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Assessment of heavy metal contamination in common fish species from Central Vietnam and the potential human risk

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LIST OF ABBREVIATIONS AND ACRONYMS

AAS:	Atomic Absorption Spectrometer
ANOVA:	Analysis of Variance
b.w:	Body weight
Cd:	Cadmium
Cu:	Copper
d.w:	Dry weight
DOF:	Directorate Of Fish
EDI:	Estimated Daily Intake
EFSA:	The European Food Safety Authority
FAO:	Food Agricultural Organization
FDA:	United States Food and Drug Administration
Fe:	Iron
Fig:	Figure
GSO:	General Statistics Office
Hg:	Mercury
HI:	Hazard Index
HSD:	Honest Significant Difference
Hue:	Thua Thien Hue
IOSPONRE:	Institute of Strategy and Policy on Natural Resources and Environment
Max:	Maximum
Min:	Minimum
MOH:	The Ministry of Health of Vietnam
MONRE:	Ministry of Natural resources and Environment of Vietnam
Pb:	Lead
PTDI:	Provisional Tolerable Daily Intake
SD:	Standard Deviation
SE:	Standard Error
SOCP:	Secretariat of the Communist Party of Vietnam
THQ:	Target Hazard Quotient
UN:	United Nations
VASEP:	Vietnam Association of Seafood Exporters and Producers
w.w:	Wet weight
WHO:	World Health Organization
Zn:	Zinc

I. INTRODUCTION

1. Heavy metals in the environment

Heavy metals are metallic elements with atomic weights and densities exceeding 5 g/cm^3 (Simeonov et al., 2011). Throughout history and the advancement of civilization, the discovery, development, and application of metals have driven the way that people live and societies have been organically shaped (Arthur, 2011). Today, with technological advancement, contaminants are widely utilized to assist and improve the living standards of the modern world (Arif et al., 2015). New challenges concerning environmental safety have appeared. Once unrestrained industrialisation and urbanisation processes without appropriate emission control and pollution abatement have put human lives at risk (Bennett et al., 2003). Especially in the developing countries the need for economic growth that generally relies on agricultural and industrial development has not complied or failed to meet environmental protection guidelines (Ikhuoria & Okieimen, 2000; Sahu & Arora, 2003). It is well known that some heavy metals, such as copper (Cu), zinc (Zn) and iron (Fe), serve as micronutrients at low concentrations but they are only toxic when in excess (Park et al., 2011; Vodyanitskii, 2013), while other heavy metals and metalloids, such as lead (Pb), cadmium (Cd), mercury (Hg), inorganic arsenic (As), aluminum (Al) and nickel (Ni), which are toxic even at very low concentrations, are of particular health concern (Schwartz et al., 2010; Pandey & Madhuri, 2014).

1. 1. Cadmium (Cd)

Cadmium is a non-essential element known to have toxic potential (EFSA, 2009). It is and responsible for various cases of poisoning through food. Small quantities of cadmium cause adverse changes in the arteries of the human kidney. Cadmium replaces zinc biochemically and causes high blood pressure and kidney damage. It interferes with enzymes and causes a painful disease called Itai-Itai (Rajappa et al., 2010).

The main routes of exposure to cadmium are via inhalation or cigarette smoke, and ingestion of food. Skin absorption is rare. Human exposure to cadmium is possible through several sources including the employment in primary metal industries, eating contaminated food, smoking cigarettes, and working in cadmium-contaminated workplaces, with smoking being a major contributor (Paschal et al., 2000). Besides, industrial activities such as mining, smelting and manufacturing of batteries are also cadmium emission sources (ATSDR, 2008).

Cadmium is persistent and once absorbed by an organism, remains resident for many years (over decades for humans) although it is eventually excreted (Tirkey et al., 2012). High exposure leads to obstructive lung disease and can even cause lung cancer. Cadmium produces bone defects in humans and animals (Tirkey et al., 2012).

1. 2. Lead (Pb)

Lead is one of the most abundant natural substances on earth. Owing to its physical properties, including low melting point and high malleability, it has widespread industrial use. In terms of usage, it ranks fifth on the list of metals (Karrari et al., 2012). Its use is associated with more than 900 industries, including mining, smelting, refining, battery manufacturing, etc. (Karrari et al., 2012; Malekirad et al., 2010). In addition to industry, it has applications in agriculture fertilizers and pesticides, and in the improvement of the gasoline octane rating in vehicular traffic systems (Hernberg, 2000). As a result of rapid industrialization, a rise in the effluent discharge from industrial units located close to rivers has increased lead amount in water bodies (Karrari et al., 2012).

Along with this, the application of sewage sludge directly or as a part of irrigation from contaminated water bodies, as an exhaust product of leaded gasoline due to increased traffic activities in urban settings and increased use as part of fertilizers and pesticide for agricultural purposes, has resulted in the pollution of soils, which has had a serious environmental impact (Jalali et al., 2008; Parizanganeh et al., 2010). Together, these (agricultural, industrial, and municipal) activities have resulted in the contamination of groundwater resources (Ebrahimi et al., 2011).

Lead exposure occurs mainly via breathing in lead-contaminated dust pieces or aerosols, and ingestion of lead-contaminated food, water, and paints (ATSDR, 2019). Adults take up from 35 to 50% of lead through drinking water while the absorption rate for children may be greater than 50%. Lead absorption is influenced by factors such as age and physiological status. In the human body, the greatest percentage of lead is taken into the kidney, followed by the liver and other soft tissues such as the heart and brain, however, the lead in the skeleton represents the major body fraction (Cascas & Sordo, 2006). The nervous system is the most vulnerable target of lead poisoning. Headache, poor attention span, irritability, loss of memory and dullness are the early symptoms of the effects of lead exposure on the central nervous system (CDCP, 2001).

1. 3. Mercury (Hg)

Mercury, considered the most toxic heavy metal, has become the part of the environment owing to the anthropogenic activities including agriculture, municipal wastewater discharge, mining, incineration, and discharges of industrial wastewater (Chen et al., 2012; Jan et al., 2009). Mercury exists in nature as an elemental or metallic form, in inorganic salts, and as organomercurial compounds. In its elemental form, mercury essentially exists as a liquid metal. Although used extensively in measuring equipment (pyrometers, thermometers, etc.), mercury arc and fluorescent lamps, and as a catalyst, its use as a component of batteries, in industries (pulp and paper), and mostly as amalgams in dental preparations is worth mentioning. Metallic mercury finds its entry into (1) air mainly through mining and burning processes; and to (2) water and soil through erosion of natural depots, discharges from industries and runoff from landfill sites. Inside the body, the average half-life of inhaled mercury is approximately 60 days (Chang, 1977).

Symptoms attributed to high-level exposure to metallic mercury include lung damage (pulmonary toxicity), mucous membrane changes, vomiting, diarrhea, nausea, skin rashes, increased heart rate or blood pressure (hypertension), renal dysfunction (nephrotoxicity), and severe neurologic abnormalities (Asano et al., 2000; Bates, 2003). The intellectual disorder that leads to behavioral changes, anxiety, depression, tremors, and reduced coordination of muscles are common neurological symptoms. Once released into the environment, inorganic mercury is acted on by bacteria, causing its transformation to methylmercury, having the ability to bioaccumulate in fish and other animal tissues (Hintelmann et al., 2010). In addition to the accumulation of neurotoxic molecules such as aspartate, serotonin, and glutamate, toxicities associated with methylmercury include microtubule destruction, lipid peroxidation and damage to mitochondria (Patrick, 2002). Compared to methylmercury, ethylmercury is rapidly metabolized into inorganic salts, thereby proceeding through nephrotoxicity (Carneiro et al., 2014).

On encountering mercury, humans are reported to develop a disorder, commonly referred to as acrodynia or pink disease (ASTDR, 2015). Symptoms associated with this disease include rashes, itching, redness and peeling of the skin from hands, nose and soles of the feet, sleeplessness, and/or weakness. Owing to its health hazardous effects, current levels set by Environmental Protection Act (EPA) and World Health Organization for drinking water are 0.002 mg/L and 0.001 mg/L, respectively (WHO, 2003).

1. 4. Zinc (Zn)

Zinc, a ubiquitous trace element, essential as a catalytic, structural, and regulatory ion, is indispensable for the growth and development of microorganisms, plants, and animals (Mocchegiani et al., 2000a). The average human intake of zinc ranges between 2.5 and 10 mg/day (Letavayova et al., 2006). It is well known for its role as a cofactor for superoxide dismutase (SOD), and it protects biological structures from damage caused by free radicals by maintaining adequate levels of SOD and metallothioneins, as well as preventing interaction between chemical groups with iron. It is a part of the zinc-dependent thymic hormone that is essential for thymic functions such as T-cell maturation and differentiation (Mocchegiani et al., 2000b).

Its antioxidant function is attributed to its function of blocking the negatively charged sites, thereby preventing lipid peroxidation. Its deficiency has mostly been associated with an increase in the levels of lipid peroxidation of mitochondrial and microsomal membranes along with osmotic fragility of the erythrocyte membrane. Zinc-binding proteins such as metallothioneins (MTs) are present in virtually all living organisms. These proteins play an important role in zinc uptake, distribution, storage and release, and are protective in situations of stress (exposure to oxyradicals), exposure to toxic metals, and low Zn nutrition (Vasak et al., 2000; Coyle et al., 2002). Zn as a part of MTs improves the excretion of metals such as Pb, As, etc. from the body. Besides having positive effects, supplementation of Zn has also been found associated with the displacement of essential metals to substitute normal physiological activities (Briner, 2014). On the other hand, where the supplementation of Zn seems to protect against oxidative damage of iron in the instance of iron supplementation, long term or higher dosage treatment of Zn has been associated with the depletion of copper (Maret & Sandstead, 2006). As such, a balanced approach in the supplementation of these metal ions is necessary to prevent unwanted complications.

1. 5. Copper (Cu)

Copper, an important trace metal, acts as a cofactor for a variety of proteins and enzymes required for maturation of cytoplasmic cuproproteins and assembly of enzymes in different cell organelles (ceruloplasmin and tyrosinase in case of Golgi apparatus and cytochrome c oxidase with respect to mitochondria). Copper uptake occurs in a tightly regulated process through specific high-affinity plasma membrane copper transporters or low-affinity permeases (DeFeo et al., 2007; Kim et al., 2008). Binding to chaperone proteins results in the transfer of copper to its final destination or any intermediate location from which its transport to other cell compartments or efflux out of cells can occur in cases in which concentration exceeds the optimum level.

Acting as a cofactor for a wide range of metal-binding enzymes, it fluctuates between the oxidized Cu (II) and reduced Cu (I) forms. In humans, its average intake ranges between 260 and 700 μg/day. Although adequate intake of copper provides protection against lead, higher intake has been associated with increased lead absorption (Millier rt al., 1990).

Its presence in excess amounts led to its involvement in the generation of highly reactive oxidative species (such as hydroxyl radicals), well known for their devastating effects in cells, particularly DNA damage and oxidation of proteins and lipids (Halliwell & Gutteridge, 1990). Cu(I) and Cu(II) that hold a high affinity for protein sites having cysteine, methionine, and histidine side chains act as potential ligands that led to the displacement of essential metal ions from their active sites, thereby resulting in the misfolding of proteins. As such, its uptake, followed by distribution and utilization, and finally, excretion from the body needs to be tightly regulated (O'Halloran & Culotta, 2000).

1. 6. Iron (Fe)

Iron in the form of hemoproteins and iron-sulfur centers is the most abundant transition metal in the body. Having unusual flexibility to serve both as an electron donor and acceptor, it contributes significantly to cellular reactions within living organisms. However, the property that makes it an essential element also contributes to its potential toxicity by catalyzing reactions that generate free radicals. Required for various functions, average dietary intake ranges from 3–8 mg/day, except for pregnant women who usually require a higher amount for the proper development of the child (National Academy of Sciences, 2000). Biological reductants such as O_2 and ascorbic acid are known to regulate free iron (Fe2+) concentrations (0.2–0.5 lM) (Valko et al., 2006; Fang et al., 2002). Representing an essential trace element, it is required for optimal physical and cognitive performance. Iron essentiality to the human body stems from the fact that its deficiency continues to be the most prevalent micronutrient deficiency that acts as one of the leading factors behind disability and mortality worldwide (Stoltzfus, 2001). In a survey conducted by the World Health Organization (WHO), 47.4% of children in the age group of 1–5 years, 25.4% of school children, 41.8% of pregnant and 30.2% of non-pregnant women, 12.7% of men, and 23.9% of the elderly population suffer from anemia arising out of iron deficiency (WHO, 2008). Upon stimulation by transition metal ions, Fe2+ mediated lipid peroxidation occurs after binding Fe2+ to negatively charged phospholipids, which results in the alteration of the physical properties of the phospholipid bilayer, and is followed by the initiation and propagation reactions of lipid peroxidation (Oteiza et al., 2004).

It is worth mentioning that iron, as a part of hemoglobin, counteracts the toxic effects of Pb. Its deficiency is associated with exacerbation of the toxicity of Pb to the hemopoietic system (Briner, 2014). Iron deficiency of anemic persons has been reported to demonstrate the significant impact of Pb compared with a normal iron load (Briner, 2014). At the intestinal surface, it competes with the deleterious metals for uptake and, as such, prevents the body from toxic effects. Its increased presence also helps in overcoming the competitive binding of heavy metals to the active sites of the enzymes that render them unable to perform their function (Skoczynska, 2008; Ahamed et al., 2007). A good example of this is the δ-aminolevulinic acid dehydratase (δ-ALAD) and ferrochelatase that are more prone to the inhibition by Pb.

2. Metal uptake by aquatic organisms

Metal uptake by aquatic organisms is a two-phased process, which involves initial rapid adsorption or binding to the surface, followed by a slower transport into the cell interior (Crist et al., 1988). In epithelial tissues, the last step is the rate-limiting factor in the transepithelial movement of metals (Foulkes, 1988). Transport of metals into the intracellular compartment may be facilitated by either diffusion of the metal ion across the cell membrane or by active transport by a carrier protein (Brezonik et al., 1991).

Many xenobiotics are accumulated in the organism. Some of them are quickly detoxified, but the vast majority is stored in tissues and organs. The bioconcentration and bioaccumulation factors and trophic transfer factor measure the levels of metals retained in an organism (Jakimska et al., 2011). Many data indicate that the dynamics of metal concentrations in various organs during exposure and depuration may be different. At the beginning of waterborne exposure metal concentrations in the gills rapidly increase, and then usually decline. After the exposure, metals are rapidly removed from the gills. In the case of dietary exposure, metal levels in the gills increase much slower and usually reach lower values. During dietary management, metal concentrations in the digestive tract rise and remain high until the end of the exposure and quickly reduce during depuration. In the case of waterborne exposure, metal levels in the digestive tract are usually low (Jezierska & Witeska, 2006).

Bioconcentration is the process resulting in the concentration of contaminants in an organism that is higher than in the surrounding environment (Shun-Xing et al., 2007). This term is also applicable to an organism studied under laboratory conditions, where a xenobiotic enters via the respiratory pathways or the skin. This process is characterized by the bioconcentration factor, i.e. the ratio of the concentration of a metal in an organism to its concentration in water (Deforest et al., 2007). In contrast, the bioaccumulation factor differs from the bioconcentration factor in that the xenobiotic concentration is the total amount of contaminant that has entered the organism by all possible pathways, i.e. via food intake, respiratory pathways, skin penetration, etc. (Deforest et al., 2007). It happens that the level of metal in the tissues of one organism does not elicit any toxic effect at all, but may constitute a danger to its predators. The concentration of a metal in the tissues of a predator may be higher (bioaccumulation) or lower (biodilution) than in the tissues of its victims (Deforest et al., 2007). The bioaccumulation of a metal resulting from the consumption of other organisms is characterized by the trophic transfer factor, i.e. the ratio of the concentration of a metal in an organism (predator) to its concentration in the food that organism consumes. The trophic transfer factor is useful for assessing the extent to which metal has been transferred from an organism on a lower trophic level to an organism on a higher one.

3. The effect of metals on marine organisms

Despite the progress made in the environmental waste management, marine ecosystems are exposed to a great variety of contaminants, heavy metals included (Jakimska et al., 2011). Heavy metals can remain in the environment for a long time and non-biodegradable, they may affect living organisms even when their environmental concentrations are small (Wepener et al., 2001). Their harmfulness is due not only to the degree of contamination of the environment but also to the biochemical role in metabolic processes and the extent to which they are absorbed and excreted by marine organisms (Jakimska et al., 2011).

During metal ingestion, metals are specifically transported by lipoproteins into different body compartments, where they can be specifically oriented to different centers: (i) action centers where the toxic metal interacts with an endogen macromolecule (protein or ADN) or a certain cellular structure inducing toxic effects for all body; (ii) metabolism centers where the metal is processed by detoxified enzymes; (iii) storage centers where the metals are collected in a toxic inactive state; and (iv) excretion centers where the metals are disposed (Gheorghe et al., 2017).

The heavy metal overload has inhibitory effect on the development of aquatic organisms (phytoplankton, zooplankton, and fish) (Atici et al., 2010; Bere et al., 2012). The metallic compounds could disturb the oxygen level and mollusks development, the formation of byssus, as well as the reproductive processes. Several histological changes such as gill necrosis or fatty degeneration of the liver occur in the fish and crustaceans (Bere et al., 2012; Sevcikova et al., 2016).

In fish, heavy metals in water are particularly dangerous for fish juveniles and may considerably reduce the size of fish populations or even cause the extinction of the entire fish population in the polluted reservoirs. The data of many authors indicate that heavy metals reduce the survival and growth of fish larvae. They also cause behavioral anomalies (such as impaired locomotors performance resulting in increased susceptibility to predators) or structural damages (mainly vertebral deformities) (Stomiñska & Jezierska, 2000).

4. Heavy metals in fish

It is well known that fish plays an important role in the human diet. Fish is not only a source of proteins and essential fats, but also a source of important nutrients, including long-chain omega-3 fatty acids, iodine, vitamin D, and calcium (FDA, 2006; Kruzikova et al., 2013; Vicarova et al., 2015). However, it can represent a dangerous source of some heavy metals (Kruzikova et al., 2013).

Aquatic biota has been used to monitor heavy metal pollution in aquatic ecosystems for decades (Etesin & Benson, 2007; Kamaruzzaman et al., 2011). The choice of biota depends on several factors like the heavy metal accumulating potential of the organism, utility, economic value, etc. (Ndimele & Kumolu-Johnson, 2012). One of the most efficient methods of assessing chemical pollution in aquatic ecosystems is the determination of the accumulated pollutant loads in organs of different animals populating the site of interest (Salánki & Salama, 1987). In such biological monitoring studies, fish proved to be the most appropriate indicator organisms, due to their longer life span and the top position in the aquatic food chain (Rashed, 2001; Oguzie & Izevbigie, 2009). Therefore, fish are useful as bio-monitors of metal pollution because they can help to understand the risk to the aquatic ecosystem and to humans (Peakall & Burger, 2003). The use of wild and cultured food fish as bio-monitors of metal pollution in aquatic ecosystems is becoming popular throughout the world (Yi et al., 2011; Copat et al., 2012; Saha & Zaman, 2013; Jaishankar et al., 2014; Alam et al., 2015; Frías-Espericueta et al., 2016; Rajan, 2017; Saher et al., 2018).

Heavy metals entre in fish bodies in three possible ways: by gills, digestive tract, and body surface. The gills are considered a significant site for direct uptake of metals from water (Beijer & Jernelov, 1986), though the body surface is normally estimated to take a minor part in the uptake of heavy metals in fish (Selda & Nurşah, 2012). The toxic effects of heavy metals can affect the individual growth rates, physiological functions, mortality and reproduction in fish (Amundsen et al., 1997).

Metal existence in fish tissues depends not only on their feeding habits, ecological needs, metabolism but also on the age and size of fish (Peakall & Burger, 2003; Marcovecchio, 2004). Heavy metals accumulate in many important organs (Golovanova, 2008) such as muscle and often result in high concentrations in the liver, gills, kidney, gills (Dural et al., 2007; Golovanova, 2008).

Diet is the main route of exposure to heavy metals in the case of the population not exposed to them. Therefore, contaminants received through the food chain as a result of pollution are probable chemical hazards, threatening the consumers. The aftereffect of heavy metal contaminations can be hazardous to man through his food. Therefore, it is important to assess and monitor heavy metal in aquatic environments (water, sediment, and fish).

5. Vietnam fishery sector

The fishery is a key national economic sector of Vietnam, taking into account its contribution to the achievement of food security, the alleviation of poverty, creation of sustainable livelihood and the generation of rural employment. In 2018, the fishery accounted for about 4-5% of Gross Domestic Product (GDP) and 9-10% of total national turnover, creating jobs for about 4 million people. According to a report by FAO (2019), Vietnam is one of the top 5 seafood export countries in the world with a high export rate to the world's top markets such as the United States of America, Europe, Japan.

The total fishery production has kept rising over the last ten years from 5 million tonnes in 2008 to nearly 7.74 million tonnes in 2018, a 7.13% year-on-year increase, of which 4.15 million tonnes was from aquaculture and 3.59 million tonnes from capture production (GSO, 2018). The most important seafood products for export are shrimp, pangasius, tuna, squid and octopus. In 2018, Vietnam's seafood exports increased by 8.4% to nearly USD 9 billion, led by shrimp exports which account for 39.8%, followed by pangasius with 25.1%. Under this plan, the seafood industry is expected to contribute 30-35% of the agro-forestry-fisheries sector's GDP and the total fisheries production to reach 6.5-7 million tonnes, with the aquaculture production of 65-70%, by the end of 2020 (DOF, 2019).

5. 1. Capture fishery

Geographically, there are four main fishing areas in Vietnam: Gulf of Tonkin (shared with China); Central Vietnam; South-Eastern Vietnam; and South-Western Vietnam (part of the Gulf of Thailand).

Marine catches are the highest in Central and Southern Vietnam, especially from the Khanh Hoa Province to the Ca Mau Province.

According to VASEP (2019), as of May 2018, the total number of fishing vessels nationwide is 108,504, the reduction of 1,158 vessels compared to 2017. In particular:

- Vessels with the engine under 90 HP: 70,437
- Vessels with the engine from 90 to under 400 HP: 20,231
- Vessels with the engine of 400 HP or more: 17,836

The fishing vessels are mostly made of wood (about 99%), while steel and composite boats have been built recently, under the Government's support policies for fishery development, but comprise only a negligible number (VASEP, 2019).

Currently, there are about 40 kinds of marine fishing gears in Vietnam, which could be grouped into six, including gillnet, trawl, purse seine, hook and line, stick-falling net, and others (DOF, 2019).

The production from marine capture fisheries has developed rapidly over recent years, in 2018 reaching 3.37 million metric tonnes, up 5.67% year-on-year (DOF, 2019).

Besides focusing on offshore fishing, inland capture fisheries have also developed in Vietnam, which has an ancient tradition for both collecting and capturing fish direct from the coastal, shallow mangroves, estuaries, lagoons, river, lakes, particularly in the floodplains and fields along of the Mekong and Red River deltas, where they have been providing an important source of aquatic products for rural people's seasonal nutrition.

A variety of simple, as well as sophisticated, fishing gear, such as gillnet, long-line, lift-net, push net (illegal) and traps, is used for the capture of all kinds of fish and shellfish species. According to DOF (2019), in addition to the 8 million people whose livelihood depends on these fisheries as the household primary income source, there are another 12 million who get a part of their revenue or subsistence from fisheries.

The inshore fishery (often up to 4-5 miles from the coast) depends on a fleet of about 28,000 non-mechanized canoes and boats, and approximately 45,000 smaller mechanized boats with long-tail or one-cylinder diesel engines up to approximately 20hp. Almost all of these vessels operate directly from the beach or estuaries and harbor facilities (GSO, 2018).

5. 2. Culture fishery

Aquaculture production according to GSO (2018), aquaculture production has been growing considerably during the period from 2006 to 2016 with the average annual growth rate of 8%, making significant contributions to the country's total fisheries production. As of 2018, Vietnam's aquaculture production increased by 8.2% year-on-year to 4.15 million tonnes. Pangasius and shrimp continue to be the two major products.

6. The status of pollution of the marine environment in Vietnam

Vietnam is a coastal country located on the Indochinese peninsula of South East Asia. With a coastline of over 3,260 km stretching from North to South, it is ranked 27th among 157 coastal countries, island nations and territories in the world (Costa-Böddeker et al., 2017). Vietnam's sea has been acknowledged as a country with high biodiversity, with more than 3,000 aquatic creatures that have been identified in the interior wetlands. The tropical marine with more than 20 typical ecosystems is also home to more than 11,000 sea creatures (MONRE, 2008). Besides the great contribution to the development and economic growth of the country, Vietnam's sea has been facing a series of environmental problems. According to the research results of the Institute of Strategy and Policy on Natural Resources and Environment Vietnam, in recent years, the marine environment is facing threats from (i) Pollution originating from the continent and the sea, (ii) Destroying natural habitats, (iii) Exploitation and overfishing.

6. 1. The increasing sources of marine pollution

In recent years, the increase in waste sources from the continent, especially along with the river flows to the sea, has led to the deterioration of marine quality in many places. Many estuaries have been polluted by industrial and urban wastewater. The discharge of untreated or substandard waste is becoming more and more complicated even in coastal provinces, causing great economic, life and livelihood losses of the community coastal and unpredictable damage to ecosystems and marine life (MONRE, 2018). According to the report of the first state of the marine environment released by the United Nations in 2016 (UN, 2016), over 80% of marine pollution comes from the mainland. Another source comes from marine activities from boats, platforms, seabed. The main origin of wastewater to the sea includes the domestic wastewater coming from urban areas and industrial zones which flow into the river and then to the sea. Since Vietnam has a dense river system, these are the roads to transport garbage and waste from inland to the ocean (GSO, 2018). Besides, wastewater, waste oil, and chemicals of ships at sea are also a notable source of pollution to the marine environment (Villegas et al., 2016).

According to Law (2017), in 12 countries which have a significant impact on the marine environment, Southeast Asia has 5 representatives, including Thailand, Vietnam, Malaysia, Indonesia, and the Philippines. The dangerous thing is that compared to the current economic development position, the developing countries have been the leading cause of the environmental pollution in recent decades. In 2017, Indonesia, China, Philippines, Thailand, and Vietnam dump more plastic waste into the sea than all the rest of the world combined (Duong, 2019).

The representative of the United Nations Environment Program announced that in 2018, Vietnam, emitting to the oceans from 0.28 to 0.73 million tons of plastic waste every year (accounting for 6% of the world’s total), ranked the fourth in the world. The pollution of marine waste not only affects the quality of the environment and ecosystems but also affects the economic development and coastal communities (Li et al., 2016, Duong, 2019).

The pollution of organic matter, oil and grease has been taking place quite commonly in the coastal provinces of Vietnam, especially in the estuaries of Northern provinces and along the coastal strip of the Mekong Delta. Although organic pollution is only local, it is quite high and exceeds the permissible level near the tourist areas, densely stretching from the North to the South, comprising Cua Luc, Sam Son, Da Nang, Nha Trang, and Vung Tau. In these areas, eutrophication, red tide, and toxic algae have been a prominent environmental problem (Hoang, 2018). There is no denying the economic benefits from oil and gas exploration or the shipping industry, however, a large amount of waste from these activities is released into the environment every year. They generate about 5.600 tons of petroleum waste, more than 15.000 tons of floating oil and grease, 23-30% of which are untreated hazardous solid physical waste annually (Hoang, 2018). In addition, the oil spill is also one of the causes affecting the marine environment. According to statistics of the Ministry of Natural Resources and Environment in the period of 2010 to 2017, there were over 100 oil spills from large ships (MONRE, 2018).

6. 2. Unsustainable exploitation

Marine resources are being over-exploited and unsustainable, the destruction of coral reefs, seagrass beds, and mangroves is increasing in many places. According to estimates, all the country's waters from Quang Ninh to Ha Tien have lost about 40-60% of seagrass, mangroves have lost up to 70%, and about 11% of coral reefs have been destroyed, unable to recover by themselves. Pristine mangroves are almost gone. The serious decline of the mangrove area has led to the decline of marine biodiversity, especially the loss of spawning grounds and habitat of aquatic species (SOCP, 2018). The seagrass ecosystems are ones of the most important marine ecosystems but are now in danger of being damaged and degraded. They are distributed from the North to the South and along with the islands, at the depth of 0-20m, currently only about 5.583ha. In some areas, seagrass beds have almost no opportunity to recover naturally due to too many impacts like tourism and aquaculture activities in this area (Cat Ba, Ha Long, Quang Nam) (SOCP, 2018). As a result, marine species living in the seagrass ecosystem are at risk of shrinking habitat, directly threatening biodiversity.

6. 3. Exploitation and overfishing

To date, about 100 of marine species in our country are becoming rare and endangered, and have been included in the Vietnam Red Book and on the IUCN Red List as requiring protection measures (37 species of marine fish, 6 corals, 5 species of echinoderms, 4 species of dragon shrimp, 1 species of horseshoe crab, 21 species of snails, 6 species of bivalve shells, 3 species of squid) (IOSPONRE, 2018). Research results of FAO and some other international organizations in recent years have shown that about 80% of fish on coastal and offshore areas of Vietnam have been exploited, 25% of the fish is overfished or depleted and the catches decreased significantly. Many other marine species are at risk of extinction (IOSPONRE, 2018).

Open access regime is a key problem leading to overcapacity and overfishing which needs to be more significantly addressed. Due to open access, everyone can approach the fishing areas and there is no effective mechanism to control the fishing entry. Therefore, too many people, but not enough fish are the factors leading to the competition and destruction of fishery resources and habitats, contributing significantly to illegal, unreported and unregulated fishing. Under the current system of state-management, there is a shortage of resources (HR, finance) to effectively manage, monitor and regulate the fisheries. There is also a lack of management information (number of vessels, the position of operation, catch) and a shortage of officials (quantity and quality) (VASEP, 2019).

II. RESEARCH AIMS

The purpose of the study is to evaluate and compare metal accumulation in 7 common fish species to diagnose environmental pollution and determine the impact of habitat. At the same time the aim is to determine the absorption and biological distribution of 6 metals (mercury, cadmium, lead, iron, zinc and copper) in the organs and tissues of 7 fish species in 5 different areas. From this, we can compare the ability to absorb and distribute heavy metals between these 7 fish species, identify issues that can reduce the ability of metal accumulation, as well as show the relationship between metals and tissues in researched fish species. At the same time we will assess the potential health risks of consumers through the Estimated daily intake, Target hazard quotient, and Hazard index.

The hypotheses are reached as follows:

- There is a significant difference in the concentrations of heavy metals in the same fish species from 5 station studies.

- There is a significant difference in the concentrations of heavy metals between 7 fish species.

- The highest metal concentrations were accumulated in the liver, lower – in the muscles in all studied fish species.

- The levels of heavy metal contaminants in the selected food fish species do not exceed the WHO set limits for food for human consumption.

- The Estimated daily intake values of all heavy metals in all tested fish were below the recommended threshold.

- The THQ and HI values of individual metals in all fish species are lower than 1.

III. MATERIALS AND METHODS

1. Study subjects

1. 1. Yellowfin seabream (*Acanthopagrus latus*)

Yellowfin seabream (*Acanthopagrus latus*) belongs to the genus Acanthopagrus, the family Sparidae, the order Perciformes. They are demersal inhabitants found in warm coastal waters, from shallow to relatively deeper ones, widely distributed throughout the Indo-Pacific (Lee & Al-Baz, 1989; Xia et al., 2008; Iwatsuki, 2013). In Vietnam, where it is also widely found in the coastal estuaries, it was recorded as a commercially and ecologically important species (Xia et al., 2008; Tran et al., 2019).

Figure 1. *Acanthopagrus latus*

1. 2. Dotted gizzard shad (*Konosirus punctatus*)

Dotted gizzard shad (*Konosirus punctatus*) belongs to the family Clupeidae, the order of Clupeiformes and is the only member of the genus Konosirus. It is a common pelagic fish in the East China Sea found in a lot of the coasts and estuaries. It tends to migrate into shallower brackish water for breeding (Kong et al. 2004; Song et al., 2017). *K. punctatus* size is not too large. It lives in groups and its diet includes small numbers of rhizopods, tintinnids, euglenoids, copepods, larval bivalves, dinoflagellates, diatoms, and unidentified materials. *K. punctatus* is recorded as a fish of economic value in Vietnam (Thiep et al., 2017).

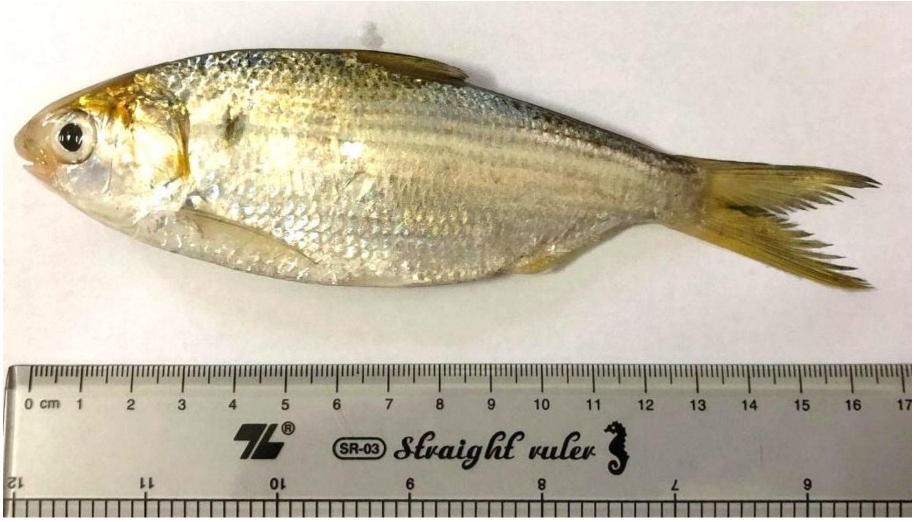

Figure 2. *Konosirus punctatus*

1. 3. The flathead grey mullet (*Mugil cephalus*)

The flathead grey mullet (*Mugil cephalus*) is an important food fish species in the mullet Mugilidae family, the Mugiliformes order (Thomson, 1997). It is a coastal fish found in temperate, subtropical and tropical regions within marine, brackish and freshwater habitats worldwide (Piras et al., 2018). Adult mullet often enters estuaries and freshwater environments, it forms huge schools near the surface over sandy or muddy bottoms (Silva & Silva, 1981). It was found to feed mainly on diatoms, algae, copepods, decayed organic matter, sand & mud (Islam et al., 2009).

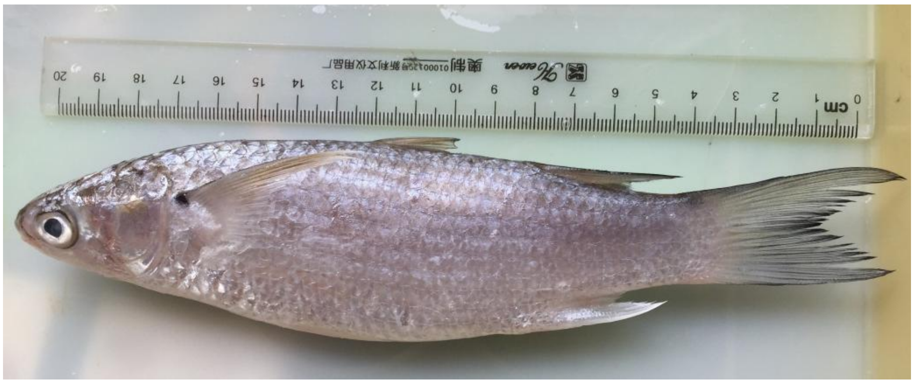

Figure 3. *Mugil cephalus*

1. 4. Mottled spinefoot (*Siganus fuscescens*)

Mottled spinefoot (*Siganus fuscescens*) is in a single genus Siganus, the only genus in the family Siganidae, the order Perciformes (Froese & Pauly, 2019). It is widely distributed in shallow coastal habitats throughout the Indo-Pacific Ocean and the eastern Mediterranean Sea. *S. fuscescens* has delicious meat, which is a popular target in Vietnam, so it is not only the object of interest of fishermen in the structure of commercial farming in Vietnam but also in the world (Soliman et al., 2009; Hsu et al., 2011; Linh et al., 2018).

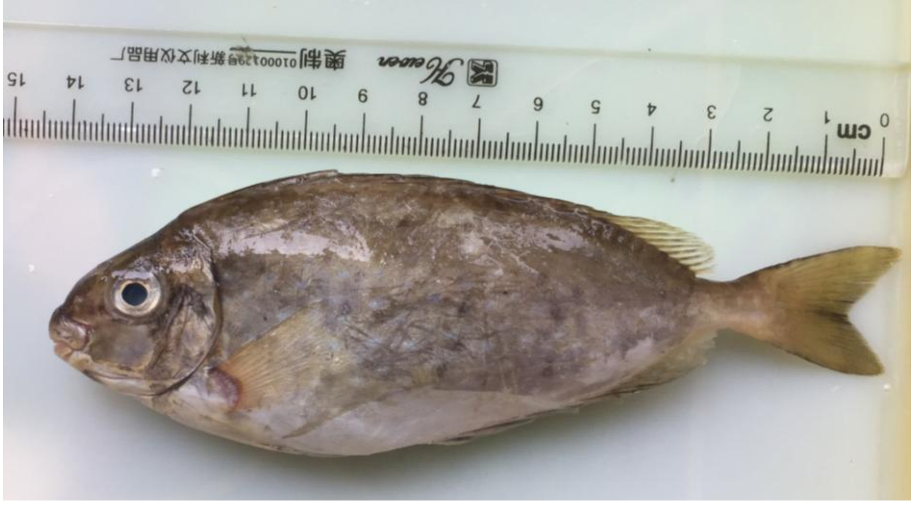

Figure 4. *Siganus fuscescens*

1. 5. Silver sillago (*Sillago sihama*)

Silver sillago (*Sillago sihama*) belongs to the genus Sillago, which is the most widespread and abundant member of the family Sillaginidae, the order Perciformes (Liu et al., 2012). It distributes in the Indo-Pacific region and is common along beaches, sandbars, mangrove creeks and estuaries (Allen et al., 2002). It has been found as carnivorous fish with crustacean, teleosts, mollusks, echinoderms and polychaetes feeder in its diet (Khan et al., 2014). It was recorded as one of the commercially most important fish species (Hou et al. 2011).

Figure 5. *Sillago sihama*

1. 6. Sulphur goatfish (*Upeneus sulphureus*)

Sulphur goatfish (*Upeneus sulphureus*) of the genus Upeneus, the family Mullidae, the order Perciformes are predominantly benthivores which live in marine and brackish water habitats above sandy or muddy bottoms, their food ingredients are mainly crustaceans (Ramteke et al., 2015). Their main distribution in East Africa to southeast Asia, north to China, south to northern Australia (Mohamed & Resen, 2010).

Figure 6. *Upeneus sulphureus*

1. 7. Whipfin silver-biddy (*Gerres filamentosus*)

Whipfin silver-biddy (*Gerres filamentosus*), belonging to the order Perciform, the family Gerreidae, the genus Gerres, are widespread in all the warm seas of the Indo-Pacific, from the east coast of Africa through the Indo-Malayan Archipelago, South China Sea, Northern Australia and the west Pacific islands also westward to the east and South Africa (Iwatsuki et al., 2015). They have been reported to be very common in coastal and estuarine fish species in Central Vietnam (Hoang & Duc, 2012).

Figure 7. *Gerres filamentosus*

2. Study area

The main study site is at 5 large estuaries, the sampling range at each position equals approximately 40 km^2 (Fig. 8); the coordinates at S1, S2, S3, S4, and S5 were 18°46'06.8"N 105°46'05.0"E, 18°16'01.6"N 106°07'10.8"E, 17°42'19.0"N 106°29'30.8"E, 17°00'55.7"N 107°06'47.3"E, 16°34'37.0"N 107°37'16.2"E, respectively). The study area is located in the North Central region of Central Vietnam and is one of the country's 7 key economic zones. Currently, with the available potentials, the process of industrialization and modernization is taking place rapidly and strongly, leading to the confrontation with the risks of the environmental pollution.

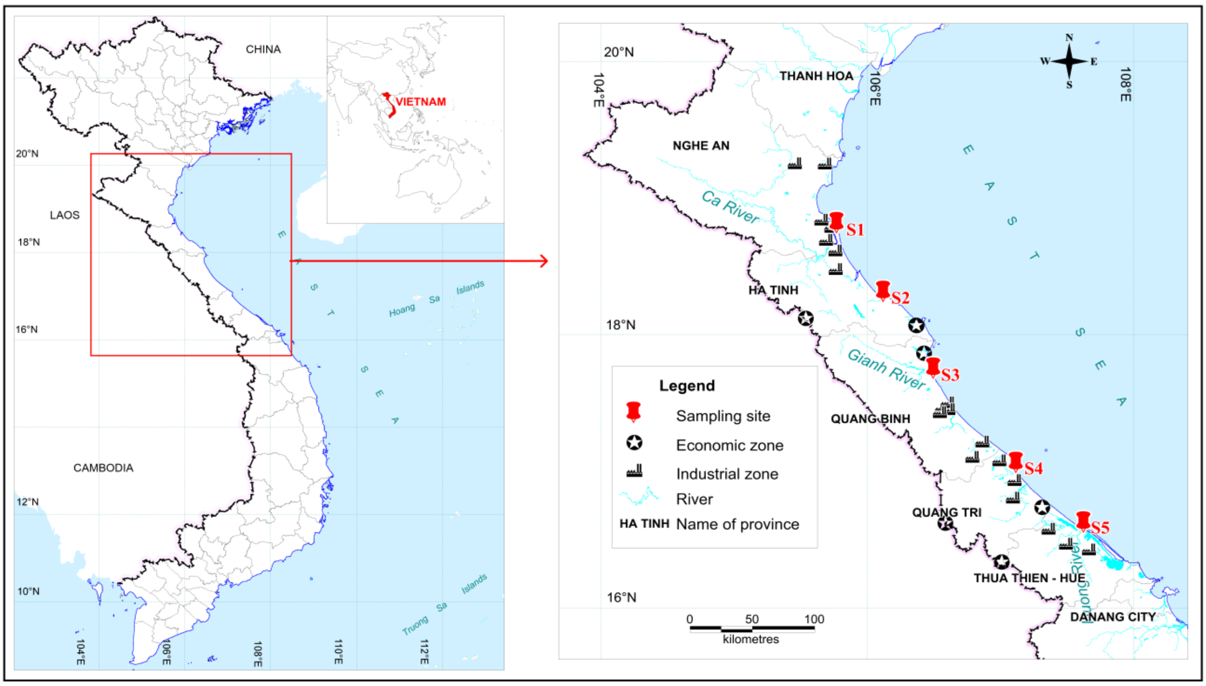

Figure 8. Fish sampling area

3. Sample collection

A total of 351 individuals of 7 species (*A. latus* (50), *K. punctatus* (50), *M. cephalus* (50), *S. fuscescens* (50), *S. sihama* (50), *U. sulphureus* (50), and *G. filamentosus* (51)) was collected at five different locations in the coasts of Central Vietnam in 2018 (Fig. 8). These fish species are abundant on the shoreline and in the estuaries in Central Vietnam. They possess high-quality meat, wide salinity, temperature tolerance and have a low position in the food chain. Besides, they are heavily consumed by the locals.

The five sampling locations are the five major estuaries of the five central provinces of Vietnam (Nghe An, Ha Tinh, Quang Binh, Quang Tri, and Hue). Fish samples were collected at fish markets, and from local fishermen in that area. Selected individuals were still fresh. They were measured for length and weight, then scales, muscles, liver, and gills were dissected, put into marked plastic bags before the transfer to storage in the laboratory of the Quang Binh University. During the transport from Vietnam to Poland, the samples were also kept in an ice chest at -4°C. They were stored at -20°C in the laboratory at the Institute of Biology, Pedagogical University of Krakow (Poland) until the time of the analysis.

4. Determination of metal concentrations

All the analyses were conducted in the Institute of Biology, Pedagogical University of Krakow (Poland). The concentrations of Cd, Cu, Fe, Pb and Zn were measured in ca. 2g of wet weight, firstly oven-dried (60°C, SUP-100W dryer, WAMED), then mineralized with hot nitric acid (65%, Baker Analyzed, JT Baker, USA) in the open mineralization system (Velp Scientifica DK20).

Mineralized solutions were diluted up to 10 mL with ultrapure water (18.2 MΩ cm at 25°C, Direct-Q 3, Merck-Millipore, Germany) and analyzed with a flame atomic absorption spectrometer (AAnalyst 200, PerkinElmer, USA). Final results, after the comparison with the LoQ and the calculations were presented as a µg of metal per 1g of the dry sample (µg/g d.w).

Hg concentrations were measured with a cold vapor atomic absorption spectrometer (MA-2, NIC, Japan) in ca. 100 mg of fresh sample. The results obtained in µg/g of wet weight (w.w).

All the analyses were run twice and the average was considered as the final result. If the RSD between repeats was higher than 15%, the analysis was checked again. Every ten samples, the spike and quality control solutions with the known concentrations were tested. All the recoveries were ranged from 90 to 110% for each metal (Table 1).

Table 1. Characteristics of the analytical method used: wavelength, limits of quantification (LoQ) established for semen samples; recoveries for certified reference materials (CRM) with relative standard deviation (RSD) of 10 replicates

Metal	Wavelength λ (nm)	LoQ (mg L^{-1})	CRM	Recovery (%)	RSD (%)
Hg	253.7	0.208*	BCR-463	97.1	2.6
Cd	228.8	0.010	SRM1577b	91.6	4.3
Pb	283.3	0.107	SRM1577b	93.7	6.2
Fe	248.3	0.415	SRM1577b	101.6	6.2
Cu	324.8	0.035	SRM1577b	99.6	2.7
Zn	213.9	0.024	SRM1577b	106.2	5.3

* LoQ value for Hg expressed as a ng per sample.

5. Human health risk

5. 1. The estimated daily intake (EDI)

EDI (µg/kg b.w per day) provides an estimation of expected dietary exposure and was calculated for Vietnamese fish consumption (Ahmed et al., 2015). The parameter was calculated as follows:

$$\mathrm{EDI} = \frac{\mathrm{CM\ x\ CONS}}{\mathrm{BW}}$$

CM – metal concentration measured (µg/g w.w, considering that the d.w represents 23–33% of the corresponding w.w (Burgera & Gochfeld, 2005; Rahman et al., 2012);

CONS – the consumption rate (g/day, 45.85 per inhabitant of Central Vietnam (Needham & Funge-Smith, 2015));

BW – body weight (kg, 54.6 per an adult inhabitant of Central Vietnam (Pham et al., 2019)).

5. 2. Target hazard quotient (THQ) for non-carcinogenic risk assessment

THQ is an indicator of the subject's risk after pollutant exposure, which is expressed as a ratio of metal EDI to its reference dose (RfD) (USEPA, 2019). If the ratio is less than 1, no health risk should be observed. The THQ was determined according to the following equation:

$$\mathrm{THQ} = \frac{\mathrm{EDI}}{\mathrm{RfD}} \mathrm{x}10^{-3}$$

Where EDI stands for estimated daily intake and RfD for the reference dose (RfDs of Hg, Cd, Pb, Fe, Zn, and Cu are 0.0003, 0.001, 0.004, 0.7, 0.04, and 0.3, respectively (USEPA, 2019)).

5. 3. Hazard index (HI)

A summation of the target hazard quotients (THQ), for all the metals an individual is exposed to, was used to estimate the hazard index (HI):

$$\mathrm{HI} = \mathrm{THQ_{Hg}} + \mathrm{THQ_{Cd}} + \mathrm{THQ_{Pb}} + \mathrm{THQ_{Fe}} + \mathrm{THQ_{Zn}} + \mathrm{THQ_{Cu}}$$

Where THQ_{-} stands for the target hazard quotients (calculated for Hg, Cd, Pb, Fe, Zn and Cu, respectively).

6. Statistical analysis

Mean, standard deviation, standard error, minimum and maximum of heavy metals of the samples were calculated using Statistical 13.3 (StatSoft, Poland). The heavy metal concentrations in the samples were tested for normality using the Shapiro-Wilk test. To identify any significant differences in the accumulation of heavy metals between organs in one fish species, between other fish species and between location studies analysis of variance were checked. When the distribution was normal parametric ANOVA test were performed, if Levene's test showed that variances are not equal non-parametric Kruskall-Wallis test were used. For non-normal distribution Kruskal-Wallis test were used. The Tukey post hoc (HSD/Tukey HSD for unequal N) (for normal distribution) and multiple comparisons of mean ranks tests (for non-normal distribution) were made. Data were considered significant at $p \leq 0.05$

IV. RESULTS

1. Concentration of heavy metals in *Acanthopagrus latus*

1. 1. Hg concentration

The mean, SE, SD, min and max values of the total Hg content in the muscle tissue, liver and gills of *A.latus* are presented in Table 2 and Fig. 9.

Table 2. The level of Hg in liver, muscles, gills of *A. latus* ($\mu \cdot g^{-1}$w.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.305±0.059	0.089±0.004	0.142±0.013
	Min-Max	0.105-0.634	0.065-0.105	0.084-0.219
	Shapiro-Wilk test (p)	0.250	0.239	0.738
	Levente test	F=15.293; p<0.001		
	Kruskal-Wallis test	H=17.459; p<0.001		
Ha Tinh (n=10)	Mean±SE	0.215±0.068	0.088±0.019	0.274±0.042
	Min-Max	0.058-0.723	0.038-0.220	0.128-0.463
	Shapiro-Wilk test (p)	0.003	0.042	0.068
	Kruskal-Wallis test	H=11.812; p=0.003		
Quang Binh (n=10)	Mean±SE	0.314±0.061	0.051±0.005	0.546±0.087
	Min-Max	0.086-0.643	0.035-0.089	0.257-1.095
	Shapiro-Wilk test (p)	0.138	0.021	0.058
	Kruskal-Wallis test	H=21.071; p<0.001		
Quang Tri (n=10)	Mean±SE	0.313±0.059	0.100±0.016	0.525±0.091
	Min-Max	0.105-0.634	0.056-0.219	0.219-1.095
	Shapiro-Wilk test (p)	0.378	0.012	0.042
	Kruskal-Wallis test	H=17.920; p<0.001		
Hue (n=10)	Mean±SE	0.391±0.148	0.085±0.016	0.149±0.028
	Min-Max	0.046-1.148	0.036-0.198	0.051-0.282
	Shapiro-Wilk test (p)	0.002	0.088	0.080
	Kruskal-Wallis test	H=3.665; p=0.160		

The analyses showed that the levels of Hg in organs of *A. latus* were different in Nghe An. The differences were statistically significant, which was shown by ANOVA Kruskal – Wallis test (H=17.459, p<0.001). The highest level of Hg was in the liver, the lowest was in muscles. This difference was strongly significant in what was shown after being checked by the effect of multiple comparisons of mean ranks, which is illustrated by number 1 in Fig. 9.

At Ha Tinh, the highest mean concentration of Hg was found in the gills, and the lowest – in the muscles. Statistical analysis showed that the differences between the Hg content in the gills and muscles are significant. The results of the Kruskal-Wallis analysis and the effect of multiple comparisons of the mean ranks of Hg in the tissues of *A. latus* are summarized by number 2 in Fig. 9 (p=0.002). At Quang Binh, the highest Hg level was found in gills, followed by liver and muscles. The result of the test by the effect of multiple comparisons of mean ranks showed that the Hg accumulation in gills and liver was significantly higher in muscles (number 3, 4 in Fig. 9). At Quang Tri, the Hg concentrations were the highest in gills, the lowest in muscles. There were significant differences in the Hg levels among liver to muscles (p=0.007) and gills to muscles (p<0.001), proved by multiple comparisons of mean ranks for 3 groups (number 5, 6 in Fig. 9). There was no significant difference between groups (Kruskal-Wallis test, p>0.05) from Hue.

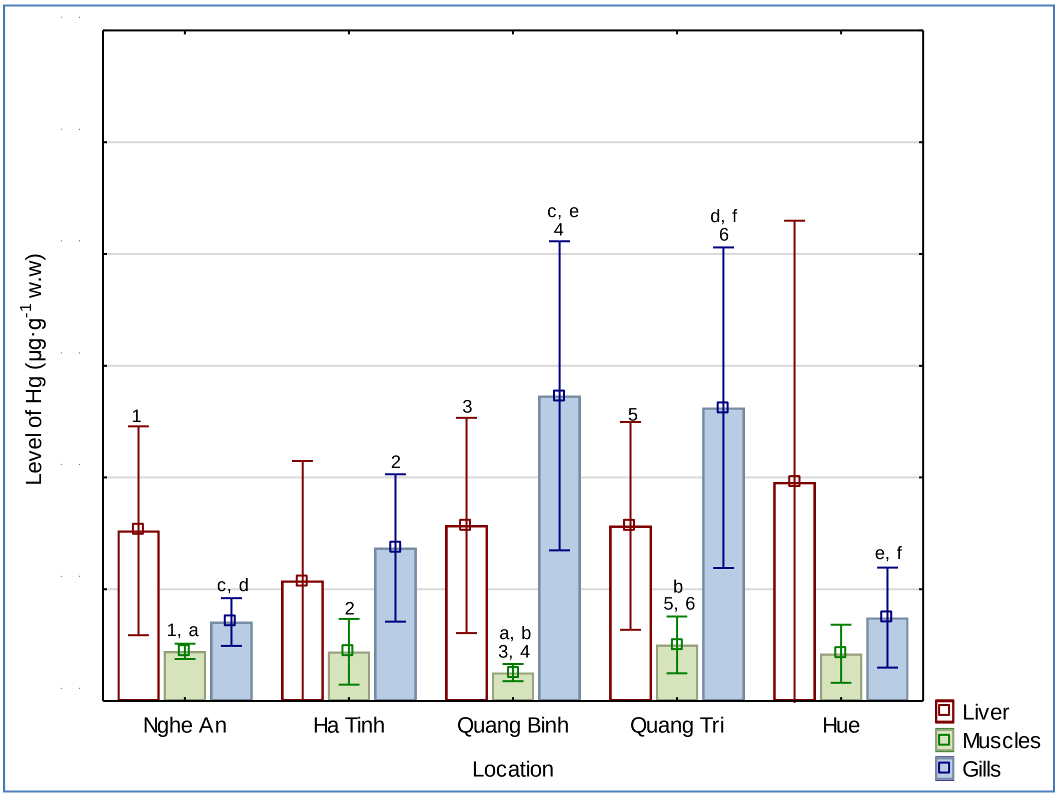

Figure 9. The mean, SD of Hg concentration in tissues of *A. latus*
(1, p<0.001; p=0.002; 3, p=0.007; 4, p<0.001; 5, p=0.019; 6, p<0.001; a, p=0.027; b, p=0.038; c, p=0.001; d, p=0.002; e, p<0.001; f, p=0.002)

The Hg concentrations in the liver were the highest at Hue (Kruskal-Wallis test, p=0.042), compared to all other locations, but there were no significant differences in the Hg levels among the 5 locations. The levels of Hg in muscles were the lowest in Quang Binh and the highest in Quang Tri. According to the presented effect of multiple comparisons of mean ranks in muscles, there was a significant difference between Quang Binh and Nghe An (a, p=0.027), and Quang Tri and Quang Binh (b, p=0.038). Unlike Hg in the liver and muscles, Hg concentrations in gills were found to be the lowest in Nghe An and Hue. Hg concentrations in Quang Binh were significantly higher than those in Nghe An (e, p=0.001) and Hue (e, p<0.001), and the accumulation of Hg in Quang Tri was significantly different in Nghe An (d, p=0.002) and Hue (f, p=0.002).

1. 2. Cd concentration

The mean, SE, SD, min and max amounts of Cd ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *A. latus* are summarized in Table 3 and Fig. 10.

In Nghe An, the Cd concentrations in the liver were significantly higher than in muscles (1, p=0.008) compared to all tissues, but there were no significant differences in the Cd levels among the liver and gills, as well as among muscles and gills. The Cd concentrations were not significantly (p>0.05) different among liver, muscles, and gills from Ha Tinh. Meanwhile, in Quang Binh, the Cd content in the liver was significantly higher in muscles (2, p<0.001), and in gills (3, p=0.002). In Quang Tri and Hue, the difference was only found for the Cd level in liver and muscles (4, 5).

Besides, the analysis of the effect of multiple comparisons of mean ranks of Cd in tissues of *A. latus* showed that the Cd concentrations in the liver were higher in Quang Binh as compared to Ha Tinh (a, p=0.008), but there were no significant differences in the Cd levels among other locations (Nghe An, Ha Tinh, Quang Tri, and Hue). The results also suggest that the accumulation of Cd in muscle and gills is similar in all other locations.

Table 3. The level of Cd in liver, muscles, gills of *A. latus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.026±0.003	0.015±0.001	0.018±0.002
	Min-Max	0.016-0.047	0.008-0.020	0.008-0.031
	Shapiro-Wilk test (p)	0.010	0.430	0.435
	Kruskal-Wallis test		H=9.794; p=0.007	
Ha Tinh (n=10)	Mean±SE	0.022±0.003	0.015±0.001	0.018±0.002
	Min-Max	0.007-0.049	0.009-0.022	0.009-0.029
	Shapiro-Wilk test (p)	0.036	0.831	0.835
	Kruskal-Wallis test		H= 4.704; p=0.095	
Quang Binh (n=10)	Mean±SE	0.042±0.004	0.015±0.002	0.016±0.002
	Min-Max	0.028-0.059	0.007-0.026	0.005-0.021
	Shapiro-Wilk test (p)	0.035	0.152	0.081
	Kruskal-Wallis test		H=19.856; p<0.001	
Quang Tri (n=10)	Mean±SE	0.034±0.005	0.015±0.001	0.021±0.002
	Min-Max	0.017-0.059	0.012-0.018	0.014-0.031
	Shapiro-Wilk test (p)	0.083	0.850	0.156
	Levene test		F=29.545; p<0.001	
	Kruskal-Wallis test		H=14.525, p<0.001	
Hue (n=10)	Mean±SE	0.026±0.005	0.015±0.002	0.016±0.002
	Min-Max	0.007-0.059	0.009-0.026	0.009-0.027
	Shapiro-Wilk test (p)	0.141	0.202	0.632
	Levene test		F=3.945; p=0.031	
	Kruskal-Wallis		H=6.531; p=0.038	

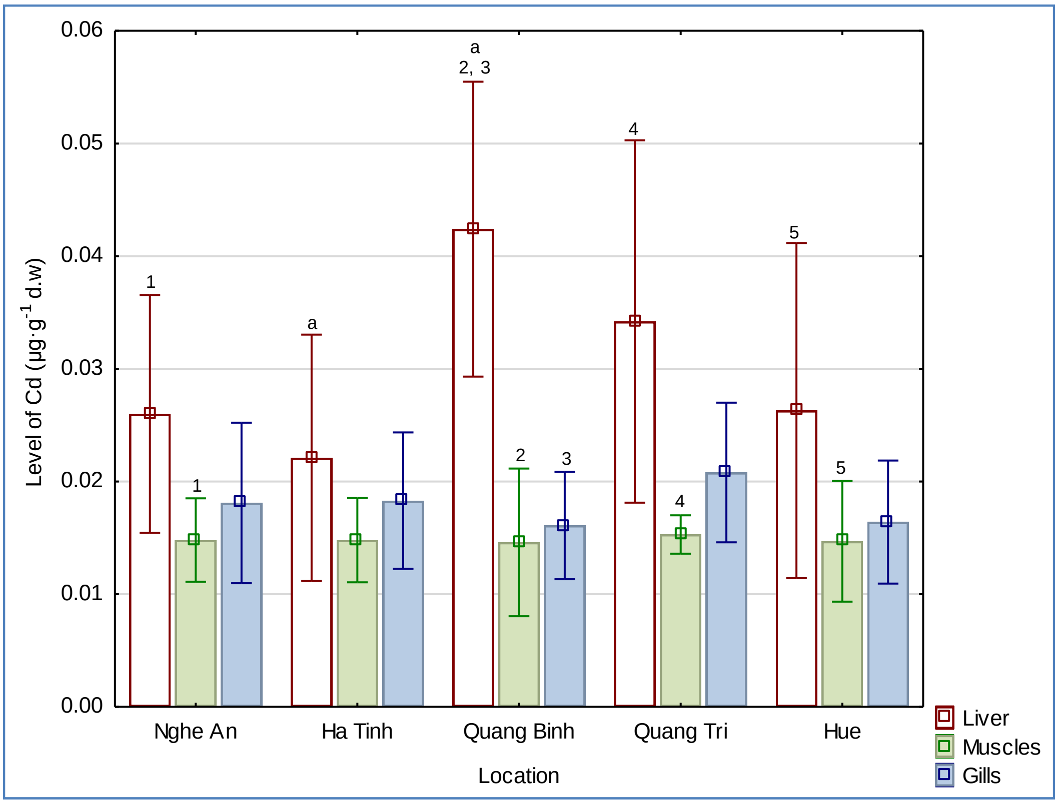

Figure 10. The mean, SD of Cd concentration in tissues of *A. latus*
(1, p=0.008; 2, p<0.001; 3, p=0.002; 4, p<0.001; 5, p=0.040; a, p=0.008)

1. 3. Pb concentration

The mean, SE, SD, min and max amounts of Pb ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *A. latus* are compiled in Table 4 and Fig. 11

The obtained results indicate that the liver of *A. latus* from Quang Tri has the highest concentration of Pb (0.334 $\mu \cdot g^{-1}$ d.w), while the muscles from Nghe An have the lowest Pb concentration (0.177 $\mu \cdot g^{-1}$ d.w). There were no strong differences in the accumulation of Pb in the liver, muscles, and gills between the research locations.

The range of Pb concentration in the organs of *A. latus* was in the order of liver>gills>muscles in all the research areas.

In Ha Tinh, Quang Binh, and Quang Tri, the accumulation of Pb in the liver was significantly higher than the accumulation in muscles and gills, which has been demonstrated by the multiple comparisons of mean ranks tests (3, p=0.016; 4, p=0.032; 5, p= 0.040; 6, p=0.005, 7, p=0.037).

In Nghe An and Hue, the concentration in muscle was significantly lower than in the liver (1, p<0.001; 8, p=0.009), but there was no significant difference for the Pb content in gills from Hue.

Table 4. The level of Pb in liver, muscles, gills of *A. latus* ($\mu\cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.280±0.027	0.119±0.016	0.177±0.023
	Min-Max	0.096-0.412	0.057-0.194	0.063-0.287
	Shapiro-Wilk test (p)	0.291	0.409	0.698
	ANOVA		F=13.361; p<0.001	
	Levene test		F=0.516; p=0.603	
Ha Tinh (n=10)	Mean±SE	0.318±0.033	0.181±0.024	0.194±0.024
	Min-Max	0.069-0.390	0.086-0.311	0.087-0.306
	Shapiro-Wilk test (p)	0.002	0.585	0.477
	Kruskal-Wallis test		H=9.540; p=0.008	
Quang Binh (n=10)	Mean±SE	0.329±0.030	0.220±0.031	0.222±0.025
	Min-Max	0.095-0.418	0.120-0.450	0.091-0.384
	Shapiro-Wilk test (p)	0.011	0.041	0.262
	Kruskal-Wallis test		H=7.707; p=0.021	
Quang Tri (n=10)	Mean±SE	0.334±0.032	0.151±0.037	0.180±0.025
	Min-Max	0.069-0.418	0.057-0.450	0.063-0.306
	Shapiro-Wilk test (p)	0.001	0.003	0.687
	Kruskal-Wallis test		H=11.007; p=0.004	
Hue (n=10)	Mean±SE	0.301±0.032	0.167±0.026	0.210±0.030
	Min-Max	0.096-0.418	0.063-0.312	0.063-0.384
	Shapiro-Wilk test (p)	0.459	0.651	0.920
	ANOVA		F=5.426; p=0.010	
	Levene test		F=0.119; p=0.888	

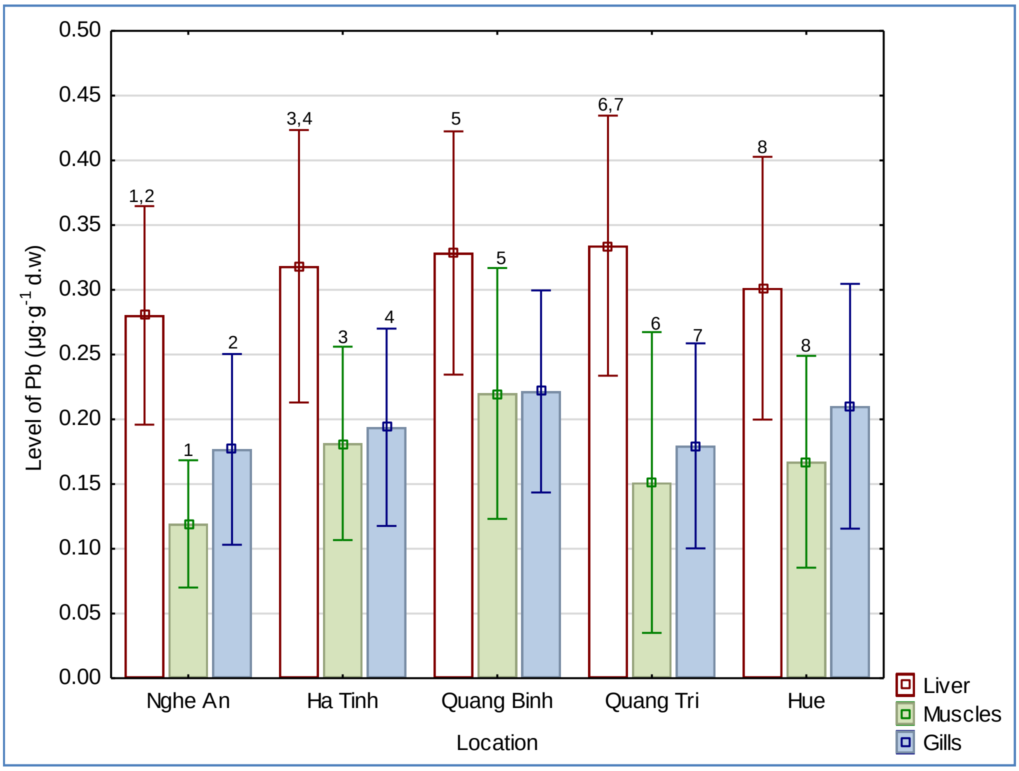

Figure 11. The mean, SD of Pb concentration in tissues of *A. latus*
(1, p<0.001; 2, p=0.008; 3, p=0.016; 4, p=0.032; 5, p=0.040; 6, p=0.005; 7, p=0.037; 8, p=0.009)

1. 4. Fe concentration

The mean, SE, SD, min and max levels of Fe ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *A. latus* are shown in Table 5 and Fig. 12.

The concentration of Fe in the *A. latus* liver ranged from 12.537 to 34.672 $\mu \cdot g^{-1}$ d.w, with the mean lowest content of approximately 18.874 $\mu \cdot g^{-1}$ d.w found in Quang Tri, and the mean highest content found in Quang Binh with 22.563 $\mu \cdot g^{-1}$ d.w. It was statistically shown that there was no significant difference between the researched areas.

Table 5. The level of Fe in liver, muscles, gills of *A. latus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	19.275±2.158	2.892±0.471	13.119±1.164
	Min-Max	12.537-32.100	1.229-6.430	8.868-21.936
	Shapiro-Wilk test (p)	0.077	0.011	0.044
	Kruskal-Wallis test	H=21.835; p<0.001		
Ha Tinh (n=10)	Mean±SE	20.596±1.537	2.238±0.239	13.153±1.629
	Min-Max	12.242-24.888	1.124-3.532	7.857-21.983
	Shapiro-Wilk test (p)	0.004	0.808	0.091
	Kruskal-Wallis test	H=23.081; p<0.001		
Quang Binh (n=10)	Mean±SE	22.563±1.747	3.481±0.393	17.342±1.653
	Min-Max	13.389-34.672	1.391-5.493	12.319-24.328
	Shapiro-Wilk test (p)	0.170	0.978	0.010
	Kruskal-Wallis test	H=20.968; p<0.001		
Quang Tri (n=10)	Mean±SE	18.874±1.528	1.857±0.162	10.765±0.805
	Min-Max	12.870-24.210	1.124-2.432	7.857-14.483
	Shapiro-Wilk test (p)	0.024	0.035	0.098
	Kruskal-Wallis test	H=23.907; p<0.001		
Hue (n=10)	Mean±SE	20.948±1.361	3.368±0.356	15.232±1.642
	Min-Max	13.633-25.854	2.154-5.493	8.868-23.957
	Shapiro-Wilk test (p)	0.049	0.385	0.182
	Kruskal-Wallis test	H=22.165; p<0.001		

While the levels of Fe in muscles ranged from 1.124 to 6.430 $\mu \cdot g^{-1}$ d.w, with the mean lowest content of approximately 1.857 $\mu \cdot g^{-1}$ d.w found in Quang Tri, the mean highest content was found in Quang Binh with 3.481 $\mu \cdot g^{-1}$. Statistically significant differences were discovered in Quang Tri and Quang Binh (a, p=0.022), between Hue and Quang Tri (b, p=0.028).

The content of Fe in gills ranged from 7.857 to 24.328 $\mu \cdot g^{-1}$ d.w, the mean accumulation of Fe in the liver from Quang Tri was the lowest (10.765 $\mu \cdot g^{-1}$ d.w), and from Quang Binh the highest (17.342 $\mu \cdot g^{-1}$ d.w). The result of the test by the effect of multiple comparisons of mean ranks in gills showed that there was a significant difference between Quang Tri and Quang Binh (c, p=0.034). According to statistics, the accumulation of Fe in the liver was significantly higher than in muscles and gills from all the research areas (1-9, Fig. 12).

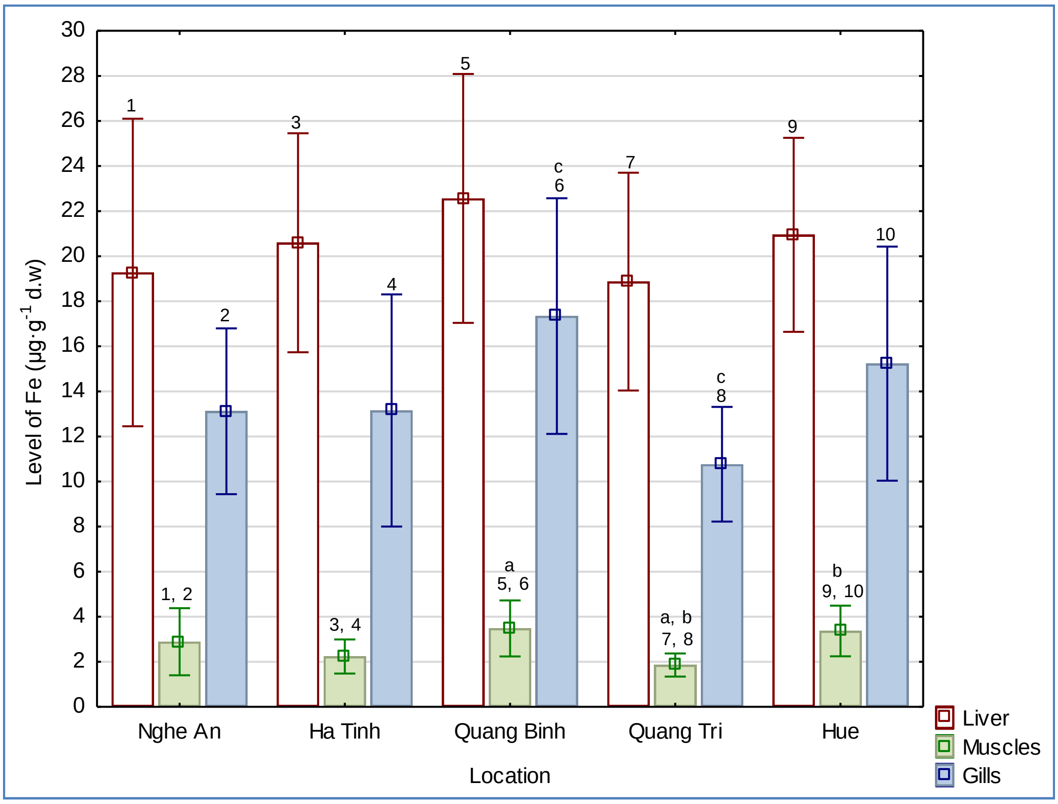

Figure 12. The mean, SD of Fe concentration in tissues of *A. latus*
(1, p<0.001; 2, p=0.008; 3, p<0.001; 4, p=0.013; 5, p<0.001; 6, p=0.005; 7, p<0.001; 8, p=0.018; 9, p<0.001; 10, p=0.009; a, p=0.022; b, p=0.028; c, p=0.034)

1. 5. Zn concentration

The mean, SE, SD, min and max amounts of Zn (μ·g^{-1} d.w) in the liver, muscles and gills of *A. latus* are summarized in Table 6 and Fig. 13.

The gills of *A. latus* had the highest concentrations of 1.969 μ·g^{-1} d.w and the lowest was 1.330 μ·g^{-1} d.w from Quang Binh and Quang Tri, respectively. There was a strong difference in the lowest and the highest concentration of Zn in the liver from Nghe An and from Ha Tinh (a, p=0.005). In muscles, the highest level found from Quang Binh was 0.964 μ·g^{-1} d.w, the lowest – from Quang Tri, was 0.530 μ·g^{-1} d.w.

The effect of multiple comparisons of mean ranks of Zn in tissues of *A. latus* from Ha Tinh and Hue showed that the differences were statistically significant between gills – liver (1, p=0.001) and gills – muscles (6, p=0.009). Besides, the Tukey test disclosed significant differences among the gills, the liver and muscles from Quang Binh (2, p<0.001; 3, p<0.001) and Quang Tri (4, p=0.036; 5, p<0.001).

Table 6. The level of Zn in liver, muscles, gills of *A. latus* (μ·g[-1] d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	1.274±0.179	0.961±0.118	1.363±0.126
	Min-Max	0.302-2.306	0.447-1.456	0.863-1.963
	Shapiro-Wilk test (p)	0.156	0.189	0.331
	ANOVA		F=2.166; p=0.134	
	Levene test		F=0.196; p=0.823	
Ha Tinh (n=10)	Mean±SE	0.537±0.095	0.846±0.086	1.693±0.244
	Min-Max	0.312-1.281	0.520-1.421	0.705-2.924
	Shapiro-Wilk test (p)	0.002	0.337	0.482
	Kruskal-Wallis test		H=16.316; p=0.003	
Quang Binh (n=10)	Mean±SE	0.689±0.117	0.964±0.133	1.969±0.182
	Min-Max	0.102-1.243	0.407-1.529	0.639-2.642
	Shapiro-Wilk test (p)	0.582	0.179	0.157
	ANOVA		F=21.102; p<0.001	
	Levene test		F=0.583; p=0.565	
Quang Tri (n=10)	Mean±SE	0.945±0.096	0.530±0.086	1.330±0.144
	Min-Max	0.447-1.420	0.102-0.903	0.705-1.959
	Shapiro-Wilk test (p)	0.519	0.502	0.369
	ANOVA		F=12.840; p<0.001	
	Levene test		F=2.521; p=0.099	
Hue (n=10)	Mean±SE	0.992±0.131	0.860±0.151	1.809±0.205
	Min-Max	0.482-1.529	0.326-1.488	0.871-2.673
	Shapiro-Wilk test (p)	0.141	0.042	0.513
	Kruskal-Wallis test		H=9.685; p=0.008	

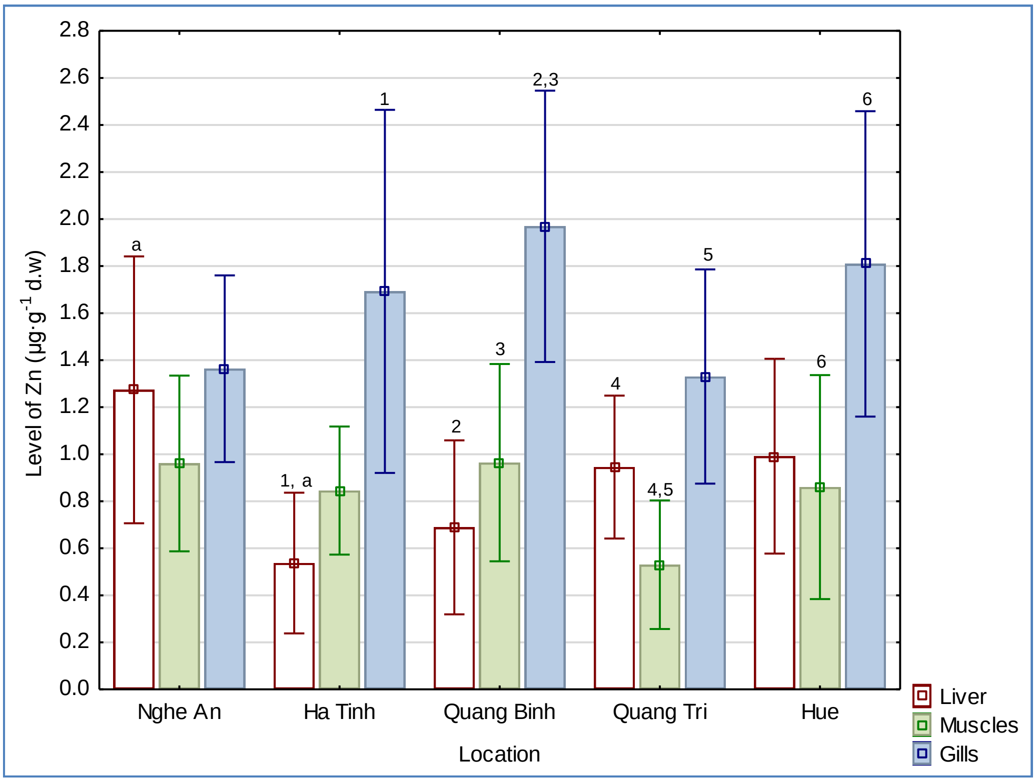

Figure 13. The mean, SD of Zn concentration in tissues of *A. latus*
(1, p=0.001; 2, p<0.001; 3, p<0.001; 4, p=0.036; 5, p<0.001; 6, 0.009; a, p=0.005)

1. 6. Cu concentration

The mean, SE, SD, min and max amounts of Cu ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *A. latus* are presented in Table 7 and Fig. 14.

From the data shown, it was observed that the concentration of Cu in muscles was significantly lower than in the liver and gills, which was proven by the Tukey HSD test and multiple comparisons of mean ranks, shown by numbers 1-11 in Fig. 14. Besides, statistical analysis results also showed that there was no noticeable difference in the Cu content among the studied areas.

Table 7. The level of Cu in liver, muscles, and gills of *A. latus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	1.313±0.122	0.431±0.091	0.798±0.061
	Min-Max	0.682-1.970	0.010-0.830	0.545-1.133
	Shapiro-Wilk test (p)	0.203	0.406	0.797
	ANOVA		F=21.818; p<0.001	
	Levene test		F=0.855; p=0.436	
Ha Tinh (n=10)	Mean±SE	1.195±0.130	0.363±0.068	1.007±0.065
	Min-Max	0.410-2.001	0.080-0.634	0.673-1.409
	Shapiro-Wilk test (p)	0.434	0.183	0.801
	ANOVA		F=22.002; p<0.001	
	Levene test		F=1.111; p=0.344	
Quang Binh (n=10)	Mean±SE	0.869±0.166	0.254±0.046	1.084±0.175
	Min-Max	0.352-2.008	0.109-0.510	0.603-2.453
	Shapiro-Wilk test (p)	0.064	0.045	0.008
	Kruskal-Wallis test		H=18.038; p<0.001	
Quang Tri (n=10)	Mean±SE	1.229±0.124	0.207±0.054	0.777±0.060
	Min-Max	0.689-2.001	0.010-0.600	0.545-1.094
	Shapiro-Wilk test (p)	0.808	0.143	0.577
	Levene test		F=3.871; p=0.033	
	Kruskal-Wallis test		H=22.121; p<0.001	
Hue (n=10)	Mean±SE	1.066±0.131	0.337±0.035	0.934±0.077
	Min-Max	0.410-1.594	0.129-0.510	0.655-1.409
	Shapiro-Wilk test (p)	0.388	0.812	0.062
	Levene test		F=11.446; p<0.001	
	Kruskal-Wallis test		H=18.681; p<0.001	

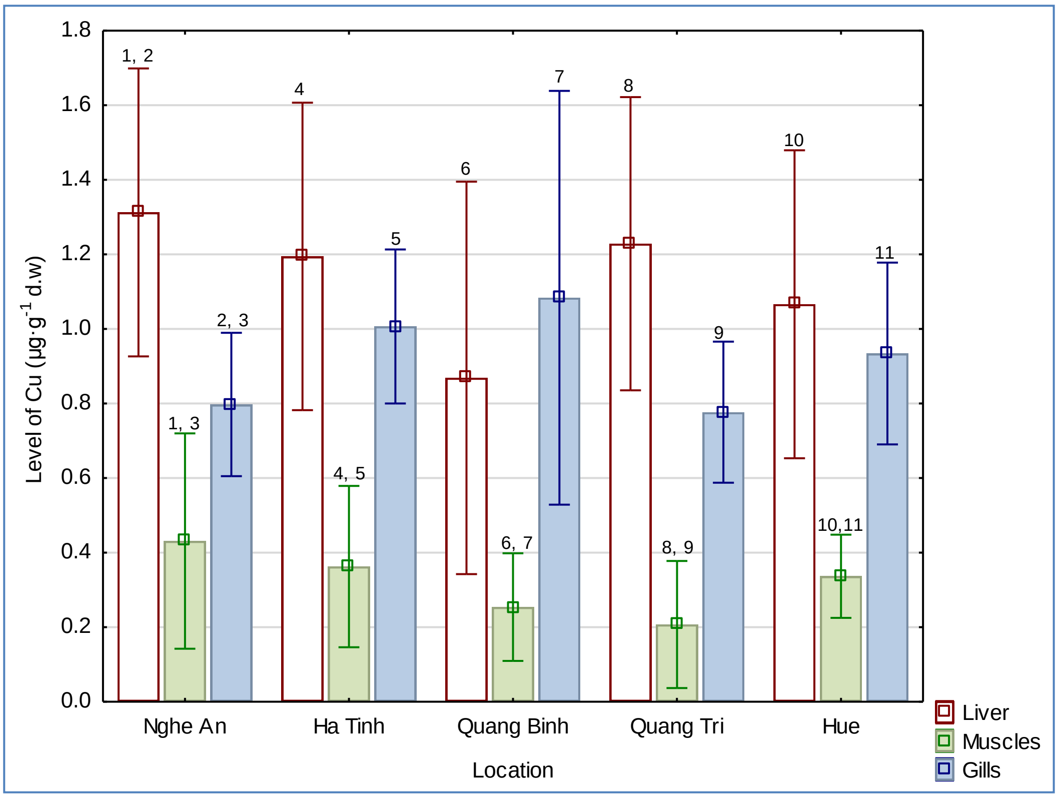

Figure 14. The mean, SD of Cu concentration in tissues of *A. latus*
(1, p<0.001; 2, p=0.002; 3, p=0.029; 4, p<0.001; 5, p<0.001; 6, p=0.006; 7, p<0.001; 8, p<0.001; 9, p=0.016; 10, p<0.001; 11, p=0.001)

2. Concentration of heavy metals in *Konorsirus punctatus*

2. 1. Hg concentration

The mean, SE, SD, min and max amounts of Hg ($\mu \cdot g^{-1}$ w.w) in the liver, muscles and gills of *K. punctatus* are presented in Table 8 and Fig. 15.

The highest concentration of mercury gills of *K. punctatus* was found in Nghe An (0.586 $\mu \cdot g^{-1}$ w.w), the lowest – in Hue (0.221 $\mu \cdot g^{-1}$ w.w), however, there was no noticeable difference between regions. The Hg content in the muscles of *K. punctatus* was similar in all the study sites. Meanwhile, the accumulation of Hg in gills significantly differed between Nghe An to Ha Tinh (a, p=0.005), Quang Tri (b, p=0.012), and Hue (c, p=0.038), which was shown by the effect of multiple comparisons of mean ranks. Within the study sites, the amount of mercury in the liver is always higher than in the gills and muscles. However, the effect of multiple comparisons of the mean ranks of Hg showed that the difference between the liver and muscles was found from Ha Tinh, Quang Binh and Quang Tri (numbers 1-4).

Table 8. The level of Hg in liver, muscles, and gills of *K. punctatus* ($\mu \cdot g^{-1}$ w.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.586±0.148	0.132±0.015	0.273±0.043
	Min-Max	0.046-1.164	0.081-0.219	0.087-0.449
	Shapiro-Wilk test (p)	0.054	0.351	0.239
	Levene test		F=48.830; p<0.001	
	Kruskal-Wallis test		H=6.329; p=0.042	
Ha Tinh (n=10)	Mean±SE	0.298±0.049	0.151±0.016	0.201±0.031
	Min-Max	0.139-0.543	0.092-0.243	0.028-0.393
	Shapiro-Wilk test (p)	0.022	0.394	0.881
	Kruskal-Wallis test		H=7.669; p=0.022	
Quang Binh (n=10)	Mean±SE	0.249±0.026	0.112±0.038	0.218±0.034
	Min-Max	0.118-0.386	0.034-0.330	0.061-0.363
	Shapiro-Wilk test (p)	0.446	0.006	0.272
	Kruskal-Wallis test		H=7.436; p=0.024	
Quang Tri (n=10)	Mean±SE	0.286±0.026	0.082±0.013	0.094±0.023
	Min-Max	0.170-0.434	0.049-0.182	0.023-0.220
	Shapiro-Wilk test (p)	0.724	0.004	0.037
	Kruskal-Wallis test		H=16.943; p<0.001	
Hue (n=10)	Mean±SE	0.221±0.034	0.191±0.041	0.111±0.031
	Min-Max	0.087-0.393	0.032-0.428	0.028-0.324
	Shapiro-Wilk test (p)	0.235	0.441	0.038
	Kruskal-Wallis test		H=5.757; p=0.056	

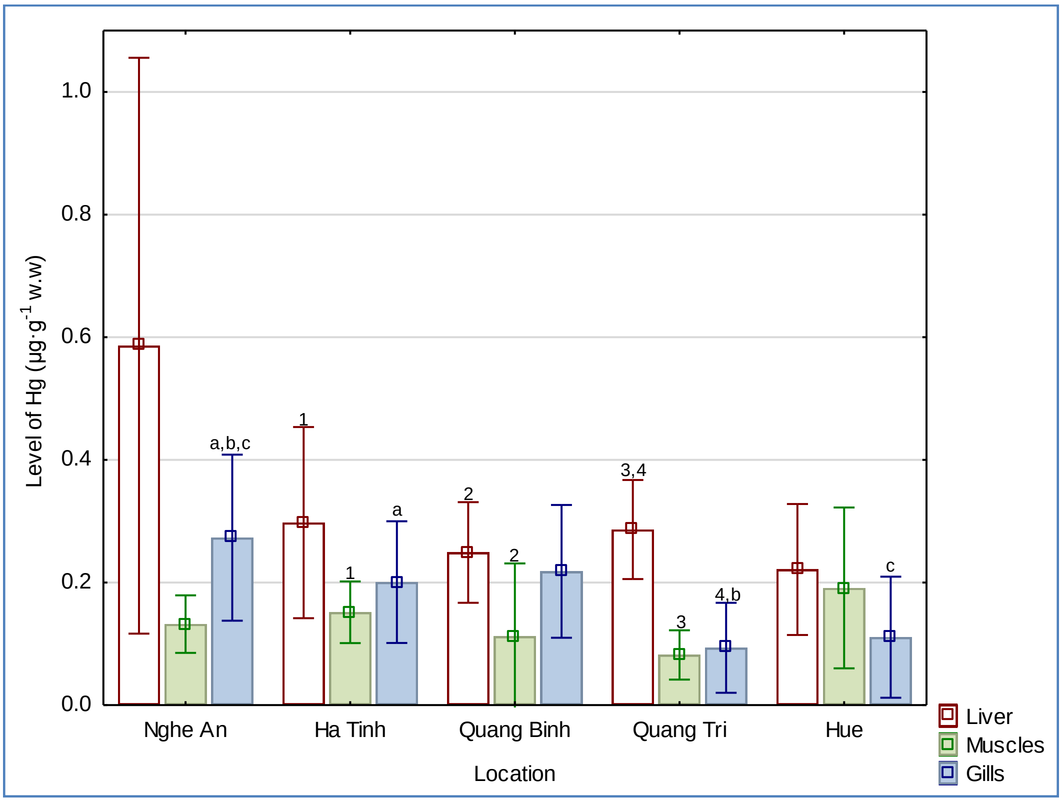

Figure 15. The mean, SD of Hg concentration in tissues of *K. punctatus*
(1, p=0.017; 2, p=0.031; 3, p=0.001; 4, p=0.001; a, p=0.005; b, p=0.012; c, p=0.038)

2. 2. Cd concentration

The mean, SE, SD, min and max levels of Cd ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *K. punctatus* are shown in Table 9 and Fig. 16.

In Nghe An, we found that the average value of Cd in the muscles is higher than in the gills and the liver however, there only is a statistically significant difference between muscles and liver (1, p=0.031).

In Ha Tinh, Cd accumulation in gills was significantly higher than in muscles and liver, which was shown by the Tukey HSD test (1, p=0.020; 2, p=0.048). In Quang Binh, Quang Tri and Hue, the average value of Cd displays in descending order: liver> gills> muscles, but does not show any statistically significant difference.

Table 9. The level of Cd in liver, muscles, gills of *K. punctatus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.010±0.002	0.019±0.003	0.013±0.003
	Min-Max	0.003-0.022	0.010-0.033	0.006-0.035
	Shapiro-Wilk test (p)	0.217	0.105	0.010
	Kruskal-Wallis test		H=6.708; p=0.035	
Ha Tinh (n=10)	Mean±SE	0.014±0.002	0.015±0.002	0.021±0.001
	Min-Max	0.004-0.023	0.005-0.025	0.014-0.027
	Shapiro-Wilk test (p)	0.566	0.908	0.461
	ANOVA		F=4.899; p=0.015	
	Levene test		F=0.941; p=0.403	
Quang Binh (n=10)	Mean±SE	0.022±0.003	0.016±0.002	0.017±0.002
	Min-Max	0.013-0.037	0.007-0.029	0.007-0.030
	Shapiro-Wilk test (p)	0.264	0.391	0.719
	ANOVA		F=2.468; p=0.104	
	Levene test		F=1.319; p=0.284	
Quang Tri (n=10)	Mean±SE	0.018±0.003	0.009±0.002	0.017±0.003
	Min-Max	0.006-0.037	0.004-0.022	0.005-0.033
	Shapiro-Wilk test (p)	0.091	0.085	0.296
	ANOVA		F=3.119; p=0.060	
	Levene test		F=1.377; p=0.269	
Hue (n=10)	Mean±SE	0.020±0.003	0.015±0.002	0.016±0.002
	Min-Max	0.009-0.035	0.007-0.030	0.009-0.024
	Shapiro-Wilk test (p)	0.487	0.299	0.382
	ANOVA		F=0.985; p=0.386	
	Levene test		F=1.282; p=0.294	

The concentration of Cd in the liver varies among the study areas. Specifically, the level of Cd in the liver from Quang Binh is significantly higher than in Nghe An. The concentration of Cd in the muscles from Nghe An is much higher than that from Quang Tri. The Cd content in the gills from the Ha Tinh region is higher than in Nghe An. The data have been confirmed by the effect of multiple comparisons of mean ranks (a, p=0.010; b, p=0.041; c, p=0.026).

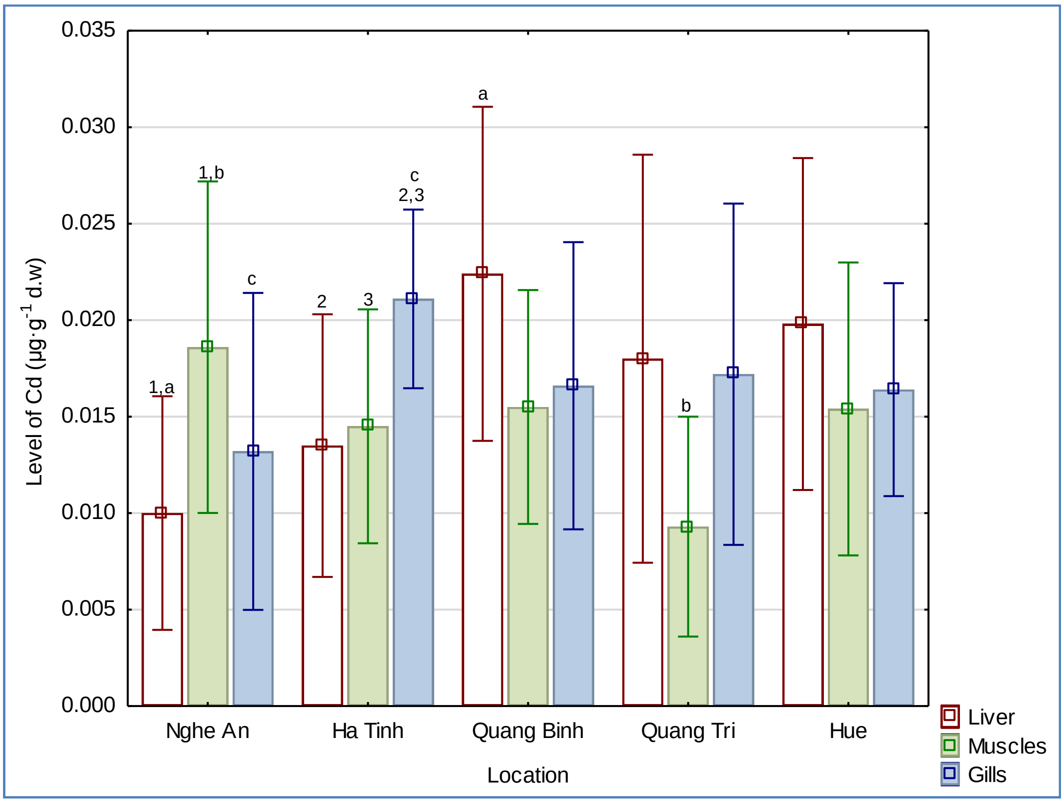

Figure 16. The mean, SD of Cd concentration in tissues of *K. punctatus*
(1, p=0.031; 2, p=0.020; 3, p=0.048; a, p=0.010; b, p=0.041; c, p=0.026)

2. 3. Pb concentration

The mean, SE, SD, min and max amounts of Pb ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *K. punctatus* are summarized in Table 10 and Fig. 17.

In all the research locations, the accumulation of Pb in the liver is much higher than in the muscle, which is confirmed by the effect of multiple comparisons of the mean ranks test (1, p<0.001; 2, p<0.001; 4, p=0.028; 6, p=0.011; 7, p<0.001).

In Ha Tinh and Quang Binh, Pb concentrations detected in gills were significantly lower than in the liver, which was proven by the effect of multiple comparisons of the mean ranks test (3, p=0.011; 5, p=0.012).

There was no significant difference in the Pb contents in liver, muscles, and gills compared to the remaining study areas.

Table 10. The level of Pb in liver, muscles, gills of *K. punctatus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.421±0.049	0.134±0.022	0.268±0.013
	Min-Max	0.195-0.675	0.067-0.264	0.189-0.327
	Shapiro-Wilk test (p)	0.288	0.129	0.558
	Levene test		F=10.979; p<0.001	
	Kruskal-Wallis test		H=15.595; p<0.001	
Ha Tinh (n=10)	Mean±SE	0.496±0.038	0.121±0.034	0.273±0.022
	Min-Max	0.356-0.783	0.018-0.315	0.083-0.325
	Shapiro-Wilk test (p)	0.083	0.099	<0.001
	Kruskal-Wallis test		H=22.704; p<0.001	
Quang Binh (n=10)	Mean±SE	0.512±0.036	0.355±0.080	0.291±0.008
	Min-Max	0.385-0.698	0.096-0.791	0.241-0.342
	Shapiro-Wilk test (p)	0.090	0.051	0.155
	Levene test		F=14.453; p<0.001	
	Kruskal-Wallis test		H=10.118; p=0.006	
Quang Tri (n=10)	Mean±SE	0.453±0.065	0.225±0.065	0.287±0.010
	Min-Max	0.095-0.783	0.019-0.710	0.245-0.327
	Shapiro-Wilk test (p)	0.958	0.070	0.160
	Levene test		F=14.453; p<0.001	
	Kruskal-Wallis test		H=8.581; p=0.014	
Hue (n=10)	Mean±SE	0.477±0.055	0.147±0.030	0.279±0.023
	Min-Max	0.095-0.697	0.018-0.315	0.083-0.342
	Shapiro-Wilk test (p)	0.417	0.569	0.001
	Kruskal-Wallis test		H=18.101; p=0.001	

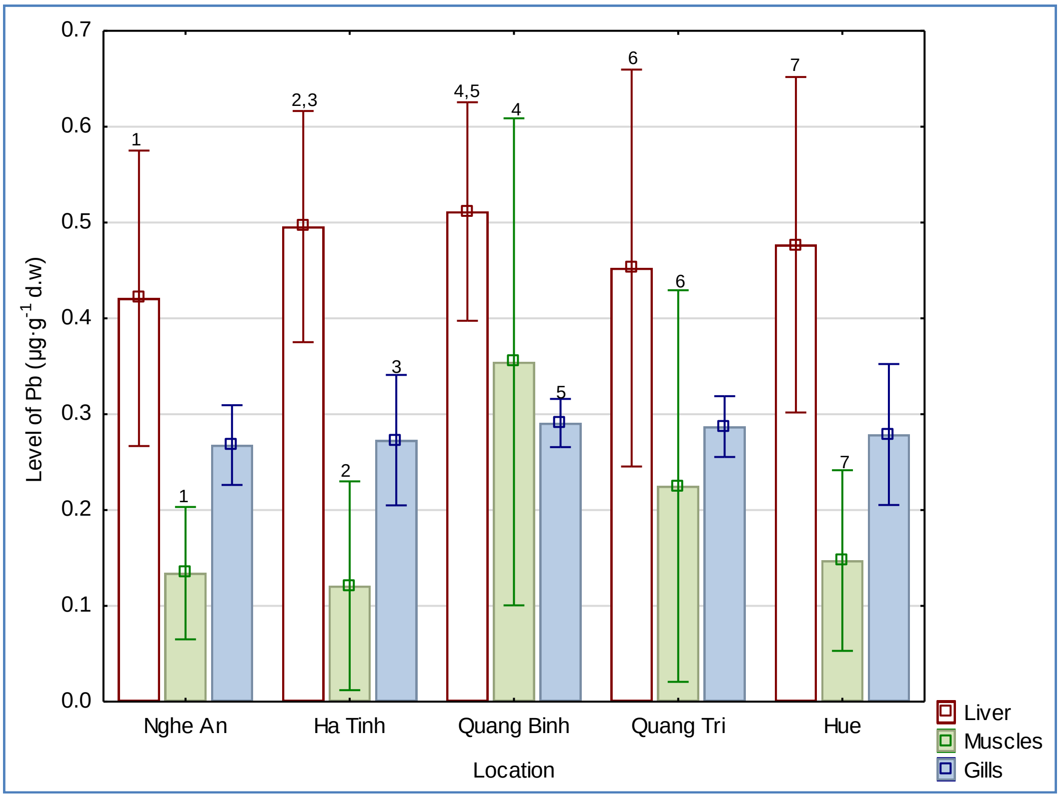

Figure 17. The mean, SD of Pb concentration in tissues of *K. punctatus*
(1, p<0.001; 2, p<0.001; 3, p=0.011; 4, p=0.028; 5, p=0.012; 6, p=0.011; 7, p<0.001)

2. 4. Fe concentration

The mean, SE, SD, min and max amounts of Fe ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *K. punctatus* are summarized in Table 11 and Fig.18.

The data have shown that the highest detected average value of Fe in the liver came from Quang Tri (15.397 $\mu \cdot g^{-1}$ d.w), the lowest from Quang Binh (12.702 $\mu \cdot g^{-1}$ d.w). The highest Fe content in muscles was found in Quang Binh (5.068 $\mu \cdot g^{-1}$ d.w), and lowest in Ha Tinh (3.071 $\mu \cdot g^{-1}$ d.w). Concurrently, the lowest Fe concentration in gills was found in Nghe An (9.833 $\mu \cdot g^{-1}$ d.w), the highest in Ha Tinh (13.770 $\mu \cdot g^{-1}$ d.w). However, there were no significant differences between the research regions in terms of the Fe content in these organs.

For each study region, the differences were shown by the Tukey HSD test in Nghe An (1, $p<0.001$; 2, $p=0.003$) and Hue (9, $p<0.001$; 10, $p<0.001$). The differences in Fe contents in tissues of *K. punctatus* from Quang Binh, Quang Tri, Ha Tinh and Hue were proven by the effect of multiple comparisons of the mean ranks. They were presented by numbers 3-8 in Fig. 18.

Table 11. The level of Fe in liver, muscles, gills of *K. punctatus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	13.267±1.644	3.523±0.542	9.833±1.211
	Min-Max	5.817-22.193	0.678-5.783	5.642-16.399
	Shapiro-Wilk test (p)	0.252	0.598	0.188
	ANOVA		F=14.420; p<0.001	
	Levene test		F=2.051; p=0.148	
Ha Tinh (n=10)	Mean±SE	14.449±1.096	3.071±0.539	13.770±1.098
	Min-Max	10.575-21.099	0.660-5.891	8.970-18.794
	Shapiro-Wilk test (p)	0.038	0.591	0.509
	Kruskal-Wallis test		H=19.481; p<0.001	
Quang Binh (n=10)	Mean±SE	12.702±1.151	5.068±1.280	12.410±1.207
	Min-Max	9.090-18.627	0.927-12.380	6.392-16.160
	Shapiro-Wilk test (p)	0.084	0.037	0.073
	Kruskal-Wallis test		H=12.390; p=0.002	
Quang Tri (n=10)	Mean±SE	15.397±1.369	4.336±1.010	10.696±1.346
	Min-Max	10.575-22.193	0.660-12.380	5.642-16.399
	Shapiro-Wilk test (p)	0.008	0.021	0.150
	Kruskal-Wallis test		H=18.320; p<0.001	
Hue (n=10)	Mean±SE	14.676±0.988	3.397±0.541	11.994±1.239
	Min-Max	9.282-19.816	0.678-5.783	5.828-16.093
	Shapiro-Wilk test (p)	0.611	0.519	0.178
	ANOVA		F=18.410; p<0.001	
	Levene test		F=1.761; p=0.191	

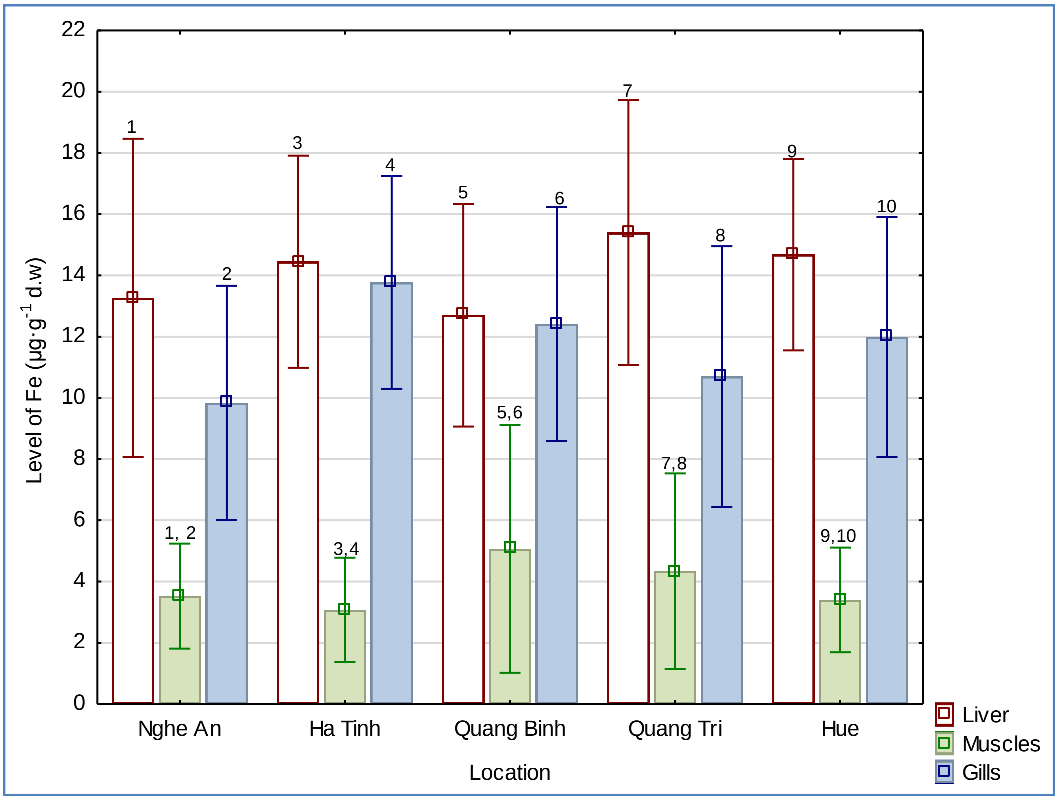

Figure 18. The mean, SD of Fe concentration in tissues of *K. punctatus*
(1, p<0.001; 2, p=0.003; 3, p<0.001; 4, p=0.001; 5, p=0.001; 6, p=0.006; 7, p<0.001; 8, p=0.027; 9, p<0.001; 10, p<0.001)

2. 5. Zn concentration

The mean, SE, SD, min and max amounts of Zn ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *K. punctatus* are summarized in Table 12 and Fig. 19.

The highest mean value of Zn in the liver of *K. punctatus* was detected in Quang Binh (2.437 $\mu \cdot g^{-1}$ d.w), the lowest in Nghe An (0.834 $\mu \cdot g^{-1}$ d.w). The effect of multiple comparisons of mean ranks test of Zn in the liver showed a significant difference between Quang Binh and Nghe An (a, p=0.001).

The content of Zn in muscles was similar in all the study areas. Also, the accumulation of Zn in gills has a significant difference between the regions of Quang Binh and Nghe An (b, p=0.001), Quang Tri (d, p=0.017), and between Ha Tinh and Quang Tri (c, p=0.031).

The concentration of Zn in the liver, muscles, and gills in each study region displayed noticeable differences only in Quang Binh (1, p=0.004) and Quang Tri (2, p=0.011), which was proven by the multiple comparisons p values.

Table 12. The level of Zn in liver, muscles, gills of *K. punctatus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.834±0.141	1.045±0.138	1.324±0.213
	Min-Max	0.477-1.822	0.456-1.675	0.680-2.854
	Shapiro-Wilk test (p)	0.010	0.541	0.067
	Kruskal-Wallis test		H=4.560; p=0.102	
Ha Tinh (n=10)	Mean±SE	1.871±0.335	0.895±0.114	1.593±0.197
	Min-Max	0.487-3.266	0.382-1.403	0.800-2.732
	Shapiro-Wilk test (p)	0.180	0.577	0.556
	Levene test		F=8.253; p=0.002	
	Kruskal-Wallis test		H=6.978; p=0.031	
Quang Binh (n=10)	Mean±SE	2.437±0.310	1.262±0.173	1.610±0.184
	Min-Max	0.928-4.218	0.782-2.473	0.960-2.489
	Shapiro-Wilk test (p)	0.822	0.019	0.139
	Kruskal-Wallis test		H=0.014; p=0.018	
Quang Tri (n=10)	Mean±SE	1.824±0.277	1.331±0.205	0.853±0.115
	Min-Max	0.487-3.266	0.680-2.854	0.513-1.404
	Shapiro-Wilk test (p)	0.955	0.045	0.020
	Kruskal-Wallis test		H=8.764; p=0.013	
Hue (n=10)	Mean±SE	1.206±0.147	1.037±0.205	1.600±0.216
	Min-Max	0.456-1.926	0.513-2.473	0.680-2.854
	Shapiro-Wilk test (p)	0.542	0.006	0.847
	Kruskal-Wallis test		H=5.122; p=0.078	

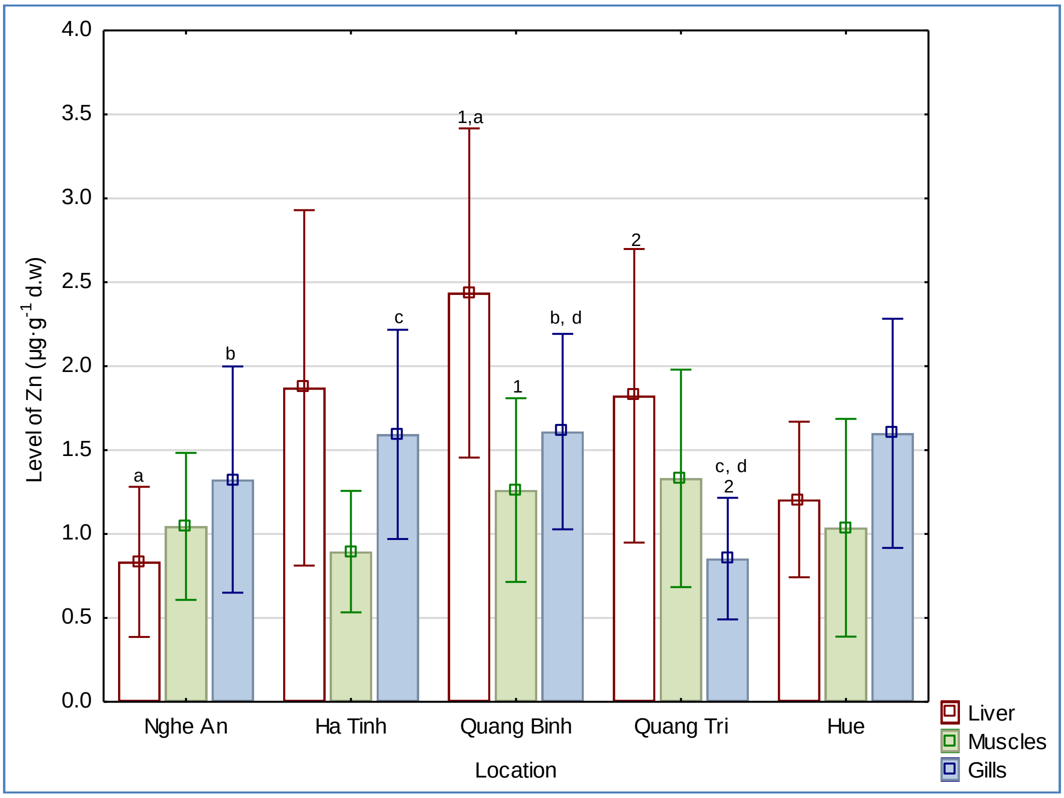

Figure 19. The mean, SD of Zn concentration in tissues of *K. punctatus*
(1, p=0.014; 2, p=0.011; a, p=0.001; b, p=0.001; c, p=0.031; d, p=0.017)

2. 6. Cu concentration

The mean, SE, SD, min and max amounts of Cu ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *K. punctatus* are presented in Table 13 and Fig. 20.

In Nghe An and Hue, the average value of Cu in the tissues was found to be in descending order: liver> gills> muscles, whereas in Ha Tinh, Quang Binh and Quang Tri, the order of the Cu accumulation in tissues was as follows: liver>muscles>gills. However, the statistical analysis showed that there was no significant difference between the tissues in the same region, and between different regions.

Table 13. The level of Cu in liver, muscles, gills of *K. punctatus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	1.375±0.365	0.600±0.107	0.796±0.062
	Min-Max	0.250-3.560	0.140-1.109	0.511-1.181
	Shapiro-Wilk test (p)	0.092	0.597	0.761
	Levene test		F=11.342; p<0.001	
	Kruskal-Wallis test		H=3.770; p=0.152	
Ha Tinh (n=10)	Mean±SE	1.336±0.296	0.976±0.096	0.643±0.111
	Min-Max	0.222-2.774	0.643-1.548	0.155-1.108
	Shapiro-Wilk test (p)	0.166	0.013	0.326
	Kruskal-Wallis test		H=3.222; p=0.199	
Quang Binh (n=10)	Mean±SE	1.642±0.309	0.883±0.082	0.854±0.104
	Min-Max	0.225-3.209	0.487-1.299	0.100-1.298
	Shapiro-Wilk test (p)	0.731	0.562	0.147
	Levene test		F=11.412; p<0.001	
	Kruskal-Wallis test		H=5.910; p=0.052	
Quang Tri (n=10)	Mean±SE	1.379±0.324	0.933±0.097	0.640±0.123
	Min-Max	0.222-2.960	0.511-1.492	0.155-1.109
	Shapiro-Wilk test (p)	0.241	0.785	0.110
	Levene test		F=10.232; p<0.001	
	Kruskal-Wallis test		H=3.822; p=0.148	
Hue (n=10)	Mean±SE	1.540±0.328	0.655±0.094	0.889±0.083
	Min-Max	0.421-3.560	0.100-1.082	0.644-1.492
	Shapiro-Wilk test (p)	0.346	0.913	0.205
	Levene test		F=9.352; p<0.001	
	Kruskal-Wallis test		H=5.493; p=0.064	

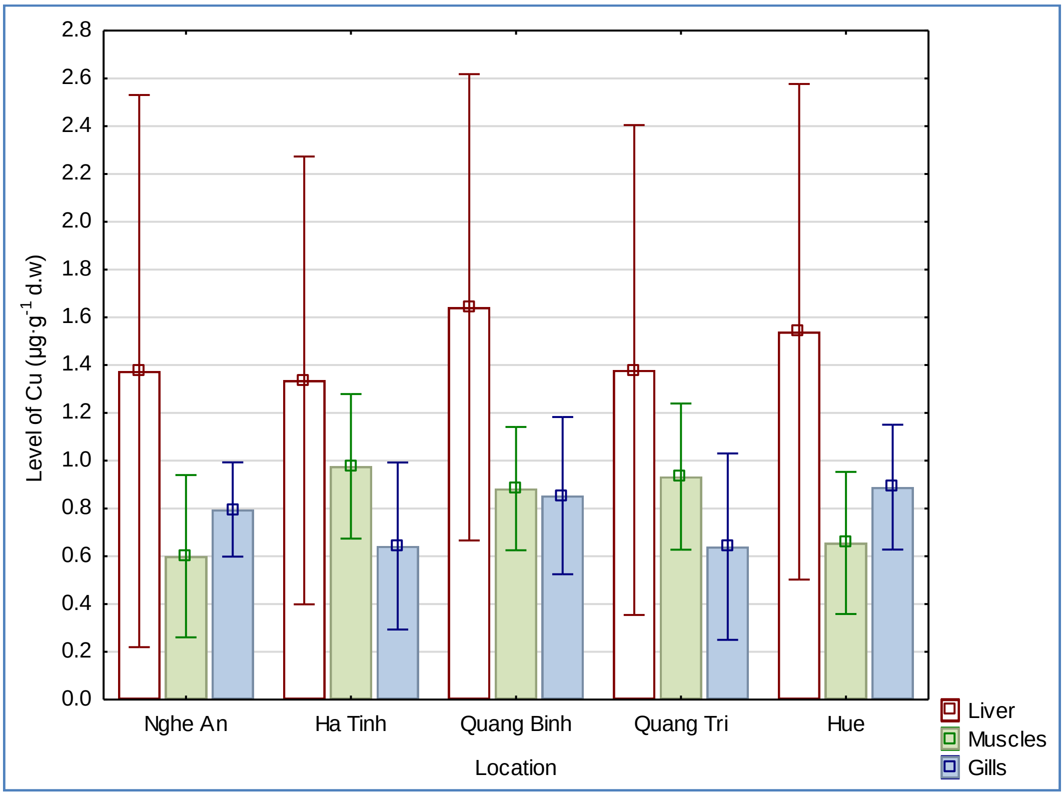

Figure 20. The mean, SD of Cu concentration in tissues of *K. punctatus*

3. Concentration of heavy metals in *Mugil cephalus*

3. 1. Hg concentration

The mean, SE, SD, min and max amounts of Hg (μ·g^{-1} w.w) in the liver, muscles and gills of *M. cephalus* are presented in Table 14 and Fig. 21.

In the Nghe An and Ha Tinh regions, the levels of Hg were observed to remain in the same order: liver>muscles>gills. The results of the effect of multiple comparisons of the mean ranks test showed a significantly higher Hg accumulation in the liver compared to muscles and gills (1, p=0.006; 2, p<0.001; 3, p=0.002; 4, p=0.004).

In Quang Binh, Quang Tri and Hue, the Hg content displayed in the order as follows: liver>gills>muscles. In Quang Binh, the Hg concentration in the muscles was much lower than in the liver and gills, which was confirmed by the effect of multiple comparisons of mean ranks of Hg in all the organs (5, p<0.001; 6, p=0.009).

In Quang Tri and Hue, the Hg concentrations in the liver were significantly higher than the Hg contents in muscles and gills, which was observed by the multiple comparisons p values (7, p<0.001; 8, p<0.001; 9, p=0.005).

Table 14. The level of Hg in liver, muscles, gills of *M. cephalus* (µ·g^{-1} w.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.327±0.037	0.147±0.028	0.108±0.015
	Min-Max	0.180-0.494	0.051-.340	0.051-0.184
	Shapiro-Wilk test (p)	0.242	0.087	0.256
	Levene test		F=3.632; p=0.040	
	Kruskal-Wallis test		H=16.616; p=0.002	
Ha Tinh (n=10)	Mean±SE	0.206±0.026	0.090±0.017	0.087±0.005
	Min-Max	0.111-0.372	0.039-0.165	0.065-0.105
	Shapiro-Wilk test (p)	0.386	0.005	0.134
	Kruskal-Wallis test		H=14.512; p=0.007	
Quang Binh (n=10)	Mean±SE	0.171±0.013	0.050±0.005	0.129±0.012
	Min-Max	0.126-0.233	0.030-0.075	0.057-0.181
	Shapiro-Wilk test (p)	0.095	0.228	0.805
	Levene test		F=4.421; p=0.022	
	Kruskal-Wallis test		H=19.732; p=0.001	
Quang Tri (n=10)	Mean±SE	0.130±0.013	0.042±0.007	0.076±0.011
	Min-Max	0.074-0.202	0.015-0.072	0.027-0.125
	Shapiro-Wilk test (p)	0.662	0.032	0.497
	Kruskal-Wallis test		H=17.259; p<0.001	
Hue (n=10)	Mean±SE	0.136±0.015	0.040±0.005	0.100±0.011
	Min-Max	0.063-0.224	0.024-0.070	0.077-0.181
	Shapiro-Wilk test (p)	0.471	0.105	<0.001
	Kruskal-Wallis test		H=20.796; p<0.001	

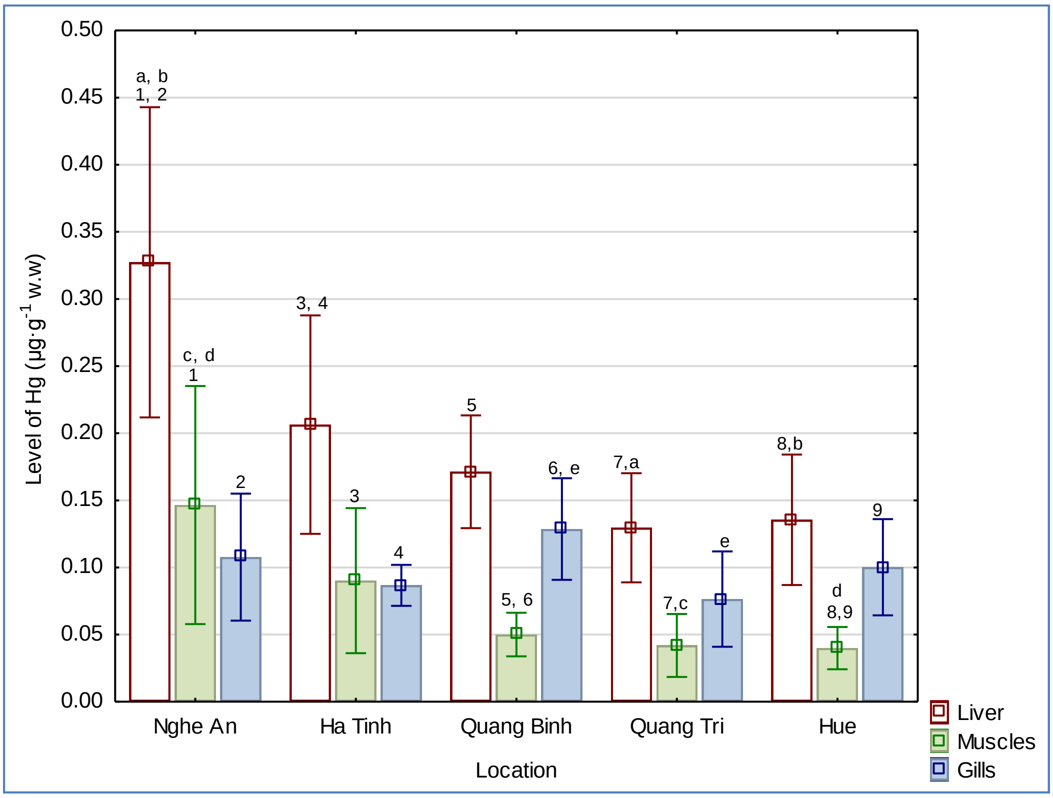

Figure 21. The mean, SD of Hg concentration in tissues of *M. cephalus*
(1, p=0.006; 2, p<0.001; 3, p=0.002; 4, p=0.004; 5, p<0.001; 6, p=0.009; 7, p<0.001; 8, p<0.001; 9, p=0.005; a, p<0.001; b, p=0.001; c, p=0.006; d, p=0.002; e, p=0.028)

Moreover, the effect of multiple comparisons of mean ranks of Hg in liver and muscles showed that the accumulation of Hg in liver and muscles in Nghe An was significantly higher than in Quang Tri and Hue (a, p<0.001; b, p=0.001; c, p=0.006; d, p=0.002). The difference in the Hg content in gills was only detected in Quang Binh and Hue (e, p=0.028).

3. 2. Cd concentration

The mean, SE, SD, min and max amounts of Cd ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *M. cephalus* are summarized in Table 15 and Fig. 22.

In Nghe An, the average value of Cd in the liver was significantly higher than in muscles and gills, which was confirmed by the Tukey HSD test (1, p=0.002; 2, p<0.001). Simultaneously, in Ha Tinh and Quang Binh, there was no significant difference between the levels of Cd in these organs. In Hue and Quang Tri, the Cd concentration in muscles was significantly lower than in the liver and gills, which was determined by the effect of multiple comparisons of mean ranks (3, p=0.002; 4, p=0.002; 5, p<0.001; 6, p=0.034).

Table 15. The level of Cd in liver, muscles, gills of *M. cephalus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.022±0.002	0.012±0.002	0.010±0.001
	Min-Max	0.013-0.033	0.003-0.022	0.004-0.015
	Shapiro-Wilk test (p)	0.693	0.578	0.852
	ANOVA		F=12.269; p<0.001	
	Levene test		F=1.614; p=0.218	
Ha Tinh (n=10)	Mean±SE	0.021±0.003	0.014±0.002	0.017±0.002
	Min-Max	0.009-0.037	0.004-0.026	0.006-0.027
	Shapiro-Wilk test (p)	0.202	0.537	0.577
	ANOVA		F=1.987; p=0.157	
	Levene test		F=1.086; p=0.352	
Quang Binh (n=10)	Mean±SE	0.028±0.003	0.020±0.003	0.022±0.003
	Min-Max	0.017-0.043	0.008-0.034	0.011-0.037
	Shapiro-Wilk test (p)	0.504	0.190	0.210
	ANOVA		F=1.627; p=0.215	
	Levene test		F=0.526; p=0.597	
Quang Tri (n=10)	Mean±SE	0.054±0.025	0.013±0.002	0.013±0.003
	Min-Max	0.019-0.280	0.004-0.023	0.004-0.028
	Shapiro-Wilk test (p)	<0.001	0.250	0.120
	Kruskal-Wallis test		H=15.220; p=0.001	
Hue (n=10)	Mean±SE	0.022±0.003	0.008±0.001	0.015±0.002
	Min-Max	0.009-0.035	0.003-0.013	0.007-0.026
	Shapiro-Wilk test (p)	0.653	0.698	0.198
	Levene test		F=3.809; p=0.035	
	Kruskal-Wallis test		H=16.347; p=0.003	

In addition, the results also showed that the Cd accumulation in the muscles from Quang Binh (0.020 $\mu \cdot g^{-1}$ d.w) was significantly higher than the Cd level in the muscles from

Hue (0.008 μ·g^{-1} d.w). The concentration of Cd in the gills from Nghe An (0.010 μ·g^{-1} d.w) was significantly lower than the Cd content in the gills from Quang Binh (0.022 μ·g^{-1} d.w). They are shown by a (p=0.031) and b (p=0.016) in Fig. 22.

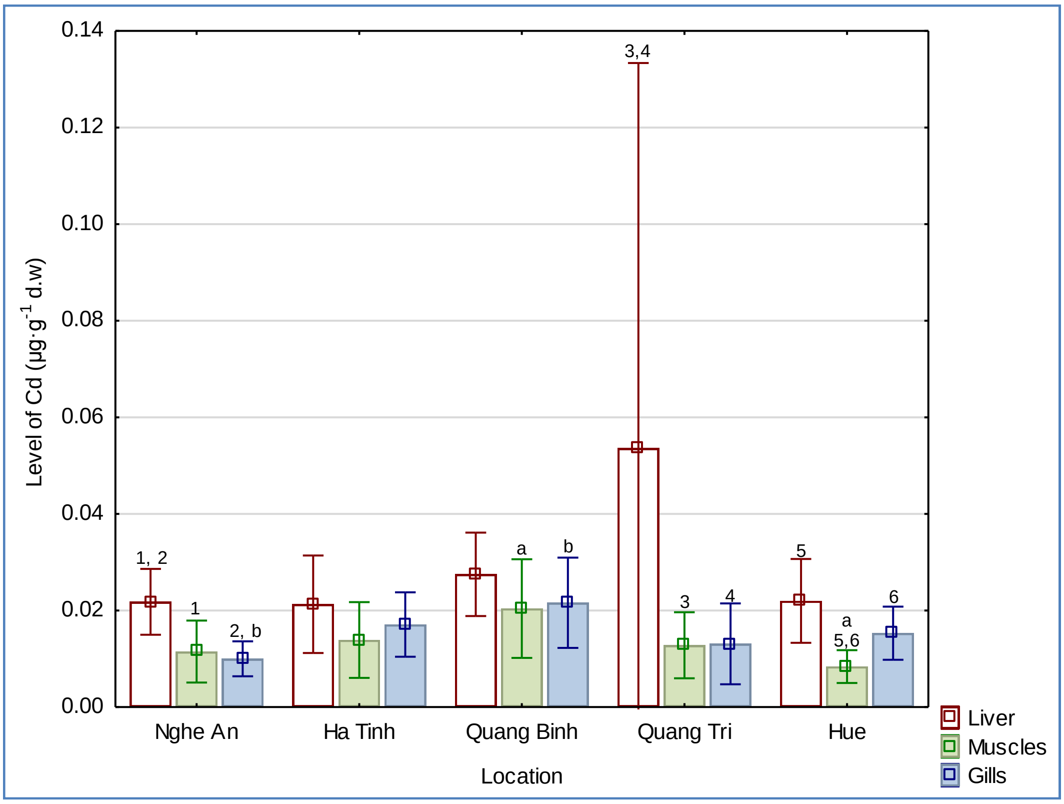

Figure 22. The mean, SD of Cd concentration in tissues of *M. cephalus*
(1, p=0.002; 2, p<0.001; 3, p=0.002; 4, p=0.002; 5, p<0.001; 6, p=0.034; a, p=0.031; b, p=0.016)

3. 3. Pb concentration

The mean, SE, SD, min and max amounts of Pb (μ·g^{-1} d.w) in the liver, muscles and gills of *M. cephalus* are summarized in Table 16 and Fig. 23.

The highest average concentration of Pb was found in the gills from Nghe An (0.226 μ·g^{-1} d.w), which was significantly higher than the Pb level in the gills from Quang Binh (0.102 μ·g^{-1} d.w). The lowest content was found in the muscles from Ha Tinh (0.016 μ μ·g^{-1} d.w), which was significantly lower than the Pb accumulation in the muscles from Quang Binh (0.030 μ·g^{-1} d.w). The differences were determined by the effect of multiple comparisons of mean ranks test, which are shown by a (p=0.040) and b (p=0.025) in Fig. 23.

In Nghe An, Ha Tinh and Hue, the Pb concentration in muscles was significantly lower than the Pb concentration in the liver and gills, which was supported by the effect of multiple comparisons of mean ranks test (1, p=0.031; 2, p<0.001; 3, p=0.043; 4, p=0.001; 7, p<0.001; 8, p=0.011).

Table 16. The level of Pb in liver, muscles, gills of *M. cephalus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.127±0.032	0.020±0.003	0.226±0.034
	Min-Max	0.003-0.364	0.007-0.039	0.116-0.443
	Shapiro-Wilk test (p)	0.079	0.351	0.264
	Levene test	F=6.699; p=0.004		
	Kruskal-Wallis test	H=18.665; p<0.001		
Ha Tinh (n=10)	Mean±SE	0.104±0.037	0.016±0.004	0.124±0.018
	Min-Max	0.008-0.315	0.005-0.043	0.017-0.185
	Shapiro-Wilk test (p)	0.016	0.028	0.128
	Kruskal-Wallis test	H=13.113; p=0.001		
Quang Binh (n=10)	Mean±SE	0.147±0.036	0.030±0.004	0.102±0.015
	Min-Max	0.010-0.322	0.008-0.048	0.011-0.160
	Shapiro-Wilk test (p)	0.218	0.877	0.403
	Levene test	F=20.357; p<0.001		
	Kruskal-Wallis test	H=7.942; p=0.019		
Quang Tri (n=10)	Mean±SE	0.122±0.035	0.021±0.003	0.151±0.023
	Min-Max	0.003-0.281	0.007-0.043	0.062-0.327
	Shapiro-Wilk test (p)	0.061	0.648	0.048
	Kruskal-Wallis test	H=10.318; p=0.006		
Hue (n=10)	Mean±SE	0.188±0.032	0.021±0.003	0.113±0.017
	Min-Max	0.069-0.364	0.007-0.042	0.011-0.210
	Shapiro-Wilk test (p)	0.296	0.706	0.890
	Levene test	F=12.574; p<0.001		
	Kruskal-Wallis test	H=17.751; p<0.001		

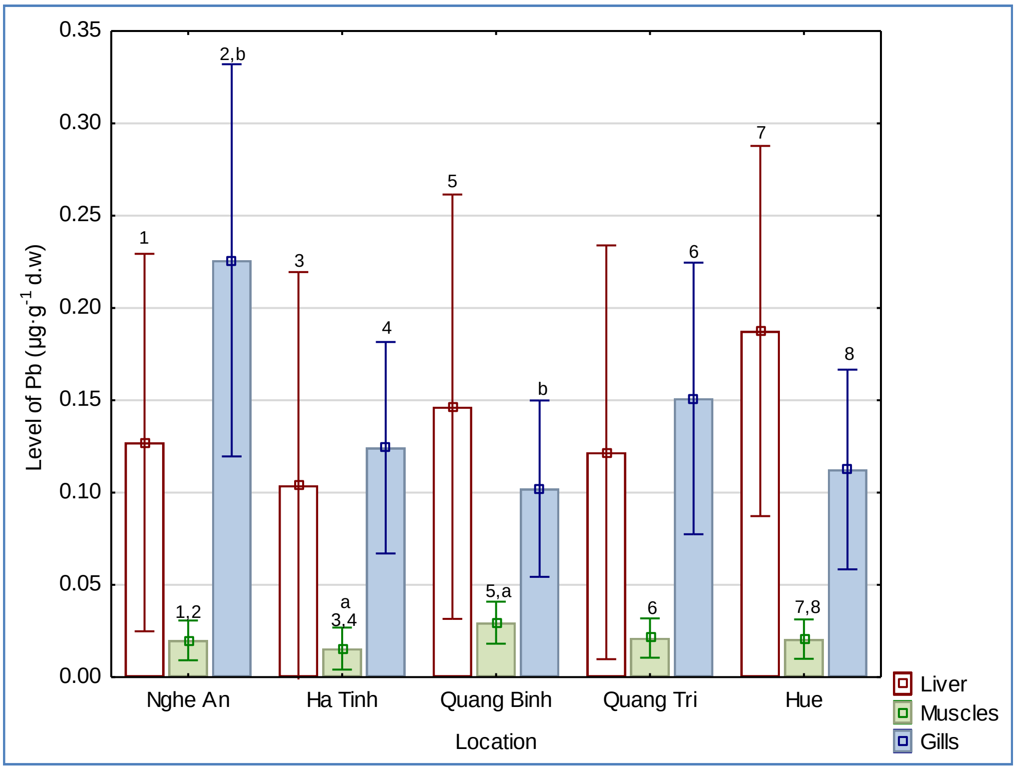

Figure 23. The mean, SD of Pb concentration in tissues of *M. cephalus*
(1, p=0.031; 2, p<0.001; 3, p=0.043; 4, p=0.001; 5, p=0.038; 6, p=0.005; 7, p<0.001; 8, p=0.011; a, p=0.040; b, p=0.025)

In Quang Binh, the Pb content in the liver was much higher than in the muscles. In Quang Tri, the Pb concentration in the muscles was many times lower than in the gills, as shown by the effect of multiple comparisons of mean ranks test (5, p=0.038; 6, p=0.005).

3. 4. Fe concentration

The mean, SE, SD, min and max amounts of Fe ($\mu\cdot g^{-1}$ d.w) in the liver, muscles and gills of *M. cephalus* are summarized in Table 17 and Fig. 24.

In all the researched regions, the highest Fe content was found in the gills in the following order: Nghe An>Ha Tinh>Quang Tri>Hue>Quang Binh. However, there was no statistical difference.

The Fe content in the liver was displayed in the following order: Nghe An>Hue>Quang Binh>Quang Tri>Ha Tinh, in which the statistically significant difference was detected between the Nghe An and Ha Tinh regions (a, p=0.018).

Table 17. The level of Fe in liver, muscles, gills of *M. cephalus* ($\mu\cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	3.303±0.361	2.523±0.561	6.133±0.864
	Min-Max	1.287-5.817	0.652-4.822	3.180-11.530
	Shapiro-Wilk test (p)	0.168	0.042	0.018
	Kruskal-Wallis test		H=11.708; p=0.003	
Ha Tinh (n=10)	Mean±SE	1.525±0.286	1.971±0.443	5.704±0.979
	Min-Max	0.575-2.922	0.660-4.958	1.383-12.296
	Shapiro-Wilk test (p)	0.068	0.033	0.553
	Kruskal-Wallis test		H=13.566; p=0.001	
Quang Binh (n=10)	Mean±SE	2.882±0.480	3.403±0.557	3.769±0.481
	Min-Max	0.485-5.092	0.186-6.382	1.635-5.782
	Shapiro-Wilk test (p)	0.711	0.909	0.392
	ANOVA		F=0.772; p=0.472	
	Levene test		F=0.077; p=0.926	
Quang Tri (n=10)	Mean±SE	2.414±0.577	2.905±0.843	4.928±0.295
	Min-Max	0.598-5.817	0.186-8.392	3.180-6.058
	Shapiro-Wilk test (p)	0.087	0.158	0.646
	Levene test		F=5.222; p=0.012	
	Kruskal-Wallis test		H=7.798; p=0.020	
Hue (n=10)	Mean±SE	3.168±0.447	2.655±0.540	4.513±0.593
	Min-Max	1.287-5.817	0.678-4.653	1.635-7.794
	Shapiro-Wilk test (p)	0.278	0.027	0.685
	Kruskal-Wallis test		H=5.345; p=0.069	

The concentration of Fe in the muscles was observed in the order: Quang Binh>Quang Tri>Hue>Nghe An>Ha Tinh, which also did not show any statistically significant differences between the study areas.

In Nghe An and Ha Tinh, the accumulation of Fe in gills was significantly higher than in the liver and muscles, as shown by the effect of multiple comparisons of the mean ranks test (1, p=0.010; 2, p=0.001; 3, p=0.001; 4, p=0.010). In Quang Tri, the difference of Fe concentration was detected in the liver and gills. While in Quang Binh, there were no significant differences between the Fe content in the liver, gills and muscles.

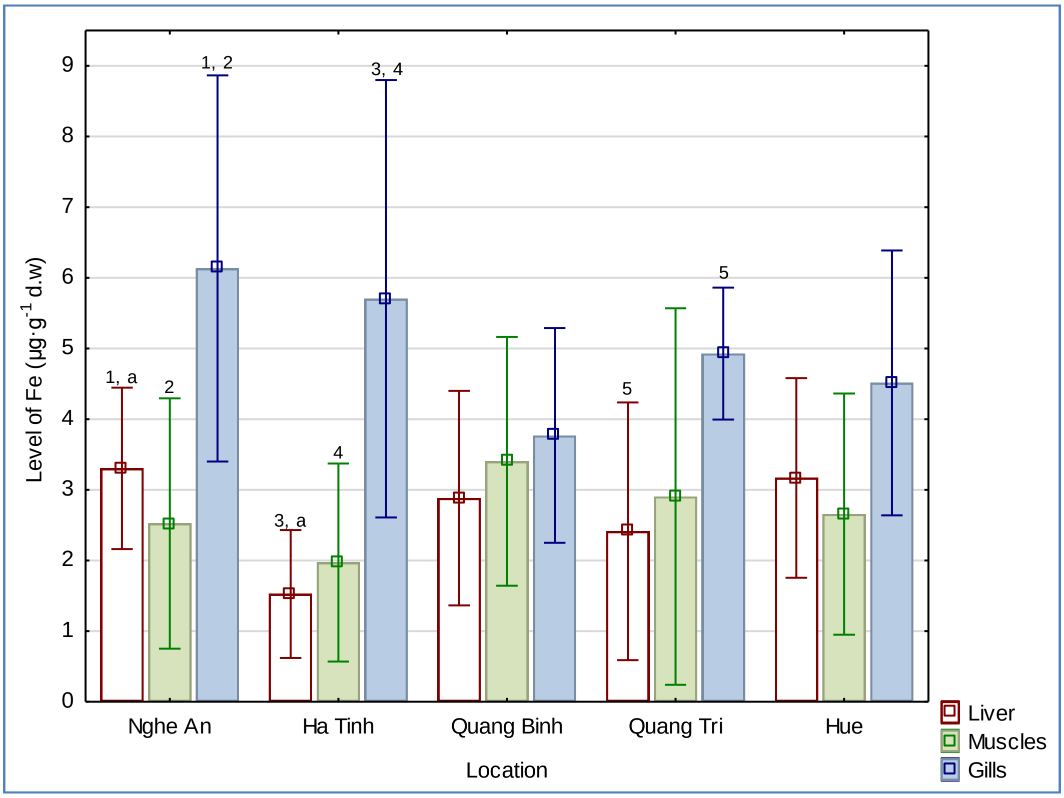

Figure 24. The mean, SD of Fe concentration in tissues of *M. cephalus*
(1, p=0.021; 2, p=0.005; 3, p=0.001; 4, p=0.010; 5, p=0.031; a, p=0.018)

3. 5. Zn concentration

The mean, SE, SD, min and max amounts of Zn ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *M. cephalus* are presented in Table 18 and Fig. 25.

The concentration of Zn in the tissues of *M. cephalus* was also observed to remain in the order: liver>gills>muscles in all the study regions. In the liver, the highest mean of Zn was found in Quang Binh (1.837 $\mu \cdot g^{-1}$ d.w), the lowest – in Nghe An (1.045 $\mu \cdot g^{-1}$ d.w).

In muscles, the highest mean of the Zn content was found in Quang Binh (0.651 $\mu \cdot g^{-1}$ d.w), and the lowest in Ha Tinh (0.393 $\mu \cdot g^{-1}$ d.w). In gills, the highest value came from Nghe An (0.774 $\mu \cdot g^{-1}$ d.w), and the lowest from Ha Tinh (0.636 $\mu \cdot g^{-1}$ d.w). However, the accumulation of Zn in the liver, muscles, and gills displayed no differences between the study areas.

Table 18. The level of Zn in liver, muscles, gills of *M. cephalus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	1.045±0.138	0.607±0.048	0.774±0.079
	Min-Max	0.456-1.675	0.463-0.900	0.543-1.268
	Shapiro-Wilk test (p)	0.542	0.026	0.029
	Kruskal-Wallis test		H=7.443; p=0.024	
Ha Tinh (n=10)	Mean±SE	1.071±0.193	0.393±0.040	0.636±0.075
	Min-Max	0.297-2.266	0.254-0.599	0.169-0.899
	Shapiro-Wilk test (p)	0.536	0.127	0.248
	Levene test		F=7.224; p=0.003	
	Kruskal-Wallis test		H=11.856; p=0.003	
Quang Binh (n=10)	Mean±SE	1.837±0.277	0.651±0.082	0.707±0.121
	Min-Max	0.552-.218	0.220-0.982	0.109-1.231
	Shapiro-Wilk test (p)	0.833	0.487	0.735
	Levene test		F=6.797; p=0.004	
	Kruskal-Wallis test		H=12.340; p=0.002	
Quang Tri (n=10)	Mean±SE	1.215±0.218	0.498±0.094	0.660±0.089
	Min-Max	0.456-2.266	0.048-0.982	0.109-1.201
	Shapiro-Wilk test (p)	0.118	0.379	0.329
	Levene test		F=10.780; p<0.001	
	Kruskal-Wallis test		H=7.673; p=0.022	
Hue (n=10)	Mean±SE	1.554±0.229	0.595±0.062	0.679±0.082
	Min-Max	0.595-2.928	0.382-0.903	0.252-1.201
	Shapiro-Wilk test (p)	0.737	0.059	0.920
	Levene test		F=5.504; p=0.010	
	Kruskal-Wallis test		H=14.042; p=0.001	

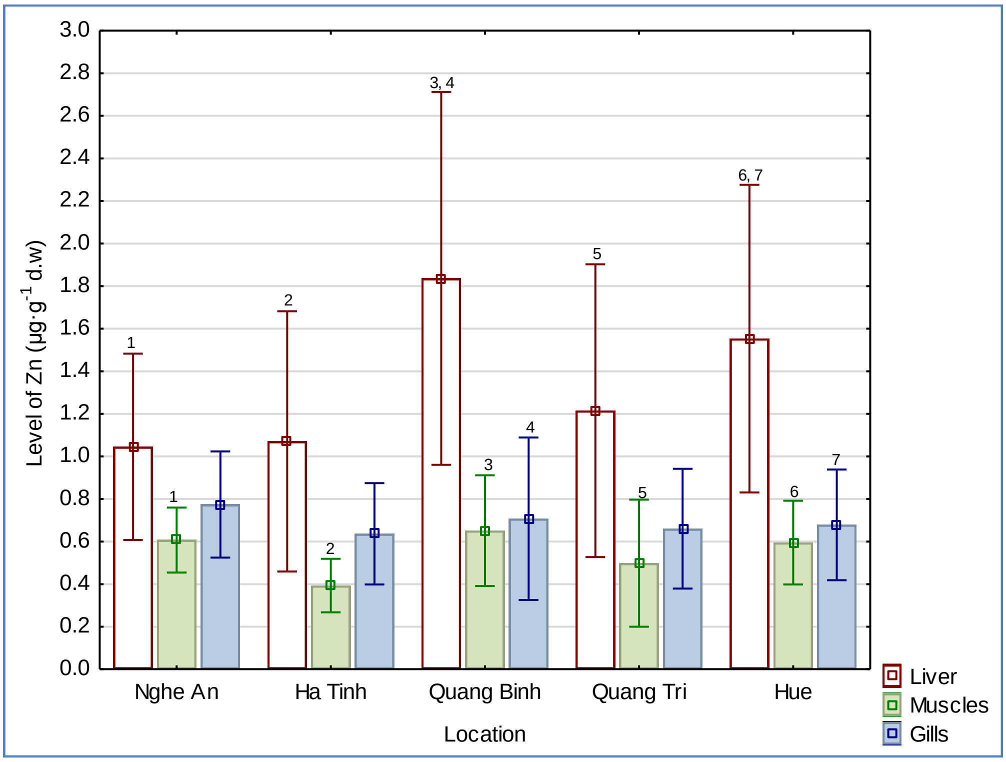

Figure 25. The mean, SD of Zn concentration in tissues of *M. cephalus*
(1, p=0.021; 2, p=0.002; 3, p=0.003; 4, p=0.018; 5, p=0.017; 6, p=0.001; 7, p=0.018)

In Nghe An, Ha Tinh and Quang Tri, the Zn contents in the liver were significantly higher than in the muscles. In Quang Binh and Hue, the levels of Zn in the muscles and gills were significantly lower than in the liver. They were confirmed by the effect of multiple comparisons of mean ranks test (1, p=0.021; 2, p=0.002; 3, p=0.003; 4, p=0.018; 5, p=0.017; 6, p=0.001; 8, p=0.018).

3. 6. Cu concentration

The mean, SE, SD, min and max levels of Cu ($\mu\cdot g^{-1}$ d.w) in the liver, muscles and gills of *M. cephalus* are shown in Table 19 and Fig. 26.

The highest mean value of Cu in the liver was found in Quang Binh (1.235 $\mu\cdot g^{-1}$ d.w), the lowest came from Nghe An (0.694 $\mu\cdot g^{-1}$ d.w). The differences were not meaningful between the regions. In muscles, the highest Cu level was found in Quang Binh (1.142 $\mu\cdot g^{-1}$ d.w), the lowest – in Nghe An (0.475 $\mu\cdot g^{-1}$ d.w). Again, there were no noticeable differences. Simultaneously, in the gills, the highest Cu content was found in Quang Binh (0.803$\mu\cdot g^{-1}$ d.w) and the lowest in Ha Tinh (0.446 $\mu\cdot g^{-1}$ d.w). The effect of multiple comparisons of mean ranks of Cu showed a significant difference (a, p=0.026).

Table 19. The level of Cu in liver, muscles, gills of *M. cephalus* ($\mu\cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.694±0.108	0.475±0.107	0.658±0.095
	Min-Max	0.250-1.140	0.120-1.113	0.144-1.181
	Shapiro-Wilk test (p)	0.187	0.135	0.354
	ANOVA		F=1.285; p=0.293	
	Levene test		F=0.396; p=0.677	
Ha Tinh (n=10)	Mean±SE	0.705±0.123	0.736±0.165	0.446±0.062
	Min-Max	0.155-1.121	0.222-2.011	0.228-0.925
	Shapiro-Wilk test (p)	0.072	0.026	0.047
	Kruskal-Wallis test		H=3.243; p=0.198	
Quang Binh (n=10)	Mean±SE	1.235±0.153	1.142±0.269	0.803±0.098
	Min-Max	0.590-2.100	0.225-3.209	0.427-1.270
	Shapiro-Wilk test (p)	0.420	0.055	0.333
	ANOVA		F=1.473; p=0.247	
	Levene test		F=1.824; p=0.181	
Quang Tri (n=10)	Mean±SE	1.012±0.225	0.811±0.279	0.534±0.057
	Min-Max	0.250-2.812	0.190-3.100	0.144-0.783
	Shapiro-Wilk test (p)	0.019	0.001	0.148
	Kruskal-Wallis test		H=3.544; p=0.170	
Hue (n=10)	Mean±SE	0.812±0.152	0.747±0.153	0.732±0.082
	Min-Max	0.250-1.902	0.223-1.627	0.427-1.209
	Shapiro-Wilk test (p)	0.206	0.184	0.480
	ANOVA		F=0.102; p=0.903	
	Levene test		F=1.454; p=0.251	

In Nghe An, the highest Cu value was found in the liver, the lowest – in the muscles. In Ha Tinh, the lowest content was found in the gills and the highest in the muscles. In Quang Binh, Quang Tri and Hue, however, the highest concentrations of Cu were concentrated in the liver and the lowest in the gills.

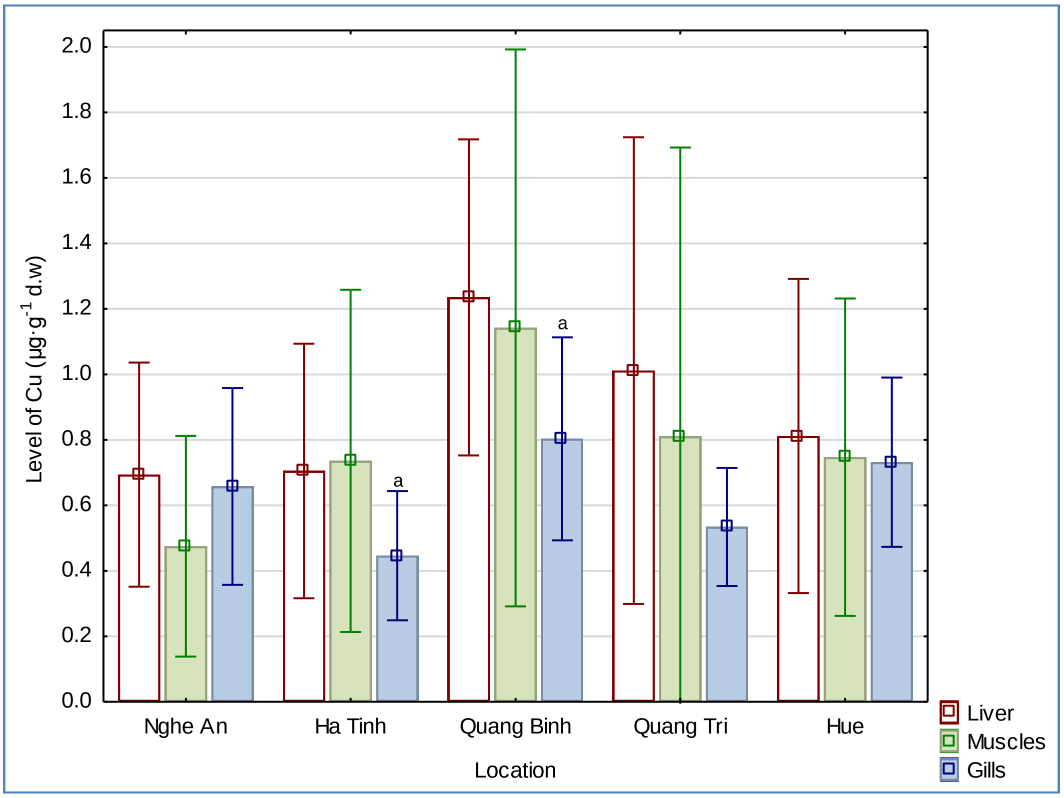

Figure 26. The mean, SD of Cu concentration in tissues of *M. cephalus*
(a, p=0.026)

4. Concentration of heavy metals in *Siganus fuscescens*

4. 1. Hg concentration

The mean, SE, SD, min and max measure of Hg ($\mu \cdot g^{-1}$ w.w) in the liver, muscles and gills of *S. fuscescens* are presented in Table 20 and Fig. 27.

In Nghe An, the highest Hg level was found in the muscles (0.092 $\mu \cdot g^{-1}$ w.w), while the lowest in the gills (0.042 $\mu \cdot g^{-1}$ w.w). The mean of Hg in the gills was significantly lower than the level of Hg in the liver and muscles, which was shown by the effect of multiple comparisons of mean ranks test (1, 2).

In Ha Tinh, the highest of Hg concentration was found in the liver (0.072 $\mu \cdot g^{-1}$ w.w) and the lowest in the gills (0.034 $\mu \cdot g^{-1}$ w.w). The average of Hg accumulation in the gills was also substantially lower than the Hg content in the liver and muscles. The differences were confirmed by the effect of multiple comparisons of mean ranks test (3, 4).

Table 20. The level of Hg in liver, muscles, gills of *S. fuscescens* ($\mu\cdot g^{-1}$ w.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.073±0.004	0.092±0.010	0.042±0.003
	Min-Max	0.058-0.100	0.038-0.143	0.026-0.061
	Shapiro-Wilk test (p)	0.283	0.994	0.653
	Levene test		F=4.386; p=0.022	
	Kruskal-Wallis test		H=17.525; p<0.001	
Ha Tinh (n=10)	Mean±SE	0.072±0.007	0.063±0.005	0.034±0.003
	Min-Max	0.049-0.117	0.038-0.084	0.025-0.055
	Shapiro-Wilk test (p)	0.057	0.672	0.047
	Kruskal-Wallis test		H=17.821; p<0.001	
Quang Binh (n=10)	Mean±SE	0.167±0.047	0.075±0.008	0.025±0.002
	Min-Max	0.032-0.459	0.036-0.106	0.019-0.037
	Shapiro-Wilk test (p)	0.032	0.319	0.068
	Kruskal-Wallis test		H=18.917; p<0.001	
Quang Tri (n=10)	Mean±SE	0.145±0.052	0.092±0.009	0.030±0.002
	Min-Max	0.032-0.555	0.055-0.143	0.018-0.039
	Shapiro-Wilk test (p)	<0.001	0.687	0.740
	Kruskal-Wallis test		H=17.980; p<0.001	
Hue (n=10)	Mean±SE	0.070±0.005	0.064±0.007	0.037±0.004
	Min-Max	0.049-0.087	0.036-0.106	0.022-0.061
	Shapiro-Wilk test (p)	0.119	0.213	0.098
	Levene test		F=4.033; p=0.029	
	Kruskal-Wallis test		H=13.241; p=0.001	

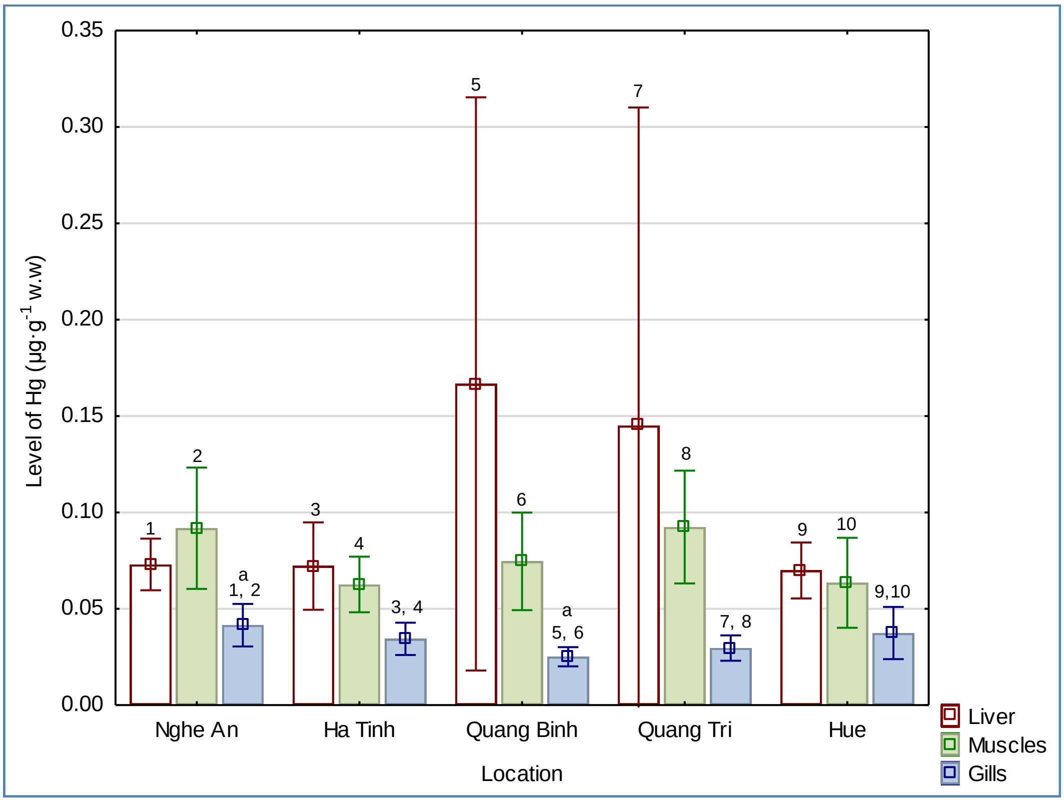

Figure 27. The mean, SD of Hg concentration in tissues of *S. fuscescens*

(1, p=0.007; 2, p<0.001; 3, p<0.001; 4, p=0.003; 5, p<0.001; 6, p=0.002; 7, p=0.002; 8, p<0.001; 9, p=0.001; 10, p=0.029; a, p=0.002)

Homologous to Ha Tinh, the mean of the Hg contents in the gills from Quang Binh, Quang Tri and Hue were strongly lower than the Hg accumulation in the liver and muscles, which was determined by the effect of multiple comparisons of mean ranks test (5-10).

4. 2. Cd concentration

The mean, SE, SD, min and max amounts of Cd ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *S. fuscescens* are presented in Table 21 and Fig. 28.

In Nghe An, the mean value of Cd in gills was the highest (0.026 $\mu \cdot g^{-1}$ d.w), while the lowest value was detected in the muscles (0.020 $\mu \cdot g^{-1}$ d.w). However, there are no noticeable differences between them.

In Ha Tinh, the Cd accumulation in the liver was unquestionably higher than the accumulation in muscles (a, p=0.007), however, there was no difference between the muscles and gills or the gills compared to the liver.

Concurrently, in Quang Binh, Quang Tri and Hue, the Cd values in the liver were always at the highest level, but there was no difference in the statistical significance.

Table 21. The level of Cd in liver, muscles, gills of *S. fuscescens* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.022±0.003	0.020±0.002	0.026±0.002
	Min-Max	0.016-0.043	0.010-0.028	0.014-0.034
	Shapiro-Wilk test (p)	0.014	0.830	0.186
	Kruskal-Wallis test		H=4.163; p=0.125	
Ha Tinh (n=10)	Mean±SE	0.038±0.008	0.016±0.002	0.021±0.002
	Min-Max	0.012-0.089	0.007-0.024	0.009-0.032
	Shapiro-Wilk test (p)	0.020	0.181	0.805
	Kruskal-Wallis test		H=9.418; p=0.009	
Quang Binh (n=10)	Mean±SE	0.030±0.004	0.023±0.003	0.021±0.002
	Min-Max	0.015-0.054	0.009-0.034	0.012-0.035
	Shapiro-Wilk test (p)	0.490	0.900	0.426
	ANOVA		F=2.469; p=0.104	
	Levene test		F=2.879; p=0.073	
Quang Tri (n=10)	Mean±SE	0.026±0.004	0.019±0.002	0.022±0.003
	Min-Max	0.015-0.052	0.012-0.026	0.009-0.035
	Shapiro-Wilk test (p)	0.046	0.074	0.217
	Kruskal-Wallis test		H=1.999; p=0.370	
Hue (n=10)	Mean±SE	0.032±0.007	0.023±0.003	0.025±0.002
	Min-Max	0.008-0.077	0.009-0.042	0.014-0.034
	Shapiro-Wilk test (p)	0.015	0.764	0.326
	Kruskal-Wallis test		H=0.565; p=0.754	

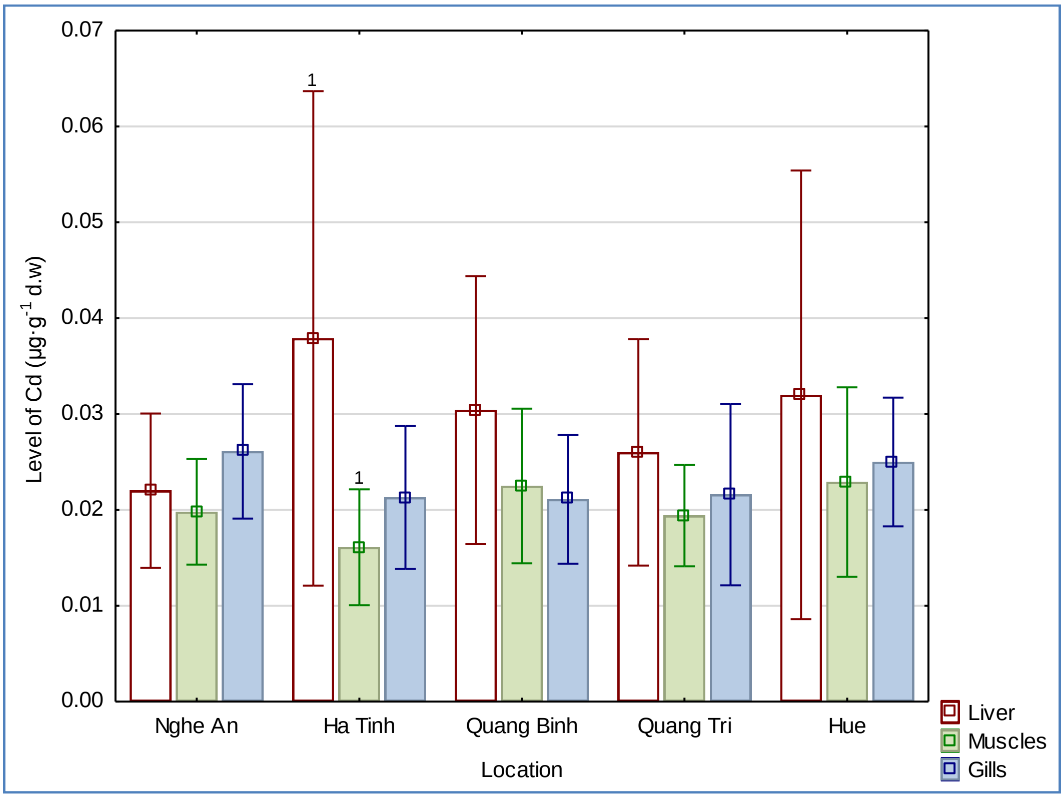

Figure 28. The mean, SD of Cd concentration in tissues of *S. fuscescens*
(1, p= 0.007)

4. 3. Pb concentration

The mean, SE, SD, min and max amounts of Pb ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *S. fuscescens* are outlined in Table 22 and Fig. 29.

There was no compelling difference in the Pb concentration in the liver, muscles, and gills in the study areas, except for the Pb concentration in the liver from Hue which was greater than the Pb concentration in the liver from Quang Binh (a, p=0.028).

The average value of Pb in the liver, muscles, and gills was observed to follow the order: Hue>Nghe An=Ha Tinh>Quang Tri>Quang Binh; Quang Binh>Nghe An>Quang Tri>Ha Tinh>Hue; Quang Binh>Hue>Nghe An>Quang Tri>Ha Tinh, respectively.

The difference was only observed in Quang Binh when the Pb content was in muscle and brought higher in the liver (1, p=0.023; 2, p=0.014).

Table 22. The level of Pb in liver, muscles, gills of *S. fuscescens* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.040±0.013	0.029±0.010	0.019±0.005
	Min-Max	0.009-0.116	0.003-0.094	0.003-0.053
	Shapiro-Wilk test (p)	0.003	0.009	0.098
	Kruskal-Wallis test		H=2.294; p=0.317	
Ha Tinh (n=10)	Mean±SE	0.040±0.012	0.015±0.004	0.016±0.004
	Min-Max	0.006-0.098	0.001-0.044	0.004-0.039
	Shapiro-Wilk test (p)	0.016	0.151	0.048
	Kruskal-Wallis test		H=2.881; p=0.237	
Quang Binh (n=10)	Mean±SE	0.010±0.001	0.071±0.043	0.026±0.004
	Min-Max	0.004-0.018	0.009-0.450	0.007-0.050
	Shapiro-Wilk test (p)	0.441	<0.001	0.891
	Kruskal-Wallis test		H=10.084; p=0.007	
Quang Tri (n=10)	Mean±SE	0.017±0.004	0.019±0.009	0.018±0.005
	Min-Max	0.004-0.044	0.003-0.098	0.004-0.050
	Shapiro-Wilk test (p)	0.150	<0.001	0.067
	Kruskal-Wallis test		H=0.623; p=0.732	
Hue (n=10)	Mean±SE	0.045±0.013	0.013±0.002	0.022±0.005
	Min-Max	0.009-0.108	0.006-0.028	0.005-0.053
	Shapiro-Wilk test (p)	0.006	0.016	0.405
	Kruskal-Wallis test		H=5.209; p=0.074	

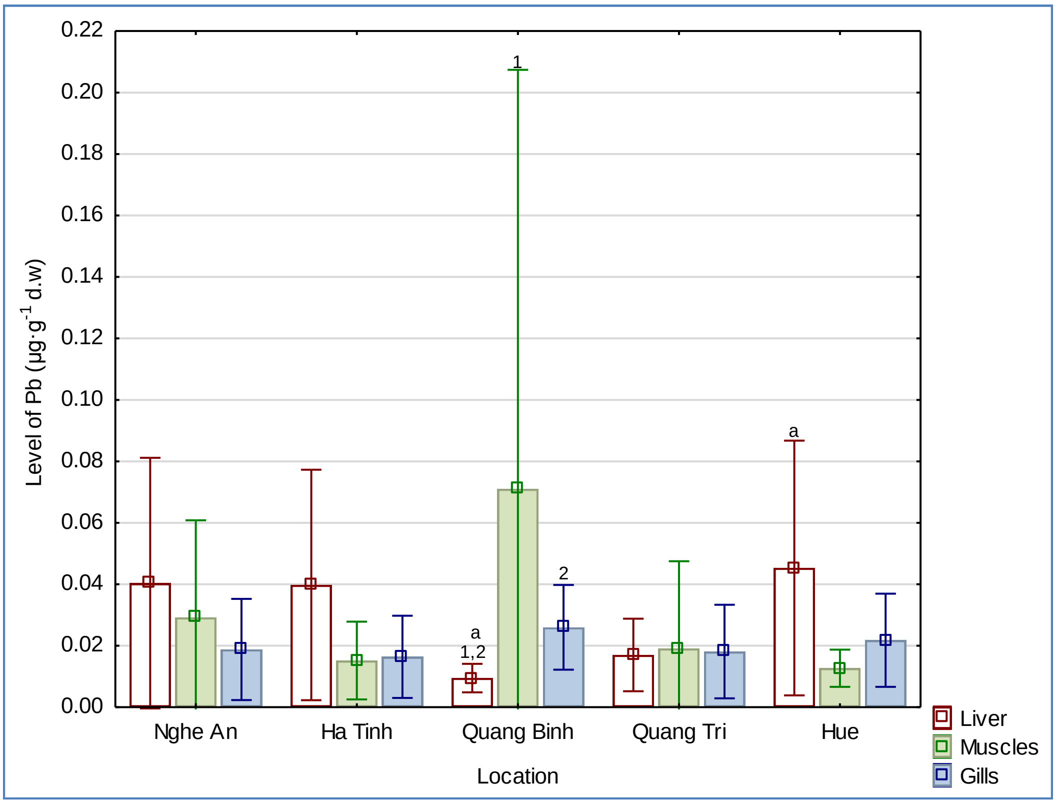

Figure 29. The mean, SD of Pb concentration in tissues of *S. fuscescens*
(1, p=0.023; 2, p=0.014; a, p=0.028)

4. 4. Fe concentration

The mean, SE, SD, min and max amounts of Fe ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *S. fuscescens* are presented in Table 23 and Fig.30.

In Nghe An, Ha Tinh, and Hue, the accumulation of Fe in muscles was found to be pressingly lower than the accumulation in the liver and gills, which was confirmed by the effect of multiple comparisons of mean ranks test (1, p=0.010; 2, p=0.012; 6, p=0.012). In Quang Binh, the highest Fe value was detected in the liver, which was considerably higher than the level in the muscles. The differences were demonstrated by the Tukey HSD test (3). Simultaneously, in Quang Tri, the highest concentration was detected in the gills (3.580 $\mu \cdot g^{-1}$ d.w). The data also showed that the accumulation of Fe in the muscles was much lower than in the liver and gills (4, p=0.002; 5, p<0.001).

Compared to the other study sites, the Fe content in the muscles from Quang Tri was strongly lower than the Fe concentration in the muscles from Quang Binh (a, p=0.012) and Hue (b, p=0.004).

Table 23. The level of Fe in liver, muscles, gills of *S. fuscescens* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	3.675±0.495	1.792±0.287	3.119±0.255
	Min-Max	2.100-6.745	1.158-3.430	1.936-4.569
	Shapiro-Wilk test (p)	0.088	0.001	0.019
	Kruskal-Wallis test		H=9.618; p=0.008	
Ha Tinh (n=10)	Mean±SE	3.196±0.269	2.038±0.357	2.223±0.278
	Min-Max	2.198-4.888	1.111-4.532	1.378-4.538
	Shapiro-Wilk test (p)	0.476	0.027	0.003
	Kruskal-Wallis test		H=9.092; p=0.011	
Quang Binh (n=10)	Mean±SE	3.481±0.390	2.108±0.198	2.802±0.408
	Min-Max	1.904-5.493	1.200-3.189	1.328-5.304
	Shapiro-Wilk test (p)	0.558	0.407	0.369
	ANOVA		F=3.958; p=0.031	
	Levene test		F=2.618; p=0.091	
Quang Tri (n=10)	Mean±SE	2.836±0.239	1.227±0.036	3.580±0.437
	Min-Max	1.730-4.210	1.111-1.432	1.378-5.304
	Shapiro-Wilk test (p)	0.899	0.206	0.285
	Levene test		F=15.527; p<0.001	
	Kruskal-Wallis test		H=19.685; p<0.001	
Hue (n=10)	Mean±SE	3.439±0.406	2.097±0.144	2.417±0.172
	Min-Max	2.153-5.854	1.366-2.754	1.637-3.087
	Shapiro-Wilk test (p)	0.176	0.640	0.178
	Levene test		F=7.018; p=0.004	
	Kruskal-Wallis test		H=8.242; p=0.016	

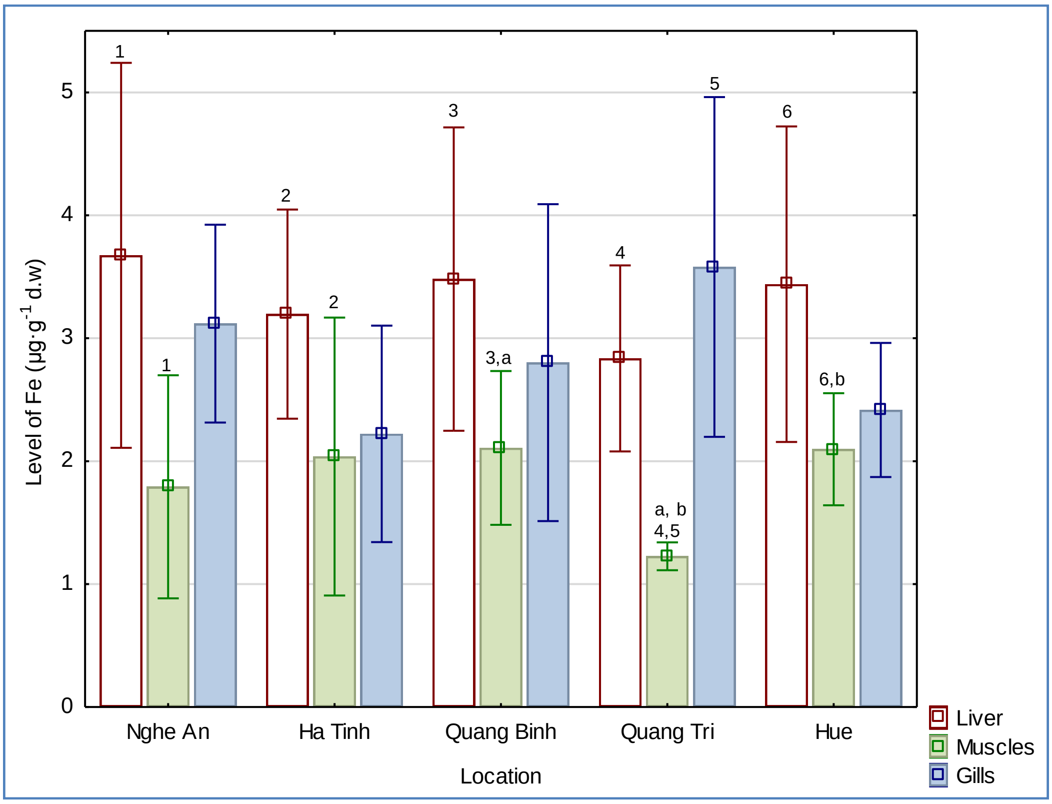

Figure 30. The mean, SD of Fe concentration in tissues of *S. fuscescens*
(1, p=0.010; 2, p=0.012; 3, p=0.024; 4, p=0.002; 5, p<0.001; 6, p=0.012; a, p=0.012; b, p=0.004)

4. 5. Zn concentration

The mean, SE, SD, min and max amounts of Zn ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *S. fuscescens* are presented in Table 24 and Fig. 31.

In Nghe An, Ha Tinh, Quang Binh, and Quang Tri, the highest values of Zn are detected in the liver. At the same time, the lowest quantities concentrated in the muscles. However, in Hue, the lowest value of Zn was found to be low in the gill, although the highest rate was detected in the liver.

The Tukey HSD test showed that the accretion of Zn in the liver was substantially higher than the concentration of Zn stored in the muscles and gills in Nghe An (1, p=0.001; 2, p=0.014). At the same time, the effect of multiple comparisons of the mean ranks test also confirmed that the Zn content in the muscle was significantly lower than the accumulation in the liver in Ha Tinh (3, p=0.038), Quang Binh (4, p=0.036) and Quang Tri (5, p=0.023; 6, p=0.029) and Hue (7, p=0.011)

The data in Fig. 31 shows that there was no appreciable difference between the study areas for the Zn accumulation in the organs of *S. fuscescens*.

Table 24. The level of Zn in liver, muscles, gills of *S. fuscescens* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.553±0.045	0.333±0.022	0.401±0.035
	Min-Max	0.396-0.840	0.253-0.467	0.247-0.562
	Shapiro-Wilk test (p)	0.206	0.399	0.395
	ANOVA		F=10.112; p=0.001	
	Levene test		F=2.368; p=0.113	
Ha Tinh (n=10)	Mean±SE	0.476±0.071	0.283±0.029	0.437±0.049
	Min-Max	0.128-0.912	0.159-0.421	0.281-0.809
	Shapiro-Wilk test (p)	0.897	0.436	0.024
	Kruskal-Wallis test		F=1.837; p=0.179	
Quang Binh (n=10)	Mean±SE	0.689±0.103	0.358±0.059	0.410±0.044
	Min-Max	0.243-1.213	0.124-0.642	0.198-0.632
	Shapiro-Wilk test (p)	0.510	0.291	0.745
	Levene test		F=5.582; p=0.009	
	Kruskal-Wallis test		H=7.022; p=0.030	
Quang Tri (n=10)	Mean±SE	0.624±0.089	0.328±0.033	0.335±0.043
	Min-Max	0.312-1.102	0.128-0.447	0.176-0.639
	Shapiro-Wilk test (p)	0.223	0.194	0.177
	Levene test		F=9.376; p=0.001	
	Kruskal-Wallis test		H=9.233; p=0.010	
Hue (n=10)	Mean±SE	0.488±0.030	0.325±0.039	0.304±0.046
	Min-Max	0.326-0.602	0.198-0.490	0.159-0.642
	Shapiro-Wilk test (p)	0.251	0.024	0.078
	Kruskal-Wallis test		H=9.163; p=0.010	

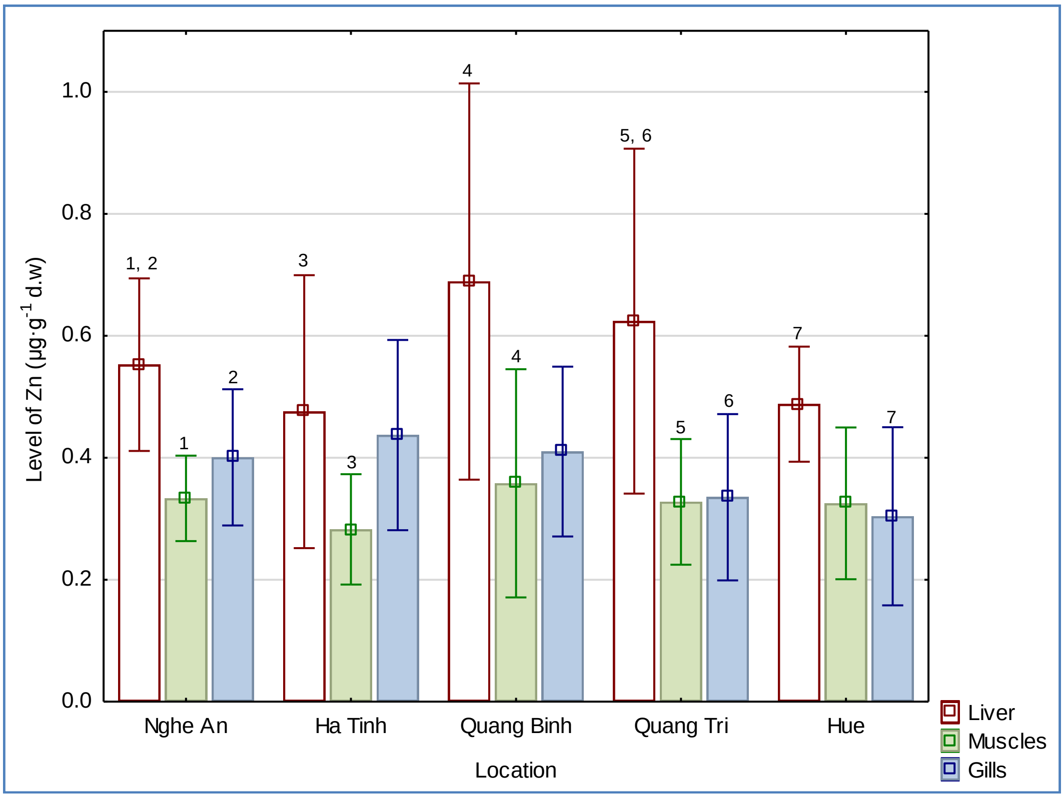

Figure 31. The mean, SD of Zn concentration in tissues of *S. fuscescens*
(1, p=0.001; 2, p=0.014; 3, p=0.038; 4, p=0.036; 5, p=0.023; 6, p=0.029; 7, p=0.011)

4. 6. Cu concentration

The mean, SE, SD, min and max volumes of Cu ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *S. fuscescens* are given in Table 25 and Fig. 32.

In general, the Cu accumulation in the organs of *S. fuscescens* at the study areas followed the order: liver>gills>muscles (except for the Hue province: liver>muscles>gills).

In the liver, Ha Tinh recorded the highest aggregation of Cu (0.687 $\mu \cdot g^{-1}$ d.w), while Hue recorded the lowest (0.511 $\mu \cdot g^{-1}$ d.w). However, according to the statistical analysis, there was no remarkable difference between them.

In the muscle, the highest values were found in Ha Tinh (0.263 $\mu \cdot g^{-1}$ d.w), while the lowest statistics were observed in Quang Tri (0.107 $\mu \cdot g^{-1}$ d.w), however, there were no obvious differences between the studied areas. In the gill, the highest value of Cu was discovered in Nghe An (0.371 $\mu \cdot g^{-1}$ d.w), while the lowest was recorded in Hue (0.152 $\mu \cdot g^{-1}$ d.w), the change was only identified between Quang Tri and Hue (a, p=0.018).

In each study area, the Cu levels in the liver were observed higher than the levels in muscles, which were shown by number 1-8 in Fig. 32 as verified by the Tukey HSD test (in Ha Tinh) and the effect of multiple comparisons of mean ranks test (for Nghe An, Quang Binh, Quang Tri and Hue).

Table 25. The level of Cu in liver, muscles, gills of *S. fuscescens* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.513±0.070	0.118±0.021	0.371±0.113
	Min-Max	0.283-0.970	0.010-0.254	0.010-0.930
	Shapiro-Wilk test (p)	0.230	0.902	0.016
	Kruskal-Wallis test	H=12.751; p=0.002		
Ha Tinh (n=10)	Mean±SE	0.687±0.100	0.263±0.054	0.307±0.094
	Min-Max	0.384-1.201	0.080-0.534	0.022-0.932
	Shapiro-Wilk test (p)	0.059	0.174	0.094
	ANOVA	F=7.451; p=0.003		
	Levene test	F=2.481; p=0.103		
Quang Binh (n=10)	Mean±SE	0.588±0.068	0.222±0.041	0.314±0.063
	Min-Max	0.352-1.000	0.109-0.456	0.165-0.760
	Shapiro-Wilk test (p)	0.107	0.012	0.007
	Kruskal-Wallis test	H=12.746; p=0.002		
Quang Tri (n=10)	Mean±SE	0.644±0.117	0.107±0.015	0.348±0.042
	Min-Max	0.214-1.230	0.010-0.190	0.165-0.498
	Shapiro-Wilk test (p)	0.078	0.792	0.071
	Levene test	F=24.929; p<0.001		
	Kruskal-Wallis test	H=20.181; p<0.001		
Hue (n=10)	Mean±SE	0.511±0.065	0.212±0.033	0.152±0.069
	Min-Max	0.283-0.889	0.090-0.400	0.022-0.760
	Shapiro-Wilk test (p)	0.420	0.326	<0.001
	Kruskal-Wallis test	H=15.501; p<0.001		

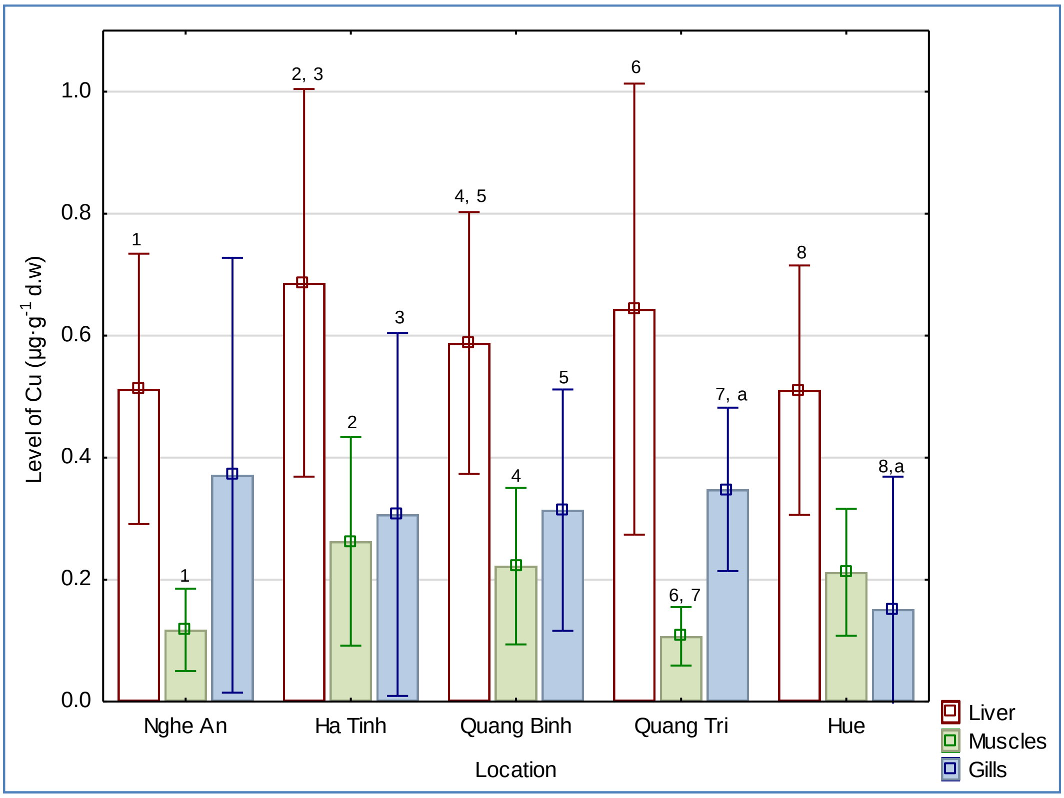

Figure 32. The mean, SD of Cu concentration in tissues of *S. fuscescens*
(1, p=0.001; 2, p=0.005; 3, p=0.011; 4, p=0.002; 5, p=0.027; 6, p<0.001; 7, p=0.004; 8, p<0.001; a, p=0.018)

5. Concentration of heavy metals in *Sillago sihama*

5. 1. Hg concentration

The mean, SE, SD, min and max amounts of Hg ($\mu\cdot g^{-1}$ w.w) in the liver, muscles, gills of *S. fuscescens* are presented in Table 26 and Fig. 33.

There was a similarity in the mean standards of Hg at the study sites, in the descending order of: liver> muscle> gills. Notably, the aggregation of Hg in the liver was unquestionably higher than the level of Hg in the gills, which has been verified by the effect of multiple comparisons of mean ranks test, presented by the number of symbols 1-6 in Fig. 33. More than that, in Ha Tinh the accumulation in Hg in the liver was also much higher than the accumulation in the muscles.

Table 26. The level of Hg in liver, muscles, gills of *S. sihama* (μ·g^{-1} w.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.821±0.144	0.273±0.043	0.132±0.015
	Min-Max	0.056-1.283	0.087-0.449	0.081-0.219
	Shapiro-Wilk test (p)	0.032	0.239	0.351
	Kruskal-Wallis test		H=10.570; p=0.005	
Ha Tinh (n=10)	Mean±SE	0.805±0.114	0.201±0.031	0.131±0.018
	Min-Max	0.096-1.312	0.028-0.393	0.081-0.260
	Shapiro-Wilk test (p)	0.846	0.880	0.054
	Levene test		F=10.952; p<0.001	
	Kruskal-Wallis test		H=15.950; p<0.001	
Quang Binh (n=10)	Mean±SE	0.655±0.108	0.231±0.028	0.154±0.021
	Min-Max	0.045-1.100	0.118-0.386	0.081-0.260
	Shapiro-Wilk test (p)	0.091	0.371	0.160
	Levene test		F=8.241; p=0.002	
	Kruskal-Wallis test		H=9.235; p=0.010	
Quang Tri (n=10)	Mean±SE	0.756±0.146	0.308±0.021	0.165±0.021
	Min-Max	0.056-1.312	0.208-0.393	0.081-0.260
	Shapiro-Wilk test (p)	0.386	0.230	0.350
	Levene test		F=17.565; p<0.001	
	Kruskal-Wallis test		H=12.794; p=0.002	
Hue (n=10)	Mean±SE	0.512±0.107	0.209±0.032	0.125±0.018
	Min-Max	0.045-0.958	0.087-0.386	0.081-0.260
	Shapiro-Wilk test (p)	0.244	0.222	0.008
	Kruskal-Wallis test		H=7.568; p=0.023	

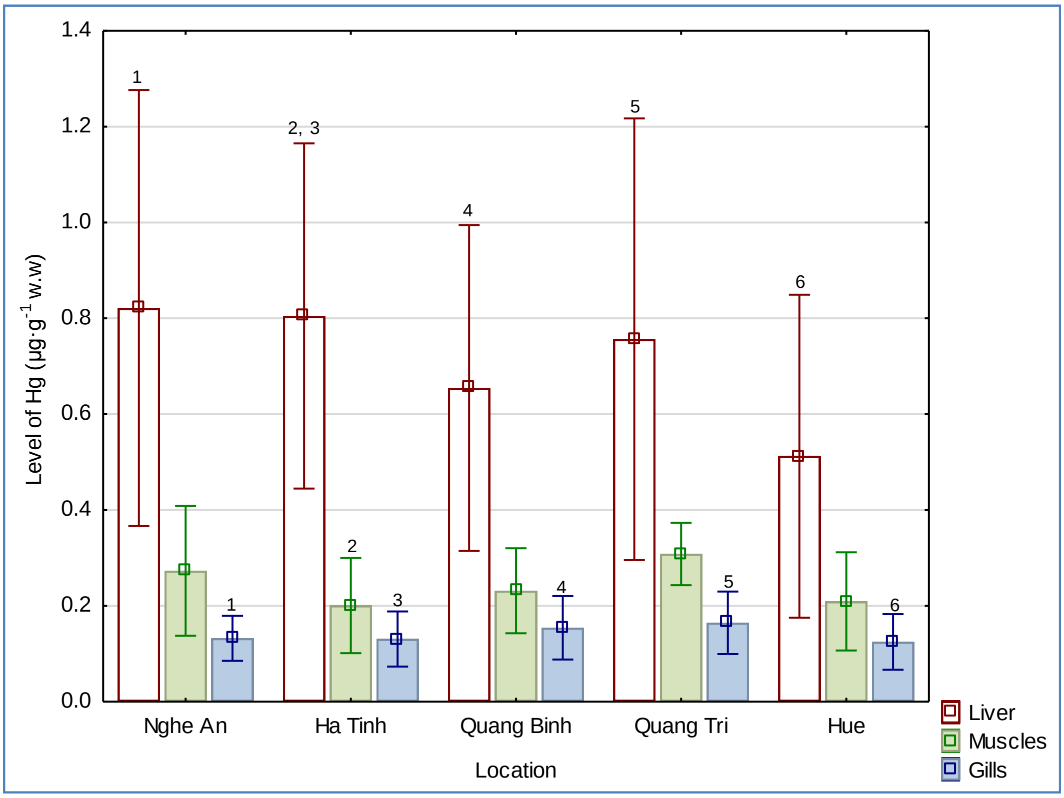

Figure 33. The mean, SD of Hg concentration in tissues of *S. sihama*
(1, p=0.003; 2, p=0.033; 3, p<0.001; 4, p=0.008; 5, p=0.001; 6, p=0.018)

5. 2. Cd concentration

The mean, SE, SD, min and max amounts of Cd ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *S. sihama* are presented in Table 27 and Fig. 34.

In Nghe An, the Cd accretion was the highest in the liver. The lowest was established in the muscles. In Ha Tinh the highest data was recorded in the liver but the lowest was found in the gills. Simultaneously, the data in Quang Binh showed the highest concentration in the gills and the lowest in the muscles. In both Quang Tri and Hue, the highest concentrations of Cd were recorded in the liver and the lowest in the gills. However, there was no analytically significant difference between them.

Table 27. The level of Cd in liver, muscles, gills of *S. sihama* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.021±0.006	0.014±0.005	0.015±0.002
	Min-Max	0.001-0.057	0.002-0.050	0.008-0.029
	Shapiro-Wilk test (p)	0.084	0.001	0.129
	Kruskal-Wallis test	H=2.771; p=0.250		
Ha Tinh (n=10)	Mean±SE	0.056±0.032	0.014±0.004	0.011±0.002
	Min-Max	0.007-0.330	0.004-0.051	0.003-0.021
	Shapiro-Wilk test (p)	<0.001	0.001	0.680
	Kruskal-Wallis test	H=2.589; p=0.274		
Quang Binh (n=10)	Mean±SE	0.026±0.006	0.014±0.003	0.049±0.038
	Min-Max	0.011-0.060	0.007-0.034	0.003-0.390
	Shapiro-Wilk test (p)	0.017	0.005	<0.001
	Kruskal-Wallis test	H-5.273; p=0.072		
Quang Tri (n=10)	Mean±SE	0.023±0.006	0.011±0.003	0.010±0.002
	Min-Max	0.007-0.057	0.004-0.034	0.003-0.028
	Shapiro-Wilk test (p)	0.007	0.001	0.019
	Kruskal-Wallis test	H=5.513; p=0.064		
Hue (n=10)	Mean±SE	0.054±0.027	0.016±0.005	0.012±0.002
	Min-Max	0.010-0.292	0.007-0.050	0.004-0.028
	Shapiro-Wilk test (p)	<0.001	<0.001	0.113
	Kruskal-Wallis test	H=5.969; p=0.051		

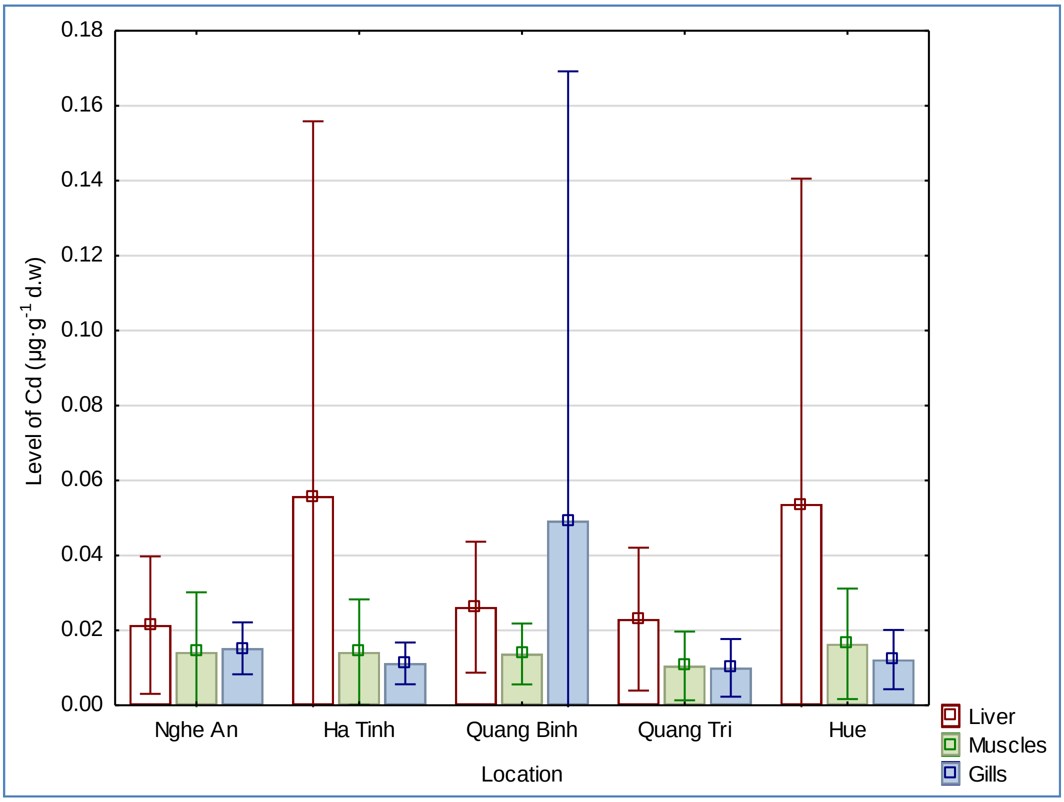

Figure 34. The mean, SD of Cd concentration in tissues of *S. sihama*

5. 3. Pb concentration

The mean, SE, SD, min and max amounts of Pb ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *S. sihama* are presented in Table 28 and Fig. 35.

The centralization of Pb still indicated a great concentration in the liver and gills.

In Nghe An, Ha Tinh, and Hue, the Pb content followed the order: liver>gills>muscles, while in Quang Binh and Quang Tri, the order of the Pb levels was: gills>liver>muscles.

Remarkably, in Ha Tinh and Quang Tri, the accumulation of Pb in muscles was much lower than the volume in the liver and gills, which was confirmed by the effect of multiple comparisons of the mean ranks test (2-4). Similarly, in Nghe An showed that the Pb value concentrated in the muscles was doubtlessly lower than the concentration in the liver and gills, in Hue showed the Pb level in muscles was lower than in gills, which was proven by the effect of multiple comparisons of the mean ranks test (1, $p=0.023$; 5, $p=0.047$).

Table 28. The level of Pb in liver, muscles, gills of *S. sihama* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.135±0.016	0.064±0.014	0.110±0.041
	Min-Max	0.064-0.251	0.006-0.121	0.024-0.360
	Shapiro-Wilk test (p)	0.452	0.129	<0.001
	Kruskal-Wallis test	H=8.144; p=0.017		
Ha Tinh (n=10)	Mean±SE	0.101±0.017	0.025±0.008	0.101±0.009
	Min-Max	0.013-0.229	0.007-0.080	0.052-0.133
	Shapiro-Wilk test (p)	0.032	0.001	0.552
	Kruskal-Wallis test	H=16.275; p<0.001		
Quang Binh (n=10)	Mean±SE	0.126±0.041	0.068±0.016	0.154±0.050
	Min-Max	0.046-0.490	0.004-0.170	0.005-0.587
	Shapiro-Wilk test (p)	<0.001	0.699	<0.001
	Kruskal-Wallis test	H=4.777; p=0.092		
Quang Tri (n=10)	Mean±SE	0.100±0.013	0.027±0.010	0.162±0.056
	Min-Max	0.046-0.171	0.006-0.115	0.005-0.587
	Shapiro-Wilk test (p)	0.412	<0.001	0.006
	Kruskal-Wallis test	H=11.638; p=0.003		
Hue (n=10)	Mean±SE	0.132±0.041	0.064±0.017	0.114±0.015
	Min-Max	0.013-0.490	0.006-0.170	0.005-0.171
	Shapiro-Wilk test (p)	<0.001	0.145	0.134
	Kruskal-Wallis test	H=6.062; p=0.048		

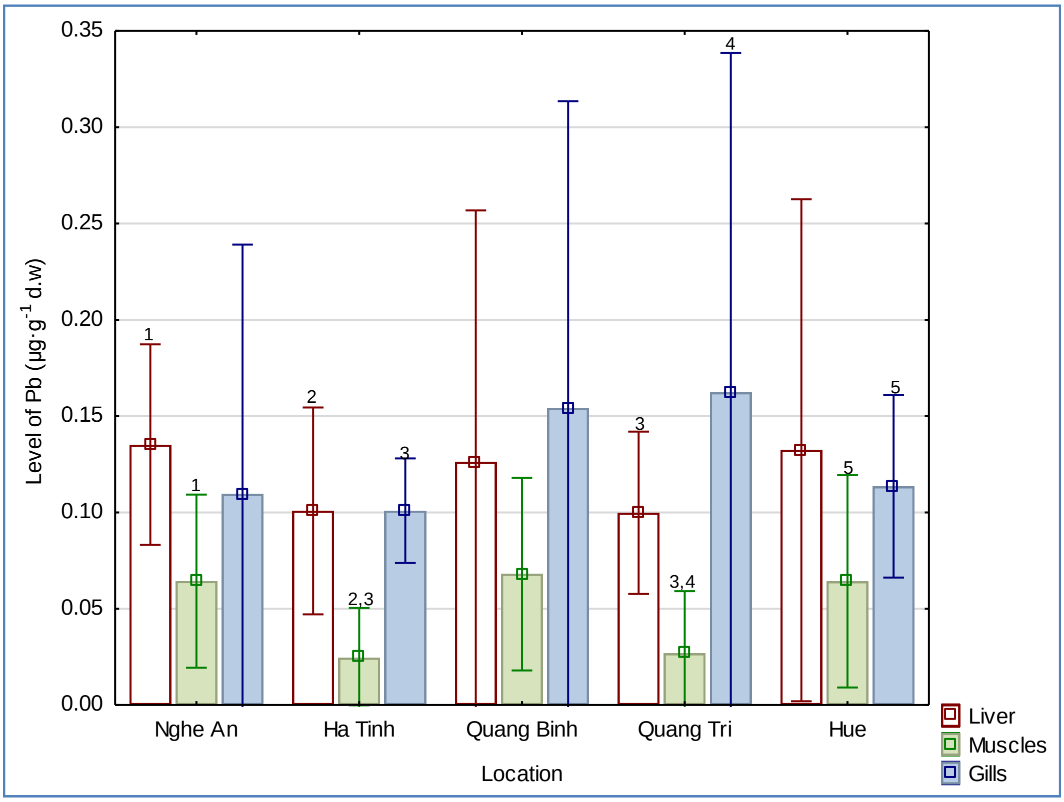

Figure 35. The mean, SD of Pb concentration in tissues of *S. sihama*
(1, p=0.023; 2, p=0.004; 3, p=0.001; 3, p=0.011; 4, p=0.008; 5, p=0.047)

5. 4. Fe concentration

The mean, SE, SD, min and max amounts of Fe ($\mu\cdot g^{-1}$ d.w) in the liver, muscles and gills of *S. sihama* are presented in Table 29 and Fig. 36.

The average content of Fe observed in the tissues of *S. sihama* followed the order: liver>gills>muscles.

In the liver, the highest Fe measure was reported in Quang Tri (4.669 $\mu\cdot g^{-1}$ d.w), whereas the lowest value was established in Quang Binh (3.344 $\mu\cdot g^{-1}$ d.w).

Contrary to the above, the data showed that the highest measure of Fe in the muscles was encountered in Quang Binh (2.021 $\mu\cdot g^{-1}$ d.w), while the lowest volume was recorded in Quang Tri (1.693 $\mu\cdot g^{-1}$ d.w). As for the Fe accumulation in the gills, the highest concentration was detected in Nghe An (3.211 $\mu\cdot g^{-1}$ d.w), while the lowest value was listed in Hue (2.149 $\mu\cdot g^{-1}$ d.w).

Notably, in Nghe An (1, p=0.011; 2, p=0.014), Ha Tinh (3, p=0.004), and Quang Tri (4, p<0.001; 5, p=0.016) the status of Fe in the muscles was lower than its content in the liver and gills, which was confirmed by the effect of multiple comparisons of the mean ranks test.

Table 29. The level of Fe in liver, muscles, gills of *S. sihama* ($\mu\cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	4.036±0.903	1.864±0.199	3.211±0.342
	Min-Max	1.119-11.183	1.389-3.515	2.094-5.911
	Shapiro-Wilk test (p)	0.029	0.001	0.019
	Kruskal-Wallis test	H=10.893; p=0.004		
Ha Tinh (n=10)	Mean±SE	4.200±0.766	1.798±0.318	2.573±0.143
	Min-Max	1.149-8.710	0.748-4.507	2.083-3.483
	Shapiro-Wilk test (p)	0.664	<0.001	0.267
	Kruskal-Wallis test	H=10.965; p=0.004		
Quang Binh (n=10)	Mean±SE	3.344±0.650	2.021±0.308	2.463±0.176
	Min-Max	0.720-6.860	1.312-4.559	1.731-3.711
	Shapiro-Wilk test (p)	0.728	<0.001	0.240
	Kruskal-Wallis test	H=3.812; p=0.149		
Quang Tri (n=10)	Mean±SE	4.669±0.765	1.693±0.057	2.743±0.201
	Min-Max	0.860-8.710	1.505-2.011	2.021-3.911
	Shapiro-Wilk test (p)	0.748	0.080	0.172
	Levene test	F=10.907; p<0.001		
	Kruskal-Wallis test	H=17.294; p<0.001		
Hue (n=10)	Mean±SE	3.374±0.654	1.874±0.119	2.149±0.274
	Min-Max	1.114-7.680	1.484-2.704	0.177-3.302
	Shapiro-Wilk test (p)	0.379	0.065	0.077
	Levene test	F=7.209; p=0.003		
	Kruskal-Wallis test	H=4.900; p=0.086		

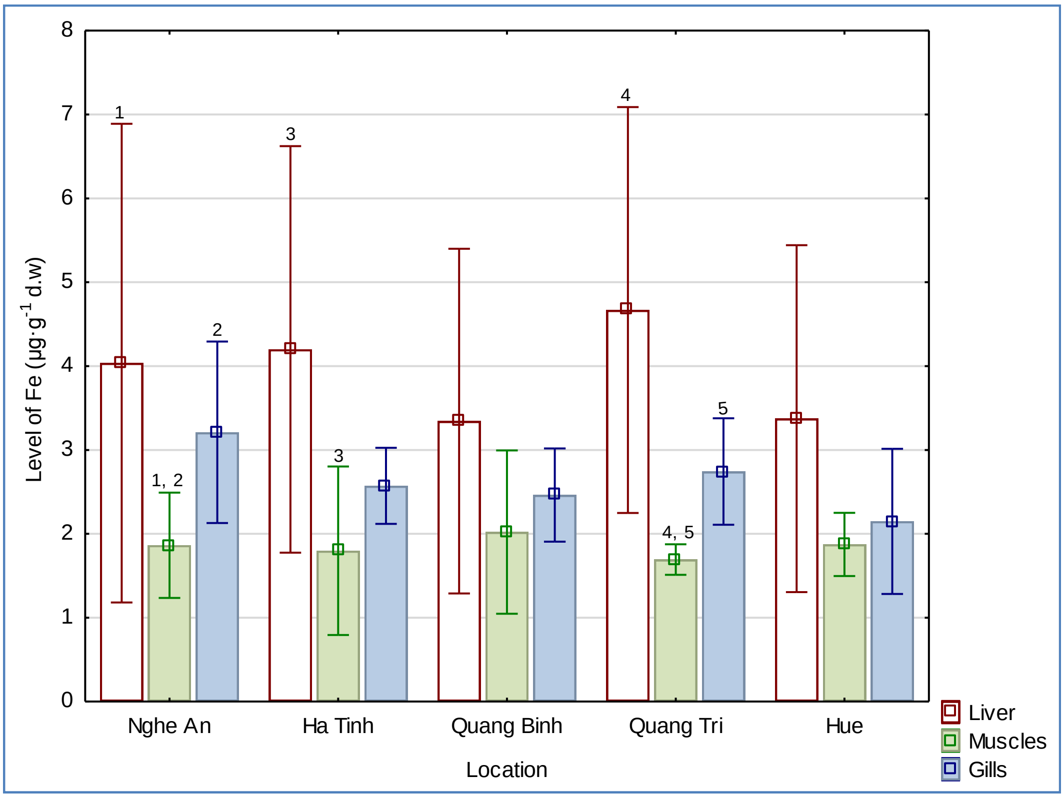

Figure 36. The mean, SD of Fe concentration in tissues of *S. sihama*
(1, p=0.011; 2, p=0.014; 3, p=0.004; 4, p<0.001; 5, p=0.016)

5. 5. Zn concentration

The mean, SE, SD, min and max amounts of Zn ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *S. sihama* are presented in Table 30 and Fig. 37.

In Nghe An, the recorded concentration of Zn in the muscles was appreciably lower than the Zn level in the liver and gills, which was supported by the effect of multiple comparisons of the mean ranks test (1, p=0.008; 2, p=0.001). In addition, the accumulated Zn content in the muscles was considerably lower than the volume of Zn in the gills, which was detected in Ha Tinh (3, p=0.013), Quang Tri (4, p=0.025) and Hue (5, p=0.010). Concurrently, the Zn values were not noticeably different in the liver, muscles, and gills from Quang Binh.

Statistical analysis showed that the Zn total existing in the muscles from Nghe An was substantially lower than the Zn total in the muscles from Quang Binh (a, p=0.031).

Table 30. The level of Zn in liver, muscles, gills of *S. sihama* ($\mu\cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	1.388±0.305	0.395±0.022	1.588±0.293
	Min-Max	0.298-3.452	0.278-0.521	0.448-3.258
	Shapiro-Wilk test (p)	0.169	0.995	0.057
	Levene test		F=9.487; p=0.001	
	Kruskal-Wallis test		H=15.494; p<0.001	
Ha Tinh (n=10)	Mean±SE	1.519±0.403	0.502±0.037	1.266±0.159
	Min-Max	0.289-4.032	0.345-0.706	0.443-2.352
	Shapiro-Wilk test (p)	0.097	0.714	0.589
	Levene test		F=11.317; p<0.001	
	Kruskal-Wallis test		H=9.030; p=0.011	
Quang Binh (n=10)	Mean±SE	2.146±0.585	0.626±0.078	0.899±0.174
	Min-Max	0.401-6.350	0.351-1.182	0.338-2.141
	Shapiro-Wilk test (p)	0.080	0.140	0.073
	Levene test		F=9.317; p<0.001	
	Kruskal-Wallis test		H=5.577; p=0.062	
Quang Tri (n=10)	Mean±SE	0.774±0.195	0.563±0.065	1.271±0.235
	Min-Max	0.032-2.113	0.348-0.882	0.413-2.709
	Shapiro-Wilk test (p)	0.177	0.080	0.163
	Levene test		F=3.624; p=0.040	
	Kruskal-Wallis test		H=7.474; p=0.024	
Hue (n=10)	Mean±SE	1.645±0.581	0.537±0.051	1.247±0.242
	Min-Max	0.160-6.350	0.351-0.858	0.413-3.258
	Shapiro-Wilk test (p)	0.004	0.324	0.002
	Kruskal-Wallis test		H=9.462; p=0.009	

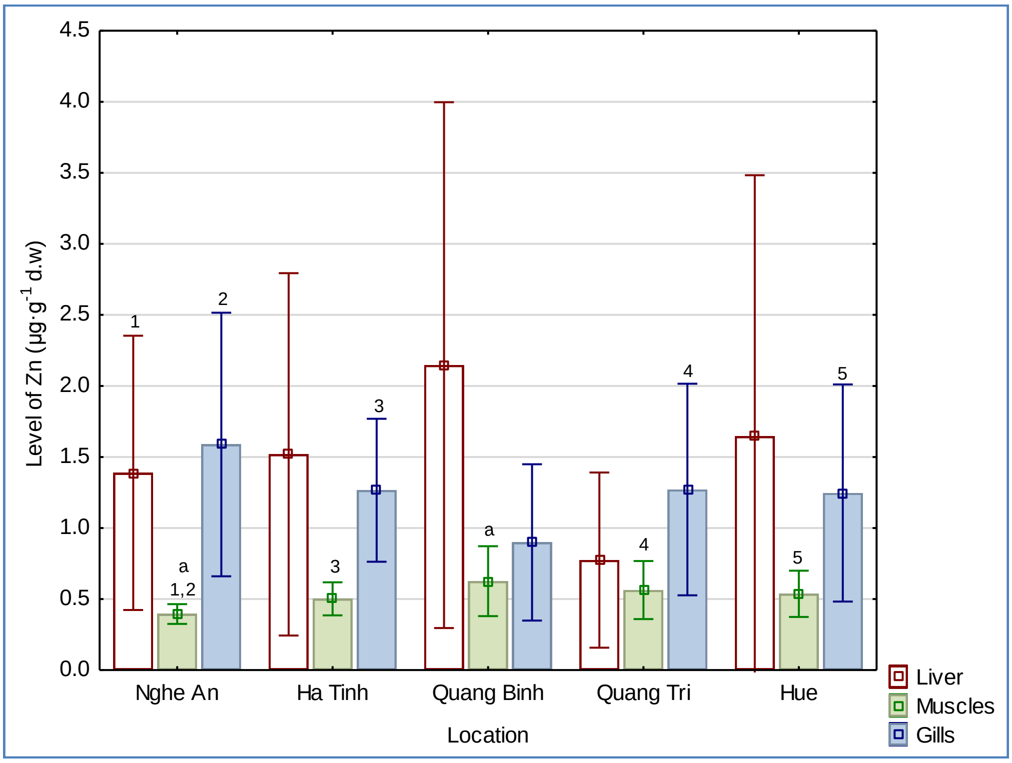

Figure 37. The mean, SD of Zn concentration in tissues of *S. sihama*
(1, p=0.008; 2, p=0.001; 3, p=0.013; 4, p=0.025 ; 5, p=0.010 ; a, p=0.031)

5. 6. Cu concentration

The mean, SE, SD, min and max amounts of Cu ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *S. sihama* are given in Table 31 and Fig. 38.

In Nghe An, although the average of Cu in the liver was higher than the Cu value in muscles and gills, they did not differ statistically. Remarkably, the Cu accumulation in the muscles from Nghe An was obviously higher than the Cu content in the muscles from Quang Binh (a, p=0.007) and Hue (b, p=0.004).

In Ha Tinh, the Cu concentration in the muscles was found decidedly lower than the Cu amount in the liver and gills (2, p=0.003; 3, p=0.037). At the same time, the Cu results in the muscles from Ha Tinh were appreciably larger than the content of Cu in the muscles from Hue (c, p=0.043).

Furthermore, the values of Cu from Quang Binh and Hue showed that the Cu levels in the liver were noticeably higher than the cumulative levels in the muscles, in Nghe An and Quang Tri showed that the level of Cu in muscles were lower than in gills, which has been verified by the the effect of multiple comparisons of mean ranks test (1, p=0.010; 4, p<0.001; 5, p=0.031; 6, p=0.027).

Table 31. The level of Cu in liver, muscles, gills of *S. sihama* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.452±0.186	0.182±0.024	0.396±0.027
	Min-Max	0.040-1.890	0.051-0.254	0.269-0.524
	Shapiro-Wilk test (p)	0.002	0.026	0.745
	Kruskal-Wallis test		H=10.071; p=0.007	
Ha Tinh (n=10)	Mean±SE	1.179±0.326	0.188±0.028	0.359±0.027
	Min-Max	0.110-3.210	0.054-0.332	0.196-0.467
	Shapiro-Wilk test (p)	0.253	0.248	0.735
	Levene test		F=16.090; p<0.001	
	Kruskal-Wallis test		H=12.011; p=0.003	
Quang Binh (n=10)	Mean±SE	1.375±0.382	0.108±0.034	0.246±0.021
	Min-Max	0.220-4.320	0.029-0.386	0.159-0.387
	Shapiro-Wilk test (p)	0.013	0.002	0510
	Kruskal-Wallis test		H=21.383; p<0.001	
Quang Tri (n=10)	Mean±SE	0.742±0.286	0.149±0.033	0.328±0.034
	Min-Max	0.015-2.459	0.029-0.386	0.179-0.512
	Shapiro-Wilk test (p)	0.017	0.149	0.907
	Kruskal-Wallis test		H=6.599; p=0.037	
Hue (n=10)	Mean±SE	1.451±0.444	0.197±0.036	0.233±0.017
	Min-Max	0.100-4.320	0.061-0.386	0.159-0.298
	Shapiro-Wilk test (p)	0.126	0.108	0.165
	Levene test		F=16.134; p<0.001	
	Kruskal-Wallis test		H=6.900; p=0.032	

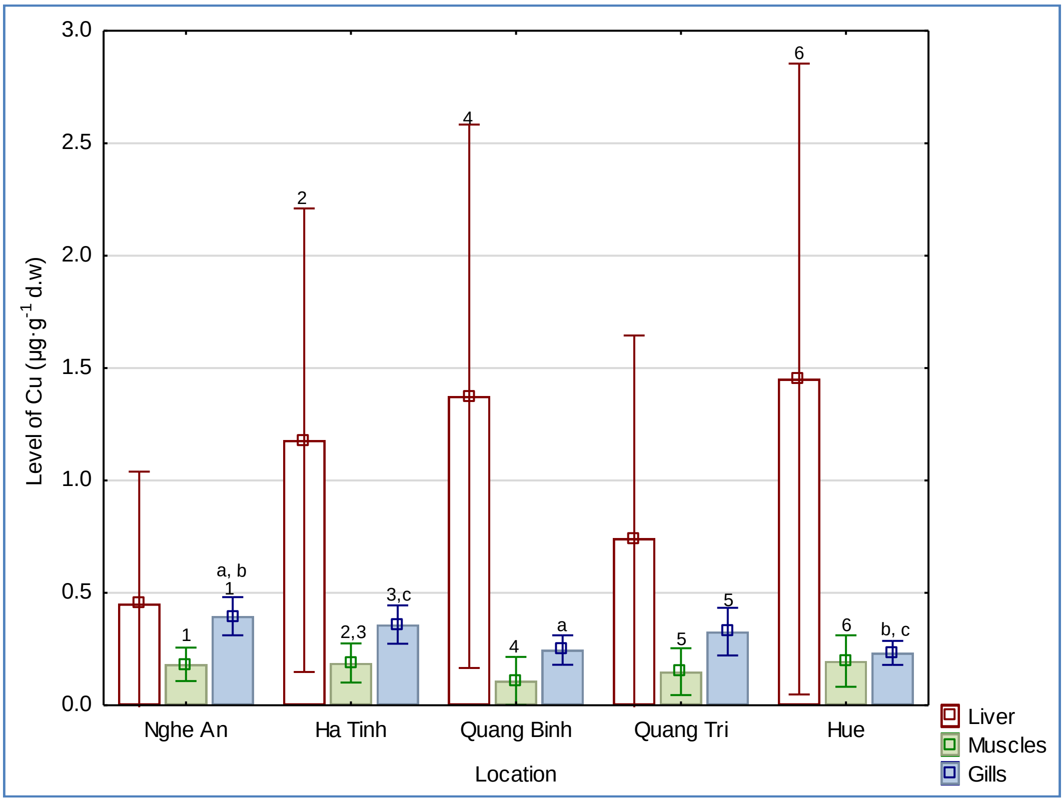

Figure 38. The mean, SD of Cu concentration in tissues of *S. sihama*
(1, p=0.010; 2, p=0.003; 3, p=0.037; 4, p<0.001; 5, p=0.031; 6, p=0.027; a, p=0.007; b, p=0.004; c, p=0.043)

6. Concentration of heavy metals in *Upeneus sulphureus*

6. 1. Hg concentration

The mean, SE, SD, min and max amounts of Hg ($\mu \cdot g^{-1}$ w.w) in the liver, muscles and gills of *U. sulphureus* are presented in Table 32 and Fig. 39.

The degrees of Hg in the tissues of *U. sulphureus* at the study sites followed the order: liver>gills>muscles. Notably, the Hg concentration in the liver from Nghe An was significantly lower than the Hg content in the liver in Ha Tinh.

At the same time, the Hg content in the liver from Ha Tinh was markedly higher than the Hg level in the liver from Quang Binh. All of them have been analyzed with the effect of multiple comparisons of mean ranks test (a, p=0.015; b, p=0.004).

Besides that, all the study sites showed disagreement of Hg levels in the liver, muscles, and gills. Detailly, in Nghe An and Hue, the total value of Hg in the liver was higher than the level of Hg in the muscles and gills. Along with that, in Ha Tinh and Quang Binh, the Hg content in muscles was undoubtedly lesser than in the liver and gills.

Table 32. The level of Hg in liver, muscles, gills of *U. sulphureus* ($\mu \cdot g^{-1}$ w.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.365±0.031	0.082±0.010	0.151±0.020
	Min-Max	0.233-0.557	0.038-0.137	0.078-0.263
	Shapiro-Wilk test (p)	0.849	0.827	0.230
	Levene test		F=4.846; p=0.016	
	Kruskal-Wallis test		H=21.769; p<0.001	
Ha Tinh (n=10)	Mean±SE	0.632±0.075	0.079±0.018	0.298±0.063
	Min-Max	0.369-1.095	0.036-0.220	0.086-0.643
	Shapiro-Wilk test (p)	0.169	0.004	0.090
	Kruskal-Wallis test		H=20.934; p<0.001	
Quang Binh (n=10)	Mean±SE	0.340±0.022	0.105±0.015	0.258±0.065
	Min-Max	0.233-0.417	0.049-0.220	0.096-0.643
	Shapiro-Wilk test (p)	0.073	0.033	0.001
	Kruskal-Wallis test		H=17.189; p<0.001	
Quang Tri (n=10)	Mean±SE	0.457±0.061	0.091±0.011	0.240±0.054
	Min-Max	0.292-0.965	0.038-0.137	0.078-0.634
	Shapiro-Wilk test (p)	0.001	0.460	0.078
	Kruskal –Wallis test		H=18.478; p<0.001	
Hue (n=10)	Mean±SE	0.508±0.067	0.093±0.017	0.219±0.071
	Min-Max	0.252-0.965	0.043-0.220	0.078-0.643
	Shapiro-Wilk test (p)	0.073	0.029	<0.001
	Kruskal-Wallis test		H=16.817; p<0.001	

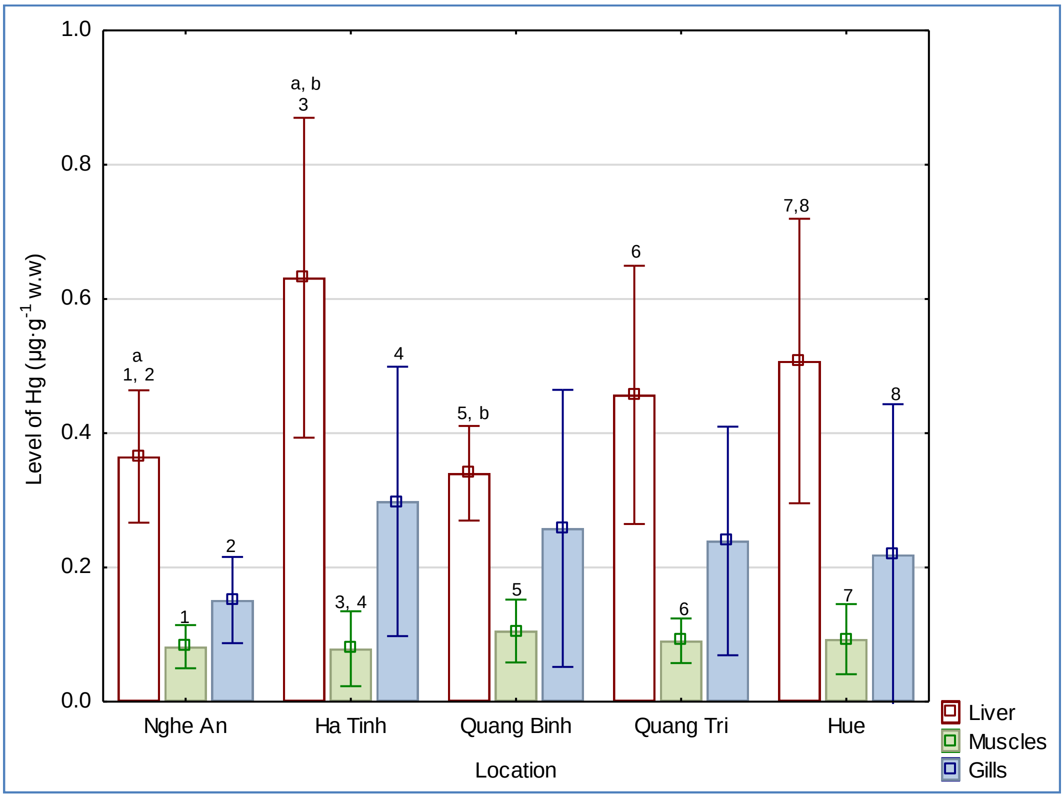

Figure 39. The mean, SD of Hg concentration in tissues of *U. sulphureus*
(1, p<0.001; 2, p=0.013; 3, p<0.001; 4, p=0.044; 5, p<0.001; 6, p<0.001; 7, p<0.001; 8, p=0.038; a, p=0.015; b, p=0.004)

All of these differences have been verified by the effect of multiple comparisons of mean ranks test (1, p<0.001; 2, p=0.013; 3, p<0.001; 4, p=0.044; 5, p<0.001; 6, p<0.001; 7, p<0.001; 8, p=0.038).

6. 2. Cd concentration

The mean, SE, SD, min and max amounts of Cd ($\mu\cdot g^{-1}$ d.w) in the liver, muscles and gills of *U. sulphureus* are presented in Table 33 and Fig. 40.

For the Cd content in the liver, the highest value was recorded in Hue (0.051 $\mu\cdot g^{-1}$ d.w), while the minimum mean was found in Quang Binh (0.030 $\mu\cdot g^{-1}$ d.w). Although in muscles, the maximum mean was detected in Ha Tinh (0.014 $\mu\cdot g^{-1}$ d.w), the lowest mean was disclosed from Quang Tri (0.011 $\mu\cdot g^{-1}$ d.w).

For the concentration of Cd in gills, the highest value was observed at Quang Tri (0.039 $\mu\cdot g^{-1}$ d.w), while the lowest value was reported at Hue (0.027 $\mu\cdot g^{-1}$ d.w).

Table 33. The level of Cd in liver, muscles, gills of *U. sulphureus* ($\mu\cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.036±0.007	0.012±0.002	0.033±0.006
	Min-Max	0.010-0.070	0.006-0.022	0.005-0.065
	Shapiro-Wilk test (p)	0.371	0.242	0.670
	Levene test		F=6.353; p=0.005	
	Kruskal-Wallis test		H=8.864; p=0.012	
Ha Tinh (n=10)	Mean±SE	0.042±0.006	0.014±0.003	0.034±0.006
	Min-Max	0.010-0.064	0.004-0.033	0.004-0.057
	Shapiro-Wilk test (p)	0.603	0.095	0.390
	Levene test		F=5.841; p=0.008	
	Kruskal-Wallis test		H=11.474; p=0.003	
Quang Binh (n=10)	Mean±SE	0.030±0.003	0.013±0.003	0.029±0.006
	Min-Max	0.017-0.049	0.003-0.033	0.004-0.067
	Shapiro-Wilk test (p)	0.726	0.236	0.338
	Levene test		F=3.960; p=0.031	
	Kruskal-Wallis test		H=9.897; p=0.007	
Quang Tri (n=10)	Mean±SE	0.031±0.004	0.011±0.002	0.039±0.006
	Min-Max	0.010-0.051	0.003-0.022	0.005-0.065
	Shapiro-Wilk test (p)	0.964	0.396	0.665
	Levene test		F=7.350; p=0.003	
	Kruskal-Wallis test		H=13.908; p=0.001	
Hue (n=10)	Mean±SE	0.051±0.010	0.012±0.002	0.027±0.006
	Min-Max	0.011-0.090	0.005-0.021	0.004-0.067
	Shapiro-Wilk test (p)	0.154	0.457	0.077
	Levene test		F=15.672; p<0.001	
	Kruskal-Wallis test		H=13.195; p=0.001	

There were no significant differences between each same organ at the research sites, which were only detected between different tissues in the same study area. Specifically, in Nghe An, Quang Binh, and Hue, the Cd value in the liver was greatly higher than the volume of Cd in muscles, which was shown by numbers 1, 4, 7 in Fig. 40. Likewise, in Ha Tinh and Quang Tri, the Cd content in muscles was significantly lower than the Cd accumulated in the liver and gills, which were confirmed by the effect of multiple comparisons of mean ranks test (2, 3, 5, 6 in Fig. 40).

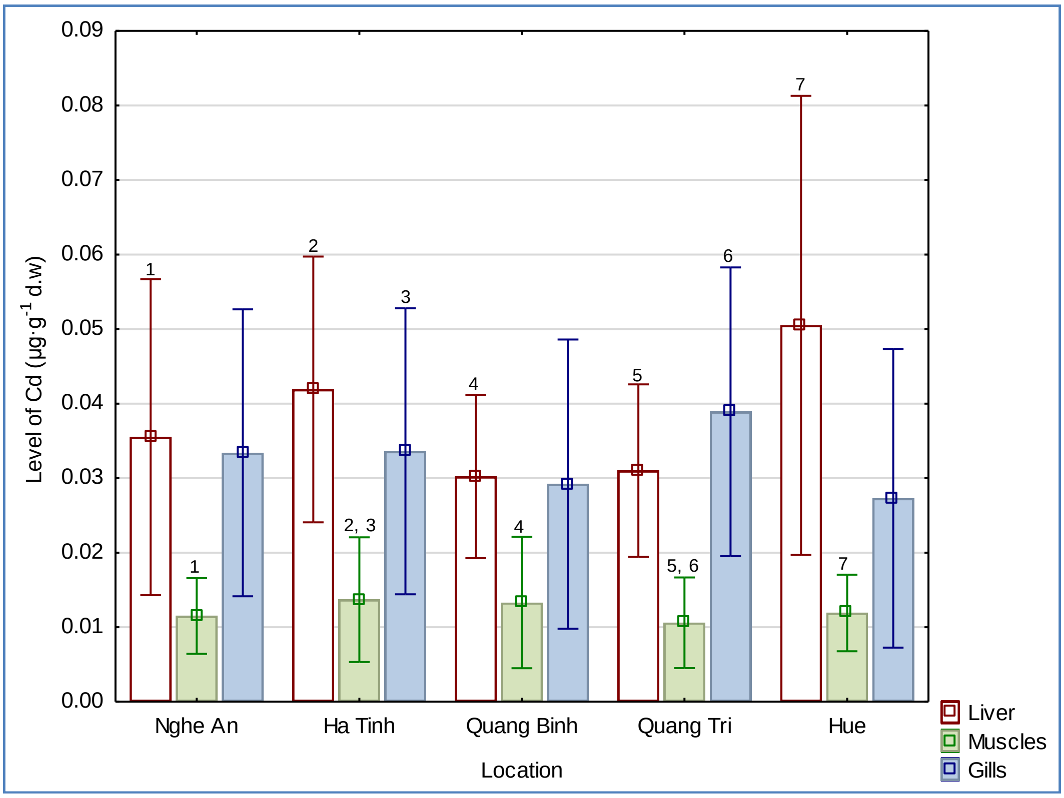

Figure 40. The mean, SD of Cd concentration in tissues of *U. Sulphureus*
(1, p=0.018; 2, p=0.003; 3, p=0.048; 4, p=0.007; 5, p=0.009; 6, p=0.002; 7, p=0.001)

6. 3. Pb concentration

The mean, SE, SD, min and max levels of Pb ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *U. sulphureus* are granted in Table 34 and Fig. 41.

At all the research areas, the Pb rank in tissues followed the order: gills>liver>muscles, except for Hue: liver>gills>muscles.

The maximum average of Pb was established in the gill at Quang Tri (0.327 $\mu \cdot g^{-1}$ d.w), and the minimum average was recorded in the muscles at Hue (0.124 $\mu \cdot g^{-1}$ d.w). Notably, the Pb content in the liver from Quang Tri was sizeably lower than the Pb content in the liver form Hue (a, p=0.021), the others did not show any differences between the same tissues in the study areas.

Table 34. The level of Pb in liver, muscles, gills of *U. sulphureus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	0.198±0.028	0.132±0.004	0.314±0.050
	Min-Max	0.080-0.365	0.115-0.156	0.023-0.507
	Shapiro-Wilk test (p)	0.499	0.628	0.605
	Levene test		F=9.209; p=0.001	
	Kruskal-Wallis test		H=8.714; p=0.013	
Ha Tinh (n=10)	Mean±SE	0.277±0.027	0.156±0.015	0.304±0.053
	Min-Max	0.125-0.412	0.113-0.237	0.011-0.505
	Shapiro-Wilk test (p)	0.777	0.008	0.326
	Kruskal-Wallis test		H=7.691; p=0.021	
Quang Binh (n=10)	Mean±SE	0.267±0.029	0.156±0.022	0.300±0.045
	Min-Max	0.118-0.422	0.092-0.321	0.109-0.507
	Shapiro-Wilk test (p)	0.643	0.009	0.534
	Kruskal-Wallis test		H=7.823; p=0.020	
Quang Tri (n=10)	Mean±SE	0.179±0.016	0.139±0.012	0.327±0.052
	Min-Max	0.110-0.226	0.091-0.231	0.023-0.505
	Shapiro-Wilk test (p)	0.005	0.025	0.193
	Kruskal-Wallis test		H=7.192; p=0.027	
Hue (n=10)	Mean±SE	0.293±0.030	0.124±0.004	0.263±0.045
	Min-Max	0.121-0.441	0.097-0.140	0.098-0.505
	Shapiro-Wilk test (p)	0.699	0.479	0.298
	Levene test		F=11.018; p<0.001	
	Kruskal-Wallis test		H=3.212; p=0.201	

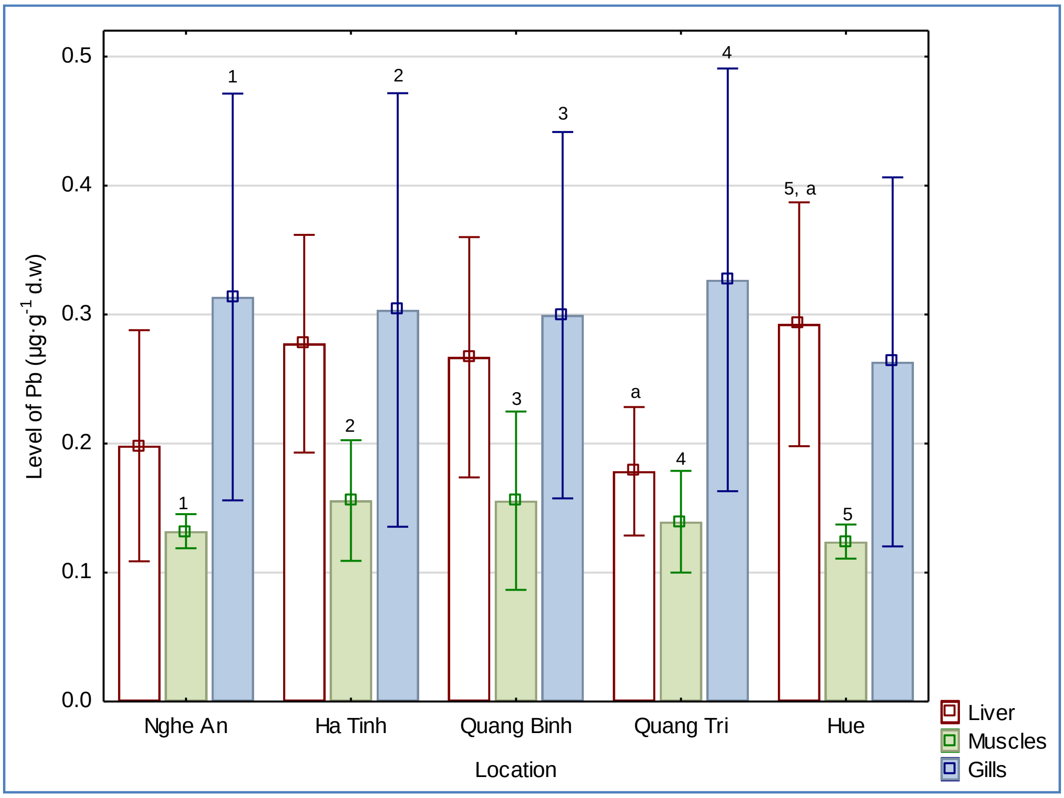

Figure 41. **The mean, SD of Pb concentration in tissues of *U. Sulphureus***
(1, p=0.010; 2, p=0.044; 3, p=0.041; 4, p=0.024; 5, p<0.001; a, p=0.021)

In each study site, Nghe An, Ha Tinh, Quang Binh, and Quang Tri, the Pb concentration in the gills was much higher than in the muscles. In addition, in Hue, the Pb concentration in the muscles was greatly lower than in the liver. These differences have been demonstrated by the effect of multiple comparisons of mean ranks test (1-5).

6. 4. Fe concentration

The mean, SE, SD, min and max amounts of Fe (μ·g^{-1} d.w) in the liver, muscles and gills of *U. sulphureus* are presented in Table 35 and Fig.42.

In Ha Tinh and Hue, the concentration of Fe in the tissues followed the order: liver>muscles>gills. The concentration of Fe in the liver was significantly higher than in the gills (presented by number 3 and 6 in Fig. 42).

In Nghe An, Ha Tinh, Quang Binh and Hue, the concentration of Fe in the tissues followed the order: liver>muscle>gill. The difference of the Fe content was apparently expressed in the liver and gills from these two locations, which was confirmed by the effect of multiple comparisons of mean ranks test (1, p<0.001; 2, p=0.018; 3, p=0.015; 4, p=0.023; 6, p=0.001).

Table 35. The level of Fe in liver, muscles, gills of *U. sulphureus* (μ·g^{-1} d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	4.971±0.763	1.979±0.165	2.558±0.268
	Min-Max	3.057-11.484	1.272-3.092	1.414-4.261
	Shapiro-Wilk test (p)	<0.001	0.541	0.544
	Kruskal-Wallis test		H=18.124, p<0.001	
Ha Tinh (n=10)	Mean±SE	5.652±0.857	3.029±0.408	2.613±0.389
	Min-Max	2.892-9.950	1.372-5.302	1.098-4.298
	Shapiro-Wilk test (p)	0.082	0.514	0.215
	Levene test		F=4.952; p=0.015	
	Kruskal-Wallis test		H=8.693; p=0.013	
Quang Binh (n=10)	Mean±SE	4.325±0.719	2.403±0.273	3.325±0.310
	Min-Max	1.750-9.950	1.098-3.478	1.921-5.302
	Shapiro-Wilk test (p)	0.028	0.206	0.836
	Kruskal-Wallis test		H=5.679; p=0.058	
Quang Tri (n=10)	Mean±SE	5.489±0.781	2.264±0.312	2.862±0.350
	Min-Max	1.750-9.950	1.272-4.472	1.098-4.298
	Shapiro-Wilk test (p)	0.268	0.072	0.454
	ANOVA		F=10.635; p<0.001	
	Levene test		F=2.613; p=0.092	
Hue (n=10)	Mean±SE	5.985±0.767	3.577±0.387	2.236±0.339
	Min-Max	3.100-9.950	1.921-5.302	1.098-4.298
	Shapiro-Wilk test (p)	0.132	0.529	0.248
	Levene test		F=4.888; p=0.015	
	Kruskal-Wallis test		H=14.234; p=0.001	

Simultaneously, the Fe levels from Quang Tri followed the order: liver>gills>muscles, and showed markedly higher Fe accumulation in the liver as compared to the muscles. They have been evidenced by the Tukey HSD test (5, p=0.001).

Besides, the Fe value in the muscles in Nghe An was also significantly lower than that in Hue (a, p=0.031).

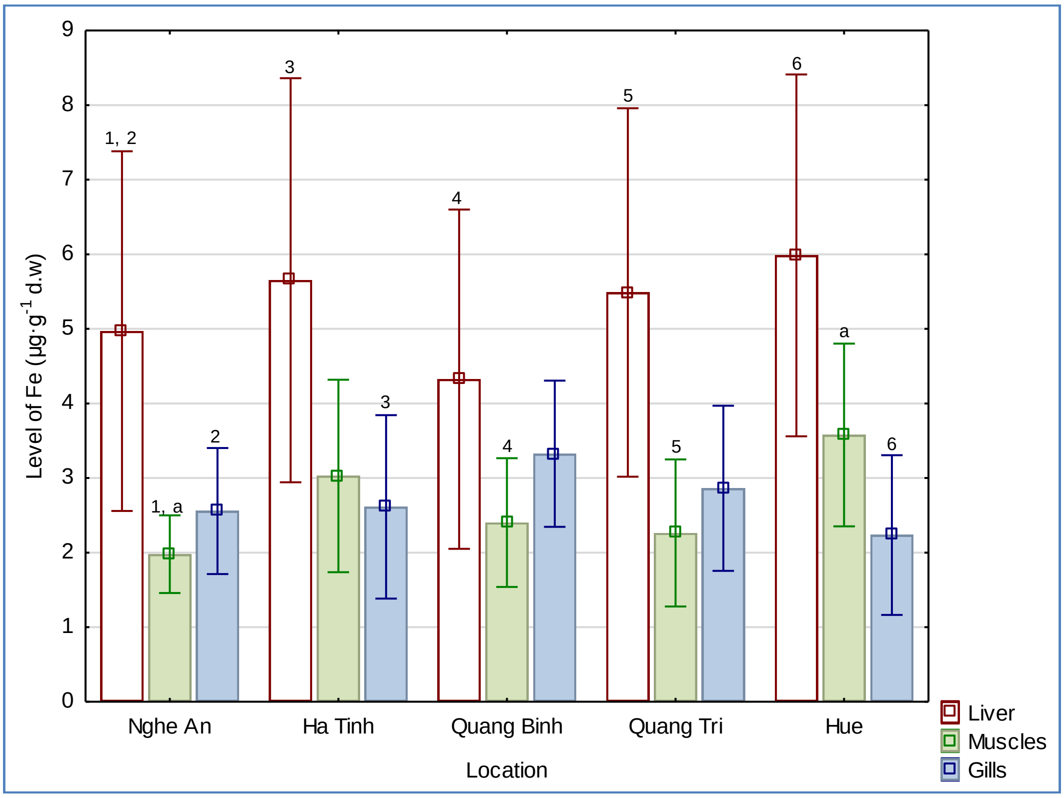

Figure 42. The mean, SD of Fe concentration in tissues of *U. Sulphureus*
(1, p<0.001; 2, p=0.018; 3, p=0.015; 4, p=0.023; 5, p=0.001; 6, p=0.001; a, p=0.031)

6. 5. Zn concentration

The mean, SE, SD, min and max amounts of Zn ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *U. sulphureus* are presented in Table 36 and Fig. 43.

High Zn concentration was found in the gills, while in the muscles – low concentration was detected in all the study areas (apart from Hue, where high content of Zn was found in the liver).

Within the liver, the highest value was detected in Hue (2.407 $\mu \cdot g^{-1}$ d.w), while the lowest was recorded in Quang Tri (0.873 $\mu \cdot g^{-1}$ d.w). The differences were shown by the a and b symbols in Fig. 43.

In muscles, the maximum value was confirmed in Ha Tinh (0.776 $\mu \cdot g^{-1}$ d.w), while the minimum value was disclosed in Nghe An (0.542 $\mu \cdot g^{-1}$ d.w), but there were no noticeable differences between them.

Table 36. The level of Zn in liver, muscles, gills of *U. sulphureus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	1.246±0.231	0.542±0.068	2.128±0.247
	Min-Max	0.308-2.430	0.358-0.987	0.945-3.662
	Shapiro-Wilk test (p)	0.615	0.060	0.862
	Levene test		F=4.847; p=0.016	
	Kruskal-Wallis test		H=15.505; p<0.001	
Ha Tinh (n=10)	Mean±SE	1.118±0.233	0.776±0.137	2.310±0.279
	Min-Max	0.405-2.828	0.109-1.200	1.097-3.921
	Shapiro-Wilk test (p)	0.017	0.034	0.755
	Kruskal-Wallis test		H=14.457; p=0.001	
Quang Binh (n=10)	Mean±SE	1.243±0.268	0.757±0.114	2.514±0.290
	Min-Max	0.098-2.828	0.209-1.200	1.232-3.921
	Shapiro-Wilk test (p)	0.712	0.383	0.482
	Levene test		F=3.452; p=0.046	
	Kruskal-Wallis test		H=15.669: p<0.001	
Quang Tri (n=10)	Mean±SE	0.873±0.115	0.736±0.110	2.045±0.216
	Min-Max	0.368-1.385	0.109-1.200	0.945-2.999
	Shapiro-Wilk test (p)	0.242	0.866	0.354
	ANOVA		F=21.603; p<0.001	
	Levene test		F=2.884; p=0.073	
Hue (n=10)	Mean±SE	2.407±0.277	0.709±0.139	1.163±0.193
	Min-Max	1.097-3.921	0.109-1.200	0.442-2.430
	Shapiro-Wilk test (p)	0.739	0.067	0.159
	ANOVA		F=17.407; p<0.001	
	Levene test		F=1.222; p=0.310	

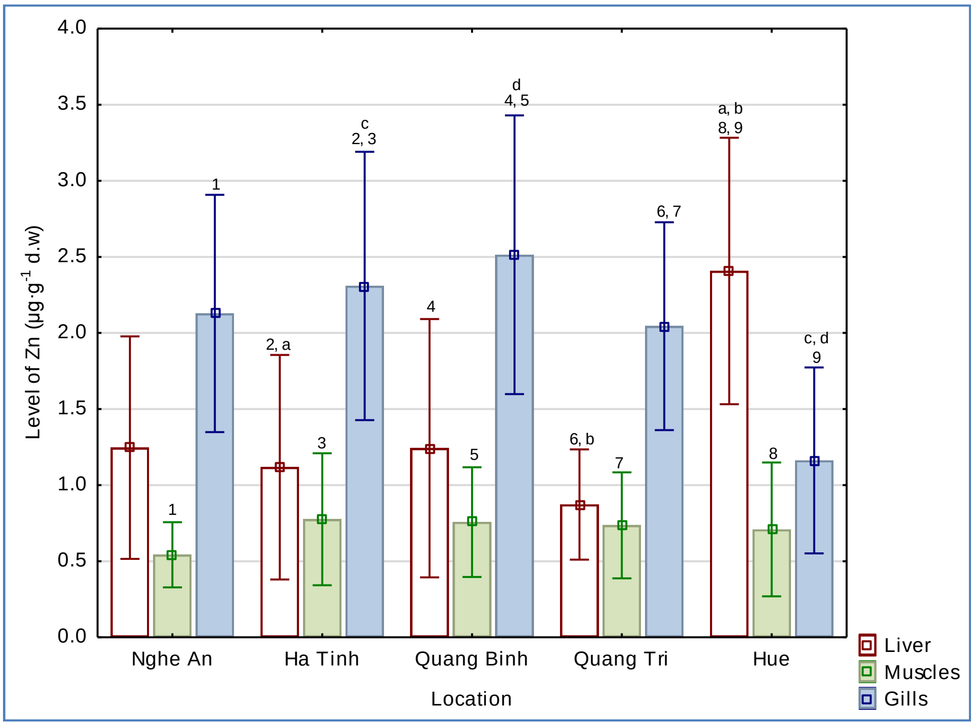

Figure 43. The mean, SD of Zn concentration in tissues of *U. Sulphureus*

(1, p<0.001; 2, p=0.002; 3, p<0.001; 4, p=0.029; 5, p<0.001; 6, p<0.001; 7, p<0.001; 8, p<0.001; 9, p=0.001; a, p=0.027; b, p=0.005; c, p=0.025; d, p=0.011)

At the same time, in gills, the highest content was set in Quang Binh (2.514 $\mu \cdot g^{-1}$ d.w), while the lowest was found in Hue (1.163 $\mu \cdot g^{-1}$ d.w). The statistical analysis showed that Zn accumulation in gills was much lower at Hue as compared to Ha Tinh and Quang Binh (c, d).

In the study areas, the concentration of Zn in muscles was significantly lower than the level in the liver and gills. Specifically they have been confirmed by the effect of multiple comparisons of mean ranks test (1- 5) and the Tukey HSD test (6-9).

6. 6. Cu concentration

The mean, SE, SD, min and max amounts of Cu ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *U. sulphureus* are presented in Table 37 and Fig. 44.

At all study sites, there was a high accumulation of Cu in the liver and low in the muscles. The highest Cu content was detected in the liver at Quang Binh (2.434 $\mu \cdot g^{-1}$ d.w), while the lowest Cu value was recorded in the muscles at Nghe An (0.638 $\mu \cdot g^{-1}$ d.w), however, they did not show any significant difference.

Table 37. The level of Cu in liver, muscles, gills of *U. sulphureus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=10)	Mean±SE	1.645±0.297	0.638±0.106	1.020±0.141
	Min-Max	0.260-3.260	0.063-1.113	0.371-1.560
	Shapiro-Wilk test (p)	0.587	0.691	0.026
	Levene test		F=8.692; p=0.001	
	Kruskal-Wallis test		H=8.185; p=0.017	
Ha Tinh (n=10)	Mean±SE	1.528±0.239	0.906±0.189	1.167±0.272
	Min-Max	0.738-2.721	0.299-1.700	0.098-3.212
	Shapiro-Wilk test (p)	0.029	0.016	0.053
	Kruskal-Wallis test		H=1.350; p=0.509	
Quang Binh (n=10)	Mean±SE	2.434±0.421	1.242±0.236	1.547±0.265
	Min-Max	1.208-5.584	0.223-2.380	0.570-3.247
	Shapiro-Wilk test (p)	0.041	0.600	0.154
	Kruskal-Wallis test		H=6.357; p=0.042	
Quang Tri (n=10)	Mean±SE	2.417±0.438	0.845±0.179	0.961±0.281
	Min-Max	0.584-4.399	0.223-1.700	0.063-3.212
	Shapiro-Wilk test (p)	0.377	0.086	0.011
	Kruskal-Wallis test		H=8.232; p=0.016	
Hue (n=10)	Mean±SE	2.399±0.513	0.929±0.167	1.673±0.283
	Min-Max	0.438-5.038	0.371-1.700	0.879-3.247
	Shapiro-Wilk test (p)	0.210	0.040	0.009
	Kruskal-Wallis test		H=4.680; p=0.096	

Nghe An, Ha Tinh and Quang Tri showed that the presence of Cu in the liver was significantly higher than in the muscles. The Cu levels were confirmed by the effect of multiple comparisons of mean ranks test (1, p=0.014; 2, p=0.034; 3, p=0.029). Simultaneously, in the remaining regions, there were no noticeable differences.

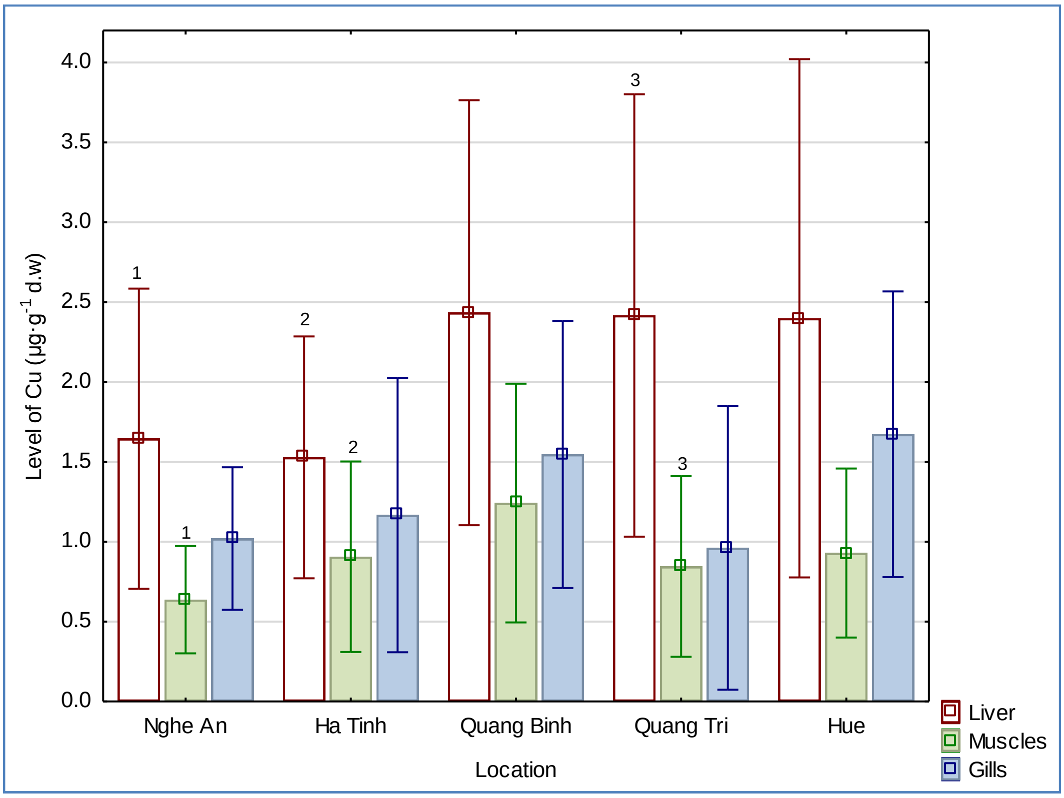

Figure 44. The mean, SD of Cu concentration in tissues of *U. Sulphureus*
(1, p=0.014; 2, p=0.034; 3, p=0.029)

7. Concentration of heavy metals in *Gerres filamentosus*

7. 1. Hg concentration

The mean, SE, SD, min and max levels of Hg ($\mu \cdot g^{-1}$ w.w) in the liver, muscles and gills of *G. filamentosus* are given in Table 38 and Fig. 45.

The Hg content was found in all the tissues at the study sites, in descending order: liver>gills>muscles. The highest average Hg value was detected in the liver at Quang Binh (0.628 $\mu \cdot g^{-1}$ w.w), while the lowest value was recorded in the muscle at the Quang Tri region (0.068 $\mu \cdot g^{-1}$ w.w). The Tukey Unequal test was performed to verify the difference of Hg concentration in the same organs from different locations and the results showed that there was no difference between the study areas.

Table 38. The level of Hg in liver, muscles, gills of *G. filamentosus* ($\mu \cdot g^{-1}$ w.w)

Locations		Liver	Muscles	Gills
Nghe An (n=11)	Mean±SE	0.374±0.030	0.084±0.009	0.145±0.019
	Min-Max	0.233-0.557	0.038-0.137	0.078-0.263
	Shapiro-Wilk test (p)	0.908	0.850	0.098
	Levene test		F=5.948; p=0.007	
	Kruskal-Wallis test		H=22.360; p<0.001	
Ha Tinh (n=10)	Mean±SE	0.557±0.084	0.089±0.017	0.353±0.067
	Min-Max	0.302-1.095	0.038-0.220	0.096-0.643
	Shapiro-Wilk test (p)	0.019	0.044	0.080
	Kruskal-Wallis test		H=18.992; p<0.001	
Quang Binh (n=12)	Mean±SE	0.628±0.075	0.087±0.016	0.278±0.064
	Min-Max	0.369-1.095	0.038-0.220	0.096-0.643
	Shapiro-Wilk test (p)	0.075	0.018	0.001
	Kruskal-Wallis test		H=22.490; p<0.001	
Quang Tri (n=10)	Mean±SE	0.473±0.080	0.068±0.009	0.200±0.051
	Min-Max	0.233-1.095	0.036-0.118	0.093-0.634
	Shapiro-Wilk test (p)	0.028	0.145	<0.001
	Kruskal-Wallis test		H=22.149; p<0.001	
Hue (n=8)	Mean±SE	0.364±0.020	0.089±0.009	0.285±0.080
	Min-Max	0.252-0.417	0.049-0.118	0.086-0.643
	Shapiro-Wilk test (p)	0.072	0.266	0.015
	Kruskal-Wallis test		H=13.457; p=0.001	

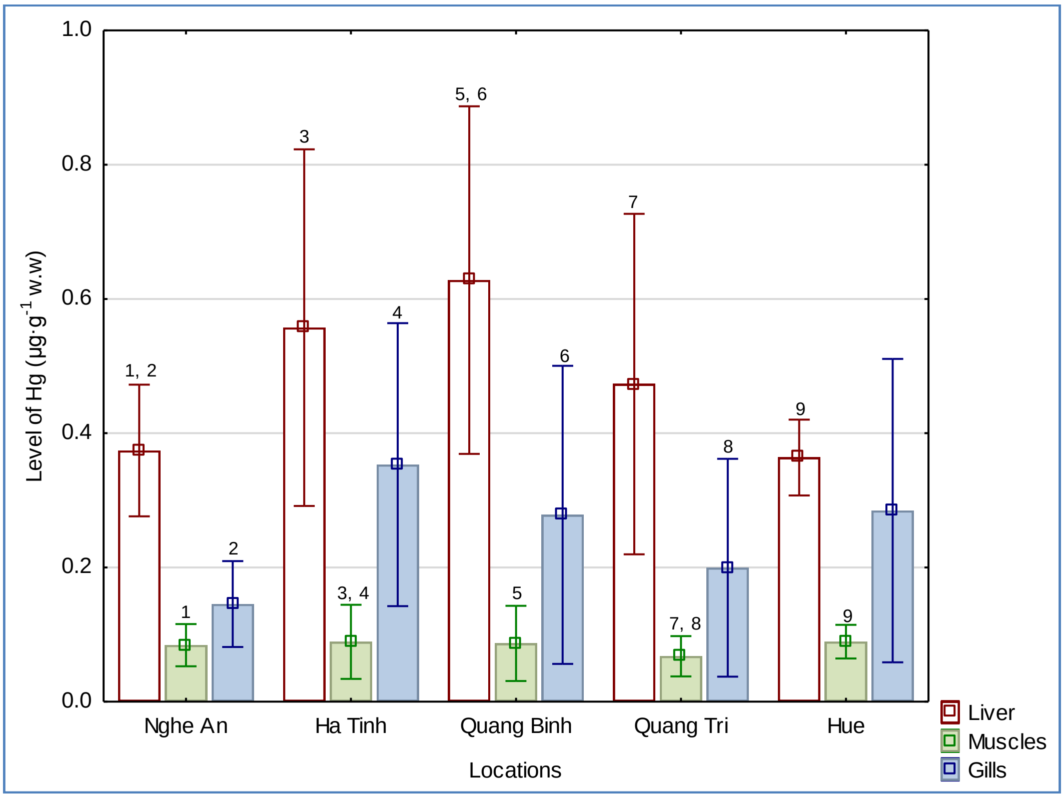

Figure 45. The mean, SD of Hg concentration in tissues of *G. filamentosus*
(1, p<0.001; 2, p=0.027; 3, p<0.001; 4, p=0.011; 5, p<0.001; 6, p=0.033;
7, p=0.001; 8, p=0.018; 9, p=0.001)

At the same time, in Nghe An and Quang Binh, the Hg concentration in the liver was significantly higher than in the muscles and gills (1, $p<0.001$; 2, $p=0.027$; 5, $p<0.001$; 6, $p=0.033$). Besides, in Ha Tinh and Quang Tri, the level of Hg in the muscles showed significantly lower levels than in the liver and gills (3, $p<0.001$; 4, $p=0.011$; 7, $p=0.001$; 8, $p=0.018$), whereas in Hue, it showed a significantly higher concentration in the liver than in the muscles (9, $p=0.001$), but there was no difference as compared to the gills.

7. 2. Cd concentration

The mean, SE, SD, min and max amounts of Cd ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *G. filamentosus* are presented in Table 39 and Fig. 46.

The data showed that, in Ha Tinh, Quang Binh, Quang Tri and Hue, Cd accumulates in organs in the order of liver>gills>muscles. At the same time, in Nghe An, the order was: liver>muscles>gills.

Table 39. The level of Cd in liver, muscles, gills of *G. filamentosus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=11)	Mean±SE	0.025±0.003	0.013±0.002	0.008±0.001
	Min-Max	0.003-0.036	0.003-0.019	0.004-0.018
	Shapiro-Wilk test (p)	0.136	0.375	0.017
	Kruskal-Wallis test		H=15.016; p=0.001	
Ha Tinh (n=10)	Mean±SE	0.017±0.001	0.009±0.002	0.012±0.002
	Min-Max	0.009-0.023	0.003-0.017	0.002-0.025
	Shapiro-Wilk test (p)	0.492	0.588	0.795
	Levene test		F=0.838; p=0.443	
	Kruskal-Wallis test		H=8.650; p=0.013	
Quang Binh (n=12)	Mean±SE	0.022±0.003	0.011±0.001	0.013±0.002
	Min-Max	0.007-0.049	0.005-0.017	0.004-0.025
	Shapiro-Wilk test (p)	0.202	0.418	0.447
	Levene test		F=4.586; p=0.017	
	Kruskal-Wallis test		H=9.883; p=0.007	
Quang Tri (n=10)	Mean±SE	0.025±0.002	0.008±0.002	0.010±0.002
	Min-Max	0.013-0.036	0.003-0.017	0.005-0.019
	Shapiro-Wilk test (p)	0.723	0.160	0.075
	ANOVA		F=26.911; p<0.001	
	Levene test		F=1.185; p=0.321	
Hue (n=8)	Mean±SE	0.017±0.003	0.009±0.002	0.014±0.002
	Min-Max	0.003-0.030	0.003-0.015	0.006-0.025
	Shapiro-Wilk test (p)	0.988	0.270	0.693
	ANOVA		F=2.883; p=0.078	
	Levene test		F=1.178; p=0.327	

In Nghe An and Quang Tri, the average value of Cd in the liver was unquestionably higher than the volume in the muscles and gills. Concurrently, in Ha Tinh and Quang Binh, the average value of Cd in the muscles showed a significant difference compared to the level in the liver. It has been confirmed by the effect of multiple comparisons of mean ranks test, which was presented by numbers 1 to 6 in Fig. 46. Although in Hue, no noticeable differences were detected between them.

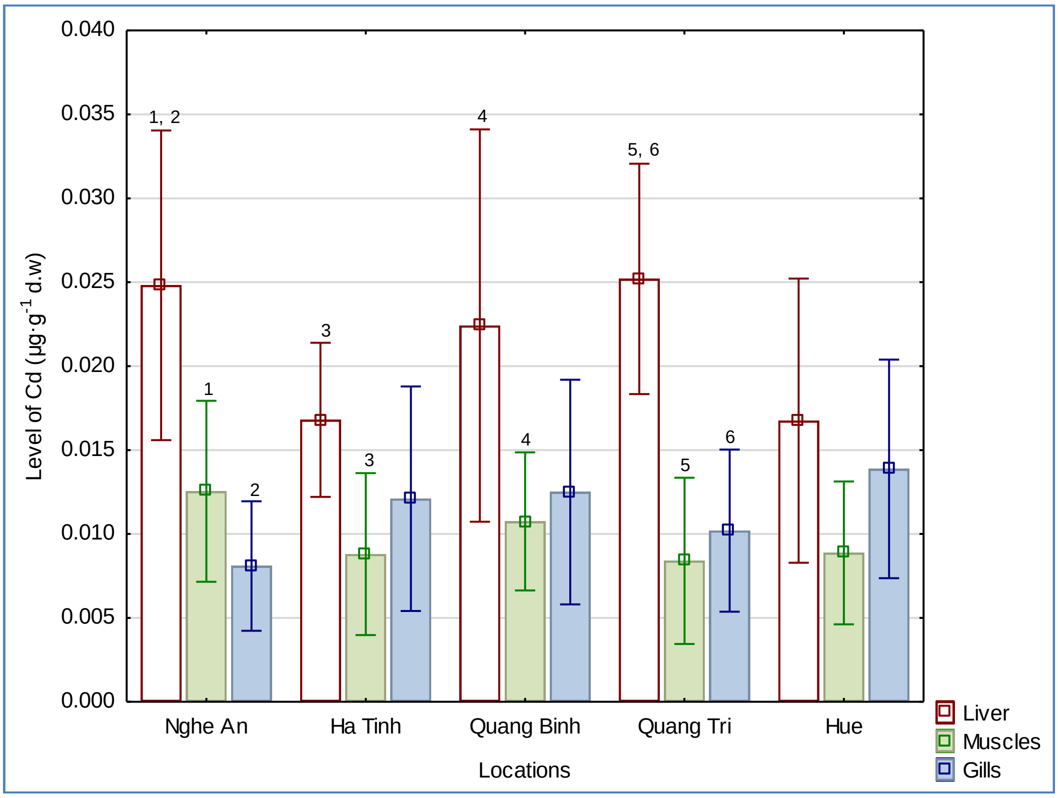

Figure 46. The mean, SD of Cd concentration in tissues of *G. filamentosus*
(1, p=0.001; 2, p<0.001 3, p=0.011; 4, p=0.006; 5, p<0.001; 6, p<0.001)

7. 3. Pb concentration

The mean, SE, SD, min and max amounts of Pb ($\mu\cdot g^{-1}$ d.w) in the liver, muscles and gills of *G. filamentosus* are presented in Table 40 and Fig. 47.

There were precise differences between the levels of Pb in the liver, gills, and muscles between the study areas.

In Nghe An, the Pb concentration in muscles was significantly lower than the Pb content in the liver and muscles (1, p<0.001; 2, p=0.049). At the same time, in Ha Tinh, the Pb aggregation in the liver was pretty higher than the Pb in the gills and muscles (confirmed by the multiple comparisons p values).

In Quang Binh, the difference of the Pb level in the liver and muscles was obvious. Besides that, in Quang Tri and Hue, the accumulation of Pb in the liver, muscles, and gills was different. It was the highest in the liver, followed by gills and muscles (6-10).

Table 40. The level of Pb in liver, muscles, gills of *G. filamentosus* (μ·g^{-1} d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=11)	Mean±SE	0.568±0.067	0.047±0.008	0.118±0.015
	Min-Max	0.108-0.933	0.005-0.083	0.069-0.225
	Shapiro-Wilk test (p)	0.888	0.596	0.033
	Kruskal-Wallis test		H=23.731; p<0.001	
Ha Tinh (n=10)	Mean±SE	0.612±0.050	0.033±0.007	0.144±0.062
	Min-Max	0.332-0.898	0.010-0.077	0.009-0.690
	Shapiro-Wilk test (p)	0.984	0.101	<0.001
	Kruskal-Wallis test		H=20.771; p<0.001	
Quang Binh (n=12)	Mean±SE	0.513±0.059	0.049±0.007	0.152±0.051
	Min-Max	0.198-0.933	0.013-0.086	0.034-0.690
	Shapiro-Wilk test (p)	0.970	0.887	<0.001
	Kruskal-Wallis test		H=20.055; p<0.001	
Quang Tri (n=10)	Mean±SE	0.593±0.066	0.042±0.006	0.094±0.005
	Min-Max	0.198-0.933	0.013-0.077	0.069-0.112
	Shapiro-Wilk test (p)	0.956	0.947	0.275
	Levene test		F=14.689; p<0.001	
	Kruskal-Wallis test		H=25.138; p<0.001	
Hue (n=8)	Mean±SE	0.408±0.068	0.053±0.005	0.178±0.074
	Min-Max	0.108-0.681	0.032-0.067	0.069-0.690
	Shapiro-Wilk test (p)	0.959	0.133	<0.001
	Kruskal-Wallis test		H=17.788; p<0.001	

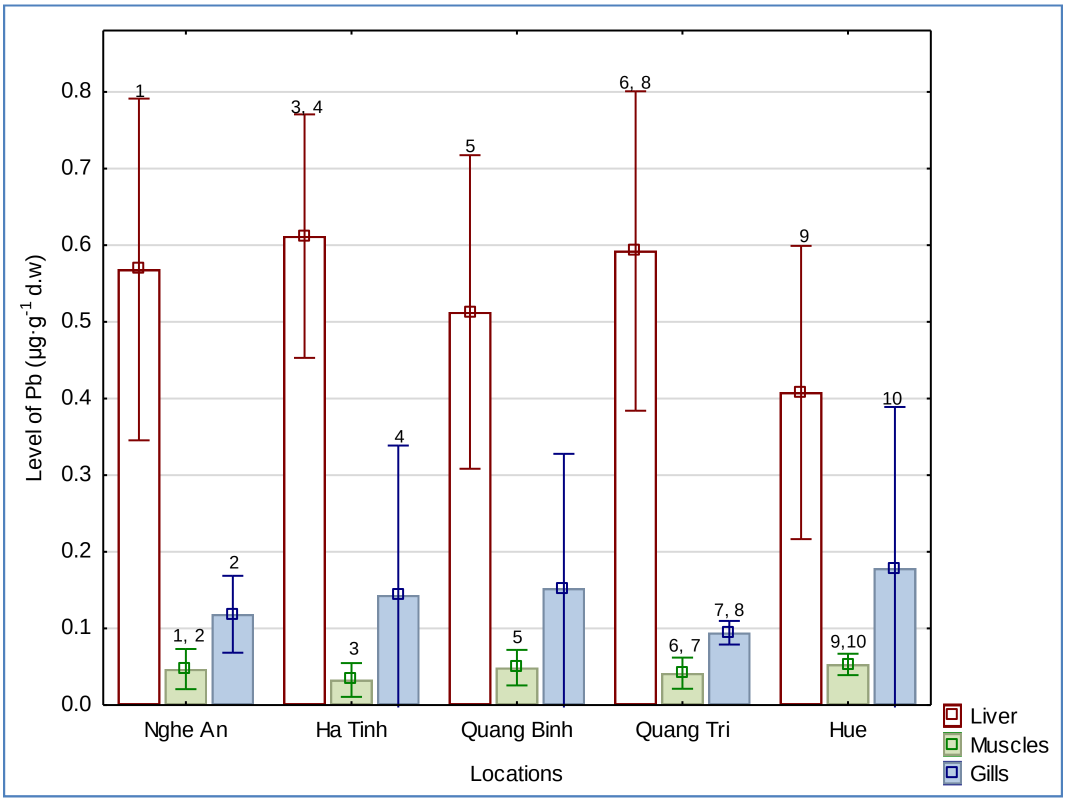

Figure 47. The mean, SD of Pb concentration in tissues of *G. filamentosus*
(1, p<0.001; 2, p=0.049; 3, p<0.001; 4, p=0.033; 5, p<0.001; 6, p<0.001; 7, p=0.029; 8, p=0.044; 9, p<0.001; 10, p=0.027)

7. 4. Fe concentration

The mean, SE, SD, min and max amounts of Fe ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *G. filamentosus* are given in Table 41 and Fig. 48.

In Nghe An, the mean value of Fe in the gills was much lower than the Fe amount in the liver and muscles. In the remaining locations, the value of Fe in the liver was significantly higher than the amount of Fe accumulated in the gills and muscles. The values were confirmed by the effect of multiple comparisons of mean ranks test, and represented by the numbers from 1 to 10 in Fig.48.

The largest Fe value was detected in the liver in Hue (9.438 $\mu \cdot g^{-1}$ d.w), while the lowest concentration was detected in the gills in Quang Binh and Quang Tri (2.530 $\mu \cdot g^{-1}$ d.w).

Table 41. The level of Fe in liver, muscles, gills of *G. filamentosus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=11)	Mean±SE	6.639±0.995	4.411±0.283	3.134±0.205
	Min-Max	3.210-13.484	3.089-6.470	2.495-4.802
	Shapiro-Wilk test (p)	0.135	0.562	0.008
	Kruskal-Wallis test	H=15.850; p<0.001		
Ha Tinh (n=10)	Mean±SE	9.352±0.716	3.549±0.238	2.720±0.143
	Min-Max	6.555-14.950	2.720-4.808	1.973-3.603
	Shapiro-Wilk test (p)	0.048	0.143	0.833
	Kruskal-Wallis test	H=22.699; p<0.001		
Quang Binh (n=12)	Mean±SE	9.165±0.897	4.034±0.227	2.530±0.123
	Min-Max	2.750-13.484	2.907-5.288	1.973-3.148
	Shapiro-Wilk test (p)	0.814	0.754	0.295
	Levene test	F=13.353; p<0.001		
	Kruskal-Wallis test	H=19.716; p<0.001		
Quang Tri (n=10)	Mean±SE	9.165±0.897	4.034±0.227	2.530±0.123
	Min-Max	2.750-13.484	2.907-5.288	1.973-3.148
	Shapiro-Wilk test (p)	0.890	0.072	0.810
	Levene test	F=4.843; p=0.016		
	Kruskal-Wallis test	H=19.967; p<0.001		
Hue (n=8)	Mean±SE	9.438±1.391	3.882±0.262	2.900±0.306
	Min-Max	5.104-16.057	2.720-4.808	1.973-4.802
	Shapiro-Wilk test (p)	0.511	0.734	0.078
	Levene test	F=11.760; p<0.001		
	Kruskal-Wallis test	H=17.368; p<0.001		

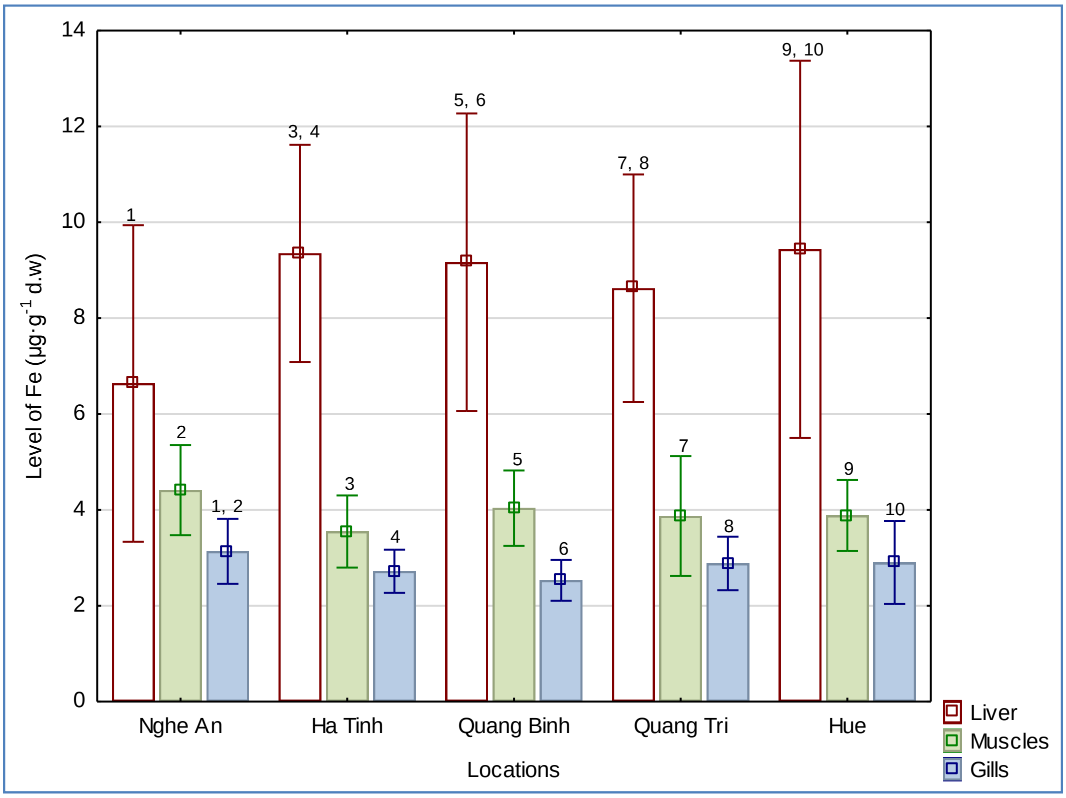

Figure 48. The mean, SD of Fe concentration in tissues of *G. filamentosus*
(1, p<0.001; 2, p=0.019; 3, p=0.011; 4, p<0.001; 5, p=0.014; 6, p<0.001; 7, p=0.005; 8, p<0.001; 9, p=0.022; 10, p<0.001)

7. 5. Zn concentration

The mean, SE, SD, min and max levels of Zn ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *G. filamentosus* are presented in Table 42 and Fig. 49.

In Ha Tinh and Quang Tri, it was found that the Zn concentrations were the highest in the gills, followed by the liver and finally the muscles. The difference is also evident between the concentration of Zn in the muscles as compared to the liver and gills, which has been demonstrated by the Tukey HSD test (3, p=0.001; 4, p=0.010) and the effect of multiple comparisons of mean ranks test (6, p=0.006; 7, p=0.003).

In Nghe An, on the other hand, the accumulation of Zn in muscles was significantly lower than that in the liver and gills (1, p=0.002; 2, p=0.004).

In Quang Binh and Hue, although the highest Zn mean value was still concentrated in the liver, the differences were only expressed in the muscles and liver (5, p<0.001; 8, p=0.002).

Table 42. The level of Zn in liver, muscles, gills of *G. filamentosus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=11)	Mean±SE	2.139±0.282	0.523±0.074	2.362±0.328
	Min-Max	0.841-3.428	0.296-0.981	0.201-3.681
	Shapiro-Wilk test (p)	0.333	0.021	0.236
	Kruskal-Wallis test	H=14.867; p=0.001		
Ha Tinh (n=10)	Mean±SE	1.849±0.222	0.691±0.088	1.556±0.231
	Min-Max	0.248-2.809	0.355-1.142	0.231-2.980
	Shapiro-Wilk test (p)	0.357	0.404	0.795
	ANOVA	F=9.840; p=0.001		
	Levene test	F=1.686; p=0.204		
Quang Binh (n=12)	Mean±SE	2.011±0.235	0.626±0.086	1.470±0.304
	Min-Max	0.928-3.129	0.252-1.290	0.231-3.139
	Shapiro-Wilk test (p)	0.181	0.405	0.076
	Levene test	F=7.218; p=0.003		
	Kruskal-Wallis test	H=14.829; p=0.001		
Quang Tri (n=10)	Mean±SE	2.224±0.210	0.724±0.081	2.320±0.356
	Min-Max	0.928-3.129	0.326-1.142	0.231-3.350
	Shapiro-Wilk test (p)	0.603	0.790	0.017
	Kruskal-Wallis test	H=13.707; p=0.001		
Hue (n=8)	Mean±SE	2.211±0.270	0.466±0.074	1.825±0.408
	Min-Max	0.928-3.428	0.326-0.933	0.231-3.681
	Shapiro-Wilk test (p)	0.992	0.004	0.848
	Kruskal-Wallis test	H=11.920; p=0.003		

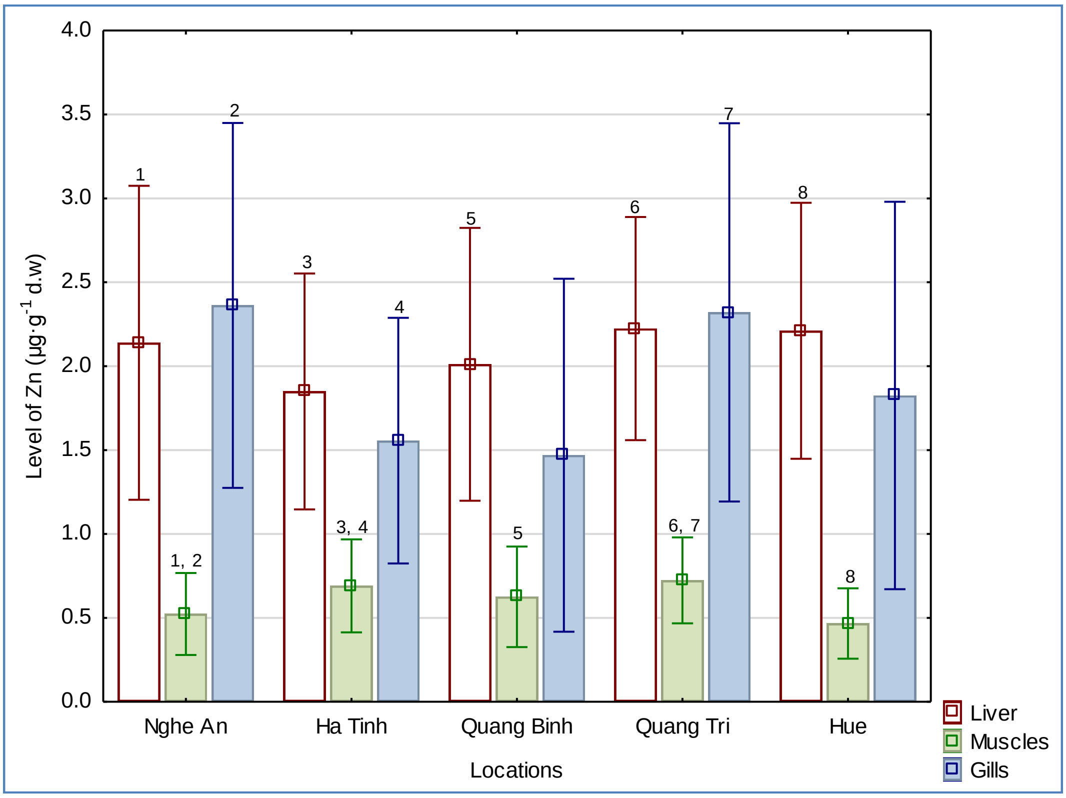

Figure 49. The mean, SD of Zn concentration in tissues of *G. filamentosus*
(1, p=0.002; 2, p=0.004; 3, p=0.001; 4, p=0.010; 5, p<0.001; 6, p=0.006; 7, p=0.003; 8, p=0.002)

7. 6. Cu concentration

The mean, SE, SD, min and max amounts of Cu ($\mu \cdot g^{-1}$ d.w) in the liver, muscles and gills of *G. filamentosus* are given in Table 43 and Fig. 50.

The collected data showed that the Cu concentrations were the highest in the liver, the lowest in the gills. In Nghe An and Quang Tri, the average value of Cu in the liver was significantly higher than the cumulative Cu value in the gills and muscles. The values were presented as numbers 1, 2, 6, and 7 in Fig.50. At the same time, Ha Tinh indicates that the value of Cu in the gills was significantly lower than the level of Cu in the liver, and muscles, as shown by numbers 3 and 4. In Quang Binh and Hue, the Cu content in the gills was significantly lower than the Cu level in the liver (5, 8).

Table 43. The level of Cu in liver, muscles, gills of *G. filamentosus* ($\mu \cdot g^{-1}$ d.w)

Locations		Liver	Muscles	Gills
Nghe An (n=11)	Mean±SE	1.015±0.046	0.636±0.094	0.404±0.043
	Min-Max	0.750-1.154	0.289-1.114	0.251-0.700
	Shapiro-Wilk test (p)	0.003	0.098	0.051
	Kruskal-Wallis test	H=15.027; p=0.001		
Ha Tinh (n=10)	Mean±SE	1.852±0.517	0.745±0.099	0.403±0.045
	Min-Max	0.710-5.481	0.421-1.210	0.223-0.700
	Shapiro-Wilk test (p)	0.001	0.079	0.258
	Kruskal-Wallis test	H=17.390; p<0.001		
Quang Binh (n=12)	Mean±SE	2.284±0.527	0.768±0.138	0.425±0.071
	Min-Max	0.511-5.750	0.312-1.910	0.221-1.090
	Shapiro-Wilk test (p)	0.019	0.023	0.001
	Kruskal-Wallis test	H=14.753; p=0.001		
Quang Tri (n=10)	Mean±SE	1.336±0.250	0.693±0.079	0.422±0.041
	Min-Max	0.750-3.481	0.398-1.114	0.311-0.700
	Shapiro-Wilk test (p)	<0.001	0.109	0.029
	Kruskal-Wallis test	H=19.837; p<0.001		
Hue (n=8)	Mean±SE	1.256±0.324	0.671±0.093	0.434±0.061
	Min-Max	0.678-3.481	0.398-1.100	0.251-0.700
	Shapiro-Wilk test (p)	<0.001	0.344	0.051
	Kruskal-Wallis test	H=12.660; p=0.002		

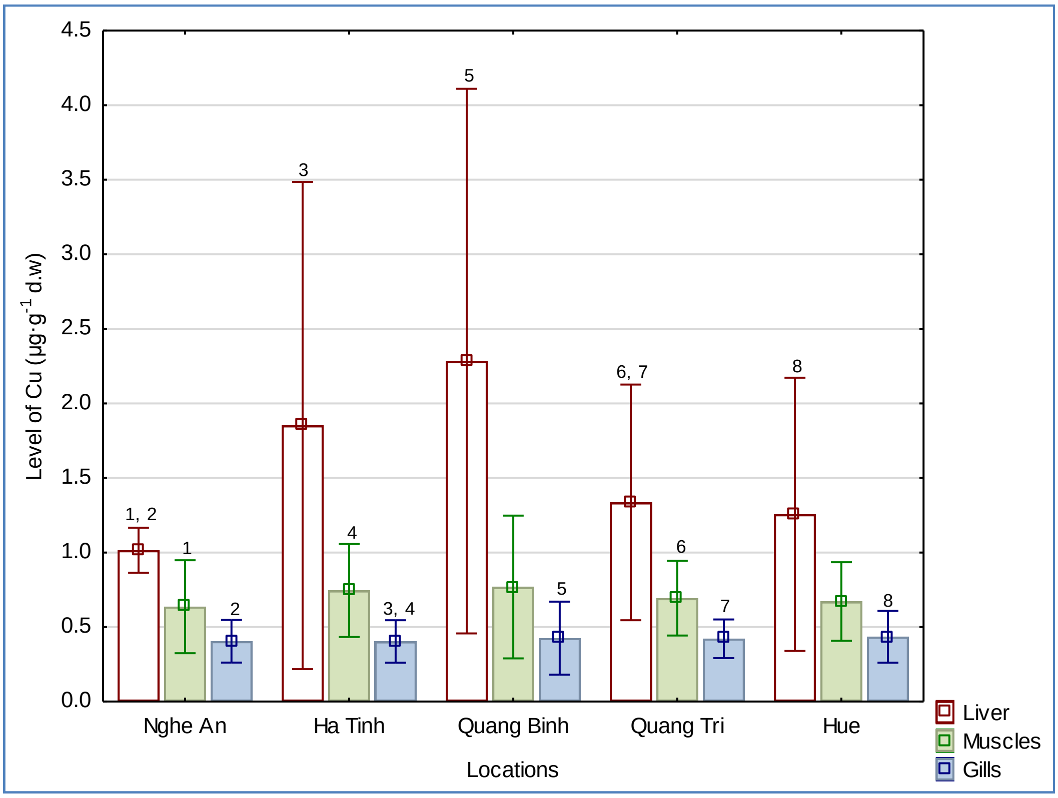

Figure 50. The mean, SD of Cu concentration in tissues of *G. filamentosus*
(1, p=0.020; 2, p=0.001; 3, p<0.001; 4, p=0.049; 5, p=0.001; 6, p=0.048; 7, p<0.001; 8, p=0.001)

8. Human health risk

The mean concentration of heavy metals in fish muscle ($\mu\cdot g^{-1}$ w.w) was used to determine the EDI, THQ, and HI of heavy metals through fish consumption for local inhabitants in Central Vietnam (Table 44, Fig. 51, 52). (The Cd, Pb, Fe, Zn and Cu content in the muscle of the studied fish species was recalculated to change from dry weight to wet weight based on moisture (25%)).

The calculated EDI for heavy metals in the seven fish species decreased in the following sequences: for Hg: *S. sihama* > *K. punctatus* > *U. Sulphureus* > *A. latus* = *G. filamentosus* > *S. fuscescens* > *M. cephalus*; for Cd: *S. fuscescens* > *A. latus* = *K. punctatus* > *S. sihama* > *M. cephalus* > *U. Sulphureus* > *G. filamentosus*; for Pb: *K. punctatus* > *A. latus* > *U. Sulphureus* > *G. filamentosus* > *S. sihama* > *S. fuscescens* > *M. cephalus*; for Fe: *G. filamentosus* > *K. punctatus* > *A. latus* > *M. cephalus* > *U. Sulphureus* > *S. fuscescens* > *S. sihama*; for Zn: *K. punctatus* > *A. latus* > *U. Sulphureus* > *G. filamentosus* > *M. cephalus* > *S. sihama* > *S. fuscescens*; for Cu: *U. Sulphureus* > *K. punctatus* > *M. cephalus* > *G. filamentosus* > *A. latus* > *S. fuscescens* > *S. sihama*. The EDI value of Fe was the highest in *G. filamentosus*, while the EDI value of Cd was the lowest in *G. filamentosus*. Compared to the Provisional tolerable daily intake (PTDI), the EDI in these fish is within the permitted levels.

Table 44. Estimated daily intake (EDI), Target hazard quotients (THQ) for individual metals and Hazard index (HI) of total heavy metals by consumption of fish.

		Hg	Cd	Pb	Fe	Zn	Cu	HI
A. latus	EDI	0.070	0.050	0.564	9.294	2.978	1.068	
	THQ	0.233	0.050	0.141	0.013	0.074	0.004	0.516
K. punctatus	EDI	0.113	0.050	0.658	13.029	3.987	2.717	
	THQ	0.377	0.050	0.165	0.019	0.100	0.009	0.719
M. cephalus	EDI	0.061	0.044	0.074	9.039	1.965	2.627	
	THQ	0.203	0.044	0.019	0.013	0.049	0.009	0.337
S. fuscescens	EDI	0.065	0.067	0.097	6.221	1.163	0.618	
	THQ	0.217	0.067	0.024	0.009	0.029	0.002	0.348
S. sihama	EDI	0.205	0.047	0.168	6.214	1.879	0.554	
	THQ	0.683	0.047	0.042	0.009	0.047	0.002	0.830
U. sulphureus	EDI	0.076	0.040	0.474	8.901	2.519	3.063	
	THQ	0.253	0.040	0.119	0.013	0.063	0.010	0.498
G. filamentosus	EDI	0.070	0.034	0.151	13.375	2.169	2.361	
	THQ	0.233	0.034	0.038	0.019	0.054	0.008	0.386
PTDI ($\mu g \cdot kg^{-1}$ b.w per day)		0.714[a]	1.00[b]	3.571[c]	800[d]	300-1000[e]	500[e]	

[a](Feeley et al., 2011); [b](Mueller et al., 2011); [c](Benford et al., 2011); [d](FAO/WHO, 1983); [e](FAO/WHO, 1982)

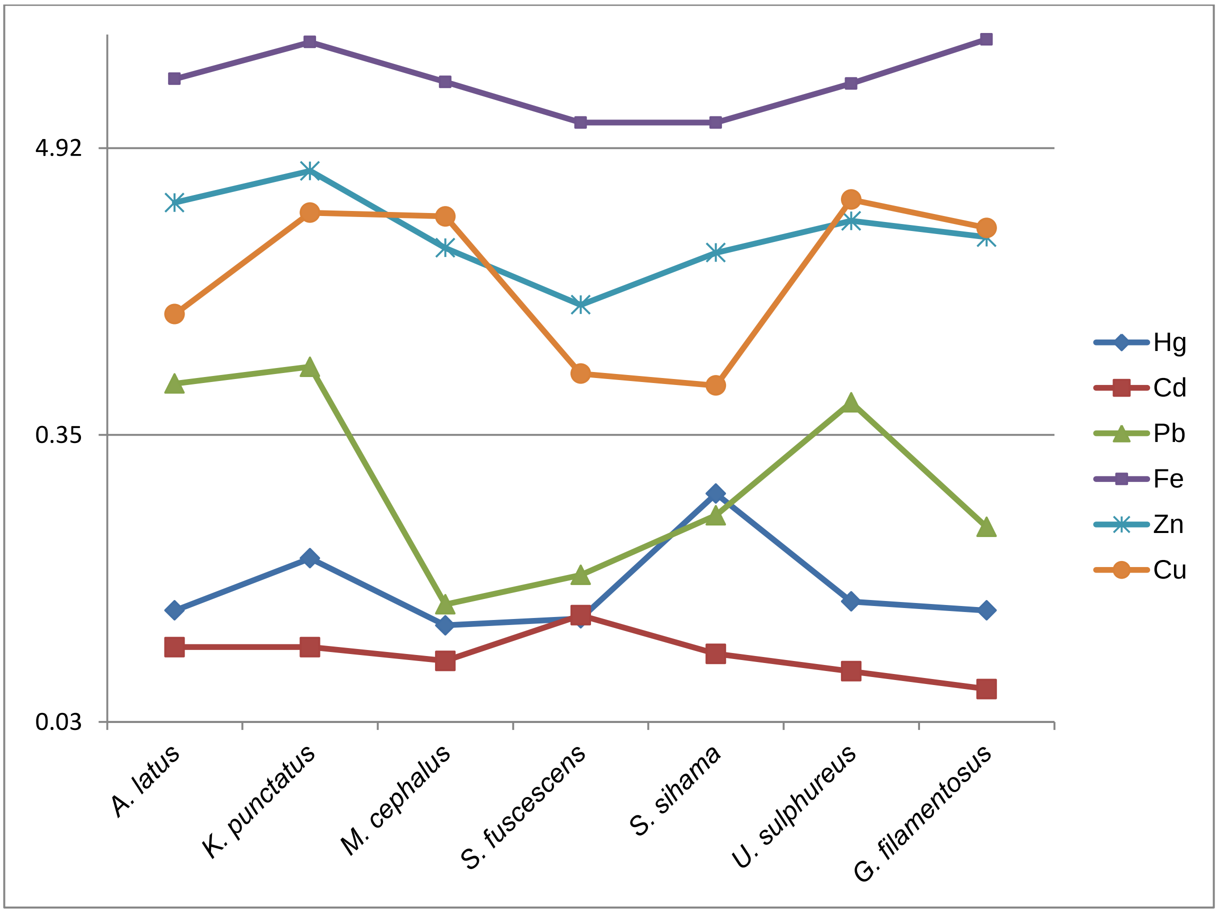

Figure 51. The Estimated Daily Intake of heavy metals through fish consumption

The calculated THQ for individual metal decreased in *A. latus*, *K. punctatus*, and *U. sulphureus* the following sequence: Hg > Pb > Zn > Cd > Fe > Cu; while in *M. cephalus*, *S. sihama*, and *G. filamentosus* in the following order: Hg > Zn > Cd > Pb > Fe > Cu; for *S. fuscescens*: Hg > Cd > Zn > Pb > Fe > Cu. In the present study, the THQ and HI were lower than 1 for all the heavy metals in the seven fish species. This indicates that the local residents are not subject to significant health risks from consumption within Central Vietnam. Nevertheless, the values of Hg account for a large proportion of the total THQ (45, 155, 52.434, 60.237, 62.356, 82.289, 50.803, and 60.363 % in *A. latus*, *K. punctatus*, *M. cephalus*, *S. fuscescens*, *S. sihama*, *U. Sulphureus*, and *G. filamentosus*, respectively), which signals that this is a higher risk contributor to the consumers' health.

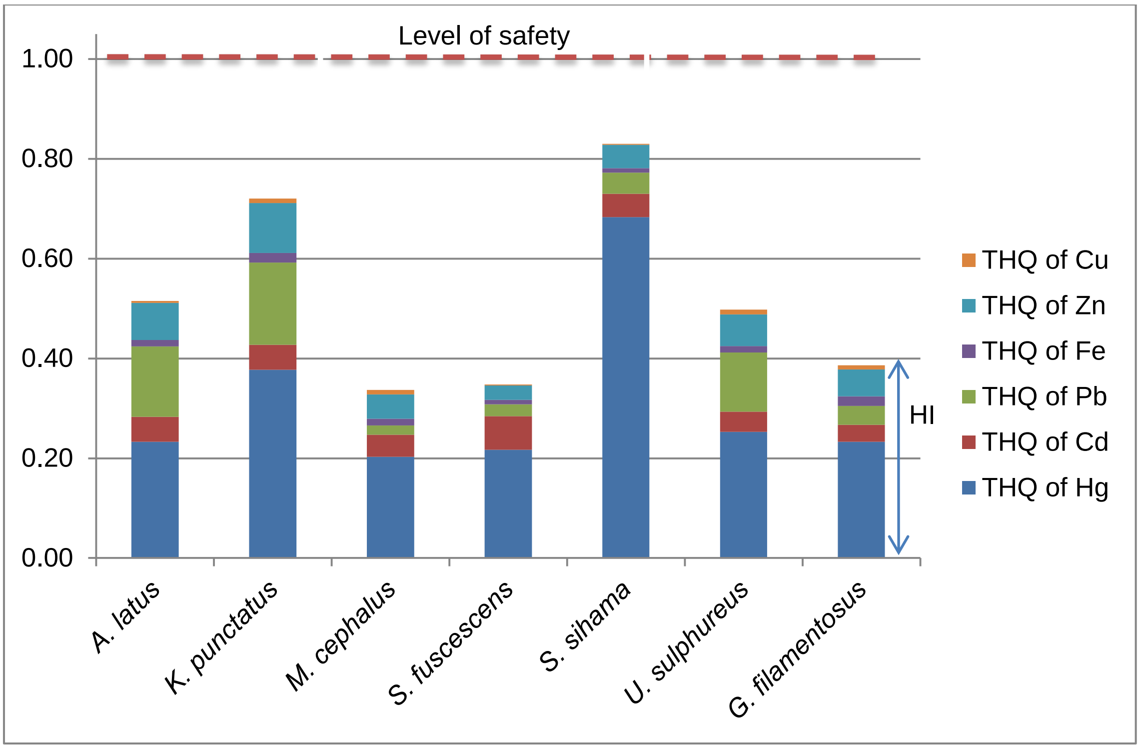

Figure 52. The Target Hazard Quotients (THQ), Hazard Index (HI) for individual metals

V. DISCUSSION

Population growth and expanded industrialization combined with agriculture development lead to the increased pressure on water quality, giving rise to water pollution and environmental deterioration, which are issues that need to be resolved in Vietnam (FAO, 2017; Liyanage & Yamada, 2017; MONRE, 2018). It is well-known that fish are an important food resource for humans and a major item of the aquatic ecosystems, thus evaluation of the heavy metal content in fish species is particularly important to the consumers (Tidwell & Allan, 2001; WHO/FAO, 2011; Tacon & Metian, 2013). On the other hand, fish have been usually used in biological monitoring and have determined the heavy metals safety levels in the environment (Tulonen et al., 2006; Naigaga et al., 2011; Plessl et al., 2017). In this study, we found that all of the tested metals were presented in the liver, muscles, and gills of *G. filamentosus, S. sihama*, *M. cephalus*, *S. fuscescens*, *U. sulphureus, A. latus* and *K. punctatus*. In all the studied species, there was a tendency to accumulate high levels of metal in the liver, then in the gills. Muscles are the places with the lowest metal accumulation. It is well known that muscles appear to be a temporary tissue in the pathway of metal uptake and storage, gills act as the primary point of exposure of organs to dissolved metals, whereas the liver specializes in metal storage and detoxification (Mazon et al., 2002; Van der Oost et al., 2003). Therefore, contradictory to muscles, liver, and gills act as main organs for metabolism, respiration and can be deliberated as destination organs for the accretion of contaminants (George et al. 2012). The results showed that metal contents in these fish species were significantly different in their tissues, across the researched species and locations. These variations might result from the differences in the species nutrition, metabolic activities, bioaccumulation processes and varied positions in the food chain. Besides, metal accumulation is also affected by other factors in the aquatic system, as well as by pH and temperature (Dhanakumar et al., 2015).

1. Hg concentration in fish

Hg is well known as a particularly hazardous metal among the studied heavy metals and the exposure to this element could permanently harm the brain, kidney, and muscles (Stancheva et al., 2013; Zhao et al., 2017; Kumar et al., 2019). Besides, it cannot only cause interruption to the membrane potential but also break off the intracellular calcium homeostasis (Jaishankar et al., 2014; Zhang et al. 2019; Esposito et al., 2020). Similarly to the WHO (2006), the EFSA (EC, 2006) and the FDA (Evans et al.,2002), the Ministry of health Vietnam (MOH, 2011) have also developed regulations to set the maximum amount of Hg in fish muscle as 0.5 mg·kg^{-1} w.w. Despite that, some species have the maximum level of 1.0 mg·kg^{-1} w.w (MOH, 2011). In this study, neither predatory fish nor non-predatory fish from the five sampling locations showed the muscles Hg levels exceeding the specified threshold, suggesting that these species reflect the low mercury bioavailability of the central Vietnam coastal waters. As observed in the previous research studies, the carnivorous and omnivorous fish species contain higher concentrations of Hg than the herbivorous fish (Dringer et al., 2015; Souza-Araujo at al., 2016).

The order of Hg levels in the muscles for the whole study area is as follows: *S. sihama* > *K. punctatus* > *G. filamentosus* > *A. latus* > *U. sulphureus* > *M. cephalus* > *S. fuscescens*.

Not surprisingly, since these tissues are the major part of the human diet, most research studies focus on the muscles. *S. sihama* has been classified as carnivore based on the contents analysis from stomachs (Khan et al., 2014). It is easily understandable that the Hg content in the muscles is quite high compared to other species. Chen (2002) discovered that the level of Hg in the muscles of *S. sihama* in the Chi-ku lagoon in Southwest Taiwan equalled 0.025 μ·g^{-1}w.w. In the South China Sea, Zhu et al. (2013) and Chen et al. (2018) detected 0.062 and 0.065 μ·g^{-1}w.w, while in the Chi-ku lagoon in Southwest Taiwan, Pan et al. (2014) have recorded 0,035 μ·g^{-1}w.w. These detections were much lower than the data presented in this work, even below the lowest value which was recorded at Ha Tinh and equalled 0.201 μ·g^{-1}w.w.

K. punctatus tends to be carnivorous in feeding habits as the common food found in its stomach comprised rhizopods, tintinnids, euglenoids, copepods, larval bivalves, dinoflagellates, diatoms, (Choi et al., 2015). Hg accumulation in the muscles of *K. punctatus* in this study shows similar results with Oh et al. (2010) in Lake Shihwa, Korea. On the other hand, many authors have reported that the Hg content is lower than the data in this study (Sho et al., 2000; Ham, 2002; Hwang & Park, 2007; Kim et al., 2007; Mok et al., 2009; Choi et al., 2012; Kim et al., 2012; Zhu et al., 2013; Hwang et al., 2017). Besides, in the South China Sea, Chen et al. (2018) have recorded the concentration of Hg in the muscles of 0.052 μ·g^{-1}w.w.

The content of Hg in the muscles of *G. filamentosus* in this study was quite low despite the fact that it is an omnivorous species and tends to bottom-feed (Uskelwar et al., 2019). Although Chen (2002) and Pan et al. (2014) have also declared a similar result in Taiwan and Southern China, Agusa et al. (2007) and Le et al. (2018) recorded a quite high Hg content in Southeast Asia.

Due to the presence in the stomach of food items of both animal and plant origins, *A. latus* could be considered omnivorous (Sourinejad et al., 2015). As with previous studies, the accumulation of Hg stored in the muscles of *A. latus* is quite small (Agusa et al., 2007; Hosseinkhezri & Tashkhourian, 2011).

Even though, Ramteke et al. (2015) reported that *U. sulphureus* is a carnivorous species feeding mainly on crustaceans and occasionally on bivalve mollusks, the concentration of Hg in its muscles was not high (0.09 $\mu \cdot g^{-1}$w.w). Concurrently, in Malaysia, Sivalingam & Sani (1980) revealed that the accumulation of Hg in the muscles is approximately at the allowed threshold.

M. cephalus is a bottom feeder, as well as a herbivorous species as its food is mainly composed of fresh and decaying plant matter (Islam et al., 2009). It is therefore not surprising that the Hg content in muscles is not too high (0.074 $\mu \cdot g^{-1}$w.w). This has also been earlier confirmed by many authors. Specifically, Stancheva et al. (2013) recorded 0.08 $\mu \cdot g^{-1}$w.w in the Black Sea, while Zhu et al. (2013), Pan et al. (2014) and Chen et al. (2018) confirmed 0.104, 0.044 and 0.016 $\mu \cdot g^{-1}$w.w, respectively, in the China Sea; in Korea, Oh et al. (2010) determined 0.098 $\mu \cdot g^{-1}$w.w.

As a herbivorous, *S. fuscescens* tends to accumulate not particularly high amounts of Hg in the muscles (Pillans et al., 2004) (except when they live in heavily polluted areas). In this study, the average value of Hg in muscles was discovered at 0.07 $\mu \cdot g^{-1}$w.w. It is higher than previously published in China by Onsanit et al. (2012) (0.030 $\mu \cdot g^{-1}$w.w), Pan et al. (2014) (0.005 $\mu \cdot g^{-1}$w.w), Chen et al. (2018) (0.018 $\mu \cdot g^{-1}$w.w), and Li et al. (2019) (0.041 $\mu \cdot g^{-1}$w.w).

The order of the Hg level in the liver for the whole study area is as follows: *S. sihama* > *G. filamentosus* > *U. sulphureus* > *K. punctatus* > *A. latus* > *M. cephalus* > *S. fuscescens*. *S. sihama*, *G. filamentosus* and *U. sulphureus* showed significantly higher levels than the remaining species ($p < 0.05$). There are many studies, which have shown that the metal concentration in the liver is high (Cogun et al., 2005; Nabavi et al., 2012; Mahboob et al., 2016; Asare et al., 2018; Ferreira da Silva & de Oliveira Lima, 2020). The liver acts as a detoxifying filter storing heavy metals led to this most important target and storage tissue in fish (Vinodhini & Narayanan, 2008). Unfortunately, there are not many documents that published the content of Hg in the liver of these fish species. In Chi-ku Lagoon, Southernwest Taiwan, Chen (2002) had announced that the Hg level in the liver of *S. sihama* was much lower than the one noted in this study. Agusa et al. (2007), however, had declared a similar level in the liver of *G. filamentosus* in Southeast Asia. Moreover, all the amounts of Hg found by Hosseinkhezri & Tashkhourian (2011) in the liver of *A. latus* (0.14 $\mu \cdot g^{-1}$w.w), by Li &

Wang (2019) in *S. fuscescens*'s liver (0. μ·g^{-1}w.w) and by Chen (2002) in the liver of *M. cephalus* (0,044 μ·g^{-1}w.w) were lower than the data presented in this work.

The concentrations of Hg in the gills for the whole study area of the selected fish were as follows: *A. latus* > *G. filamentosus* > *U. sulphureus* > *K. punctatus* > *S. sihama* > *M. cephalus* > *S. fuscescens*. The gills are an important site for the entry of heavy metals and their accumulation is relatively high in the gills of fish (Javed, 2005; Vinodhini & Narayanan, 2008). Since they are in direct contact with the external environment, gills can absorb and reabsorb contaminants present in water (Huang et al., 2007; Jesus et al., 2011). Hosseinkhezri & Tashkhourian (2011) have also announced the concentration of Hg in the gills of *A. latus* at 0.12 μ·g^{-1}w.w in the Bushehr seaport, Iran, as lower than the data in the central coastal region of Vietnam. At the same time, Stancheva et al. (2013) determined the level of Hg in the gills of *M. cephalus* as equivalent to the value detected in this study (0.12 μ·g^{-1}w.w).

2. Cd concentration in fish

Cadmium is a non-essential, highly toxic and ecotoxic metal (Meador et al., 2005; Stancheva et al., 2013; Geng & Wang, 2019). There have been different regulations on the maximum permissible content of Cd in fish muscles. The European Community (No 78/2005) recommended the maximum levels permitted for Cd in sea fish as 0.05 μ·g^{-1}w.w (EC, 2005). The FAO/WHO (2011) stipulated limits of 0.20 μ·g^{-1}w.w for the Cd concentrations in the organs of fish species. In Vietnam, the Ministry of Health (MOH, 2011) also developed a regulation to set the maximum amount of Cd in fish muscle as 0.10 μ·g^{-1} w.w for *U. sulphureus*, *K. punctatus*, and *M. cephalus*; *S. sihama*, *S. fuscescens*, *A. latus* and *G. filamentosus* must not exceed 0.05 μ·g^{-1}w.w.

The average concentration of Cd in muscles for the whole study area followed the order: *S. fuscescens* > *K. punctatus* > *S. sihama* > *M. cephalus* > *S. sulphureus* > *A. latus* > *G. filamentosus*. The mean concentration of Cd in the muscles of *S. fuscescens* was significantly higher than in all the other species ($p<0.05$) with the value of 0.020± 0.002 μ·g^{-1} d.w. Consider converting dry weight to wet weight based on moisture (Burgera & Gochfeld, 2005), the levels of Cd recorded in *A. latus*, *S. fuscescens*, and *S. sihama* exceeded the limit threshold set by the Vietnamese Ministry of Health for safe consumption.

The Cd level in the muscles of *S. fuscescens* in the present study was higher than those determined by Liu et al. (2015) but significantly lower than the ones published by Zhang & Wang (2012) in the China Sea, who indicated that the Cd mean in the muscles of *S. fuscescens* reached 0.135 μ·g^{-1} d.w. For *K. punctatus*, Mok et al. (2009) published the Cd content of 0.026 $\mu \cdot g^{-1}$ d.w for the Korean coasts, which is higher than the data published in this study. On the other hand, in the Southern Sea of Korea, Hwang et al. (2017) have recorded the accumulation of Cd of approximately 0.002 $\mu \cdot g^{-1}$ d.w, which is many times lower than the Cd level in the central coastal region of Vietnam. Jayaprakash et al. (2015) recorded higher levels of Cd in the muscles of *S. sihama* (0.06 $\mu \cdot g^{-1}$ d.w) in Chennai, southeast coast of India as compared to this study. The authors suggested that the higher values of Cd in muscles are attributed to the indiscriminate disposal of unused lead-based motor batteries and the coal combustion released to the environment from a thermal power plant.

M. cephalus is one of the fish species assessed to reflect well the sediment environment status due to relation to its mobility and food preferences (Stancheva et al., 2013; Ennouri et al., 2013). For that reason, *M. cephalus* has been extensively studied in many parts of the world. In the North-East Mediterranean (Turkey), Canli & Atli (2003) published the data with 0.66 $\mu \cdot g^{-1}$ d.w in muscles. While in the Estuary of Saint Louis, Senegal, Diop et al. (2016) recorded 2.31 $\mu \cdot g^{-1}$ d.w in muscles. At the same time, in the East Algerian coast, the data recorded by Ouali et al. (2018) equalled 0.475 $\mu \cdot g^{-1}$ d.w. These references are significantly higher than the figures in this study (0.013 $\mu \cdot g^{-1}$ d.w). Similarly, Medeiros et al. (2012) reported relatively low levels of Cd in the muscles of *M. cephalus* on the Coast of Rio de Janeiro, Brazil (0.001 $\mu \cdot g^{-1}$ d.w).

Contrary to the results of this research, the previous studies reported that the number of Cd in *U. sulphureus* was quite high. In Mombasa, Kenya, Mwashote (2003) encountered 1.6 $\mu \cdot g^{-1}$ d.w in muscles, while Zhang & Wang (2012) reported 0.053 $\mu \cdot g^{-1}$ d.w in the China Sea.

For *A. latus*, the highest Cd value in muscles was detected by Hosseinkhezri & Tashkhourian (2011) at the coastal of Persian Gulf, Iran (0.12 $\mu \cdot g^{-1}$ d.w). Also, at the Persian Gulf, Iran, Saei-Dehkordi & Fallah (2011) recorded the Cd value of 0.072 $\mu \cdot g^{-1}$ d.w. At the same time, Agusa et al. (2007) determined that the Cd value reached 0.056 $\mu \cdot g^{-1}$ d.w in the *A. latus* muscles in Malaysia. Compared to these data, this study found that the Cd levels in the muscles were relatively low.

In the report of Agusa et al. (2007), concentrations of 20 trace elements were determined in the muscles and liver of 34 species of marine fish collected from the coastal areas of Cambodia, Indonesia, Malaysia and Thailand. They have published the levels of Cd in the muscles of *G. filamentosus* reaching only 0.001 $\mu \cdot g^{-1}$ d.w, which was much lower than the data in this work (0.01 $\mu \cdot g^{-1}$ d.w).

The order of the Cd levels in the liver for the whole study area is as follows: *U. sulphureus* > *S. sihama* > *A. latus* > *S. fuscescens* > *M. cephalus* > *G. filamentosus* > *K. punctatus*. However, with the results of the Tukey HSD for unequal N test, the only noticeable difference was observed in the Cd concentrations between *U. sulphureus* and *K. punctatus*. Unfortunately, there is no previously published literature regarding the Cd contents in the liver of *U. sulphureus* and *K. punctatus*. However, the results in this study once again confirm higher concentration of Cd in the liver as compared to the other organs, which is consistent with the views of Al-Yousuf et al. (2000) and Has-Schön et al. (2007). The concentration of Cd in the liver was relatively high, which was reported by Agusa et al. (2007) in the liver of *A. latus* and *G. filamentosus* in Southeast Asia (10.8 and 0.492 $\mu \cdot g^{-1}$ d.w, respectively). Chen (2002), however, confirmed lower levels of Cd in the liver of *S. sihama* and *M. cephalus* in Taiwan (0.025 and 0.018 $\mu \cdot g^{-1}$ d.w, respectively). What is more, Liu et al. (2015) also noted that the concentration of Cd in the liver of *S. fuscescens* reached 0.04 $\mu \cdot g^{-1}$ d.w, i.e. exceeded the Cd levels in this study.

The order of the Cd levels in the gills for the whole study area was as follows: *U. sulphureus* > *S. fuscescens* > *S. sihama* > *A. latus* > *K. punctatus* >*M. cephalus* > *G. filamentosus*. However, the result of the Tukey HSD for unequal N test shows that only the Cd content in the gills of *U. sulphureus* was significantly larger than that of *A. latus*, *K. punctatus*, *M. cephalu*s, and *G. filamentosus*. Previously, data on Cd in the gills of *U. sulphureus*, *S. fuscescens*, *A. latus*, and *M. cephalus* were recorded by scientists at different locations. In Mombasa Kenya, Mwashote et al. (2003) confirmed that the concentration of Cd in the gills of *U. sulphureus* was lower than the value in this study. In the South China Sea, Liu et al. (2015) published the Cd data in the gill of *S. fuscescens* equivalent to the values from this work (0.023 mg). However, in the Bushehr seaport (the coastal of the Persian Gulf), Iran, Hosseinkhezri & Tashkhourian (2011) recorded the level of Cd in the gills of *A. latus*, which was much higher than the one in this study. In addition, in the Black Sea, Stancheva et al. (2013) also confirmed the accumulation of Cd in *M. cephalus* liver as lower than in this publication.

3. Pb concentration in fish

Due to Pb toxicity, the Ministry of Health of the People's Republic of China (2012) stipulated its standard maximum levels in fish muscles as 0.5 $\mu \cdot g^{-1}$w.w. The EC (2008), FAO/WHO (2011) and the Vietnamese Ministry of Health (MOH, 2011) have established the maximum levels of Pb in muscles of fish as 0.3$\mu \cdot g^{-1}$w.w. In our study, the Pb content in fish was measured by dry weight, considering that the dry weight represents 23–33% of the corresponding wet weight (Burgera & Gochfeld, 2005). The concentration of Pb in muscles of *K. punctatus, A. latus* and *U. sulphureus* exceeded the threshold for consumer safety.

The mean concentration of Pb in the muscles for the whole study area was in order: *K. punctatus* > *A. latus* > *U. sulphureus* > *S. sihama* > *G. filamentosus* > *S. fuscescens* > *M. cephalus*. The order in the liver was as follows: *G. filamentosus* > *K. punctatus* > *A. latus* > *U. sulphureus* > *M. cephalus* > *S. sihama* > *S. fuscescens*. The range in the gills followed a different order: *U. sulphureus* > *K. punctatus* > *A. latus* > *M. cephalus* > *G. filamentosus* > *S. sihama* > *S. fuscescens*. In these species, high Pb concentration accumulates in the liver, then in the gills, whereas the muscles have the lowest Pb accumulation. Zuluaga et al. (2015) showed high concentration of Pb in fish in the areas close to mining activities and the areas with the presence of this metal-based industry. Therefore, many publications show significant differences of Pb levels within the same object but in different positions.

Earlier, in the Bushehr seaport, Iran, Hosseinkhezri & Tashkhourian (2011) recorded the contents of Pb in the muscles, liver, and gills of *A. latus* reaching 0.11, 0.21 and 0.152 $\mu \cdot g^{-1}$ d.w, respectively. Moreover, Agusa et al. (2007) showed that in Southeast Asia the accumulation of Pb in the liver of *A. latus* was significantly higher (0.877 $\mu \cdot g^{-1}$ d.w) than in the muscle (0.072 $\mu \cdot g^{-1}$ d.w). In another study, which focused on the accumulation of lead, cadmium, copper, and zinc in the flesh of twelve of the most valuable pelagic and demersal fish species from the Persian Gulf, Iran, (Saei-Dehkordi & Fallah, 2011), the authors detected high level of Pb in the muscles of *A. latus* (0.118 $\mu \cdot g^{-1}$ d.w).

Mok et al. (2009) and Hwang et al. (2017) showed a relatively low accumulation of Pb content in the muscles of *K. punctatus*. The studies were conducted on the Korean coasts (0.027 $\mu \cdot g^{-1}$ d.w and 0.007 $\mu \cdot g^{-1}$ d.w, respectively) and their results differed from the results of this study.

In the study of Mwashote (2003) in the Tudor and Makupa creeks in Mombasa, Kenya, much affected by anthropogenic activities, the results determined relatively high levels of Pb in muscles and gills of *U. sulphureus* (0.5 $\mu \cdot g^{-1}$ d.w and 0.875 $\mu \cdot g^{-1}$ d.w, respectively), which were higher than the results of this study. Trace elements were examined on the marine wild fish in eight stations along with the coastal areas in China (Zhang & Wang, 2012), the research showed that the level of Pb in the muscle of *U. suphureus* was equal to the levels of this study (0.128 $\mu \cdot g^{-1}$ d.w).

There are not many published data on Pb accumulated in *S. fuscescens*. Earlier studies by Liu et al. (2015) in the South China Sea showed the highest Pb accumulation in the gills

(0.055 $\mu\cdot g^{-1}$ d.w), followed by the liver (0.028 $\mu\cdot g^{-1}$ d.w) and finally the muscles (0.013 $\mu\cdot g^{-1}$ d.w). However, in this study, the Pb content in the muscles, gills, and liver of *A. fuscescens* was not significantly different. In coastal China, Zhang & Wang (2012) confirmed the Pb level in the muscles reaching 0.068 $\mu\cdot g^{-1}$ d.w.

Similarly to the previous research, in *M. cephalus* the studies were also mostly focused on the muscles, while the data in the liver and gills were scarce. Chen (2002) and Mwakalapa et al. (2019) have announced the concentration of Pb in the liver reaching 0.017 and 0.865 $\mu\cdot g^{-1}$ d.w, respectively. Besides, Stancheva et al. (2013) announced the content of Pb in the gills to reached 0.02 $\mu\cdot g^{-1}$ d.w in the south part of the Bulgarian Black Sea coast. However, the highest value of Pb in the muscles was detected in the East Algerian coast by Ouali et al. (2018) (1.21 $\mu\cdot g^{-1}$ d.w), followed by the report by Yilmaz (2005) in the Iskenderun Bay, Turkey (0.897 $\mu\cdot g^{-1}$ d.w). Mwakalapa et al. (2019) confirmed the levels of 0.347 $\mu\cdot g^{-1}$ d.w in the muscles in Tanzania. Medeiros et al. (2012) announced 0.2 $\mu\cdot g^{-1}$ d.w in Rio de Janeiro State, Brazil. On the Senegalese coast, Diop et al. (2016) discovered 0.08 $\mu\cdot g^{-1}$ d.w in the muscles and Kamal et al. (2013) also disclosed the value of 0.043 $\mu\cdot g^{-1}$ d.w in Palestine. Moreover, two previous publications showed lower values than the Pb levels in this study. At the Bulgarian Black Sea coast, Stancheva et al. (2013) recorded relatively low values (0.017 $\mu\cdot g^{-1}$ d.w). Likewise, Squadrone et al. (2013) also found only 0.016 $\mu\cdot g^{-1}$ d.w in the muscles of *M. cephalus* in the Ligurian Sea, the North-West Mediterranean, Italy.

The announcement of Hung et al. (1999) on the accumulation of heavy metals in ten species of demersal fish from the Taiwan south-eastern coast revealed that the concentration of Pb in the muscles of *S. sihama* was very high (5 $\mu\cdot g^{-1}$ d.w). This result is many times higher than the one published by Dehqhani (2017) in the Persian Gulf, Iran (0.12 $\mu\cdot g^{-1}$ d.w). Earlier Jayaprakash et al. (2015) also revealed that the concentration of Pb in muscles of *S. sihama* from the Southeast coast of India was approximately 1 $\mu\cdot g^{-1}$ d.w. In the İskenderun Bay, Turkey, Kaya & Turkoglu (2017) reported significantly lower Pb concentrations than the above studies. Besides, Chen (2002) also noted a relatively low Pb accumulation in the liver of *S. sihama* in the Southwest Taiwan (0.017 $\mu\cdot g^{-1}$ d.w).

In the study at Cochin backwaters, India, Nair et al. (2006) revealed very high levels of Pb in the gills of *G. filamentosus* (15.15 $\mu\cdot g^{-1}$ d.w). According to the authors, the gills are in continuous contact with the aqueous medium and the contaminants contained therein. In the present study, we noted that the highest concentrations of Pb occur in the liver in the Ha Tinh region, reaching 0.539 $\mu\cdot g^{-1}$ d.w, which is approximately 14 times lower than the one observed by Nair et al. (2006) in the liver of *G. filamentosus* in India (7.57 $\mu\cdot g^{-1}$ d.w).

Similarly, Nair et al. (2006) also showed that the level of Pb in the muscles was very high, exceeding the allowed threshold set by the FAO/WHO (2011) by 100 times. Lower concentrations of Pb found in Southeast Asia by Agusa et al. (2007) showed 0.001 $\mu\cdot g^{-1}$ d.w in the muscles and 0.032 $\mu\cdot g^{-1}$ d.w in the gills. What is more, Chen (2002) also found a lower accumulation of Pb in the muscles (0,017 $\mu\cdot g^{-1}$ d.w) in Southwest Taiwan.

4. Fe concentration in fish

It is well known that Fe is an essential nutrient to almost all organisms (Cairo et al., 2006; Bertini et al., 2007). Fe is involved not only in the oxygen transfer, but also in the respiratory chain reactions, DNA synthesis, and immune system functions (Wood et al., 2012). Fe can be damaging when it accumulates in the tissues and can also produce toxic effects when the metal intake is excessively elevated (Jaishankar et al. 2014). Therefore, the FAO and WHO (FAO/WHO, 2011) have established the permissible consumption limit of Fe in fish muscles (450 $mg\cdot kg^{-1}$ w.w). In this study, the Fe level showed low concentrations in tissues of all the analyzed fish species as compared to the threshold. The highest average value of Fe was detected in the liver of *A. latus* at Quang Binh (22.563 $\mu\cdot g^{-1}$ d.w), while the lowest value was found in the muscle of *S. fuscescens* in Quang Tri (1.227 $\mu\cdot g^{-1}$ d.w). The results again reinforce the tendency for the metal to accumulate more in the liver and carry more in the muscles (Sayadi et al. 2020). The mean concentration of Fe in the muscles for the whole study area followed the order: *G. filamentosus* > *K. punctatus* > *A. latus* > *M. cephalus* > *U. sulphureus* > *S. fuscescens* > *S. sihama*. The order in the liver was as follows: *A. latus* > *K. punctatus* > *G. filamentosus* > *U. sulphureus* > *S. sihama* > *S. fuscescens* > *M. cephalus*. The range in the gill varied: *A. latus* > *K. punctatus* > *M. cephalus* > *S. fuscescens* > *G. filamentosus* > *U. sulphureus* > *S. sihama*.

Iran, Koshafar & Velayatzadeh (2015) presented the concentration of Fe in the *A. latus* muscles from the Bahmanshir River of approximately 4.964 $\mu\cdot g^{-1}$ d.w, which was higher than our findings in this study (2.767 $\mu\cdot g^{-1}$ d.w).

In the investigation of multi-elemental accumulation (twelve trace elements) in twenty-nine marine wild fish from the China Seas, Zhang & Wang (2012) showed that the Fe accumulation was not really high in the muscles of *U. sulphureus* and *S. fuscescens* (4.938 and 6.445 $\mu\cdot g^{-1}$ d.w, respectively), similar to this study. We also noted low Fe accumulation in the muscles of these 2 fish species in Vietnam (2.650 and 1.852 $\mu\cdot g^{-1}$ d.w, respectively).

Moreover, many studies focus on the *M. cephalus* species. Diop et al. (2016) found a relatively high concentration of Fe in the muscles on the Senegalese coast, which reached

150.25 $\mu \cdot g^{-1}$ d.w. Ouali et al. (2018) recorded 147.73 $\mu \cdot g^{-1}$ d.w in the East Algerian coast Another study was conducted at the Iskenderun Bay, Turkey, and showed the results of 12,675 $\mu \cdot g^{-1}$ d.w (Yilmaz, 2005). Moreover, Medeiros et al. (2012) disclosed the Fe volume in the muscles of *M. cephalus* of roughly 4.0 $\mu \cdot g^{-1}$ d.w, as observed in Rio de Janeiro State, Brazil. Recently, Mwakalapa et al. (2019) also presented their results (0.448 $\mu \cdot g^{-1}$ d.w), which showed lower than in this work. Chen (2002) revealed that the Fe content in the liver of *M. cephalus* was significantly higher than in our study. In Southwest, Taiwan, Chen (2002) reported the amount of Fe of 110.5 $\mu \cdot g^{-1}$ d.w, while in the coasts of Tanzania, Mwakalapa et al. (2019) declared a greatly lower value of approximately 0.065 $\mu \cdot g^{-1}$ d.w.

Compared with the species in this study, the Fe accumulation in the organs of *S. sihama* was comparatively low (1.85, 3.925 and 2.628 $\mu \cdot g^{-1}$ d.w in the muscles, liver, and gills, respectively). Along with that, Gammal et al. (2016) showed that the Fe content was much lower than the results in this publication (0.008, 0.45 and 0.019 $\mu \cdot g^{-1}$ d.w in the muscles, liver, and gills, respectively). Similarly, Mirmohammadvali & Solgi (2018) also declared a low Fe level in the muscles of *S. sihama* in the Arabian Gulf, Saudi Arabia. What is more, Chen (2002) showed high levels of Fe accumulating in the liver, but rather low ones in the muscles (24.75 and 0.338 $\mu \cdot g^{-1}$ d.w in the liver and muscles, respectively). At the same time, Chen (2002) declared analogous results for the Fe concentrations in the liver and muscles of *G. filamentosus* (52.25 and 0.425 $\mu \cdot g^{-1}$ d.w, respectively).

5. Zn concentration in fish

Undeniably, Zn is an element essential to all living organisms and it has an important role as a co-factor of several enzymes (Vallee & Auld, 1990; Silva et al., 2018). However, the high concentration of zinc exerts detrimental effects on the growth and pigment contents (Chakraborty & Mishra, 2020). The FAO/WHO (2011) has also established a safe threshold for the Zn concentration in the fish muscles (40 $mg \cdot kg^{-1}$ w.w). In this study, the average value of Zn in the whole study area followed the descending order: liver > gills > muscles (Except in *A. latus* and *U. sulphureus* with the order of: gills > liver > muscles). The highest mean was found in the liver of *K. punctatus* at Quang Binh (2.437 $\mu \cdot g^{-1}$ d.w), while the lowest mean was found in the muscles of *S. fuscescens* at Ha Tinh (0.283 $\mu \cdot g^{-1}$ d.w). This also shows that all the values of the studied species were below the FAO/WHO (2011) stipulated limit. The mean concentration of Zn in the muscles for the whole study area was in the following order: *K. punctatus* > *A. latus* > *U. sulphureus* > *G. filamentosus* > *M. cephalus* > *S. sihama* > *S. fuscescens*. The order in the liver was as follows: *G. filamentosus* > *K. punctatus* > *S. sihama*

> *U. sulphureus* > *M. cephalus* > *A. latus* > *S. fuscescens*. The range in the gill followed the order: *U. sulphureus* > *G. filamentosus* > *A. latus* > *K. punctatus* > *S. sihama* > *M. cephalus* > *S. fuscescens*.

One research at the Bushehr seaport (coastal of the Persian Gulf), Iran on *A. latus* by Hosseinkhezri & Tashkhourian (2011) revealed that the concentration of Zn in the liver was higher than in the gills and muscles, which in the liver equalled 1.700 $\mu \cdot g^{-1}$ d.w, in the gills – 0.975 $\mu \cdot g^{-1}$ d.w, and in the muscles – 0.575 $\mu \cdot g^{-1}$ d.w. Saei-Dehkordi & Fallah (2011) confirmed the Zn traces in the muscles of *A. latus* at Bandar Abbas, Iran (2.57 $\mu \cdot g^{-1}$ d.w), which was higher than the levels noted by Hosseinkhezri & Tashkhourian (2011). Surprisingly, before that Agusa et al. (2007) discovered a high level of Zn in the liver and muscles in Southeast Asia, which were above the FAO/WHO recommended limit (196 and 33.6 $\mu \cdot g^{-1}$ d.w in the liver and muscles, respectively). Recently, Beg et al. (2015) further recorded the Zn contents in the liver and gills reaching 37.075 and 3.11 $\mu \cdot g^{-1}$ d.w in Kuwait Bay. In the present study, however, the mean of Zn in *A. latus* in the muscles, liver, and gills equalled 0.823, 0.887 and 1.633 $\mu \cdot g^{-1}$ d.w, respectively.

Many researchers confirmed the levels of Zn in *K. punctatus*, *U. sulphureus* and *S. fuscescens*. In order to understand the accumulation pattern of heavy metals in wild fish from the Southern Sea of Korea, Hwang et al. (2017) analyzed and declared the Zn content in the muscles of *K. punctatus* reaching 6.46 $\mu \cdot g^{-1}$ d.w. In the Chinese coastal waters, Zhang & Wang (2012) noted the Zn volume in muscles of *U. sulphureus* and *S. fuscescens* reaching 0.148, 0.273 $\mu \cdot g^{-1}$ d.w, respectively. Besides, Liu et al. (2015) also confirmed that the Zn concentrations in China were not too high in *S. fuscescens* (30.23, 3.325 and 3.375 $\mu \cdot g^{-1}$ d.w in the muscles, liver, and gills, respectively).

For *M. cephalus*, most of the research focused on trace Zn in the muscles (Chen, 2002; Yilmaz, 2005; Nair et al., 2006; Medeiros et al., 2012; Kamal et al., 2013; Hwang et al., 2017; Ouali et al., 2018; Mwakalapa et al., 2019). Ouali et al. (2018) have revealed that the content of Zn in the muscles from the East Algerian coast was quite high, much higher than the FAO/WHO (2011) standards. Except for the report of Nair et al. (2006), who studied the Cochin backwaters, India (42.7 $\mu \cdot g^{-1}$ d.w) and Diop et al. (2016), who researched the Senegalese coast (24.4 $\mu \cdot g^{-1}$ d.w), the remaining publications showed that the Zn content in muscles of *M. cephalus* was at an average level. As for the results of Mwakalapa et al. (2019), it was roughly low (0.22 $\mu \cdot g^{-1}$ d.w), lower than in this study. In addition, Nair et al. (2006) showed a high accumulation of Zn in the liver and gills (111.450 and 65.000 $\mu \cdot g^{-1}$ d.w in the liver and gills, respectively).

Similarly to previous studies, we also showed that the accumulation of Zn in the liver of *S. sihama* was higher than in other tissues. Chen (2002) reported 14,125 $\mu \cdot g^{-1}$ d.w in the liver, while in the muscles it was only 1.425 $\mu \cdot g^{-1}$ d.w. Mohammadnabizadeh et al. (2012), however, confirmed the Zn concentration in the liver of 6.385 $\mu \cdot g^{-1}$ d.w, in the gills of 4.213 $\mu \cdot g^{-1}$ d.w, and in muscles of approximately 1.830 $\mu \cdot g^{-1}$ d.w. In the Arabian Gulf, Saudi Arabia, Gammal et al. (2016) revealed that the cumulative Zn content in the liver was 27.096 $\mu \cdot g^{-1}$ d.w, in gills it equalled 19.772 $\mu \cdot g^{-1}$ d.w, while in the muscles – 10.723 $\mu \cdot g^{-1}$ d.w. Most notably, Nair et al. (2006) disclosed very high traces of Zn in the muscles, up to 167.17 $\mu \cdot g^{-1}$ d.w, while in the liver and gills only 48.62 and 40.62 $\mu \cdot g^{-1}$ d.w, respectively. Dehghani (2017) also presented high Zn content in the muscles of *S. sihama* in the Persian Gulf, Iran (67.5 $\mu \cdot g^{-1}$ d.w). Jayaprakash et al. (2015) and Mirmohammadvali & Solgi (2018), however, did not show high accumulation of Zn in the muscles (3.81 and 2.067 $\mu \cdot g^{-1}$ d.w, respectively).

Studies on *G. filamentosus* have all shown higher Zn deposition in the liver (Chen, 2002; Agusa et al., 2007) than in the muscle. However, in their report in the Cochin backwaters, India, Nair et al. (2006) once again revealed very high values of Zn in the muscles, gills, and liver.

6. Cu concentration in fish

In spite of the fact that Cu is an essential element for living organisms (Festa & Thiele, 2011; Lata Bansal & Asthana, 2018), it is also a very potent toxicant due to the production of reactive oxygen species when the cellular Cu levels are elevated (Solomon & Lowery, 1993; Harris & Gitlin, 1996). Thus, the FAO/WHO (2011) had established the allowable limits of Cu in the muscles of fish (40 $mg \cdot kg^{-1}$ w.w). In this study, we found that the Cu content tends to be highly concentrated in the liver, gills and muscles. The highest Cu values were obtained in Quang Tri in the liver of *U. sulphureus* (2.417 $\mu \cdot g^{-1}$ d.w), while the lowest Cu average concentrations were recorded in Quang Tri in the muscles of *S. fuscescens* (0.107 $\mu \cdot g^{-1}$ d.w). All the Cu values in the species in this study were below the FAO/WHO (2011) recommended threshold. The concentration of Cu in the muscles of fish species from central Vietnam decreased in the following order: *U. sulphureus* > *K. punctatus* > *M. cephalus* > *G. filamentosus* > *A. latus* > *S. fuscescens* > *S. sihama*. In the liver: *U. sulphureus* > *G. filamentosus* > *K. punctatus* > *A. latus* > *S. sihama* > *M. cephalus* > *S. fuscescens*. In the gills it followed the order: *U. sulphureus* > *A. latus* > *K. punctatus* > *M. cephalus* > *G. filamentosus* > *S. sihama* > *S. fuscescens*.

Previous studies on *A. latus* showed the accumulation of high Cu concentration in the liver. Beg et al. (2015) found the highest concentration of Cu in the liver (28.05 $\mu \cdot g^{-1}$ d.w), although the concentration was low in the muscles (1.248 $\mu \cdot g^{-1}$ d.w), the study was conducted in Kuwait Bay. Similarly, in Southeast Asia, Agusa et al. (2007) reported that Cu in the liver of *A. latus* reached 12.7 $\mu \cdot g^{-1}$ d.w, while in the muscles it was recorded only at 1.31 $\mu \cdot g^{-1}$ d.w. Saei-Dehkordi & Fallah (2011) reported lower Cu content in the muscles (0.621 $\mu \cdot g^{-1}$ d.w), which was similar to Hosseinkhezri & Tashkhourian (2011) (0.234, 0.575 and 0.475 $\mu \cdot g^{-1}$ d.w in the muscles, liver, and gills, respectively) and observed in the Persian Gulf, Iran.

Relatively low concentrations of Cu were also confirmed for the species of *K. punctatus*, *U. sulphureus*, and *S. fuscescens*. Accordingly, Mok et al. (2009) presented the level of Cu in *K. punctatus* (1,006 $\mu \cdot g^{-1}$ d.w), which was higher than the Cu value determined in this study (0.809 $\mu \cdot g^{-1}$ d.w). However, in the Southern Sea of Korea, Hwang et al. (2017) noted that the level of Cu in the muscle of *K. punctatus* reached 0.66 $\mu \cdot g^{-1}$ d.w. Another aspect, Zhang et al. (2012) revealed was that the trace of Cu in the muscles of *U. sulphureus* and *S. fuscescens* was quite low (0.002 $\mu \cdot g^{-1}$ d.w in both species). These results were significantly lower than the data in this study (0.912 and 0.184 $\mu \cdot g^{-1}$ d.w, respectively). In South China Sea, Liu et al. (2015) also reported that the Cu data in *S. fuscescens* was relatively low (0.07, 0.568 and 0.225 $\mu \cdot g^{-1}$ d.w in the muscle, liver and gills, respectively), similarly to the results in this report from central Vietnam. Also, Bramandito et al. (2018) found that in Indonesia the level of Cu in the muscles of *S. fuscescens* equalled 5.22 $\mu \cdot g^{-1}$ d.w.

There have not been found any references related to the Cu concentration in the gills of *M. cephalus* yet. Mostly, studies have focused on traces of Cu in the muscles and liver rather than in gills. Earlier, Chen (2002) and Mwakalapa et al. (2019) reported Cu traces in the liver of *M. cephalus*, which were higher than in this report. It is worth noting that, the report of Diop et al. (2006) and Ouali et al. (2018), which have shown a fairly high Cu trace are present in the muscle (45 and 8.1 $\mu \cdot g^{-1}$ d.w, respectively). Meanwhile, other reports showed that Cu tends to accumulate low in the muscle (Yilmaz, 2005; Kamal et al., 2013).

An earlier finding showed that the Cu values were very high in the muscles of *S. sihama*, as reported by Hung et al. (1999) in the Southern Coast of Taiwan (550 $\mu \cdot g^{-1}$ d.w). According to the author, the reason behind it might be the rainy season that dominates the distributions of trace metals. They were collected in February, the dry season in Taiwan, when the metal absorption in the environment is higher than in the rainy season, leading to the high accumulation in the fish organs. This aspect occurred in several studies establishing that mean heavy metal concentrations in fish were higher in the dry season (Fufeyin, 1998; Idodo-Umeh, 2000; Oguzie, 2003; Obasohan & Eguavoen, 2008). Besides, Dehghani et al. (2017) also noted relatively high concentrations of Cu in the muscle (60 $\mu \cdot g^{-1}$ d.w) in the Persian Gulf, Iran. Other studies show that the accumulation of Cu in the *S. sihama* muscles is low, below the recommended FAO/WHO threshold (2011) (Jayaprakash et al., 2015; Gammal et al., 2016). Other reports also confirmed that the accumulation of Cu in the liver was higher than in the gills and muscles, Chen (2002) determined that the Cu levels in the gills and muscles were 0.425 and 0.033 $\mu \cdot g^{-1}$ d.w, respectively, while Mohammadnabizadeh et al. (2012) reported the Cu levels in the liver, gills, and muscles of 2.385, 1.513 and 0.29 $\mu \cdot g^{-1}$ d.w, respectively. George et al. (2012) reported sequentially that Cu in the liver, gills, and muscles reached 8.375, 4.970 and 1.220 $\mu \cdot g^{-1}$ d.w, respectively, in Cochin Backwaters, India.

Similarly to this work, other reports also showed a higher Cu accumulation in the liver than in the muscles of *G. filamentosus*. In Southeast Asia, Agusa et al. (2007) reported that the Cu levels presented in the liver and muscles were 9.01 and 1.52, respectively. Also, Chen (2000) reported Cu levels of 0.72 and 0.038 in the gills and muscles, respectively. On the other hand, Anandkumara et al. (2018) and Gashkina et al. (2020) showed that Cu tended to accumulate high in the liver.

7. Human health risk

In recent years, the confirmation of heavy metal contents in fish has been used to appraise the potential risk for human health. The health risk evaluations are carried out based on the assumption of the most chemicals with non-cancerous influence (Yadav et al., 2019). An important aspect to assess consumption health risk is the knowledge of the dietary intake of potentially harmful chemicals in food (Storelli, 2008). For Hg, Cd, Pb, Fe, Zn, and Cu, the WHO and FAO have established the Provisional Tolerable Daily Intake (PTDI) as safe intake levels: 0.714, 1.00, 3.571, 800, 300-1000, and 500 $\mu g \cdot kg^{-1}$ b.w per day (FAO/WHO, 1982; FAO/WHO, 1983; Feeley et al., 2011; Mueller et al., 2011; Benford et al., 2011). In our research, all heavy metal daily intakes through the consumption of fish (Table 44) were lower than the daily intake threshold limits, indicating that there is no potential health hazard for consumption from this location. The highest EDI values of the 7 fish species are detected for Fe, while the lowest value is EDI for Cd. The EDI values for Fe, Cu and Zn are significantly higher than the EDI values for Pb, Hg, and Cd, which agree with many previous statements. Raknuzzaman et al. (2016) reported the daily intake in commercial fish at the coastal area of Bangladesh in the order of Zn > Cu > As > Cr > Pb > Cd. Mok et al. (2015), declared the daily intake in kinds of seafood from the Southern Coast of Korea as follows Zn > Cu > As >

Cd > Pb > Hg. Besides that, Mol et al. (2017) reported the EDI in three fish species from the Southwest Black Sea also as follows Zn > Cu > Pb > Cd > Hg. Stoyanova et al. (2015) found the EDI values of Zn > Ni > Pb > Cd in fish muscles from the coasts of Bulgaria to be below their corresponding allowable tolerable daily intake for human consumption. However, it should be noted that it was not a long-term measurement process to conclude “acceptable limit” and “unacceptable limit”, based on doses lower than the recommended daily intake/RfD (Vu et al., 2017; Baki et al., 2018).

We also calculated the THQ with Reference dose set by USEPA (2019) for Hg, Cd, Pb, Fe, Zn, and Cu (Table 44). THQ results indicate that there were no THQ values for all individual metals above 1 through the consumption of these seven fish species, which means the exposed residents are unlikely to experience obvious adverse influences (Saha & Zaman, 2013; Ezemonye et al., 2019). In general, the THQ values of Hg account for the largest proportion of total THQ of metals in most fish species, along with the previous research opinion of Ruelas-Inzunza et al. (2020).

A similar scenario is turned up with the Hazard Index (HI), the HI value of this study was lower than 1, suggesting minimal exposure risk of no significant health consequence. A similar scenario occurred with the Hazard Index (HI), the HI value of this study was less than 1 suggesting minimal exposure risk of no significant health consequence. Previous studies performed by several authors in similar conditions were in line with our work (Saha et al., 2016; Dadar et al., 2017; Zafarzadeh et al., 2017; Khemis et al., 2017; Ahmed et al., 2019; Maurya et al., 2019). Besides, there are also many reports showing that the HI value is higher than 1 in some different fish species (Mok et al., 2015; Zhang et al., 2019; Ezemonye et al., 2019; Solgi & Beigzadeh-Shahraki, 2019). Nevertheless, humans are prone to suffer from simultaneous deleterious effects of combined pollutants (Li et al., 2013). Therefore, this is an indirect way of assessing potential hazards, and should not be considered a direct measurement of risk concern for the health of fish consumers.

VI. CONCLUSIONS

The determination of essential (Cu, Fe, and Zn) and non-essential (Cd, Hg, and Pb) metal concentrations in the organs of common fish species in central Vietnam is a necessity due to their utility as an essential food for the local residents. The results showed that:

There is no significant difference in the content of some metals between the study areas (e.g: Pb, Zn and Cu in *A. latus*; Pb, Fe, and Cu in *K. punctatus*; Zn in *M. cephalus*; Cd and Zn in *S. fuscescens*; Hg, Cd, Pb and Fe in *S. sihama*; Cd and Cu in *U. sulphureus*; Cu, Fe, Zn, Cd, Hg, and Pb in *G. filamentosus*). It does not fully confirm the hypothesis put forward. The habitat of the species was located near the coast and estuaries. The five locations are the five largest estuaries of the five study areas, where a lot of pollutants from the runoff of rivers were found.

The accumulation of heavy metals in the 7 species studied had a significant difference. *K. punctatus*, *U. sulphureus*, *A. latus*, and *G. filamentosus* tend to accumulate pollutants in their tissues higher than *M. cephalus*, *S. sihama*. Especially *S. fuscescen* shows a relatively low accumulation of heavy metals in their tissues. This reinforces the initial hypothesis that metals tend to concentrate more in the liver, rather than in the gills and the muscles.

The Cd contents in the muscles of *A. latus*, *S. fuscescens*, and *S. sihama*, and the levels of Pb in the muscles of *A. latus*, *K. punctatus*, and *U. sulphuresus* exceeded the WHO established threshold. Nevertheless, the estimated daily intakes of these metals were also below the PTDI guidelines. Furthermore, the estimated THQ and HI for all the heavy metals of these fish were also lower than 1. Hence, there is no long-term significant health risk from the consumption at the present condition but it must be noted that enormous intake of these products could also establish long-term health threats.

SUMMARY

Vietnam is located on the eastern part of the Indochinese Peninsula and borders with Cambodia, China, Laos, as well as the Gulf of Thailand, the Gulf of Tonkin and the South China Sea. There are dense river systems and a long coastline of over 3260 km in length, which is very convenient to develop fishing and aquaculture activities. However, in recent years, the demographic growth, the high rate of urbanization, industrialization, and modernization negatively affected environmental quality. Thus, the pollution of Vietnamese aquatic and terrestrial ecosystems with toxic heavy metals is an urgent problem. Despite that, there is still a lack of research related to the metals levels assessment in animals, which constitutes a part of the human diet.

This study aimed to determine the concentration of Cd, Cu, Fe, Hg, Pb, and Zn in gills, liver, and muscles of common fish species from Central Vietnam (*Acanthopagrus latus*, *Gerres filamentosus*, *Konosirus punctatus*, *Mugil cephalus*, *Siganus fuscescens, Sillago sihama,* and *Upeneus sulphureus*) to assess the potential risk to consumer health. The concentrations of Cd, Cu, Fe, Pb, and Zn were determined with a flame atomic absorption spectrometer (AAnalyst 200, PerkinElmer, USA), while the Hg concentrations were measured with a cold vapor atomic absorption spectrometer (MA-2, NIC, Japan).

The highest Hg, Cd, Pb, Fe, Zn, and Cu values were detected in the liver of *S. sihama* (2.840 $\mu \cdot g^{-1}$d.w), *U. sulphureus* (0.038 $\mu \cdot g^{-1}$d.w), *G. filamentosus* (0.538 $\mu \cdot g^{-1}$d.w), *A. latus* (20.451 $\mu \cdot g^{-1}$d.w), *G. filamentosus* (2.087 $\mu \cdot g^{-1}$d.w), and *U. sulphureus* (2.085 $\mu \cdot g^{-1}$d.w), respectively. The lowest Cd, Fe, and Pb levels were found in the muscles of *G. filamentosus* (0.010 $\mu \cdot g^{-1}$d.w), *S. sihama* (1.85$\mu \cdot g^{-1}$d.w) and *M. cephalus* (0.022 $\mu \cdot g^{-1}$d.w). However, the lowest levels of Cu, Hg, and Zn were determined in the muscles (0.184 and 0.325 $\mu \cdot g^{-1}$d.w for Cu and Zn) and gills of *S. fuscescens* (0.136 $\mu \cdot g^{-1}$d.w for Hg). *K. punctatus, U. sulphureus, A. latus*, and *G. filamentosus* tend to accumulate higher metals levels than *M. cephalus, S. sihama*, and *S. fuscescen*s. The results also showed that the muscles Cd levels of *A. latus*, *S. fuscescens*, and *S. sihama*, and muscles Pb levels of *A. latus*, *K. punctatus*, and *U. sulphuresus* have exceeded the WHO established thresholds. THQ and HI values of each species tested were lower than 1, implying negligible non-carcinogenic risk via fish consumption in the study area.

KEYWORDS: Central Vietnam, fish, heavy metals, HI, potential risk, THQ

STRESZCZENIE

Wietnam leży nad Morzem Południowochińskim i jego zatokami: Tajlandzką i Tonkińską. Dobrze rozwinięte sieci rzeczne oraz linia brzegowa o długości 3260 km umożliwiły rozwój akwakultury oraz rybołówstwa. Wyż demograficzny, rozwój urbanizacji i uprzemysłowienia, negatywnie wpływają na jakość środowiska naturalnego. Pomimo tego ilość i zakres badań nad zanieczyszczeniem środowiska, między innymi metalami ciężkimi są niewystarczające.

Celem badań była ocena zawartości Cd, Cu, Fe, Hg, Pb i Zn w mięśniach, skrzelach i wątrobie siedmiu gatunków ryb (*Acanthopagrus latus, Gerres filamentosus, Konosirus punctatus, Mugil cephalus, Siganus fuscescens, Sillago sihama* i *Upeneus sulphureus*) występujących w trzech miejscach na obszarze centralnego Wietnamu (Nghe An, Ha Tinh, Quang Binh, Quang Tri i Hue) oraz oszacowanie potencjalnego ryzyka dla zdrowia ludzi, dla których ryby te są istotnym składnikiem diety.

Zawartości rtęci oznaczano za pomocą spektrometrii absorpcji atomowej z generowaniem zimnych par (MA-2, NIC, Japonia), zaś pozostałych metali za pomocą spektrometrii absorpcji atomowej z atomizacją w płomieniu (AAnalyst 200, PerkinElmer, USA).

Wątroba *S. sihama* kumulowała najwyższe ilości rtęci (2,840 $\mu\cdot g^{-1}$s.m.), *U. sulphureus* kadmu (0,038 $\mu\cdot g^{-1}$s.m.), *G. filamentosus* ołowiu (0,538 $\mu\cdot g^{-1}$s.m.), *A. latus* żelaza (20,451 $\mu\cdot g^{-1}$s.m.), *G. filamentosus* cynku (2,087 $\mu\cdot g^{-1}$s.m.), a miedzi *U. sulphureus* (2,085 $\mu\cdot g^{-1}$s.m.). Najniższe stężenia Cd (0,010 $\mu\cdot g^{-1}$s.m.), Fe (1,85 $\mu\cdot g^{-1}$s.m.) i Pb (0,022 $\mu\cdot g^{-1}$s.m.) odnotowano w mięśniach, odpowiednio u *G. filamentosus, S. sihama* i *M. cephalus.* Najniższe zawartości Cu (0,184 $\mu\cdot g^{-1}$s.m.) i Zn (0,325 $\mu\cdot g^{-1}$s.m.) obserwowano w mięśniach *S. fuscescens*, a rtęci (0,136 $\mu\cdot g^{-1}$s.m.) w skrzelach tego samego gatunku. U *K. punctatus, U. sulphureus, A. latus* i *G. filamentosus* stwierdzono wyższe zawartości metali ciężkich w badanych narządach niż w narządach pozostałych gatunków. Zawartości kadmu w mięśniach *A. latus, S. fuscescens* i *S. sihama* oraz ołowiu w mięśniach *A. latus, K. punctatus* i *U. sulphuresus* przekraczały wartości graniczne ustanowione przez Światową Organizację Zdrowia. Wartości całkowitego ilorazu zagrożenia (ang. total hazard quotient, THQ) oraz indeksu zagrożenia (ang. hazard index, HI) wyliczone dla każdego z badanych gatunków wynosiły mniej niż 1, wskazując na nieznaczne ryzyko nierakotwórcze poprzez spożywanie ryb z badanego obszaru.

SŁOWA KLUCZOWE: metale ciężkie, potencjalne ryzyko, ryby, THQ, HI

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ANNEX

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