



Effects of alkaline fertilizer and rice cultivation (*Oryza sativa* L.) on remediation of soils polluted with cadmium (Cd)

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ABSTRACT

Objective: The objective of this study was to develop a simple and feasible methodology which makes possible restoration of soils polluted by cadmium using two types of fertilizers (alkaline and conventional), and the technique of phytoremediation (low rice variety, and hyper accumulator of cadmium).

Methodology and results: Its application mode was experimented at Guangdong Provincial Research Center for Environmentally Friendly Fertilizer Engineering Technology in Guangzhou, republic of china. The experiment was carried out in a greenhouse, with an average daytime temperature of 25.5°C, in the city of Guangzhou (China). Polyethylene pots with an inner diameter of 25 cm and a height of 25 cm were each filled with 5 kg of air-dried soil and passed through a 1cmx1cm mesh of a sieve. Four factors were studied, soil contamination level (soil contaminated with Cd, soil with low Cd contamination), pH of the fertilizer (high, pH = 9.5, normal pH 5.8), dosage of the fertilizer applied (low level, medium level, high level) and low Cd accumulating rice variety (Xiangwanxian 12) and hyper Cd accumulating rice (Yuzhenxiang). Soil samples taken from a depth of 0 to 30 cm were delivered to the laboratory for analyses. Results obtained showed that the soil pH was higher with the treatment of alkaline fertilizer compared to that of normal fertilizer. The pH in the soil contaminated with Cd was 5.80 (alkaline fertilizer applied), higher than that of 5.74 (normal fertilizer applied), alkaline fertilizer improve pH soil, cure Cd pollution, bring to rice specific nutrients, at specific moment that rice need to growth and achieve the effect of inhibiting soil pollution, more in hyper accumulation rice than that low accumulation one.

Conclusion and application of results: The results should be helpful in determining appropriate amounts of alkaline fertilizer rice farmers should use to return farm land to productive food crop use, beyond that it was thought cane would be helpful in cadmium uptake from soil. Some rice farmers were reluctant to follow this transition and insisted on growing their rice for survival, alkaline fertilizers can better stop soil pollution and increase the soil pH value. The application of alkaline fertilizer achieves the effect of "controlling Cd pollution with fertilizer".

Key words: alkaline fertilizer, pH, Cd, normal pH fertilizer, accumulation

INTRODUCTION

Cadmium (Cd) is a toxic metal recognized throughout the world, due to its high bio-toxicity for both humans and plants (Aoun, 2009). It is a primary soil pollutant that accumulates in the soil as a result of anthropogenic actions such as Zinc (Zn) extraction, metal smelting and industrial wastewater irrigation (Phaenark *et al.*, 2009; Bauddh *et al.*, 2016), which represents a real human health problem. For example, Itai-itai disease appeared after the population ingested contaminated rice with Cd in 1970 in Japan (Franz *et al.*, 2008; Kobayashi *et al.*, 2008). Studies have shown that 70 to 80% represents the rate of human contamination by Cd through the absorption of vegetables grown on the ground (Olsson *et al.*, 2002; Wang *et al.*, 2016; Yang *et al.*, 2017). Ingestion of large amounts of Cd causes severe damage to vital organs, like the lungs and liver, and is the source of cancer and other fatal health problems (Adriano, 2001; Moynihan *et al.*, 2017). Consequently, reducing the concentration of Cd in the soil is imperative to preserve human health (Miquel, 2001). Phytoremediation is an inexpensive and environmentally friendly technique that reduces the toxicity of heavy metals accumulated in the soil such as Cd (Elizabeth 2005; Ji *et al.*, 2011). Larger, hyper-Cd accumulating vegetables such as *Thalia caerulea* L. (Alligator-fag), *Brassica napus* L. (rape), and *Solanum nigrum* L. (black nightshade) (Zhao *et al.*, 2003; Grispen *et al.*, 2006; Ji *et al.*, 2011) are considered to be potential

phytoremediators of Cd-contaminated soils. Most Cd-accumulating plants are not commercial on a large-scale (Li *et al.*, 2012; Nie *et al.*, 2016). On the other hand, *Oryza sativa* L., is a species known to have a great ability to accumulate Cd because of its ability to grow in marshy environments (Niu *et al.*, 2013; Zhang *et al.*, 2013; Chen *et al.*, 2015; Lu *et al.*, 2015; Zhang *et al.*, 2015 a; b). Cd is a toxic element that have a relatively high risk of transfer from paddy soil to rice grain. Reducing his accumulation in rice grain is important for food safety and human health (Zhao, *et al.*, 2020). This study was conducted to assess the effects of alkaline fertilizer and rice cultivation on Cd reduction in soils. Specifically, this involves evaluating the impact of low-Cd accumulation rice (Hunan late indica 12, Hunan rice) and hyper-accumulation rice (Yujianxiang, Hunan Academy of Agricultural Sciences) on soil pollution. Three research questions was formulated:

- does the application of alkaline fertilizer increase soil pH compared to conventional fertilizer?
- does the application of alkaline fertilizer contribute to the reduction of Cadmium in the soil?
- does the cultivation of rice (*Oryza sativa* L.) have the capacity to accumulate Cadmium stored in the soil?

MATERIALS AND METHODS

Materials: A new type of alkaline NPK fertilizer (28-8-15) of pH = 8.5, recognized for its ability to improve soil acidity, by its slow release (Fan Xiaolin, 2014) was tested. Also evaluated were two varieties of rice namely, Cd lower accumulation rice (Xiangwanxian 12), but also Cd-hyperaccumulation rice (Yuzhenxiang) from the Department of Rice, Ministry of Agriculture of republic of china, the criteria for grain selection was similar size and weight as well as germination percentage that must be greater than 90.

Methods: The experiment was carried out in a greenhouse, with an average daytime temperature of 25.5°C, in the city of Guangzhou (China). Polyethylene pots with an inner diameter of 25 cm and a height of 25

cm were each filled with 5 kg of air-dried soil and passed through a 1cm x1 cm mesh of a sieve. Four factors were studied. The level of soil contamination (soil contaminated with Cd, soil with low Cd contamination), the pH of the fertilizer (high, pH = 9.5, normal pH 5.8), the dosage of the fertilizer applied (low level, medium level, high level) and low Cd accumulating rice variety (Xiangwanxian 12) and hyper Cd accumulating rice (Yuzhenxiang). Soil samples taken from a depth of 0 to 30cm were delivered to the laboratory for analysis. The soil was air dried, then thoroughly mixed and sieved through 1cm x 1cm mesh. These samples were analysed to estimate the following soil parameters: pH, nitrogen content (N), phosphorus (P), potassium (K) and

cadmium (Cd). The properties of two soils tested are presented in Table 1.

Table 1: Soil properties

Soil types	pH	Nitrogen (mg/kg)	Phosphorus (mg/kg)	Potassium (mg/kg)	Cd (mg/kg)
Soil with Cd	4.89	20.7	6.3	52.61	3.09
Soil without Cd	5.07	4.88	3.4	22.61	0.93

Cd dosage: Harvesting took place 125 days after transplanting. Each plant was subdivided into roots, stems and leaves. The different samples were washed with tap water to remove any debris and then rinsed three times with ultra-pure water. For the determination of the dry mass, these plant samples were subsequently dried in an oven at 75°C and then ground to obtain particles with an individual size of less than 0.5 mm. After drying and grinding, the different compartments were weighed separately inside a 0.5 g flask and digested with HNO₃ and HClO₄ concentrated in the proportions of 5: 1 (v/v). The digested samples were diluted with 50 ml of ultrapure water. The Cd concentration of the samples was measured by inductive plasma mass spectrometry (ICP-MS, Elan DRcE, Perkin Elmer and USA).

Sampling and determination indicators: The soil samples were taken as follows: the first soil sample was taken before transplanting and 24 hours after fertilization, the second at 10 days after fertilization, the third at 30 days after fertilization, the fourth, at 60 days after fertilization, the fifth, at 80 days after transplanting and the sixth at harvest.

Methods of collecting plant samples: Before transplanting three samples of replicas of rice plants were collected, the first sample was collected at the transplanting stage, the 2nd sample, 24 hours after the first application of the fertilizer, the 3rd sample, 24 hours after the second top dressing, the 4th sample took place 24 hours after the third top dressing, the 5th sample of plants at 80 days after transplanting, the 6th plant sample was collected at the time of harvest. At the last sample, in addition to the seedling biomass of rice plants

and the biomass of the roots, the sample of each organ including grain (seed), leaves, stems and roots was collected, from the 2nd to the 5th plant sample. Two plants from each hole were collected and 8 seedlings from each pot.

Analytical methods: The methods used were to test the soil pH of each sample, the cadmium of the soil and the cadmium content of the different organs of the rice, the soil of each pot was sampled and homogenized to be air dried, the pH of the soil was measured using a soil / ultrapure water ratio of 1: 5 (w/v) by a pH meter. To assess the soil Cd concentration, the soils were digested with concentrated HCl. The extraction of Cd from soil was based on analysis of the concentrations of Cd available in soils (GB / T 23739-2009). A total of 25 ml of DTPA extract (0.005 mol.L-1DTPA, 0.1 mol.L-1TEA and 0.01 mol.L-1CaCl₂) was added to 5g of air-dried soil in a 50ml polypropylene centrifuge tube. The tubes were shaken end to end for 2 h at 25°C, then centrifuged at 5000 rpm for 10min, the concentrations of Cd and extractable cadmium were determined using inductive plasma mass spectrometry (ICP- MS, Elan DRcE, Perkin Elmer and USA).

Statistical analyses: Analysis of variance (ANOVA) for the various parameters was performed at the 95% level. A comparison of means following Duncan's multiple plaque test was subsequently performed. The data in operational solutions were analysed using the SPSS statistical software package. The various means obtained are presented in the form of histograms with standard deviation bars.

RESULTS

Effects of alkaline and conventional fertilizers on the pH of soils contaminated with cadmium: The results of the analysis of variance indicate significant differences between the fertilizer treatments (conventional and alkaline), concerning the pH values obtained over time at the alpha level = 0.05 (DMRT, P <0.05, n = 5). The

results obtained show that the contribution of alkaline fertilizers increased by 0.97 (SE: 0.34), and that the contribution of conventional fertilizers increased by 0.91 (SE: 0.32) mean of pH value of soils contaminated with cadmium during the growth period (Figures 1 and 2).

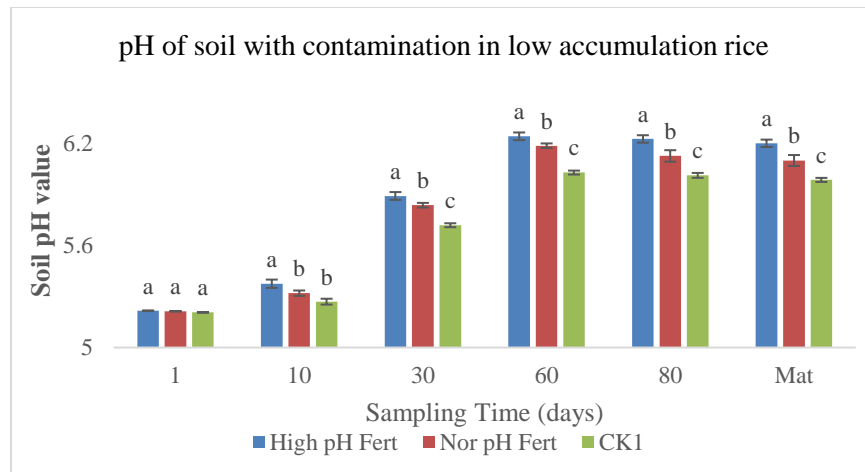


Figure 1: Effect of alkaline and conventional fertilizers on pH in soils with high Cd contamination. Bars represent standard deviation intervals and different letters distinguish means which are significantly different.

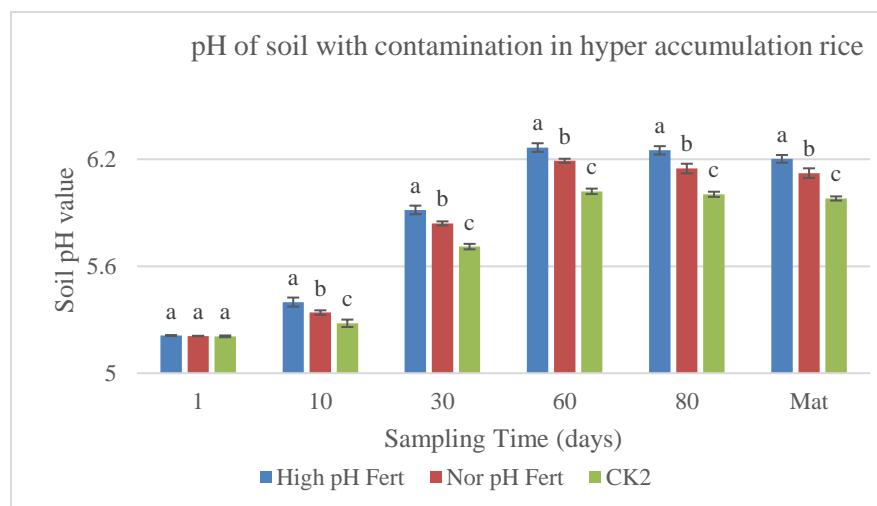


Figure 2: Effect of alkaline and conventional fertilizers on pH in soils with high Cd Contamination. Bars represent standard deviation intervals and different letters distinguish means which are significantly different

The results also revealed that for both varieties of rice, the soil pH was higher ($P < 0.05$) for treatment with alkaline fertilizer than with conventional fertilizer. Regarding the treatment based on alkaline fertilizer applied, the average pH obtained is 5.85 (SE: 0.01) during the growth period, higher ($P < 0.05$) than the treatment based on a conventional fertilizer applied 5.79 (SE: 0.01) for varieties of rice with a high accumulation of cadmium. Likewise, for the variety of rice with low cadmium accumulation, the average soil pH recorded is 5.87 (SE: 0.02) units concerning the treatment with alkaline fertilizer applied, also higher ($P < 0.05$) than the pH 5.80 (SE: 0.02) of the conventional fertilizer treatment applied.

Effects of alkaline and conventional fertilizers on the pH of soils slightly contaminated by cadmium:

Treatment with alkaline fertilizers increased by 0.92 (SE: 0.32) indicate significant differences ($P < 0.05$) compared to the input of conventional fertilizers increased by 0.81 (SE: 0.28) the average pH value of soils contaminated by cadmium during the growing season (Figures 3 and 4). The results obtained show that for both varieties, the soil pH was higher for the treatment with an alkaline fertilizer than that carried out with a conventional fertilizer. The pH was 6.02 units on average for the treatment of an applied alkaline fertilizer ($P < 0.05$), higher than that of a conventional fertilizer applied 5.91 units for varieties of rice with high cadmium accumulation. Similarly, for the low cadmium accumulation rice variety, the soil pH was 5.97 units on average for the alkaline fertilizer treatment applied ($P < 0.05$) higher than the pH of treatment with conventional fertilizer applied, pH 5.86 units.

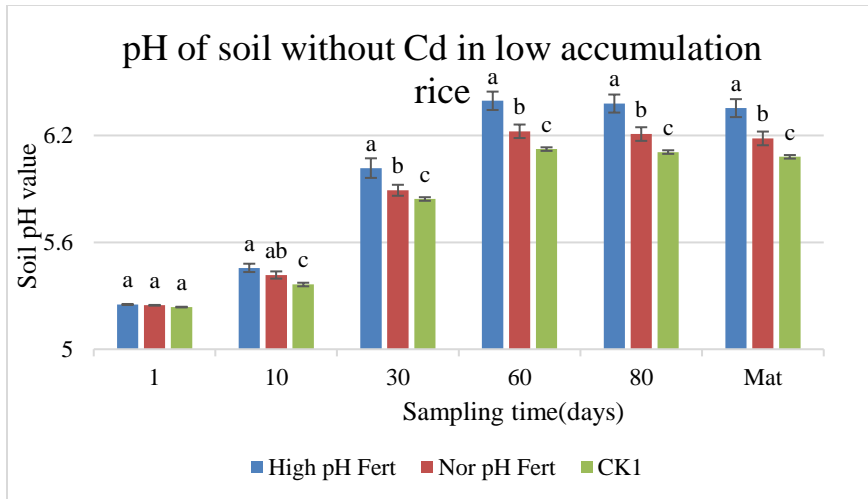


Figure 3: Effects of alkaline and conventional fertilizers on Cadmium accumulation in highly contaminated. Bars represent standard deviation intervals and different letters distinguish means which are significantly different.

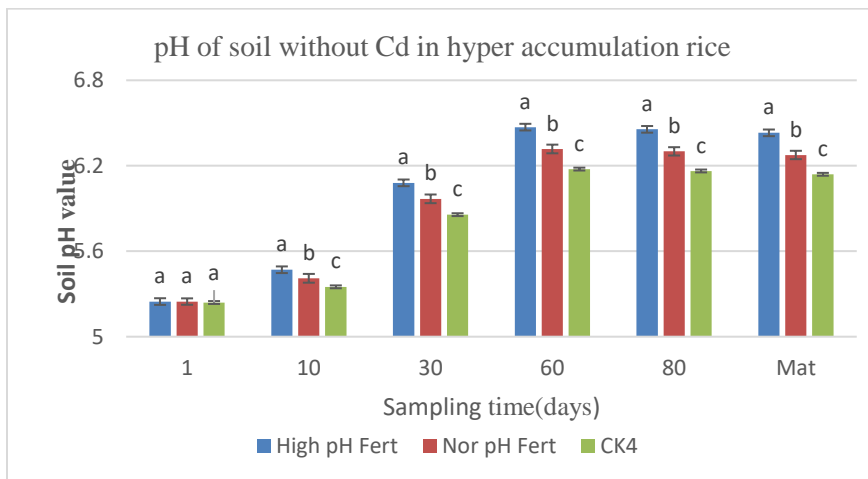


Figure 4: Effects of alkaline and conventional fertilizers on Cadmium accumulation in highly contaminated soils. Bars represent standard deviation intervals and different letters distinguish means which are significantly different.

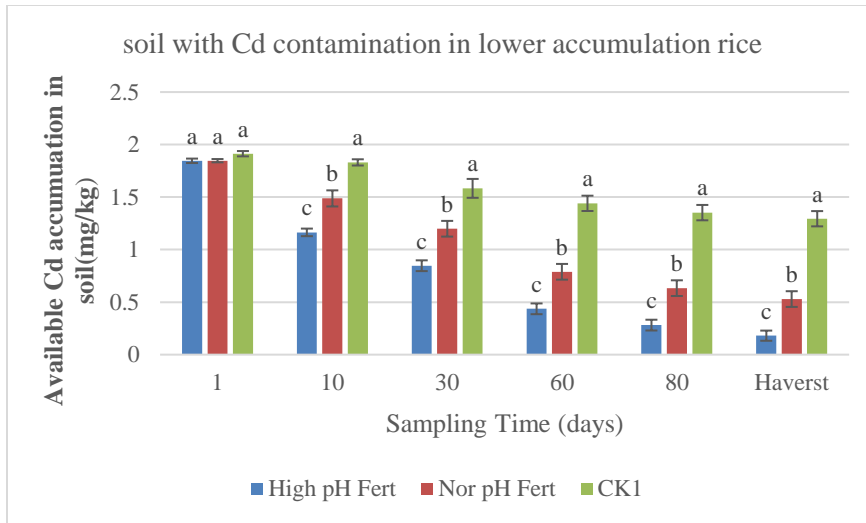


Figure 5: Effect of alkaline and conventional fertilizers on Cd accumulation in soil with high Contamination. Bars represent standard deviation intervals and different letters distinguish means which are significantly different.

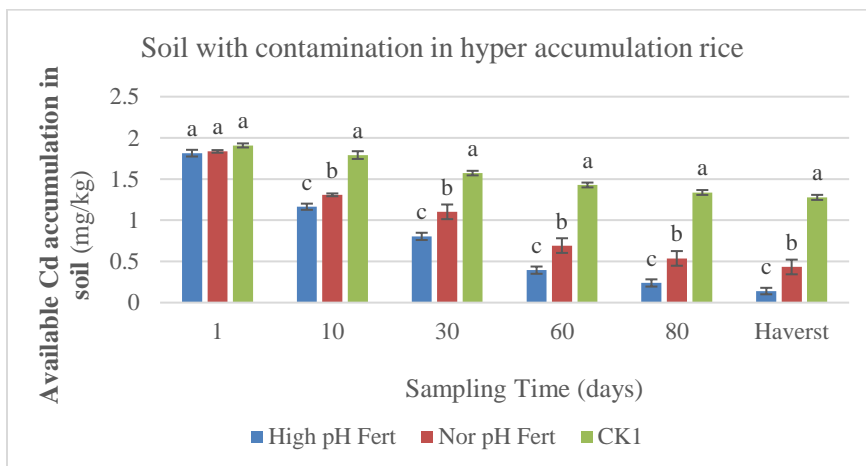


Figure 6: Effect of alkaline and conventional fertilizers on Cd accumulation in soil with high Contamination. Bars represent standard deviation intervals and different letters distinguish means which are significantly different.

Figures (5 and 6) reports the effects of alkaline and conventional fertilizer on cadmium accumulation of contaminated soils, compared to conventional fertilizer treatment, alkaline fertilizer treatment could significantly reduce cadmium in the soil of the medium growth phase to the final stage of rice. The results showed that for both varieties the contaminated soil cadmium was significantly reduced with treatment of alkaline fertilizers indicating significant differences ($P < 0.05$) compared to treatment of conventional fertilizers. For the treatment with alkaline fertilizer cadmium was 0.75 mg / kg (SE: 0.04) on average from the period of growth until maturity considerably reduced ($P < 0.05$) than the cadmium of the treatment with Conventional fertilizer which was 0.98

mg/Kg (SE: 0.06) for the hyper accumulating rice variety. As well, the cadmium in the soil was 0.79 mg / kg (SE: 0.04) on average from the growth phase to the maturity phase with the alkaline fertilizer treatment applied, also lower ($P < 0.05$) than the conventional fertilizer treatment applied which was 1.08 mg/kg (SE: 0.06) for the low accumulation rice variety. Application of alkaline fertilizer could significantly reduce the level or quantity of Cadmium available in contaminated soils.

Effects of alkaline and conventional fertilizers on the accumulation of cadmium in lightly contaminated soils: Figures (7 and 8) illustrates the effects of alkaline and conventional fertilizer on the accumulation of cadmium in contaminated soils. Compared with

conventional fertilizer treatment, alkaline fertilizer treatment would significantly reduce cadmium in the soil from the middle growth stage to the final stage of rice. The results showed that the cadmium in low

contamination soil was significantly reduced with the treatment of alkaline fertilizers compared to conventional fertilizers.

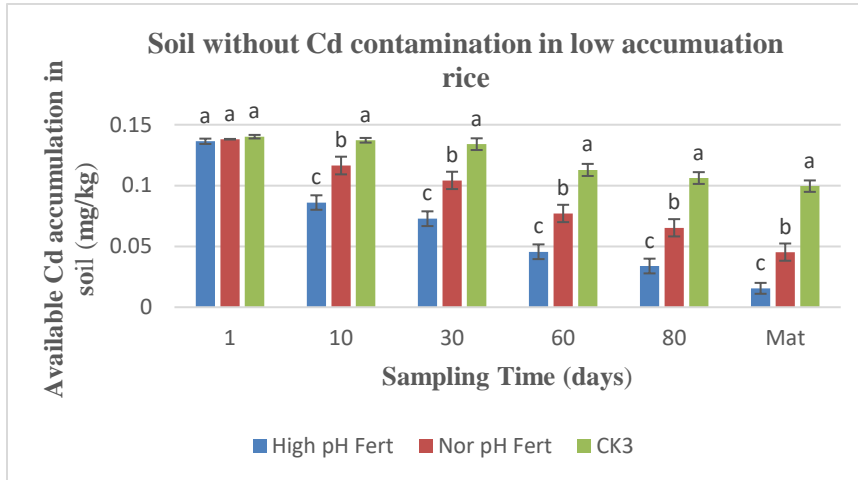


Figure 7: Effect of alkaline and conventional fertilizers on Cd accumulation in soil with less contamination; Bars represent standard deviation intervals and different letters distinguish means which are significantly different.

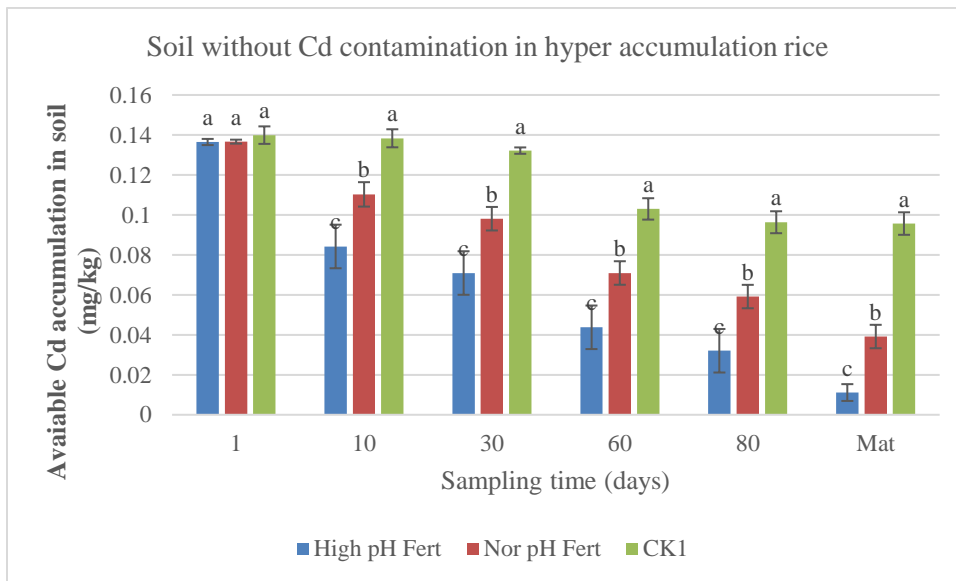


Figure 8: Effect of alkaline and conventional fertilizers on Cd accumulation in soil with less contamination. Bars represent standard deviation intervals and different letters distinguish means which are significantly different.

Regarding the application of alkaline fertilizers, an average value of 0.06 mg/kg (SE: 0.005) was recorded from the period of growth to maturity considerably reduced than the treatment with the application of conventional fertilizers which recorded a value of 0.08 mg/Kg (SE: 0.005) for the hyper accumulation rice variety. Regarding the low accumulation rice variety the

average cadmium value is 0.06 mg / kg (SE: 0.008) from the growth phase to the maturity phase following the application of alkaline fertilizer lower in cadmium concentration in the soil than the application of conventional fertilizers having a value of 0.09mg/Kg (SE: 0.005). Application of alkaline fertilizer could significantly

reduce the level or quantity of cadmium available in contaminated soils.

Effects of alkaline fertilizers on the accumulation of Cd in different organs of rice (mg / kg): Table 2 shows the effects of fertilizers on Cd accumulation in different plant regions of rice. Compared to conventional fertilizers, the Cd content of rice organs decreased with the application of alkaline fertilizer. The results showed for both varieties that the accumulation of cadmium in

different rice organs was reduced with the application of alkaline fertilizers. With regard to the highly contaminated soils, the cadmium accumulates in the different organs of rice, was more reduced during the maturation phase with the application of the alkaline fertilizer applied, than the application of the normal fertilizers for the variety of rice. With strong cadmium accumulation, however, similar results were observed in soil with low cadmium contamination.

Table 2: Effects of alkaline fertilizer on the accumulation of Cd in various organs of rice plant (mg/kg)

Treatments		Organs				
Soils Types	Rice Types	Types Fertilizers	Root	Stem	Leaf	Grain
Soils with Cd contamination	Low accumulation rice	High pH Fert	0.53±0.14c	0.86±0.085c	0.71±0.11c	5.70±0.097c
		Nor pH Fert	1.49±0.29b	1.13±0.07b	1.72±0.29b	6.76±0.31b
		CK1	2.65±0.56a	1.39±0.03a	2.76±0.18a	7.52±0.02 a
	Hyper accumulation rice	High pH Fert	0.48±0.11c	0.8±0.08c	0.67±0.12c	5.64±0.097c
		Nor pH Fert	1.48±0.29b	1.10±0.09b	1.70±0.29b	6.70±0.31b
		CK2	2.64±0.58a	1.43±0.04a	2.76±0.07a	7.50±0.007a
Soils without Cd contamination	Low accumulation rice	High pH Fert	0.05±0.012c	0.22±0.01c	0.53±0.01c	0.51±0.01c
		Nor pH Fert	0.31±0.05b	0.36±0.04b	0.27±0.08b	0.77±0.06b
		CK1	0.93±0.06a	0.42±0.01a	0.45±0.007 a	1.035±0.07a
	Hyper accumulation rice	High pH Fert	0.04±0.01c	0.22±0.01c	0.04±0.01c	0.50±0.01c
		Nor pH Fert	0.30±0.05b	0.36±0.02b	0.29±0.09b	0.76±0.06b
		CK2	0.92±0.07a	0.42±0.01a	0.47±0.06a	1.02±0.07a

Legend: _Nor: Normal, _Nor pH Fert: Normal pH fertilizer, _High pH Fert: High pH fertilizer
CK: Witness (without using fertilizer)

The accumulation of cadmium in the various organs of the rice was less with the application of the alkaline fertilizer compared to the application of conventional fertilizers. Application of alkaline fertilizer promotes

growth (height), increases length, stalk leaves, grain, roots of rice during the growing period, the Cd content of each organ of the rice at harvest was in the order root> stem> grain>leaf.

DISCUSSION

This study found that compared to conventional fertilizers, alkaline fertilizers can increase soil pH and reduce soil Cd. The application of alkaline fertilizer increases the pH of soils with the neutralization time. This study proved that the continued application of alkaline fertilizers within 6 months of growing potted rice will continue to significantly increase the pH value of the soil. Therefore, in agricultural production, fertilizers composed of alkaline fertilizers should be used instead of fertilizers composed of conventional fertilizers, not only can it affect the growth of crops, but also can play an alkaline suppressing property acid soil can achieve the goal of restoring soil neutrality through the long-term

application of alkaline fertilizers, there by resolving the damage caused to the soil by the long-term application of physiological acid fertilizers such as urea. When soil acidity is improved, it is to fundamentally control heavy metal pollution like Cd caused by soil acidification. It was found that exchangeable Cd in soil is significantly ($p < 0.05$) negatively correlated with soil pH, and the content of exchangeable Cd in soil decreases with increasing soil pH (Muhammad *et al.*, 2012; Liang Qiaofeng, 2010; Wang *et al.*, 2015) found that heavy metals in river sediments are mainly released under acidic conditions and their release rate decreases rapidly with increasing soil pH. The pH reduction of the precipitate can increase

the rate of release of Cd from weakly acid-extracted and reducible Cd (Wang Yaping, 2012). The pH value of the leach solution is also inversely proportional to the Cd content of the stone charcoal filtrate (Song Mingyi *et al.*, 2011). The use of lime and other alkaline substances to treat and passivate Cd pollution is used, for example, the application of lime and alkaline wastes in the paddy field reduced the effective Cd content of the soil by 33.2% ~ 38.7% 60 days after transplanting rice, and the Cd content of brown rice was reduced by 27.1% ~ 65.1% compared to the treatment without applying the modifier (Liu Zhaobing *et al.*, 2011). Soil pH value increased with increasing amount of alkaline serpentine powder, Cd content exchangeable in soil with alkaline serpentine powder decreased by 17.42% ~ 53.72% compared to the witness (Wang Xue *et al.*, 2015). Application of lime can reduce the effective Cd activity of soil (Zhou Xiangyu, 2012), and increasing the amount of lime can increase Cd bound to iron manganese oxide and Pb bound to carbonate, reducing thus the bioavailability of soil Cd and Pb (Liang Qiaofeng, 2010). Jiao-Feng Gu (2019) showed that after application of Cd and As fertilizers, the concentrations of the exchangeable fraction and the extraction of TCLP in the soil decreased with the growth of the rice plants. Cd concentrations in rice tissue decreased during tillering, filling and ripening in situ

CONCLUSION AND APPLICATION OF RESULTS

This study is part of a perspective of assessing and eliminating the environmental risks inherent in soils polluted by cadmium, the objective is to develop a simple and feasible methodology, which makes it possible to restore soils polluted by cadmium using two types of fertilizers (alkaline, conventional), and the technique of phytoremediation (low rice variety, and hyper accumulator of cadmium). Analysis of the results obtained after cultivation, confirms the decrease of Cd in soils highly and slightly contaminated by cadmium. This fact demonstrates the effectiveness of alkaline fertilizers, and rice varieties to absorb, fix, and reduce the Cd in soil. Our results showed also the decrease of 0.79mg/Kg (alkaline fertilizer), a decrease of 1.08 mg/Kg (conventional fertilizer) with regard to highly polluted

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sanitation stages, while as concentrations in rice tissue decreased only at ripening stage. The results of (Yasir *et al.*, 2018) showed that the treatment of fertilizers showed the best effects on reducing the phyto availability of Cd and Pb in the soil and absorption by early rice. (Xueling yin *et al.*, 2017) state that the natural sepiolite remediation mechanisms for Cd pollutant in paddy soils could be summarized as the collective effects of soil chemistry and plant physiology. The results of (Huan Jing ke Xue, 2020) show that the application of calcium-magnesium-phosphorus fertilizer improves the effect of reducing the Cd content of brown rice by lime associated with sepiolite. Finally, it can both meet the quality safety requirements of brown rice and increase the yield of brown rice in Cd contaminated paddy soils. Although the application of alkaline substances such as lime can passivate the activity of Cd heavy metals to some extent and reduce their biological efficiency, the long-term application of lime and other alkaline wastes will inevitably result in compaction of the soil and lead to other pollution. Increase the soil load. Previous research results from this research group show that the application of alkaline fertilizers can significantly improve soil acidity and reduce the Cd content of rice while increasing the 1000 grain weight of rice (Zhang Liang *et al.*, 2016).

soils, there is also a decrease of 0.06 mg/kg (alkaline fertilizer), a decrease of 0.08 mg/Kg (conventional fertilizer) for soils slightly contaminated by cadmium. These results showed that alkaline fertilizers can be used as long-term compound fertilizers for growing nutrient crops, as soil amendments to treat acidified soils, solve the problem of Cd soil pollution, unlike to conventional fertilizers, which are prone to defertilization. Ultimately, to fully exploit the nutritional effect of long-acting alkaline fertilizers and the characteristics of soil improvement, it is necessary to understand and master the properties of fertilizers and to apply appropriate fertilization methods to fully exert their effects.

experimental network room of environmental friendly fertilizer engineering technology research center of Guangdong University of South China agricultural University to finish the work.

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