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Near Real Time Biomonitoring of Mercury Copper and Silver in Port Dickson Coastal Water Using a Ficin Inhibition Assay

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Abstract

Frequent coastal-water surveillance is often constrained by the cost, transport requirements, and turnaround time of element-specific laboratory analysis. This study evaluated a portable ficin-inhibition assay as an effect-based screening tool for near-real-time monitoring of coastal water at Port Dickson, Negeri Sembilan, Malaysia. Ficin-catalysed casein hydrolysis was quantified indirectly using the Bradford dye-binding response. Surface water was collected hourly from 10:00 to 16:00 during a single field campaign and analysed at 30 ± 1 °C with a portable spectrophotometer. Mercury (Hg) was subsequently determined by flow-injection mercury analysis, while copper (Cu) and silver (Ag) were measured by inductively coupled plasma optical emission spectrometry. Mean ficin inhibition remained below 10% at every sampling time and did not reach the prespecified 20% positive-screening threshold. Instrumental metal concentrations varied during the six-hour period, but these changes were not proportional to the enzyme response, consistent with differences between total measured concentrations and the bioavailable fraction interacting with ficin. The assay produced results within approximately 1 h using small sample volumes and simple field equipment. It should, however, be used as a first-tier screening method rather than a substitute for element-specific analysis because salinity, pH, colour, turbidity, metal speciation, and mixture effects may alter enzyme activity. Broader validation across seasons, tidal conditions, sites, and matrix-matched fortified samples is required before routine or regulatory application.

INTRODUCTION

Coastal water quality can change within hours as tidal exchange, storm runoff, boating, shoreline development, wastewater discharge, and sediment resuspension generate short-lived contaminant pulses. Intermittent grab sampling may therefore overlook exposures that occur between scheduled surveys. This concern is relevant to Port Dickson, a heavily used coastal corridor on the Strait of Malacca where recreation, urban development, industrial activity, and riverine inputs converge. Metals warrant attention because they persist, partition among dissolved, colloidal, and particulate phases, and may accumulate in sediments and organisms even when water-column concentrations are modest [1–6]. Mercury and silver can be toxic at low bioavailable concentrations, whereas copper changes from an essential micronutrient to a toxicant as exposure increases [7–10]. Element-specific methods, including cold-vapour mercury analysis and inductively coupled plasma optical emission

spectrometry (ICP-OES), remain indispensable for identifying and quantifying individual metals. Nevertheless, total concentration does not directly measure the biological effect of the fraction accessible to an organism or enzyme. Metal speciation, dissolved-organic-matter complexation, competition from major ions, pH, hardness, and salinity can substantially modify toxicity without an equivalent change in total concentration [2–6]. Effect-based assays address a complementary question: whether a sample, under defined test conditions, disrupts a biological process. As first-tier screens, they can support higher sampling frequencies and direct confirmatory analysis towards samples showing an adverse response [11–18].

Enzyme-inhibition assays are attractive for field screening because they require small reaction volumes, provide rapid responses, and can be measured with portable visible-range instruments. Their principal limitation is low chemical

selectivity. Reduced catalytic activity demonstrates perturbation of the enzyme system but does not identify the responsible compound. Matrix constituents and mixtures may either suppress or enhance inhibition, while assay pH, temperature, substrate concentration, exposure duration, and metal speciation influence the measured response [19–25]. Reliable field use consequently requires an uninhibited enzyme control, a positive inhibition control, consistent reaction timing, technical replication, and confirmatory chemical measurements.

Ficin (EC 3.4.22.3) is a cysteine endopeptidase obtained from fig latex. Like papain and bromelain, it depends on an intact catalytic thiol environment that can be disrupted by thiol-reactive metal ions [26–30]. In the present assay, ficin hydrolyses casein and the residual intact protein is quantified using Bradford reagent. Inhibition preserves more casein and consequently increases the dye-binding response relative to the uninhibited control. Previous optimization demonstrated responses to Hg, Ag, and Cu under controlled conditions and established a miniaturized format suitable for field deployment [26,29,30]. The combination of a plant-derived enzyme, inexpensive reagents, and portable absorbance measurement could therefore enable denser temporal surveillance than laboratory analysis alone.

This study assessed whether the optimized ficin assay could be deployed during a six-hour coastal monitoring exercise at Port Dickson and whether its response was consistent with laboratory measurements of Hg, Cu, and Ag. A positive screening result was defined *a priori* as at least 20% inhibition. The work was conceived as a proof-of-application study: it evaluated field practicality and short-term response patterns rather than regulatory compliance, long-term trends, or spatial water quality.

MATERIALS AND METHODS

Study site and sample collection

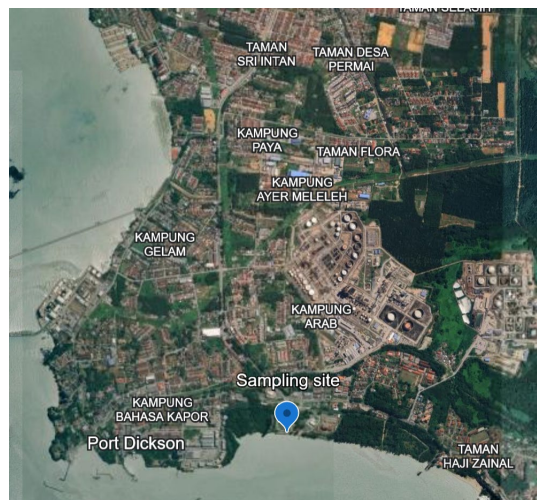
Surface seawater was collected in February 2019 at Port Dickson, Negeri Sembilan, Malaysia (2°31'23.23" N, 101°48'25.42" E; **Fig. 1**). Samples were obtained approximately 50 cm below the surface at hourly intervals from 10:00 to 16:00 during one six-hour campaign. Acid-washed 500 mL high-density polyethylene bottles were used. Temperature, dissolved oxygen, conductivity, pH, and salinity were measured *in situ* with a calibrated multiparameter YSI instrument. Portions intended for the ficin assay were passed through a 0.45 µm polytetrafluoroethylene syringe filter and analysed promptly.

Remaining portions were kept in an insulated ice container, transported to Universiti Putra Malaysia, and submitted immediately for instrumental metal analysis. Total suspended solids were determined gravimetrically by the total-solids-dried method. Tidal state, weather, instrument model, calibration records, holding times, and the total-suspended-solids result were not available for the present analysis.

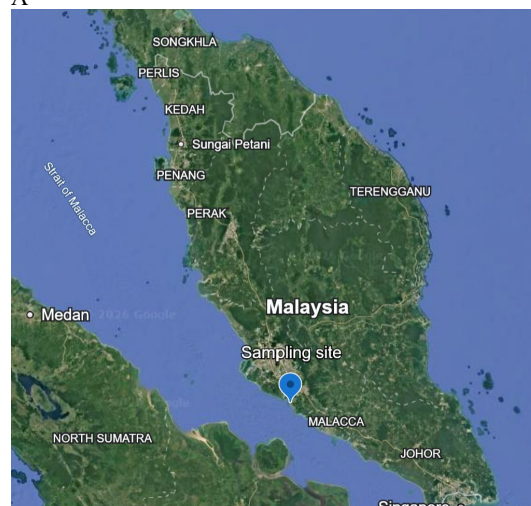
Reagents and preparation of casein and ficin

Analytical-grade reagents and deionized water were used throughout. Casein (Sigma-Aldrich) was prepared at 20 mg mL⁻¹ by dispersing 2.0 g in 100 mL water, adjusting the suspension to pH 8.0 with 5 M NaOH or HCl, and stirring overnight at 60 °C. Insoluble material was removed by filtration through several layers of cheesecloth and centrifugation at 10,000 × g and 4 °C. Protein in the clarified supernatant was quantified by the Bradford method using crystalline bovine serum albumin as the standard. The preparation was stored at 4 °C for immediate use or at -20 °C for longer storage.

Commercial ficin from crude dried fig latex (Sigma-Aldrich; EC 3.4.22.3; product F4165; stated activity, 0.5 U mg⁻¹) was dissolved at 10 mg mL⁻¹ in 20 mM sodium phosphate buffer, pH 6.77, at 4 °C. Fresh ficin (2.0 mg mL⁻¹) and casein (10 mg mL⁻¹) working solutions were prepared on the day of analysis.



A



B

Fig. 1. Coastal sampling location at Port Dickson, Negeri Sembilan, Malaysia: (A) local setting and (B) position on Peninsular Malaysia. Source imagery: Google Earth.

Ficin-inhibition assay

For the positive inhibition control, 50 µL ficin working solution was combined with 50 µL Hg solution to give final reaction concentrations of 0.6 mg mL⁻¹ ficin and 0.040 mg L⁻¹ Hg, as previously described [26]. After incubation for 10 min at 30 °C, 50 µL casein solution was added (final concentration, 2.36 mg mL⁻¹). In the uninhibited control, the Hg solution was replaced with 20 mM sodium phosphate buffer, pH 6.77. Immediately after substrate addition, a 20 µL aliquot was mixed with 200 µL Bradford reagent, held for 5 min at room temperature, and measured at 595 nm. A second 20 µL aliquot was processed identically after a further 30 min of hydrolysis. Sample reactions contained 50 µL filtered field water in place of the Hg exposure solution. All reactions were performed in triplicate.

Field assay operation

The assay temperature was maintained at 30 ± 1 °C in a portable 30 W incubator powered through a 12 V DC-to-220 V AC vehicle inverter. Absorbance was measured using a portable M6+ spectrophotometer (Axiom, Germany). An uninhibited control and Hg-positive control were included to verify enzyme activity and assay responsiveness during the field exercise.

Instrumental metal analysis

Mercury was determined using a PerkinElmer FIMS 400 flow-injection mercury system. Copper and Ag were measured by ICP-OES with a PerkinElmer Optima 8300 instrument (PerkinElmer, Waltham, MA, USA). The available analytical record did not contain sufficient information on preservation or digestion, calibration ranges, blanks, certified reference materials, spike recovery, detection and quantification limits, or whether dissolved or total-recoverable metal was reported. Instrumental concentrations were therefore interpreted descriptively and not as a regulatory-compliance dataset [31–34].

Calculation and statistical analysis

Ficin activity was calculated from the change in Bradford response between the initial and 30 min measurements. Inhibition (%) was calculated as

$$\%Inhibition = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100\%$$

where Abs control is the activity of the buffer control and Abs sample is the activity measured after exposure to field water. A result of at least 20% inhibition was defined a priori as a positive screening response. Values are reported as mean $\pm$ standard deviation for three technical replicates. Because the campaign comprised one site monitored for only six hours, the analysis was descriptive and no inference was made concerning seasonal, tidal, daily, or spatial water quality.

RESULTS

The measured in situ variables are summarized in **Table 1**. The water was mildly alkaline (pH 8.14), with a temperature of 29.7 °C, salinity of 29.6 ppt, and dissolved oxygen concentration of 4.8 mg L⁻¹. These observations provide essential matrix context for interpreting enzyme activity but cannot establish compliance or temporal representativeness from a single sampling campaign.

Table 1. Selected water-quality measurements at the Port Dickson sampling site in situ.

Parameter	Measured value	Unit	Interpretive note
pH	8.14	pH units	Mildly alkaline coastal water
Temperature	29.7	°C	Single field measurement; no ambient baseline comparison
Salinity	29.6	ppt	Brackish-to-marine coastal range
Dissolved oxygen	4.8	mg L ⁻¹	Contextual value from one monitoring campaign

Ficin response and mercury profile

Mean ficin inhibition remained below 10% at every hourly measurement and therefore did not reach the 20% positive-screening threshold (**Fig. 2**). The Hg concentrations plotted on the secondary axis were in the sub-microgram-per-litre range and varied modestly during the monitoring period. Variability among technical replicates was greatest at 10:00 and 13:00, but the corresponding mean responses remained well below the decision threshold. Thus, the field assay remained operational and did not detect substantial inhibition by ficin-reactive constituents during the six-hour observation window.

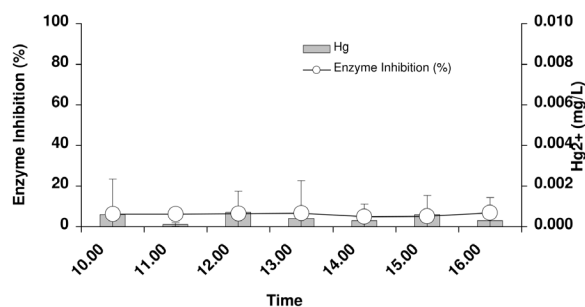


Fig. 2. Hourly ficin inhibition and Hg concentration in Port Dickson coastal water. The open-circle line represents mean enzyme inhibition, grey bars represent instrumental Hg concentration on the secondary axis, and error bars denote standard deviation (n = 3 technical replicates).

Copper and silver profiles

The Cu profile varied more widely than Hg, reaching its highest plotted value at 11:00 before declining later in the day (**Fig. 3**). Silver also varied during the sampling period, with the largest plotted bars at approximately 13:00 and 16:00 (**Fig. 4**). Ficin inhibition remained below 10% and did not change proportionally with either metal series. This divergence is plausible for a nonspecific biochemical endpoint in saline water because total instrumental concentration is not equivalent to the fraction available to interact with the enzyme, and each metal may contribute differently to a mixture response [2–6,9,10].

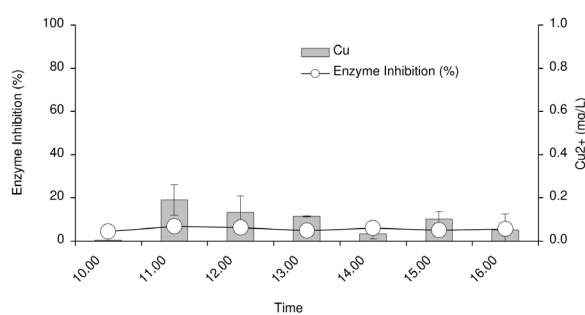


Fig. 3. Hourly ficin inhibition and Cu concentration in Port Dickson coastal water. The open-circle line represents mean enzyme inhibition, grey bars represent instrumental Cu concentration on the secondary axis, and error bars denote standard deviation (n = 3 technical replicates).

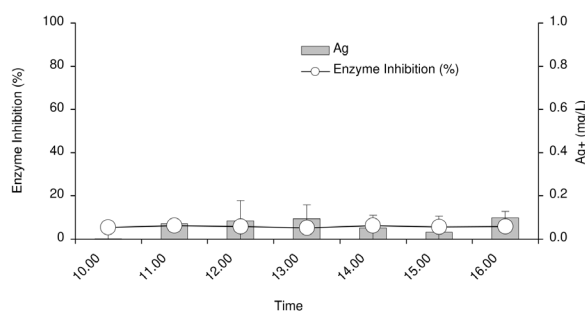


Fig. 4. Hourly ficin inhibition and Ag concentration in Port Dickson coastal water. The open-circle line represents mean enzyme inhibition, grey bars represent instrumental Ag concentration on the secondary axis, and error bars denote standard deviation (n = 3 technical replicates).

DISCUSSION

This study demonstrates the field deployment of a ficin-casein inhibition assay at Port Dickson, but its principal conclusion is operational rather than regulatory. The procedure could be completed near the sampling location using a portable incubator and spectrophotometer, and every mean response remained below the predetermined 20% threshold. A negative screen indicates that the sample did not substantially inhibit ficin under the specified assay conditions. It does not demonstrate the absence of metals or other contaminants, nor does it establish suitability for recreation, fisheries, aquaculture, or consumption. Such decisions require chemical, microbiological, and ecotoxicological criteria appropriate to the intended use.

The low inhibition observed is compatible with the chemistry of coastal water. Chloride, carbonate, dissolved organic matter, and suspended particles can complex metals and reduce the free-ion fraction available to bind an enzyme [2–6]. Conversely, salinity and sample pH may independently alter enzyme kinetics. Cu and Ag varied over the six-hour period without a parallel enzyme response. This mismatch illustrates the complementary roles of the two approaches: FIMS and ICP-OES identify and quantify selected elements, whereas the ficin assay integrates the net perturbation of one catalytic system by the tested sample [11–18].

Ficin is a biologically plausible metal-sensitive recognition element because cysteine proteases depend on a reactive catalytic thiol. Soft metal ions, particularly Hg and Ag, bind strongly to sulfur-containing groups and can disrupt protein conformation or catalytic function [7–10,26–30]. Copper may inhibit through coordination or oxidative mechanisms, although its response depends strongly on buffer composition and competing ligands. The same broad sensitivity that makes the assay useful for preliminary hazard screening prevents attribution of a positive response to one metal without selective masking, fractionation, or instrumental confirmation.

Compared with whole-cell biosensors, purified-enzyme assays avoid culture maintenance and may shorten preparation and response times [11–25]. They are also amenable to immobilization, reagent stabilization, and microfluidic integration. An isolated enzyme, however, lacks the membrane transport, detoxification, repair, and metabolic integration of an intact organism. The ficin endpoint should therefore be interpreted as a defined biochemical response rather than a direct prediction of ecological injury. A test battery combining ficin with a microbial reporter, algal-growth endpoint, or acetylcholinesterase assay would broaden biological coverage [19–25,35–42].

The narrow sampling design is the principal limitation. A single six-hour series cannot characterize tidal, daily, monsoonal, or spatial variation along the Port Dickson coast. Some field metadata and essential analytical quality-control information were unavailable, precluding regulatory comparison. Future validation should use matrix-matched Hg, Cu, and Ag fortification over realistic salinity ranges and determine recovery, repeatability, intermediate precision, selectivity, carryover, detection capability, and reagent stability. Coloured and turbid waters should be tested for optical interference. Blanks and positive controls should accompany every sampling interval, and the 20% threshold should ultimately be evaluated by receiver-operating-characteristic analysis against a sufficiently large collection of chemically characterized samples [31–34,43–50].

A practical monitoring programme could position ficin as a triage assay. Samples below the screening threshold would remain under routine surveillance, whereas positive samples would be re-tested, examined for dilution-related matrix effects, and submitted for confirmatory multielement analysis. Repeated positives could trigger denser sampling near drainage outlets, upstream sources, or across tidal stages. This tiered approach does not replace laboratory chemistry; it uses rapid biological evidence to allocate limited analytical resources more efficiently.

CONCLUSION

The portable ficin-inhibition assay functioned throughout a six-hour monitoring exercise at Port Dickson, and mean inhibition remained below 10% at every sampling time, well under the prespecified 20% positive-screen threshold. Laboratory measurements showed time-varying Hg, Cu, and Ag concentrations, but these profiles were not proportional to enzyme inhibition. The method is therefore best positioned as a rapid, low-cost, effect-based screen that complements rather than replaces FIMS or ICP-OES. Its wider application requires matrix-matched validation, complete analytical quality control, seasonal and tidal replication, broader spatial coverage, and an explicit decision pathway for confirmatory analysis.

DECLARATION OF COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this article.

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AUTHOR CONTRIBUTIONS

G.U. and M.M.: conceptualization and methodology. G.U.: investigation, data curation, formal analysis, and writing—original draft. M.M.: supervision and writing—review and editing. Both authors approved the final manuscript.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

DECLARATION OF GENERATIVE AI USE

Generative artificial-intelligence tools were used for language editing and refinement during manuscript preparation. The authors reviewed and edited the resulting text and accept full responsibility for the accuracy and integrity of the final article.

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