This paper presents a critical analysis about the water quality implications considering the complexity of the pollution sources matrix in a Brazilian critical basin characterized by a strong highly density with very low rate of wastewater treatment. The case study was the Iguaçu River at the Upper Iguaçu Basin, covering an area of 2,800 km², with 7 monitoring points in 107 km of river length, and serves as an important water resource for the southeastern area of Parana State, Brazil. Currently, about 3 million people reside within the basin, and an additional number intake drinking water from the river. During the last several decades, the combination of rapid population growth coupled with industrial and urban development, mainly with irregular occupation of flood plains, has resulted in a serious deterioration of water quality. Major pollution sources include domestic sewage, industrial wastewater, and urban and agricultural runoff. As a consequence of this process, problems have been encountered in the water supply, sanitary sewage treatment and urban drainage systems, which did not keep up with the growth of cities, negatively affecting the environment and people’s quality of life. This study proposes to use fluorescence and visible ultraviolet spectroscopy, to better assess the origin and sources of organic matter. Additionally, a complementary monitoring plan is proposed to improve the assessment of the origin and dynamics of organic matter for the river system analyzed, based on conventional water quality parameters, biochemical oxygen demand, chemical oxygen demand, dissolved oxygen, total organic carbon, nitrogen, phosphorus and solids.