

**Bioinspired stormwater control measure for the enhanced removal of
truly dissolved polycyclic aromatic hydrocarbons and heavy metals
from urban runoff**

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Abstract

Stormwater harvesting (SWH) addresses the UN's Sustainable Development Goals (SDGs). Conventional stormwater control measures (SCMs) effectively remove particulate and colloidal contaminants from urban runoff; however, they fail to retain dissolved contaminants, particularly substances of concern like polycyclic aromatic hydrocarbons (PAHs) and heavy metals (HMs), thereby hindering the SWH applicability. Here, inspired by protein folding in nature, we reported a novel biomimetic SCM for the efficient removal of dissolved PAHs and HMs from urban runoff. Lab-scale tests were conducted together with a more mechanistic investigation on how the contaminants were removed. By integrating hydrophobic organic chains with low-cost hydrophilic flocculant matrixes, our biomimetic flocculants achieved a 1.4-9.5 times removal of all detected dissolved PAHs and HMs, while enhancing the removal of a wide-spectrum of particulate and colloidal contaminants, compared to existing SCMs. Ecotoxicity, as indicated by newborn *Daphnia magna* as experimental organisms, was reduced from "acute toxicity" of the original runoff sample (toxic unit of ~2.6) to "non-toxicity" (toxic unit < 0.4) of the treated water. The improved performance is attributed to the protein-folding-like features of the bioinspired flocculants providing: (i) stronger binding to PAHs (*via* hydrophobic association) and HMs (*via* coordination), and (ii) the ability of spontaneous aggregation. The bio-inspired approach in this work holds strong promise as an alternative or supplementary component in SCM systems, and is expected to contribute to sustainable water management practices in relation to SDGs.

Keywords: Stormwater control measures; Flocculation; Dissolved contaminants; Polycyclic aromatic hydrocarbons; Heavy metals; Hydrophobic-modified flocculants

1. INTRODUCTION

To address the worldwide water challenges caused by climate change, population growth, and underinvestment in water and sanitation, the UN's Sustainable Development Goal 6 (SDG-6) seeks to ensure access to sufficient, safe and affordable water (UN, 2023). Stormwater, originating from nature, has the potential to be a sustainable water source. Stormwater harvesting (SWH), involving the receiving, storage, treatment and reuse of stormwater (Hamilton et al., 2019), has long been considered a practical contribution to achieving the SDG-6, and continues to stimulate research interest in the development of current and new technologies (Chen et al., 2019; Nguyen et al., 2019; Nogradý, 2022; Palmer et al., 2015; Zhang et al., 2020). Specific goals for SWH have been proposed in various urban stormwater management concepts, including the Sponge Cities (SCs) in China, the Low Impact Development (LID) in the USA, and the Sustainable Urban Drainage Systems (SUDS) and the Blue-Green Cities (BGCs) in the UK (Campisano et al., 2017; Chan et al., 2018; Lin et al., 2016). For example, according to the Construction Guidelines of SCs, up to 80% of stormwater in urban areas of China is to be reused by 2030 (Chan et al., 2018).

The expansion of infrastructure construction for stormwater diversion systems has strengthened the promising role of SWH in augmenting water supply and alleviating waterlog (Larsen et al., 2016; Lund et al., 2019; Wang et al., 2021). However, the increasing amounts of runoff with deteriorated water quality, arising from the increase in impermeable surface cover during urbanization (Xia et al., 2017), have been identified as a potential obstacle to the security of SWH (Chen et al., 2019; Pamuru et al., 2022). In particular, the initial portion of stormwater runoff, containing substantially high concentrations of hazardous contaminants and great toxicity due to the "first flush" of deposited pollutants, is a major pollution pathway with potential adverse effects on

human health and urban ecosystems (Kayhanian et al., 2012). Although conventional stormwater control measures (SCMs), involving bioretention ponds, settling tanks, constructed wetlands, etc., have demonstrated some effectiveness in the removal of particulate and colloidal contaminants (Lund et al., 2019), smaller colloids and soluble contaminants generally escape these SCMs, and therefore represent an exposure risk during water reuse (Kayhanian et al., 2012; Nystrom et al., 2020; Pamuru et al., 2022). Prominent inorganic and organic high-risk dissolved contaminants in stormwater runoff are heavy metals (HMs) and petroleum-based compounds (such as polycyclic aromatic hydrocarbons (PAHs)) (Fig. 1a), which originate from vehicles, building materials, asphalt-based roads, etc. These are of considerable concern due to their potential health risk and ecological toxicity, even at trace concentrations (Fairbairn et al., 2018; Lapointe et al., 2022; Masoner et al., 2019; McDonald et al., 2022). The development of cost-effective SCMs for the removal of dissolved HMs and PAHs is thus identified as a key requirement.

Flocculation is a natural-occurring process affecting the transport of materials in surface water, and by the addition of flocculants, it is extensively employed as a principal treatment technology in water pollution control engineering (Ho et al., 2022). Through the destabilization and aggregation of particulate and colloidal contaminants to form large, settleable flocs (Campisano et al., 2017), flocculation has been shown to be effective in the removal of a variety of contaminants, with advantages of facile operation, low cost and easy upgradability (Demissie et al., 2021). Some flocculation-based systems have been established and are in operation for SWH in the USA, EU, China and India (Clary et al., 2020; Kayhanian et al., 2012; Kumar et al., 2019; Nystrom et al., 2020). However, the ineffective reduction of dissolved contaminants has limited their reliability (Clary et al., 2020; Nystrom et al., 2020). Conventional flocculants,

either inorganic (principally aluminum or iron hydrolysates) or organic (such as synthetic polyacrylamide (PAM) and eco-friendly natural polymer chitosan (CS)), are not able to sufficiently aggregate dissolved nanometer-scale contaminants to form mesoscale flocculation nuclei, and hence, further growth to millimeter-scale flocs (macroscale condensed matter) is inhibited (Liu et al., 2019).

Protein folding is a ubiquitous phenomenon in living organisms, by which soluble peptide chains emerging from ribosome undergo aggregation and phase transition processes to form an active native state of proteins with hierarchical structures (Dill et al., 2008). A major contributing interaction for protein folding is the entropy-driven water-mediated hydrophobic association of hydrophobic groups of amino acids, through which the relatively nonpolar parts of peptide aggregate to hydrophobic cores, while the polar parts form the water-exposed surface (Baldwin, 1986; Walti et al., 2016). Therefore, proteins with characteristics of condensed matter achieve microphase separation from water (Eisenberg and Sawaya, 2016). Inspired by this nature, to enhance the removal of dissolved PAHs, we designed biomimetic flocculants by the integration of hydrophobic organic chains (poly (N-n-propylacrylamide), PNNPAM) (Li et al., 2023; Yang et al., 2020) with a conventional hydrophilic matrix (aluminum hydrolysates or CS; Fig. 1b). Like that in protein folding, hydrophobic association between the flocculants and PAHs, as well as among hydrophobic groups within the flocculants, is hypothesized to promote aggregation and microphase separation to form flocculation nuclei.

-Fig. 1-

In addition to the organic components, more than one third of proteins are metal-dependent. Metal ions, as cofactors, widely participate and influence the folding of proteins (such as the well-known “zinc finger”) *via* a coordination effect (Holm et al.,

1996). This brought another inspiration in that the above-mentioned biomimetic flocculants might be effective also in HMs removal, whereby the abundant oxygen- or nitrogen-containing groups in the bioinspired flocculants naturally possess a coordination potential with HMs. Thus, during the proposed primary nucleus formation process, HMs are bound tightly into the condensed nucleus. In this scenario, significantly enhanced removal of both PAHs and HMs from stormwater runoff is achieved. At the same time, the conventional hydrophilic matrix (such as aluminum hydrolysates or CS) of the flocculants is expected to be still effective in the removal of particulate and colloidal contaminants in simple one-stage treatment. To verify the above hypotheses, proof-of-concept lab-scale tests were conducted together with a more mechanistic investigation on how the contaminants (taking PAHs and HMs in runoff as representative organic and inorganic high-risk contaminants) were removed in this work.

2. MATERIALS AND METHODS

2.1 Materials and runoff sampling

Conventional flocculants AlCl_3 , PAM (weight-average molecular weight (MW) of 1,000 kDa) and CS (viscosity-average MW of 820 kDa; deacetylation degree of 86.0%) were purchased from Sinopharm Chemical Reagent Co., Macklin Co., and Shandong Aokang Biotechnol. Co., respectively. The biomimetic flocculants, hydrophobic-organic-chains-modified CS (HOC-CS) and aluminum hydrolysates (HOC-Al), were prepared according to the methods in Supplementary Text S1 and their molecular structures were identified in Supplementary Fig. S1. All other chemicals were purchased from Sinopharm Chemical Reagent Co.

Prior to stormwater runoff collection, all equipment and containers for sampling

were rinsed using dilute HCl (0.01 mol/L) and then deionized water. Runoff arising from heavy-traffic roads adjacent to a petroleum refinery in an industrial area (Site A: 32°7'32" N, 118°51'51"E) and a university in a college town (Site B: 32°6'14" N, 118°54'19"E), as shown in Fig. 2a, were manually collected in the first 20 min of a rainfall event from stormwater inlets on the road surface. The runoff samples were sieved through a 4.6-mm sieve for the removal of gravels, rocks, tree barks, leaves, and other large debris, and stored, without any chemical treatment, at 4 °C in a dark environment before use.

-Fig. 2-

2.2 Decontamination experiments

Static and continuous-flow water treatment tests were carried out respectively. For static tests, a programmable flocculator (Wuhan Meiyu MY3000–6B) was used. A known volume of the flocculant stock solution was dosed into 1 L of stormwater runoff. Then, the treatment process included 5 min of rapid stirring (200 revolutions per minute (rpm)) for full contact between the flocculant and contaminants, 15 min of slow stirring (50 rpm) for floc growth, and finally 30 min without stirring for floc sedimentation (long enough for the supernatant turbidity to reach a stable value). Supernatant water and flocs were sampled carefully for subsequent water quality analysis.

Continuous-flow flocculation tests were conducted using a custom-made apparatus (Supplementary Fig. S5a; 0.5 L/h), representing a scaled down version of real facilities at water treatment works. Both stormwater runoff and flocculant stock solution (optimized in jar tests) were pumped with pre-designated flow rates into the first flocculation tank. The stirring speeds of the four flocculation tanks in series were 200, 150, 100, and 50 rpm, respectively. The obtained flocs were allowed to settle in an

inclined plate settling tank. The hydraulic residence time (HRT) of each flocculation tank and the settling tank was 5 min and 1 h, respectively. The produced water was collected every 1 h for water quality analysis.

2.3 Characterizations

Infrared (IR) spectra, ^1H nuclear magnetic resonance (^1H NMR) spectra, and water contact angles of flocculants were obtained using an IR spectrometer (Bruker IFS 66/S), NMR (Bruker AVANCE Model DRX-500) spectrometer, and telescopic goniometer (Rame-Hart-100), respectively. The zeta potentials (ZPs) of flocculants in aqueous solution and colloidal contaminants in runoff were determined using a Zetasizer (Malvern Nano-Z). Fluorescence images were obtained using a fluorescence microscope (Nikon CF160) and photographic images of flocs were captured by an optical microscope. QCM-D monitoring was performed using QCM-D (Biolin Scientific QSense E4) with piezoelectric quartz crystal sensors (Qsx 301) with PAHs or flocculants-coated surfaces. Isothermal microcalorimetry (IMC) tests were conducted using an isothermal titration calorimeter (MicroCal iTC-200) at 25 °C. After the equilibrium was achieved, the resulting binding isotherms provided information on the equilibrium dissociation constant (K_d) and ΔH values. ΔG and ΔS were calculated from the following expressions:

$$\Delta G = R T \ln K_d \quad (\text{Eq. 1})$$

$$\Delta H = \Delta G - T \Delta S \quad (\text{Eq. 2})$$

2.4 Water quality and floc property analyses

Turbidity was measured using a turbidimeter (Shanghai Xinrui WGZ-200). The DOC of solution after filtration by a 0.45- μm membrane was determined by a total

organic carbon analyzer (Analytik Jena multi N/C 3100). For either HMs or PAHs, particle-bound (PB) components were filtered out using 0.45- μ m membranes, and the filtrate components were further separated into colloidal-bound (CB) and truly dissolved (TD) fractions using membranes with a molecular-weight-cut-off (MWCO) of 1 kDa. The concentrations of PAHs and HMs in each fraction were determined by a gas chromatograph coupled with mass spectrometer (Shimadzu GCMS-QP2020 NX) and an Inductively coupled plasma atomic emission spectrometer (PerkinElmer Avio 500), respectively. Each water quality parameter was the average value from triplicated parallel tests.

Acute toxicity tests were carried out using newborn (6-24 h) *Daphnia magna* (a 62 Dm pure strain; cultured for more than 3 generations) as experimental organisms to determine the median effective dose after 48 h (48h-ED₅₀). Inhibition ratio was defined as the percentage of *Daphnia magna* without response to gentle agitation for 15 s. During the 48 h, *Daphnia magna* were not fed. 48h-ED₅₀ values were transformed to toxic units (TU) as follows:

$$TU = 100\% / (48h-ED_{50}) \quad (\text{Eq. 3})$$

Capillary suction time (CST) and specific filtration resistance (SFR) were measured using a CST tester (Triton 304M) and the vacuum filtration method (standard Buchner funnel was used at a pressure of 0.07 MPa). The water content of flocs after dewatering (using a mechanical pressure filter device with procedures including 400 kPa for 15 min, 600 kPa for 50 min, and 900 kPa for 30 min) was calculated from the sample weight loss at 105 °C.

2.5 Statistical analyses

Correlation analysis and principal component analysis (PCA) were performed

using SPSS® Statistics 20 (IBM) and Origin® 2021 (OriginLab) software. The linear correlation was evaluated using SPSS® Statistics 20 based on Pearson's correlation coefficient (r), and the probability (p) value below 0.05 indicated that the correlation was statistically significant. To analyze the correlation among REs of PB, CB, and TD fractions of different contaminants, and to mark the 95% confidence ellipses of different data groups, PCA was employed by using the Principal Component Analysis plug-in in Origin® 2021.

2.6 Density functional theory and molecular dynamics computations

Density functional theory (DFT) computations were performed using Gaussian® 09 software to gain insight into the interactions between the various flocculants, PAHs, and HMs. The geometries of the molecules and complexes were optimized at M062x/6-31+G(d) level. The strength of pair-wise binding interactions was evaluated by the absolute value of the binding energy (BE):

$$BE = |E_{\text{Complex(A-B)}} - E_A - E_B| \quad (\text{Eq. 4})$$

Molecular dynamics (MD) computations of molecular structures of different systems were performed using Materials Studio® 2019 software. For the morphological simulation, 10 flocculant chains and 2000 water molecules, with or without 10 Nap molecules and 10 Cu ions, were added into a box with the size of 100×100×100 Å. The structure of each molecule was firstly optimized using the task "Geometric Optimization" in the Forcite package. COMPASS II forcefield was selected and the max iteration was 10000. Smart algorithm was utilized to eliminate the situations of too close atomic spacing or overlap. Then, the simulations were performed using the task "Dynamics" in the Forcite package. The temperature was set to be 298 K. Canonical ensemble (NVT) was selected for the simulation with a step of 1 fs and a duration of

1,000 ps.

3. RESULTS AND DISCUSSION

3.1 Characteristics of the bioinspired flocculants and water quality of initial runoff

Water contact angles of HOC-CS and HOC-Al were larger than those of the hydrophilic aluminum hydrolysate and CS (Supplementary Fig. S2), due to the introduction of hydrophobic PNNPAM chains. Zeta potentials (ZPs) of both HOC-Al and HOC-CS were positive within the common pH range (5-8) of runoff (Supplementary Fig. S3), implying that both flocculants have the ability to destabilize negatively-charged colloids and particulates.

To investigate the universality of the bioinspired flocculation, six typical HMs and sixteen priority PAHs (Azadgoleh et al., 2022; Masoner et al., 2019) (Supplementary Tables S1-S2) were determined. For total HMs and PAHs, runoff at Site A was more seriously polluted with higher contaminant concentrations (Fig. 2b). Six HMs were detected, among which Cr, Pb and Zn were predominant. Five and three of the sixteen PAHs, were detected in samples at Sites A and B, respectively; these were mainly small molecular weight (MW) compounds with 2-4 benzene rings. Among them, the smallest, Nap (details of compounds – see Supplementary Table S1), accounted for the highest proportion. For different fractions (PB, CB, and TD) of HMs and PAHs, the amounts of the three fractions followed the sequence of PB>CB>TD in most cases. The TD fractions are the most difficult to remove, as previously reported (Fairbairn et al., 2018), and this was verified by the water treatment tests. For PAHs, larger molecules with a higher octanol/water partition coefficient (K_{ow}) (Supplementary Table S2) engender lower TD proportions.

3.2 Stormwater runoff decontamination performance

Decontamination of the collected runoff samples was carried out using HOC-Al, HOC-CS and three conventional flocculants (AlCl_3 , CS and PAM). As HOC-Al is a chemically-bound inorganic-organic composite with benefits of both inorganic and organic parts, for a better comparison, the physically combined dosing of the inorganic AlCl_3 and the organic HOC-CS (AlCl_3 +HOC-CS) was also tested.

For conventional water quality parameters, AlCl_3 , CS and PAM removed 80%-91% of turbidity and 19%-79% of dissolved organic matter (DOC) (Fig. 3a and Supplementary Fig. S4). In contrast, the bioinspired flocculants (HOC-Al, HOC-CS and AlCl_3 +HOC-CS) demonstrated greater removal efficiencies (REs), reaching up to 82%-99% of turbidity and 88%-96% of DOC. Among them, the combined flocculant (AlCl_3 +HOC-CS) achieved the highest REs. While the low-priced AlCl_3 has a widely-acknowledged ability to removal turbidity and DOC, as demonstrated here, the bioinspired HOC-CS in the combined dosing mode (AlCl_3 +HOC-CS) can provide an enhanced performance for specific contaminants and situations, as described as follows.

-Fig. 3-

The removal of the HMs and PAHs, mainly present in concentrations in the order of magnitude of 10^0 to 10^2 $\mu\text{g/L}$ in runoff, was also determined. According to the overall REs of total HMs and total PAHs, the AlCl_3 +HOC-CS system always produced the best performance, with REs within the range of 91%-99% (Fig. 3a). Such outstanding performance was also much greater than most of the reported SCMs (Supplementary Table S3). It was also demonstrated in Table S3 that, while the removal of the total amounts of PAHs and HMs was addressed, rare attention was given to the differences of removal efficiencies of various fractions (PB, CB and TD) of the contaminants. In this work, among the PB, CB, and TD fractions, the contaminant removal difficulty

292 followed the sequence of PB<CB<TD (Fig. 3b and 3c). Compared to the conventional
293 flocculants, the bioinspired flocculants demonstrated slight, moderate, and substantial
294 improvement in the REs of PB, CB, and TD fractions, respectively. The REs for TD
295 HMs and PAHs by the AlCl₃+HOC-CS system were 1.4-9.5 times that of the REs by
296 conventional flocculants. Compared to the HOC-CS or HOC-Al system, the improved
297 removal for TD fractions of the AlCl₃+HOC-CS system can be explained as follows:
298 when single HOC-Al or HOC-CS was dosed, parts of the flocculant molecules were
299 consumed in the destabilization and binding of particulate and colloidal contaminants,
300 whereas the remaining HOC-Al or HOC-CS was utilized in binding TD fractions as
301 designed; by comparison, for the AlCl₃+HOC-CS system, the AlCl₃ coagulant
302 principally played the role in destabilizing particulate and colloidal contaminants, and
303 the main role of the subsequently dosed HOC-CS was to remove the TD fractions of
304 contaminants. In this way, HOC-CS was used where it was most effective. In most cases,
305 the AlCl₃+HOC-CS system achieved significantly high REs of 80%-95% for TD
306 fractions. These results were confirmed by the continuous-flow treatment operation
307 (Supplementary Fig. S5), which was a scaled down simulation of a full-scale
308 flocculation process in practice. The significantly enhanced removal of TD fractions
309 was clearly visualized in tests using water containing only a single dissolved probe
310 contaminant (3,3'-diethylthiacyanine iodide (DTCI, with a LogK_{ow} value of 4.21,
311 similar to those of PAHs with 2-4 benzene rings) or Cu²⁺ to represent organic PAHs or
312 inorganic HMs, respectively) (Fig. 4 and Supplementary Fig. S6). A high concentration
313 (10 mg/L) of the probe materials was used in order to clearly demonstrate the
314 aggregation of them into flocs formed by the bioinspired flocculants. It was found that,
315 the dissolved probes were incorporated into flocs giving an intense fluorescent green
316 (for DTCI) or distinct blue (for Cu²⁺) appearance when the bioinspired flocculants were

dosed, whereas no apparent aggregation of the probes are found with the conventional flocculants.

-Fig. 4-

Among the REs for the TD fractions of HMs and PAHs, there were strong positive correlations ($r>0.87$; Supplementary Fig. S7), indicating the simultaneous removal of both contaminants. This implies that HMs and PAHs may occupy different flocculation sites (the hydrophobic sites for PAHs and the coordination sites for HMs) on the bioinspired flocculants, respectively, which was confirmed, as described, in the following section.

3.3 Mechanistic origin of decontamination performance

Zeta potential (ZP) monitoring showed that ZP values always increased with flocculant dosage. Except for the neutral non-charged PAM, other positively charged flocculants (Supplementary Fig. S8) increased the ZP to near zero within the optimal dosage (OD) ranges where the highest REs of turbidity and DOC occurred. This suggested that charge neutralization predominated in the removal of negatively charged particles and colloids (such as the ubiquitous suspended solids (SS) and natural organic matter (NOM) as the main turbidity- and DOC-causing contaminants, respectively) (Li et al., 2023). Although not being the dominant role in NOM removal, hydrophobic association is involved in the binding of NOM, which has been found in authors' and other researchers' previous work (Golea et al., 2020; Wang et al., 2022; Yuan et al., 2017). In this way, when single flocculant HOC-Al or HOC-CS was dosed, parts of the hydrophobic chains of the flocculant were consumed by NOM, as previously discussed in Section 3.2.

Principal component analysis (PCA) of different REs revealed that principal

component 1 (PC1) explained the major part (> 74%) of the total variance (Fig. 5a and 5b). Different REs were divided into three groups, with 95% confidence ellipses marked (Liu et al., 2022). For Group 1, the strongly centralized data points indicated that the REs of PB fractions had a high similarity with RE (turbidity). For Group 2, the REs of CB fractions had a moderate similarity with RE (DOC) as well. Hence, when charge neutralization promotes the removal of turbidity and DOC, the PB and CB fractions of HMs and PAHs are simultaneously removed. By contrast, the REs of TD fractions were highly scattered, suggesting a low similarity among the removal of TD fractions. Given the surface charge nature of TD HMs (cations) and PAHs (non-charged molecules), charge neutralization cannot explain their removal either.

-Fig. 5-

To clarify why the bioinspired flocculant was able to achieve an enhanced removal of TD fractions, chemical computations were performed. Pairwise binding energies (BEs) between representative fragments of the flocculants and contaminants (Fig. 5c and Supplementary Figs. S9-S13), computed by DFT, demonstrated that HMs were mostly likely to be captured by the hydrophilic segments (the metal hydrolysates or the CS chains) *via* coordination, whereas PAHs were all favored by the hydrophobic PNNPAM parts *via* hydrophobic association. This is consistent with the aforementioned hypothesis. For each contaminant, the bioinspired flocculants always had much larger BEs (when the contaminant is in the preferred binding configuration) than conventional AlCl_3 and PAM. Further Pearson correlation analyses (Fig. 5d) revealed that BEs of different flocculant-HM or flocculant-PAH pairs were positively correlated with corresponding REs. Therefore, BE is recognized as one of the key indicators for predicting the enhanced decontamination of different TD contaminants.

However, BE is not the only factor determining the decontamination performance.

For example, although the hydrophilic CS segment of HOC-CS has much higher BEs towards HMs, the CS flocculant alone cannot effectively remove TD HMs. MD simulations (Fig. 5d and Supplementary Figs. S14-S19) show that HOC-CS and HOC-Al, behaving like protein folding, can cause microphase separation from water to form a nuclei-like structure within 1000 ps, regardless of the presence of contaminants. This is mainly due to the hydrophobic association of PNNPAM chains. As a result, when HMs (represented by Cu^{2+} in Fig. 5d) are involved in HOC-CS flocculation, they can participate in the fast microphase separation (primary nucleus formation) and be removed. Differently, the aggregation of CS is much weaker (Supplementary Figs. S18-S19). Consequently, the ability of spontaneous aggregation of a flocculant itself is another key factor.

The thermodynamic nature of the coordination- and hydrophobic association-induced binding of contaminants was further analyzed. Quartz crystal microbalance with dissipation (QCM-D) monitoring using flocculant-modified sensor surfaces and single-contaminant aqueous solutions displays the affinities of different pairs of flocculant-contaminant. The deposited mass of HMs and PAHs on the bioinspired flocculants-modified surface were always larger than those on conventional flocculants (Fig. 6a), which agrees with the calculated BE values in Fig. 5c. The shifts of energy dissipation factor (ΔD , where D is defined as the loss of energy per oscillation period divided by the total energy stored in the system) and resonance frequency (Δf) curves of the deposition of contaminants on the sensor surface were obtained as shown in Supplementary Fig. S20. The “ $-\Delta D/\Delta f$ ” values further provide information about the deposited layer stiffness (Fig. 6b), whereby the binding layer of flocculant-contaminant is rigid if the value $< 1 \times 10^{-7}/\text{Hz}$ (empirical stiff-viscoelastic limit), otherwise, the layer is soft and flexible (Lapointe et al., 2020). According to this criterion, the coordination

between HMs and the bioinspired flocculants is rigid, whereas hydrophobic association leads to a flexible binding of PAHs. IMC tests further give information about changes of thermodynamic state functions (ΔH , ΔG and ΔS) about the binding process of flocculant-contaminant (Fig. 6c and Supplementary Fig.S21). Although the binding of contaminants by the bioinspired flocculants was always spontaneous, there were significant differences in the binding of HMs and PAHs: for HMs, the binding was predominantly driven by enthalpy drop ($|\Delta H| > |-T\Delta S|$), agreeing with the phenomena of coordination effect; whereas the latter, the hydrophobic association with PAHs, was driven by entropy increase ($|\Delta H| < |-T\Delta S|$), which was consistent with the reported results in protein folding (Baldwin, 1986).

-Fig. 6-

3.4 Other aspects of performance

In addition to the decontamination performance as discussed before, other aspects of performance that are of concern, involving treated runoff ecotoxicity, safety of the use of the bioinspired flocculants, floc dewaterability, and cost, are evaluated and discussed.

In the treatment of stormwater, ecotoxicity reduction is of equal importance to contaminant removal. Acute toxicity tests, using *Daphnia magna* as the model organism, demonstrated that the ecotoxicity of runoff samples treated by the bioinspired flocculants was largely reduced with remarkably increased 48h-ED₅₀, compared to that of raw (untreated) runoff or stormwater samples treated by conventional flocculants (Fig. 7a and 7b). According to the toxicity classification (Guo et al., 2021; Persoone et al., 2003), the initial designation of “acute toxic” for the raw runoff changed to “slight toxic” (treated by HOC-CS), or even “nontoxic” (treated by HOC-Al or AlCl₃+HOC-

CS) (Fig. 7c). Thus, although the impacts of the discharge of new chemicals are commonly of concern, the public health acceptability of the SWH by use of the bioinspired approach was ensured, based on following aspects: firstly, the enhanced removal of highly-toxic HMs and PAHs, especially those in the TD fractions (more easily absorbed by organisms) was achieved; secondly, the PNNPAM branches in the flocculants are one type of N-alkyl-substituted PAM, which is widely applied in vivo drug delivery and proven to have high biocompatibility and eco-safety (Phillips and Gibson, 2015); thirdly, the bioinspired flocculants had much lower toxicity with significantly higher 48h-ED₅₀ than conventional chemicals (Supplementary Fig. S22).

Floc dewaterability, another important factor determining the feasibility and benefit of employing the bioinspired approach, was evaluated by CST, SFR and water content after mechanical compression filtration. Among different flocculation systems, the three bioinspired flocculation processes all generated flocs with better dewaterability characteristics (lower CST, SRF and water content) than conventional flocculants (Fig. 7d). Thus, both the difficulty and cost of further floc (sludge) treatment and disposal can be largely mitigated (Wu et al., 2020).

-Fig. 7-

With respect to the cost of acquiring and dosing the flocculants, the unit costs for HOC-Al and HOC-CS are estimated to be ~3,844 and ~11,317 US dollar/t, respectively (Supplementary Text S2). Although these values are higher than those of conventional flocculants (PAM: 1,000-3,000 US dollar/t; alum: 100-300 US dollar/t), it is important to emphasize that conventional flocculants are inadequate in the removal of high-risk contaminants or in the reduction of ecotoxicity. It is also noted that there are other organic contaminants besides PAHs in different stormwater runoff, such as pesticides, pharmaceuticals and personal care products (PPCPs), etc. In this work, PAHs, as a

representative class of high-risk organic pollutants, were effectively removed due to both the strong hydrophobic association of flocculant-PAHs and the ability of spontaneous aggregation of the bio-inspired flocculants. It could be expected that other organic contaminants with similar functional groups that have strong hydrophobic association with the bio-inspired flocculants can be effectively removed as well. The greater degree of treatment achieved by the bioinspired approach substantially decreases the difficulty and complexity of subsequent processes in an integrated SCM system, thereby reducing the cost of the overall SCM.

4. Conclusions

This study, inspired by the ubiquitous protein-folding process, validated the use of biomimetic flocculation approach for SWH as effective in the wide-spectrum removal of contaminants (especially for the TD fractions of organic PAHs and inorganic HMs) from stormwater runoff in a single step: In addition to greater REs for turbidity, DOC, and PB and CB fractions of PAHs and HMs than conventional SCMs, the bio-inspired approach has a significantly enhanced removal of TD PAHs and HMs, which are more challenging pollutants to remove and of greater public health concern. The improved performance is attributed to protein-folding-like features of the bioinspired flocculants providing: (i) stronger binding to PAHs (via hydrophobic association) and HMs (via coordination), and (ii) the ability of spontaneous aggregation. Coupled with other advantages involving the substantial reduction of ecotoxicity and facile sludge dewaterability, the novel approach holds strong promise as an alternative or supplementary component in SCM systems, for improving water quality and environmental/public health safety, and can make an important contribution to sustainable water management practices in relation to SDGs 6 (water quality

improvement), 11 (sustainable urban development), 12 (pollution prevention for responsible consumption and production), 13 (climate resilience enhancement) and 14 (environmental protection for aquatic ecosystems).

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Conceptualization: Z.Y., N.J.D.G.; Methodology: Z.Y., L.Z., T.L., Z.T., and B.B.; Software: R.C. and Z.T.; Resources: Z.Y., Z.T. and B.B.; Investigation and validation: L.Z., T.L., and R.C.; Formal analysis: Z.Y., L.Z., T.L., and R.C.; Visualization: Z.Y., L.Z., T.L. and R.C.; Writing - Original Draft: Z.Y., L.Z., and T.L.; Writing - Review & Editing: Z.Y. and N.J.D.G.; Supervision: Z.Y. and N.J.D.G.; Funding acquisition: Z.Y. and B.B.

Competing interests

The authors declare no competing interests.

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Captions

Fig. 1. Schematic diagram of the proposed bioinspired flocculant structure.

Fig. 2. (a) The two runoff sampling sites and corresponding water quality parameters; (b) Concentrations and percentages of particle-bound (PB), colloidal-bound (CB) and totally dissolved (TD) fractions of detected HMs and PAHs.

Fig. 3. (a) Removal efficiencies of turbidity, DOC, and total concentrations of HMs and PAHs for the treatment of both runoff samples at corresponding optimal flocculant dosages where the highest DOC occurred in Supplementary Fig. S4. (b and c) Removal efficiencies of PB, CB, and TD fractions of HMs and PAHs for the treatment of the two samples.

Fig. 4. (a) Dark- and (b) bright-field images of flocs formed by flocculation of 3,3'-diethylthiacyanine iodide (10 mg/L) solution using different flocculants.

Fig. 5. (a and b) Principal component analysis (PCA) plots demonstrating the correlations among removal efficiencies for the two runoff samples (95% confidence ellipses were marked); (c) Binding energies (BEs) of different pairs of flocculant-contaminant at the corresponding optimized configurations, and (d) their Pearson correlations with removal efficiencies of TD fractions of contaminants; (e) Spatial distribution of different substances (10 characteristic fragments of HOC-Al, 10 copper cations (blue), and 10 naphthalene molecules (yellow)) in 2000 water molecules in a $100\times100\times100$ Å box at 0 and 1000 ps.

Fig. 6. (a) Equilibrium deposition mass of different contaminants on sensor surfaces and (b) $-\Delta D/\Delta f$ values of sensors with the deposition of contaminants; (c) Changes of Gibbs free energy, enthalpy, and entropy calculated according to isothermal micro-calorimetry results.

Fig. 7. (a) D. magna inhibition ratios as functions of liquid percentages, (b) 48h-ED₅₀,

and (c) TU of raw runoff and treated samples. (d) CST and SFR of flocs formed by different flocculants and water content of flocs after mechanical compression filtration.

Figure 1

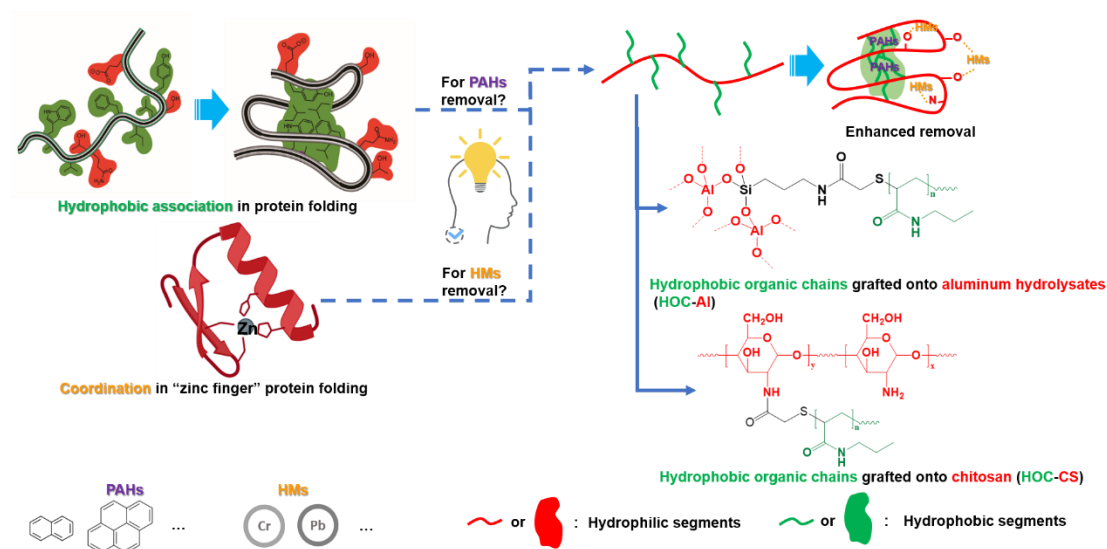


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Figure 2

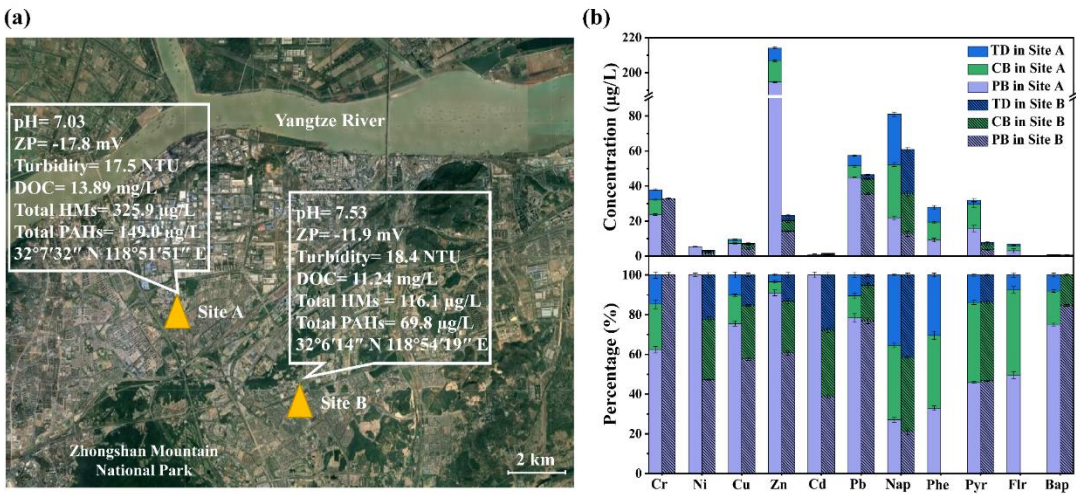


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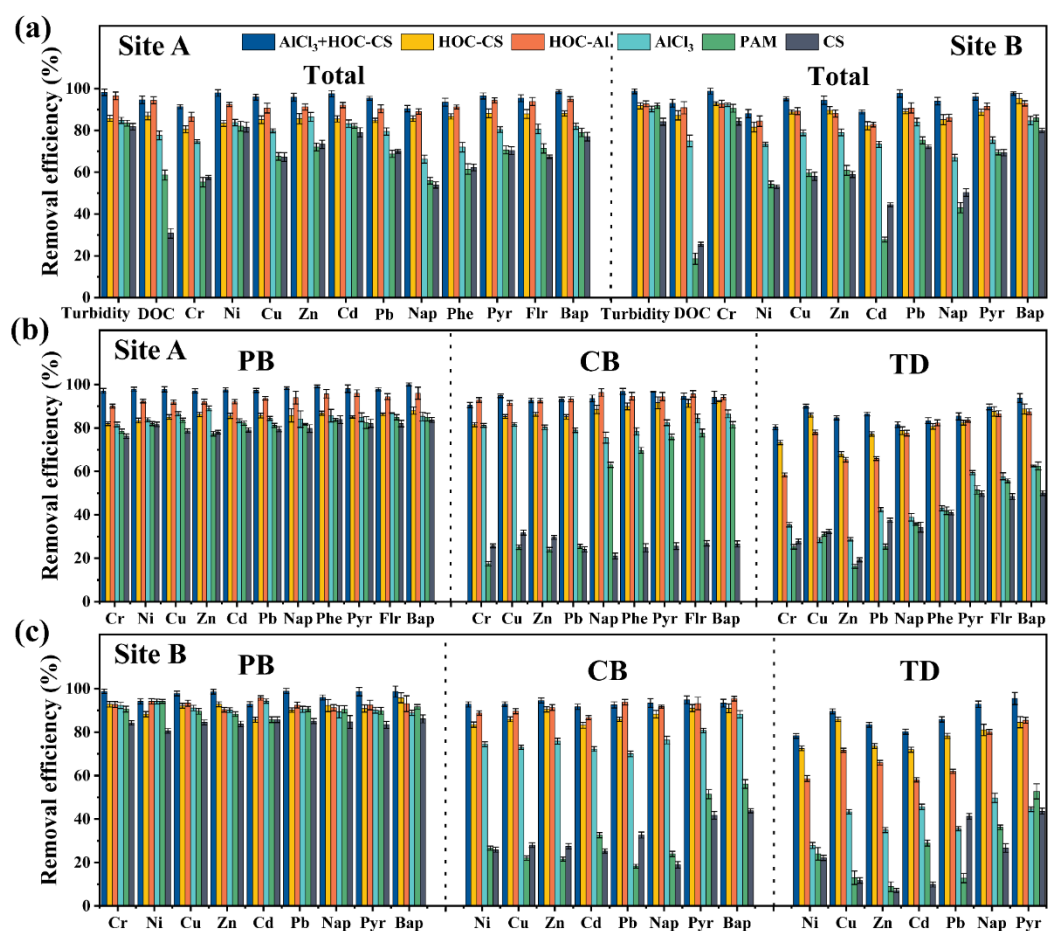


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Figure 4

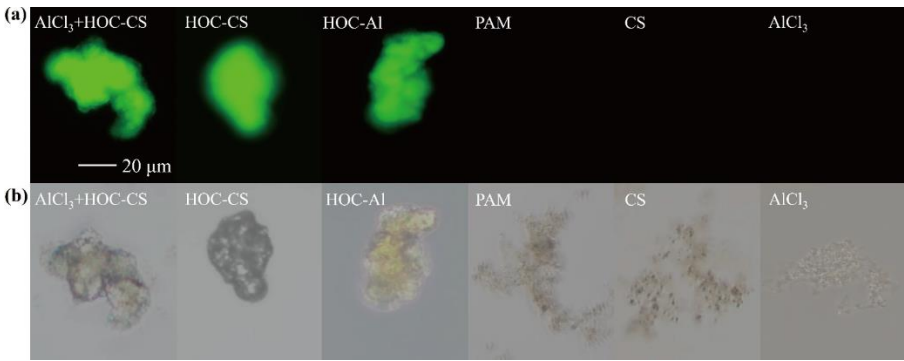


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Figure 5

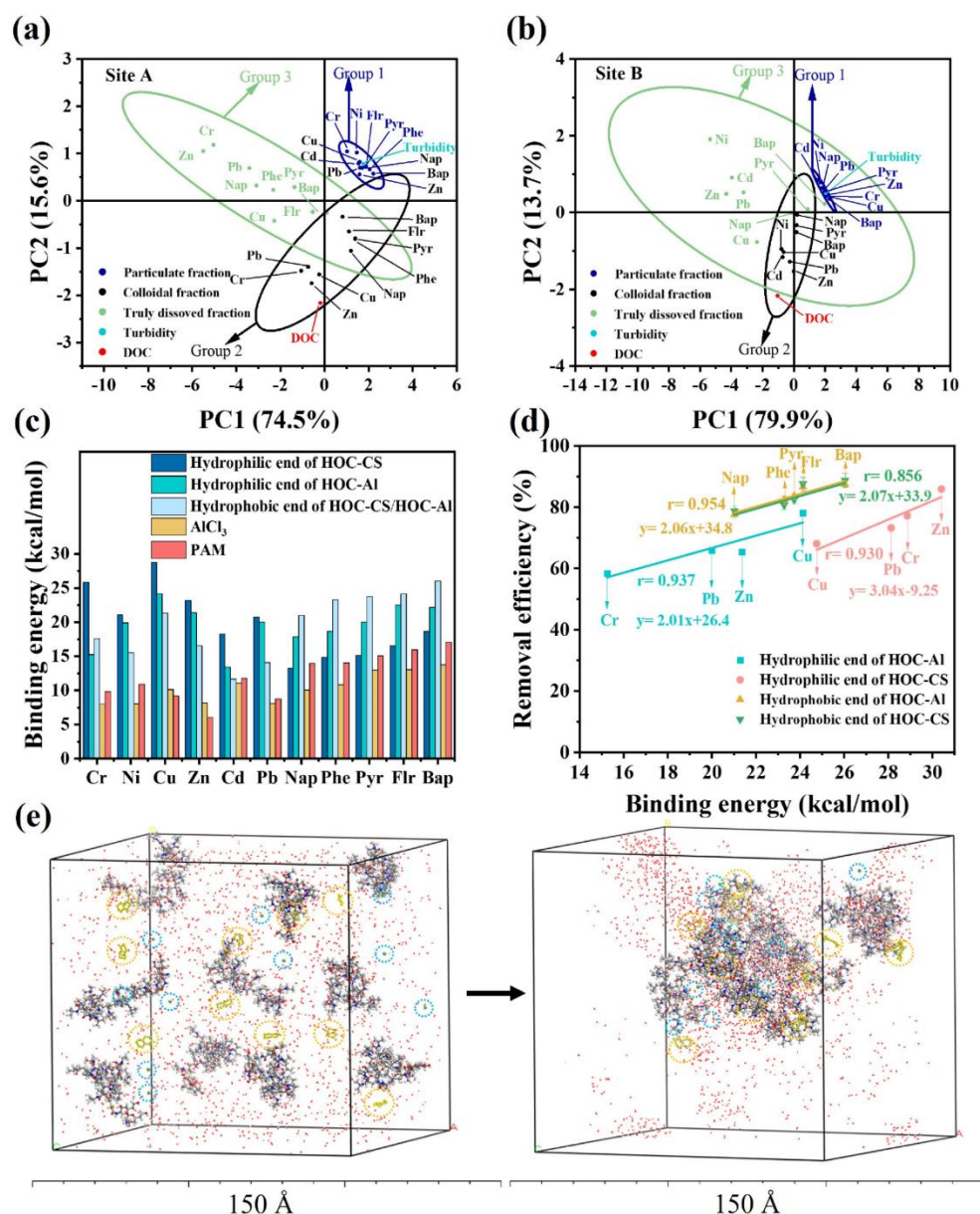


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Figure 6

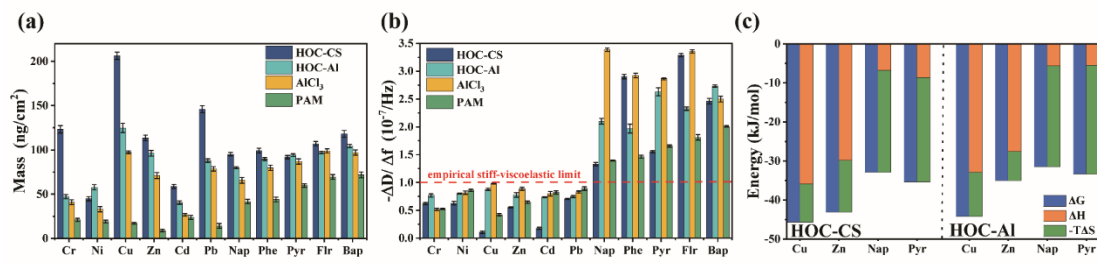


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Figure 7

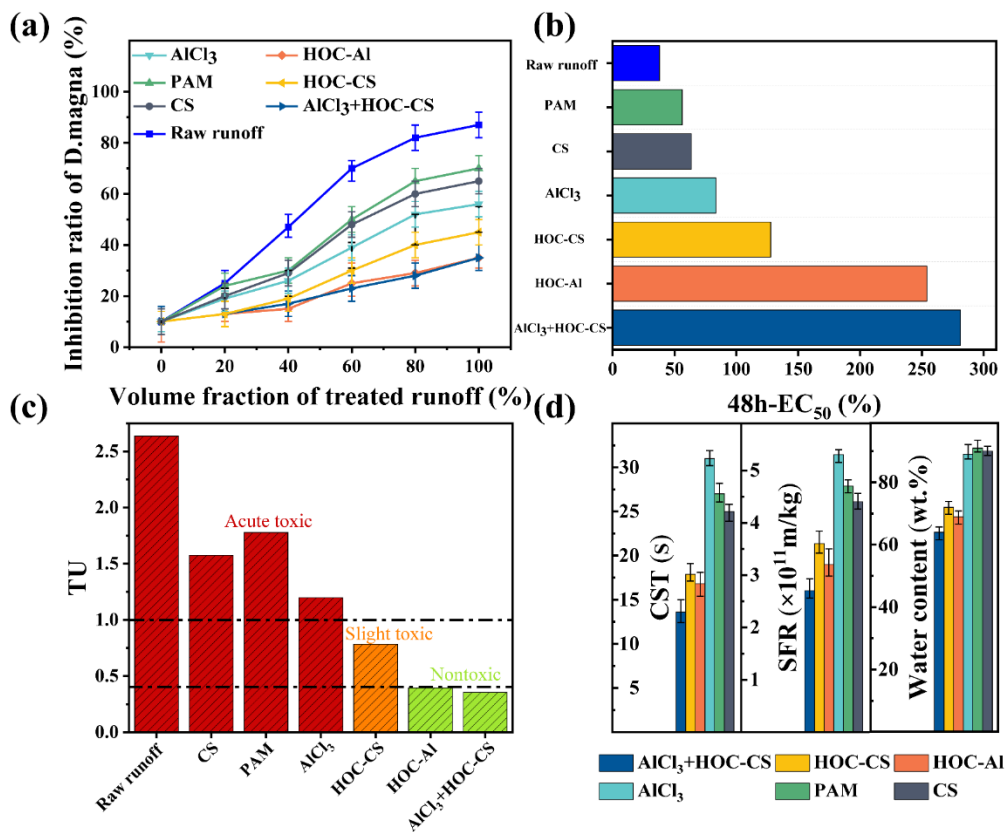
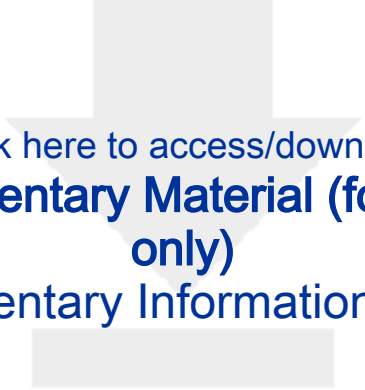


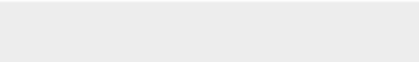
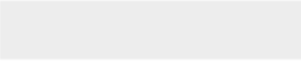
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**Electronic Supplementary Material (for online publication
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Supplementary Information R1.docx



Author contributions

Conceptualization: Z.Y., N.J.D.G.; Methodology: Z.Y., L.Z., T.L., Z.T., and B.B.; Software: R.C. and Z.T.; Resources: Z.Y., Z.T. and B.B.; Investigation and validation: L.Z., T.L., and R.C.; Formal analysis: Z.Y., L.Z., T.L., and R.C.; Visualization: Z.Y., L.Z., T.L. and R.C.; Writing - Original Draft: Z.Y., L.Z., and T.L.; Writing - Review & Editing: Z.Y. and N.J.D.G.; Supervision: Z.Y. and N.J.D.G.; Funding acquisition: Z.Y. and B.B.