The Guadalupe River begins its journey as a typical spring-fed river, flowing over the Edwards Plateau in Kerr County. It then moves over rocky land in Kendall and Comal counties as it approaches the Balcones Escarpment. Before reaching the escarpment, the river fills Canyon Reservoir, built in the 1960s. This large reservoir (over 8,000 acres) was built to control flooding downstream and to provide a water supply for people and businesses in the basin.

After leaving Canyon Reservoir, the river begins a steep downhill slope over the Balcones Escarpment. In a 25-mile stretch, the river drops more than 150 feet in elevation. Shortly after the escarpment, the Edwards Aquifer spring-fed Comal River joins up with the Guadalupe River.

The Guadalupe River flows south through the Blackland Prairie region, in Guadalupe and Gonzales counties. Two rivers to the north in Hays and Caldwell counties follow the same route. The Blanco River begins in the Hill Country, and joins up with the spring-fed San Marcos River immediately after the escarpment. These rivers join together in the Blackland Prairie.

The Post Oak Savannah is dominant throughout Gonzales County. This is where the San Marcos River flows into the Guadalupe River. Because of a gradual decrease in the slope of the land and increase in the amount of water in the river, the Guadalupe becomes a much slower, lazier river throughout this region.

The Guadalupe River begins its descent into the Gulf Coastal Prairies and Marshes as it flows through DeWitt County and into Victoria County. In this region it is still a slow-moving river, but it increases in size because of tributaries, including the San Antonio River. As the river approaches the San Antonio Bay in Refugio and Calhoun counties, it travels through a coastal estuary.