Temporal and spatial changes in concentrations of selected radionuclides (tritium, radiostrontium and radiocaesium) were assessed in the parts of the Vltava and Elbe river basins affected by the operation of the Temelin Nuclear Power Plant. Concentrations of radionuclides were evaluated in surface water, sediments, fish and aquatic flora both affected and unaffected by waste water discharges from the Temelin Nuclear Power Plant before and during the operation of the plant. The assessment included residual contamination from atmospheric tests of nuclear weapons in the last century and the Chernobyl accident in 1986. Results of long-term monitoring (1990–2014) were used for derivation of effective ecological half-lives in surface water, sediments, fish and aquatic flora. Possible impact of waste waters discharged from the Temelin plant on tritium, radiocaesium and radiostrontium concentrations in the Vltava River was assessed by using data observed over the period of 2001–2014.