Reprint

International Journal of Experimental Agriculture

(Int. J. Expt. Agric.)

Volume: 10

Issue: 1

January 2020

Int. J. Expt. Agric. 10(1): 38-43 (January 2020) **RESPONSE OF RICE PLANT TO DIFFERENT LEVELS OF CADMIUM UNDER** DIFFERENT SOIL TEXTURE, AND ACIDITY TO PRODUCE SAFE FOOD

M.S. ISLAM, M.J. ABEDIN, A.K.M.M. HOSSAIN, A.K.M.A. BARI AND A.B. MONDAL



RESPONSE OF RICE PLANT TO DIFFERENT LEVELS OF CADMIUM UNDER DIFFERENT SOIL TEXTURE, AND ACIDITY TO PRODUCE SAFE FOOD

M.S. ISLAM¹*, M.J. ABEDIN², A.K.M.M. HOSSAIN³, A.K.M.A. BARI⁴ AND A.B. MONDAL⁵

¹Senior Scientific Officer, Bangladesh Institute of Research and Training on Applied Nutrition (BIRTAN), Rangpur; ²Professor, Department of Soil Science, Bangladesh Agricultural University, Mymensigh; ³Professor, Department of Soil Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur; ⁴Associate Professor, Department of Crop Science and

Technology, Rajshahi University; ⁴Ph.D Fellow, Agrotechnology Discipline, Khulna University.

*Corresponding author & address: Md. Sadequl Islam, E-mail: sadequl2009@gmail.com Accepted for publication on 7 January 2020

ABSTRACT

Islam MS, Abedin MJ, Hossain AKMM, Bari AKMA, Mondal AB (2020) Response of rice plant to different levels of cadmium under different soil texture, and acidity to produce safe food. *Int. J. Expt. Agric.* 10(1), 38-43.

A pot experiment was to evaluate the effects of cadmium on growth, yield and nutrient concentration of rice grown under different soil texture and acidity to produce safe food for his nation. The experiment was laid out in a completely randomized design with three replications. Two levels of cadmium such as 0 and 20 ppm were tested in two types of soils viz. silty loam and silty clay under either neutral (pH 7.0) or acidic (pH 4.3) conditions. There were three replications there were 24 pots for the experiments. The pots were divided into two groups for two different soils (silt loam and silty clay soil). The 12 pots of each soil were filled with 5 kg of each soil. The pots selected for each soil was again divided into two groups for two different acidity level (pH 7 and pH 4.3). Three healthy rice seedlings (BRRI dhan28) of 35 days old were transplanted in each pot. The parameters studied were plant height, panicle length, filled grains panicle⁻¹, 1000 grain weight and yields. Plant samples were analyzed for P, K, S and Cd. Results showed that addition of Cd, decreased the yield and yield contributing characteristics irrespective of soil texture and acidity. The detrimental effects of cadmium on yield were more prominent in silt loam and acid soils. The application of Cd in general decreased the concentration of P in grain and straw. Grain K content decreased but straw K content increased due to addition of Cd. The higher concentration of Cd in grain than straw of rice was found in acidic condition than grown in neutral condition. On the other hand, bioavailability and uptake of Cd were higher in silt loam soil than in silty clay soil. In acidic soils are not feasible to produce our safe staple food. Before rice crop production in acid soil should be properly manage soil pH and used balanced fertilizer to safe from heavy metal contamination of our food as well as our nation. Hence, heavy metals accumulation in rice grains in acidic condition is a big concern in south Asia where people's daily meal largely contains rice or rice based products.

Key words: cadmium, soil texture, acid soil, safe food

INTRODUCTION

Rice is most important staple food for about 50% of the world's population (Muthayya et al. 2014) and it supplies about 30% of the dietary energy and 20% of the dietary protein in Asia (WHO 2002). However, rice may contain significant amounts of contaminants such as arsenic (As), cadmium and lead (Meharg et al. 2013; Watanabe et al. 1996). Serious concerns over heavy metal accumulation in rice grains have been addressed in recent years (Divabalanage et al. 2016). Elevated concentration of As (Abedin et al. 2002) and Cd in rice grain have been reported (Meharg et al. 2013). Heavy metal contamination of food is one of the most important assessment parameters of food quality assurance (Wang et al. 2005; Khan et al. 2008). It has been reported that over 20 million peoples in Bangladesh suffer from kidney disease, especially chronic kidney disease (UNB 2011). The toxic concentration of Cd is usually considered 3 mg kg⁻¹ for soils, 5-10 mg kg⁻¹ for plants and 0.5-1.0 mg kg⁻¹ for the diet of animals (Das 2005). It is common belief that higher Cd intake through food chain may be one of the major reasons for such a high number of kidney patients. At extremely high cadmium concentrations, however, the precipitation of phosphates and carbonates could be expected (Alloway 1995). Under anaerobic conditions, cadmium in soil solution may be controlled by the formation of insoluble sulphides (Kabata-Pendias and Mukherjee, 2007). The above discussion indicated that the availability and uptake of Cd are influenced by a number of factors such as texture, pH, Cd content of soil, organic matter, redox potential, fertilizers and irrigation etc (Jahiruddin et al. 2017). Research work on Cd is very limited in our country. Halim et al. (2015) reported higher concentrations of heavy metals in rice grains from contaminated soils. The present study was undertaken to determine the levels of Cd in irrigated rice in low pH soils and to assess its risk exposure to through typical safe rice production (daily dietary intake levels) of Bangladeshi adults.

MATERIALS AND METHODS

A pot experiment was conducted at the net house of the Department of Soil Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during the Boro season of 2013 to evaluate the effect of cadmium on growth, yield and nutrient concentration of boro rice. The boro rice BRRI dhan28 was considered the research work.

Plant material: After threshing the rice grain and straw weights were recorded per harvested pot. Representative samples of grain and straw were collected and dried in an oven at 65°C.

Soil texture and Acidity: The experiment was conducted with two different soils, silt loam soil of (Bangladesh Agricultural University farm Soil) the river old Brahmaputra flood plain and silty clay loam soil of Kushdaha, Nawabgonj, Dinajpur soil. Soil sample collected and prepared as per the scientific procedure. The pH of the soil

Islam et al.

was adjusted at desired level (4.3 and pH 7) by adding sulfuric acid or lime almost 90 days before starting the experiment. An amount of 500 g soil was preserved for initial physical and chemical analysis. The physical and chemical properties of the soil are presented in Table 1.

Characteristics	BAU farm soil	Dinajpur soil	
Mechanical fractions			
Sand (%)	17.36	21.36	
Silt (%)	68	50	
Clay (%)	14.64	28.64	
Textural class	Silt loam	Silty clay	
pH	7.0	4.3	
Organic carbon (%)	1.08	1.05	
Total nitrogen (%)	0.13	0.08	
Available phosphorus (mg/kg)	8.0	7.03	
Available potassium (me100g ⁻¹)	0.08	0.11	

Table 1. Physical and chemical characteristics of Brahmaputra and Dinajpur soil

Cadmium levels: Here S, L, C, N, A and Cd indicates silty, loam, clay, neutral, acidic soil and cadmium respectively. The source for Cd was Cd(NO₃)₂.4H₂O. All the pots received 100 ppm N, 25 ppm P, 40 ppm K and 25 ppm S from urea, triple super phosphate, muriate of potash and gypsum, respectively.

Pot Preparation: Plastic pots (10 L) were divided into two groups for two different soils. The twelve pots of each group were filled with 5 kg of each soil. Again each of two groups was divided into two groups for pH (7 and 4.3). The experiment under CRD and there were altogether 24 pots comprising of 8 different treatments of cadmium with 3 replications (Table 2).

Table 2. Description of the experimental treatments studied in this study

Symbol	Soil texture	Acidity Cd levels (ppi		
SLACd ₀	Silty loam	Acidic (pH 4.3)	0	
SLACd ₂₀	Silty loam	Acidic (pH 4.3)	20	
SCACd ₀	Silty clay	Acidic (pH 4.3)	0	
SCACd ₂₀	Silty clay	Acidic (pH 4.3)	20	
SLNCd ₀	Silty loam	Neutral (pH 7.0)	0	
SLNCd ₂₀	Silty loam	Neutral (pH 7.0)	20	
SCNCd ₀	Silty clay	Neutral (pH 7.0)	0	
SCNCd ₂₀	Silty clay	Neutral (pH 7.0)	20	

Fertilizer management: All the pots received 100 ppm N, 25 ppm P, 40 ppm K and 25 ppm S from urea, triple super phosphate, Muriate of potash and gypsum, respectively. The amounts of all fertilizer like N, P, K, S as per FRG 2005 on STB. Nitrogen was added in three equal splits at transplanting, 45 and 60 days after transplanting.

Cultivation procedure: One hill consisting three healthy seedlings of thirty-five days old were transplanted in each of the pots on 1st March, 2013. Six cm water was added and allowed to dry until where hair cracking was observed. This process was continued up to panicle initiation stage. Weeding and loosening of soils around the hills were done when felt necessary. Top dressing of urea was done at the maximum tillering and panicle initiation stages. At the grain filling stage, the pots were covered with net to protect the grains from the attack of bird. The crop was harvested at full maturity on 31st May 2013 each pot separately for taking data on yield and yield parameters at laboratory. Recorded all agronomical and chemical data by standard method.

Determination of mineral PK and Cd in soils and plants:

Phosphorus: one ml digest (both in grain and straw) from 20 ml extract was taken in a 50 ml volumetric flask. Then the samples were shaken with 0.5 M NaHCO₃ solution at pH 8.5 following Olsen method (Olsen *et al.* 1954). The extracted phosphorus was determined by developing blue color by $SnCl_2$ reduction of phosphomolybdate complex and measuring the intensity of color spectrophotometrically at 660 nm wavelength.

Potassium: Two ml of digest each of grain and straw was taken and diluted 50 ml. The K was determined from the extract by using flame photometer.

Sulphur: Two ml digest (both in grain and straw) was taken in a 50 ml beaker. One ml acid seed solution (20 ppm S as K_2SO_4 in 6N HCl) and 0.5 g BaCl₂ crystals were added in it. The intensity of turbidity was measured by spectrophotometer at 420 nm wave length. (Page *et al.* 1989).

Cadmium: Total cadmium concentration was determined from the digest by SHIMADJU AA 7000 atomic absorption spectrophotometer.

Statistics: Data were analyzed statistically using analysis of variance (ANOVA) to examine the treatment effects and the mean comparisons of the treatments were made by the Duncan's Multiple Range Test (DMRT). Correlation statistics were performed to examine the interrelationship among the plant characters under study.

RESULTS

Results on yield and yield contributing characters such as plant height, panicle length, number of tillers pot⁻¹, number of grains panicle⁻¹, 1000 grain weight, and nutrient contents and nutrient uptake by rice have been presented in this chapter (Table 3).

Treatments*	Tiller Nos.	Plant height (cm)	Panicle length (cm)	1000-grain weight (g)	Weight of grain g pot ⁻¹ (g)	Weight of straw g pot ⁻¹ (g)
SLACd ₀	23.3bc	99abc	19.68ab	18.50ab	29.13cd	30.73cd
SLACd ₂₀	20.6c	95c	18.7b	16.12b	22.16e	24.48e
SLNCd ₀	40a	104.67ab	21.58a	19.48ab	46.34a	48.05a
SLNCd ₂₀	31ab	100abc	20.58ab	17.54ab	37.61b	38.95b
SCACd ₀	29.44bc	100.5abc	19.95ab	18.89ab	31.19c	33.9c
SCACd ₂₀	25.66bc	96bc	19.57ab	17.09ab	26.44d	29.02de
SCNCd ₀	31.33ab	106a	20.54ab	19.91a	36.6b	39.24b
SCNCd ₂₀	24.33bc	101.36abc	19.74ab	18.86ab	31.06c	34.63bc
P value	0.000	0.010	0.045	0.020	0.000	0.000

Table 3. Single effects of texture, pH and Cd on agronomical parameters of rice var. BRRI dhan28

*Treatment symbols are elaborated in Table 2.

Values within columns followed by same letters do not differ significantly at 5% level by DMRT

Grain and straw yields of BRRI dhan28:

The grain and straw yields of rice varied significantly due to different treatments combinations under study (Table 3). The result varied from 22.16 to 46.34 g/pot for grain and 24.48 to 48.05 g/pot for straw irrespective of treatments. The percent reduction in yield indicated that grain yield was more affected by Cd than straw yield. The mean effect of Cd on grain and straw yields also showed remarkable variation due to soil texture and acidity (Table 3). In both cases (texture and acidity) the addition of Cd decreased the yield of rice. Considering the effects of Cd on grain and straw yields of rice in neutral and acid soil it appears from the result that the yields were higher in neutral soil than in acid soil but the rate of reduction in yield due to Cd was higher in acidic condition than in neutral condition. The lower yield as found in acid soil indicate there Cd toxicity was higher under acidic condition than in neutral condition (Table 3).

Plant analysis: Grain and straw samples collected at the time of harvest were analyzed for P, K and S. Result showed a remarkable variation between grain and straw samples as well as between treatments (Table 4). The concentration of P in grain straw decreased over control due to application of Cd in acidic silt loam soils. Similar trend was also observed under neutral condition of the same soil except the straw sample where addition of Cd did not show any effect on straw P. In silty clay soil, like silt loam soil, the addition of Cd decreased the grain and straw P over control in acidic condition as a result yield decrease from other soils. But under neutral condition, the addition of Cd in the same soil did not show any effects on grain P but in case of straw P an increase was noted due to added Cd. In general, the concentration of P was higher in grain than in straw samples. Another observation over the data revealed that under acidic condition the grain P was higher in silt loam soil. But under neutral condition the grain P concentration was higher in silty clay soil than in silt loam soil. The concentration of K in grain and straw also show remarkable variations between the soils. Different treatments did not show any definite trend on grain and straw K. In general, the concentration was higher in silt loam soil was higher than in silty clay soil. The concentration of S in grain and straw showed some effects of Cd, texture as well as soil acidity. But no definite trend of plant S was noted due to different treatment.

Islam et al.

Treatments*	%P		%K		%S	
	Grain	Straw	Grain	Straw	Grain	Straw
SLACd ₀	0.204a	0.11a	0.359a	3.193a	0.244abcd	0.229ab
SLACd ₂₀	0.193a	0.107a	0.318a	3.677a	0.298a	0.248a
SLNCd ₀	0.168a	0.1a	0.267a	3.575a	0.258abc	0.215ab
SLNCd ₂₀	0.148a	0.1a	0.267a	3.856a	0.196cd	0.187bc
SCACd ₀	0.191a	0.137a	0.257a	0.0847b	0.27ab	0.217ab
SCACd ₂₀	0.177a	0.108a	0.206a	1.127b	0.258abc	0.203ab
SCNCd ₀	0.185a	0.104a	0.267a	1.204b	0.177d	0.132c
SCNCd ₂₀	0.19a	0.178a	0.277a	1.484b	0.229bcd	0.187bc
P value	0.897	0.448	0.175	0.000	0.002	0.002

Table 4. Interaction effects of Cd, texture and acidity on plant analysis of rice

*Treatment symbols are elaborated in Table 2.

Values within columns followed by same letters do not differ significantly at 5% level by DMRT

Cadmium concentration

The concentration of Cd in grain and straw showed a strong variation due to levels of Cd, soil texture as well as soil acidity. In general the addition of Cd increased its concentration in grain and straw samples where the values obtained from straw samples were 5 to more than 13 times higher than that of grain irrespective of treatments (Table 5). An observation over the data reveals that the concentration in grain was almost 2 times higher in silt loam soil than in silty clay soils both under Cd treated and untreated pots. This was probably due to higher adsorption of Cd by clay particles of silty clay soil than silt loam soil. Similar trend was also noted in case of straw. A striking difference in grain and straw Cd concentration was also noted due to acidity of soils. Both in Cd treated and untreated pot the concentration was higher in acid soils than in neutral soils (Table 5).

Cadmium uptake: The uptake of Cd by grain and straw also varied considerably due to different treatment combinations.

Treatments*	Cadmium con	nc. (mg pot ⁻¹)	Cadmium uptake (µg pot ⁻¹)		
	Grain	Straw	Grain	Straw	
SLACd ₀	0.46c	2.48d	13.40c	76.21b	
SLACd ₂₀	6.028a	65.99a	133.58a	1615.44a	
SLNCd ₀	0.178c	1.90d	8.25c	91.30b	
SLNCd ₂₀	4.55a	30.64b	171.31a	1193.23ab	
SCACd ₀	0.244c	2.12d	7.61c	71.70b	
SCACd ₂₀	3.81ab	21.27c	100.74	617.11ab	
SCNCd ₀	0.108c	1.42d	3.95c	55.52b	
SCNCd ₂₀	1.028bc	6.31d	31.93c	218.34b	
P value	0.000	0.000	0.000	0.007	

Table 5. Effects of Cd concentration (mg pot⁻¹) and Cd uptake (µg pot⁻¹) in rice

Values within columns followed by same letters do not differ significantly at 5% level by DMRT

The concentration of grain and straw and the yields mainly influenced the uptake of cadmium. In general, the uptake increased due to application of Cd showing a good relation with concentration. It appears that the rate of increase in cadmium uptake due to its application was lower than the increase in concentration in all cases. This variation in rate of increase between concentration and uptake due to texture and acidity followed the trend of concentration (Table 5).

DISCUSSION

The pot experiment on "availability and accumulation of cadmium in rice grown in soil of variable properties" was conducted to see the effects of cadmium on yield and yield contributing characters of rice under variable texture and soil acidity. A remarkable variation on the effects of Cd was noticed due to variation in soil properties. Cadmium has been marked as poisonous heavy metal both for plants and animals (Holmgren *et al.* 1993; Das *et al.* 1997). The addition of Cd decreased the growth, yield and yield contributing characters. There are many reports that application of Cd can affect crop growth and yield. According to Sarkunan *et al.* (1991), rice yield drastically decreased due to the application of 20 ppm Cd. Similar results were reported by Alloway (1995); Dixit and Gupta (1992). Cadmium concentration above 20 ppm in soil reduces rice plant biomass by

poisoning the roots and restricting growth (Herawati *et al.* 2000). Plant analysis showed higher concentration of Cd in grain and straw of Cd treated pots. This finding also supports the negative effects of Cd on crops. Adverse effects of heavy metals on crops have been reporter by Singh and Nayyar, 1994; Sarkunan *et al.* (1991).

The higher values of tiller, plant height, field grain, panicle length found in Cd treated silty clay soil also support it (Dube and Zbytniewski, 2001). The lower reduction of grain yield due to addition of Cd in silty clay soil (15%) than in silt loam soil (20.8%) also support that the added Cd was adsorbed by the clay particles as a result its toxic effect 3as reduced. However, the lower grain and straw yields found in silty clay soil than silt loam soil was due to unfavorable physical condition.

Soil acidity or pH also shows a remarkable influence on the effects of Cd. The parameter under study was in general higher in neutral condition (pH 7) than in acidic condition (pH 4.3). The low yield was showed in low pH soil as well as Cd added plot. The previous work revealed that higher acidity increased the solubility as well as toxicity of heavy metals (Lacatusa *et al.* 1996). In the present study lower values of different parameter as found under acidic condition than neutral condition was probably due to higher bioavailability of Cd. Plant analysis showed high concentration of Cd in grain and straw of rice grown under acidic condition than grown in neutral condition. This increased Cd uptake under acidic condition was mainly the reasons for the obtained low pH values in acid soil. So it is clearly shown that low pH soil is not feasible for healthy and safe rice production in Bangladesh.

CONCLUSION

In the light of the results obtained from the present study and the above discussion the following conclusion may be made:

- i. Addition of Cd decreased the yield and yield parameters of rice
- ii. The concentration of Cd was much higher in straw than in grain
- iii. Bioavailability and uptake of Cd was higher in acid soil
- iv. Soil acidic condition are not feasible to produce safe and healthy rice production for a nation

REFERENCES

Abedin MJ, Cotter-Howels J, Mehrag AA (2002) Arsenic uptake and accumulation in rice (*Oryza sativa* L.) irrigated with contaminated water. Plant and Soil. 240:311-319.

Alloway BJ (1995) Cadmium. In Heavy Metals in Soils (2nd edn.). (ed. B.J. Alloway). London: Blackie Academic and Professional 368.

Das DK (2005) Inorganic Pollutants: Their Behavior in soils and plants and Possible Mitigation Options. Dept. of Agril. Chemistry and Soil Science BCKV, Mohanpur, Nadia.

Das P, Samantaray S, Rout GR (1997) Studies on cadmium toxicity in plants: a review. *Environmental Pollution* 98, 29–36.

Dixit ML, Gupta VK (1992) Influence of Soil Applied Cadmium on Growth and Nutrient Composition of Plant Species. *Journal of Indian Society Soil Science* 40, 878-880.

Diyabalanage S, Navarathna T, Abeysundara HTK, Rajapakse S, Chandrajth R (2016) Trace elements in native and improved paddy rice from different climatic regions of Sri Lanka: implications for public health. SpringerPlus 5:1864 (DOI 10.1186/s40064-016-3547-9)

Dube A, Zbytniewski T (2001) Adsorption and Migration of heavy metals in soil. *Polish Journal of Environment studies*. 10, 1-10.

FRG, BARC (Bangladesh Agricultural Research Council) (2005) Fertilizer Recommendation Guide. Soil Publication no. 45. Farmgate, Dhaka.

Halim MA, Majumder RK, Zaman MN (2015) Paddy soil heavy metal contamination and uptake in rice plants from the adjacent area of Barapukuria coal mine, northwest Bangladesh. *Arab J Geosci.* 8:3391-3401.

Herawati N, Suzuki S, Hayashi K, Rivai IF, Koyama H (2000) Cadmium copper and zinc levels in rice and soils of Japan, Indonesia, and China by soil type. *Bulletin of Environment Contamination and Toxicology* 64, 33-39.

Holmgren GGS, Meyer MW, Chaney RL, Daniel RB (1993) Cadmium, lead, zinc, copper, and nickel in agricultural soils of the United States of America. *Journal of Environmental Quality*. 22, 335–348.

Jahiruddin M, Xie Y, Ozaki A, Islam MR, Nguyen TV, Kurosawa K (2017) Arsenic, cadmium, lead and chromium concentrations in irrigated and rain-fed rice and their dietary intake implications. *Australian J. of Crop Sci.* 11(07), 806-812.

Kabata-pendias A, Mukherjee AB (2007) Trace Elements from Soil to Human. Berlin Springer-Verlag.

Islam et al.

Khan S, Cao Q, Zheng M, Huang YZ, Zhu YG (2008) Health risk of heavy metals in contaminated soils and food crops irrigated with waste water in Beijing, China. Environ Pollut. 152:686-692.

Lacatuşa R, Rauţa C, Cârstea S, Ghelase I (1996) Soil-plant-man relationships in heavy metal polluted areas in Romania. *Applied Geochem.* 11, 105–107.

Meharg AA, Norton G, Deacon C, Williams P, Adomako EE, Price A, Zhu Y, Li G, Zhao F, McGrath S, Villada A, Sommella A, Magala P, de Silva CS, Brammer H, Dasgupta T, Islam MR (2013) Variation in rice cadmium related to human Exposure. Environ Sci Technol. 47:56135618.

Muthayya S, Sugimoto JD, Montgomery S, Maberly GF (2014) An overview of global rice production, supply, trade, and consumption. Ann. NY Acad Sci. 1324:7-14.

Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture Circle. pp. 939.

Page AL, Miller RH, Keeney DR (1989) Methods of Soil Analysis part 2, 2nd edition. American Society of Agronomy, Madison, Wisconsin, USA. pp. 245-256.

Sarkunan V, Misra AK, Nayar PK (1991) Effect of compost lime and phosphorus on cadmium toxicity in rice. *Journal Indian Society Soil Science* 39, 595-597.

Singh SP, Nayyar VK (1994) Accumulation characteristics of cadmium in selected forage species. *Journal of Indian Soil Science* 42, 96-100.

UNB (2011) 2 crore people suffering from kidney diseases in Bangladesh: experts. Available from: <u>http://www.unbconnect.com/component/news/task- show/id- 43116</u>. Accessed Sept. 26, 2011.

Wang XL, Sato T, Xing BS, Tao S (2005) Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. Sci. Total Environ. 350:28-37.

Watanabe T, Shimbo S, Moon CS, Zhang ZW, Ikeda M (1996) Cadmium contents in rice samples from various areas in the world. Sci Total Environ. 184:191-196.

WHO (2002) Diet, nutrition and the prevention of chronic diseases. World Health Organization/Food and Agriculture Organization of the United Nations, Geneva.