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## PROFILE OF FUNGAL CONTAMINANTS OF MAIZE (*ZEA MAYS*) INTENDED FOR CONSUMPTION AND THEIR POTENTIAL HEALTH IMPLICATIONS IN THE HO MUNICIPALITY OF GHANA

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## ABSTRACT

Maize is a principal food crop used extensively by both humans and animals in Africa and across the globe. Unfortunately, maize is highly susceptible to fungal contamination, especially with toxicogenic species. The contamination is exacerbated subsequently by mycotoxins of these fungi, which is indeed a major concern to governments and the international community, as it renders the food unsafe for human and animal consumption. Whole maize was sampled from 10 different sites in the Ho municipality, Ghana, and evaluated for moisture contents, fungal count, and species diversity. The fungal analysis was conducted at three points per location. Fungal species were cultured and identified on the two media used; Sabouraud Dextrose Agar (SDA) and Dichlor Rose Bengal Chloramphenicol (DRBC). A total of sixteen (16) fungal species belonging to eleven (11) genera were identified in this study. They included *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. tamarii*, *A. ochraceus*, and *A. parasiticus*, *Cladosporium herbarium*, *Curvularia lunata*, *Penicillium citrinum*, *Fusarium moniliforme*, *Eurotium* sp., *Mucor racemosus*, *Rhizopus stolonifer*, *Paecilomyces variotii*, *Neurospora sitophila* and *Rhodotorula* sp. The genus *Fusarium* was found to be the most overriding fungus. The overall decreasing order of ranking of occurrence was *Fusarium*>*Penicillium*>*Aspergillus*. Fungal counts of the maize samples ranged between  $2.77 \pm 1.01$ -  $4.1 \pm 0.81$  Log<sub>10</sub> CFU/g and  $3.00 \pm 1.13$ - $4.08 \pm 1.22$  Log<sub>10</sub> CFU/g for SDA and DRBC respectively and showed no significant differences ( $p > 0.05$ ). The moisture content of the maize grains ranged between  $12.06 \pm 1.17$ -  $16.71 \pm 2.65$  %. Generally, there was a weak association between moisture content and fungal counts, which showed a poor fit to the linear equations ( $R^2 = 0.1989$ ,  $R^2 = 0.0047$  for SDA and DRBC respectively). Our results underscore that consumers and farmers should be up-to-date on the danger of fungal contamination in maize. The outcomes of this paper would be worthwhile in advising policy makers to particularly stress on in adopting international legislations on food quality parameters and to use tools that will change the frame of mind of the population on risks involving fungal intoxication.

**Key words:** Maize, fungi, mycoflora, health, Ghana, toxicogenic fungi, pathogenic fungi

## INTRODUCTION

Fungi are inevitable to encounter in man's daily activities owing to their ubiquitous nature. Fungi have a host-pathogen relationship which could either be beneficial or harmful to the host. Harmful fungi cause diseases in plants, animals, and humans, as well as spoilage in food and feed [1]. Among the fungi classified as microorganisms, the filamentous fungi have the greatest economic significance. They do not only cause food spoilage during pre-and postharvest stages of production but may release various toxic secondary metabolites, referred to as mycotoxins [1]. Filamentous fungi pose serious health risks and have been implicated in the incidence of many diseases of Public Health concern.

Fungal infection of grains before and after harvest remains a major problem of food safety in most parts of Africa. Problems associated with this infection include loss of germination, mustiness, moldy smell [2, 3], and mycotoxin contamination [4-6]. These problems are, however, not effectively dealt with in most developing countries where no careful commodity screening and improved storage conditions are provided.

Maize (*Zea mays L.*), a staple crop for billions across the globe [7], is commonly consumed fresh or processed into cooked or fermented, milled, and beverage products [8, 9] especially in Africa. Intake levels of approximately 43- 46 kg/person/day of household consumption of maize in rural subsistence farming communities in Ghana have been reported by FAOSTAT [10] and [11]. Shiferaw *et al.* [12] emphasized that maize is consumed by over 200 million people in different forms globally. This consumption rate is increasing annually with FAO predicting that human and animal consumption demand for maize will be greater than before at nearly 300 million tons by 2030 [13]. Most importantly, it is frequently accompanied by groundnuts in complementary food formulations to help combat protein energy malnutrition although it contains some amino acids [14].

Maize is a stable, important, and very popular worldwide crop probably due to its great nutritional value. It is endowed with abundant macronutrients such as starch, fibre, protein, and fat as well as micronutrients like vitamin B complex,  $\beta$ -carotene, and essential minerals such as magnesium, phosphorus, zinc, copper, and others. [15]. These nutrients make maize prone to colonization by different fungus species both in the field and postharvest. In particular, maize can be a good substrate for some of the best known mycotoxigenic fungi such as *Aspergillus flavus*, *Fusarium verticillioides*, and *Fusarium graminearum*. These mycotoxigenic fungi produce some of the most hazardous substances for humans and animals. The

environment during field cultivation or postharvest can determine the conditions in which fungal species are more likely to develop [16].

In warm agricultural areas, the maize crop is frequently infested by *Aspergillus* section Flavi fungi [17] and contaminated with mycotoxins before, during, and after harvest [18]. Mycotoxins are highly toxic and carcinogenic compounds that negatively impact the health of both humans and livestock [19].

Although there have been some reported works done on the fungal contamination of maize in other parts of Ghana [20, 21], there is a paucity of data regarding the range of fungal contamination of maize in the Volta region. This study therefore aimed at investigating the fungal profile of maize sold and consumed in Ho Volta Region, Ghana.

## MATERIALS AND METHODS

### Study site

The Ho Municipal is one of the 260 Metropolitan, Municipal and District Assemblies (MMDAs) in Ghana. The population of Ho Municipality according to the 2021 population and Housing Census is 180, 420 (Fig. 1). The Municipality shares boundaries with Adaklu and Agotime-Ziope Districts to the South, Ho West District to the West, Hohoe Municipality to the North, and the Republic of Togo to the East. This study was quantitative and purely experimental. Microbiological analysis was conducted at the Microbiology Laboratory, School of Allied Health Sciences, University of Health and Allied Sciences, Ho, Volta Region, Ghana.



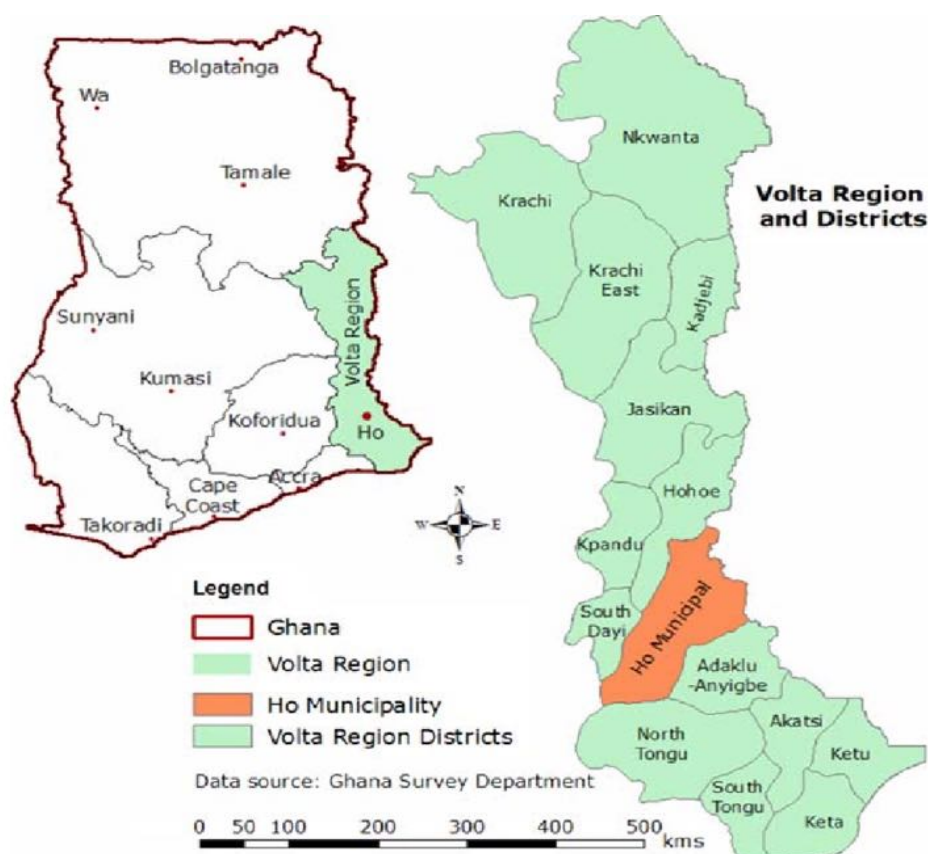


Figure 1: Ho Municipality in the Volta Region of Ghana. Adapted from [22]

### Sample collection

The maize sellers in each market (location) were conveniently sampled. About one kilogram (1kg) of raw maize samples were purchased from February to September 2020. Twenty (20) grams of each of the maize samples were fetched into sterile bags, placed on ice, and transported to the laboratory within the same day. Samples were then stored in a deep freezer at  $-20^{\circ}\text{C}$  until ready for mycological analysis [23].

### Estimation of viable fungal colonies

Exactly 50 g of sample was weighed into 100 ml 0.1% peptone water in 250 ml Erlenmeyer flasks using an electronic weighing balance (OHAUS®, UK) with a readability of 0.01g. The samples were shaken in a Gallenkamp Orbital shaker (140 rev/minute) for 30 seconds. From the stock suspension, decimal serial dilutions up to  $1:10^3$  were prepared. Exactly 1ml aliquot of each dilution level was put into 20 ml of Sabouraud Dextrose Agar (SDA) or Dichlor Rose Bengal Chloramphenicol (DRBC) as previously outlined by Kortei *et al.* [24]. There were triplicate samples for each media and dilution level. The plates were incubated at  $28-30^{\circ}\text{C}$  for up to 7 days until the fungi grew.

### Fungal Enumeration and Identification

Fungal enumeration was done by a colony counter (STAR 8500, Funke Gerber, Germany) and then calculated as Colony-forming Unit per gram sample (CFU/g) using equation (1). Data obtained in standard units were transformed into the logarithmic form and presented as  $\log_{10}$  CFU/g samples [24]

$$\text{CFU/g} = \frac{\text{No. of colonies} \times \text{reciprocal dilution factor}}{\text{The volume of the culture plate.}} \quad (1)$$

Molds and yeasts that appeared were identified by their cultural and morphological characteristics (Table 1) using standard identification manuals [25]. Percentage occurrence of fungal species was calculated using the formula;

$$\text{Percentage (\%)} \text{ occurrence of fungal species} = \frac{\text{Number of species}}{\text{Total number of fungi isolated}} \times 100. \quad (2)$$

The overall occurrence of fungal species on maize samples were calculated using (3)

$$\text{The overall occurrence of fungal species} = \frac{\text{Total fungal occurrence}}{\text{Number of appearances}} \quad (3)$$

### Determination of Moisture Content (MC)

The MC of the maize samples used was determined based on the procedure outlined by Gyimah *et al.* [26]. Five grams of the crushed homogenate of the maize samples were weighed into Petri dishes and dried overnight (16 h) in an oven at 105 °C. Cooling of Petri dishes was done in a desiccator and the final weight was recorded with an Accu Lab ALC-150.3. The MC was determined using the following equation:

$$= \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where; W1 is the weight of an empty Petri dish

W2 is the weight of sample + petri dish before drying

W3 is the weight of sample + petri dish after drying

### Data Analysis

All procedures were carried out in triplicate and the data collected were subjected to a single-factor analysis of variance (ANOVA). Differences among means were separated using Duncan's multiple range test (DMRT) and significances were

accepted at a 5% level ( $P < 0.05$ ) using Statistical Package for the Social Sciences (SPSS) software version 22. The analysis was done using the mean counts expressed in standard forms which were transformed into logarithmic values and results reported as means + standard deviation. Linear Regression analysis was used to determine the association of fungal counts and moisture contents

## RESULTS AND DISCUSSION

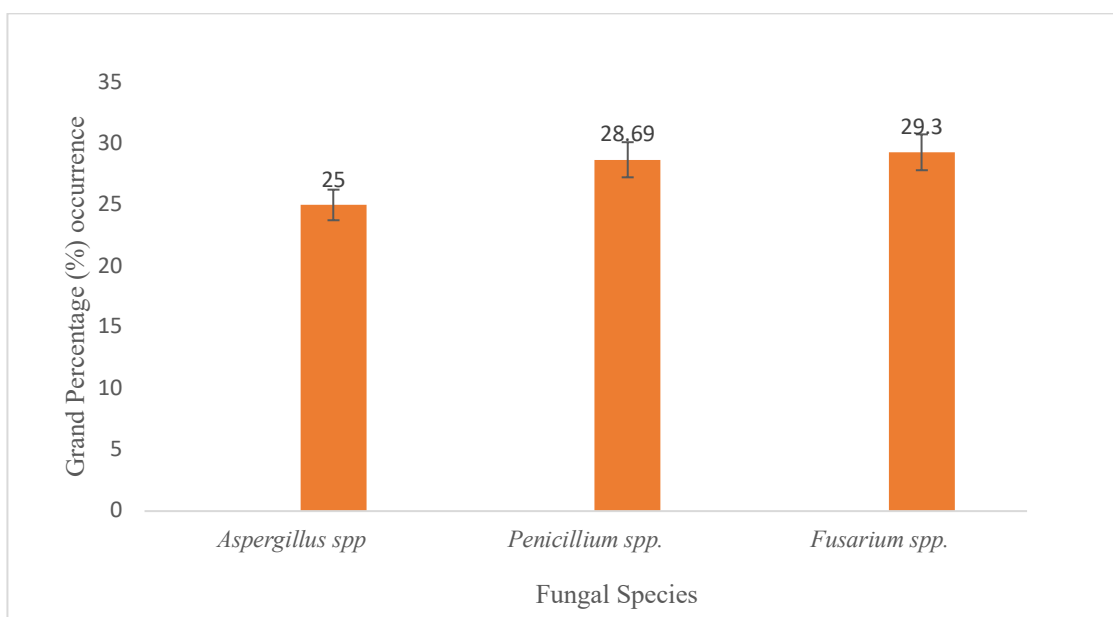
The moisture content of all maize samples ranged between  $12.06 \pm 1.17$ - $16.71 \pm 2.65\%$  (Table 2), with the moisture content of HM1 being significantly not different ( $p > 0.05$ ) from the majority of maize samples from the different locations of the municipality except for HM4, HM7, HM9 and HM10 where it differed ( $p < 0.05$ ).

The results of the fungal counts of maize obtained from different locations in the Ho municipality of Ghana have been summarized in Table 3. Fungal counts on the SDA ranged between  $2.77 \pm 1.01$ - $4.10 \pm 0.81 \text{ Log}_{10} \text{ CFU/g}$  corresponding to maize obtained from Ho Municipality areas 1 and 4 (HM1 and HM4) respectively. Statistically, there were no significant differences ( $p > 0.05$ ). On DRBC, the values of fungal counts recorded were within the range of  $3.00 \pm 1.13$ - $4.08 \pm 1.22 \text{ Log}_{10} \text{ CFU/g}$  corresponding to HM1 and HM3 respectively. Again, all values were comparable ( $p > 0.05$ ). The fungal counts concerning location followed no particular trend.

Tables 4 and 5 show a total of sixteen (16) fungal species belonging to the eleven genera identified in this study. They included *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. tamarii*, *A. ochraceus*, *A. parasiticus*, *Cladosporium herbarium*, *Curvularia lunata*, *Penicillium citrinum*, *Fusarium moniliforme*, *Eurotium sp.*, *Mucor racemosus*, *Rhizopus stolonifer*, *Paecilomyces variotii*, *Neurospora sitophila* and *Rhodotorula sp.*

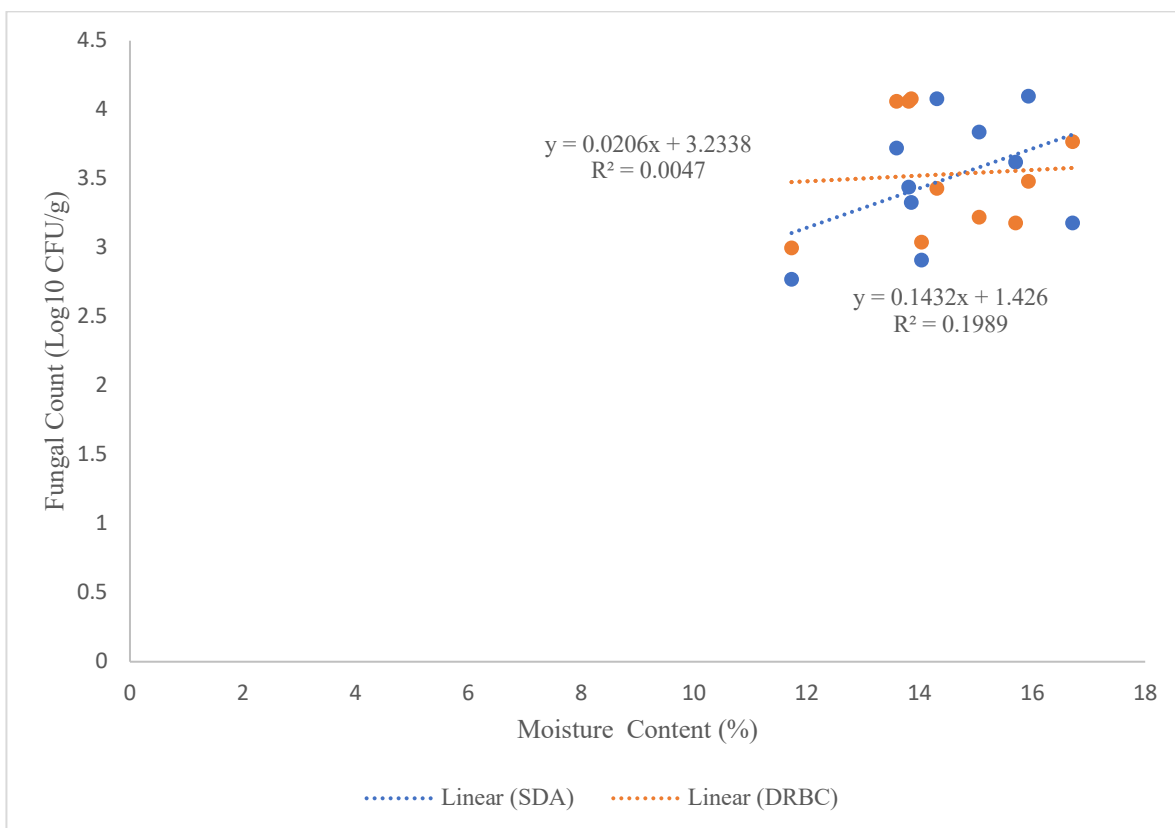
The species of fungi recorded in this study as dominant included *Fusarium spp.* (29.3%), *Penicillium spp.* (28.59%) and *Aspergillus spp.* (25.0%) and were ranked in decreasing order of *Fusarium spp.* > *Penicillium spp.* > *Aspergillus spp.* Statistically, *Aspergillus spp.* was significantly lower ( $p < 0.05$ ) than *Penicillium spp.* and *Fusarium spp.* (Fig. 2)





**Figure 2: Overall percentage (%) occurrence of the most dominant fungal species of maize of Ho municipality of Ghana**

A poor fit to linear the equations ( $R^2 = 0.1989$ ,  $R^2 = 0.0047$  for SDA and DRBC respectively) (Fig. 3) were derived from the regression analysis obtained from the plot of fungal counts and moisture contents in maize obtained from the Ho municipality.



**Figure 3: Relationship of the fungal count and moisture content of maize**

### Moisture Content

Cereal grains are common substrates that support the growth of a diversity of fungi, especially during storage [27]. The growth of most filamentous fungi and their subsequent mycotoxin production are to a large extent, influenced by environmental factors such as moisture, temperature, and relative humidity [28]. The results were in the same range of values recorded by Al-Shikli *et al.* [28] and Danso *et al.* [29] who reported 13.6-18% and 12.4-19% respectively. Findings from this study suggest that the range of moisture content recorded supports fungal growth, especially on the field before harvest.

In many African countries, especially Ghana, the ears of maize are traditionally left in the field on the plants after ripening until they are dry. Throughout this period, maize grains are exposed to unpredictable events, such as tropical downpours, which may occur daily and cause contamination [30]. However, more research should be conducted in the Ho municipality on the handling conditions of maize grains from harvest to storage/market, to confirm the possibility of contamination or otherwise.

Muga *et al.* [31] investigated the aflatoxin contamination of maize kernels at selected temperature, relative humidity, and moisture content levels. Samples of maize kernels at greater moisture content levels of 14, 15, 16, 18, and 20% (wb) were inoculated with *Aspergillus flavus* spores and incubated in a climatic test chamber for ten days at 20°C and 30°C, and a relative humidity of 60% and 90%. Their results indicated that aflatoxin contamination was significantly affected by temperature and relative humidity, whereas moisture content had no significant effect. In a related study, Raudiene *et al.* [32] showed grain with high MC has a high rate of respiration and Rogovskii *et al.* [33] also predicted an increase in temperature in grains with high MC. Likhayo *et al.* [34] also noted warm temperatures and high MC can result in rapid deterioration of maize and promote the growth and development of microorganisms and insects.

Inadequate storage techniques and environmental conditions trigger fungal growth and mycotoxin contamination of maize. The complex interaction of biotic and abiotic factors within the grain storage ecosystem determines the severity of fungal contamination of stored maize [35]. The primary factors that promote fungal contamination of stored maize are high temperature, grain moisture content, and relative humidity of the surrounding air [36].

### Fungal Counts

The survival of fungi on dehydrated products is well known. Maize grains from different locations of the municipality resulted in varied ( $p < 0.05$ ) fungal counts on the media used (Table 2). Fungal counts obtained in this present study were slightly lower than fungal counts obtained from some previous studies. Fungal counts obtained by [37] were in the range of  $10^4$  ( $4.0 \log_{10}$ ) CFU/g. Jonathan *et al.* [38] also recorded a range of  $4.43$ - $4.92 \log_{10}$  CFU/ml from steeping water from soaked maize. However, the same range of values were reported by Fasoyiro *et al.* [39] recorded counts within a range of  $1.69$ - $2.78 \log_{10}$  CFU/g. While recently, Sserumaga *et al.* [40] recorded a range of  $0$ - $4.97 \log_{10}$  CFU/g from maize samples in Uganda.

Greater fungal counts were observed by Hackman [41] who recorded an initial fungal population in the mixed grain variety was  $4.8$  -  $5.4 \log_{10}$  CFU/g and observed a decrease by  $0.4$  -  $1.3 \log$  cycle after 2 months in maize grains obtained from the Ghana Food Distribution Corporation (GFDC) Warehouse at Balduzzi, Kumasi. In Ethiopia, Ayalew *et al.* [42] recorded mean levels of total fungal density which ranged from  $3.46$ -  $5.53 \log_{10}$  CFU/g in maize samples from Dire Dawa, Adama, and Ambo respectively. In South Africa, Ekwomadu *et al.* [43] recorded a range of  $1.04$ - $6.07 \log_{10}$  CFU/g for maize samples meant for consumption in

commercial and small-scale settings. Agbetiameh *et al.* [44] reported a count of range 0.6-5.73 log<sub>10</sub> CFU/g in maize samples in Ghana. Jespersen *et al.* [45] also recorded a range of 3.91-6.18 log<sub>10</sub> CFU/g in maize kennels under different fermentation treatments.

The results suggested that environmental conditions and characteristics exceptional to each type of maize grain can exert an impact on microbial communities existing in that environment [24]. Furthermore, the variation in fungal counts may depend on the mycological medium used to culture the fungi since they are composed of different ingredients which have different capacities to support fungal growth [24].

### Fungal Species

The contamination of agricultural products from the field, during storage and transportation are often linked to the genera *Aspergillus*, *Fusarium*, and *Penicillium* (toxicogenic fungi), presumably due to favorable environmental conditions of growth and proliferation in the tropics. They have the capacity to produce mycotoxins which have adverse health effects on humans and animals when ingested. Fungal contamination of grains during storage and transportation occurs commonly in the intercontinental trade of cereals. Spores from fungi are transferred across boundaries during these times. Wheat, rice, barley, corn, and some other cereals are delimited for their mold (physical) and mycotoxin (chemical) contamination by the quarantine service of export and import harbors due to the danger they pose which is a major problem in food safety.

Fungal species identified in this present study corroborated with findings from similar previous studies globally. Organisms identified alongside *A. flavus* species by Dadzie *et al.* [37] included *A. niger*, *Rhizopus sp.*, *Fusarium moniliforme*, *Penicillium sp.*, *Verticillium sp.*, *Curvularia lunata*, *Trichoderma sp.*, *Bipolaris sp.*, *Trichothecium sp.*, *Botryodiplodia sp.* and *Nigrospora sp.* Among the fungal groups identified, five (*A. flavus*, *A. niger*, *Rhizopus sp.*, *Penicillium sp.* and *Fusarium sp.*) were commonly found on the maize samples in most of the locations they surveyed.

Kieh [46] recorded a plethora of fungi causing maize ear rot in the major maize growing areas in the Ashanti Region of Ghana which included *A. flavus*, *Penicillium sp.*, *Fusarium sp.*, *A. niger*, *Trichoderma*, and *Curvularia spp.* with *Colletotrichum sp.* as the most prevalent fungus.

Benson-Obuor *et al.* [47] isolated fungal species *Aspergillus flavus*, *Colletotrichum gleosporioides*, *Fusarium* sp., *Lasiodiplodia theobromae*, *Penicillium* sp. and *Rhizopus* sp. on maize grains before storage and *A. flavus*, *A. niger*, *Fusarium* sp., *L. theobromae*, *Penicillium* sp. and *Rhizopus* sp. No *Colletotrichum gleosporioides* was identified. Six different storage fungal species were again isolated from the maize samples after six months of storage from three maize varieties; “Obaatanpa”, “aburohemaa”, and “abontem” from the Brong-Ahafo region of Ghana.

Interestingly, a mixed flora comprising *Candida*, *Saccharomyces*, *Trichosporon*, *Kluyveromyces*, and *Debaryomyces* species were isolated from raw maize, during steeping and early phases of fermentation, and later *Penicillium*, *Aspergillus*, and *Fusarium* species, including potential mycotoxin producers, were isolated from raw maize for ‘kenkey’ production in Ghana by Jespersen *et al.* [45]. The findings are in line with other studies done in Ghana [37, 41, 44, 46]. Hackman [41] also isolated fifteen different fungal species, *Aspergillus flavus*, *A. niger*, *A. sulphureus*, *A. tamarii*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. citrinum*, *P. cyclopium*, *P. digitatum*, *P. glabrum*, *P. oxalicum*, *Cladosporium herbarum*, *Fusarium moniliforme*, *F. roseum* and *Mucor haemalis* were isolated from maize grains obtained from the Ghana Food Distribution Corporation (GFDC) Warehouse at Balduzzi, Kumasi where *Aspergillus* species (*A. flavus*, *A. niger*, *A. sulphureus*, *A. tamarii*) and *Penicillium* species (*P. brevicompactum*, *P. chrysogenum*, *P. citrinum*, *P. cyclopium*, *P. digitatum*) preponderated. The toxigenic mycobiota recorded in Haitian maize kernels confirmed the well-known existence of favorable conditions for *Aspergillus* spp. infection in tropical and subtropical latitudes [44].

Several studies by researchers from other African countries [42] (Ethiopia), [43] (South Africa), [48] (Cameroun) have highlighted the mycoflora of maize and implicated the same three toxigenic genera (*Aspergillus*, *Fusarium* and *Penicillium*) of fungi which dominate the contamination of maize grains. Jonathan *et al.* [38] also isolated *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Rhizopus* spp. and *Saccharomyces cerevisiae* from stored maize and the isolation of *Aspergillus* spp. and *Penicillium* spp. from fresh maize. In many instances, most *Aspergillus* sp is recorded as the most dominant.

Contrariwise, the results of this study agreed with the findings of Kpodo [6] who isolated different species (*verticilloides*, *semitectum*, *equiseti*, *oxysporum*, and *graminaerum*) of the *Fusaria* genus from maize grains in Ghana which was a deviation from the norm were the dominant genera have been *Aspergillus* which were always known to contaminate maize meant for consumption in Ghana.



Likewise, the published findings of [49, 50, 51] all pointed to *Fusarium verticillioides*, *F. graminearum*, *F. subglutinans* as the most prevalent fungal species of maize.

The predominance of *Penicillium spp.* in the maize mycobiota has also been previously found by other authors [41, 52] in association with nonoptimal conditions during storage. Similarly, Ngoko *et al.* [48] also recorded *Nigrospora sp* as the most prevalent fungus in maize from humid forest and western highlands of Cameroun.

Studies have also reported that maize samples with greater occurrence of *Fusarium verticillioides* are less likely to be infested with *A. flavus* and have been shown to be negatively linked with other fungal species [53]. This could be an explanation to the overall lower incidence of *Aspergillus* and *Penicillium sp.* as well as some other fungal isolates as reported in this study.

Rheedar *et al.* [53] emphasized climatic factors (temperature, precipitation, drought, and atmospheric carbon dioxide, a biological environment of crops such as the abundance of pests and plant pathogens) greatly impact agriculture and gradually but surely affect the quality of grains produced. Darfour and Rosentrater [11] also explained that storage of maize is a common practice in the market where maize samples were collected is characteristic of most markets in Ghana and in Sub Saharan Africa. This practice of hording is usually done to preserve maize grains until the time that fresh ones will come out in the subsequent season. However, maize grains are often contaminated by fungi during storage when fungal spores in the air enter the maize grains through the pores of the storage materials that are used to store the grains. Therefore, there is a need to unremittingly monitor the quality of grain to assess possible climatic effects and be able to take measures before any outbreak. This is because several genera and species of filamentous fungi produce mycotoxins that have significant agricultural, epidemiological, economic, and health bearings. It is worthy to note that the impacts of climate change on growth and development of fungi and their direct bearing on food safety and food security are stern issues which require devotion. A spot light on the health implications of the major genera of toxigenic fungi (*Fusarium*, *Aspergillus*, and *Penicillium*) associated with maize in this present study is considered.

The genus *Aspergillus* is almost synonymous with aflatoxin production. Aflatoxins are secondary metabolites produced by toxic strains of *A. flavus* and *A. parasiticus* and to a lesser extent by *A. nomius*. The presence of aflatoxins in maize is well

known. There are five different types; aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, and M<sub>1</sub> produced primarily in cow milk by cows eating contaminated silage. Prolonged consumption of aflatoxins has been reported to cause impaired immune function, malnutrition and stunted growth in children, teratogenic, mutagenic disabilities, and eventual death [40, 54]. Aflatoxin as a well-categorized class 1 carcinogenic toxin (IARC) is known to have adverse effects on reproductive health and associated with cirrhosis, hepatitis B and C infections, and liver cancer [54]. Recently, Richard *et al.* [55] reported on the novel neurotoxic and neuro-immunotoxic capabilities of aflatoxins on the nervous system. There has also been a reported correlation between hepatitis B and aflatoxin consumption in Africa and liver damage occurrence by aflatoxins, which differed over a 5-fold range and was strongly associated with estimated levels of aflatoxins [56].

Varietal differences in *A. flavus* infection and aflatoxin production in foods have been documented [5, 44, 57, 58]. This probably partly explains the differences in the susceptibility of the local maize varieties to infection by *A. flavus* especially those obtained from other regions of Ghana. Future studies with focus on varietal differences in susceptibility to potential mycotoxigenic fungi from the different agro-ecological zones of Ghana will provide a clear understanding of this phenomenon. Although *A. niger* is not known to produce aflatoxins, it possesses the ability to produce other toxins such as ochratoxin A, malformin, and nigerone [59]. Ochratoxin A is lipid soluble produced by *A. alutaceus* (= *A. ochraceus*) and *A. carbonarius* and is not expelled efficiently and thus accumulates in meat which exposes humans to health risk after entering contaminated meat [60].

*Penicillium citinum* was among the frequently isolated contaminants in stored maize in this present study. More than 80 *Penicillium* species are documented toxin producers [58]; the most important are ochratoxin A, citreoviridin, penitrem A, roquefortine, and secalonic acid. The genus has major importance in the natural environment as well as food and drug production industry. The first citrinin producer described was *Penicillium citrinum*, other species such as *P. miczynski*, *P. hirsutum*, *P. verrucosum*, *P. westling*, *P. expansum*, *P. stechii*, *P. cyclopium* have been reported to also produce citrinin [60]. Besides, several species included in the genera *Aspergillus* and *Monascus* have also been reported to be able to produce this toxin. Other variants or species of *Penicillium* such as *P. verrucosum*, *P. viridicatum* produce ochratoxins, cyclopiazonic acid, penicillic acid and citrinin. Observed in higher latitudes, *P. verrucosum* is the primary producer of ochratoxin A and this toxin is relatively ten times more toxic than citrinin [62]. The chief target organ of citrinin is the kidney, and its ingestion is related to weight loss because of renal degeneration. This nephrotoxin causes damage in the proximal tubules of the

kidney, and it is considered one possible cause of porcine nephropathy. Few reports are available about the acute toxicity of citrinin. Oral LD<sub>50</sub> in mice has been established as 110 and 134 mg kg<sup>-1</sup> in rabbits [63].

*Fusarium* species isolated from maize seeds produce several mycotoxins such as biologically active trichothecenes, which when ingested in high concentrations, cause vomiting and diarrhea in humans. Trichothecenes are also associated with reduced weight gain and immune dysfunction in animals [64]. Human uterotrophic (anti-reproduction) effects are caused in animals and pigs by zearalenone [58, 65]. Another *Fusarium* species, *F. verticillioides* (*F. moniliforme*), isolated in this study produces fumonisins which have been reported to have a neurotoxic effects in animals and is associated with oesophageal cancer in Sub-Saharan -Africa [66]. Consumers are entreated to be cautious of the kind of maize they choose in the preparation of their meals and beverages since improperly treated (dried and stored) maize grains are a cause for alarm. Toxigenic fungi have a penchant for growing on maize and besides their presence, also exude some natural metabolites (mycotoxins) which are detrimental to the health of both humans and animals.

Results showed a wider range of mycotoxigenic fungi that contaminate local maize and there is a need to widen the scope of mycotoxin analysis to cover possible mycotoxins such as fumagillin (*A. fumigatus*) Ochratoxin A (*P. verrucosum*), Fumonisin (*Fusarium spp.*) which have demonstrated to have human health implications. Furthermore, *F. oxysporum* isolated in this study ranks among the ten (10) most wilt destroying fungal pathogens worldwide [65]. Its presence in maize seeds could devastate crop productivity in the field.

Good agricultural practice (GAP), good manufacturing practices (GMP), as well as good hygiene practices (GHP), are vital to avert the growth of filamentous fungi and possibly mycotoxins in the field and during storage. By discouraging fungal growth and subsequent mycotoxin formation in maize, public health is protected and economic losses can be avoided. Monitoring maize for the presence of possible fungal contamination in a consistent manner is judicious to evaluate the public level of awareness.

## CONCLUSION

The results obtained were able to ascertain the degree of contamination and occurrence of a wide range of filamentous fungal species in maize destined for human and animal consumption in Ho municipality of Ghana. The findings on the

fungal diversity of maize from this area of the country may be an addition to the microbial assemblage in the ecosystems of Ghana and Africa at large. It can be construed from this present work that local maize was contaminated with a total of sixteen (16) fungal species belonging to eleven (11) genera were identified in this study. They included *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. tamarii*, *A. ochraceus*, *A. parasiticus*, *Cladosporium herbarium*, *Curvularia lunata*, *Penicillium citrinum*, *Fusarium moniliforme*, *Eurotium* sp., *Mucor racemosus*, *Rhizopus stolonifer*, *Paecilomyces variotii*, *Neurospora sitophila* and *Rhodotorula* sp. The genus *Fusarium* was found to be the most overriding fungus.

Previous studies have focused on mycotoxin analysis of maize narrowing down to the aflatoxin groups by and large, but not on other equally potent mycotoxins. Results from this study showed that a broader spectrum of mycotoxigenic fungi infect maize grown in the study area. This suggests the need to widen the scope of mycotoxin analysis to cover possible mycotoxins such as fumagillin (*A. fumigatus*), ochratoxin A (*P. verrucosum*), and fumonisin (*Fusarium* spp.), which have been confirmed to have human adverse health implications. Furthermore, *F. moniliforme* isolated in this study ranks among the 10 most destructive wilt fungal pathogens worldwide. Its presence in maize seeds could devastate crop productivity in the field.

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## AUTHOR CONTRIBUTIONS

NKK, DA, W-KM, EKE, COT, AAA and TA performed the experiments and wrote the manuscript. EKE, JGD, DA, and COT were responsible for statistical analysis. W-KM, JGD and NKK helped conceive the experiments and prepare the manuscript. NKK, TA, and AAA conceived the original study and COT, EKE, NKK, and AAA led the sampling and study in Ghana. All authors read and approved the final manuscript.

**Table 1: Cultural and morphological characteristics of identified fungi**

Fungal Specie	Cultural Characteristics	Morphological Characteristics
<i>Mucor spp</i>	Large white colonies which turn into black later.	Erect sporangiophores are formed. Sporangiophore swell at the tip to form sporangia which are globular shaped. Columella is present.
<i>Rhizopus spp.</i>	White cottony mycelia with black dots and covers the entire plate.	Sporangiospores are produced inside a spherical sporangium. Columella is present on the top of the sporangiophore. Root-like rhizoids are found
<i>Penicillium spp.</i>	Fast-growing colonies in green color with dense felt conidiophore	Branched conidiophores with chains of conidia look like a brush.
<i>Aspergillus spp.</i>	Yellow to green, and black colonies with a distinct margin	Conidiophores arise from a foot cell. Club shaped vesicles on top of the conidiophores. Conidia are found in chains
<i>Curvularia Spp.</i>	Fast-growing colonies of suede-like with downy, brown to blackish	Conidiophores are erect, straight to flexuous, septate, often geniculate (producing conidia in sympodial succession)
<i>Cladosporium spp</i>	Colonies are mostly greyish-olive appearance and later powdery	Conidiophores arising laterally or terminally from the hyphae. Bears chains of conidia
<i>Fusarium spp.</i>	White-pink, sparse aerial mycelia becoming felty	Macro conidia sparse, borne on phalides with branched conidiophores (Septate banana-shaped).
<i>Rhodotorula spp.</i>	Soft, smooth, moist, and mucoid	Round or oval-shaped budding cells.
<i>Eurotium spp.</i>	Broad zones of flat dull green to greyish green colonies	Asci are globose to subglobose. Ascospores single-celled, lenticular, small, smooth-rough surface
<i>Neurospora spp.</i>	Ascomata are ostiolate, dark brown to black, smooth or downy with loose hyphae	Ascospores are uniseriate or somewhat overlapping, initially hyaline, becoming yellowish-brown to black with maturity, one-celled ellipsoidal or elongate, ascospores wall surface with global ornate pits

Sources: [36, 37]



**Table 2: Moisture content of maize samples obtained from various locations in Ho**

Location	Moisture Content (%)	Mean $\pm$ standard deviation
HM1	13.42 11.38 11.41	12.06 $\pm$ 1.17 <sup>a</sup>
HM2	11.24 14.27 15.28	13.59 $\pm$ 2.10 <sup>ab</sup>
HM3	15.22 14.18 12.17	13.85 $\pm$ 1.55 <sup>ab</sup>
HM4	14.48 16.68 16.65	15.93 $\pm$ 1.25 <sup>bc</sup>
HM5	13.11 15.16 13.17	13.81 $\pm$ 1.16 <sup>ab</sup>
HM6	10.14 16.97 14.99	14.03 $\pm$ 3.51 <sup>ab</sup>
HM7	15.84 13.69 15.09	15.06 $\pm$ 1.19 <sup>bc</sup>
HM8	11.63 16.21 15.09	14.31 $\pm$ 2.38 <sup>ab</sup>
HM9	17.77 18.67 13.69	16.71 $\pm$ 2.65 <sup>d</sup>
HM10	15.78 16.21 15.11	15.70 $\pm$ 0.55 <sup>bc</sup>

Means in a column with the same superscript letters are not statistically different ( $p > 0.05$ )

HM1= Ho Municipality area 1, HM2= Ho Municipality area 2, HM3= Ho Municipality area 3, HM4= Ho Municipality area 4, HM5= Ho Municipality area 5, HM6= Ho Municipality area 6, HM7= Ho Municipality area 7, HM8= Ho Municipality area 8, HM9= Ho Municipality area 9 HM10= Ho Municipality area 10

**Table 3: Fungal counts of maize enumerated on two (2) different media (SDA and DRBC) incubated for 5-7 days at 36±1 °C**

	SDA			DRBC		
	Log <sub>10</sub> CFU/g			Log <sub>10</sub> CFU/g		
	Mean + Standard Deviation	Grand Standard deviation	Mean +	Mean + Standard deviation	Grand Standard deviation	Mean +
HM1	2.83±0.61 2.92±1.20 2.58±1.20	2.77±1.01 <sup>a</sup>		2.77±1.21 2.79±1.42 3.44±0.70	3.00±1.13 <sup>ab</sup>	
HM2	3.03±0.30 4.73±1.11 3.42±1.32	3.72±0.90 <sup>a</sup>		3.72±0.83 4.55±1.20 3.72±0.11	4.06±0.81 <sup>ab</sup>	
HM3	3.83±0.50 3.05±0.70 3.11±1.21	3.33±0.81 <sup>a</sup>		4.30±1.10 4.55±1.21 3.38±1.21	4.08±1.22 <sup>ab</sup>	
HM4	3.80±0.90 5.20±1.22 3.31±0.50	4.10±0.81 <sup>a</sup>		3.43±1.70 3.72±0.81 3.28±0.51	3.48±1.04 <sup>ab</sup>	
HM5	3.19±0.50 3.90±0.90 3.25±0.41	3.44±0.71 <sup>a</sup>		3.92±1.11 2.84±0.20 2.80±0.40	4.07±1.11 <sup>ab</sup>	
HM6	3.45±1.10 3.05±1.42 2.25±0.30	2.91±0.90 <sup>a</sup>		3.42±1.60 3.30±1.20 2.74±0.82	3.04±0.81 <sup>ab</sup>	
HM7	3.78±1.10 3.26±1.10 4.49±0.62	3.84±1.41 <sup>a</sup>		2.99±0.92 3.35±1.51 3.31±0.81	3.21±1.12 <sup>ab</sup>	
HM8	4.30±1.11 4.55±1.00 3.38±1.90	4.08±1.41 <sup>a</sup>		3.06±0.73 3.50±0.71 3.75±0.61	3.43±0.52 <sup>ab</sup>	
HM9	3.92±1.10 2.84±0.22 2.80±0.71	3.19±0.60 <sup>a</sup>		4.27±1.50 3.39±0.62 3.69±0.82	3.78±0.90 <sup>ab</sup>	
HM10	3.72±0.81 3.01±0.92 4.13±0.70	3.62±0.80 <sup>a</sup>		2.57±0.91 4.17±0.53 2.81±0.42	3.18±0.61 <sup>ab</sup>	

Means in a column with the same superscript letters are not statistically different (p>0.05)

HM1= Ho Municipality area 1, HM2= Ho Municipality area 2, HM3= Ho Municipality area 3, HM4= Ho Municipality area 4, HM5= Ho Municipality area 5, HM6= Ho Municipality area 6, HM7= Ho Municipality area 7, HM8= Ho Municipality area 8, HM9= Ho Municipality area 9 HM10= Ho Municipality area 10

**Table 4: Fungal species and their percentage (%) occurrence in maize from various locations in Ho cultured on two (2) different media (SDA and DRBC) and incubated for 5-7 days at 36±1 °C**

	Location of sampling																			
	HM1		HM2		HM3		HM4		HM5		HM6		HM7		HM8		HM9		HM10	
	S %	D %	S %	D %	S %	D %	S %	D %	S %	D %	S %	D %	S %	D %	S %	D %	S %	D %	S %	D %
Fungi recorded																				
<i>Aspergillus flavus</i>	43.5	24.5			17.4	6.3	14.8	17.2	45.7		34.6	24.1			44.0	23.9		37	2.4	
<i>A.niger</i>	2.0	4.1		20.5	25.5	13.8		24.2	6.0				38.1	21.7			5.0	6.8	15	5.0
<i>A.fumigatus</i>			44.8			13.8	11.1	13.8		65.2	18.3	13.8		24.6						
<i>A.tamarii</i>	4.8	4.1		13					3.0									3.5		
<i>A.ochraceous</i>			17.1								8.2	3.4				7.3				
<i>A.parasiticus</i>				5.1					3.0	12.2									4.6	
<i>Cladosporium herbarum</i>			6.9			3.6			6.0		7.6				16	20				
<i>Curvularia lunata</i>					4.1		7.4	3.4			8.2	3.4		5.6						

<i>Penicillium citrinum</i>	46.3	51.9	17.2	10		20.8	29.7	24.2		22.6	16.3	17.2	9.5		26	15.7			78	45
<i>Fusarium moniliforme</i>			6.9	46.3	31.7	24.2	30.0	20.8	6.0			13.7	28.6	35.2		5.1	95	31.8		35
<i>Eurotium sp.</i>			1.3															5.0		
<i>Mucor racemosus</i>				5.1			7.0		22.5			10.5								
<i>Rhizopus stolonifer</i>		7.7	6.9		15.5	17.5							23.8		14	28				15
<i>Paecilomyces variotii</i>	3.4				4.3	3.4			7.8		2.0	3.4								
<i>Neurospora sitophila</i>					1.5													10.8		
<i>Rhodotorula sp.</i>		7.7				3.4					5.2			22.9				5.0		

S= Sabouraud Dextrose Agar (SDA), D= Dichlor Rose Bengal Chloramphenicol (DRBC)

HM1= Ho Municipality area 1, HM2= Ho Municipality area 2, HM3= Ho Municipality area 3, HM4= Ho Municipality area 4, HM5= Ho Municipality area 5, HM6= Ho Municipality area 6, HM7= Ho Municipality area 7, HM8= Ho Municipality area 8, HM9= Ho Municipality area 9 HM10= Ho Municipality area 10.



**Table 5: Pooled data of total fungi isolated from maize obtained from the Ho municipality, Ghana**

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<i>Aspergillus niger</i> Van Tieghem	<sup>HM1, HM2, HM3, HM4, HM5, HM7, HM9, HM10</sup>
<i>A. flavus</i> Link	<sup>HM1, HM3, HM4, HM5, HM6, HM8, HM9, HM10</sup>
<i>A. fumigatus</i> Fresen	<sup>HM2, HM3, HM4, HM5, HM6, HM7</sup>
<i>A. ochraceus</i> Wilhelm	<sup>HM2, HM6, HM8</sup>
<i>A. parasiticus</i> Speare	<sup>HM2, HM5</sup>
<i>A. tamarii</i> Kita	<sup>HM1, HM2, HM5, HM9</sup>
<i>Cladosporium herbarum</i> (Pers.) Link	<sup>HM2, HM3, HM5, HM6, HM8</sup>
<i>Curvularia lunata</i>	<sup>HM3, HM4, HM6, HM7</sup>
<i>Penicillium citrinum</i> Thom	<sup>HM1, HM2, HM3, HM4, HM5, HM6, HM7, HM8, HM10</sup>
<i>Fusarium moniliforme</i> Schlecht	<sup>HM2, HM3, HM4, HM5, HM6, HM7, HM8, HM9, HM10</sup>
<i>Eurotium</i> sp. Mangin	<sup>HM2, HM9</sup>
<i>Mucor racemosus</i> Fres.	<sup>HM2, HM4, HM5, HM6</sup>
<i>Rhizopus stolonifer</i> (Ehrenb.) Lind.	<sup>HM1, HM2, HM3, HM7, HM8, HM10</sup>
<i>Paecilomyces variotii</i> Bain	<sup>HM1, HM3, HM5, HM6</sup>
<i>Neurospora sitophila</i>	<sup>HM3, HM9</sup>
<i>Rhodotorula</i> sp. (A. Jorg) F.C. Harrison	<sup>HM1, HM3, HM6, HM7, HM9</sup>

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Note: Superscripts show the treatments in which the fungal species appeared in

HM1= Ho Municipality area 1, HM2= Ho Municipality area 2, HM3= Ho Municipality area 3, HM4= Ho Municipality area 4, HM5= Ho Municipality area 5, HM6= Ho Municipality area 6, HM7= Ho Municipality area 7, HM8= Ho Municipality area 8, HM9= Ho Municipality area 9 HM10= Ho Municipality area 10



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