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FUNGAL PATHOGENIC INFESTATION IN RELATION TO HEAVY METAL CONTAMINATION IN RICE

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Abstract

Rice is a staple food for many people and is vulnerable to contamination from soil and irrigation water, which poses significant health and trade concerns, particularly in countries like Pakistan. Therefore, the occurrence of heavy metal contaminations in relation to fungal pathogens was determined considering type, variety and location of rice. All the samples were compliant with the permissible limits set by the WHO. However, only 2.5% of the total samples had mercury levels slightly higher than the EU limit. The location imparted significant ($p<0.01$) influence on arsenic levels in rice. About half of the paddy and few brown rice samples showed incidence of fungal pathogens, primarily *Tilletia barclayana*. Effects of rice type, variety and location were found to be significant ($p<0.001$) on the incidence and severity of fungal pathogens on rice. However, the most effective source of variation was an interaction of rice type and variety. The severity of fungal pathogens was moderately related (r= -0.58) to the arsenic contamination levels. A weak correlation (r=0.31) found between arsenic and mercury, indicating that their uptake in rice plant is independent of each other. Compared to rice grain forms, the co-products viz. broken rice and rice bran showed higher levels of mercury contamination, while indifferent in arsenic and fungal incidence. The rice variety IRRI-9 demonstrated the highest and statistically significant ($p<0.05$) levels of mercury contamination and the most severe infestation by fungal pathogens, requiring specific preventative measures or control strategies compared to other varieties.

Key words: Rice, Paddy, Mercury, Arsenic, Fungal Pathogen.

Introduction

Rice is one of the most widely grown and consumed crops worldwide, serving as a main source of energy and nutrition, especially in Asia. Beyond its nutritional value, rice holds cultural and economic significance as well. Rice cultivation provides livelihood for millions of farmers, and the rice industry is a major contributor to many countries' economies. It is also a significant export crop for countries like Pakistan, and also playing a crucial role in the global economy. However, it is commonly grown in areas that may be susceptible to environmental contaminants such as heavy metals, pesticides, and microorganisms. Rice plant absorbs these contaminants from the soil and water, posing a potential threat to human health when consumed and can also have significant negative impacts on trade (Mitra *et al*., 2022).

The current state of the global climate continues to worsen, resulting in high levels of carbon emissions and heavy metal pollution that pose serious threats to human health and civilization (Lu *et al*., 2020) Human activities such as the disposal of solid waste, gasoline, and various substances like paint, manure, and fertilizer, have led to the accumulation of heavy metals in the soil and increase their presence in food (Akoury *et al*., 2023; Guo *et al*., 2022). Exposure to high levels of heavy metals can have serious health consequences, including damage to the nervous system, kidneys, and other organs (Rehman *et al*., 2018). Similarly, fungal pathogens in rice can have a substantial negative impact by causing reductions in crop yields, degradation of grain quality, and contribute to a decline in economic stability for those relying on rice production for their livelihood. The incidence of heavy metal contamination and fungal pathogens in rice is crucial for ensuring food safety and security, and for the development of effective strategies for controlling these issues.

The occurrence of heavy metals has been identified in almost all major rice growing countries like China (Guo *et al*., 2022; Xie *et al*., 2023), India (Akoury *et al*., 2023), Thailand (Satachon, Keawmoon, Rengsungnoen, Thummajitsakul and Silprasit, 2019). Arsenic and mercury have been reported in rice grown in certain areas of Pakistan (Ali, Khan and Ilahi, 2019; Aslam *et al*., 2020). However, the levels of contamination varies from country to country. The variability could be due to the differences in environmental conditions as these metals can come from a variety of sources, including industrial pollution, agricultural pesticides, and natural mineral deposits in the soil (Akhtar *et al*., 2021). Other factor that may influence the contamination level is the genetic makeup of the rice (Kumarathilaka, Seneweera, Meharg and Bundschuh, 2018). It is rather common for identifying the resistance of rice plant against a particular pathogen or disease but a limited attempt has been made to evaluate the influence of varietal differences on metal uptake (Liu *et al*., 2021). Similarly, the variability in contaminant levels in different rice forms and their presence in co-products has not been explored comprehensively. Few studies have highlighted the relationship of arsenic contamination with fungal pathogen incidence in rice and suggested that arsenic showed certain activity against fungal pathogens. Like Sultana *et al*., 2023 reported that arsenic rich environment lead to decrease in blast incidence compared to the rice with no or less contamination of arsenic (Sultana *et al*., 2023).

Similarly, Griffith *et al*., (2021) found that rice treated with arsenic was less susceptible to M. oryzae infection than control (Griffith *et al*., 2021). Other study found no impact of arsenic on fungal pathogens (Li *et al*., 2021).

Given the widespread cultivation and consumption of rice, it is important to ensure the safety and quality of the rice supply. Contaminants such as heavy metals and fungal pathogens can pose significant health risks, and it is essential to understand and manage the factors that influence their occurrence in rice. Therefore, this study aimed to examine the presence of heavy metal contamination and fungal pathogens in rice and its by-products, considering the impact of factors such as rice type, variety, and location on the accumulation of these contaminants in rice. This study further explored different forms of rice, including paddy, brown parboiled, and polished rice, emphasizing the importance of ensuring rice safety and quality, as it serves as a staple food for a substantial portion of the population.

Material and Methods

Sampling and sample description: In the period between 2019 and 2022, this study was conducted in which 129 samples were collected from various sources such as consumer markets, farmer fields, and rice mills situated in six different locations in the province of Sindh, Pakistan. These samples (at least 1.5 kg of each) comprised of polished rice, brown rice, and parboiled rice, and included both Basmati and non-Basmati varieties like Super Basmati (26), IRRI 6 (43), D-98 (31), and IRRI-9 (29). Additionally, samples of rice processing by-products like rice bran (15) and broken rice (23) were also collected to evaluate the presence of contaminants in different rice fractions. According to information provided by the representative at the sample collection site, the samples collected from the field and mills were not older than one month, while the consumer market samples represented lots stored for up to six months.

Cleaning of samples: All samples were cleaned manually and by using sieves to remove foreign matter, including stones, straw and dust. Two representative sub-samples of ~0.5 kg each were drawn from respective sample for the analysis of heavy metal contamination and fungal pathogen incidence, separately.

Sample digestion: Prior to analysis all glassware were soaked in chromic acid followed by rinsing with double distilled water. Approximately 1.0 g of each sample in triplicate were taken into digestion tubes and soaked overnight in a mixture of perchloric and nitric acid (1:3). Samples were digested at 120°C for 2hrs followed by heating at 180°C till the solution became clear and the volume reduced to 2-3 ml. Then cooled and shifted into volumetric flask and makeup the mark with 0.1 M HNO_3 (AOAC, 2000). The digested samples were then analyzed for arsenic and mercury as described in section below.

Estimation of heavy metals: Mercury and arsenic were determined through Vapor Generation Assay by using Atomic Absorption Spectrophotometry (AAS) and following the method described JOAC Vol 77 (01) (1994) with slight modifications. LOD of mercury and arsenic were 0.5 and 1.0 µgkg-1, respectively.

Assessment of fungal infection: The AACC 42-70 (microscopic method) was implemented for pathological contaminations of rice samples. Samples were assessed virtually and by using appropriate sieves for incidence of insect infestation (Chemists & Committee, 1983).

Statistical analysis

At least triplicate attempts on each sample were analyzed for estimating mean and standard deviation. A 3-way ANOVA for a factorial design (the factors were variety at 4 levels, rice type at 4 levels; and location at 6 levels) was performed to analyze the sources of variation in mercury, arsenic and fungal infection in rice. Duncan's test (at $p<0.05$) was used to separate samples within variety/location or rice type. Pearson's correlation (r) was determined between arsenic and mercury and also with the severity of fungal pathogens in rice. All the statistical analysis was undertaken using SPSS software (SPSS version 21, Inc., USA).

Results and Discussion

Occurrence of arsenic and mercury contaminations in rice: Table 1 shows the arsenic and mercury contaminations in rice samples. It has been observed that 99% samples were contaminated with mercury while 85% of samples were contaminated with arsenic. Heavy metal contamination of rice has been a concern in several countries. Studies on Chinese rice have suggested that a significant portion is contaminated with mercury (Xie *et al*., 2023). Similarly, a study by Le Wang *et al*., (2021) found more than 80% of rice samples from Nepal were contaminated with mercury (Wang *et al*., 2021). However, the level of mercury was found to be double compared to arsenic contamination in rice. They were ranged between 0.6 to 20.62 µg/kg (10.009µg/kg) and 0.04 to 17.83µg/kg (5.25µg/kg), respectively.

Rice-type-wise variability: Statistically, insignificant ($p>0.05$) differences were found within the rice types for mercury and arsenic contaminations (Table 1). Maximum concentration of mercury was found in polished rice followed by brown rice and paddy. This could be due to a variety of factors, such as the type of soil in which the rice is grown or the methods used to process and polish the rice. On other side, arsenic was found maximum in paddy. During the milling process, which removes the outer layers of the rice grain to produce polished rice, much of the arsenic is removed along with the bran and germ. The remaining endosperm, which is used to produce white rice, has lower levels of arsenic. Mercury and arsenic were found minimum in parboiled rice. This could be due to the parboiling process, which involves soaking, boiling and drying the rice before milling, can remove some of the contaminants, including mercury and arsenic, present in the outer layers of the grain. Contrary to our results, Bielecka *et al*., 2020 conducted research on different rice type and reported that parboiled rice showed maximum level of mercury contamination compared to white rice (Bielecka *et al*., 2020).

Variety-wise variability: Mercury contamination was found significantly (p<0.05) higher in IRRI- 9 compared to other varieties. This could be due to the presence of certain genes that control the absorption from the soil and water, and further transport and storage of these contaminants within the plant. However, insignificant differences (p>0.05) were found amongst rice varieties in arsenic varieties. The varietal influence on arsenic and lead contaminations was investigated in Zamfara State, Nigeria and reported similar to our study that variety has no such effect on the accumulation of arsenic content (Mandal *et al*., 2022).

Location-wise variability: Maximum contaminations of mercury and arsenic were found in L6 and L1, respectively (Table 1). Within same location, arsenic levels were higher in L1 and L2 compared to mercury whereas rest of locations showed higher contamination levels of mercury compared to arsenic. Similar type of results were reported in different rice growing areas of China (Cai *et al*., 2019). Significant variations in the levels of mercury and arsenic found in different locations could be due to factors such as differences in soil composition, the use of irrigation water, agricultural practices, and the presence of industrial pollution in the area.

Incidence of fungal infection in rice: Table 2 shows the incidence of fungal infection in rice samples. Out of the samples studied, about 12% were found to be infected with fungal pathogens, and this infection was limited only to paddy and brown rice. This indicates that the incidence of fungal infection is relatively low, but it is still present in these two types of rice. The samples studied were infected with three different fungal pathogens: *Tilletia barclayana*, *Alternaria pedwikii,* and *Bipolaris oryzae*. However, the most common pathogen found was *Tilletia barclayana* (black smut of rice), with levels ranging from 0.001 to 0.109 grams per 250 grams of rice sample (Khanal *et al*., 2022).

Rice-type-wise variability: The maximum incidence of fungal infection was found in paddy, with 53% of the samples studied showing signs of infection. This indicates that paddy is particularly susceptible to fungal pathogens, and may require additional measures to control or prevent the spread of fungal infections. Brown rice, which is rice that has had only the outermost husk removed, also showed some incidence of fungal infection. On the other hand, polished and parboiled rice, which are further processed forms of rice, were found to be free of any fungal incidence.

Variety-wise variability: All the varieties of rice studied were found to have some level of fungal infection, with an average of 12% of the samples showing signs of infection. However, the severity of the incidence was found to be highest in the IRRI-9 variety. Additionally, the infection level in IRRI-9 was significantly (p<0.05) different from that of the other varieties, indicating that IRRI-9 may be more susceptible to fungal pathogens than the other varieties. Younas *et al*., 2023 supported our research findings and reported that variety plays significant role and amongst one of the best approaches to reduce the pathogenic infestation (Younas *et al*., 2023).

Location-wise variability: The fungal infection was found in rice obtained from 50% of the locations included in the study. Additionally, the severity of infection was highest in rice obtained from location 6 (Qambar Shahdadkot) and the level of infection in this location was significantly different from the levels found in other locations. This indicates that the growing conditions in Qambar Shahdadkot may be particularly conducive to the growth of fungal pathogens, making the rice grown in this location more susceptible to fungal infections. The intensity of smut disease in selected areas of China was studied in 2021 and they found maximum smut infection in the country (An *et al*., 2021)

Incidence of fungal infection and metal contamination in rice co-products: Rice is often processed to produce some co-products such as rice bran and broken rice. Both broken rice and rice bran contained insignificantly (p>0.05) different mercury levels from each other with the mean values of 18.14µg/kg and 17.03µg/kg, respectively (Fig. 1). However, broken rice and rice bran samples had shown higher levels of mercury contamination compared to paddy, brown, polished and parboiled rice. This means that these co-products may retain more of the contaminants present in the outer layers of the grain, including mercury. Further, broken rice and rice bran are co-products of the milling process and are not subjected to the same level of cleaning and processing as the main products. The contaminations of arsenic in these co-products were slightly lower than rice types. Previous study recorded higher levels of arsenic in rice bran and its products compared to rice grains (Weber *et al*., 2021).

Isolated fungi were identified using cultural and morphological features illustrated by Gaire *et al*., 2023. *T. barclayana* were detected in rice bran in the range between 0.013 and 0.066g with the mean value of 0.046g/250g (Fig. 2). Results showed that *T. barclayana* can survive and grow on rice co-products such as broken rice, rice bran and can reduce the shelf-life of these products (Li *et al*., 2022). However, rice co-products are considered to be safe as they are undergone multiple stages of cleaning and purification process (Spaggiari *et al*., 2021).

Samples exceeded regulatory limits: All the samples were compliant with the permissible limits set by the world health organization for Arsenic and mercury. However, only 2.5% of the total samples exceeded the limit of 20µg/kg for mercury with the levels slightly higher than the limit set by EU. Whereas, none of the samples exceeded the limit for arsenic contamination. A previous study on determining mercury in Pakistani rice also found only 2% samples that exceeded the limit of 20µg/kg (Aslam *et al*., 2020). In the present study, none of the samples exceeded the Codex-adopted Pakistan standard for rice (PS: 3342-2007), which sets the permissible limit for arsenic at 200 µg/kg.

Table 1. Occurrence of mercury and arsenic contaminations in rice.

Rice type-wise		Mercury (µg/kg)			Arsenic (µg/kg)		
		Mean	Range	SD	Mean	Range	SD
Paddy	16	9.489^{a}	0.86-20.01	± 6.02	6.818^{a}	0.38-16.35	± 4.97
Brown	31	9.861^{a}	1.17-18.84	± 6.09	4.181^{a}	0.04-9.43	± 2.87
Polished	56	10.609^{a}	0.60-20.62	± 6.40	6.049^{a}	0.26-17.83	± 4.77
Parboiled	26	6.547^{a}	4.49-10.15	± 2.16	3.778^{a}	1.81-6.18	± 1.78
Location-wise							
L1	13	6.9575^{a}	1.36-17.41	± 6.23	11.8863^{c}	1.41-17.43	± 5.60
L2	11	8.0943^{a}	0.6-15.49	± 6.32	10.3243^{c}	1.4-16.97	± 5.25
L3	28	8.7407^{ab}	3.15-19.44	± 4.68	2.2453^{a}	0.26-4.74	± 1.49
L4	18	9.9593^{ab}	1.96-19.09	± 6.25	5.1393^{b}	1.13-9.13	± 2.41
L5	30	10.0068^{ab}	0.6-20.62	± 6.98	4.2100^{ab}	0.04-9.43	± 2.54
L6	29	13.8380^{b}	4.6-18.46	± 4.15	4.4547^{ab}	0.26-8.93	± 3.24
Variety-wise							
Super basmati	26	8.9772^{a}	2.59-18.84	± 5.05	3.2359^{a}	0.26-9.13	± 2.27
IRRI-6	43	9.5737^{a}	0.0-20.62	± 7.13	7.8030^{b}	0.91-17.83	± 5.18
D-98	31	9.6382^{a}	0.62-17.24	± 6.63	5.2959^{a}	0.04-15.41	± 3.98
IRRI-9	29	14.2475^{b}	4.6-19.13	± 4.21	4.8808^{a}	0.26-8.93	± 3.13

n= 129, L1=Badin; L2=Thatta; L3=Karachi; L4=Shikarpur; L5=Larkana; L6= Qambar Shahdadkot
Different superscript letters within each row are significantly different at $p<0.05$

Table 2. Incidence of fungal pathogens in rice.

Rice-type-wise	No. of samples	Infected samples (%)	Fungal infection (g / 250 g)		
			Mean	Range	SD
Paddy	16	57	0.0381^{a}	0.001-0.109	± 0.039
Brown	31	10	0.046^{b}	0.013- 0.053	± 0.019
Polished	56	ND	ND	ND	ND
Parboiled	26	ND	ND	ND	ND
Location-wise					
L1	13	ND	ND	ND	ND
L2	11	ND	ND	ND	ND
L3	28	ND	ND	ND	ND
L4	18	7	0.023^{a}	0.001- 0.034	± 0.034
L5	30	13	0.004^{a}	0.013-0.053	± 0.019
L6	29	29	0.021^{b}	0.001- 0.109	± 0.044
Variety-wise					
Super basmati	26	12	0.004^{a}	0.001-0.066	± 0.032
IRRI-6	43	11	0.007^{a}	0.033-0.109	± 0.034
D-98	31	11	0.004^{a}	0.013-0.015	± 0.027
IRRI-9	29	14	0.014^{b}	0.093-0.106	± 0.042

n= 129, ND= Not Detected, L1=Badin; L2= Thatta; L3=Karachi; L4= Shikarpur; L5= Larkana; L6= Qambar Shahdadkot

Table 3. Analysis of variance of metal contaminations in rice.

Parameters	R^2	*F* value							
		Corrected model	T	V	L	T × V	T × L	V × L	T × V × L
Mercury	0.25	1.038ns	1.55ns	0.64ns	1.058ns	1.77ns	0.494ns	1.517ns	1.413ns
Arsenic	0.50	3.125***	0.012 ns	1.061ns	4.846**	0.215ns	0.182ns	0.288ns	0.172ns
Fungal infection	0.85	13.242***	7.534***	9.133***	7.108***	11.639***	0.645ns	0.315ns	0.213ns

F values used type III mean squares; ns, non-significant; *, $p<0.05$; ** $p<0.01$ and ***, $p<0.001$
T = Rice-type; V = Variety; and L = Locations

Analysis of variance (ANOVA): Table 3 show the results of ANOVA that was performed to determine the effect of rice type, variety and location on the contaminations of mercury, arsenic and fungal pathogens in rice. Only location had a significant ($p<0.01$) influence on arsenic contamination levels in rice. Contrary to our results, there was no clear spatial pattern in total arsenic concentration in northeastern region of China (Chen *et al*., 2018). However, the results of present study suggested that rice type and variety had statistically insignificant ($p<0.05$) influence on the mercury and arsenic contamination in rice.

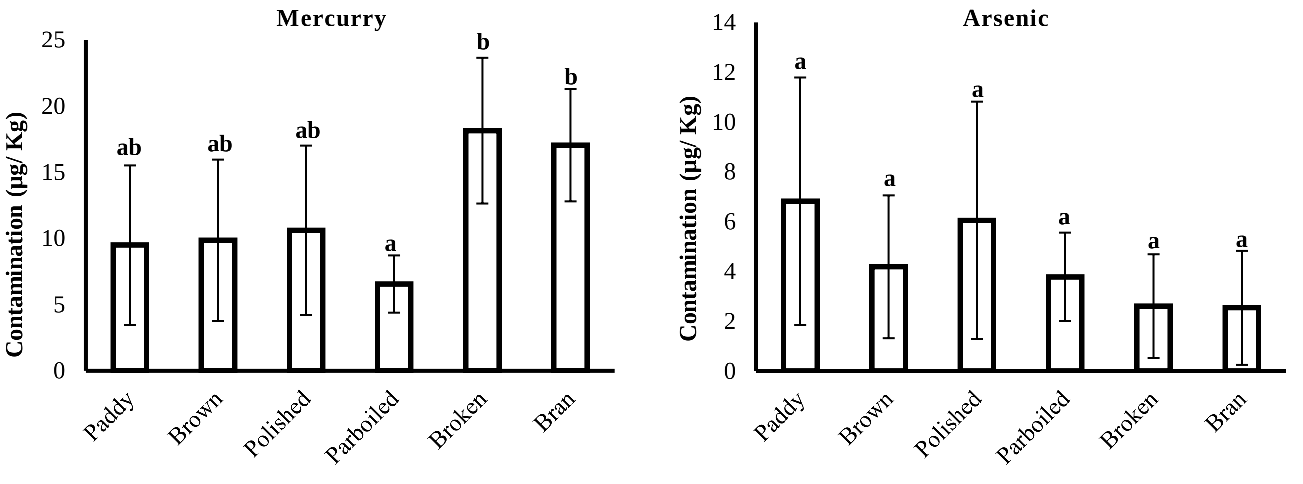
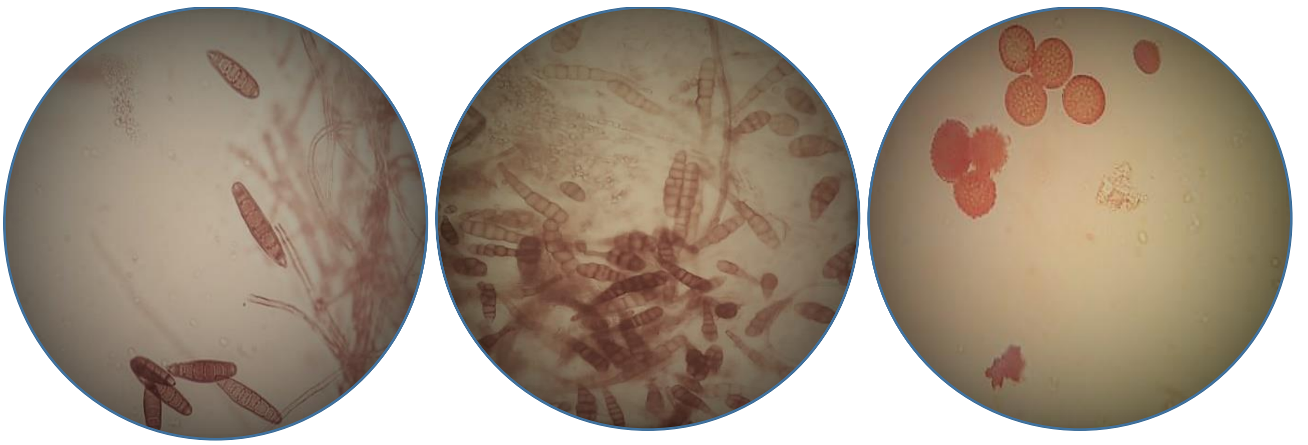


Fig. 1. Mercury and arsenic contaminations in rice co-products.



(a) **(b)** **(c)**

Fig. 2. Representative micrographs of (a) *Alternaria pedwikii* **(b)** *Bipolaras oryzea* and **(c)** *T. Barclayana.*

Effects of rice type, variety and location were found to be significant (p<0.001) on the incidence and severity of fungal pathogens on rice. However, the most effective source of variation was an interaction of rice type and variety. Rice varieties may have varying levels of resistance to certain pathogens, and growing conditions in different locations may also affect the susceptibility of the crop to infection. Additionally, the interaction of rice type and variety may indicate that certain combinations of these factors are particularly effective in reducing the incidence and severity of fungal pathogens. Previous study showed that rice variety and location played a vital role in resistance to the pathogenic fungi (Liu *et al*., 2021).

Relationships amongst mercury, arsenic and fungal infection: A significantly (p<0.05) moderate negative correlation (r=-0.58) was found between arsenic and fungal infection in paddy. The presence of arsenic in paddy may inhibit the growth of fungal pathogens, acting as a natural fungicide. Arsenic is known to have antimicrobial properties and can disrupt the metabolism of fungal cells, making it difficult for them to survive and reproduce. Another reason could be that the fields that are more prone to fungal infections may have lower levels of arsenic in the soil. Fungal pathogens thrive in environments that are warm, moist and have high organic matter, which may not be suitable for the growth of arsenic-tolerant plants. In such fields, farmers may not use arsenic-containing pesticides or fertilizers, leading to lower levels of arsenic in the rice grown there. However, further research is needed to exploit the relationship between arsenic and fungal infection in rice.

Arsenic contamination was weakly correlated with the mercury contamination in paddy (r=0.31) samples only. Since both mercury and arsenic can be taken up by rice plants through their roots and the soils where the rice is grown have similar characteristics that would result in a similar level of contamination in paddy.

Further, we have neither found any relationships between fungal severity and mercury contamination nor between the levels of arsenic and mercury in brown, polished and parboiled rice samples.

Conclusion

Although most of the samples were contaminated with mercury and arsenic but only 2.5% samples exceeded the limit of EU for mercury (i.e. 20sµg/Kg) with the levels slightly higher than the limit. Neither the rice type nor the rice variety imparted significant influence on heavy metal contamination in rice. Only, location influenced significantly the arsenic levels in rice. On other side, rice type, variety and location significantly influenced the incidence and severity of fungal pathogens on rice. About half of the samples of paddy and some of brown rice showed incidence of fungal infection mainly with *Tilletia barclayana* indicating that this could be a significant problem for rice crops and should be a priority for control and prevention measures. The rice variety IRRI-9 showed maximum mercury contamination and severity of fungal pathogens and was significantly different from other varieties in these respects. This result may indicate that IRRI-9 requires specific preventative measure or control strategy compared to the other varieties. The higher levels of arsenic does not mean the increased levels of mercury in paddy, but the arsenic contamination may inhibit the fungal attack or growth in rice plant. However, more efforts are needed to understand the underlying mechanisms and the impact of arsenic on fungal infections in rice.

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