The Use of Smart Devices for the Detection of Aflatoxin in Ground Corn Feeds

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THE USE OF SMART DEVICES FOR THE DETECTION OF AFLATOXIN IN GROUND CORN FEEDS

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Abstract

Aflatoxin contaminates agricultural commodities, plants or animal-derived food, in warm and humid conditions primarily in tropical countries such as the Philippines. Although the type and degree of contamination are dependent on its concentration, its effect becomes critical when biomagnified. In this study, a rapid, simple, and portable detection was developed. A smart-device sensor was used to measure the pH of the samples with aflatoxin and compared it with the pH of pure samples. Concentrations in parts per billion (ppb) were calculated for each of the samples from the obtained pH readings; Cyclic voltammetry was also conducted to further study the electrochemical properties of the mixture with aflatoxin.

***Keywords**:* Aflatoxin, voltammogram, pH sensor, detection, computer-based software

Aflatoxins are toxic and carcinogenic secondary metabolites produced predominantly by two fungal species: *Aspergillus flavus* and *Aspergillus parasiticus* (Gourama, H., & Bullerman, L., 1995). These fungal species are contaminants of food crops as well as animal feeds and are responsible for aflatoxin contamination of these agricultural products. The toxicity and potency of aflatoxins make them the primary health hazard as well as responsible for losses associated with contaminations of processed foods and feeds (Gourama, H. & Bullerman, L., 1995). Determination of

aflatoxins concentration in food crops and animal feeds is thus very important for Food Safety Regulatory Agencies (FRSA) to create effective policies (Shane, S.H. & Groopman, J.D., 1994). However, the current mechanism of aflatoxin detection does not provide an immediate result, requires technical expertise, and are costly (Paniel, N., Radoi, A. & Marty, J., 2010).

In several studies, determination of aflatoxin requires a variety of complex sample preparations, characterization, and analysis. Such methods include high-performance liquid chromatography (HPLC), thin layer chromatography, fluorescence, and immunoenzymatic assays. However, detection using a smart device has not been fully explored when it comes to detecting toxins. The use of smart devices received relative attention in the research field due to its simple and abrupt mechanism of detection with its application as a real-time evaluation instrument and currently being explored in different fields. This research developed a simple method for aflatoxin extraction and detection in ground corn feeds obtained from the Bureau of Animal Industry (BAI). However, the study was limited to the detection of Aflatoxin B1 ($C_{17}H_{12}O_6$).

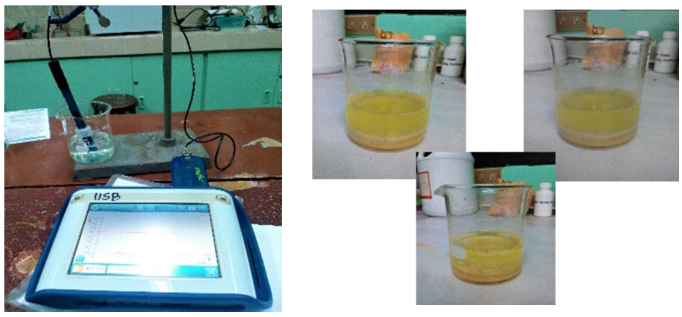
Methodology

Ground corn feeds were obtained from the Bureau of Animal Industry (BAI). Three bottles of different concentrations were provided with the specificity made unknown to us to remove any biases in the experiment.

Figure 1. Each bottle contains a different concentration of aflatoxin (1 with less than 20 ppb, 1 with a low concentration (21-100 ppb), and 1 with a high (above 100 ppb) concentration of aflatoxin).

Two tablespoons of aflatoxin from each bottle were taken, and the samples were mixed in a 100 mL water-methanol solution with 1:4 ratio as shown in Figure 2. In the study conducted by Wacoo, A., Wendiro, D., Vuzi, P. & Hawumba, J., (2014), aflatoxin can be extracted using a polar protic solvent such as acetone, ethanol, methanol, or acetonitrile. Among these organic solvents, methanol has the least effect on toxins; thus, the natural composition of the toxin is somewhat preserved (McPherson, M., 2015). All preparations were done under a fume hood with observed safety precautions for aflatoxin as indicated in Appendix A**.**

The samples were studied using a smart device pH sensor that is composed of a monitor and a pen-type connector as shown in the setup below.



(a) (b)

Figure 2. (a) Setup of the smart device pH sensor dipped in 100 mL of pure distilled water. (b) Samples with 100 mL of water-methanol solution and 2 tablespoons of aflatoxin from the three bottles obtained from BAI.

As a reference sample, 100 mL of distilled water and a solution of water-methanol were studied in comparison to the samples of aflatoxin. Cyclic voltammetry was conducted to further study the electrochemical properties of the prepared electrolytes.

Results

Using the smart device, the pH of each sample was measured with 30 seconds running time. Five trials were made for all samples to show a consistency of data. The pen-type connector from the smart device was submerged in acetone for 30 seconds before the next test was made to remove any impurities from the previous analyte.

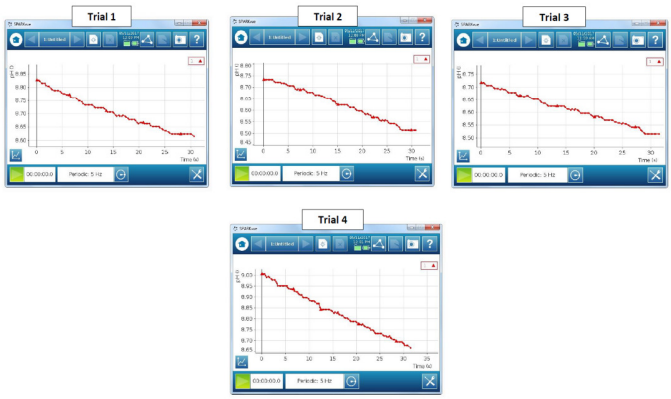


Figure 3. pH reading of five trials with 100 mL distilled water.

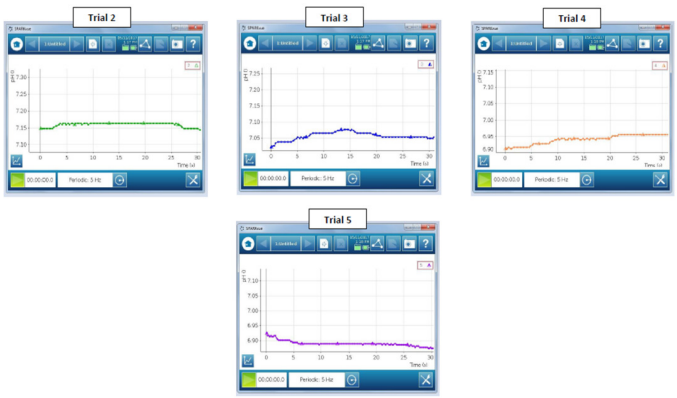


Figure 4. pH reading of five trials with 100 mL solution of distilled water and methanol.

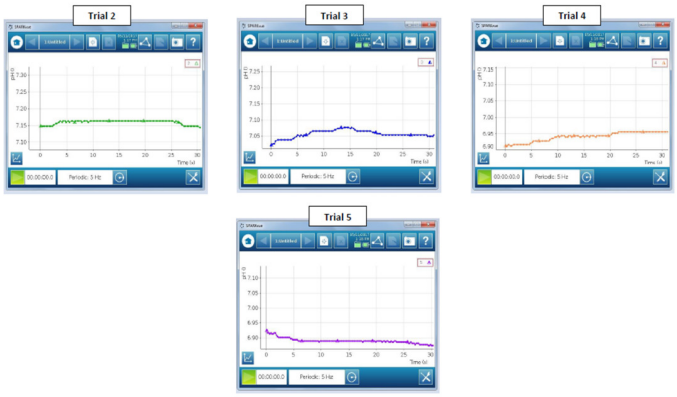


Figure 5. pH reading of five trials with 100 mL solution of distilled water and methanol and 2 tablespoons of aflatoxin from Bottle 1.

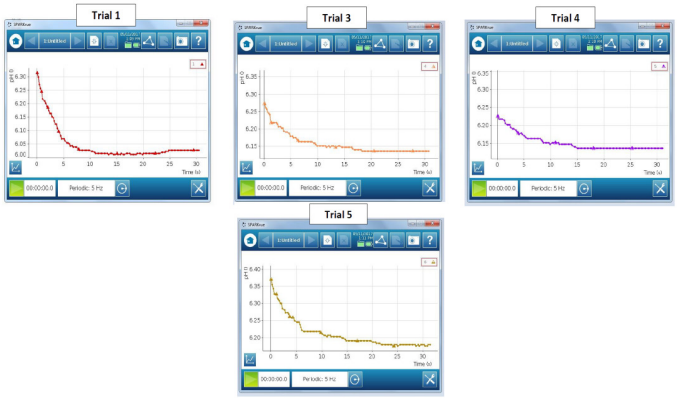


Figure 6. pH reading of five trials with 100 mL solution of distilled water and methanol and 2 tablespoons of aflatoxin from Bottle 2.

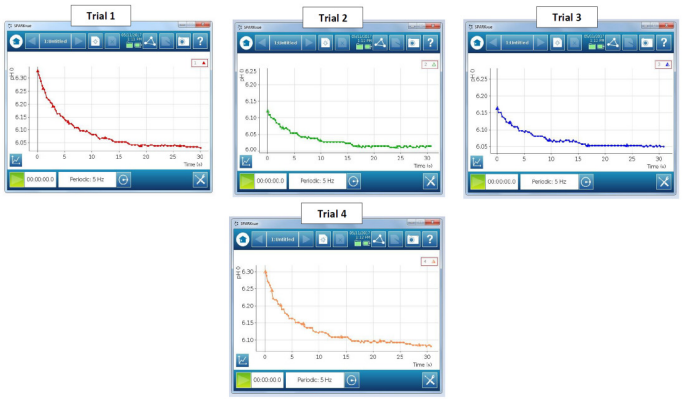


Figure 7. pH reading of five trials with 100 mL solution of distilled water and methanol and 2 tablespoons of aflatoxin from Bottle 3.

The average pH reading was calculated for each sample and was plotted with the concentration of aflatoxin in corn feed samples where the pH was converted to its equivalent parts per billion with respect to time. The concentration was converted using the formula:

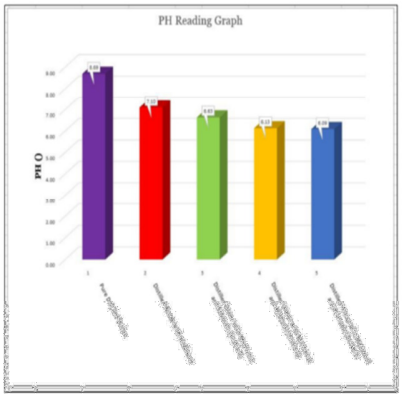
$$[\,concentration] = \; 10^{-pH}$$

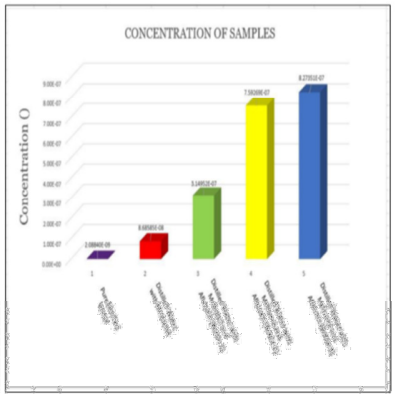
To get the concentration to its equivalent parts per billion (ppb), we used the following relation.

$$[\,concentration] = \frac{moles}{L}\; x\, \frac{molar\ mass\ (\frac{g}{mol})}{1}\; x \frac{1000\ mg}{1g}\; x \frac{1ppm}{1mg/L}\; x \frac{1000\ ppb}{1\ ppm}$$

Aflatoxin B_1 is found to be the most abundant and toxic among all other types of aflatoxin present among feed crops (Paniel, N., Radoi, A. & Marty, J., 2010). Thus, to make the calculations simpler, we limited all solutions to aflatoxin B_1. We calculated the molar mass of B_1 using the formula:

$$\text{Molar Mass or Aflatoxin } B_1\ (C_{17}H_{12}O_6) = 312\,\frac{g}{mol}$$





SET UP	pH	CONCENTRATION (ppb)
Pure Distilled Water	8.69	0.65
Distilled Water with Methanol	7.10	27.10
Distilled Water with Methanol and Aflatoxin (Bottle 1)	6.63	98.27
Distilled Water with Methanol and Aflatoxin (Bottle 2)	6.13	236.89
Distilled Water with Methanol and Aflatoxin (Bottle 3)	6.09	258.13

Figure 8. Average pH measurements of each setup (left) and concentration plot (right).

The graph shows that the highest pH reading was measured from the sample with pure distilled water followed by the sample with 1:4 water and methanol solution. Amongst the three bottles that contain aflatoxin, Bottle 1 has the highest pH while Bottle 3 has the lowest. The concentration in parts per billion (ppb) showed a reverse reading from the pH graphs. The higher the pH, the lower its concentration. Bottle 1 showed the least content of aflatoxin B_1 concentration while Bottle 3 has the highest amount of toxin.

To confirm observations and results, cyclic voltammetry (CV) test was conducted. A CV plot provided the electrochemical properties of the sample analyte. The CV device is composed of three working electrodes: counter, reference, and the working electrode. The morphological structure of the three electrodes was examined using the Double Unit Phenom Pro with advanced imaging system for Scanning Electron Microscopy (SEM). It showed that flake-like microstructures could be found in the counter and working electrode while irregularly shaped microstructures and a few irregular orthorhombic structures were found in the reference electrode.

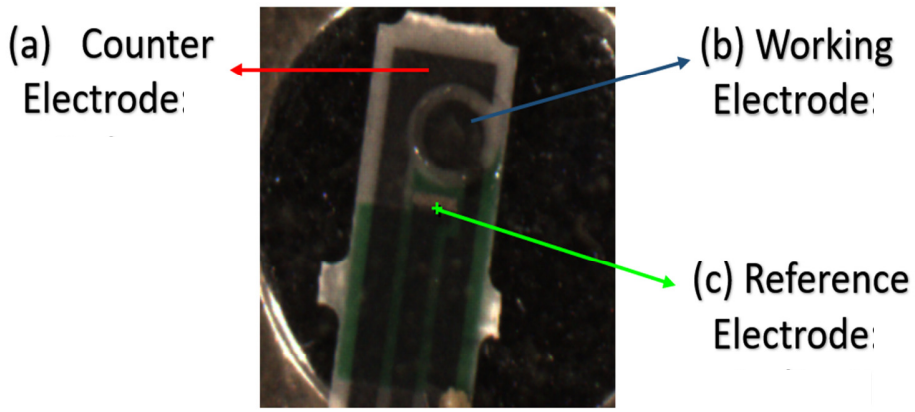


Figure 9. Three electrodes of the cyclic voltammetry device.

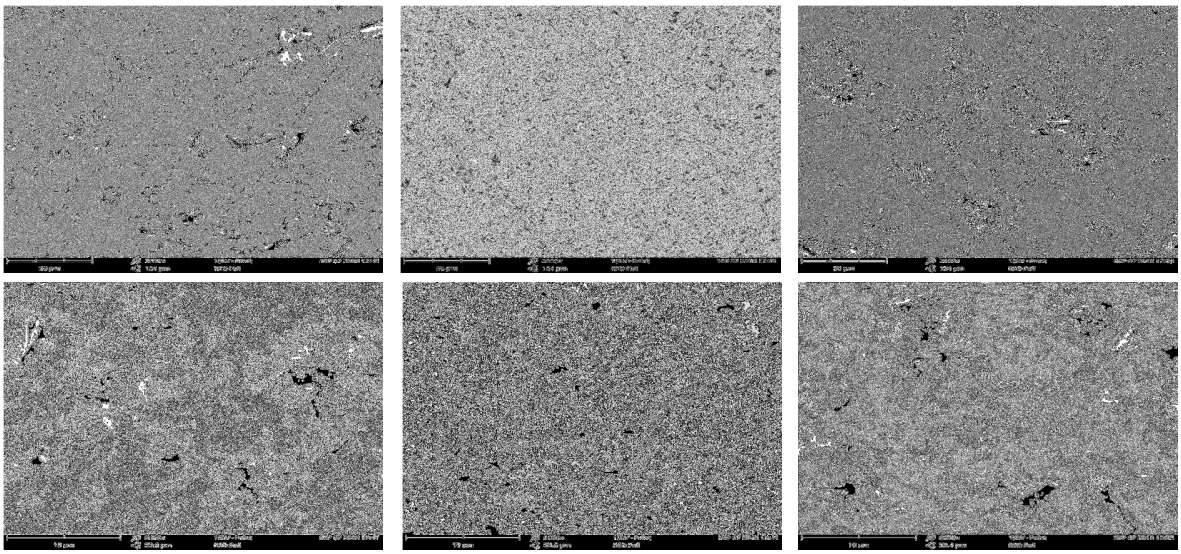


Figure 10. Scanning Electron Microscopy (SEM) images of (a) counter electrode, (b) reference electrode and (c) working electrode. The top row shows SEM images with 2000 magnification and the bottom row shows 8000 magnification.

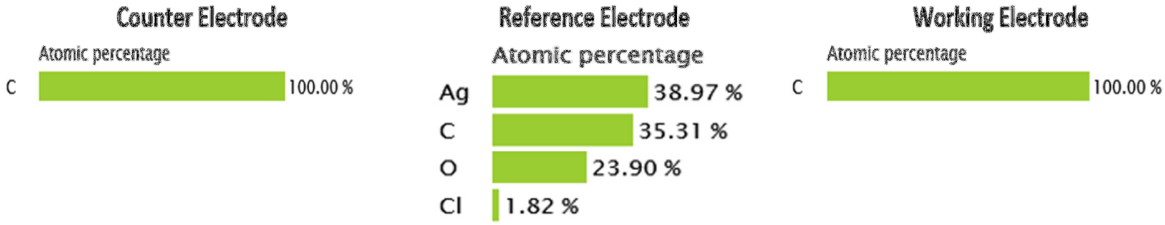


Figure 11. Atomic percent composition of the three electrodes using Energy Dispersive X-Ray (EDX).

The elemental composition of the electrodes was characterized using Energy Dispersive X-Ray (EDX). It was found that the counter and working electrodes have 100% carbon in atomic composition while the reference electrode has 38.97 % silver, 35.31 % carbon, 23.90 % oxygen, and 1.82 % chlorine.

Readings from the cyclic voltammogram showed that the current vs. voltage plot for the three bottles that contain different concentrations of Aflatoxin has an insignificant

difference as compared to that of pure distilled water and water-methanol solution as shown in Figure 12.

Based on the presented results, the mixture of water-methanol with or without aflatoxin is irreversible; thus, transfer of electrons from the analyte to the electrodes are slow. The potential also dropped when the aflatoxin sample was added to the mixture of water and methanol. This is possibly due to the breakage of the intermolecular forces of the aflatoxin molecules. Thus, extraction of aflatoxin was successful using the water-methanol solution.

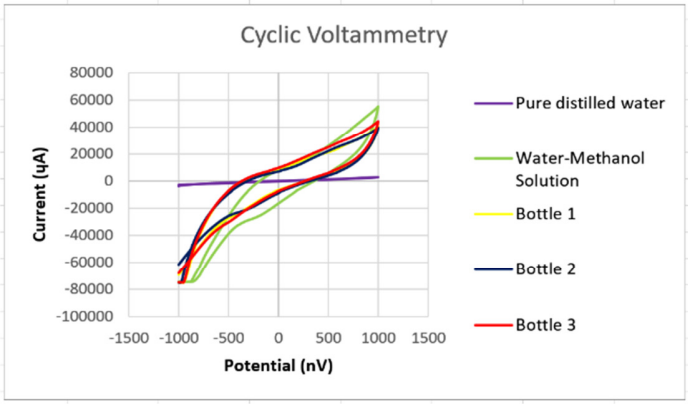


Figure 12. Average current vs. voltage cyclic voltammetry plot of all samples.

In addition, the results from the obtained pH and calculated ppb concentrations were comparable to the standards set by the United States Food and Drug Administration (USFDA) as shown in the next figure.

Aflatoxin leve (in parts per billion)	Commodities and species
10	All products, except milk, designated for humans
0.5	Milk
20	Corn for immature animals and dairy cattle
100	Corn for breeding beef cattle, swine and mature poultry
200	Corn for finishing swine
300	Corn for finishing beef cattle
300	Cottonseed meal (as a feed ingredient)
20	All feedstuff other than corn

Conclusion and Recommendations

Extraction of aflatoxin from corn feeds can be performed using a simple solution of pure distilled water and methanol with 1:4 ratio. Detection of aflatoxin among feed samples can be obtained using a simple mechanism with the aid of a smart device. The concentration of aflatoxin in corn feed samples can be obtained using a conversion of the pH reading of the samples to its equivalent parts per billion.

For further studies, the other type of aflatoxin (i.e., B_2, M_1, etc.) should be considered in the calculation. Also, use of other organic solvents could be explored as an alternative to methanol.

Acknowledgment

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References

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APPENDIX A:

Safety Precautions for Aflatoxin

A. Safety Measures for Handling Mold Cultures and Contaminated Commodities

1. Workers should always wear a face mask or respirator and disposable surgical gloves when working with the mold cultures.

2. Inoculation of growth media/substrates and transferring of A. flavus/A. parasiticus spores should be done under a laboratory hood that is first sprayed inside with a disinfectant.
3. Never handle the contaminated seeds with bare hands; always use surgical gloves when examining seeds for external or internal fungal infection. Seeds should be examined for infection under an exhaust hood.
4. The seed for dry seed resistance testing should be prepared under an exhaust hood to draw the dust away from the worker.
5. When it is necessary to work with any contaminated material, workers should wear face masks to avoid breathing in spores or dust that may be associated with the contaminated commodity.
6. Samples of commodities for aflatoxin analysis should be prepared cautiously as the grinding of dry samples may result in airborne dust. Even if no aflatoxin is present, there is potential harm from inhalation of mold spores or allergic responses to inhaled dust. Use of a protective mask is essential.
7. Disposable laboratory coats are also helpful in protecting workers' clothing from accidental contamination with mold spores and aflatoxins.

B. Safety Measures for Handling Aflatoxins

Aflatoxins are highly toxic and carcinogenic substances, and stringent safety measures must be taken when it is necessary to work with pure aflatoxins or with extract preparations. The greatest risk of laboratory contamination comes from handling dry aflatoxins because of their electrostatic nature and resulting tendency to disperse in working areas.

1. When dry aflatoxins are handled, the worker should use a respirator or disposable face mask to prevent inhalation of the toxin. Disposable surgical gloves should be worn.

2. Whenever possible, perform manipulations within a laboratory hood. Use a glove box if available.
3. Whenever possible, the aflatoxins should be handled in solution.
4. Low-cost paper laboratory coats should be used to protect workers' clothing from contamination with mycotoxins. These paper laboratory coats should be destroyed upon accidental contamination.
5. Workers must use a rubber bulb pipetting device—not the mouth when pipetting toxin solutions to prevent any oral exposure to them.
6. Contamination of the mouth should be treated with a wash composed of 1% sodium perborate and 1% sodium bicarbonate in water (a supply of this wash should be kept available in the laboratory).
7. If mycotoxins contaminated substances or cultures come in contact with the skin, the affected area should immediately be washed with full strength domestic bleach (e.g., Clorox which contains 5.25% sodium hypochlorite). Any bleach solution which contains around 5% of sodium hypochlorite will suffice. Follow this treatment by washing the skin with germicidal soap or detergent and then rinse thoroughly in tap water. If the skin is too sensitive to wash with sodium hypochlorite solution, a solution (5%) of sodium perborate may be used instead (supplies of the above solutions should be kept in a readily accessible position in the laboratory).
8. Protective goggles and face mask should be used when viewing chromatographic plates and mini-columns under ultraviolet light for the presence of aflatoxins. Similar protection is required when removing aflatoxin “spots” from silica gel or when removing the gel from chromatographic plates. These processes should be carried out in a laboratory hood.

C. Decontamination Procedures for Laboratory Glassware and Spillage

1. Mold cultures in flasks should be sterilized by autoclaving or by introducing a small amount of chloroform, replacing the cotton-wool plug, and heating in a steam bath until chloroform vapor can be seen condensing in the plug (ca. 10–15 minutes).
2. Mold cultures in Petri plates should be placed in a bleach solution (5% sodium hypochlorite). Allow at least 1 hour for effective decontamination.
3. Infected seeds in the Petri plates should be kept in a bleach solution (5% sodium hypochlorite) for 1 hour for decontamination.
4. Use surgical gloves (throw-away type) and face mask when it is necessary to handle mold cultures and contaminated seeds.
5. For general "dish-washing," a solution of bleach diluted tenfold with water (0.5% sodium hypochlorite) should be used. Make sure that the entire surfaces of the glassware are wetted. All aflatoxins are destroyed by alkalis, strong acids, and oxidizing agents. Clean-up procedures involving the use of such reagents should be sufficient for decontamination. Sodium hypochlorite is a strong oxidizing agent, very effective in destroying aflatoxins and in detoxification. Ordinary liquid household bleaches contain 5 to 6% active sodium hypochlorite and are very effective in destroying aflatoxins in solution, in fungal spores, and in mycelia.
6. Swab accidental spills of aflatoxin or toxic substances with 5% sodium hypochlorite solution.
7. Contaminated waste materials such as filter papers, adsorbents, and so forth should be kept in tightly closed containers, should not be comingled with other waste materials, and should be incinerated. If burning is not practical, such material may be decontaminated by soaking in 5% sodium hypochlorite bleach for an hour, care being taken to ensure thorough contact.

8. Prior to disposal, toxin-containing solutions should also be treated with bleach or strong acids.
9. Adsorbents on TLC plates should be soaked with bleach before they are removed from the plates.
10. Contaminated garments which are to be laundered should be soaked for an hour in 5% sodium carbonate solution or 1% bleach solution depending upon fabric and coloring.

D. General Safety Precautions for Handling of Aflatoxins

1. Laboratory areas where work with A. flavus and aflatoxins takes place should be clearly demarcated. Such rooms should have warning signs: DANGER - AFLATOXIN - CARCINOGEN HANDLING AREA.
2. The work surfaces should be of glass or decolam sheet.
3. Laboratory floors, walls, and ceilings should be cleaned using wet cloths and not by dry sweeping. A vacuum cleaner may be used to remove dust, powder, and so forth from inaccessible areas.
4. Possible contamination of working areas should be monitored frequently by means of a portable long-wave ultraviolet lamp, the presence of aflatoxins being indicated by their characteristic fluorescence.
5. The glass vessel containing aflatoxin should be kept within a metal container packed with adsorbent material. The container should be clearly labeled and bear a warning notice.
6. Eating, drinking, smoking, and chewing (beetle, gum, or tobacco) should be strictly prohibited in all rooms in which work with *Aspsrgillus flavus* and aflatoxins is carried out.
7. Laboratory workers should receive regular medical examinations.