

Despite the growing awareness in freshwater conservation, and the sociocultural significance of the Osun River in the designation of the Osun-Osogbo Sacred Grove as a UNESCO World Heritage Site, there is dearth of information on the biological water quality and conservation value of the river. In this study, the conservation value and biological water quality of the river which defines a UNESCO protected environment are assessed. Benthic macroinvertebrates and physicochemical water condition were studied quarterly from June 2015 to March 2016. The river's naturalness was also measured based on habitat alteration, land use and hydrological modifications. A total of 27 macroinvertebrate taxa were recorded, and the fauna was dominated by the Ephemeroptera, Odonata and Trichoptera (EOT) group. Taxa richness and/or abundance of macroinvertebrates showed a significant inverse response to vegetation removal, farming, total suspended solids, flow velocity, nitrate and phosphate. There is a notable occurrence of *Margaritifera margaritifera* which is considered a flagship and umbrella species in the river. This study further reveals the natural property of the sacred grove in addition to its cultural property as a WHS; hence, it may be re-considered as a WHS based on mixed properties