The excellent biocompatibility and capability to improve the electron transfer make porous silicon extremely attractive for applying in biosensing applications. In order to engineer its performance this work describes the use of Horseradish Peroxidase (HRP) attached electrostatic onto the surface of polyaniline (PANI) deposited electrochemically onto the electrode surface. PANI has the capacity to bind biomolecules, tuning their biocatalytic properties, rapid transferring of electrons and direct production of analytical signals. Different parameters were investigated including pH solution, PANI concentration, enzyme concentration and numbers of cyclic voltammetry. As-synthesized and deposited, the film was characterized by FTIR spectroscopies. XRD and TEM/FEG were employed to study structure and morphology of modified cladding. The biosensing principle in this work was based on the characterization of hydroquinone responses before and after incubation in glyphosate standard solutions. Glyphosate inhibited the activity of HRP causing a decrease in its response. In order to study the performance of the proposed electrode, sensing parameters, such as sensing response, selectivity and stability were determined and evaluated. The results obtained for glyphosate in water sample using the proposed method are in close agreement with those using a high performance liquid chromatography procedure at the 95% confidence level. Results illustrate that HRP/PANI biosensor retain catalytic activity and was of considerable interest due to its simple procedure in practical application and a promise methodology for the lack of monitoring data on these contaminants.