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STUDY OF FUNGI FROM THE CONTAMINATED SOILS OF PERI-URBAN AGRICULTURAL AREAS

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Abstract

The micro-fungal flora of heavy metals contaminated peri-urban agricultural fields of Pakistan were investigated in terms of their diversity by soil serial dilution method. A total of 30 micro-fungi were isolated from 6 sampling sites. Of these isolates 24 belong to phylum Ascomycota, 3 to phylum Zygomycota, 2 to phylum Basidiomycota and 1 to phylum Deuteromycota. The most widespread genus was *Aspergillus* and common species *Aspergillus niger*. Frequency percentage showed that Kasur is rich in fungal population as compared to other peri urban areas while Wah Cantt showed maximum fungal Colony Forming Unit (CFU). The aim of present investigation was to see the diversity of fungi in heavy metal contaminated soils of peri-urban agricultural areas and study them in future for heavy metal tolerance and biosorption analysis in reference to bioremediation.

Introduction

Soil resources are critical to the environment as well as to food and fiber production. Soil is the habitat for many organisms including bacteria, fungi, algae, viruses and protozoa. It supports the growth of a variety of unstressed plants, animals and soil microorganisms usually by providing a diverse physical, chemical and biological habitat (Yoder, 1937; Cihacek *et al*., 1996). Microorganisms are found in large numbers in soil-usually between one and ten million microorganisms are present per gram of soil with bacteria and fungi being the most prevalent (Yoder, 1937). Estimated numbers of soil species include 30,000 bacteria, 1,500,000 fungi, 60,000 algae, 10,000 protozoa, 500,000 nematodes and 3,000 earthworms (Pankhurst, 1997). Agricultural production systems in urban and peri-urban areas can pose risks to public health and the environment. These arise from the inappropriate or excessive use of agricultural inputs-including pesticides, nitrogen and raw organic matter containing heavy metal residues-which may leach or runoff into drinking water sources, microbial contamination of soil, water and air pollution. In particular, leafy vegetables can be contaminated through overuse of chemical sprays, while zoonotic diseases and veterinary public health problems can arise from intensive livestock production (Evan, 2002). In the present study the diversity of fungi of the heavy metals contaminated peri-urban agricultural soils of Lahore, Faisalabad, Multan, Islamabad, Kasure and Wah Cantt was studied because in peri-urban areas the use of wastewater for agriculture is common. Industrial waste or effluents have no proper planning for their disposals and wastewater directly enter into the water bodies as a result huge amount of toxic chemicals and heavy metals are added in irrigation water. Wastewater is often the only source of water for irrigation in these areas. Farmers of big cities where water from natural surface rain is not easily available use sewerage water and water of natural drains for crop production, due to their being less expensive. The reality is that wastewater generated in Pakistan receives no treatment at all. The use of wastewater for irrigation may affect the whole biological community, including species diversity and accumulation of toxic contaminates in food chain. The main objective of this study was to study the mycoflora of peri-urban agriculture soils with the possibility of heavy metal contamination and use them for tolerance and biosorption analysis in reference to bioremediation. .

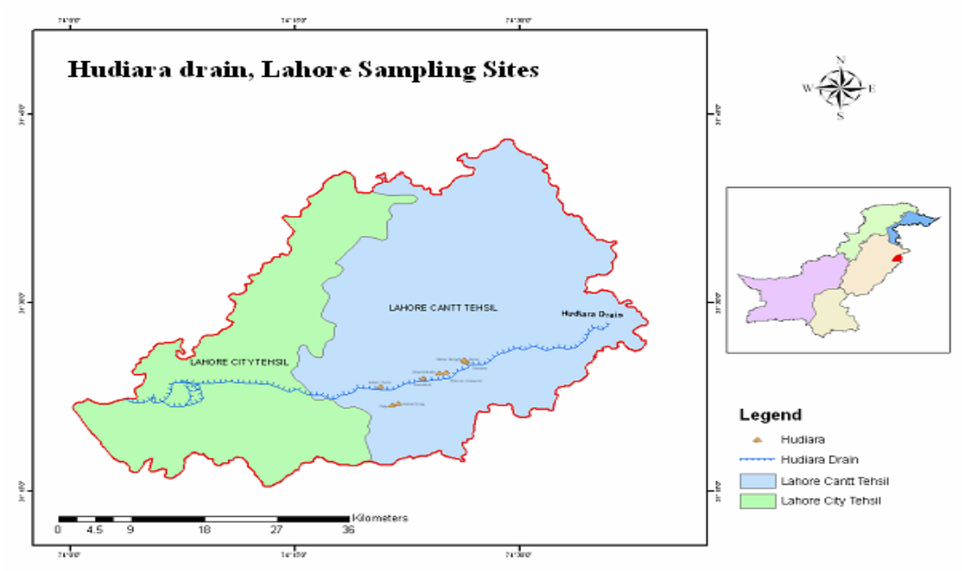
Materials and Methods

Study area and samples collection: Soil was collected from peri-urban agricultural fields of Lahore, Faisalabad, Multan, Kasur, Islamabad and Wah Cantt (Fig. 1). Soil samples were randomly collected from the top (3-5 cm) and thoroughly mixed. After sample collection, samples were brought in laboratory for the study of diversity of fungi.

Media preparation: Potato dextrose agar (PDA) media was used for the isolation of fungi (Razak *et al.*, 1999). Potatoes (200g) were peeled, sliced and boiled, and then sieved through a clean muslin cloth to get a broth in which agar (15g) and dextrose sugar (15g) was added. The media was then autoclaved. To suppress the bacterial growth, 30mg/L of streptomycin was added in the medium (Martin, 1950).

Soil dilutions method: The soil samples were processed with isolation procedure using the soil dilution plate method (Waksman, 1922). One g of soil was mixed in 10 ml sterilized distilled water to form an aliquot then 1/1000 dilutions of sample were prepared and inoculated on to prepared potato dextrose agar plates.

After incubation distinct colonies were counted and identified at generic level on the basis of macroscopic and microscopic characteristics (Zafar *et al.*, 2006). Fungi were preserved on PDA slants for further research.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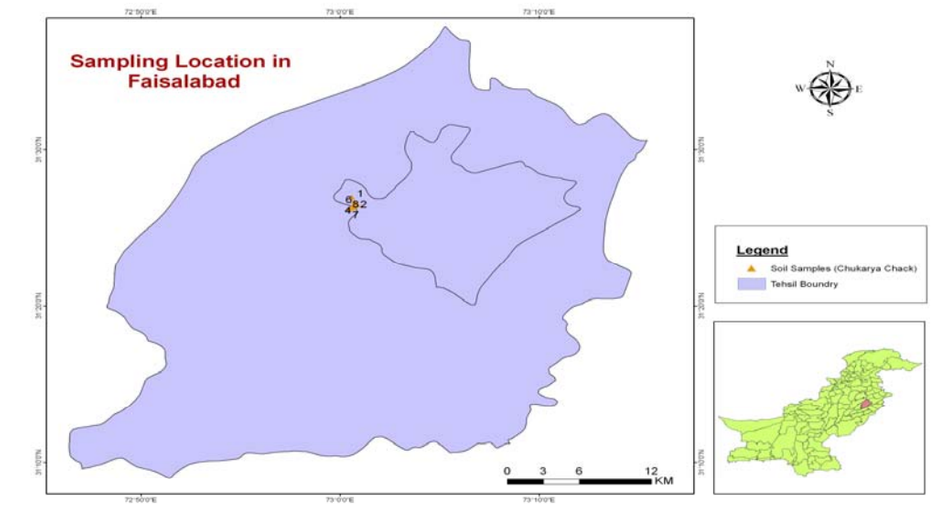
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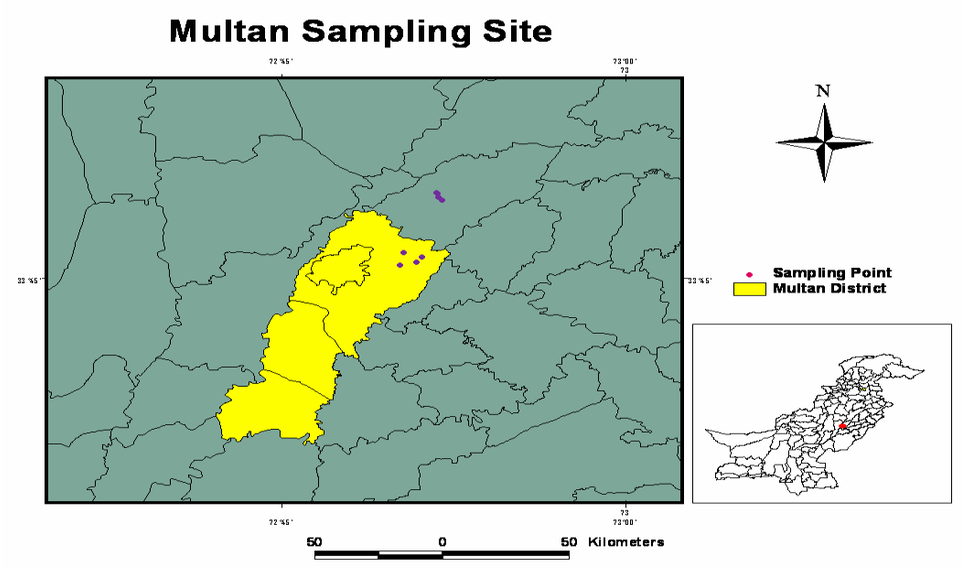
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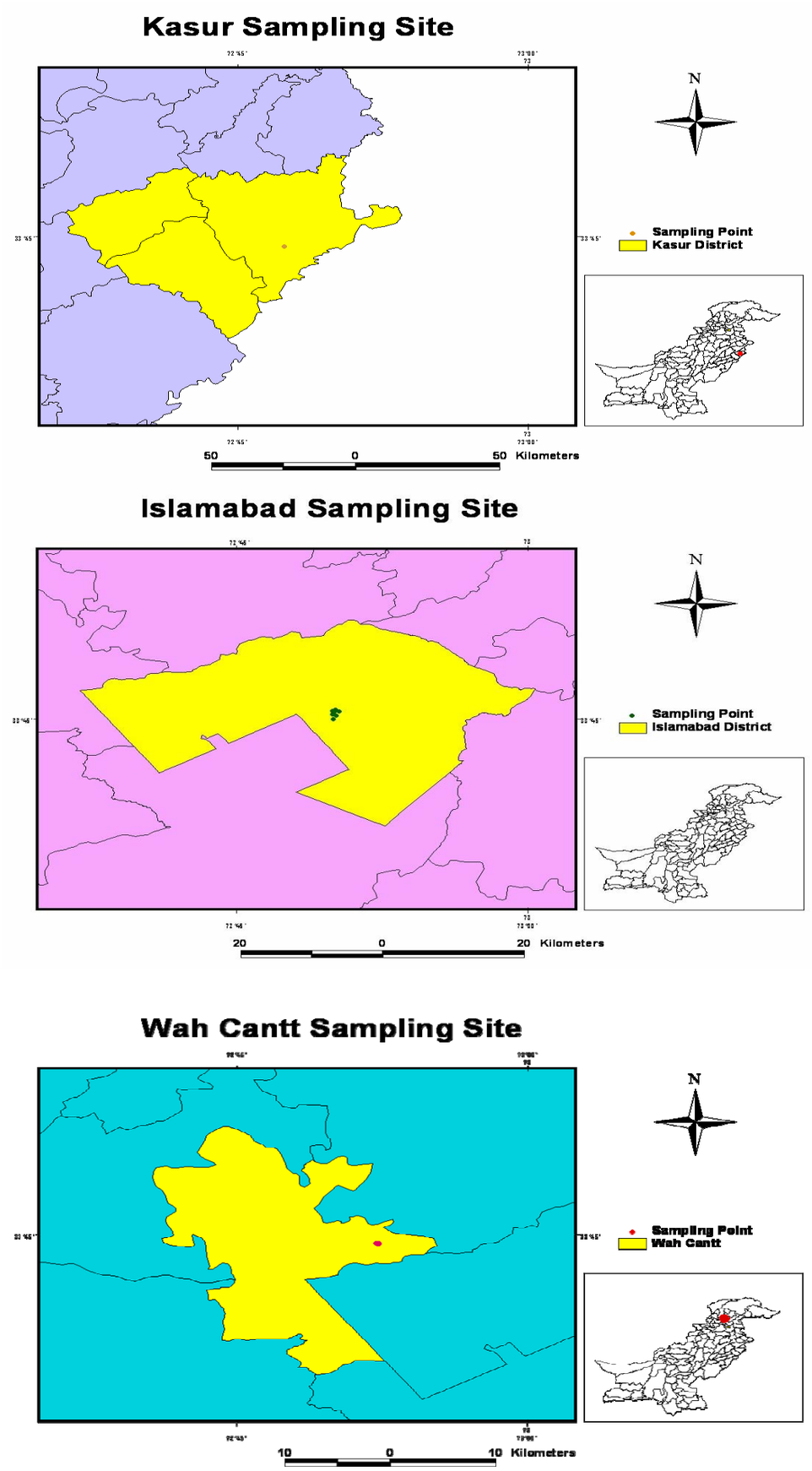

Fig. 1. Soil samples collection from the contaminated peri-urban agricultural fields.

Results and Discussion

Maximum fungal diversity was found in Kasur flowed by Multan, Islamabad and Faisalabad (Table 1). At Lahore and Wah Cantt the fungal diversity was same. *Aspergillus* spp., were isolated from all the sampling sites but from Multan soil samples only *Aspergillus niger* was isolated. In the present investigation the richest class was Ascomycota (24 genera), Zygomycota (3 genera), Basidiomycota (2 genera) and Deutromycota (1 genus). Azaz (2003) reported that 1 g of fertile land soil micro fungi are around 400,000. In the present study few types of fungi were isolated because peri-urban areas are contaminated of heavy metals and sources are automobiles exhaust, sewage water, industrial, tanneries waste and it can be argued that the peri urban fields of present investigation are poor in quality with highest level of contamination. Major differences among the species in terms of both numbers of spores and tolerance to metals suggest that fungi follow different strategies to establish symbiosis and probably *Aspergillus* sp., was preferentially found in soil samples with the differences in functioning (Johnson *et al.,* 1992; Allen *et al.,* 1995; Bever *et al.,* 1996). The increase in fungus propagule diversity could be a fungal stress response whereby fungal ecotypes are better adapted to unpolluted soil but affected at intermediates rates of contamination allow other fungi, probably less competitive in non stressed soils but better adapted to heavy metals to colonize the roots and complete their life cycles. Thus, the number of fungal ecotypes in these soils can be increased. However, at the highest levels of soil pollution, both indices diminished sharply. Certain soil fungus species are better adapted to the disturbance produced by the addition of metals would overcome the stress situation and complete their life cycles. The relationship between genetic diversity within populations and heavy-metal stress in soils may lead to an increase in diversity with a moderate metal loading, followed by a sharp decrease at higher levels of stress (Giller *et al.*, 1998). The reasons underlying stress-related changes in the diversity of soil fungus populations, particularly those due to the presence of heavy metals, are not completely understood. It is well known that heavy metals cannot be chemically degraded. A better understanding of the mechanisms behind these changes in fungal diversity, and particularly of those on which fungal adaptation and tolerance to metals are based, is important, since such an understanding could facilitate the management of these soil microorganisms for a restoration and/or bioremediation program (Comis, 1996).

Table 1. Diversity of fungi of the contaminated peri-urban agricultural fields.

Fungi	Faisalabad	Lahore	Kasur	Wah Cantt	Multan	Islamabad
Class Ascomycota						
Acremonium sp.	-	-	+	-	+	-
Aureobasidium sp.	-	-	+	-	-	-
Alternaria sp.	-	-	+	-	-	-
Aspergillus sp.	+	+	+	+	-	+
Aspergillus flavus	+	+	+	+	-	-
Aspergillus fumigatus	-	-	-	+	-	+
Aspergillus nigar	+	+	-	+	+	+
Aspergillus versicolor	+	+	+	-	+	-
Botrytis sp.	+	+	-	+	-	+
Chaetomium sp.	-	+	+	+	-	+
Coniothyrium sp.	+	-	-	-	-	-
Curvularia sp.	-	+	+	+	+	-
Fusarium sp.	+	-	+	+	-	-
Geotichum sp.	-	-	+	-	+	-
Histoplasma sp.	-	+	-	-	-	+
Humicola sp.	-	-	+	-	+	-
Monilia sp.	-	-	-	-	+	-
Monocillium sp.	-	-	-	-	+	-
Helminthosporium sp.	+	-	-	-	-	-
Neottiospora sp.	-	-	-	-	-	-
Oidiodendron cerealis	+	-	+		+	+
Paecilomyces sp.	-	-	+	-	-	-
Pseudeurotium sp.	-	-	+	-	-	-
Scopulariopsis sp.	+	-	-	-	-	-
Class Zygomycota						
Mortierella sp.	+	-	-	-	-	+
Mucor sp.	-	-	+	-	-	-
Rhizopus sp.	-	-	-	-	+	+
Class Basidiomycota						
Rhizoctonia sp.	-	-	-	-	+	-
Sclerotium sp.	-	-	-	-	+	-
Class Deutromycota						
Penecillium sp.	-	+	+	+	-	+
Non-Sporulating fungi	-	-	+	-	+	-

Frequency percentage of isolated fungi: The frequency distribution of fungi is shown in Figure 2. The maximum frequency of fungi was shown in Kasur (50%) and Multan samples (40%) followed by Wah Cantt (33%), Islamabad (35%) and Faisalabad (31%), Lahore (35%).

A present study concludes, even though it was performed with soil fungi isolates, suggests that fungi from metal-environments differ in metal sensitivities and that some of these fungi may survive metal stress by avoiding soil microhabitats with toxic metal ion concentrations. This ability may be of particular importance for fungi introduced during restoration practices into habitats with a history of heavy metal pollution. The localization of the metal effects on soil fungi indicates that metal-sensitive fungi may be able to survive and propagate in metal-polluted environments by thriving in relatively uncontaminated soil micro sites (Balsberg, 1982; Turner & Dickinson, 1993).

Colony forming unit of isolated fungi: Figure 3.2 shows about the colony forming unit of the collected soil samples. Maximum CFU was showed by Wah Cantt and Islamabad fungal isolates whereas minimum CFU was observed by Multan isolates.

Microorganisms like fungi, bacteria etc are known to tolerate and accumulate heavy metals. The short-term effects on microbial activity clearly indicate that bacteria and fungi were affected differently by the addition of the metals. The finding that fungal activity was less affected was in accordance with earlier studies indicating that fungi were less sensitive to heavy metals than bacteria (Maliszewska *et al.*, 1985; Hiroki, 1992; Korthals *et al.*, 1996; Müller *et al.*, 2001; Khan & Scullion, 2002). The opposite effect of heavy metals on these groups of organisms was further evident by the drastic decrease in CFU for bacteria but not for fungi found in the previous study and by the increase in the relative fungal/bacterial ratio with increasing metal levels. Finally to general concern, less information has been gathered in Pakistan heavy metal effect on fungal diversity in peri-urban agriculture soil. Only preliminary findings are present in this concern. Further investigations are needed to provide sufficient information in this context, especially because peri-urban agriculture is of utmost importance for sustainable use of resources, as Pakistan is an agricultural country. Hope this investigation will lay basis for further investigations for large scale operation like tolerance, biosorption and genetic analysis in reference to bioremediation of contaminated soil and water.

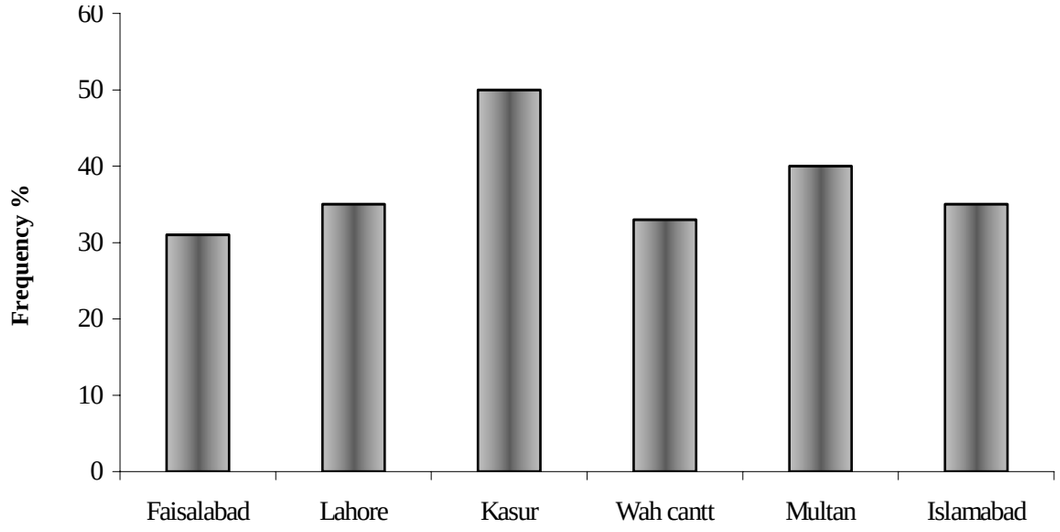

Fig. 2. Frequency percentage of isolated fungi of peri-urban agricultural fields.

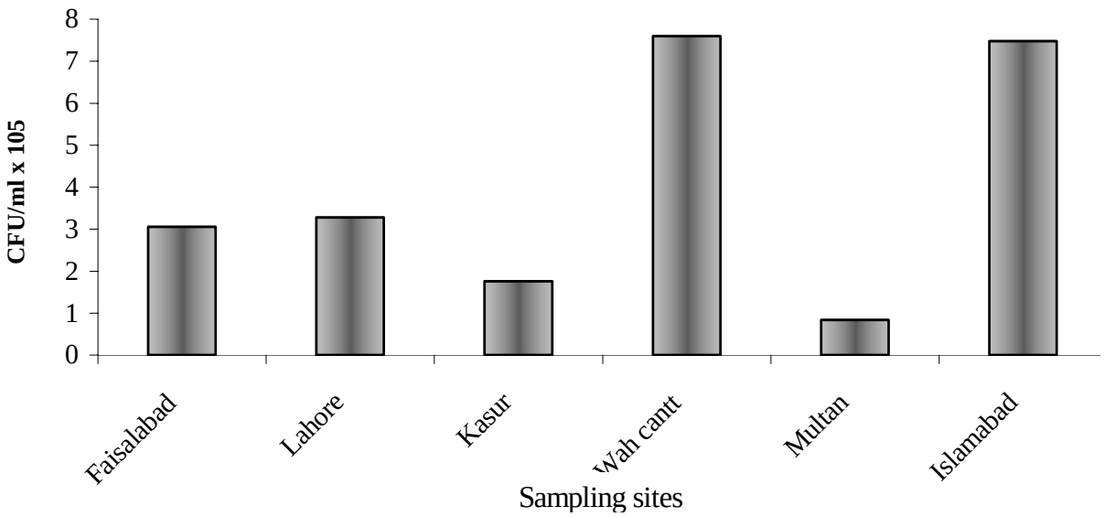

Fig. 3. Colony forming unit (CFU) of isolated fungi of peri-urban agricultural fields.

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