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Detection of heavy metals in *Chanos chanos* (Bangus) from selected fish ponds of Northern Samar, Philippines

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Abstract
This study was conducted to determine presence of heavy metals (Cd, Cr, As, Pb, Hg) in *Chanos chanos* (Bangus) obtained from selected fish ponds in Northern Samar, Philippines. Environmental parameters of fish pond waters were also determined. Fish pond in Bobon has pH of 8.5, salinity of 390 ppt, turbidity of 1,26m, and temperature of 33, 2°C. San Jose has pH of 8.5, salinity of 380 ppt, turbidity of 2.40m, and 35.1°C and Lavezares has pH of 8.5, salinity of 360 ppt, turbidity of 1,54m, and 33,1°C The heavy metals in samples were analyzed using qualitative and quantitative procedures. Qualitative tests showed that only lead (Pb) metal contamination was detected from bangus meat extract from Bobon and San Jose. Quantitative analysis using Atomic Absorption Spectrophotometer (AAS) revealed very low lead concentration of 0.00003 mg/L in Bobon, 0.00003 mg/L in San Jose and 0.00002 mg/L in Lavezares. The said lead concentration is below the permissible level set by the International Standard Maximum Permissible Level (MPL). The researcher recommends that every municipality must perform educational campaigns on local residents on proper waste disposal to avoid further contamination of marine products.

Keywords: heavy metals, bangus, detection

1. Introduction
Heavy metals in living organisms and bio magnifications describe process and pathways of pollutants from one trophic level to another. Various species of fish are mostly used as bio-indicators of heavy metals contamination. Fishes have been recognized as a good accumulator of organic and inorganic pollutants. Pollution of the aquatic environment by inorganic and organic chemicals has been considered a major threat to the aquatic organism including fishes. The agricultural drainage water containing pesticides and fertilizers and effluents of industrial activities and runoffs in addition to sewage effluents supply the water bodies and sediment with huge quantities of inorganic anions and heavy metals.

The presence of toxic heavy metals in fish can invalidate their beneficial effects. Several unfavorable effects of heavy metal to human health have been known for a long time. This includes serious threats like renal failure, liver damage, cardiovascular diseases, and even death.

2. Materials and Methods
2.1 Determination of Environmental Parameters
The following environmental parameters were determined in three trials: pH, salinity, turbidity and fish pond water temperature. Salinity determination was done using refractometer, pH using pH meter, temperature using digital thermometer and turbidity using Secchi disk.

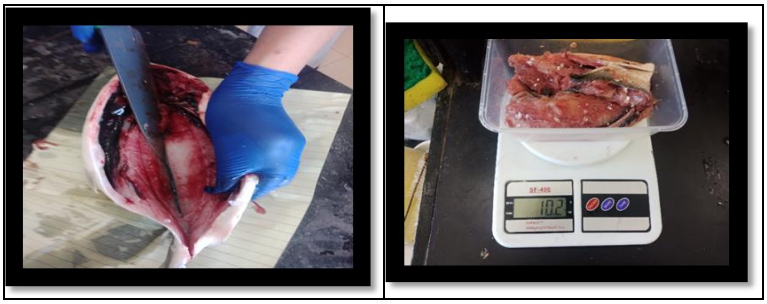

Fig 1: Bangus sample

2.2 Preparation of Bangus Sample

The samples were collected from selected fish ponds of San Jose, Bobon, and Lavezares, Northern Samar. A total of 12 bangus samples were used for the conduct of the study.

After gathering the samples from sampling sites, Bangus samples were placed in cooler and brought to the College of Science Laboratory for digestion. Bangus samples were taken from abdominal part of the fish. All bangus meat samples were cleaned, weighed and turned into solution by grinding into pieces with water. Fish meat solutions were placed in a labeled bottle for further analysis, Detection of heavy metals was immediately conducted.

2.3 Qualitative Detection of Cd, Cr, As, Pb, Hg

Qualitative Detection of Heavy Metals procedure by Yoger, C. (2010) [7], was used in detecting the presence of heavy metals.

Cadmium

Cadmium forms a yellow precipitate with sulfide ion either from a neutral solution containing free Cd^{2+} or from an ammoniacal solution of $[Cd\ (NH_3)_4]^{2+}$. Since most sulfides are insoluble in water, and many of them are black, the presence of other metal ions may make it difficult to detect the yellow color of CdS. Therefore, separations must be complete as possible before testing for Cd^{2+}

From the prepared fish samples solution, the detection of Cadmium followed the following procedures: To the prepared fish sample 0.1 M Na_2S Solution was added, dropwise. The formation of a yellow precipitate confirms the presence of Cadmium ions.

Chromium (Cr)

Chromium can be taken through a series of colored tests which leaves no doubt as to its identity. Chromium (III) form a steel green hydroxide which dissolves in excess strong base to give a deeply green colored solution of the hydroxyl complex. Treating this complex with 3% hydrogen peroxide gives the yellow solution of the chromate ion, which upon acidification with dilute nitric acid gives the orange color of dichromate. Treatment of the cold solution of the dichromate with 3% hydrogen peroxide gives the intense blue color of a peroxide of chromium.(the actual composition of this peroxide readily decomposes to the pale violet color of the original hydrated chromium (III) ion. in low concentration of dichromate the blue color is fleeting and attention must be focused on the test tube during the addition of the hydrogen peroxide to missing the color change. the following color changes are all indicative of Cd^{3+}. An excess of 6 M NaOH was added about one mL of the fish sample solution. To this solution 10 drops of 3% H_2O_2 was added. Then the test tube was heated in the bath until the excess H_2O_2 was destroyed as indicated by cessation of bubbles. The yellow solution was acidified with 3 M HNO_3. The resulting orange was cooled in an ice bath. Finally, to the cooled solution a drop or two of 3%H_20_2 was added and the immediate fleeting blue/steel green color was observed. This fleeting blue/steel green color indicates the presence of chromium ion.

Arsenic (As)

To the prepared clam samples solution, 3F HCl was added until it is barely acid. It was centrifuged for 3 minutes, and then the centrifugate was discarded. Then, on the residue, 10 drops of concentrated HCl was added. The solution was then stirred and heated in a hot water bath for 1 minute and then the centrifugate was removed.

The residue was washed with a mixture of 8 drops of water and 4 drops of concentrated HCl. It was then centrifuged and added to the centrifugate of the concentrated HCl treatment. The residue and the centrifugate were separated.

The residue was washed with hot water for three times, then 4 drops of concentrated HNO_3 was added, and heated for 5 minutes in water bath.

After heating, 5 drops of 0.5F $AgNO_3$ was added and then stirred. To clear the centrifugation, 15 drops of 2.5F NaAc solution was added. The formation of a reddish-brown precipitate indicates the presence of arsenic.

Lead (Pb)

Although $PbCl_2$ is insoluble at room temperature, its solubility is increased dramatically at higher temperatures; it dissolves readily in boiling water, Pb^{2+} also forms an insoluble white sulfate, which dissolves in a solution containing acetate ion due to the formation of the weak electrolyte, $Pb(CH_3COO)_2$. The addition of chromate ion to this lead acetate solution yields a precipitate of yellow lead chromate.

To the clams solution 3M HCl was added drop wise. (A large excess of HCl must be avoided because of the formation of the soluble chloro complex, $PbCl_4^{2-}$.) Centrifuged and the supernatant liquid from the white precipitate were removed.

Hot water was added to the precipitate and then stirred. If the precipitate dissolves, Pb^{2+} is indicated. To the hot solution 3M H_2SO_4 was added. Centrifuged and the supernatant liquid from the white precipitate ($PbSO_4$) were removed.

To the precipitate, 3M NH_4CH_3COO was added and then stirred. A few drops of 0.5M K_2CrO_4 were added to the solution. A yellow precipitate of $PbCrO_4$ indicates the presence of Pb^{2+}.

Mercury (Hg)

When Hg_2Cl_2 is treated with aqueous NH_3 a reaction occurs in which free mercury and amino chloro mercury (II) are formed.

About 2 mL of the prepared clam samples solution was used in the detection of mercury ions in the clam samples.

To the prepared clam samples solution 3M HCl was added. If a white precipitate forms, it was centrifuged and the supernatant liquid removed. Then, to the precipitate, 6M NH_3 was added and stirred. The appearance of gray to black precipitate is positive to mercury ion.

2.4 Quantitative Analysis of Heavy Metals

This study used the methods of Pak, J. (2011) to detect the heavy metals of fish using Atomic Absorption Spectroscopy (A.A.S). Samples found positive for heavy metals in the qualitative analysis were forwarded to the University of Santo Tomas (UST) Chemistry Laboratory.

3. Results & Discussion

This study determined the environmental parameters of fish pond waters in terms of pH, salinity, turbidity and temperature. Table 1 shows the average results of the different analysis done on the three sampling sites.

Table 1: Environmental Parameters of Fish Pond Waters

Sampling Sites	pH	Salinity (ppt)	Turbidity (m)	Temperature (°C)
Bobon	8.5	390	1.26	33.2
San Jose	8.5	380	2.40	35.1
Lavezares	8.5	360	1.54	33.1

The three sampling sites have almost similar properties except on turbidity value of San Jose fish pond. Such result indicates higher amount of suspended particulates in the water as compared to other sampling sites. The researchers however were not able to obtain from Department of Environmental and Natural Resources (DENR) standard values for the environmental parameters tested in this study. It is likely that standard values were not yet set for these parameters on fish pond waters.

Bangus meat samples from the three sampling sites were tested for heavy metals using the procedure from Yoger, C. (2010) [7]. The formation of a yellow precipitate confirms the presence of Cadmium ions. Presence of mercury is indicated by the formation of a black or grey precipitate. Fleeting blue/steel green color indicates the presence of chromium ion. Arsenic is detected by the formation of reddish-brown precipitate and lead showed a yellow precipitate. Results of qualitative tests are shown in Table 2.

Table 2: Presence of Heavy Metals in Samples Using Qualitative Analysis

Sampling Sites	As	Cd	Cr	Hg	Pb
Bobon	Negative	Negative	Negative	Negative	Positive
San Jose	Negative	Negative	Negative	Negative	Positive
Lavezares	Negative	Negative	Negative	Negative	Negative

Table 2 shows the qualitative analysis of heavy metal contamination (As, Cd, Cr, Hg and Pb) in three different sampling areas. Only lead (Pb) was detected in Bobon and San Jose sampling sites.

The quantitative analysis focused only on quantity of Pb in bangus fish samples. Samples were forwarded to the University of Santo Tomas (UST) Laboratory for Atomic Absorption Spectroscopy (AAS). Result shows the following lead (Pb) content in bangus fish samples: Bobon has 0.00003 mg/L, San Jose has 0.00003 mg/L and Lavezares has 0.00002 mg/L. Such values are below permissible level set by the International Standard Maximum Permissible Level (MPL).

4. Conclusion

From the data gathered throughout the conduct of this study, the following conclusions were formulated by the researchers:

- The different sampling sites have almost similar pH, salinity and water temperatures. San Jose fish pond contains higher amount of suspended materials as compared to other sampling sites.
- Only lead is present in Bobon and San Jose bangus samples as detected by qualitative analysis.
- Quantitative analysis of the amount of Pb contamination showed very low amounts of Pb in bangus samples.
- Bangus from the three sampling sites are safe for human consumption in terms of heavy metal contamination.

5. Acknowledgments

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