Effect of Exposure to Decabromodiphenyl Ether and Tetrabromobisphenol A in combination with Lead and Cadmium on Soil Enzyme Activity

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Abstract

The two most widely used brominated flame retardants (BFRs), decabromodiphenyl ether (BDE209) and tetrabromobisphenol A (TBBPA), co-exist prevalently with heavy metals (HM), at e-waste recycling sites (EWRSSs). The laboratory incubation we conducted, which 50 g of dried soil spiked with Pb, Cd, BDE209 and TBBPA, were incubated 180 d in the dark at 25°C, and kept the soil moisture at 60%. The results demonstrated that the inhibition ratio of 4 kinds of the enzyme (catalase, dehydrogenase, polyphenol oxidase and urease) in the combined of BFRs and HMs treatments were -127% – 66%, -72% – 49%, -52% – 76% and 54% – 107% respectively, and in TBBPA or BDE209 contaminant treatments were -135% – 18%, -16% – 31%, -18% – 15% and -17% – 83% respectively. Urease was more sensitive to the combined contamination than catalase, dehydrogenase and Polyphenol oxidase. BDE209 exhibited a higher eco-toxicological potential to soil enzymatic activity than TBBPA. The co-existence of

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HM induces a stronger response of enzymatic activity to the artificial contamination. The availability of the residual fraction in the later stage of incubation, along with the results of principal component analysis demonstrated that there may be different mechanisms responsible for the generation of residual and extractable fractions of HM.

Keywords: Combined exposure, Decabromodiphenyl ether, Tetrabromobisphenol A, Lead , Cadmium , Ecotoxicological effects

1. Introduction

Electronic waste (e-waste) treatment has recently become an issue of significant concern, especially in developing countries since 1990s (Chan et al., 2007; Zhang et al., 2012). It has been reported that 50%-80% of e-wastes worldwide were being exported to Asia and Africa legally or illegally, approximately 90% of which were sent to China since 2002 (Ni et al., 2013). A lack of effective recycling techniques has led to the continual release of various hazardous chemicals, including heavy metals (HM) and flame retardants (FRs) from e-waste recycling sites (EWRSSs) in China. Many studies on the effects of these harmful chemicals examine only a single chemical, while very few studies have examined joint exposure to FRs and HM, such as polybrominated diphenyl esters (PBDEs) with tetrabromobisphenol A (TBBPA), PBDEs with HM (i.e., cadmium and copper), and TBBPA with HM (i.e., lead) in soils. Available information indicates that exposure to a combination of these chemicals may have different ecological effects on soil microbial communities, compared with the effects of exposure to FRs alone (Zhang et al., 2012a, b; Zhang et al., 2016; Zhang et al., 2014). It has been hypothesized that, because it contains two phenolic hydroxyl groups, the mobility and bioavailability of TBBPA in the environment would be different from that of non-ionic FRs (Xue et al., 2009), such as PBDEs. A recent study revealed insight into the sorption process of TBBPA and suggested that it is dependent on pH (Tong et al., 2015), which is also believed to be one of the most important factors controlling mobility and bioavailability of HM in soils. To date, the impact of HM on the ecotoxicity of TBBPA and PBDEs remains largely unknown.

Soil microorganisms respond rapidly to ecosystem perturbation and therefore are considered to be sentinels of soil pollution. Soil enzymatic activity reflects the direction and strength of biochemical processes in soil (Yang et al., 2006). Hence, it is widely used as a biological indicator for estimating the adverse effects of various pollutants on soil quality (Gao et al., 2010). Dehydrogenase and catalase, two common intracellular enzymes, are considered to be more sensitive to chemical toxicity than extracellular enzymes (Chu et al., 2003; García-Gil et al., 2000; Caldwell, 2005). The activity of urease is also frequently used for determining the influence of various pollutants (e.g., HM, pesticides, and crude oil) on the microbiological

quality of soil (Margesin et al., 2000). Polyphenol oxidase, which could oxidize the aromatic compound (eg.PAHs) to quione, which reacts with protein, amino acids, glucide, minerals, and small organic matter compounds, plays an important role in the cycling of aromatic compounds in soil (Fang et al., 2015). The objectives of this study were: to explore the joint toxicological effects of BFRs (BDE209 and TBBPA) and two frequently studied HM (Pb and Cd) on soil enzyme activity, and to examine the impact of combined exposure to BFRs on HM speciation, which is closely related to HM bioavailability in soil. The results will provide further information about the interactions between of BFRs and HM on the ecological risk assessments of EWRSSs.

2. Materials and methods

2.1 Chemicals

Decabromodiphenyl ether (commercial Deca-BDE, purity of BDE209 >99%) and TBBPA (>98% in purity) were purchased from J&K Scientific (USA). Cadmium chloride (CdCl_2 , purity $\geq 98\%$ of A.R. grade) and lead acetate ($(\text{CH}_3\text{COO})_2\text{Pb}$, purity >99.8% of A.R. grade) were obtained from Sinopharm Chemical Reagent Co., Ltd. (China). All organic solvents used in the study were of HPLC grade (J.T. Baker Inc., USA). BDE209 and TBBPA stock solution were prepared by dissolving the commercial product powder in toluene and methanol, respectively. Pb and Cd stock solution were prepared by dissolving $(\text{CH}_3\text{COO})_2\text{Pb}$ and CdCl_2 in de-ionized water, respectively.

2.2 Incubation experiments

A silty clay soil (pH 6.0, organic matter content of 3.2%, and cation exchange capacity $10.9 \text{ cmol}\cdot\text{kg}^{-1}$) was sampled from a forest field located in Qingyuan, Guangdong province, China. The soil was air-dried, separated from plants residues and stones, and then sieved through a 2 mm sieve before use. The background levels of the investigated contaminants in pristine soil were determined: $3.3 \text{ }\mu\text{g}\cdot\text{g}^{-1}$ for Pb, $0.14 \text{ }\mu\text{g}\cdot\text{g}^{-1}$ for Cd, $0.029 \text{ ng}\cdot\text{g}^{-1}$ for BDE209 and not detectable for TBBPA, respectively. The background concentrations of the chemicals were far below the spiked levels. Incubation experiments were carried out as follows: about 50 g of dried soil was spiked with BDE209 and TBBPA in dichloromethane, Pb^{2+} and Cd^{2+} in water.

Before joined Pb and Cd, it must be removed dichloromethane by evaporation in a fume hood for 30 min. The mixture was thoroughly combined with the submerged soil, resulting in a final BDE209 and TBBPA concentration of $10 \text{ mg} \cdot \text{kg}^{-1}$ soil (dry weight). Table 1 presents the contaminant of the treatments. The control received the same amounts of distilled water instead of the contaminant solution. Total of 15 beakers were incubated in the dark at 25°C . Deionized water was added to keep the soil moisture at 60% of field capacity every 3 days. Soil samples were collected for further analysis on day 0, 5, 10, 30, 60, 120 and 180 after incubation.

2.3 Enzymatic activity assay

Catalase activity was determined using a titrimetric method (Roberge, 1978). Two gram of the soil was put into a 150 mL Erlenmeyer flask, and then 40 mL of distilled water and 5 mL of 0.3% H_2O_2 were added into the flask. The flask was sealed and shaken, and then gently placed in a water bath at 37°C for 30 min. Then 5 mL of $1.5 \text{ mol} \cdot \text{L}^{-1}$ H_2SO_4 was added to terminate the reaction. And the light absorbance was measured using the spectrophotometer at 240 nm. The amount of surplus H_2O_2 from 25 mL of the filtrate was determined by titration using $0.02 \text{ mol} \cdot \text{L}^{-1}$ KMnO_4 .

Dehydrogenase activity was determined using the measurement of triphenyl formazan (TPF) produced from the microbial reduction of 2, 3, 5-triphenyltetrazolium (TTC) (Chaperon and Sauve, 2007). Samples of 2 g soil were placed in 50 mL EP tubes. To each tube, 2 mL of 1% TTC, 2 mL of $0.1 \text{ mol} \cdot \text{L}^{-1}$ glucose solution and 2 mL of Tris-HCl buffer (pH7.4) were added. After being mixed on a vortex, the tubes were stoppered and incubated in the dark for 24 h at 37°C . After incubation, 0.5 mL of methanal was added and additional 5 mL of methylbenzene were added. Then the samples were shaken in a water bath for 30 min and stood for 30 min. The samples were separated with a centrifuge for 5 min in $4000 \text{ r} \cdot \text{min}^{-1}$. The red-colored complexes were measured within 1 h using the spectrophotometer at 485 nm.

Urease activity was determined using the method of measurement of $\text{mg NH}_4^+\text{-N}$ produced from dry soil $\text{g}^{-1} \cdot \text{d}^{-1}$ (May and Douglas, 1976). Two grams of the soil were incubated with 5 mL 10% urea and 10 mL citrate buffer at 37°C for 24 h, then diluted with 38°C distilled water to 50 mL. One mL filtered liquor was put into a 50 mL volumetric flask, and then diluted with

distilled water to 10 mL. Then 4 mL of sodium phenate and 3 mL sodium hypochlorite were added. Finally the flask was sealed and shaken, and the light absorbance was measured using the spectrophotometer at 578 nm 20 min later.

Polyphenol oxidase activity was determined using the method of measurement of mg gallnut produced from dry soil $\text{g}^{-1} \cdot \text{d}^{-1}$ (Vepsäläinen et al., 2001). One gram of the soil was incubated with 10 mL of 1% pyrogallol at 30°C for 2 h and the samples were shaken for 30 min. Then 4.5 mL of citric acid-phosphate buffer and 35 mL of diethyl ether were added into the flask. Finally the flask was sealed and shaken, and the light absorbance was measured using the spectrophotometer at 430 nm.

2.4 Pb and Cd speciation assay

The concentrations of lead (Pb) and cadmium (Cd) in soil were measured using the relevant national standard methods (GB/T25282-2010). The fractions of Pb and Cd were determined using sequential extraction proposed by Bureau Community of Reference (BCR) (Ure et al, 1993). Soil samples (0.2 g of dry soil) were extracted by shaking in different solvents according to the following steps:

Step 1: A total of 20 ml of 0.11 M acetic acid was added to the soil and shaken for 16 h. The extraction, regarded as acid-extractable fraction (Extr.-fraction), was separated from the residue by centrifugation at 3000 g for 20 min.

Step 2: The residue from Step 1 was extracted with 20 mL of 0.5 $\text{mol} \cdot \text{L}^{-1}$ hydroxylamine hydrochloride by shaking for 16 h. The extraction was separated by centrifugation as Step 1 and was identified as reducible fraction (Red.-fraction).

Step 3: The residue from Step 2 was treated with 5 mL of H_2O_2 (pH2-3) at room temperature with intermittent shaking for 1 h. A second 5 mL of H_2O_2 (pH2-3) was added for subsequent digestion in water bath at $85 \pm 2^\circ\text{C}$ for 1 h. After cooling, 25 mL of 1.0 $\text{mol} \cdot \text{L}^{-1}$ ammonium acetate (pH2) was added to the solid residue and shaken for 16 h. The extraction was separated by centrifugation as above and was identified as oxidizable fraction (Oxid.-fraction).

Step 4: The residue from Step 3 was digested by concentrated $\text{HNO}_3\text{-HClO}_4\text{-HF}$ mixture followed the procedure described in GB/T25282-2010, and was identified as residual fraction

(Res.-fraction).

All the fractions (supernatants) above were analyzed by Graphite Furnace Atomic Absorption Spectrum (GFAAS). For quality assurance and quality control (QA/QC) purpose, certified reference material GSS-6 (CRM soil, National Institute of Standard and Technology, China) were used. The measured values for total content of Pb and Cd were $101 \pm 2.7\%$ compared to the certified values, indicating the accuracy of the analytical method. The recovery of the sequential extraction procedure, which is defined by calculating the ratio of the sum of metal concentration in individual fraction to the total metal content in soils, was $87 \pm 28\%$ for Pb and $112 \pm 41\%$ for Cd, respectively.

2.5 Statistical analysis

The inhibition ratio (IR) of enzyme activity was calculated as $[(Y-X)/Y]$, where X is the value of the contaminated soil and Y is the value of the control soil. The results were expressed as the mean of three paralleled determinations of the triplicates. Statistical analysis was carried out using SPSS 17.0. Multi-comparison for the measured variables were conducted by one-way analysis of variance (ANOVA) accompanied by least significant difference (LSD) tests. The level of significance through the present study was set $\alpha = 0.05$.

3. Results and discussions

3.1 Effect of exposure to BFRs and heavy metals on soil enzyme activity

Oxidoreductases, such as catalase, dehydrogenase, and polyphenol oxidase, are an important group of enzymes often used in soil ecological studies (Liu et al., 2015). During the 180-d incubation period, the activity of catalase in all the exposed groups displayed similar restrained-activated-restrained response, except for the TBBPA-only group (Group T, Fig. 1(a)), in which catalase was consistently inhibited, but a minor effect of TBBPA on catalase activity. Zhang et al. (2014) also suggested that BDE209 influenced catalase activity more significantly than did TBBPA. Compare with the group exposure to only PBDE, the inhibition and induction effects were stronger in the presence of Pb and Cd in addition to PBDE (Fig.1), indicating a possible synergistic interaction between Pb, Cd, and BDE209.

Dehydrogenase is another soil enzyme that is frequently investigated in study of the

ecotoxicology of organic pollutants, in particular with respect to its role in energy transfer process and as a microbiological status indicator. In the BFR-treated groups (Group T, P, and PT), the activity of dehydrogenase was generally augmented, except in some samples exposed to TBBPA alone (Fig. 1(b), Fig. S1). This may be in part because BDE209 is a carbon and energy source that facilitates growth of soil microbial populations, resulting in an increase in dehydrogenase activity (Zhang et al., 2016). In contrast, the decrease in dehydrogenase activity by TBBPA is in line with results of a previous study in which dehydrogenase was completely inhibited during the incubation period (Shu and Su, 2011). We observed a greater limited inhibitory effect in this study, which may be related to the lower level of TBBPA exposure (100 mg·kg⁻¹ soil) compare with Shu and Su's (2011) study (range of 50-2000 mg·kg⁻¹ soil). It is clear that the addition of Cd and Pb at a high dose strongly decreased the activity of dehydrogenase (from 45.1% to 65.8% over the incubation period). However, at the low and moderate dose levels, the impact of Pb and Cd on dehydrogenase activity in the FR-contaminated soil was not significant. Both Pb and Cd have previously been found to have adverse effects on dehydrogenase even at a relatively low level (Gao et al., 2010). For polyphenol oxidase, a similar trend in inhibition ratios were observed among Group P, PT, CA, and CB, in which enzymatic activity exhibited an increase followed by a decrease in the early stage (first 30 days) of incubation, followed by a constant trend of enhanced activity that lasted until the end of the experiment (Fig. 1(c), Fig. S1). However, at the high dose level of the HM-treated group (Group CC), polyphenol oxidase was consistently inhibitory ($p < 0.01$) for almost the entire incubation period, from 63.3% on day 0 to 33.3% on day 120. Sustained pollution stress may be responsible for the significant reduction in enzymatic activity. It has been suggested that TBBPA could activate polyphenol oxidase activity, and that there is a close relationship between TBBPA dose and its activation rate (Shu and Su, 2011). However, we did not observe an enhancement of polyphenol oxidase activity in TBBPA-treated soil, and we did observe a slight decrease in activity on Day 10 (Fig. 1(c), Fig. S1). TBBPA might be transformed into its ionic form under higher pH conditions, which would lead to a reduction in soil sorption (Tong et al, 2015); however, the change in pH we observed on Day 10 was low (Fig. S2). This association between the observed species distribution of TBBPA and the minor response of polyphenol oxidase activity needs to be

further investigated.

As shown in Fig. 1(d) and Fig. S1, urease activity was significantly inhibited throughout the experimental period in all contaminated soil groups compared with the control group, with the minor exception being the later stages (>60 days) of the incubation period of the TBBPA-treated group. Moreover, urease activity following exposure to a combination of HM and BFRs generally decreased with an increase in the concentration of Pb and Cd. Previous studies have demonstrated that the enzyme activities were decreased as the concentration of BDE-209, Pb or Cd increased at the same incubation time,(Yang et al., 2006; Huang et al., 2011). It could be due to HM exhibit toxic activity towards soil biota, which may lead to the decrease of the number and the activity of soil microorganisms, thus the enzyme activity was primarily decreased by direct suppression of microbial growth in these contaminated sites. Alternatively, a portion of the reduction in enzyme activity may possibly have been caused by direct interactions between enzymes and HM (Chen et al., 2005).

In this study, the co-existence of BDE209 and HM weakened the dose-effect relationship between urease and Pb/Cd, which is in agreement with the findings of Zhang et al. (2015).

The above results indicated that urease was more vulnerable than the other three enzymes. Similar results have been reported; for example, urease showed greater susceptibility to BDE209 combined with either heavy metal (Pb and Cd) or TBBPA (Zhang et al., 2012b; Zhang et al., 2016; Zhang et al., 2014). There were only minor impacts on the activity of most enzymes in soil exposed to TBBPA alone, while exposure to BDE209 more significantly impacted the soil enzymes. The similarity in the responses of enzymatic activity between Group P and Group PT suggests that BDE209 had a major contribution to the observed ecotoxicological effects on soil enzymes, compared with TBBPA (Fig. 1). The effect of combined pollution of BRFs adding Pb and Cd on enzyme activity was stronger than amended with TBBPA and BDE209 separately. It could be due to sulfhydryl groups of enzymes serve as catalytic sites or as groups involved in maintaining the correct conformation of the protein. Metals can react with sulfhydryl groups causing inactivation or inhibition of enzyme activity (Yang et al., 2006).

The species distribution of TBBPA in soils, which is influenced by pH (Tong et al., 2015) as well as the redox status (Liu et al., 2013), might impact the mobility and availability of

TBBPA, leading to the differences in the ecotoxicity of TBBPA to microorganisms compared with HM and other nonionic organic contaminants.

3.2 Effect of exposure to BFRs on heavy metal speciation

We conducted a BCR sequential extraction to assess heavy metal speciation in soil samples (Fig. 2). Both Pb and Cd aged from the Extr.-fraction to Res.-fraction during the incubation period. Specifically, Cd occurred predominantly as Extr.-Cd in all treatments, while Pb occurred primarily in the acid-extractable and reducible fractions (Fig. 2), which is due to the greater mobility and bioavailability of Cd compare with Pb (Zhang et al., 2016; Zhang et al., 2012). After a period of decline, the proportion of Pb occurring in the Extr.-fraction rebounded on day 60, while the proportion in the Res.-fraction decreased sharply. This phenomenon was particularly significant in the group exposed to low doses of Pb. The change in soil pH during the incubation period may have resulted in the observed changes in the proportion of Cd and Pb occurring in the Extr.-fraction, especially the carbonates-bound fraction, in soils (Zhang et al., 2016). In this study, the increase in the proportion of Pb occurring in the Extr.-fraction might be due to the increase in soil pH from day 30 to day 60 (Fig. S2). It has been reported that the level of contamination did not influence the proportion of Pb and Cd occurring in the Res.-fraction soils (Zheng et al., 2010). Using Tessier's sequential extraction scheme, Zhang et al. (2015) also demonstrated that the proportion of Pb in the Res.-fraction remained constant throughout the incubation period. In this study, however, the proportion of Cd and Pb occurring in the Res.-fraction was variable, and was negative dose-dependent (Fig. 2). It is thought that the activity of micro-organisms is the primary factor responsible for the release of bound residues (Gevao et al., 2000; Liu et al., 2013). Though we did not measure soil microbial biomass in this study, the promotion of dehydrogenase activity could partly mirror the status of the microbes. Thus, the observed changes in the proportion of Cd and Pb occurrence in the Res.-fraction in the early and later stages of the incubation. It could be due to Extr.-fraction of the HM depends on the initial concentration, and a proportion of the sulfhydryl groups of enzyme-active sites reacts with the Extr.-fraction (Yang et al., 2006). So, we suspect that it may be due to their different sources for the Res.-fraction in the early and later stages of the incubation.

Because results from the TBBPA-only group exhibited trends in enzymatic activity that were distinct from all the other groups (Fig. 1), the potential impact of TBBPA co-existence on HM speciation should not be neglected. Further investigation is necessary to explore whether the release of HM from the Res.-fraction related to changes in the extractable-bound form of TBBPA (Liu et al., 2013).

3.3 Correlationship between toxicological properties and speciation characteristics in soil co-contaminated with FRs and HM.

Principal Component Analysis (PCA) is usually used to reduce the complexity of a dataset into fewer independent factors. As such, the underlying relationships between HM speciation and enzyme activity in different treatments in soil can be elucidated (Fig. 3). We chose the Extr.-fraction and Res.-fraction to represent the highest and lowest species of bioavailability, respectively. We standardized enzymatic activity and HM speciation using SPSS v17.0 prior to analysis. The factor loadings, representing the correlation between the individual factor and the principal components (PCs), were plotted as points, while the mean factor scores for each dose were presented as dots with standard errors (SEs). The first two PCs accounted for 83.59% of the total variability. The biplot of PCA clearly displays two clusters. Samples from the joint exposure group with the low and moderate HM dose fell into Cluster I, and samples from the high dose treatment dominated in Cluster II, suggesting that the factors controlling enzymatic activity in groups with high exposure to HM with FRs were different from those in the low and moderate-exposure groups, which was in agreement with our observations of the enzymatic activity and speciation (Fig. 1 and Fig. 2). In the factor-loading plot, catalase activity partially showed a closer association with the Extr.-fraction of Pb and Cd in Cluster II, while activities of other enzymes were mostly influenced by the contribution of Res.-fraction of Pb and Cd in Cluster I. The separation of the Extr.-fraction and Res.-fraction into two clusters may indicate their different sources, which supports our hypothesis. The high loadings for both Res.-Pb and dehydrogenase activity (Table 2) suggest that their correlation is distinct from the correlation between Res.-Pb and other enzymes.

4. Conclusions

We measured the activity of soil enzyme, including urease, catalase, dehydrogenase, and polyphenol oxidase. The results clearly demonstrated that inhibition ratios were enhanced by the presence of BDE209, TBBPA, Cd, and Pb. Urease was the most sensitive to the exposure of both FRs and HM. BDE209 exhibited a higher eco-toxicological potential to soil enzymatic activity than TBBPA. The Res.-fraction contribution was variable and was with negative dose-dependent influence of pH. Results of the PCA suggest that the exposure levels of HM with FRs were in line with our observation on enzymatic activity and speciation of Cd and Pb. Results of the enzyme assays of the TBBPA-only group varied from those of the other treatments, suggesting that it is important to further explore the environmental behaviors and joint effects of TBBPA-analogous compounds with HM in compounds other than non-ionic organic pollutants in soils.

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Fig. 1 Inhibition ratios of catalase (a), dehydrogenase (b), polyphenol oxidase (c) and urease (d) in different treatments during the incubation period.

Fig. S1 Catalase, dehydrogenase, polyphenol oxidase and urease activities in different treatments after sole or co-exposure to BDE209, TBBPA, Pb and Cd. Each column represents the mean of three replicates with standard deviations on top of the each bar. Single and double asterisks (*) indicate significance at the $p < 0.05$ and $p < 0.01$ level compared to controlled treatments (CK) (LSD test).

Fig. 2 Speciation distribution of Pb and Cd in different treatments during the incubation period.

Fig. 3 Biplot of the factor loadings (symbols) and the mean factor scores (with SE bars) for toxicological and speciation parameters analyzed.

Fig. S2 Dynamic of soil pH in different soil samples during the incubation period. Each column represents the mean of three replicates with error bars as standard deviations.

Table 1 Experimental design of PBDEs, TBBPA, Pb and Cd at different concentrations used in this study

Treatments	Concentrations ($\mu\text{g/g dw}$, 25°C, 60% water-holding capacity)			
	PBDEs	TBBPA	Pb	Cd
CK	0	0	0	0
P	10	0	0	0
T	0	10	0	0
PT	10	10	0	0
CA	10	10	100	10
CB	10	10	1000	100
CC	10	10	5000	500

Table 2 PCA factor loading (varimax rotated factor matrix) of PC1 for investigated HM speciation and soil enzyme activities during the incubation period

	Res.-Pb	Res.-Cd	Extr.-Pb	Extr.-Cd	Dehydrogenase	Catalase	Polyphenol oxidase	Urease
day 0	.901	.402	-.843	-.024	.773	.020	.484	-.237
day 5	.994	.977	-.822	-.956	.924	.193	.524	.061
day 10	.986	-.322	-.836	-.856	.635	.210	.512	.240
day 30	.831	-.522	-.769	-.928	.254	-.695	.287	.315
day 60	.897	-.471	-.769	-.823	.905	-.602	.600	.301
day 120	.960	.977	-.931	-.984	.678	-.148	.559	.509
day 180	.963	.951	-.921	-.985	.773	-.801	-.053	.640

Values > 0.6 are highlighted in red

Values > 0.5 are highlighted in black