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► To cite this version:

Ilenia Cattani, Marco Romani, Raffaella Boccelli. Effect of cultivation practices on cadmium concentration in rice grain. *Agronomy for Sustainable Development*, 2008, 28 (2), pp.265-271. hal-00886419

HAL Id: hal-00886419

<https://hal.science/hal-00886419>

Submitted on 1 Jan 2008

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Original article

Effect of cultivation practices on cadmium concentration in rice grain

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(Accepted 26 July 2007)

Abstract – Cadmium (Cd) is one of the most toxic heavy metals, polluting the general environment. The application of sewage sludge, wastewaters and Cd-containing fertilizers causes an increase in Cd content in agricultural soils. Cd is easily taken up by plants and then enters the food chain, resulting in a serious health issue for humans. There is increasing concern regarding the occurrence of cadmium in rice, not only in the rice-growing areas of the Far East, but also in Europe. In this work we highlighted that, even when the agricultural soil is unpolluted and the concentration of Cd is low, e.g. 0.96 mg kg^{-1} , the Cd content of rice may still exceed the regulatory limit of 0.2 mg kg^{-1} . To reduce the uptake of Cd by rice, paddy-field flooding and soil amendment with lime and compost were tested in a field trial during 2003 and 2004 in Rosate, near Milan, Italy. We found that submersion was the main factor decreasing the Cd concentration in rice grain, producing maximum concentrations of 0.14 mg kg^{-1} in 2003 and 0.06 mg kg^{-1} in 2004. By comparison, Cd concentrations was at least two times higher for rice cultivated by irrigation only. Moreover, the addition of lime decreased the Cd concentration of rice by about 25% versus control under dry conditions. Lime addition thus appears to be a promising technique to reduce the bioavailability of soil Cd and minimize Cd concentrations in the produced rice. In contrast, the application of compost alone does not produced a significant effect. Differences in uptake over the years, with concentrations up to 40% lower in 2004, can be explained by differences in transpiration. These results shows that the influence of climatic conditions on Cd uptake in plants should not be underestimated. Such agronomic information represents a very helpful tool for rice growers, in particular in the case of cultivation of Cd-polluted soils and production of Cd-contaminated rice grain.

paddy field / rice / water management / fertilizers

1. INTRODUCTION

Cadmium is one of the most toxic heavy metals reaching the food chain (Adriano, 2001) without being essential for plant function. Cadmium appears to be absorbed passively, translocated freely and is generally strongly assimilated by crops (Liu et al., 2003). Cadmium distribution between plant parts varies among plant species. For example, graminaceous and leguminous plants accumulate more cadmium in the aerie apical organs than leafy vegetables, such as lettuce and cabbage (Grant et al., 1999). Cadmium in soils, readily taken up and translocated by plants, is not phytotoxic at concentrations found in crops but this uptake can significantly increase human exposure (Adriano, 2001). There is increasing concern about the effect of Cd on human health. It is well known that rice-growing areas in the Far East are characterized by an irrefutable relationship between Cd exposure due to long-term rice consumption – rice is the staple food in these areas- and human diseases, such as renal dysfunction and Itai-Itai disease (Simmons et al., 2005). Some authors (Welch and Norwell,

1999) report that rice plants absorb Cd at a higher rate than water and concentrate it into tissues from the nutrient solution. The European limit for the concentration of Cd in rice grain is 0.2 mg kg^{-1} (Council regulation, 2001) and a lower limit in foodstuffs – particularly in baby food – is suggested. It has been established that cadmium-rich soils generally produce cadmium-rich foods (Suzuki et al., 1988). Consequently, information on trace element intake from different sources is useful for the prevention and control of diseases that are caused by mineral intake. Cadmium in soils may be atmospheric, geological or agricultural in origin, such as wastewaters, pesticides, fertilizers (Gimeno-García et al., 1996; Lugon-Moulinat et al., 2006) or sewage sludge utilization (McBride, 1995). The agricultural soil solution typically contains Cd at a concentration of 0.1 to $4.7 \mu\text{g L}^{-1}$ (John et al., 1972), but soil factors such as pH, organic matter content, cation exchange capacity, clay content, and the presence of microorganisms and other ions may play a role in the solubilization and mobilization of Cd (Chanmugathas and Bollag, 1987). Technologies to minimize the likelihood of Cd reaching the food chain are primarily aimed at reducing its uptake by plants and animals. Chemical

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Table I. Selected properties of soil collected from the experimental field (mean of 48 replicates). CEC: cation exchange capacity, OM: organic matter, DTPA: diethylenetriaminepentaacetate, Tot: total, SD: standard deviation.

	Sand (g kg ⁻¹)	Silt (g kg ⁻¹)	Clay	pH (cmol ⁺ kg ⁻¹)	CEC (g kg ⁻¹)	OM (mg kg ⁻¹)	Cd _{DTPA} (mg kg ⁻¹)	Cd _{tot}
mean	512	376	112	6.21	14.0	2.59	0.151	0.96
SD	93	110	32	0.16	2.6	0.19	0.032	0.16

Table II. Cd distribution through the soil profile (mean of four replicates, mg kg⁻¹).

Layer (m)	0–0.14	0.14–0.24	0.24–0.54	0.54–0.84	0.84–1.20
Cd (mg kg ⁻¹)	1.13 ± 0.010	1.18 ± 0.11	1.29 ± 0.15	1.42 ± 0.16	1.32 ± 0.22

and biological immobilization using a range of soil amendments, such as lime (Grant et al., 1999; Bolan et al., 2003a), zinc (Hassan et al., 2005) and phosphate (Bolan et al., 2003b) compounds, stabilized alkaline biosolid compost (Bolan et al., 2003c) and zeolite (Oste et al., 2002), phytoremediation (Su and Wong, 2004) and the selection of rice varieties characterized by reduced uptake and translocation (Arao and Ae, 2003) may be useful for achieving this. Organic matter may play a dual role in influencing Cd availability in soil: low-molecular-weight organic components serve as carriers of Cd in the soil solution, whilst high-molecular-weight organic components may be very effective at rendering Cd unavailable, functioning as a sink (McBride, 1995; Christensen and Haug, 1999). Some agronomic practices, i.e. soil dressing, soil amendment and paddy-field flooding, may reduce or increase Cd levels in grains. For example, if rice is cultivated under submerged conditions, the reducing redox potential results in a low correlation between the Cd content of the soil and grain (Daum et al., 2001). In 2003 and 2004, a field trial was performed in Italy, in a site located in the northern region (Po Valley), where rice cultivation is mostly located and where, according to previous unpublished studies, the Cd concentration in the produced brown rice grain exceeded the European regulatory limit. The aims of the study were to evaluate the origin of Cd in paddy field soils and the influence of some agricultural practices (soil treatment and water management) on Cd uptake by plants.

2. MATERIALS AND METHODS

The experimental trial was performed on a site in the locality of Rosate, 25 km south-west of Milan (Italy). The site was a field of 2 ha, cultivated in monosuccession for more than ten years. According to the Soil Taxonomy (Soil Survey Staff, 2006), the soil was classified as an Inceptisol Aquept. Forty-eight 25-cm cores were collected at nodes of a 40 × 20 m grid.

Soil characteristics and atmospheric deposition

Soil properties (Tab. I) were measured using official analytical methods (SSIS, 2000).

The mean Cd concentration in the topsoil – measured following nitric-hydrochloric acid digestion – is 0.96 mg kg⁻¹,

Figure 1. The experimental design: “dry” and “flooded” correspond to the main plots (type of water management), “compost”, “compost + lime”, “lime” and “control (untreated)” correspond to the subplots (type of soil amendment) and the gray squares numbered from 1 to 48 correspond to the collection points.

whereas the mean diethylenetriaminepentaacetate (DTPA)-extractable fraction is 0.15 mg kg⁻¹. The low standard deviation for these values indicates a reasonable soil homogeneity. In order to evaluate the possible geological origin of Cd, the metal distribution through one soil profile was determined in samples taken from different layers, to a depth of 1.20 meters. The results of this analysis are shown in Table II.

The atmospheric contribution to Cd concentrations in the superficial soil layers was measured using a Bergerhoff instrument. Wet and dry deposits were collected on a monthly basis in 2003 and 2004, for two consecutive years. Cd concentration was determined using the method proposed by Thöni et al. (1999), by means of ICP-OES at 228.80 nm wavelength. The bulk deposition (mean of two years) was 1.53 ± 0.72 μg m⁻² d⁻¹, corresponding to a supply of about 5 μg per kg of topsoil.

Experimental design and cultivation

The rice selected for cultivation was *Oryza sativa*, var. Baldo. The experimental design (Fig. 1) comprised two main plots (60 × 124 m each) corresponding to the different rice-sowing practices and field water-management strategies (dry-seeded with rotational irrigation and water-seeded with permanent flooding, respectively). The main plots were

separated by an embankment. The distance between the test $\times$ dry subplot and the compost $\times$ flooded subplot was 30 m. The mean concentration of total Cd was 1.01 ± 0.06 in the dry plot and 0.96 ± 0.06 in the flooded plot, and the mean concentrations of DTPA-extractable Cd were 0.13 ± 0.02 and 0.15 ± 0.04 , respectively, these parameters, as well as the remaining soil characteristics, indicating a strong similarity of soil between the plots. The subplots (15×124 m each) corresponded to four treatments (compost, compost and lime, lime and control (not treated with lime or compost)). In both years, amendments were applied to the selected subplots two months prior to sowing (and flooding) to avoid as much as possible any lateral migration among subplots. Compost was applied at 30 t ha^{-1} and lime (80% CaO and 15% MgO) at 1 t ha^{-1} . Table III shows the mean results of Cd concentration in the compost and lime amendments as well as in the fertilizers, such as KCl and urea, used in all subplots indifferently (at 66 and 240 kg ha^{-1} , respectively). Each treatment was performed only following Cd analysis. Only in the case of “compost + lime” subplots were all the amendments and fertilizers used, with the maximum Cd supply among the subplots: $3 \mu\text{g}$ per kg of topsoil.

Water management was conditioned by a co-operative distribution and, especially in 2003, the very hot summer temperatures (Fig. 2) resulted in several 4-day periods of dryness between irrigation events.

Sample collection and field measurement

To individualize the collection points, each subplot was divided into eight square parts and the points were fixed in the middle of six squares, after having excluded those at the two ends. The six equidistant points (Fig. 2, numbered from 1 to 48), set 30 m apart, were georeferenced for the measurement of topsoil pH, which was performed twice a year, at panicle initiation and heading, corresponding to the beginning of July and of August. At the same points, collection of plants (straw) and soil solution was realized three times a year during the trial, comprising the panicle initiation, heading and maturity, around the middle of October. During the growing period, 6 plants were collected from each collection point. In order to extract the soil solutions, cores of soil were collected at the same points. Cores collected under flooded conditions were kept under anaerobic conditions and dried under vacuum to avoid any oxidation or alteration of the redox status, which was negative at the moment of collection, due to anaerobic conditions typical of submersion. For samples collected under flooding conditions, anaerobiosis was preserved only in 2004: data from 2003 related to this plot were certainly misrepresentative. Samples collected under dry oxidized conditions in both years were dried according to official methods as well. Soil solution was extracted from all samples after grinding to 2 mm, centrifugation for 30 min at 10000 rpm (revolutions per minute) (water/soil: 2.5/1) and filtration through a $0.45 \mu\text{m}$ filter (Millipore).

Brown rice grain was collected from each subplot at the end of the experiment and mixed before analysis. At maturity, the produced dry biomass (mean of the subplots) was: grain

Table III. Cd content in soil amendments and fertilizers (mg kg^{-1}) used in 2003 and 2004.

Fertilizer	Cd 2003 (mg kg^{-1})	Cd 2004 (mg kg^{-1})
Compost	0.241 ± 0.042	0.36 ± 0.10
Lime	< 0.31	1.112 ± 0.058
KCl	< 0.31	2.5 ± 1.5
Urea	< 0.31	< 0.31

Figure 2. Variation in air temperature in the experimental site of Rosate (Italy) during 2003 and 2004.

$6780 \pm 540 \text{ kg ha}^{-1}$ and straw $8960 \pm 810 \text{ kg ha}^{-1}$ under dry conditions, and grain $6850 \pm 720 \text{ kg ha}^{-1}$ and straw $9450 \pm 370 \text{ kg ha}^{-1}$ under flooded conditions, indicating that the potential diluting effects caused by the interaction between yield and treatments, and between yield and Cd concentration, was not significant.

In 2004, pH and redox potential were monitored using 4 pairs of electrodes, permanently fitted at a depth of 30 cm, in the control and lime subplots of the dry and flooded main plots, respectively.

Irrigation water was analyzed monthly. The mean Cd concentration was always below the limit of detection ($<1 \text{ ppb}$).

Sample analysis

The harvested plant samples were dried at 105°C to constant weight, ground using a stainless steel grinder and digested by nitric acid microwave-assisted digestion (ETHOS, Milestone, FKV), performed using a modified US EPA SW 846 test method 3051 (Shute and Macfie, 2006). Analysis of Cd concentration was performed using Inductively-Coupled Plasma Optical Emission Spectrometry (ICP-OES, Spectro CIROS^{CCD}). Soil solution Cd was measured by ICP-OES as well, after the extraction reported in the previous paragraph. Statistical analysis of the results was performed using SAS (SAS Institute, 1996).

3. RESULTS AND DISCUSSION

3.1. Origin of Cd in soil

The mean concentration of Cd in the superficial soil layer of the experimental field is $0.96 \pm 0.16 \text{ mg kg}^{-1}$. The recorded data of the atmospheric depositions, as well as those of the supply by the irrigation water and applied amendments, suggest that any anthropic contribution is inconsistent.

Table IV. Comparison of pH, Cd concentration in soil solution and Cd in brown rice resulting from the interaction treatment and water management in 2003 and 2004 (mean of monthly measurements). Means with the same letters are not significantly different ($P < 0.01$) using the Duncan test.

2003 Fertilizer × water management	Soil solution pH		Soil solution Cd ($\mu\text{g L}^{-1}$)		Brown grain Cd (mg kg^{-1})	
Compost × dry	6.06	C	3.93	A	0.25	A
Control × dry	6.03	C	5.27	A	0.25	A
Lime × dry	6.24	BC	3.27	A	0.18	B
(Compost + lime) × dry	6.40	AB	3.95	A	0.12	BCD
Compost × flooded	6.18	BC	3.63	A	0.09	CD
Control × flooded	6.13	C	4.23	A	0.14	BC
Lime × flooded	6.25	BC	3.68	A	0.07	D
(Compost + lime) × flooded	6.51	A	3.95	A	0.07	D

2004 Fertilizer × water management	Soil solution pH		Soil solution Cd ($\mu\text{g L}^{-1}$)		Brown rice Cd (mg kg^{-1})	
Compost × dry	6.71	C	5.68	AB	0.15	A
Control × dry	6.50	D	5.94	A	0.13	A
Lime × dry	6.91	AB	4.93	AB	0.10	B
(Compost + lime) × dry	6.91	AB	4.20	B	0.10	B
Compost × flooded	6.86	B	1.36	C	0.06	C
Control × flooded	6.72	C	1.97	C	0.05	C
Lime × flooded	6.87	B	1.00	C	0.05	C
(Compost + lime) × flooded	7.05	A	1.14	C	0.05	C

As cadmium is generally fairly immobile in the soil profile (Adriano, 2001), the investigation of the soil profile seems to be in agreement with this assertion. These findings conflict with what was reported by Singh and McLaughlin (1998), who found significant increases in soil Cd concentrations over time, due to atmospheric or agricultural inputs. Nonetheless, even if the concentration of total Cd in the investigated field is similar to what is reported for non-contaminated soils (Adriano, 2001), we found that the Cd content of the produced rice may still exceed the regulatory limit of 0.2 mg kg^{-1} (Fig. 4). For this reason, we tested the effect of flooding and soil amendment with lime and compost on the reduction of Cd by rice.

3.2. Effect of water management

Table IV shows that submersion was very useful for reducing the Cd concentration in rice grain in both years, with a great decrease (50% or more) in Cd assimilation compared with dry sowing. In 2004, the effect of flooding conditions was stronger than any of the treatments, with no significant differences between control and treatments, and concentration in grain of 0.06 mg kg^{-1} or less. This outcome depending on the water management could be due to a redox potential stable around -400 mV under flooded conditions and fluctuating from $+300$ to -400 mV under dry conditions (Fig. 3): under flooded conditions, corresponding to an Eh under -200 mV , sulfate ions are reduced to sulfide ions that may form complexes with Cd and other metals, immobilizing them as sulfide salts (e.g. CdS , Gimeno-García et al., 1996), and sub-acid soils are generally neutralized (Sequi, 1989). In 2004, the variation in redox potential from oxidizing to reducing conditions was

Figure 3. Modifications of (a) redox potential (Eh) and (b) pH in 2004 as affected by water management in control and lime subplots.

due to irrigation schedule, conditioned by a co-operative distribution of water, and produced a significant enhancement of the uptake of Cd by rice, with concentrations two times higher at least than for rice cultivated under submersion (0.12 versus

Figure 4. Cd content (mean value, mg kg^{-1}) in plants and brown rice, during the two experimental years. The graphs compare flooding and non-flooding conditions: means with the same letters in the same year are not significantly different ($P < 0.01$) using the Duncan test. The European regulatory limit for brown grain is 0.2 mg kg^{-1} .

0.05 mg kg^{-1} , Fig. 4). These data partially confirmed what was reported by Daum et al. (2001), who showed that the alternation of submersion and drainage increased the Cd concentration in the whole plant and in rice grains compared with the usual strategy of continuous flooding until shortly before harvest. In 2003, the substantial uptake of Cd, resulting in rice grain concentrations meanly in excess of the regulatory limit (Fig. 4), with values of 0.25 mg kg^{-1} in compost and control subplots for plants cultivated under dry conditions, is probably due to this alternation as well. Also, the high temperatures measured in this year (Fig. 2) could have improved what was observed: in effect, the concentration in brown rice generally increases in proportion to the amount absorbed by straw (Fig. 4), even if the data from 2004 show that accumulation over time is not necessarily linear. Some authors emphasized that the rice plant has no system for controlling the movement of cadmium into the acrian parts (Kashem and Singh, 2001) and others (e.g. Chino, 1981) noted the influence of the evapotranspiration rate on increasing both the lymphatic flux and the translocation of Cd. Differences in transpiration (and therefore in climate) could also explain differences in uptake over years.

The soil solution pH measured in the laboratory (Tab. IV) appears to reflect mostly the pH measured in the field (Fig. 3). Also, these data indicate that water management modified the effect of the amendments. For flooded conditions, the lack of fluctuation over time and the higher pH compared with dry conditions are typical consequences of a long-term submersion. Flooding usually produces an increase in the solubility of Fe and Mn and, consequently, an increase in immobilization of Cd by sorption in soil free sites provided by hydrous Fe and Mn oxides (Brown et al., 1989).

3.3. Effect of lime and compost addition

Soil treatments mainly affect soil pH (Fig. 3), although pH does not increase regularly over time and variations are smaller than 1 pH unit. Nevertheless, the addition of compost and lime, as well as the addition of lime alone, and their effect on the pH revealed a good efficacy regarding the concentration of

Figure 5. Rice Cd content (mean value, mg kg^{-1}) in plants and brown rice, during the two experimental years. The graphs compare treatments: means with the same letters in the same year are not significantly different ($P < 0.01$) using the Duncan test. The European regulatory limit for brown grain is 0.2 mg kg^{-1} .

Cd in brown grain, in particular in 2003, for dry-seeded rice (Tab. IV). In fact, we observed a reduction of the bioavailability of soil Cd, so that Cd concentrations in the produced rice were about 25% lower than for control and below the regulatory limit of 0.2 mg kg^{-1} . Lime, dominated by variable-charge components, produces a rise in cation adsorption capacity (Bolan et al., 2003a) and also a precipitation of Cd as CdCO_3 (Holm et al., 2003). Both of these processes reduce the availability of Cd to plants.

Compost application seems to be ineffectual on Cd availability (expressed as Cd concentration in solution or in grain): in both years the results do not show a clear effect of compost (Fig. 5 and Tab. IV).

This is consistent with the existing literature, which shows variable and contrary effects of compost organic matter on Cd uptake or solubility of soil Cd. For example, Kashem and Singh (2001) and Bolan et al. (2003c) found the application of humified organic matter (cow manure and biosolid compost) to decrease the total uptake of Cd by rice plants, due to an increased complexation of the metal in soil solution (Adriano, 2001). On the contrary, Antoniadis and Alloway (2002) and Christensen and Haug (1999) found that metals bound by soil components can be released into the soil solution following the decomposition of organic matter by soil microorganisms and the release of low-molecular-weight organic components. It has been observed (Grant et al., 1999) that this dual effect on metal availability to plants may be due to the variable composition of organic matter: low molecular fractions, such as hydrophilic bases, have a strong affinity towards forming soluble Cd complexes. Cd-DOC complexes are more labile in soil. The prevalence of high or low molecular fractions depends on the specific composition and on the aging of the considered organic soil amendment.

The application of compost with lime produced an effect on Cd assimilation by rice which was undetected in the case of application of compost alone and more similar to that of lime alone. The unexpected outcome of the compost and lime treatment could be due to an increased strength of the bond between Cd and organic matter.

In 2003, due to the incorrect conservation of samples from the flooded plot, we did not measure any difference between Cd in soil solution under aerobic and anaerobic conditions (Tab. IV). As a consequence, the calculation of correlation

Table V. Effects of Cd concentration and pH of soil solution on Cd concentration in brown rice grain. Correlation coefficients (r) and their significance. * and ** indicate significance at $P < 0.05$ and $P < 0.01$ levels, respectively; n.s. = not significant.

Variable	Brown rice Cd	Significance
Soil solution Cd July 2003	0.18	n.s.
Soil solution Cd Aug. 2003	0.20	n.s.
Soil solution pH July 2003	-0.32	*
Soil solution pH Aug. 2003	-0.36	*
Soil solution Cd July 2004	0.75	**
Soil solution Cd Aug. 2004	0.57	**
Soil solution pH July 2004	-0.34	*
Soil solution pH Aug. 2004	-0.44	**

coefficients (Tab. V) was based only on values obtained under dry conditions.

In our study, the correlation between Cd concentration in brown rice and the pH of the soil solution was negative. We also found an increase in Cd in soil solution corresponding to a slight drop in pH. In general, our results demonstrate the effect of soil amendments on the pH of soil solution and the effect of soil solution pH on Cd bioavailability in soil (Tab. IV). Increasing pH leads to the adsorption of Cd to soil organic matter and inorganic colloids (King, 1988; Simmons et al., 2005). Some authors (Kashem and Singh, 2001) reported that the affinity of Cd for soil sorption sites increased at high pH as Cd^{2+} forms $\text{Cd}(\text{OH})^+$ by hydrolysis.

Although it is well known that the distribution of Cd between the soil solid phase and the soil solution is a key issue in assessing Cd assimilation (Holm et al., 2003), in our case the concentration in the soil solution was not always the only predictor of rice grain content. In effect, although there were differences in soil solution concentrations of Cd between the different water management techniques, these differences were either not significant or misrepresentative between the soil amendment treatments. In addition, the concentration of soluble Cd was greater in 2004, when uptake was lower (Tab. IV).

4. CONCLUSION

The soil studied here was non-polluted with a concentration of total Cd lower than 1 mg kg^{-1} . Nevertheless, the content of Cd in the brown rice may exceed the regulatory limit of 0.2 mg kg^{-1} and could become hazardous to human health, particularly for children. Our results show that Cd concentration in rice is mainly affected by water management and amendments, both of which are strongly effective on redox potential and pH, and consequently on Cd in soil solution. The anaerobic conditions, typical for flooding, are strongly effective at minimizing Cd concentrations in soil solution and in plant parts. Our results suggest that a redox potential below -200 mV should be preserved to improve the safety of rice. Lime treatment generated only a small increase in the pH of soil and soil solution, but it was very useful for reducing the accumulation of Cd, especially if the soil was not submerged. This suggests that,

if submersion is not possible, lime may be used to ensure the concentrations of Cd in rice are below the regulatory limit. Compost treatment, on the contrary, seems to be unsuccessful regarding the bioaccumulation of the investigated metal. Nevertheless, it should be noted that the synergism of lime and compost could increase the effect produced by lime alone. As the effect of humified organic matter, such as cow manure and biosolid compost, on the total uptake of Cd by rice plants depends on the composition of the organic matter and its interaction with soil properties, we recommend accurate selection and characterization of organic amendments. High temperatures, together with the alternation of submersion and drying, may substantially increase assimilation under dry conditions. Moreover, the dependence of Cd accumulation by rice on the climatic conditions in a particular year should not be overlooked. This work contributes to the understanding of agricultural techniques and of factors affecting Cd mobilization. This would aid in the development of guidelines for rice farmers. The employment of precautions useful for reducing the availability of Cd to rice could allow the production of high-quality rice crops, particularly in areas where rice contamination by Cd is a significant agricultural problem.

Acknowledgements: This research was financially supported by the Regione Lombardia and Ente Nazionale Risi within the projects CEREALOMB 2003 and RISOLOMB 2004.

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