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Proficiency Testing Scheme for Toxic Elements in Milkfish (*Chanos chanos*) in the Philippines: Ensuring Food Safety and Quality Control

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Abstract **– Milkfish (*Chanos chanos*) is a staple aquaculture product in the Philippines, making food safety and quality assurance essential for consumer protection. This study presents an accuracy-based proficiency testing (PT) scheme to assess the capability of Philippine chemical testing laboratories to quantify toxic elements in milkfish. Philippine Reference Material (PRM) 2002 – As, Cd, Hg & Pb in Milkfish was prepared and distributed to participating laboratories for independent analysis. Results were evaluated using *z'-scores* and other statistical performance metrics.**

A total of 26 local testing laboratories participated in the PT scheme, with 23 submitting results. Among them, 42 % of the 12 participants for total As, 62 % of the 21 participants for Cd, 64 % of the 14 participants for total Hg, and 82 % of the 22 participants for Pb achieved an "acceptable" *z'-score* performance. These findings highlight areas for improvement in laboratory proficiency, underscoring the importance of continuous development and active participation in accuracy-based proficiency testing (PT) schemes to enhance the reliability and accuracy of toxic element analysis in Philippine laboratories.

I. INTRODUCTION

Toxic elements such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) are persistent and hazardous environmental contaminants that can accumulate in aquatic organisms, including fish consumed by humans [1]. In the Philippines, milkfish (locally known as "bangus") is a widely consumed aquaculture species and a vital component of national food security and rural livelihoods [2]. However, contamination of aquatic environments due to industrial discharge, agricultural runoff, and domestic waste has raised concerns about the presence of toxic elements in farmed fish [1].

To ensure food safety and protect public health, it is essential to monitor these toxic elements and assess their concentrations against established food safety standards. The Bureau of Agriculture and Fisheries Standards (BAFS) of the Philippines established the Philippine National Standard (PNS) for milkfish, which specifies maximum allowable limits (MALs) for toxic metals. According to this standard, the MALs for Cd, Hg, and Pb in milkfish are 0.5 mg/kg, 0.5 mg/kg, and 0.3 mg/kg, respectively [3]. In addition, the Food Standards Australia New Zealand (FSANZ) has set a limit of 2 mg/kg for arsenic in fish and crustacean products.

Accurate and reliable measurement of toxic elements is critical and requires competent analytical laboratories equipped with validated methods, robust quality assurance systems, and traceability of measurement results to the International System of Units (SI). Ensuring metrological traceability enhances confidence in test results, supports comparability across laboratories, and strengthens the integrity of food safety monitoring programs [4].

Recognizing this need, the National Metrology Laboratory (NML) of the Philippines organized an accuracy-based proficiency testing (PT) scheme for toxic elements in milkfish, in accordance with ISO/IEC 17043, the international standard for the competence of PT providers [5]. This initiative aimed to evaluate and strengthen the analytical capabilities of local testing laboratories, promote result comparability, and support regulatory compliance. Participation in such PT schemes enables laboratories to assess their performance, identify areas for improvement, demonstrate technical competence, and establish traceability of their measurement results to the SI [6]

Accuracy-based PT schemes offer the added advantage of using reference values traceable to SI units or established using higher-order methods, allowing participating laboratories to directly evaluate the trueness of their results. This promotes measurement reliability, harmonization of data across testing facilities, and informed decision-making in food safety regulation and public health protection [6].

II. MATERIALS AND METHODS

A. Organization of the proficiency testing scheme

Local laboratories across the country were invited to participate in the PT scheme at no cost. Each participant was provided with one (1) bottle of the PT item in a tightly sealed corrugated box containing a temperature-monitoring strip and was dispatched by a local courier. Upon receipt, participants were asked to store the PT item at -20 °C. Participants were asked to use the analytical method of their choice. PT item is recommended to be equilibrated to room temperature and homogenize before using for analysis. Participants were requested to perform at least three (3) independent measurements and report the mean value of replicate measurements on an as-received basis at mg/kg unit with associated expanded uncertainty with a coverage factor (k) of 2 (approximately 95 % confidence level). Participants were given 35 working days to perform the analysis.

B. Preparation of proficiency test material

As described in detail in a separate publication [2], the proficiency test material, designated as Philippine Reference Material (PRM) 2002 – As, Cd, Hg & Pb in Milkfish, was prepared by lyophilizing deboned milkfish meat, followed by defatting with petroleum ether, air-drying, and milling to a particle size of < 250 µm. A portion of the lyophilized material was spiked with known concentrations of toxic elements, freeze-dried, and blended with the unspiked bulk to ensure homogeneity. Approximately 10 g of the final mixture was transferred into pre-cleaned, sterilized amber glass bottles, which were then sealed, labelled, and stored at –80 °C.

C. Homogeneity and stability testing of PT material

Homogeneity testing was performed on eleven (11) bottles selected via stratified random sampling. Each bottle was analyzed in triplicate using validated methods: hydride vapor generation–atomic absorption spectrophotometry (HVG-AAS) for total arsenic, direct mercury analyzer (DMA) for total mercury, inductively coupled plasma–optical emission spectrometry (ICP-OES) for cadmium, and graphite furnace–atomic absorption spectrophotometry (GF-AAS) for lead. Gravimetric techniques were employed during sample preparation and processing. Data were statistically evaluated in 2023 following ISO Guide 35:2017 [7]. The PT material met the following criteria for all test parameters: no significant bottling trend as determined by regression analysis, absence of outliers based on Cochran's test, and acceptable homogeneity confirmed by one-way analysis of variance (ANOVA).

Short-term stability testing was conducted to assess suitable transportation duration and environmental conditions [7]. An isochronous study design was employed using two test temperatures (4 °C and 50 °C) and a control temperature (-80 °C). Regression analysis indicated no significant change in the mass fractions of all target analytes at both test temperatures over 3 weeks.

Long-term stability testing was carried out to evaluate analyte stability during storage. The study followed the isochronous approach, with randomly selected bottles stored at -20 °C and analyzed at defined intervals. Regression analysis showed no significant change in the mass fractions of all target analytes in the PT item for up to six (6) months of storage at -20 °C.

D. Assignment of reference values

Characterization of the reference material was performed by the National Institute of Metrology, Thailand (NIMT) using isotope dilution-inductively coupled plasma mass spectrometry (ID-ICP-MS) for Cd, total Hg, and Pb, while the gravimetric standard addition ICP-MS method was used for total As. The analytical instruments were calibrated by certified reference materials from the National Institute of Standards and Technology (NIST).

The assigned value (*X*) or the PT reference value (PTRV) and associated uncertainties were determined by NML (Table 1). The standard uncertainty of the assigned value (u_{X}) was estimated following ISO Guide 35:2017 [7] by combining the uncertainties from characterization (u_{char}), homogeneity (u_{bb}), short-term stability (u_{sts}), and long-term stability (u_{lts}).

$$u_{\mathrm{X}} = \sqrt{u_{\mathrm{char}}^2 + u_{\mathrm{bb}}^2 + u_{\mathrm{sts}}^2 + u_{\mathrm{lts}}^2} \qquad (1)$$

The expanded uncertainties (U_{X}) stated are obtained by multiplying each analyte's standard uncertainty by the corresponding coverage factor k = 2. The uncertainties were under the "JCGM 100:2008 Evaluation of measurement data - Guide to the expression of uncertainty in measurement". The value of the measurand lies within the assigned range of values with a probability of approximately 95 %.

The SDPAs for this PT scheme were derived using the Horwitz function. This general model demonstrates the mathematical relationship between predicted reproducibility standard deviation and the assigned value of an analyte expressed in unitless value.

$$\sigma_{pt} = 0.02 \cdot (X)^{0.8495} \tag{2}$$

Table 1. Assigned value and associated uncertainties of PRM 2002

Analyte	Assigned value, X (mg/kg)	U_X (mg/kg)	σ_{pt}
Total Arsenic	2.36	0.29	0.33
Cadmium	0.55	0.06	0.10
Total Mercury	0.49	0.07	0.09
Lead	3.03	0.35	0.41

III. RESULTS AND DISCUSSION

A. Scores and Evaluation Criteria

The participants' results were assessed through the z'-*score*. This is applied when the standard uncertainty of the assigned value is greater than $0.3\sigma_{pt}$ [8]. The z'-*score* was calculated using the formula:

$$z' = \frac{x - X}{\sqrt{\sigma_{pt}^2 + u_X^2}} \tag{3}$$

where: x = participant's result
X = assigned value
σ_{pt} = standard deviation for proficiency assessment
u_X = standard deviation of the assigned value

Interpretation of z'-*score* is as follows:

$|z'| \leq 2.0$ indicates "acceptable" performance
$2.0 < |z'| < 3.0$ indicates "warning signal" performance
$|z'| \geq 3.0$ indicates "unacceptable" performance

B. PT Participants' results

The number and percentage of z'-*scores* in the acceptable range, warning signal range and unacceptable range are presented in Table 2. Twelve (12) participants submitted results for the determination of arsenic mass fraction. Forty-two percent (42 %) of the participants achieved results within the acceptable range, 17 % received a warning signal, and 42 % fell within the unacceptable range. A graphical representation of the participants' z'-*scores* is shown in Fig. 1.

Most of the participants employed HVG-AAS (50 %) and ICP-OES (25 %) for the determination of total arsenic. Visual inspection showed wide variation (55 % RSD) in the reported results within the same instrument and among all the instruments used, as shown in Fig. 2. The plot also showed that participants' results were underestimated since the robust mean of participants' results for total arsenic is 1.48 mg/kg, which is below the assigned value.

Table 2. Summary of participants' performance score per analyte

Analyte	Number of participants (percentage) Acceptable	Warning signal	Unacceptable	Total
Total Arsenic	5 (42 %)	2 (17 %)	5 (42 %)	12 (100 %)
Cadmium	13(62 %)	5 (24 %)	3 (14 %)	21 (100 %)
Total Mercury	9 (64 %)	3 (21 %)	2 (14 %)	14 (100 %)
Lead	18 (82 %)	0 (0 %)	4 (18 %)	22 (100 %)

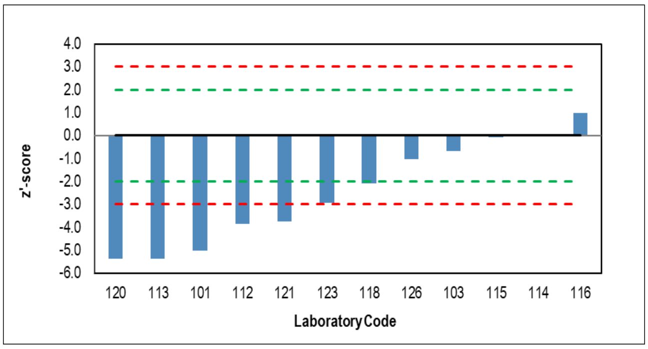

Fig. 1. Participants z'-scores for total arsenic

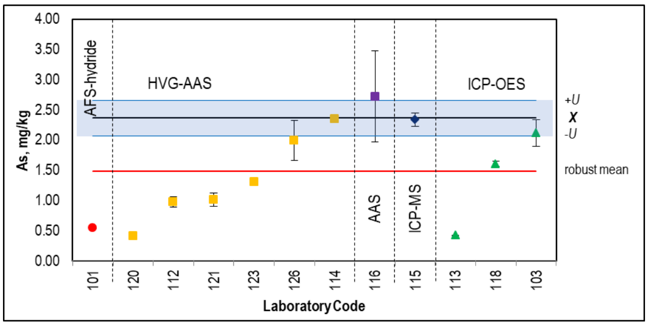

Fig. 2. Participants' results grouped by instrument used for total arsenic determination (X = assigned value; U = expanded uncertainty of the assigned value)

For cadmium, twenty-one (21) participants submitted results for the determination of the mass fraction of cadmium. Sixty-two percent (62 %) of the participants are within the acceptable range, 24 % obtained a warning signal, and 14 % fall within the unacceptable range. A graphical plot of participants' z'-*scores* for cadmium is presented in

Fig. 3. In terms of methodology, 62 % of the participants employed AAS (AAS, Flame-AAS and GF-AAS) (Fig. 4). For this PT scheme, Flame-AAS results show better performance as compared to GF-AAS. Similar to arsenic, there is a high variation of results (101 % RSD),

especially for AAS and ICP-OES. The robust mean of participants' results for cadmium is 0.48 mg/kg, which still falls outside the range of the assigned value. However, it is much closer compared to the arsenic results.

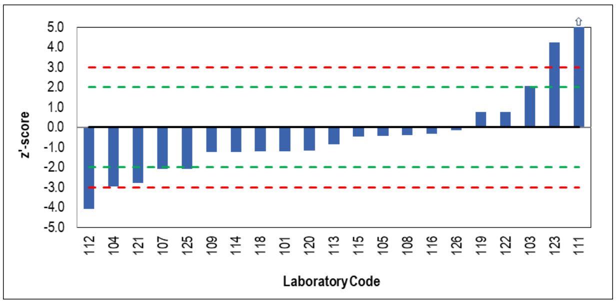

Fig. 3. Participants z'-scores for cadmium

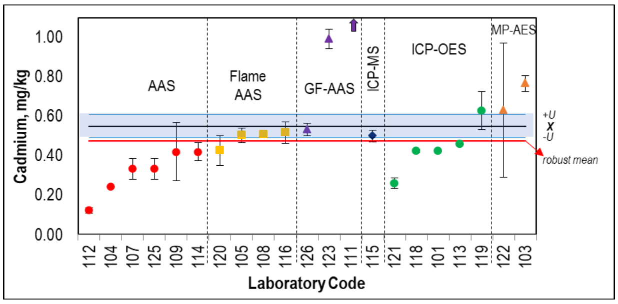

Fig. 4. Participants' results grouped by the instrument used for cadmium

Fourteen (14) participants submitted results for the determination of total mercury. Sixty-four per cent (64 %) are within the acceptable range, 21 % obtained a warning signal, and 14 % fall within the unacceptable range. A graphical plot of the participants' *z'-scores* is presented in Fig. 5. Five (5) out of fourteen (14) participants employed cold vapor CV-AAS for the determination of total mercury (Fig. 6). Similar to arsenic and cadmium, there is a high variation of results (41 % RSD) among the analytical instruments used. Furthermore, a similar trend in the variability of results was also observed, specifically for AAS. The robust mean of participants' results for total mercury is 0.42 mg/kg, which is close to the assigned value.

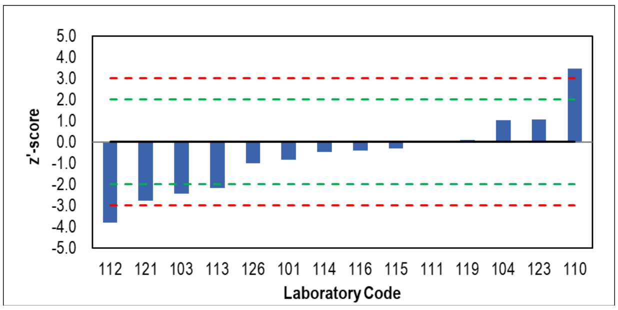

Fig. 5. Participants' z'-scores for total mercury

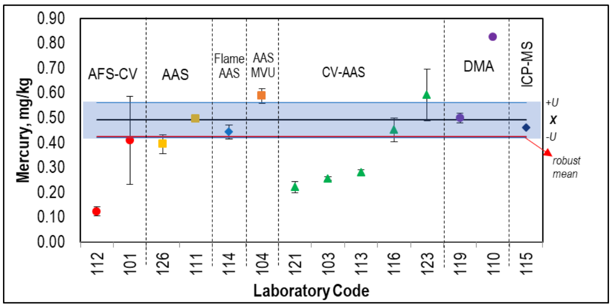

Fig. 6. Participants' results grouped by the instrument used for total mercury

In the determination of Pb, 22 participants submitted results. Eighty-two per cent (82 %) of the participants are within the acceptable range, and 18 % fall within the unacceptable range. None of the participants obtained a warning signal. A graphical plot of the participants' *z'-scores* is presented in Fig. 7. Thirty-two (32 %) of the participants employed ICP-OES for the determination of Pb, all of which obtained acceptable performance. ICP-OES performed well in Pb determination compared to other instruments (Fig. 8). A total of six (6) analytical instruments were used, and the reported values varied compared to the assigned value. The robust mean of participants' results for lead is 2.89 mg/kg, which is comparable to the assigned value.

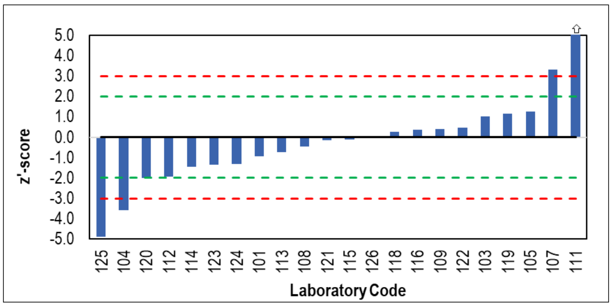

Fig. 7. Participants' z'-scores for lead

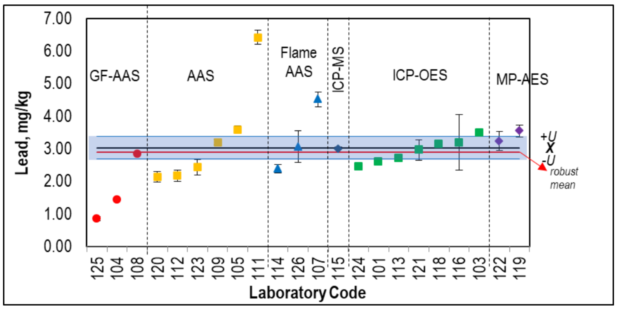

Fig. 8. Participants' results grouped by instrument used for lead determination

In this PT scheme, participants' results were compared to a metrologically traceable assigned value derived from a high-order method. This allowed laboratories to demonstrate and strengthen the traceability of their measurements to the International System of Units (SI). Participation also enabled comparison of technical competence and access to recommendations and shared information that could improve measurement methods. Continued and increased participation in such accuracy-based PT schemes can enhance the overall capability of chemical testing laboratories in the country.

Participants in total arsenic determination with warning signals or unacceptable *z'-scores* are encouraged to review their sample digestion process, which is critical to the analysis. A pre-reduction step, converting arsenic (V) to arsenic (III) using reagents like potassium iodide, is recommended. If this step is already part of the method, optimizing the reaction time should be considered, and effectiveness can be verified using certified reference materials (CRMs) or recovery checks through spiking. Consistent use of quality control and quality assurance measures is also advised to ensure accuracy and reliability.

For cadmium, total mercury, and lead determinations, most participants achieved acceptable *z'-scores* using various sample preparation techniques and instruments. Those who received warning signals or unacceptable results despite using similar methods should reassess their processes for possible sources of sample loss or contamination during analysis.

IV. CONCLUSION

The accuracy-based PT scheme organized by NML successfully assessed the capability of local laboratories in the Philippines in determining toxic elements in milkfish using metrologically traceable assigned values. Of the 26 registered laboratories, 23 submitted results within the deadline. Assigned values were established using high-accuracy methods: ID-ICP-MS for cadmium, mercury, and lead, and gravimetric standard addition ICP-MS for arsenic. Most participants demonstrated satisfactory performance for cadmium, total mercury, and lead. However, the overall performance for total arsenic determination indicated a need for methodological improvement, particularly in sample preparation. These results underscore the importance of continued participation in PT schemes to strengthen analytical competence and traceability in chemical measurements.

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V. CITATIONS AND REFERENCES

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