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Microbial Quality of Paddy fields at Alfurat Alawsat Area, Iraq and the Effect of Milling Process on the Rice Contamination Level

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Abstract:

Fifty-Four paddy samples, 45 soil samples, and nine irrigation water samples of some paddy fields at Alfurat Alawsat Area, Iraq were collected to investigate the microbial quality including total count bacteria (TCB), molds and yeast, total coliform (TC), and *E. coli* bacteria. Paddy samples were processed at the laboratory level to produce rice at 32 and 36 whiteness, and the microbial quality of the produced rice was conducted too. The results showed that all irrigation water samples were overpassed the FDA limit for *E. coli*. All paddy samples were exceeded the good limit of TCB, but no sample exceeded the acceptable limit. Most paddy samples were exceeded the FDA limit for molds and yeast, and few paddy samples exceeded the total coliform limit. The milling process reduced microbial levels for some samples, while the effect was undetectable for other samples. Ten rice samples at 32 whiteness and eight rice samples at 36 whiteness out of 13 paddy samples overpassed FDA and Iraqi Quality Standardization (IQS) for molds and yeast. All produced rice at both whiteness levels was exceeded the total coliform of IQS, while only six and three samples out of 13 rice samples at 32 and 36 whiteness respectively overpassed the FDA limit. In conclusion, paddy samples taken from Alfurat Alawsat fields were overpassed some microbial levels from fields without the effect of storage or milling processing. Irrigation water was probably the main source of fields' contamination, therefore, the paddy fields need to be monitored and controlled in terms of their microbial contamination.

Keywords: Microbial contamination, Paddy fields, Irrigation water, *E. coli*, rice, Mills.

Introduction:

Rice is the seed of the grass species *Oryza sativa* that contains many species; only two of them were classified as cultivated rice[1]. Rice is one of the most important staple foods worldwide[2], and it is considered the second most commonly used grain after wheat[3]. Rice is a strategic crop for Iraq, which is a staple food for the majority of the Iraqi people[4]. The variety Anber and variety of Iraq because of its unique flavor, aromatic character[5], while Yassamine is the major rice variety of Iraq, about 97% of the produced rice of Iraq was Yassamine. Alfurat Alawsat Area, Alnajaf, Aldiwania, and Babel Governorates, is the main area of planting and producing paddy and Rice respectively in Iraq[6].

Microorganisms could contaminate rice during planting, harvesting, and other agricultural operations such as processing, handling, and shipping. The contamination either attacks rice plants at a growth stage or infects grains after harvest. In both cases, the quantity losses and the quality decreases in rice crops occurred[7]. Contamination occurs mostly before harvesting, however, the storage of rice is an important step in terms of keeping the rice of good quality. Microbial contamination of fresh products can present a severe risk to public health. The main source of contamination before harvesting is irrigation water that may greatly affect the quality of the fresh agricultural products. The potential for contamination via irrigation water is increased in developing countries as untreated wastewater is used for irrigation[8]. The surface irrigation water was contaminated with *Escherichia coli*, *Salmonella* spp., and somatic coliphages[9]. In the UK, 71% of irrigation water is obtained from surface waters, which receive sewage effluent[10]. Although irrigation water is considered the main source of soil contamination, organic wastes that came from using fertilizers, are the other source of soil contamination, which leads to contaminating crops directly[11]. The ability of the pathogen to survive in the environment

impacts the probability of crop contamination and pathogen viability at harvest through consumption. Originally, the pathogen must survive in the propagation environment until crops are planted out, or inorganic wastes were used[12].

The most common types of paddy and rice microflora include bacteria, mold, and yeast[7]. The main indicators of bacteria for cereal contamination are coliform, fecal coliform and *E. coli* in addition to total bacteria and molds and yeast[13]. There were many species of fungi associated with rice seeds such as *Aspergillus flavus, Bipolaris oryzae, Curvularia lunata, Fusarium moniliforme, F. oxysporum and Rhizopus* spp[14]. Recently, a survey study on rice contamination from some markets in Iraq revealed that domestic and imported rice samples were contaminated with *Aspergillus* spp, *pencillium* spp, *Rhizupus* spp, *fusarium* spp, *Alternaria* spp, and *Mucor* spp. Also some samples were exceeded the IQS accepted limit for fungi, which considered dangerous for human health[15].

In a previous study[16], the microbial quality of some paddy and rice samples taken from several mills at Alfurat Alawsat area overpassed the FDA and IQS limits. The rice samples were taken directly from mills without a storage period hence the contamination of paddy from fields issue was raised with the probability of paddy contamination during the storage period. Therefore, this study aimed to determine the paddy safety in terms of its microbial quality from fields to eliminate the effect of the storage on paddy contamination. The effect of irrigation water source on the paddy planting was studied too by taking paddy samples near, middle, and far away from the irrigation water source. Also, the microbial quality of irrigation water and soils of some paddy fields were investigated.

Materials and Methods

Samples Collection

Fifty-four paddy samples, 45 soil samples, and nine irrigation water samples were collected from seven paddy fields of Alnajaf and Al diwania (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 were collected. 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 in the middle, and part 3 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, nine samples of Anber, and 9 samples of Yassamine were collected to detect the variety effect on the paddy contamination. The other fields, 4 and 6, were harvested, therefore, three paddy samples were taken from the paddy bulk of each field, while the three soil samples of fields, 4 and 6, were collected from the three parts as divided above, sample of each part. Three sources of irrigation water were considered. The first source represented 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 Alfurat River, while the third source was taken from a pump machine belonging to the fields.

Preparing of Samples

Irrigation water samples were kept in a refrigerator until the analysis. Paddy and soil samples were dried to suitable moisture content for paddy, 15%, and to be dry for soil. Soil samples were crashed before being analyzed.

Moisture Content

The moisture content of paddy samples was measured by using a moisture tester (Seedburo Equipment Company, Des Plaines, IL, USA) following AACC (44-11) method[17].

Milling Process

The milling process was done as described in the Alhendi et al. study[6]. Briefly, two huller machines were used. The first one removed the paddy husk (SATAKE Corporation, Bangkok, Japan), and the second machine was used to obtain the required whiteness of rice, which was set to be 32, and 36. The minimum whiteness required for Iraqi domestic rice is 32 (Data from Grain Board of Iraq).

Rice whiteness

Rice whiteness tester (Rice whiteness Tester C-600, Kett Electric Laboratory, Tokyo, Japan) was used to determine the produced rice whiteness after calibration with a whiteness standard plate at white, 88.7.

Culture media

Many culture media were used in the current study to enumerate the different microorganisms. Nutrient Agar (N.A)was used for detection of total count bacteria (TCB), Violet Red Bile Agar (VRBA) was used to enumerate total coliform (TC), Sabouraud Dextrose Agar (SDA) was used to detect molds and yeast, and lauryl broth, E.C. broth and eosin methylene blue (EMB) were used to enumerate *E.coli*. All culture media were prepared according to the manufacturing company's instructions. The media was on a hot surface with

stirring to dissolve all contain completely, and then sterilized by autoclaving at 121 ºC for 15 min, except for VRBA were prepared without autoclaving

Enumeration of Microbial Organisms

Total bacteria, coliform, *E.coli*, and molds and yeasts were conducted. Ten grams of paddy, rice, or soil were added to 90 ml of peptone water, or 1 ml of irrigation water was added to 9 ml of peptone water. Then serial dilutions, 10^2,10^3,10^4, and 10^5 were made[18]. One ml of serially diluted sample was placed in each petri dish, and approximately 20ml of the suitable media was added. Most Parable Number (MPN) method, three tubes, was followed to enumerate *E. coli* bacteria. The cultures of bacteria were incubated at 37 ºC for 24 h, while the plates of fungi were incubated at 25 ºC for 5 days.

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.

Results:

The microbial quality of irrigation water that was used to irrigate the fields of the current study was stated in Table. 1. Some fields were significantly different from others, for example, irrigation water of fields 4, 5, and 6 were significantly higher than other irrigation water sources for TCB. Also, it was insignificantly higher than other sources for total coliform. Molds and yeast count was significantly higher for fields 1 and 2 than other irrigation water. All irrigation water had *E. coli* bacteria more than the FDA limit, and there was no significant difference between fields (Table 1).

Table 1. Microbial quality of irrigation water.

Source of Fields	Total platecount 10^3 (cfu/ml)	Molds and yeast (10^2 cfu/ml)	Total coliform (10^2 cfu/ml)	*E. coli* (cell/100ml)
1 & 2	9.3 ± 0.4 b	8.0 ± 0.2 a	0.9 ± 0.7 a	1210 ± 410 a
3	7.9 ± 0.1 b	0.4 ± 0.3 b	2.3 ± 2.7 a	920 ± 0 a
4, 5, & 6	13.0 ± 1.4 a	1.3 ± 0.6 b	41.5 ± 54.4 a	550 ± 269 a
Limit (FDA)*	-	-	-	410

Values are expressed as a mean ± SD. Means with different letters within the same column are significantly different at $P < 0.05$. *FDA = Food and Drug Administration[13].

The microbial quality of soil and paddy of the studied fields was placed in Table. 2. There was a significant difference between soil samples for total count bacteria and molds and yeast, while there was no significant difference for total coliform. Only four out of 54 soil samples had *E. coli* bacteria. All paddy samples were exceeded the good limit of the total count of bacteria, while all samples were within the FDA accepted limit. The mean of molds and yeast were exceeded the good and the accepted limit for all fields except paddy of field 4. Noticeably, yeast formed the majority of the molds and yeast count. Coliform bacteria of paddy samples were within the accepted limit except for the paddy sample of field two, while all paddy samples exceeded the FDA good limit. *E. coli* bacteria had not been detected for all paddy samples at a 3 cell/g detection limit. The statistical analysis showed that there were no significant differences between the three parts of the same field for total count bacteria, molds and yeast, and total coliform. The data has not been shown. There was no significant difference between microorganisms' count of Yassamin and Anber[6] verities of field 1, which they taken from the same area.

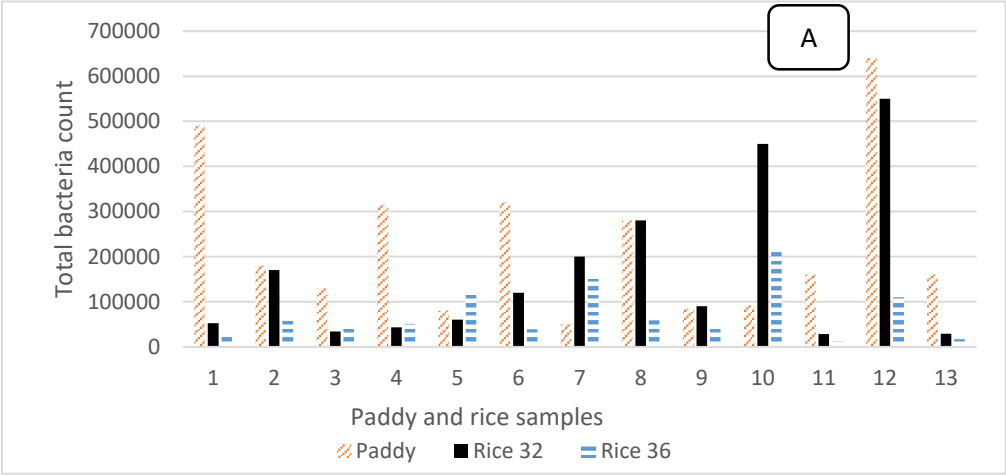
Table 2. Microbiological quality of soil and paddy

Fields	Total plate count (cfu/g)		Molds and yeast (cfu/g)		Total coliform (cfu/g)		*E. coli* (cell/g)	
	Soil (10^5)	Paddy (10^5)	Soil (10^4)	Paddy (10^4)	Soil (10^4)	Paddy (10^3)	Soil	Paddy
1 Yassamin[6]	7.0 ± 3.6a	1.4 ± 1.2ab	1.3 ± 1.2b	1.4 ± 1.5bc	1.2 ± 2.0a	3.6 ± 3.7b	155 ± 214*	< 3
1 Anber[6]	---------	1.2 ± 0.9ab	---------	1.5 ± 0.9bc	---------	4.0 ± 3.9b		< 3
2	3.3 ± 2.5cd	1.0 ± 1.2b	6.3 ± 4.9b	1.8 ± 1.1bc	0.6 ± 0.6a	14.2 ± 27.0a	< 3	< 3
3	4.0 ± 1.3bcd	1.3 ± 1.0ab	5.0 ± 8.2b	2.5 ± 1.0ab	0.05 ± 0.04a	8.7 ± 8.9ab	< 3	< 3
4	4.5 ± 3.0abcd	1.6 ± 0.5ab	23.6 ± 23.9a	0.9 ± 0.3c	2.2 ± 2.5a	8.0 ± 5.5ab	9.2**	< 3
5	4.8 ± 2.3abc	1.9 ± 0.7a	2.7 ± 1.6b	3.5 ± 2.4a	0.2 ± 0.2a	3.4 ± 2.7b	< 3	< 3
6	6.3 ± 4.1ab	0.8 ± 0.3b	3.3 ± 2.1b	1.0 ± 0.7c	1.0 ± 0.5a	3.1 ± 1.2b	> 1100**	< 3
7	2.2 ± 0.8d	0.9 ± 0.8b	1.3 ± 0.8b	2.3 ± 1.6abc	0.6 ± 0.3a	6.1 ± 5.0ab	< 3	< 3
FDA limit[1]	-	10^2– 10^6	-	10^2 – 10^4	-	10^2 – 10^4	-	10^2 – 10^4

Values are expressed as a mean ± SD. Means with different letters within the same column are significantly different at P < 0.05., FDA = Food and Drug Administration Circular[13].
*Only two samples out of 9 was positive, **One sample out of three was positive, [1]good and accepted limit.

Some paddy samples were chosen to be milled to produce rice at 32 and 36 whiteness to determine the effect of milling processing on the microorganisms count. Fig. 1, expressed total bacteria count, molds and yeast, and total coliform for paddy and its counterpart's rice at 32 and 36 whiteness. Although total bacteria count was considerably reduced for rice at 32 and 36 whiteness compared to their paddy samples for samples number 1, 3, 4, 6, 11, 12, and 13 (Fig. 1A). The reduction was undetectable for 2, 8, and 9 samples. Furthermore, some rice samples had total bacteria count more than their counterpart's paddy samples, sample numbers 5, 7, and 10 (Fig. 1A). Almost the same observation was noticed for molds and yeast (Figure 1B) and total coliform bacteria (Fig. 1C).

The number of samples that exceeded the FDA and IQS limit for molds and yeast and total coliform to the total number of paddy samples that were used to produce rice was placed in Fig. 2. All paddy and rice samples were higher than the good limit, 10^2 cfu/g, of total bacteria count but less than the accepted limit, 10^6 cfu/g, therefore the data was not placed in Fig. 2. Eleven paddy samples, ten rice at 32 whiteness sample, and eight rice samples at 36 whiteness out of 13 samples were higher than the accepted FDA and IQS limits, 10^4cfu/g, for molds and yeast (Fig. 2). For total coliform bacteria, six paddy samples, six rice samples at 32 whiteness, and three rice samples at 36 whiteness out of 13 samples were overpassed the FDA limit, 104 cfu/g, while all produced rice samples were exceeded the IQS limit, 10^3 cfu/g. There is no IQS limit for paddy.



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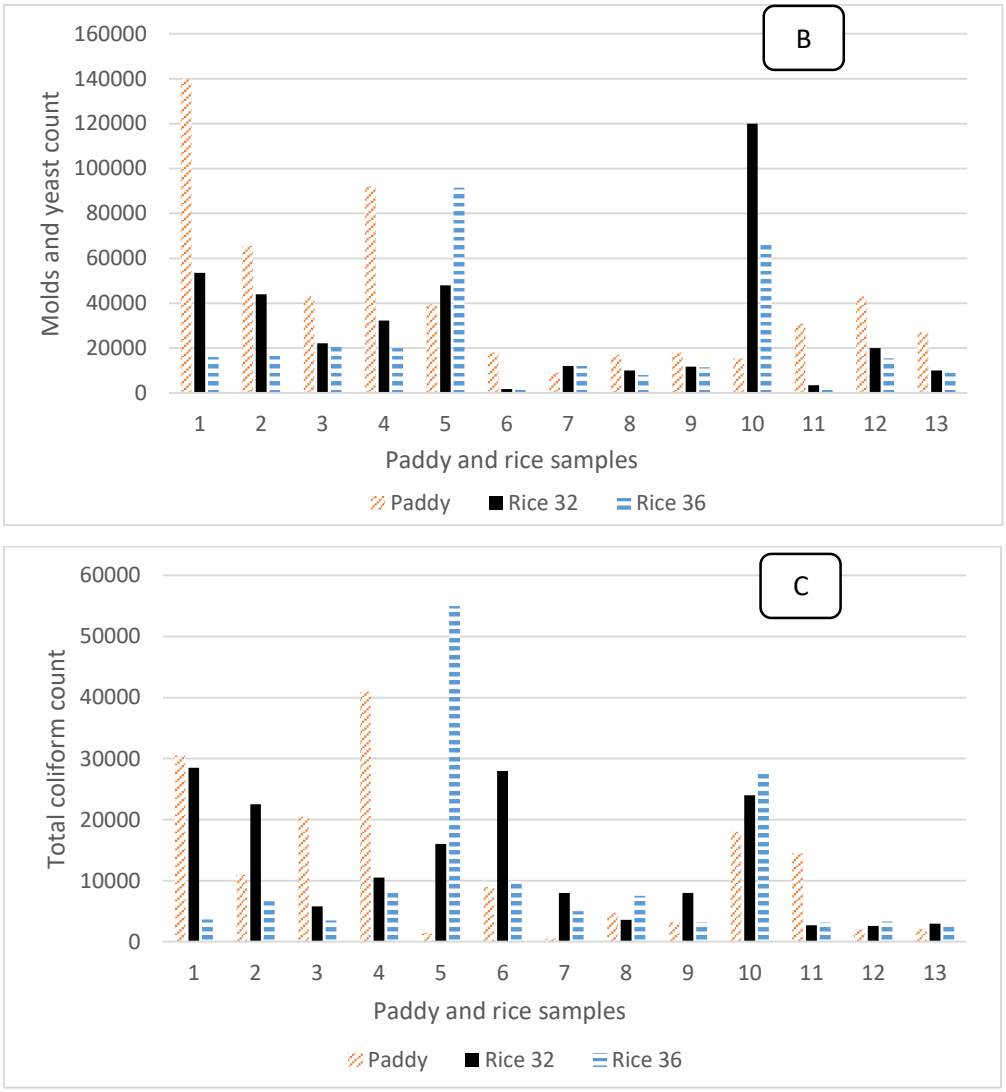

Figure 1. Microbiological quality of paddy, rice at 32 whiteness, and rice at 36 whiteness. A: Total bacteria count, B: molds and yeast, and C: total coliform.

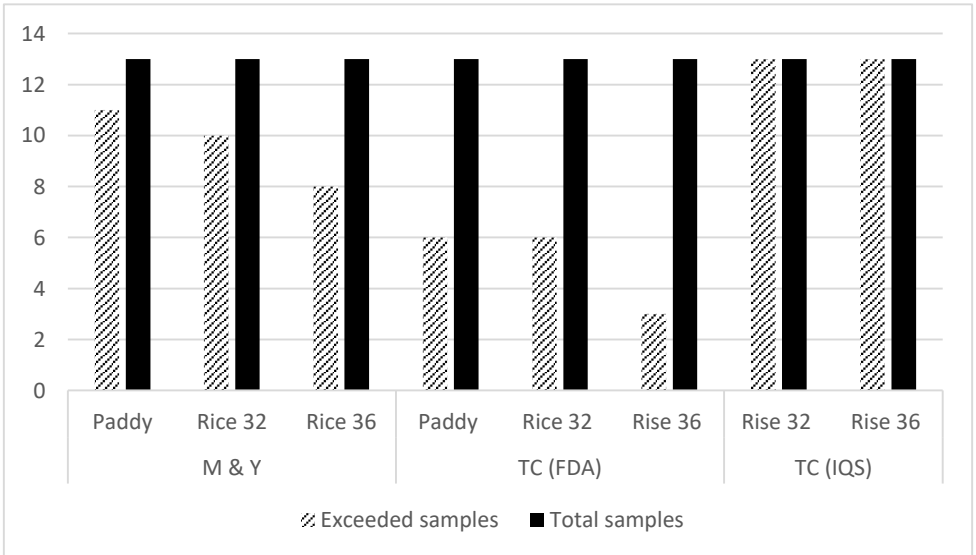

Figure 2. Number of exceeded samples to FDA and IQS limits to the total sample number. M & Y: molds and yeast, TC: total coliform. Molds and yeast limit 10^4 for both FDA and IQS. Total coliform FDA limit 10^4 and IQS limit 10^3. IQS = Iraqi Quality Standardization[19].

Discussion:

In Iraq, if domestic rice was inspected for its safety, it will be after milling processing, which is definitely after harvesting of paddy and a storage period. Therefore, monitoring paddy safety from fields is an important step to determine the source of the contamination. In a previous study[16], some paddy and rice samples produced from several mills of Iraq, Alfurat Al-Awsat, were exceeded the count of molds and yeast and total coliform bacteria. Generally, the microbial count of paddy was more than the produced rice and the number of exceeded samples was more in paddy samples compared to rice samples. The authors highlighted that it is important to figure out the source of the contamination by referring to the fields' sources such as irrigation water and soil. Microbial contamination can occur during any steps involved in the production such as planting, harvesting, processing, and packaging[20].

In the current study, paddy samples taken from fields and rice samples were produced at laboratory level and under controlled conditions were overpassed some microorganisms limits, which refers to the fields contamination. Irrigation water of the tested fields was analyzed for total bacteria count, molds and yeast, coliform, and *E. coli* to determine the microbial quality. *E. coli* of all irrigation water were more than the FDA limit, which indicated the fecal contamination. Although the irrigation water was contaminated with *E. coli* bacteria, all paddy and rice samples were free of the bacteria. The irrigation water contamination was an indicator of its quality[21]. In a recent study[22], irrigation water of some paddy fields of Diyala and Salah Al-Din provinces, east and north of Iraq respectively, had 700 to 1000 *E. coli* cell/ 100 ml irrigation water, which is within the range of the current study, 550 to 1210 cell/ 100 ml. While all other microorganisms were much higher than the current study. The source of irrigation water is different in both studies, in their study the source of irrigation water was Tigris River, while the source of irrigation water of this study was the Euphrates River. Also, the fact that the irrigation water of field crops in Iraq was not monitored by a specific organization, and some untreated wastewater engages directly to the irrigation water, therefore, contamination was expected. Recently, the determination of the biological quality of the irrigation water of some places of Europe were done. The study group[23] found that human fecal pollution was found in the most of the samples of the irrigation water sources, and E. coli level was not at the accepted limit (100 cell/100 ml).

The microbial quality of irrigation water of the third source was more contaminated compared to other sources, which they were taken from the river branches. The reason behind this result was probably because the irrigation water was taken from the pump machine after passing several tunnels, and these tunnels were contaminated as results of the activity of human and animals around them. The irrigation water source of the study area was mainly from Alfurat River, and several branches divided from it. Then there were tunnels full of water from these branches, and so on. The irrigation water of tunnels appeared unclean, and several unwanted plants were grown. Therefore, one can conclude that the more irrigation water pass through tunnels, the more contaminated became.

The microbial quality of paddy from fields was more contaminated than wheat taken from several fields of Iraq[24]. For example, the highest total coliform and molds and yeast were observed 900 cfu/g and 1700 cfu/g for wheat and 14200 cfu/g and 35000 cfu/g for paddy respectively. The main reason behind this fact is probably the difference of paddy and wheat need for irrigation water. Paddy planting requires more irrigation water than wheat or beans[25], and irrigation water is one of the main source of microbial contamination of fields in addition to manure, insect, wild and domestic animals, etc[26]. An interesting study about the effect of flies on the rice contamination by *E. coli*, the group[27] of the study found that there was a significant increase ($p < 0{\cdot}001$) of *E. coli* cells for rice where flies landed on than no files landed on the rice.

Although there was some microbial reduction for some produced rice at 32 and 36 whiteness (Fig. 1), some other produced rice samples had no reduction. Furthermore, some produced rice samples had a microbial count more than their counterpart paddy samples. This result disagreed with the Alhendi et al.[16] and Alla et al.[22] studies. In the former study, the authors measured the microbial count of paddy and rice from several mills of Alfurat Alawsat Area, Iraq while in the second study, paddy samples were taken from fields of east and north of Iraq and processed at the laboratory level. They mentioned that paddy processing reduced microbial count, while it had no clear effect on mycotoxins and heavy metals concentration. In comparisons between the microbial count of paddy taken from mills[16], and paddy were taken from fields, the current study, for the same region. Total count bacteria were higher from fields, molds and yeast count were almost similar, and total coliform bacteria were higher for some paddy samples of mills while other samples were similar with paddy taken from fields. The variety of microbial count of samples taken from the same field was high, sometimes the variety was double (Table 1), therefore, determining a pattern between paddy and rice samples was not applicable.

One reason why some rice samples had a microbial count more than paddy samples was probably because the surface area of rice samples increased after paddy processing compared to paddy surface area. Also, some broken rice that was produced as a result of paddy processing increased the surface area, which led to increased microbial count.

Conclusion:

The microbial level of irrigation water and paddy taken from some Alfurat Alawsat fields were exceeded the FDA authorized level. Paddy taken from fields was contaminated with microorganisms without being affected by storage or processing of paddy. Rice produced at 32 and 36 whiteness at a laboratory level and under controlled conditions overpassed some microbial limits too. Therefore, paddy fields need to be monitored and controlled in terms of its microbial contamination, and irrigation water was probably the main source of the contamination.

Authors' Declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in the Iraqi Ministry of Trade.

Authors Contribution:

A S. A, Conceptualization; project administration; methodology; formal analysis; resources; supervision; writing – original draft; writing – review and editing. A M. A, conceptualization; project administration; methodology; resources; supervision; writing – original draft; writing – review and editing. A K. M, methodology.

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المحتوى المايكروبي لحقول الشلب في منطقة الفرات الاوسط/ العراق وتاثير العملية التصنيعية للشلب على مستوى التلوث المايكروبي للرز

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الخلاصة:
شملت الدراسة تحليل اربعة وخمسون عينة شلب، خمسة واربعون عينة تربة، وتسع عينات مياه ري اخذت من منطقة الفرات الاوسط/ العراق. حللت من الناحية المايكروبية وشملت تحليل العدد البكتيري الكلي، عدد الخمائر والاعفان، عدد بكتريا القولون، وعدد بكتريا *E. coli*. تم تصنيع عينات الشلب على مستوى مختبري لانتاج رز ذا درجة بياض 32 و 36 وتم تحديد المحتوى المايكروبي للرز المصنع. اظهرت النتائج ان كل عينات مياه الري تجاوزت الحد المسموح به لمنظمة الغذاء والدواء الامريكية (FDA) لبكتريا *E. coli* وان كل عينات الشلب تجاوزت الحد الجيد للعدد الكلي للبكتيريا بينما لم تتجاوت اي عينة الحد المسموح به للعدد الكلي للبكتريا. معظم عينات الشلب تجاوزت العدد المسموح به للخمائر والاعفان وعدد قليل من النماذج تجاوزت العدد الكلي لبكتريا القولون. قللت العملية التصيعية للشلب الحمل المايكوبي لبعض عينات الشلب بينما التاثير لم يكن واضحا مع عينات اخرى وزداد المحتوى المايكروبي لعينات اخرى بعد العملية التصنيعية. عشر عينات رز على درجة بياض 32 وثمان عينات رز على درجة بياض 36 من اصل 13 عينة تجاوت الحد المسموح به للخمائر والاعفان بالنسبة لمواصفة الغذاء والدواء الامريكية والمواصفة القياسية العراقية. كل عينات الرز المصنة تجاوت الحد المسموح به لبكتريا القولون بالنسبة للمواصفة القياسية العراقية بينما فقط ستة عينات وثلاث عينات من اصل 13 عينة تجاوز الحد المسموح به لذات البكتريا بالنسبة لمواصفة منظمة الغذاء والدواء الامريكية لرز على درجة بياض 32 و36 على التوالي. نستنتج من الدراسة ان عينات الشلب الماخوذة من حقول الشلب في منطقة الفرات الاوسط تجاوزت بعض الحدود المايركروبية من الحقل دون تاثير للعملية التصنيعية او الخزن عليها وان مياه الري اعتبرت المصدر الرئيسي لتلوث الحقول لذلك يجب مراقبة حقول الشلب للسيطرة على التلوث المايكروبي.

الكلمات المفتاحية: التلوث المايكروبي، حقول الشلب، مياه الري، بكتريا *E. coli*، رز، تصنيع الشلب.