Interactions between environmental copper, microbial community structure and histamine levels in edible crustaceans

Water pollution, particularly metal and microbial, is a global problem. When the pollution occurs in aquacultural areas, it may negatively impact the edible species and eventually threaten food quality and safety. Indonesia, one of the top ten aquaculture producers in the world, also experiences severe water pollution. Aquaculture is economically important throughout Indonesia, like for Semarang – a major city on the Northern coast of Central Java – known as one of aquaculture producers. One of the most important aquaculture activities in Semarang is shrimp culture. Many ponds used for shrimp and fish culture are situated along the coast line and are influenced by metal pollution from a variety of sources and microbial pollution from city drainage. For that reason, we studied the influence of metal pollution and microbial contamination in an edible crustacean species. The main aim was to determine the interaction between copper and microorganisms in the aquatic environment and their impact on crustaceans. To deal with the problem, several approaches were selected, i.e. metal bioaccumulation, histamine formation in the animal triggered by metals exposure, bacterial community structure under the influence of metal contamination and histamine accumulation in the crustacean’s edible tissue during storage.

The metal of interest in this study was copper, referring to the situation found in shrimp ponds along the Semarang coast line (Chapter 2). In three sampling sites, copper concentrations were significantly higher than those of the other sampling sites, up to twice the local background concentration,
which is approximately 40 µg/g dry sediment. Cadmium concentrations in sediment samples taken from different aquacultural ponds in the Semarang coastal area were below the detection limit (0.03 µg/g dry weight), while zinc, nickel and iron concentrations varied among locations but were generally also low. Sediments taken from two shrimp ponds located close to the intercity highway of Semarang had lead (Pb) concentrations that were significantly higher than at the other locations. In addition to copper and lead, at some sites nickel and chromium also showed elevated sediment concentrations. According to the Sediment Quality Guidelines (SQGs) applied in several countries, the Semarang coastal pond sediments can be classified as mid-range (medium range) contaminated. The contamination may partly be attributed to anthropogenic inputs, such as intensification of agricultural and aquacultural practices, and metal-contaminated waste from industrial and household activities.

The interaction between copper, microbial community structure and histamine level was studied by performing a set of experiments. Because of its accessibility for biological experiments, i.e. ease of culturing and producing high numbers of genetically identical offspring, the freshwater marbled crayfish (*Procambarus* sp.) was chosen as a model of edible crustacean species (Chapters 3, 4 and 5). Our study on copper toxicokinetics in marbled crayfish (*Procambarus* sp.) showed there was no clear uptake in animals exposed to 0.031 mg Cu/L, suggesting that the animals at low copper exposure levels are able to regulate copper concentrations in their body to a fairly constant level. However, at higher exposure levels (0.38 mg Cu/L) the internal copper concentration was not regulated at the same level, at least not in all organs. The exoskeleton, gills and muscle tissues accumulated copper relatively fast and reached equilibrium within 10 days of
exposure. Copper accumulation was highest in the hepatopancreas as uptake in this storage organ steadily increased with time and did not reach equilibrium within the 14-day exposure period (Chapter 3). The copper accumulation levels in the marbled crayfish found in this study were hepatopancreas > gills > exoskeleton > muscle. In terms of food safety, the highest copper concentrations measured in the marbled crayfish muscles (meat) were 40 µg/g dry weight (~10 µg/g wet weight). This level does not exceed the recommendations set by the Australian National Health and Medical Research Council (ANHMRC) for seafood, which is 10 µg/g wet weight.

In Chapter 4, we observed that metal accumulation in marbled crayfish organs affected the concentrations of histamine, an important indicator of food spoilage used in food safety research. The higher the copper exposure concentrations, the higher the histamine levels were in the hepatopancreas. A rapid built-up of histamine in the hepatopancreas started right from the beginning of the copper exposure of the crayfish. Copper exposure to average concentrations of 0.031 and 0.38 mg Cu/L did not affect histamine concentrations in the crayfish muscle. In contrast, histamine concentrations in the hepatopancreas of crayfish exposed to 0.38 mg Cu/L was significantly higher than in crayfish exposed to 0.031 mg Cu/L and reached approximately 10 mg histamine/kg fresh weight. Histamine is a well-known neuromodulator (besides dopamine) in the animal nervous system. Histamine is specifically associated with modulation of muscle action in intestinal tissues. Why histamine levels in the crayfish would increase with copper accumulation is unknown. This is an interesting phenomenon but so far has not been reported in the literature. Two proposed mechanisms are (1) a stress response of the animal, or (2) up-regulation of histidine production followed
by decarboxylation reactions. Histamine concentrations in the muscle in all cases never exceeded 2 mg histamine/kg fresh weight, which is much lower than the maximum level of histamine in seafood of 50 mg histamine/kg fresh weight set by the United States Food and Drug Administration (US FDA). Our experiment on histamine formation in the marbled crayfish triggered by copper exposure therefore indicated that in terms of the meat, copper exposure itself did not pose any threat for seafood safety.

In Chapter 5, the bacterial community structures under the influence of copper contamination and their effect on the histamine accumulation in the marbled crayfish meat during storage are described. Cluster analysis of 16S rRNA gene-based microbial community fingerprints revealed copper toxicity to the freshwater bacterial community. Further, we observed a relation between bacteria in the water and bacteria playing role in deteriorative process in crayfish meat during storage. Histamine concentrations in the meat of the marbled crayfish exposed to 0.5 mg Cu/L upon storage were significantly lower and did not increase as rapidly compared to those in the control marbled crayfish. After 10 days of storage, meat from crayfish exposed to 0.5 mg Cu/L contained approximately 7.5 mg histamine/kg fresh weight, significantly less than the meat of animals incubated in copper-free water, which approximated 22 mg/kg fresh weight. We suggest that copper exposure can slow down histamine accumulation in crayfish meat during storage through affecting the composition of bacterial communities and the associated histamine production.

According to our findings on the copper accumulation in the marbled crayfish and by extrapolating of these results to other crustaceans (Chapter 3), it is recommended to consume only muscle tissue (meat) rather than the
whole body of crustaceans. Consuming small size edible crustaceans, where it is hard to avoid eating the gills and hepatopancreas, which usually contain the highest metal concentrations, indeed may pose a hazard for food safety. Histamine levels induced by copper exposure, however, generally are much lower than the threshold level for food safety.

Furthermore, copper concentrations in the water up to 0.5 mg Cu/L have been shown to have a large impact on the microbial community structure in both the culturing water and in the stored marbled crayfish meat. Copper exposure can decrease histamine levels in crayfish meat during storage through affecting the bacterial community-associated histamine production (Chapter 5). In spite of these findings and recommendations, still several uncertainties remain. Future research therefore might focus e.g. on (1) the mechanism(s) of histamine formation triggered by copper exposure, (2) copper uptake and internal distribution in different species of crustaceans, especially species living in brackish water, (3) determination of sources of bacterial contamination – from the aquatic environment or from seafood handling – that play a major role in histamine accumulation, (4) impact of other chemicals of concern for the coastal areas of Java, such as Semarang on microbial communities in crustaceans, especially on the histamine-producing microorganisms.