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# Poultry Farm Hygiene: Microbiological Quality Assessment of Drinking Water Used in Layer Chickens Managed under the Battery Cage and Deep Litter Systems at Three Poultry Farms in Southwestern Nigeria

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Abstract: Water troughs from deep litter and caged chicken water troughs (drinkers) fixed to each of the different 3-tier cages containing layer chickens in Farms A, B and C were subjected to a 7-day study which involved the monitoring of poultry farm hygiene. Drinkers were washed before filling with water on Day 1. For Days 3, 5 and 7 water was served without prior washing. The occurrence and characterization of the bacteria isolates were investigated and data obtained were analyzed and compared. For the bacterial count on Day 1, for layer chickens on cage system, no significant differences (p>0.05) among the farms and between the farms tier interactions. On Day 3, no significant difference (p>0.05) among the parameters. On Day 5, there was significant difference (p<0.05) among the farms and on Day 7, there was high significant difference (p<0.01) among the farms. On Days 5 and 7, there were no significant differences (p>0.05) among the tiers nor between the interactions of the farms and tiers. The bacterial count in water troughs of layer chickens in deep litter system, on Day 1, had no significant differences (p>0.05) between the farms, water troughs and their interactions. On Day 3, no significant difference (p>0.05) among the parameters. On Days 5 and 7, there were significant difference (p<0.05) and a high significant difference (p<0.01) between the farms respectively. On Days 5 and 7, no significant differences between the water troughs and between the interaction of the farms and the water troughs. Farm A isolates contained Staphylococcus aureus, Proteus vulgaris, Streptococcus faecalis, Escherichia coli, Staphylococcus epidermis, Klebsiella sp., Salmonella sp., Bacillus subtilis, Lactobacillus salivarius and Corynebacterium sp. Farm B had Staphylococcus aureus, S. epidermis, Bacillus subtilis, Corynebacterium sp., Escherichia coli, Streptococcus faecalis and Klebsiella sp. while for Farm C, apart from the prevalent bacteria isolates obtained in Farms A and B, additional 2 bacterial isolates, Lactobacillus salivarius and Pseudomonas aeuriginosa were found. In conclusion, water troughs when cleaned on daily basis carry minimum bacterial load. Those left for 3, 5 and 7 days uncleaned had progressively high bacterial loads, suggesting that the flock of birds and the consumers of the eggs and meat from the chickens are at risk of bacterial infection unless strict farm hygiene is ensured through regular monitoring.

**Key words:** Chicken water troughs, bacterial infection, egg production

### INTRODUCTION

The importance of water is universally accepted by poultry producers in its use as an electrolyte replacement therapy and in treatment with drugs, where water medication is particularly important. However, the visual purity of water can mask many problems that may eventually affect the profitability of poultry enterprises. Assessment of water quality therefore, can provide insight into the suitability of water supply. Water can be contaminated with dirt or impurities from bad handling. Many a time, when birds eat, the digesta may be deposited into their water. Also, the water drinkers may not be thoroughly washed by the attendants, thus

enhancing the formation of slimy materials which could encourage the proliferation of micro-organisms.

Many livestock farmers in Nigeria, have no formal education and therefore have relatively little knowledge of the effect of poor hygiene on their livestock and the public. They place little or no emphasis on clean water for their chickens, let alone the daily and regular cleaning of the water troughs, but only ensure that water is available for them to drink. To many of them, infectious disease outbreak is more of spiritual than developments arising from poor farm hygiene.

Water is the most essential nutrient birds receive, yet the quality of water is often overlooked (Dragas and Tratnik, 1975). According to Donald *et al.* (2000), water could be contaminated in two ways. These, according the author, could be from the water source, most especially when the water source is from the well water or surface water, where there are chances of bacterial contamination. This is especially true with shallow wells and surface water from ponds and rivers that might be subjected to surface run-off or tidal contaminations and also from pipelines contamination, where overtime, scale, dust, algae and dirt can collect in water-lines, if proper maintenance does not take place. Build-up of such is an ideal place for microorganisms to become established and slime-forming bacteria helps in such build-up. However, whatever may be the contamination source, every time birds drink the water they are exposed to microbial load and immune challenges. Besides the damage to watering system, they cause diseases like Colibacillosis, Omphalitis in chicks, Salmonellosis, necrotic enteritis and many others, which result in severe economic losses (Mangash, 2002).

Two major housing systems used in commercial egg production include the deep litter system (where the birds are restricted to the rearing house) and the battery cage system, where the hens are kept indoors in cages within a large controlled environment (Hughes and Black, 1974). The birds are housed in individual cage compartments or cells (Oluyemi and Roberts, 2000). This individual cage compartment has the egg nest and water troughs. The cage system, which may be one step (1 tier), 2 step (2 tier) or 3 steps (3 tier) is more efficient than the litter system (Dun *et al.*, 1991) and mostly used for commercial layer-chicken production.

Caged layer-chicken water troughs (aluminium or plastics) deserve frequent cleaning because the regurgitated feed particles are favourable medium for microbial growth especially when left un-cleaned. The source of water for the chicken also determines the type of microorganisms occurring in the trough and consequently has poultry disease implications such as reduced egg production and even death (in the flock): beyond negative poultry production effect, the consumer of the products may also be affected when such disease organisms overcome the normal human body defences and invade the tissues (Purohit, 1990). This work was designed to investigate the progressive occurrence and characteristics of bacteria in the caged and deep litter managed layer chicken water troughs un-cleaned for seven days continuously but filled with clean water daily for the seven days.

### MATERIALS AND METHODS

**Project site (Farms A, B and C):** In Farm A, One hundred and twenty (120) layer chickens were housed (20 each) in

each of the 3- tier of the battery cages, with another one hundred layer chickens on deep litter system.

Farm B had One hundred and eighty (180) layer chicken (60 each) in each of the 3-tier of the battery cages, with another sixty (60) layer chickens on the deep litter.

In Farm C, One hundred and fifty (150) layer chickens were housed (50) in each of the 3-tier of the battery cages, with another sixty (60) layer chickens on the deep litter. All the 3 farms were within Akure metropolis, Nigeria. The floors of the poultry houses in farms A, B and C were concretized. Asbestos and corrugated iron roofing sheets were used in all the farms respectively. Each of the farms sunk underground well as water source. A general assessment of the hygiene status of each farm was made using stated yardsticks which were scored and recorded (Table 1).

Sample collection: Three poultry farms (A, B and C), where layer chickens were managed under the battery cage and deep litter systems were visited for sample collection. In the battery cage system, samples were taken from all the 36 water troughs (drinkers) attached to the poultry cages accommodating layer-chickens housed in twos per cell. Approximately, 12 water troughs located on the same side of the battery cages were sampled in each of the farms. Similarly, samples were taken from the water troughs and water source used for the birds managed under the deep litter system. Constant availability of water in the troughs was ensured throughout the experimental period. Four sets of twelve samples were collected from each farm. The first set was collected on the day when the troughs were washed (Day 1), the second set, two days after the washing (Day 3), the third, four days after washing (Day 5) and the last set, six days after washing (Day 7). The samples were collected with sterile swabs dipped into the middle and both ends of the water troughs (where much regurgitated feed in the troughs had accumulated). This procedure was repeated for all the water troughs throughout the period of sampling. The sampling process was carried out under sterile conditions.

**Isolation of bacteria:** Each sample collected was diluted using normal saline. One milliliter of the sample was aseptically withdrawn and added to 9 mL of saline to give 10. Serial dilution was carried out from 10<sup>1</sup> to 10<sup>1</sup>. One milliliter of the dilutions was placed in separate sterile Petri dishes to which sterile nutrient agar medium cooled to 45°C was added and properly rotated for uniform distribution. The plates were allowed to set and were later observed for bacterial growth after 24 h at 37°C. Pure colonies of the bacterial isolates present were collected.

**Identification of bacteria:** Bacteria were identified on their morphology, stain and biochemical characteristics through the modified methods of Cowan and Steel (1974).

Statistical analysis: Data obtained for the bacteria count were subjected to one-way Analysis of Variance (ANOVA) and 2x3 factorial analysis after square root transformation (Steel and Torrie, 1980). Where results obtained within the mean of a set of data were significantly different, they were further compared using Duncan's multiple range tests.

### RESULTS AND DISCUSSION

Farm observation: During the course of this study, it was observed that the floor in Farm A was in good shape while that of farm B and C had cracks, messy with numerous holes due to old age of the concrete. The roof of the poultry house in Farm A was in good condition while that of farm B had leaky roof and that of Farm C, apart from being leaky, were covered with cob webs. The amount of regurgitated feed in the water troughs was relatively less in Farm A, high in Farm B and highest in Farm C. the percentage assessment score for the parameters of hygiene used for the farms is shown in Table 1.

**Bacteria count:** Table 2 shows the bacteria loads in the samples collected from the water troughs in the 3 tiers of cages in the three farms. On day 1, the 3rd tier cage in Farm A, had the highest bacteria load of  $1.45\pm0.13\times10^8$  CFU mL<sup>-1</sup>, Farm B had had  $1.43\pm0.23\times10^8$  CFU mL<sup>-1</sup> and Farm C recorded  $1.42\pm0.22\times10^8$  CFU mL<sup>-1</sup> bacteria load. The mean bacterial load for tier 1 from all the farms on Day 1 had a value of  $1.32\times10^8$  CFU mL<sup>-1</sup>. This load progressively increased through Day 3, 5 and 7. Tier 3 had the highest values throughout the study period.

The persistently high value of bacterial load observed in tier 3 for Farms A, B and C in this study could

Table 1: Percentage assessment score for the various comparative parameters of hygiene on the farms

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Hygienic factor considered (%)	Farm A	Farm B	Farm C
Structure	70	40	35
Roof and roofing status	77	45	40
Poultry house floor and surrounding	80	43	37
Cleanliness of chickens' water troughs	65	40	35

be attributed specifically to the height of the water trough in tier 3 which makes them relatively inaccessible to the attendants for effective cleaning compared to those in tiers 1 and 2. This factor is very vital in the farmers' choice of cage type for layer-chicken production. In a comparative investigation into egg production using 3 and 2 tier cages, it was reported (Awoniyi, 2003) that (egg-production) in the uppermost tier (tier 3) of the 3-tier cage in houses with corrugated metal roof was more negatively affected than under the asbestos roofing sheets in the tropic (Nigeria) especially in the dry season.

Water troughs that are not properly washed create favourable environment for the growth and proliferation of bacterial organisms when subsequently refilled without cleaning. As shown in Table 2, birds in the 3rd tier may have been exposed to a greater risk of bacterial infection, compared to other birds in the lower tiers.

Comparing the mean bacterial load in the water troughs on Days 3,5 and 7 for the 3 farms. Farm A had lower values than farms B and C. The disparity could be attributed to the varied levels of hygiene in these farms. Comparative bacterial load in the water troughs for the 3-tier cages in Farms A, B and C as shown in Table 3 revealed that on days 1 and 3, there were no significant (p>0.05) differences between the farms the cage tiers and the farm-tier interaction. The significant difference (p<0.01) between farms on day 5 and (p<0.05) on day 7 is an indication of poor hygienic status of Farm B and C which was further confirmed by the high value for the bacterial load in their respective water troughs which was caused by a build-up of regurgitated feed providing a favourable environment for bacteria proliferation in their troughs. This finding substantiates the reports of Awoniyi (2003) that layer-chickens occupying tier 3 of the 3-tier cages stand the risk of reduced productivity because they could suffer stress infection as a result of improper management by attendants when enough feed is not supplied or when water troughs are not cleaned.

Table 4 presents the bacterial loads in the samples collected from the water troughs of layer-chickens in deep litter system. On day 1, the 3rd sampling recorded the highest bacteria load of  $2.53\pm0.32\times10^8$  CFU mL<sup>-1</sup>, Farm B had  $2.50\pm0.41\times10^8$  CFU mL<sup>-1</sup> and Farm C recorded  $2.52\pm0.32\times10^8$  CFU mL<sup>-1</sup> bacterial load. The mean bacterial load for samples collected from Farms A, B and C on Day 1 stood at  $2.36\times10^8$  CFU mL<sup>-1</sup>. This load progressively

Table 2: Mean bacterial count in	ı (×10 <sup>-</sup> ° CFU mL <sup>-</sup> ') water	troughs in the 3 tiers of cages in Farms A, B and C	

	Day 1 Day 2				Day 3				,			
	 Fапп A	 Farm B	Farm C	Farm A	 Farm B	Farm C	Farm A	 Farm B	Farm C	Farm A	 Farm B	Farm C
1	1.32±0.40	1.32±0.41	1.33±0.42	1.61±0.32	1.60±0.32	1.62±0.43	1.72+0.33	2.31±0.33	2.40±1.02	2.00±0.32	2.60±0.41	2.56±0.2
2	$1.43\pm0.12$	1.36±0.52	$1.39\pm0.33$	$1.58\pm0.13$	$1.71\pm0.21$	1.69±0.22	$1.78\pm0.41$	$2.28\pm0.43$	2.52±0.32	2.10±0.44	2.64±0.52	$2.60\pm0.32$
3	$1.45\pm0.13$	1.43±0.23	$1.42\pm0.22$	$1.86\pm0.33$	$1.80\pm0.33$	$1.96\pm0.25$	$1.98\pm0.33$	$2.52\pm0.41$	$2.60\pm0.41$	2.22±0.32	2.92±0.32	$2.83\pm0.41$

increased through Day 3, 5 and 7. Samples obtained from the 3rd sampling had the highest values throughout the study period.

The persistently high value of bacterial load observed in the 3rd sampling of the water troughs in deep litter system could be adduced to the possibility of high level of contamination by the birds. This, many a times apart from being soiled with their digesta could also be contaminated with their feaces and even when the birds dust-bath, litter materials could enter and further contaminate the water troughs. When the water troughs are not properly washed, could create favourable environment for the growth and proliferation of bacterial organisms when subsequently refilled without cleaning.

Comparing the mean bacterial load in the water troughs on Days 3,5 and 7 for the 3 farms, Farm A had least values, followed by Farm B and the highest was recorded for Farm C. the disparity could be attributed to the varied levels of hygiene in the farms. Comparative bacterial load in the water troughs layer-chicken managed under the deep litter system in the 3 farms as shown in Table 5 revealed that on days 1 and 3, there were no significant differences (p>0.05) between the farms, the water troughs used and the farms-water troughs used interaction. The significant difference (p<0.01) between farms on day 5 and (p<0.05) on day 7 is an indication of poor hygienic status of farm B and C which was further confirmed by the extremely high value obtained for the bacterial load in the water troughs which was caused by a build-up of regurgitated feed feacal contamination and litter materials, providing a favourable environment for bacteria proliferation in the trough.

## Probable bacteria occurrence in the water troughs:

Table 6 depicts the various bacterial reactions to the tests carried out. The different bacteria identified are listed under the table. As presented in Table 6, it was observed that the longer the drinkers were left without being washed, the more the variation of the bacterial organisms occurring in the culture from the water troughs. In Farm A,

Table 3: Statistical significance of the bacterial load between farms, tiers and their interaction

	Day 1	Day 3	Day 5	Day 7						
Farm	ns	ns	*	**						
Tier	ns	ns	ns	ns						
<u>Farm×Tier</u>	ns	ns	ns	ns						
ns: Not significant*, **p<0.01, p<0.05										

the culture from Day 1 sample, four bacterial isolates (Staphylococcus and aureus, S. epidermis, Bacillis subtilis and Corynebacterium sp.) were isolated while from Day 3 sample, additional two bacteria-Streptococcus faecalis and Escherichia coli were observed. Salmonella sp and Klebsiella sp. were isolated from Day 5 sample cultures while on Day 7, Proteus vulgaris and Lactobacillus salivarius were found in addition to those already isolated.

In farm B, Day 1 sample showed a prevalence of Staphylococcus aureus, S. epidermis, Bacillus subtilis, Corynebacterium sp. Day 3 sample culture contained the same 4 isolates which were identified in the Day 1 samples with 3 additional isolates identified as Esherichia coli, Streptococcus faecalis and Klebsiella sp. Nine bacteria isolates were altogether identified from the Day 5 sample culture, they included the 7 isolates from Day 3 and two new ones comprising Salmonella sp and Proteus vulgaris. A total of 11 bacteria were isolated from the Day 7 sample culture. They include all those identified in Day 5 samples as well as Lactobacillus salivarius and Pseudomonas aeruginosa.

In Farm C, the prevalent bacteria in the samples collected from Day 1 included the likes of Staphylococcus aureus, S. epidermis, Bacillus subtilis and Corynebacterium sp. Day 3 samples contained the same 4 isolates which were identified in the Day 1 samples with 3 additional isolates identified as Escherichia coli, Streptococcus faecalis and Klebsiella sp. Day 5 sample produced nine bacteria isolates which included the likes of the 7 isolates for Day 3 and two new ones comprising Salmonella sp. and Proteus vulgaris. Day 7 sample culture produced 11 bacteria isolates. They included all those identified in Day 5 samples as well as Lactobacillus salivarius and Pseudomonas aeuriginosa.

The bacterial organisms in the samples collected from these three farms showed some differences. On day 1, bacterial isolates found in Farm A samples would have been similar to those of Farm B and C, but for the occurrence of *Klebsiella* sp. in Farm B samples and *Proteus Vulgaris*, *Lactobacillus salivarius* and *Pseudomonas aeruginosa* in Farm C samples. Samples taken from the three Farms on Day 3 presented all those isolates earlier identified in Day 1 samples with *E. coli* and *Streptococcus faecalis* as new isolates from the farms.

Table 4: Mean bacterial count in (×10<sup>-8</sup> CFU mL<sup>-1</sup>) water troughs in the deep litter systems in Farms A, B and C

	Day 1			Day 3			Day 5			Day 7		
	Farm A	 Farm B	Farm C	Farm A	 Farm B	Farm C	Farm A	Farm B	Farm C	Farm A	Farm B	Farm C
1	2.34±0.35	2.36±0.21	2.36±0.32	3.40±0.31	3.60±0.28	3.72±0.41	3.82±0.30	4.90±0.32	4.63±0.31	4.92±0.23	5.00±0.22	5.10±0.33
2	$2.46\pm0.21$	$2.34\pm0.31$	$2.31\pm0.31$	$3.46\pm0.42$	$3.43\pm0.22$	$3.68\pm0.23$	$3.75\pm0.22$	$4.80\pm0.23$	$4.72\pm0.20$	$4.98\pm0.24$	$5.14\pm0.32$	$5.22\pm0.34$
3	$2.53\pm0.32$	2.50±0.41	2.52±0.32	3.33±0.27	3.52±0.33	$3.62\pm0.22$	$3.70\pm0.34$	4.90±0.33	4.83±0.24	4.79±0.22	5.20±0.23	5.30±0.23

Klebsiella sp. was the unique isolate identified in farms B and C and on Day 3. Salmonella sp. and Proteus vulgaris appeared in samples from Farms B and C on Day 5. A total of eight and nine bacterial isolates were found in the samples collected from Farms A, B and c respectively.

On day 7, only *Lactobacillus salivarius* and *Pseudomonas aeruginosa* were found in Farms A, B and c samples, all other bacterial organisms isolated from the 3 farms samples were similar.

**Implications of bacterial prevalence as revealed by the study:** Most of the bacterial organisms identified in this study are Gram positive and Gram negative (Table 7) and are of medical importance (Cruickshank *et al.*, 1973). Farm

Table 5: Statistical significance of the bacterial load between farms, water troughs and their interaction

	Day 1	Day 3	Day 5	Day 7
Farm	ns	ns	*	**
Water troughs	ns	ns	ns	ns
Farm*water troughs	ns	ns	ns	ns

ns: Not significant\*, \*\*p<0.01, p<0.05

Table 6: Characteristics of the bacterial isolated from the water troughs Characteristics 1 Gram staining Shape of cells  $^{\rm C}$  $\mathbf{C}$  $\mathbf{C}$ R R R R Motility Catalase Oxidase Spores Indole Coagulase Glucose AG Α AG AG AG Lactose Maltose Manitol Sucrose Arabinose Frutose

- +: Positive, -: Negative, R: Rod, C: Coci, A: Acid, Ag: Acid and gas
- Staphylococcus aureus 2. Proteus vulgaris, 3. Pseudomonas aeruginosa
   Streptococcus faecalis 5. Escherichia coli 6. Staphylococcus epidermis
- 7. Klebsiella sp. 8. Salmonella sp., 9. Bacillus subtilis 10. Lactobacillus salivarius. 11. Corvne bacterium sp.

products or equipment contaminated by these organisms e.g., egg, battery cages, faecal materials are the indirect means of their transmission to the animals or the handlers.

In the poultry houses, man (the attendant or the final consumer of the poultry products) becomes infected depending on his immune status or the virulence of the organisms. Card and Nesheim (1975) emphasized the paramount importance of maintaining high level of hygiene in the watering system of poultry especially layers, suggesting that for effective control, there is the need to understand basic management principle that since chickens in the cages drink from water trough conventionally, the water trough placed above the feed trough should be suspended such that the level of the water in the trough in the same as the back level of the birds when in normal standing position. The height of the water troughs should be adjusted as the birds grow, this is to minimize organic accumulation in the trough.

Secondly, water troughs must be cleaned daily to remove regurgitated feed particles and other organic matter. By this hygiene practice, the build-up of the filth which favour bacterial proliferation is prevented. Most times, the 3rd tier trough of cages is improperly washed thus, the layer-chickens in this tier cage are comparatively more exposed to microbial risk. It was reported by Atlas and Bartha (1981), that bacteria especially coliforms are the main contaminants of water. Although there are acceptable counts of these organisms per 100 mL of water (Solberg et al., 1976), higher counts result in bacterial challenges for the birds and may result in disease outbreaks. The quality of metal used to fabricate the drinkers also determines the water quality.

The assertion by Jay et al. (1997) that policy plan for water supply must be well considered for a poultry production enterprise, to enable safe egg production for public consumption is relevant to the findings in this study, more so that water troughs in some poultry farms are left uncleaned for 5-7 days, even longer. Bacterial

Table 7: Occurrence of bacterial organism in water troughs of layer chickens

	Day 1			Day 3	Day 3			Day 5			Day 7		
Bacterial organism	FA	FB	FC	FA	FB	FC	FA	FB	FC	FA	FB	FC	
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	+	+	
Staphylococcus epidermis	+	+	+	+	+	+	+	+	+	+	+	+	
Bacillus subtilis	+	+	+	+	+	+	+	+	+	+	+	+	
Corynebacterium	+	+	+	+	+	+	+	+	+	+	+	+	
Escherichia coli				+	+	+	+	+	+	+	+	+	
Streptococci faecalis				+	+	+	+	+	+	+	+	+	
Klebsiella sp.					+	+	+	+	+	+	+	+	
Salmone lla sp.							+	+	+	+	+	+	
Proteus vulgaris								+	+	+	+	+	
Lactobacilus salivarius										+	+	+	
Pseudomonas aeruginosa											+	+	

FA: Farm A, FB: Farm B, FC: Farm C, +: Positive, -: Negative

infections occur because of poor management which creates an environment that favours microbial growth or activity (Vandemark and Batzing, 1987). Drinkers (water trough) which were not washed everyday greatly influenced microbial population as indicated in this 7-day study. The appearance and growth of certain bacteria and algae in such polluted water following their death and decomposition present disagreeable odour and taste.

Based on this, it was recommended that water toughs should be cleaned and sanitized to reduce microbial population; water samples should be collected from the drinkers for laboratory assay to determine the microbial load and the quality of water being supplied for drinking.

### CONCLUSION

In this study, it was established that bacteria occur in water troughs used in the layer-chicken's farms and the bacteria load was highest in the 3rd tier of the cages for birds managed in cages and in the third phase sampling of the water troughs of laying birds kept in deep litter system.

Low level of hygiene accounted for the high bacterial counts and most remarkable in the 3rd tier of the cages due probably to its inaccessibility, which resulted from the cage height and infrequent cleaning in the case of the water troughs of laying birds on deep litter system. Most of the bacteria characterized have public health significance because they do affect the health of the birds and consequently their product and the consumers. The findings showed why better hygiene should be maintained in poultry farms and from these, it was recommended that:

- Drinkers are better washed daily to reduce the growth of microbes which affect the purity of water provided for the birds; troughs are better made of aluminium rather than galvanized iron which corrodes fast and pollutes the water
- The use of 3-tiers cages should be discouraged in order to ensure reasonable level of hygiene of the troughs because the 2-tier cages are comparatively easier to access and clean than the 3-tier
- The water troughs in layer-chickens on deep litter should be placed on platforms or be suspended, so as to minimize the level of contamination and washed daily

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