**HEAVY METAL ACCUMULATION IN CULTURED MUD CRABS(*Scylla serrata*) FROM SELECTED MUNICIPALITIES IN NORTHERN SAMAR**

**ABSTRACT**

This study investigated the accumulation of heavy metals (arsenic, cadmium, chromium, lead, and mercury) in mud crabs (*Scylla serrata*) from selected municipalities in Northern Samar, Philippines. The results revealed favorable water parameters, with pH levels ranging from 8.04 to 9.0, salinity levels between 5.7 and 9.0 ppt, and water temperatures averaging between 27.5°C and 29.5°C. The water turbidity levels were also within acceptable ranges, from 0.7 to 4.2 NTU. However, the assessment also revealed concerning levels of heavy metals in the soil and crab samples. The soil substrates were found to contain high concentrations of arsenic, cadmium, chromium, lead, and mercury, with levels generally higher in the soil than in the crab meat. The crab samples also tested positive for these heavy metals, with levels exceeding the maximum standards set by FAO/WHO. The findings suggest that while the water conditions in the fish ponds are suitable for mud crab cultivation, the presence of heavy metals poses significant health risks to consumers and environmental concerns. The study highlights the need for further investigation and mitigation strategies to address the issue of heavy metal contamination in mud crab cultivation in Northern Samar.

**INTRODUCTION**

Mangrove crabs (Scylla spp.) or mud crabs or locally referred to as “alimango,” inhabit muddy and sandy bottom in marine environments. The mud crab is a portunid crab, belonging to the family Portunidae and is a valuable component of coastal fisheries in many Asian countries (SEAFDEC, 2016). Mud crabs are a species of crab found in the estuaries and mangroves where they inhabit brackish water, a mixture of salt and freshwater (Roysfarm, 2023).

Mud crab burrows deep into soft substrates in mangrove swamps shallow waters, and intertidal zones. Mud crabs live in the muddy substrates of river mouths, coasts, and mangrove areas, and they have the potential to be utilized as bio-indicators of pollution, particularly heavy metals. As a result, pollutants can enter the rivers and contaminate species that live in the river's mouth and surrounding areas. Measuring the levels of heavy metals contained in crabs is essential for public health (Soegianto, *et. al.*, 2022).

Heavy metals are metallic chemical elements with a high density that are toxic or poisonous at low concentration. They normally occur in nature and are essential to life but, can become toxic through accumulation in organisms (Koller, *et. al*., 2018). Heavy metal pollution that enters the aquatic environment dissolves in the water and accumulates in sediments, and can increase over time depending on the environmental conditions of the waters. Heavy metals can move from the environment to organisms and from one organism to another through the food chain (Fatryani, *et. al.,* 2022). The bioaccumulation and biomagnification tendencies of toxic heavy metals can cause health issues in living organisms, including humans (William, *et. al.*, 2022).

Heavy metal contamination in the environment becomes concentrated as a consequence of human caused activities. Heavy metal contamination in aquaculture farm sediments may pose a major risk to ecosystem and human health via the food web, resulting from poor handling, incorrect waste management, and excessive use of artificial feed. Mining and industrial waste, lead-acid batteries, vehicle emissions, fertilizers, paints and treated wood are well-known anthropogenic sources of heavy metals. (Hossain, *et. al*., 2023).

Heavy metals in crabs are a significant concern due to their potential toxicity and bioaccumulation in these crustaceans. As benthic organisms, crabs are particularly sensitive to heavy metals, which can accumulate in their organs and tissues. Common heavy metals found in crabs include copper (Cu), which is essential for crab biology but can be toxic at excessive levels, cadmium (Cd), which is toxic even at low concentrations and can cause health issues in humans, lead (Pb), which is neurotoxic and carcinogenic, and can accumulate in crab tissues, zinc (Zn), which is important for crab physiology but can be detrimental at high levels, iron (Fe), which is crucial for crab biology, but can lead to toxicity with excessive accumulation, and chromium (Cr), which can be toxic to crabs and humans, particularly in its hexavalent form (Massou, *et. al.,* 2022).

Essential metals can also produce toxic effects at high concentrations. Only a few metals with proven hazardous nature are to be completely excluded from food for human consumption. Specifically, five metals - arsenic, lead, cadmium, chromium and mercury have been identified as posing significant risks.

Northern Samar is a significant player in the Philippines' crab industry, particularly in the production of mangrove crabs. The province has established itself as a hub for crab farming and aquaculture, with various initiatives and projects aimed at promoting sustainable crab production. Crabs are a vital source of food for many communities worldwide, providing a rich source of protein, nutrients, and income. Crabs are a nutritious and flavorful food source, but it's essential to be aware of potential food safety concerns and cultural significance.

Crab farmers in Northern Samar encounter several problems, including environmental challenges, such as pollution from agricultural runoff, industrial activities, or other sources which can harm crab health.

Since Northern Samar is one of the top crab-producing provinces, we need to determine the presence of heavy metals in crabs to prevent diseases and maintain good quality farming practices.

The municipalities of Biri, Capul, Lapinig and Palapag were selected as sampling sites because they have crab farm owners who produce mud crabs. Sample collection were conducted during the wet season due to environmental factors, including increased rainfall, which lead to more runoff from agricultural and industrial areas, carrying heavy metals into water bodies where crab lives.

Hence, this study aims to determine the accumulation of heavy metals, particularly cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), and chromium (Cr), in the meat of mud crabs.

**METHODOLOGY**

**Locale of the Study**

Crab samples were collected from fish ponds in selected municipalities in Northern Samar, specifically Biri, Capul, Lapinig, and Palapag, where crab farm owners were actively engaged in mud crab (*Scylla serrata*) farming. In Biri, the sampling sites were located at Sitio Cawayan, Barangay Kauswagan. In Capul, the sampling sites were located at Barangay San Luis. In Lapinig, the sampling sites were located at Barangay Imelda. Lastly, in Palapag, the sampling sites were located at Barangay Sinalaran. The samples were then transported to the Chemistry Laboratory, College of Science, University of Eastern Philippines, University Town, Catarman, Northern Samar.

|  |
| --- |
| **Physical Parameters**  Environmental quality is one of the most critical factors to measure. Local meteorological conditions, such as air temperature, rainfall, and sunlight, significantly impact the behavior of terrestrial organisms. According to the Natural Resource Institute, these conditions influence the distribution and activity of animals and plants. Assessing environmental quality also involves evaluating water parameters, including current and turbulence, dissolved oxygen levels, water temperature, salinity, and substrate types. These factors encompass physical and chemical characteristics essential for understanding environmental quality, as noted by Fondriest Environmental Inc. (2020).  pH, or "power of hydrogen," measures how acidic or basic a solution is. It's calculated based on the concentration of hydrogen ions. The pH scale ranges from 0 to 14, with 7 being neutral. Values below 7 are acidic, while those above 7 are basic. pH affects aquatic life significantly. Most aquatic organisms thrive in a pH range of 6.5 to 9.0. If the pH level deviates from this range, it can stress the organisms, reduce their survival rates, and increase toxicity. Humans are also affected by pH levels. While we can tolerate a wider pH range, extreme levels can cause skin and eye irritation, and even damage organs. Additionally, pH levels can impact the effectiveness of water treatment and the safety of our pipes. Both natural and human factors influence water pH. Natural changes occur due to interactions with rocks and materials, while human activities like waste water discharge and mining also impact pH levels. Even CO2 concentrations can affect pH levels, making regulation complex (Fondriest Environmental Inc., 2020).  Salinity of water in a pond varies depending on the specific needs of the aquatic organisms living there. For freshwater ponds, the salinity level is typically between 0-0.5 parts per thousand (ppt). Brackish water ponds, on the other hand, have a mix of fresh and saltwater, with salinity levels ranging from 0.5-35 ppt. Saltwater ponds, similar to seawater, usually have salinity levels between 35-40 ppt. Mud crabs, in particular, thrive in brackish water with optimal salinity levels between 10-25 ppt. While they can adapt to varying salinity levels between 5-35 ppt, maintaining suitable levels is crucial for their health and well-being. It's worth noting that salinity levels can fluctuate naturally due to factors like rainfall, evaporation, or tidal influences. Therefore, regular monitoring and maintenance are necessary to ensure the health and well-being of aquatic organisms in the pond. By maintaining optimal salinity levels, you can create a healthy environment for your aquatic life to thrive (SEAFDEC, 2016).  Temperature is a crucial factor when it comes to crabs. The 2016 Water Quality Guidelines don't provide exact temperatures for crabs, and that's because different species have different needs. Generally, many crab species thrive in temperatures between 20-30°C (68-86°F). However, some crabs require specific conditions, so it's essential to research the particular needs of your species. Temperatures above 30°C (86°F) can be a concern.  Turbidity - or water clarity - plays a crucial role in supporting aquatic life when it comes to ponds. The ideal turbidity level varies depending on the pond's purpose and the species that call it home. For clear water ponds, a turbidity range of 0-5 NTU is ideal, making it perfect for aquatic plants, fish, and other sensitive species. Moderate turbidity ponds, with levels between 5-20 NTU, can support a diverse range of aquatic life, including mud crabs. Ponds with higher turbidity levels, ranging from 20-50 NTU, might be suitable for species that can tolerate high sediment loads or algal growth. However, it's essential to consider the specific needs of the aquatic life and monitor water quality regularly. High turbidity can be a sign of poor water quality or excessive sedimentation, which can harm aquatic life. By managing turbidity levels, you can maintain a healthy and thriving ecosystem in your pond (SEAFDEC, 2016).  Soil substrate is a crucial factor when it comes to pond aquaculture. The type of soil can greatly impact the ecosystem, and different soils have different benefits. For example, clay soils can help retain water and reduce seepage, while sandy soils might require extra measures to prevent water loss. In mud crab farming, the soil substrate plays a vital role. Mud crabs thrive in soft, muddy substrates that allow them to burrow and hide. The sediment should be rich in organic matter and have adequate nutrient levels to support their growth.  In crab farming, the soil substrate is crucial for creating a suitable environment. Mangrove crabs thrive in muddy and sandy bottoms, so a mix of sand and mud is often used. In hatcheries, sand substrate is commonly used in tanks for broodstock maintenance. For nursery and grow-out phases, earthen ponds with clay or clay-loam soil are recommended, as they retain water and provide a natural setting (SEAFDEC, 2016).  The Southeast Asian Fisheries Development Center (SEAFDEC) 2016 standards for heavy metal concentrations in soil are not readily available.  **Research Design**  Generally, this study employed an experimental research design, where all gathered data were analyzed. The necessary data were acquired using methods adapted from the literature. No statistical treatments were applied, as the study primarily focused on determining the accumulation of heavy metals in mud crabs. Two analytical methods were utilized: qualitative analysis to detect the presence of heavy metals (arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg)) in crab meat samples, and quantitative analysis to measure the concentration of these heavy metals in both crab meat samples and soil substrate using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).  **Date Gathering Procedure**  **Assessment and documentation for the area covering the production of the cultured mud crab**  **pH (Power of Hydrogen)**  The pH levels of water from different areas were determined using a digital pH meter, which was directly submerged into the fish ponds where crabs were collected. The reading from the digital pH meter was recorded after one minute. This procedure was repeated three times for each sampling site.  **Salinity**  To analyze the salinity of water from the fish pond, a refractometer was utilized. A single drop of water sample was placed on the refractometer's prism, providing a measurement in percentage, which was then converted to parts per thousand (ppt) using a specific formula.  **ppt = n/100 x 1000**  Where; n = percentage seen in the refractometer  **Temperature**  The water temperature was determined using an infrared thermometer, which emitted a beam of light directly into the fish ponds where the crabs were collected. Measurements were taken at 2 PM and 2 AM to obtain the standard temperatures of the area. The readings from the infrared thermometer were recorded. This procedure was repeated three times for each sampling site.  **Turbidity**  In the determination of turbidity, a Nephelometer was used in this analysis. A 330 mL of water sample will be collected and transported to the laboratory for turbidity analysis.  **Substrate**  Prior to pond preparation, soil samples were collected from the entire area, including noticeable corners and the center. At each site, three replicate samples of 50g each were gathered and stored in sterilized plastic containers. Samples were kept at 40C during transport (Camille, *et. al*., 2015).    **Gathering of the Crab Samples**  Crab samples were gathered directly from the fish ponds of selected municipalities in Northern Samar: Biri, Capul, Palapag, and Lapinig. For each municipality, 1kg mud crab was required, consisting of four (4) mature individuals. The sample crabs had to be in live condition and had reached market size or maturity (Erivera, *et. al.,* 2023). The samples were kept at 4°C during transport and were immediately frozen in separate containers (Camille, *et. al.,* 2015).  **Preparation of Crab Samples**  After gathering the samples from the sampling area, they were immediately brought to College of Science, Chemistry Laboratory for the removal of the “meat”. The crab meat was stored separately in a clean vial for further analysis (Erivera, *et. al,* 2023).  **Detection of Heavy Metals present (Qualitative Analysis)**  Qualitative analysis was carried out to detect the presence of heavy metals such as arsenic, cadmium, chromium, lead, and mercury.  **Detection of Arsenic (As)**  To the prepared crab samples, 3F HCl was added until they were barely acidic. The mixture was centrifuged for three minutes, and the centrifugate (supernatant liquid) was discarded. Then, ten drops of concentrated HCl were added to the residue, and the solution was stirred. It was then heated in a hot water bath for one minute, centrifuged, and the centrifugate was removed, leaving only the residue. The residue was washed with a mixture of eight drops of water and four drops of concentrated HCl, centrifuged, and the residue was separated from the supernatant liquid. The residue was washed with enough hot water three times. Four drops of concentrated HNO3 were added, and the mixture was heated for five minutes in a water bath (Erivera, 2023). After heating, five drops of 0.5F AgNO3 were added and stirred. To clear the centrifugate (supernatant liquid), fifteen drops of 2.5F NaAc solution were added. The formation of a reddish-brown precipitate indicated the presence of arsenic.  **Detection of Cadmium (Cd)**  Cadmium forms a yellow precipitate with sulfide ions in either a neutral solution containing free Cd²⁺ or an ammoniacal solution containing Cd (NH₃) ₄²⁺. Since most sulfides are insoluble and many are black, the presence of other metal ions may make it difficult to detect the yellow color of CdS. Therefore, separations must be as complete as possible before testing for Cd²⁺. To a solution of Cd²⁺ or a solution thought to contain Cd (NH₃) ₄²⁺, add 0.1 M Na₂S solution dropwise. The formation of a yellow precipitate confirms the presence of Cd²⁺.  **Detection of Chromium (Cr)**  To the prepared crab sample, ten (10) drops of saturated NH₄Ac were added, followed by 3F HAc until acidic, and then 3F ammonia was added until the solution was decidedly ammoniacal. The solution was then centrifuged, and the residue and centrifugate were separated. To the centrifugate, 1F Ba (Ac)₂ was added until precipitation was complete. The solution was centrifuged, and the residue and centrifugate were separated. To the residue, ten (10) drops of 3F HCl were added. It was then warmed for 1 minute in a water bath, centrifuged, and the white residue was discarded (Erivera, *et. al.,* 2023).  Two (2) drops of centrifugate were placed on a piece of filter paper. Then, two (2) drops of H₂O₂ were added to the same filter paper. The formation of a blue color that fades rapidly confirms the presence of chromium.  **Detection of Lead (Pb)**  To the prepared crab sample, add 3M HCl dropwise. (A large excess of HCl must be avoided because of the formation of the soluble chloro complex, PbCl₄²⁻). Centrifuge and remove the supernatant from the white precipitate (PbCl₂). Add hot water to the precipitate and stir. If the precipitate dissolves, Pb²⁺ is indicated. Add 3M H₂SO₄ to the hot solution. Centrifuge and remove the supernatant liquid from the white precipitate (PbSO₄). To the precipitate, add 3M NH₄(CH₃COO) and stir. Add a few drops of 0.5M K₂CrO₄ to the solution. A yellow precipitate of PbCrO₄ indicates the presence of Pb²⁺.  **Detection of Mercury (Hg)**  When Hg₂Cl₂ is treated with aqueous NH₃, a reaction occurs in which free mercury and aminochloromercury (II) are formed. About 2 mL of the prepared crab sample was used in the detection of mercury ions. The following procedures were followed: 3M HCl was added to the prepared crab sample solution. If a white precipitate formed, it was centrifuged and the supernatant liquid was removed. Then, 6M NH₃ was added to the precipitate and stirred. The appearance of a gray to black precipitate indicates the presence of mercury ions.  **Soil processing and digestion**  Substrate/soil samples from the same site were pooled together, air-dried to dryness, and then sieved by passing through a 1 mm nylon sieve. Fractions less than 1 mm in size were ground in an agate mortar until the entire sample was homogenized. Two (2) grams of the ground substrate/soil sample underwent a 3-step digestion using 10 mL of concentrated hydrochloric/nitric acid (1:1), 10 mL of 3:1 hydrochloric/nitric acid, and 10 mL of 5% nitric acid. Digestion and evaporation of acids for each step were done by heating at 400°C. The final products were re-dissolved in 50 mL of 1% HNO₃ and filtered, then stored in polyethylene bottles (Camille, *et. al.,* 2015). The digested samples were analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).  **Tissue preparation and digestion**  The tissue samples were prepared and digested. The tissues were oven-dried at 50°C for 48 hours and then pulverized. Digestion was performed in two rounds using 1% HNO₃ (1st round: 2 mL; 2nd round: 10 mL). The breakdown of meat tissue and evaporation of solvents were facilitated by heating the samples. The resulting products were re-dissolved in 25 mL of distilled deionized water after complete evaporation of solvents. The solution was then sealed in a Falcon tube and stored (Camille, *et. al.,* 2015).  **Quantification of Heavy Metals concentration (Quantitative Analysis)**  To determine the total concentration or quantity of heavy metals in crab and substrate/soil samples, the prepared digested samples were analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), and the data obtained were interpreted. |

**PRESENTATION, DISCUSSION AND INTERPRETATION OF DATA**

This study aimed to assess environmental parameters in the area surrounding the production of cultured mud crab (*Scylla serrata*) from four (4) sampling areas and to determine the presence and concentration of heavy metals (arsenic, cadmium, chromium, lead, and mercury) in different municipalities in Northern Samar.

Tests were conducted on environmental parameters (pH, salinity, temperature, type of substrate, and turbidity). Qualitative analysis was performed using the method adopted from Erivera et al. (2023) in a preliminary test for the presence of heavy metals. Inducted Coupled Plasma Optical Emission Spectrometry (ICP-OES) was used for quantitative analysis to quantify/measure the concentration of heavy metals in the digested samples of mud crabs (*Scylla serrata)*.

**I. Assess and document the area covering the production of the cultured mud crab (*Scylla serrata*)**

**pH level**

Table 1a presents the pH levels of four selected fish ponds in municipalities in Northern Samar. The pH levels from the fish ponds in Biri, Lapinig, Palapag, and Capul, Northern Samar had average of 9.0, 8.40, 8.04, and 8.90, respectively. Most aquatic organisms thrive in a pH range of 6.5 to 9.0, which is suitable for marine life survival and reproduction (Fondriest Environmental, Inc., 2020). These results imply that the pH levels in these fish ponds fall within the normal pH range for mud crab health.

**Table 1a. pH level of water from the four fishponds.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Para-**  **Meter** | **Sampling**  **Area** | **Trial 1** | **Trial 2** | **Trial 3** | **Ave-**  **rage** | **Inter-**  **pretation** | **Standard value** |
| pH | BNS-FP | 9.0 | 9.01 | 9.0 | 9.0 | Slightly  basic | 6.5-9.0 |
| LNS-FP | 8.39 | 8.37 | 8.39 | 8.40 | Slightly basic |
| PNS-FP | 8.05 | 8.04 | 8.05 | 8.04 | Slightly basic |
| CNS-FP | 8.91 | 8.89 | 8.91 | 8.90 | Slightly basic |

\*Legend:

**BNS - FP** – Biri Northern Samar Fish Pond

**LNS – FP** - Lapinig Northern Samar Fish Pond

**PNS - FP** – Palapag Northern Samar Fish Pond

**CNS – FP** – Capul Northern Samar Fish Pond

**Salinity**

Table 1b presents the salinity values of four selected fish ponds in municipalities in Northern Samar. The salinity from the fish ponds in Biri, Lapinig, Palapag, and Capul, Northern Samar had average of 8.7 ppt, 5.7 ppt, 9.0 ppt, and 9.0 ppt, respectively. Brackish water ponds have a mix of fresh and saltwater, with salinity levels ranging from 0.5 to 35 ppt. Mud crabs, in particular, thrive in brackish water with optimal salinity levels between 10 and 25 ppt. While they can adapt to varying salinity levels between 5 and 35 ppt, maintaining suitable levels is crucial for their health and well-being (SEAFDEC, 2016). These results imply that the salinity levels in these fish ponds are within the suitable salinity range for mud crab health.

**Table 1b.** Salinity of water from the four fish ponds.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Para-**  **meter** | **Sampling**  **Area** | **Trial 1** | **Trial 2** | **Trial 3** | **Ave-**  **rage** | **ppt conversion** | **Standard Value** |
| Salinity  (ppt) | BNS-FP | 0.9% | 0.9% | 0.8% | 0.87% | 8.7 ppt | 0.5-35 |
| LNS-FP | 0.6% | 0.5% | 0.6% | 0.57% | 5.7 ppt |
| PNS-FP | 1.0% | 0.9% | 0.8% | 0.0% | 9.0 ppt |
| CNS-FP | 0.9% | 0.8% | 1.0% | 0.9% | 9.0 ppt |

\*Legend:

**BNS - FP** – Biri Northern Samar Fish Pond

**LNS – FP** - Lapinig Northern Samar Fish Pond

**PNS - FP** – Palapag Northern Samar Fish Pond

**CNS – FP** – Capul Northern Samar Fish Pond

**Temperature**

Table 1c presents the water temperatures of four selected fish ponds in municipalities in Northern Samar. The temperature from the fish ponds in Biri, Lapinig, Palapag, and Capul, Northern Samar had average of 29.5, 27.5, 29.0, and 28.5, respectively. Mud crab species thrive in temperatures between 20oC and 30oC. Temperatures above 30oC can be a concern based on the Water Quality Guidelines of 2016. These results imply that the water temperatures in these fish ponds fall within the normal temperature range for mud crab health.

**Table 1c. Temperature of water from the four fish ponds.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Sampling**  **Area** | **Time**  **2 PM** | **Time**  **2 AM** | **Average** | **Standard Value** |
| Temperature  (0C) | BNS-FP | 32 | 27 | 29.5 | 20- 30 |
| LNS-FP | 30 | 25 | 27.5 |
| PNS-FP | 31 | 26 | 29 |
| CNS-FP | 30 | 27 | 28.5 |

\*Legend:

**BNS - FP** – Biri Northern Samar Fish Pond

**LNS – FP** - Lapinig Northern Samar Fish Pond

**PNS - FP** – Palapag Northern Samar Fish Pond

**CNS – FP** – Capul Northern Samar Fish Pond

**Turbidity**

Table 1d presents the water turbidity of four selected fish ponds in municipalities in Northern Samar. The turbidity from the fish ponds in Biri, Lapinig, Palapag, and Capul, Northern Samar had average of 0.7 NTU, 0.8 NTU, 4.2 NTU, and 1.8 NTU, respectively. Moderate turbidity ponds, with levels between 5-20 NTU, which support a diverse range of aquatic life, including mud crabs (SEAFDEC, 2016). These results imply that the turbidity in these fish ponds fall within the normal turbidity range for mud crab health.

**Table 1d.** Turbidity of water from the

four fish ponds.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Sampling**  **Area** | **Time**  **2 PM** | **Trial 2** | **Trial 3** | **Average** | **Standard Value** |
| Turbidity  (NTU) | BNS-FP | 0.7 NTU | 0.7 NTU | 0.7 NTU | 0.7 NTU | 5-20 |
| LNS-FP | 0.8 NTU | 0.8 NTU | 0.8 NTU | 0.8 NTU |
| PNS-FP | 4.2 NTU | 4.2 NTU | 4.2 NTU | 4.2 NTU |
| CNS-FP | 1.8 NTU | 1.8 NTU | 1.8 NTU | 1.8 NTU |

\*Legend:

**BNS - FP** – Biri Northern Samar Fish Pond

**LNS – FP** - Lapinig Northern Samar Fish Pond

**PNS - FP** – Palapag Northern Samar Fish Pond

**CNS – FP** – Capul Northern Samar Fish Pond

**Type of Substrate**

Table 1e presents the type of substrates found in four selected fish ponds in municipalities in Northern Samar. The substrate in the fishponds in Biri, Northern Samar, consists of sandy-clay soil, while those in Lapinig, Palapag, and Capul, Northern Samar, are composed of clay-loam soil. Mud crabs prefer soft, muddy substrates for burrowing and hiding, while mangrove crabs thrive in mixed muddy and sandy environments, according to SEAFDEC (2016). These results imply that the type of substrates in these fish ponds are suitable for mud crab health.

**Table 1e.** Type of Substrate of the four fish ponds.

|  |  |
| --- | --- |
| **Sampling area** | **Type of Substrate** |
| BNS-FP | Sandy-clay soil |
| LNS-FP | Clay-loam soil |
| PNS-FP | Clay-loam soil |
| CNS-FP | Clay-loam soil |

\*Legend:

**BNS - FP** – Biri Northern Samar Fish Pond

**LNS – FP** - Lapinig Northern Samar Fish Pond

**PNS - FP** – Palapag Northern Samar Fish Pond

**CNS – FP** – Capul Northern Samar Fish Pond

**II. Determination of heavy metals found in soil substrate.**

Table 2 presents the presence of heavy metals in soil substrates from four selected fish ponds in municipalities in Northern Samar (Biri, Lapinig, Palapag, and Capul), as analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Alfian, *et. al.,* 2017). The results show that all soil substrates contain heavy metals, specifically arsenic (As), chromium (Cr), cadmium (Cd), lead (Pb), and mercury (Hg), indicating the presence of these five heavy metals in the soil samples. The municipality with the highest concentration of heavy metals (arsenic and chromium) in soil substrate is Palapag, with the highest concentration of heavy metal (mercury) in soil substrate is Biri, and with the highest concentration of heavy metals (lead and cadmium) in soil substrate is Lapinig. Conversely, the municipality with the lowest concentration of heavy metals (arsenic, lead, and mercury) in soil substrate is Capul, while with the lowest concentration of heavy metals (cadmium and chromium) in soil substrate is Biri (Refer to Table 4. Quantification and concentration of heavy metals accumulated in cultured mud crab (*Scylla serrata*) and the soil substrate using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)).

This suggest that the concentration of heavy metals in soil substrate is higher than in crab samples. The difference in exposure pathways contributes to this disparity: soil is directly exposed to heavy metal sources, whereas crabs are exposed through the food chain or water (Hossain, *et. al.,* 2023). As a results soil tends to accumulate higher concentrations of heavy metals over time due to various natural and anthropogenic factors (Camille, *et. al.,* 2015).

**Table 2. Heavy Metals found in soil substrate using ICP-OES.**

|  |  |
| --- | --- |
| **Sample Description** | **Detected Heavy Metals** |
| BNS-S | Arsenic, Chromium, Cadmium, Lead, Mercury |
| LNS-S | Arsenic, Chromium, Cadmium, Lead, Mercury |
| PNS-S | Arsenic, Chromium, Cadmium, Lead, Mercury |
| CNS-S | Arsenic, Chromium, Cadmium, Lead, Mercury |

\*Legend:

**BNS - FP** – Biri Northern Samar Fish Pond

**LNS – FP** - Lapinig Northern Samar Fish Pond

**PNS - FP** – Palapag Northern Samar Fish Pond

**CNS – FP** – Capul Northern Samar Fish Pond

**III. Determine the qualitative heavy metal present in cultured mud crab *(Scylla serrata)*.**

Preliminary tests for the presence of heavy metals in mud crabs (*Scylla serrata*) were conducted using the method of Erivera, (2023). The heavy metals assessed were as follows:

**Arsenic (As)**

Table 3a presents the presence of arsenic in crab meat samples from four selected municipalities in Northern Samar (Lapinig, Palapag, Capul, and Biri). The results show that crab meat samples from Lapinig, Palapag, and Capul exhibited a reddish-brown precipitate, indicating a positive result for arsenic, while Biri showed an absence of arsenic.

**Table 3a.** Detection of Arsenic.

|  |  |  |  |
| --- | --- | --- | --- |
| **Heavy metal** | **Sample description** | **Crab Meat** | **Remarks** |
| Arsenic | LNS | + | Positive (+) if there’s a reddish-brown precipitate form.  Negative (-) of there is no reddish-brown precipitate form. |
| PNS | + |
| CNS | + |
| BNS | - |

\*Legend:

**BNS - FP** – Biri Northern Samar

**LNS – FP** - Lapinig Northern Samar

**PNS - FP** – Palapag Northern Samar

**CNS – FP** – Capul Northern Samar

**Cadmium (Cd)**

Table 3b presents the presence of cadmium in crab meat samples from four selected municipalities in Northern Samar (Lapinig, Palapag, Capul, and Biri). The results show

that crab meat samples from Lapinig, Palapag, and Capul exhibited a yellow precipitate form, indicating a positive result for cadmium, while Biri showed an absence of cadmium.

**Table 3b.** Detection of Cadmium.

|  |  |  |  |
| --- | --- | --- | --- |
| **Heavy metal** | **Sample description** | **Crab Meat** | **Remarks** |
| Cadmium | LNS | + | Positive (+) if there’s a yellow precipitate form.  Negative (-) of there’s no yellow precipitate form. |
| PNS | + |
| CNS | + |
| BNS | - |

\*Legend:

**BNS - FP** – Biri Northern Samar

**LNS – FP** - Lapinig Northern Samar

**PNS - FP** – Palapag Northern Samar

**CNS – FP** – Capul Northern Samar

**Chromium (Cr)**

Table 3c presents the presence of chromium in crab meat samples from four selected municipalities in Northern Samar (Lapinig, Palapag, Capul, and Biri). The results show that crab meat samples from Lapinig and Capul exhibited a blue color form that fades rapidly, indicating a positive result for chromium, while Palapag and Biri showed an absence of chromium.

**Table 3c.** Detection of Chromium.

|  |  |  |  |
| --- | --- | --- | --- |
| **Heavy metal** | **Sample description** | **Crab Meat** | **Remarks** |
| Chromium | LNS | + | Positive (+) if there’s a blue color form that fades rapidly.  Negative (-) of there’s no blue color form. |
| PNS | - |
| CNS | + |
| BNS | - |

\*Legend:

**BNS - FP** – Biri Northern Samar

**LNS – FP** - Lapinig Northern Samar

**PNS - FP** – Palapag Northern Samar

**CNS – FP** – Capul Northern Samar

**Lead (Pb)**

Table 3d presents the presence of lead in crab meat samples from four selected municipalities in Northern Samar (Lapinig, Palapag, Capul, and Biri). The results show that crab meat samples from Lapinig, Palapag, and Capul exhibited a yellow precipitate form, indicating a positive result for lead, while Biri showed an absence of lead.

**Table 3d.** Detection of Lead.

|  |  |  |  |
| --- | --- | --- | --- |
| Heavy metal | Sample description | Crab Meat | Remarks |
| Lead | LNS | + | Positive (+) if there’s a yellow precipitate form.  Negative (-) of there’s no yellow precipitate form. |
| PNS | + |
| CNS | + |
| BNS | - |

\*Legend:

**BNS - FP** – Biri Northern Samar

**LNS – FP** - Lapinig Northern Samar

**PNS - FP** – Palapag Northern Samar

**CNS – FP** – Capul Northern Samar

**Mercury (Hg)**

Table 3e presents the presence of mercury in crab meat samples from four municipalities in Northern Samar (Lapinig, Palapag, Capul, and Biri). The results show that all crab meat samples exhibited a gray-to-black precipitate, indicating a positive result for mercury.

**Table 3e.** Detection of Mercury.

|  |  |  |  |
| --- | --- | --- | --- |
| **Heavy metal** | **Sample description** | **Crab**  **Meat** | **Remarks** |
| Mercury | LNS | + | Positive (+) if there’s a gray-to-black precipitate form.  Negative (-) no gray-to-black precipitate form. |
| PNS | + |
| CNS | + |
| BNS | + |

\*Legend:

**BNS - FP** – Biri Northern Samar

**LNS – FP** - Lapinig Northern Samar

**PNS - FP** – Palapag Northern Samar

**CNS – FP** – Capul Northern Samar

**IV. Determine the quantify of heavy metals in cultured mud crab (*Scylla serrata*) using ICP-OES.**

To quantify the concentration of heavy metals accumulated in cultured mud crab (*Scylla serrata*), Inductively Coupled Plasma Optical Emission Spectrometry was used.

Table 4 presents the results of ICP-OES analysis of mud crab and soil samples from four sampling sites. All five heavy metals (arsenic, cadmium, chromium, lead, and mercury) were detected in both crab meat and soil samples. Notably, the crab meat samples from the four municipalities (Lapinig, Palapag, Capul, and Biri) exceeded the maximum standards set by FAO/WHO, which are: arsenic (0.5-1.0 mg/kg), cadmium (0.3 mg/kg), chromium (0.5 mg/kg), lead (0.5 mg/kg), and mercury (0.001 mg/kg).

The results show that the municipality with the highest concentration of heavy metals varied: Lapinig had the highest levels of arsenic, cadmium, and mercury, while Palapag had the highest levels of chromium and lead. Conversely, the municipality with the lowest concentration of heavy metals also varied: Capul had the lowest levels of arsenic, lead, mercury, and chromium, while Biri had the lowest level of cadmium.

Similarly, the soil samples contained all five heavy metals, with results indicating higher contamination levels in soil compared to crab meat. These imply that high levels of heavy metals (arsenic, cadmium, chromium, lead, and mercury), exceeding FAO/WHO standards, poses health risks to consumers, environmental concerns, and economic implications for the fishing industry.

As per SEAFDEC guidelines (2016), standard values for heavy metal concentrations in soil samples have not been established.

**Table 4.** Quantification and concentration results of heavy metals accumulated in cultured mud crab (*Scylla serrata*) and the soil substrate using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample Name** | **Analyte**  **Concentration (mg/Kg sample or ppm)\*** | | | | |
| As | Cd | Cr | Pb | Hg |
| BNS-CM | 13.37 | 3.93 | 17.81 | 3.89 | 0.0005 |
| BNS-S | 237 | 39.98 | 86.91 | 156.43 | 0.0025 |
| CNS-CM | 7.71 | 8.14 | 13.56 | 1.45 | 0.0002 |
| CNS-S | 109.16 | 40.23 | 88.19 | 131.22 | 0.0016 |
| LNS-CM | 34.66 | 14.13 | 16.81 | 2.13 | 0.0009 |
| LNS-S | 242.67 | 41.22 | 89.36 | 168.11 | 0.0021 |
| PNS-CM | 29.32 | 5.66 | 15.44 | 4.57 | 0.0008 |
| PNS-S | 287.88 | 41.03 | 90.04 | 137.13 | 0.0018 |
| **Standard Value for CM**  **mg/kg** | 0.5-1.0 | 0.3 | 0.5 | 0.5 | 0.001 |

\*Legend:

**BNS - CM** – Biri Northern Samar-Crab Meat

**BNS – S** - Biri Northern Samar-soil

**LNS – CM** - Lapinig Northern Samar-Crab Meat

**LNS – S** - Lapinig Northern Samar-Soil

**PNS - CM** – Palapag Northern Samar-Crab Meat

**PNS – S** - Palapag Northern Samar-Soil

**CNS – CM** – Capul Northern Samar-Crab Meat

**CNS – S** - Capul Northern Samar-Soil

**SUMMARY**

The environmental conditions in four selected fish ponds in Northern Samar were assessed, and the results indicate a favorable environment for mud crab cultivation. The pH levels in these ponds were suitable, ranging from 8.04 to 9.0, which falls within the optimal range of 6.5 to 9.0, indicating a favorable environment for mud crab growth and development (Fondriest Environmental Inc., 2020). The salinity levels were assessed, with readings of 8.7 ppt in Biri, 5.7 ppt in Lapinig, and 9.0 ppt in both Palapag and Capul. Although slightly below the optimal range of 10-25 ppt, these levels fall within the adaptable range of 5-35 ppt, suggesting suitable water conditions for mud crab health and well-being (SEAFDEC, 2016). The water temperatures averaged between 27.5°C and 29.5°C, comfortably within the ideal range of 20°C to 30°C. With none exceeding 30°C, the conditions are favorable for mud crab cultivation, aligning with the Water Quality Guidelines of 2016. The water turbidity levels ranged from 0.7 NTU to 4.2 NTU, which, although below the moderate range of 5-20 NTU, still falls within acceptable ranges. This suggests suitable water clarity for mud crab health and well-being (SEAFDEC, 2016). The substrate composition varied, with Biri's ponds featuring sandy-clay soil and the others having clay-loam soil. Given mud crabs' preference for soft, muddy substrates, these soil types seem suitable, providing a conducive environment for them to thrive (SEAFDEC, 2016).

Soil substrates were analyzed using Inductively Coupled Optical Emission Spectroscopy (ICP-OES), which revealed the presence of heavy metals, specifically arsenic, chromium, cadmium, lead, and mercury. The municipality of Palapag had the highest concentrations of arsenic and chromium, while Biri had the highest concentration of mercury, and Lapinig had the highest levels of lead and cadmium. Conversely, Capul had the lowest concentrations of arsenic, lead, and mercury, while Biri had the lowest levels of cadmium and chromium. Notably, the concentration of heavy metals was higher in the soil substrates than in the crab samples, likely due to the soil's direct exposure to heavy metal sources, whereas crabs are exposed through the food chain or water (Camille, *et. al.,* 2015). Over time, soil tends to accumulate higher concentrations of heavy metals due to various natural and anthropogenic factors (Hossain, *et. al.,* 2023).

Preliminary tests were conducted to detect the presence of heavy metals in mud crabs (*Scylla serrata*). The tests, based on the method of Erivera (2023), revealed varying results for different heavy metals. For arsenic, crab meat samples from Lapinig, Palapag, and Capul showed a reddish-brown precipitate, indicating a positive result, while Biri showed no presence of arsenic (Table 3a). Similarly, for cadmium, Lapinig, Palapag, and Capul exhibited a yellow precipitate, confirming its presence, whereas Biri tested negative (Table 3b). Chromium was detected in crab meat samples from Lapinig and Capul, which turned blue, although the color faded rapidly. Palapag and Biri, however, showed no signs of chromium (Table 3C). Lead was present in samples from Lapinig, Palapag, and Capul, indicated by a yellow precipitate, but was absent in Biri (Table 3d). Notably, all crab meat samples tested positive for mercury, displaying a gray-to-black precipitate (Table 3e). These findings suggest that heavy metals are present in varying degrees across the sampled municipalities.

The concentration of heavy metals in cultured mud crabs (*Scylla serrata*) and soil samples was quantified using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The results revealed that all five heavy metals - arsenic, cadmium, chromium, lead, and mercury - were detected in both crab meat and soil samples. However, the levels of these heavy metals in the crab meat samples exceeded the maximum standards set by FAO/WHO. Lapinig had the highest levels of arsenic, cadmium, and mercury, while Palapag had the highest levels of chromium and lead. In contrast, Capul had the lowest levels of most heavy metals, except for cadmium, which was lowest in Biri. The soil samples also contained high levels of heavy metals, with contamination levels generally higher than in the crab meat. The presence of these heavy metals at levels exceeding FAO/WHO standards poses significant health risks to consumers, environmental concerns, and economic implications for the fishing industry.

**CONCLUSION**

Based on our study's findings, contrasting results were revealed regarding mud crab cultivation in Northern Samar. On one hand, the environmental conditions in the four selected fish ponds were favorable for mud crab cultivation, characterized by suitable pH levels, salinity, water temperatures, turbidity, and substrate composition. These conditions align with established guidelines and standards, indicating that the selected fish ponds have the potential to support healthy mud crab farming practices. However, our analysis also revealed concerning levels of heavy metals in both soil substrates and mud crab samples, including arsenic, chromium, cadmium, lead, and mercury. The presence of these heavy metals poses potential environmental and health risks. Notably, the levels of these heavy metals in crab meat samples exceeded FAO/WHO standards, highlighting significant health risks to consumers and economic implications for the fishing industry. The varying concentrations of heavy metals across municipalities and the higher accumulation in soil compared to crab samples underscore the importance of monitoring and managing soil quality. Furthermore, the detection of mercury in all crab samples emphasizes the need for further investigation and monitoring to ensure the safety and sustainability of mud crab farming. Ultimately, our findings emphasize the need for urgent attention and action to mitigate heavy metal contamination, ensure food safety, and protect the environment and public health.

**RECOMMENDATIONS**

1. Regularly monitor water quality parameters to ensure that the favorable conditions are maintained.

1.1 Promote sustainable aquaculture practices to ensure long-term viability and minimize environmental impacts.

1.2 Conduct further research to identify potential areas for improvement and optimize mud crab farming practices in the region.

2. Implement regular monitoring of soil quality in fish ponds to track heavy metal concentrations and identify potential sources of contamination.

2.1 Develop and implement strategies to manage soil quality, such as reducing heavy metal inputs, improving water circulation, and using soil remediation techniques.

2.2 Conduct thorough risk assessments to determine the potential impacts of heavy metal contamination on aquatic life and human health.

3. Conduct further research to determine the sources and pathways of heavy metal contamination in mud crabs.

3.1 Establish a regular monitoring program to track heavy metal concentrations in mud crabs and identify potential hotspots.

3.2 Develop and implement strategies to mitigate heavy metal contamination in mud crab farming, such as improving water quality, reducing feed contamination, and implementing best management practices.

4. Conduct regular inspections and monitoring of mud crab farming areas to identify sources of contamination.

4.1 Implement soil remediation strategies to reduce heavy metal concentration in farming areas.

4.2 Conduct further research to understand the sources and pathways of heavy metal contamination in mud crab farming.

**REFERENCES**

Alfian, Z., E. Z. Nst, & M.

Taufiq M (2017). CODEN (USA): PCHHAX Qualitative and Quantitative Analysis of Heavy Metal and Mineral of Volcanic Ashmount Sinabung by Using Inductively Coupled Plasma (ICP-OES). 9 (5), 18-22.

Briffa, J., E. Sinagra, & R.

Blundell, R. (2020). Heliyon Heavy metalpollurion in the environment and their toxicological effects on humans. Heliyon, 6(June), e04691. https://doi.org.10.1016/j.heliyon.2020.e04691

Camille, C., E. V. Cruz, G.

Ramos, & M. C. Ablan-Lagman, (2015) Heavy Metal Levels in mud Crabs (Scylla spp.) from East Bataan coast. 3, 2-7.

Charles, C., H. Robinson, C.

Rumisha, & Leermakers, M. (2017). Bioaccumulation and Public Health Implications of Trace Metals in edible Tissues of the Crustaceans Scylla serrate and Penaeus monodon from the Tranzanian coast. <https://doi.org/10.1007/s10661-017-6248-0>

Department of Environment and Natural

Resources, Administrative Order No. 26-08 on “Water Quality Guidelines and General Effluent Standards, 2016.

DOST.gov.ph. Mangrove Crab – Industry

Strategic Science and Technology Plans. (https://ispweb.pcaarrd.dost.gov.ph).

Erivera, J. L., M. L. C. Alvarez, T.

J. L. Abobo, K. R. L. Diaz, L., M. C., Manla, K. M. C. Lim, (2023). Health Risks Assessment of Heavy Metal Concentration in Cultured *Chanos chanos* (Bangus) and *Scylla serrata* (Mudcrab) in Selected Municipalities in Northern Samar. Asian Journal of Chemical Sciences, Volume 14, Issue 2, Page 113-130, 2024; Article no. AJ0CS.113303 ISSN: 2456-7795.

Food and Agriculture Organization,

(2023). Improving Pond Quality. https://www.fao.org/fishery/docs/CDrom/FAO\_Training/General/xh6709e2.htm.

Food and Agriculture Organization,

(2023). Soils and Freshwater Fish Culture.https://fao.org/fishery/docs/CDrom/FAo\_Training/General/x6706e/x6706e12.htm#top.

Fatryani, D., (2022). Heavy Metal

Content of Hg, Cd, Pb, and Cu in Mud Crab (*Scylla Serrata*) in Bantan Bay, Indonesia. IOP Conf. Ser.: Earth Environ. Sci. 1083 012057.

Fondriest Environmental, Inc. (2020)

“Water Quality” Fundamentals of the Environmental Measurements. <https://www>. Fondriest.com/environmental-measurements/parameters/water-quality/ph/>

Hossain, M. B., J. Sultana, F. H. Pingki, A. U. Nur, M. S. Mia

M. A. Bakar, J. Yu, B. A. Paray, T. Arai, (2023). Accumulation and contamination assessment of heavy metals in sediments of commercial aquaculture farms from a coastal area along the northern Bay of Bengal. https://www.frontiersin.org>fenvs.2023.1148360.

Journal of King Saud University –

Science Volume 34, Issue 1, January 2022. Health and environmental effects of heavy metals. <https://doi.org/10.1016/j.jksus.2021.101653>

Kamaruzzaman, B. Y., J. B. Akbar, B.

Z. Maryam, K. C. A. Jalal, and S. Shahbuddin, (2012). Bioaccumulation of Heavy Metals (cd, Pb,Cu, and Zn) in Scylla serrata collected from sungai Penor, Pahang-Malaysia.

Koller, M., & H. M. Saleh (2018).

Introductory Chapter: Introducing Heavy Metals. 27 June 2018. https://www.ncbi.nlm.nih.gov>articles>PMC4144270.

Marslab, (2016). “A Name of Quality

Laboratory Furniture” Mars Laboratory Instruments Center, Ortigas Center, Pasig City, 127-152.

Massou, et. al., (2022). Heavy Metal

Accumulation in the Edible crab *(Cardisoma armatum)* (Brachyura: Gecarcinidae) and implications for huma health. Journal of Environment Science and Health, Part B.

National Cancer Institute (.gov),

2022. Cadmium-Cancer- Causing Substances -NCI. 05 December 2022. https://www..cancer.gov>causes-prevention>risk>cadmium.

PhilAtlas (2020) “Municipalities of

Northern Samar” 21 June 2020. <https://www.philatlas.com/visayas/r08/northern->samar/laoang/html

Quinitio, E. T. (2017). Overview of

the mud crab industry in the Philippines. In E. T. Quinitio, F. D. Parado-Estepa, & R. M. Coloso (Eds.), Philippines: In the forefront of the mud crab industry development: proceedings of the 1st National Mud Crab Congress, 16-18 November 2015, Iloilo City, Philippines (pp. 1-12). Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

Roysfarm (2023) Mud Crab:

Characteristics, Diet, Uses, Facts. 06 November 2023. https://www.roysfarm.com>mud-crab.

Saher, N. U., & N. Kanwal, (2018).

Some Biomonitoring Studies of Heavy Metals in commercial species of Crustacean Along Karachi Coast, Pakistan Noor Us Saher \* and Nayab Kanwal. 15 (2), 269-275.

Sarah, R., B. Tabassum, N. Idrees, &

A. Hashem, (2019). Bioaccumulation of Heavy Metals in Channa Punctatus (Bloch) in river Ramganga (U.P.), India. Saudi Journal of Biological Sciences, 26(5), 979-984. <https://doi.org/10.1016/j.sjbs.2019.02.009>

SEAFDEC Aquaculture Department,

2016. Mangrove crab hatchery and nursery operations [Brochure]. Tigbauan, Iloilo, Philippines: Author.

Soegianto, A., H. I. Wahyuni, B.

Yulianto, L. A. Manaf, (2022). Health risk assessment of metals in mud crab *(Scylla serrata)* from the East Java Estuaries of Indonesia. Environmental Toxiology and Pharmacology, Volume 90, 103810. <https://www.sciencedirect.com/science/article/abs/pii/S1382668922000035>.

Vikaspedia (2020). ”Mud Crab

Culture”.<https://vikaspedia./inagriculture/fisheries/brackish->water fisheries/culture-fisheries/mud-crab-culture.

Williams, S. E., Priya, L. V., &

Karim, R. L., (2022). Watershed Ecology and the Environment. Vol. 4 pp. 59-65. <https://doi.org/10.1016/j.wsee.2022.06.001>

WHO (World Health Organization),

2022. Arsenic. https://www..who.int.Newsroom>Fact sheets>Detail

WHO (World Health Organization),

2023. Lead poisoning. 11 August

2023.https://www.who.int>Newsroom>Fact sheets>Detail

Zheng, X., K. Wu, P. Sun, S.

Zhouyang, Y. Wang, H. Wang, Y. Zheng, and Q. Li, (2021). Effects of Substrate Types on the Transformation of Heavy Metal speciation and Bioavailability in an Anaerobic Digestion. Journal of environmental Sciences, 101: 361-372. <https://doi.org/10.1016/j>.jes.2020.08.032