Supplemental Material (Figures) for the paper:

Linear and non-linear modelling of the cytotoxicity of TiO_2 and ZnO nanoparticles by empirical descriptors

by Ester Papa*, Jean Pierre Doucet, and Annick Doucet-Panaye

Figure S1: PCA on the available descriptors (PC1 E.v.=40%, PC2 E.v.=37%) performed for the complete dataset (42 NPs). Large ZnO NPs (IDs 34 – 42) are clearly isolated in the lower part of the graph (i.e. PC2 values <-1).

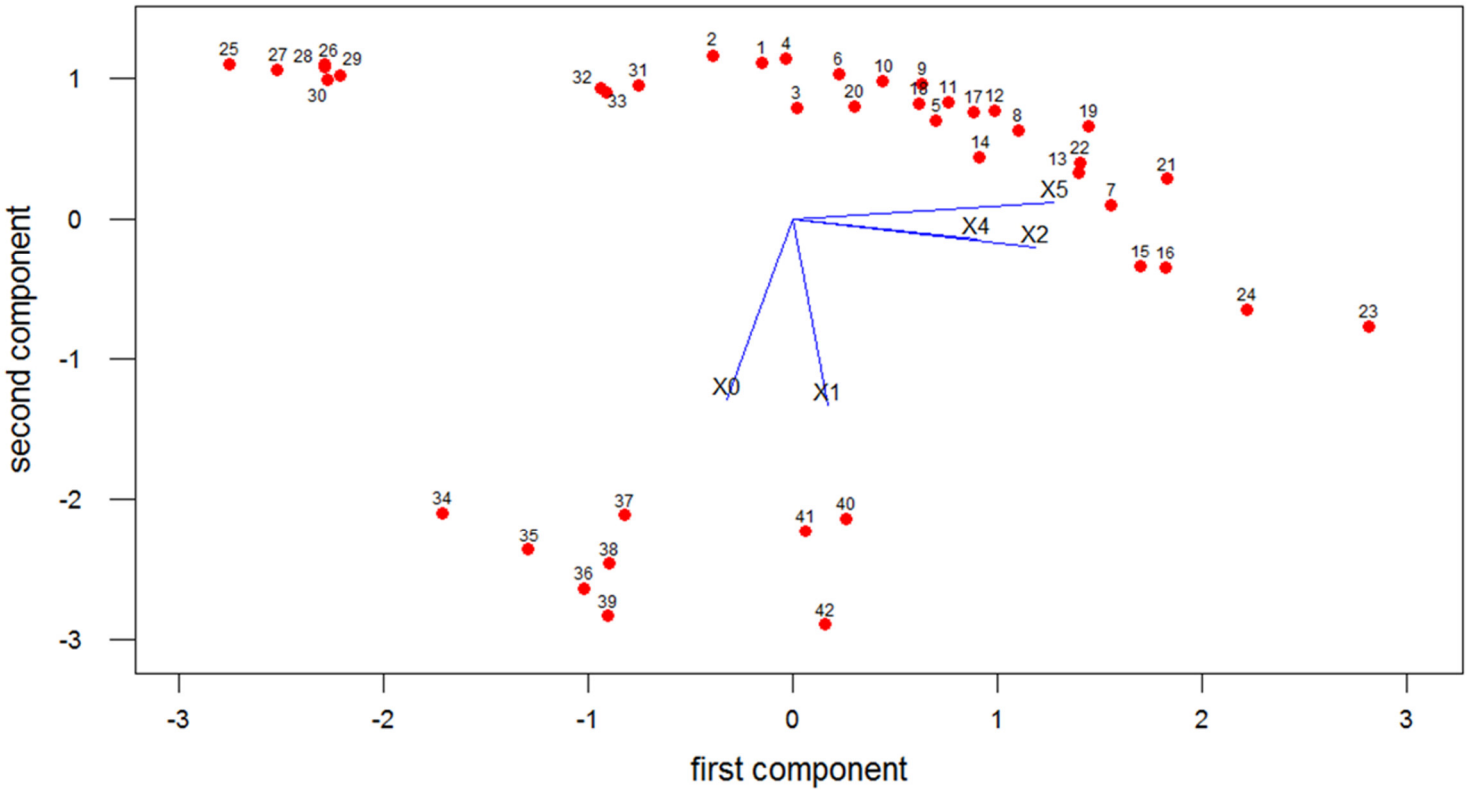
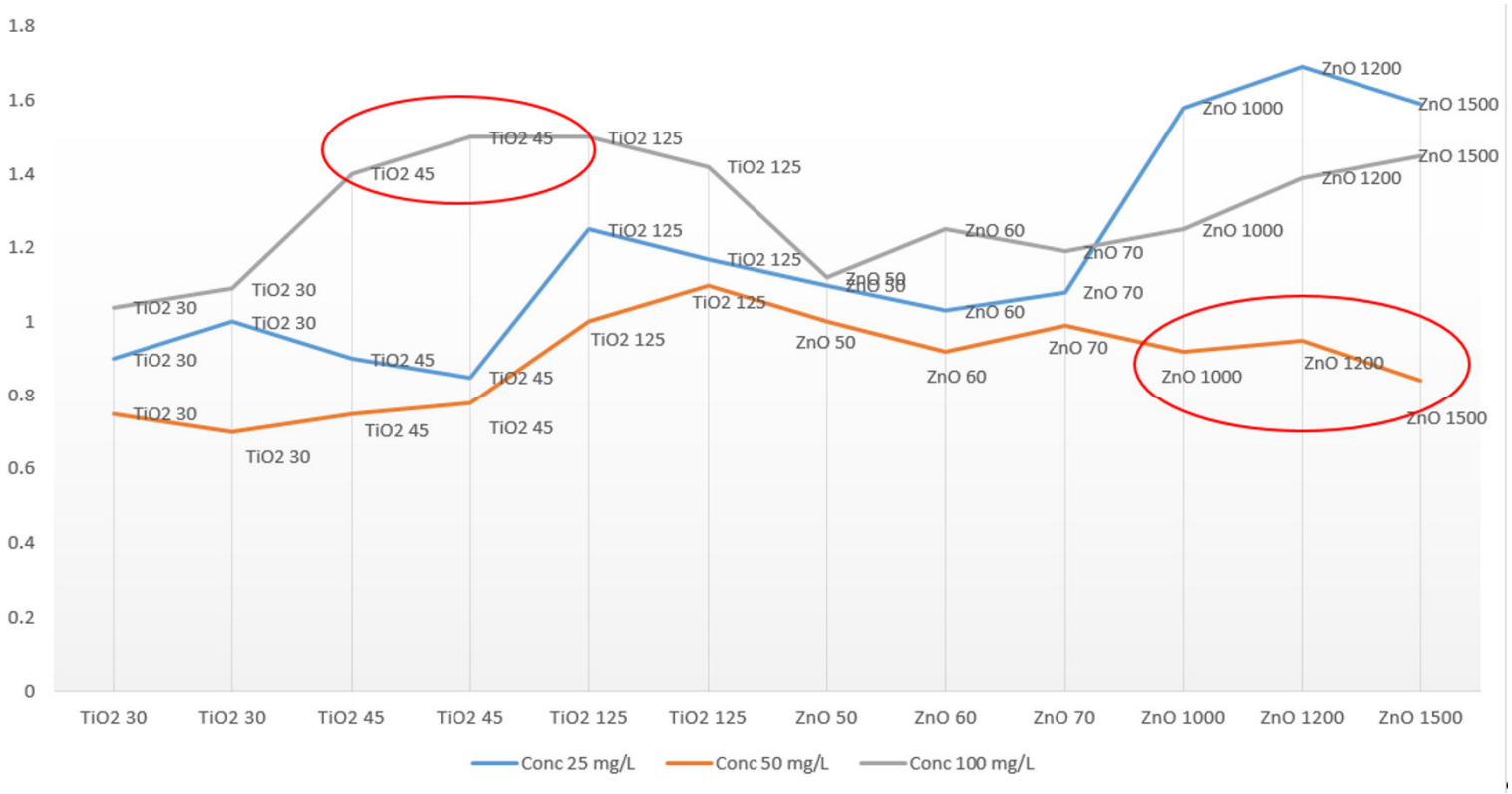


Figure S2. Comparison of experimental values measured at different concentrations. Red circles are anomalies in the general trend (i.e. ID 13 and 14 measured at 100mg/L and IDs 37, 38, 39 measured at 50 mg/L).



Influence of Agglomeration Rate on TiO_2 and ZnO cytotoxic activity

The agglomeration rate (AgR) calculated as the ratio size in water / engineered size, was studied in order to gain additional insight from the available data. The values of correlation among AgR and the response of activity LDH were calculated for ZnO, TiO_2+ZnO and TiO_2 datasets with values of -10%, 16% and 31% respectively. Additionally, we verified that AgR did not influence the results of the regression by including this new variable in the pool of available descriptors. No models with better performances than those included in the manuscript were identified by including AgR in the variable selection procedure.

We performed a simple analysis of the Structure-Activity Relationship (SAR) between AgR and LDH by using the software Excel (correlation function and scatter plots). In general, we observed that less aggregated and larger NPs within each concentration group, had the largest LDH values (Figure S3A-B)

Figure S3A

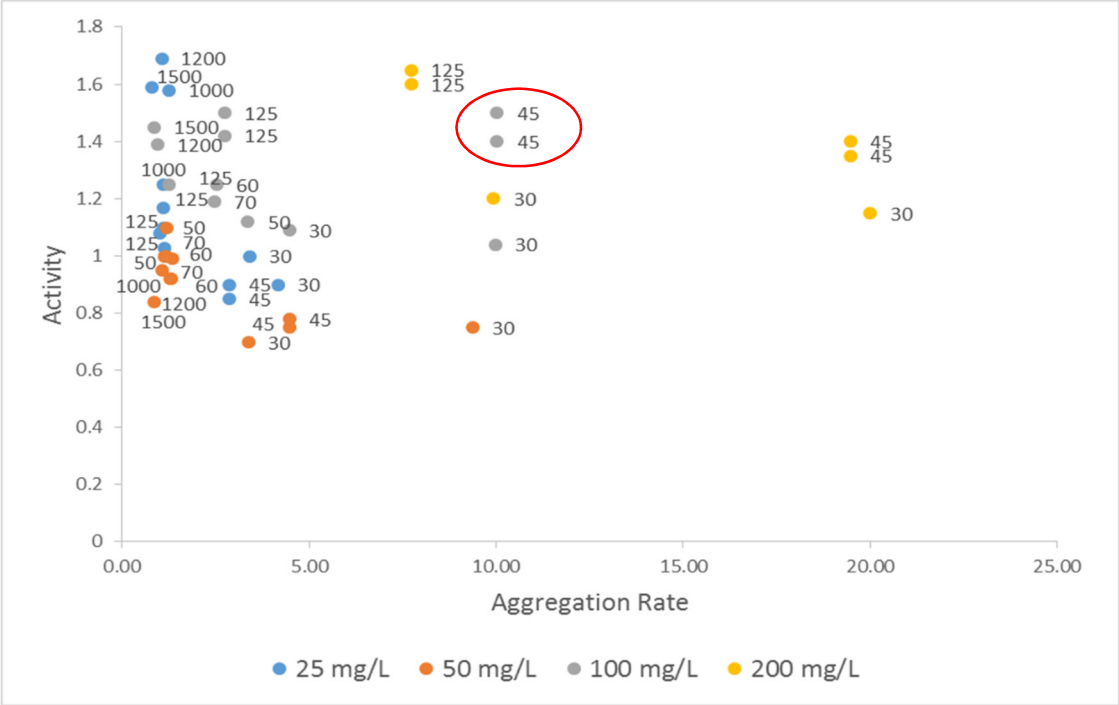
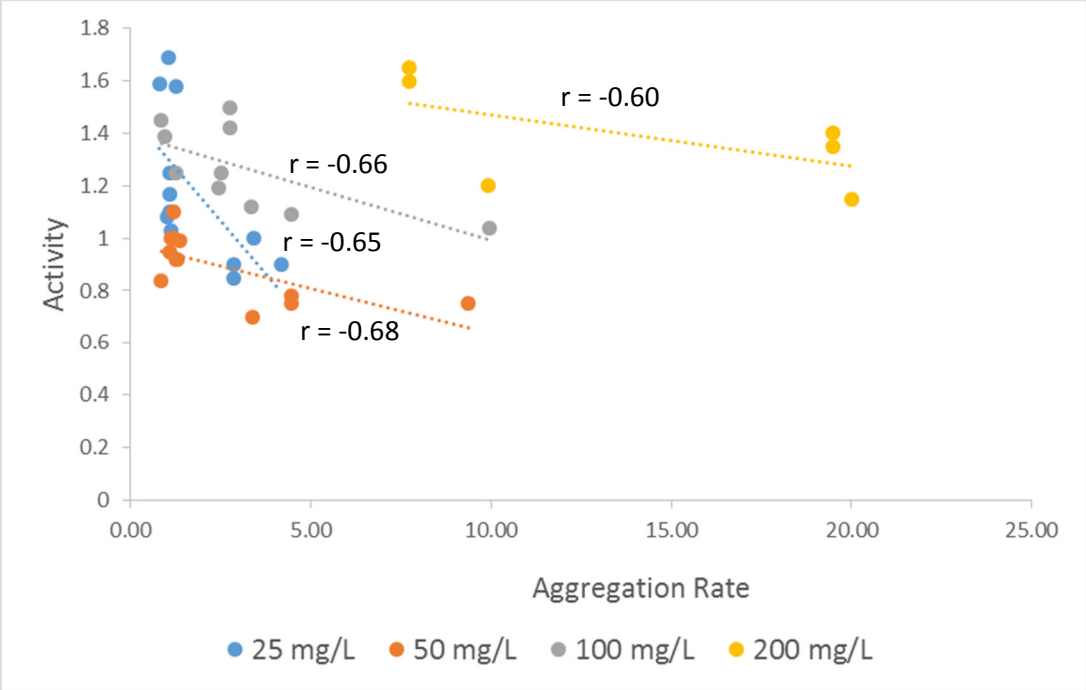


Figure S3B



AgR was inversely related to the increase of LDH cytotoxicity (Figure S3A-B). In particular, correlations between AgR and LDH, evaluated separately for the different concentrations were always > -0.6, with the exception of samples measured at 100mg/L for which we calculated correlation of – 0.07. However, after exclusion of NPs 13 and 14 (NPs in the red circle in Figure S3A) the correlation raised to -0.66 (correlation coefficients reported in figure S3B). This suggests an anomaly in the aggregation state of NPs 13 and 14, which are highlighted as outliers. No additional experimental details were available in the original literature (Sayes and Ivanov, 2010) on factors, which may be helpful to explain these anomalies in aggregation rates (such as pH). TiO_2 NPs tested at 200 mg/L were the most aggregated and with cytotoxicity values always >1.1 (leaky membranes). The aggregation-toxicity trend observed before was found also within this group of NPs, i.e the less aggregated NPs were the most toxic.

The analysis of the Structure-Activity Relationship among Agglomeration Rate (AgR) and the response of toxicity LDH, suggested that AgR plays a clear role in defining the ability of the studied NPs to disrupt cellular membranes. In particular less aggregated and larger NPs within each concentration group, have the largest LDH values (Figure S3B).

Figure S4. Plot of experimental vs predicted values and Williams plot (AD) generated for Eq. 2.

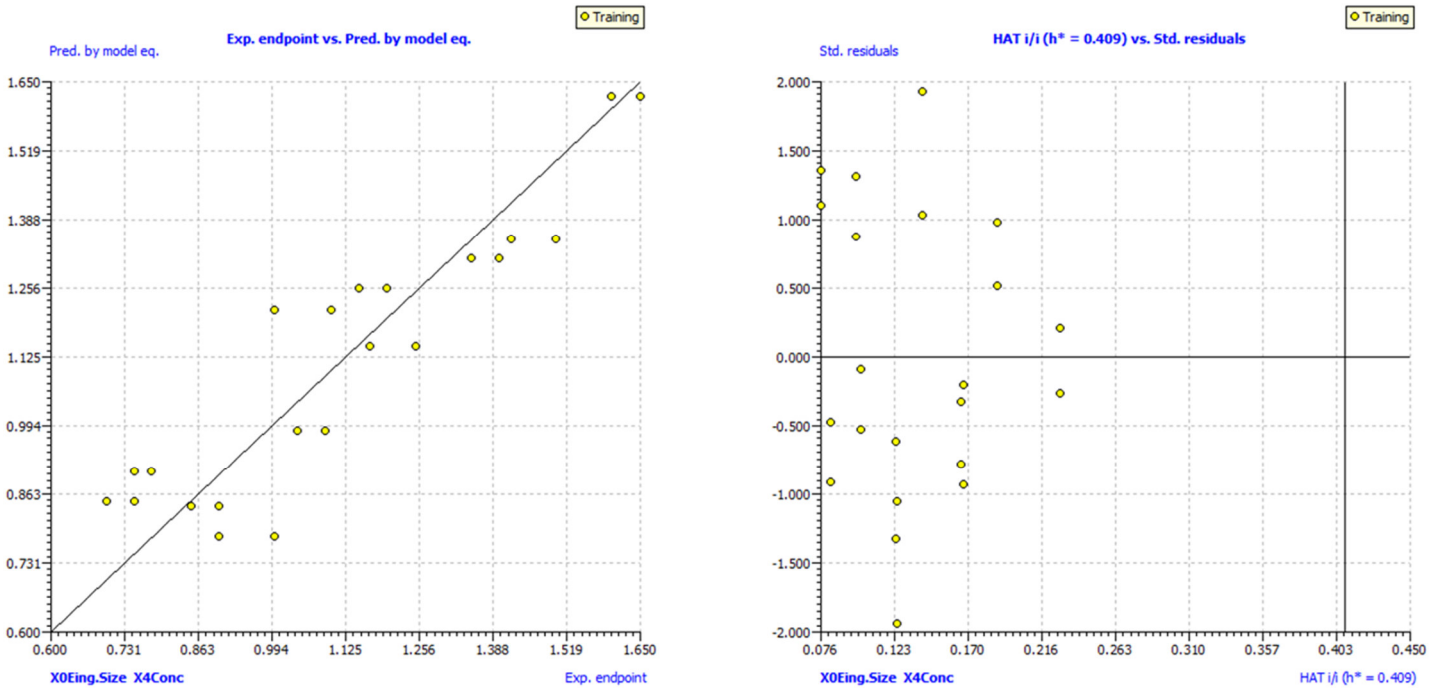


Figure S5. Plot of experimental vs predicted values and Williams plot (AD) generated for Eq. 3.

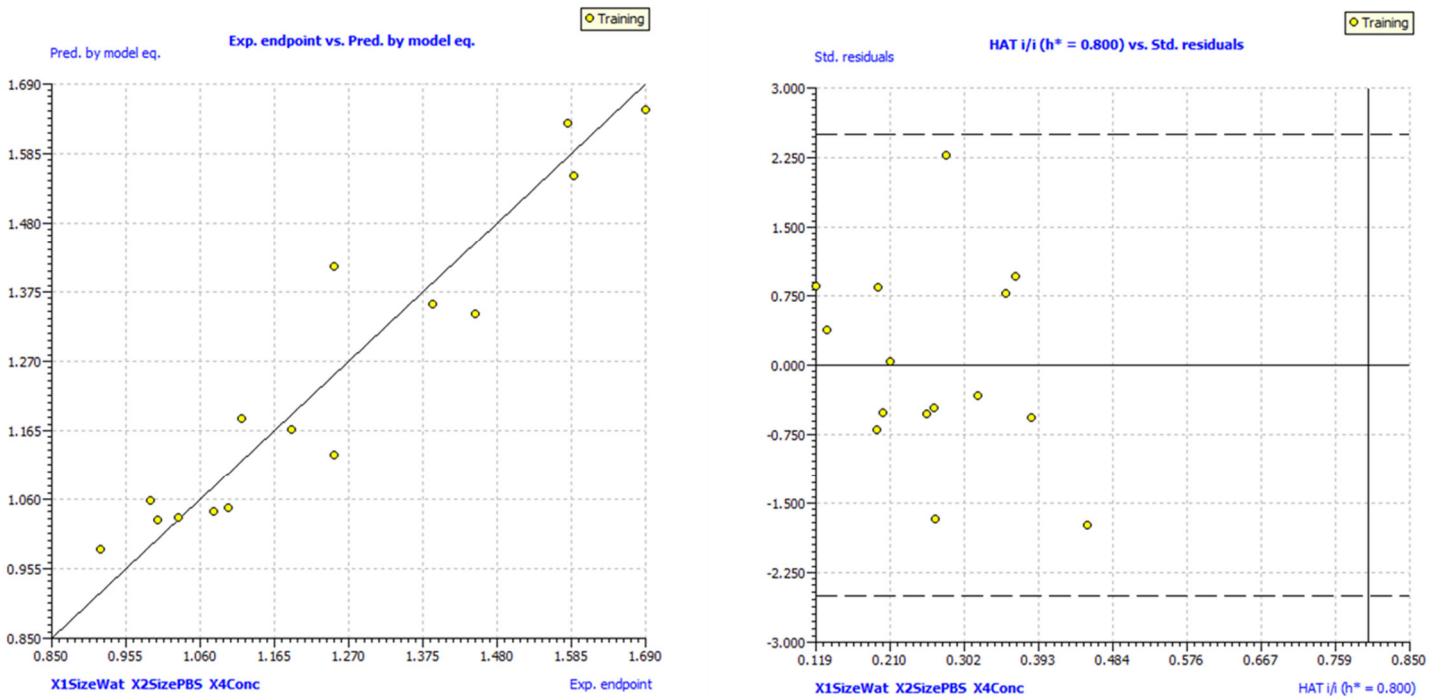


Figure S6 A-D. Comparison of performances of MLR and non-linear models developed for the complete dataset.

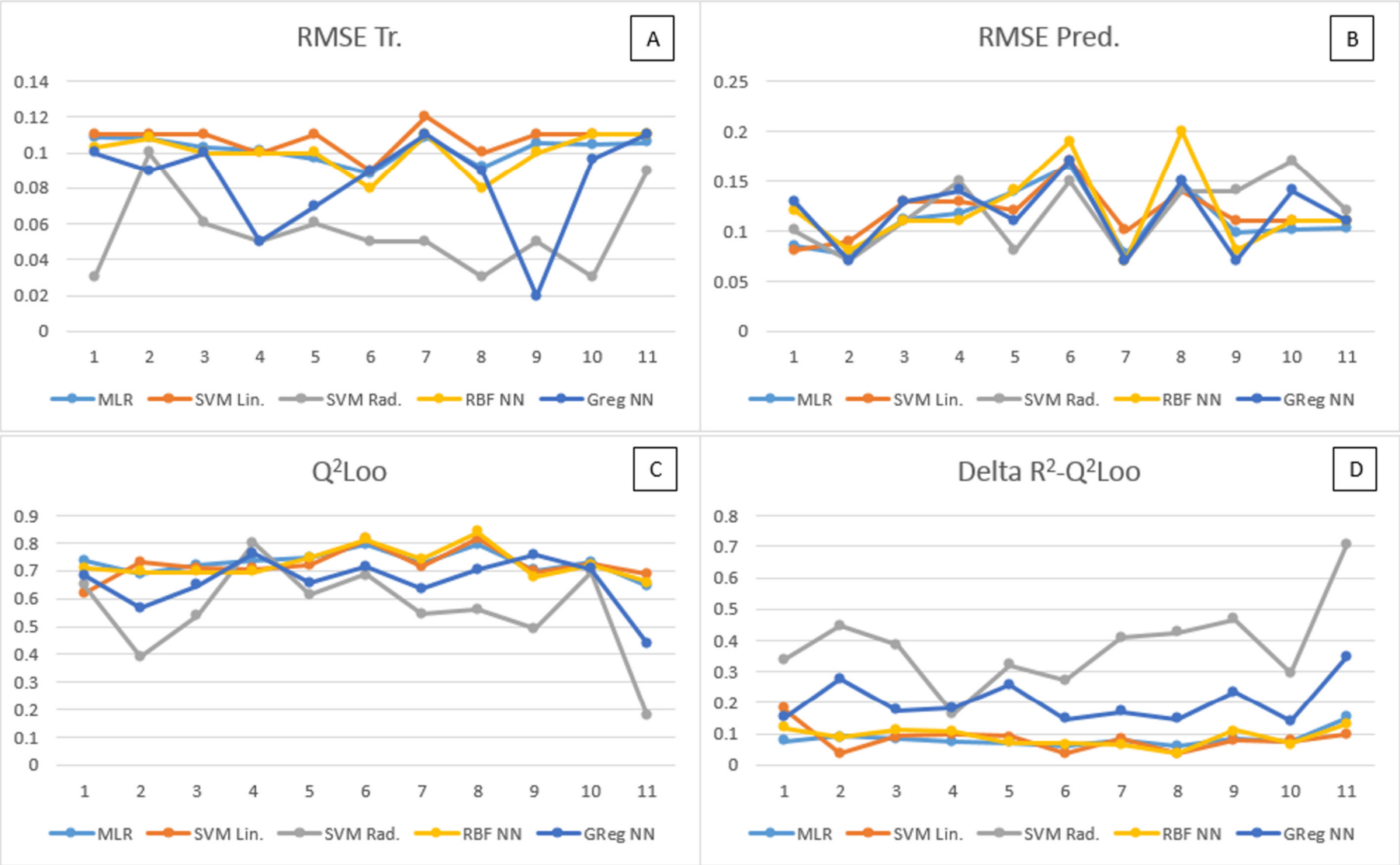


Figure S7 A-D. Comparison of performances of MLR and non-linear models developed for the TiO_2 dataset.

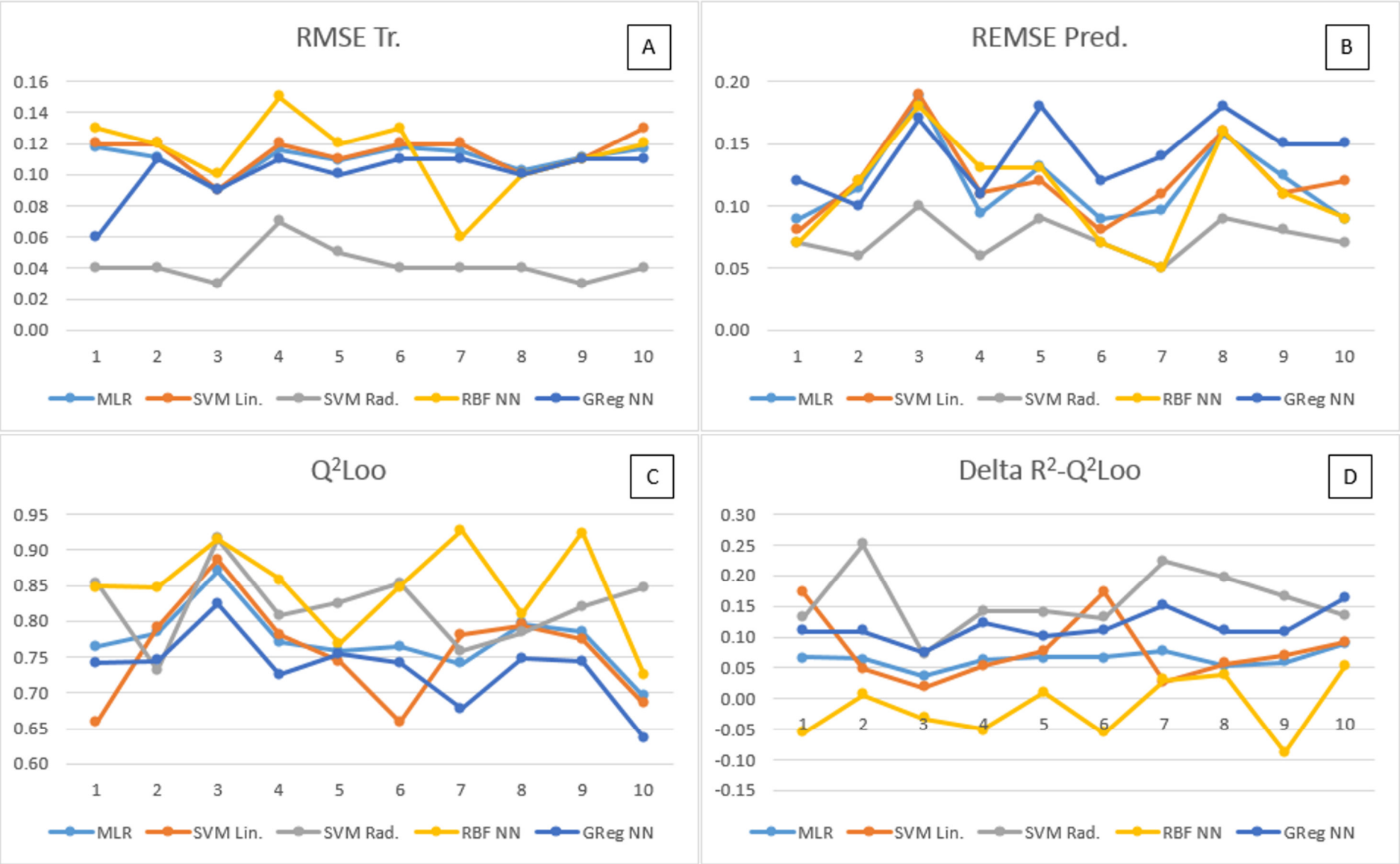


Figure S8 A-D. Comparison of performances of MLR and non-linear models developed for the ZnO dataset.

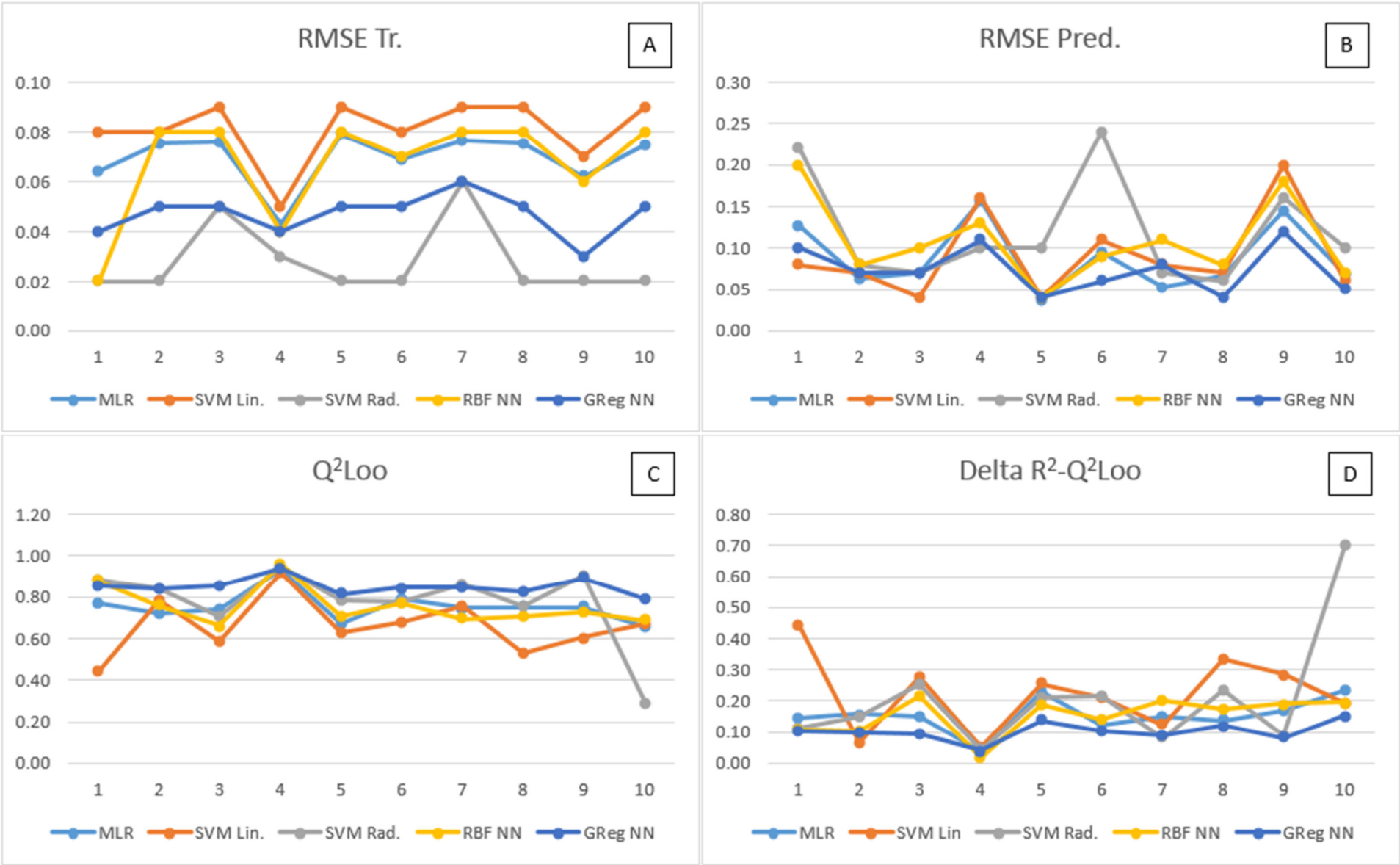


Figure S9. PCA on model descriptors (Equation 1) (PC1 E.V.=54%, PC2 E.V.=33%), performed for the complete dataset (after exclusion of outliers).

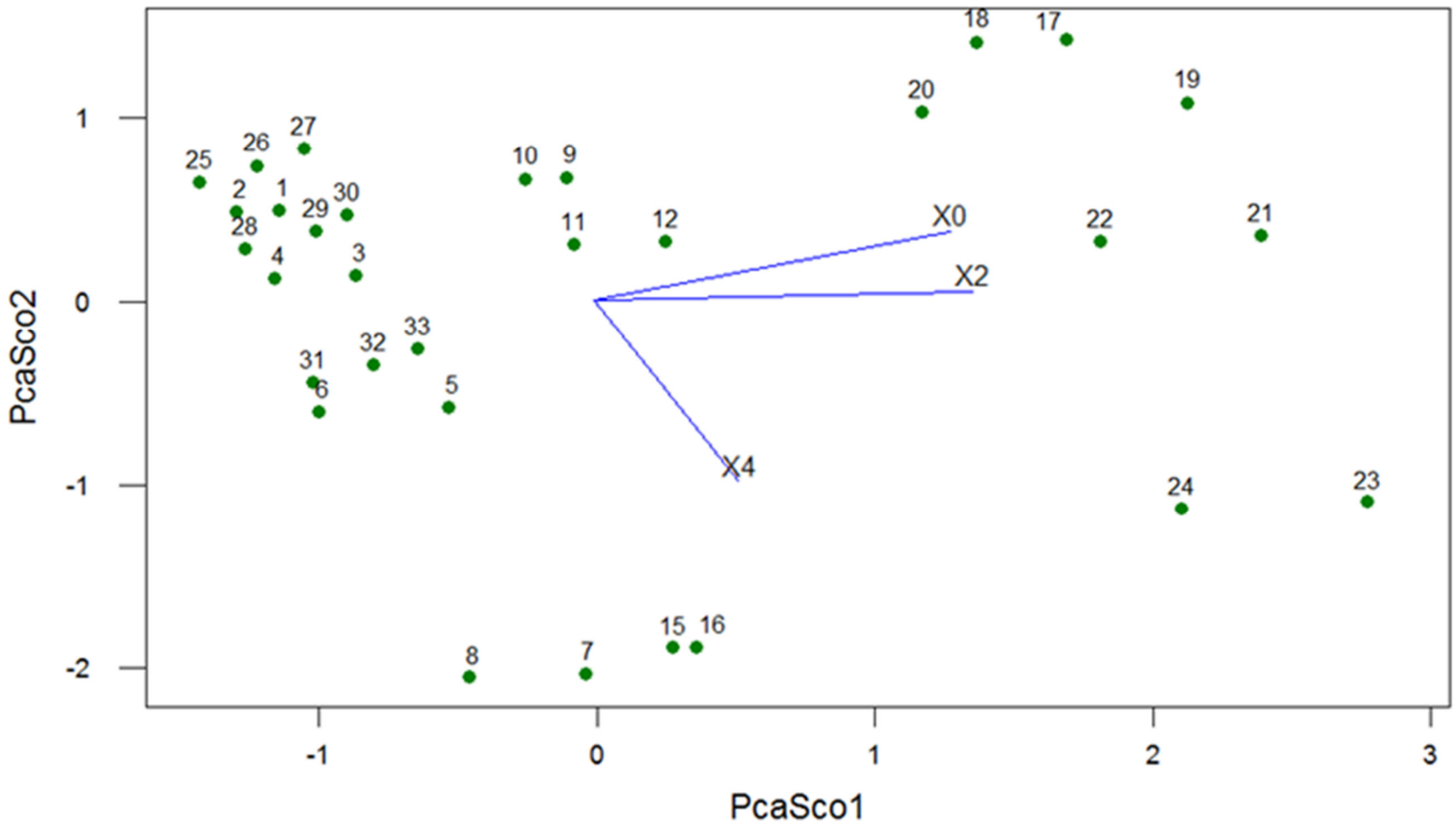


Figure S10. PCA on model descriptors (Equation 2) (PC1 E.v.=50%, PC2 E.v.=50%), performed for the TiO_2 dataset.

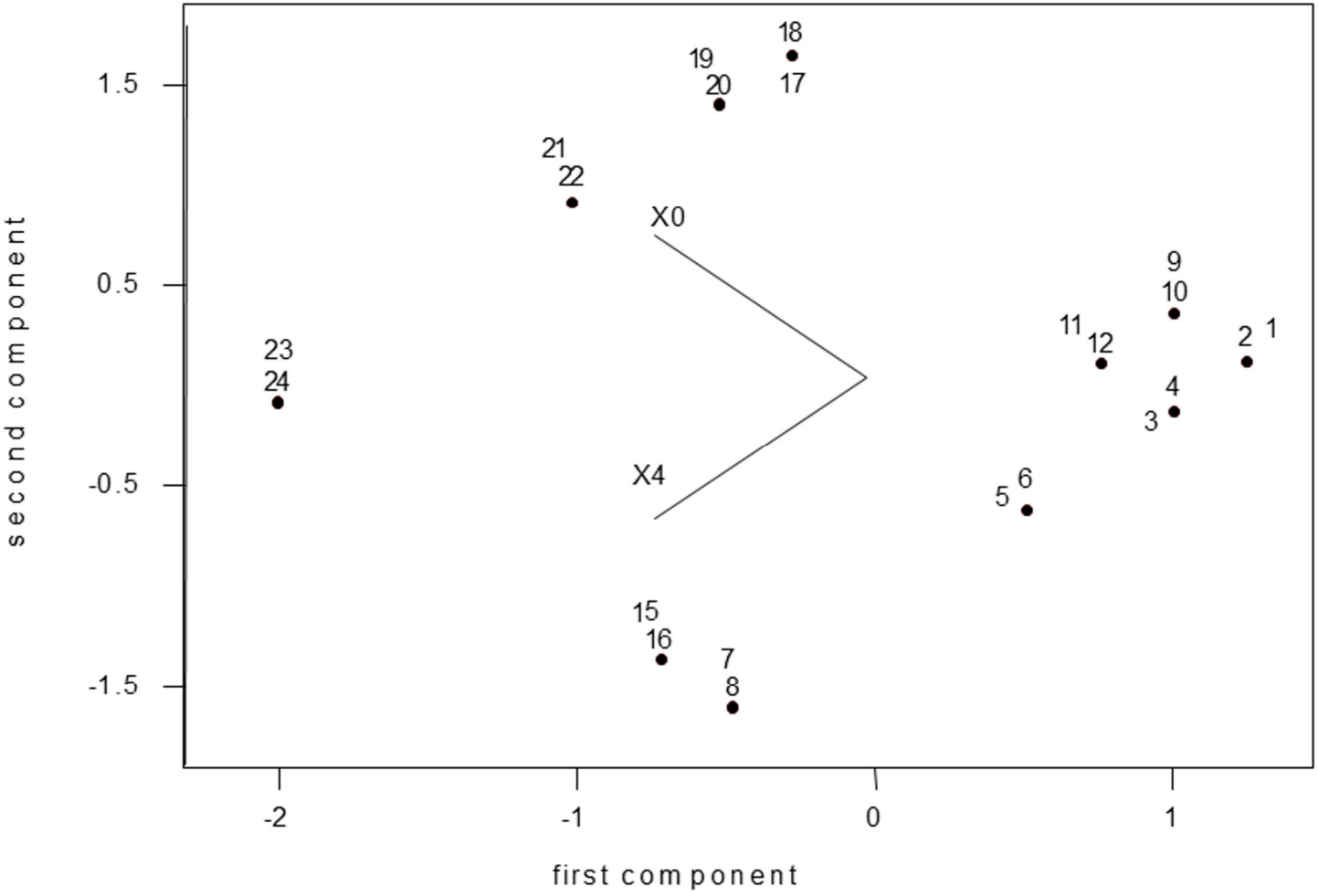


Figure S11. PCA on model descriptors (Equation 3) (PC1 E.v. = 68%, PC2 E.v. = 32%), performed for the ZnO dataset.

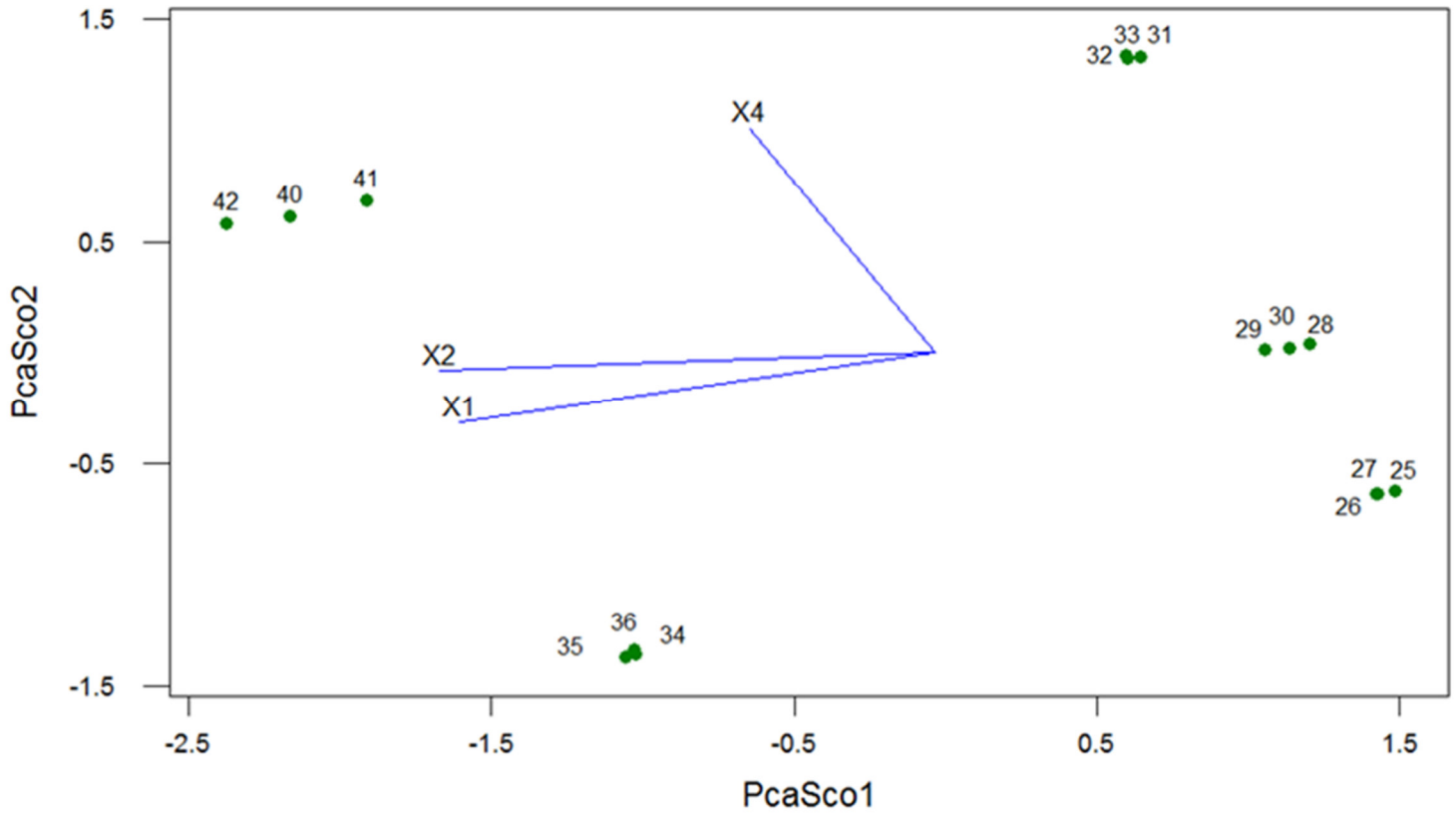
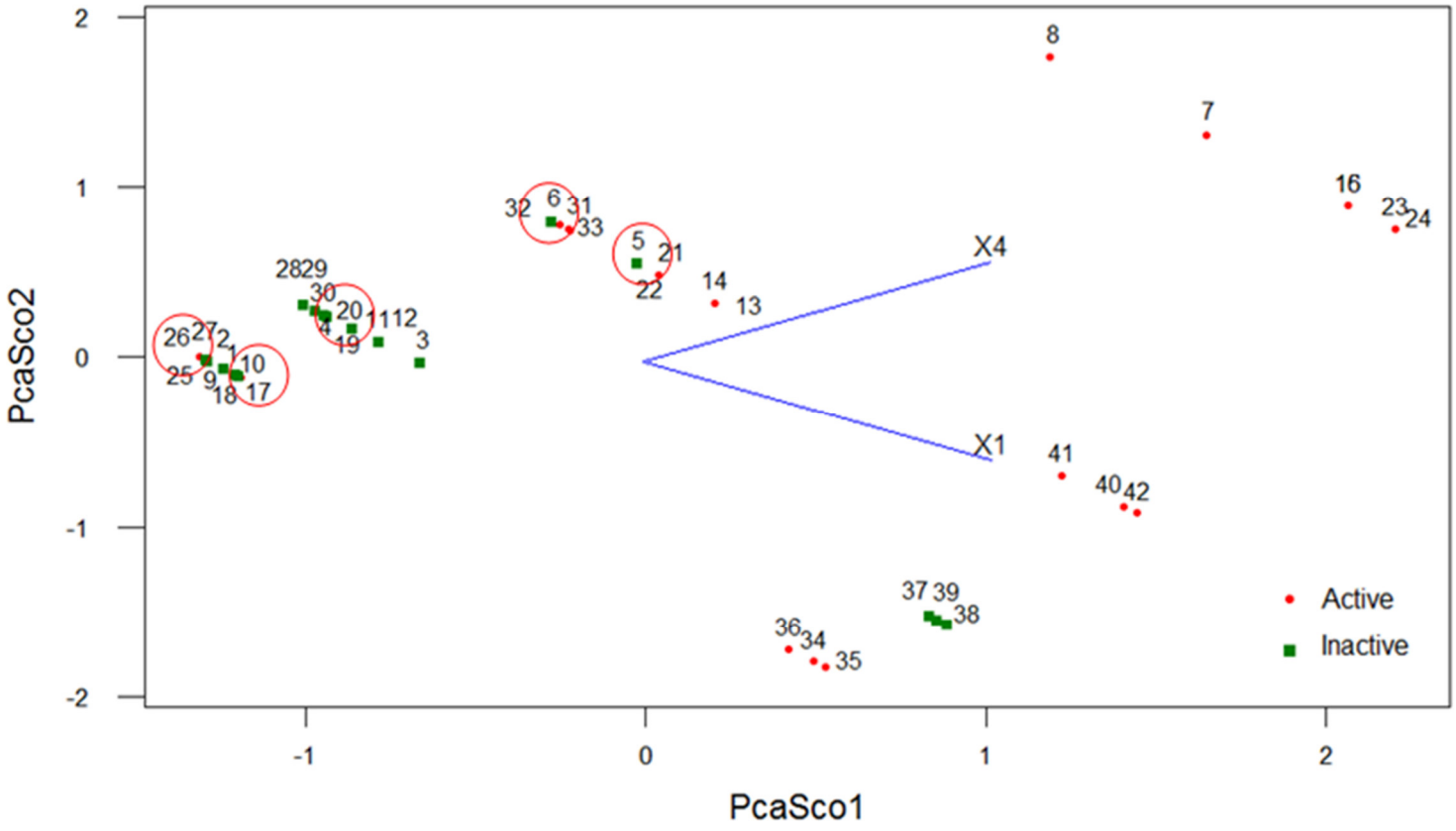



Figure S12. PCA on model descriptors used in classification (PC1 E.v.=63%; PC2 E.v.=37%), performed for the complete dataset (42 NPs). Misclassified NPs have been highlighted in red circles.