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Detection of Arsenic In Chickens And Ducks

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ABSTRACT: The present study was undertaken for the detection of arsenic in chickens and ducks. Arsenic was detected qualitatively by Reinsch test, semiquantitatively by Arsen test kit and quantitatively by Perkin-Elmer Hydride Generation AAS 300 system (HGQF-AAS) in seven organs and faeces of each of fifty live chickens and ducks collected randomly from different chickens and duck farms and markets from various places of each of five districts of Bangladesh i e. Chandpur, Mymensingh, Rajshahi, Khulna and Barisal. The distribution of arsenic concentration was highest in liver and lowest in faeces of chickens and ducks.

Key words: Detection, arsenic, chickens, ducks.

INTRODUCTION

The peoples of Bangladesh have been suffering from serious public health problem arising from drinking arsenic contaminated groundwater (1). Nearly 62, out of 64 districts of the country's tube wells contain dangerous levels of inorganic arsenic, tube wells, which are serving as main sources for drinking and cooking purposes. People who are drinking this inorganic arsenic contaminated water are developing various pathological manifestations in their bodies (2). Manifestation starts from hyper pigmentation of the skin and mucous membrane and leads to death from mutation of cells in the body (3). The general populations are exposed to arsenic through drinking water, dust, fumes, and dietary sources. The highest concentrations of arsenic were reported in seafood, rice, mushrooms and poultry in U.S.A. (4).

Arsenic is ubiquitous in the biosphere and occurs naturally in both organic and inorganic forms in water, food, soil, dust, wood and other materials (5, 6). Inorganic arsenic is more toxic than organic arsenical compounds and arsenic trioxide is more toxic than arsenic pentoxide (7). Arsenic is stored mainly in liver, kidney and spleen, and most of it is excreted through urine and if the salt is not readily absorbed, much of it is eliminated in the feces (8). Chronic arsenic exposure in the range of 0.01-0.04 mg/kg/day has been associated with skin cancer in Taiwan (9). Arsenic was present in meat and meat products, eggs, honey, milk and milk products, fresh water fishes, marine fishes and other marine organisms collected from Slovenia between 1985 and 1995 (10). Contamination of farm animals and fishes from Slovenia with heavy metals and sulfonamides were studied. Arsenic was only detected in 9 (15%) marine fishes (11).

An organic arsenical compound Roxarsone is used widely in poultry production to control coccidian intestinal parasites. It was reported that roxarsone can be degraded biotically and abiotically, to produce more toxic inorganic forms of arsenic, such as arsenite and arsenate (12).

It has been reported that every U.S people may ingest 3.6-5.2 µg/ inorganic arsenic daily from chicken alone consuming in an average 60 g chickens/day (13). Levels of arsenic and other metals in the livers and bones of five waterfowl species were studied after the Aznalcollar mine spill. Dry weight bone concentrations ranged from n.d-1.76 mg/kg arsenic and wet weight liver concentrations ranged from n.d-0.34 mg/kg arsenic (14). Highest concentrations of arsenic were found in seafood, followed by meats and grain. Fruit, vegetables and dairy products tend to have lower concentrations (15).

Chickens and ducks are the main source of meat in our Bangladesh. Chickens and duck meats may contain arsenic through arsenic contaminated water, growth promoters containing arsenicals and through arsenic medication. Arsenic is concentrated by many species of fish and shellfish and is used as a feed additive for poultry and livestock; fish and meat are therefore the main sources of dietary intake of almost 78.9%, according to a recent U.S. survey (16).

Groundwater contamination by arsenic is a serious threat to mankind all over the world. Humans are exposed to this toxic arsenic primarily from air, food, and water (17).

In the context of the above situation, the present study was undertaken with the following objective:

i) Detection of arsenic in different organs of chickens and ducks in five selected districts (Chandpur, Mymensingh, Rajshahi, Khulna and Barisal) of Bangladesh.

BODY TEXT

The present study was undertaken for the detection of arsenic in chickens and ducks in natural conditions. Chandpur, Mymensingh, Rajshahi, Khulna and Barisal districts of Bangladesh were selected as sites for sample collection.

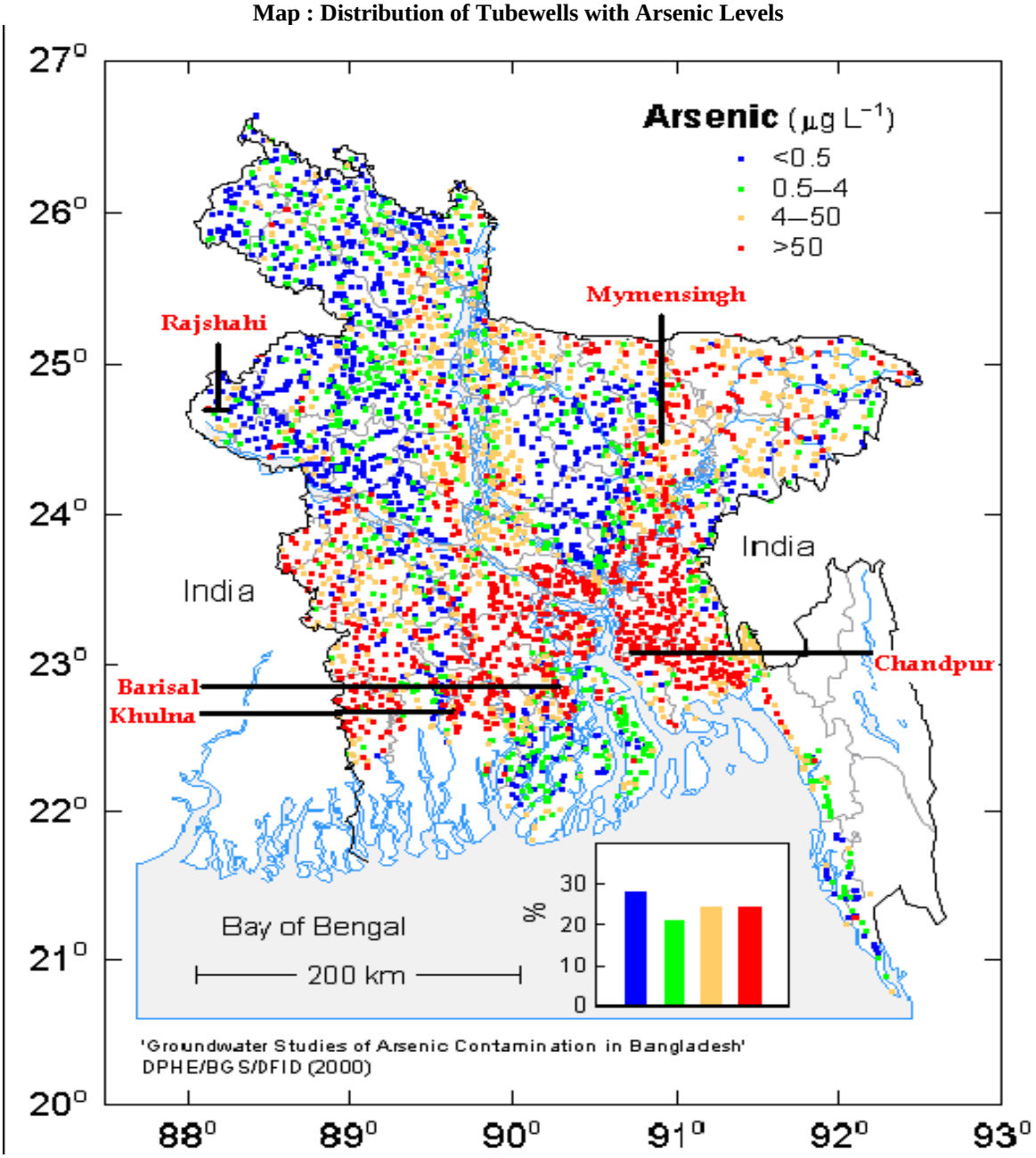
Collection of samples for determination of arsenic in different organs of chickens and ducks:

Fifty live chickens and fifty live ducks were collected randomly from different chicken and duck farms and markets from various places of each of five districts. Samples of liver, kidney, heart, small intestine, thigh muscle, femur, brain, and faeces of chickens and ducks were collected. The samples were preserved in frozen conditions (-20°C) and transported to the laboratory and kept frozen until analysis.

Detection of arsenic in different organs of chickens and ducks:

After thawing the preserved samples, liver, kidney, heart, small intestine, thigh muscle, femur, brain, and faeces of chickens and ducks, tissue homogenates were prepared as per following procedure:

Map : Distribution of Tubewells with Arsenic Levels



Preparation of tissue homogenate:

2-3 grams of the individual organs or faeces was grinded with the aid of pestle and mortar in demineralized water and then the tissue homogenates were taken in different test tubes placed on the rack. Demineralized water was added to the tissues at the ratio 4: 1 (4 ml water + 1 gm tissue) for the preparation of tissue homogenates.

Qualitative determination of arsenic in the tissue homogenates by Reinsch test (18):

Principle:

The Reinsch test is based upon the fact that when moderately acid solution (weak HCl) containing ionized salts of arsenic, mercury are heated with metallic copper, these elements are deposited on the copper surface.

Procedure:

1. Half a dozen of copper wires were cleaned by warming with 1:4 nitric acids (HNO_3) and then washed thoroughly with distilled water.
2. 5 ml of tissue homogenates as sample was taken in a 250 ml beaker.

3. Six copper wires half inch length were placed in the beaker.
4. 1:4 hydrochloric acid was then added as double of the sample.
5. The mixture was allowed to boil gently with the help of an electric heater for about 30 minutes.
6. The wires were obtained by decanting off the liquid and washed thoroughly by decantation using distilled water, then ethyl alcohol and finally by pure ether.
7. The copper wires were then placed on a watch glass and dried at room temperature in desiccator.
8. Change of colour of copper wires to dark brown or black indicated the presence of arsenic in the tissue homogenate.

Arsenic determination by "Merck Arsen Test" kit (Semiquantitative method):

Reaction principle

The trivalent and pentavalent arsenic compounds in the solution to be tested are converted to arsenic by adding zinc and hydrochloric acid which turns the reaction zone, containing mercury (II) bromide in the headspace above the solution, yellow to brown. Mixed arsenic mercury halogenides, e.g. As A_2H_2HgBr are formed.

Materials required:

To estimate the concentration of arsenic by "Merck Arsen Test Kit" the following materials were used:

1. Analytical test strips
2. Reaction vessel
3. Plastic syringe
4. Measuring spoon
5. Reagent 1 (Zinc powder)
6. Reagent 2 (hydrochloric acid)
7. Samples to be tested (1gram tissue grinded in 4 ml demineralized water).

Procedure:

1. Holding the reaction zone of the test strip downwards, the test strip was inserted through slit in the cap of the reaction vessel, in such a way that the cap divides the strip into two approximately equal segments.
2. 5 ml of the solution to be tested was transferred to the reaction vessel using the syringe; one measuring spoonful of reagent 1 was added and shaken.
3. 5 drops of reagent 2 was added and immediately the reaction vessel was closed with the cap.
4. It was left to react for 30 minutes, gentle swirling 2 or 3 times.
5. The test strip was removed, briefly dipped into water, shaken off excess liquid and compared the reaction zone with the colour scale and was multiplied by five.

The reaction zone of the strip indicated amount (mg/lit.) of arsenic was present in the tested solution.

Quantitative detection of arsenic in tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrophotometer (HGQFAAS):

Tissue digestion procedure:

One gram of tissue was removed from the bulk sample using a stainless steel scalpel or stainless steel bone cutters, and weighed to an accuracy of 0.001 g into an acid cleaned 50 ml conical flask. To each flask, 5 ml of analytical grade 70% nitric acid was added; the flask was closed and left overnight to digest. In total, 5 ml of analytical grade 30% hydrogen peroxide was then added and the digest flasks were heated in a hot air woven step by step up to a final temperature of 160^0C. This temperature was then maintained for 4 h, until the sample was fully digested (14). The digested solution was then decanted into 25 ml glass sample vial and made up to 20 ml with demineralized water. These solutions were stored in refrigerator until analysis.

Arsenic determination:

Arsenic was determined in the CIMMYT Arsenic Laboratory, BARI, Gazipur, Bangladesh using a Perkin-Elmer Hydride Generation AAS 300 system. Arsenic was determined after pre-reduction of an aliquot of the sample with a solution of 10% potassium iodide, 10% hydrochloric acid, and 5% ascorbic acid. All the tissue data was presented in mg/kg.

Statistical Analysis

The experimental data were analyzed statistically with the help of the MSTAT software (19).

RESULTS AND DISCUSSION

Collected samples were processed for laboratory analysis followed by standard methodology. The data were computed and tabulated as follows.

Qualitative determination of arsenic in chickens and ducks by Reinsch test

The results of the qualitative determination of arsenic in chickens and ducks by Reinsch test is presented in Table 1. Initially 50 livers of chickens and 50 livers of ducks from each of 5 districts i.e. total 250 samples of chickens and 250 samples of ducks tested for presence of arsenic. In chickens, among the 50 samples (Liver) of each of 5 districts tested, arsenic was detected in 26%, 22%, 14%, 12% and 10% in Chandpur, Mymensingh, Rajshahi, Khulna and Barisal, respectively. Similarly, in ducks, among the 50 samples (Liver) of each of 5 districts tested, arsenic was detected in 16%, 12%, 10%, 10% and 12% in Chandpur, Mymensingh, Rajshahi, Khulna and Barisal, respectively.

Result:

Table 1: Qualitative determination of arsenic in liver samples by Reinsch test

Sites	Chickens			Ducks		
	No. of samples	Positive samples	%	No. of samples	Positive samples	%
Chandpur	50	13	26%	50	8	16%
Mymensingh	50	11	22%	50	6	12%
Rajshahi	50	7	14%	50	5	10%
Khulna	50	6	12%	50	5	10%
Barisal	50	5	10%	50	6	12%

Semiquantitative determination of arsenic in tissue samples of chickens by Arsen Test kit

The results of the semiquantitative determination of arsenic in chickens by Arsen Test kit are presented in Table 2. The concentration of arsenic was highest in liver in all five districts followed by kidney, small intestine and thigh muscle. Concentration of arsenic in liver, kidney, thigh muscle and small intestine was highest in Chandpur district. Arsenic was not found in heart, femur and brain tissues and faeces of tested chickens by Arsen test kit .

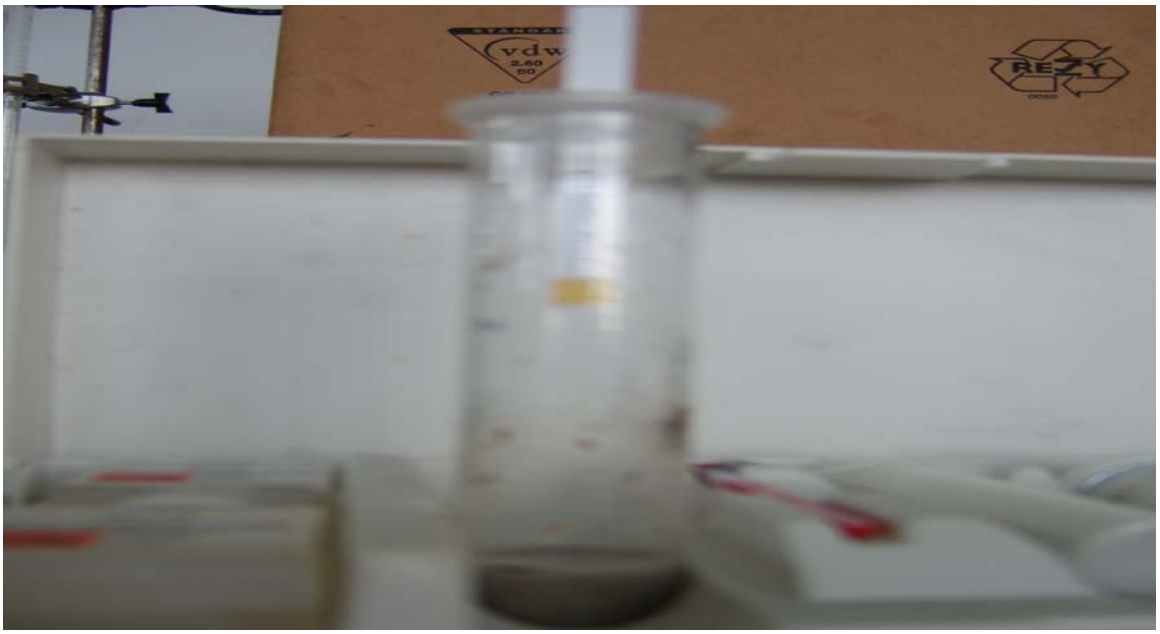
Table 2: Determination of arsenic in tissue samples (mg/kg WW) of chickens by Arsen Test kit (Semiquantitative)

Sites	Liver	Kidney	Thigh muscle	Small intestine	Heart	Femur	Brain	Faeces
Chandpur	0.122 ± 0.04	0.106 ± 0.03	0.058 ± 0.02	0.074 ± 0.02	ND	ND	ND	ND
Mymensingh	0.104 ± 0.03	0.093 ± 0.04	0.054 ± 0.01	0.068 ± 0.02	ND	ND	ND	ND
Rajshahi	0.111 ± 0.04	0.094 ± 0.02	0.053 ± 0.01	0.066 ± 0.02	ND	ND	ND	ND
Khulna	0.105 ± 0.02	0.085 ± 0.02	0.053 ± 0.01	0.067 ± 0.01	ND	ND	ND	ND
Barisal	0.114 ± 0.04	0.096 ± 0.03	0.054 ± 0.01	0.068 ± 0.02	ND	ND	ND	ND

Values indicate the mean ± SD
ND = Non- Detectable

Determination of arsenic in tissue samples of ducks by Arsen Test kit (Semiquantitative method)

The results of the semiquantitative determination of arsenic in ducks by Arsen Test kit are presented in Table 3. The concentration of arsenic was highest in liver in all five districts followed by kidney, small intestine and thigh muscle. Concentration of arsenic in liver, kidney, thigh muscle and small intestine was highest in Chandpur district. Arsenic was not found in heart, femur and brain tissues and faeces of tested ducks by Arsen test kit.



Photograph 1: Arsen kit test in positive sample

Table 3: Determination of arsenic in tissue samples (mg/kg WW) of ducks by Arsen Test kit (Semiquantitative)

Sites	Liver	Kidney	Thigh muscle	Small intestine	Heart	Femur	Brain	Faeces
Chandpur	0.093 ± 0.03	0.09 ± 0.03	0.051 ± 0.01	0.068 ± 0.02	ND	ND	ND	ND
Mymensingh	0.091 ± 0.02	0.086 ± 0.03	0.048 ± 0.01	0.065 ± 0.02	ND	ND	ND	ND
Rajshahi	0.088± 0.03	0.084 ± 0.03	0.046 ± 0.01	0.062 ± 0.01	ND	ND	ND	ND
Khulna	0.09 ± 0.03	0.084 ± 0.03	0.046 ± 0.02	0.064 ± 0.03	ND	ND	ND	ND
Barisal	0.085 ± 0.02	0.078 ± 0.03	0.047 ± 0.01	0.061 ± 0.01	ND	ND	ND	ND

Values indicate the mean ± SD
ND = Non- Detectable

Quantitative determination of arsenic in tissue samples of chickens by Hydride Generation Quartz Furnace Atomic Absorption Spectrophotometer (HGQFAAS)

The results of the quantitative determination of arsenic in tissue samples of chickens by HGQFAAS are presented in Table 4. In quantitative determination arsenic concentration in liver (0.104 ± 0.029 mg/kg WW), kidney (0.098 ± 0.024 mg/kg WW), thigh muscle (0.048 ± 0.021 mg/kg WW), small intestine (0.069 ± 0.022 mg/kg WW), heart (0.022 ± 0.009 mg/kg WW), femur (0.014 ± 0.007 mg/kg WW),

brain (0.0031 ± 0.001 mg/kg WW) and faeces (0.0026 ± 0.001 mg/kg WW) was highest in Chandpur district. However, the lowest concentration of arsenic in liver (0.095 ± 0.020 mg/kg WW), kidney (0.091 ± 0.019 mg/kg WW) and brain (0.0021 ± 0.001 mg/kg WW) were recorded in Rajshahi. Similarly, the lowest concentration of arsenic in thigh muscle (0.042 ± 0.016 mg/kg WW), small intestine (0.058 ± 0.019 mg/kg WW), heart (0.017 ± 0.009 mg/kg WW), femur (0.010 ± 0.006 mg/kg WW) and faeces (0.0016 ± 0.001 mg/kg WW) were found in Barisal.

Table 4: Quantitative determination of arsenic in tissue samples (mg/kg WW) of chickens by HGQFAAS

Sites	Liver	Kidney	Thigh muscle	Small intestine	Heart	Femur	Brain	Faeces
Chandpur	0.104 ± 0.03	0.098 ± 0.02	0.048 ± 0.02	0.069 ± 0.02	0.022 ± 0.01	0.014 ± 0.01	0.003 ± 0.01	0.002 ± 0.01
Mymensingh	0.099 ± 0.02	0.094 ± 0.02	0.047 ± 0.2	0.067 ± 0.01	0.019 ± 0.01	0.012 ± 0.01	0.002 ± 0.01	0.002 ± 0.01
Rajshahi	0.095 ± 0.02	0.091 ± 0.01	0.046 ± 0.02	0.063 ± 0.01	0.018 ± 0.01	0.011 ± 0.01	0.002 ± 0.01	0.002 ± 0.01
Khulna	0.102 ± 0.02	0.095 ± 0.01	0.043 ± 0.01	0.061 ± 0.02	0.018 ± 0.01	0.008 ± 0.01	0.002 ± 0.02	0.001 ± 0.01
Barisal	0.096 ± 0.01	0.092 ± 0.02	0.042 ± 0.01	0.058 ± 0.01	0.017 ± 0.01	0.010 ± 0.01	0.002 ± 0.01	0.001 ± 0.01

Values indicate the mean ± SD

Quantitative determination of arsenic in tissue samples of ducks by HGQFAAS

The results of the quantitative determination of arsenic in tissue samples of ducks by HGQFAAS are presented in Table 5. In quantitative determination arsenic concentration in liver (0.091 ± 0.021 mg/kg WW), kidney (0.089 ± 0.024 mg/kg WW), thigh muscle (0.049 ± 0.018 mg/kg WW), small intestine (0.065 ± 0.016 mg/kg WW), heart (0.021 ± 0.011 mg/kg WW), femur (0.013 ± 0.006 mg/kg WW), brain (0.0028 ± 0.001 mg/kg WW) and faeces (0.0023 ± 0.001 mg/kg WW) was highest in Chundpur district. However, the lowest concentration of arsenic in liver (0.086 ± 0.021 mg/kg WW), kidney (0.077 ± 0.027 mg/kg WW), thigh muscle (0.041 ± 0.012 mg/kg WW), small intestine (0.056 ± 0.017 mg/kg WW) and faeces (0.0015 ± 0.001 mg/kg WW) were recorded in Barisal. Similarly, lowest concentration of arsenic in heart (0.015 ± 0.009 mg/kg WW) and brain (0.0022 ± 0.002 mg/kg WW) were observed in Rajshahi district and femur (0.008 ± 0.002 mg/kg WW) in Khulna district.

Table 5: Quantitative determination of arsenic in tissue (mg/kg WW) samples of ducks by HGQFAAS

Sites	Liver	Kidney	Thigh muscle	Small intestine	Heart	Femur	Brain	Faeces
Chandpur	0.091 ± 0.02	0.089 ± 0.02	0.049 ± 0.02	0.065 ± 0.01	0.021 ± 0.01	0.013 ± 0.01	0.002 ± 0.01	0.002 ± 0.01
Mymensingh	0.088 ± 0.03	0.087 ± 0.02	0.047 ± 0.01	0.063 ± 0.01	0.018 ± 0.01	0.012 ± 0.01	0.002 ± 0.01	0.002 ± 0.01
Rajshahi	0.092 ± 0.02	0.088 ± 0.01	0.042 ± 0.02	0.060 ± 0.02	0.015 ± 0.01	0.011 ± 0.01	0.002 ± 0.02	0.001 ± 0.01
Khulna	0.089 ± 0.03	0.082 ± 0.02	0.044 ± 0.02	0.059 ± 0.02	0.017 ± 0.01	0.008 ± 0.01	0.002 ± 0.01	0.001 ± 0.01
Barisal	0.086 ± 0.02	0.077 ± 0.03	0.041 ± 0.01	0.056 ± 0.01	0.017 ± 0.01	0.012 ± 0.01	0.002 ± 0.01	0.001 ± 0.01

Values indicate the mean ± SD

Quantitative determination of arsenic in tissue samples of ducks by HGQFAAS

The results of the quantitative determination of arsenic in tissue samples of ducks by HGQFAAS are presented in Table 5. In quantitative determination arsenic concentration in liver (0.091 ± 0.021 mg/kg WW), kidney (0.089 ± 0.024 mg/kg WW), thigh muscle (0.049 ± 0.018 mg/kg WW), small intestine (0.065 ± 0.016 mg/kg WW), heart (0.021 ± 0.011 mg/kg WW), femur (0.013 ± 0.006 mg/kg WW), brain (0.0028 ± 0.001 mg/kg WW) and faeces (0.0023 ± 0.001 mg/kg WW) was highest in Chundpur district. However, the lowest concentration of arsenic in liver (0.086 ± 0.021 mg/kg WW), kidney (0.077 ± 0.027 mg/kg WW), thigh muscle (0.041 ± 0.012 mg/kg WW), small intestine (0.056 ± 0.017 mg/kg WW) and faeces (0.0015 ± 0.001 mg/kg WW) were recorded in Barisal. Similarly, lowest concentration of arsenic in heart (0.015 ± 0.009 mg/kg WW) and brain (0.0022 ± 0.002 mg/kg WW) were observed in Rajshahi district and femur (0.008 ± 0.002 mg/kg WW) in Khulna district.

At present, arsenic is found to be distributed in different food chains in Bangladesh including livestock products. In the present study arsenic was detected in different tissues of chickens and ducks. Similar to present findings, as per quantitative and semiquantitative method of arsenic determination, arsenic was detected in different organs of chickens and goat at low concentration in different regions of Bangladesh (20). Similarly arsenic was determined (21) in poultry and fowl species in Pakistan. In accordance to the present findings they also observed highest concentration of arsenic in liver followed by kidney and muscle. Likewise, arsenic was also detected (22) in poultry liver. In their study, they observed that the mean arsenic in liver was below 50 µg/kg body weight in 95% of samples. On the other hand, scientists (23) detected arsenic in gizzard (0.131 ppm), liver (0.091 ppm) and muscle (0.076 ppm) of poultry. In the present study gizzard was not tested for arsenic content. However, scientists (23) found highest concentrations of arsenic in gizzard. It needs further study in Bangladesh context.

Similar to present findings, other than chickens, arsenic was also detected in ducks by several authors (24, 25). Arsenic was also detected in various animals by several workers. Pig liver had the highest average content of arsenic (0.49 ppm) followed by muscle (0.076 ppm), muscle of cattle contained 0.11 ppm and Roe deer muscle contained 0.008 ppm (23). Similarly scientists (26) detected over 2.0 µg/g arsenic in 0.9% of swine liver and 0.3% of swine kidneys indicating very high concentration of arsenic in these two organs.

From the available data it indicates that arsenic is present in most organs of livestock including chickens and ducks in variable concentrations in Bangladesh. In most cases, the concentrations of arsenic in different organs are below the dangerous levels. However, the level of arsenic may exceed the safe level in near future in some or all organs which might be a serious concern for livestock products.

In the present findings arsenic content of different tissues varied between semi quantitative and quantitative method. The quantitative method of arsenic determination is most sensitive and more accurate. As a result minor variations in the amount of arsenic in different organs were observed. However, following quantitative method, it was possible to detect the accurate arsenic content indicating that for accurate determination of arsenic concentration in different samples, quantitative method is the best and most appropriate for all samples.

In the semi quantitative method, arsenic was not detected in four tested samples i.e. heart, femur, brain and faeces. However, in quantitative method arsenic was detected in very low concentration in these four organs (heart, femur, brain and faeces) of all samples tested. It is assumed that in the semi quantitative method, the sensitivity of the strips was not high enough to detect such a low concentration of arsenic in these four samples.

In Bangladesh, incidence and concentrations of occurring arsenic are reported to be increasing day by day (27). Ground water is not only source of all potable waters, but also used for irrigation of crops and drinking to poultry and dairy stock mainly in urban areas of the country (28). In the rural areas, the most important animal exposure to arsenic is through rice-straw and husk which are used as feed for cattle (29). Rice straw and husk contain more amount of arsenic than other vegetables (30). Moreover, dairy milk and beef are also reported to contain arsenic (31). In Nazirpur thana of Pirujpur district of Bangladesh, few cattle died of arsenic poisoning by drinking arsenic contaminated water and milk of affected dairy cows contained arsenic (20). Arsenic in poultry mostly passes through faeces (excreta) and these excreta are used as fish meal and fertilizers. So, in Bangladesh arsenic is a threat not only to human being; but also to livestock and poultry through different food chains which needs an extensive study.

CONCLUSION

The findings of the present research work reveal that arsenic is present in different organs and faeces of chickens and ducks but the level is below the danger limit. Highest concentration of arsenic was observed in liver followed by kidney, small intestine, thigh muscle, heart, femur, brain and faeces. So, in Bangladesh arsenic might be a threat in near future not only to human being also to livestock and poultry through different food chains which needs an extensive study.

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