

Analytical Evaluation of Aflatoxins and Heavy Metals Contamination of Some Paddy fields in the Middle Euphrates Iraq

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Abstract

Total aflatoxins, aflatoxin B1, and heavy metals, lead (Pb) and cadmium (Cd), were estimated in some paddy, soil, and irrigation water sample taken from seven fields of Al-Diwaniyah and Al-Najaf Al-Ashraf provinces of Iraq. Fifty-one paddy samples, 45 soil samples, and 9 irrigation water samples were analyzed for total aflatoxins and aflatoxine B1 while, 32 samples of paddy, 24 samples of soil, and 6 samples of irrigation water were investigated for heavy metals. Five paddy samples were processed to produce rice at 32 degrees of whiteness. High performance liquid chromatography (HPLC) was used to detect aflatoxins, and Atomic Absorption Spectrophotometer (AAS) was used to detect heavy metals. Most of the paddy samples contained total aflatoxins and aflatoxin B1, which ranged from N.D-4.6770 µg/kg for total aflatoxins, N.D-1.8593 for aflatoxin B1. Two out of 51 paddy samples were overpassed the permissible limit for total aflatoxin. All produced rice samples were free of total aflatoxin and aflatoxin B1. The ranged of Lead was from 84-190 µg/kg for paddy samples, 90-147 µg/kg for rice samples and 77-155 µg/kg for soil samples. The results of cadmium concentrations ranged from 25-66 µg/kg for paddy samples, 59-72 µg/kg for rice samples and 20-99 µg/kg for soil samples. The manufacturing process of rice had a role in reducing the concentration of total aflatoxins and aflatoxin B1, while it had no specific role in reducing lead and cadmium pollution. In conclusion, paddy produced in the study area was mostly safe in terms of its content of aflatoxins and heavy metals, Pb and Cd, for 2021 season.

Keywords: Heavy metals, aflatoxins, Paddy, Rice, HPLC, Atomic Absorption Spectrophotometer

1. Introduction

Mycotoxins are secondary metabolites from molds that can contaminate foods and cause serious health problems in consumers (Oliveira 2018). Grains are very susceptible to mycotoxin contamination and their metabolites have been linked to toxicological effects ranging from mild gastroenteritis to cancer disease (Anjorin 2021). Aflatoxins (AFs) are mycotoxins formed by *Aspergillus* species such as *A. parasiticus* and *A. flavus* that are toxic to humans and animal. Aflatoxigenic molds can cause a decrease in production, a loss of nutritional value, and a diminution of the market value of agricultural products. (Ren et al. 2020) Aflatoxin B1 (AFB1) is the most common and carcinogenic member of the aflatoxin family. The International Agency for Research on Cancer (IARC) has classified AFB1 as a Class I carcinogen (Huiying Li 2018). Aflatoxin B1 is produced by *Aspergillus flavus* and related fungi that grow on improperly stored foods, such as corn, rice, and peanuts (Smela et al. 2001).

Heavy metals are metals with densities greater than 5 g/cm³. Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), and Lead (Pb) are some examples of heavy metals, and they are non-degradable toxic elements (Kayastha 2015). Heavy metals are carcinogenic and mutagenic compounds in nature (Gola et al. 2016). Heavy metals are highly

persistent in the atmosphere because they are non-biodegradable or non-thermally degradable, which is allowing them to accumulate to toxic levels (Chandrajith, Dissanayake, and Tobschall 2005). Heavy metals are harmful for human and animals when they are present in food and feed at high concentration. For example, lead causes headaches, nausea, vomiting, decreasing hemoglobin synthesis, impaired renal function, deafness, blindness, and retardation. While Cadmium causes organs toxicity such as liver and kidney, pulmonary, osteoporosis, carcinoma. (Feng et al. 2020; Zhang et al. 2021). Some metals such as calcium, iron, magnesium, and zinc are essential nutrients that become harmful and toxic when their concentrations exceed recommended standards (Lu et al. 2015).

Rice is one of the most important staple foods worldwide, and it is a staple food for more than half of the world's population. Rice is the second most commonly used grain after wheat. Rice is grown on an area of approximately 167 million hectares under diverse climatic conditions in tropical and subtropical regions. It is cultivated in 144 out of 196 countries around the world (Ali and Jakhsi 2020; Song and Mansaray 2018).

For Iraq, rice is a strategic and crop, a staple food for the majority of the Iraqi people. The Amber variety is one of the most favorite types of local Iraqi rice, and it is characterized by high quality in

terms of flavor and aromatic character.(Anon 2020c). Rice crops are subjected to be infected by many factors that may be biotic (pathogens) or abiotic (environmental factors) (Hassanein, Shoala, and Gouda 2018). These diseases either attack rice plants at a growth stage or infect rice grains after harvest, and in both cases, the quantity and quality losses in rice crops have occurred(Fageria 2013). Therefore, proper pre-harvest and post-harvest management of the rice is crucial to maintain the grain quality and safety(Soraya Shafiekhani 2018). Contamination occurs mostly before harvesting, however, the storage of rice is an important step in terms of keeping rice in a good quality and safe(Joshi et al. 2015). Therefore, the aim of this study was to determine the safety of the paddy and rice from fields before storage. Also, determining the source of the contamination. The study included a series of analyses carried out to detect contamination of aflatoxins and heavy metals in paddy, soil, and irrigation water of several fields in Iraq. In addition, the effect of the paddy processing on the contamination level was detected too.

Sample collection

Fifty-one paddy samples, 51 soil samples, and nine irrigation water samples were collected from seven paddy fields of Al-diwania and Al-Najaf (Alfurat Alawsat Area) governorates/Iraq during November 2021. Some paddy samples were taken from 1, 2, 3, 5, and 7 fields before harvesting, which were nine samples of paddy and nine samples of soil of each field. The unharvested fields. were divided into three parts depending on the distance of the irrigation source, where part 1 was the nearest to the water source, part 2 was in the middle of a field, and part 3 was the farthest area from the irrigation water source. Three paddy and soil samples were collected from each part. For field one, there were anber and yassamine varieties planted beside each other, therefore, six samples of anber, and 9 samples of yassamine were collected to detect the variety effect on the paddy contamination. for fields, 4 and 6, which were harvested, three paddy samples were taken from the paddy bulk of each field, while three soil samples of fields, 4 and 6, were collected, sample of each part of the three parts of a fields as divided (Fig 1. Three sources of irrigation water were considered. The first source was for fields 1 and 2. The second source represented field 3, and the third source represented fields 4, 5, and 6. Three samples were taken from each source. There were some difficulties to obtain samples for the irrigation water of field 7. The first and second sources of irrigation water were taken from a branch of Euphrates River, while the third source was taken from a pump machine belonging to the field. This pumping machine transports water to the fields from one of the

branches of the Euphrates River.

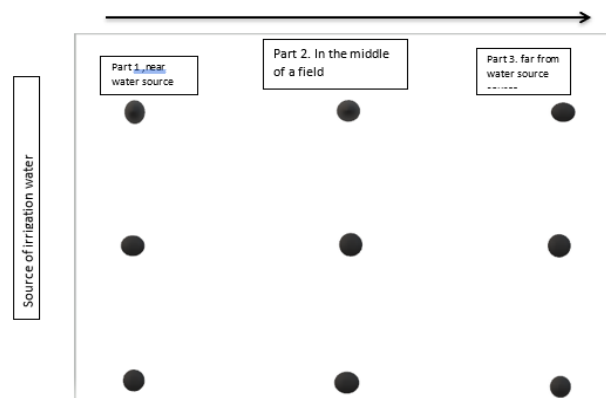


Figure 1. Places of collecting paddy and soil samples of unharvested fields.

Estimation of Total Aflatoxin and Aflatoxin B1

Total aflatoxins and aflatoxins B1 was measured by weighing 0.1g of milled rice, paddy or soil sample was weighed and 10 mL of 50% ammonium acetate, 20% methanol, and 30% acetonitrile solution was added at pH=4. The solution was placed in an ultrasonic device for 30 min then the solution was filtered by using filter paper (1) and filtered again by using microfilters (0.45 μ m). The solution was injected after filtration through the injection system of Reverse phase high-performance liquid chromatography device in a stream of mobile phase (50% acetyl acetate, 30 % acetonitrile and 20% methanol) at pH=4 on a column type ODS - C18 (15 cm L * 0.46 cm I.D. and 5 μ m particle size diameter) at a flow rate 1.5 ml/min. The measurement time was 5 min, and the UV detector was used. Ten μ l of total aflatoxin standard 1 ng/mL and aflatoxin B1 standard 1 ng/ml, were injected through the HPLC injection system under optimal conditions. Four partially separated peaks were appeared for total aflatoxin standard, which refers to G1, G2, B1and B2, while one sharp peak was appeared for aflatoxin B1 standard. The area of the peaks was calculated to determine the total aflatoxin and aflatoxin B1 for samples(Mazaheri 2009;Albert et al. 2021).

Heavy metals measurement

Irrigation water samples were filtered by using a Whatman filter paper(1).Then few drops of strong nitric acid were added to samples (60 ml) to inhibit metal precipitation, and then Atomic Absorption Spectrophotometer (AAS) was performed to quantify heavy metals for Cd and Pb (Assubaie 2015).

Heavy metals of soil samples were measured by adding

15 ml of 30% HCL and 70% HNO₃ (30:70 ratio) to one gram of dried soil and left-over night. Then the mixture was placed on a hot plate for 45 min in a fume hood (chamber). The solution was quantitatively transferred to a volumetric flask and the volume was completed to 100 ml by adding ion-

free distillate water. The solution was filtered by filter papers#(1) and heavy metals were measured by AAS device (Mankoula et al. 2021).

Heavy metals of paddy and rice samples were measured by placing

10 g of well-ground paddy or rice sample in a crucible and burned in the oven at a temperature of 600 °C for 6 h. Then 5 ml of HCl (5.5M) were added and placed on a hot plate, and 20 ml of HNO₃ (0.2M) was added and left for 90 min. The solution was filtered by filter papers#(1) for measuring heavy metals in the AAS device(Zeng, F. et al 2015).

Atomic Absorption Spectrophotometer Analysis (AAS)

The metals were measured by using an Atomic Absorption Spectrophotometer (AAs; AA-7000 Atomic Absorption Spectrophotometer, Shimadzu, Japan), which was used an air acetylene flame. The AAS measured the elements, Lead (Pb), Cadmium (Cd). The calibration curves for the two metals were created individually using varied concentrations of standard solutions. By running the reagent blanks. The standard solutions were produced right before the analyses. R² of lead standard curve was (0.9992) and for cadmium was (0.9995). The prepared

samples were inhaled into the nebulizer and turned into aerosol before entering the atomizer (dos Santos Costa and Coelho 2021;Habibollahi et al. 2019).

2. Statistical Analysis

One-way analysis of variance (ANOVA) was performed for statistical analysis of data. Tukey's test of means was implemented by using IBM SPSS Statistics –Version 23.0. Significant differences were considered at $\alpha = 0.05$ level.

3. Results

Table 1. shows the average concentration of total aflatoxins and aflatoxin B1 and the standard deviation for the Paddy and soil samples of Al-Diwaniyah and Najaf governorates. All the taken paddy samples of Al-Diwaniyah and Najaf governorates had AFs and AFB1 less than the permissible limit of the World Health Organization (WHO). Also, all soil samples had low amount of and AFB1. The results showed that there was no significant differences between paddy samples and soil samples of different fields for AFs, while there were a significant differences for paddy and soil samples for AFB1 of different fields.

Field number/ Rice variety		Total Aflatoxin		Aflatoxin B1	
		Paddy	Soil	Paddy	Soil
Field1	Yassamin	0.74±1.35a	0.58±0.43a	0.02±0.07b	0.04±0.06c
	Anber	0.92±1.44a	0.58±0.43a	N.D b	0.04±0.06c
Field 2/Rashet		0.29±0.13a	0.45±0.06a	0.24±0.16ab	N.D c
Field 3/Yassamin		0.22±0.38a	0.65±0.19a	0.06±0.05b	0.03±0.08c
Field 4/Anber		0.09±0.03a	0.74±0.14a	0.07±0.06ab	0.06±0.09ab
Field 5/Anber		0.14±0.05a	0.58±0.13a	0.09±0.08b	0.12±0.11ab
Field 6/Yassamin		0.01±0.02a	0.63±0.06a	N.D b	0.04±0.07ab
Field 7/Yassamin		0.82±1.82a	0.15±0.05b	0.38±0.73a	0.14±0.05a
Limits WHO and European standard		4 $\mu\text{g}/\text{kg}$	-----	2 $\mu\text{g}/\text{kg}$	-----

Values are expressed as a mean \pm SD. Means with different letters within the same category and column are significantly different at $P < 0.05$. N.D. Not detected.

Table 2. shows the average concentration of AFs and AFB1 and the of paddy samples that taken from the three parts of field as mentioned in the method section. The results showed that there were some

significant differences between different parts of fields, however, there was no specific pattern. In other words, the differences was not behalf the begging or the end of the fields.

Paddy	Total Aflatoxin			Aflatoxin B1		
	Line 1	Line 2	Line 3	Line 1	Line 2	Line 3
Field 1	0.07±0.12a	N.D a	N.D a	N.D a	N.D a	N.D a
Field 3	0.09±0.01a	0.09±0.02a	0.41±0.65a	0.08±0.01b	0.09±0.03a	N.D c
Field 5	N.D b	0.17±0.05a	0.06±0.05b	N.D b	0.17±0.04a	N.D b
Field 7	2.30±3.20a	0.09±0.02a	N.D a	1.00±1.23a	0.10±0.01a	N.D a
WHO Limits	4 $\mu\text{g}/\text{kg}$			2 $\mu\text{g}/\text{kg}$		

Values are expressed as a mean \pm SD. Means with different letters within the same category and raw are significantly different at $P < 0.05$.

Table 3. Reports the average concentration of AFs and AFB1 and in the soil samples for the three parts of the fields. The results showed that

there were a significant difference between soil samples of the three parts of a field for AFs, but there was no specific pattern. For AFB1, there

were no significant differences between the field's parts.

Table 3. Average concentration of total aflatoxins and aflatoxins B1 (µg/kg) and standard deviation of soil samples (for the three parts of the field) first line close to the water source

Soil	Total Aflatoxin			Aflatoxin B1		
	Line 1	Line 2	Line 3	Line 1	Line 2	Line 3
Field 1	0.53±0.37a	0.48±0.38a	0.71±0.63a	N.D a	N.D a	0.12±0.05a
Field 3	0.54±0.15b	0.64±0.21ab	0.85±0.07a	N.D a	N.D a	N.D a
Field 5	0.66±0.05a	0.46±0.15b	0.61±0.07ab	0.30±0.05a	N.D a	N.D a
Field 7	0.20±0.04a	0.10±0.02a	N.D a	0.20±0.04a	0.10±0.02a	N.D a

Values are expressed as a mean ± SD. Means with different letters within the same category and raw are significantly different at P < 0.05.

Table 4. shows the average concentrations of cadmium and lead and the standard deviation in paddy and soil samples of Diwaniyah and Najaf fields. The concentration of paddy and soil samples did not exceed the permissible limits of

the WHO and the Iraqi Quality Standards (IQS) of cadmium and lead. There were significant differences between paddy and soil samples for the provinces for the concentration of cadmium and lead.

Table 4. The average concentration of cadmium and lead (µg/kg) and the standard deviation of the straw and soil samples of Diwaniyah and Najaf fields

Field number/ Rice variety	Cd		Pb		
	Paddy	Soil	Paddy	Soil	
Field1	Yassamin	44.13±13.69ab	32.25±11.24c	138.38±37.92b	97.50±13.44b
	Anber	35.00±11.312bc	32.25±11.24c	163.00±29.00a	97.50±13.44b
Field 2/Rashet	N.D d	75.75±26.00ab	N.D d	115.00±21.68ab	
Field 3/Yassamin	50.17±9.26a	57.00±4.00b	99.67±8.60c	103.00±10.23b	
Field 4/Anber	N.D d	46.00±28.30c	N.D d	105.00±4.24ab	
Field 5/Anber	37.33±3.01bc	76.00±14.00ab	91.17±4.79c	110.75±14.43ab	
Field 6/Yassamin	N.D d	84.00±2.83ab	N.D d	138.50±4.95a	
Field 7/Yassamin	28.00±2.16c	88.50±6.81a	101.25±10.63c	102.75±25.07b	
Limits WHO and IQS	400 µg/kg	800 µg/kg	200 µg/kg	85000 µg/kg	

Values are expressed as a mean ± SD. Means with different letters within the same category and column are significantly different at P < 0.05.

Table 5. demonstrates the average concentration of cadmium and lead of the samples for the parts of the

field, and the results showed that there were no significant differences between the fields parts.

Table 5. The average concentration of cadmium and lead (µg/kg) and the standard deviation of the paddy samples for the first line, near of the irrigation water, and the second line far from the water source.

Paddy	Cd		Pb	
	Line 1	Line 2	Line 1	Line 2
Field 1	29.00±2.00b	56.67±5.69a	132.33±44.00a	148.70±53.00a
Field 3	43.67±2.52a	56.67±9.02a	100.00±8.00a	99.33±11.02a
Field 5	36.67±1.53a	38.00±4.36a	90.00±5.57a	92.33±4.73a
Field 7	30.00±17.32a	27.33±2.08a	102.00±55.43a	103.00±12.30a
Limits WHO and IQS	400 µg/kg		200 µg/kg	

Values are expressed as a mean ± SD. Means with different letters within the same category and raw are significantly different at P < 0.05.

Table 6. shows the average concentration and the standard deviation of cadmium and lead of soil

samples for the parts of the field, and the results showed that there were no significant differences between the fields parts.

Table 6. The average concentration of cadmium and lead (µg/kg) and the standard deviation of soil samples for the first line, near of the irrigation water, and the second line, far from the water source.

Soil	Cd		Pb	
	Line 1	Line 2	Line 1	Line 2
Field 1	31.00±3.00a	34.00±19.10a	98.00±13.44a	132.50±13.44a
Field 3	57.00±3.00a	56.00±6.00a	111.50±0.71a	105.00±4.24a
Field 5	74.00±7.07a	78.00±22.63a	120.00±16.00a	138.50±5.00a
Field 7	93.00±3.00a	84.00±7.07a	124.00±10.61a	141.00±20.51a
Limits WHO	800 µg/kg		85000 µg/kg	

Values are expressed as a mean ± SD. Means with different letters within the same category and raw are significantly different at P < 0.05.

4. Discussion

All paddy samples had total aflatoxins below the permissible limits of the World Health Organization, 4µg/kg, except two out of 51 paddy samples that contained a concentration of total aflatoxins above the permissible limits. The lowest and highest value of total aflatoxins for this study ranged from not detected (ND) to 4.7 µg/kg respectfully, which was lower than ranged of total aflatoxin, 2.2 to 10.2 µg/kg of paddy samples that taken from seven mills at the same area of this study during 2018 processing season (Alhendi et al. 2020). The difference of these results might be because the total aflatoxin increased more during the storage period because in their study the samples were taken from mills not from fields. Also, the level of total aflatoxin of this study was much less than the results of the study done by Chyad 2022 which was conducted on 75 samples of paddy collected from different fields of Diyala and Salah al-Din, east and north of Iraq. The range of their study was from 1190 to 16400 µg/kg and they mentioned that the cause of the pollution was the irrigation water (Alaa A. Chyad 2022). The different results could be because of the paddy contamination from fields varies from season to other or might be because the irrigation water source is different from their study the current study. The main source of irrigation water of this study was Euphrates River, while in their study Tigris River was the source of the irrigation water. All the paddy samples of this study had aflatoxin B1 less than the permissible limits of WHO, 2 µg/kg. The range was from N.D to 1.9 µg/kg. Samples of paddy were processed, and aflatoxins of produced rice were measured. The rice samples were free from any detection of total aflatoxins and aflatoxin B1 Karagipani 2011 mentioned the range of aflatoxin B1 of rice samples collected from 100 samples from different regions of Iran, which was from 0.34 to 0.58 µg/kg (Karajibani, Merkazee, and Montazerifar 2011). Nganou Donkeng 2022 reported that aflatoxin B1 of 12 rice samples collected from Cameroon were ranged from 0.3 to 0.8 µg/kg (Nganou Donkeng et al. 2022). The reason for this is the effect of the manufacturing process on reducing the contamination of rice with aflatoxins thus, the pollution of rice in the fields is less than the pollution from the packing and storage operations.

Total aflatoxins and aflatoxins B1 were detected in the most soil and the amount of aflatoxins were low concentration. The contamination range was from 0.07 to 1.35 µg/kg for total aflatoxins and from N.D to 0.30 µg/kg for aflatoxin B1. To our knowledge, there was no permissible limits for total aflatoxins and aflatoxin B1 in the agricultural soil, specification worldwide. In Comparison with other study Jayaratne et al. 2020 estimated aflatoxins in soil samples collected from fields in Sri Lanka, and the range was 350-400 micrograms/kg, which the contamination level was high compared to our study (Jayaratne et al. 2020). The level of aflatoxins in soil depends on

several factors, and the main factor is moisture content therefore the differences between fields occurred.

The contamination level of lead in paddy samples were less than the permissible limit of the World Health Organization and Iraqi quality standards, which is 200 µg/kg. The range of lead in the two study areas ranged from 84 to 190 µg/kg, which was less than the results obtained from the paddy samples taken from the same area of this study. (Hussain and Abdullah 2018; Alhendi et al. 2020). The first study mentioned that the lead concentration reached to 778 µg/kg, and the second study reported that the lead concentration of paddy samples were between 97 to 525 µg/kg. The difference of lead concentration in these studies is probably because of the contamination level of fields because the storage of paddy have no effect on heavy metal concentration. Globally, (Ochoa et al. 2020) mentioned that the amount of lead in most of the paddy samples of Ecuador was about 1300 µg / kg, and Satpathy et al. (2014) reported that the lead concentration of paddy taken from fields in India was from 10 to 1000 µg / kg.

For rice samples the range of lead in the rice samples were was from 90 to 147 µg/kg, which is an approach from the results of a study conducted on samples of rice produced in Babylon, Iraq 2020 by Al-Mayahi and Al-Jarrah, and the amount of lead was from 70 – 243 µg/kg (Almayah et al. 2020). Also, (Alhendi et al. 2020) mentioned that the lead concentration of rice samples taken from mills were from 93 to 339 µg/kg, which is more than the results of this study. The results of this study declared that the paddy planted in the area of the study and the produced rice were safe in term of its content of Pb.

The lead range in this study for the soil samples lead from 77 to 155 µg/kg, which is less than the amount of lead in the soil of Iraqi Kurdistan, was about 14670 µg/kg for soil depth of 20 cm, while it was 6500 µg/kg for the top layer of soil (Hama and Darwesh 2019). Also the concentration was less than the lead concentration of paddy fields of Diyala and Salah al-Din, which was about 30.000 µg/kg (Chyad et al., 2022). (Satpathy, et al. 2014) found that the lead concentration of some paddy fields in India ranged from 20 to 600 µg/kg.

The cadmium concentration in the paddy samples in study area was less than the permissible limit, 400 µg / kg of the World Health Organization WHO and IQS. The cadmium concentrations in this study ranged in both governorates from 25-66 µg / kg which is similar to the results of the study that was conducted In 2020 in Iraq by (Alhendi et al. 2020) to estimate cadmium in samples of paddy, where the results of cadmium ranged from 55-66 µg/kg. Also (Chyad et al. (2022) detected Cd in paddy samples taken from north and east of Iraq and the contamination level was between 42 to 72 µg/kg. Globally, Satpathy et al. (2014) found that Cd concentration was between 20 to 50 µg/kg in paddy samples planted in India. For the produced rice samples, cadmium

concentration was ranged from 59 to 72 µg/ kg. The result agreed with the (Almayahi et. all 2020).study that was done on Anber rice planted Nasiriyah, Iraq, and the concentration was 47 µg/kg. Alhendi et al (2020) mentioned that Cd concentration of rice planted in Iraq was from 55 to 66 µg/ kg. The results of this study confirmed that the paddy planted in Iraq and the produced rice were safe in term of its content of Cd.

Cadmium concentration of soil samples in the study area for this study ranged from 20 to 99 µg/kg, which was much lower than the cadmium concentration in soil for a study conducted in Al-Najaf in 2018, which ranged from 210 - 330 µg / kg (Hussain and Abdullah 2018). Also, Chyad et al. (2022b) mentioned that Cd concentration was about 1000 µg/ kg in the soil sample collected from north and east of Iraq. Worldwide, Alam et al. (2020) measured Cd in soil samples of some fields in Bangladesh, and they found the concentration was about 50 µg/ kg. Satpathy et al. (2014) reported that the Cd contamination in some paddy soils of India was from 20 to 60 µg/ kg.

5. Conclusions

The study showed that most of the paddy samples within the study area were suitable for human consumption in terms of their content of aflatoxins and heavy metals, lead and cadmium. Although the paddy samples contained total aflatoxins, aflatoxin B1, and heavy metals, the concentrations were less than the permissible limit of the World Health Organization and Iraqi standards. The soil samples of paddy fields within the study area, Al-Diwaniyah and Najaf Al-Ashraf, were suitable for cultivation of paddy because they had total aflatoxins, aflatoxin B1, cadmium, and lead within the acceptable limits. The industrial process of paddy had an effective role in reducing the pollution of aflatoxins, while it had no clear role in reducing the pollution of heavy metals. The paddy and rice within the study area were unpolluted and safe in terms of their content of aflatoxins and heavy metals for the year 2021.

References

- Alaa A. Chyad^{1, 2}, Ahmed M. Saeed¹, Abeer S. Alhendi². n.d. "Heavy Metals Content of Irrigation Water, Soil, Paddy, and Produced Rice of Some Paddy Fields of Iraq(2021)." 1–12.
- Alaa A. Chyad¹, Abeer S. Alhendi² and Ahmed M. Saeed¹. 2022. "MICROORGANISMS and AFLATOXINS CONTENT OF IRRIGATION WATER , SOIL , MICROORGANISMS AND AFLATOXINS CONTENT OF IRRIGATION WATER , SOIL , PADDY AND PRODUCED RICE OF SOME PADDY FIELDS." (July).
- Albert, Julius, Camilla A. More, Niklaus R. P. Dahlke, Zacharias Steinmetz, Gabriele E. Schaumann, and Katherine Muñoz. 2021. "Validation of a Simple and Reliable Method for the Determination of Aflatoxins in Soil and Food Matrices." *ACS Omega* 6(29):18684–93. doi: 10.1021/acsomega.1c01451.
- Alhendi, A. S., Mhaibes, A. A., Al-Rawi, S. H., & Mohammed, A. K. (2020). Occurrence of microorganisms, aflatoxin, ochratoxin, and heavy metals in paddy and rice produced in Iraq. *Thai Journal of Agricultural Science*, 53(3), 109-119.
- Ali, Jaafar A., and Nadhim Sulaiman A. Jakhsi. 2020. "Determination of the Aflatoxin B1 Level in Imported Milled Rice by ELISA in Duhok Province."
- Anon. 2018a. "Huiying Li, Lei Xing, Muchen Zhang, Jiaqi Wang, and Nan Zheng The Toxic Effects of Aflatoxin B1 and Aflatoxin M1 on Kidney through Regulating L-Proline and Downstream Apoptosis." 1–12.
- Anon. 2018b. "Oliveira Elias, Susana de, Tiago Baptista Noronha, and Eduardo Cesar Tondo. 2018. 'Assessment of Salmonella Spp. and Escherichia Coli O157:H7 Growth on Lettuce Exposed to Isothermal and Non-Isothermal Conditions.' *Food Microbiology* 72: 206–13. <https://doi.org/>
- Anon. 2018c. "Soraya Shafiekhani, Shantae A. Wilson, Griffiths G. Atungulu Impacts of Storage Temperature and Rice Moisture Content on Color Characteristics of Rice from Fields with Different Disease Management Practices." 1–9.
- Anon. 2020a. "Alam, R., Ahmed, Z. & Howladar, M. F. Evaluation of Heavy Metal Contamination in Water, Soil and Plant around the Open Landfill Site Mogla Bazar in Sylhet, Bangladesh. *Groundw. Sustain. Dev.* 10, 100311, 2020."
- Anon. 2020b. "Almayahi, B. A. & Aljarrah, N. Heliyon Relationship between Heavy Metals and Alpha Particles as a Marker of Environmental Pollution in Rice Consumed in Najaf , Iraq. *Heliyon* 6, E03134 (2020)."
- Anon. 2020c. "Hayba Badro , Agnelo Furtado and Robert Henry Relationships between Iraqi Rice Varieties at the Nuclear and Plastid Genome Levels Hayba." 1–14.
- Anon. 2020d. "Which Is Similar to the Results of the Study That Was Conducted In 2020 in Iraq by Al-Hindi and Others to Estimate Cadmium in Samples of Rye, Where the Results of Cadmium Ranged from 55-66 Mg/Kg A. S., Mhaibes, A. A., Rawi, S. H. Al & Mohammed, A. K. Occu."
- Anon. 2021. "Anjorin, T. S., Ariyo, A. L., Peter, A. O., Sulyok, M. & Krska, R. Co-Occurrence of Mycotoxins, Aflatoxin Biosynthetic Precursors, and Aspergillus Metabolites in Garlic (*Allium Sativum* L) Marketed in Zaria, Nigeria. *Food Addit. Contam. Part B Surveill.* 14."
- Anon. n.d. "Zeng, F. et Al. Heavy Metal Contamination in Rice-Producing Soils of Hunan Province, China and Potential Health Risks. *Int. J. Environ. Res. Public Health* 12, 15584–15593(2015)."
- Assubaie, Fahad N. 2015. "Assessment of the Levels of Some Heavy Metals in Water in Alahsa Oasis Farms, Saudi Arabia, with Analysis by Atomic Absorption Spectrophotometry." *Arabian Journal of Chemistry* 8(2):240–45.
- Chandrajith, Rohana, Chandra B. Dissanayake, and Heinz J. Tobschall. 2005. "The Abundances of Rarer

- Trace Elements in Paddy (Rice) Soils of Sri Lanka." *Chemosphere* 58(10):1415–20. doi: 10.1016/j.chemosphere.2004.09.090.
- Chyad, Alaa A., Abeer S. Alhendi, and Ahmed M. Saeed. "MICROORGANISMS AND AFLATOXINS CONTENT OF IRRIGATION WATER, SOIL, PADDY AND PRODUCED RICE OF SOME PADDY FIELDS OF IRAQ." *Journal of Science*, 63 (11).
- Chyad, Alaa A., Ahmed M. Saeed, and Abeer S. Alhendi. (2022) Determination of Heavy Metals Content of Irrigation Water, Soil, Paddy, and Produced rice of Some Paddy Fields of Iraq, *Iraqi Journal of Science*, 63 (11).
- Feng, Sheng Jun, Xue Song Liu, Li Ya Ma, Irfan ullah khan, Justice Kipkoir Rono, and Zhi Min Yang. 2020. "Identification of Epigenetic Mechanisms in Paddy Crop Associated with Lowering Environmentally Related Cadmium Risks to Food Safety." *Environmental Pollution* 256. doi: 10.1016/j.envpol.2019.113464.
- Gola, Deepak, Anushree Malik, Ziauddin Ahammad Shaikh, and T. R. Srekrishnan. 2016. "Impact of Heavy Metal Containing Wastewater on Agricultural Soil and Produce: Relevance of Biological Treatment." *Environmental Processes* 3(4):1063–80.
- Habibollahi, Mohammad Hossein, Kamaledin Karimyan, Hossein Arfaenia, Nezam Mirzaei, Yahya Safari, Reza Akramipour, Hooshmand Sharafi, and Nazir Fattahi. 2019. "Extraction and Determination of Heavy Metals in Soil and Vegetables Irrigated with Treated Municipal Wastewater Using New Mode of Dispersive Liquid–Liquid Microextraction Based on the Solidified Deep Eutectic Solvent Followed by GFAAS." *Journal of the Science of Food and Agriculture* 99(2):656–65.
- Hama, Rawa Hatam, and Dalshad Azeez Darwesh. 2019. "Heavy Metals Evaluation in Soil of Agricultural Field around a Pond of Gas Plant in the Kurdistan Region of Iraq." *Zanco Journal of Pure and Applied Sciences* 31(5). doi: 10.21271/zjpas.31.5.4.
- Hassanein, Naziha M., Tahsin Shoala, and Shaymaa A. Gouda. 2018. "Microbiology Research Article 2018 | Volume 3 | Issue 2 | 43-54 In Vitro Studies on Biological Control of Drechslera Species Causing Brown Spot Disease in Rice Plants." *Journal of Food and Agriculture* 99(2):656–65.
- Hussain, Raghda F., and Enaam J. Abdullah. 2018. "Risk Assessment of Heavy Metals Contamination in Kenyan Major Estuaries." *Environmental Pollution* 26(6):124–34.
- Jayarathne, W. M. S. C., A. H. M. A. K. Abeyratne, H. K. S. De Zoysa, D. M. R. B. N. Dissanayake, T. C. Bamunuarachchige, V. Y. Waisundara, and S. Chang. 2020. "Detection and Quantification of Aflatoxin B1 in Corn and Corn-Grown Soils in the District of Anuradhapura, Sri Lanka." *Heliyon* 6(10):4–9. doi: 10.1016/j.heliyon.2020.e05319.
- Joshi, Ritu, Changyeun Mo, Wang-Hee Lee, Seung Hyun Lee, and Byoung-Kwan Cho. 2015. "Review of Rice Quality under Various Growth and Storage Conditions and Its Evaluation Using Spectroscopic Technology." *Journal of Biosystems Engineering* 40(2):124–36.
- Karajibani, Mansour, Azita Merkazee, and Farzaneh Montazerifar. 2011. "Determination of Aflatoxin in the Imported Rice in Zahedan, South-East of Iran, 2011."
- Kayastha, Sadhana Pradhanang. 2015. "Heavy Metal Pollution of Agricultural Soils and Vegetables of Bhaktapur District, Nepal." *Scientific World* 12(12):48–55.
- Lu, Yintao, Hong Yao, Dan Shan, Yichen Jiang, Shichao Zhang, and Jun Yang. 2015. "Heavy Metal Residues in Soil and Accumulation in Maize at Long-Term Wastewater Irrigation Area in Tongliao, China." *Journal of Chemistry* 2015.
- Mankoula, Asmaa F., Walid Tawfik, Joel E. Gagnon, Brian J. Fryer, F. El-Mekawy, and Mohamed E. Shaheen. 2021. "Assessment of Heavy Metals Content in the Agricultural Soils of Kafr El-Zayat Egypt Using Laser Ablation Inductively Coupled Plasma Mass Spectrometry and Inductively Coupled Plasma Optical Emission Spectroscopy." *Egyptian Journal of Chemistry* 64(3):1167–77.
- Mazaheri, Mansooreh. 2009. "Determination of Aflatoxins in Imported Rice to Iran." *Food and Chemical Toxicology* 47(8):2064–66. doi: 10.1016/j.fct.2009.05.027.
- Nganou Donkeng, Nadège, Alphonse Sokamte Tegang, Eliane Tchinda Sonwa, Thierry Marcel Ntsamo Beumo, Francky Steve Nodem Sohanang, Nikaise Forestine Douanla Nodem, Thierry Noumo Ngamou, and Leopold Tatsadjieu Ngoune. 2022. "Fungal Diversity and Occurrence of Aflatoxin B1, Citrinine, and Ochratoxin A in Rice of Cameroon." *Journal of Food Processing and Preservation* 46(4). doi: 10.1111/jfpp.16429.
- Ochoa, Martin, Wladimir Tierra, Diego Santiago Tupuna-Yerovi, Danilo Guanoluiza, Xosé Luis Otero, and Jenny Ruales. 2020. "Assessment of Cadmium and Lead Contamination in Rice Farming Soils and Rice (*Oryza Sativa* L.) from Guayas Province in Ecuador." *Environmental Pollution* 260. doi: 10.1016/j.envpol.2020.114050.
- Ren, Xianfeng, Qi Zhang, Wen Zhang, Jin Mao, and Peiwu Li. 2020. "Control of Aflatoxigenic Molds by Antagonistic." *Toxins* 12(24):1–21.
- dos Santos Costa, Bruno Elias, and Nívia Maria Melo Coelho. 2021. "Selective Determination of As(III) and Total Inorganic Arsenic in Rice Sample Using in-Situ μ -Sorbent Formation Solid Phase Extraction and FI-HG AAS." *Journal of Food Composition and Analysis* 95(October 2020). doi: 10.1016/j.jfca.2020.103686.
- Satpathy, D., Reddy, M. V., & Dhal, S. P. (2014). Risk assessment of heavy metals contamination in paddy soil, plants, and grains (*Oryza sativa* L.) at the East Coast of India. *BioMed research international*, 2014.
- Smela, M. E., S. S. Currier, E. A. Bailey, and J. M. Essigmann. 2001. "The Chemistry and Biology of Aflatoxin B1: From Mutational Spectrometry to Carcinogenesis." *Carcinogenesis* 22(4):535–45.
- Song, Peilin, and Lamin R. Mansaray. 2018. "ISPRS Journal of Photogrammetry and Remote Sensing Mapping Paddy Rice Agriculture over China Using AMSR-E Time Series Data." *ISPRS Journal of Photogrammetry and Remote Sensing*

144(March):469–82.

Zhang, Yong, Chengyong Wu, Hongxin Liu, Mohammad Rizwan Khan, Zhifeng Zhao, Guiping He, Aimin Luo, Jiaqi Zhang, Ruijie Deng, and Qiang He. 2021. "Label-Free DNAzyme Assays for Dually Amplified and One-Pot Detection of Lead Pollution." *Journal of Hazardous Materials* 406(December 2020):124790. doi: 10.1016/j.jhazmat.2020.124790.