

RAMAN SCATTERING-BASED INVESTIGATION OF PESTICIDE-INDUCED ALTERATIONS IN TILAPIA LIVER TISSUE BIOMARKERS.

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The intensive use of herbicides in agricultural areas has raised increasing concern regarding their persistence in aquatic environments and their potential toxic effects on non-target organisms, particularly fish species that are widely used as bioindicators of environmental contamination. In this context, the present study evaluated the morphological and biochemical effects of the herbicide imazapic (IMZ) on the hepatic tissue of *Oreochromis niloticus* (tilapia), aiming to investigate sublethal alterations induced by acute and prolonged exposure scenarios. Tilapias were exposed to IMZ under controlled laboratory conditions, considering two exposure periods: acute exposure (24 h) and prolonged exposure (96 h), in addition to a non-exposed control group. Liver tissues were collected and analyzed using conventional histological techniques combined with Raman spectroscopy and multivariate statistical analysis. This integrated approach allowed the investigation of both structural and molecular-level alterations associated with herbicide exposure. Histological analysis revealed that IMZ exposure induced a progressive disorganization of hepatic

architecture when compared to the control group. The main morphological alterations included cytoplasmic vacuolization of hepatocytes, increased collagen deposition, and activation of inflammatory responses, evidenced by mast cell degranulation. Vacuolization was more evident in exposed groups and suggests disturbances in cellular metabolism, possibly associated with lipid accumulation or glycogen depletion. The increased deposition of collagen fibers indicates the early onset of tissue remodeling and fibrotic processes, while the inflammatory response reflects the activation of defense mechanisms typically associated with hepatic stress. These alterations were observed even after 24 h of exposure and became more pronounced after 96 h, indicating a time-dependent progression of sublethal hepatic damage. Complementary to histological findings, Raman spectroscopy proved to be a sensitive and non-destructive technique for detecting biochemical changes in liver tissue induced by IMZ exposure. Spectral analysis revealed significant modifications in bands associated with lipids, proteins, glycogen, and nucleic acids. Alterations in lipid-related bands suggest disruptions in membrane integrity and lipid metabolism, while changes in protein-associated bands indicate possible conformational modifications and structural damage. Variations in glycogen and nucleic acid signals point to impairments in energy metabolism and cellular homeostasis. These molecular-level changes are consistent with oxidative stress processes and lipid peroxidation, which are commonly reported mechanisms of herbicide-induced toxicity. To further evaluate the systematic nature of these biochemical alterations, Principal Component Analysis (PCA) was applied to the Raman spectral data. PCA results corroborated the spectroscopic findings, promoting a discrimination among the control, IMZ 24 h, and IMZ 96 h groups. The formation of distinct clusters demonstrates that the biochemical effects induced by IMZ are not random but directly related to exposure time, reinforcing the sensitivity of Raman spectroscopy combined with multivariate analysis for identifying sublethal toxic effects. Overall, the results obtained in this study confirm the hepatotoxic potential of IMZ in *Oreochromis niloticus*, even under acute exposure conditions, and highlight the cumulative nature of its effects with prolonged exposure. The integration of histological analysis, Raman spectroscopy, and PCA constitutes a complementary approach for ecotoxicological assessment, enabling the detection of early morphological and biochemical biomarkers of toxicity. This methodology provides insights for environmental monitoring programs and contributes to a better understanding of the impacts associated with the intensive use of herbicides in aquatic

ecosystems, particularly in regions characterized by intensive agricultural activity.

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Palavras-chave: raman scattering; biomarkers; pesticides.