# Metabolomics applied to ecotoxicology: a field study analyzing the blood plasma of tilapia (Oreochromis niloticus)

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The Maceió Stream, in Fortaleza-CE, has been impacted by urban growth, receiving a high load of domestic effluents without adequate wastewater treatment, affecting the quality and ecological balance of this aquatic resource. Despite the evident pollution, the local survival of more resistant and adapted organisms, such as tilapia, has been observed, which is captured and consumed by a part of the poorest local population. Metabolomics applied to environmental studies has emerged as a promising area for understanding how stressors affect the metabolic profile of organisms. However, the application of this technique in ecotoxicological studies is still very scarce in Brazil. This study is the first to analyze the metabolites in fish from the Maceió Stream and aims to compare the metabolic profile with that of tilapia (Oreochromis niloticus) obtained from the fish farm from the Department of Fisheries Engineering of the Federal University of Ceará. The fish were anesthetized with MS-222 for blood sampling and after the sample was centrifuged to obtain the plasma. Two extraction protocols were applied: one using pure methanol and another with a separation phase system (MeOH, H?O and chloroform in a 3:1:2 ratio) to optimize metabolite detection. The samples were analyzed by HPLC-HRMS (QTOF) and subjected to statistical analysis. The results showed that 431 features were detected in total, in which 19 metabolites were differentially expressed between the sites. In fish from the Maceió stream, 10 metabolites showed higher intensity compared to farmed fish. Among them, three lipids were identified, metabolites associated with plant processing and pharmaceutical production were also detected, and another related to high microbial activity in the aquatic environment, indicating compound degradation. Guanosine, a biomarker of cellular damage, was also identified, suggesting stress by chemical contamination. For farmed fish, 9 metabolites with more intense signals were found, many of which are not typical of farmed fish. Among these, those associated with research and pharmacological compounds, stood out. These metabolites may be related to possible contamination from laboratories within the University, where the fish farm is located. In addition, metabolites related to the fish's diet, such and stress conditions, such as sudden changes in temperature, poor water quality or overcrowding, which can influence metabolite levels, were identified. Therefore, it was concluded that the significant differences in the metabolic composition between the two groups are related to both exposure to different contaminants, as well as by the niche of the organisms in their environment. Therefore, the metabolomics technique used proved to be an effective tool for assessing the impacts on fish health in environmental studies.

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