had a significantly higher CEC than all other treatments except the soil treated with swine compost. The soil of the swine compost treatment had a significantly higher Cu level but Cd and Zn levels in the soils were not significantly different between the treatments.

3.4. Cd concentrations extracted with 0.05 $mol\,L^{-1}$ $Ca(NO_3)_2$

The exchangeable Cd concentration of each treated soil before sowing and after harvesting was shown in Fig. 2. The exchangeable Cd concentrations both before sowing and after harvesting were slightly lower in some of the AWC-treated soils than in the CF control. Before sowing, the exchangeable Cd concentrations were 25% ($P > 0.1$), 46% ($P = 0.084$), and 66% ($P = 0.042$) lower than the CF control in cattle, swine, and poultry treatments, respectively; the corresponding differences after harvesting were 4% ($P > 0.1$), 11% ($P > 0.1$), and 76% ($P = 0.081$).

3.5. Cd input to soil in AWC and CF

The cattle, swine, and poultry compost used in this study contained Cd at concentrations of 0.42 $mg\,kg^{-1}$, 0.53 $mg\,kg^{-1}$, and 0.74 $mg\,kg^{-1}$, respectively (Table 4). These are similar to typical Cd contents of AWC produced in Japan [27]. Although the Cd concentration of the CF used in our study was higher than that of the AWC, the Cd input was less than that from AWC (43% to 60% of the AWCs) owing to the lower application rate of CF. The 4 years of applying either AWC or CF did not lead to Cd accumulation in the soil (Table 6).

4. Discussion

A review by Kirkham [40] cited several studies in which increasing pH reduced the amount of Cd in plants. In the present study, the pH value of the soil in the poultry compost treatment before sowing was significantly higher than that of the CF control, but the pH values of the soils treated with cattle and swine compost were not significantly different from that of the CF control (Table 6). Besides, the pH value of the soil in the poultry compost treatment after harvesting was highest among AWC treatments but the difference from that of the CF control was not significant (Table 6). However, Cd concentration in the cattle and swine compost treatments was also significantly lower than in the control (Fig. 1). This shows that factors other than pH are needed to explain the change in Cd phytoavailability caused by AWC amendment.

Early report showed that the decreased Cd phytoavailability associated with the addition of organic matter was predominantly due to increased soil CEC [41]. He and Singh [42] also reported that plant Cd content was highly but negatively correlated with soil CEC. However, in our study, a significant increase in CEC was found only in the treatment with cattle compost (Table 6).

AWC-treated soils had significantly higher C levels than the controls (Table 6). Li et al. [43] found that Cd adsorption was increased by biosolids-application in long-term field studies and removal of organic C reduced Cd adsorption for the soils, but increased adsorption attributed to biosolids-application was still present compared to the control soil. Hettiarachchi et al. [44] demonstrated that Fe/Mn fractions were important factor that maintained Cd adsorption in corporation with the organic C. The AWC used in this study contained substantial amount of Mn and Fe (Table 4). These inorganic elements would cause the Cd binding to the AWC and thus decrease the Cd phytoavailability of spinach leaves.

Manure composts contain a substantial amount of humic substances [45]. Cadmium may be tightly bound to insoluble organic matter such as large humic acid molecules and humin, thereby increasing the capacity of soil to adsorb Cd [46]. Humic substances contain a variety of functional groups, including COOH, phenolic OH, enolic OH, alcoholic OH, quinone, hydroxyquinone, lactone, and ether [47]. Xia and Rayson [48] reported that the binding of Cd^{2+} to various organic materials was associated with carboxylate functionalities. The kinetic experiment showed the evidence that the Cd binding occurs via an ion exchange as well as by electrostatic interaction between carboxylate groups and Cd^{2+} [49]. The esterification of carboxyl groups and hydrolysis of ester groups in the native biomass provided strong evidence that the carboxyl functionality is the main group responsible for binding Cd [50]. We speculate, then, that soil Cd in the exchangeable form would have become bound to functional group(s) of the AWC applied to the soils.

The mild neutral salt extraction using $Ca(NO_3)_2$ showed that the Cd concentrations in the exchangeable Cd form of the AWC-treated soils were lower than those of the CF control (Fig. 2). Trace elements in soluble or weakly adsorbed pools are regarded as more phytoavailable than those in strongly adsorbed and occluded forms [51]. Therefore, the decrease of exchangeable Cd form in the AWC-treated soils could be one of the main reasons to explain the decrease of Cd uptake by spinach grown on these soils. However, because the original Cd concentrations in the exchangeable form were so low, and there was a lack of significant differences between some of the treatments, further investigation is needed to confirm the relationship between the AWC-application and the decrease of exchangeable Cd.

It has been noted that the application of AWC could pose a risk of Cd accumulation in the soil because AWC contains endogenous Cd [23,24]. However, our results showed that the total Cd concentrations in the AWC-treated soils were similar to those of the control soils (Table 6). Further, the Cd concentrations were lower in spinach from the AWC treatments than from the controls (Fig. 1). Orihara et al. [27] surveyed heavy metal content in a range of AWCs and found them to contain Cd, As, Hg, and Pb at relatively low rates that posed no risk of environmental pollution. Brown and Chaney [52] reported that significantly less Cd was taken up by lettuce grown on long-term biosolids-amended soil collected even after cessation of amendment for over 10 years than that grown on soil amended with equivalent rates of Cd salts. Similar results were also obtained by Kukier et al. [53] in which the slope of Cd uptake of lettuce shoots with increasing Cd addition was remarkably reduced for the biosolids-amended soil collected after cessation of amendment up to 24 years compared to the control soil. These findings indicate that Cd release from biosolids, which contain substantial organic carbon and inorganic elements like AWC, would not occur with time after cessation of application and hazards do not increase over time. Karaca [20] reported that the Cd concentrations in the soils to which organic matters were applied were higher after 6 months of incubation than in the first day of incubation, probably due to degradation of the organic matter. However, the present study was conducted after 4 years application of AWC. Therefore the duration is long enough to evaluate the degradation of AWC and the occurrence of the release of Cd. Thus, AWC might be used as soil amendment to reduce Cd risk of spinach without a potential risk of Cd accumulation in the soil.

Podar and Ramsey [19] demonstrated that the concentration of Cd in the lettuce leaves grown in the Cd-contaminated soil ($20\,mg\,kg^{-1}$) showed no significant variation with increasing the level of Zn ($<500\,mg\,kg^{-1}$) in soil when soil pH was 7.0 or below. The Zn concentrations between AWC-treated soils and the controls in this study were not significantly different (Table 6).

The background Zn level in Japan has been reported to be approximately $100\,mg\,kg^{-1}$ [54–56]. Our data on Zn levels were 1.4–1.5 times higher than those shown in these articles (Table 6). Because the significant difference in Zn values was not found between the NA control and the other treatments, the higher Zn values in all treatments could be attributed to the original Zn concentration of soil in this experimental site, rather than to the result of historic application of AWC or fertilizer. The soil of the swine compost treatment had a significantly higher Cu level than that of the NA control (Table 6). This could be due to the historic application of swine compost that had higher Cu content than the other composts (Table 4). However, the background Cu level in Japan has been reported to be approximately from $30\,mg\,kg^{-1}$ [56] to $50\,mg\,kg^{-1}$ [54] and our data on Cu levels were even lower than those found in the reports.

We confirmed that the P and K contents of the AWC were sufficient to prevent any negative effect on spinach yield (Table 5). Further, the repeated application of AWC significantly increased available N in the soils compared to the controls (Table 6). However, P derived from swine and poultry compost accumulated in the soils (Table 6), presumably because they contain substantially more P than cattle compost (Table 3). The mean value of available P in the upland field of Japan has been reported to be $292\,mg\,kg^{-1}$ and 20% of soil samples contained available P over $435\,mg\,kg^{-1}$ ($N = 4915$) [57]. The soil treated with cattle compost did not cause P accumulation and the value was under $325\,mg\,kg^{-1}$ (Table 6), which had been proposed as the maximum allowable P level for vegetable production in Japan [57]. Cattle compost should therefore be preferred as a soil amendment to reduce the Cd uptake by spinach because it does not lead to P accumulation. Considering that P is an essential and limited nutrient in agriculture, the P contained in AWC should be utilized in a way that achieves the maximum possible substitution of AWC for chemical fertilizer in rotational vegetable production.

5. Conclusions

This study confirmed that the application of AWC reduces Cd uptake in spinach. This phenomenon could be attributed to the high capacity of AWC for adsorbing Cd. Although the Cd concentration in the spinach in the chemical fertilizer control was below the Codex regulation, the application of AWC would be a cost-effective method of decreasing the potential risk of elevated Cd concentrations in spinach. To avoid excessive P accumulation in the cropped soil, cattle compost is the preferred soil amendment to reduce Cd uptake by spinach.

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